Commission Implementing Regulation (EU) 2015/1833 of 12 October 2015 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis

COMMISSION IMPLEMENTING REGULATION (EU) 2015/1833

of 12 October 2015

amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EU) No 1308/2013 of the European Parliament and of the Council of 17 December 2013 establishing a common organisation of the markets in agricultural products and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007[(1)], and in particular point (d) of the first paragraph and the second paragraph of Article 91 thereof,

Whereas:

(1) Commission Regulation (EEC) No 2568/91[(2)] defines the physico-chemical and organoleptic characteristics of olive and olive-pomace oil and lays down methods of assessing those characteristics. Those methods are regularly updated on the basis of the opinion of chemical experts and in line with the work carried out within the International Olive Council (IOC).

(2) In order to ensure the implementation at Union level of the most recent international standards established by the IOC, certain methods of analysis set out in Regulation (EEC) No 2568/91 should be updated.

(3) In the light of the experience it appears that the method for the detection of extraneous vegetable oils in olive oils may produce false positives. Therefore, references to that method should be deleted.

(4) Regulation (EEC) No 2568/91 should therefore be amended accordingly.

(5) The measures provided for in this Regulation are in accordance with the opinion of the Committee for the Common Organisation of the Agricultural Markets,

HAS ADOPTED THIS REGULATION:

Article 1

Regulation (EEC) No 2568/91 is amended as follows:

(1) Article 2(1) is amended as follows:

(a) the first subparagraph is amended as follows:

(i) point (g) is replaced by the following:

(g) for the determination of the fatty acid composition, the method set out in Annex X;;

(ii) point (l) is replaced by the following:

(l) for the determination of the aliphatic and triterpenic alcohols content, the method set out in Annex XIX;;

(b) the second subparagraph is deleted;

(2) the summary of the Annexes is amended as follows:

(a) the references to Annex X A and Annex X B, including the titles of those Annexes, are replaced by the following single reference:

Annex X Determination of fatty acid methyl esters by gas chromatography;

(b) in the reference to Annex XIX, the title is replaced by the following: Determination of aliphatic and triterpenic alcohols content by capillary gas chromatography;

(c) the reference to Annex XXa is deleted;

(3) Appendix 1 to Annex Ib is amended in accordance with Annex I to this Regulation;

(4) Annex V is amended in accordance with Annex II to this Regulation;

(5) Annex IX is replaced by the text set out in Annex III to this Regulation;

(6) Annexes X A and X B are replaced by the text set out in Annex IV to this Regulation;

(7) Annex XII is amended in accordance with Annex V to this Regulation;

(8) Annex XIX is amended in accordance with Annex VI to this Regulation;

(9) Annex XXa is deleted.

Article 2

This Regulation shall enter into force on the third day following that of its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 12 October 2015.

For the Commission

The President

Jean-Claude JUNCKER

ANNEX I

In Appendix 1 to Annex Ib to Regulation (EEC) No 2568/91, the table of equivalence is amended as follows:

(1) the rows relating to trans isomers of fatty acids and fatty acids content are replaced by the following:

—	Trans isomers of fatty acids	Annex X	Determination of fatty acid methyl esters by gas chromatography
—	Fatty acids content	Annex X	Determination of fatty acid methyl esters by gas chromatography

(2) the row relating to alipahatic alcohols is replaced by the following:

—	Aliphatic and triterpenic alcohols	Annex XIX	Determination of aliphatic and triterpenic alcohols content by capillary gas chromatography

ANNEX II

In Annex V to Regulation (EEC) No 2568/91, point 6.2 is replaced by the following:

6.2. Calculate the percentage of each individual sterol from the ratio of the relevant peak area to the total peak area for sterols:

$$sterol_x = \frac{A_x}{\sum A} \times 100$$

where:

A_x = peak area for x;
ΣA = total peak area for sterols.

ANNEX III

ANNEX IX

SPECTROPHOTOMETRIC INVESTIGATION IN THE ULTRAVIOLET

FOREWORD

Spectrophotometric examination in the ultraviolet can provide information on the quality of a fat, its state of preservation and changes brought about by technological processes. The absorption at the wavelengths specified in the method is due to the presence of conjugated diene and triene systems resulting from oxidation processes and/or refining practices. These absorptions are expressed as specific extinctions

$E_{1\,\%}^{1\,cm}$
(the extinction of 1 % w/v solution of the fat in the specified solvent, in a 10 mm cell) conventionally indicated by K (also referred to as "extinction coefficient").

1. SCOPE

This Annex describes the procedure for performing a spectrophotometric examination of olive oil in the ultraviolet region.

2. PRINCIPLE OF THE METHOD

A sample is dissolved in the required solvent and the absorbance of the solution is measured at the specified wavelengths with reference to pure solvent.

The specific extinctions at 232 nm and 268 nm in iso-octane or 232 nm and 270 nm in cyclohexane are calculated for a concentration of 1 % w/v in a 10 mm cell.

3. EQUIPMENT

3.1. A spectrophotometer suitable for measurements at ultraviolet wavelengths (220 nm to 360 nm), with the capability of reading individual nanometric units. A regular check is recommended for the accuracy and reproducibility of the absorbance and wavelength scales as well as for stray light.

3.1.1. *Wavelength scale:* This may be checked using a reference material consisting of an optical glass filter containing holmium oxide or a holmium oxide solution (sealed or not) that has distinct absorption bands. The reference materials are designed for the verification and calibration of the wavelength scales of visible and ultraviolet spectrophotometers having nominal spectral bandwidths of 5 nm or less. The measurements are carried out against an air blank over the wavelength range of 640 to 240 nm, according to the instructions enclosed with the reference materials. A baseline correction is performed with an empty beam path at every slit width alteration. The wavelengths of the standard are listed in the certificate of the reference material.

3.1.2. *Absorbance scale:* This may be checked using commercially available sealed reference materials consisting of acidic potassium dichromate solutions, in certain concentrations and certified values of absorbance at its λmax (of 4 solutions of potassium dichromate in perchloric acid sealed in four UV quartz cells to measure the linearity and photometric accuracy reference in the UV). The potassium dichromate solutions are measured against a blank of the acid used, after baseline correction, according to the instructions enclosed with the reference material. The absorbance values are listed in the certificate of the reference material.

