International Organisation of Vine and Wine

RESOLUTIONS
of the 7th GENERAL ASSEMBLY

(adopted by consensus)

Zagreb (Croatia) – 3rd July 2009

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Summary of resolutions adopted by the 7th General Assembly of the OIV - Zagreb (Croatia)

The 7th General Assembly of the International Organisation of Vine and Wine (OIV), held in Zagreb (Croatia), adopted a total of 37 resolutions. The objective of this summary of the principle resolutions is to provide an insight into the most important decisions made by the General Assembly of the OIV.

Decisions concerning OIV Rules

The General Assembly of the OIV decided by consensus to modify the general provisions of the Internal Rules of Procedure, in order to enable international intergovernmental organisations made up of sovereign states to have the option to request a particular status. **(Resolution OIV/Comex 05/2009)**.

The General Assembly adopted guidelines which define the criteria for OIV sponsorship for wine and spirituous beverages of vitivinicultural origin competitions. In order to benefit from the patronage of the OIV, the organisers of international competitions must comply with the new OIV standard for international wine competitions and spirituous beverages of vitiviniculture origin and these guidelines in force when the request is made. **(Resolution OIV/Concours 332B/2009)**.

Decision concerning the OIV competition standard

The General Assembly likewise decided to replace the OIV international wine competitions standard adopted in 1994 and the spirituous beverages of vitivinicultural origin competition adopted in 1999 by a new standard. This standard applies to competitions which request OIV patronage for which the granting conditions are defined by guidelines as set out in the Internal Rules of Procedure of the OIV. This new standard takes into account the evolution of international standards and validates a single tasting sheet carried out in cooperation with the International Union of Oenologists. **(Resolution OIV/Concours 332A/2009)**.

Decision concerning Viticulture

The 7th General Assembly approved a resolution related to an evaluation protocol of vines obtained through genetic engineering. The research works, carried out in several countries, are focused on improving existing vine varieties using genetic engineering techniques and are in the process of producing genetically modified vines (« GM vines »). This resolution recommends that the member states adapt, and where relevant, integrate the guidelines found in this protocol in accordance with their respective regulatory regimes. This protocol integrates the general bases and objectives for the evaluation of genetically modified vines in addition to the commercial use of genetically modified vines. **(Resolution OIV/Viti 355/2009)**.

Decisions concerning oenological practices

Among the decisions adopted by the General Assembly, several resolutions concerning new oenological practices complete the International Code of Oenology of the OIV. These include:

- Treatment by silver chloride in order to reduce olfactory defects due to hydrogen sulphide and certain mercaptans. The dose used must not exceed 1g/hl. The precipitate must be eliminated by

sedimentation and/or filtration and the silver content limit in wine msut be under 0,1 mg/l. Residues must be treated by a sector specialised in residue treatment. **(Resolution OIV/Oeno 145/2009)**.

- The maceration of raisined grape or their marc on wine. The objective of this new practice is to increase sugar content of wine, phenolic and aromatic compounds. **(Resolution OIV/Oeno 278/2009)**. Parallel to this resolution, is a general sheet on maceration, which was adopted and which combines different techniques admitted in this field. **(Resolution OIV/Oeno 196/2009)**.

- The fining of musts and wine by chitosan or chitine glucan of fungal origin. The objective of these practices is to admit these two compounds for musts and wine in order to facilitate racking and clarification in addition to carrying out preventive treatments for protein haze for musts **(Resolution OIV/Oeno 336A/2009, OIV/Oeno 336B/2009) and for wine**, to reduce turbidity by precipitating particles in suspension
  and to carry out preventive treatment on protein haze **(Resolution OIV/Oeno 337A/2009, OIV/Oeno 337B/2009)**. These two compounds will also be added to list of admitted fining products **(Resolution OIV/Oeno 339A/2009, OIV/Oeno 339B/2009)**.

- The objective of wine treatment by chitin glucan of fungal origin is to reduce heavy metal content particularly iron, lead, cadmium and copper, to prevent iron haze, copper haze, to reduce possible contaminants, particularly ochratoxin A. In addition to these objectives, chitosan treatment, also adopted, enables the reduction of undesirable micro-organisms, particularly *Brettanomyces*. Maximum dose is set in accordance with objectives to be reached. **(Resolution OIV/Oeno 338A/2009, OIV/Oeno 338B/2009)**.

Decisions concerning specifications of oenological products

A certain number of decisions concern monographs complete the International Oenological Codex. These include:

- A new monograph concerning hemicellulase enzymes. These enzymes are used during grape maceration. This activity can be estimated by the hydrolysis of potato galactans. Enzymatic preparations containing these activities come from directed fermentations of *Aspergillus niger* and/or a mixture of *Aspergillus niger* – *Trichoderma reesei*. This monograph details the object, origin and field of application in addition to purity criteria and the methods of determination of enzymatic activity **(Resolution OIV/Oeno 313/2009)**.

- Another monograph concerns pectinelyase enzymes. These activities are used to promote the maceration of grapes, for the clarification of musts and wines, to improve filterability of musts and wine and grape pressing. Enzymatic preparations containing these activities come from directed fermentations of *Aspergillus*. This monograph details the object, origin and field of application in addition to purity criteria and the methods of determination of enzymatic activity. **(Resolution OIV/Oeno 314/2009)**.

- A new monography on lactic bacteria which replaces the existing monography in the International Oenological Codex. This monography details all the prescriptions which apply to lactic bacteria in addition to certain mediums used for analysis and control. **(Resolution OIV/Oeno 328/2009)**

- A new monograph on active dry yeast which replaces the existing monograph in the International Oenological Codex. This monograph details all the prescriptions which apply to active dry yeast in addition to certain mediums used for analysis and control **(Resolution OIV/Oeno 329/2009)**

- A monograph related to the determination of the ability of an enzymatic preparation to interrupt pectic chains by measuring viscosity. This is a purely technological measurement designed to test the true clarifying efficiency of the enzyme. It essentially measures the pectinase activity, which cannot be directly deduced from the release of galacturonic acid in the medium. **(Resolution OIV/Oeno 351/2009).**

- The modification of the existing monograph on oenological tannins by adding a differentiation of commercial oenological tannins by GC-MS analysis of monosaccharides and polyalcohols. The method described here is suitable for the differentiation of commercial oenological tannins from different origins. The concentration of monosaccharides and polyalcohols in tannin samples is thus determined by gas chromatography-mass spectrometry (GC-MS) after their previous derivatization into their trimethylsilyl ethers. **(Resolution OIV/Oeno 352/2009)**.

- A modification of the existing monography on enzymatic preparations as laid out in the International Oenology Codex. This resolution describes all enzymatic preparations susceptible of being used during various operations that can be applied to grapes and their derivatives. **(Resolution OIV/Oeno 365/2009)**.

- A new monograph concerns carboxymethylcellulose. This monograph describes all prescriptions which apply to carboxymethylcellulose, particularly its origin and different limits. Cellulose gum (carboxymethyl cellulose) for oenological use is prepared exclusively from wood by treatment with alkali and monochloroacetic acid or its sodium salt. **(Resolution OIV/Oeno 366/2009)**

- Two new monographs which concern chitin-glucan **(Resolution OIV/Oeno 367/2009)** and chitosan **(Resolution OIV/Oeno 368/2009)**. Chitin-glucan is of fungus origin and is a natural polymer, the main component of the cellular walls of *Aspergillus niger*. Chitosan is a polysaccharide prepared based on fungal origin. It is extracted and purified from fungal food sources or sure and abundant biotechnologies such as *Agaricus bisporus* or *Aspergillus niger*. This monograph likewise describes all prescriptions which apply to chitin-glucan and chitosan.

Decisions concerning methods of analysis

Finally, during this same session the member states adopted new analysis methods which are encompassed in the International Compendium of Method of Analysis of Wine and Musts of the OIV, including:

- The new classification of methods of analysis of musts and wines of the OIV. The Member states of the OIV consider the need to classify certain methods of analysis according to new classification criteria mentionned in Resolution 9/2000. The methods included in the Compendium of Methods of Analysis of Wine and Musts will be, if required, consequently modified. Moreover, certain methods of analysis which are no longer used will be eliminated from the Compendium of International Methods of Musts and Wine. **(Resolution OIV/Oeno 377/2009)**

- Recommendations related to correcting the recovery rate which must be included in the Compendium of Methods of Analysis. The OIV recommends certain procedures concerning proceedings reports related to the recovery rate of analytical results. **(Resolution OIV/Oeno 392/2009)**.

- The method of determination of releasable 2,4,6-trichloroanisole (TCA) by cork stoppers measures the quantity of TCA released by a sample of cork stoppers macerated in a aqueous-alcoholic solution. The aim of this method is to evaluate the risk of releasing by the lot of analyzed cork stoppers and to provide a method for controlling the quality of cork stoppers.
  The method aims to simulate 2,4,6-trichloroanisole (TCA) migration phenomena susceptible of being produced between the cork stopper and wine in bottles. Cork stoppers are macerated in a wine or a aqueous-alcoholic solution, until a balance is obtained. The TCA of the head space is sampled from an appropriate part of the macerate by the solid-phase micro-extraction technique (SPME), then analyzed by gas chromatography, with detection by mass spectrometer (GC/MS) or by electron-capture detector (GC/ECD). **(Resolution OIV/Oeno 296/2009)**

- Determination method of glutathion content in musts and wine by capillary electrophoresis. The separation of a mixture's solutes by capillary electrophoresis is obtained by differential migration in an electrolyte. The capillary tube is filled with this electrolyte. This method makes it possible to determine the glutathion content of musts and wines in a concentration range of 0 to 40 mg/L. It

uses capillary electrophoresis (CE) associated with fluorimetric detection (LIF). **(Resolution OIV/Oeno 345/2009)**

- Analysis of biogenic amines in musts and wines using HPLC. This type II method can be applied to the analysis of biogenic amines in musts and wine. Seventeen biogenic amines can be separated by this method. The biogenic amines are directly measured by HPLC using a C18 column following an O-phtaldialdéhyde (OPA) derivation and detected by fluorimetry. (**Resolution OIV/Oeno 346/2009)**

- The modification of the current type II method of the $^{18}O/^{16}O$ isotope ratio of water from wine in Annex A of the Compendium of International Methods of Analysis of Wine and Must. The method describes the determination of the $^{18}O/^{16}O$ isotope ratio of water from wine and must after equilibration with CO_2, using the isotope ratio mass spectrometry (IRMS). The method is based on the isotopic equilibration of water in samples of wine or must with a CO_2 standard gas. After equilibration the carbon dioxide in the gaseous phase is used for analysis by means of Isotopic Ratio Mass Spectrometry (IRMS) where the $^{18}O/^{16}O$ isotopic ratio is determined on the CO_2 resulting from the equilibration. **(Resolution OIV/Oeno 353/2009).**

- Supplement to the « Total dry extract » method contained in the « Compendium of Methods of Analysis of Wine and Musts» with the adding of a note related to the calculation of total dry extract taking separately into account quantities of glucose and fructose (reducing sugars) and the quantity saccharose. **(Resolution OIV/Oeno 387/2009).**

- A modification of a method of analysis currently contained in the « Compendium of Methods of Analysis of Wine and Musts» of the OIV on the research and dosage of polychlorophenols and polychloroanisols, in wine, stoppers, wood and bentonites used as atmospheric traps. The principle of this type IV method is based on the dosage of 2,4,6-trichloroanisole, and 2,4,6-trichlorophenol, 2,3,4,6-tetrachloroanisole, 2,3,4,6-tetrachlorophenol, pentachloroanisole and pentachlorophenol by chromatography in the gaseous phase by injection of a wine hexane extract and ether/hexane of solid samples to be analysed and internal sampling. **(Resolution OIV/Oeno 374/2009)**

- The revision of the Compendium of International Methods of Analysis of Spirituous beverages of vitivinicultural origin, alcohol and the aromatic fraction of beverages. In effect, four resolutions concerning this revision were adopted taking into account that certain methods of analysis are no longer used and that they should be eliminated from the Compendium. The evolution of methods and the availability of inter laboratory parameters since 1994 should likewise be taken into account. **(Resolutions OIV/Oeno 379/2009, OIV/Oeno 380/2009, OIV/Oeno 381/2009, OIV/Oeno 382A/2009)**. As such, these decisions shall lead to the OIV publishing a new « Compendium of International Methods of Analysis of Spirituous beverages of vitiviniculture origin ».

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RESOLUTION OIV/COMEX 05/2009

MODIFICATION OF PARAGRAPH 1 OF ARTICLE 4 OF CHAPTER 1 OF THE INTERNAL RULES OF PROCEDURE

The General Assembly of the International Organisation of Vine and Wine,

Considering that a sovereign state, that wishes to attend, intervene or participate in the activities of the OIV shall have the option to request Observer or Member status,

Considering that this option is only possible for international inter-governmental organisations made up of Sovereign states that have authority transferred by its Member States,

Considering that the Member states of the OIV, during the establishment of the OIV, specifically requested that international inter-governmental organisations shall have particular status by creating a Chapter VI « Participation of international inter-governmental organisations » in the Agreement of 3 April 2001,

Considering that this Chapter VI, is made up Article 8 which provides that « An international inter-governmental organisation may participate in the works of the OIV or be a member with a financial contribution to the Organisation under conditions to be set on case by case basis by the General Assembly on the proposal of the Executive Committee. »

DECIDES to modify paragraph 1 of article 4 of Chapter 1 « Nature of the Organisation, membership conditions, withdrawal conditions » of Title I « General provisions » of the Internal Rules of Procedure, to enable international inter-governmental organisations made up of sovereign states to have the option to request particular or member status.

In Article 4 point 1 the words « that does not fulfil all the conditions set out in Article 2.2 paragraph (b) » are deleted.

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

RESOLUTION OIV/CONCOURS 332B/2009

GUIDELINES FOR GRANTING OIV PATRONAGE OF INTERNATIONAL WINE AND SPIRITUOUS BEVERAGES OF VITIVINICULTURAL ORIGIN COMPETITIONS

The General Assembly of the International Organisation of Vine and Wine,

Adopts the following document and requests the secretariat to implement:

Guidelines for granting OIV patronage of international wine and spirituous beverages of vitivinicultural origin competitions

In accordance with article 24.3 of the Internal Rules, the International Organisation of Vine and Wine may grant patronage to international or national wine and spirits of vitivinicultural origin competitions, provided that their organisation and internal rules procedures are in accordance with the international standards of the O.I.V.
The present document sets the guidelines which apply to granting patronages for International competitions (Title I) and national competitions (Title II).

Title I: International competitions

1. SUBJECT: Definition of conditions and procedure for granting sponsorship of the O.I.V. for international wine and spirited beverages of vitivinicultural origin competitions in accordance with article 24.3 of the Internal Rules.
There can be two types of international competitions; general, that being open to all categories as provided for in the standard to international competitions, or by category, that being limited to one or several categories of wine or spirituous beverages of determined vitivinicultural origin.

2. CONDITIONS FOR GRANTING PATRONAGE:

2.1 Organisers of international wine and spirituous beverages of vitivinicultural origin must comply with standards of the O.I.V. for international competitions for wine and spirituous beverages of vitivinicultural origin and the present guidelines in force when the request is made.

2.2 The competition must have an international outlook.

- Participation in a previous competition with samples from at least 8 countries for general competitions or at least 5 for category competitions is required;
- At least 20 % for general competitions or at least 15 % for category competitions of total number of samples presented at the previous competition must come from countries other than the organiser's.

2.3 Each competition shall require the presentation of over 500 samples for general competitions or 300 for category competitions at a previous competition.

Certified in conformity
Zagreb, 3rd July 2009
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However, in derogation of this rule, international competitions which have the accreditation or tutelage of public bodies of organiser countries may be exempted from observing the limits mentioned in points 2.2 and 2.3 to request patronage of the O.I.V.

2.4 A previous edition of the competition (without patronage) must be organised with the participation of an observer, appointed by the O.I.V., to review and report on operations. This trial observation shall not be required for an already sponsored competition set up by the same organiser on another territory. An international competition given to a new organiser shall loose O.I.V. patronage and shall be observed in order to be granted this patronage once again.

2.5 Organisers of international competitions shall share the administrative expenses of patronages by paying a contribution amount to the O.I.V., set by the Executive Committee of the O.I.V., per sample.

2.6 However, for request for patronage for competitions which are members of the VINOFED and which are observers of the O.I.V. shall be made directly through the Vinofed secretariat. Based on the payment of an observer contribution by Vinofed, competitions which are members are exempt from paying a contribution amount per sample provided that observer status is maintained.

3. **DOCUMENTS TO BE PROVIDED BY THE ORGANISER**:

The organiser shall supply all required documents as set out in the attached form in addition any information the organiser deems useful, along with the application form in one of the official languages of the O.I.V.

4. REVIEW OF REQUEST:

4.1 The request for patronage shall be addressed to the Director General of the O.I.V. with the available information using the corresponding application form for competitions.

4.2 The Director General of may request any information he/she deems necessary for the review of the request.

4.3 When the event is organised in a member's country or by a person of a member country, the request with the supplied information shall be conveyed by the Director General of the O.I.V. to the delegate(s) of these members of the Executive Committee for opinion.

4.4 The Director General shall convey documents provided by the organiser, in addition to the appointed observer's report (in the case of a previous edition) or from the commissioner expert (for future editions) to the members of the Scientific and Technical Committee of the O.I.V. for opinion and to the delegates of the Executive Committee.

4.5 In order to be reviewed by the next CST and COMEX of the O.I.V., the Director General must receive requests by 31 January at the very latest for review in March, or by 15 September for review in October; that being at least four months before the event takes place. On an exceptional basis, when the decision making bodies of the O.I.V. are unable to render a decision within the time delay, the Director General, when the above-mentioned criteria has been fulfilled, following written consultation of the delegate(s) or the member(s) concerned by the event, shall address the members of the Steering Committee for decision. The Director General shall convey this decision of the Steering Committee to all members of the Executive Committee.

4.6 The name, acronym or logo of the International Organisation of Vine and Wine shall in no case be used in documents, information or news releases done by the organiser of international competitions. Their use is subject to being granted patronage. In no case shall the fact of referring to the O.I.V. authorise prior usage.

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The General Director of the OIV
Secretary of the General Assembly

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5. GRANTING PATRONAGE:

5.1 The decision to grant or not grant patronage shall be made by the Executive Committee after opinion of the Scientific and Technical Committee, or under specific conditions as set out in above-mentioned 4.5, by the Steering Committee.

5.2 The decision shall be conveyed by the Director General.

5.3 This decision shall be definitive and may not be appealed.

5.4 Patronage for an international competition shall be granted for one edition.

6. BENEFITS AND OBLIGATIONS LINKED TO GRANTING PATRONAGE

6.1 Approval given by the O.I.V. shall imply that reference of this patronage be included on all background documents related to the competition with the mentions « under the auspices of or the high patronage of the International Organisation of Vine and Wine ». The O.I.V. initials shall not be translated or modified. The logo shall be used in reference with this patronage.

6.2 Approval given by the O.I.V. commits the competition organiser to bear the expenses of the expert commissioner appointed by the O.I.V. to monitor the application of the standards of the competition. Bearing expenses under the same conditions as those given to all the members of the jury includes travel expenses, housing, and registration fees for the person appointed by the O.I.V. These obligations shall be applied mutatis mutandis to the observer appointed by the O.I.V. for a first request for patronage.

6.3 The agreement given by the O.I.V. commits the organiser of the event to address to the O.I.V. all acts and notably list of winners, which shall be published for the event.

6.4 The agreement given by the O.I.V. commits the organiser of the competition to pay the contribution as provided in point 2.5 of the present guidelines to the O.I.V.

Title II: National competitions (to be drafted)

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The General Director of the OIV
Secretary of the General Assembly

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REQUEST FOR PATRONAGE
OFFICIAL APPLICATION FORM

- Name of organiser:

Requests O.I.V. patronage for an international wine and/or spirituous beverages of vitivinicultural origin competition entitled:

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which shall be conducted in compliance with the enclosed application form.

I pledge to respect the O.I.V. international wine and spirituous beverages of vitivinicultural origin competition standard and guidelines for granting sponsorships.

Done at: on

Signature

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

REQUEST FORM FOR PATRONAGE

INTERNATIONAL WINE AND SPIRITUOUS BEVERAGES OF VITIVINICULTURAL ORIGIN COMPETITION

- Name of competition:

- Date: Place: Country:

- Organiser in charge:
  (Complete name, phone number and address)

  Telephone Fax E-mail

- Rules and procedure for the competition: (mandatory document must be enclosed)

- List of wine tasters (confirmed or programmed):

- Other requested patronages:

- Medium and means of broadcasting winners and/or acts programmed:

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The General Director of the OIV
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RESOLUTION OIV/CONCOURS 332A/2009

OIV STANDARD FOR INTERNATIONAL WINE AND SPIRITUOUS BEVERAGES OF VITIVINICULTURAL ORIGIN COMPETITIONS

THE GENERAL ASSEMBLY,

Taking note of the works of the « International wine and spirituous beverages of vitivinicultural origin competition » ad hoc group,

DECIDES:
To replace the OIV international wine competition standard, adopted in 1994, and the international spirituous beverage of vitivinicultural origin competition standard, adopted in 1999, with the following standard:

OIV STANDARD FOR INTERNATIONAL WINE COMPETITIONS AND SPIRITUOUS BEVERAGES OF VITIVINICULTURAL ORIGIN

Article 1: DEFINITION AND OBJECTIVES

The present standard provides that “competitions” refer to competitions with previously registered wine or spirituous beverages of vitivinicultural origin which are eligible to be granted an award based on quality. The quality is evaluated by a qualified jury and the competition is carried out in strict compliance to the provisions set out in the present standard.
The objective of the international competition shall be:

- to promote knowledge of wine and spirituous beverages of vitivinicultural of outstanding quality,
- to encourage their production and responsible consumption as an active part of civilisation,
- to make known and present characteristic types of wine and spirituous beverages of vitivinicultural origin, produced in various countries, to the public,
- to raise the technical and scientific level of producers,
- to contribute to the expansion of their production,

Article 2: FIELD OF APPLICATION

2-1. Competitions sponsored by the OIV

Without prejudice to stricter rules, the present standard applies to competitions which call upon the sponsorship of the O.I.V. The conditions for granting sponsorship are defined by the guidelines as provided in the Internal Rules of the O.I.V.
For these competitions, the O.I.V. appoints an expert commissioner for the competitions. He/she participates in the works of the different juries. He/she has the duty of verifying that

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rules enacted by the present standard are abided. The Organiser must, prior to or simultaneously to the conducting of the competition, allow the commissioner to access all useful information by putting a technically competent person at his/her disposal. He/she draws up a detailed report for the O.I.V. on the conducting of the competition. Based on this report, the O.I.V. may make recommendations and may cancel sponsorship before the forecasted deadline.

Only those competitions sponsored by the O.I.V. are authorised to use the name, abbreviation the logo of the International Organisation of Vine and Wine under the conditions set out in the above-mentioned guidelines. The competitions are presented on the O.I.V. internet site.

2-2: Other competitions

The organisers of other competitions which use all or part of the present standard may under no conditions make reference to the O.I.V.
The O.I.V. shall take action it deems opportune to prevent the improper use of it name, abbreviation or logo.

Article 3: PRODUCTS ELIGIBLE TO COMPETE

The competition is open, without discrimination, to all wines and vitivinicultural spirits, in accordance with the definitions of the "International Code of Oenological Practices" of the O.I.V. All products must have an indication of the country of origin and of the place where grapes were harvested and where the spirituous beverages of vitivinicultural origin were made.
All these products must be destined for sale and must be from a homogeneous batch of at least 1000 litres. On an exceptional basis, a reduced volume, of at least 100 litres, may be admitted upon justification of a particularly low production. All samples must be presented with labels and commercial presentation. If the product was packaged specifically for the competition, the sample shall be accompanied by explanatory documents justifying the status.

Article 4: REGISTRATION PROCEDURE

The registration form for each sample must include:

- the complete and exact identification of the participant who has the right to market or distribute the batch corresponding to the samples
- the exact designation of the product, in accordance with the regulations of the country of origin, the colour as concerns wines, and if possible, the vintage and indication of age and any possible contact with wood
- the category of the product, in accordance with Annex 1, (CATEGORISATION OF SAMPLES IN DIFFERENT CATEGORIES), sub-groups may be further divided or regrouped, at the discretion of the organiser,

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- the analysis certificates, in conformity with Annex II, carried out by an accredited laboratory of by a competent laboratory in conformity with the laws of the country or certified by an oenologist[1] in charge of the wine cellar,
- grape variety (ies) and their percentage in wine and, if necessary, in spirituous beverages of vitivinicultural origin,
- the quantity of the sample available.

Article 5: TESTING AND STORAGE OF SAMPLES RECEIVED

1. The organiser shall verify the receipt of samples with the registration form and the official documents accompanying them. Attention shall be paid to the correct use of appellations of origin and geographic indications. Samples which do not respond with the provisions of the present standard shall be rejected.

2. Samples must be stored in a secure place under temperature and atmospheric conditions guaranteeing preservation.

Article 6: CLASSIFICATION AND ORGANISATION OF SAMPLES PRESENTED

After testing, based on the registration documents and analysis certificates, subject to the accuracy of the registration in each category, the samples shall generally be presented to the jury, in each category, in the order of vintages whilst taking into account the contents of sugars and, where relevant, according to the wooded or unwooded characteristics.

Article 7: DESIGNATION OF JURORS

1. The organiser responsible for the competition shall designate the jurors. The organiser may ask the O.I.V. to suggest names of experts from different countries.

[1] International definition of oenologist (Resolution 7/76)

A- Definition

An oenologist is a person, based on his/her scientific and technical skills with a corresponding diploma, is capable of carrying out, the duties as defined hereinafter, with due respect to good and fair practices

B- Functions of an oenologist

An oenologist shall have the following duties:

a) to rationally apply information received or information found in scientific and technical revues, and where relevant, to carry out technological research
b) to collaborate with conception of technological material and for equipping wine cellars
c) to collaborate with setting up and growing vineyards
d) to be responsible for making grape juice, wine and grape based products and to ensure proper storage
e) to carry out analyses (physical, chemical, microbiological or organoleptic) for the above mentioned products and to interpret results
f) to be able to assess existing relations between vitivinicultural economy and legislation and oenological techniques, and to organise product distribution

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2. The samples shall be evaluated by international juries, whose numbers shall be set according to the nature of wine and spirituous beverages of vitivinicultural origin presented at the competition.

3. In a jury, it is appropriate to respect a great geographic dispersion in the origin of the jurors. Within the same jury, the absolute majority of the jurors may not come from the organiser country. It is desirable that one of the jurors represent a mainly consumer country.

4. In principle, each jury shall be composed of 7 jurors, of which a maximum of three nationals may be from the organising country. In any case, the number of jurors can not be less than 5 of which a maximum of two nationals from the organising country.

All the jurors shall have tasting technique skills and the majority of jurors shall be oenologists or persons with an equivalent diploma in the field of wine or spirituous beverages of vitivinicultural origin. Persons having demonstrated a high qualification for tasting in the field of wine and spirituous beverages of vitivinicultural origin may complete the jury. The organiser must ensure that they have data about jurors, which is constantly kept up to date, to ensure their constant evaluation.

5. The juries shall function under the authority of a President, who should be an oenologist, and who may be a member of the jury or a person with an equivalent diploma in the field of wine or spirituous beverages. One single President may likewise be envisaged. The President shall ensure, in liaison with the organiser, the general functioning of the jury, in accordance with article 10.

6. The jurors shall be designated "intuitu personae". Therefore, they may only be replaced by the authority which designated them. The organiser must take care that a juror does not participate in a jury if he/she has a commercial relationship in relation to one of the samples presented.
Likewise, a juror must not be invited to participate according to his/her contribution in terms of samples but based on his/her skills.

Article 8: DUTIES AND RESPONSABILITIES OF THE ORGANISER

The organiser of the competition shall be solely responsible for the material running of the competition and its' inherent risks. He/she may be assisted in his/her duties by a legal expert.

The organiser shall ensure correct preparations, examination of samples and communication of the results as well as constant evaluation of the jurors. The organiser shall endeavour:
- to oversee the absolute anonymity of samples submitted to the jurors as well as the secrecy of the results until the close of the competition,
- to test the organisation of the tasting for which it has responsibility before constitution of the juries. Notably it shall verify the order in which the samples shall be presented,
- to test the opening of the samples in an adjoining room, their temperature, their identity and their anonymity for the members of the jury; to also oversee correct handling and serving,

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Secretary of the General Assembly

Federico CASTELLUCCI

- to test the running of the secretariat responsible for the examination of the results; distribution and collection of score sheets, verification of identification of samples, calculations, posting of the results,
- to provide an information session beforehand to all jurors on the use of the score sheet and its descriptors, correctly illustrated by one or two practical examples,
- to ensure that any comments from the tasting which may be circulated are the result of tasting during the competition,
- to allow a second tasting of a sample, notably when:
- the majority of jurors request it,
- and each time the President of jury deems it useful, to allow on an exceptional basis, a tasting by another jury.

Article 9: GENERAL FUNCTIONNING OF THE JURIES

1. Discipline

Absolute anonymity shall be a fundamental principle of competitions, consequently:

1.1 The jurors shall remain silent and make no gestures or facial expressions indicative of their impressions during the wine tasting and rating.

1.2 Before each sample shall be served, a data sheet of samples presented and corresponding score sheets shall be placed at the disposal of the jurors. Technical indications related to samples shall be indicated on the score. The name of the juror or his/her identification and his/her signature shall be included on the score sheets. For computerised competitions, a data sheet of the samples with the jury's average score must be provided to the President of the jury j every day for control purposes and signature.

1.3 The personnel collecting the sheets shall ensure that score sheets are correctly completed. The president shall sign the sheets for authentication and to verify the correct entry of data for electronic score sheets entries for computer entries.

1.4 Duplicates of score sheets shall not be left with the jurors.

1.5 The organiser shall provide jurors with a consolidated list with the geographic origin and, if possible, grape varieties of each sample, at the end of the daily tasting sessions.

2. Material functioning

Once the jury is formed, the jurors shall meet for one or more preliminary explanatory meetings and for a group tasting. Comparisons of results shall be made with different jurors. Special attention shall be paid to score sheet instructions and to the significance of descriptors.

2.1 The jury or juries shall be seated in an isolated, quiet, well-lit, well-ventilated, odour-free room. Access shall be in principle prohibited to all persons who are not essential to the organisation of the tasting. Ambient temperature must be maintained, to the extent possible, between 20 and 24 °C.
Smoking shall be forbidden. In addition, the juries must avoid using perfumes, which may disturb the sessions. Mobile phones must be turned off.

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2.2 A second adjacent room, outside the jurors' view, shall be reserved for opening bottles and for concealing all signs enabling the sample to be identified. Smoking is forbidden. Strict discipline and silence shall be observed.

2.3 In all cases, the bottles shall be placed, beforehand, in packaging which conceals the form of the bottle and guarantees the anonymity of the sample. This packaging must guarantee anonymity for the duration of the tasting. When necessary, the original stoppers shall be replaced by an anonymous closing system; In addition, the jurors must never know the identity of the samples presented.
The number of the service on the packaging must be different than the registration number. The expert commissioner of the O.I.V. shall ensure the manner by which the anonymity of the sample is guaranteed.

After controlling the service number and the agreement of the President of the Jury, the glasses shall be filled in the tasting room in front of each juror.

2.4 Each juror shall receive a permanent number. The juror shall be provided with a seat and an individual table with a white surface bearing his/her number and with:

- a carafe of cold water
- pieces of bread
- paper towels or handkerchiefs
- discard receptacle.

2.5 Each sample must be presented in a glass corresponding to the category, at least the standardised international type (ISO 3591: 1977). It is recommended that glasses be changed for every sample.
2.6 The tasting session shall preferably take place in the morning. Each juror shall taste at the rate of no more than 45 samples per day, without prejudice to possible tastings asked again by the President of the Jury, in three sessions of approximately 15 samples each of dry wine or two sessions of 15 samples of dry wine to which may be added a session of 10 samples from other categories.
For spirituous beverages of vitivinicultural origin, the taste-testing shall be done at the rate of no more than 30 samples per day, in five sessions of approximately 6 samples each. In the case of going over these levels and within the limit of 50 samples per day, detailed information must be explicitly mentioned in the competition regulations.

3. Presentation of wine and spirituous beverages of vitivinicultural origin.

Each product shall be tasted individually and not in comparison.

4. Breaks

There shall be a 15 minute minimum break between each session, during which the organiser shall ensure that the jurors have beverages and food available which shall not interfere in pursuing taste-testing.

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Article 10: ORDER OF PRESENTATION OF SAMPLES AND TEMPERATURE

1. The objective of categorising wine and spirituous beverages of vitivinicultural origin shall essentially be to present homogenous, successive series of samples based on the following categories and criteria: geographic provenance, vine varieties[2], vintage, sugar contents, wooded or unwooded characteristics. These series must be reviewed in a rational order.

1.1 The wines shall be tasted by jurors during sessions, in principle in the following order:

1. Sparkling whites
2. Still whites
3. Sparkling rosés
4. Still rosés
5. Sparkling reds
6. Still reds
7. Wine under a film of yeasts
8. Naturally sweet wine
9. Ice wine
10. Liqueur wine
11. Mistelles

1.2 Spirituous beverages of vitivinicultural origin shall, in principle, be tasted in the following order:

1. Wine spirits,
2. Brandy/weinbrand
3. Grape spirits,
4. Raisin spirits,
5. Grape marc wine spirits,
6. Wine lees spirits

2. The organiser shall ensure the dispatching of the samples between the juries.

3. Every morning, before the first tasting session, the jurors must be presented, under the same conditions as per the tasting, with a product preferably of the same type as the forecasted series in view of their "mouth feel". The tasting and the scoring must be discussed together within the jury.

4. Much effort needs to be paid in order that the wine and spirituous beverages of vitiviniculture origin are tasted by the jurors at the following temperatures:

1. white and rosé wines: 10/12 °C
2. red wines: 15/18 °C
3. sparkling wines: 8/10 °C
4. naturally sweet wine, ice wine, liqueur wine and mistelles: 10/14 °C
5. spirituous beverages of vitivinicultural origin: 12/16 °C

It is essential that all products of the same type within the same session, shall be tasted at the same temperature.

[2] The O.I.V. shall propose a list of aromatic vine varieties.

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Article 11: DESCRIPTION OF THE SCORE SHEET (see Annexes 3.1, 3.2 and 3.3)

Each expert shall have the score sheet corresponding to a sample to be tasted and the definitions of descriptors used.

Score sheets shall be drawn up in languages susceptible of being understood by the jurors.

A space shall be reserved for possible observations concerning each organoleptic characteristic.

Each sheet must likewise include the jury number.

The operating procedure of the score sheet describing the detailed organoleptic characteristics must be addressed to the jurors (Annex 3.4.). The objective shall be to ensure an identical understanding of terms used by all the jurors.

1. Wine score sheet:

For tasting still wines, the O.I.V.-U.I.OE. sheet model used shall be in accordance with the sheet hereinafter (Annex 3.1) *[3].
For tasting sparkling and fizzy wines, the O.I.V.-U.I.OE. sheet model used shall be in accordance with the sheet hereinafter (Annex 3.2) *[3].

2. Score sheet for spirituous beverages of vitivinicultural origin:

The O.I.V.-U.I.OE. sheet model used is in accordance with the sheet hereinafter (Annex 3.3)*[3] shall be used for tasting spirituous beverages of vitivinicultural origin. For specific cases, the model sheet may be modified with regard to the weighting of criteria within the 12 months of the standard entering into force.

Article 12: ROLE OF JURORS

The jurors shall verify or complete, if necessary, the information on the rating sheet relative to the sample.

After tasting the sample, each juror shall tick the box on each line corresponding to the appraisal of a given characteristic. Concerning the O.I.V.-U.I.OE. rating sheet, each box corresponds to a number of points, indicated on the sheet which enables the juror to make a rating.
The taster can make possible observations in the appropriate space, signs the sheet and then he/she can submit the sheet or validate his/her choice, in the case of computerised competitions.

Article 13: TRANSCRIPTION AND CALCULATION OF RESULTS

The secretariat shall verify that the sheet is completely filled out and carry out or check the total score allotted by the juror.

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*[3] As a transitional measure and until 31 December 2010, sheets authorized by 1994 standards (Wine) and 1999 standards (Spirituous beverages of vitivinicultural origin) are admitted.

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Secretary of the General Assembly

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When a wine or a spirituous beverage of vitivinicultural origin is marked « eliminated » due to a major defect by at least two jurors, the sample shall, under no circumstances receive an award by this jury.

Each sample shall be rated based on the average of the scores resulting from the calculation of appraisals from each of the jurors. It is recommended to eliminate figures which differ by more or less than seven points ranging from the average rating. The jurors must be informed beforehand of the choice of method to be used.
If the President of the jury deems useful, he/she may ask the director of the competition for a second tasting of the sample by another jury. In the case in which the director of the competition responds to this request, only the score of the second jury shall be taken into account.

Article 14: GRANTING AWARDS

The sum of all the medals awarded to the samples must not exceed 30 % of the total of samples presented at the competition. If this percentage is exceeded, the samples that obtained the lowest score are eliminated.

The samples having obtained a determined number of points for a tasting shall be classified according to the following award level categories:

- Grand gold – at least 92 points
- Gold - at least 85 points
- Silver - at least 82 points
- Bronze - at least 80 points.

Subject to informing the producers beforehand in the regulations for each competition;

- The awards may be divided into groups particularly for wine for example, in accordance with sugar content (for example more than 45 g/l), content of CO_2, colour and vintage. In this case, the sum of the awards in each group must not exceed 30 % of the total of samples presented in each group:

- The award winner may be limited to a single winner and for each award level. In this case, the winners will be ranked solely in accordance with their level.

In the event of overrunning 30 %, the samples having received the lowest scores are not taken into account.

The organiser shall have the possibility to limit the type of award and in so far as the sample received at least 80 points, within the same limit of 30 %, to grant other types of awards. Within the same limit of 30 % of awards, the organiser may likewise provide supplementary awards particularly by country, by grape variety, vintage, or vinification type or by wine maturing in barrels, provided that this does not cause confusion with regard to awards cited in the first paragraph.

Article 15: DOCUMENTATION OF AWARDS

Awards granted are necessarily accompanied by documentary proof, or a "Diploma", established by the body, which has been appointed as being responsible for the competition. This diploma must indicate the exact designation of the sample which received the award and

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The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

the exact identification of the corresponding producer or merchant. In no case, should another certificate or diploma of participation be granted. The organiser must withdraw all awards granted if it is demonstrated that the labelling does not conform to the laws of the country of origin or unfair use or in the event of illegal handling.

Awards obtained may be represented in the form of a label, as a sign of an award or in the form of a back label, duly authorised and quantified by the organiser to enable the organiser to identify all relevant data.

If the award is in the form of a medal, the medals must indicate the year of the competition. The number of medals granted shall be strictly limited to the declared available number at the time of registration for the wine or spirituous beverage of vitivinicultural origin competition.

In order to ensure traceability of granting awards, the organisers shall keep all the relevant documents with regard to sample registration, score sheets, in addition to a copy of the samples which received awards, for at least one year after the competition was held.

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Secretary of the General Assembly

Federico CASTELLUCCI

Annex I

CLASSIFICATION OF SAMPLES IN DIFFERENT CATEGORIES

CATEGORY I – WHITE WINES OF NON-AROMATIC GRAPE VARIETIES	
Group A – Still wines (*) These wines may have carbon dioxide overpressure under 0.5 bar at 20 °C.	
Sub-group of wines containing not more than 4 g/L of sugar	I-A-1
Sub-group of wines containing between 4.1 g/L to 12 g/L of sugar	I-A-2
Sub-group of wines containing between 12.1 g/L to 45 g/L of sugar	I-A-3
Sub-group of wines containing more than 45 g/L of sugar	I-A-4
Group B – Pearl wines These wines may have a carbon dioxide overpressure from 0.5 to 2.5 bar at 20 °C.	
Sub-group of wines containing not more than 4 g/L of sugar	I-B-5
Sub-group of wine containing more than 4 g/L of sugar	I-B-6
Group C – Sparkling wines These wines may have a carbon dioxide overpressure above 2.5 bar at 20 °C	
Sub-group of sparkling wines containing not more than 12 g/L of sugar with a tolerance of +3 g/L	I-C-7
Sub-group of sparkling wines containing between 12.1 g/L to 32 g/L of sugar with a tolerance of +3 g/L	I-C-8
Sub-group of sparkling wines containing between 32.1 g/L to 50 g/L of sugar	I-C-9
Sub-group of sparkling wines containing more than 50 g/L of sugar	I-C-10

CATEGORY II – ROSÉ WINES	
Group A – Still wines (*) These wines may have carbon dioxide overpressure under 0.5 bar at 20 °C.	
Sub-group of wine containing not more than 4 g/L of sugar	II-A-11
Sub-group of wine containing between 4.1 g/L to 12 g/L of sugar	II-A-12
Sub-group of wine containing between 12.1 g/L to 45 g/L of sugar	II-A-13
Sub-group of wine containing more than 45 g/L of sugar	II-A-14
Group B – Pearl wines These wines may have a carbon dioxide overpressure from 0.5 to 2.5 bar at 20 °C.	
Sub-group of wines containing not more than 4 g/L of sugar	II-B-15
Sub-group of wine containing more than 4 g/L of sugar	II-B-16
Group C – Sparkling wine. These wines may have a carbon dioxide overpressure above 2.5 bar at 20 °C	
Sub-group of sparkling wines containing not more than 12 g/L of sugar with a tolerance of +3 g/L	II-C-17
Sub-group of sparkling wines containing between 12.1 g/L to 32 g/L of sugar with a tolerance of +3 g/L	II-C-18
Sub-group of sparkling wines containing between 32.1 g/L to 50 g/L of sugar	II-C-19
Sub-group of sparkling wines containing more than 50 g/L of sugar	II-C-20

CATEGORY III – RED WINES	
Group A – Still wines (*) These wines may have carbon dioxide overpressure under 0.5 bar at 20 °C.	
Sub-group of wines containing not more than 4 g/L of sugar	III-A-21
Sub-group of wines containing more than 4 g/L of sugar	III-A-22
Group B – Pearl wines These wines may have carbon dioxide overpressure from 0.5 to 2.5 bar at 20 °C.	
Sub-group of wines containing not more than 4 g/L of sugar	III-B-23
Sub-group of wine containing more than 4 g/L of sugar	III-B-24
Group C – Sparkling wines These wines may have a carbon dioxide pressure above 2.5 bar at 20 °C	III-C-25

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Secretary of the General Assembly

Federico CASTELLUCCI

CATEGORY IV – WINES OF AROMATIC GRAPE VARIETIES	
Group A – Still wines (*) These wines may have carbon dioxide overpressure under 0.5 bar at 20 °C.	
Sub-group of wines containing not more than 4 g/L of sugar	IV-A-26
Sub-group of wines containing from 4.1 g/L to 12 g/L of sugar	IV-A-27
Sub-group of wine containing from 12.1 g/L to 45 g/L of sugar	IV-A-28
Sub-group of wines containing more than 45 g/L of sugar	IV-A-29
Group B – Pearl wines These wines may have a carbon dioxide overpressure from 0.5 to 2.5 bar at 20 °C.	
Sub-group of wines containing not more than 4 g/L of sugar	IV-B-30
Sub-group of wines containing more than 4 g/L of sugar	IV-B-31
Group C – Sparkling wines. These wines may have a carbon dioxide overpressure above 2.5 bar at 20 °C	
Sub-group of sparkling wines containing not more than 12 g/L of sugar with a tolerance of +3 g/L	IV-C-32
Sub-group of sparkling wines containing between 12.1 g/L to 32 g/L of sugar with a tolerance of +3 g/L	IV-C-33
Sub-group of sparkling wines containing between 32.1 g/L to 50 g/L of sugar	IV-C-34
Sub-group of sparkling wines containing more than 50 g/L of sugar	IV-C-35

CATEGORY V – WINES UNDER A FILM OF YEAST	
Group A - wines containing not more than 4 g/L of sugar	
Sub-group of wines with alcoholic content not more than 15 %	V-A-36
Sub-group of wines with alcoholic content above 15 %	V-A-37
Group B – wines containing from 4.1 to 20 g/L of sugar	
Sub-group of wines with alcoholic content not more than 15 %	V-B-38
Sub-group of wines with alcoholic content above 15 %	V-B-39
Group C – wines containing more than 20 g/L of sugar	
Sub-group of wines with alcoholic content not more than 15 %	V-C-40
Sub-group of wines with alcoholic content above 15 %	V-C-41

CATEGORY VI - NATURALLY SWEET WINE (Ex. Late harvest, botrytised wine, ice wine…)	
Group A - Non aromatic grape varieties	VI-A-42
Group B – Aromatic grape varieties	VI-B-43

CATEGORY VII – LIQUEUR WINE	
Group A - Non aromatic grape varieties (Ex: Porto, Marsala, Madera, Mistelles, Tokay Aszu….)	
Sub-group of liqueur wines containing not more than 6 g/L of sugar	VII-A-44
Sub-group of liqueur wines containing between 6.1 to 40 g/L of sugar	
With an alcoholic content not more than or equal to 18 %	VII-A-45
With an alcoholic content above 18 %	VII-A-46
Sub-group of liqueur wines containing between 40,1 to 80 g/L of sugar	
With an alcoholic content not more than or equal to 18 %	VII-A-47
With an alcoholic content above 18 %	VII-A-48
Sub-group of liqueur wines containing more than 80 g/L of sugar	
With an alcoholic content not more than or equal to 18 %	VII-A-49
With an alcoholic content above 18 %	VII-A-50
Groupe B – Aromatic grape varieties (Ex. Muscats…)	VII-B-51
Group C – Liqueur wines under a film of yeast (Ex. Jerez, Fino, Montilla-Morilles)	VII-C-52

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CATEGORY VIII - MISTELLES	
	VIII-A-53

CATEGORY IX – SPIRITUOUS BEVERAGES OF VITIVINICULTURAL ORIGIN		
	Group A – wine spirits	IX-A-54
	Group B – Brandy/Weinbrand	IX-B-55
	Group C – grape spirits	IX-C-56
	Group D – raisin spirits	IX-D-57
	Group E – grape marc spirits	IX-E-58
	Group F – wine lees spirits	IX-F-59

(*) EXEMPTION

Wines which are used to be presented with gas overpressure above 0.5 bar and not above 1 bar can be classified in Group A – Still wines, provided that these wines are tasted after other still wines.

REMARK: If in the sub-groups there are only a few samples, these samples can be included in another sub-group.

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Annex II

ANALYSIS CERTIFICATE OF WINE

Samples must be accompanied by an analysis certificate drawn up by an accredited laboratory of by a competent laboratory in conformity with the laws of the country or certified by an oenologist[4] in charge of the wine cellar with, at least, the hereinafter specifications:

1. Alcoholic strength by volume at 20 degrees Centigrade vol. %
2. Sugar (glucose + fructose) ..... g/L
3. Total acidity ..... meq/L
4. Volatile acidity ..... meq/L
5. Sulphur dioxide (SO_2) total ..... mg/L
6. Sulphur dioxide (SO_2) free ..... mg/L
7. Concerning sparkling and pearl wine:

Pressure in the bottle..... (bars)
..... hPa

The methods of analysis used are provided in the Compendium of International Methods of Analysis and Appraisal of Wine and Musts.

ANALYSIS CERTIFICATES OF SPIRITUOUS BEVERAGES OF VITIVINICULTURAL ORIGIN

Samples must be accompanied by an analysis certificate drawn up by an accredited laboratory of a competent laboratory in conformity with the laws of the country or certified by an oenologist in charge of the wine cellar and with, at least, the hereinafter specifications:

1. Alcohol strength by volume at 20 degrees C. ..... % vol.
2. Sugars ..... g/l
3. Content of volatile substances..... g/hL of alcohol for 100 % vol.
4. Content of methanol ..... g/hL of alcohol for 100 % vol.

The methods of analysis used are posted in the Compendium of Methods of Analysis of Spirituous Beverages of Vitivinicultural Origin.

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Annex 3.1

SCORE SHEET	STILL WINES

Jury	N°	Sample	N°	Category	N°

		Excellent +				Inadequate -	Observations
Visual	Limpidity	☐ (5)	☐ (4)	☐ (3)	☐ (2)	☐ (1)	
	Aspect other than limpidity	☐ (10)	☐ (8)	☐ (6)	☐ (4)	☐ (2)	
Nose	Genuineness	☐ (6)	☐ (5)	☐ (4)	☐ (3)	☐ (2)	
	Positive intensity	☐ (8)	☐ (7)	☐ (6)	☐ (4)	☐ (2)	
	Quality	☐ (16)	☐ (14)	☐ (12)	☐ (10)	☐ (8)	
Taste	Genuineness	☐ (6)	☐ (5)	☐ (4)	☐ (3)	☐ (2)	
	Positive intensity	☐ (8)	☐ (7)	☐ (6)	☐ (4)	☐ (2)	
	Harmonious persistence	☐ (8)	☐ (7)	☐ (6)	☐ (5)	☐ (4)	
	Quality	☐ (22)	☐ (19)	☐ (16)	☐ (13)	☐ (10)	
Harmony – Overall judgement		☐ (11)	☐ (10)	☐ (9)	☐ (8)	☐ (7)	

Total	+	+	+	+	=	

Eliminated due to major defect						0

Signature of juror

Signature of President of the jury

Annex 3.2

OIV Organisation Internationale de la Vigne et du Vin	SCORE SHEET	SPARKLING AND PEARL WINES

UNION INTERNATIONALE DES ŒNOLOGUES

Jury	N°	Sample	N°	Category	N°

		Excellent +				Inadequate -	Observations
Visual	Limpidity	☐ (5)	☐ (4)	☐ (3)	☐ (2)	☐ (1)	
	Aspect other than Limpidity	☐ (10)	☐ (8)	☐ (6)	☐ (4)	☐ (2)	
	Effervescence	☐ (10)	☐ (8)	☐ (6)	☐ (4)	☐ (2)	
Nose	Genuineness	☐ (7)	☐ (6)	☐ (5)	☐ (4)	☐ (3)	
	Positive intensity	☐ (7)	☐ (6)	☐ (5)	☐ (4)	☐ (3)	
	Quality	☐ (14)	☐ (12)	☐ (10)	☐ (8)	☐ (6)	
Taste	Genuineness	☐ (7)	☐ (6)	☐ (5)	☐ (4)	☐ (3)	
	Positive intensity	☐ (7)	☐ (6)	☐ (5)	☐ (4)	☐ (3)	
	Harmonious persistence	☐ (7)	☐ (6)	☐ (5)	☐ (4)	☐ (3)	
	Quality	☐ (14)	☐ (12)	☐ (10)	☐ (8)	☐ (6)	
Harmony – Overall judgement		☐ (12)	☐ (11)	☐ (10)	☐ (9)	☐ (8)	

Total	+	+	+	+	=	

Eliminated due to major defect						0

Signature of juror

Signature of the President of the Jury

Annex 3.3

SCORE SHEET	SPIRITUOUS BEVERAGES OF VITIVINICULTURAL ORIGIN

Jury	N°	Sample	N°	Category	N°

		Excellent +				Inadequat -		Observations
Visual	Limpidity	☐ (5)	☐ (4)	☐ (3)	☐ (2)	☐ (1)		
	Colour	☐ (5)	☐ (4)	☐ (3)	☐ (2)	☐ (1)		
Nose	Typicality*	☐ (6)	☐ (5)	☐ (4)	☐ (3)	☐ (2)		
	Quality	☐(15)	☐ (13)	☐ (11)	☐ (9)	☐ (7)		
	Positive intensity	☐ (9)	☐ (7)	☐ (5)	☐ (3)	☐ (1)		
Taste	Typicality*	☐ (8)	☐ (7)	☐ (6)	☐ (5)	☐ (4)		
	Quality	☐ (20)	☐ (18)	☐ (14)	☐ (10)	☐ (6)		
	Harmonious persistence	☐ (12)	☐ (10)	☐ (8)	☐ (6)	☐ (4)		
Harmony – Overall judgement		☐ (20)	☐ (18)	☐ (14)	☐ (10)	☐ (6)		

Total	+	+	+	+	=	

Eliminated due to major defect						0

Signature of juror

Signature of the President of the Jury

Annexe 3.4

1. **VISUAL / eye**
Discrimination of differences in outside world with sensory impressions from visible light rays

2. **NOSE / nose**
Sensations perceived by the olfactory organ when stimulated by certain volatile substances

3. **TASTE / mouth**
Full spectrum of sensations perceived with wine mouthfeel.

Limpidity
Definition: Measure of cloudiness.

This descriptor allows cloudiness intensity of wine to be measured

LIMPIDITY	DESCRIPTOR SHEET		
	TW	SW	SB
Excellent limpiditY	5	5	5
Limpid	4	4	4
Ambiguous limidity	3	3	3
Moderate cloudiness	2	2	2
Very cloudy	1	1	1

Aspect:
Definition: to determine the full spectrum of visible properties of a product

This descriptor evaluates the intensity, the main colour of the product, its nuances (secondary colours), its viscosity…not taking into account its limpidity.

ASPECT NOT INCL LIMIDITY	DESCRIPTOR SHEET		
	TW	SW	SB
Excellent impression	10	10	5
Very good impression	8	8	4
Good impression	6	6	3
Fairly good impression	4	4	2
Bad impression	2	2	1

Positive intensity
Definition: degree (magnitude) of full spectrum of **qualitative** odours perceived by nose and taste.

This descriptor evaluates the influence of the spectrum of olfactory and gustatory perceptions which contribute to enhancing the qualitative perception perceived by the nose and taste

POSITIVE INTENSITY	DESCRIPTOR SHEET		
	TW	SW	SB
Very strong qualitative intensity	8	7	9
Strong intensity	7	6	7
Average intensity	6	5	5
Light intensity	4	4	3
Very light intensity	2	3	1

Genuineness:
Definition: to measure degree of sensation perceived (magnitude) by the nose and taste, of a viticulture, oenological defect of product

This descriptor allows the taster to evaluate the genuineness or the cleanliness of wine. In penalising the genuineness the taster should be able to identify viticulture based defects, oenological or foreign to wine, which the taster perceives by nose or by taste. Plant, animal notes (etc) for vine varieties, in addition to wooded notes are evaluated in the quality section.

Origins of defects:
Raw material: grapes: rotten, hailed, degraded…
Contamination: volatile solvents, volatile phenols, plastic, paper, TCA-mould-, dust, negative influence of containers (concrete vats, steel, polyester, plastic, coopered wood vat, barrels)
Microbial: volatile phenols (stable, gouache, ink), volatile acidity, volatile acidity esters, acetone

Oxydo-reduction: SO_2, all thiols and sulphide (rubber, cabbage, rotten eggs, alliaceous, sweat, lees, beer, soap, stagnated) lack of cleanliness, ethanol, oxidation.

GENUINENESS	DESCRIPTOR SHEET		
	TW	SW	
Total absence of defects	6	7	
Very low intensity of defects	5	6	
Low intensity of defects	4	5	
Average intensity of defects	3	4	
Strong intensity of defects	2	3	

Quality:
Definition: spectrum of properties and characteristics of a wine that gives an aptitude to satisfy, nose and taste, implicit or expressed needs

This descriptor enables the overall judgement of product on an olfactory and gustatory level. The taster can express in a meaningful manner his/her personal preferences and cultural references.
Nose, this descriptor takes into account **in priority complexity,** which corresponds to the richness of the aromatic palette by the perception of several different and changing odours, associated with the **finesse of** odours.

NOSE QUALITY	DECRIPTOR SHEET		
	TW	SW	SB
Excellent impression of quality	16	14	15
Very good impression of quality	14	12	13
Good impression of quality	12	10	11
Fairly good impression of quality	10	8	9
Bad impression of quality	8	6	7

Taste, the descriptor takes into account, in **priority richness,** which corresponds to the overall sensation in mouth integrating aromas (complexity), structure (acid, tannins, alcohol), coating elements (fatty), residual sugars, bitterness.

TASTE QUALITY	DESCRIPTOR SHEET		
	TW	SW	SB
Excellent impression of quality	22	14	20
Very good impression of quality	19	12	18
Good impression of quality	16	10	14
Fairly good impression of quality	13	9	10
Bad impression of quality	10	8	6

Persistence:
Definition: To measure the length of residual olfacto-gustatory sensation, corresponding to the sensation perceived when the product is in mouth and length of time is measured.

This descriptor is equal to one time measurement. This is calculated in seconds (caudalie) and starts once the product has left the mouth.
Counting is done by chewing and discreetly opening the lips and exerting a small depression in the mouth to allow air to enter. Slow chewing corresponds to approximately 1 second.

HARMONIOUS PERSISTANCE		DESCRIPTOR SHEET		
		TW	SW	SB
Excellent persistance:	> 6''	8	7	12
Very good persistance:	5'' à 6''	7	6	10
Good persistance:	3'' à 4''	6	5	8
Fairly good persistance:	2''	5	4	6
Bad persistance:	1''	4	3	4

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Zagreb, 3rd July 2009
The General Director of the OIV
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General impression or overall judgement:
Definition: corresponds to overall appraisal of a product.

This descriptor allows the taster to express the impression that the product leaves all together. This gives the possibility of grading high or low.
Depending on the type of competition, and the information given to the tasters, this descriptor also enables the analysis of the difficult issue of typicality and appraisal potential of the wine to evolve over time.

