Commission Implementing Regulation (EU) No 299/2013 of 26 March 2013 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) No 299/2013

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THE EUROPEAN COMMISSION,

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

Having regard to Council Regulation (EC) No 1234/2007 of 22 October 2007 establishing a common organisation of agricultural markets and on specific provisions for certain agricultural products (Single CMO Regulation)[(1)], and in particular Article 113, paragraph 1, point (a), and Article 121, first paragraph, point (a), in conjunction with Article 4 thereof,

Whereas:

(1) 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[(2)] defines the chemical and organoleptic characteristics of olive and olive-residue oil and stipulates methods of assessing these characteristics. Those methods should be updated on the basis of the opinion of chemical experts and in line with the work carried out within the International Olive Council (hereinafter 'IOC').

(2) Pursuant to Article 113(3) of Regulation (EC) No 1234/2007, Member States are to check whether olive oils and olive-residue oils conform to the marketing standards laid down in Regulation (EEC) No 2568/91 and are to apply penalties as appropriate. Articles 2 and 2a of Regulation (EEC) No 2568/91 provide detailed rules for those conformity checks. Those rules should ensure that olive oil for which a quality standard has been laid down effectively complies with that standard. The rules should be further detailed, including a risk analysis. For the purpose of those conformity checks, the term 'marketed olive oil' should be defined.

(3) Experience has shown certain risks of fraud impeding the full effect of the consumer protection offered by Regulation (EEC) No 2568/91. Holders of olive oil should therefore keep entry and withdrawal for each category of oils in a register. In order to avoid excessive administrative burdens without undermining the objectives of the olive oil register, the gathering of information should be limited until the stage of bottling of olive oil.

(4) In order to ensure the follow-up and evaluate the measures of Regulation (EEC) No 2568/91, Member States should notify the Commission not only of the national implementing measures, but also report results of the conformity checks.

(5) In order to continue the process of harmonisation with the international standards laid down by the IOC certain methods of analysis laid down in Regulation (EEC) No 2568/91 should be updated. Consequently, the method of analysis provided for in Annex XVIII to that Regulation should be replaced by a more efficient method. It is also appropriate to remedy some inconsistencies and imperfections of the methods of analysis provided for in Annex IX thereto.

(6) A transitional period is needed for Member States in order to apply the new rules laid down by this Regulation.

(7) The Commission has developed an information system that allows managing documents and procedures electronically in its own internal working procedures and in its relations with the authorities involved in the common agricultural policy. It is considered that the notification obligations provided for in Regulation (EEC) No 2568/91 can be fulfilled via that system in accordance with Commission Regulation (EC) No 792/2009 of 31 August 2009 laying down detailed rules for the Member States' notification to the Commission of information and documents in implementation of the common organisation of the markets, the direct payments' regime, the promotion of agricultural products and the regimes applicable to the outermost regions and the smaller Aegean islands[(3)].

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

(9) The Management Committee for the Common Organisation of Agricultural Markets has not delivered an opinion within the time limit set by its chairman,

HAS ADOPTED THIS REGULATION:

Article 1

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

(1) Article 2a is replaced by the following:

Article 2a

1 For the purpose of this Article, "olive oil marketed" means total quantity of olive oil and olive pomace oil of a relevant Member State that is consumed in that Member State or exported from that Member State.

2 Member States shall ensure that conformity checks are carried out selectively, based on a risk analysis, and with appropriate frequency, so as to ensure that the olive oil marketed is consistent with the category declared.

3 The criteria to assess the risk may include:

a the category of oil, the period of production, the price of oils in relation to other vegetable oils, the blending and packing operations, the storage facilities and conditions, the country of origin, the country of destination, the means of transport or the volume of the lot;

b the position of the operators in the marketing chain, the volume and/or value marketed by them, the range of oil categories they market, the type of business carried out such as milling, storage, refining, blending, packaging or retail sale;

c findings made during previous checks including the number and type of defects found, the usual quality of oils marketed, the performance of technical equipment used;

d the reliability of operators' quality assurance systems or self-checking systems related to the conformity to marketing standards;

e the place where the check is carried out, in particular if it is the first point of entry into the Union, the last point of exit from the Union or the place where the oils are produced, packaged, loaded or sold to the final consumer;

f any other information that might indicate a risk of non-compliance.

4 Member States shall lay down in advance:

a the criteria for assessing the risk of non-conformity of lots;

b on the basis of a risk analysis for each risk category, the minimum number of operators or lots and/or quantities which will be subject to a conformity check.

At least one conformity check per thousand tonnes of olive oil marketed in the Member State shall be carried out per year.

5 Member States shall verify compliance by:

a carrying out, in any order, the analyses set out in Annex I; or

b following the order set out in Annex Ib on the decision tree until one of the decisions appearing in the decision tree is reached.;

(2) Article 3 is replaced by the following:

Article 3

Where it is found that an oil does not correspond to its category description, the Member State concerned shall, without prejudice to any other penalties, apply effective, proportionate and dissuasive penalties to be determined in the light of the seriousness of the irregularity detected.