Another possibility in order to check the response of the photocell and the photomultiplier is to proceed as follows: weigh 0,2000 g of pure potassium chromate for spectrophotometry and dissolve in 0,05 N potassium hydroxide solution in a 1 000 ml graduated flask and make up to the mark. Take precisely 25 ml of the solution obtained, transfer to a 500 ml graduated flask and dilute up to the mark using the same potassium hydroxide solution.

Measure the extinction of the solution so obtained at 275 nm, using the potassium hydroxide solution as a reference. The extinction measured using a 1 cm cuvette should be 0,200 ± 0,005.

3.2. Rectangular quartz cuvettes, with covers, suitable for measurements at the ultraviolet wavelengths (220 to 360 nm) having an optical path-length of 10 mm. When filled with water or other suitable solvent the cuvettes should not show differences between them of more than 0,01 extinction units.

3.3. One- mark volumetric flasks, capacity 25 ml, class A.

3.4. Analytical balance, capable of being read to the nearest 0,0001 g

4. REAGENTS

During the analysis, unless otherwise stated, use only reagents of recognised analytical grade and distilled or demineralised water or water of equivalent purity.

Solvent: Iso-octane (2,2,4 trimethylpentane) for the measurements at 232 nm and 268 nm and cyclohexane for the measurements at 232 nm and 270 nm, having an absorbance less than 0,12 at 232 nm and less than 0,05 at 270 nm against distilled water, measured in a 10 mm cell.

5. PROCEDURE

5.1. The sample must be perfectly homogeneous and without suspended impurities. If not, it must be filtered through paper at a temperature of approximately 30 °C.

5.2. Weigh accurately approximately 0,25 g (to the nearest 1 mg) of the sample so prepared into a 25 ml graduated flask, make up to the mark with the specified solvent and homogenise. The resulting solution must be perfectly clear. If opalescence or turbidity is present, filter quickly through paper.

NOTE: Generally, a mass of 0,25 to 0,30 g is sufficient for absorbance measurements of virgin and extra virgin olive oils at 268 nm and 270 nm. For measurements at 232 nm, 0,05 g of sample are usually required, so two distinct solutions are usually prepared. For absorbance measurements of olive pomace oils, refined olive oils and adulterated olive oils, a smaller portion of sample, e.g. 0,1 g is usually needed due to their higher absorbance.

5.3. If necessary, correct the baseline (220-290 nm) with solvent in both quartz cells (sample and reference), then fill the sample quartz cell with the test solution and measure the extinctions at 232, 268 or 270 nm against the solvent used as a reference.

The extinction values recorded must lie within the range 0,1 to 0,8 or within the range of linearity of the spectrophotometer which should be verified. If not, the measurements must be repeated using more concentrated or more dilute solutions as appropriate.

5.4. After measuring the absorbance at 268 or 270 nm, measure the absorbance at λmax, λmax + 4 and λmax – 4. These absorbance values are used to determine the variation in the specific extinction (ΔK).

NOTE: λmax is considered to be 268 nm for isooctane used as solvent and 270 nm for cyclohexane.

6. EXPRESSION OF THE RESULTS

6.1. Record the specific extinctions (extinction coefficients) at the various wavelengths calculated as follows:

$$K\lambda = \frac{E\lambda}{c \times s}$$

where:

Kλ	=	specific extinction at wavelength λ;
Eλ	=	extinction measured at wavelength λ;
c	=	concentration of the solution in g/100 ml;
s	=	path length of the quartz cell in cm;

expressed to two decimal places.

6.2. Variation of the specific extinction (ΔK)

The variation of the absolute value of the extinction (ΔK) is given by:

$$\Delta K = \left| Km - \left(\frac{K\lambda m-4 + K\lambda m+4}{2} \right) \right|$$

where Km is the specific extinction at the wavelength for maximum absorption at 270 nm and 268 nm depending on the solvent used.

The results should be expressed to two decimal places.

ANNEX IV

ANNEX X DETERMINATION OF FATTY ACID METHYL ESTERS BY GAS CHROMATOGRAPHY1.SCOPE

This Annex gives guidance on the gas chromatographic determination of free and bound fatty acids in vegetable fats and oils following their conversion into fatty acid methyl esters (FAME).

The bound fatty acids of the triacylglycerols (TAGs) and, depending on the esterification method, the free fatty acids (FFA), are converted into fatty acid methyl esters (FAME), which are determined by capillary gas chromatography.

The method described in this Annex allows the determination of FAME from C_{12} to C_{24}, including saturated, cis- and transmonounsaturated and cis- and trans-polyunsaturated fatty acid methyl esters.

2.PRINCIPLE

Gas chromatography (GC) is used for the quantitative analysis of FAME. The FAME are prepared according to Part A. They are then injected into and vaporised within the injector. The separation of FAME is performed on analytical columns of specific polarity and length. A Flame Ionisation Detector (FID) is used for the detection of the FAME. The conditions of analysis are given in Part B.

Hydrogen or helium may be used as the carrier gas (mobile phase) in the gas chromatography of FAME with FID. Hydrogen speeds up separation and gives sharper peaks. The stationary phase is a microscopic layer of a thin liquid film on an inert solid surface made of fused silica.

As they pass through the capillary column the volatilised compounds being analysed interact with the stationary phase coating the inner surface of the column. Due to this different interaction of different compounds, they elute at a different time, which is called the retention time of the compound for a given set of analysis parameters. The comparison of the retention times is used for the identification of the different compounds.

PART APREPARATION OF THE FATTY ACID METHYL ESTERS FROM OLIVE OIL AND OLIVE-POMACE OIL1.SCOPE

This part specifies the preparation of the methyl esters of fatty acids. It includes methods for preparing fatty acid methyl esters from olive and olive-pomace oils.

2.FIELD OF APPLICATION

The preparation of the fatty acid methyl esters from olive oils and olive-pomace oils are performed by transesterification with methanolic solution of potassium hydroxide at room temperature. The necessity of purification of the sample prior to the trans-esterification depends on the sample's free fatty acids content and the analytical parameter to be determined, it can be chosen according to the following table:

Category of oil	**Method**
Virgin olive oil with acidity ≤ 2,0 %	1. Fatty acids 2. *trans*-Fatty acids 3. ΔECN42 (after purification with silica-gel SPE)
Refined olive oil	
Olive oil composed of refined olive oil and virgin olive oils	
Refined olive pomace oil	
Olive pomace oil	
Virgin olive oil with acidity > 2,0 % Crude olive pomace oil	1. Fatty acids (after purification with silica-gel SPE) 2. *trans*-Fatty acids (after purification with silica-gel SPE) 3. ΔECN42 (after purification with silica-gel SPE)

3.METHODOLOGY3.1.Trans-esterification with methanolic solution of potassium hydroxide at room temperature3.1.1.Principle

Methyl esters are formed by trans-esterification with methanolic potassium hydroxide as an intermediate stage before saponification takes place.
3.1.2.Reagents3.1.2.1.