GENERAL IMPRESSION	DESCRIPTOR SHEET		
	TW	SW	SB
Excellent general impression	11	12	20
Very good general impression	10	11	18
Good general impression	0	10	14
Satisfactory general impression	8	9	10
Inadequate general impression	7	8	6

Effervescence:
Definition: bubbling formed as gas escapes from a liquid

Three descriptors concerning bubbles are used to evaluate effervescence in terms of sight and taste:
- Finesse of bubbles (= size of bubbles)
- Abundance of bubbles (= quantity of bubbles)
- Persistence of bubbles (= length of time bubbles are perceived)

Fine bubbles, not too impetuous, regular and persistent are evaluated positively.
On the contrary, large bubbles, aggressive, irregular and not very persistent are evaluated negatively.
To visual appreciation, the foam collar is evaluated, that being the foam which forms on the surface of the wine. A positive evaluation is a foam collar made up of 3-4 levels of bubbles. The bubbles must be fine, small and the foam collar must last a long time.

EFFERVESCENCE	DESCRIPTOR SHEET		
		SW	
Excellent		10	
Very good		8	
Good		6	
Sufficient		4	
Insufficient		2	

Typicity:

This descriptor is used to evaluate if a given product corresponds to typical characteristics in the spirited beverages of vitivinicultural origin category

ODOUR TYPICITY	DESCRIPTOR SHEET		
			SB
Excellent			6
Very good			5
Good			4
Sufficient			3
Insufficient			2

TASTE TYPICITY	DESCRIPTOR SHEET		
			SB
Excellent			8
Very good			7
Good			6
Sufficient			5
Insufficient			4

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The General Director of the OIV
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RESOLUTION OIV/VITI 355/2009

OIV PROTOCOL FOR THE EVALUATION OF GRAPEVINES OBTAINED BY GENETIC TRANSFORMATION

The GENERAL ASSEMBLY,

On the proposal of the Viticulture Commission I,

TAKING NOTE of the work developed by the « GENETIC RESOURCES AND VINE SELECTION» (GENET) expert group, focused on harmonising evaluation systems for new vine varieties obtained by using genetic transformation techniques,

CONSIDERING the recommendations of Resolution Viti 1/2006 on « Vine genomes and genetically modified varieties », particularly the recommendation that « compared to the initial variety/clone any change in characteristics due to a genetic modification should be clearly described (through transcriptomic, proteomic and metabolomic studies, in addition to all new appropriate methods which will be developed.) »,

DECIDES to adopt guidelines of the OIV Protocol for the evaluation of grapevines obtained by genetic transformation »,

RECOMMENDS Member states to adopt and integrate the guidelines in “the OIV protocol for the evaluation of grapevines obtained by genetic transformation”, in accordance with their regulatory scheme.

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

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OIV PROTOCOL FOR THE EVALUATION OF GRAPEVINES OBTAINED BY GENETIC TRANSFORMATION

A - PREAMBLE

Research work aiming at an improvement of existing grapevine cultivars by transgenic approaches is carried out in several countries resulting in genetically modified vines (GM-vines).

Genetic modifications applied may have various objectives, e.g.:

- improvement of resistance against pest and diseases such as fungus diseases of the berry, foliage and wood (e.g. downy mildew, powdery mildew, eutypa dieback, esca), viruses and their vectors (in particular nematodes), bacteria, phytoplasms and insects (e.g. grape moths);
- tolerance to the abiotic stresses, in particular to thermal stress, draught, salinity of the soil and freezing;
- tolerance to certain inputs (herbicides);
- modification of the physiological and phenological characteristics (seedlessness, early ripening) and quality traits (product composition of berry components and their relations);

Non-applied scientific purposes may exist.

B - FUNDAMENTALS AND GENERAL OBJECTIVES OF THE EVALUATION

In addition to the usual criteria taken into consideration in the selection of new varieties, the development and use of GM-vines or products thereof implies that it is necessary to assess and evaluate risks and benefits.

To evaluate potential risks and benefits of cultivating a GM vine, the following issues should be taken into account:

- the nature of the genetic modification
- the gene construct (target gene, marker gene)
- the species, variety and clone of vines subjected to transformation
- the growing conditions and the ecosystem
- the application, for food or industrial purposes

Based on these factors, the GM-vines should be evaluated according to the following items:

- health effects for consumers (e.g. toxic and allergenic potential)
- effects on the grape's composition
- environmental effects;
- potential commercial value

Socio-economic aspects could also be taken into consideration

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Finally the evaluation procedure should clearly point out the potential risks as well as the potential benefits of a GM-vine.

All evaluation procedures should be based on scientifically sound hypotheses. Scientific knowledge coming from other genetically modified crops should likewise be taken into account. The evaluation protocol for the GM-vines must be flexible. Thus, for example, depending on the modified characteristics (e.g. qualitative, resistance, or phenological characteristics) the priorities of the evaluation procedures should be performed on a **case by case basis.**

In spite of the preferential autogamous fertilization of hermaphrodite vine flowers, the pollination of non GM-vines by the pollen of GM-vines located in the vicinity can not be excluded. Within the Vitis genus, the risk of uncontrolled spreading of genes incorporated in other varieties is very low due to the asexual commercial propagation of varieties and clones. However the possible risk of introgression of the incorporated genes into wild populations of Vitis genus depends on the continent and the geographic region. There is no risk in grape-growing areas without any wild vines like Australia, South America and most parts of Africa. Risk is very limited in Europe where the only one wild grape, *Vitis vinifera subsp. sylvestris*, is rarely found and the existing habitats are usually located far away from commercial vine-growing areas.

C – GENERAL FRAMWORKS FOR THE EVALUATION OF TRANSGENIC VINES

1. Evaluation procedure control institution

Each country appoints a control institution in charge of supervising the different evaluation procedures described according to point 2.1-2.6 which reacts in due respect to criteria established in its respective country. This control institution can be, for example, a corresponding research centre, a technical institute, a university, particularly those institutions qualified to carry out studies and surveys on genomes. The control institution may appoint other organizations and/or laboratories qualified for executing certain special subtasks. The control institution periodically checks corresponding field trials, collects all corresponding data and assesses the results of all the experiments and draws up annual reports and provides a summary in a final report.

2. Evaluation procedures

For each new GM vine obtained the laboratories that have operated, as well as all the *in vitro* multiplication procedures, acclimatisation steps and propagation and multiplication interventions should be described and drawn up in a document in accordance with recommendations of resolution VITI 1/2006.

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Secretary of the General Assembly

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2.1 Phenotypic evaluation

a) Requirements on field trials

The designing of field trials may vary depending on the usage of GM vines (wine, table grape, raisin, rootstock) and from the modified characteristics(s). In any case, these trials should be in a way which leads to reliable results. As a minimum requirement, these field trials should be carried out, at different periods and in distinctly different sites (different climatic regions) with a number of plants and repetitions in accordance to the analysis, for at least three harvesting periods. Furthermore, original untransformed cultivar/clone should be used as a control test.
During the trial period of genetically modified vines, the established conditions (for example instructions for handling the pruning material, the defoliation, the gathering) should be fulfilled.

b) Evaluation of the target characteristic(s)

Based on the field trials and eventually on other available data, the influence of the modification on target characteristic(s) should be precisely described. This (e.g. modification of resistance level to diseases and pests, modification in sugar or acid content, qualitative and/or quantitative modification of anthocyanins or aroma compounds, etc.). The description should be made through transcriptomic, proteomic and metabolomic studies, in addition to all new appropriate methods which will be developed. In any case, analytical evaluations of the final product (wine, table grape, raisin) should be carried out and completed by organoleptic tests.

In the case of root stock, the compatibility with grafts should be checked.

c) Evaluation of other characteristics beside the target characteristic(s)

It has to be verified whether other important viticultural characteristics beside the target characteristic(s) are influenced by the genetic modification. If so, the relevant features as well as the degree of alterations compared to the original untransformed cultivar/clone has to be described in detail.

The ampelographic and ampelometric characteristics of GM vines should be evaluated and compared to appropriate data from the original untransformed cultivar/clone. This has to be done using scientifically appropriate and internationally accepted methods and/or descriptors (i.e. OIV, UPOV). Any significant alterations should be reported.

d) Stability of phenotype

The stability of the transformed gene has to be proved over several propagation cycles by asexual propagation. It should be confirmed that adult plants, after repeated multiplications, show no other significant alteration than the sought and identified in preliminary tests.

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2.2. Genotypic evaluation

The genetic transformation itself should be confirmed by adequate molecular techniques (e.g. PCR, border sequencing, ...). Furthermore, the number of copies, as well as the number of insertion locations should be investigated (e.g. Southern Blot). For studies on traceability (refer to point 2.3.) it is necessary to establish appropriate molecular tools (e.g. SCAR-markers).

2.3. – Final product traceability of genetic modification

It should be verified whether the incorporated gene construct(s), parts of the gene construct(s), new metabolites due to the incorporated gene(s) are traceable in the final product (for example wine, table grape, raisin, oil from seed). This data is of special importance with regard to labelling in the case of later commercial use of genetically modified vines. In certain cases, it may be particularly interesting to investigate in the course of the production process that the incorporated genes or the corresponding metabolites are traceable (ex.: wine production: fresh grapes -> must -> during fermentation -> several steps after fermentation up until bottling). This should be done by using appropriate analytical tools which are registered and made available to the control institution.

2.4. Evaluations of possible interactions of transgenic vines on ecosystem

Depending on the kind of modification (case by case basis) reliable results of monitoring investigations on the influence of the ecosystem should be presented (for example possible occurrence of horizontal and/or vertical gene transfer, effects on flora and/or fauna, qualitative and/or quantitative changes in metabolism and their possible effects). The methods for evaluating these parameters should be based on scientifically international accepted protocols.

2.5. – Evaluations of possible effects of transgenic vines on the health of consumers

In the case that the genetic transformation leads to qualitative and/or quantitative changes of the metabolites in the final product, possible influence on human health should be checked.

2.6. – Evaluations of possible effects of transgenic vines on technological aspects

It has to be evaluated whether the genetic modification leads to an impact on technological parameters (e.g. influence on the fermentation process).

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Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

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D – COMMERCIAL USE OF A GENETICALLY MODIFIED VINE

1. Registration of a genetically modified grapevine

Based on the data revealed by the investigations described in the aforementioned section C and certified by the control institution, the official institution in charge of reviewing the genetically modified vines will be able to make a decision by:

a) accepting an application without obligations,
b) accepting an application with specific obligation(s)
c) rejecting an application

In case of a) it will be possible to propagate and disseminate the new genetically modified grapevine. In case of b) it may be possible, with some restrictions and recommendations, to propagate and disseminate the new genetically modified grapevine.

In both cases the further propagation and dissemination can be stopped if further research results (carried out for example with newly developed, formerly unknown research techniques) gives evidence of some kind of possible “danger”. GMO matter should be indicated as such on the label.

2. Phytosanitary status

As for existing varieties and clones, a genetically modified grapevine – when introduced to the market – should fulfill all the existing obligations in the individual Member countries of OIV concerning the phytosanitary status of the propagation material.

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

RESOLUTION OIV/OENO 145/2009

TREATMENT WITH SILVER CHLORIDE

THE GENERAL ASSEMBLY,

Considering Article 2 paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

Considering the work of the « International Code of Oenological Practices » experts group,

Considering the favourable opinion issued by the "Food safety" group at its 14th session,

DECIDES: Upon the proposal of Commission II « Oenology » to introduce in the aforementioned « International Code of Oenological Practices », the following oenological practices and treatments:

DECIDES to update the relevant OIV document with the maximal residue level of silver in wine

PART II

Chapter 3: « Wines »

Treatment with silver chloride

Definition:

Addition of silver chloride to wine

Objective:

To reduce odour defects due to hydrogen sulphide and some mercaptans.

Prescriptions

a) The dose used must not be over 1 g/hl
b) The silver chloride must be previously applied to an inert support, like kieselguhr (diatomaceous earth) or kaolin
c) The main operation must be preceded by trials to determine the amount of product to add.
d) The precipitate must be eliminated by any appropriate physical procedure
e) Residues must be treated by specialised sector
f) Treated wine must be analysed to insure that the maximal residue level do not exceed 0.1 mg/L in silver
g) The treatment must be carried out under the responsibility of an oenologist or a specialised technician
h) Silver chloride must comply with the provisions of the International Oenological Codex

Recommendation of the OIV:

Admitted.

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

RESOLUTION OIV/OENO 278/2009

MACERATION OF RAISINED GRAPES OR THEIR POMACE IN WINE

THE GENERAL ASSEMBLY

Considering Article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine

Taking note of the works of the «Technology» expert group,

DECIDES, on the proposal of Commission II « Oenology », to introduce in part II of the "*International Code of Oenological Practices*" the following oenological practices:

PART II

Chapter 3

Maceration of raisined grapes or their pomace in wine.

Definition:

Procedure which involves the maceration in wine of raisined grapes or grapes affected by noble rot or their pomace following fermentation.

Objectives:

a) To increase content of:
* sugar in wine
* phenolic compounds in wine,
* aromatic compounds in wine.

Prescriptions:

a) The length of maceration depends on the characteristics of the wine, the grape variety and the style of wine desired.
b) Avoid excessive extraction of phenolic compounds brought on by a too lengthy maceration.
c) The wine, raisined grapes and pomace obtained following fermentation must all be from the same vintage.

Recommendation of the OIV

Admitted

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

RESOLUTION OIV/OENO 196/2009

MACERATION (General sheet)

THE GENERAL ASSEMBLY,

in view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

and having learned of the work carried out by the "Technology" group of experts,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to introduce into Part II of the "*International Code of Oenological Practices*" the following oenological practices and treatments:

PART II

Section

MACERATION

Definition

Process consisting of leaving the solid and liquid parts of harvested grapes in contact for a more or less extended period of time. Maceration takes place before, simultaneously with or after fermentation.

Objective

Dissolution of substances contained within grapes, notably phenolic compounds, aromas and their precursors.

Prescriptions

The objective may be achieved by:
a) Maceration according to the traditional technique of fermentation on skins (II 1.6)
b) Carbonic maceration (II 1.7)
c) Maceration after heating the harvested grapes (II 1.8)
d) Prefermentative cold maceration for making white wine (II 1.14)
e) Prefermentative cold maceration for making red wine (II 1.15)
f) Warm post-fermentation of red grapes, known as warm final maceration (II 2.3.9)
g) Maceration of raisined grapes or their pomace in wine (II 2.3.10)

OIV recommendation:

Refer to the practices and treatments indicated above.

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

RESOLUTION OIV/OENO 336A/2009

MUSTS – FINING USING CHITOSAN

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

taking into account the favourable opinion of the "Food Security" group of experts,

taking note of the work carried out by the "Technology" group of experts,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to introduce into Part II of the "*International Code of Oenological Practices*" the following oenological practices and treatments:

PART II

Section 2: Musts

2.1.22. Fining using chitosan

Definition:

Addition of chitosan of fungal origin for the purpose of fining musts

Objectives:

a) To facilitate settling and clarification
b) To carry out a treatment to prevent protein haze

Prescriptions:

a) The doses to be used are determined after preliminary testing. The recommended dose used should be less than or equal to 100 g/hl.
b) Chitosan must comply with the requirements of the International Oenological Codex.

OIV recommendations:

Accepted

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

RESOLUTION OIV/OENO 336B/2009

MUSTS – FINING USING CHITIN-GLUCAN

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

taking into account the favourable opinion of the "Food Security" group of experts,
taking note of the work carried out by the "Technology" group of experts,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to introduce into Part II of the "*International Code of Oenological Practices*" the following oenological practices and treatments:

PART II

Section 2: Musts

2.1.23. Fining using chitin-glucan

Definition:

Addition of chitin-glucan of fungal origin for the purpose of fining musts

Objectives:

a) To facilitate settling and clarification
b) To carry out a treatment to prevent protein haze

Prescriptions:

a) The doses to be used are determined after preliminary testing. The recommended dose used should be less than or equal to 100 g/hl.
b) Chitin-glucan complex must comply with the requirements of the International Oenological Codex.

OIV recommendations:

Accepted.

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

RESOLUTION OIV/OENO 337A/2009

WINES – FINING USING CHITOSAN

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

taking into account the favourable opinion of the "Food Security" group of experts,

taking into account the work carried out by the "Technology" group of experts,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to introduce into Part II of the "*International Code of Oenological Practices*" the following oenological practice:

PART II

Section 3: Wines

3.2.12. Fining using chitosan

Definition:

Addition of chitosan of fungal origin for the purpose of fining wines

Objectives:

a) To reduce turbidity by precipitating particles in suspension.
b) To carry out a treatment to prevent protein haze by the partial precipitation of excess proteinaceous matter.

Prescriptions:

a) The doses to be used are determined after preliminary testing. The maximum dose used must not exceed 100 g/hl.
b) Sediments are eliminated by physical procedures.
c) Chitosan of fungal origin may be used alone or together with other admitted products.
d) Chitosan must comply with the requirements of the International Oenological Codex.

OIV recommendations:
Accepted.

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

RESOLUTION OIV/OENO 337B/2009

WINES – FINING USING CHITIN-GLUCAN COMPLEX

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

taking into account the favourable opinion of the "Food Security" group of experts,

taking into account the work carried out by the "Technology" group of experts,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to introduce into Part II of the "*International Code of Oenological Practices*" the following oenological practice:

PART II

Section 3: Wines

3.2.13. Fining using chitin-glucan complex

Definition:

Addition of chitin-glucan complex of fungal origin for the purpose of fining wines

Objectives:

a) To reduce turbidity by precipitating particles in suspension
b) To carry out a treatment to prevent protein haze by the partial precipitation of excess proteinaceous matter.

Prescriptions:

a) The doses to be used are determined after preliminary testing. The maximum dose used must not exceed 100 g/hl.
b) Sediments are eliminated by physical procedures.
c) Chitin-glucan complex of fungal origin may be used alone or together with other admitted products.
d) Chitin-glucan complex must comply with the requirements of the International Oenological Codex.

OIV recommendations:

Accepted.

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

RESOLUTION OIV/OENO 339A/2009

WINES – FINING: MODIFICATION OF THE EXISTING SHEET - CHITOSAN

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing International Office of Vine and Wine,

taking into account the favourable opinion of the "Food Security" group of experts,

taking into account the work carried out by the "Technology" group of experts,

and in view of draft resolution OENO/TECHNO/07/337A Wines – Fining using chitosan,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to introduce into Part II of the "*International Code of Oenological Practices*" the following treatment:

PART II

Section 3: Wines

3.2.1. Fining

The addition to prescription b), chitosan

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

RESOLUTION OIV/OENO 339B/2009

WINES – FINING: COMPLEX MODIFICATION OF THE EXISTING SHEET - CHITIN GLUCAN

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

taking into account the favourable opinion of the "Food Security" group of experts,

taking into account the work carried out by the "Technology" group of experts,

and in view of draft resolution OENO/TECHNO/07/337B Wines – Fining using chitin-glucan complex,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to introduce into Part II of the "*International Code of Oenological Practices*" the following treatment:

PART II

Section 3: Wines

3.2.1. Fining

The addition to prescription b), chitin-glucan.

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

RESOLUTION OIV/OENO 338A/2009

WINES – TREATMENT USING CHITOSAN

THE GENERAL ASSEMBLY,

in view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

taking into account the favourable opinion of the "Food Security" group of experts,

taking into account the work carried out by the "Technology" group of experts,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to introduce into Part II of the "*International Code of Oenological Practices*" the following oenological treatment:

PART II

Section 3: Wines

3.4.16. Treatment using chitosan

Definition:

Addition of chitosan of fungal origin to wines

Objectives:

a) To reduce heavy metal content, notably iron, lead, cadmium, copper,
b) To prevent iron haze, copper haze,
c) To reduce possible contaminants, especially ochratoxin A,
d) To reduce undesirable micro-organisms, notably *Brettanomyces*.

Prescriptions:

a) The doses to be used are determined after preliminary testing. The maximum dose used must not exceed :
    - 100 g/hl for the objectives a) and b)
    - 500 g/hl for the objective c)
    - 10 g/hl for the objective d)
b) Sediments are eliminated by physical procedures.
c) Chitosan of fungal origin may be used alone or together with other admitted products.
d) Chitosan must comply with the requirements of the International Oenological Codex.

OIV recommendations:
Accepted

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

RESOLUTION OIV/OENO 338B/2009

WINES – TREATMENT USING CHITIN-GLUCAN COMPLEX

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

taking into account the favourable opinion of the "Food Security" group of experts,

taking into account the work carried out by the "Technology" group of experts,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to introduce into Part II of the "*International Code of Oenological Practices*" the following oenological treatment:

PART II

Section 3: Wines

3.4.17. Treatment using chitin-glucan complex

Definition:

Addition of chitin-glucan complex of fungal origin to wines

Objectives:
a) To reduce heavy metal content, notably iron, lead, cadmium, copper,
b) To prevent iron haze, copper haze,
c) To reduce possible contaminants, especially ochratoxin A.

Prescriptions:

a) The doses to be used are determined after preliminary testing. The maximum dose used must not exceed :
    - 100 g/hl for the objectives a) and b)
    - 500 g/hl for the objective c)

b) Sediments are eliminated by physical procedures.
c) Chitin-glucan complex of fungal origin may be used alone or together with other admitted products.
d) Chitin-glucan complex must comply with the requirements of the International Oenological Codex.

OIV recommendations:
Accepted.

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

RESOLUTION OIV/OENO 313/2009

CODEX - HEMI CELLULASES

The GENERAL ASSEMBLY

In view of article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

Following a proposal made by the Sub-Commission of Methods of Analysis and the expert group Specifications of Oenological Products**,**

HAS HEREBY DECIDED to add the following monograph to the international Oenological Codex:

HEMICELLULASES
(galactanase activity)
(EC 3.2.1.89 – CAS no. 58182-40-4)

General specifications

These enzymes are not found in a pure state but they are present within an enzymatic complex. Unless otherwise stipulated, the specifications must comply with Oeno resolution concerning "the general specifications for enzymatic preparations" included in the International Oenological Codex.

1. Origin and scope

Hemicellulases catalyse the decomposition of hemicelluloses. (galactans, xyloglucans, arabinoxylans, glucuronoarabinoxylans, mannans, glucomannans) They are used during the maceration of grapes. This activity can be estimated by the hydrolysis of potato galactans.

The enzymatic preparations containing these activities come from directed fermentations of *Aspergillus niger* and/or a mixture of *Aspergillus niger – Trichoderma reesei.*

Principal activities accompanying Hemicellulases activities (arabanase, galactanase, xylanase, rhamnosidase):

- Polygalacturonases
- Pectin / pectate-lyase
- Pectinmethylesterase

Secondary activities: proteases, cellulases, beta-glucosidases; the 50 % clause is applicable to these activities (monograph on enzymatic preparations 4.1)

2. Scope

The determination method was developed using a commercially available galactanase. The conditions and the method were developed for application to the commercial enzymatic preparations such as those found on the oenological market.

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3. Principle

The galactanases cut the chains of arabinogalactan, thereby releasing the reducing ends of the constitutive sugars. Measurement of the galactanase activity is based on determination the galactose according to the NELSON method (1994). In an alkaline medium, the pseudoaldehydic group of sugars reduces the cupric ions Cu^{2+}. The latter react with the arsenomolybdic reagent producing a blue colour, whose absorbance, measured at 520 nm, varies linearly with the concentration in starch hydrolysates (between 0 and 400 µg/mL).

4. Apparatus

4.1 Heating magnetic stirrer
4.2 water bath at 40 °C
4.3 water bath at 100 °C
4.4 100-mL cylindrical flask
4.5 centrifuge capable of housing 15-mL glass test tubes
4.6 chronometer
4.7 100-mL graduated flask
4.7.1 500-mL graduated flask
4.8. 200-µL precision syringe
    4.8.1 1-mL precision syringe
4.9 10-mL straight pipette graduated to 1/10 mL
4.10 spectrophotometer
4.11 15-mL glass test tubes
4.12 shaker of the vortex type
4.13 500-mL amber glass bottle
4.14 chamber at 4 °C
4.15 drying oven at 37 °C
4.16 carded cotton
4.17 Kraft paper
4.18 pH-meter
4.19 metal rack for 15-mL test tubes
4.20 cuvets with a 1-cm optical path length, for single use, for spectrophotometer, for measurement in the visible spectrum

5. Reagents and products

5.1 Sodium acetate (CH_3COONa 99 % pure - PM = 82g/mole)
5.2 acetic acid (CH_3COOH 96 % pure - PM = 60 g/mole, density = 1.058)
5.3 potato galactan (Megazyme, batch 71201) as an example. If this substrate is not available alternative substrates must be validated for this essay.
5.4 anhydrous sodium sulphate (NaC_2SO_4 99.5 % pure - PM = 142 g/mole)
5.5 anhydrous sodium carbonate (Na_2CO_3 99.5 % pure - PM = 105.99 g/mole)
5.6 sodium and potassium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$ 99 % pure - PM = 282.2 g/mole)
5.7 anhydrous sodium hydrogenocarbonate ($NaHCO_3$ 98 % pure - PM = 84.01 g/mole)
5.8 penta-hydrated copper sulfate ($CuSO_4 \cdot 5H_2O$ 99 % pure - PM = 249.68 g/mole)
5.9 concentrated sulphuric acid (H_2SO_4 98 % pure)
5.10 ammonium heptamolybdate ($(NH_4)_6MO_7O_{24} \cdot 4H_2O$ 99 % pure - PM = 1235.86 g/mole)
5.11 sodium hydrogenarsenate ($Na_2HAsO_4 \cdot 7H_2O$ 98.5 % pure - PM = 312.02 g/mole) g/mole). Given the toxicity of this product, special attention must be paid during manipulation. Waste material must be treated in an appropriate manner.
5.12 D-galactose ($C_6H_{12}O_6$ 99 % pure - PM = 180.16 g/mole)
5.13 distilled water
5.14 commercial enzymatic preparation for analysis.

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6. Solutions

6.1 Reagents of the oxidizing solution
These reagents must be prepared first, taking into account the 24 hours lead-time for solution D.
6.1.1 Solution A: place in a 100-mL cylindrical flask (4.4) successively
20 g of anhydrous sodium sulphate (5.4)
2.5 g of anhydrous sodium carbonate (5.5)
2.5 g of sodium and potassium tartrate (5.6)
2 g of anhydrous sodium hydrogenocarbonate (5.7)
Dissolve in 80 mL of distilled water (5.13). Heat (4.1) until dissolution and decant into a 100-mL flask (4.7). Make up to the gauge line with distilled water (5.13). Maintain at 37 °C (4.15); if a deposit is formed, filter using a folded filter.

6.1.2 Solution B:
Dissolve 15 g of penta-hydrated copper sulfate (5.8) in 100 mL of distilled water (5.13) and add a drop of concentrated sulphuric acid (5.9).

6.1.3 Solution C:
This solution is prepared extemporaneously in order to have a satisfactory proportionality between the density of colour and the quantity of glucose by mixing 1 mL of solution B (6.1.2) with 24 mL of solution A (6.1.1).

6.1.4 Solution D:
In a 500-mL graduated flask (4.7.1), dissolve 25 g of ammonium heptamolybdate (5.10) in 400 mL of water (5.13). Add 25 mL of concentrated sulphuric acid (5.9) (cooled under cold running water).
In a 100-mL cylindrical flask (4.4), dissolve 3 g of sodium hydrogenarsenate (5.11) in 25 mL of water (5.13) and quantitatively transfer into the 500-mL graduated flask (4.7.1) containing ammonium molybdate (5.10).
Make up with water (5.13) to have a final volume of 500 mL.
Place at 37°C (4.15) for 24 hours then maintain at 4 °C (4.14) in a 500 mL amber glass bottle (4.13).

6.2 Sodium acetate buffer (pH 4.2, 100 mmol/L)
It consists of solutions A and B.
6.2.1 Solution A: sodium acetate 0.1 M: dissolve 0.5 g of sodium acetate (5.1) in 60 mL of distilled water (5.13)
6.2.2 Solution B: acetic acid 0.1 M: dilute 1 mL of acetic acid (5.2) with 175 mL of distilled water (5.13)
6.2.3 Preparing the sodium acetate buffer: mix 23.9 mL of solution A (6.2.1) + 76.1 mL of solution B (6.2.2).
Check the pH of the buffer using a pH-meter (4.18).
The solution must be maintained at 4 °C (4.14).

6.3 Solution of potato galactan at 1 % (p/v)
In a 100-mL graduated flask of (4.7) dissolve 1 g of potato galactan (5.3) in 100 mL of sodium acetate buffer (6.2).

6.4 Stock solution of Galactose with 400 µg/mL
Dissolve 0.040 g of galactose (5.12) in 100 mL of distilled water (5.13).

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7. Preparation of the standard range of galactose

A standard range is produced using the stock solution of galactose (from 0 to 400 µg/mL) (6.4) as indicated in table 1.

Galactose (µg/mL)	**0**	**50**	**100**	**150**	**200**	**250**	**300**	**400**
Galactose (µmole/mL)	0	0.278	0.555	0.833	1.110	1.388	1.665	2.220
Vol. stock solution (µL) (6.4)	0	125	250	375	500	625	750	1000
Vol. distilled water (µL) (5.13)	1000	875	750	625	500	375	250	0

Table 1. Standard range of galactose

8. Preparation of the sample

It is important to homogenise the enzymatic preparation before sampling, by upturning the container for example. The enzymatic solution and the blanks will have to be prepared at time of use.

8.1 Enzymatic solution with 2 g/L to be prepared just before use.
Place 200 mg of commercial preparation (5.14) in a 100-mL graduated flask (4.7), make up with distilled water (5.13), and stir in order to obtain a homogeneous mixture.

8.2. Blank denatured by heating to be prepared just before use
Place 10 mL of the enzymatic solution at 2 g/L (8.1) in a 15 mL test tube (4.11), plug with carded cotton (4.16) covered with Kraft paper (4.17) and immerse the test tube for 5 minutes in the water bath at 100 °C (4.3). Chill and centrifuge for 5 min at 6500 g.

9. Procedure

9.1 Enzymatic kinetics: The test tubes are produced at least in duplicate.
In 5 x 15-mL test tubes (4.11) numbered from 1 to 5, placed in a rack (4.19) in a water bath at 40 °C, introduce
200 µL of the enzymatic solution at 2 g/L (8.1), using the precision syringe (4.8),
400 µL of sodium acetate buffer (6.2), using the precision syringe (4.8.1),
600 µL of potato galactan (6.3) beforehand warmed at 40 °C in water bath, start the chronometer (4.6)

After shaking (4.12), the test tubes plugged with carded cotton (4.16) and Kraft paper (4.17) are replaced in the water bath at 40 °C (4.2)
for 1 min for test tube no.1
for 2 min for test tube no.2
for 5 min for test tube no.3
for 10 min for test tube no.4
for 15 min for test tube no.5.

The reaction is stopped by placing each of the test tubes numbered from 1 to 5 immediately after they have been removed from the water bath at 40 °C, in the water bath at 100 °C (4.3) for 10 min.

The test tubes are then cooled under running cold water.

Note: Kinetic point at 10 min enables the evaluation of sought after enzymatic activity

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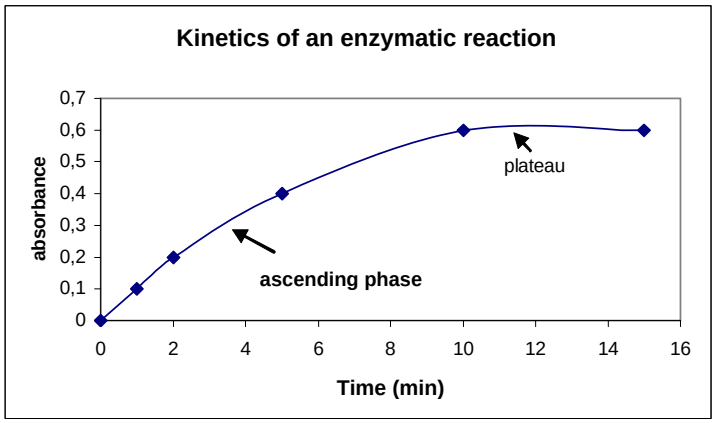
9.2 Determination of the reducing substances released
In a 15-mL test tube (4.11)
Place 1 mL of the reaction medium (9.1)
Add 1 mL of solution C (6.1.3)
After shaking (4.12), the test tube is placed in the water bath at 100 °C (4.3) for 10 min. The test tube is then cooled under running cold water.
Add 1 mL of solution D (6.1.4)
Add 9.5 mL of water (5.14) using the straight 10 mL pipette (4.9)
Wait 10 min for the colour to stabilise.
Centrifuge (4.5) each test tube at 2430 g for 10 min.
Place the supernatant liquid in a tank (4.20).
Zero the spectrophotometer using distilled water.
Immediately measure the absorbance at 520 nm, using a spectrophotometer (4.10).

9.3 Blanks
Proceed as described in 9.1, replacing the enzymatic solution at 2 g/L (8.1) by the blank denatured by heat (8.2). For each kinetic point, the enzymatic reaction of each blank is carried out at the same time as that of the enzymatic solution.

9.4 Standard range
Proceed as described in 9.2, replacing the reaction medium (9.1) by the various mediums of the standard range of galactose from 0 to 400 µg/mL (7).

10. Calculations

10.1 Determining the kinetics
In general, calculating the enzymatic activity can only be done when the substrate and the enzyme are not in limiting quantities. This therefore refers to the ascending phase of the kinetic representation: the enzymatic activity is linear in time. Otherwise, the activity would be underestimated (Figure 1).



The kinetics are determined over 15 minutes. The activity concerned is measured at T=1 min T=2 min, T=5 min, T=10 min, T=15 min.
After determining the kinetics of the enzymatic reaction, plot the curve for the variation in absorbance in relation to reaction time. *The absorbance* corresponds to the difference between the absorbance at time T of the enzymatic preparation and that of the corresponding blank.

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Then calculate the equation (1) of the straight regression line, taking into account only the points of the ascending phase (see figure 1).

10.2 Producing the calibration line

The calibration line corresponds to plotting a graph whose X-coordinates are the various concentrations of the standard range of galactose (from 0 to 0.693 µmol/mL) and whose Y-coordinates are the corresponding values of optical densities, obtained in 9.4. Then calculate the straight regression line (2) resulting from the linearity of the data of the graph.

10.3 Calculating the enzymatic activity

Based on the straight regression line (1) calculate the absorbance for an average time T (for example 4 min. in the case of figure 1) deduct from it the quantity Q of galactose released (in µmoles) for this intermediate time using equation (2).

The formula used to calculate the enzymatic activity in U/g of the preparation is as follows

$$\text{Activity in U/g} = 1000 \times (Q/T)/(V \times C)$$

Where Q: quantity of galactose released in µmol during time T (min)

V: quantity of enzymatic solution introduced (mL) here 0.2 mL

C: concentration of the enzymatic solution (g/L) here 2 g/L

It is then possible to express the enzymatic activity in nanokatals. This unit corresponds to the number of nanomoles of product formed per second under the conditions defined by the determination protocols and therefore:

$$\text{Activity in nkat/g} = (\text{activity in U/g}) \times (1000/60)$$

11. Characteristics of the method

r= 0,056

R= 0,056

Sr= 0,02

SR= 0,02

The repeatability of the method is estimated using the mean standard deviation of the absorbance values resulting from the same sampling of the enzymatic preparation, proportioned 5 times. In this way, to proportion the galactanase the mean standard deviation of the values is 0.02 with a percentage error of 9.7, in which the % error corresponds to:

$$\frac{\text{(mean standard deviation of values x 100)}}{\text{mean test value}}$$

In this way, the determination method as presented is considered repeatable.

The reproducibility tests were carried out using 2 enzymatic preparations with 5 samplings for each.

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2 tests were used in order to determine the satisfactory reproducibility of the method:

- variance analysis (the study of the probability of the occurrence of differences between samplings). Variance analysis is a statistical method used to test the homogeneity hypothesis of a series of K averages. Performing the variance analysis consists in determining if the "treatment" effect is "significant or not". The standard deviation of reproducibility given by this variance analysis is 0.02.
- the power of the test for the first species of risk α (5 %) – first species of risk α is the risk of deciding that identical treatments are in fact different.

  If the power is low ($\cong$ 20 %), this means that no difference has been detected between treatments, but there is little chance of seeing a difference if one did in fact exist.

  If the power is high ($\cong$ 80 %), this means that no difference has been detected between the treatments, but, if there was one, we have the means of seeing it.

The results are given in table 2.

Determination	**Variance analysis hypotheses**	**Probability**	**Power of test (α= 5 %)**	**Newman-Keuls test (*)**	**Bonferroni test (**)**
Galactanase	Adhered to	0.00087	93 %	Significant	Significant

Table 2: Variance analysis – study of the sampling effect

* Newmann-Keuls test: this comparison test of means is used to constitute homogeneous groups of treatments: those belonging to the same group are regarded as not being different to risk α of the first species selected

** Bonferroni test: also referred to as the "corrected T test", the Bonferroni test is used to carry out all the comparisons of pairs of means, i.e., (t (t-1))/2 comparisons before treatments, respecting the risk α of the first species selected.

In this way, the tests set up are used to see a difference if there really is one (high power test); in addition, the determination method involves a probability of occurrence of a discrepancy in activity (between samplings) lower than 5%, reinforced by belonging to the same group (Newmann-Keuls test not significant) and considered not to be different to the first species of risk α (Bonferroni test not significant).

12. Bibliography

NELSON N, A photometric adaptation of the SOMOGYI method for the determination of glucose. The May Institute for medical research of the Jewish hospital, 1944. p 375-380.

Thierry Doco, et al. Polysaccharides from grape berry cell walls. Part II. Structural characterization of the xyloglucan polysaccharides, Carbohydrate Polymers, Volume 53, Issue 3, 15 August 2003, Pages 253-261.

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RESOLUTION OIV/OENO 314/2009

CODEX – PECTINLYASE

The GENERAL ASSEMBLY

In view of article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

Following a proposal made by the Sub-Commission of Methods of Analysis and the Specifications of Oenological Products expert group**,**

HAS HEREBY DECIDED to add the following monograph to the international Oenological Codex:

PECTINLYASE
(PECTINLYASE activity)
EC. 4.2.2.10. – CAS no. 9033-35-6)

General specifications

These enzymes are not found in a pure state but they are present within an enzymatic complex. Unless otherwise stipulated, the specifications must comply with the resolution concerning the general specifications for enzymatic preparations included in the international oenological Codex.

1. Origin and oenological scope

These activities are used to support the maceration of grapes, the clarification of musts and wines, to improve the filterability of musts and wines and the pressing of grapes.
The enzymatic preparations containing these activities come from directed fermentations of *Aspergillus niger.*

Principle activities accompanying lyase pectin activity include:

- Polygalacturonase
- Pectin methyl esterase

Secondary activities: Various hemicellulases such as xylanases as well as cellulases can be considered as secondary activities, but are also extremely useful for the hydrolysis of pectic substances. In this case, given their utility, it is not appropriate to apply the clause of the resolution on enzymatic preparationsto these activities requiring that the sum of secondary activities should not be higher than 50 % of the sum of activities needed by the required function since they usefully contribute to reaching that objective.
On the other hand, the 50 % clause can be applied for the following secondary activities: Protease, beta glucosidase

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2. Scope

The determination method was developed using a commercially available pectinlyase (5.5). The conditions and the method were developed for application to the commercial enzymatic preparations such as those found on the oenological market.

3. Principle

This enzymatic activity results in the decomposition of highly methylated pectins by the β-elimination of methylated galacturonic acids. In so doing, a system of highly delocalised conjugated double bonds is created, absorbing in the ultraviolet range.

4. Apparatus

4.1 magnetic stirrer
4.2 water bath at 25 °C
4.3 water bath at 100 °C
4.4 1000-mL graduated flask
4.4.1 100-mL graduated flask
4.5 chronometer
4.6 quartz cuvets with a 1-cm optical path length, for spectrophotometer, for measurement in the UV spectrum
4.7 pH-meter
4.8 100-µL precision syringes
4.8.1 1000-µL precision syringes
4.9 spectrophotometer
4.10 15-mL test tubes
4.11 shaker of the vortex type
4.12 metal rack for 15-mL test tubes
4.13 chamber at 4 °C
4.14 carded cotton
4.15 Kraft paper

5. Products

5.1 Citrus fruit pectin with a 63-66 % degree of esterification (Pectin from citrus peel, Fluka, Ref. 76280), as an example.
5.2 Sodium hydroxide (NaOH, 99 % pure - PM = 40 g/mole)
5.3 Citric acid ($C_6H_8O_7 \cdot H_2O$, 99.5 % pure - PM = 210.14 g/mole)
5.4 Sodium dihydrogenophosphate ($NaH_2PO_4 \cdot 2H_2O$, 99 % pure - PM = 156.01 g/mole)
5.5 Distilled water
5.6 Commercial enzymatic preparation for analysis

6. Solutions

6.1 Solution of sodium hydroxide 1M
Introduce 40 g of sodium hydroxide (5.2) into a 1000-mL graduated flask (4.4) and make up with distilled water (5.5).

6.2 Mc Ilvaine buffer (Devries *et al).*
It consists of solutions A and B.

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6.2.1 Solution A: acid citric at 100 mM: dissolve 4.596 g of citric acid (5.3) in 200 mL of distilled water (5.5)
6.2.2 Solution B: sodium dihydrogenophosphate at 200 mM: dissolve 6.25 g of sodium dihydrogenophosphate (5.4) in 200 mL of distilled water (5.5).
6.2.3 Preparation of the Mac Ilvaine buffer
Mix 50% of solution A (6.2.1) + 50 % of solution B (6.2.2) and adjust pH to 6 using the solution of sodium hydroxide (6.1).
The solution must be maintained at 4 °C (4.13). Check the pH of the buffer using a pH-meter (4.7)

6.3 Solution of citrus fruit pectin at 1 % (p/v)
Dissolve 0.5 g of pectin (5.1) in 50 mL of Mc Ilvaine buffer (6.2).

7. Preparation of the sample
It is important to homogenise the enzymatic preparation before taking a sample by turning over the recipient, for example. The enzymatic solutions and blanks should be prepared at time of use.

7.1 Enzymatic solution at 10 g/L to be prepared just before use.
Place 1g of commercial preparation (5.6) in a 100-mL graduated flask (4.4.1), make up with distilled water (5.5), stir (4.1) in order to obtain a homogeneous mixture.

7.2. Blank denatured by heating to be prepared just before use
Place 10 mL of the enzymatic solution at 10 g/L (7.1) in a 15-mL test tube (4.10), plug with carded cotton (4.14) covered with Kraft paper (4.15) and immerse the tube for 5 minutes in the water bath at 100°C (4.3). Then chill and centrifuge 5 min at 6500 g.

8. Procedure

8.1 Enzymatic reaction: The test tubes are produced at least in duplicate.
In 5 x 15-mL test tubes (4.10) numbered from 1 to 5, placed in a rack (4.12) in a water bath at 25°C, introduce
400 µL of Mc Ilvaine buffer (6.2) using a 1000-µL precision syringe (4.8.1)
100 µL of the enzymatic solution at 10 g/L (7.1) using a 100-µL precision syringe (4.8)
500 µL of citrus fruit pectin solution (6.3) beforehand warmed at 25°C in water bath; start the chronometer (4.5)

After stirring (4.11), the tubes plugged with carded cotton (4.14) and Kraft paper (4.15), are placed in the water bath at 25 °C (4.2)
for 1 min for tube no.1
for 2 min for tube no.2
for 5 min for tube no.3
for 10 min for tube no.4
for 15 min for tube no.5

The reaction is stopped by rapid (30 seconds max) heating by placing each tube numbered from 1 to 5 in the water bath at 100 °C (4.3) and adding acid or basic concentrated solutions as stop reagent. The tubes are then cooled under running cold water.

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8.2 Determination of released substances
The reactional medium (8.1) is diluted to one tenth with distilled water (5.5). The dilution is placed in a cuvet (4.6) with an optical path of 1 cm.
Zero spectrophotometer using distilled water.
Immediately measure the absorbance at 235 nm, using a spectrophometer (4.9).

8.3 Blank
Proceed as described in 8.1, replacing the enzymatic solution by the blank denatured by heating (7.2). For each kinetic point, the enzymatic reaction of each blank is carried out at the same time as that of the enzymatic solution.

9. Calculations

9.1 Determining the kinetics
In general, calculating the enzymatic activity can only be done when the substrate and the enzyme are not in limiting quantities. This therefore refers to the ascending phase of the kinetic representation: the enzymatic activity is linear in time. Otherwise, the activity would be underestimated (Figure 1).

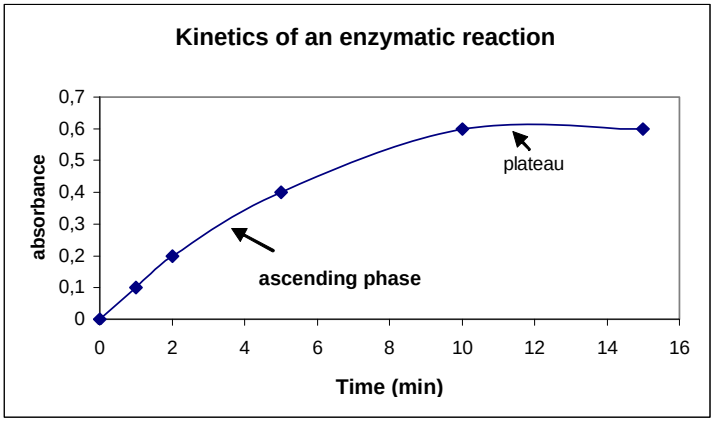

Figure 1: kinetics of enzymatic reaction

The kinetics are determined over 15 minutes. The activity concerned is measured at T=1 min T=2 min, T=5 min, T=10 min, T=15 min.
After determining the kinetics of the enzymatic reaction, plot the curve for the variation in absorbance in relation to reaction time. The absorbance corresponds to the difference between the absorbance at time T of the enzymatic preparation and that of the corresponding blank. Then calculate the DO/T slope (1) of the straight regression line, taking into account only the points of the ascending phase (see figure 1).

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9.2 Calculating the enzymatic activity

The enzymatic activity of the pectinlyase is calculated using the molar extinction coefficient of the molecule formed (ε = 5500 $M^{-1}cm^{-1}$). The formula to be applied is as follows:

$$\text{Activity in U/g} = (DO_T / T) / (0.1/ V) \times (1000/(5.5/C))$$

Where DO_T: absorbance value at time T (min)
V: quantity of enzymatic solution introduced (mL): in this case, 0.1 mL
C: concentration of the enzymatic solution (g/L): in this case 10 g/L

It is then possible to express the enzymatic activity in nanokatals. This unit corresponds to the number of nanomoles of product formed per second under the conditions defined by the determination protocols and therefore:

$$\text{Activity in nkat/g} = (\text{activity in U/g}) \times (1000/60)$$

10. Characteristics of the method

r= 0,028
R= 0,112
Sr= 0,01
SR= 0,04

The repeatability of the method is estimated using the mean standard deviation of the absorbance values resulting from the same sampling of the enzymatic preparation, proportioned 5 times. In this way, to proportion the pectinlyase the mean standard deviation of the values is 0.01 with a percentage error of 4.66, in which the % error corresponds to:

$$\frac{\text{(mean standard deviation of values x 100)}}{\text{mean test value}}$$

In this way, the determination method as presented is considered repeatable.

The reproducibility tests were carried out using 2 enzymatic preparations with 5 samplings for each.

2 tests were used in order to determine the satisfactory reproducibility of the method:

- variance analysis (the study of the probability of the occurrence of differences between samplings). Variance analysis is a statistical method used to test the homogeneity hypothesis of a series of K averages. Performing the variance analysis consists in determining if the "treatment" effect is "significant or not". The standard deviation of reproductibility given by this variance analysis is 0,04.

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- the power of the test for the first species of risk α (5 %) – first species of risk α is the risk of deciding that identical treatments are in fact different.

  If the power is low ($\cong$ 20 %), this means that no difference has been detected between treatments, but there is little chance of seeing a difference if one did in fact exist.

  If the power is high ($\cong$ 80 %), this means that no difference has been detected between the treatments, but, if there was one, we have the means of seeing it.

The results are given in table 1.

Determination	**Variance analysis hypotheses**	**Probability**	**Power of test (α= 5 %)**	**Newman-Keuls test (*)**	**Bonferroni test (**)**
PL	Adhered to	0.00725	87 %	Significant	Significant

Table 1: Variance analysis – study of the sampling effect

* Newmann-Keuls test: this comparison test of means is used to constitute homogeneous groups of treatments: those belonging to the same group are regarded as not being different to risk α of the first species selected

** Bonferroni test: also referred to as the "corrected T test", the Bonferroni test is used to carry out all the comparisons of pairs of means, i.e., (t (t-1))/2 comparisons before treatments, respecting the risk α of the first species selected.

In this way, the tests set up are used to see a difference if there really is one (high power test); in addition, the determination method involves a probability of occurrence of a discrepancy in activity (between samplings) lower than 5 %.

11. Bibliography

DE VRIES J.A., F. M. ROMBOUTS F.M., VORAGEN A.g.J., PILNIK W. Enzymic degradation of apple pectins. Carbohydrate Polymers, 2, 1982, 25-33.

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Secretary of the General Assembly

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RESOLUTION OIV/OENO 328/2009

LACTIC ACID BACTERIA- Modification

The GENERAL ASSEMBLY,

Considering Article 2, paragraph iii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

Upon proposal of the group of experts "Microbiology" and the group of experts "Specifications of oenological products",

DECIDES to replace the existing monograph (Oeno 15/2003) by the following monograph in the International Oenological Codex and to adapt the resolution accordingly (17/2003):

LACTIC ACID BACTERIA

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Lactic acid bacteria are used in oenology to perform malolactic fermentation. The lactic acid bacteria must belong to the Oenococcus, *Leuconostoc*, *Lactobacillus* and *Pediococcus* genus and must be isolated from grapes, musts, wine or have been derived from these bacteria.

The use of genetically modified bacteria will be governed by the currently applicable legislation.

The strains of lactic acid bacteria must be kept under conditions which most favour their genetic stability.

Lactic acid bacteria used in oenology must transform the malic acid in must and wine into lactic acid and carbon dioxide. This should produce biogenic amines in the smallest possible quantities, and must not produce an off taste.

2. LABELLING

The following information must be indicated on the label:
- The genus name and specie(s) in addition to the reference(s) of the strain(s) in the case that there is a registration body.
- Selecting body
- Operating instructions method and possible reactivation additives recommended by the manufacturer.
- The minimum number of viable cells per gram of preparation that is guaranteed by the manufacturer,

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- The manufacturing batch number, in addition to the expiration date and storage conditions with a storage temperature recommended by the manufacturer.
- Where relevant, the indication that lactic acid bacteria were obtained by genetic modifications and their modified character(s).
- The additives.

3. CHARACTERISTICS

Lactic acid bacteria are marketed in liquid, frozen or powder form obtained by lyophilisation or drying, in pure culture or in association with pure cultures.

4. TEST TRIALS

4.1 – Humidity for lyophilisated or dried bacteria

Measured by the weight loss of 5 g of the product, dried at 105 °C until constant weight (about 3 hours).
Maximum content should not exceed 8 %.

4.2 - Lead

Proceed with the determination according to the method in chapter II of the International Oenological Codex.
Content should be less than 2 mg/kg of dry matter.

4.3 - Mercury

Proceed with the determination according to the method in chapter II of the International Oenological Codex.
Content should be less than 1 mg/kg of dry matter.

4.4 - Arsenic

Proceed with the determination according to the method in chapter II of the International Oenological Codex.
Content should be less than 3 mg/kg of dry matter.

4.5 - Cadmium

Proceed with the determination according to the method in chapter II of the International Oenological Codex.
Content should be less than 1 mg/kg of dry matter.

4.6 – Viable lactic acid bacteria

Proceed with counting according to the method in chapter II of the International Oenological Codex. (method in annex of the present resolution)
The number should be more or equal to 10^8 CFU/ml for frozen or liquid bacteria.
The number should be more or equal to 10^{11} CFU/g for lyophilisated or dried bacteria.

4.7 - Mould

Proceed with counting according to the method in chapter II of the International Oenological Codex.
The number should be less than 10^3 CFU/g.

4.8 – Contaminant acetic acid bacteria

Proceed with counting according to the methods in chapter II of the International Oenological Codex.

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The number of acetic bacteria should be less than 10^3 CFU/g for frozen or liquid lactic acid bacteria or 10^4 CFU/g for lyophilisated or dried lactic acid bacteria.
The sum of *Acetobacter* + *Gluconobacter* should be less than 10^3 CFU/ml for frozen or liquid lactic acid bacteria or 10^4 CFU/g for lyophilisated or dried lactic acid bacteria.

4.9 – Yeasts contaminants Proceed with counting according to the methods in chapter II of the International Oenological Codex
The number of viable cells of total contaminant yeasts must be less than 10^3 CFU/g for lyophilisated or dried lactic acid bacteria or 10^2 CFU/ml for frozen or liquid lactic acid bacteria.

4.10 - Salmonella
Proceed with counting according to the method in chapter II of the International Oenological Codex
Absence should be checked on a 25 g sample.

4.11 - *Pseudomonas aeruginosa*[1]

4.12 - Escherichia coli
Proceed with counting according to the method in chapter II of the International Oenological Codex using a selective differential medium for Escherichia coli. MET in the annex. A lactic acid bacteria stock suspension is carried out in a trypone salt solution using 1 g of lactic acid bacteria for 10 ml of solution (total volume). 2 ml of stock solution is transferred to each dish using 5 different dishes. Absence should be checked on 1 g sample[2].

4.13 - Staphylococci
Proceed with counting according to the method in chapter II of the International Oenological Codex. The presence of staphylococci is evaluated by an enrichment culture in a liquid Giolitti and Cantoni medium followed by a confirmation on a solid Baird Parker medium in the annex.
A lactic acid bacteria stock suspension is carried out in a salt tryptone solution using 1 g of lactic acid bacteria for 10 ml of solution (total volume). 10 ml of stock suspension is used to inoculate a Giolitti and Cantoni medium to Tween 80 double concentration. Cultures are incubated 48 hours at 37 °C.
In the case that the Giolitti and Cantoni medium gives positive results, the presence of Staphylococci is confirmed by isolation on a solid Barid Parker medium. A positive culture medium loop is used to inoculate solid BP mediums to obtain isolated colonies.

Absence should be checked on 1 g sample[3].

4.14 - Coliforms
Proceed with counting according to the method in chapter II of the International Oenological Codex using a selective differential medium for coliforms, desoxycholate gelose in the annex. A lactic acid bacteria stock suspension is carried out in a salt tryptone solution using 1 g of lactic acid bacteria for 10 ml of solution (total volume). 2 ml of stock solution are transferred is each dish using 5 different dishes.

The number of coliforms should be less than 10^2 CFU/g.[4]

[1] Point to be studied at a later date by the expert group "Microbiology".
[2] Annex 1
[3] Annex 1
[4] Annex 1

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5. ADDITIVES

They must be in conformity with regulations in force.

6. STORAGE CONDITIONS

Always refer to manufacturer's recommendations.

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MICROBIOLOGICAL ANALYSIS METHODS
(amendment of the resolution 17/2003
in chapter II of the International Oenological Codex).

A: point 1

1. Preliminary rehydration of lactic acid bacteria

- weigh 1 g of ADB under **aseptic** conditions;
- add 100 ml **of 5 % saccharose solution in water at 36-40 °C** under sterile conditions;
- slowly homogenise using a rod **or** a magnetic stirrer for 5 min;
- stop stirring and allow to stand for 20 minutes at a temperature of **36-40 °C**;
- homogenise again at room temperature for 5 minutes;
- take 10 ml under sterile conditions and then proceed with micro-biological controls on the homogenised reference solution.

B: replace in all the milieu composition Agar by Bacteriological Agar

C: Add the following paragraphs

7.2 – For research of *Acetobacter*

Acb/s agar environment

Composition	
Yeast extract	30 g
Alcohol 95 % per volum after sterilisation	20 ml
Bromocresol green (sol. 2.2 %)	1 ml
Bacteriological Agar	2 %
Water	up to 1000 ml

Sterilisation at 120 °C for 20 min.

Add directly to Petri dish 0.1 ml of penicillin solution at 0.25 % in pure alcohol.
Add directly to Petri dish 0.2 ml of pimaricine hydroalcoholic solution at 25 % m/v.
Incubate under aerobic conditions at 25 °C for 7 days.

7.3 - Search for *Gluconobacter*

Gcb/s agar medium

Composition	
yeast autolysate	10 g
glucose	3 g
$CaCO_3$	**3g**
Bacteriological agar	2 %
water	up to 1000 ml

Sterilisation at 120 °C for 20 min.

Add directly to Petri dish 0.1 ml of penicillin solution at 0.25 % in pure alcohol.
Add directly to Petri dish 0.2 ml of pimaricine hydroalcoholic solution at 25 % m/v.
($CaCO_3$ facilitates the recognition of *Gluconobacter* colonies which dissolve and produce a lighter circular zone around the colony.)
Aerobic incubation at 25 °C for 7 days.