Where checks reveal significant irregularities, Member States shall increase the frequency of checks in relation to marketing stage, oil category, origin, or other criteria.;

(3) the following Article 7a is inserted:

Article 7a

Natural or legal persons and groups of persons who hold olive oil and olive pomace oil from the extraction at the mill up to the bottling stage included, for whatever professional or commercial purposes, shall be required to keep entry and withdrawal registers for each category of such oils.

Member State shall ensure that the obligation laid down in the first paragraph is duly complied with.;

(4) Article 8 is replaced by the following:

Article 8

1 Member States shall notify the Commission of the measures implementing this Regulation. They shall inform the Commission of any subsequent amendments.

2 No later than 31 May of each year, Member States shall transmit to the Commission a report on the implementation of this Regulation during the previous

calendar year. The report shall contain at least the results of the conformity checks carried out on olive oils as per the templates set out in Annex XXI.

3 The notifications referred to in this Regulation shall be made in accordance with Commission Regulation (EC) No 792/2009[4].;

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

(6) Annex XVIII is replaced by the text set out in Annex II to this Regulation;

(7) Annex XXI, the text of which is set out in Annex III to this Regulation, is added.

Article 2

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

It shall apply from 1 January 2014. However, Article 8(2) shall apply from 1 January 2015.

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

Done at Brussels, 26 March 2013.

For the Commission

The President

José Manuel BARROSO

ANNEX I

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 in it by technological processes.

The absorption at the wavelengths specified in the method is due to the presence of conjugated diene and triene systems. These absorptions are expressed as specific extinctions $E^{1\%}_{1\ cm}$ (the extinction of 1 % solution of the fat in the specified solvent, in a thickness of 1 cm) conventionally indicated by K (also referred to as 'extinction coefficient').

1. SCOPE

The method describes the procedure for performing a spectrophotometric examination of olive oil (as described in the Appendix) in the ultraviolet.

2. PRINCIPLE OF THE METHOD

The fat in question is dissolved in the required solvent and the extinction of the solution is then determined at the specified wavelengths with reference to pure solvent. Specific extinctions are calculated from the spectrophotometer readings. The specific absorbance at 232 nm and 268 nm in iso-octane or 232 nm and 270 nm in cyclohexane for a concentration of 1 g per 100 ml in a 10 mm cell is calculated.

3. EQUIPMENT

3.1. A spectrophotometer for measuring extinction in the ultraviolet between 220 and 360 nm, with the possibility of reading individual nanometric units. Before use it is recommended that the wavelength and absorbance scales of the spectrometer be checked as follows.

3.1.1. *Wavelength scale:* This may be checked using a reference material consisting of an optical glass filter containing holmium oxide which has distinct absorption bands. The reference material is 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 holmium glass filter is measured in the absorbance mode against an air blank, over the wavelength range of 640 to 240 nm. For each spectral bandwidth (0,10 – 0,25 – 0,50 – 1,00 – 1,50 – 2,00 and 3,00), a baseline correction is performed with an empty cell holder. The wavelengths of the spectral bandwidth are listed in the certificate of the reference material in ISO 3656.

3.1.2. *Absorbance scale:* This may be checked using a reference material consisting 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 filled cells (40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml) are measured against a perchloric acid blank. The net absorbance values are listed in the certificate of the reference material in ISO 3656.

3.2. Rectangular quartz cells, with covers, having an optical length of 1 cm. When filled with water or other suitable solvent the cells should not show differences between them of more than 0,01 extinction units.

3.3. 25 ml graduated flasks.

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

4. REAGENTS

Use only reagents of recognized analytical grade, unless otherwise stated.

Solvent: Iso-octane (2,2,4-trimethylpentane) for the measurement at 232 nm and 268 nm or cyclohexane for the measurement at 232 nm and 270 nm, having an absorbance less than 0,12 at 232 nm and less than 0,05 at 250 nm against distilled water, measured in a 10 mm cell.

5. PROCEDURE

5.1. The sample in question must be perfectly homogeneous and without suspected impurities. Oils which are liquid at ambient temperature are to be filtered through paper at a temperature of approximately 30 °C, hard fats are to be homogenized and filtered at a temperature of not more than 10 °C above the melting point.

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 solvent specified and homogenize. The resulting solution must be perfectly clear. If opalescence or turbidity is present filter quickly through paper.

5.3. Fill a quartz cell with the solution obtained and measure the extinctions at an appropriate wavelength between 232 and 276 nm, using the solvent used as a reference.

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

NOTE: It may not be necessary to measure the absorbance over the full wavelength range.