Methanol containing not more than 0,5 % (m/m) water.
3.1.2.2.

Hexane, chromatographic quality.
3.1.2.3.

Heptane, chromatographic quality.
3.1.2.4.

Diethyl ether, stabilised for analysis.
3.1.2.5.

Acetone, chromatographic quality.
3.1.2.6.

Elution solvent for purifying the oil by column/SPE chromatography, mixture hexane/diethyl ether 87/13 (v/v).
3.1.2.7.

Potassium hydroxide, approximately 2M methanolic solution: dissolve 11,2 g of potassium hydroxide in 100 ml of methanol.
3.1.2.8.

Silica gel cartridges, 1 g (6 ml), for solid phase extraction.

3.1.3.Apparatus3.1.3.1.

Screw-top test tubes (5 ml volume) with cap fitted with a PTFE joint.
3.1.3.2.

Graduated or automatic pipettes, 2 ml and 0,2 ml.
3.1.4.Purification of oil samples

When necessary, the samples will be purified by passing the oil through a silica gel solid-phase extraction cartridge. A silica gel cartridge (3.1.2.8) is placed in a vacuum elution apparatus and washed with 6 ml of hexane (3.1.2.2); washing is performed without vacuum. Then a solution of the oil (0,12 g approximately) in 0,5 ml of hexane (3.1.2.2) is loaded onto the column. The solution is pulled down and then eluted with 10 ml of hexane/diethyl ether (87:13 v/v) (3.1.2.6). The combined eluates are homogenised and divided in two similar volumes. An aliquot is evaporated to dryness in a rotary evaporator under reduced pressure at room temperature. The residue is dissolved in 1 ml of heptane and the solution is ready for fatty acid analysis by GC. The second aliquot is evaporated and the residue is dissolved in 1 ml of acetone for triglyceride analysis by HPLC, if necessary.
3.1.5.Procedure

In a 5 ml screw-top test tube (3.1.3.1) weigh approximately 0,1 g of the oil sample. Add 2 ml of heptane (3.1.2.2), and shake. Add 0,2 ml of the methanolic potassium hydroxide solution (3.1.2.7), put on the cap fitted with a PTFE joint, tighten the cap, and shake vigorously for 30 seconds. Leave to stratify until the upper solution becomes clear. Decant the upper layer containing the methyl esters. The heptane solution is ready for injection into the gas chromatograph. It is advisable to keep the solution in the refrigerator until gas chromatographic analysis. Storage of the solution for more than 12 hours is not recommended.
PART BANALYSIS OF FATTY ACID METHYL ESTERS BY GAS CHROMATOGRAPHY1.SCOPE

This part gives general guidance for the application of capillary column gas chromatography to determine the qualitative and quantitative composition of a mixture of fatty acid methyl esters obtained in accordance with the method specified in Part A.

The part is not applicable to polymerised fatty acids.
2.REAGENTS2.1.Carrier gas

Inert gas (helium or hydrogen), thoroughly dried and with an oxygen content of less than 10 mg/kg.
Note 1:

Hydrogen can double the speed of analysis but is hazardous. Safety devices are available.
2.2.Auxiliary gases2.2.1.

Hydrogen (purity ≥ 99,9 %), free from organic impurities.
2.2.2.

Air or oxygen, free from organic impurities.
2.2.3.

Nitrogen (purity > 99 %).
2.3.Reference standard

Mixture of methyl esters of pure fatty acids, or the methyl esters of a fat of known composition, preferably similar to that of the fatty matter to be analysed. Cis and trans isomers of octadecenoic, octadecadienoic and octadecatrienoic methyl esters are useful for the identification of trans isomers of unsaturated acids.

Care should be taken to prevent the oxidation of polyunsaturated fatty acids.
3.APPARATUS

The instructions given are for the usual equipment used for gas chromatography, employing capillary columns and a flame-ionisation detector.
3.1.Gas chromatograph

The gas chromatograph shall comprise the following elements.
3.1.1.Injection system

Use an injection system with capillary columns, in which case the injection system should be specially designed for use with such columns. It may be of the split type or the splitless on-column injector type.
3.1.2.Oven

The oven shall be capable of heating the capillary column to a temperature of at least 260 °C and of maintaining the desired temperature to within 0,1 °C. The last requirement is particularly important when a fused silica tube is used.

The use of temperature-programmed heating is recommended in all cases, and in particular for fatty acids with less than 16 carbon atoms.
3.1.3.Capillary column3.1.3.1.

Tube, made of a material inert to the substances to be analysed (usually glass or fused silica). The internal diameter shall be between 0,20 to 0,32 mm. The internal surface shall undergo an appropriate treatment (e.g. surface preparation, inactivation) before receiving the stationary phase coating. A length of 60 m is sufficient for fatty acid and cis and trans isomers of fatty acids.
3.1.3.2.

Stationary phase, polar polysiloxane (cyanopropylsilicone) bonded (cross-linked) columns are suitable.
Note 2:

There is a risk that polar polysiloxanes may give rise to difficulties in the identification and separation of linolenic acid and C_{20} acids.

The coatings shall be thin, i.e. 0,1 to 0,2 μm.
3.1.3.3.Assembly and conditioning of the column

Observe the normal precautions for assembling capillary columns, i.e. arrangement of the column in the oven (support), choice and assembly of joints (leak tightness), positioning of the ends of the column in the injector and the detector (reduction of dead-spaces). Place the column under a flow of carrier gas (e.g. 0,3 bar (30 kPa) for a column of length 25 m and internal diameter 0,3 mm).

Condition the column by temperature programming of the oven at 3 °C/min from ambient temperature to a temperature 10 °C below the decomposition limit of the stationary phase. Maintain the oven at this temperature for one hour until stabilisation of the baseline. Return it to 180 °C to work under isothermal conditions.
Note 3:

Suitably pre-conditioned columns are available commercially.
3.1.4.Flame ionisation detector and converter-amplifier3.2.Syringe

The syringe shall have a maximum capacity of 10 µl, graduated in 0,1 µl divisions.
3.3.Data acquisition system

Data acquisition system connected online with the detectors and employed with a software program suitable for peak integration and normalisation.
4.PROCEDURE

The operations described in 4.1 to 4.3 are for the use of a flame-ionisation detector.
4.1.Test conditions4.1.1.Selection of optimum operating conditions for capillary columns

Owing to the efficiency and permeability of capillary columns, the separation of the constituents and the duration of the analysis are largely dependent on the flow-rate of the carrier gas in the column. It will therefore be necessary to optimise the operating conditions by adjusting this parameter (or simply column head loss) depending on whether the aim is to improve separation or speed up analysis.