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ANNEX 1

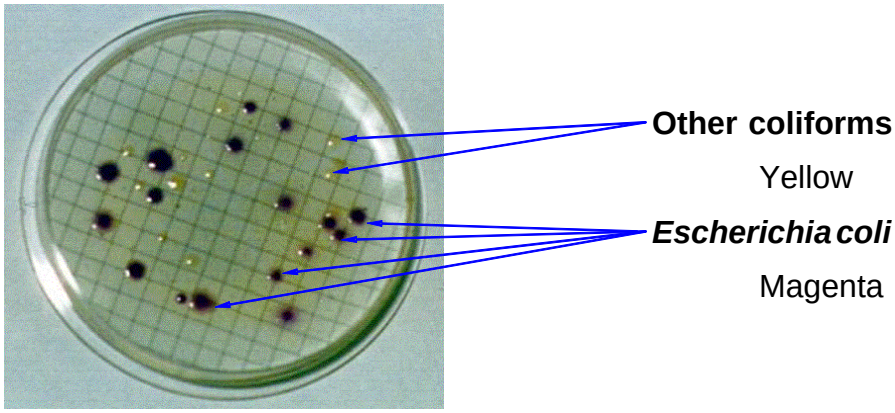
REVIEW OF METHODS OF COLIFORM RESEARCH
Escherichia coli and *Staphylococcus*

SELECTIVE-DIFFERENTIAL MEDIUM FOR COLIFORMS. DESOXYCOLATE AGAR

Ingredients/l	
Peptone	10.0 g
Lactose	10.0 g
Sodium desoxycolate	**1.0 g (Inhibition of the flora accompanying coliforms)**
Sodium chloride	5.0 g
Dipotassium phosphate	2.0 g
Ferric ammonium citrate	1.0 g
Sodium citrate	1.0 g
Agar	15.0 g
Neutral red	0.03 g

SELECTIVE-DIFFERENTIAL MEDIUM FOR *Escherichia coli*. MET

Sodium laurisulphate and sodium desoxycolate are used as selective factors, in accordance with their properties to inhibit the development of Gram-positive cocci and sporulated bacteria. The differential nature of the method is provided by the chromogen 5-bromo, 6-chloro-indolyl-β-D-glucuronide.



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SELECTIVE-DIFFERENTIAL MEDIAS FOR *Staphylococcus*

Giolitti and Cantoni medium

Composition (g) for 1 litre of medium:

Tryptone:	10,0.
Meat extract:	5,0.
Autolytic yeast extract:	5,0.
Glycine :	1,2.
Mannitol :	20,0.
Sodium piruvate:	3,0.
Sodium chloride:	5,0.
Lithium chloride:	5,0.
Tween 80 :	1,0.
pH medium:	6,9 ± 0,2.

Baird Parker solid medium

Composition (g/l)

Tryptone :	10,0.
Meat extract:	5,0.
Autolytic yeast extract:	1,0.
Sodium pyruvate :	10,0.
Glycine :	12,0.
Lithium chloride:	5,0.
Bacteriological agar:	20
Egg yolk emulsion:	47 ml.
Potassium tellurite at 3,5 %:	3 ml.
Sulfamehazine:	0,05 g/l (if necessary inhibit *Proteus*)
pH medium:	7,2 ± 0,2.

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RESOLUTION OIV/OENO 329/2009

CODEX - ACTIVE DRY YEASTS - Modification

The GENERAL ASSEMBLY,

Considering Article 5, paragraph 2 iii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

Upon the proposal of the group of expert "Microbiology" and the "Method of Analysis" Sub-commission,

DECIDES to replace the existing monograph (Oeno 16/2003) by the following monograph in the International Oenological Codex and to adapt the resolution (17/2003) accordingly:

ACTIVE DRY YEASTS (A.D.Y.) *Saccharomyces spp.*

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Yeasts are used for the inoculation of musts and wine. They are proposed under dehydrated form
The rate of inoculation is at the user's discretion.
Yeasts used must be isolated from grapes, musts or wine or result from hybridisation, or have been derived from these same yeasts. The use of genetically modified oenological yeasts will be submitted to prior authorisation of competent authorities.
Oenological yeasts must be kept under conditions which most favour its genetic stability.

2. LABELLING

The following information must be indicated on the label:
- The genus name and specie(s) name in addition to the reference(s) of the strain(s) in the case that there is a registration body.
- Selecting body.
- Operating instructions or method and reactivation media recommended by the manufacturer.
- The minimum number of viable cells per gram of powder (CFU as determined in the annex) guaranteed by the manufacturer, with a storage temperature lower than 15 °C.
- The manufacturing batch number, the expiration date and storage conditions.
- Where relevant, the indication that the yeasts were obtained through genetic modifications and their modified character(s).
- Additives, including substances used during drying operations

3. CHARACTERISTICS

Active dry yeast is in **typically in** the form of round or vermiculated pellets obtained by drying a concentrated yeast culture.

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4. TEST TRIAL METHODS AND LIMITS

4.1 - Humidity
Measured by the weight loss of 5 g of product dried at 105 °C until it reaches a constant weight (about 3 hours).
Maximum level should be less than 8 %.

4.2 - Lead
Proceed with the determination according to the method in chapter II of the International Oenological Codex.
Content should be less than 2 mg/kg of dry matter.

4.3 - Mercury
Proceed with the determination according to the method in chapter II of the International Oenological Codex.
Content should be less than 1 mg/kg of dry matter.

4.4 - Arsenic
Proceed with the determination according to the method in chapter II of the International Oenological Codex.
Content should be less than 3 mg/kg of dry matter.

4.5 - Cadmium
Proceed with the determination according to the method in chapter II of the International Oenological Codex.
Content should be less than 1 mg/kg of dry matter.

4.6 – Viable yeasts
Proceed with counting according to the method in chapter II of the International Oenological Codex.
Content should be above or equal to 10^{10} CFU/g.

NB: Counting is not applied when marketed yeasts are not *Saccharomyces spp*. or if they are mixtures of *Saccharomyces spp* and non *Saccharomyces.*

4.7 – Yeasts of species different from the species indicated on the label
Proceed with counting according to the method in chapter II of the International Oenological Codex.
Content should less than 10^5 CFU/g.

4.8 - Moulds
Proceed with counting according to the method in chapter II of the International Oenological Codex.
Content should be less than 10^3 CFU/g of powder.

4.9 – Lactic acid bacteria
Proceed with counting according to the method in chapter II of the International Oenological Codex.
Content should be less than 10^5 CFU/g.

4.10 – Acetic acid bacteria
Proceed with counting according to the method in chapter II of the International Oenological Codex.
Content should be less than 10^4 CFU/g.

4.11 - Salmonella
Proceed with counting according to the method in chapter II of the International Oenological Codex.

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Absence should be checked on a 25 g sample.

4.12 - *Escherichia coli*
Proceed with counting according to the method in chapter II of the International Oenological Codex using the selective differential medium for *Escherichia coli* MET in annex. A lactic bacteria stock solution is carried out in a Tryptone salt solution with 1g of lactic bacteria for 10 ml of solution (total volume). 2 ml of stock solution are transferred to each dish using 5 different dishes.
Absence should be checked on a 1 g sample.

4.13 - Staphylococci
Proceed with counting according to the method in chapter II of the International Oenological Codex. *The presence of staphylococci is evaluated by an enrichment culture in a liquid Giolitti and Cantoni medium followed by a confirmation on a solid Baird Parker medium in the annex.*
A lactic bacteria stock suspension is carried out in a salt tryptone solution using 1 g of lactic bacteria for 10 ml of solution (total volume). 10 ml of stock suspension is used to inoculate a Giolitti and Cantoni medium to Tween 80 double concentration. Cultures are incubated 48 hours at 37 °C.
In the case that the Giolitti and Cantoni medium gives positive results, the presence of Staphylococci is confirmed by isolation on a solid Barid Parker medium. A positive culture medium loop is used to inoculate solid BP mediums to obtain isolated colonies.
Absence should be checked on a 1 g sample.

4.14 - Coliforms
Proceed with counting according to the method in chapter II of the International Oenological Codex. *using a selective differential medium for coliforms, desoxycholate gelose in the annex. A lactic bacteria stock suspension is carried out in a salt tryptone solution using 1 g of lactic bacteria for 10 ml of solution (total volume). 2 ml of stock solution are transferred is each dish using 5 different dishes.*

Number should be less than 10^2 CFU/g[1].

5. ADDITIVES

They must be in conformity with regulations in force.

6. STORAGE CONDITIONS

Storage should be below 15 °C in unopened packs.

Always refer to manufacturer's recommendations.

[1] See annex 1

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METHODS OF MICROBIOLOGICAL ANALYSIS
(amendment of the resolution 17/2003
in chapter II of the International Oenological Codex)

A: point 1

1. Preliminary rehydration of active dry yeasts (ADY)

- weigh 1 g of ADY under **aseptic** conditions;
- add 100 ml **of 5 % saccharose solution in water at 36-40 °C** under sterile conditions;
- slowly homogenise using a rod **or** a magnetic stirrer for 5 min;
- stop stirring and allow to stand for 20 minutes at a temperature of between **30-40 °C referring to the manufacturer's recommendations**;
- homogenise again at room temperature for 5 minutes;
- take 10 ml under sterile conditions and then proceed with micro-biological controls on the homogenised reference solution.

B: replace in all the "Environment" paragraphs replace Bacteriological agar agarby Bacteriological agar

C: Add the following paragraphs

7.2 – For research of *Acetobacter*

Acb/s agar environment

Composition

Yeast extract	30 g
Bromocresol green (sol. 2.2 %)	1 ml
Bacteriological Agar	2 %
Water	up to 1000 ml

Sterilisation at 120 °C for 20 min.
Add 20 ml of alcohol 95 % per volume
Add directly to Petri dish 0.1 ml of penicillin solution at 0.25 % in pure alcohol.
Add directly to Petri dish 0.2 ml of pimaricine hydroalcoholic solution at 25 % m/v.
Incubate under aerobic conditions at 25 °C for 7 days.

7.3 - Search for *Gluconobacter*

Gcb/s agar medium

Composition

yeast autolysate	10 g
glucose	3 g
$CaCO_3$	3 g
Bacteriological agar	2 %
water	up to 1000 ml

Sterilisation at 120 °C for 20 min.

Add directly to Petri dish 0.1 ml of penicillin solution at 0.25 % in pure alcohol.
Add directly to Petri dish 0.2 ml of pymaricine hydroalcoholic solution at 25 % m/v.
($CaCO_3$ facilitates the recognition of *Gluconobacter* colonies which dissolve and produce a lighter circular zone around the colony.)
Aerobic incubation at 25 °C for 7 days.

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ANNEX 1

REVIEW OF METHODS OF COLIFORM RESEARCH
Escherichia coli* and *Staphylococcus

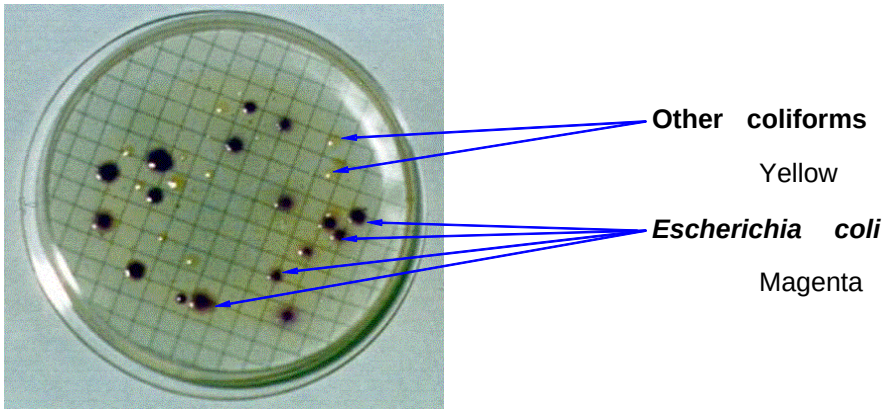
SELECTIVE-DIFFERENTIAL MEDIUM FOR COLIFORMS. DESOXYCOLATE AGAR

Ingredients/l	
Peptone	10.0 g
Lactose	10.0 g
Sodium desoxycolate	1.0 g
Sodium chloride	5.0 g
Dipotassium phosphate	2.0 g
Ferric ammonium citrate	1.0 g
Sodium citrate	1.0 g
Agar	15.0 g
Neutral red	0.03 g

(Inhibition of the flora accompanying coliforms)

SELECTIVE-DIFFERENTIAL MEDIUM FOR *Escherichia coli*. MET

Sodium laurisulphate and sodium desoxycolate are used as selective factors, in accordance with their properties to inhibit the development of Gram-positive cocci and sporulated bacteria. The differential nature of the method is provided by the chromogen 5-bromo, 6-chloro-indolyl-β-D-glucuronide.



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SELECTIVE-DIFFERENTIAL MEDIA FOR *Staphylococcus*

Giolitti and Cantoni medium

Composition (g) for 1 litre of medium:

Tryptone:	10,0.
Meat extract:	5,0.
Autolytic yeast extract:	5,0.
Glycine :	1,2.
Mannitol :	20,0.
Sodium piruvate:	3,0.
Sodium chloride:	5,0.
Lithium chloride:	5,0.
Tween 80 :	1,0.
pH medium:	6,9 ± 0,2.

Baird Parker solid medium

Composition (g/l)

Tryptone :	10,0.
Meat extract:	5,0.
Autolytic yeast extract:	1,0.
Sodium pyruvate :	10,0.
Glycine :	12,0.
Lithium chloride:	5,0.
Bacteriological agar:	20.
Egg yolk emulsion:	47 ml.
Potassium tellurite at 3,5 % :	3 ml.
Sulfamehazine:	0,05 g/l (if necessary inhibit *Proteus*)
pH medium:	7,2 ± 0,2.

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RESOLUTION OIV/OENO 351/2009

DETERMINATION OF THE ABILITY OF AN ENZYMATIC PREPARATION TO INTERRUPT PECTIC CHAINS BY MEASURING VISCOSITY

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 iv of the Agreement of 3 April 2001, establishing the International Organisation of Vine and Wine,

following a proposal made by the sub-commission for "Methods of Analysis" and the "Specifications of Oenological Products" group of experts,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to add the following analytical techniques to Section II of the International Oenological Codex:

1. PRINCIPLE

Here, it is proposed to measure the quantity of enzyme needed to halve the viscosity of a standard solution with a given pH, temperature and time.
This is a purely technological measurement designed to test the true clarifying efficiency of the enzyme. It essentially measures the pectinase activity, which cannot be directly deduced from the release of galacturonic acid in the medium.

Comment
To measure the enzyme's activity, there are two possible approaches:
- Either the time it takes a given concentration of the enzyme to halve the viscosity of the pectin solution,
- Or, the concentration of enzyme needed in order for the pectin solution's viscosity to be halved in a given period of time.

Tests show that, as long as the substrate is not limiting:
- In the first case, the viscosity logarithm (flow time) is inversely proportional to the reaction time and,
- In the second case, the viscosity logarithm is inversely proportional to the quantity of enzyme in the medium.

In either case, it is easy to find either the time or the quantity of enzyme needed to halve the viscosity on the basis of a judiciously chosen spectrum.

2. REAGENT CONDITIONS

70 mmol/l phosphate buffer medium 70 mmol/l and 30 mmol/l citrate
Substrate: 70-75 % esterified apple pectin (e.g. Sigma P 8471), diluted to 10 g/l in the buffer solution.

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pH = 3.5
Temperature: 30 °C
Reaction time: 15 minutes.
Pectinase: spectrum of concentrations covering approximately 10 mg/l of enzyme dry weight in the sample; i.e., for example, 0.5 mg in 50 ml of substrate, which corresponds to the quantity of enzyme that is liable to halve the substrate's viscosity in 15 minutes in the conditions described above.

3. APPARATUS

3.1 Bath or water circulation thermostat (30 °C ± 1 °C)
3.2 Capillary flow viscometer (A.3.1: Fig. 2) with a water value (the time for water to flow between the two marks) of approximately 18 to 20 seconds (i.e. a capillary tube roughly 0.5 to 0.6 mm in diameter)
3.3 Timer
3.4 Analytical balance (sensitivity 0,001 g)
3.5 pH meter
3.6 Magnetic stirrer, conventional laboratory glassware
3.7 Rapid paper filters
3.8 Micro-pipettes or micro-syringes for dispensing volumes from 5 to 500 µl

4. PURE PRODUCTS

4.1 Pure citric acid (99,5 %)
4.2 Pure disodium hydrogenophosphate (Na_2 $HPO_4 \bullet 2H_2O$) (99,0 %)
4.3 70-75 % esterified apple pectin with more than 90 % purity (e.g. Sigma P 8471)
4.4 Distilled or deionized water
4.5 Pure sodium hydroxide (98 %)
4.6 Pure hydrochloric acid (11.5 M) (33,5 %)
4.7 Pectinase the activity of which is to be measured.

5. SOLUTIONS

Each solution should be homogenised before using

5.1 2 M sodium hydroxide
Weigh out 80 g pure sodium hydroxide (4.5) in a 100-ml volumetric flask and dissolve in deionized water (4.4). Top up to the filler mark after complete dissolution and cooling.

5.2 2M hydrochloric acid
In a 100-ml volumetric flask half-filled with deionized water, place enough pure hydrochloric acid (4.6) to obtain a 2 M solution, (after having topped up to the filler mark).

5.3 47 mmol/l phosphate buffer, 53 mmol/l citrate, pH 3.5
5.3.1 Put 800 ml deionized water (4.4) in a 1,000-ml volumetric flask
5.3.2 Weigh out 11.22 g citric acid (4.1)
5.3.3 Weigh out 8.30 g pure disodium hydrogenophosphate (Na_2 $HPO_4 \bullet 2H_2O$) (4.2)
5.3.4 Transfer the quantitatively-weighed chemical products to the 1,000 ml volumetric flask, stirring all the time

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5.3.5 Mix until completely dissolved
5.3.6 Adjust the pH to 3.50 ± 0.05, at ambient temperature, with 2 M sodium hydroxide (5.1) or 2M hydrochloric acid (5.2), depending on the initial pH
5.3.7 Top up to the filler mark with deionized water (4.4). Mix
Stability: 8 days at ambient temperature.

5.4. Substrate: Apple pectin (4.3),
5.4.1 Put a 400-ml cylindrical container into a bath of water with a temperature of 40° C± 3° on a rotating stirrer
5.4.2 Add 250 ml of buffer with a pH of 3.5 (5.3), measured exactly, to the cylindrical container
5.4.3 Keep stirring gently at 40 °C
5.4.4 Weigh out 2,500 g ± 0,01 g of pectin (4.3)
5.4.5 Slowly add the pectin whilst stirring vigorously
5.4.6 Then stir slowly for 60 minutes, maintaining the temperature at 40° C
5.4.7 Stop stirring and cool to 30 °C ± 3 °C
5.4.8 Filter with rapid filter paper (3.8) if necessary (if lumpy)
Stability: 24 hours at ambient temperature.

5.5 100 g/l dry weight pectinase solution (4.7)
5.5.1 Weigh out 2.50 g ±0.01 g of powdered or granulated pectinase
5.5.2 Transfer to a 25-ml volumetric flask
5.5.3 Top up to the filler mark with buffer solution at pH 3.5 (5.3)
5.5.4 Dissolve by stirring for 20 minutes using a magnetic stirrer.
Filter through rapid filter paper if the enzyme is immobilised on an insoluble substance using a rapid filter (3.7)
5.5.5 In the case of a liquid enzymatic preparation, use it directly.
Stability: 4 hours at ambient temperature.

6. MEASUREMENTS

6.1 Put the viscometer in the bath of water at 30 °C or use any device that makes it possible to measure the viscosity at 30 °C.
6.2 Measure the viscosity (the flow time between the two marks on the viscometer) of the buffer solution at pH 3.5; that is, t_o. This time should be approximately 20 seconds for a capillary tube 0.5 to 0.6 mm in diameter.
6.3 Measure the flow time of the 10 g/l pectin solution, that is, $T_{p.}$ This time should be approximately 200 seconds or more.
6.4 Prepare a series of 4 volumetric flasks containing 50 ml of 10 g/l pectin and put them in the bath of water at 30 °C.
6.5 Add 5 µl of the 100 g/l enzyme solution to the first flask and homogenize.
Then, approximately every 15 minutes, successively add to the other flasks:
15 µl, 35 µl and 100 µl of the 100 g/l enzyme solution and homogenize.
6.6 Measure the time taken by the various solutions to flow between the two marks on the viscometer exactly 15 minutes after adding the enzyme.

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7. GRAPHIC REPRESENTATION OF THE MEASURED VALUES

Deduct the t_o value corresponding to the buffer at pH 3.5 alone from the flow time.
Produce a graph to represent the flow time logarithm as a function of enzyme concentration. There must be at least three points in a line corresponding to the strongest dilutions. If this is not the case, use a more diluted enzyme solution - 50 g/l or even 10 g/l, for example.

8. INTERPRETATION OF THE RESULTS

Find the regression line equation passing through the three aligned points:
$T = ax + b$
Deduct from this the necessary concentration of enzyme C to halve the pectin solution's viscosity $(T_p - t_o)/2$; that is, $T_{0,5}$.

9. EXAMPLES

9.1 Determination of the necessary enzyme concentration to halve the viscosity of the pectin solution. (Table 1)
Flow time of the buffer alone t_o = 19.3 s

Table 1:

Vol (µl) of 100 g/l enzyme /50 ml of pectin	Concentration (g/l)	Flow time(s)	Corrected time(s)	Corrected time log.
0	0	230 (Tp)	210.7 (Tp - to)	2.32
5	0.01	190	170.7	**2.23**
25	0.05	107	87.7	**1.94**
100	0.2	32.8	13.5	**1.13**
500	1	23.8	4.5	0.65*

Corrected time = flow time – flow time of buffer with a pH of 3.5
* value not taken into consideration

regression line equation (Fig.1) $y = -5.8366x+2.2844$

$(T_p - t_o) /2 = 105$ s.
Log 105 = 2.02 → C = (2.28-2.02)/5.84 = 0.044
Therefore, 0.044 g/l of enzyme are needed to halve the viscosity of a 10 g/l apple pectin solution at 30 °C during 15 minutes.

It has been shown that 1 g/l of enzyme was sufficient to almost totally reduce the viscosity of the pectin solution in 15 minutes.

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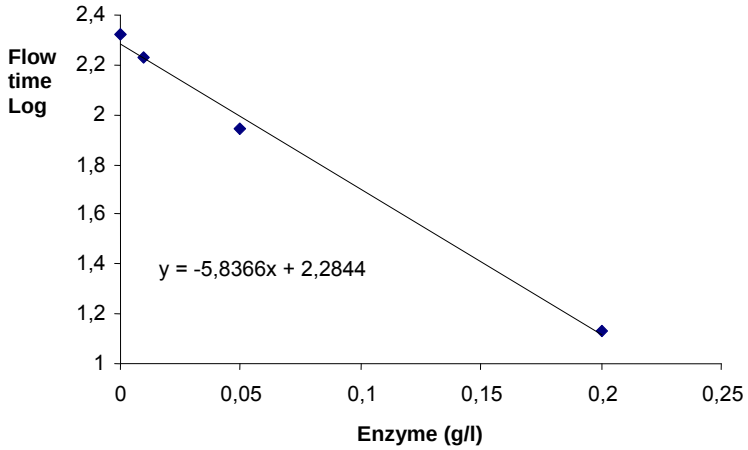

Fig.1 Reduction in the viscosity of a pectin solution as a function of enzyme concentration.

9.2 Reduction in the viscosity of a 10 g/l pectin solution as a function of the reaction time at 30 °C of an enzyme with a concentration of 0.1 g/l. (Fig. 2) – *For information only*

The buffer flow time was 19.6 seconds.

Table 2:

Reaction time (mn)	Flow time(s)	Corrected flow time(s)*	Flow time log.
0	170 (T_p)	150.7 ($T_p - t_o$)	2.18
11.5	101.4	82.1	1.91
15.6	86	66.7	1.82
21.08	72.8	53.5	1.73
29	59.83	40.53	1.61
40.31	48.79	29.49	1.47
57	40.08	20.78	1.32
90	32.25**	12.95	1.11
167	26.25**	6.95	0.84

* Corrected flow time
**Values not taken into consideration since the remaining quantity of pectin limits the reaction.

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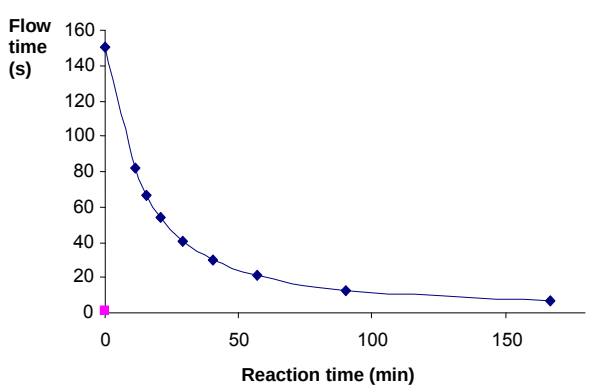

Fig. 2. Changes in the viscosity of a 10 g/l pectin solution as a function of the reaction time of a 0.1 g/l enzyme at 30 °C

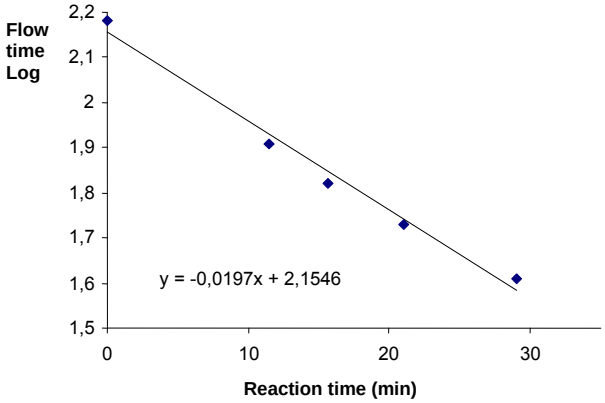

Fig.3. Changes in the viscosity of a 10 g/l pectin solution as a function of the reaction time logarithm of a 0.1 g/l enzyme at 30 °C.

Interpretation of the results

The values in table 4 show that a T/2 reaction time of 13.3 minutes is needed to halve the viscosity of the 10 g/l pectin solution at 30 °C.

For the calculation, on the basis of the regression line in Fig. 3:
Log75.35 = 1.877
Hence, $T_0/2$ = (2.1545 – 1.877)/0.0197 =14.1 minutes.

10. BIBLIOGRAPHY

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RESOLUTION OIV/OENO 352/2009

DIFFERENTIATION METHOD FOR COMMERCIAL OENOLOGICAL TANNINS - AMENDMENT TO THE MONOGRAPH

THE GENERAL ASSEMBLY

In view of Article 2 paragraph 2 iii of the Agreement establishing the International Organisation of Vine and Wine

Having studied the research by the "Methods of Analysis" Sub-commission and the group of experts "Specification of Oenological products"

Considering resolution Oeno 12/2002 concerning the oenological tannins monograph

DECIDES, upon the proposal of Commission II "Oenology" to amend and complete the existing monograph with the addition of an annex related to the following method

DIFFERENTIATION OF COMMERCIAL ENOLOGICAL TANNINS BY GC-MS ANALYSIS OF MONOSACCHARIDES AND POLYALCOHOLS

1. Introduction

According to the International Enological Codex of the O.I.V., the enological tannins should be extracted from gall nuts (of *Quercus,* such as Aleppo galls, and of Tara, also called *Caesalpina Spinosa*), oak wood (*Quercus* sp.), grape seeds and skins (*Vitis vinifera*) and the wood of certain trees such as quebracho (*Schinopsis balansae)* and chestnut (*Castanea* sp.).

2. Scope

The method described here is suitable for the differentiation of commercial enological tannins from different origins (plant galls, seed and skin grape, oak wood, chestnut and quebracho).

3. Principle

The concentration of monosaccharides (arabinose, xylose, fructose and glucose) and polyalcohols (arabitol, quercitol, pinitol, *chiro*-inositol, *muco*-inosotol, *scyllo*-inositol and *meso*-inositol) in tannin samples was determined by gas chromatography-mass spectrometry (GC-MS) after their previous derivatization into their trimethylsilyl ethers.

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4. Reagent and materials

Reagents

Trimethylsilylimidazole (TMSI) 97 % pure
Trimethylchlorosilane (TMCS)
Dried pyridine 99.5 % pure
High purity water produced in a Milli-Q synthesis A10 system

Standards

Phenyl-β-glucoside (internal standard): 1 mg/mL prepared in 70 % methanol

Preparation of the standard solutions (of monosaccharides and polyalcohols)

Standard solutions of glucose, fructose, arabinose, xylose, arabitol, pinitol, *meso*-inositol, *scyllo*-inositol, *muco*-inositol and c*hiro*-inositol were disolved in methanol: water 30:70 at concentrations varying between 0.05 and 0.5 mg/mL of each standard. As quercitol and bornesitol are not commercially available, aqueous extracts were prepared from oak acorns of *Quercus* sp. and from leaves of *Echium vulgare*. The extracts were evaporated at low temperature under vacuum, silylated and injected as described below. Carbohydrate composition (in triplicate, RSD$\leq$ 5 %) of oak extract was 68 % quercitol, 20 % fructose and 18 % glucose and 20 % fructose, 33 % glucose, 27 % bornesitol, 2 % meso-inositol and 19 % saccharose for the *Echium* extract.
Note: All standard solutions have to be prepared working daily and preferably stored cold in a refrigerator prior to injection. All samples have to be derivatised and analysed in the day.

5. Samples

Twenty eight samples of different commercial tannins, including oak wood (O; n=4), grape seed (S; n=6), grape skin (H; n=2), plant galls (G; n=6), chestnut (Ch; n=3), quebracho (Q; n=3), gambier (GMB; n=1) and mixtures of grape+quebracho (GQ; n=1), quebracho+chestnut+plant gall (QChG; n=1) and chestnut+quebracho (ChQ; n=1) tannins, were directly purchased in the market or supplied by the manufacturers.

6. Apparatus

- Fume cupboard
- Laboratory glassware: beakers, vessels, etc.
- Micropipets
- Rotaevaporator
- Vortex
- Domestic mill
- Centrifuge

-Gas chromatograph equipped with a flame ionisation detector (FID)
-Gas chromatograph coupled to a quadrupole mass spectrometry detector operating in electronic impact (EI) mode at 70 eV. MS data were registered from 40 to 700 m/z.

- Column: 25 m x 0.25 mm i.d. x 0.25 µm film thickness fused silica column coated with crosslinked methyl silicone.

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7. Procedure

Derivatization procedure

50 mg of tannins are dissolved in 5 mL of deionized water and filtered through Whatman No. 1 or similar filter paper. 1 mL of the sample is mixed with 1 mL of phenyl-β-glucoside, as internal standard. This mixture is evaporated under vacuum and trimethylsilyl derivates were formed by addition of 100 µL of anhydrous pyridine, 100 µL of TMSI and 100 µL of TMCS, shaking after each addition. Extraction of the trimethylsilyl (TMS) derivatives is carried out using 100 µL of hexane and 200 µL of water.

GC analysis

1µL of the hexane upper layer is injected on the GC. Identity of each compound is confirmed by comparison of their retention times and mass spectra using GC-MS method with those of standards. The typical chromatographic profile of each tannin origin is shown in Figure 1.

GC-FID analysis: *chromatographic conditions*

Injections are made in splitless mode. Injector and detector temperature are 300 ºC. Oven temperature is maintained at 100 ºC for 1 min, then programmed with a heating rate of 30 ºC/min up to 200 ºC kept for 15 min and finally programmedat a heating rate of 15 ºC/min up to 270 ºC maintained for 20 min. Carrier gas is nitrogen.

GC-MS analysis: *chromatographic conditions*

Injections are made in splitless mode. The injector is at 300ºC and the oven temperature is maintained at 100 ºC for 1 min, then programmed with a heating rate of 30 ºC/min up to 200 ºC kept for 15 min and finally programmed at a heating rate of 15 ºC/min up to 270 ºC maintained for 20 min. Carrier gas is He at 1 mL/min.

8. Calculation (Results)

Quantitative analysis is carried out using the response factor (RF) of each standard relative to phenyl-β-D-glucoside (internal standard) over the expected range. Reproducibility of the method is evaluated analyzing one sample on five different days. However this method does not allow to distinguish quebracho tannins from those of skin grape.

For example the limits of detection (LOD) and quantification (LOQ) (Tables 1 and 2) are calculated for each compound according to Foley and Dorsey (1984). Mean values of 0.42 ng and 1.41 ng injected were obtained for LOD and LOQ, respectively. Concentrations of polyols and monosaccharides in tannins analysed are respectively in tables 3 and 4.

This method allows the classification of tannins according to the scheme suggested in Figure 2. The presence of quercitol is indicative of tannins from oak wood, whereas pinitol is mainly indicator of tannins from tara galls and bornesitol of tannins from gambier. The absence of arabinose and xylose in gall tannins can also help to the characterization of these samples. Therefore, bornesitol, quercitol, pinitol, arabinose and xylose could be used to unequivocally differentiate these products, and furthermore, to distinguish these tannins from the rest of the products analyzed. Tannins from galls and grapes can be easily differentiated from tannins of other origins due to the absence of arabinose

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and xylose in their monosaccharide composition. Referring to grape tannin samples, fructose could be observed in seed grape tannins, whereas it was absent in skin grape tannin. The presence of *muco*- and *chiro*-inositol could be useful to distinguish tannins from chestnuts from those of quebracho or grape skin.

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Table 1. Repeatability of the GC method for the determination of carbohydrates in tannins (sample Q3).

	Mean value	Standard deviation
Xylose	0.17	0.01
Arabinose	0.43	0.03
Arabitol	0.04	0.00
Quercitol	0.00	0.00
Fructose	0.32	0.04
Glucose	0.60	0.02
***Muco*-inositol**	0.02	0.00
***Chiro*-inositol**	0.00	0.00
***Scyllo*-inositol**	0.00	0.00
***Meso*-inositol**	0.05	0.00

Table 2. Limit of detection (LOD) and of quantification (LOQ) of the GC method for the determination of carbohydratesand of plyols in oenological tannins samples by means of gas-chromatography (expressed in injected ng)

	LOD (ng)	LOQ (ng)
Xylose	0.50	1.66
Arabinose	0.66	2.21
Arabitol	0.21	0.70
Fructose	1.11	3.70
Glucose	0.51	1.70
***Muco*-inositol**	0.16	0.52
***Chiro*-inositol**	0.22	0.74
***Scyllo*-inositol**	0.20	0.68
***Meso*-inositol**	0.24	0.80

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Table 3. Concentration of polyols (mg/100g, tr=traces) in commercial tannins

		mg/100g							
		Arabitol	Quercitol	Pinitol	Bornesitol	*Muco*-inositol	*Chiro*-inositol	*Scyllo*-inositol	Meso-inositol
Oak wood	O1	0.06	6.92	-	-	0.10	0.10	0.52	0.49
	O2	0.06	4.49	-	-	0.11	0.11	0.57	0.55
	O3	0.05	1.57	-	-	0.04	0.02	0.13	0.12
	O4	0.09	3.14	-	-	0.14	0.17	0.17	0.30
Gall plant	G1	-	-	0.73	-	-	-	-	-
	G2	-	-	0.26	-	-	-	-	tr
	G3	-	0.03	0.07	-	-	-	0.03	tr
	G4	-	0.06	0.06	-	-	-	0.04	-
	G5	-	-	1.35	-	-	-	-	0.02
	G6	-	-	-	-	-	-	-	-
Seed grape	S1	-	-	-	-	-	-	tr	0.16
	S2	-	-	-	-	-	-	tr	0.01
	S3	-	-	-	-	-	-	0.38	2.34
	S4	-	-	-	-	-	-	tr	0.01
	S5	-	-	-	-	-	-	-	0.01
	S6	0.64	-	-	-	-	-	tr	0.25
Skin grape	H1	-	-	-	-	-	-	-	-
	H2	-	-	-	-	-	-	-	tr
Chestnut	Ch1	0.08	-	-	-	0.14	0.55	-	0.62
	Ch2	0.04	-	0.49	-	0.03	0.33	-	0.05
	Ch3	0.07	-	-	-	0.19	0.52	-	0.49
Quebracho	Q1	tr	-	-	-	-	-	-	0.01
	Q2	0.02	0.05	0.09	-	-	-	-	tr
	Q3	0.03	-	-	-	0.02	-	-	0.05
Gambier	GMB	0.01	-	tr	0.02	-	-	-	0.03
Grape+quebracho	GQ	0.10	-	0.19	-	0.02	0.06	-	0.07
Quebracho+chestnut+gall	QChG	0.03	-	0.19	-	0.03	0.12	-	0.12
Chestnut+quebracho	ChQ	0.05	-	-	-	0.13	0.56	-	0.53

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Table 4. Concentration of monosaccharides (mg/100g, tr= traces) in commercial tannins

		mg/100g			
		Xylose	Arabinose	Fructose	Glucose
Oak wood	O1	0.29	1.18	-	0.22
	O2	0.57	2.53	-	0.07
	O3	0.37	0.85	0.12	0.58
	O4	0.41	1.84	1.82	2.69
Gall plant	G1	-	-	0.26	0.42
	G2	-	-	0.07	0.17
	G3	-	-	0.05	0.05
	G4	-	-	0.11	0.16
	G5	-	-	0.50	0.63
	G6	-	-	-	-
Seed grape	S1	-	-	10.01	9.59
	S2	-	-	0.64	0.50
	S3	-	-	45.23	32.46
	S4	-	-	0.61	0.46
	S5	0.13	-	-	0.03
	S6	-	-	1.22	tr
Skin grape	H1	-	-	-	0.07
	H2	0.31	0.48	0.30	0.67
Chestnut	Ch1	0.50	1.46	1.15	0.78
	Ch2	0.41	1.04	0.95	0.91
	Ch3	0.65	1.55	0.28	0.69
Quebracho	Q1	0.30	0.44	0.22	0.20
	Q2	0.07	0.10	0.05	0.10
	Q3	0.16	0.42	0.32	0.59
Gambier	GMB	0.02	-	0.42	0.12
Grape+quebracho	GQ	0.07	0.11	0.25	0.28
Quebracho+chestnut+gall	QChG	0.04	0.07	0.17	0.30
Chestnut+quebracho	ChQ	0.29	1.29	1.34	1.46

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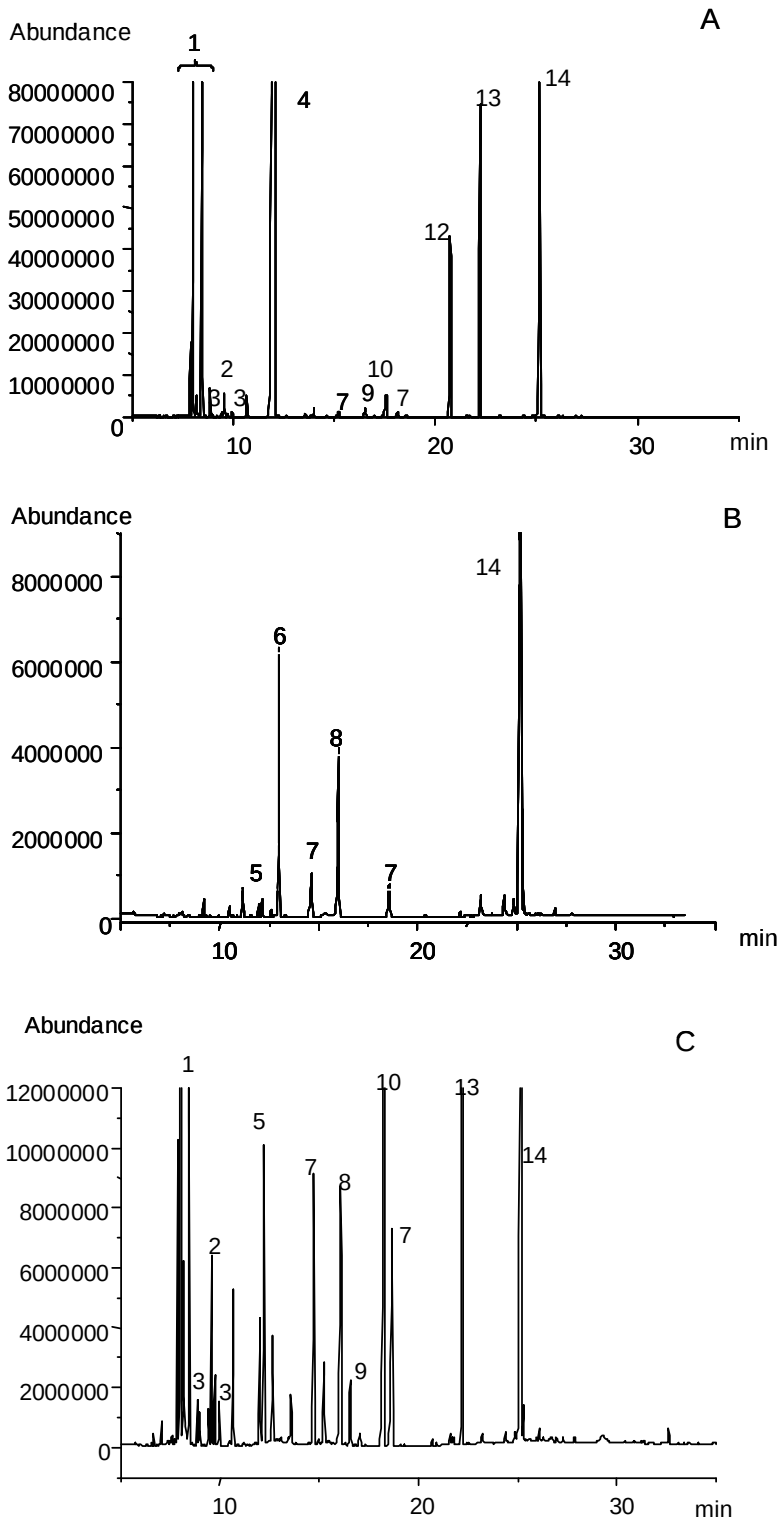

Figure 1. Gas chromatographic profiles of polyalcohols and carbohydrates in commercial tannins of A) oak wood, B) plant gall, C) chestnut wood, D) seed grape, E) skin grape, F) quebracho wood, G) Gambier. 1-Arabinose, 2-Arabitol, 3-Xylose, 4-Quercitol, 5-Fructose, 6-Pinitol, 7-Glucose, 8-Gallic acid, 9-*Muco*-inositol, 10-*Chiro*-inositol, 11-Bornesitol, 12- *Scyllo*-inositol, 13-*Meso*-inositol, 14-Phenyl-β-D-glucoside (i.s.)

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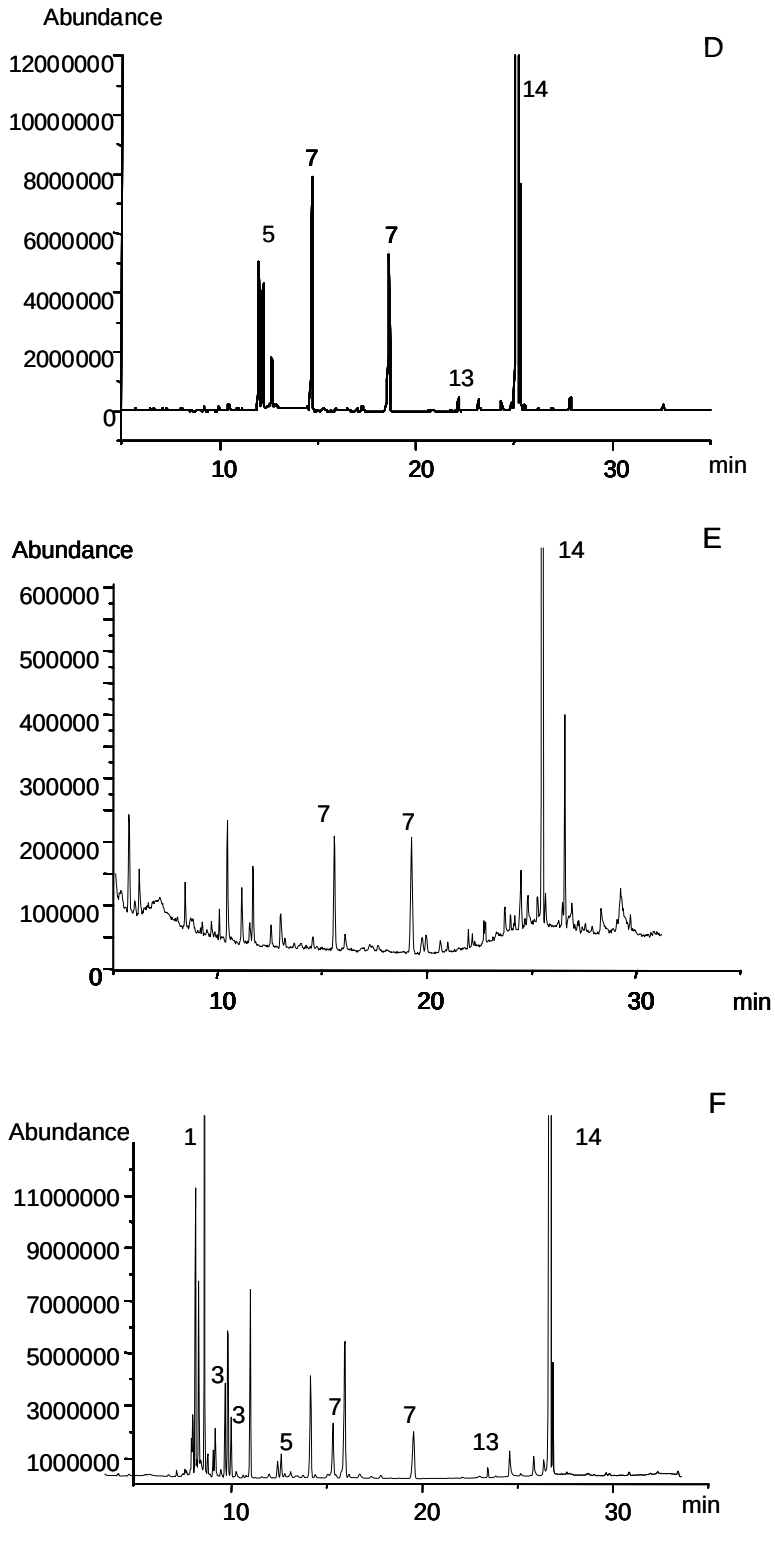
Figure 1. continuation



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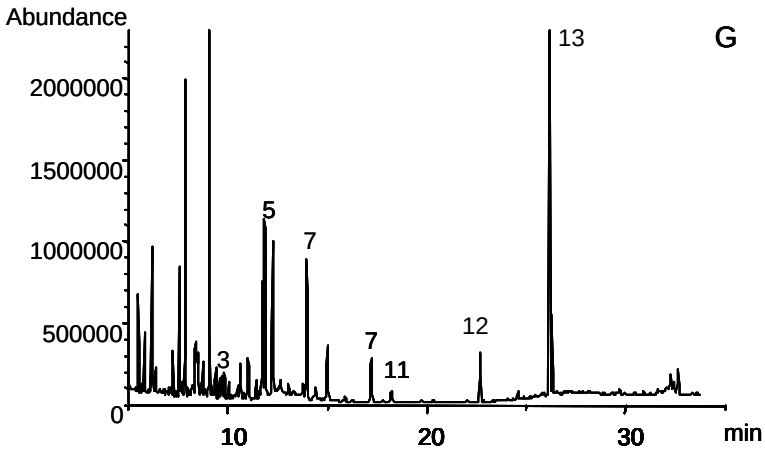

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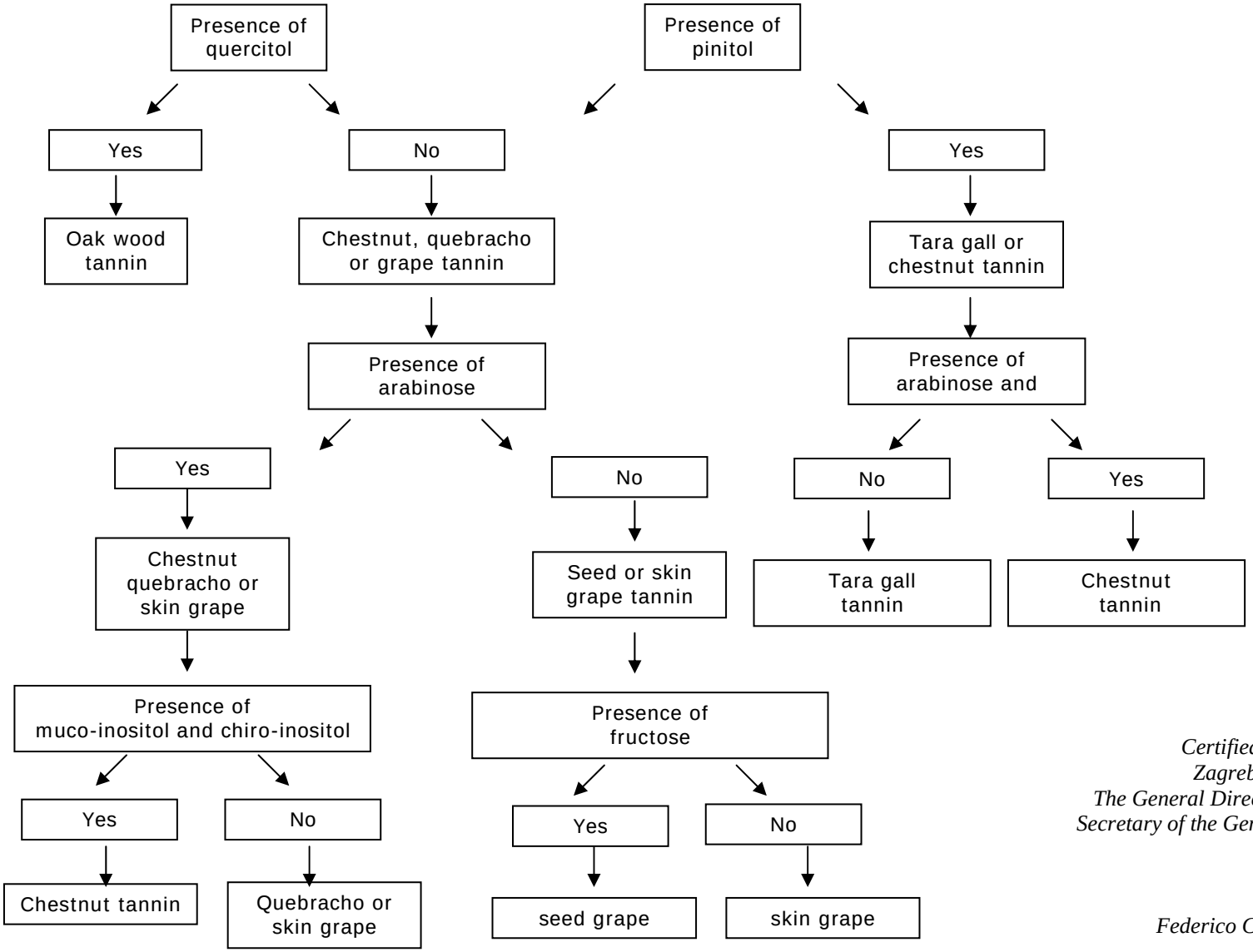

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Figure 2. Scheme of tannins classified according to their monosaccharide and polyalcohol composition

RESOLUTION OIV/OENO 365/2009

REVISION OF THE MONOGRAPH ON ENZYMATIC PREPARATIONS (OENO 14/2003)

The GENERAL ASSEMBLY,

In view of article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine was founded,

Taking note of the works of the "Specification of Oenological Products" expert group,

Considering resolution Oeno 14/2003 adopted by the OIV

Considering the explanatory items mentioned in the introductory report in the annex

DECIDES on the proposal of Commission II "Oenology" to modify resolution Oeno 14/2003 in the International Oenological Codex by the following monograph:

ENZYMATIC PREPARATIONS

The prescriptions described below concern all enzymatic preparations susceptible of being used during various operations that can be applied to grapes and their derivatives.

The prescriptions are based on the recommendations from the "General Specifications and Considerations for Enzymes used in Food Processing" drafted by the "*Joint FAO/WHO Expert Committee on Food Additives (JECFA), 67th Session, Rome 20 -29 June 2006* published in 2006 in the *FAO JECFA monographs.*

1. GENERAL CONSIDERATONS

Enzymatic preparations can be made from micro-organisms or plants.
When looking for synergies between various enzymatic activities including pectinase, cellulase and hemicellulase, mixtures of preparations made from different strains can be carried out. These preparations can contain one or more active compounds, in addition to supports, diluents, preservatives, antioxidants and other substances compatible with the good manufacturing practices and in accordance with local regulations. In certain cases, preparations can contain cells or cell fragments. Furthermore they can be in either liquid or solid form. The active substances can also be immobilised on a support admitted for food use.

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2. LABELLING

The labelling of enzymatic preparations must at least specify the nature of the preparation (ex. pectinolytic enzyme), the activity (in units by g or ml), the batch number and the expiration date. Enzymatic preparations with multiple technological activities (cf. 4.1) must be mentioned as such on the label (for example: Enzymatic preparation with clarifying or aromatic enhancer properties).

If there is available space, it is desirable that the label has the following information: the main activity the preparation was standardised for, recommended dose and implementation conditions, storage condition for maintaining stability, the nature of additives and carriers used, the nature of enzymatic activities, batch number and expiration date. If there is not enough space, this information shall be indicated on the technical data sheet of the preparation.
The indication that enzymatic preparations were obtained by genetically modified organisms must be mentioned, where relevant.

3. ADMITTED ENZYMATIC PREPARATIONS

All enzymatic preparations with activities presenting a technological interest duly proven in practice and meeting the conditions and criteria mentioned above, are accepted for the treatment of grapes and their by-products.
Enzymatic preparations used must not contain any substance, microorganism, nor enzymatic activity that:

- is harmful to health,
- is harmful to the quality of the products manufactured,
- can lead to the formation of undesirable products,
- or that will give rise or facilitate fraud.

4. ENZYMATIC ACTIVITIES

4.1 General considerations

Enzymatic preparations contain many enzymatic activities. Other than the main enzymatic activities, (activities for which, respectively, the enzymatic preparation has been standardised) whose technological interest has been duly proven, secondary enzymatic activities are only tolerated if they are set within the technological constraint limits for manufacturing of enzymatic preparations. They must be as limited as possible. Activities which have a negative effect on wine (ex. Cinnamoyl esterase) should not be present in commercialised enzymatic products in oenology. Generally speaking, secondary activities must likewise comply with the requirements formulated above (point 3).
Generally speaking, the secondary activities present in a given preparation must not become the main reason to use the said preparation unless this preparation is declared as multiple technological effects. On a technological level, there is a distinction between the following preparations:
Maceration preparations: facilitate extraction of compounds such as colour, tannins,...
Clarification preparations: facilitate clarification and filtration of musts and wine
Aroma enhancers: reinforces and/or modifies aromatic profile of musts and wine
Stabilisation preparations: facilitates extraction of macromolecules or other substances with a stabilising effect on wine (yeast mannans).

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When an enzymatic preparation generates multiple technological effects, duly noted in a practice, (ex. Clarification and aroma enhancer enzymes), whether they are the result of a main and/or secondary activity, they must be declared as such on the label. Different enzymatic activities responsible for these effects must be measured and indicated in the technical preparation data sheet.

Activities are expressed in nKat. (nkat = 1 nmole of transformed substrate or product formed per second by g or ml of the preparation).

4.2 Activity measurement

The enzymatic activities presented are measured under the conditions corresponding to their biochemical characteristics. (pH, temperature) and if possible, the closest to activities encountered in the practice (grape juice, must or wine). The methods implemented must correspond to state of the art in analytical terms and , if possible, be validated in accordance with appropriate international standards (for example: ISO 78-20; ISO 5725).
Results are expressed in nanokatal/g or nanokatal/ml.

When the sought out technological effect results from the action of different enzymes within the same preparation, it is necessary to measure each enzymatic activity. Each of these activities will require special sheets, with the details of the analytical method.

5. SOURCES OF ENZYMES AND FERMENTATION ENVIRONMENT

The microbial sources of enzymes must be non-pathogenic, non-toxic and genetically stable, and the fermentation broth should not leave harmful residues in enzymatic preparations. In the case of microorganisms, a safety study must be conducted in order to ensure that enzymatic preparation produced by a microorganism species *(e.g. Aspergillus niger)* does not present any health risk. This study can be based on principles brought forth on food enzyme guidelines published by the Scientific Committee for Food (SCF), or other equivalent organisations.
The techniques implemented must be compatible with good manufacturing practices and the prescriptions of the International Oenological Codex if yeast and/or lactic bacteria are used.

6. CARRIERS , DILUENTS, PRESERVATIVES AND OTHER ADDITIVES

Substances used as carriers , diluents, preservatives or other additives must not, with a "carry over" effect, disseminate compounds in the grapes and derivative products, which are not compatible with regulations in force in different countries. Moreover, these compounds must not have a negative effect on the organoleptic properties of wine. In the case of immobilised enzymes, the carriers used must comply to standards on material in contact with foodstuffs. For this type of preparation, the content of compounds of the carriers used, susceptible to enter the musts and wine, should be determined and indicated on the label of the enzymatic preparation.
Preservatives such as KCl are added in the liquid enzyme concentrate during manufacturing**.** These substances prevent the development of micro-organisms during the different formulation operations of products**.** These substances can be found not only in liquid preparations but also in solid preparations. Given the inevitable "carry over"

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effect, only preservatives which are compatible to regulations in force in the different countries are authorised.
These substances must be clearly identified and indicated on the label or on the technical data sheet of the commercial product.

7. HYGIENE AND MAXIMAL LEVEL OF CONTAMINANTS

Enzymatic preparations must be produced in accordance with good manufacturing practices:

7.1 Lead

Proceed with the determination according to the method described in chapter II of the International Oenological Codex.
Content less than 5 mg/kg.

7.2 Mercury

Proceed with the determination according to the method described in chapter II of the International Oenological Codex.
Content less than 0.5 mg/kg.

7.3 Arsenic

Proceed with the determination according to the method described in chapter II of the International Oenological Codex.
Content less than 3 mg/kg.

7.4 Cadmium

Proceed with the determination according to the method described in chapter II of the International Oenological Codex.
Content less than 0.5 mg/kg.

7.5 *Salmonella* sp

Proceed with counting according to the method described in chapter II of the International Oenological Codex.
Absence checked on a 25 g sample.

7.6 Total coliforms

Proceed with counting according to the method described in chapter II of the International Oenological Codex.
Content less than 30/per gram of preparation.