6. EXPRESSION OF THE RESULTS

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

$$K_\lambda = \left(\frac{E_\lambda}{c \cdot s}\right)$$

where:

Kλ	=	specific extinction at wavelength λ,
Eλ	=	extinction measured at wavelength λ;
c	=	concentration of the solution in g/100 ml;
s	=	thickness of the quartz cells in cm.

The results are to be expressed to two decimal places.

6.2. **Variation of the specific extinction (ΔK)**

Spectrophotometric analysis of olive oil in accordance with the official method in the Union legislation involves also the determination of the variation of the absolute value of the specific extinction (ΔK), which is given by:

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

where Km is the specific extinction at wavelength m, the wavelength for maximum absorption depends on the solvent used: 270 for cyclohexane and 268 for iso-octane.

AppendixOLIVE OIL CHARACTERISTICSTotal isomers which could (or could not) be separated by capillary column.Or where the median defect is less than or equal to 3,5 and the fruity median is equal to 0.Oils with a wax content of between 300 mg/kg and 350 mg/kg are considered to be lampante olive oil if the total aliphatic alcohol content is less than or equal to 350 mg/kg or if the erythrodiol and uvaol content is less than or equal to 3,5 %.Oils with a wax content of between 300 mg/kg and 350 mg/kg are considered to be crude olive-pomace oil if the total aliphatic alcohol content is above 350 mg/kg and if the erythrodiol and uvaol content is greater than 3,5 %.K 270 if solvant is cyclohexane, K 268 if solvant is iso-octane.CategoryFatty acid methyl esters (FAMES) and fatty acid ethyl esters (FAEEs)Acidity (%) (*)Peroxide index mEq 02/kg (*)Waxes mg/kg (**)2 glyceril monopalmitate (%)Stigmastadiene mg/kgDifference: ECN42 (HPLC) and ECN42 (theoretical calculation)K232 (*)K270 (*) 'K 270 or K 268'Delta-K (*)Organoleptic evaluation Median defect (Md) (*)Organoleptic evaluation Fruity median (Mf) (*)1.Extra virgin olive oilΣ FAME + FAEE ≤75 mg/kg or 75 mg/kg <Σ FAME + FAEE ≤150 mg/kg and (FAEE/FAME) ≤1,5≤ 0,8≤ 20≤ 250≤ 0,9 if total palmitic acid % ≤ 14 %≤ 0,10≤ 0,2≤ 2,50≤ 0,22≤ 0,01Md = 0Mf > 0≤ 1,0 if total palmitic acid % > 14 %2.Virgin olive oil—≤ 2,0≤ 20≤ 250≤ 0,9 if total palmitic acid % ≤ 14 %≤ 0,10≤ 0,2≤ 2,60≤ 0,25≤ 0,01Md ≤ 3,5Mf > 0≤ 1,0 if total palmitic acid % > 14 %3.Lampante olive oil—> 2,0—≤ 300≤ 0,9 if total palmitic acid % ≤ 14 %≤ 0,50≤ 0,3———Md > 3,5—≤ 1,1 if total palmitic acid % > 14 %4.Refined olive oil —≤ 0,3≤ 5≤ 350≤ 0,9 if total palmitic acid % ≤ 14 %—≤ 0,3—≤ 1,10≤ 0,16——≤ 1,1 if total palmitic acid % > 14 %5.Olive oil composed of refined and virgin olive oils —≤ 1,0≤ 15≤ 350≤ 0,9 if total palmitic acid % ≤ 14 %—≤ 0,3—≤ 0,90≤ 0,15——≤ 1,0 if total palmitic acid % > 14 %6.Crude olive-pomace oil———> 350≤ 1,4—≤ 0,6 ————7.Refined olive-pomace oil—≤ 0,3≤ 5> 350≤ 1,4—≤ 0,5—≤ 2,00≤ 0,20 ——8.Olive-pomace oil≤ 1,0≤ 15> 350≤ 1,2—≤ 0,5—≤ 1,70≤ 0,18——

ANNEX II

ANNEX XVIII

DETERMINATION OF THE DIFFERENCE BETWEEN ACTUAL AND THEORETICAL CONTENT OF TRIACYLGLYCEROLS WITH ECN 42

1. SCOPE

Determination of the absolute difference between the experimental values of triacylglycerols (TAGs) with equivalent carbon number 42 ($ECN42_{HPLC}$) obtained by determination in the oil by high performance liquid chromatography and the theoretical value of TAGs with an equivalent carbon number of 42 ($ECN\ 42_{theoretical}$) calculated from the fatty acid composition.

2. FIELD OF APPLICATION

The standard is applicable to olive oils. The method is applicable to the detection of the presence of small amounts of seed oils (rich in linoleic acid) in every class of olive oils.

3. PRINCIPLE

The content of triacylglycerols with ECN 42 determined by HPLC analysis and the theoretical content of triacylglycerols with ECN 42 (calculated on the basis of GLC determination of fatty

acid composition) correspond within a certain limit for genuine olive oils. A difference larger than the values adopted for each type of oil points out that the oil contains seed oils.