The following conditions have proved to be suitable for the separation of FAMEs (C_4 to C_{26}). Examples of chromatograms are shown in Appendix B:

Injector temperature:	250 °C
Detector temperature:	250 °C
Oven temperature:	165 °C (8 min) to 210 °C at 2 °C/min
Carrier gas hydrogen:	column head pressure, 179 kPa
Total flow:	154,0 ml/min;
Split ratio:	1:100
Injection volume:	1 µl

4.1.2.Determination of the resolution (see Appendix A)

Calculate the resolution, R, of two neighbouring peaks I and II, using the formula:

$R = 2 \times ((d_{dr(II)} - d_{r(I)})/(\omega_{(I)} + \omega_{(II)}))$ or $R = 2 \times ((t_{r(II)} - t_{r(I)})/(\omega_{(I)} + \omega_{(II)}))$ (USP) (United States Pharmacopeia),

or

$R = 1{,}18 \times ((t_{r(II)} - t_{r(I)})/(\omega_{0,5(I)} + \omega_{0,5(II)}))$ (EP, BP, JP, DAB), (JP (Japanese Pharmacopeia), EP (Pharmacopée Européenne), BP (British Pharmacopeia))

where:

$d_{r(I)}$ is the retention distance of peak I;
$d_{r(II)}$ is the retention distance of peak II;
$t_{r(I)}$ is the retention time of peak I;
$t_{r(II)}$ is the retention time of peak II;
$\omega_{(I)}$ is the width of the base of peak I;
$\omega_{(II)}$ is the width of the base of peak II;

$\omega_{0,5}$ is the peak width of the specified compound, at mid-height of the peak;

If $\omega_{(I)} \approx \omega_{(II)}$, calculate R using the following formulas:

$R = (d_{r(II)} - d_{r(I)})/\omega = (d_{r(II)} - d_{r(I)})/4\sigma$

where:

σ is the standard deviation (see Appendix A, Figure 1).

If the distance dr between the two peaks $d_{r(II)}$ - $d_{r(I)}$ is equal to 4σ, the resolution factor R = 1.

If two peaks are not separated completely, the tangents to the inflection points of the two peaks intersect at point C. In order to completely separate the two peaks, the distance between the two peaks must be equal to:

$d_{r(II)}$ - $d_{r(I)}$ = 6 σ from where R = 1,5 (see Appendix A, Figure 3).
5.EXPRESSION OF RESULTS5.1.Qualitative analysis

Identify the methyl ester peaks of the sample from the chromatogram in Appendix B, figure 1, if necessary by interpolation, or by comparison with those of the methyl esters reference mixtures (as indicated at point 2.3).
5.2.Quantitative analysis5.2.1.Determination of the composition

Calculate the mass fraction w_i of the individual fatty acid methyl esters, expressed as a percentage by mass of methyl esters, as follows:
5.2.2.Method of calculation5.2.2.1.General case

Calculate the content of a given component i, expressed as a percentage by mass of methyl esters, by determining the percentage represented by the area of the corresponding peak relative to the sum of the areas of all the peaks, using the following formula:

$w_i = (A_i/\Sigma A) \times 100$

where:

A_i is the area under the peak of the individual fatty acid methyl ester *i*;

ΣA is the sum of the areas under all the peaks of all the individual fatty acid methyl esters.

The results are expressed to two decimal places.
Note 4:

For fats and oils, the mass fraction of the fatty acid methyl esters is equal to the mass fraction of the triacylglycerols in grams per 100 g. For cases in which this assumption is not allowed, see 5.2.2.2.
5.2.2.2.Use of correction factors

In certain cases, for example in the presence of fatty acids with less than eight carbon atoms or of acids with secondary groups, the areas shall be corrected with specific correction factors (Fci). These factors shall be determined for each single instrument. For this purpose suitable reference materials with certified fatty acid composition in the corresponding range shall be used.
Note 5:

These correction factors are not identical to the theoretical FID correction factors, which are given in Appendix A, as they also include the performance of the injection system etc. However, in the case of bigger differences, the whole system should be checked for performance.

For this reference mixture, the mass percentage of the FAME *i* is given by the formula:

$w_i = (m_i / \Sigma m) \times 100$

where

m_i is the mass of the FAME **i** in the reference mixture;

Σm is the total of the masses of the various components as FAMEs of the reference mixture.

From the chromatogram of the reference mixture, calculate the percentage by area for the FAME *i* as follows:

$w_i = (A_i/\Sigma A) \times 100$

where:

A_i is the area of the FAME *i* in the reference mixture;

ΣA is the sum of all the areas of all the FAMEs of the reference mixture.

The correction factor F_c is then

$F_c = (m_i \times \Sigma A)/(A_i/\Sigma m)$

For the sample, the percentage by mass of each FAME *i* is:

$w_i = (F_i \times A_i)/\Sigma (F_i \times A_i)$

The results are expressed to two decimal places.
Note 6:

The calculated value corresponds to the percentage of mass of the individual fatty acid calculated as triacylglycerols per 100 g fat.
5.2.2.3.Use of an internal standard

In certain analyses (for example where not all of the fatty acids are quantified, such as when acids with four and six carbons are present alongside acids with 16 and 18 carbons, or when it is necessary to determine the absolute amount of a fatty acid in a sample) it is necessary to use an Internal Standard. Fatty acids with 5, 15 or 17 carbons are frequently used. The correction factor (if any) for the Internal Standard should be determined.

The percentage by mass of component i, expressed as methyl esters, is then given by the formula:

$w_i = (m_{IS} \times F_i \times A_i)/(m \times F_{IS} \times A_{IS})$

where:

A_i is the area the FAME *i*;

A_{IS} is the area of the internal standard;

F_i is the correction factor of the fatty acid *i*, expressed as FAME;

F_{IS} is the correction factor of the internal standard;

m is the mass of the test portion, in milligrams
m_{IS} is the mass of the internal standard, in milligrams.