7.7 *Escherichia coli*

Proceed with counting according to the method described in chapter II of the International Oenological Codex.
Absence checked on a 25 g sample.

7.8 ANTIMICROBIAL ACTIVITY

Non-detectable

7.9 SPECIFIC MYCOTOXINS OF DIFFERENT PRODUCTION STRAINS

Non-detectable

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8. TECHNICAL DATA SHEET TO BE SUPPLIED BY MANUFACTURER

Each type of enzymatic preparation must be defined using a technical data sheet.
It must contain at least the following information:

- Name of enzyme and biological origin
  (e.g. pectolytic enzymes of *Aspergillus niger* or pectolytic enzyme of *A. oryzae* expressed as *A. niger*),

- Declared activity (in nKat/g or nKat/ml of preparation)
- Fields and application mode (technological effects and useful details for the implementation of the preparation) ,
- Stability of the preparation and expiration date period based on production date guaranteeing the maintaining of activity, under the given storage conditions (temperature),
- Types of reactions catalysed by the main enzymatic activities,
- Main enzymatic activities with IUB number (for example Tannase 3.1.1.20),
- Secondary enzymatic activities with, if possible, the IUB number
- Types of carriers, diluents, preservatives and additives used and their respective contents,

If deemed useful, further information can be added to this technical data sheet.

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RESOLUTION OIV/OENO 366/2009

MONOGRAPH ON CARBOXYMETHYLCELLULOSE (CELLULOSE GUM)

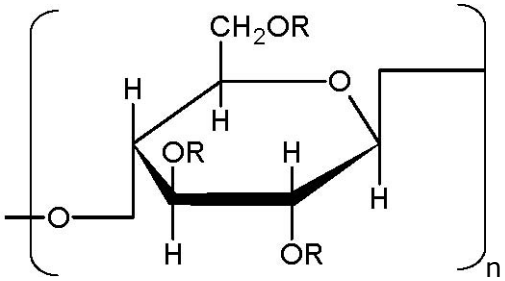
The GENERAL ASSEMBLY

In view of article 2, paragraph 2 iv of the Agreement of 3 April 2001, establishing the International Organisation of Vine and Wine,

Considering resolution 2/2008 adopted by the OIV in 2008 concerning the addition of carboxymethylcellulose to white wines and sparkling wines in order to enhance their tartaric stabilisation following a proposal made by the "Specifications of oenological products" experts group

HAS HEREBY DECIDED to add the following monograph to the international Oenological Codex:

CARBOXYMETHYLCELLULOSE
(cellulose gum)
(CMC)
INS no. 466
CAS [9004-32-4]



where R = H or CH_2COONa

1. SUBJECT, ORIGIN AND SCOPE
Carboxymethylcellulose (cellulose gum) for oenological use is prepared exclusively from wood by treatment with **alkali** and monochloroacetic acid or its sodium salt. Carboxymethylcellulose inhibits tartaric precipitation through a "protective colloid" effect. A limited dose is used.

2. SYNONYMS
Cellulose gum, CMC, Sodium CMC, Sodium salt of a carboxymethyl ether of cellulose, NaCMC

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3. LABELLING

Labelling must mention that the carboxymethylcellulose is for use in food, as well as safety and preservation conditions.

4. CHARACTERISTICS

4.1 Description

Granular or fibrous powder, blank or slightly yellowish or greyish, slightly hygroscopic, odourless and tasteless. This may be proposed in the form of a concentrate for solution in wine prior to use. Solutions must contain at least 3,5 % carboxymethylcellulose.

4.2 Chemical formula

The polymers contain anhydroglucose units substituted with the following general formula: $[C_6H_7O_2(OH)x(OCH_2COONa)y]n$ where
N is the degree of polymerisation
x = from 1.50 to 2.80
y = from 0.2 to 1.50
x + y = 3.0
(y = degree of substitution)
Note: Only the carboxymethylcellulose possessing a degree of substitution between 0.6 and 1.0 are completely soluble.

4.3 Degree of substitution

Evaluate the degree of substitution using the method described below. The degree of substitution must lie between 0.60 and 0.95.

4.4 Molecular weight

Ranges from 17,000 to 300,000 (degree of polymerisation from 80 to 1,500). The molecular weight can be evaluated through measurement of viscosity.

The viscosity of a 1 % solution must lie between 10 and 15 $mPa \cdot s^{-1}$, or between 20 and 45 $mPa \cdot s^{-1}$ for a 2 % solution, or between 200 and 500 $mPa \cdot s^{-1}$ for a 4 % solution.

4.5 Composition

Measure the carboxymethylcellulose composition using the method described below. The carboxymethylcellulose content must be at least 99.5 % of the anhydrous substance.

5. TESTS

5.1 Solubility

Forms viscous colloidal solution with water. Insoluble in ethanol.

5.2 Foam test

Vigorously shake a 0.1 % solution of the sample. No layer of foam appears (this test distinguishes sodium carboxymethylcellulose from other cellulose ethers and from alginates and natural gums).

5.3 Precipitate Formation

To 5 mL of an 0.5% solution of the sample add 5 mL of a 5 % solution of copper sulfate or of aluminium sulfate. No precipitate appears. (This test permists the distinction of sodium carboxymethyl cellulose ethers from other cellulose ethers, and from gelatine, carob bean gum and tragacanth gum)

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5.4 Colour reaction
Add 0.5 g of powdered carboxymethylcellulose sodium to 50 mL of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced. To 1 mL of the solution, diluted with an equal volume of water, in a small test tube, add 5 drops of 1-naphthol. Incline the test tube, and carefully introduce down the side of the tube 2 mL of sulfuric acid so that it forms a lower layer. A red-purple colour develops at the interface.

5.5 Moisture - Loss on drying
Measure the loss on drying using the method described below. Not more than 12 % after drying.

5.6 pH of a 1 % solution
No less than 6 and no more than 8.5 pH units.

5.7 Arsenic
Quantifythe arsenic using the method described in chapter II. The arsenic content must be lower than 3 mg/kg

5.8 Lead
Quantify the lead using the method described in chapter II. The lead content must be lower than 2 mg/kg

5.9 Mercury
Quantify the mercury using the method described in chapter II. The mercury content must be lower than 1 mg/kg

5.10 Cadmium
Quantify the cadmium using the method described in chapter II. The cadmium content must be lower than 1 mg/kg

5.11 Free Glycolate
Quantify the glycolate using the method described below. The carboxymethylcellulose should not contain more than 0.4 % (calculated in sodium glycolate percentage of the anhydrous substance).

5.12 Sodium
Quantify the sodium using the method described in chapter II. The sodium content must be lower than 12.4 % of the anhydrous substance

5.13 Sodium chloride
Quantify the sodium chloride using the method described below. The carboxymethylcellulose must not contain more than 0.5 % of the anhydrous substance.

5.14 Loss on drying

5.14.1 Objective
This test determines the volatile part of carboxymethylcellulose. The result of this test is used to calculate the total solids of the sample and by extension, all the volatile substances at the test temperature are regarded as moisture.

5.14.2 Interest and use The measurement of water content (by taking account of the purity) is used to measure the quantity of carboxymethylcellulose in commercial products.

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5.14.3 Equipment
5.14.3.1 Drying oven at 105 °C ± 3 °C;
5.14.3.2 Weighing bottle 50 mm in internal diameter and 30 mm in height or equivalent;
5.14.3.2 Precision balance
5.14.4 Test
5.14.4.1 Weigh between 3 and 5 g of sample to ± 1 mg, in a weighing bottle which has already been tared.
5.14.4.2 Place the weighing bottle without its lid in the drying oven for four hours. Let cool in a desiccator, replace the lid and weigh.
5.14.4.3 Continue the process until constant weight

5.14.5 Calculation
5.14.5.1 Calculate the percentage of the water content M according to the formula:
$M = (A/B) \times 100$ where
A = loss of weight by drying (in g); B = initial mass of sample.

5.15 Sodium Glycolate

5.15.1. Objective
This test covers the determination of sodium glycolate contained in the purified carboxymethylcellose containing not more than 2 % sodium glycolate.

5.15.2 Summary of the test method
Carboxymethyl cellulose dissolved in acetic acid (50 %), precipitated with acetone in the presence of sodium chloride and the insoluble is eliminated by filtration. The filtrate containing the glycolate sodium (in the form of glycolic acid) is treated to remove the acetone and reacts with 2,7-dihydroxynaphthalene. The resulting colour is measured at 540 nm with a calibrated spectrophotometer using solutions of known concentrations.

5.15.3 Interest and use
This test method (along with moisture and sodium chloride) is must been used when measuring the quantity of polymer in the substance. It must be used to check the purity of carboxymethylcellulose required by public health regulations.

5.15.4 Equipment
5.15.4.1 Spectrophotometer capable of carrying out analysis at 540 nm;
5.15.4.2 Spectrophotometer cells, 1 cm of optical path
5.15.4.3 Aluminium paper in squares approximately 50 × 50 mm;
5.15.4.4 Precision balance

5.15.5 Reagents
5.15.5.1 Acetic acid, glacial (purity ≥ 99 %);
5.15.5.2 Acetone (purity ≥ 99 %);
5.15.5.3 2,7-dihydroxynaphtalene solution (0.100 g/L): Dissolve 100 mg ± 1 mg of 2,7-dihydroxynaphthalene (naphthalenediol) in 1 L of sulphuric acid. Before using, allow the solution to stand until the initial yellow colo disappears. If the solution is dark, eliminate it and prepare a new one with a different supply of sulphuric acid. This solution remains stable for one month when stored in a dark bottle;
5.15.5.4 Standard glycolic acid solution at 1 mg/mL: dry several grams of glycolic acid in a desiccator for at least sixteen hours at room temperature. Weigh 100 mg ± 1 mg, pour into a 100 mL graduated flask, dissolve with water, adjust with water to the filling mark. Do not keep solution longer than 30 days;

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5.15.5.5 Sodium chloride (NaCl, purity ≥ 99 %);
5.15.5.6 Sulphuric acid concentrate (H_2SO_4 purity ≥ 98 %, ρ≥ 1.84).

5.15.6 Preparation of the calibration curve
5.15.6.1 In a series of five graduated 100 mL volumetric flasks, pour 0, 1, 2, 3 and 4 mL of the glycolic acid reference solution (to 1 mg / mL). Into each flask, add 5 mL of water, then 5 mL of glacial acetic acid, make up with acetone to the filling mark and mix. These flasks contain respectively, 0, 1, 2, 3 and 4 mg of glycolic acid.
5.15.6.2 Pipet 2 mL of each of these solutions and transfer them into five 25 mL graduated flasks. Evaporate the acetone by heating the open graduated flasks, laid out vertically, in a water bath for exactly 20 min. Remove from the water bath and let cool at room temperature.
5.15.6.3 Add 5 mL of 0.100 g/L 2,7-dihydroxynaphtalene solution, mix thoroughly, then add an additional 15 mL of 2,7-dihydroxynaphtalene solution and mix. Cover the mouth of the flasks with a small piece of aluminium foil, place the flasks upright in the water bath for 20 min. Remove from the water bath, let cool at room temperature and add sulphuric acid to the filling mark.
5.15.6.4 Measure the absorbance of each sample at 540 nm against the blank using 1 cm optical depth cells. Plot the absorbance curve according to the corresponding quantity of glycolic acid (in mg) in each flask.

5.15.7 Test method
5.15.7.1 Weigh 0.500 g ± 0.001 g of sample and transfer into a 100 mL beaker. Moisten the sample entirely with 5 mL of acetic acid, followed by 5 mL of water, stir with a glass rod until dissolution is complete (usually requires approximately 15 minutes). Slowly add 50 mL of acetone while stirring, then approximately 1 g of sodium sulphate. Continue to stir for several minutes to ensure complete completely precipitation the carboxymethylcellulose.
5.15.7.2 Filter using a paper filter previously soaked with small amount of acetone, and collect the filtrate in a 100 mL graduated flask. Use 30 mL of acetone to facilitate transfer of solid matter and to wash the filter cake. Make up to the filling mark with acetone and mix.
5.15.7.3 In another 100 mL graduated flask, prepare a blank with 5 mL of water, 5 mL of glacial acetic acid, then make up to the filling mark with acetone and mix.
5.15.7.4 Pipet 2 mL of the sample solution and 2 mL of the blank solution and pour them into two 25 mL graduated flasks. Evaporate the acetone as before (5.15.6.2).
5.15.7.5 Measure the absorbance of the sample and infer the quantity of glycolic acid (in mg) using the calibration curve (5.15.6.4).

5.15.8 Calculation: Calculate the content C (in %) of sodium glycolate (free glycolate) contained using the formula:

$$C(\%\ \ sodium\ \ glycolate) \ = \ \frac{B \times 0.129}{W \times (100 - M)}$$

where
B = glycolic acid (in mg) inferred using the calibration curve;
W = quantity of weighed carboxymethylcellulose (in g);
M = water content of the sample (in %);

0.129 = (ratio of the molecular weight of sodium glycolate compared to the molecular weight of the glycolic acid)/10.
Note: if the test is carried out with pre-dehydrated carboxymethylcellulose, the formula becomes:

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Secretary of the General Assembly

Federico CASTELLUCCI

$$C(\%\ \ sodium\ \ glycolate)\ =\ \frac{B\times 0.129}{W}$$

W = quantity of carboxymethylcellulose (dry) weighed (in g).

5.16 Sodium chloride

5.16.1 Objective This test method determines the sodium chloride content of the purified carboxymethylcellulose (> 98 %).

5.16.2 Summary of the test method The sodium carboxymethylcellulose is dissolved in water and titrated by potentiometry with a silver nitrate solution. Hydrogen peroxide is added to reduce the viscosity of the solution.

5.16.3 Importance and use This test method (along with moisture and sodium glycolate content) is used to calculate the degree of substitution of carboxymethylcellulose. It must be used to analyse highly purified grades of sodium carboxymethylcellulose (> 98 %).

5.16. 4 Equipment
5.16.4. 1 pH-meter capable of reading voltage (in mV), equipped with a silver electrode and a mercury sulphate reference electrode saturated with potassium sulphate.
5.16.4.2 buret, 10 mL
5.16.4.3 Precision balance.
5.16.4.4 250 mL Erlenmeyer flask.

5.16. 5. Reagents
5.16.5. 1 Concentrated hydrogen peroxide (30 % in mV) (H2O2).
5.16.5.2 Concentrated nitric acid (HNO_3) (ρ 1.42).
5.16.5.3 Silver nitrate, standard solution (0.1 N) - Dissolve 17.0 g of silver nitrate ($AgNO_3$) in 1 L of water. Store in an amber glass bottle. Standardise the solution as follows: Dry the sodium chloride (NaCl) for 2 hrs at 120 °C. Weigh 0.65 g ± 0.0001 g in a 250 mL beaker and add 100 mL of water. Place on a magnetic stirrer, add 10 mL of HNO_3, and immerse the electrodes of the pH-meter. Using a buret, add by 0.25 mL fractions the theoretical quantity of the $AgNO_3$ solution. After each addition, wait approximately 30 seconds before carrying out readings of the corresponding voltages. When approaching the endpoint, decrease the additions to 0.05 mL. Record the voltage (in millivolts) according to the volume (in mL) of the titration solution, continue titration a few mL beyond the endpoint. Trace the potential values obtained in relation to the corresponding volumes of titrated solution, and determine the potential of the equivalence point according to the singular point of the curve obtained.
Calculate the normality, N, as follows:

$$N = (A \times 1000) / (B \times 58{,}45)$$

where
A = NaCl used in g,
B = added $AgNO_3$ solution in mL,

58.45 = molecular mass of the NaCl in g,
5.16.5.4 Sodium chloride (NaCl, purity ≥ 99%).

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5.16. 6 Test method
5.16.6 1 Weigh 5 g ± 0.0001 g of sample in a 250 mL beaker. Add 50 mL of water and 5 mL of H_2O_2 (30 %). Place the beaker on a steam bath, stirring occasionally until the solution is fluid. If dissolution does not occur within 20 min, add 5 mL of H_2O_2 and heat until dissolution is complete.
5.16.6 2 Cool the beaker, add 100 mL of water and 10 mL of HNO_3. Place it on the magnetic stirrer and titrate with the 0.1 N $AgNO_3$ solution (5.16.5.3) up to the equivalence point.

5.16. 7 Calculation
5.16.7. 1 Calculate the sodium chloride content C (in %) as follows:

$$C = (AN \times 584{,}5) / [G \times (100 - B)]$$

Where:
A = volume of $AgNO_3$ solution added (in mL);
N = Normality of the $AgNO_3$ solution;
G = weight of the sample used (in g),
B = Moisture, given extemporaneously (in %) as per paragraph 5.14 and 584.5 = molecular mass of NaCl ×10 (in g).

5.17 Degree of substitution

5.17.1 Objective This method is used to determine the degree of etherification (of substitution) of the carboxymethylcellulose used.

5.17.2 Summary of the test method Pre-purified carboxymethylcellulose mineralises in the presence of sulphuric acid. The weight of the residual sodium sulphate enables inference of the sodium content and by extension the degree of substitution.

5.17.3 Importance and use This test method is used to determine the number of substituent groups added to the basic cellulose backbone.

5.17.4 Equipment
5.17.4. 1 500 mL Erlenmeyer flask.
5.17.4. 2 Precision balance.
5.17.4. 3 Sintered glass filter.
5.17.4. 4 Filter-flask.
5.17.4. 5 Porcelain crucible.
5.17.4. 6 Drying oven at 110 °C.
5.17.4. 7 Desiccator.
5.17.4. 8 Bunsen burner or muffle furnace at 600 °C.

5.17.5 Reagents
5.17.5.1 Methanol or ethanol (purity ≥ 98 %)
5.17.5.2 0.1 N silver nitrate ($AgNO_3$)
5.17.5.3 Acetone (purity ≥ 99 %)
5.17.5.4 Sulphuric acid (purity ≥ 96 %)
5.17.5.5 Ammonium carbonate (NH_4HCO_3)

5.17. 6 Preparation of the sample (This step is not necessary if the sample is assumed to contain at least 99.5 % of of carboxymethylcellulose)Weigh 5 g of the sample ± 0.1 mg, and transfer into a 500 mL conical flask. Add 350 mL of methanol or ethanol (80 % volume). Stir the suspension for 30 min. Decant trough a tared glass filtering crucible under gentle suction. At the

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The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

end of filtration, avoid drawing in air through the crucible. Repeat the treatment until the 0.1 N silver nitrate test for the chloride ions is negative for the crucible. Normally, three washings suffisant. Transfer the carboxymethylcellulose into the same crucible. Eliminate the traces of alcohol by rinsing with acetone. Let the acetone evaporate into the air (under a hood) then in a drying oven at 110 °C until constant weight. Weigh for the first time after two hours. Cool the crucible each time in a desiccator and during the weighing, pay attention to the fact that sodium carboxymethyl cellulose is slightly hygroscopic.

5.17. 7 Test method In a porcelain crucible tared beforehand, weigh 2 g ± 0.1 mg of dried substance following the preparation above. Char with the Bunsen burner, first carefully with a small flame and then for 10 min with a large flame. Cool, then pour 3 to 5 mL of concentrated sulphuric acid onto the residue. Heat carefully with the fuming is finished. After cooling, add about 1 g of ammonium carbonate by pouring the powder onto the entire contents of the crucible. Reheat, initially with a small flame until no more smoke is released then at deep red for 10 min. Repeat the sulphuric acid and ammonium carbonate washing if the residual sodium sulphate still contains carbon. Let the crucible cool in a desiccator and weigh. In place of adding the ammonium carbonate and heating by flame, the crucible can be placed in an over for one hour at approximately 600 °C.

5.17. 8 Calculate the sodium content of the sample extracted from alcohol by the formula

$$\%\ sodium\ =\ \frac{a \times 32.38}{b}$$

a = weight of residual sodium sulphate
b = weight of the sample extracted from dry alcohol

5.17. 9 Calculate the degree of substitution using the formula:

$$Degré\ \ de\ \ substitution\ =\ \frac{162 \times \%\, sodium}{2300 - (80 \times \%\, sodium)}$$

5.18 Composition in carboxymethyl cellulose

Calculate the percentage of sodium carboxymethyl cellulose in the sample by deducting 100 % of the sum of percentages of sodium and sodium glycolate (free glycolate), determined separately by the procedures above.
Carboxymethyl cellulose content (in %) = 100 – (% NaCl + % sodium glycolate)

5.19 Measurement of viscosity

5.19.1. Objective
5.19.1. 1 This test method determines the viscosity of aqueous carboxymethylcellulose solutions within ranging from 10 to 10 000 mPa/s at 25 °C.
5.19.1 2 The concentration to be used for the test must be such that determination of the solution viscosity will be possible within the limits of the test.
5.19.1 3 The results of the carboxymethylcellulose viscosity measurement by the present test method are not necessarily identical to the results obtained with other types of instruments used for the measurement of viscosity.
5.19.1 4 The determinations are calculated on a dry weight, which requires knowledge of the water content of carboxymethylcellulose (see §5.14).
5.19.1 5 The recommended Brookfield spindles and the speeds are shown in table 1, but they can be adapted for greater convenience.

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TABLE 1: Spindles and speeds required by the viscometer

Domaine de viscosité, (en mPa/s)	mobile n°	vitesse (en tr/min)	Echelle	Facteur
10 to 100	1	60	100	1
100 to 200	1	30	100	2
200 to 1000	2	30	100	10
1000 to 4000	3	30	100	40

5.19.2. Interest and use This test method is used to estimate the molecular weight of carboxymethylcellulose

5.19.3. Equipment
5.19.3. 1 Brookfield viscometer.
5.19.3. 2 Glass container, approximately 64 mm (2 ½ inches) in diameter and 152 mm (6 inches) tall, straight edged, 40 g capacity (12 oz).
5.19.3. 3 Precision balance
5.19.3. 4 Mechanical stirrers with a stainless steel blade fastened to a variable speed motor capable of functioning at 900 ± 100 r/min under different load conditions.
5.19.3. 5 Water bath, at 25 °C ± 0.5 °C.
5.19.3. 6 Precision thermometer capable of reading temperatures ranging from 20 to 30 °C ± 0.1 °C.

5.19.4. Test method
5.19.4. 1 Determine the water content following § 5.14.
5.19.4. 2 Calculate the dry weight of the sample in grams, M, required to prepare 240 g of the test solution as follows:

$$M = 100\ A / (100 - B)$$

where:
A = desired dry mass of the sample in g, and
B = the water content of the sample in %.

5.19.4. 3 Calculate the quantity of distilled water as follows:

$$V = 240 - S$$

where:
V = volume of distilled water in mL and
S = mass of the sample in g.

5.19.4. 4 Add the quantity of water calculated in the jar. Position of the stirrer must allow a minimal clearance between the stirrer and the bottom of the container.
5.19.4. 5 Begin stirring and to slowly add the carboxymethylcellulose. Adjust stirring speed to approximately 900 ± 100 r/min and mix for 2 hrs. Do not allow the stirring speed exceed 1,200 r/min as higher speeds tend to affect the viscosity of certain carboxymethylcellulose solutions.
NOTE: If the sample is added too quickly, an agglomeration will occur, which could prevent the complete dissolution of the sample in the indicated time interval.

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5.19.4. 6 Remove the stirrer and transfer the container containing the sample to the water bath until a constant temperature is reached (approximately one hour). Check the temperature of the sample with a thermometer at the end of one hour and make sure that the test temperature has been reached.
5.19.4. 7 Remove the container containing the sample from the water bath and stir vigorously for 10 sec. Measure viscosity with the Brookfield viscometer, choosing the spindle and speed following table 1. Let the spindle turn for three minutes before carrying out the reading.

5.19.5. Calculation
28.1 Calculate viscosity, V, in millipascals per second (mP/s) as follows:
V = reading × factor

5.19.6. Expression of results Express the result of Brookfield viscosity at 25 °C by indicating the concentration of the solution, the spindle, and the spindle speed used.

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RESOLUTION OIV/OENO 367/2009

MONOGRAPH ON CHITIN–GLUCANE

The GENERAL ASSEMBLY

In view of article 2, paragraph 2 IV of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

Considering the works of the Specifications of Oenological Products experts group,

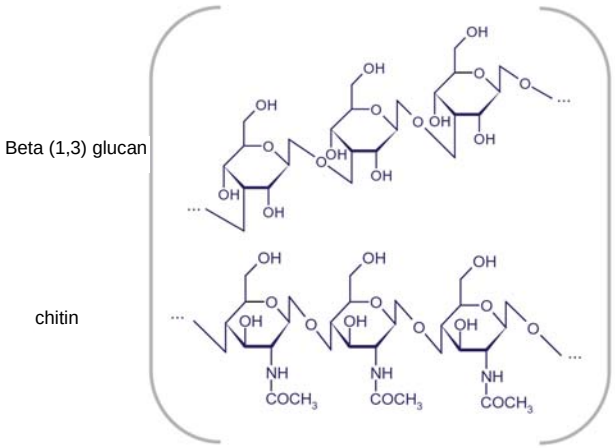
DECIDE to add in the International Oenological Codex the following monograph:

CHITIN-GLUCAN

$[C_6H_{10}O_5]_m$ - $[C_8H_{13}NO_5]_n$

CAS number Chitin: **[1398-61-4]**

CAS number β-glucan: **[9041-22-9]**



1 PURPOSE, ORIGIN AND SCOPE

Chitin-glucan is of fungus origin and is a natural polymer, the main component of the cellular walls of *Aspergillus niger*. It is initially extracted and purified from the mycelium of *Aspergillus niger*. This fungal resource is a by-product of the citric acid produced for the food and pharmaceutical markets.

Chitin-glucan is composed of polysaccharides chitin (repeat units N-acetyl-D-glucosamine) and 1,3-ß-glucan (repeat unit D-glucose). The two polymers are covalently connected and form a three-dimensional network. The chitin/glucan ratio ranges from 25:75 to 60:40 (m/m).
It is used as a fining agent of musts during racking in order to reduce the colloid content and cloudiness.

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It is also used for stabilising wines prior to bottling after alcoholic fermentation. This polymer has a stabilising capacity with respect to ferric breakages. It also helps eliminate undesirable compounds such as heavy metals (lead, cadmium), mycotoxins, etc.

2 SYNONYMS

Poly(N-acetyl-D-glucosamine)-poly(D-glucose) and 1,3-ß-glucan

3 LABELLING

The following information must be stated on the packaging label: fungal origin, product for oenological use, use and conservation conditions and use-by date.

4 CHARACTERS

4.1 Aspect

Chitin-glucan comes in the form of a white, odourless and flavourless powder. Chitin-glucan is almost completely insoluble in aqueous or organic medium.

4.2 Purity and soluble residues

The purity of the product must be equal to or higher than 95 %.
Dissolve 5 g of chitin-glucan in 100 ml of bidistilled water and agitate for 2 minutes. Filter after cooling on a fine mesh filter or membrane.
Evaporate the filtrate and dry at 100-105 °C. The content of solubles should not be higher than 5 %.

5 TESTS

5.1 Identification and chitin-glucan ratio

5.1.1 Determination of the chitin-glucan ratio
The chitin/glucan ratio is determined using the ^{13}C NMR spectrum in solid phase, by comparison with the spectrum of a pure chitin reference sample.
This method is detailed in appendix I.

5.2 Loss during desiccation

In a glass cup, previously dried for 1 hour in an oven at 100-105 °C and cooled in a desiccator, place 10 g of the analyte. Allow to desiccate in the drying oven at 100-105 °C to constant mass. Weigh the dry residue amount after cooling in the desiccator.

The weight loss must be lower than 10 %.

Note: all the limits stated below are reported in dry weight except for the microbiological analyses

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5.3 Ashes

Incinerate without exceeding 600°C the residue left from the determination of the loss during desiccation as described in 5.2. Allow to calcine for 6 hours. Allow the crucible to cool in a desiccator and weigh.

The total ash content should not be higher than 3 %.

5.4 Preparation of the test solution

Before determining the metals, the sample is dissolved by acid digestion (HNO_3, H_2O_2 and HCl). Mineralisation is performed in a closed microwave system. The sample undergoes neither crushing nor drying before mineralisation.
The reagents used for the mineralisation of chitin-glucan are as follows: HNO_3 (65 %) (Suprapur), HCl (37 %) (Suprapur), H_2O_2 (35 %). The 0.5 to 2 g sample of chitin-glucan is placed in a flask to which are added 25 ml of HNO_3, 2 ml of HCl and 3 ml of H_2O_2. This is submitted to microwave digestion (Power of 60 % for 1 min, 30 % for 10 min, 15 % for 3 min, and 40 % for 15 min). The solution is diluted in a volumetric flask with bidistilled water to a final volume of 25.0 ml.
The metal contents can then be determined.

5.5 Lead

Lead is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The lead content must be lower than 1 mg/kg.

It is also possible to achieve lead determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.6 Mercury

Mercury is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The mercury content must be lower than 0.1 mg/kg.

It is also possible to achieve mercury determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.7 Arsenic

Arsenic is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The arsenic content must be lower than 1 mg/kg.

It is also possible to achieve arsenic determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.8 Cadmium

Cadmium is determined by atomic absorption spectrophotometry, using the method described in appendix II.

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The cadmium content must be lower than 1 mg/kg.

It is also possible to achieve cadmium determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.9 Chromium

Chromium is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The chromium content must be lower than 10 mg/kg.

It is also possible to achieve chromium determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.10 Zinc

Zinc is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The zinc content must be lower than 50 mg/kg.

It is also possible to achieve zinc determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.11 Iron

Iron is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The iron content must be lower than 100 mg/kg.

It is also possible to achieve iron determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.12 Copper

Copper is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The copper content must be lower than 30 mg/kg.

It is also possible to achieve copper determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.13 MICROBIOLOGICAL CONTROL

5.13.1 Total bacteria count

The total bacteria count is performed according to the horizontal method by means of the colony count technique at 30 °C on the PCA medium described in appendix III.
Less than 1000 CFU/g of preparation.

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It is also possible to carry out the enumeration as described in chapter II of the International Oenological Codex.

5.13.2 Enterobacteria

The enumeration of *Enterobacteria* is carried out according to the horizontal method by means of the colony count technique at 30 °C described in appendix IV.
Less than 10 CFU/g of preparation.

5.13.3 Salmonella

Carry out the enumeration as described in chapter II of the International Oenological Codex.
Absence checked on a 25 g sample.

5.13.4 Coliform bacteria

Carry out the enumeration as described in chapter II of the International Oenological Codex.
Less than 100 CFU/g of preparation.

5.13.5 Yeasts

The enumeration of yeasts is carried out according to the horizontal method by means of the colony count technique at 25 °C on the YGC medium described in appendix V.
Less than 100 CFU/g of preparation.

It is also possible to carry out the enumeration as described in chapter II of the International Oenological Codex.

5.13.6 Moulds

The enumeration of moulds is carried out according to the horizontal method by means of the colony count technique at 25 °C on the YGC medium described in appendix VI.
Less than 100 CFU/g of preparation.

It is also possible to carry out the enumeration as described in chapter II of the International Oenological Codex.

6 OCHRATOXIN A TESTING

Prepare an aqueous solution (distilled water) of chitin-glucan at 1 % and agitate for 1 hour, then carry out determination using the method described in the Compendium of International Methods of Analysis of Wine and Musts.
Less than 5 µg/kg.

7 STORAGE

Keep container closed and store in a cool and dry place.

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Appendix I
Determination of the chitin/glucan ratio

1. PRINCIPLE

This method consists in determining the chitin/glucan ratio using the ^{13}C RMN spectrum in solid phase.

2. RÉACTIFS ET MATÉRIEL

2.1. Chitin glucan sample
2.2. Osmosis purified water
2.3. Hydrochloric acid 1 M
2.4. Pure ethanol
2.5. Pure chloroform
2.6. Pure methanol
2.7. Acetone
2.8. Standard laboratory material, pipettes, cylindrical glass vases, porosity filters 30 µm…
2.9. Rotary shaker
2.10. Laboratory centifuge
2.11. Conductimeter
2.12. Nuclear Magnetic resonance apparatus

3. SAMPLE PREPARATION

Before the determination, samples are prepared according to a precise protocol as described below:

- 3.1 Washing with HCl 1 M (2.3)

This step consists in mixing 2 g of chitin-glucan (2.1) and 40 ml of HCl 1 M in a tube flask.
This mixture is agitated for 30 min at 320 rpm then centrifuged at 4000 rpm for 10 min. The supernatant is eliminated.
This step is repeated once.

- 3.2 Washing with osmosis purified water

This step consists in mixing the sediment from the previous step with 40 ml of osmosis purified water (2.2).
This mixture is centrifuged for 10 min at 4000 rpm. The supernatant is eliminated.
This step is repeated until the supernatant conductivity is lower than 100 µS/cm.

- 3.3 Washing with ethanol

This step consists in mixing the sediment from the previous step with 40 ml of ethanol (2.4).

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This mixture is centrifuged for 10 min at 4000 rpm. The supernatant is eliminated.
This step is repeated once.

- 3.4 Washing with chloroform/methanol

This step consists in mixing the sediment from the previous step with 40 ml of a of 50/50, v/v of chloroform (2.5) and methanol (2.6) mixture.
This mixture is agitated for 30 min at 320 rpm then centrifuged at 4000 rpm for 10 min. The supernatant is eliminated.
This step is repeated once.

- 3.5 Washing with acetone and drying

This step consists in mixing the sediment from the previous step with 40 ml of acetone (2.7).
This mixture is agitated for 30 min at 320 rpm then centrifuged at 4000 rpm for 10 min.
After centrifugation, pour the supernatant on a 30 µm filter, rinse the tube flask with acetone (2.7) and pour everything on the filter.
Place the material located on the filter in a crystallising dish and allow to dry.
After drying, the product is ready to be analysed by NMR.

4. PROCEDURE

The prepared samples are then analysed on the Brücker Avance DSX 400WB nuclear magnetic resonance instrument (or the equivalent).
The analysis conditions are as follows:

- Magnetic field: 9.04 Tesla
- Larmor frequency: 83 kHz
- Time interval between 2 magnetic pulses: 5s
- Time period during which the magnetic pulse is applied: 5,5ms
- Number of magnetic pulse sequences: 3000

5. EXPRESSION OF THE RESULTS

5.1 The beta-glucan content is determined from the area of the four resonance bands.
5.2 The results are expressed in mol %.

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Appendix II
METAL DETERMINATION BY ATOMIC EMISSION SPECTROSCOPY

1. PRINCIPLE

This method consists in measuring atomic emission by an optical spectroscopy technique.

2. SAMPLE PREPARATION

Before the determination of metals, the sample is dissolved by acid digestion (HNO_3, H_2O_2 and HCl). Mineralisation takes place in closed microwave system. The sample undergoes neither crushing nor drying before mineralisation.
The reagents used for the mineralisation of chitosan are as follows: HNO_3 (65 %) (Suprapur), HCl (37 %) (Suprapur), H_2O_2 (35 %). The 0.5 to 2 g sample of chitin-glucan is placed in a flask to which are added 25 ml of HNO_3, 2 ml of HCl and 3 ml of H_2O_2. The whole is then submitted to microwave digestion (Power of 60 % for 1 min, 30 % for 10 min, 15 % for 3 min, and 40 % for 15 min). The solution is then diluted in a volumetric flask with bidistilled water to a final volume of 25.0 ml.
The metal contents can then be determined.

3. PROCEDURE

The dissolved samples are nebulised and the resulting aerosol is transported in a plasma torch induced by a high frequency electric field. The emission spectra are dispersed by a grating spectrometer and the line intensity is evaluated by a detector (photomultiplier). The detector signals are processed and controlled by a computer system. A background noise correction is applied to compensate for the background noise variations.

4. EXPRESSION OF THE RESULTS

The metal concentrations in the oenological products are expressed in mg/kg

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Appendix III
Total bacteria count by counting the colonies obtained at 30 °C

PCA medium

Composition:

Peptone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Agar-agar	15 g
Adjusted to	pH 7.0
Water	complete to 1000 ml

The medium is sterilised before use in an autoclave at 120 °C for 20 min.

The Petri dishes are inoculated by pour plate method and spiral plating method. After inoculation, they are incubated at 30 °C in aerobiosis for 48 to 72 hours. Count the CFU number.

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Appendix IV

Enumeration of *Enterobacteria* is carried out according to the horizontal method by means of the colony count technique at 30 °C

VRBG medium

Composition:

Peptone	7 g
Yeast extract	3 g
Glucose	10 g
Sodium Chloride	5 g
Crystal Violet	0.002 g
Neutral Red	0.03 g
Agar-agar	13 g
Bile salts	1.5 g
Adjusted to	pH 7.4
Water	complete to 1000 ml

The medium is sterilised before use in an autoclave at 120 °C for 20 min.

The Petri dishes are inoculated by pour plate method and spiral plating method. After inoculation, they are incubated at 30 °C in aerobiosis for 18 to 24 hours. Count the CFU number.

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Appendix V
Enumeration of yeasts by counting

YGC medium

Composition:

Yeast extract	5.0 g
D-glucose	20 g
Agar-agar	14.9 g
Choramphenicol	0.1 g
Adjusted to	pH 6.6
Water	complete to 1000 ml

The medium is sterilised before use in an autoclave at 120 °C for 20 min.

The Petri dishes are inoculated by pour plate method and spiral plating method.
After inoculation, they are incubated at 25 °C in aerobiosis for 3 to 5 days without being turned over.
Count the number of yeasts.

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Appendix VI
Enumeration of the moulds by counting

YGC medium

Composition:

Yeast extract	5.0 g
D-glucose	20 g
Agar-agar	14.9 g
Choramphenicol	0.1 g
Adjusted to	pH 6.6
Water	complete to 1000 ml

The medium is sterilised before use in an autoclave at 120 °C for 20 min.

The Petri dishes are inoculated by pour plate method and spiral plating method.
After inoculation, they are incubated at 25 °C in aerobiosis for 3 to 5 days without being turned over.
Count the number of moulds.

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RESOLUTION OIV/OENO 368/2009

MONOGRAPH ON CHITOSAN

The GENERAL ASSEMBLY

In view of article 2, paragraph 2 IV of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

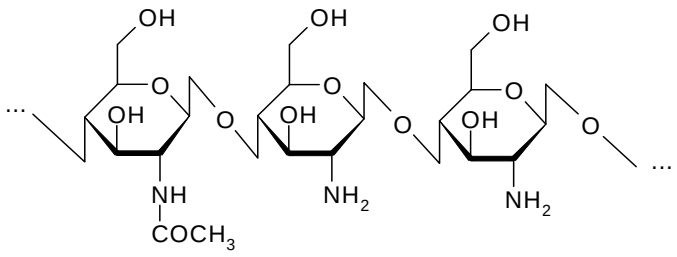
Considering the works of the group of experts "Specifications of Oenological Products",

DECIDE to add in the International Oenological Codex the following monograph:

CHITOSAN

$[C_6H_{11}NO_4]_n$

CAS number Chitosan: **[9012-76-4]**



Chitosan

1 PURPOSE, ORIGIN AND APPLICABILITY

Chitosan, a natural polysaccharide prepared of fungal origin, is initially extracted and purified from reliable and abundant food or biotechnological fungal sources such as *Agaricus bisporus* or *Aspergillus niger*.
Chitosan is obtained by hydrolysis of a chitin-rich extract. Chitin is a polysaccharide composed of several N-acetyl-D-glucosamine units interconnected by ß→ (1.4) type linkages.
Chitosan is composed of glucosamine sugar units (deacetylated units) and N-acetyl-D-glucosamine units (acetylated units) interconnected by ß→ (1.4) type linkages.

It is used as a fining agent in the treatment of musts for flotation clarification to reduce cloudiness and the content of unstable colloids.
It is also used for stabilising wines. This polymer actually helps eliminate undesirable micro-organisms such as *Brettanomyces.*

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2 SYNONYMS

Poly(N-acetyl-D-glucosamine)-poly(D-glucose).

3 LABELLING

The following information must be stated on the packaging label: exclusively fungal origin, product for oenological use, use and conservation conditions and use-by date.

4 CHARACTERS

4.1 Aspect and solubility

Chitosan comes in the form of a white, odourless and flavourless powder. Chitin-glucan is almost completely insoluble in aqueous or organic medium.

4.2 Purity and soluble residues

The purity of the product must be equal to or higher than 95 %.
Dissolve 5 g of chitin-glucan in 100 ml of bidistilled water and agitate for 2 minutes. Filter after cooling on a fine mesh filter or membrane.
Evaporate the filtrate and dry at 100-105 °C. The content of solubles should not be higher than 5 %.

5 TESTS

5.1 Determination of the acetylation degree and chitosan origin

5.1.1 Determination of the acetylation degree
The acetylation degree is determined by potentiometric titration, using the method described in Appendix I.

5.1.2 Determination of the source
Chitosan, as a natural polymer, is extracted and purified from fungal sources; it is obtained by hydrolysis of a chitin-rich extract. This chitosan is considered identical to chitosan from shellfish in terms of structures and properties.
An identification of the origin of chitosan is made based on 3 characteristics: content of residual glucans (refer to method in annex II), viscosity of chitosan in solution 1 % and settled density (following settlement).
Only fungal origin chitosan has both contents of residual glucan > at 2 %, a settled density ≥ at 0,7 g/cm^3 and viscosity in solution 1 % in acetic acid 1 % < at 15 cPs

5.2 Loss during desiccation

In a glass cup, previously dried for 1 hour in an oven at 100-105 °C and cooled in a desiccator, place 10 g of the analyte. Allow to desiccate in the drying oven at 100-105 °C to constant mass. Weigh the dry residue amount after cooling in the desiccator.

The weight loss must be lower than 10 %.

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Note: all the limits stated below are reported in dry weight except for the microbiological analyses

5.3 Ashes

Incinerate without exceeding 600 °C the residue left from the determination of the loss during desiccation as described in 5.2. Allow to calcine for 6 hours. Allow the crucible to cool in a desiccator and weigh.

The total ash content should not be higher than 3 %.

5.4 Preparation of the test solution

Before determining the metals, the sample is dissolved by acid digestion (HNO_3, H_2O_2 and HCl). Mineralisation is performed in a closed microwave system. The sample undergoes neither crushing nor drying before mineralisation.
The reagents used for the mineralisation of chitosan are as follows: HNO_3 (65 %) (Suprapur), HCl (37 %) (Suprapur), H_2O_2 (35 %). The 0.5 to 2 g sample of chitosan is placed in a flask to which are added 25 ml of HNO_3, 2 ml of HCl and 3 ml of H_2O_2. This is submitted to microwave digestion with a maximum power of 1200 watts; Power of 60 % for 1 min, 30 % for 10 min, 15 % for 3 min, and 40 % for 15 min). The solution is diluted in a volumetric flask with bidistilled water to a final volume of 25.0 ml.
The metal contents can then be determined.

5.5 Lead

Lead is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The lead content must be lower than 1 mg/kg.

It is also possible to achieve lead determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.6 Mercury

Mercury is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The mercury content must be lower than 0.1 mg/kg.

It is also possible to achieve mercury determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.7 Arsenic

Arsenic is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The arsenic content must be lower than 1 mg/kg.

It is also possible to achieve arsenic determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

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5.8 Cadmium

Cadmium is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The cadmium content must be lower than 1 mg/kg.

It is also possible to achieve cadmium determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.9 Chromium

Chromium is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The chromium content must be lower than 10 mg/kg.

It is also possible to achieve chromium determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.10 Zinc

Zinc is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The zinc content must be lower than 50 mg/kg.

It is also possible to achieve zinc determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.11 Iron

Iron is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The iron content must be lower than 100 mg/kg.

It is also possible to achieve iron determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.12 Copper

Copper is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The copper content must be lower than 30 mg/kg.

It is also possible to achieve copper determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

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5.13 MICROBIOLOGICAL CONTROL

5.13.1 Total bacteria count

The total bacteria count is performed according to the horizontal method by means of the colony count technique at 30 °C on the PCA medium described in appendix III.
Less than 1000 CFU/g of preparation.

It is also possible to carry out the enumeration as described in chapter II of the International Oenological Codex.

5.13.2 Enterobacteria

The enumeration of *Enterobacteria* is carried out according to the horizontal method by means of the colony count technique at 30 °C described in appendix IV.
Less than 10 CFU/g of preparation.

5.13.3 Salmonella

Carry out the enumeration as described in chapter II of the International Oenological Codex.
Absence checked on a 25 g sample.

5.13.4 Coliform bacteria

Carry out the enumeration as described in chapter II of the International Oenological Codex.
Less than 100 CFU/g of preparation.

5.13.5 Yeasts

The enumeration of yeasts is carried out according to the horizontal method by means of the colony count technique at 25 °C on the YGC medium described in appendix VI.
Less than 100 CFU/g of preparation.

It is also possible to carry out the enumeration as described in chapter II of the International Oenological Codex.

5.13.6 Moulds

The enumeration of moulds is carried out according to the horizontal method by means of the colony count technique at 25 °C on the YGC medium described in appendix VII.
Less than 100 CFU/g of preparation.

It is also possible to carry out the enumeration as described in chapter II of the International Oenological Codex.

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6 OCHRATOXIN A TESTING

Prepare an aqueous solution (distilled water) of chitosan at 1 % and agitate for 1 hour, then carry out determination using the method described in the Compendium of International Methods of Analysis of Wine and Musts.
Less than 5 µg/kg.

7 STORAGE

Keep container closed and store in a cool and dry place.

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Appendix I

DETERMINATION OF THE ACETYLATION DEGREE

1. PRINCIPLE

This method consists in determining the acetylation degree of chitosan by titration of the amino groups. The acetylation degree is the ratio of the number of N-acetyl-glucosamine units to the number of total monomers.
This method is based on the method described by Rinaudo et al., (1999).
The titration of a chitosan solution by means of NaOH at 0.1 M must be performed in order to identify two pH jumps from 0 to 14.
Chitosan is dissolved in 0.1M HCl, the amino groups (on the deacetylated glucosamine units (G)) are positively charged (HCl in excess)).
The chitosan solution (of known quantity) is titrated by NaOH of known concentration. In the first part of the reaction, the excess quantity of HCl is determined:
1.1. HCl (excess)+NaOH + $NH_3^+Cl^-$ --> NaCl + H_2O + $NH_3^+Cl^-$
After the first pH jump, the quantity of charged amino groups is determined:
1.2. HCl + H_2O + $NH_3^+Cl^-$+ NaOH --> NH_2 + $2H_2O$ + 2NaCl
13. After the second pH jump, the excess quantity of NaOH is measured.
The determination of the NaOH volume between the two jumps makes it possible to identify the quantity of charged amines.

1. SAMPLE PREPARATION

Before determination, the samples are prepared according to the protocol described hereafter:
100 mg of chitosan are placed into a cylindrical flask to which 3 ml of 0.3 M HCl and 40 ml of water are added. Agitate for 12 hours.

2. PROCEDURE

First introduce the pH electrode of the pH-meter as well as the temperature sensor into the cylindrical flask. Check that the pH value is lower than 3.
To bring to pH = 1, add a V1 volume (ml) of HCl 0.3 M and agitate.
Then to bring to pH = 7 with a V2 volume (ml) of 0.1 M NaOH
These operations can be carried out using an automatic titrator.

3. EXPRESSION OF RESULTS

The acetylation degree of chitosan is expressed in %. This formula is the ratio of the mass of acetylated glucosamine (aG) units in g actually present in the sample, to the mass in g that would be present if all the groups were acetylated, where:
Q = (V_{NaOH} X 0.1) / (1000 X M_{cs})
= specific concentration in amino groups

Mcs: dry weight of chitosan in g

$\mathbf{V_{NaOH}}$ = V2 – V1
= volume of 0.1 M NaOH between 2 pH jumps in ml

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For a 1 g sample

With G = Glucosamine part; a = acetylated part

aG weight actually present (in g) =
1g – (Number of moles of G groups/g) x G molecular weight =
1g – Q x 162

aG weight if all the deacetylated groups were acetylated (in g) =
1g + (Number of moles of G groups/g) x molecular weight a =
1g + Q x 42

The acetylation degree will be equal to DA, where:

$$\mathbf{DA} = (1-162 \times Q) / (1+ 42 \times Q)$$

Rinaudo, M., G. Pavlov and J. Desbrieres. 1999. Influenced of acetic acid concentration on the solubilization of chitosan. *Polym.* 40, 7029-7032

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Appendix II

DETERMINATION OF THE RESIDUAL GLUCAN CONTENT

1. PRINCIPLE

This method consists in determining the content of residual glucans in chitosan by means of spectrophotometry.
This method is based on a colorimetric reaction with a response depending on the degradation of the starch hydrolysates by hot concentrated sulphuric acid.
This degradation gives a brown yellow compound with a colour intensity proportional to the content of residual glucans.

2. PREPARATION OF THE STANDARD RANGE

A stock solution of glucan (glucan with a purity of 97 % is provided by the company Megazyme) is prepared according to the precise protocol described hereafter:
500 mg of glucan are introduced into a volumetric flask of 100 ml into which 6 ml of ethanol and 80 ml of distilled water are added.
Agitate and boil out to allow glucan dissolution
Allow to cool, adjust to the filling mark with water
Agitate for 30 minutes.
Pour 1 ml of this solution into a 50 ml volumetric flask and adjust to the filling mark with 1 % acetic acid.
The solution is ready to use to produce the standard range according to the protocol hereafter.

Stock solution V (ml)	Water V (ml)	Glucan M (µg)
0	1	0
0.1	0.9	10
0.3	0.7	30
0.5	0.5	50
0.7	0.3	70

3. SAMPLE PREPARATION

Before determination, the samples are prepared according to the protocol described hereafter:
100 mg of chitosan are placed into a 50 ml volumetric flask to which 25 ml of 1 % acetic acid are added.
Agitate for 12 hours then adjust to the filling mark.

4. PROCEDURE

In a test tube, add 1 ml of the analyte solution, 1 ml of phenol at 5 % and 5 ml of concentrated sulphuric acid.
Agitate this mixture using a vortex for 10 s, then allow to cool for 1 hour.
The absorbance A is measured at 490 nm.

5. EXPRESSION OF THE RESULTS

Determine the glucan content in µg/g from the calibration curve (0-70 µg). This content is expressed in µg/g of chitosan.

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Appendix III
METAL DETERMINATION BY ATOMIC EMISSION SPECTROSCOPY

1. PRINCIPLE

This method consists in measuring atomic emission by an optical spectroscopy technique.

2. SAMPLE PREPARATION

Before the determination of metals, the sample is dissolved by acid digestion (HNO_3, H_2O_2 and HCl). Mineralisation takes place in closed microwave system. The sample undergoes neither crushing nor drying before mineralisation.
The reagents used for the mineralisation of chitosan are as follows: HNO_3 (65 %) (Suprapur), HCl (37 %) (Suprapur), H_2O_2 (35 %). The 0.5 to 2 g sample of chitosan is placed in a flask to which are added 25 ml of HNO_3, 2 ml of HCl and 3 ml of H_2O_2. The whole is then submitted to microwave digestion (Power of 60 % for 1 min, 30 % for 10 min, 15 % for 3 min, and 40 % for 15 min). The solution is then diluted in a volumetric flask with bidistilled water to a final volume of 25.0 ml.
The metal contents can then be determined.

3. PROCEDURE

The dissolved samples are nebulised and the resulting aerosol is transported in a plasma torch induced by a high frequency electric field. The emission spectra are dispersed by a grating spectrometer and the line intensity is evaluated by a detector (photomultiplier). The detector signals are processed and controlled by a computer system. A background noise correction is applied to compensate for the background noise variations.

4. EXPRESSION OF THE RESULTS

The metal concentrations in chitosan are expressed in mg/kg.

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Appendix IV
Total bacteria count by counting the colonies obtained at 30 °C

PCA medium

Composition:

Peptone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Agar-agar	15 g
Adjusted to	pH 7.0
Water	complete to 1000 ml

The medium is sterilised before use in an autoclave at 120 °C for 20 min.

The Petri dishes are inoculated by pour plate method and spiral plating method. After inoculation, they are incubated at 30 °C in aerobiosis for 48 to 72 hours. Count the CFU number.

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Appendix V

Enumeration of *Enterobacteria* is carried out according to the horizontal method by means of the colony count technique at 30 °C

VRBG medium

Composition:

Peptone	7 g
Yeast extract	3 g
Glucose	10 g
Sodium Chloride	5 g
Crystal Violet	0.002 g
Neutral Red	0.03 g
Agar-agar	13 g
Bile salts	1.5 g
Adjusted to	pH 7.4
Water	complete to 1000 ml

The medium is sterilised before use in an autoclave at 120 °C for 20 min.

The Petri dishes are inoculated by pour plate method and spiral plating method. After inoculation, they are incubated at 30 °C in aerobiosis for 18 to 24 hours. Count the CFU number.

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Appendix VI
Enumeration of yeasts by counting

YGC medium

Composition:

Yeast extract	5.0 g
D-glucose	20 g
Agar-agar	14.9 g
Choramphenicol	0.1 g
Adjusted to	pH 6.6
Water	complete to 1000 ml

The medium is sterilised before use in an autoclave at 120 °C for 20 min.

The Petri dishes are inoculated by pour plate method and spiral plating method.
After inoculation, they are incubated at 25 °C in aerobiosis for 3 to 5 days without being turned over.
Count the number of yeasts.

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Appendix VII
Enumeration of the moulds by counting

YGC medium

Composition:

Yeast extract	5.0 g
D-glucose	20 g
Agar-agar	14.9 g
Choramphenicol	0.1 g
Adjusted to	pH 6.6
Water	complete to 1000 ml

The medium is sterilised before use in an autoclave at 120 °C for 20 min.

The Petri dishes are inoculated by pour plate method and spiral plating method.
After inoculation, they are incubated at 25 °C in aerobiosis for 3 to 5 days without being turned over.
Count the number of moulds.

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RESOLUTION OIV/OENO 377/2009

UPDATE OF THE OIV COMPENDIUM OF METHODS OF ANALYSIS OF WINE AND MUSTS – CLASSIFICATION OF METHODS

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

IN VIEW OF the actions of the OIV strategic plan 2009-2012

CONSIDERING the work carried out by the "Methods of analysis" sub-commission,

CONSIDERING resolution 9/2000 adopted by the OIV, defining the internationally recognised classification of analytical methods of the OIV,

CONSIDERING that this resolution mentions the four following classification categories:

Category I (Criterion reference method): A method which determines a value that can be reached only by implementing the method per se and which serves, by definition, as the only method for establishing the accepted value of the parameter measured (e.g., alcoholometric content, total acidity, volatile acidity).

Category II (Reference method): A category II method is designated as the Reference Method in cases where category I methods cannot be used. It should be selected from category III methods (as defined below). Such methods should be recommended for use in cases of disputes and for calibration purposes (e.g., potassium, citric acid).

Category III (Approved alternative methods): A category III Method meets all of the criteria specified by the Sub-Committee on Methods of Analysis and is used for monitoring, inspection and regulatory purposes (e.g., enzymatic determinations of glucose and fructose).

Category IV (Auxiliary method): A category IV Method is a conventional or recently-implemented technique, with respect to which the Sub-Committee on Methods of Analysis has not as yet specified the requisite criteria (e.g., synthesized colouring agents, measurement of oxidation-reduction potential).

CONSIDERING that some analytical methods adopted by the OIV do not comply with this new classification but follow the former classification, adopted in 1990, which classifies methods either as reference methods or as usual methods,

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CONSIDERING the necessity to classify old methods based on the new classification criteria mentioned in resolution 9/2000,

CONSIDERING that some analytical methods are no longer used and should be removed from the International Compendium of Musts and Wines,

DECIDES, following a proposal made by Commission II "Oenology", to adopt the new classification of the following analytical methods currently included in the International Compendium of Musts and Wines,

DECIDES that the methods included in the Compendium of International Methods of Analysis of Wine and Musts will be subsequently modified, as needed.

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Part 1: Methods already adopted in Category I, II, III or IV by the OIV – For information only

These methods have already been adopted according to the new definitions of analytical methods of the OIV. This classification is provided for reference only and shall not be modified.

1. A: Methods already adopted in Category I by the OIV – For information only

TITLE	REFERENCE	PRINCIPLE	TYPE METHOD	ADOPTION YEAR
Chromatic characteristics	AS2-11-CARCHR	Spectrophotometry	I	2006
Overpressure measurement	AS314-02-SURPRES	Aphrometry	I	2003

1. B: Methods already adopted in Category II by the OIV – For information only

TITLE	REFERENCE	PRINCIPLE	TYPE METHOD	ADOPTION YEAR
Shikimic acid	AS313-17-ACSHIK	HPLC	II	2004
Carbon dioxide proportioning	AS314-01-DIOCAR	Reference method: Titrimetry	II	2006
Determination of the carbon isotope ratio $^{13}C/^{12}C$ of CO_2	AS314-03-CO2MOU	MS-IR	II	2005
Carbon dioxide	AS314-04-CO2MAN	Manometry	II	2006
Determination of nine major Anthocyanins	AS315-11-ANCYAN	HPLC	II	2003/2007
Fluorides	AS321-03-FLUORU	Specific electrode	II	2004
Determination of 3-Methoxypropane-1,2-diol and cyclic diglycerols	AS315-15-GLYCCYC	Gas chromatography/mass spectrometry	II	2007
Sugars	AS311-03-SUCRES	HPLC	II	2003
Lead (Criteria)	AS322-12-CHIPLO	Atomic absorption spectrometry	II	2006

1. C: Methods already adopted in Category II by the OIV – For information only

None

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1. D: Methods already adopted in Category IV by the OIV – For information only

TITLE	REFERENCE	PRINCIPLE	TYPE METHOD	ADOPTION YEAR
Plant proteins	AS315-12-PROVEG	Electrophoresis	IV	2004
Proportioning of Organic Acids and Mineral Anions	AS313-16-ORGION	Ionic Chromatography	IV	2004
Sorbic acid	AS313-18-SORCAP	Capillary electrophoresis	IV	2006
Proportioning of Sorbic, Benzoic, Salicylic acids	AS313-20-SOBESA	HPLC	IV	2006
Determination of the presence of metatartaric acid	AS313-21-METTAR	Spectrometry	IV	2007
Polychlorophenols, polychloroanisols	AS315-13-PCAPCP	Gas Chromatography	IV	2006
Lysozyme	AS315-14-LYSOZY	HPLC	IV	2007
Polyols derived from sugars	AS311-06-POLYOL	Gas Chromatography	IV	2006

Part 2: Methods for which a new classification is proposed

These methods are adopted and published in accordance with the old characterisation of analytical methods. In some cases, for a single compound, two or more principles may appear (depending on the reference method or the usual method) under the same reference. It is thus proposed to classify these methods under category I, II, III or IV, bearing in mind that for category II methods, intra-laboratory validation parameters apply.