4. METHOD

The method for the calculation of the theoretical content of triacylglycerols with ECN 42 and of the difference with respect to the HPLC data is essentially made by the coordination of analytical data obtained by means of other methods. It is possible to distinguish three phases: determination of fatty acid composition by capillary gas chromatography, calculation of theoretical composition of triacylglycerols with ECN 42, HPLC determination of ECN 42 triacylglycerols.

4.1. **Apparatus**

4.1.1. Round-bottomed flasks, 250 and 500 ml.

4.1.2. Beakers 100 ml.

4.1.3. Glass chromatographic column, 21 mm internal diameter, 450 mm length, with cock and normalised cone (female) at the top.

4.1.4. Separating funnels, 250 ml, with normalised cone (male) at the bottom, suitable for connection to the top of the column.

4.1.5. Glass rod, 600 mm length.

4.1.6. Glass funnel, 80 mm diameter.

4.1.7. Volumetric flasks, 50 ml.

4.1.8. Volumetric flasks, 20 ml.

4.1.9. Rotary evaporator.

4.1.10. High performance liquid chromatograph, allowing thermostatic control of column temperature.

4.1.11. Injection units for 10 µl delivery.

4.1.12. Detector: differential refractometer. The full scale sensitivity should be at least 10^{-4} units of refractive index.

4.1.13. Column: stainless steel tube 250 mm length x 4,5 mm internal diameter packed with 5 µm diameter particles of silica with 22 to 23 % carbon in the form of octadecylsilane.

4.1.14. Data processing software.

4.1.15. Vials, of about 2 ml volumes, with Teflon-layered septa and screw caps.

4.2. **Reagents**

The reagents should be of analytical purity. Elution solvents should be de-gassed, and may be recycled several times without effect on the separations.

4.2.1. Petroleum ether 40– 60 °C chromatographic grade or hexane.

4.2.2. Ethyl ether, peroxide-free, freshly distilled.

4.2.3. Elution solvent for purifying the oil by column chromatography mixture petroleum ether/ethyl ether 87/13 (v/v).

4.2.4. Silica gel, 70-230 mesh, type Merck 7734, with water content standardised at 5 % (w/w/).

4.2.5. Glass wool.

4.2.6. Acetone for HPLC.

4.2.7. Acetonitrile or propionitrile for HPLC.

4.2.8. HPLC elution solvent: acetonitrile + acetone (proportions to be adjusted to obtain the desired separation; begin with 50:50 mixture) or propionitrile.

4.2.9. Solubilisation solvent: acetone.

4.2.10. Reference triglycerides: commercial triglycerides (tripalmitin, triolein, etc.) may be used and the retention times then plotted in accordance with the equivalent carbon number, or alternatively reference chromatograms obtained from soya oil, mixture 30:70 soya oil — olive oil and pure olive oil (see notes 1 and 2 and figures 1 to 4).

4.2.11. Solid phase extraction column with silica phase 1 g, 6 ml.

4.3. **Sample preparation**

As a number of interfering substances can give rise to false positive results, the sample must always be purified according to IUPAC method 2.507, used for the determination of polar compounds in frying fats.

4.3.1. *Chromatographic column preparation*

Fill the column (4.1.3) with about 30 ml of elution solvent (4.2.3), then introduce inside the column some glass wool (4.2.5) pushing it to the bottom of the column by means of the glass rod (4.1.5).

In a 100 ml beaker, suspend 25 g of silica gel (4.2.4) in 80 ml of elution mixture (4.2.3), then transfer it to the column by means of a glass funnel (4.1.6).

To ensure the complete transfer of the silica gel to the column, wash the beaker with the elution mixture and transfer the washing portions to the column too.

Open the cock and let the solvent elute from the column until its level is about 1 cm over the silica gel.

4.3.2. *Column chromatography*

Weigh with the accuracy of 0,001 g, 2,5 ± 0,1 g of oil, previously filtered, homogenised and anhydrified, if necessary, in a 50 ml volumetric flask (4.1.7).

Dissolve it in about 20 ml of elution solvent (4.2.3). If necessary, slightly heat it to make the dissolution easily. Cool at room temperature and adjust the volume with elution solvent.

By means of a volumetric pipette, introduce 20 ml of solution inside the column prepared according to 4.3.1, open the cock and let the solvent elute to the silica gel layer level.

Then elute with 150 ml of elution solvent (4.2.3), adjusting the solvent rate at about 2 ml/min (150 ml will take about 60-70 minutes to pass through the column).

The eluate is recovered in a 250 ml round-bottomed flask (4.1.1) previously tared in an oven and exactly weighed. Eliminate the solvent at reduced pressure in a rotary evaporator (4.1.9)

and weigh the residue that will be used to prepare the solution for HPLC analysis and for methyl ester preparation.