The results are expressed to two decimal places.
6.TEST REPORT

The test report shall specify the methods used for the preparation of the methyl esters and for the gas chromatographic analysis. It shall also mention all operating details not specified in this Standard Method, or regarded as optional, together with details of any incidents which may have influenced the results.

The test report shall include all the information necessary for complete identification of the sample.
7.PRECISION7.1.Results of interlaboratory test

Details of an interlaboratory test on the precision of the method are set out in Annex C to standard IOC/T.20/Doc. No 33. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.
7.2.Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than r given in tables 1 to 14 in Annex C to standard IOC/T.20/ Doc. No 33.
7.3.Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than R given in tables 1 to 14 in Annex C to standard IOC/T.20/Doc. No 33.

Appendix A

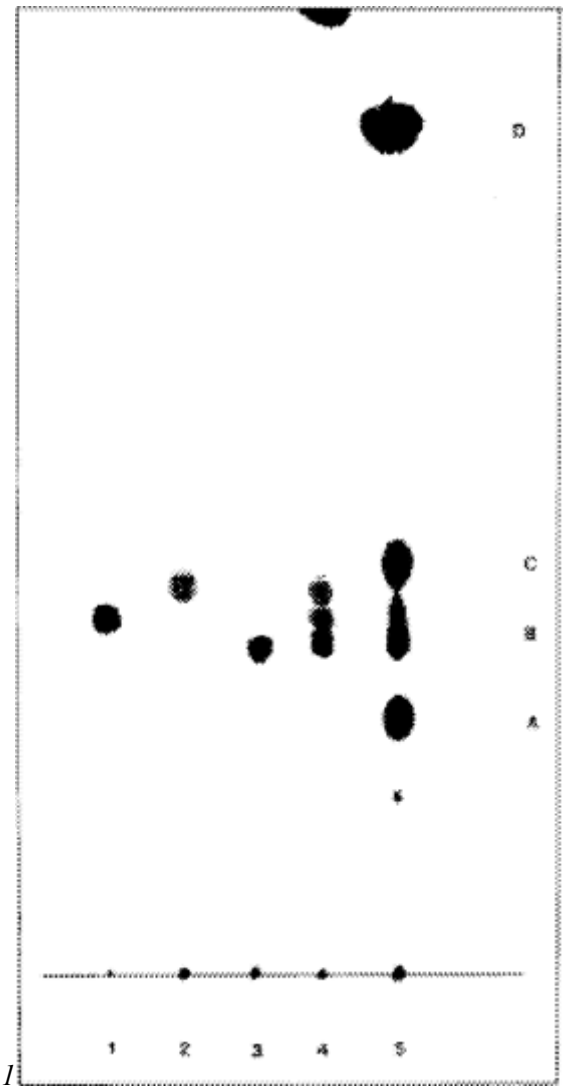
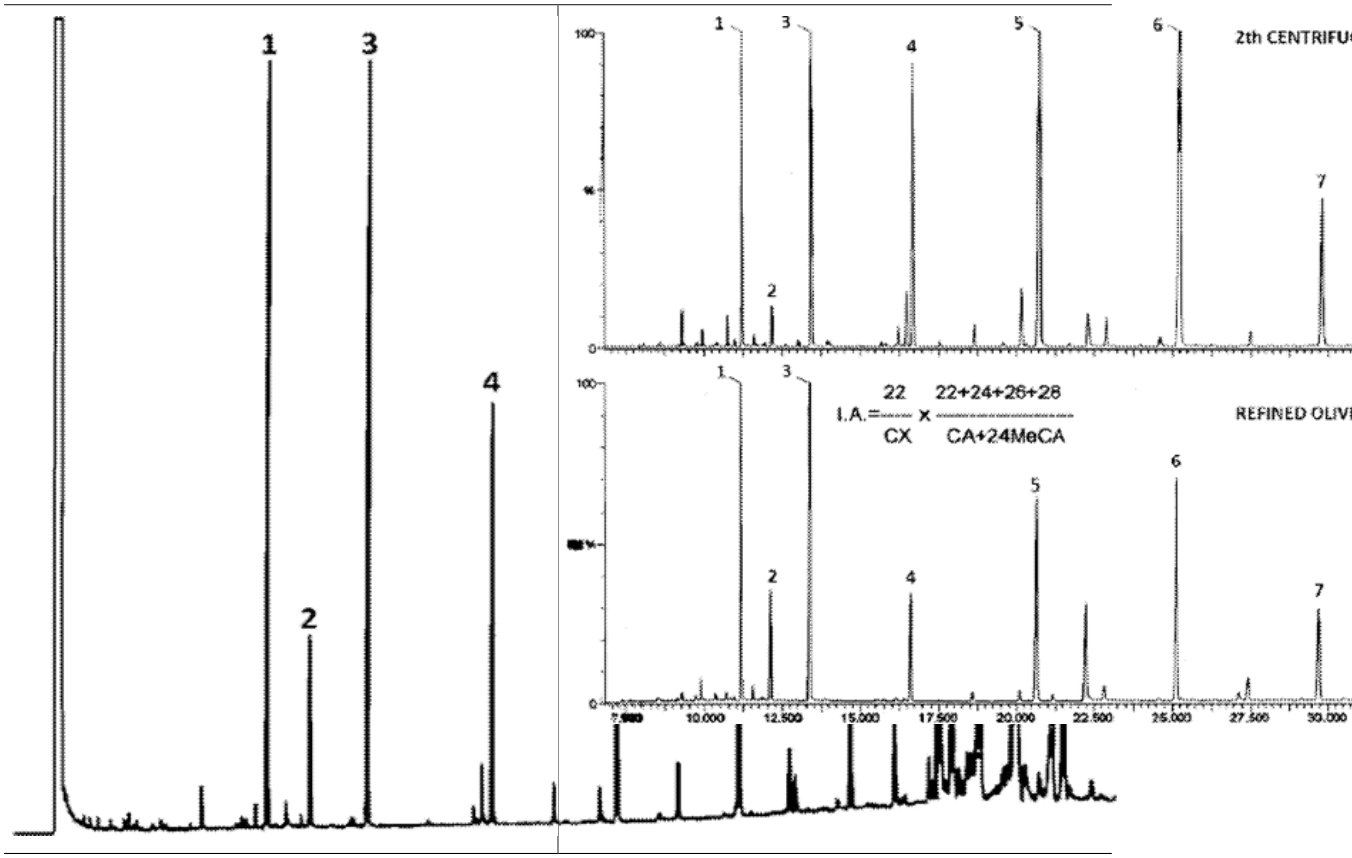