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TITLE	REFERENCE	PRINCIPLE	Proposed TYPE
Acetaldehyde (ethanal)	AS315-01-ETHANA	Colorimetry	IV
Total acidity	AS313-01-ACITOT	Titrimetry	II
Fix acidity		Calculation	II
Volatile acidity	AS313-02-ACIVOL	Titrimetry after distillation	II
Alcoholic strength by volume	AS312-01-TALVOL	Reference method: pycnometry	I
Alcoholic strength by volume	AS312-01-TALVOL	Reference method: electronic densimetry	I
Alcoholic strength by volume	AS312-01-TALVOL	Reference method: hydrostatic balance	I
Alkalinity of ash	AS2-05-ALCCEN	Titrimetry	IV
Ammonium	AS322-01-AMMONI	Titrimetry	IV
Arsenic	AS323-01-ARSENI	Reference method: Atomic absorption spectrometry	IV
Arsenic	AS323-01-ASSAA	Atomic absorption spectrometry	IV
Arsenic	AS323-01-ARSENI	Usual method: Colorimetry	Remove
Artificial colorants	AS315-08-COLSYN	TLC	IV
Artificial sweeteners	AS315-07-EDUSYN	Reference TLC	IV
Artificial sweeteners	AS315-07-EDUSYN	Usual TLC	IV
L- ascorbic acid	AS313-13-ALASCO	Reference method: Spectrofluorimetry	IV
L- ascorbic acid	AS313-13-ALASCO	Usual method: TLC + Spectrophotometry	Remove
Ash	AS2-04-CENDRE	Gravimetry	I
Boron	AS323-03-BORE	Spectrophotometry	IV
Total Bromide	AS321-01-BROTOT	Colorimetry	IV
Cadmium	AS322-10-CADMIU	Atomic absorption spectrometry	IV
Calcium	AS322-04-CALCIU	Atomic absorption spectrometry	II
Chlorides	SA321-02-CHLORU	Specific Electrode	II
Chromatic Characteristics	AS2-07-CACHR2	Usual method	IV
Citric acid	AS313-08-ACICHI	Oxidation, iodometry	IV
Citric acid	AS313-09-ACIENZ	Enzymatic	II
Copper	AS322-06-CUIVRE	Atomic absorption spectrometry	IV
Cyanide derivatives	AS315-06-DERCYA	Colorimetry	II
Density at 20°C	AS2-01-MASVOL	Reference method: pycnometry	I
Density at 20°C	AS2-01-MASVOL	Usual method: aerometry	IV
Density at 20°C	AS2-01-MASVOL	Usual method: densimetry (hydrostatic balance)	I
Detecting enrichment of musts, concentrated grape musts, rectified concentrated grape musts and wine by ^{2}H-RMN	AS311-05-ENRRMN	SNIF NMR	I
Detection of preservatives and fermentation inhibitors	AS4-02-RECANT	HPLC	IV

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Detection of preservatives and fermentation inhibitors	AS4-02-RECANT	sorbic, benzoic, p-chlorobenzoic acids	IV
Detection of preservatives and fermentation inhibitors	AS4-02-RECANT	p-hydroxybenzoic acid, sodium azide	IV
Detection of preservatives and fermentation inhibitors	AS4-02-RECANT	GC ethyl pyrocarbonate	IV
Determination of isotopic ratio of ethanol	AS312-06-ETHANO	Reference method SNIF NMR	II
Diethylene glycol	AS315-09-DIEGLY	Gas chromatography	IV
Differentiation of fortified musts and sweet fortified wines	AS5-01-DIFMIS		IV
Total dry matter	AS2-03-EXTSEC	Reference method: gravimetry	I
Total dry matter	AS2-03-EXTSEC	Usual method: densimetry	IV
Ethyl acetate	AS315-02-ACEETH	Reference method: Gas chromatography	IV
Ethyl acetate	AS315-02-ACEETH	Usual method: Titrimetry	IV
Ethyl carbamate	AS315-04-CARETH	Chromatography gas-MS	II
Evaluation by refractometry of the sugar concentration in grape musts, concentrated grape musts and rectified concentrated grape musts	AS2-02-SUCREF	Refractometry	I
Folin-Ciocalteu Index	AS2-10-INDFOL	Colorimetry	IV
Glycerol	AS312-05-GLYENZ	Enzymatic	IV
Glycerol and 2,3- butanediol	AS312-04-GLYBUT	Reference method: Colorimetry	IV
Hydroxymethylfurfural	AS315-05-HYDMFF	Colorimetry	IV
Hydroxymethylfurfural	AS315-05-HYDMFF	High performance liquid chromatography	IV
Iron	AS322-05-FER	Reference method: Atomic absorption spectrometry	IV
Iron	AS322-05-FER	Usual method: Colorimetry	IV
Lactic acid	AS313-06-ALACHI	Colorimetry	Remove
Lactic acid	AS313-07-ALAENZ	Enzymatic	II
Magnesium	AS322-07-MAGNES	Atomic absorption spectrometry	II
D-malic acid: enzymatic method	AS313-12-ADMENZ	Enzymatic	II
D-malic acid: low concentrations	AS313-12-ADMEZ2	Enzymatic	IV
L-malic acid: enzymatic method	AS313-11-ALMENZ	Enzymatic	II
Total malic acid: usual method	AS313-10-AMALTO	Colorimetry	IV
Malvidin diglucoside	AS315-03-DIGMAL	Colorimetry	IV
Mercury	AS323-06-MERCUR	Atomic absorption spectrometry	IV
Methanol	AS312-03-METHAN	Reference: Gas chromatography	IV
Methanol	AS312-03-METHAN	Usual method	IV
Method for isotopic ratio 18O/16O	AS2-09-MOUO18	Mass spectrometry (isotopic ratio)	II

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Total nitrogen	AS323-02-AZOTOT	Kjeldhahl method	IV
Total nitrogen – Dumas method	AS323-02-AZOTDU	Dumas method	II
Ochratoxin A	AS315-10-OCHRAT	HPLC	II
Organic acids	AS313-04-ACIORG	HPLC	IV
Organic acids	AS313-19-ACORG2	Capillary electrophoresis	II
Oxidation-reduction potential	AS2-06-POTOXY	Potentiometry	IV
pH	AS313-15-PH	Potentiometry	I
Total phosphorus	AS321-04-PHOTOT	Colorimetry	IV
Potassium	AS322-02-POTASS	Reference method: Atomic absorption spectrometry	II
Potassium	AS322-02-POTASS	Usual method: flame photometry	III
Potassium	AS322-02-POTASS	Gravimetry	Remove
Silver	AS322-09-ARGENT	Atomic absorption spectrometry	IV
Sodium	AS322-03-SODIUM	Reference method: Atomic absorption spectrometry	II
Sodium	AS322-03-SODIUM	Usual method: flame photometry	III
Sorbic acid	AS313-14-ACISOR	Spectrophotometry	IV
Sorbic acid	AS313-14-ACISOR	Gas chromatography	IV
Sorbic acid	AS313-14-ACISOR	TLC	IV
Glucose and fructose	AS311-02-GLUFRU	Enzymatic	II
Glucose and fructose	AS311-07-GLCFR2	pH-metry	III
Glucose, fructose et saccharose	AS311-08-SACCHA	pH-metry	IV
Sulfates	AS321-05-SULFAT	Reference method: gravimetry	II
Sulfates	AS321-05-SULFAT	Usual method: Titrimetry	Remove
Sugars: reducing sugars	AS311-01-SUCRED	Clarification reference method: Titrimetry (clarification)	Remove
Sugars: reducing sugars	AS311-01-SUCRED	Clarification usual method: Titrimetry (clarification)	IV including replacement of the term "reducing sugars" with "reductive substances"
Sugars: reducing sugars	AS311-01-SUCRED	Reference method	Remove
Sulfur dioxide – wine	AS323-04-DIOSOU	Titrimetry (reference)	II
Sulfur dioxide – wine	AS323-04-DIOSOU	Iodometry (fast)	IV
Sulfur dioxide – wine	AS323-04-DIOSOU	Molecular method	IV
Sulfur dioxide – grape juice	AS323-05-SO2JUS	Titrimetry	IV
Tartaric acid	AS313-05-ACITAR	Reference method: gravimetry	IV
Tartaric acid	AS313-05-ACITAR	Usual method: Spectrophotometry	Remove
Turbidity measurement	AS2-08-TURBID	Nephelometry	IV
Zinc	AS322-08-ZINC	Atomic absorption spectrometry	IV

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RESOLUTION OIV/OENO 392/2009

DISCUSSION ON UNCERTAINTIES AND RECOVERY RATE

The GENERAL ASSEMBLY

In view of article 2 of the Agreement of 3 April 2001establishing the International Organisation of Vine and Wine was founded,

Following a proposal made by the Methods of Analysis Sub-Commission

CONSIDERING that scientifically there is a broad consensus on the principle of correcting by the recovery and the transparent reporting thereof. However whether or not a result is corrected for recovery may have considerable implications for other stakeholders outside of the immediate analytical community.

CONSIDERING the works of many international organisation in the field of recovery correction

RECOMMENDS that a further sub-group investigates the implications of recovery correction (and Measurement Uncertainty) on the interpretation and the setting of limits, reports and makes clear recommendations based on risk management and statistical principles taking account of uncertainties of estimation of recovery correction and measurement uncertainty.

DECIDES to adopt the following recommendations and text related to the recovery correction which must be included in the OIV compendium of Methods of Analysis.

Recovery

"The OIV recommends the following practice with regards to reporting recovery of analytical results.

- Analytical results are to be expressed on a recovery corrected basis where appropriate and relevant, and when corrected it has to be stated.

- If a result has been corrected for recovery, the method by which the recovery was taken into account should be stated. The recovery rate is to be quoted wherever possible.

- When laying down provisions for standards, it will be necessary to state whether the result obtained by a method used for analysis within conformity checks shall be expressed on a recovery-corrected basis or not."

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RESOLUTION OIV/OENO 296/2009

DETERMINATION OF RELEASABLE 2,4,6-TRICHLOROANISOLE IN WINE BY CORK STOPPERS

The GENERAL ASSEMBLY

Considering Article 2 paragraph 2 iv of the Agreement of April 3, 2001 establishing the International Organisation of Vine and Wine,

Upon the proposal of the Sub-commission of Methods of Analysis,

DECIDES to complete Annex A of the Compendium of International Methods of Analysis of Wine and Must by the following type IV method:

Title	Type of Method
DETERMINATION OF RELEASABLE 2,4,6-TRICHLOROANISOLE IN WINE BY CORK STOPPERS	**IV**

1 SCOPE:
The method of determination of releasable 2,4,6-trichloroanisole (TCA) by cork stoppers measures the quantity of TCA released by a sample of cork stoppers macerated in a aqueous-alcoholic solution. The aim of this method is to evaluate the risk of releasing by the lot of analyzed cork stoppers and to provide a method for controlling the quality of cork stoppers.

2 PRINCIPLE
The method aims to simulate 2,4,6-trichloroanisole migration phenomena susceptible of being produced between the cork stopper and wine in bottles. Cork stoppers are macerated in a wine or a aqueous-alcoholic solution, until a balance is obtained. The TCA of the head space is sampled from an appropriate part of the macerate by the solid-phase micro-extraction technique (SPME), then analyzed by gas chromatography, with detection by mass spectrometer (or by electron-capture detector).

3 REAGENTS AND PRODUCTS
3.1 White wine with an alcoholic strength ranging between 10 and 12 % vol. (It can be replaced by an aqueous-alcoholic solution with an alcoholic strength of 12 % vol). The wine and/or the aqueous-alcoholic solution must be free of TCA.
3.2 Sodium chloride ≥ 99.5 %
3.3 2,4,6-trichloroanisole (TCA)-d_5 purity ≥ 98% for GC/MS; 2,6-dibromoanisole or 2,3,6-trichloroanisole purity ≥ 99 % for GC/ECD

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3.4 2,4,6-trichloroanisole (TCA) purity ≥ 99.0%
3.5 Absolute ethanol
3.6 Pure de-ionised water void of TCA (Standard EN ISO 3696)
3.7 Aqueous-alcoholic solution at 12 % vol.
Prepared using absolute ethanol (3.5) and de-ionised water void of TCA (3.6).
3.8 Internal standard stock solution (500 mg/L)

Add either 0.050 g of 2,4,6-trichloroanisole-d_5 (or 2,6-dibromoanisole or 2,3,6-trichloroanisole (3.3) to approximately 60 ml of absolute ethanol (3.5). After dissolution, adjust the volume to 100 mL with absolute ethanol (3.5). It can be kept in a glass bottle with a metallic or glasscover.

3.9 Intermediate solution of internal standard (5.0 mg/L)
Add 1 mL of a solution of either 2,4,6-trichloroanisole-d_5 (or 2,6-dibromoanisole or 2,3,6-trichloroanisole) at 500 mg/L (3.8) to approximately 60 mL of absolute ethanol (3.5). Adjust the volume to 100 mL with absolute ethanol (3.5). It can be kept in a glass bottle with a metallic or glass cover.
3.10 Internal standard solution (2.0 µg/L)
Add 40 µL of a solution of either 2,4,6-trichloroanisole-d_5 (or 2,6-dibromoanisole or 2,3,6 trichloroanisole) at 5.0 mg/L (3.9) to approximately 60 mL of absolute ethanol (3.5). Adjust the volume to 100 ml with absolute ethanol (3.5). It can be kept at an ambient temperature in a glass bottle with a metallic or glass cover.

3.11 Stock solution of TCA standard (40 mg/L)
Add 0.020g of 2,4,6-trichloroanisole to approximately 400 ml of absolute ethanol (3.5). Following dissolution, adjust volume to 500 mL with absolute ethanol (3.5).
3.12 Intermediate solution A of TCA standard (80 µg/L)
Add 1 mL of 2,4,6-trichloroanisole solution at 40 mg/L (3.11) to approximately 400 mL of absolute ethanol (3.5). Following dissolution, adjust volume to 500 mL with absolute ethanol (3.5).
3.13 Intermediate solution B of TCA standard (160 ng/L)
Add 1 mL of solution 2,4,6-trichloroanisole at 80 µg/L (3.12) to approximately 400 mL of pure de-ionised water (3.6). Following dissolution, adjust the volume to 500 mL with pure de-ionised water (3.6)
3.14 Use the standard-addition technique to make up a range of standard solutions of TCA. Standard solutions in the range from 0.5 ng/L to 50 ng/L can be used, by making additions with a solution of 2,4,6-trichloroanisole at 160 ng/L (3.13) to 6 ml of absolute ethanol (3.5). Following dissolution, adjust volume to 50 mL with pure de-ionised water (3.6) The calibration curve obtained should be evaluated regularly and in any case whenever there is a major change in the GC/MS or GC/ECD systems.
3.15 Carrier gas: Helium, chromatographic purity (≥ 99.9990 %)

4. APPARATUS

4.1 Laboratory glassware
4.1.1 Graduated 100-mL flask
4.1.2 100-µL microsyringe
4.1.3 Wide-neck glass jar of a capacity adapted to the sample size, closed with a glass or metallic stopper or a material which does not bind TCA.

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4.1.4 20-mL glass sample bottle closed with a perforated capsule and a liner with one side Teflon-coated.

4.2 Solid-phase microextraction system (SPME) with a fiber coated with a polydimethylsiloxane film 100 µm thick

4.3 Heating system for sample bottle (4.1.4)

4.4 Stirring system for sample bottle (4.1.4)

4.5 Gas chromatograph equipped with a "split-splitless" injector and a mass spectrometer detector (MS) or an electron-capture detector (ECD)

4.6 Data-acquisition system

4.7 If required, an automatic sampling and injection system operating with an SPME system

4.8 Capillary column coated with an apolar stationary phase, of the phenylmethylpolysiloxane type (e.g.: 5 % phenyl methylpolysiloxane, 30 m x 0,25 mm x 0,25 µm film thickness or equivalent.)

5. SAMPLE PREPARATION

The corks are placed whole in a glass closed container. The container capacity (4.1.3), the same as the quantity of wine or aqueous-alcoholic solution (3.1 or 3.7), must be chosen in accordance to the sample size while ensuring that the corks are completely covered and immersed in the maceration container.
Example 1: 20 corks (45x24) mm, in a 1 L container;
Example 2: 50 corks (45x24) mm, in a 2 L container.

Most of the TCA released during maceration of the groups of stoppers is generally derived from a very low percentage of these stoppers. In order to obtain the best representation of a batch of stoppers, a number of appropriate analyses according to sampling rules and risk with regard to wine contamination should be carried out.

6. OPERATING METHOD

6.1 Extraction

After macerating at ambient temperature for (24 ± 2) hours under laboratory ambient temperature conditions, the maceration is homogenized by inversion. A part of the aliquot of the 10ml maceration solution (5) is transferred to a glass sample bottle (4.1.4)

To increase extraction efficiency and subsequent sensitivity of the method, a quantity of approximately 1 g of sodium chloride (3.2) can be added. 50 µL of the internal standard solution at 2.0 µg/L (3.10) are immediately added, then the bottle is closed using a perforated metal capsule fitted with a silicone / Teflon-coated liner. The capsule is crimped. The contents of the bottle are homogenized for 10 minutes by mixing using a stirring system (4.4) or by using an automatic system (4.7).

The bottle containing the sample is placed in the heating system (4.3) set to 35 °C ± 2 °C, with stirring (4.4). The extraction of the headspace is carried out using the SPME system (4.2) for at least 15 minutes.

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6.2 Analysis

The fiber is then desorbed at 260 °C for at least 2 minutes in the injector of a gas chromatograph, in splitless mode (4.5). The separation is carried out using a capillary column with a non-polar stationary phase (4.8). The carrier gas is helium with a constant flow of 1 ml/min. A temperature program from 35 °C (for 3 min) to 265 °C (at 15 °C/min) is given as an example.

6.3 Detection and quantification

Detection is carried out by mass spectrometry with a selection of specific ions for the 2,4,6-trichloroanisole (ions m/z 195, 210, 212), quantified on the m/z 195 ion, and the internal standard 2,4,6-trichloroanisol-d_5 (ions m/z 199, 215, 217) quantified on ion m/z 215..

For the determination of ECD, identify the analyte and internal standard (2,6-dibromoanisole or 2,3,6 trichloroanisole) in the chromatogram, by comparing the retention time of the sample peak corresponding to that of the standard solution peak.

7. CALCULATIONS

The area of the chromatographic peak obtained for the 2,4,6-trichloroanisole is corrected by the area obtained for the chromatographic peak of the internal standard. The content in 2,4,6-trichloroanisole of each sample is obtained using a calibration curve. The points on this curve are obtained by tracing the relative responses of the 2,4,6-trichloroanisole/internal standard, obtained for aqueous-alcoholic solutions (3.7) containing known concentrations of 2,4,6-trichloroanisole, as a function of the concentrations of these solutions (3.14).

The results are given in ng/L of TCA present in the maceration, rounded off to the nearest 0.1 ng/L.

8. CHARACTERISTICS OF THE METHOD

As an indication, the detection limit of the analysis of the macerations must be lower than 0.5 ng/L, and the quantification limit close to 1 ng/L. The coefficient of variation is lower than 5% for 5 ng/L, when the selected internal standard is the deuterated analogue TCA-d_5.

An interlaboratory trial was carried out in order to validate the method. This interlaboratory trial was not carried out according to the OIV protocol and the validation parameters mentioned in the FV 1224.

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FV 1224 - Résultats de l'analyse collaborative Ring test 3-TCA SPME.

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RESOLUTION OIV/OENO 345/2009

DETERMINATION OF GLUTATHIONE IN MUSTS AND WINES BY CAPILLARY ELECTROPHORESIS COUPLED WITH A LASER-INDUCED FLUORESCENCE DETECTOR (LIF)

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 iv of the Agreement of 3 April 2001, establishing the International Organisation of Vine and Wine,

following a proposal made by the sub-commission "Methods of Analysis",

HAS HEREBY DECIDED to add the following type IV method to Appendix A of the *International Compendium of Methods of Analysis:*

Title	Type of method
Determination of glutathione in musts and wines by capillary electrophoresis	**IV**

1. Scope

This method makes it possible to determine the glutathione content of musts and wines in a concentration range of 0 to 40 mg/L. It uses capillary electrophoresis (CE) associated with fluorimetric detection (LIF).

2. Principle

The method used, which proceeds by capillary electrophoresis, is an adaptation of the method developed by Noctor and Foyer (1998) to determine non-volatile thiols in poplar leaves using HPLC coupled with fluorimetric detection.
The separation of a mixture's solutes by capillary electrophoresis is obtained by differential migration in an electrolyte. The capillary tube is filled with this electrolyte.
The sample to be separated is injected into one end of the capillary tube. As a result of electrical field activity generated by the electrodes immersed in the electrolyte, the solutes separate due to differences in migration speed and are detected near the other end of the capillary tube in the form of peaks. In given operating conditions, migration times constitute a criterion for the identification of chemical species and the peak area is proportional to the quantity injected.

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3. Products and reagents

3.1 List of products

3.1.1 Glutathione (GSH, > 98 %)
3.1.2 Dithiothreitol (DTT, > 99 %)
3.1.3 Anhydrous monobasic sodium phosphate (NaH_2PO_4, > 99 %)
3.1.4 Anhydrous dibasic sodium phosphate (Na_2HPO_4, > 99 %)
3.1.5 2-(N-cyclohexylamino)ethanesulfonic acid (CHES, > 98 %),
3.1.6 Monobromobimane (MBB, 97 %)
3.1.7 Ethylenediamine tetraacetic acid sodium salt (EDTA, > 99 %)
3.1.8 Sodium hydroxide
3.1.9 Hydrochloric acid (35 %)
3.1.10 Acetonitrile (99.5 %)
3.1.11 Ultra-pure water with a resistance of >18 MΩ·cm.

3.2 List of solutions
All solutions are homogenised prior to use

3.2.1 Electrophoretic buffer: phosphate buffer, 50 mM, pH 7
This buffer is prepared using two solutions - A and B
3.2.1.1 Solution A: 3 mg of anhydrous monobasic phosphate (3.1.3) taken up by 250 ml ultra-pure water (3.1.11)
3.2.1.2 Solution B: 3.55 mg of anhydrous dibasic phosphate (3.1.4) taken up by 250 ml ultra-pure water (3.1.11)
The phosphate buffer is obtained by the addition of 40 ml of solution A (3.2.1.1) and 210 ml of solution B (3.2.1.2) and then made up to 500 ml with ultra-pure water (3.1.11). The buffer's pH is then adjusted to 7 using hydrochloric acid (3.1.9).

3.2.2 Monobromobimane solution (MBB) - 50 mM
25 mg of monobromobimane (MBB) (3.1.6) are taken up by 1,850 µl of acetonitrile (3.1.10). Stored in the dark at -20 °C, this reagent remains stable for three months.

3.2.3 0.1 M sodium hydroxide solution
0.4 g of sodium hydroxide (3.1.8) are put into a 100-ml volumetric flask and taken up by 100 ml of ultra-pure water (3.1.11).

3.2.4 5 M sodium hydroxide solution
20 g of sodium hydroxide (3.1.8) are put into a 100-ml volumetric flask and taken up by 100 ml of ultra-pure water (3.1.11).

3.2.5 CHES buffer: 0.5 M, pH 9.3
2.58 g of 2-(N-cyclohexylamino) ethanesulfonic acid (CHES) (3.1.5) are dissolved in approximately 20ml of ultra pure water (3.1.11). The pH buffer is adjusted to 9.3 by the addition of sodium hydroxide 5 M (3.2.4). The volume is then adjusted to 25 ml with ultra pure water (3.1.11). This buffer is divided between the 1.5-ml test tubes (Eppendorf type) with 1 ml per tube. Stored at –20 °C, the CHES aqueous solution may be kept for several months.

3.2.6 Dithiothreitol solution (DTT) - 10 mM
15.4 mg of dithiothreitol (3.1.2) is dissolved in 10 mL of ultra pure water (3.1.11) then this solution is divided in 1.5-ml test tube (Eppendorf type) with 1 ml per tube
Stored at –20 °C, this DTT aqueous solution may be kept several months.

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4. Apparatus

4.1 Capillary electrophoresis

Capillary electrophoresis equipped with a hydrostatic-type injector is coupled with a laser-induced fluorescence detector with an excitation wavelength similar to the absorption wavelength of the MBB-GSH adduct: e.g.= 390 nm (e.g. Zetalif detector).

4.2 The capillary tube

The total length of the non-grafted silica capillary tube is 120 cm. Its effective length is 105 cm, and its internal diameter is 30 µm.

5. Preparation of samples

The method of determination used consists of the derivatization of the SH functions by the monobromobimane (MBB) (Radkowsky & Kosower, 1986). Samples of musts or non-bottled wines are clarified by centrifugation prior to analysis. Bottled wines are analysed without prior clarification.

Preparation of samples:
In a 1.5-ml test tube (Eppendorf type), put successively:
- 200 µl of the sample,
- 10 µl of the DTT solution (3.2.4) - final concentration of 0.25 mM,
- 145 µl of CHES (3.2.3) - final concentration of 179 mM,
- 50 µl of MBB (3.2.2) - final concentration of 6.2 mM.

After stirring the reagent mixture, the derivatization of thiol functions by the MBB requires a 20-minute incubation period in the dark at ambient temperature. In these analytical conditions, the MBB-SR derivatives thus formed are relatively unstable; CE-LIF determination should be carried out immediately after incubation.

6. Procedure

6.1 Capillary tube preparation

Before being used for the first time and as soon as migration times increase, the capillary tube (4.2) should be treated in the following way:
6.1.1. Rinse with 0.1 M sodium hydroxide (3.2.5) for 3 minutes,
6.1.2. Rinse with ultra-pure water (3.1.12) for 3 minutes,
6.1.3. Rinse with the electrophoretic phosphate buffer (3.2.1) for 3 minutes.

6.2 Migration conditions

6.2.1 Injection of the sample is of the hydrostatic type; 3 s at 50 kPa.
This is followed by injection of 50 mb electrophoretic buffer (3.2.1) to improve peak resolution (Staking).

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6.2.2 Analysis.
A voltage of +30 kV, applied throughout separation, generates a current of 47 µA. These conditions are reached in 20 s. Separation is carried out at a constant temperature of 21 °C.
6.2.3 Rinsing the capillary tube
The capillary tube should be rinsed after each analysis, successively with:
- 0.1M sodium hydroxide (3.2.5) for 3 minutes,
- ultra-pure water (3.1.12) for 3 minutes,
- electrophoretic phosphate buffer (3.2.1) for 3 minutes.

7. Results
At the concentration ultimately used in the sample, the presence of DTT during derivatization makes it possible to stabilise the unstable functions of thiols that have an alkaline pH and are very easily oxidized by quinines produced by phenolic compound auto-oxidation, but does not break the disulphide bonds. Thus, under these analytical conditions, the reduced glutathione content (GSH) found in a wine with or without the addition of 10 mg/l of oxidized glutathione (GSSG) is strictly comparable (Figure 1). This method therefore makes it possible to determine glutathione content in its reduced form alone.

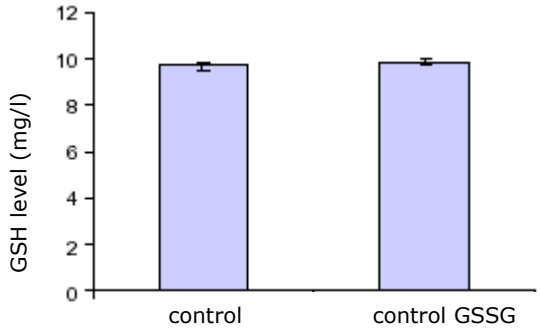

Figure 1: Demonstration of the stability of disulphide bonds according to the conditions of derivatization described. (DTT, ultimately 0.25 mM).

Figure 2 shows the electrophoretic profile of a white grape must sample (Sauvignon) in which cysteine, glutathione, N-acetyl-cysteine and sulphur dioxide are identified. The first peak corresponds to excess reagents (DTT, MBB). The separation of non-volatile thiols takes less than 20 minutes. Only certain peaks could be identified (Figure 2, A) (Newton et al., 1981). These thiols, apart from the sulphur dioxide, are generally present in varying quantities in grapes (Cheynier et al., 1989), fruit and vegetables (Mills et al., 2000).

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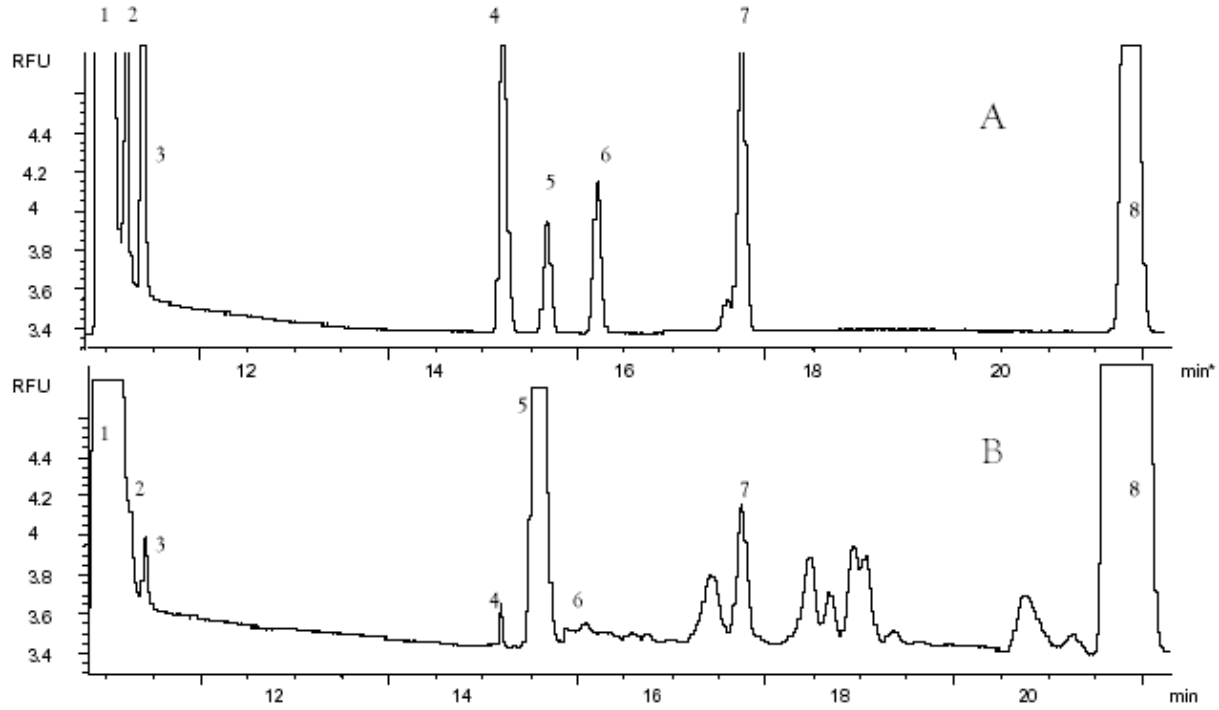

Figure 2: Example of the separation of the known non-volatile thiols in an HCl/EDTA solution (A) 1 and in a grape must (B): DTT; 2: homocysteine; 3: cysteine; 4: Cys-Gly; 5: GSH; 6: g Glu-Cys; ,7: NAC; 8: SO_2 .

In these analytical conditions, MBB-RS adduct retention times are as follows: MBB-homocysteine 10.40 mins; MBB-cysteine 10.65 mins, MBB-GSH 14.14 mins; MBB-NAC 15.41mins; MBB-SO2 18.58mins.

8. Characteristics of the method

Certain internal elements of validation were determined, but do not constitute formal validation according to the protocol for the design, conducts and interpretation of methods of analysis performance studies (OIV 6/2000).

Wine is used as a matrix to produce calibration curves and repeatability tests for each compound. Each concentration is calculated based on the average of three determinations obtained by using the right of the calibration curb regression. Results are expressed in mg/L.
Linear regressions and correlation coefficients are calculated according to the least squares method. The stock solutions of the various thiols are produced from an HCl/EDTA solution, allowing them to be stored at +6 °C for several days with no loss. Successive dilutions of these solutions allow the threshold limits for detection in wine to be estimated, for a signal-to-noise ratio of three of more.

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The linearity spectrum varies according to thiols (Table 1).

Table 1: Linearity spectrum, linear regression properties for each thiol in solutions prepared in exactly the same way as that of the glutathione.

	Linearity spectrum	Linear regression	Correlation coefficient
Homocysteine	0 - 15 mg/l	Y= 0.459X – 0.231	0.9987
Cysteine	0 - 15 mg/l	Y = 0.374X – 0.131	0.9979
Glutathione	0 - 40 mg/l	Y = 0.583X – 0.948	0.9966
N-acetyl-cysteine	0 - 10 mg/l	Y = 0.256X – 0.085	0.9982

These analytical conditions make it possible to eliminate interference caused by MBB hydrolysis products, unlike the reported findings of other works (Ivanov et al., 2000).

The method's repeatability is calculated on the basis of ten analyses of the same sample of wine. For a thiol concentration of 10 mg/l, the coefficient of variation is 6.0 % for the glutathione; besides this, it is 3.2 % for the homocysteine, 4.8 % for the cysteine and 6.4 % for the N-acetyl-cysteine.

The limit for detecting glutathione is 20 µg/l and the quantification limit is 60 µg/l.

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RESOLUTION OIV/OENO 346/2009

ANALYSIS OF BIOGENIC AMINES IN MUSTS AND WINES USING HPLC

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 iv of the Agreement of 3 April 2001, establishing the International Organisation of Vine and Wine,

following a proposal made by the "Methods of Analysis" Sub-commission

HAS HEREBY DECIDED to add the following type II method to Appendix A of the *International Compendium of Methods of Analysis*:

Title	Type of method
Analysis of biogenic amines in musts and wines using HPLC	**II**

1. SCOPE

This method can be applied for analysing biogenic amines in musts and wines:
Ethanolamine: up to 20 mg/l
Histamine: up to 15 mg/l
Methylamine: up to 10 mg/l
Serotonin: up to 20 mg/l
Ethylamine: up to 20 mg/l
Tyramine: up to 20 mg/l
Isopropylamine: up to 20 mg/l
Propylamine: normally absent
Isobutylamine: up to 15 mg/l
Butylamine: up to 10 mg/l
Tryptamine: up to 20 mg/l
Phenylethylamine: up to 20 mg/l
Putrescine or 1,4-diaminobutane: up to 40 mg/l
2-Methylbutylamine: up to 20 mg/l
3-Methylbutylamine: up to 20 mg/l
Cadaverine or 1,5-diaminopentane: up to 20 mg/l
Hexylamine: up to 10 mg/l

2. DEFINITION

The biogenic amines measured are:
Ethanolamine: C_2H_7NO – CAS [141 – 43 – 5]
Histamine: $C_5H_9N_3$ - CAS [51 – 45 – 6]
Methylamine: CH_5N – CAS [74 – 89 – 5]
Serotonin: $C_{10}H_{12}N_2O$ – CAS [153 – 98 – 0]

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Ethylamine: C_2H_7N – CAS [557 – 66 – 4]
Tyramine: $C_8H_{11}NO$ - CAS [60 – 19 – 5]
Isopropylamine: C_3H_9N - CAS [75 – 31 – 0]
Propylamine: C_3H_9N – CAS [107 – 10 – 8]
Isobutylamine: $C_4H_{11}N$ – CAS [78 – 81 – 9]
Butylamine: $C_4H_{11}N$ – CAS [109 – 73 – 9]
Tryptamine: $C_{10}H_{12}N_2$ – CAS [61 – 54 – 1]
Phenylethylamine: $C_8H_{11}N$ – CAS [64 – 04 – 0]
Putrescine or 1,4-diaminobutane: $C_4H_{12}N_2$ – CAS [333 – 93 – 7]
2-Methylbutylamine: $C_5H_{13}N$ - CAS [96 – 15 – 1]
3-Methylbutylamine: $C_5H_{13}N$ - CAS [107 – 85 – 7]
Cadaverine or 1,5-diaminopentane: $C_5H_{14}N_2$ – CAS [1476 – 39 – 7]
1,6-Diaminohexane: $C_6H_{16}N_2$ – CAS [124 – 09 – 4]
Hexylamine: $C_6H_{15}N$ – CAS [111 – 26 – 2]

3. PRINCIPLE

The biogenic amines are directly determined by HPLC using a C_{18} column after O-phthalaldehyde (OPA) derivatization and fluorimetric detection.

4. REAGENTS AND PRODUCTS

4.1 High purity resistivity water (18MΩ·cm)
4.2 Dihydrate disodium hydrogenophosphate - purity ≥ 99 %
4.3 Acetonitrile - Transmission minimum at 200 nm - purity ≥ 99 %
4.4 O-phthalaldehyde (OPA) - Application for fluorescence - purity ≥ 99 %
4.5 Disodium tetraborate decahydrate - purity ≥ 99 %
4.6 Methanol - purity ≥ 99 %
4.7 Hydrochloric acid 32 %
4.8 Sodium hydroxide pellets - purity ≥ 99 %
4.9 Ethanolamine - Purity ≥ 99 %
4.10 Histamine dichlorhydrate - Purity ≥ 99 %
4.11 Ethylamine chlorhydrate - Purity ≥ 99 %
4.12 Serotonin - Purity ≥ 99 %
4.13 Methylamine chlorhydrate – Purity ≥ 98 %
4.14 Tyramine chlorhydrate - Purity ≥ 99 %
4.15 Isopropylamine purity ≥99 %
4.16 Butylamine - Purity ≥ 99 %
4.17 Tryptamine chlorhydrate - purity ≥ 98 %
4.18 Phenylethylamine - Purity ≥ 99 %
4.19 Putrescine dichlorhydrate - Purity ≥ 99 %
4.20 2-Methylbutylamine - Purity ≥ 98 %
4.21 3-Methylbutylamine - Purity ≥ 98 %
4.22 Cadaverine dichlorhydrate - Purity ≥ 99 %
4.23 1-6-Diaminohexane - Purity ≥ 97 %
4.24 Hexylamine - Purity ≥ 99 %
4.25 Nitrogen (maximum impurities: $H_2O \leq 3$ mg/l; $O_2 \leq 2$ mg/L; $C_nH_ms \leq 0.5$ mg/l)
4.26 Helium (maximum impurities: $H_2O \leq 3$ mg/l; $O_2 \leq 2$ mg/L; $C_nH_m \leq 0.5$ mg/l)

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Preparation of reagent solutions:

4.27 Preparation of eluents

Phosphate solution A: Weigh 11.12 g ± 0.01 g of di-basic sodium phosphate (4.2) in a 50-ml beaker (5.5) on a balance (5.27). Transfer to a 2-litre volumetric flask (5.9) and make up to 2 litres with high purity water (4.1). Homogenize using a magnetic stirrer (5.30) and filter over a 0.45 µm membrane (5.17). Put in the 2-litre bottle (5.12).

Solution B: The acetonitrile (4.3) is used directly.

4.28 OPA solution – Daily preparation

Weigh 20 mg ± 0.1 mg of OPA (4.4) in a 50-ml flask (5.7) on the precision balance (5.27). Make up to 50 ml with methanol (4.6). Homogenize.

4.29 Preparation of the borate buffer (4.29) – Weekly preparation

Weigh 3.81 g ± 0.01 g of $Na_2B_4O_7 \cdot 10H_2O$ (4.5) in a 25-ml beaker (5.6) on the precision balance (5.27). Transfer to a 100-ml volumetric flask (5.8) and make up to 100 ml with demineralised water (4.1). Homogenize with a magnetic stirrer (5.30), transfer to a 150-ml beaker (5.4) and adjust to pH 10.5 using a pH meter (5.28 and 5.29) with 10 N soda (4.8).

4.30 0.1 M hydrochloric acid solution: Put a little demineralised water (4.1) into a 2-litre volumetric flask (5.9). Add 20 ml of hydrochloric acid (4.7) using a 10-ml automatic pipette (5.24 and 5.25)

4.31 Calibration solution in 0.1 M hydrochloric acid

Guideline concentration of the calibration solution - weigh at ± 0.1 mg

	Indicative final concentration in the calibration mix in mg/l
Ethanolamine	5
Histamine	5
Methylamine	1
Serotonin	20
Ethylamine	2
Tyramine	7
Isopropylamine	4
Propylamine	2.5
Isobutylamine	5
Butylamine	5
Tryptamine	10
Phenylethylamine	2
Putrescine	12
2- Methylbutylamine	5
3- Methylbutylamine	6
Cadaverine	13
1.6 Diaminohexane	8
Hexylamine	5

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The true concentration of the calibration solution is recorded with the batch number of the products used.
Certain biogenic amines being in salt form, the weight of the salt needs to be taken into account when determining the true weight of the biogenic amine.
The stock solution is made in a 100-ml volumetric flask (5.8).
The surrogate solution is made in a 250-ml volumetric flask (5.10).

4.32 1,6 Diaminohexane internal standard
Weigh exactly 119 mg in a 25-ml Erlenmeyer flask (5.1) on a balance (5.26). Transfer to a 100-ml volumetric flask (5.8) and top up to the filling mark with 0.1 N hydrochloric acid (4.30).
4.33 2-Mercaptoethanol - Purity ≥ 99 %.

5. APPARATUS

5.1 25-ml Erlenmeyer flasks
5.2 250-ml Erlenmeyer flasks
5.3 100-ml beakers
5.4 150-ml beakers
5.5 50-ml beaker
5.6 25-ml beaker
5.7 50-ml volumetric flasks
5.8 100-ml volumetric flasks
5.9 2,000-ml volumetric flasks
5.10 250-ml volumetric flask
5.11 1-litre bottles
5.12 2-litre bottle
5.13 2-ml screw cap containers suitable for the sample changer
5.14 50-ml syringe
5.15 Needle
5.16 Filter holder
5.17 0.45 µm cellulose membrane
5.18 0.8 µm cellulose membrane
5.19 1.2 µm cellulose membrane
5.20 5 µm cellulose membrane
5.21 Cellulose pre-filter
5.22 1-ml automatic pipette
5.23 5-ml automatic pipette
5.24 10-ml automatic pipette
5.25 Cones for 10-ml, 5-ml and 1-ml automatic pipettes
5.26 Filtering system
5.27 Balances for weighing 0 to 205 g at ± 0.01 mg
5.28 pH meter
5.29 Electrode
5.30 Magnetic stirrer
5.31 HPLC pump
5.32 Changer-preparer equipped with an oven
Note: An oven is indispensable, if a changer-preparer is used for injecting several samples one after another. This operation may likewise be done manually) the results may be less precise;
5.33 Injection loop
5.34 5 µm C_{18} column, 250 mm × 4 (which must lead to a similar chromatogram as presented in annex B);

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5.35 Fluorimetric detector
5.36 Integrator
5.37 Borosilicic glass tube with a stopper and closure cap covered with PTFE (ex Sovirel 15).

6. PREPARATION OF SAMPLES

Samples are previously purged of gas with nitrogen (4.25).

6.1 Filtering
Filter approximately 120 ml of the sample over membrane:
- for a wine: 0.45 µm (5.17),
- for a must or non-clarified wine: 0.45 (5.17) – 0.8 (5.18) – 1.2 (5.19) - 5 µm (5.20) + pre-filter (5.21), pile filters in the following order, the sample pushed by the top: 0.45 µm (5.17) + 0.8 µm (5.18) + 1.2 µm (5.19) + 5 µm (5.20) + prefiltered (5.21)

6.2 Preparation of the sample
Put 100 ml of the sample (6.1) into a 100-ml volumetric flask (5.8);
Add 0.5 ml of 1-6-diaminohexane (4.32) at 119 mg/100 ml using a 1-ml automatic pipette (5.21 and 25);
Draw off 5 ml of the sample using the pipette (5.23 and 5.25); pour this into a 25-ml Erlenmeyer flask (5.1);
Add 5 ml of methanol to this (4.6) using the pipette (5.23 and 5.25);
Stir to homogenize;
Transfer to containers (5.13);
Start the HPLC pump (5.31), then inject 1 µl (5.32 and 5.33)

6.3 Derivatisation
In a borosilicic glass tune (5.37), pour 2 ml of OPA solution (4.28), 2 ml of borate buffer (4.29), 0,6 ml of 2-mercaptoethanol (4.33). Close, mix (5.30). Open and pour 0,4 ml of sample. Close, mix (5.30). **Inject immediately, as the derivitive is not stable.** Rinse recipient immediately after injection, due to odour.
Note: Derivatisation can be carried out by an automatic changer-preparer. In this case, the process will be programmed to come close to the proportion of manual derivisation

6.4 Routine cleaning
Syringe (5.13) and needle (5.14) rinsed with demineralised water (4.1) after each sample; filter holder (5.16) rinsed with hot water, then MeOH (4.6). Leave to drain and dry.

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7. PROCEDURE

Mobile phase (5.31)
- A: phosphate buffer (4.2)
- B: acetonitrile (4.3)

Elution gradient:

time (in mins)	% A	% B
0	80	20
15	70	30
23	60	40
42	50	50
55	35	65
60	35	65
70	80	20
95	80	20

Note: The gradient can be adjusted to obtain a chromatogram close to the one presented in annex B

Flow rate: 1 ml/min;
Column temperature: 35 °C (5.32);
Detector (5.35): Exc = 356 nm, Em = 445 nm (5.30);

Internal calibration
The calibration solution is injected for each series;
Calibration by internal standard;

Calculation of response factors:

$$RF = Ccis \times \text{area i} / \text{area is} \times Cci$$

Cci = concentration of the component in the calibration solution and
Ccis = concentration of the internal standard in the calibration solution (1-6-diaminohexane).
Area i = area of the product peak present in the sample
Area is = area of the internal standard peak in the sample

Calculation of concentrations:

$$Cci = (XF \times \text{area i})/ (\text{area is} \times RF)$$

Area i = area of the product peak present in the sample
Area is = area of the internal standard peak present in the sample
XF = quantity of internal calibration added to samples for analysis
$XF = 119 \times 0.5/100 = 5.95$.

8. EXPRESSION OF RESULTS

Results are expressed in mg/l with one significant digit after the decimal point.

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9. RELIABILITY

	r (mg/l)	R (mg/l)
Histamine	0.07x + 0.23	0.50x + 0.36
Methylamine	0.11x + 0.09	0.40x + 0.25
Ethylamine	0.34x - 0.08	0.33x + 0.18
Tyramine	0.06x + 0.15	0.54x + 0.13
Phenylethylamine	0.06x + 0.09	0.34x + 0.03
Diaminobutane	0.03x + 0.71	0.31x + 0.23
2-methylbutylamine et 3-methylbutylamine	0.38x + 0.03	0.38x + 0.03
Diaminopentane	0.14x + 0.09	0.36x + 0.12

The details of the interlaboratory trial with regard to reliability of the method are summarised in appendix A.

10. OTHER CHARACTERISTICS OF THE ANALYSIS

The influence of certain wine components: amino acids are released at the beginning of the analysis and do not impede in detection of biogenic amines.

The limit of detection (LOD) and limit of quantification (LOQ) according to an intralaboratory study

	LOD (in mg/l)	LOQ (in mg/l)
Histamine	0,01	0,03
Methylamine	0,01	0,02
Ethylamine	0,01	0,03
Tyramine	0,01	0,04
Phenylethylamine	0,02	0,06
Diaminobutane	0,02	0,06
2-methylbutylamine	0,01	0,03
3-methylbutylamine	0,03	0,10
Diaminopentane	0,01	0,03

11. QUALITY CONTROL

Quality controls may be carried out with certified reference materials, with wines the characteristics of which result from a consensus or spiked wines regularly inserted into analytical series and by following the corresponding control charts.

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Annex A

Statistical data obtained from the results of interlaboratory trials

The following parameters were defined during an interlaboratory trial. This trial was carried out by the Oenology Institute of Bordeaux (France) under the supervision of the National Interprofessional Office of Wine (ONIVINS – France).

Year of interlaboratory trial: 1994

Number of laboratories: 7

Number of samples: 9 double blind samples

(Bulletin de l'O.I.V. November-December 1994, 765-766, p.916 to 962) numbers recalculated in compliance with ISO 5725-2:1994.

Types of samples: white wine (BT), white wine (BT) fortified = B1, white wine (BT) fortified = B2, red wine n°1 (RT), red wine fortified = R1, red wine (RT) fortified = R2, red wine n°2 (CT), red wine (CT) fortified = C1 and red wine (CT) fortified = C2. fortified in mg/l.

	HistN	MetN	EthN	TyrN	PhEtN	DiNbut	IsoamN	DiNpen
wine B1	wine BT + 0,5	vine BT + 0,12	wineBT + 0,13	wine BT + 0,36	vine BT + 0,15	wine BT + 0,5	wine BT + 0,28	wineBT + 0,25
wine B2	wine BT + 2	wine BT + 0,40	wine BT + 0,50	wine BT + 1,44	wine BT + 0,60	wine BT + 2	Wine BT + 0,1,74	wine BT + 1,04
wine C1	wine CT + 2	wine CT + 0,1	wine CT + 0,18	wine CT + 0,72	wine CT + 0,15	wine CT + 2	wine CT + 0,29	wine CT + 0,26
wineC2	wine CT + 4	wine CT + 0,41	wine CT + 0,50	wine CT + 2,90	wine CT + 0,58	wine CT + 8	wine CT + 1,14	wine CT + 1,04
wine R1	wine RT + 2	wine RT + 0,14	wine RT + 0,13	wine RT + 1,45	wine RT + 0,19	wine RT + 3	wine RT + 0,0,57	wine RT + 0,51
wine R2	wine RT + 5	wine RT + 0,41	wine RT + 0,50	wine RT + 2,88	wine RT + 0,59	wine RT + 10	wine RT + 2,28	wine RT + 2,08

HistN : histamine, MetN : methylamine, EthN : ethylamine, TyrN : tyramine, PhEtN : phenylethylamine, DiNbut : diaminobutane, IsoamN : isoamylamine and DiNpen : diaminopentane.

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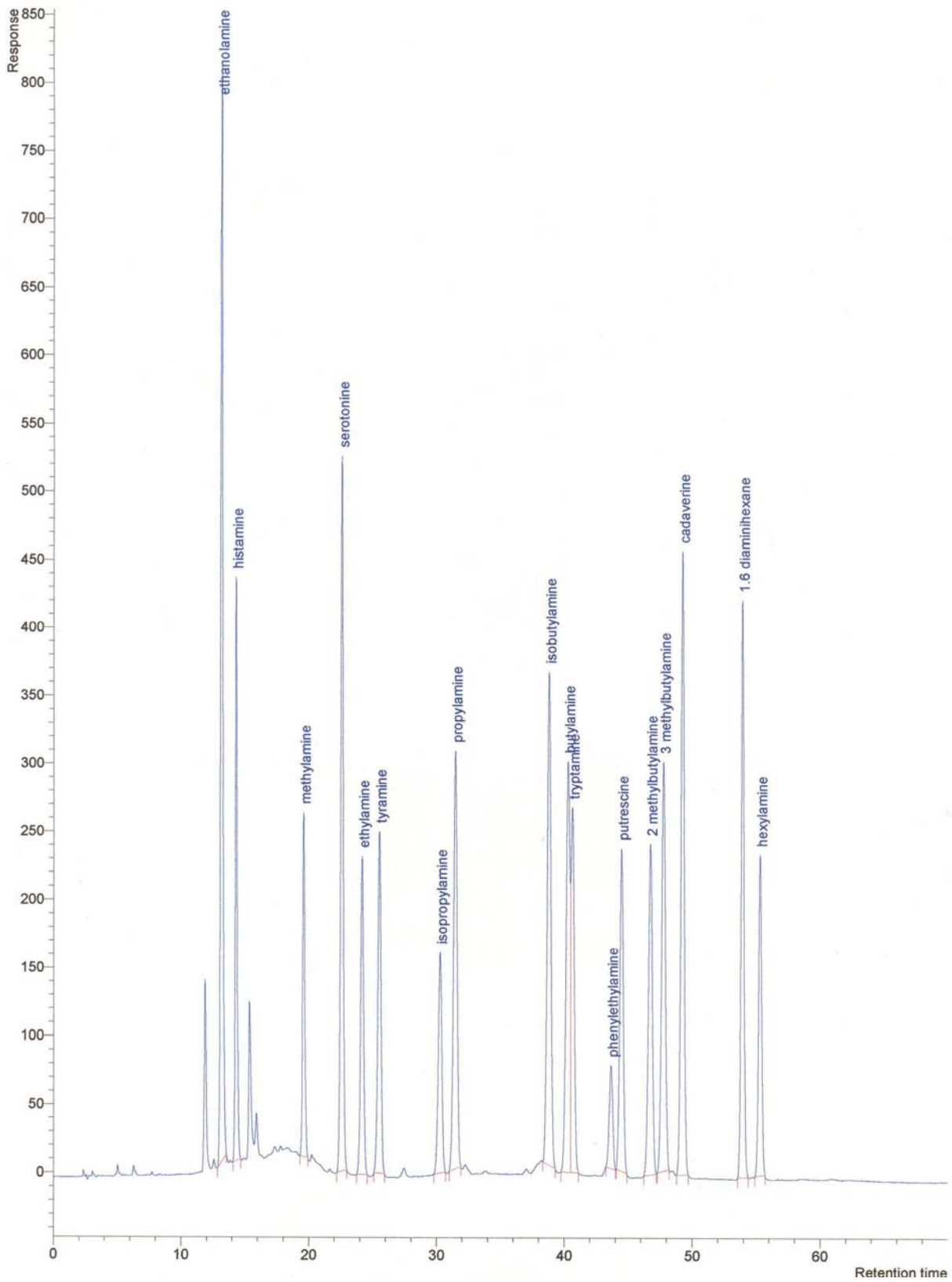
Annex B : Chromatogram model obtained by this method



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RESOLUTION OIV/OENO 353/2009

METHOD FOR $^{18}O/^{16}O$ ISOTOPE RATIO DETERMINATION OF WATER IN WINE AND MUST

The GENERAL ASSEMBLY,

Considering Article 2 paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

Considering the resolution Oeno 2/96 related to the determination method of the isotopic ratio $^{18}O/^{16}O$ of the water content in wines and the collaborative inter-laboratory study,

Upon the proposal of the "Methods of Analysis" Sub-commission,

DECIDES to replace the existing type II method for isotopic ratio $^{18}O/^{16}O$ determination of water content in wines in Annex A of the Compendium of International Methods of Analysis of Wine and Must with the following method:

Title	Type of the method
Method for $^{18}O/^{16}O$ isotope ratio determination of water in wine and must	**II**

1. SCOPE

The method describes the determination of the $^{18}O/^{16}O$ isotope ratio of water from wine and must after equilibration with CO_2, using the isotope ratio mass spectrometry (IRMS).

2. REFERENCE STANDARDS

ISO 5725:1994: Accuracy (trueness and precision) of measurement methods and results: Basic method for the determination of repeatability and reproducibility of a standard measurement method.
V-SMOW: Vienna-Standard Mean Ocean Water ($^{18}O/^{16}O = R_{V\text{-}SMOW} = 0.0020052$)
GISP Greenland Ice Sheet Precipitation
SLAP Standard Light Antarctic Precipitation

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3. DEFINITIONS

$^{18}O/^{16}O$ Isotope ratio of oxygen 18 to oxygen 16 for a given sample

$\delta^{18}O_{V\text{-}SMOW}$ Relative scale for the expression of the isotope ratio of oxygen 18 to oxygen 16 for a given sample. $\delta^{18}O_{V\text{-}SMOW}$ is calculated using the following equation:

$$\delta^{18}O_{V-SMOW} = \left[\frac{\left(\frac{^{18}O}{^{16}O}\right)_{sample} - \left(\frac{^{18}O}{^{16}O}\right)_{standard}}{\left(\frac{^{18}O}{^{16}O}\right)_{standard}} \right] \times 1000 \quad [‰]$$

using the V-SMOW as standard and as reference point for the relative δ scale.

BCR Community Bureau of Reference
IAEA International Atomic Energy Agency (Vienna, Austria)
IRMM Institute for Reference Materials and Measurements
IRMS Isotope Ratio Mass Spectrometry
m/z mass to charge ratio
NIST National Institute of Standards & Technology
RM Reference Material

4. PRINCIPLE

The technique described thereafter is based on the isotopic equilibration of water in samples of wine or must with a CO_2 standard gas according to the following isotopic exchange reaction:

$$C^{16}O_2 + H_2{}^{18}O \leftrightarrow C^{16}O^{18}O + H_2{}^{16}O$$

After equilibration the carbon dioxide in the gaseous phase is used for analysis by means of Isotopic Ratio Mass Spectrometry (IRMS) where the $^{18}O/^{16}O$ isotopic ratio is determined on the CO_2 resulting from the equilibration.