The sample recovery from the column must be 90 % at least for the extra virgin, virgin, ordinary, refined and olive oil categories, and a minimum of 80 % for lampante and olive-pomace oils.

4.3.3. *SPE purification*

Silica SPE column is activated by passing 6 ml of hexane (4.2.3) under vacuum, avoiding dryness.

Weigh to an accuracy of 0,001 g, 0,12 g in a 2 ml vial (4.1.15) and dissolve with 0,5 ml of hexane (4.2.3).

Load the SPE column with the solution and elute with 10 ml of hexane-diethyl ether (87:13 v/v) (4.2.3) under vacuum.

The collected fraction is evaporated to dryness in a rotary evaporator (4.1.9) under reduced pressure at room temperature. The residue is dissolved in 2 ml of acetone (4.2.6) for triacylglycerol (TAG) analysis.

4.4. **HPLC analysis**

4.4.1. *Preparation of the samples for chromatographic analysis*

A 5 % solution of the sample to be analysed is prepared by weighing 0,5 ± 0,001 g of the sample into a 10 ml graduated flask and making up to 10 ml with the solubilisation solvent (4.2.9).

4.4.2. *Procedure*

Set up the chromatographic system. Pump elution solvent (4.2.8) at a rate of 1,5 ml/min to purge the entire system. Wait until a stable base line is obtained.

Inject 10 µl of the sample prepared as in point 4.3.

4.4.3. *Calculation and expression of results*

Use the area normalisation method, i.e. assume that the sum of the areas of the peaks corresponding to TAGs from ECN 42 up to ECN 52 is equal to 100 %.

Calculate the relative percentage of each triglyceride using the formula:

$$\% \text{ triglyceride} = \text{area of peak} \times 100 / \text{sum of peak areas}$$

.

The results should be given to at least two decimal places.

See notes 1 to 4.

4.5. **Calculation of triacylglycerols composition (moles %) from fatty acid composition data (area %)**

4.5.1. *Determination of fatty acid composition*

Fatty acid composition is determined by ISO 5508 by means of a capillary column. The methyl esters are prepared according to COI/T.20/Doc. No 24.

4.5.2. *Fatty acids for calculation*

Glycerides are grouped by their Equivalent Carbon Number (ECN), taking into account the following equivalencies between ECN and fatty acids. Only fatty acids with 16 and 18 carbon atoms were taken into consideration, because only these are important for olive oil. The fatty acids should be normalised to 100 %.

Fatty acid (FA)	**Abbreviation**	**Molecular weight(MW)**	**ECN**
Palmitic acid	P	256,4	16
Palmitoleic acid	Po	254,4	14
Stearic acid	S	284,5	18
Oleic acid	O	282,5	16
Linoleic acid	L	280,4	14
Linolenic acid	Ln	278,4	12

4.5.3. *Conversion of area % into moles for all fatty acids (1)*

$\text{moles P} = \frac{\text{area \% P}}{\text{MW P}}$	$\text{moles S} = \frac{\text{area \% S}}{\text{MW S}}$	$\text{moles Po} = \frac{\text{area \% Po}}{\text{MW Po}}$
$\text{moles O} = \frac{\text{area \% O}}{\text{MW O}}$	$\text{moles L} = \frac{\text{area \% L}}{\text{MW L}}$	$\text{moles Ln} = \frac{\text{area \% Ln}}{\text{MW Ln}}$

4.5.4. *Normalisation of fatty acid moles to 100 % (2)*

$$\text{moles \% P (1,2,3)} = \frac{\text{moles P} * 100}{\text{moles(P + S + Po + O + L + Ln)}}$$

$$\text{moles \% S (1,2,3)} = \frac{\text{moles S} * 100}{\text{moles(P + S + Po + O + L + Ln)}}$$

$$\text{moles \% Po (1,2,3)} = \frac{\text{moles Po} * 100}{\text{moles(P + S + Po + O + L + Ln)}}$$

$$\text{moles \% O (1,2,3)} = \frac{\text{moles O} * 100}{\text{moles(P + S + Po + O + L + Ln)}}$$

$$\text{moles \% L (1,2,3)} = \frac{\text{moles L} * 100}{\text{moles(P + S + Po + O + L + Ln)}}$$

$$\text{moles \% Ln (1,2,3)} = \frac{\text{moles Ln} * 100}{\text{moles(P + S + Po + O + L + Ln)}}$$

The result gives the percentage of each fatty acid in moles % in the overall (1, 2, 3–) position of the TAGs.

Then the sum of the saturated fatty acids P and S (SFA) and the unsaturated fatty acids Po, O, L and Ln (UFA) are calculated (3):

$$\text{moles \% SFA} = \text{moles \% P} + \text{moles \% S}$$

$$\text{moles \% UFA} = 100 - \text{moles \% SFA}$$

4.5.5. *Calculation of the fatty acid composition in 2- and 1, 3- positions of TAGs*

The fatty acids are distributed to three pools as follows: one for 2- position and two identical for 1- and 3- positions, with different coefficients for the saturated (P and S) and unsaturated acids (Po, O, L and Ln).