Figure 1

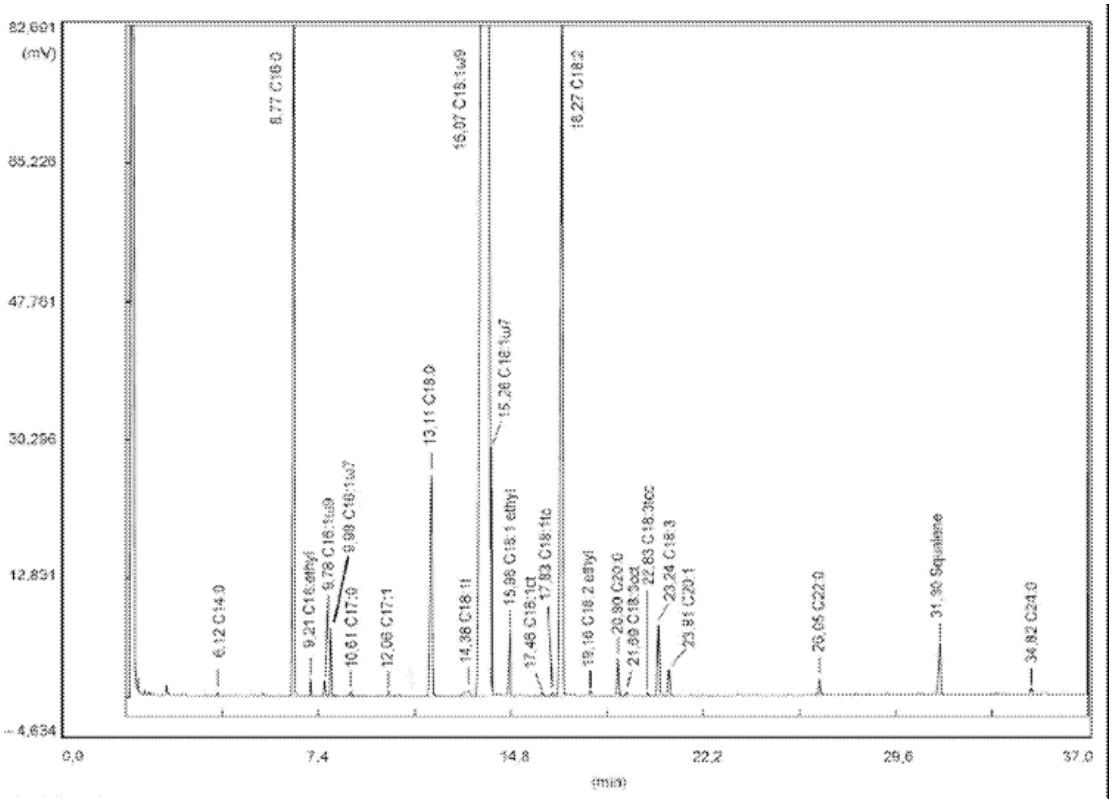
$\omega_{0,5}$ width at half height of the triangle (ABC) and b width at half height of the triangle (NPM).

Figure 2	*Figure 3*



Appendix B *Figure 1*

Gas chromatographic profile obtained by the cold methylation method from olive-pomace oil



The chromatographic peaks correspond to the methyl and ethyl esters except where otherwise indicated.

ANNEX V

Annex XII to Regulation (EEC) No 2568/91 is amended as follows:

(1) point 1 is replaced by the following:

1. PURPOSE AND SCOPE

The purpose of the international method described in this Annex is to determine the procedure for assessing the organoleptic characteristics of virgin olive oil within the meaning of point 1 of Part VIII of Annex VII to Regulation (EU) No 1308/2013 of the European Parliament and of the Council[(3)] and to establish the method for its classification on the basis of those characteristics. It also provides indications for optional labelling.

The method described is applicable only to virgin olive oils and to the classification or labelling of such oils according to the intensity of the defects perceived and of the fruitiness, as determined by a group of tasters selected, trained and monitored as a panel.

The IOC standards mentioned in this Annex are used in their last available version.;

(2) points 3.2, 3.3 and 3.4 are replaced by the following:

3.1.1. *Other negative attributes*

Heated or Burnt	Characteristic flavour of oils caused by excessive and/or prolonged heating during processing, particularly when the paste is thermally mixed, if this is done under unsuitable thermal conditions.
Hay-wood	Characteristic flavour of certain oils produced from olives that have dried out.
Rough	Thick, pasty mouthfeel sensation produced by certain old oils.
Greasy	Flavour of oil reminiscent of that of diesel oil, grease or mineral oil.
Vegetable water	Flavour acquired by the oil as a result of prolonged contact with vegetable water which has undergone fermentation processes.
Brine	Flavour of oil extracted from olives which have been preserved in brine.
Metallic	Flavour that is reminiscent of metals. It is characteristic of oil which has been in prolonged contact with metallic surfaces during crushing, mixing, pressing or storage.
Esparto	Characteristic flavour of oil obtained from olives pressed in new esparto mats. The flavour may differ depending on whether the mats are made of green esparto or dried esparto.
Grubby	Flavour of oil obtained from olives which have been heavily attacked by the grubs of the olive fly (*Bactrocera oleae*).
Cucumber	Flavour produced when an oil is hermetically packed for too long, particularly in tin containers, and which is attributed to the formation of 2,6 nonadienal.

3.2. **Positive attributes**

Fruity	Set of olfactory sensations characteristic of the oil which depends on the variety and comes from sound, fresh olives,

	either ripe or unripe. It is perceived directly and/or through the back of the nose.
Bitter	Characteristic primary taste of oil obtained from green olives or olives turning colour. It is perceived in the circumvallate papillae on the "V" region of the tongue.
Pungent	Biting tactile sensation characteristic of oils produced at the start of the crop year, primarily from olives that are still unripe. It can be perceived throughout the whole of the mouth cavity, particularly in the throat.

3.3. **Optional terminology for labelling purposes**

Upon request, the panel leader may certify that the oils which have been assessed comply with the definitions and ranges corresponding to the following adjectives according to the intensity and perception of the attributes.

Positive attributes (fruity, bitter and pungent): According to the intensity of perception:

— *Intense*, when the median of the attribute is more than 6,

— *Medium*, when the median of the attribute is between 3 and 6,

— *Light*, when the median of the attribute is less than 3.