5. REAGENTS AND MATERIALS

The materials and consumables depend on the method used (see chapter 6). The systems generally used are based on the equilibration of water in wine or must with CO_2.
The following reference materials, working standards and consumables can be used:

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5.1 Reference materials

Name	issued by	$\delta^{18}O$ versus V-SMOW
V-SMOW, RM 8535	IAEA / NIST	0 ‰
BCR-659	IRMM	-7.18 ‰
GISP, RM 8536	IAEA / NIST	-24.78 ‰
SLAP, RM 8537	IAEA / NIST	-55.5 ‰

5.2 Working Standards
5.2.1 Carbon dioxide as a secondary reference gas for measurement (CAS 00124-38-9).
5.2.2 Carbon dioxide used for equilibration (depending on the instrument this gas could be the same as 5.2.1 or in the case of continuous flow systems cylinders containing gas mixture helium-carbon dioxide can also be used)

5.2.3 Working Standards with calibrated $\delta^{18}O_{V\text{-}SMOW}$ values traceable to international reference materials.

5.3 Consumables
Helium for analysis (CAS 07440-59-7)

6. APPARATUS

6.1 Isotope ratio mass spectrometry (IRMS)
The Isotope ratio mass spectrometer (IRMS) enables the determination of the relative contents of ^{13}C of CO_2 gas naturally occurring with an internal accuracy of 0.05‰. Internal accuracy here is defined as the difference between 2 measurements of the same sample of CO_2.
The mass spectrometer used for the determination of the isotopic composition of CO_2 gas is generally equipped with a triple collector to simultaneously measure the following ion currents:

- m/z = 44 ($^{12}C^{16}O^{16}O$)
- m/z = 45 ($^{13}C^{16}O^{16}O$ and $^{12}C^{17}O^{16}O$)
- m/z = 46 ($^{12}C^{16}O^{18}O$, $^{12}C^{17}O^{17}O$ and $^{13}C^{17}O^{16}O$)

By measuring the corresponding intensities, the $^{18}O/^{16}O$ isotopic ratio is determined from the ratio of intensities of m/z = 46 and m/z = 44 after corrections for isobaric species ($^{12}C^{17}O^{17}O$ and $^{13}C^{17}O^{16}O$) whose contributions can be calculated from the actual intensity observed for m/z= 45 and the usual isotopic abundances for ^{13}C and ^{17}O in Nature.

The isotope ratio mass spectrometry must either be equipped with:
- a double introduction system (dual inlet system) to alternately measure the unknown sample and a reference standard.
- or a continuous flow system that transfers quantitatively the CO_2 from the sample vials after equilibration but also the CO_2 standard gas into the mass spectrometer.

6.2 Equipment and Materials
All equipments and materials used must meet stated requirements of the used method / apparatus (as specified by the manufacturer). However, all equipments and materials can be replaced by items with similar performance.

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6.2.1 Vials with septa appropriate for the used system
6.2.2 Volumetric pipettes with appropriate tips
6.2.3 Temperature controlled system to carry out the equilibration at constant temperature, typically within ±1 °C
6.2.4 Vacuum pump (if needed for the used system)
6.2.5 Autosampler (if needed for the used system)
6.2.6 Syringes for sampling (if needed for the used system)
6.2.7 GC Column to separate CO_2 from other elementary gases (if needed for the used system)
6.2.8 Water removal device (e.g. cryo-trap, selective permeable membranes)

7. SAMPLING

Wine and must samples as well as reference materials are used for analysis without any pre-treatment. In the case of the possible fermentation of the sample, benzoic acid (or another anti-fermentation product) should be added or filtered with a with a 0,22 µm pore diameter filter.

Preferably, the reference materials used for calibration and drift-correction should be placed at the beginning and at the end of the sequence and inserted after every ten samples.

8. PROCEDURE

The descriptions that follow refer to procedures generally used for the determination of the $^{18}O/^{16}O$ isotopic ratios by means of equilibration of water with a CO_2 working standard and the subsequent measurement by IRMS. These procedures can be altered according to changes of equipment and instrumentation provided by the manufacturers as various kind of equilibration devices are available, implying various conditions of operation. Two main technical procedures can be used for introduction of CO_2 into the IRMS either through a dual inlet system or using a continuous flow system. The description of all these technical systems and of the corresponding conditions of operation is not possible. Note: all values given for volumes, temperatures, pressures and time periods are only indicative. Appropriate values must be obtained from specifications provided by the manufacturer and/or determined experimentally.

8.1 Manual equilibration

A defined volume of the sample/standard is transferred into a flask using a pipette. The flask is then attached tightly to the manifold.
Each manifold is cooled down to below – 80 °C to deep-freeze the samples (manifold equipped with capillary opening tubes do not require this freezing step). Subsequently, the whole system is evacuated. After reaching a stable vacuum the gaseous CO_2 working standard is allowed to expand into the various flasks. For the equilibration process each manifold is placed in a temperature controlled water-bath typically at 25°C (± 1 °C) for 12 hours (overnight). It is crucial that the temperature of the water-bath is kept constant and homogeneous.
After the equilibration process is completed, the resulting CO_2 is transferred from the flasks to the sample side bellow of the dual inlet system. The measurements are performed by comparing

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several times the ratios of the CO_2 contained in the sample side and the standard side (CO_2 reference standard gas) of the dual inlet. This approach is repeated till the last sample of the sequence has been measured.

8.2 Use of an automatic equilibration apparatus

A defined volume of the sample/standard is transferred into a vial using a pipette. The sample vials are attached to the equilibration system and cooled down to below – 80 °C to deep-freeze the samples (systems equipped with capillary opening tubes do not require this freezing step). Subsequently, the whole system is evacuated.
After reaching a stable vacuum the gaseous CO_2 working standard is expanded into the vials. Equilibrium is reached at a temperature of typically 22 ± 1 °C after a minimum period of 5 hours and with moderate agitation (if available). Since the equilibration duration depends on various parameters (e.g. the vial geometry, temperature, applied agitation ...), the minimum equilibrium time should be determined experimentally.
After the equilibration process is completed, the resulting CO_2 is transferred from the vials to the sample side bellow of the dual inlet system. The measurements are performed by comparing several times the ratios of the CO_2 contained in the sample side and the standard side (CO_2 reference standard gas) of the dual inlet. This approach is repeated till the last sample of the sequence has been measured.

8.3 Manual preparation manual and automatic equilibration and analysis with a dual inlet IRMS

A defined volume of sample / standard (eg. 200 µL) is introduced into a vial using a pipette. The open vials are then placed in a closed chamber filled with the CO_2 used for equilibration (5.2.2). After several purges to eliminate any trace of air, the vials are closed and then placed on the thermostated plate of the sample changer. The equilibration is reached after at least 8 hours at 40 °C. Once the process of equilibration completed, the CO_2 obtained is dried and then transferred into the sample side of the dual inlet introduction system. The measurements are performed by comparing several times the ratios of the CO_2 contained in the sample side and the standard side (CO_2 reference standard gas) of the dual inlet. This approach is repeated till the last sample of the sequence has been measured.

8.4 Use of an automatic equilibration apparatus coupled to a continuous flow system

A defined volume of the sample/standard is transferred into a vial using a pipette. The sample vials are placed into a temperature controlled tray.
Using a gas syringe the vials are flushed with mixture of He and CO_2. The CO_2 remains in the headspace of the vials for equilibration.
Equilibrium is reached at a temperature typically of 30 ± 1 °C after a minimum period of 18 hours.
After the equilibration process is completed the resulting CO_2 is transferred by means of the continuous flow system into the ion source of the mass spectrometer. CO_2 reference gas is also introduced into the IRMS by means of the continuous flow system. The measurement is carried out according to a specific protocol for each kind of equipment.

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9. CALCULATION

The intensities for m/z = 44, 45, 46 are recorded for each sample and reference materials analysed in a batch of measurements. The $^{18}O/^{16}O$ isotope ratios are then calculated by the computer and the software of the IRMS instrument according to the principles explained in section 6.1. In practice the $^{18}O/^{16}O$ isotope ratios are measured against a working standard previously calibrated against the V-SMOW. Small variations may occur while measuring on line due to changes in the instrumental conditions. In such a case the $\delta\ ^{18}O$ of the samples must be corrected according to the difference in the $\delta\ ^{18}O$ value from the working standard and its assigned value, which was calibrated beforehand against V-SMOW. Between two measurements of the working standard, the variation is the correction applied to the sample results that may be assumed to be linear. Indeed, the working standard must be measured at the beginning and at the end of all sample series. Therefore a correction can be calculated for each sample using linear interpolation between two values (the difference between the assigned value of the working standard and the measurements of the obtained values).
The final results are presented as relative $\delta^{18}O_{V\text{-}SMOW}$ values expressed in ‰.
$\delta^{18}O_{V\text{-}SMOW}$ values are calculated using the following equation:

$$\delta^{18}\mathrm{O}_{\mathrm{V-SMOW}} = \left[\frac{\left(\frac{^{18}\mathrm{O}}{^{16}\mathrm{O}}\right)_{\mathrm{sample}} - \left(\frac{^{18}\mathrm{O}}{^{16}\mathrm{O}}\right)_{\mathrm{V-SMOW}}}{\left(\frac{^{18}\mathrm{O}}{^{16}\mathrm{O}}\right)_{\mathrm{V-SMOW}}} \right] \times 1000 \ \ [‰]$$

The $\delta^{18}O$ value normalized versus the V-SMOW/SLAP scale is calculated using the following equation:

$$\delta^{18}O_{V-SMOW\,/\,SLAP} = \left[\frac{\delta^{18}O_{sample} - \delta^{18}O_{V-SMOW}}{\delta^{18}O_{V-SMOW} - \delta^{18}O_{SLAP}} \right] \times 55.5 \ [‰]$$

The $\delta^{18}O_{V\text{-}SMOW}$ value accepted for SLAP is -55.5‰ (see also 5.1).

10. PRECISION

The repeatability (r) is equal to 0.24 ‰.
The reproducibility (R) is equal to 0.50 ‰.

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Summary of statistical results

	General average (‰)		Standard deviation of repeatability (‰) s_r		Repeatability (‰) r		Standard deviation of reproducibility (‰) s_R		Reproducibility (‰) R
Water									
Sample 1	-8.20		0.068		0.19		0.171		0.48
Sample 2	-8.22		0.096		0.27		0.136		0.38
Wine N° 1									
Sample 5	6.87		0.098		0.27		0.220		0.62
Sample 8	6.02		0.074		0.21		0.167		0.47
Sample 9	5.19		0.094		0.26		0.194		0.54
Sample 4	3.59		0.106		0.30		0.205		0.57
Wine N° 2									
Sample 3	-1.54		0.065		0.18		0.165		0.46
Sample 6	-1.79		0.078		0.22		0.141		0.40
Sample 7	-2.04		0.089		0.25		0.173		0.49
Sample 10	-2.61		0.103		0.29		0.200		0.56

11. INTER-LABORATORIES STUDIES

Bulletin de l'O.I.V. janvier-février 1997, 791-792, p.53 - 65.

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RESOLUTION OIV/OENO 387/2009

SUPPLEMENT TO THE METHOD OF DETERMINATION OF THE DRY EXTRACT

THE GENERAL ASSEMBLY

Considering Article 2 paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

Upon the proposal of the Methods of Analysis Sub-commission
Considering the method « Total dry extract » adopted by the OIV contained in the « Compendium of Methods of Analysis of Wine and Musts» (MA-F-AS2-03-EXTSEC).

Considering the first paragraph –Definitions- of the method indicated in the « object », the sugar-free extract is defined as a total dry extract minus total sugars, as follows:

Sugar-free extract = Total dry extract – total sugars

Considering that there is no indication on the manner in which total sugars are expressed.

Considering that in certain countries standards make a provision for a sugar-free extract limit

Considering that the lack of indications on the manner of calculating total sugars may pose a problem if the wine or the wine-based product has added saccharose, in addition to its own sugars, as is the case with flavoured wine.

DECIDES to complete the "Total dry extract" method in the Compendium of Methods of Analysis of Wine and Musts" (MA-F-AS2-03-EXTSEC) with the following note**:**

Note:

Calculate total dry extract by separately taking into account quantities of glucose and fructose (reducing sugars) and the quantity of saccharose, as follows:

Sugar-free extract = Total dry extract – reducing sugars (glucose + fructose) – saccharose

In the case that the method of analysis allows for sugar inversion, use the following formula for the calculation:

Sugar-free extract = Total dry extract – reducing sugars (glucose + fructose)- [(Sugars after inversion – Sugars before inversion) x 0,95]

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RESOLUTION OIV/OENO 374/2009

DETERMINING THE PRESENCE AND CONTENT OF POLYCHLOROPHENOLS AND POLYCHLOROANISOLS IN WINES, CORK STOPPERS, WOOD AND BENTONITES USED AS ATMOSPHERIC TRAPS

The GENERAL ASSEMBLY,

CONSIDERING Article 2 paragraph 2 iv of the Agreement of 3 April, 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING resolution OENO 8/2006 on determining the presence and content of polychlorophenols and polychloroanisols in wines, cork stoppers, wood and atmospheric traps

CONSIDERING the works of the Sub-commission of Methods of Analysis and the necessity of reviewing the above-mentioned resolution

DECIDES on proposal of the Oenology Commission to modify the AS315-13-PCAPCP Method as described currently in the Compendium of International Methods of Analysis of Wine and Must by the following type IV method:

Title	Method type
DETERMINING THE PRESENCE AND CONTENT OF POLYCHLOROPHENOLS AND POLYCHLOROANISOLS IN WINES, CORK STOPPERS, WOOD AND BENTONITES USED AS ATMOSPHERIC TRAPS	**IV**

1. SCOPE

All wines, cork stoppers, bentonites (absorption traps) and wood.

2. PRINCIPLE

Determination of 2,4,6-trichloroanisol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachloroanisol, 2,3,4,6-tetrachlorophenol, pentachloroanisol and pentachlorophenol by gas chromatography, by injecting a hexane extract of the wine and an ether/hexane extract of the solid samples to be analyzed and internal calibration.

3. REAGENTS

Preliminary remark: all the reagents and solvents must be free of the compounds to be determined listed in 2 at the detection limit.

3.1 Purity of hexane > 99 %
3.2 Purity of ethylic ether > 99 %
3.3 Ether/hexane mixture (50/50; v/v)

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3.4 or 2,5-dibromophenol purity ≥ 99 %
3.5 Pure ethanol
3.6 Pure deionized water, TCA free, type II in accordance with ISO standard EN 3696
3.7 50 % vol. aqueous-alcoholic solution. Place 100 ml of absolute ethanol (3.<5) in a graduated 200-ml flask (4.9.9), add 200 ml of deionized water (3.6), and homogenize.

3.8 Internal standard:

3.8.1 200 mg/l stock solution. Place 20 mg of internal standard (3.4) in a graduated 100-ml flask (4.9.8), add the 50 % volume aqueous-alcoholic solution (3.7) and homogenize.

3.8.2 Internal standard solution (2 mg/l). Place 1 ml of the stock solution of internal standard (3.8.1) in a graduated 100-ml flask (4.9.8), add the 50% vol aqueous-alcoholic solution (3.7) and homogenize.

3.8.3 Internal standard solution (20 µg/l). Place 1 ml of stock solution of internal standard (3.8.2) in a 100 ml graduated flask (4.9.8), add with 50 % vol aqueous-alcoholic solution

3.9 Pure products

- 3.9.1 2,4,6-trichloroanisole: ≥ 99 %, case: 87-40-1
- 3.9.2 2, 4, 6-trichlorophenol: ≥ 99.8 %, case: 88-06-2
- 3.9.3 2,3,5,6-tetrachloroanisole: ≥ 99 %, case: 6936-40-9 (note: the product sought in the samples is 2,3,4,6-tetrachloroanisole but is does not exist on the market)
- 3.9.4 2, 3, 4, 6-tetrachlorophenol: ≥ 99 %, case: 58-90-2
- 3.9.5 pentachloroanisole: ≥ 99 %, case: 1825-21-1
- 3.9.6 pentachlorophenol: 99 %, case: 87-86-5

3.10 Reagents for derivatisation - Piridine: acetic anydride (1:0,4) vol.

3.10.1 Piridine: ≥ 99 %

3.10.2 Acetic anydride: ≥ 98 %

3.11 Calibration stock solution at 200 mg/l
In a graduated 100-ml flask (4.9.8), place approximately 20 mg of the pure reference products (3.9.1 to 3.9.6) but whose exactly weight is known (4.7), add absolute ethanol (3.5). Homogenize.

3.12 Intermediate calibration solution at 200 µg/l
In a graduated 100-ml flask (4.9.8) filled with absolute ethanol (3.5), add 100 µl of the calibration stock solution at 200 mg/l (3.11) using the 100-µl micro-syringe (4.9.1) and homogenize.

3.13 Calibration surrogate solution at 4 µg/l
In a graduated 50-ml flask (4.9.7) containing 50 % vol aqueous-alcoholic solution (3.7) add 1 ml of the intermediate calibration solution at 200 µg/l (3.11) using a 1-ml pipette (4.9.6). Add to volume 50 ml with pure ethanol (3.5) and homogenize.

3.14 Calibration solutions. It is possible to prepare various standard solutions with various concentrations by adding, using the 100-µl micro-syringe of (4.9.1), for example 50 µl of the surrogate calibration solution at 4 µg/l (3.12) to 50 ml of wine to enrich it with 4 ng/l of the substances to be determined.
The same reasoning can be used to prepare calibration solutions of various concentrations, either using aqueous-alcoholic solutions, or wine, or to enrich an extraction medium with a known quantity of pure products.

3.15 Commercially available Bentonite.

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4. APPARATUS

4.1 Gas phase chromatograph with Split-splitless injector coupled to an electron capture detector. (It is likewise possible to use a mass spectrometer)
4.2 Capillary tube of non-polar steady-state phénylmethylpolysiloxane type: (0.32 mm x 50 m, thickness of film 0.12 µm or the equivalent
4.3 Chromatographic conditions, as an example:

4.3.1 Injection in "split-splitless" mode (valve closing time 30 seconds)
4.3.2 Carrier gas flow rate: 30 ml/min including 1 ml in the column Hydrogen U ®[2] (It is likewise possible to use helium)
4.3.3 Auxiliary gas flow rate: 60 ml/min – Nitrogen with chromatographic purity (≥ 99,9990 %). It is also possible to use argon methane.
4.3.4 Furnace gradient temperature for information purposes:
- from 40 °C to 160 °C at a rate of 2 °C/min
- from 160 °C to 200 °C at a rate of 5 °C/min
- step at 220 °C for 10 min

4.3.5 Injector temperature: 250 °C
43.6 Detector temperature: 250 °C

4.4 Acquisition and integration: acquisition is by computer. The peaks of the various compounds identified by comparison with the reference are then integrated.
4.5 Magnetic agitator.
4.6 Vortex with adaptation for 30-ml flask (4.9.3)
4.7 Precision balance to within 0.1 mg
4.8 Manual or electric household grate
4.9 Laboratory equipment:

4.9.1 100-µl micro-syringe
4.9.2 10-µl micro-syringe
4.9.3 30-ml flask closing with a screwed plug and cover with one side Teflon-coated
4.9.4 10-ml stick pipette graduated 1/10 ml
4.9.5 5-ml stick pipette graduated 1/10 ml
4.9.6 1-ml precision pipette
4.9.7 Graduated 50-ml flask
4.9.8 Graduated 100-ml flask
4.9.9 Graduated 200-ml flask
4.9.10 100-ml separating funnel
4.9.11 Pasteur pipettes and suitable propipette pear
4.9.12 Household aluminum foil, roll-form.
4.9.13 Centrifuge

5. SAMPLE PREPARATION

5.1 The stopper is grated (4.8) or cut into pieces (dimension < 3 mm)
5.2 Wood is cut with a clipper to obtain pieces (dimension < 3 mm)
5.3 The bentonite (3.15) (30 g for example) is spread out over a strip of aluminum foil (4.9.12) of approximately 30 cm x 20 cm and is exposed to the atmosphere to be analyzed for at least 5 days.

6. OPERATING METHOD

6.1 Extraction process for solid samples:

6.1.1 Stopper: in a 30-ml flask (4.9.3), place approximately 1 g of grated stopper (5.1) but of a precisely known weight (4.7)

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6.1.2 Wood: in a 30-ml flask (4.9.3), place approximately 2 g of wood chips (5.2) but of a precisely known weight (4.7)

6.1.3 Control Bentonite: in a 30-ml flask (4.9.3), place approximately 5 g of bentonite (3.15) but of a precisely known weight (4.7)

6.1.4 Sample bentonite: in a 30-ml flask (4.9.3), place approximately 5 g of bentonite (5.3) of a precisely known weight (4.7)

6.1.5 Add 10 ml (4.9.4) of ether/hexane mixture (3.3)

6.1.7 Add with the micro-syringe (4.9.1) 50 µl of the internal standard solution (3.8.2)

6.1.8 Agitate with the vortex (4.6) for 3 min

6.1.9 Recover the ether/hexane liquid phase in a 30-ml flask (4.9.3)

6.1.10 Repeat the extraction operation on the sample with 2 times 5 ml of ether/hexane mixture (3.3)

6.1.11 Final extract: mix the 3 phases of ether/hexane.

6.2 Extraction of the wine and calibration solution

6.2.1 Sample 50 ml of wine or calibration solution (using the graduated flask (4.9.7)

6.2.2 Place them in the 100-ml graduated flask (4.9.8)

6.2.3 Add with the microsyringe (4.9.1) 50 µl of internal standard (3.8.3)

6.2.4 Add 4 ml (4.9.5) of hexane (3.1)

6.2.5 Carry out the extraction using the magnetic stirrer (4.5) for 5 min.

6.2.6 Elutriate into the funnel (4.9.10)

6.2.7 Recover the organic phase with the emulsion in a 30-ml flask (4.9.3) and aqueous phase in the 100-ml graduated flask (4.9.8)

6.2.8 Repeat the extraction of the wine or calibration solution using 2 ml of hexane (3.1)

6.2.9 Carry out the extraction using the magnetic stirrer (4.5) for 5 min.

6.2.10 Elutriate into the funnel (4.9.10)

6.2.11 Recover the organic phase with the emulsion in the same 30-ml flask mentioned in 6.2.7 (containing the organic phase obtained upon the first extraction)

6.2.12 Break the emulsion of the organic phase by centrifugation (4.9.13) by eliminating the lower aqueous phase using a Pasteur pipette (4.9.11) fitted with a propipette pear.

6.2.13 Final wine extract and calibration solutions: the residual organic extract

6.3 Analyze:

6.3.1 Add final extract (6.1.11 or 6.2.13) 100 µl (4.9.1) of the pyridine acetic anydride reagent mixture (3.10) for the derivatisation.

6.3.2 Mix using a magnetic stirrer (4.5) for 10 min.

6.3.3 Inject 2 µl of derivatised final extract (6.3.2) into the chromatograph

7. CALCULATION:

$$\text{Concentration of product} = \frac{\text{Product peak area}}{\text{Peak area of internal standard}} * \text{Response factor}$$

Response factor = concentration of calibration solution (3.13) * (Peak area of the internal standard / *(Peak area of the pure product in the calibration solution).

Check the calibration by ensuring the response factors +/- 10 %.

8. RESULTS

The results are expressed in ng/l for the wine and ng/g for the cork stoppers, bentonites and wood.

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9. CHARACERISTICS OF THE METHOD

9.1 Coverage rate

The coverage rate calculated in relation to the quantities added in terms of wood chips, polychloroanisols and polychlorophenols of 115 ng/g is:

- 2,4,6-trichloroanisol: 96 %
- 2,4,6-trichlorophenol: 96 %
- 2,3,4,6-tetrachloroanisol: 96 %
- 2,3,4,6-tetrachlorophenol: 97 %
- pentachloroanisol: 96 %
- pentachlorophenol: 97 %

9.2 Measurement repeatability

Calculated for each product, the uncertainties are as follows:

In a stopper ng/g	**Mean**	**Standard deviation**	**Repeatability**
2,4,6-trichloroanisol	1.2	0.1	0.28
2,4,6-trichlorophenol	26	3.3	9.24
2,3,4,6-tetrachloroanisol	1.77	0.44	1.23
2,3,4,6-tetrachlorophenol	2.59	0.33	0.92
pentachloroanisol	23.3	2.9	8.12
pentachlorophenol	7.39	1.91	5.35

In wood with 23 ng/g	**Standard deviation**	**Repeatability**
2,4,6-trichloroanisol	1.9	5.3
2,4,6-trichlorophenol	1.9	5.3
2,3,4,6-tetrachloroanisol	2.6	7.4
2,3,4,6-tetrachlorophenol	3.3	9.3
pentachloroanisol	2.7	7.5
pentachlorophenol	3.6	10.1

In wine with 10 ng/l	**Standard deviation**	**Repeatability**
2,4,6-trichloroanisol	0,4	1,1
2,4,6-trichlorophenol	2,1	5,9
2,3,4,6-tetrachloroanisol	0,6	1,7
2,3,4,6-tetrachlorophenol	4	11,2
pentachloroanisol	1,2	3,4
pentachlorophenol	6,5	18,2

In bentonite with15ng/g	**Standard deviation**	**Repeatability**
2,4,6-trichloroanisol	0,9	2,5
2,4,6-trichlorophenol	4	11,2
2,3,4,6-tetrachloroanisol	1,2	3,4
2,3,4,6-tetrachlorophenol	5,2	14,6
pentachloroanisol	4,3	12,0
pentachlorophenol	12,1	33,9

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9.3 Detection limits (DL) and quantification limits (QL) calculated according to the OIV method:

9.3.1 Wood

	DL in ng/g	QL in ng/g
2,4,6-trichloroanisol	0.72	2.4
2,4,6-trichlorophenol	0.62	2.0
2,3,4,6-tetrachloroanisol	0.59	2.0
2,3,4,6-tetrachlorophenol	1.12	3.74
pentachloroanisol	0.41	1.4
pentachlorophenol	0.91	3.1

9.3.2 Bentonite

	DL in ng/g	QL in ng/g
2,4,6-trichloroanisol	0.5	1
2,4,6-trichlorophenol	1	3
2,3,4,6-tetrachloroanisol	0.5	1
2,3,4,6-tetrachlorophenol	1	3
pentachloroanisol	0.5	1
pentachlorophenol	Not det.	Not det.

9.3.3 Stopper

	DL in ng/g	QL in ng/g
2,4,6-trichloroanisol	0.5	1.5
2,4,6-trichlorophenol	1	2
2,3,4,6-tetrachloroanisol	0.5	1.5
2,3,4,6-tetrachlorophenol	1	2
pentachloroanisol	0.5	1.5
pentachlorophenol	1	2

9.3.4 Wine

	DL in ng/l	QL in ng/l
2,4,6-trichloroanisol	0.3	1
2,4,6-trichlorophenol	1	3
2,3,4,6-tetrachloroanisol	0.3	1
2,3,4,6-tetrachlorophenol	0.3	1
pentachloroanisol	0.5	3
pentachlorophenol	1	3

®[2] Air Liquide

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RESOLUTION OIV/OENO 379/2009

UPDATE OF THE OIV INTERNATIONAL COMPENDIUM OF METHODS OF ANALYSIS OF SPIRIT DRINKS OF VITIVINICULTURAL ORIGIN – PART 1

THE GENERAL ASSEMBLY

CONSIDERING article 2 paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

IN VIEW OF the actions of the 2009-2012 OIV Strategic plan, particularly those focused on reorganising publications related to the vitivinicultural methods of analysis

CONSIDERING the works of the Methods of Analysis sub-commission

IN VIEW OF the 1994 publication of the Compendium of International Methods of Analysis of spirituous beverages, alcohol and the aromatic fraction of beverages

GIVEN the evolution of methods and availability of inter-laboratory validation parameters since 1994, which are already recognised by international authorities bodies, the following methods shall be described and retained as Type II methods of analysis;

CONSIDERING that certain methods of analysis are no longer used and should be removed from the Compendium of International Methods of Analysis of spirituous beverages, alcohol and the aromatic fraction of beverages,

For the purpose of the present resolution, the following terms are defined as such:

(a) repeatability limit: shall be the value less than or equal to which the absolute difference between two test results obtained under the repeatability conditions (same operator, same apparatus, same laboratory and a short interval of time) may be expected to be with a probability of 95 % {ISO 3534-1};

(b) reproducibility limit: shall be the value less than or equal to which the absolute difference between two test results obtained under the reproducibility conditions (different operators, different apparatus and different laboratories), may be expected to be with a probability of 95 % {ISO 3534-1};

(c) accuracy: shall be the closeness of agreement between a test result and the accepted reference value {ISO 3534-1}.

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DECIDES to revise the 1994 publication of the « Compendium of International Methods of Analysis of spirituous beverages, alcohol and the aromatic fraction of beverages » while retaining and describing the following methods as Type II methods of analysis. The title of the compendium shall be renamed as « Compendium of International Methods of Analysis of Spirit Drinks of Vitivinicultural Origin »

DECIDES that the methods listed in the Compendium of International Methods of Analysis of spirituous beverages, alcohol and aromatic fractions of beverages shall, if necessary, be modified accordingly.

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REFERENCE METHOD FOR THE DETERMINATION OF ALCOHOLIC STRENGTH BY VOLUME OF SPIRIT DRINKS OF VITI-VINCULTURAL ORIGIN: General Remarks

Introduction

The reference method includes two Annexes:
Annex I - Preparation of distillate
Annex II - Measurement of density of distillate by three methods A, B, and C

1. Scope

The method is suitable for the determination of the real alcoholic strength by volume of spirit drinks of viti-vinicultural origin.

2. Normative References

ISO 3696:1987 Water for analytical laboratory use - Specifications and test methods.

3. Terms and Definitions

3.1 Reference temperature:

The reference temperature for the determination of alcoholic strength by volume, density and specific gravity of spirit drinks is 20 °C.

Note 1: The term 'at t °C' is reserved for all determinations (of density or alcoholic strength by volume) expressed at a temperature other than the reference temperature of 20 °C.

3.2 Density:

The density is the mass per unit volume in vacuo of spirit drinks at 20 °C. It is expressed in kilograms per cubic metre and its symbol is $\rho_{20\,°C}$ or ρ_{20}.

3.3 Specific gravity:

The specific gravity is the ratio, expressed as a decimal number, of the density of spirit drinks at 20 °C to the density of water at the same temperature. It is denoted by the symbol $d_{20\,°C/20\,°C}$ or $d_{20/20}$, or simply d when there is no possibility of confusion. The characteristic that was measured must be specified on the assay certificate using the above-defined symbols only.

Note 2: It is possible to obtain the specific gravity from the density ρ_{20} at 20 °C:
$\rho_{20} = 998.203 \times d_{20/20}$ or $d_{20/20} = \rho_{20} / 998.203$ where 998.203 is the density of water at 20 °C.

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3.4 Real alcoholic strength by volume:
The real alcoholic strength by volume of spirit drinks is equal to the number of litres of ethyl alcohol contained in 100 l of a water-alcohol mixture having the same density as the alcohol or spirit after distillation. The reference values for alcoholic strength by volume (% vol.) at 20 °C versus density at 20 °C for different water-alcohol mixtures that are to be used are those given in the international table adopted by the International Legal Metrology Organisation in its Recommendation no. 22.

Note 3: For liqueurs and crèmes for which it is very difficult to measure volume accurately the sample must be weighed and the alcoholic strength is calculated first by mass.

Conversion formula:

$$\text{alcoholic strength by volume (\% vol)} = \frac{\text{ASM (\% mass)} \times \rho_{20}\ \text{(Sample)}}{\rho_{20}\ \text{(alcohol)}}$$

where ASM = alcoholic strength by mass,
ρ_{20} (alcohol) = 789.24 kg/m^3

4. Principle

Following distillation the alcoholic strength by volume of the distillate is determined by pycnometry, electronic densimetry, or densimetry using a hydrostatic balance.

5. Bibliography

Commission Regulation (EC) N° 2870/2000 of 19 December 2000 laying down Community reference methods for the analysis of spirits drinks, *OJEC of 29 December 2000, L333/20*

P. Brereton, S. Hasnip, A. Bertrand, R. Wittkowski, C. Guillou, Analytical methods for the determination of spirit drinks, Trends in Analytical Chemistry, Vol. 22, No. 1, 19-25, 2003

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Annex I: REFERENCE METHOD FOR THE DETERMINATION OFALCOHOLIC STRENGTH BY VOLUME OF SPIRIT DRINKS OF VITI-VINCULTURAL ORIGIN : preparation of distillate

1. Scope

The method is suitable for the preparation of distillates to be used to determine the real alcoholic strength by volume of spirit drinks.

2. Principle

The spirits are distilled to separate the ethyl alcohol and other volatile compounds from the extractive matter (substances which do not distil).

3. Reagents and Materials

3.1 Anti-bumping granules

3.2 Concentrated antifoam emulsion (for crème liqueurs)

4. Apparatus and Equipment

Usual laboratory apparatus and in particular the following.

4.1 Water bath capable of being maintained at 10 °C to 15 °C.
Water bath capable of being maintained at 20 ºC (± 0.2 ºC)

4.2 Class A volumetric flasks, 100 ml and 200 ml, that have been certified to ±0.1 % and ±0.15 % respectively.

4.3 Distillation apparatus:

4.3.1 General requirements

The distillation apparatus must meet the following specifications:

- The number of joints must be no more than the strict minimum needed to ensure the system is leak-tight.
- Inclusion of a device designed to prevent priming (entrainment of the boiling liquid by the vapour) and to regularise the distillation rate of alcohol-rich vapours.
- Rapid and complete condensation of the alcohol vapours.
- Collection of the first distillation fractions in an aqueous medium.

The heat source must be used with a suitable heat-diffuser to prevent any pyrogenic reaction involving the extractive matter.

4.3.2 As an example, a suitable distillation apparatus would include the following parts:

- Round bottomed flask, 1 litre, with a standardised ground-glass joint.
- Rectifying column at least 20 cm high (a Vigreux column, for example).
- Elbow connector with an approximately 10 cm long straight-rimmed condenser (a West-type condenser) fitted vertically.

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- Cooling coil, 40 cm long.
- Drawn out tube, taking the distillate to the bottom of a graduated collecting flask containing a small amount of water.

Note: The apparatus described above is intended for a sample of least 200 ml. However, a smaller sample size (100 ml) can be distilled by using a smaller distillation flask, provided a splash-head or some other device to prevent entrainment is used.

5. Storage of test samples

Samples are stored at room temperature prior to analysis.

6. Procedure

6.1 Distillation apparatus verification

The apparatus used must be capable of the following:

The distillation of 200 ml of a water-alcohol solution with known concentration close to 50 % vol. must not cause a loss of alcohol of more than 0.1 % vol.

6.2. Spirit drinks with alcoholic strength below 50 % vol.

Measure out 200 ml of the spirit into a volumetric flask.

Record the temperature of this liquid, or maintain at standard temperature (20 ºC).

Pour the sample into the round bottomed flask of the distillation apparatus and rinse the volumetric flask with three aliquots each of approximately 20 ml of distilled water. Add each rinse water aliquot to the contents of the distillation flask.

Note: This 60-ml dilution is sufficient for spirits containing less than 250 g of dry extract per litre. Otherwise, to prevent pyrolysis, the volume of rinse water must be at least 70 ml if the dry extract concentration is 300 g/l, 85 ml for 400 g/l dry extract, and 100 ml for 500 g/l dry extract (some fruit liqueurs or crèmes). Adjust these volumes proportionally for different sample volumes.

Add a few anti-bumping granules (3.1) (and antifoam for crème liqueurs).

Pour 20 ml of distilled water into the original 200 ml volumetric flask that will be used to hold the distillate. This flask must then be placed in a cold water bath (4.1) (10 - 15 °C for aniseed-flavoured spirit drinks).

Distil, avoiding entrainment and charring, occasionally agitating the contents of the flask, until the level of distillate is a few millimetres below the calibration mark of the volumetric flask.

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When the temperature of this distillate has been brought down to within 0.5 ºC of the liquid's initial temperature, make up to the mark with distilled water and mix thoroughly.
This distillate is used for the determination of alcoholic strength by volume (Annex II)

6.3 Spirit drinks with alcoholic strength above 50 % vol.
Measure out 100 ml of the spirit drink into a 100 ml volumetric flask and pour into the round bottomed flask of the distillation apparatus.
Rinse the volumetric flask several times with distilled water and add the washings to the contents of the round-bottomed distillation flask. Use enough water to bring the flask's contents up to approximately 230 ml.
Pour 20 ml of distilled water into a 200 ml volumetric flask that will be used to hold the distillate. This flask must then be placed in a cold water bath (4.1) (10 °C to 15 °C for aniseed-flavoured spirits).
Distil, agitating the contents occasionally, until the level of distillate is a few millimetres below the calibration mark of the 200 ml volumetric flask.
When the temperature of this distillate has been brought down to within 0.5 °C of the liquid's initial temperature, make up to the mark with distilled water and mix thoroughly.
This distillate is used for the determination of alcoholic strength by volume (Annex II)
Note: The alcoholic strength by volume of the spirit drink is twice the alcoholic strength of the distillate.

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ANNEX IIA: DETERMINATION OF REAL ALCOHOLIC STRENGTH BY VOLUME OF SPIRIT DRINKS OF VITI-VINICULTURAL ORIGIN - MEASUREMENT BY PYCNOMETRY

Type II method
Year : 2009

A.1 Principle

The alcoholic strength by volume is obtained from the density of the distillate measured by pycnometry.

A.2. Reagents and Materials

During the analysis, unless otherwise is stated, use only reagents of recognised analytical grade and water of at least grade 3 as defined in ISO 3696:1987.

A.2.1 Sodium chloride solution (2 % w/v)

To prepare 1 litre, weigh out 20 g sodium chloride and dissolve to 1 litre using water.

A.3 Apparatus and Equipment

Usual laboratory apparatus and in particular the following.

A.3.1 Analytical balance capable of reading 0.1 mg.

A.3.2 Thermometer, with ground glass joint, calibrated in tenths of a degree from 10 to 30 °C. This thermometer must be certified or checked against a certified thermometer.

A.3.3 Pyrex glass pycnometer of approximately 100 ml capacity fitted with a removable ground-glass thermometer (A.3.2). The pycnometer has a side tube 25 mm in length and 1 mm (maximum) in internal diameter ending in a conical ground joint. Other pycnometers as described in ISO 3507 e.g. 50 ml may be used if appropriate.

A.3.4 A tare bottle of the same external volume (to within 1 ml) as the pycnometer and with a mass equal to the mass of the pycnometer filled with a liquid of density 1.01 (sodium chloride solution A.2.1).

A.3.5 Thermally insulated jacket that fits the body of the pycnometer exactly.

Note 1: The method for determining the densities in vacuo of spirits calls for the use of a twin-pan balance, a pycnometer and a tare bottle of the same outside external volume to cancel out the effect of air buoyancy at any given moment. This simple technique may be applied using a single-pan balance provided that the tare bottle is weighed again to monitor changes in air buoyancy over time.

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A.4. Procedure

Preliminary remarks:

- The following procedure is described for the use of 100-ml pycnometer for determination of the alcoholic strength; this gives the best accuracy. However, it is also possible to use a smaller pycnometer, for example 50 ml.

A.4.1 Calibration of pycnometer

The pycnometer is calibrated by determining the following parameters:
tare of the empty pycnometer,
volume of the pycnometer at 20 °C,
mass of the water-filled pycnometer at 20 °C.

A.4.1.1 Calibration using a single-pan balance:

Determine:
the mass of the clean, dry pycnometer (P),
the mass of the water-filled pycnometer at t °C (P1)
the mass of the tare bottle (T0).

A.4.1.1.1 Weigh the clean, dry pycnometer (P).

A.4.1.1.2 Fill the pycnometer carefully with distilled water at ambient temperature and fit the thermometer.

Carefully wipe the pycnometer dry and place it in the thermally-insulated jacket. Agitate by inverting the container until the thermometer's temperature reading is constant

Set the pycnometer flush with the upper rim of the side tube. Read the temperature t °C carefully and if necessary correct for any inaccuracies in the temperature scale.

Weigh the water-filled pycnometer (P1).

A.4.1.1.3 Weigh the tare bottle (T0).

A.4.1.1.4 Calculation

- Tare of the empty pycnometer = P - m
  where m is the mass of air in the pycnometer.
  m = 0.0012 x (P1 - P)

Note 2: 0.0012 is the density of dry air at 20 °C at a pressure of 760 mm Hg

- Volume of the pycnometer at 20 °C:
  $V_{20\,°C} = [P1 - (P - m)] \times F_t$
  where F_t is the factor for temperature t °C taken from Table I below.
  $V_{20\,°C}$ must be known to the nearest 0.001 ml.
- Mass of water in the pycnometer at 20 °C :
  $M_{20\,°C} = V_{20\,°C} \times 0.998203$
  where 0.998203 is the density of water at 20 °C.

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Note 3: If necessary, the value 0.99715 of the density in air can be used and the alcoholic strength calculated with reference to the corresponding density in HM Customs and Excise tables in air.

A.4.1.2 Calibration method using a twin-pan balance:

A.4.1.2.1 Place the tare bottle on the left-hand pan and the clean, dry pycnometer with its collecting stopper on the right-hand pan. Balance them by placing weights on the pycnometer side: p grams. (p)

A.4.1.2.2 Fill the pycnometer carefully with distilled water at ambient temperature and fit the thermometer; carefully wipe the pycnometer dry and place it in the thermally insulated jacket; agitate by inverting the container until the thermometer's temperature reading is constant.

Accurately adjust the level to the upper rim of the side tube. Clean the side tube, fit the collecting stopper; read the temperature t °C carefully and if necessary correct for any inaccuracies in the temperature scale.

Weigh the water-filled pycnometer, with p' the weight in grams making up the equilibrium. (p')

A.4.1.2.3 Calculation

- Tare of the empty pycnometer = p + m
  where m is the mass of air in the pycnometer.
  m = 0.0012 x (p - p')
- Volume of the pycnometer at 20 °C:
  $V_{20\,°C} = (p + m - p') \times F_t$
  where F_t is the factor for temperature t °C taken from Table I below.
  $V_{20\,°C}$ must be known to the nearest 0.001 ml.
- Mass of water in the pycnometer at 20 °C:
  $M_{20\,°C} = V_{20\,°C} \times 0.998203$
  where 0.998203 is the density of water at 20 °C.

A.4.2 Determination of alcoholic strength of test sample

A.4.2.1 Using a single-pan balance.

A.4.2.1.1 Weigh the tare bottle, weight T1

A.4.2.1.2 Weigh the pycnometer with the prepared distillate (see Annex I), P2 is its weight at t °C.

A.4.2.1.3 Calculation

- dT = T1 - T0
- Mass of empty pycnometer at moment of measuring
  = P - m + dT
- Mass of the liquid in the pycnometer at t °C
  = P2 - (P - m + dT)
- Density at t °C in g/ml
- $\rho_{t\,°C} = [P_2 - (P - m + dT)]/V_{20\,°C}$
  Express the density at t °C in kilograms per m^3 by multiplying $\rho_{t\,°C}$ by 1000, the value being known as ρ_t.

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- Correct ρ_t to 20 using the table of densities ρT for water-alcohol mixtures in the Manual of Analysis Methods for Wines of the OIV.
  In the table find the horizontal line corresponding to temperature T in whole degrees immediately below t °C, the smallest density above ρ_t. Use the table difference found below that density to calculate the density ρ_t of the spirit at that temperature T in whole degrees.
- Using the whole temperature line, calculate the difference between density ρ' in the table immediately above ρ_t and the calculated density ρ_t. Divide that difference by the table difference found to the right of density ρ'. The quotient provides the decimal portion of the alcoholic strength while the integer of the alcoholic strength is found at the top of the column in which density ρ' is found (Dt, the alcoholic strength).

Note 4: Alternatively keep the pycnometer in a water bath maintained at 20 °C (± 0.2 °C) when making up to the mark.

A.4.2.1.4 Result

Using the density ρ_{20} calculate the real alcoholic strength using the alcoholic strength tables identified below:

The table giving the value of the alcoholic strength by volume (% vol.) at 20 °C as a function of the density at 20 °C of water-alcohol mixtures is the international table adopted by the International Legal Metrology Organisation in its Recommendation no. 22.

A.4.2.2 Method using a single-pan balance

A.4.2.2.1 Weigh the pycnometer with the distillate prepared (see part I), p'' is mass at t °C.

A.4.2.2.2 Calculation

- Mass of the liquid in the pycnometer at t °C
  = p + m - p''
- Density at t °C in g/ml
  $\rho_{t\,°C} = (p + m - p'')/V_{20\,°C}$
- Express the density at t °C in kilograms per m^3 and carry out the temperature correction in order to calculate the alcoholic strength at 20 °C, as indicated above for use of the single-pan balance.

A.5. Method performance characteristics (Precision)

A.5.1 Statistical results of the interlaboratory test

The following data were obtained from an international method performance study carried out on a variety of spirit drinks to internationally agreed procedures.

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Year of interlaboratory test	1997
Number of laboratories	20
Number of samples	6

Samples	A	B	C
Number of laboratories retained after eliminating outliers	19	20	17
Number of outliers (Laboratories)	1	-	2
Number of accepted results	38	40	34
Mean value ($\bar{x}$) % vol.	23.77	40.04	40.29
	26.51*		
Repeatability standard deviation (s_r) % vol.	0.106	0.176	0.072
Repeatability relative standard deviation (RSD_r) (%)	0.42	0.44	0.18
Repeatability limit (r) % vol.	0.30	0.49	0.20
Reproducibility standard deviation (s_R) % vol.	0.131	0.236	0.154
Reproducibility relative standard deviation (RSD_R) (%)	0.52	0.59	0.38
Reproducibility limit (R) % vol.	0.37	0.66	0.43

Sample types

A Fruit liqueur; split level*

B Brandy; blind duplicates

C Whisky; blind duplicates

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Samples	D	E	F
Number of laboratories retained after eliminating outliers	19	19	17
Number of outliers (Laboratories)	1	1	3
Number of accepted results	38	38	34
Mean value ($\overline{\times}$) % vol.	39.20	42.24	57.03
	42.93*	45.73*	63.03*
Repeatability standard deviation (s_r) % vol.	0.103	0.171	0.190
Repeatability relative standard deviation (RSD_r) (%)	0.25	0.39	0.32
Repeatability limit (r) % vol.	0.29	0.48	0.53
Reproducibility standard deviation (s_R) % vol.	0.233	0.238	0.322
Reproducibility relative standard deviation (RSD_R) (%)	0.57	0.54	0.53
Reproducibility limit (R) % vol.	0.65	0.67	0.90

Sample types

D grappa; split level*

E aquavit; split level*

F rum; split level*

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TABLE I

F factors by which the mass of water contained in the Pyrex pycnometer at t °C has to be multiplied in order to calculate the pycnometer volume at 20 °C

t °C	F	t °C	F	t °C	F	t °C	F	t °C	F	t °C	F	t °C	F
10,0	1,000398	13,0	1,000691	16,0	1,001097	19,0	1,001608	22,0	1,002215	25,0	1,002916	28,0	1,003704
,1	1,000406	,1	1,000703	,1	1,001113	,1	1,001627	,1	1,002238	,1	1,002941	,1	1,003731
,2	1,000414	,2	1,000714	,2	1,001128	,2	1,001646	,2	1,002260	,2	1,002966	,2	1,003759
,3	1,000422	,3	1,000726	,3	1,001144	,3	1,001665	,3	1,002282	,3	1,002990	,3	1,003787
,4	1,000430	,4	1,000738	,4	1,001159	,4	1,001684	,4	1,002304	,4	1,003015	,4	1,003815
10,5	1,000439	13,5	1,000752	16,5	1,001175	19,5	1,001703	22,5	1,002326	25,5	1,003041	28,5	1,003843
,6	1,000447	,6	1,000764	,6	1,001191	,6	1,001722	,6	1,002349	,6	1,003066	,6	1,003871
,7	1,000456	,7	1,000777	,7	1,001207	,7	1,001741	,7	1,002372	,7	1,003092	,7	1,003899
,8	1,000465	,8	1,000789	,8	1,001223	,8	1,001761	,8	1,002394	,8	1,003117	,8	1,003928
,9	1,000474	,9	1,000803	,9	1,001239	,9	1,001780	,9	1,002417	,9	1,003143	,9	1,003956
11,0	1,000483	14,0	1,000816	17,0	1,001257	20,0	1,001800	23,0	1,002439	26,0	1,003168	29,0	1,003984
,1	1,000492	,1	1,000829	,1	1,001273	,1	1,001819	,1	1,002462	,1	1,003194	,1	1,004013
,2	1,000501	,2	1,000842	,2	1,001290	,2	1,001839	,2	1,002485	,2	1,003222	,2	1,004042
,3	1,000511	,3	1,000855	,3	1,001306	,3	1,001859	,3	1,002508	,3	1,003247	,3	1,004071
,4	1,000520	,4	1,000868	,4	1,001323	,4	1,001880	,4	1,002531	,4	1,003273	,4	1,004099
11,5	1,000530	14,5	1,000882	17,5	1,001340	20,5	1,001900	23,5	1,002555	26,5	1,003299	29,5	1,004128
,6	1,000540	,6	1,000895	,6	1,001357	,6	1,001920	,6	1,002578	,6	1,003326	,6	1,004158
,7	1,000550	,7	1,000909	,7	1,001374	,7	1,001941	,7	1,002602	,7	1,003352	,7	1,004187
,8	1,000560	,8	1,000923	,8	1,001391	,8	1,001961	,8	1,002625	,8	1,003379	,8	1,004216
,9	1,000570	,9	1,000937	,9	1,001409	,9	1,001982	,9	1,002649	,9	1,003405	,9	1,004245
12,0	1,000580	15,0	1,000951	18,0	1,001427	21,0	1,002002	24,0	1,002672	27,0	1,003432	30,0	1,004275
,1	1,000591	,1	1,000965	,1	1,001445	,1	1,002023	,1	1,002696	,1	1,003458		
,2	1,000601	,2	1,000979	,2	1,001462	,2	1,002044	,2	1,002720	,2	1,003485		
,3	1,000612	,3	1,000993	,3	1,001480	,3	1,002065	,3	1,002745	,3	1,003513		
,4	1,000623	,4	1,001008	,4	1,001498	,4	1,002086	,4	1,002769	,4	1,003540		
12,5	1,000634	15,5	1,001022	18,5	1,001516	21,5	1,002107	24,5	1,002793	27,5	1,003567		
,6	1,000645	,6	1,001037	,6	1,001534	,6	1,002129	,6	1,002817	,6	1,003594		
,7	1,000656	,7	1,001052	,7	1,001552	,7	1,002151	,7	1,002842	,7	1,003621		
,8	1,000668	,8	1,001067	,8	1,001570	,8	1,002172	,8	1,002866	,8	1,003649		
,9	1,000679	,9	1,001082	,9	1,001589	,9	1,002194	,9	1,002891	,9	1,003676		

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Annexe IIB: DETERMINATION OF REAL ALCOHOLIC STRENGTH BY VOLUME OF SPIRIT DRINKS - MEASUREMENT BY ELECTRONIC DENSIMETRY (BASED ON THE RESONANT FREQUENCY OSCILLATION OF A SAMPLE IN AN OSCILLATING CELL)

Type II method
Year : 2009

B.1. Principle

The liquid's density is determined by electronic measurement of the oscillations of a vibrating U-tube. To perform this measurement, the sample is added to an oscillating system, whose specific oscillation frequency is thus modified by the added mass.

B.2. Reagents and Materials

During the analysis, unless otherwise is stated, use only reagents of recognised analytical grade and water of at least grade 3 as defined in ISO 3696:1987.

B.2.1 Acetone (CAS 666-52-4) or absolute alcohol

B.2.2 Dry air.

B.3. Apparatus and Equipment

Usual laboratory apparatus and in particular the following.

B.3.1 Digital display densimeter

Electronic densimeter for performing such measurements must be capable of expressing density in g/ml to 5 decimal places.

Note 1: The densimeter should be placed on a perfectly stable stand that is insulated from all vibrations.

B.3.2 Temperature regulation

The densimeter's performance is valid only if the measuring cell is connected to a built-in temperature regulator that can achieve the same temperature stability of ± 0.02 ºC or better.

Note 2: The precise setting and monitoring of the temperature in the measuring cell are very important, for an error of 0.1 °C can lead to a variation in density of the order of 0.0001 g/mL.

B.3.3 Sample injection syringes, auto sampler, or other equivalent system.

B.4. Procedure

B.4.1 Calibration of the densimeter

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The apparatus must be calibrated according to the instrument manufacturer's instructions when it is first put into service. It must be recalibrated regularly and checked against a certified reference standard or an internal laboratory reference solution based on a certified reference standard.

B.4.2 Determination of sample density

B.4.2.1 If required prior to measurement clean and dry the cell with acetone or absolute alcohol and dry air. Rinse the cell with the sample.

B.4.2.2 Inject the sample into the cell (using a syringe, autosampler, or other equivalent system) so that the cell is completely filled. During the filling operation make sure that all air bubbles are completely eliminated. The sample must be homogeneous and must not contain any solid particles. Any suspended matter should be removed by filtration prior to analysis.

B.4.2.3 Once the reading has stabilised, record the density ρ_{20} or the alcoholic strength displayed by the densimeter.

B.4.3 Result

When the density ρ_{20} is used, calculate the real alcoholic strength using the alcoholic strength tables identified below:

The table giving the value of the alcoholic strength by volume (% vol.) at 20 °C as a function of the density at 20 °C of water-alcohol mixtures is the international table adopted by the International Legal Metrology Organisation in its Recommendation No. 22 (Table IVa).

B.5. Method performance characteristics (Precision)

B.5.1 Statistical results of the interlaboratory test

The following data were obtained from an international method performance study carried out on a variety of spirit drinks to internationally agreed procedures.

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Year of interlaboratory test	1997
Number of laboratories	16
Number of samples	6

Samples	A	B	C
Number of laboratories retained after eliminating outliers	11	13	15
Number of outliers (Laboratories)	2	3	1
Number of accepted results	22	26	30
Mean value ($\bar{\times}$) % vol.	23.81	40.12	40.35
	26.52*		
Repeatability standard deviation (s_r) % vol.	0.044	0.046	0.027
Repeatability relative standard deviation (RSD_r) (%)	0.17	0.12	0.07
Repeatability limit (r) % vol.	0.12	0.13	0.08
Reproducibility standard deviation (s_R) % vol.	0.054	0.069	0.083
Reproducibility relative standard deviation (RSD_R) (%)	0.21	0.17	0.21
Reproducibility limit (R) % vol.	0.15	0.19	0.23

Sample types

A Fruit liqueur; split level*

B Brandy; blind duplicates

C Whisky; blind duplicates

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Samples	D	E	F
Number of laboratories retained after eliminating outliers	16	14	13
Number of outliers (Laboratories)	-	1	2
Number of accepted results	32	28	26
Mean value ($\bar{\times}$) % vol.	39.27	42.39	56.99
	43.10*	45.91*	63.31*
Repeatability standard deviation (s_r) % vol.	0.079	0.172	0.144
Repeatability relative standard deviation (RSD_r) (%)	0.19	0.39	0.24
Repeatability limit (r) % vol.	0.22	0.48	0.40
Reproducibility standard deviation (s_R) % vol.	0.141	0.197	0.205
Reproducibility relative standard deviation (RSD_R) (%)	0.34	0.45	0.34
Reproducibility limit (R) % vol.	0.40	0.55	0.58

Sample types

D Grappa; split level*

E Aquavit; split level*

F Rum; split level*

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Annexe IIC: DETERMINATION OF REAL ALCOHOLIC STRENGTH BY VOLUME OF SPIRIT DRINKS - MEASUREMENT BY DENSIMETRY USING HYDROSTATIC BALANCE

Type II method
Year : 2009

C.1. Principle

The alcoholic strength of spirits can be measured by densimetry using a hydrostatic balance based on Archimedes' principle according to which a body immersed in a liquid receives a vertical upward thrust from the liquid equal to the weight of liquid displaced.

C.2. Reagents and Materials

During the analysis, unless otherwise is stated, use only reagents of recognised analytical grade and water of at least grade 3 as defined in ISO 3696:1987.

C.2.1 Float cleaning solution (sodium hydroxide, 30 % w/v)

To prepare 100 ml weigh 30 g sodium hydroxide and make up to volume using 96 % volume ethanol.

C.3. Apparatus and Equipment

Usual laboratory apparatus and in particular the following.

C.3.1 Single-pan hydrostatic balance with a sensitivity of 1 mg.

C.3.2 Float with a volume of at least 20 ml, specially adapted to the balance, suspended with a thread of diameter not exceeding 0.1 mm.

C.3.3 Measuring cylinder bearing a level mark. The float must be capable of being contained completely within the volume of the cylinder located below the mark; the surface of the liquid may only be penetrated by the supporting thread. The measuring cylinder must have an internal diameter at least 6 mm larger than that of the float.

C.3.4 Thermometer (or temperature-measuring probe) graduated in degrees and tenths of a degree from 10 to 40 °C, calibrated to 0.05 °C.

C.3.5 Weights, calibrated by a recognised certifying body.

Note 1: Use of a twin-pan balance is also possible; the principle is described in the Manual of Analysis Methods for Wines of the OIV.

C.4. Procedure

The float and measuring cylinder must be cleaned between each measurement with distilled water, dried with soft laboratory paper which does not shed fibres and rinsed with the solution whose density is to be determined. Measurements must be made as soon as the apparatus has reached stability so as to restrict alcohol loss by evaporation.

C.4.1 Calibration of the balance

Although balances usually have an internal calibration system, the hydrostatic balance must be capable of calibration with weights checked by an official certifying body.

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C.4.2 Calibration of the float

C.4.2.1 Fill the measuring cylinder to the mark with double-distilled water (or water of equivalent purity, e.g. microfiltered water with a conductivity of 18.2 MΩ/cm) at a temperature between 15 °C and 25 °C but preferably at 20 °C.