4.5.5.1. Saturated fatty acids in 2-position [P(2) and S(2)] (4):

$$\text{moles \% P (2)} = \text{moles \% P (1,2,3)} * 0{,}06$$

$$\text{moles \% S (2)} = \text{moles \% S (1,2,3)} * 0{,}06$$

4.5.5.2. Unsaturated fatty acids in 2-position [Po(2), O(2), L(2) and Ln(2)] (5):

$$\text{moles \% Po (2)} = \frac{\text{moles \% Po(1,2,3)}}{\text{moles \% UFA}} * (100 - \text{moles \% P (2)} - \text{moles \% S (2)})$$

$$\text{moles \% O (2)} = \frac{\text{moles \% O(1,2,3)}}{\text{moles \% UFA}} * (100 - \text{moles \% P (2)} - \text{moles \% S (2)})$$

$$\text{moles \% L (2)} = \frac{\text{moles \% L(1,2,3)}}{\text{moles \% UFA}} * (100 - \text{moles \% P (2)} - \text{moles \% S (2)})$$

$$\text{moles \% Ln (2)} = \frac{\text{moles \% Ln(1,2,3)}}{\text{moles \% UFA}} * (100 - \text{moles \% P (2)} - \text{moles \% S (2)})$$

4.5.5.3. Fatty acids in 1,3-positions [P(1,3), S(1,3), Po(1,3), O(1,3), L(1,3) and Ln(1,3)] (6):

$$\text{moles \% P (1,3)} = \frac{\text{moles \% P(1,2,3)} - \text{moles \% P(2)}}{2} + \text{moles \% P (1,2,3)}$$

$$\text{moles \% S (1,3)} = \frac{\text{moles \% S(1,2,3)} - \text{moles \% S(2)}}{2} + \text{moles \% S (1,2,3)}$$

$$\text{moles \% Po (1,3)} = \frac{\text{moles \% Po(1,2,3)} - \text{moles \% Po(2)}}{2} + \text{moles \% Po (1,2,3)}$$

$$\text{moles \% O (1,3)} = \frac{\text{moles \% O(1,2,3)} - \text{moles \% O(2)}}{2} + \text{moles \% O (1,2,3)}$$

$$\text{moles \% L (1,3)} = \frac{\text{moles \% L(1,2,3)} - \text{moles \% L(2)}}{2} + \text{moles \% L (1,2,3)}$$

$$\text{moles \% Ln (1,3)} = \frac{\text{moles \% Ln(1,2,3)} - \text{moles \% Ln(2)}}{2} + \text{moles \% Ln (1,2,3)}$$

4.5.6. *Calculation of triacylglycerols*

4.5.6.1. TAGs with one fatty acid (AAA, here LLL, PoPoPo) (7)

$$\text{moles \% AAA} = \frac{\text{moles \% A(1,3)} * \text{moles \% A(2)} * \text{moles \% A(1,3)}}{10\,000}$$

4.5.6.2. TAGs with two fatty acids (AAB, here PoPoL, PoLL) (8)

$$\text{moles \% AAB} = \frac{\text{moles \% A(1,3)} * \text{moles \% A(2)} * \text{moles \% B(1,3)} * 2}{10\,000}$$

$$\text{moles \% ABA} = \frac{\text{moles \% A(1,3)} * \text{moles \% B(2)} * \text{moles \% A(1,3)}}{10\,000}$$

4.5.6.3. TAGs with three different fatty acids (ABC, here OLLn, PLLn, PoOLn, PPoLn) (9)

$$\text{moles \% ABC} = \frac{\text{moles \% A(1,3)} * \text{moles \% B(2)} * \text{moles \% C(1,3)} * 2}{10\,000}$$

$$\text{moles \% BCA} = \frac{\text{moles \% B(1,3)} * \text{moles \% C(2)} * \text{moles \% A(1,3)} * 2}{10\,000}$$

$$\text{moles \% CAB} = \frac{\text{moles \% C(1,3)} * \text{moles \% A(2)} * \text{moles \% B(1,3)} * 2}{10\,000}$$

4.5.6.4. Triacylglycerols with ECN42

The triacylglycerols with ECN42 are calculated according to equations 7, 8 and 9 and are then given in order of expected elution in HPLC (normally only three peaks).

LLL
PoLL and the positional isomer LPoL
OLLn and the positional isomers OLnL and LnOL
PoPoL and the positional isomer PoLPo
PoOLn and the positional isomers OPoLn and OLnPo
PLLn and the positional isomers LLnP and LnPL
PoPoPo
SLnLn and the positional isomer LnSLn
PPoLn and the positional isomers PLnPo and PoPLn

The triacylglycerols with ECN42 are given by the sum of the nine triacylglycerols including their positional isomers. The results should be given to at least two decimal places.