Fruity	Set of olfactory sensations characteristic of the oil which depends on the variety of olive and comes from sound, fresh olives in which neither green nor ripe fruitiness predominates. It is perceived directly and/or through the back of the nose.
Greenly fruity	Set of olfactory sensations characteristic of the oil which is reminiscent of green fruit depends on the variety of olive and comes from green, sound, fresh olives. It is perceived directly and/or through the back of the nose.
Ripely fruity	Set of olfactory sensations characteristic of the oil which is reminiscent of ripe fruit depends on the variety of olive and comes from sound, fresh olives. It is perceived directly and/or through the back of the nose.
Well balanced	Oil which does not display a lack of balance, by which is meant the olfactory- gustatory and tactile sensation where the median of the bitter and/or

	pungent attributes is two points higher than the median of the fruitiness.
Mild oil	Oil for which the median of the bitter and pungent attributes is 2 or less.;

(3) in point 7, the following point is inserted after point 7.1:

7.1.1. *Deputy panel leader*

The panel leader may, on justified grounds, be replaced by a deputy panel leader who may stand in for duties regarding the performance of the tests. This substitute must have all the necessary skills required of a panel leader.;

(4) point 7.2 is replaced by the following:

7.2. **Tasters**

The people acting as tasters in organoleptic tests carried out on olive oils must do so voluntarily. It is therefore advisable for candidates to submit an application in writing. Candidates shall be selected, trained and monitored by the panel leader in accordance with their skills in distinguishing between similar samples; it should be borne in mind that their accuracy will improve with training.

Tasters must act like real sensory observers, setting aside their personal tastes and solely reporting the sensations they perceive. To do so, they must always work in silence, in a relaxed, unhurried manner, paying the fullest possible sensory attention to the sample they are tasting.

Between 8 and 12 tasters are required for each test, although it is wise to keep some extra tasters in reserve to cover possible absences.;

(5) point 9.3 is replaced by the following:

9.3. **Use of the data by the panel leaders**

The panel leader shall collect the profile sheets completed by each taster and shall review the intensities assigned to the different attributes. Should they find any anomaly, they shall invite the taster to revise his or her profile sheet and, if necessary, to repeat the test.

The panel leader shall enter the assessment data of each panel member in a computer program like that provided by the standard IOC/T.20/Doc. No 15 with a view to statistically calculating the results of the analysis, based on the calculation of their median. See point 9.4 and the Appendix to this Annex. The data for a given sample shall be entered with the aid of a matrix comprising 9 columns representing the 9 sensory attributes and n lines representing the n panel members used.

When a defect is perceived and entered under the “others” heading by at least 50 % of the panel, the panel leader shall calculate the median of the defect and shall arrive at the corresponding classification.

The value of the robust coefficient of variation which defines classification (defect with the strongest intensity and fruity attribute) must be no greater than 20 %.

If the opposite is the case, the panel leader must repeat the evaluation of the specific sample in another tasting session.

If this situation arises often, the panel leader is recommended to give the tasters specific additional training (IOC/T.20/Doc. No 14, § 5) and to use the repeatability index and deviation index to check taster performance (IOC/T.20/Doc. No 14, § 6).;

(6) point 9.4 is replaced by the following:

9.4. **Classification of the oil**

The oil is graded as follows in line with the median of the defects and the median for the fruity attribute. The median of the defects is defined as the median of the defect perceived with the greatest intensity. The median of the defects and the median of the fruity attribute are expressed to one decimal place.

The oil is graded by comparing the median value of the defects and the median for the fruity attribute with the reference ranges given below. The error of the method has been taken into account when establishing the limits of these ranges, which are therefore considered to be absolute. The software packages allow the grading to be displayed as a table of statistics or a graph.

(a) Extra virgin olive oil: the median of the defects is 0 and the median of the fruity attribute is above 0.

(b) Virgin olive oil: the median of the defects is above 0 but not more than 3,5 and the median of the fruity attribute is above 0.

(c) Lampante olive oil: the median of the defects is above 3,5 or the median of the defects is less than or equal to 3,5 and the fruity median is equal to 0.

Note 1: When the median of the bitter and/or pungent attribute is more than 5,0, the panel leader shall state so on the test certificate.

For assessments intended to monitor compliance, one test shall be carried out. In the case of counter assessments, the panel leader must arrange for the assessment to be carried out in duplicate in different sessions; the median of the attributes will be calculated on the basis of all the profile sheet data for both tests.;

(7) Figure 1 is replaced by the following:

Figure 1 **PROFILE SHEET FOR VIRGIN OLIVE OIL**

Intensity of perception of defects		
Fusty/muddy sediment		
Musty/humid/ earthy		
Winey/ vinegary acid/sour		
Frostbitten olives (wet wood)		
Rancid		

Other negative attributes:		
Descriptor:	Metallic # Dry hay # Grubby # Rough # Brine # Heated or burnt # Vegetable water # Esparto # Cucumber # Greasy #	
Intensity of perception of positive attributes		
Fruity		
	Green #	Ripe #
Bitter		
Pungent		
Name of taster:		Taster code:
Sample code:	Signature:	
Date:		
Comments:		

ANNEX VI

Annex XIX to Regulation (EEC) No 2568/91 is amended as follows:

(1) the title is replaced by the following:
DETERMINATION OF ALIPHATIC AND TRITERPENIC ALCOHOLS CONTENT BY CAPILLARY GAS CHROMATOGRAPHY;

(2) point 1 is replaced by the following:

1. SUBJECT MATTER

This Annex describes a method for the determination of aliphatic and triterpenic alcohols content in oils and fats.;

(3) point 4.11 is replaced by the following:

4.11. Reference solution for thin-layer chromatography: C_{20}-C_{28} alcohols 0,5 % in chloroform, or a fraction of alcohols obtained as indicated in point 5.2 from the unsaponifiable matter of an olive-pomace oil.;

(4) points 5.2.5 and 5.2.6 are replaced by the following:

5.2.5. The plate is sprayed lightly and evenly with the solution of 2′, 7′-dichlorofluorescein when the plate is observed under ultra violet light. The aliphatic alcohols band can be identified through being aligned with the stain obtained from the reference solution: mark the limits of the band with a black pencil; outlining the band of aliphatic alcohols and the band immediately above that, which is the terpenic alcohols band, together (Note 4).

Note 4: The aliphatic alcohols band and the terpenic alcohols band are to be grouped together in view of the possible migration of some aliphatic alcohols into

the triterpenic alcohols band. An example of the TLC separation in given in Figure 1 of the Appendix.