C.4.2.2 Immerse the float and the thermometer, stir, read off the density of the liquid from the apparatus and, if necessary, correct the reading so that it is equal to that of the water at measurement temperature.

C.4.3 Control using a water-alcohol solution

C.4.3.1 Fill the measuring cylinder to the mark with a water-alcohol mixture of known strength at a temperature between 15 °C and 25 °C but preferably at 20 °C.

C.4.2.1 Immerse the float and the thermometer, stir, read off the density of the liquid (or the alcoholic strength if this is possible) from the apparatus. The alcoholic strength thus established should be equal to the previously determined alcoholic strength.

Note 2: This solution of known alcoholic strength can also be used to calibrate the float instead of double-distilled water.

C.4.4 Measurement of the density of a distillate (or of its alcoholic strength if the apparatus allows)

C.4.4.1 Pour the test sample into the measuring cylinder up to the graduation mark.

C.4.2.1 Immerse the float and the thermometer, stir, read off the density of the liquid (or the alcoholic strength if this is possible) from the apparatus. Note the temperature if the density is measured at t °C (ρ_t).

C.4.4.3 Correct ρ_t to 20 using the table of densities ρT for water-alcohol mixtures in the Manual of Analysis Methods for Wines of the OIV.

C.4.5 Cleaning of float and measuring cylinder

C.4.5.1 Immerse the float in the float cleaning solution in the measuring cylinder.

C.4.2.1 Allow to soak for one hour spinning the float periodically.

C.4.5.3 Rinse with copious amounts of tap water followed by distilled water.

C.4.5.4 Dry with soft laboratory paper which does not shed fibres.

Carry out this procedure when the float is first used and then regularly as required.

C.4.6 Result

Using the density ρ_{20} calculate the real alcoholic strength using the alcoholic strength tables identified below.

The table giving the value of the alcoholic strength by volume (% vol.) at 20 °C as a function of the density at 20 °C of water-alcohol mixtures is the international table adopted by the International Legal Metrology Organisation in its Recommendation no. 22.

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C.5. Method performance characteristics (Precision)

C.5.1 Statistical results of the interlaboratory test

The following data were obtained from an international method performance study carried out on a variety of spirit drinks to internationally agreed procedures.

Year of interlaboratory test	1997
Number of laboratories	12
Number of samples	6

Samples	A	B	C
Number of laboratories retained after eliminating outliers	12	10	11
Number of outliers (Laboratories)	-	2	1
Number of accepted results	24	20	22
Mean value ($\bar{x}$) % vol.	23.80 26.51*	40.09	40.29
Repeatability standard deviation (s_r) % vol.	0.048	0.065	0.042
Repeatability relative standard deviation (RSD_r) (%)	0.19	0.16	0.10
Repeatability limit (r) % vol.	0.13	0.18	0.12
Reproducibility standard deviation (s_R) % vol.	0.060	0.076	0.073
Reproducibility relative standard deviation (RSD_R) (%)	0.24	0.19	0.18
Reproducibility limit (R) % vol.	0.17	0.21	0.20

Sample types

A Fruit liqueur; split level*

B Brandy; blind duplicates

C Whisky; blind duplicates

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Samples	D	E	F
Number of laboratories retained after eliminating outliers	12	11	9
Number of outliers (Laboratories)	-	1	2
Number of accepted results	24	22	18
Mean value ($\overline{\times}$) % vol.	39.26	42.38	57.16
	43.09*	45.89*	63.44*
Repeatability standard deviation (s_r) % vol.	0.099	0.094	0.106
Repeatability relative standard deviation (RSD_r) (%)	0.24	0.21	0.18
Repeatability limit (r) % vol.	0.28	0.26	0.30
Reproducibility standard deviation (s_R) % vol.	0.118	0.103	0.125
Reproducibility relative standard deviation (RSD_R) (%)	0.29	0.23	0.21
Reproducibility limit (R) % vol.	0.33	0.29	0.35

Sample types

D Grappa; split level*

E Aquavit; split level*

F Rum; split level*

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METHOD FOR THE DETERMINATION OF TOTAL DRY EXTRACT OF SPIRIT DRINKS OF VITI-VINICULTURAL ORIGIN - GRAVIMETRIC METHOD

Type II method
Year : 2009

1. Scope

This method is suited to the determination of the total dry extract in spirit drinks of viti-vinicultural origin whichcontain less than 15 g/L of dry matter.

2. Normative References

ISO 3696:1987 Water for analytical laboratory use - Specifications and test methods.

3. Definition

The total dry extract or total dry matter includes all matter that is non-volatile under specified physical conditions.

4. Principle

Weighing of the residue left by evaporation of the spirit on a boiling water bath and drying in a drying oven.

5. Apparatus and Equipment

5.1 Flat-bottomed stainless-steel cylindrical capsule, of sufficient dimensions to avoid loss of liquid when evaporating.
5.2 Boiling water bath.
5.3 25 ml pipette, class A.
5.4 Drying oven.
5.5 Dessicator.
5.6 Analytical balance accurate to 0.1 mg.

6. Sampling and Samples.

Samples are stored at room temperature prior to analysis.

7. Procedure

7.1 Pipette 25 ml of the spirit drink into a previously-weighed cylindrical capsule (5.1) . During the first hour of evaporation the evaporating dish is placed on the lid of a boiling water bath so that the liquid will not boil, as this could lead to losses through splattering. Leave one more hour directly in contact with the steam of the boiling water bath.

7.2 Complete the drying by placing the evaporating dish in a drying oven at 105 °C ± 3 °C for two hours. Allow the evaporating dish to cool in a dessicator and weigh the evaporating dish and its contents.

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8. Calculation

The mass of the residue multiplied by 40 is equal to the dry extract contained in the spirit and it must be expressed in g/l to one decimal place.

9. Method performance characteristics (Precision)

9.1 Statistical results of the interlaboratory test

The following data were obtained from an international method performance study carried out on a variety of spirit drinks to internationally agreed procedures.

Year of interlaboratory test	1997
Number of laboratories	10
Number of samples	4

Samples	A	B	C	D
Number of laboratories retained after eliminating outliers	9	9	8	9
Number of outliers (Laboratories)	1	1	2	-
Number of accepted results	18	18	16	18
Mean value ($\bar{x}$) g/l	9.0	9.1	10.0	11.8
		7.8	9.4	11.1
Repeatability standard deviation (s_r) g/l	0.075	0.441	0.028	0.123
Repeatability relative standard deviation (RSD_r) (%)	0.8	5.2	0.3	1.1
Repeatability limit (r) g/l	0.2	1.2	0.1	0.3
Reproducibility standard deviation (s_R) g/l	0.148	0.451	0.058	0.210
Reproducibility relative standard deviation (RSD_R) (%)	1.6	5.3	0.6	1.8
Reproducibility limit (R) g/l	0.4	1.3	0.2	0.6

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Sample types

A Brandy; blind duplicates

B Rum; split levels

C Grappa; split levels

D Aquavit; split levels

10. Bibliography

Commission Regulation (EC) N° 2870/2000 of 19 December 2000 laying down Community reference methods for the analysis of spirits drinks, *OJEC of 29 December 2000, L333/20*

P. Brereton, S. Hasnip, A. Bertrand, R. Wittkowski, C. Guillou, Analytical methods for the determination of spirit drinks, Trends in Analytical Chemistry, Vol. 22, No. 1, 19-25, 2003

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DETERMINATION OF THE PRINCIPAL VOLATILE SUBSTANCES OF SPIRIT DRINKS OF VITI-VINICULTURAL ORIGIN

Type II method
Year : 2009

1. Scope

This method is suitable for the determination of the following compounds by gas chromatography in spirit drinks of viti-vinicultural origin: ethanal (acetaldehyde), both free and total (obtained from the sum of ethanal and the fraction of ethanal contained in 1,1-diéthoxyéthane), ethyl ethanoate (ethyl acetate), 1,1-diethoxyethane (acetal), methanol (methyl alcohol), butan-2-ol (sec-butanol), propan-1-ol (n-propanol), 2-methylpropan-1-ol (isobutyl alcohol), butan-1-ol (n-butanol), 2-methylbutan-1-ol (active amyl alcohol), 3-methylbutan-1-ol (isoamyl alcohol).

2. Normative References

ISO 3696:1987 Water for analytical laboratory use - Specifications and test methods.

3. Definition

Congeners are volatile substances formed along with ethanol during fermentation, distillation and maturation of spirit drinks.

4. Principle

Congeners in spirit drinks are determined by direct injection of the spirit drink, or appropriately diluted spirit drink, or its distillate, into a gas chromatography (GC) system. A suitable internal standard is added to the spirit drink prior to injection. The congeners are separated by temperature programming on a suitable column and are detected using a flame ionisation detector (FID). The concentration of each congener is determined with respect to the internal standard from response factors, which are obtained during calibration under the same chromatographic conditions as those of the spirit drink analysis.

Note: The concentrations of the analytes are expressed as grams per 100 litres of absolute alcohol; the alcoholic strength of the product must be determined prior to analysis.

5. Reagents and Materials

Unless otherwise stated, use only reagents of a purity greater than 97 %, purchased from an ISO accredited supplier with a Certificate of Purity, free from other congeners at test dilution (this may be confirmed by injection of individual congener standards at the test dilution using GC conditions as in 6.4) and only water of at least grade 3 as defined in ISO 3696. Acetal and acetaldehyde must be stored in the dark at <5 °C, all other reagents should be stored according to the supplier's instructions.

5.1 Ethanol absolute (CAS 64-17-5)

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5.2 Methanol (CAS 67-56-1)
5.3 Propan-1-ol (CAS 71-23-8)
5.4 2-methylpropan-1-ol (CAS 78-33-1)
5.5 Acceptable internal standards: pentan-3-ol (CAS 584-02-1), pentan-1-ol (CAS 71-41-0), 4-methylpentan-1-ol (CAS 626-89-1), 4-méthylpentan-2-ol (CAS 108-11-2), or methyl nonanoate (CAS 1731-84-6).
5.6 2-methylbutan-1-ol (CAS 137-32-6)
5.7 3-methylbutan-1-ol (CAS 123-51-3)
5.8 Ethyl acetate (CAS 141-78-6)
5.9 Butan-1-ol (CAS 71-36-3)
5.10 Butan-2-ol (CAS 78-92-2)
5.11 Acetaldehyde (CAS 75-07-0)
5.12 Acetal (CAS 105-57-7)
5.13 40% v/v ethanol solution
To prepare 400 ml/l ethanol solution pour 400 ml ethanol (5.1) into a 1 litre volumetric flask, make up to volume with distilled water and mix.
5.14 Preparation and storage of standard solutions (procedure suggested for the validated method: the calibration ranges should be adapted to the nature of the different types of products analysed by each laboratory).
All standard solutions must be stored at <5 °C and be prepared freshly on a monthly basis, if necessary. Masses of components and solutions should be recorded to the nearest 0.1 mg.
5.14.1 Standard solution - A
Pipette the following reagents into a 100 ml volumetric flask, containing approximately 60 ml ethanol solution (5.13) to minimise component evaporation, make up to volume with ethanol solution (5.13) and mix thoroughly. Record the weight of the flask, each component added and the total final weight of contents.

Component	Volume (ml)
Methanol (5.2)	3.0
Propan-1-ol (5.3)	3.0
2-methylpropan-1-ol (5.4)	3.0
2-methylbutan-1-ol (5.6)	3.0
3-methylbutan-1-ol (5.7)	3.0
Ethyl acetate (5.8)	3.0
Butan-1-ol (5.9)	3.0
Butan-2-ol (5.10)	3.0
Acetaldehyde (5.11)	3.0
Acetal (5.12)	3.0

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NOTE - It is preferable to add acetal and acetaldehyde last in order to minimise losses through evaporation. The solutions may be prepared individually, and the final solution and dilutions prepared subsequently.

5.14.2 Standard solution - B

Pipette 3 ml of pentan-3-ol, or other suitable internal standard, (5.5) into a 100 ml volumetric flask, containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.

Record the weight of the flask, the weight of pentan-3-ol or other internal standard added and the total final weight of contents.

5.14.3 Standard solution - C

Pipette 1 ml solution A (5.14.1) and 1 ml solution B (5.14.2) into a 100 ml volumetric flask containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.

Record the weight of the flask, each component added and the total final weight of contents.

5.14.4 Standard solution - D

In order to maintain analytical continuity and an effective quality control, prepare a quality control standard using the previously prepared standard A (5.14.1) or, preferably, prepare a control standard as indicated for standard A, but using different batches or suppliers of reagents. Pipette 1 ml solution A (5.14.1) into a 100 ml volumetric flask containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.

Record the weight of the flask, each component added and the total final weight of contents.

5.14.5 Standard solution - E

Pipette 10 ml solution B (5.14.2) into a 100 ml volumetric flask containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.

Record the weight of the flask, each component added and the total final weight of contents.

5.14.6 Standard solutions used to check the linearity of response of FID

Into separate 100 ml volumetric flasks, containing approximately 80 ml ethanol (5.13), pipette 0, 0.1, 0.5, 1.0, 2.0 ml solution A (5.14.1) and 1 ml solution B (5.14.2), make up to volume with ethanol solution (5.13) and mix thoroughly.

Record the weight of the flask, each component added and the total final weight of contents.

5.14.7 QC standard solution

Pipette 9 ml standard solution D (5.14.4) and 1 ml of standard solution E (5.14.5) into a weighing vessel and mix thoroughly.

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Record the weight of the flask, each component added and the total final weight of contents.

6. Apparatus and Equipment

6.1 Apparatus capable of measuring the density and alcoholic strength.

6.2 Analytical balance, capable of measuring to four decimal places.

6.3 A temperature programmed gas chromatograph fitted with a flame ionisation detector and integrator or other data handling system capable of measuring peak areas.

6.4 Gas chromatographic column(s), capable of separating the analytes such that the minimum resolution between the individual components (other than 2-methylbutan-1-ol and 3-methylbutan-1-ol) is, as a guide, at least 1.3, if a simple visual examination of the chromatogram is not sufficient.

NOTE - The following columns and GC conditions are given as suitable examples:

1 A retention gap 1 m x 0.32 mm i.d. connected to a CP-WAX 57 CB column 50 m x 0.32 mm i.d. 0.2 μm film thickness (stabilised polyethylene glycol) followed by a Carbowax 400 column 50 m x 0.32 mm i.d. 0.2 μm film thickness. (Columns are connected using press-fit connectors.)

Carrier gas and pressure:	Helium (135 kPa)
Column temperature:	35 °C for 17 min., 35 °C to 70 °C at 12 °C/min., hold at 70 C for 25 min.
Injector temperature:	150 °C
Detector temperature:	250 °C
Injection volume:	1 μl, split 20 to 100:1

2 A retention gap 1 m x 0.32 mm i.d. connected to a CP-WAX 57 CB column 50 m x 0.32 mm i.d. 0.2 μm film thickness (stabilised polyethylene glycol). (Retention gap is connected using a press-fit connector.)

Carrier gas and pressure:	Helium (65 kPa)
Column temperature:	35 °C for 10 min., 35 °C to 110 °C at 5 °C/min., 110 °C to 190 °C at 30 °C/min., hold at 190 °C for 2 min.
Injector temperature:	260 °C
Detector temperature:	300 °C
Injection volume:	1 μl, split 55:1

3 A packed column (5% CW 20M, Carbopak B), 2 m x 2 mm i.d.

Column temperature: 65 °C for 4 min., 65 °C to 140 °C at 10 °C/min., hold at 140 °C for 5 min., 140 °C to 150 °C at 5 °C/min., hold at 150 °C for 3 min.

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Injector temperature:	65 °C
Detector temperature:	200 °C
Injection volume:	1 µl

7. Sampling and Samples.

7.1 Laboratory sample

On receipt, the alcoholic strength of each sample is measured (6.1).

8. Procedure (used for the validated method, and given as an example; the exact procedure, and in particular the calibration range, should be adapted to the nature of the spirit drinks analysed and to the procedures validated by each laboratory)

8.1 Test portion

8.1.1 Weigh an appropriate sealed weighing vessel and record the weight.

8.1.2 Pipette 9 ml laboratory sample into the vessel and record the weight (M_{SAMPLE}).

8.1.3 Add 1 ml of standard solution E (5.14.5) and record the weight (M_{IS}).

8.1.4 Shake the test material vigorously (at least 20 inversions). Samples must be stored at less than 5 °C prior to analysis in order to minimise any volatile losses.

8.2 Blank test

8.2.1 Using a four decimal place balance (6.2), weigh an appropriate sealed weighing vessel and record the weight.

8.2.2 Pipette 9 ml 400 ml/l ethanol solution (5.13) into the vessel and record the weight.

8.2.3 Add 1 ml of standard solution E (5.14.5) and record the weight.

8.2.4 Shake the test material vigorously (at least 20 inversions). Samples must be stored at less than 5 °C prior to analysis in order to minimise any volatile losses.

8.3 Preliminary test

Inject standard solution C (5.14.3) to ensure that all of the analytes are separated with a minimum resolution of 1.3 (except 2-methylbutan-1-ol and 3-methylbutan-1-ol).

8.4 Calibration

The calibration should be checked using the following procedure. Ensure that the response is linear by successively analysing in triplicate each of the linearity standard solutions (5.14.6) containing internal standard (IS). From the integrator peak areas for each injection calculate the ratio R for each congener and plot a graph of R versus the concentration ratio of congener to internal standard (IS), C. A linear plot should be obtained, with a correlation coefficient of at least 0.99.

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$$R = \frac{\text{Peak area of congener}}{\text{Peak area of IS}}$$

$$C = \frac{\text{Concentration of congener} \quad (\mu g / g)}{\text{Concentration of IS} \quad (\mu g / g)}$$

8.5 Determination

Inject standard solution C (5.14.3) and 2 QC standard solutions (5.14.7). Follow with unknown samples (prepared according to 8.1 and 8.2) inserting one QC standard every 10 samples to ensure analytical stability. Inject one standard solution C (5.14.3) after every 5 samples.

9. Calculation

An automated system of data handling can be used, provided the data can be checked using the principles described in the method below and to good gas-chromatographic practice (calculation of response factors and/or establishment of calibration curves). Measure peak areas for congener and internal standard peaks.

9.1 Response factor calculation.

From the chromatogram of the injection of standard solution C (5.14.3), calculate response factors for each congener using equation (1).

$$\text{(1) Response factor} = \frac{\text{Peak area of IS}}{\text{Peak area of congener}} \times \frac{\text{Conc. congener } (\mu g / \text{g})}{\text{Conc. IS } (\mu \text{g} / \text{g})}$$

where:

IS	=	Internal Standard
Conc. congener	=	concentration of congener in solution C (5.14.3)
Conc. IS	=	concentration of internal standard in solution C (5.14.3).

9.2 Sample analysis

Using equation (2) below, calculate the concentration of each congener in the samples.

$$\text{(2) Congener concentrations, (µg/g)} = \frac{\text{Peak area of congener}}{\text{Peak area of IS}} \times \frac{M_{IS}\,(\text{g})}{M_{SAMPLE}\,(\text{g})} \times \text{Conc. IS } (\mu \text{g} / \text{g}) \times \text{RF}$$

where:

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M_{SAMPLE}	=	weight of sample (8.1.2);
M_{IS}	=	weight of internal standard (8.1.3);
Conc. IS	=	concentration of internal standard in solution E (5.14.5);
RF	=	response factor calculated using equation 1.

9.3 Quality control standard solution analysis

Using equation (3) below, calculate the percentage recovery of the target value for each congener in the Quality Control standards (5.14.7):

$$(3)\ \%\ \text{Recovery of QC sample} = \frac{\text{concentration of analyte in QC standard}}{\text{concentration of analyte in solution D}} \times 100$$

The concentration of the analyte in the QC standard is calculated using equations (1) and (2) above.

9.4 Final presentation of results

Results are converted from µg/g to g per 100 litres absolute alcohol for samples using equation (4):

(4) Concentration in g per 100 litres absolute alcohol

$$= \text{Conc}\ (\mu g/g) \times \rho \times 10/(\text{strength}(\%\ \text{vol.}) \times 1000)$$

where ρ = density in kg/m³.

Results are quoted to a maximum of 3 significant figures and a maximum of one decimal place e.g. 11.4 g per 100 l absolute alcohol.

10. Quality Assurance and Control (used for the validated method)

Using equation (2) above, calculate the concentration of each congener in the quality control standard solutions prepared by following the procedure as in 8.1.1 to 8.1.4. Using equation (3), calculate the percentage recovery of the target value. If the analysed results are within ± 10 % of their theoretical values for each congener, analysis may proceed. If not, an investigation should be made to find the cause of the inaccuracy and remedial action taken as appropriate.

11 Method performance characteristics (Precision)

The following data were obtained from an international method performance study carried out on a variety of spirit drinks to internationally agreed procedures.

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Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	ethanal

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	28	26	27	27	28
Number of outliers (Laboratories)	2	4	3	3	2
Number of accepted results	56	52	54	54	56
Mean value ($\bar{x}$) µg/g.	63.4	71.67	130.4	38.4	28.6
				13.8*	52.2*
Repeatability standard deviation (s_r) µg/g	3.3	1.9	6.8	4.1	3.6
Repeatability relative standard deviation (RSD_r) (%)	5.2	2.6	5.2	15.8	8.9
Repeatability limit (r) µg/g.	9.3	5.3	19.1	11.6	10.1
Reproducibility standard deviation (s_R) µg/g	12	14	22	6.8	8.9
Reproducibility relative standard deviation (RSD_R) (%)	18.9	19.4	17.1	26.2	22.2
Reproducibility limit (R) µg/g.	33.5	38.9	62.4	19.1	25.1

Sample types

A Brandy; blind duplicates

B Kirsch; blind duplicates

C Grappa; blind duplicates

D Whisky; split levels*

E Rum; split levels*

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Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	ethyl acetate

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	24	24	25	24	24
Number of outliers (Laboratories)	2	2	1	2	2
Number of accepted results	48	48	50	48	48
Mean value ($\bar{x}$) µg/g.	96.8	1046	120.3	112.5	99.1
				91.8*	117.0*
Repeatability standard deviation (s_r) µg/g	2.2	15	2.6	2.1	2.6
Repeatability relative standard deviation (RSD_r) (%)	2.3	1.4	2.1	2.0	2.4
Repeatability limit (r) µg/g.	6.2	40.7	7.2	5.8	7.3
Reproducibility standard deviation (s_R) µg/g	6.4	79	8.2	6.2	7.1
Reproducibility relative standard deviation (RSD_R) (%)	6.6	7.6	6.8	6.2	6.6
Reproducibility limit (R) µg/g.	17.9	221.9	22.9	17.5	20.0

Sample types

A Brandy; blind duplicates

B Kirsch; blind duplicates

C Grappa; blind duplicates

D Whisky; split levels*

E Rum; split levels*

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Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	acetal

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	20	21	22	17	21
Number of outliers (Laboratories)	4	3	2	4	3
Number of accepted results	40	42	44	34	42
Mean value ($\bar{x}$) µg/g.	35.04	36.46	68.5	20.36 6.60*	15.1 28.3*
Repeatability standard deviation (s_r) µg/g	0.58	0.84	1.6	0.82	1.9
Repeatability relative standard deviation (RSD_r) (%)	1.7	2.3	2.3	6.1	8.7
Repeatability limit (r) µg/g.	1.6	2.4	4.4	2.3	5.3
Reproducibility standard deviation (s_R) µg/g	4.2	4.4	8.9	1.4	3.1
Reproducibility relative standard deviation (RSD_R) (%)	12.1	12.0	13.0	10.7	14.2
Reproducibility limit (R) µg/g.	11.8	12.2	25.0	4.0	8.7

Sample types

A Brandy; blind duplicates

B Kirsch; blind duplicates

C Grappa; blind duplicates

D Whisky; split levels*

E Rum; split levels*

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Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	total ethanal

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	23	19	22	21	22
Number of outliers (Laboratories)	1	5	2	3	2
Number of accepted results	46	38	44	42	44
Mean value ($\bar{x}$) µg/g.	76.5	85.3	156.5	45.4	32.7
				15.8*	61.8*
Repeatability standard deviation (s_r) µg/g	3.5	1.3	6.5	4.4	3.6
Repeatability relative standard deviation (RSD_r) (%)	4.6	1.5	4.2	14.2	7.6
Repeatability limit (r) µg/g.	9.8	3.5	18.3	12.2	10.0
Reproducibility standard deviation (s_R) µg/g	13	15	24.1	7.3	9.0
Reproducibility relative standard deviation (RSD_R) (%)	16.4	17.5	15.4	23.7	19.1
Reproducibility limit (R) µg/g.	35.2	41.8	67.4	20.3	25.2

Sample types

A Brandy; blind duplicates

B Kirsch; blind duplicates

C Grappa; blind duplicates

D Whisky; split levels*

E Rum; split levels*

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Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	Methanol

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	26	27	27	28	25
Number of outliers (Laboratories)	4	3	3	1	4
Number of accepted results	52	54	54	56	50
Mean value ($\bar{x}$) µg/g.	319.8	2245	1326	83.0. 61.5*	18.6. 28.9*
Repeatability standard deviation (s_r) µg/g	4.4	27	22	1.5	1.3
Repeatability relative standard deviation (RSD_r) (%)	1.4	1.2	1.7	2.1	5.6
Repeatability limit (r) µg/g.	12.3	74.4	62.5	4.3	3.8
Reproducibility standard deviation (s_R) µg/g	13	99	60	4.5	2.8
Reproducibility relative standard deviation (RSD_R) (%)	3.9	4.4	4.6	6.2	11.8
Reproducibility limit (R) µg/g.	35.2	278.3	169.1	12.5	7.9

Sample types

A Brandy; blind duplicates

B Kirsch; blind duplicates

C Grappa; blind duplicates

D Whisky; split levels*

E Rum; split levels*

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Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	4
Analyte	butan-2-ol

Samples	A	B	C	E
Number of laboratories retained after eliminating outliers	21	27	29	22
Number of outliers (Laboratories)	4	3	1	3
Number of accepted results	42	54	58	44
Mean value ($\bar{\times}$) µg/g.	5.88	250.2	27.57	5.83 14.12*
Repeatability standard deviation (s_r) µg/g	0.40	2.2	0.87	0.64
Repeatability relative standard deviation (RSD_r) (%)	6.8	0.9	3.2	6.4
Repeatability limit (r) µg/g.	1.1	6.1	2.5	1.8
Reproducibility standard deviation (s_R) µg/g	0.89	13	3.2	0.87
Reproducibility relative standard deviation (RSD_R) (%)	15.2	5.1	11.5	8.7
Reproducibility limit (R) µg/g.	2.5	35.5	8.9	2.4

Sample types

A Brandy; blind duplicates

B Kirsch; blind duplicates

C Grappa; blind duplicates

E Rum; split levels*

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Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	propan-1-ol

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	29	27	27	29	29
Number of outliers (Laboratories)	2	4	3	2	2
Number of accepted results	58	54	54	58	58
Mean value ($\bar{x}$) µg/g.	86.4	3541	159.1	272.1	177.1
				229.3*	222.1*
Repeatability standard deviation (s_r) µg/g	3.0	24	3.6	2.3	3.3
Repeatability relative standard deviation (RSD_r) (%)	3.4	0.7	2.3	0.9	1.6
Repeatability limit (r) µg/g.	8.3	68.5	10.0	6.4	9.1
Reproducibility standard deviation (s_R) µg/g	5.3	150	6.5	9.0	8.1
Reproducibility relative standard deviation (RSD_R) (%)	6.1	4.1	4.1	3.6	4.1
Reproducibility limit (R) µg/g.	14.8	407.2	18.2	25.2	22.7

Sample types

A Brandy; blind duplicates

B Kirsch; blind duplicates

C Grappa; blind duplicates

D Whisky; split levels*

E Rum; split levels*

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Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	3
Analyte	butan-1-ol

Samples	A	B	C
Number of laboratories retained after eliminating outliers	20	22	22
Number of outliers (Laboratories)	4	4	6
Number of accepted results	40	44	44
Mean value ($\bar{x}$) µg/g.	3.79	5.57	7.54
Repeatability standard deviation (s_r) µg/g	0.43	0.20	0.43
Repeatability relative standard deviation (RSD_r) (%)	11.2	3.6	5.6
Repeatability limit (r) µg/g.	1.1	0.6	1.2
Reproducibility standard deviation (s_R) µg/g	0.59	0.55	0.82
Reproducibility relative standard deviation (RSD_R) (%)	15.7	9.8	10.8
Reproducibility limit (R) µg/g.	1.7	1.5	2.3

Sample types

A Brandy; blind duplicates

B Kirsch; blind duplicates

C Grappa; blind duplicates*

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Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	2-methylpropan-1-ol

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	28	31	30	26	25
Number of outliers (Laboratories)	3	0	1	5	6
Number of accepted results	56	62	60	52	50
Mean value ($\bar{x}$) µg/g.	174.2	111.7	185.0	291.0	115.99
				246.8*	133.87*
Repeatability standard deviation (s_r) µg/g	2.3	1.6	2.5	1.8	0.74
Repeatability relative standard deviation (RSD_r) (%)	1.3	1.4	1.3	0.7	0.6
Repeatability limit (r) µg/g.	6.4	4.5	6.9	5.0	2.1
Reproducibility standard deviation (s_R) µg/g	8.9	8.9	9.7	6.0	6.2
Reproducibility relative standard deviation (RSD_R) (%)	5.1	8.0	5.2	2.2	5.0
Reproducibility limit (R) µg/g.	24.9	24.9	27.2	16.9	17.4

Sample types

A Brandy; blind duplicates

B Kirsch; blind duplicates

C Grappa; blind duplicates

D Whisky; split levels*

E Rum; split levels*

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Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	2-methyl-butan-1-ol

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	25	26	25	27	25
Number of outliers (Laboratories)	3	2	3	1	2
Number of accepted results	50	52	50	54	50
Mean value ($\bar{\times}$) µg/g.	113.0	48.3	91.6	72.1	39.5
				45.2*	61.5*
Repeatability standard deviation (s_r) µg/g	2.1	1.5	1.7	2.3	2.3
Repeatability relative standard deviation (RSD_r) (%)	1.9	3.1	1.8	3.9	4.5
Repeatability limit (r) µg/g.	6.0	4.2	4.7	6.4	6.3
Reproducibility standard deviation (s_R) µg/g	7.4	3.8	6.6	4.7	4.5
Reproducibility relative standard deviation (RSD_R) (%)	6.6	7.9	7.2	8.1	8.8
Reproducibility limit (R) µg/g.	20.8	10.7	18.4	13.3	12.5

Sample types

A Brandy; blind duplicates

B Kirsch; blind duplicates

C Grappa; blind duplicates

D Whisky; split levels*

E Rum; split levels*

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Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	3-methyl-butan-1-ol

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	23	23	24	27	21
Number of outliers (Laboratories)	5	5	4	1	6
Number of accepted results	46	46	48	54	42
Mean value ($\bar{x}$) µg/g.	459.4	242.7	288.4	142.2	212.3
				120.4*	245.6*
Repeatability standard deviation (s_r) µg/g	5.0	2.4	3.4	2.4	3.2
Repeatability relative standard deviation (RSD_r) (%)	1.1	1.0	1.2	1.8	1.4
Repeatability limit (r) µg/g.	13.9	6.6	9.6	6.6	9.1
Reproducibility standard deviation (s_R) µg/g	29.8	13	21	8.5	6.7
Reproducibility relative standard deviation (RSD_R) (%)	6.5	5.2	7.3	6.5	2.9
Reproducibility limit (R) µg/g.	83.4	35.4	58.8	23.8	18.7

Sample types

A Brandy; blind duplicates

B Kirsch; blind duplicates

C Grappa; blind duplicates

D Whisky; split levels*

E Rum; split levels*

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12. Bibliography

Commission Regulation (EC) N° 2870/2000 of 19 December 2000 laying down Community reference methods for the analysis of spirits drinks, *OJEC of 29 December 2000, L333/20*

P. Brereton, S. Hasnip, A. Bertrand, R. Wittkowski, C. Guillou, Analytical methods for the determination of spirit drinks, Trends in Analytical Chemistry, Vol. 22, No. 1, 19-25, 2003

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ANETHOLE. GAS CHROMATOGRAPHIC DETERMINATION OF TRANS-ANETHOLE IN SPIRIT DRINKS OF VITI-VINICULTURAL ORIGIN

Type II method
Year : 2009

1. SCOPE

This method is suitable for the determination of trans-anethole in aniseed-flavoured spirit drinks using capillary gas chromatography.

2. NORMATIVE REFERENCES

ISO 3696: 1987 Water for analytical laboratory use - Specifications and test methods.

3. PRINCIPLE

The trans-anethole concentration of the spirit is determined by gas chromatography (GC). The same quantity of an internal standard, e.g. 4-allylanisole (estragole) when estragole is not naturally present in the sample, is added to the test sample and to a trans-anethole reference solution of known concentration, both of which are then diluted with a 45% ethanol solution and injected directly into the GC system.
An extraction is necessary before sample preparation and analysis for liqueurs that contain large amounts of sugars.

4. REAGENTS AND MATERIALS

During the analysis use only reagents of a purity of at least 98 %. Water of at least grade 3 as defined by ISO 3696 should be used.
Reference chemicals should be stored cold (ca. 4°C), away from light, in aluminium containers or in tinted (amber) glass reagent bottles. The stoppers should preferably be fitted with an aluminium seal. Trans-anethole will need to be "thawed" from its crystalline state before use, but in this case its temperature should never exceed 35°C.

4.1 Ethanol 96 % vol. (CAS 64-17-5)

4.2 1-methoxy-4- (1-propenyl) benzene; (trans-anethole) (CAS 4180-23-8)

4.3 4-allylanisole, (estragole) (CAS 140-67-0), suggested internal standard (IS)

4.4 Ethanol 45 % vol.

Add 560 g of distilled water to 378 g of ethanol 96 % vol.

4.5 Preparation of standard solutions

All standard solutions should be stored at room temperature (15-35°C) away from light in aluminium containers or in tinted (amber) glass reagent bottles. The stopper should preferably be fitted with an aluminium seal.
Trans-anethole and 4-allylanisole are practically insoluble in water, and it is therefore necessary to dissolve the trans-anethole and 4-allylanisole in some 96 % ethanol (4.1) before the addition of 45 % ethanol (4.4).
The stock solutions must be freshly prepared each week.

4.5.1 Standard solution A

Stock solution of trans-anethole (concentration: 2 g/L)

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Weigh 40 mg of trans-anethole (4.2) in a 20 mL volumetric flask (or 400 mg in 200 mL, etc.). Add some 96 % ethanol (4.1) and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

4.5.2 Internal standard solution B

Stock solution of internal standard, e.g. estragole (concentration: 2 g/L)

Weigh 40 mg of estragole (4.3) in a 20 mL volumetric flask (400 mg in 200 mL etc.). Add some 96 % vol. ethanol (4.1) make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

4.5.3 Solutions used to check the linearity response of the FID

The linearity response of the FID must be checked for the analysis taking into account a range of concentrations of trans-anethole in spirits from 0 g/L up to 2.5 g/L. In the procedure of analysis, the unknown samples of spirits to be analysed are diluted 10 times (8.3). For the conditions of the analysis described in the method, stock solutions corresponding to concentrations of 0, 0.05, 0.1, 0.15, 0.2, and 0.25 g/L of trans-anethole in the sample to be analysed are prepared as follows: take 0.5, 1, 1.5, 2, and 2.5 mL of stock solution A (4.5.1) and pipette in separate 20 mL volumetric flasks; pipette into each flask 2 mL of internal standard solution B (4.5.2) and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

The blank solutions (8.4) is used as the 0 g/L solution.

4.5.4 Standard solution C

Take 2 mL of standard solution A (4.5.1) and pipette into a 20 mL volumetric flask then add 2 mL of internal standard solution B (4.5.2) and make up to volume with 45% vol. ethanol (4.4), mix thoroughly.

5. APPARATUS AND EQUIPMENT

5.1 A capillary gas chromatograph fitted with a flame ionisation detector (FID) and integrator or other data handling system capable of measuring peak areas, and with an automatic sampler or the necessary equipment for manual sample injection.

5.2 Split/splitless injector

5.3 Capillary column, for example:

Length: 50 m

Internal diameter: 0.32 mm

Film thickness: 0.2 µm

Stationary phase: FFAP - modified TPA polyethylene glycol cross-linked porous polymer

5.4 Common laboratory equipment: A grade volumetric glassware, analytical balance (precision: ±0.1 mg).

6. CHROMATOGRAPHY CONDITIONS

The column type and dimensions, and the GC conditions, should be such that anethole and the internal standard are separated from each other and from any interfering substances. Typical conditions for the column given as an example in 5.3 are:

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6.1 Carrier gas: analytical helium.
6.2 Flow rate: 2 mL/min
6.3 Injector temperature: 250°C.
6.4 Detector temperature: 250°C.
6.5 Oven temperature conditions: isothermal, 180°C, run time 10 minutes
6.6 Injection volume: 1µL, split 1:40

7. SAMPLES

Samples should be stored at room temperature, away from light and cold.

8. PROCEDURE

8.1 Sample screening for estragole

To ensure that there is no estragole naturally present in the sample, a blank analysis should be carried out without the addition of any internal standard. If estragole is naturally present then another internal standard must be chosen (for instance menthol).

Pipette 2 mL sample into a 20 mL volumetric flask and make up to volume with 45% vol. ethanol (4.4), mix thoroughly.

8.2 Preparation of unknown samples

Pipette 2 mL sample into a 20 mL volumetric flask then add 2 mL of internal standard solution B (4.5.2) and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

8.3 Blank

Pipette 2 mL of internal standard solution B (4.5.2) into a 20 mL volumetric flask and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

8.4 Linearity test

Prior to the commencement of the analysis the linearity of the response of the FID should be checked by successively analysing in triplicate each of the linearity standard solutions (4.5.3).

From the integrator peak areas for each injection plot a graph of their mother solution concentration in g/L versus the ratio R for each.

R = trans-anethole peak area divided by the estragole peak area.

A linear plot should be obtained.

8.5 Determination

Inject the blank solution (8.3), followed by standard solution C (4.5.4), followed by one of the linearity standards (4.5.3) which will act as a quality control sample (this may be chosen with reference to the probable concentration of trans-anethole in the unknown), followed by 5 unknowns (8.2); insert a linearity (quality control) sample after every 5 unknown samples, to ensure analytical stability.

9. CALCULATION OF RESPONSE FACTOR

Measure peak areas (using an integrator or other data system) for trans-anethole and internal standard peaks.

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9.1 Response factor (RF_i) calculation

The response factor is calculated as follows

$RF_i = (C_i / area_i)*(area_{is} / C_{is})$

Where:

C_i is the concentration of trans-anethole in the standard solution A (4.5.1.)

C_{is} is the concentration of internal standard in the standard solution B (4.5.2.)

$area_i$ is the area of the trans-anethole peak

$area_{is}$ is the area of the internal standard peak

RF_i is calculated from the 5 samples of solution C (4.5.4)

9.2 Analysis of the linearity response test solutions

Inject the linearity response test solutions (4.5.3).

9.3 Analysis of the sample

Inject the unknown sample solution (8.2)

10. CALCULATION OF RESULTS

The formula for the calculation of the concentration of trans-anethole is the following:

$c_i = C_{is} * (area_i/area_{is})*RF_i$

where:

c_i	is the unknown trans-anethole concentration
C_{is}	is the concentration of internal standard in the unknown (4.5.2)
$Area_i$	is the area of the trans-anethole peak
$Area_{is}$	is the area of the internal standard peak
RF_i	is the response coefficient (calculated as in 9.1)

The trans-anethole concentration is expressed as grams per litre, to one decimal place.

11. QUALITY ASSURANCE AND CONTROL

The chromatograms should be such that anethole and the internal standard are separated from each other and from any interfering substances. The RF_i value is calculated from the results for the 5 injections of solution C (4.5.4). If the coefficient of variation (CV % = (standard deviation/mean)*100)) is within plus or minus 1 %, the RF_i average value is acceptable.

The calculation above should be used to calculate the concentration of trans-anethole in the sample selected for the quality control from the linearity control solutions (4.5.3).

If the mean calculated results from analysis of the linearity solution selected for Internal Quality Control sample (IQC) are within plus or minus 2.5 % of their theoretical value, then the results for the unknown samples can be accepted.

12. TREATMENT OF SPIRITS SAMPLE CONTAINING LARGE AMOUNT OF SUGAR AND OF LIQUEUR SAMPLE PRIOR TO GC ANALYSIS

Extraction of alcohol from spirit drink containing a large amount of sugar, in order to be able to determine the trans-anethole concentration using capillary gas chromatography.

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12.1 PRINCIPLE

An aliquot of the liqueur sample is taken and to this is added the internal standard, at a concentration similar to that of the analyte (trans-anethole) in the liqueur. To this are added sodium phosphate dodecahydrate and anhydrous ammonium sulphate. The resulting mixture is well shaken and chilled, two layers develop, and the upper alcohol layer is removed. An aliquot of this alcohol layer is taken and diluted with 45 % ethanol solution (4.4) (Note: no internal standard is added at this stage, because it has already been added). The resulting solution is analysed in gas chromatography.

12.2 REAGENTS AND MATERIALS

During the extraction use only reagents of a purity greater than 99 %.

12.2.1 Ammonium sulphate, anhydrous, (CAS 7783-20-2)

12.2.2 Sodium phosphate, dibasic, dodecahydrate, (CAS 10039-32-4)

12.3 APPARATUS AND EQUIPMENT

Conical flasks, separating flasks, refrigerator.

12.4 PROCEDURE

12.4.1 Sample screening for estragole

To ensure that there is no estragole naturally present in the sample, a blank extraction (12.6.2) and analysis should be carried out without the addition of any internal standard. If estragole is naturally present then another internal standard must be chosen.

12.4.2 Extraction

Pipette 5 mL of 96 % ethanol (4.1) into a conical flask, weigh into this flask 50 mg of internal standard (4.3), and add 50 mL of the sample. Add 12 g of ammonium sulphate, anhydrous (12.2.1), and 8.6 g of dibasic sodium phosphate, dodecahydrate (12.2.2). Stopper the conical flask.

Shake the flask for at least 30 minutes. A mechanical shaking device may be used, but not a Teflon coated magnetic stirring bar, as the Teflon will absorb some of the analyte. Note that the added salts will not dissolve completely.

Place the stoppered flask in a refrigerator ($T < 5^{o}C$) for at least two hours.

After this time, there should be two distinct liquid layers and a solid residue. The alcohol layer should be clear; if not replace in the refrigerator until a clear separation is achieved.

When the alcohol layer is clear, carefully take an aliquot (e.g. 10 mL), without disturbing the aqueous layer, place in an amber vial and close securely.

12.4.3 Preparation of the extracted sample to be analysed

Allow extract (12.4.2) to reach room temperature.

Take 2 mL of the alcohol layer of the attemperated extracted sample and pipette into a 20 mL volumetric flask, make up to volume with 45 % ethanol (4.4), mix thoroughly.

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12.5 DETERMINATION

Follow the procedure as outlined in 8.5.

12.6 CALCULATION OF RESULTS

Use the following formula to calculate the results

$C_i = (m_{is} / V)^* (area_i / area_{is}) ^*RF_i$

Where:

m_{is}	is the weight of internal standard (4.3.) taken (12.4.2) (in milligrams)
V	is the volume of unknown sample (50 mL)
RF_i	is the response factor (9.1.)
$area_i$	is the area of the trans-anethole peak
$area_{is}$	is the area of the internal standard peak

The results are expressed in grams per litre, to one decimal place.

12.7 QUALITY CONTROL AND ASSURANCE

Follow the procedure as outlined in 11 above.

13. METHOD PERFORMANCE CHARACTERISTICS (PRECISION)

Statistical results of the interlaboratory test: the following tables give the values for anethole.

The following data were obtained from an international method performance study carried out on a variety of spirit drinks to internationally agreed procedures.

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Year of interlaboratory test	1998
Number of laboratories	16
Number of samples	10
Analyte	anethole

Pastis :

Samples	A	B	C	D	E	F
Number of laboratories retained after eliminating outliers	15	15	15	13	16	16
Number of outliers (laboratories)	1	1	1	3	-	-
Number of accepted results	30	30	30	26	16	16
Mean value g/L	1.477	1.955	1.940	1.833	1.741	1.754
Repeatability standard deviation (S_r) g/L	0.022	0.033	0.034	0.017	-	-
Repeatability relative Standard deviation(RSD_r) (%)	1.5	1.7	1.8	0.9	-	-
Repeatability limit (r) g/L	0.062	0.093	0.096	0.047	-	-
Reproducibility standard deviation (s_R) g/L	0.034	0.045	0.063	0.037	0.058	0.042
Reproducibility relative Standard deviation (RSD_R) (%)	2.3	2.3	3.2	2.0	3.3	2.4
Reproducibility limit (R) g/L	0.094	0.125	0.176	0.103	0.163	0.119

Sample types:

A pastis, blind duplicates

B pastis, blind duplicates

C pastis, blind duplicates

D pastis, blind duplicates

E pastis, single sample

F pastis, single sample

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Other aniseed-flavoured spirit drinks:

Samples	G	H	I	J
Number of laboratories retained after eliminating outliers	16	14	14	14
Number of outliers (Laboratories)	-	2	1	1
Number of accepted results	32	28	28	28
Mean value g/L	0.778 0.530*	1.742	0.351	0.599
Repeatability standard deviation (S_r) g/L	0.020	0.012	0.013	0.014
Repeatability relative standard deviation (RSD_r) (%)	3.1	0.7	3.8	2.3
Repeatability limit (r) g/L	0.056	0.033	0.038	0.038
Reproducibility standard deviation (S_R) g/L	0.031	0.029	0.021	0.030
Repeatability relative standard deviation (RSD_R) (%)	4.8	1.6	5.9	5.0
Reproducibility limit (R) g/L	0.088	0.080	0.058	0.084

Sample types:

G ouzo, split levels (*)

H anis, blind duplicates

I aniseed-flavoured liqueur, duplicates

J aniseed-flavoured liqueur, duplicates

14. Bibliography

Commission Regulation (EC) N° 2091/2002 of 26 November 2002 amending Regulation (EC) No 2870/2000 laying down Community reference methods for the analysis of spirits drinks, *OJEC of 27 November 2002, L322/11*

P. Brereton, S. Hasnip, A. Bertrand, R. Wittkowski, C. Guillou, Analytical methods for the determination of spirit drinks, Trends in Analytical Chemistry, Vol. 22, No. 1, 19-25, 2003

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RESOLUTION OIV/OENO 380/2009

UPDATE OF THE OIV COMPENDIUM OF METHODS OF ANALYSIS OF SPIRIT DRINKS OF VITIVINICULTURAL ORIGIN – PART 2

THE GENERAL ASSEMBLY

CONSIDERING article 2 paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

IN VIEW OF the actions of the 2009-2012 OIV Strategic plan, particularly those focused on reorganising publications related to the vitivinicultural methods of analysis

CONSIDERING the works of the Methods of Analysis sub-commission

IN VIEW OF the 1994 publication of the Compendium of International Methods of Analysis of spirituous beverages, alcohol and the aromatic fraction of beverages

DECIDES given the evolution of methods and availability of inter-laboratory validation parameters to retain the following methods as Type II methods of analysis;

DECIDES to introduce these methods into the new edition of the "Compendium of international methods of analysis of spirituous beverages of vitivinicultural origin"

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

DETERMINATION OF THE ACIDITIES OF SPIRIT DRINKS OF VITI-VINICULTURAL ORIGIN

Type II method
Year : 2009

1. Scope

This method is suitable for the determination of the volatile, total, and fixed acidities of spirit drinks of viti-vinicultural origin.

2. Normative References

ISO 3696: 1987: Water for analytical use - Specifications and test methods

3. Definitions

3.1 Volatile acidity is made up of acetic and higher volatile aliphatic acids that are present in spirit drinks.

3.2 Total acidity is the sum of titratable acidities.

3.3 Fixed acidity is the acidity of the residue left after evaporating the spirit drink to dryness.

4. Principle

The total acidity is determined by direct titration of the sprit drink. The fixed acidity is determined by titration of the aqueous solution obtained after dissolving the residue from evaporation of the spirit drink. The volatile acidity is calculated by deducting the fixed acidity from the total acidity.

5. Reagents and Materials

During the analysis, unless otherwise stated, use only reagents of recognised analytical grade and water of at least grade 3 as defined in ISO 3696:1987

5.1 0.05 M sodium hydroxide solution

5.2 Mixed indicator solution:

Weigh 0.1 g of indigo carmine and 0.1 g of phenol red.
Dissolve in 40 mL water and make up to 100 mL with ethanol.

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6. Apparatus and Equipment

Standard laboratory apparatus, "A" grade volumetric glassware and, in particular, the following:

6.1 Equipment for applying vacuum (water pump, vacuum flask, etc.), or other system for eliminating carbon dioxide (bubbling or other).

6.2 Flat-bottomed stainless-steel cylindric capsule, of sufficient dimensions to avoid loss of liquid when evaporating.

6.3 Equipment for potentiometric titration (optional).

7. Sampling and samples

Samples are stored at room temperature prior to analysis.

8. Procedure

8.1.0 Total acidity

8.1.1 Preparation of sample

If necessary, the spirit is stirred for at least two minutes under vacuum to remove carbon dioxide, or the latter is eliminated by any other convenient method.

8.1.2 Titration

Pipette 25 mL of the spirit into a 500 mL conical flask

Add about 200 mL of cooled boiled distilled water (freshly prepared) and 2-6 drops of the mixed indicator solution (5.2).

Titrate with the 0.05 M sodium hydroxide solution (5.1) until the yellow-green colour changes to violet in the case of colourless spirit drinks, or the yellow-brown colour to red-brown in the case of brown-coloured spirit drinks.

The titration may also be carried out by potentiometry, to pH 7.5.

Let n_1 mL be the volume of the 0.05 M sodium hydroxide solution added.

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8.1.3 Calculation

The total acidity (TA) expressed in milliequivalents per L of spirit drink is equal to 2 x n_1.

The total acidity (TA') expressed in mg of acetic acid per L of spirit drink is equal to 120 x n_1.

The total acidity (TA') expressed in g of acetic acid per hL of pure 100 % vol alcohol is equal to 120 x n_1 x 10/A, where A is the alcoholic strength by volume of the spirit drink.

8.2 Fixed acidity

8.2.1 Preparation of sample

Pipette 25 mL (or a larger volume if the fixed acidity is very low) of the spirit drink into a flat-bottomed cylindrical evaporating dish (6.2). During the first hour of evaporation the evaporating dish is placed on the lid of a boiling water bath so that the liquid will not boil, as this could lead to losses through splattering.
If necessary, complete the drying by placing the evaporating dish in a drying oven at 105 °C for two hours. Allow the evaporating dish to cool in a desiccator.

8.2.2 Titration

Take up the residue left after evaporating with cooled boiled distilled water (freshly prepared), make up to a volume of about 100 mL and add 2-6 drops of the mixed indicator solution (5.2).

Titrate with the 0.05 M sodium hydroxide solution (5.1) until the yellow-green colour changes to violet if the solution is colourless, or the yellow-brown colour to red-brown if the solution is brown-coloured.

The titration may also be carried out by potentiometry, to pH 7.5.

Let n_2 mL be the volume of the 0.05 M sodium hydroxide solution added, and V mL the volume of sample evaporated.

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8.2.3 Calculation

The fixed acidity (FA) expressed in milliequivalents per L of spirit drink is equal to 2 x n_2 x 25/V.

The fixed acidity (FA') expressed in mg of acetic acid per L of spirit drink is equal to 120 x n_2 x 25/V.

The fixed acidity (FA') expressed in g of acetic acid per hL of pure 100% vol alcohol is equal to 120 x n_2 x 25/V x 10/A, where A is the alcoholic strength by volume of the spirit drink.

8. Calculation of volatile acidity

8.3.1 Expression in milliequivalents per L :

Let:
TA = total acidity in milliequivalents per L
FA = fixed acidity in milliequivalents per L

Volatile acidity,VA, in milliequivalents per L is equal to :

TA - FA

8.3.2 Expression in mg of acetic acid per L:

Let:
TA' = total acidity in mg of acetic acid per L
FA' = fixed acidity in mg of acetic acid per L

Volatile acidity,VA, in mg of acetic acid per L is equal to :

TA' - FA'

8.3.3 Expression in g of acetic acid per hL of pure 100 % vol alcohol is equal to :

$$\frac{TA'-FA'}{A} \times 10$$

where A is the alcoholic strength by volume of the spirit drink.

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9. Method performance characteristics (Precision)

The following data were obtained in 2000 from an international method-performance study on a variety of spirit drinks, carried out following internationally-agreed procedures.
Key to the tables below:

nLT	Number of laboratories (2 results per laboratory),
nL	Number of laboratories to calculate precision values,
r	repeatability limit
Sr	repeatability standard deviation
RSDr	repeatability standard deviation expressed in % of the level
R	reproducibility limit
SR	reproducibility standard deviation
RSDR	reproducibility standard deviation expressed in % of the level
PRSDR	RSDR predicted with the Horwitz formula (%)
HoR	HorRat value = RSDR / PRSDR
SH240	Aqueous-alcoholic solution: acetic acid (240 mg/L), tartaric acid (200 mg/L), sucrose (10 g/L)

All the acidities are expressed as mg of acetic acid per L of spirit drink.

9.1 Total acidity

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Rum 1	18	18	53	8	2.7	5.1	34	12	23	8.8	2.6
Slibowitz	18	17	55	10	3.7	6.7	19	6.6	12	8.8	1.4
Brandy	20	18	193	16	5.7	2.9	43	15	7.9	7.2	1.1
Brandy	18	18	194	16	5.8	3.0	38	13	6.9	7.2	1.0
Calvados	18	17	282	21	7.5	2.7	34	12	4.3	6.8	0.6
SH240	20	17	400	14	4.9	1.2	18	6.2	1.6	6.5	0.2
Marc	18	18	547	16	5.8	1.1	42	15	2.7	6.2	0.4
Armagnac	20	19	580	27	9.4	1.6	53	19	3.2	6.1	0.5
Rum 2	18	18	641	41	14.3	2.2	66	23	3.7	6.0	0.6

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9.2 Fixed acidity

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Slibowitz	18	16	9.5	5.1	1.8	19	14	4.9	52	11	4.6
Rum 1	18	18	22	6.1	2.2	9.7	28	10	45	10	4.5
Calvados	18	16	25	7.7	2.7	10.8	24	8.4	34	9.9	3.4
Rum 2	18	18	25	5.7	2.0	7.9	28	9.9	39	9.8	4.0
Marc	18	17	51	25	8.8	17	60	21	42	8.8	4.7
Brandy	18	18	87	17	6.0	6.9	47	17	19	8.2	2.3
Brandy	20	19	89	12	4.2	4.7	33	12	13	8.1	1.6
Armagnac	20	19	159	13	4.7	2.9	80	28	18	7.5	2.4
SH240	20	17	162	12	4.1	2.5	32	11	7.1	7.4	1.0

9.3 Volatile acidity

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Rum 1	18	18	30	10	3.5	12	24	8.4	28	9.6	2.9
Slibowitz	18	14	46	10	3.7	8.1	13	4.6	10	9.0	1.1
Brandy	20	18	107	23	8.0	7.5	44	16	15	7.9	1.8
Brandy	18	18	107	19	6.6	6.2	38	13	13	7.9	1.6
SH240	20	17	242	21	7.2	3.0	48	17	6.9	7.0	1.0
Calvados	18	16	257	23	8.0	3.1	24	8.5	3.3	6.9	0.5
Armagnac	20	17	418	22	7.8	1.9	62	22	5.2	6.5	0.8
Marc	18	18	492	24	8.5	1.7	69	24	5.0	6.3	0.8
Rum 2	18	18	616	42	15	2.4	71	25	4.1	6.1	0.7

10. Bibliography

R. Wittkowski, A. Bertrand, P. Brereton, C. Guillou, 2000. PROJECT SMT4-CT96-2119, Validation of analytical methods of analysis for spirit drinks. REPORT NO. 02/08-WORKSTREAM 8

P. Brereton, S. Hasnip, A. Bertrand, R. Wittkowski, C. Guillou, Analytical methods for the determination of spirit drinks, Trends in Analytical Chemistry, Vol. 22, No. 1, 19-25, 2003

FV 1322 (2009), Measurement of acidities in spirits - estimation of precision

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DETERMINATION OF SUGARS IN SPIRIT DRINKS OF VITI-VINICULTURAL ORIGIN

Type II method
Year : 2009

Introduction

Spirit drinks of viti-vinicultural origin may be sweetened by various compounds, and in certain legislations the concentrations of sweetener are subject to minimum or maximum levels.

1. Scope

This method is suitable for the determination of the glucose, fructose, and sucrose contents of spirit drinks of viti-vinicultural origin. It is not suitable for spirit drinks containing dairy products or eggs.

2. Normative References

ISO 3696:1897 Waters for analytical use - Specifications and test methods.

3. Principle

High performance liquid chromatography (HPLC) to determine the glucose, fructose, and sucrose concentrations.

This method is described as an example. It uses an alkylamine stationary phase and differential refractometry detection. Other columns/detectors may be used, for example anion exchange resins as the stationary phase.

4. Reagents and Materials

4.1 Glucose (CAS 50-99-7), at least 99 % pure.

4.2 Fructose (CAS 57-48-7), at least 99 % pure.

4.3 Sucrose (CAS 57-50-1), at least 99 % pure.

4.4 Pure acetonitrile (CAS 75-05-8) for HPLC analysis.
Acetonitrile is a highly flammable liquid. It is toxic by inhalation, in contact with skin and if swallowed. It is irritating to eyes.