5. EVALUATION OF THE RESULTS

The calculated theoretical content and the content determined by the HPLC analysis are compared. If the difference in the absolute value of the HPLC data minus the theoretical data is greater than the values stated for the appropriate oil category in the standard, the sample contains seed oil.

Results are given to two decimal figures.

6. EXAMPLE (THE NUMBERS REFER TO THE SECTIONS IN THE TEXT OF THE METHOD)

— 4.5.1. *Calculation of moles % fatty acids from GLC data (normalised area %)*

The following data are obtained for the fatty acid composition by GLC:

FA	**P**	**S**	**Po**	**O**	**L**	**Ln**
MW	256,4	284,5	254,4	282,5	280,4	278,4
Area %	10,0	3,0	1,0	75,0	10,0	1,0

— 4.5.3 *Conversion of area % into moles for all fatty acids (see formula (1))*

moles P = $\frac{10}{256,4} = 0,03900$ moles P

moles S = $\frac{3}{284,5} = 0,01054$ moles S

moles Po = $\frac{1}{254,4} = 0,00393$ moles Po

moles O = $\frac{75}{282,5} = 0,26549$ moles O

moles L = $\frac{10}{280,4} = 0,03566$ moles L

moles Ln = $\frac{1}{278,4} = 0,00359$ moles Ln

Total = 0,35821 moles TAGs

— 4.5.4 *Normalisation of fatty acid moles to 100 % (see formula (2))*

moles % P(1,2,3) = $\frac{0,03900 \text{ moles P} * 100}{0,35821 \text{ moles}} = 10,887\ \%$

moles % S(1,2,3) = $\frac{0,01054 \text{ moles S} * 100}{0,35821 \text{ moles}} = 2,942\ \%$

moles % Po(1,2,3) = $\frac{0,00393 \text{ moles Po} * 100}{0,35821 \text{ moles}} = 1,097\ \%$

moles % O(1,2,3) = $\frac{0,26549 \text{ moles O} * 100}{0,35821 \text{ moles}} = 74,116\ \%$

moles % L(1,2,3) = $\frac{0,03566 \text{ moles L} * 100}{0,35821 \text{ moles}} = 9,955\ \%$

moles % Ln(1,2,3) = $\frac{0,00359 \text{ moles Ln} * 100}{0,35821 \text{ moles}} = 1,002\ \%$

Total moles % = 100 %

Sum of the saturated and unsaturated fatty acids in the 1,2,3-position of TAGs (see formula (3)):

$\text{moles \% SFA} = 10,887\ \% + 2,942\ \% = 13,829\ \%$

$\text{moles \% UFA} = 100,000\ \% - 13,829\ \% = 86,171\ \%$

— 4.5.5 *Calculation of the fatty acid composition in 2- and 1,3-positions of the TAGs*

— 4.5.5.1 Saturated fatty acids in 2-position [P(2) and S(2)] (see formula (4))

$\text{moles \% P (2)} = 10,887\ \% * 0,06 = 0,653 \text{ moles \%}$

$\text{moles \% S (2)} = 2,942\ \% * 0,06 = 0,177 \text{ moles \%}$

— 4.5.5.2 Unsaturated fatty acids in 2-position [Po(1,3), O(1,3), L(1,3) and Ln(1,3)] (see formula (5))

$$\text{moles \% Po (2)} = \frac{1{,}097\ \%}{86{,}171\ \%} * (100 - 0{,}653 - 0{,}177) = 1{,}262 \text{ moles \%}$$

$$\text{moles \% O (2)} = \frac{74{,}116\ \%}{86{,}171\ \%} * (100 - 0{,}653 - 0{,}177) = 85{,}296 \text{ moles \%}$$

$$\text{moles \% L (2)} = \frac{9{,}955\ \%}{86{,}171\ \%} * (100 - 0{,}653 - 0{,}177) = 11{,}457 \text{ moles \%}$$

$$\text{moles \% Ln (2)} = \frac{1{,}002\ \%}{86{,}171\ \%} * (100 - 0{,}653 - 0{,}177) = 1{,}153 \text{ moles \%}$$