5.2.6. Using a metal spatula scrape off the silica gel in the marked area. Place the finely comminuted material removed into the filter funnel (3.7). Add 10 ml of hot chloroform, mix carefully with the metal spatula and filter under vacuum, collecting the filtrate in the conical flask (3.8) attached to the filter funnel.

Wash the silica gel in the flask three times with ethyl ether (approximately 10 ml each time) collecting the filtrate in the same flask attached to the funnel. Evaporate the filtrate to a volume of 4 to 5 ml, transfer the residual solution to the previously weighed 10 ml test tube (3.9), evaporate to dryness by mild heating in a gentle flow of nitrogen, make up again using a few drops of acetone, evaporate again to dryness, place in an oven at 105 °C for approximately 10 minutes and then allow to cool in a desiccator and weigh.

The residue inside the test tube is composed of the alcoholic fraction.;

(5) point 5.4.4 is replaced by the following:

5.4.4. *Peak identification.*

The identification of individual peaks is effected according to the retention times and by comparison with the standard TMSE mixture, analysed under the same conditions.

Examples of chromatogram of the alcoholic fraction of a refined olive oil is shown in Figures 2 and 3 of the Appendix.;

(6) the Appendix is replaced by the following:

‘Appendix

TLC separation example and chromatogram examples

Figure 1

Thin-layer chromatography plate of the unsaponifiable fraction from olive oil eluted with hexane/ethyl ether (65/35)

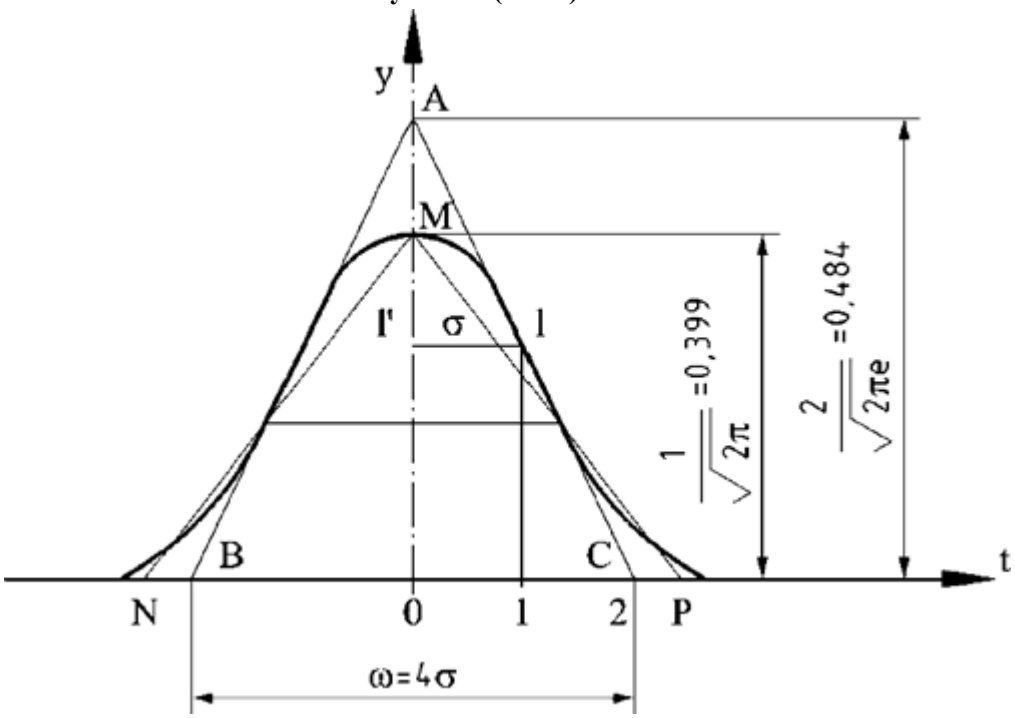


Figure 2

Chromatogram of the alcoholic fraction of a refined olive oil

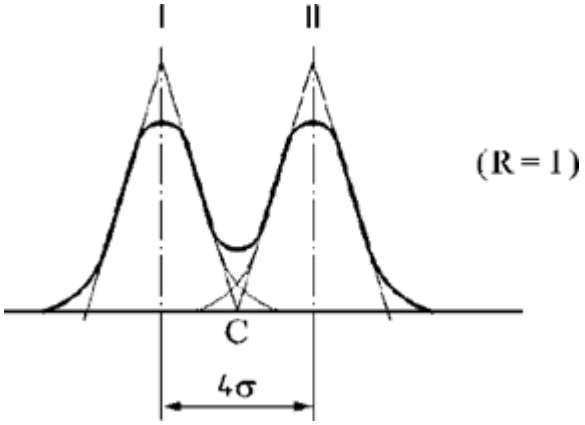
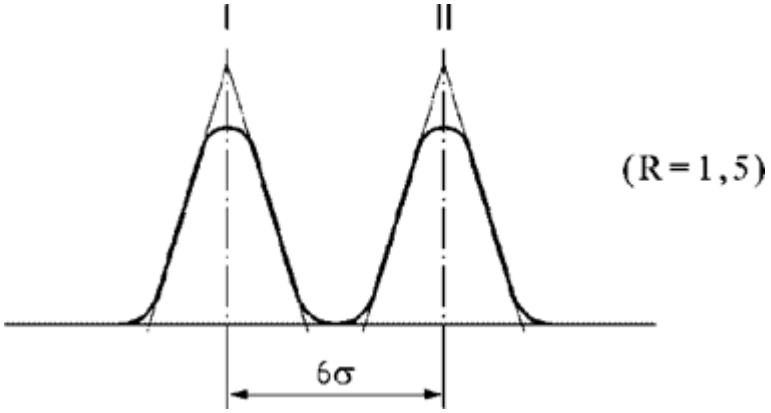


Figure 3

Aliphatic and triterpenic alcohols of a refined olive oil and a second centrifugation olive oil



(**1**) OJ L 347, 20.12.2013, p. 671.

(**2**) Commission Regulation (EEC) No 2568/91 of 11 July 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis (OJ L 248, 5.9.1991, p. 1).

(**3**) Regulation (EU) No 1308/2013 of the European Parliament and of the Council of 17 December 2013 establishing a common organisation of the markets in agricultural products and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007 (OJ L 347, 20.12.2013, p. 671).';

Changes to legislation:
There are currently no known outstanding effects for the Commission Implementing Regulation (EU) 2015/1833.