4.5 Distilled or demineralised water, preferably micro-filtered.

4.6 Solvents (example)
The elution solvent is prepared beforehand by mixing:
75 parts by volume of acetonitrile (4.4),

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25 parts by volume of distilled or demineralised water (4.5).
Pass helium through at a slow rate for 5 - 10 minutes prior to use to degas.
If the water being used has not been micro-filtered, it is advisable to pass the solvent through a filter for organic solvents with a pore size less than or equal to 0.45 µm.

4.7 Ethanol, absolute (CAS 64-17-5).

4.8 Ethanol solution (5 %, v/v).

4.9 Preparation of stock standard solution (20 g/L)
Weigh 2 g each of the sugars to be analysed (4.1 to 4.3), transfer them without loss to a 100 mL volumetric flask. Adjust to 100 mL with a 5 % vol. alcohol solution (4.8), shake and store at around +4 °C. Prepare a new stock solution once a week if necessary.

4.10 Preparation of working standard solutions (2.5, 5.0, 7.5, 10.0 and 20.0 g/L)
Dilute the stock solution, 20 g/L, (4.9) appropriately with a 5% vol. alcohol solution (4.8) to give five working standards of 2.5, 5.0, 7.5, 10.0 and 20.0 g/L. Filter with a filter of a pore size less than or equal to 0.45 µm (5.3.).

5. Apparatus and Equipment (as an example - other systems that provide equivalent performance can be used)

Standard laboratory apparatus, "A" grade volumetric glassware and, in particular, the following:

5.1. HPLC system capable of achieving baseline resolution of all of the sugars.

5.1.1 High-performance liquid chromatograph with a six-way injection valve fitted with a 10 µL loop or any other device, whether automatic or manual, for the reliable injection of micro-volumes.

5.1.2. Pumping system enabling one to achieve and maintain a constant or programmed rate of flow with great precision.

5.1.3. Differential refractometer.

5.1.4. Computational integrator or recorder, the performance of which is compatible with the rest of the set-up.

5.1.5. Pre-column:
It is recommended that a suitable pre-column is attached to the analytical column.

5.1.6. Column (example):
Material: stainless steel or glass
Internal diameter: 2-5 mm
Length: 100-250 mm (depending on the packing particle size), for example 250 mm if the particles are 5 µm in diameter

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Stationary phase: cross-linked silica with radicals containing the alkylamine functional group, maximum particle size 5 µm.

5.1.7. Chromatography conditions (example):
Elution solvent (4.6), flow rate: 1 mL/minute
Detection: Differential refractometry
To make certain that the detector is perfectly stable, it may be advisable to switch it on a few hours before use. The reference cell must be filled with the elution solvent.

5.2. Analytical balance accurate to 0.1 mg.

5.3. Filtration equipment for small volumes using a 0.45 µm membrane.

6. Sample storage

On receipt, samples are to be stored at room temperature prior to analysis.

7. Procedure

7.1. PART A: Sample Preparation

7.1.1. Shake the sample.

7.1.2. Filter the sample through a filter with a pore size less than or equal to 0.45 µm (5.3).

7.2. PART B: HPLC

7.2.1. Determination
Inject 10 µL of the standard solutions (4.10) and samples (7.1.2.). Perform the analysis under suitable chromatography conditions, for example those described above.

7.2.2. Should any peak of a sample have a greater area (or height) than the corresponding peak in the most concentrated standard, then the sample should be diluted with distilled or demineralised water and re-analysed.

8. Calculation

Compare the two chromatograms obtained for the standard solution and spirit. Identify the peaks by their retention times. Measure their areas (or heights) to calculate the concentrations by the external standard method. Take into account any dilutions made to the sample.
The final result by convention is the sum of sucrose, glucose, and fructose, in g/L.

9.. Method performance characteristics (Precision)

The following data were obtained in 2000 from an international method-performance study carried out on a variety of spirit drinks, following internationally-agreed procedures.

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Key to the tables below:

nLT	Number of laboratories (2 results per laboratory),
nL	Number of laboratories to calculate precision values,
r	repeatability limit
Sr	repeatability standard deviation
RSDr	repeatability standard deviation expressed in % of the level
R	reproducibility limit
SR	reproducibility standard deviation
RSDR	reproducibility standard deviation expressed in % of the level
PRSDR	RSDR predicted with the Horwitz formula (%)
HoR	HorRat value = RSDR / PRSDR

9.1 Glucose

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	HoR
Liqueur 1	26	24	92.4	5.4	1.9	2.1	13	4.8	5.2	1.8
Liqueur 2	24	23	93.2	9.7	3.5	3.7	28	10	11	3.8

9.2 Fructose

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	HoR
Liqueur 1	26	22	87	3.2	1.2	1.3	8.5	3.0	3.5	1.2
Liqueur 2	24	21	93	6.6	2.3	2.5	22	7.7	8.3	2.9

9.3 Saccharose

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	HoR
Liqueur 1	26	24	174	12	4.2	2.4	24	8.7	5.0	1.9
Liqueur 2	24	18	320	12	4.3	1.3	45	16	5.0	2.1
Liqueur 3	24	18	349	22	8.0	2.3	30	11	3.1	1.3
Pastis	24	19	11	0.2	0.1	0.8	2.2	0.8	7.3	1.9
Ouzo	24	19	24	2.1	0.8	3.1	2.6	0.9	3.8	1.1
Kirsch	24	20	103	6.1	2.2	2.1	12	4.2	4.0	1.4

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9.4 Sucres totaux

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	HoR
Liqueur 1	26	21	353	8.7	3.1	0.9	41	15	4.2	1.8
Liqueur 2	24	18	510	16	5.6	1.1	41	15	2.9	1.3
Liqueur 3	24	18	349	22	8.0	2.3	30	11	3.1	1.3
Pastis	24	20	11	0.4	0.1	1.2	2.2	0.8	7.3	1.8
Ouzo	24	19	24	2.1	0.8	3.1	2.6	0.9	3.8	1.1
Kirsch	24	20	103	6.1	2.2	2.1	12	4.2	4.0	1.4

10. Bibliography

R. Wittkowski, A. Bertrand, P. Brereton, C. Guillou, 2000. PROJECT SMT4-CT96-2119, Validation of analytical methods of analysis for spirit drinks. REPORT NO. 02/09 - WORKSTREAM 10.

P. Brereton, S. Hasnip, A. Bertrand, R. Wittkowski, C. Guillou, Analytical methods for the determination of spirit drinks, Trends in Analytical Chemistry, Vol. 22, No. 1, 19-25, 2003.

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RESOLUTION OIV/OENO 381/2009

UPDATE OF COMPEMDIUM OF INTERNATIONAL METHODS OF ANAYSIS OF SPIRIT DRINKS OF VITIVINICULTURE ORIGIN– PART 3

The GENERAL ASSEMBLY

Considering Article 2 paragraph 2 iv of the Agreement of 3 April 3 2001 establishing the International Organisation of Vine and Wine,

IN VIEW OF the actions of the Strategic plan of the OIV 2009-2012 particularly actions focused on reorganising publications related to vitivinicultural methods of analysis

CONSIDERING the works of the Sub-commission of Methods of Analysis and

IN VIEW OF the 1994 edition of the Compendium of International Methods of Analysis of Spirituous beverages, alcohol and aromatic fraction of beverages

IN VIEW OF the compendium of international methods of analysis of wine and musts

CONSIDERING that the methods described below are likewise described in the compendium of international methods of analysis of wine and musts with interlaboratory validation parameters.

CONSIDERING that the principle of these methods lies in measuring the parameters concerned with the ethanol fraction so obtained;

DECIDES to incorporate these methods in the new edition of the Compendium of International Methods of Analysis of Spirit Drinks of Vitivinicultural Origin

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DETERMINATION BY ISOTOPE RATIO MASS SPECTOMETRY OF THE $^{13}C/^{12}C$ RATIO OF WINE ETHANOL OF SPIRIT DRINKS OF VITIVINICULTURAL ORIGIN.

Type II method
Year: 2009

1. FIELD OF APPLICATION

The method enables the measuring of the $^{13}C/^{12}C$ isotope ratio of the ethanol of spirit drinks of vitivinicultural origin.

2. REFERENCE STANDARDS

ISO 5725 :1994 «Accuracy (trueness and precision) of measurement methods and results: Basic method for the determination of repeatability and reproducibility of a standard measurement method»

V-PDB : Vienna-Pee-Dee Belemnite (R_{PDB} = 0.0112372).

Method OIV «Detection of enriching musts, concentrated musts, grape sugar and wine by application of nuclear magnetic deuterium resonance (SNIF-NMR)»

3. TERMS AND DEFINITIONS

$^{13}C/^{12}C$: Carbon 13 and carbon 12 isotope ratio for a given sample

$\delta^{13}C$: Carbon 13 contents (^{13}C) expressed in parts per 1000 (‰)

SNIF-NMR: Site-specific natural isotope fractionation studied by nuclear magnetic resonance

V-PDB : Vienna-Pee-Dee Belemnite. or PDB, is the main reference for measuring natural variations of carbon 13 isotopic contents. Calcium carbonate comes from a Cretaceous belemnite from the Pee Dee formation in South Carolina (USA). Its isotopic ratio $^{13}C/^{12}C$ or $R_{PDB\ is}$ R_{PDB} = 0.0112372. PDB reserves have been exhausted for a long time, but it has remained the primary reference to express natural variations of Carbon 13 isotopic contents. Reference material is calibrated based on this content and is available at the International Agency of Atomic Energy (IAEA) in Vienna (Austria). The isotopic indications of naturally occurring carbon 13 are expressed by V-PDB, as is the custom.

m/z: Mass to charge ratio

4. PRINCIPLE

During photosynthesis, the assimilation of carbon dioxide by plants occurs according to 2 principle types of metabolism that are: metabolism C_3 (Calvin cycle) and C_4 (Hatch and Slack). These two means of photosynthesis present a different type of isotope fractionation. Products, such as sugars and alcohol, derived from C_4 plants and fermentation, have higher levels of Carbon 13 than from C_3 plants. Most plants, such as vines and sugar beet belong to the C_3 group. Sugar cane and corn belong to the C_4 group. Measuring the carbon 13 content enables the detection and the quantification of C_4 (sugar cane or corn isoglucose) origin sugars which are added to products derived from grapes (grape musts, wines). The combined information on carbon 13 content and information obtained from SNIF-NMR enable the quantification of mixed sugars added or alcohol of plant origin C_3 and C_4.

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The carbon 13 content is determined on carbon dioxide resulting from the complete combustion of the sample. The abundance of the main mass isotopomers 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$ et $^{12}C^{17}O^{16}O$) and 46 ($^{12}C^{16}O^{18}O$), resulting from different possible combinations of isotopes ^{18}O, ^{17}O, ^{16}O, ^{13}C et ^{12}C, are determined from ion currents measured by three different collectors of isotopic mass spectrometers. The contributions of isotopomers $^{13}C^{17}O^{16}O$ et $^{12}C^{17}O_2$ can be neglected because of their low abundance. The ion current for m/z = 45 is corrected for the contribution of $^{12}C^{17}O^{16}O$ which is calculated according to the current intensity measured for m/z = 46 while considering the relative abundance of ^{18}O and ^{17}O (Craig adjustment). The comparison with the calibrated reference and the international reference V-PDB enable the calculation of carbon 13 content on a relative scale of $\delta^{13}C$.

5. REAGENTS

The material and the consumables depend on the apparatus (6) used by the laboratory. The systems generally used are based on elementary analysers. These systems can be equipped to introduce the samples placed in sealed metal capsules or for the injection of liquid samples through a septum using a syringe.

Depending on the type of instrument used, the reference material, reagents, and consumables can be used:

- Reference material

available from the IAEA:

Name	Material	$\delta^{13}C$ versus V-PDB (9)
- IAEA-CH-6	saccharose	-10.4 ‰
-IAEA-CH-7	polyethylene	-31.8 ‰
- NBS22	oil	-29.7 ‰
- USGS24	graphite	-16.1 ‰

available from the IRMM in Geel (B) (Institut des Matériaux et Mesures de Référence) :

Name	Material	$\delta^{13}C$ versus V-PDB (9)
- CRM 656	Wine alcohol	-26.93 ‰
- CRM 657	glucose	-10.75 ‰
- CRM 660	aqueous-alcoholic solution (ABV 12 % vol.)	-26.72 ‰

Standard working standard with a known $^{13}C/^{12}C$ ratio calibrated with international reference materials.

A standard list of consumables established for continuous flow systems follows below:

- Helium for analysis (CAS 07440-59-7)
- Oxygen for analysis (CAS 07782-44-7)
- Carbon dioxide for analysis, used as a secondary reference gas for the content of carbon 13 (CAS 00124-38-9)
- Oxidation reagent for the oven and the combustion system as follows:
  copper oxide (II) for elementary analysis (CAS 1317-38-0)

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- Drying agent to eliminate water produced by combustion. For example:
  anhydrone for elementary analysis (magnesium perchlorate) (CAS 10034-81-8).

This is not necessary for apparatus equipped with a water elimination system by cryo-trapping or through selective permeable capillaries.

6. APPARATUS AND MATERIALS

6.*1. Isotope ratio mass spectrometry (IRMS)*

Isotope ratio mass spectrometry (IRMS) enables the determination of the relative contents of ^{13}C of naturally occurring CO_2 gas with an internal accuracy of 0.05‰ or expressed in relative value (9). Internal accuracy here is defined as the difference between 2 measurements of the same sample of CO_2. The mass spectrometer used to measure isotope ratios is generally equipped with a triple collector to simultaneously measure m/z = 44, 45 and 46 intensities. The isotope ratio mass spectrometry must either be equipped with a double introduction system to alternately measure the unknown sample and a reference sample, or use an integrated system that carries out quantitative combustion on samples and separates the carbon dioxide from the other combustion products before measuring the mass spectrum.

6.2. Combustion apparatus

Combustion apparatus able to quantitatively convert ethanol into carbon dioxide and capable of eliminating all other combustion products including water, without any isotopic fractionation. The apparatus can be either an integrated continuous flow system integrated with mass spectrometry (6.2.1), of an autonomous combustion system (6.2.2). The apparatus must be as precise as indicated in (11).

6.2.1. Continuous flow systems

These consist of either an elemental analyser, or a gas chromatograph equipped with an online combustion system.

The following laboratory material is used for systems equipped for the introduction of samples contained in metallic capsules :

- volumetric micropipette with appropriate cones
- balance with 1 μg accuracy or better
- tool for capsule sealing
- tin capsules for liquid samples
- tin capsules for solid samples

The following laboratory material is needed when using an elemental analyser equipped with a liquid injector or in the case of a preparation system for combustion chromatography:

- syringe for liquids
- flasks equipped with sealed closing system and inert septa

The laboratory materials indicated in the lists are examples that are susceptible of being replaced by other equivalent performance material depending on the type of combustion apparatus and of mass spectrometry used by the laboratory.

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6.2.2 Separate preparation systems

In this case the samples of carbon dioxide resulting from the combustion of samples to be analyzed and the reference sample are collected in ampoules which are then put in a double entry spectrometer system to carry out isotopic analyses. Several types of combustion apparatus described in the literature can be used:

- Closed combustion system filled with circulating oxygen gas
- Elemental analyser with helium and oxygen flows
- Sealed glass ampoule filled with copper oxide (II) used as an oxidation agent

7. PREPARATION OF SAMPLES FOR TESTS

Ethanol must be extracted from the spirit drink before isotopic testing. This is carried out by distilling the beverage as described in §3.1 of the SNIF-NMR method for the determination by NMR of the deuterium distribution in the ethanol of spirit drinks of vitivinicultural origin.

8. PROCEDURE

All preparation steps must be carried out without any significant ethanol loss through evaporation, which would change the isotopic composition of the sample.

The description that follows makes reference to the procedure generally used for ethanol sample combustion using commercial automatic combustion systems. All other methods, ensuring that ethanol samples are converted quantitatively into carbon dioxide without the evaporation of ethanol, can use the preparation of carbon dioxide for isotopic analyses. An experimental procedure based on the use of an elemental analyser:

a) Placing the samples in capsules

- use capsules, tweezers and a clean preparation tray
- take an appropriate sized capsule using tweezers
- introduce an appropriate amount of liquid into the capsule using a micropipette

Note: 3.84 mg of absolute ethanol or 4.17 mg of distillate with an alcohol content of 92 % m/m are necessary to obtain 2 mg of carbon. The appropriate quantity of distillate must be calculated in the same way according to the quantity of carbon necessary based on the mass spectrometer's sensitivity.

- close the capsule with the sealing tool.
- each capsule must be completely sealed. If not, it must be discarded and a new capsule must be prepared.
- two capsules must be prepared for every sample
- place the capsules in an appropriate place on the elemental analyser sample tray. Every capsule must be carefully identified in order by number.
- systematically place capsules containing working references at the beginning and the end of the sample series
- regularly insert a check sample in the sample series.

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b) check and adjust the elemental analysis and mass spectometry instrumentation

- adjust the temperature of the elemental analyzer ovens and the helium and oxygen gas flow for an optimal combustion of the sample;
- check the elemental analysis system and the mass spectometry system for leaks (for example by checking the ion current where m/z = 28 corresponds to N_2);
- adjust the mass spectrometer to measure the ion current intensities for m/z = 44, 45 and 46;
- check the system using known reference samples before starting to measure the samples.

c) To carry out a series of measurements

The samples that are placed on the autosampler of the elemental analyser or chromatographare introduced successively. The carbon dioxide for each sample combustion is eluted into the mass spectrometer which measures the ion current. The data system records the ion current intensities and calculates the δ values for each sample (9).

9. CALCULATION

The objective of the method is to measure the $^{13}C/^{12}C$ isotopic ratio of the ethanol extracted from spirit drinks. The $^{13}C/^{12}C$ isotopic ratio can be expressed by its deviation compared to the reference work. The carbon 13 ($\delta\,^{13}C$) isotopic ratio is calculated on a delta scale per thousand by comparing the results obtained for the sample to be measured to the working reference calibrated previously based on the primary international reference (V-PDB). The $\delta\,^{13}C$ values are expressed compared to the working reference:

$$\delta^{13}C_{ech/ref}\,‰ = 1000 \times (R_{ech}-R_{ref})/R_{ref}$$

where R_{ech} and R_{ref} are respectively the isotopic ratio $^{13}C/^{12}C$ of the sample and the working reference.

The $\delta\,^{13}C$ values are thus expressed using V-PDB:

$$\delta^{13}C_{ech/V\text{-}PDB}‰ = \delta^{13}C_{ech/ref} + \delta^{13}C_{ref/V\text{-}PDB\,+}\ (\delta^{13}C_{ech/ref} \times \delta^{13}C_{ref/V\text{-}PDB})\,/1000$$

where $\delta^{13}C_{ref/V\text{-}PDB}$ is the isotopic deviation determined beforehand for the working reference to V-PDB.

Small variations may occur while measuring on line due to changes in the instrumental conditions. In this case the $\delta\,^{13}C$ samples must be corrected according to the difference in the $\delta^{13}C$ value from the working reference and the real value, which was calibrated beforehand against V-PDB by comparison with one of the international reference materials. Between two measurements of the working reference, the variation and therefore the correction applied to the sample results may be assumed to be linear. The working reference must be measured at the beginning and at the end of all sample series. A correction can then be calculated for each sample using linear interpolation between the two values (the difference between the assigned value of the working reference and the measurement values obtained).

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10. QUALITY ASSURANCE AND CONTROL

Check that the ^{13}C value for the working reference does not differ by more than 0.5‰ of the accepted value. If not, the spectrometer must be checked and possibly readjusted.

For each sample, verify that the difference in the results between the 2 capsules measured successively is under 0.3 ‰. The final result for a given sample is the average value of the 2 capsules. If the deviation is higher than 0.3 ‰ the measurement should be repeated.

Measurement condition monitoring can be based on the ion current intensity for m/z = 44 and is proportional to the quantity of carbon injected in the elemental analyzer. Under standard conditions, the ion current intensity should be almost constant for the samples analysed. A significant deviation could be indicative of ethanol evaporation (an imperfect seal on a capsule), an instability of the elemental analyser, or the mass spectrometer.

11. METHOD PERFORMANCE CHARCTERISTICS (Precision)

One collaborative analysis (11.1) was carried out on distillates containing alcohol of vinous origin, and cane and beet alcohol, in addition to different mixtures of these three origins. This study did not take into account the distillation step, and further information from other joint laboratory studies on wine (11.2) and in particular proficiency testing (11.3) for isotopic measurements have also been considered. The results show that different distillation systems under satisfactory conditions, and in particular those used for SNIF-NMR measurements, do not have significant variability for determining the $\delta^{13}C$ of ethanol in wine. It is reasonable to suppose that this would likewise be true for the ethanol of spirit drinks. The precision parameters observed for wine are almost identical to those obtained in the joint study on distillates (11.1).

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11.1. Joint study on distillates

Year of inter laboratory study: 1996

Number of laboratories: 20

Number of samples: 6 samples in double-blind comparison

Analysis: ethanol $\delta\ ^{13}C$

Sample code	Vinous origin alcohol	Beet alcohol	Sugar cane alcohol
A & G	80%	10%	10%
B & C	90%	10%	0%
D & F	0%	100%	0%
E & I	90%	0%	10%
H & K	100%	0%	0%
J & L	0%	0%	100%

Samples	A / G	B / C	D / F	E / I	H / K	J / L
Number of laboratories retained after eliminating anomalous results	19	18	17	19	19	19
Number of results accepted	38	36	34	38	38	38
Average value ($\delta\ ^{13}C$) ‰	-25.32	-26.75	-27.79	-25.26	-26.63	-12.54
Sr^2	0.0064	0.0077	0.0031	0.0127	0.0069	0.0041
Repeatability standard deviation (Sr) ‰	0.08	0.09	0.06	0.11	0.08	0.06
Repeatability limit r ($2.8 \times S_r$) ‰	0.22	0.25	0.16	0.32	0.23	0.18
S_R^2	0.0389	0.0309	0.0382	0.0459	0.0316	0.0584
Reproducibility standard deviation (S_R) ‰	0.20	0.18	0.20	0.21	0.18	0.24
Reproducibility limit R ($2,8 \times S_R$) ‰	0.55	0.9	0.55	0.60	0.50	0.68

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11.2. Inter laboratory study on two wines and one alcohol

Year of inter laboratory trial: 1996

Number of laboratories: 14 for distillation of wine and 7 for also measuring the $\delta\ ^{13}C$ of ethanol in wine

8 for measuring the $\delta\ ^{13}C$ in the alcohol sample

Number of samples of strength 93% m/m) 3 (White wine ABV 9.3 % vol., white wine ABV 9.6 % and alcohol

Analysis: ethanol $\delta\ ^{13}C$

Samples	Red wine	White wine	Alcohol
Number of laboratories	7	7	8
Number of accepted results	7	7	8
Average value ($\delta\ ^{13}C$) ‰	-26.20	-26.20	-25.08
Reproducibility variance S_R^2	0.0525	0.0740	0.0962
Reproducibility standard deviation (S_R) ‰	0.23	0.27	0.31
Reproducibility limit R (2.8× S_R) ‰	0.64	0.76	0.87

Different distillation systems were used by the participating laboratories. The $\delta\ ^{13}C$ isotopic determinations carried out in one laboratory on all of the distillates returned by the participants, did not reveal any anomalous values or significantly distinct average values. The variation in results ($S^2 = 0.0059$) is comparable to repeatability variances Sr^2 from the collaborative study on distillates (11.1).

11.3. Results from proficiency-testing studies

Since December 1994 international proficiency testing to determine the isotopic measurements for wine and alcohol (ABV of distillates 96 % vol.) have been organized regularly. The results enable participating laboratories to check the quality of their analyses. Statistical results enable an appreciation of the variability of derterminations under reproducibility conditions. This enables an estimation of the variance parameters and the reproducibility limit. The results obtained for the wine and ethanol distillate $\delta\ ^{13}C$ determinations are summarized in the table below:

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	Wine				Distillates			
Date	N	S_R	S^2_R	R	N	S_R	S^2_R	R
Dec. 1994	6	0.210	0.044	0.59	6	0.151	0.023	0.42
June 1995	8	0.133	0.018	0.37	8	0.147	0.021	0.41
Dec. 1995	7	0.075	0.006	0.21	8	0.115	0.013	0.32
March 1996	9	0.249	0.062	0.70	11	0.278	0.077	0.78
June 1996	8	0.127	0.016	0.36	8	0.189	0.036	0.53
Sept. 1996	10	0.147	0.022	0.41	11	0.224	0.050	0.63
Dec. 1996	10	0.330	0.109	0.92	9	0.057	0.003	0.16
March 1997	10	0.069	0.005	0.19	8	0.059	0.003	0.16
June 1997	11	0.280	0.079	0.78	11	0.175	0.031	0.49
Sept 1997	12	0.237	0.056	0.66	11	0.203	0.041	0.57
Dec. 1997	11	0.127	0.016	0.36	12	0.156	0.024	0.44
March 1998	12	0.285	0.081	0.80	13	0.245	0.060	0.69
June 1998	12	0.182	0.033	0.51	12	0.263	0.069	0.74
Sept 1998	11	0.264	0.070	0.74	12	0.327	0.107	0.91
Weighted average		0.215	0.046	0.60		0.209	0.044	0.59

N: number of participating laboratories

12. BIBLIOGRAPHY

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Determination of the distribution of deuterium in ethanol of spirit drinks of vitivinicultural origin by application of nuclear magnetic resonance of deuterium (SNIF-NMR/RMN-FINS [1])

Type I method
Year: 2009

1 Definition

The deuterium contained in the sugars and the water in grape must will be redistributed after fermentation in molecules I, II, III and IV of the wine:

$CH_2D\ CH_2OH$	$CH_3CHD\ OH$	CH_3CH_2OD	HOD
I	II	III	IV

The addition of exogenous sugar (chaptalisation) before the must ferments will have an effect on the distribution of the deuterium.

As compared with the figures for parameters for a natural control wine from the same region, the enrichment of an exogenous sugar will lead to the following variations:

Parameter	$(D/H)_I$	$(D/H)_{II}$	$(D/H)^{\frac{Q}{W}}$	R
Wine – Natural ……	→	→	→	→
Wine - Enriched:				
- beet sugar ……	↘	↗	↗	↗
- cane sugar ……				
- maize sugar ……	↗	↗	↗	↘

$(D/H)_I$: isotope ratio associated with molecule I

$(D/H)_{II}$: isotope ratio associated with molecule II

$(D/H)^{\frac{Q}{W}}$: isotope ratio of the water in the wine.

$R = 2\ (D/H)_{II}/(D/H)_I$, expresses the relative distribution of deuterium in molecules I and II: R is measured directly from the *h* intensities of the signals and then $R = 3h_{II}/h_I$.

$(D/H)_I$ mainly characterizes the vegetable species which synthesized the sugar and to a lesser extent the geographical location of the place of harvest (type of water used during photosynthesis).

$(D/H)_{II}$ represents the climatology of the place of production of the grapes (type of rain-water and weather conditions) and to a lesser extent the sugar concentration of the original must.

---

[1] **Fractionnement Isotopique Naturel Spécifique étudié par Résonance Magnétique Nucléaire (Site-Specific Natural Isotope Fractionation studied by Nuclear Magnetic Resonance). - Brevet: France, 81-22710; Europe, 82-402-209-9; USA, 85-4-550-082; Japan 57-123-249.**

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$(D/H)^{\frac{Q}{W}}$ represents the climatology of the place of production and the sugar content of the original must. Henceforth, this parameter shall no longer be considered, since it is not characteristic of water of a spirituous beverage.

2. Principle

The parameters defined above (R, $(D/H)_I$ and $(D/H)_{II}$) are determined by nuclear magnetic resonance of the deuterium in the ethanol extracted from a spirituous beverage; they may be supplemented by determining the ratio $^{13}C/^{12}C$ in the ethanol.

3. Preparation of the sample for analysis

Note: Any method for ethanol extraction can be used as long as 98 to 98.5% of the total alcohol in the wine is recovered in a distillate which contains 92 to 93% (*m*/*m*) (95% vol.).

3.1 *Extraction of ethanol*

3.1.1 Apparatus and reagents

- Apparatus for extracting ethanol (Figure 1) comprising:
    - Electric heating mantle with voltage regulator,
    - One-liter round-bottom flask with ground glass neck joint,
    - Cadiot column with rotating band (moving part in Teflon),
    - 125 mL conical flasks with ground glass neck joints,
    - 125 and 60 mL bottles with plastic stoppers.
- Reagents for the determination of water by the Karl Fischer method.

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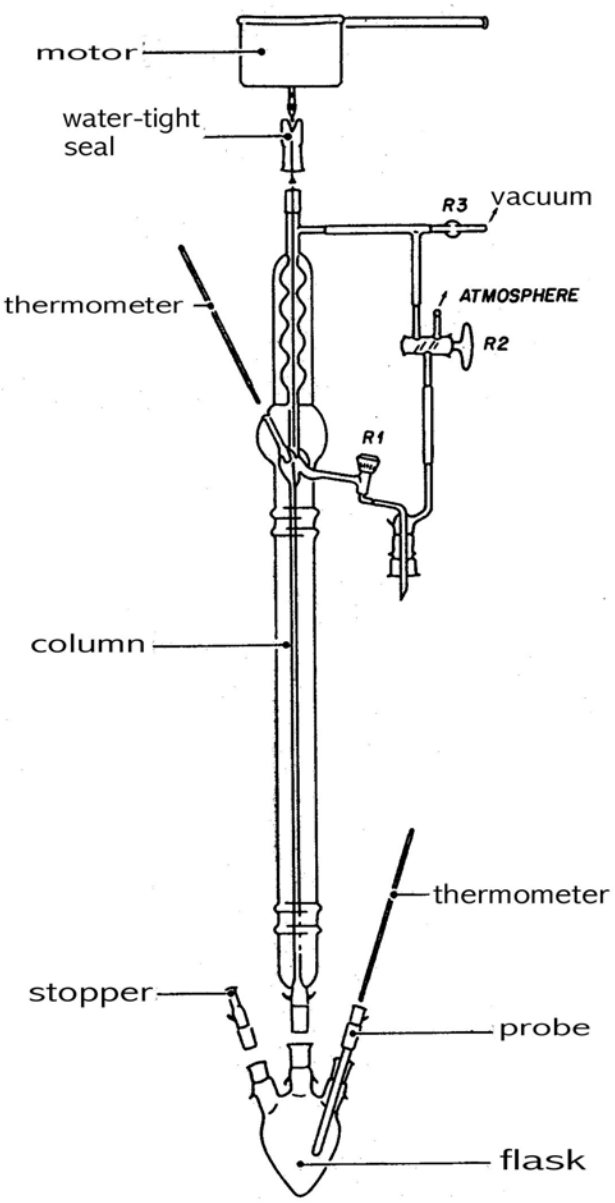

Figure 1 - Apparatus for extracting ethanol

3.1.2 Procedure

3.1.2.1 Extraction of the ethanol

Introduce a homogeneous sample of 50 to 300 mL of spirit drink (depending on its alcoholic strength) into the flask of the distillation apparatus with a constant reflux ratio of about 0.9. Place a 125 mL ground conical flask, calibrated beforehand, to receive the

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distillate. Collect the boiling liquid between 78.0 and 78.2°C, i.e. approximately 40 to 60 mL. When the temperature exceeds 78.5°C, discontinue collection for five minutes.

When the temperature returns to 78°C, recommence collecting the distillate until 78.5°C and repeat this operation until the temperature, after discontinuing collection and operating within a closed system, remains constant. Complete distillation lasts approximately five hours. This procedure enables between 98 and 98.5% of the total alcohol in the wine to be recovered in a distillate with a strength of between 92 and 93% mass (95% vol.), a strength for which the NMR conditions described in section 4 have been established. The collected ethanol is weighed.

3.1.2.2 Determination of the alcoholic strength of the alcohol extracted.

The water content (p′ g) is determined by the Karl Fischer method using a sample of about 0.5 mL of alcohol of exactly known mass p′.

The strength by mass of the alcohol is given by:

$$t\frac{D}{m} = \frac{\rho - \rho'}{\rho} \text{ x } 100$$

3.2 *Preparation of alcohol sample for NMR measurement*

3.2.1 Reagents

N,N-tetramethyl urea (TMU); use a sample of standard TMU with a given, monitored isotope ratio D/H. Such samples may be supplied by Community Bureau of Reference, Brussels, or other authoritative body.

3.2.2 Procedure

- 15 mm diameter NMR probe:

In a previously weighed bottle, collect 7 mL alcohol as in 3.1.2 and weigh it to the nearest 0.1 mg (m_A); then take a 3 mL sample of the internal standard (TMU) and weigh to the nearest 0.1 mg (m_{ST}). Homogenize by shaking.

- 10 mm diameter NMR probe:

.

3.2 mL of alcohol and 1.3 mL TMU are sufficient

Depending on the type of spectrometer and probe used (cf.. section 4), add a sufficient quantity of hexafluorobenzene as a field-frequency stabilization substance (lock).

Spectrometer	Probe	
	10 mm	15 mm
7.05T	150 µL	200 µL
9.4T	35 µL	50 µL

4 Recording of ^{2}H NMR spectra for the alcohol. Determination of isotope parameters.

4.1 *Apparatus*

- NMR spectrometer fitted with a specific "deuterium" probe tuned to a frequency v_O, characteristic of channel B_O (e.g. B_O = 7.05 T, v_O = 46.05 MHz and for B_O = 9.4 T, v_O = 61.4 MHz) having a decoupling channel (B_2) and a field-frequency stabilization channel (lock) at the fluorine frequency.

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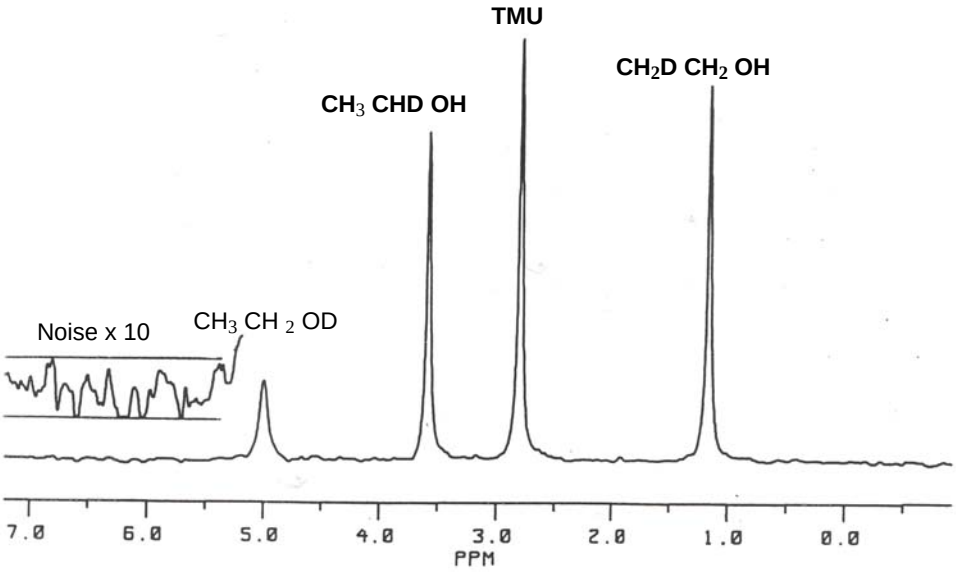

Figure 2a
^{2}H NMR spectrum of an ethanol from wine with an internal standard (TMU: N, N-tetramethylurea)

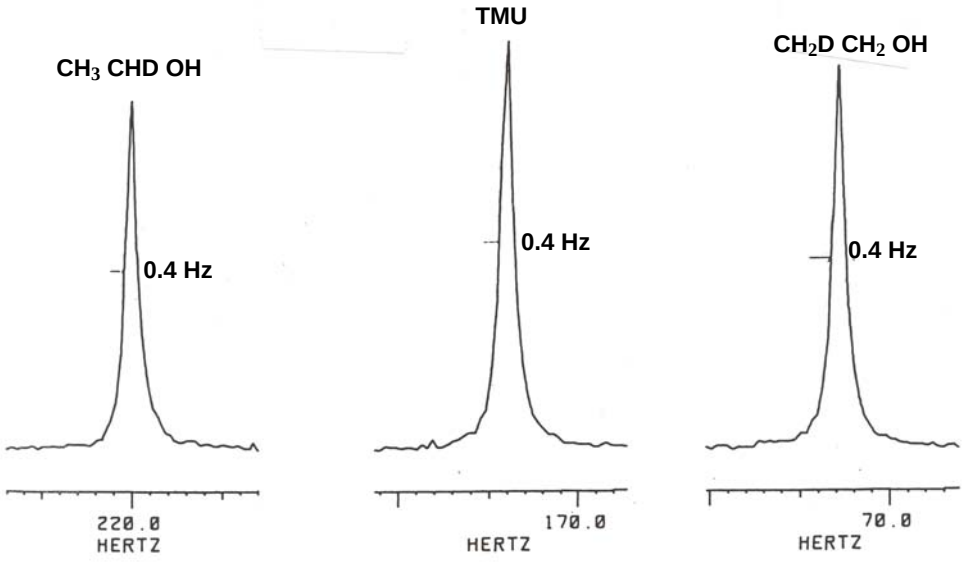

Figure 2b
^{2}H spectrum of ethanol taken under the same conditions as those of Figure 2a, but without exponential multiplication (LB = 0)

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The resolution measured on the spectrum, transformed without exponential multiplication (i.e. LB = 0) (Figure 2b) and expressed by the (half-height) of the methyl and methylene signals of ethanol and the methyl signal of TMU, must be less than 0.5 Hz. The sensitivity, measured with an exponential multiplying factor LB equal to 2 (Figure 2a) must be greater than or equal to 150 for the methyl signal of ethanol of alcoholic strength 95% vol. (93.5% mass).

Under these conditions, the confidence interval for the measurement of the signal height, calculated for a 97.5% probability (one-sided test) and 10 repetitions of the spectrum, is 0.35%.

- Automatic sample changer (optional).
- Data-processing software.
- 15 mm or 10 mm sample tubes according to spectrometer performance.

4.2 *Standardization of spectrometer and checks*

4.2.1 Standardization

Carry out customary standardization for homogeneity and sensitivity according to the manufacturer's specifications.

4.2.2 Checking the validity of the standardization

Use standard ethanol solutions designated by the letters:
C: alcohol from cane sugar or maize,
V: wine spirit,
B: alcohol from beet sugar.

These samples are supplied by the Community Bureau of Reference or other authoritative body.

Following the procedure described in 4.3, determine the isotope values of these alcohols, denoting them C_{mes}, V_{mes}, B_{mes} (see 5.3).

Compare them with the given corresponding standard values, denoted by a superscript C_{st}, B_{st}, V_{st} (see 5.3).

The standard deviation for repeatability obtained on an average of 10 repetitions of each spectrum must be less than 0.01 for the ratio R and less than 0.3 ppm for $(D/H)_I$ and $(D/H)_{II}$.

The average values obtained for the various isotopic parameters (R, $(D/H)_I$, $(D/H)_{II}$) must be within the corresponding standard deviation of repeatability given for those parameters for the three standard alcohols by the Community Bureau of References or other authoritative bodies. If they are not, carry out the checks again.

4.3 *Conditions for obtaining NMR spectra*

Place a sample of alcohol prepared as in 3.2 in a 15 mm or 10 mm tube and introduce it into the probe.

The conditions for obtaining NMR spectra are as follows:

- a constant probe temperature (e.g. 302 K);
- acquisition time of at least 6.8 s for 1200 Hz spectral width (16K memory) (i.e. about 20 ppm at 61.4 MHz or 27 ppm at 46.1 MHz);
- 90° pulse;
- adjustment of acquisition time: its value must be of the same order as the dwell time;

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- parabolic detection: fix the offset O1 between the OD and CHD reference signals for ethanol and between the HOD and TMU reference signals for water;
- determine the value of the decoupling offset O2 from the proton spectrum measured by the decoupling coil on the same tube. Good decoupling is obtained when O2 is located in the middle of the frequency interval existing between the CH_3- and CH_2- groups. Use the wide band-decoupling mode.

For each spectrum, carry out a number of accumulations NS sufficient to obtain the signal-to-noise ratio given in 4.1 and repeat this set of NS accumulations NE = 10 times. The values of NS depend on the types of spectrometer and probe used (cf. section 4). Examples of the possible choices are:

Spectrometer	Probe	
	10 mm	15 mm
7.05T	NS = 304	NS = 200
9.4T	NS = 200	NS = 128

5 Expression of results

5.1

For each of the 10 spectra (see NMR spectrum for ethanol, Figure 2a), determine:

$$R = 3\frac{h_{II}}{h_1} = 3\,\frac{\text{height of signal II } (CH_3\ CHD\ OH)}{\text{height of signal I } (CH_2D\ CH_2OH)}$$

$$\left(D/H\right)_I = 1.5866 \cdot T_I \cdot \frac{m_{st}}{m_A} \cdot \frac{\left(D/H\right)_{st}}{t_m^D}$$

$$\left(D/H\right)_{II} = 2.3799 \cdot T_{II} \cdot \frac{m_{st}}{m_A} \cdot \frac{\left(D/H\right)_{st}}{t_m^D}$$

with

$$T_I = \frac{\text{height of signal I } (CH_2D\ CH_2OH)}{\text{height of signal of internal standard (TMU)}}$$

$$T_{II} = \frac{\text{height of signal II } (CH_3CHD\ OH)}{\text{height of signal of internal standard (TMU)}}$$

- m_{st} and m_A, see 3.2.2.
- t_m^D, see 3.1.2.2.
- $(D/H)_{st}$ = isotope ratio of internal standard (TMU) indicated on the bottle supplied by the Community Bureau of Reference or other appropriate body.

The use of peak heights instead of peak area, which is less precise, supposes that peak width at half height is uniform and is a reasonable approximation if applicable (Figure 2b).

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5.2 For each of the isotope parameters, calculate the average of 10 determinations and the confidence interval.

Optional software (e.g. SNIF-NMR) suitable for the spectrometer computer enables such calculations to be carried out on-line.

Note: If, after standardization of the spectrometer, there is a systematic difference between the average values obtained for the characteristic isotopes of the standard alcohols (4.2.2) and the values indicated by the Community Bureau of Reference or other authoritative body, to within the standard deviation, the following corrections may be applied to obtain the true value for any sample X.

The interpolation will be made by taking the values for the standard sample which straddle that of the sample X.

Let $(D/H)_I^{Xmes}$ be the measured value and $(D/H)_I^{Xcorr}$ be the corrected value. This will give:

$$(D/H)_I^{Xcorr} = (D/H)_I^{Bst} + \alpha\,[\,(D/H)_I^{Xmes} - (D/H)_I^{Bmes}\,(D/H)_I^{Bmes}\,]$$

where

$$\alpha = \frac{(D/H)_I^{Vst} - (D/H)_I^{Bst}}{(D/H)_I^{Vmes} - (D/H)_I^{Bmes}}$$

Example:

Standard samples supplied and standardized by the Community Bureau of Reference or other authoritative body:

$(D/H)_I^{Vst}$ = 102.0 ppm $(D/H)_I^{Bst}$ = 91.95 ppm

Standard samples measured by the laboratory:

$(D/H)_I^{Vmes}$ = 102.8 ppm $(D/H)_I^{Bmes}$ = 93.0 ppm

Reviewed non corrected sample:

$(D/H)_I^{Xmes}$ = 100.2 ppm

Calculation: α= 1.0255 and $(D/H)_I^{Xcorr}$ = 99.3 ppm

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RESOLUTION OIV/OENO 382A/2009

UPDATE OF THE OIV INTERNATIONAL COMPENDIUM OF METHODS OF ANALYSIS OF SPIRIT DRINKS OF VITIVINICULTURAL ORIGIN – PART 4

THE GENERAL ASSEMBLY

IN VIEW OF article 2 paragraph 2b iv of the agreement dated 3 April 2001 by which the international organisation of vine and wine was founded,

IN VIEW OF the actions of the 2009-2012 OIV strategic plan, in particular those aiming to reorganise the publications relating to vitivinicultural methods of analysis

CONSIDERING the work of the sub-commission of methods of analysis

IN VIEW OF the 1994 edition of the Compendium of international methods of analysis of spirituous beverages, alcohols and the aromatic fraction of beverages

IN VIEW OF the fact that for certain methods it would be, in the very least, possible to make data available concerning the dispersion of the results, based on proficiency-testing schemes

IN VIEW OF the fact that certain methods published in the current Compendium do indeed apply to spirit drinks of vitivinicultural origin

HAS DECIDED to introduce these methods into the "Compendium of international methods of analysis of spirituous beverages of vitivinicultural origin"

HAS DECIDED to adopt certain methods already present in the current Compendium as type IV methods, and to describe the following method as a Type II method of analysis**:** Determination of the principal compounds extracted from wood during ageing of spirit drinks of vitivinicultural origin

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Part 1: Retained type IV methods which appear in the 1994 edition of the Compendium of international methods of analysis of spirit drinks, alcohols and the aromatic fraction of beverages – for information purposes only

Method Title	***Page N° of the 1994 edition of the Compendium***
Density	*47*
ABV by near-infrared spectroscopy	*66*
Indirect dry extract by calculation	*85*
pH	*113*
Ethyl carbamate	*154*
Colour intensity	*159*
Chromatic characteristics	*161*
Turbidity	*178*
Calcium	*186*
Copper	*188*
Iron	*190*
Lead	*12-92*
2-Propanol by GC	*293*
UV absorption of rectified alcohols of vitivinicultural origin	*306*
Determination of carbon-14 content by liquid scintillation spectrometry	*307*

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Part 2: Proposed new validated method

DETERMINATION OF THE PRINCIPAL COMPOUNDS EXTRACTED FROM WOOD DURING AGEING OF SPIRIT DRINKS OF VITIVINICULTURAL ORIGIN

Type II method
Year: 2009

1. PURPOSE AND APPLICABILITY.

The present method pertains to the determination of furfural, 5-hydroxymethylfurfural,5-methylfurfural, vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, gallic, ellagic , vanillic, and syringic acids, and scopoletin, by high-performance liquid chromatography.

2. PRINCIPLE.

Determination by high-performance liquid chromatography (HPLC), with detection by ultraviolet spectrophotometry at several wavelengths, and by spectrofluorimetry.

3. REAGENTS.

The reagents must be of analytical quality. The water used must be distilled water or water of at least equivalent purity. It is preferable to use microfiltered water with a resistivity of 18.2 M Ω.

3.1 96% vol. alcohol.

3.2 HPLC-quality methanol (Solvent B).

3.3 Acetic acid diluted to 0.5% vol. (Solvent A).

3.4 Mobile phases: (given only an example).
Solvent A (0.5% acetic acid) and solvent B (pure methanol). Filter through a membrane (porosity 0.45 µm). Degas in an ultrasonic bath, if necessary.

3.5 Reference standards of 99% minimum purity: furfural, 5-hydroxymethyl furfural, 5-methylfurfural, vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, gallic, ellagic, vanillic, and syringic acids, and scopoletin.

3.6 Reference solution: the standard substances are dissolved in a 50% vol. aqueous-alcoholic solution. The final concentrations in the reference solution should be of the order of:
furfural: 5 mg/L; 5-hydroxymethyl furfural: 10 mg/L; 5-methylfurfural 2 mg/L;
vanillin: 5 mg/L; syringaldehyde: 10 mg/L; coniferaldéhyde: 5 mg/L; sinapaldehyde: 5 mg/L; gallic acid: 10 mg/L; ellagic acid: 10 mg/L;
vanillic acid: 5 mg/L; syringic acid: 5 mg/L; scopoletin: 0.5 mg/L.

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4. APPARATUS.

Standard laboratory apparatus

4.1 A high-performance liquid chromatograph capable of functioning in binary gradient mode and equipped with:

4.1.1 A spectrophotometric detector capable of measuring at wavelengths from 280 to 313 nm. It is however preferable to work with a multiple wavelength detector with a diode array or similar, in order to confirm the purity of the peaks.

4.1.2 A spectrofluorimetric detector – excitation wavelength: 354 nm, emission wavelength: 446 nm (for the trace determination of scopoletin; which is also detectable at 313 nm by spectrophotometry).

4.1.3 An injection device capable of introducing 10 or 20 µL (for example) of the test sample.

4.1.4 A high-performance liquid chromatography column, RP C18 type, 5 µm maximum particle size.

4.2 Syringes for HPLC.

4.3 Device for membrane-filtration of small volumes.

4.4 Integrator-computer or recorder with performance compatible with the entire apparatus, and in particular, it must have several acquisition channels.

5. PROCEDURE.

5.1 Preparation of the injection
The reference solution and the spirit drink are filtered if necessary through a membrane with a maximum pore diameter of 0.45 µm.

5.2 Chromatographic operating conditions: Carry out the analysis at ambient temperature under the conditions defined in 4.1 using the mobile phases (3.4) with a flow of approximately 0.6 ml per minute following the gradient below (given as an example only)

Time:	0 min	50 min	70 min	90 min
solvent A (water-acid):	100 %	60 %	100 %	100 %
solvent B (methanol):	0 %	40 %	0 %	0 %

Note that in certain cases this gradient should be modified to avoid co-elutions.

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5.3 Determination

5.3.1 Inject the reference standards separately, then mixed.
Adapt the operating conditions so that the resolution factors of the peaks of all the compounds are equal to at least 1.

5.3.2 Inject the sample as prepared in 5.1, after filtering it through a membrane.

5.3.3 Measure the area of the peaks in the reference solution and the spirit drink and calculate the concentrations.

6. EXPRESSION OF RESULTS.

Express the concentration of each constituent in mg/l.

7. PERFORMANCE CHARACTERISICS OF THE METHOD (PRECISION)

The following data were obtained in 2009 from an international method-performance study on a variety of spirit drinks, carried out following internationally-agreed procedures.

Key to the tables below:

nLT	Number of participating laboratories
nL	Number of laboratories used to calculate precision data
r	repeatability limit
Sr	repeatability standard deviation
RSDr	repeatability standard deviation expressed as % of the mean
R	reproducibility limit
SR	reproducibility standard deviation
RSDR	reproducibility standard deviation expressed as % of the mean
PRSDR	RSDR predicted with the Horwitz formula (%)
HoR	HorRat value = RSDR / PRSDR

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7.1 Gallic acid

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	16	15	1.2	0.2	0.07	6.1	1.2	0.43	36	16	2.3
Brandy	15	14	0.4	0.1	0.04	8.1	0.6	0.20	47	18	2.6
Rum	16	16	2.0	0.2	0.06	2.9	1.7	0.62	31	14	2.1
Cognac 1	16	16	6.1	0.5	0.18	3.0	9.1	3.3	53	12	4.4
Bourbon	16	16	7.3	0.5	0.18	2.4	6.2	2.2	30	12	2.6
Cognac 2	16	16	21.8	1.7	0.60	2.8	21.7	7.7	35	10	3.5

7.2 5-Hydroxymethylfurfural

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	16	14	5.0	0.2	0.09	1.7	1.1	0.39	8	13	0.6
Brandy	16	14	11.1	0.3	0.09	0.8	2.8	1.01	9	11	0.8
Rum	16	14	9.4	0.3	0.09	1.0	1.4	0.50	5	11	0.5
Cognac 1	16	14	33.7	1.2	0.42	1.3	12.5	4.5	13	9	1.4
Bourbon	16	14	5.8	0.2	0.07	1.2	1.1	0.4	7	12	0.6
Cognac 2	16	14	17.5	0.4	0.13	0.8	4.6	1.6	9	10	0.9

7.3 Furfural

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	15	14	2.9	0.1	0.04	1.4	0.7	0.24	8	14	0.6
Brandy	15	12	1.2	0.2	0.05	4.5	0.5	0.18	15	16	0.9
Rum	15	13	1.7	0.1	0.04	2.3	0.3	0.09	5	15	0.4
Cognac 1	15	14	10.6	0.5	0.18	1.7	3.8	1.4	13	11	1.1
Bourbon	15	13	15.3	0.6	0.23	1.5	1.4	0.49	3	11	0.3
Cognac 2	15	13	13.9	0.6	0.20	1.5	1.9	0.69	5	11	0.5

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7.4 Vanillic acid

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	15	12	0.2	0.1	0.03	14.2	0.2	0.06	28	20	1.4
Brandy	15	11	0.2	0.1	0.04	16.5	0.1	0.05	20	20	1.0
Rum	15	14	1.5	0.1	0.03	2.3	1.4	0.51	35	15	2.3
Cognac 1	15	14	0.8	0.3	0.10	12.6	0.7	0.2	31	17	1.9
Bourbon	15	15	2.4	0.4	0.13	5.3	3.4	1.22	51	14	3.6
Cognac 2	15	14	2.7	0.6	0.21	7.7	2.0	0.70	26	14	1.9

7.5 5-Methylfurfural

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	11	11	0.1	0.0	0.01	10.7	0.1	0.03	35	24	1.5
Brandy	11	11	0.2	0.0	0.01	6.1	0.1	0.04	18	20	0.9
Rum	11	8	0.1	0.1	0.02	13.6	0.1	0.03	22	22	1.0
Cognac 1	11	11	0.5	0.1	0.02	4.7	0.5	0.18	39	18	2.2
Bourbon	11	10	1.7	0.1	0.03	2.0	0.6	0.20	12	15	0.8
Cognac 2	11	11	0.8	0.2	0.07	10.0	0.7	0.26	35	17	2.1

7.6 Syringic acid

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	16	16	0.4	0.1	0.03	6.7	0.2	0.08	19	18	1.0
Brandy	15	15	0.2	0.1	0.02	12.6	0.1	0.05	29	21	1.4
Rum	16	15	2.5	0.2	0.06	2.3	0.8	0.29	11	14	0.8
Cognac 1	16	15	1.4	0.4	0.13	9.0	0.7	0.26	18	15	1.2
Bourbon	16	16	3.4	0.2	0.08	2.3	1.2	0.43	13	13	0.9
Cognac 2	16	15	4.8	0.3	0.11	2.3	1.9	0.67	14	13	1.1

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7.7 Vanillin

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	16	16	0.5	0.1	0.03	6.8	0.3	0.09	19	18	1.1
Brandy	15	15	0.2	0.1	0.02	9.6	0.2	0.06	25	20	1.2
Rum	16	16	1.2	0.2	0.06	4.6	0.5	0.18	15	16	1.0
Cognac 1	16	16	1.2	0.3	0.11	8.9	0.8	0.27	22	16	1.4
Bourbon	16	16	3.2	0.3	0.11	3.5	1.2	0.41	13	13	0.9
Cognac 2	16	16	3.9	0.3	0.09	2.3	1.7	0.62	16	13	1.2

7.8 Syringaldehyde

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	16	13	1.0	0.1	0.03	2.6	0.2	0.08	8	16	0.5
Brandy	15	13	0.2	0.1	0.02	8.1	0.2	0.07	33	20	1.6
Rum	16	13	4.8	0.1	0.04	0.8	0.7	0.23	5	13	0.4
Cognac 1	16	12	3.2	0.2	0.08	2.6	0.5	0.19	6	14	0.4
Bourbon	16	14	10.5	0.3	0.10	0.9	1.1	0.39	4	11	0.3
Cognac 2	16	13	9.7	0.3	0.09	0.9	1.2	0.43	4	11	0.4

7.9 Scopoletin

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	10	9	0.09	0.007	0.0024	2.6	0.04	0.01	15	23	0.6
Brandy	10	8	0.04	0.002	0.0008	2.2	0.02	0.01	16	26	0.6
Rum	10	9	0.11	0.005	0.0018	1.6	0.07	0.03	23	22	1.0
Cognac 1	10	8	0.04	0.004	0.0014	3.3	0.02	0.01	17	26	0.7
Bourbon	10	8	0.65	0.015	0.0054	0.8	0.26	0.09	15	17	0.8
Cognac 2	10	8	0.15	0.011	0.0040	2.7	0.06	0.02	15	21	0.7

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7.10 Coniferaldéhyde

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	13	12	0.2	0.04	0.02	9.2	0.1	0.04	23	21	1.1
Brandy	12	12	0.2	0.04	0.02	9.8	0.1	0.04	27	21	1.3
Rum	13	13	0.6	0.07	0.03	4.6	0.3	0.11	21	18	1.2
Cognac 1	12	12	0.8	0.09	0.03	4.3	0.5	0.18	23	17	1.4
Bourbon	13	13	4.6	0.24	0.09	1.9	1.1	0.38	8	13	0.6
Cognac 2	13	13	1.3	0.16	0.06	4.5	0.7	0.25	19	15	1.2

7.11 Sinapaldehyde

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	14	14	0.3	0.06	0.02	7.5	0.2	0.09	31	19	1.6
Brandy	14	13	0.2	0.03	0.01	4.6	0.2	0.05	27	20	1.3
Rum	14	12	0.2	0.06	0.02	11.2	0.2	0.08	46	21	2.2
Cognac 1	14	13	1.6	0.17	0.06	3.7	0.6	0.20	13	15	0.8
Bourbon	15	13	8.3	0.38	0.14	1.6	2.3	0.81	10	12	0.8
Cognac 2	14	12	0.3	0.08	0.03	11.4	0.5	0.18	73	20	3.7

7.12 Ellagic acid

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	7	7	3.2	0.6	0.20	6.3	4.0	1.41	44	13	3.2
Brandy	7	7	1.0	0.4	0.16	16	1.2	0.42	43	16	2.7
Rum	7	7	9.5	0.9	0.30	3.2	11	4.0	42	11	3.7
Cognac 1	7	7	13	1.1	0.41	3.2	14	5.0	39	11	3.6
Bourbon	7	7	13	2.7	0.95	7.4	14	4.9	39	11	3.5
Cognac 2	7	6	36	1.0	0.34	1.0	40	14	40	9	4.3

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