— 4.5.5.3 Fatty acids in 1,3-positions [P(1,3), S(1,3), Po(1,3), O(1,3), L(1,3) and Ln(1,3)] (see formula (6))

$$\text{moles \% P (1,3)} = \frac{10{,}887 - 0{,}653}{2} + 10{,}887 = 16{,}004 \text{ moles \%}$$

$$\text{moles \% S (1,3)} = \frac{2{,}942 - 0{,}177}{2} + 2{,}942 = 4{,}325 \text{ moles \%}$$

$$\text{moles \% Po (1,3)} = \frac{1{,}097 - 1{,}262}{2} + 1{,}097 = 1{,}015 \text{ moles \%}$$

$$\text{moles \% O (1,3)} = \frac{74{,}116 - 85{,}296}{2} + 74{,}116 = 68{,}526 \text{ moles \%}$$

$$\text{moles \% L (1,3)} = \frac{9{,}955 - 11{,}457}{2} + 9{,}955 = 9{,}204 \text{ moles \%}$$

$$\text{moles \% Ln (1,3)} = \frac{1{,}002 - 1{,}153}{2} + 1{,}002 = 0{,}927 \text{ moles \%}$$

— 4.5.6. *Calculation of triacylglycerols*

From the calculated fatty acid composition in sn-2- and sn-1,3-positions:

FA in	1,3-pos	2-pos
P	16,004 %	0,653 %
S	4,325 %	0,177 %
Po	1,015 %	1,262 %
O	68,526 %	85,296 %
L	9,204 %	11,457 %
Ln	0,927 %	1,153 %
Sum	100,0 %	100,0 %

the following triacylglycerols are calculated:

LLL
PoPoPo
PoLL with 1 positional isomer
SLnLn with 1 positional isomer
PoPoL with 1 positional isomer
PPoLn with 2 positional isomers
OLLn with 2 positional isomers
PLLn with 2 positional isomers
PoOLn with 2 positional isomers

— 4.5.6.1. TAGs with one fatty acid (LLL, PoPoPo) (see formula (7))

$$\text{mol \% LLL} = \frac{9{,}204\ \% * 11{,}457\ \% * 9{,}204\ \%}{10\ 000}$$

, = **0,09706 mol LLL**

$$\text{mol \% PoPoPo} = \frac{1{,}015\ \% * 1{,}262\ \% * 1{,}015\ \%}{10\,000} = 0{,}00013\ \text{mol PoPoPo}$$

— TAGs with two fatty acids (PoLL, SLnLn, PoPoL) (see formula (8))

4.5.6.2

$$\text{mol \% PoLL + LLPo} = \frac{1{,}015\ \% * 11{,}457\ \% * 9{,}204\ \% * 2}{10\,000} = 0{,}02141$$

$$\text{mol \% LPoL} = \frac{9{,}204\ \% * 1{,}262\ \% * 9{,}204\ \%}{10\,000} = 0{,}01069$$

0,03210 mol PoLL

$$\text{mol \% SLnLn + LnLnS} = \frac{4{,}325\ \% * 1{,}153\ \% * 0{,}927\ \% * 2}{10\,000} = 0{,}00092$$

$$\text{mol \% LnSLn} = \frac{0{,}927\ \% * 0{,}177\ \% * 0{,}927\ \%}{10\,000} = 0{,}00002$$

0,00094 mol SLnLn

$$\text{mol \% PoPoL + LPoPo} = \frac{1{,}015\ \% * 1{,}262\ \% * 9{,}204\ \% * 2}{10\,000} = 0{,}00236$$

$$\text{mol \% PoLPo} = \frac{1{,}015\ \% * 11{,}457\ \% * 1{,}015\ \%}{10\,000} = 0{,}00118$$

0,00354 mol PoPoL

— TAGs with three different fatty acids (PoPLn, OLLn, PLLn, PoOLn) See formula (9)

4.5.6.3

$$\text{mol \% PPoLn} = \frac{16{,}004\ \% * 1{,}262\ \% * 0{,}927\ \% * 2}{10\,000} = 0{,}00374$$

$$\text{mol \% LnPPo} = \frac{0{,}927\ \% * 0{,}653\ \% * 1{,}015\ \% * 2}{10\,000} = 0{,}00012$$

$$\text{mol \% PoLnP} = \frac{1{,}015\ \% * 1{,}153\ \% * 16{,}004\ \% * 2}{10\,000} = 0{,}00375$$

0,00761 mol PPoLn

$$\text{mol \% OLLn} = \frac{68{,}526\ \% * 11{,}457\ \% * 0{,}927\ \% * 2}{10\,000} = 0{,}14556$$

$$\text{mol \% LnOL} = \frac{0{,}927\ \% * 85{,}296\ \% * 9{,}204\ \% * 2}{10\,000} = 0{,}14555$$

$$\text{mol \% LLnO} = \frac{9{,}204\ \% * 1{,}153\ \% * 68{,}526\ \% * 2}{10\,000} = 0{,}14544$$

0,43655 mol OLLn

$$\text{mol \% PLLn} = \frac{16{,}004\ \% * 11{,}457\ \% * 0{,}927\ \% * 2}{10\,000} = 0{,}03399$$

$$\text{mol \% LnPL} = \frac{0{,}927\ \% * 0{,}653\ \% * 9{,}204\ \% * 2}{10\,000} = 0{,}00111$$

$$\text{mol \% LLnP} = \frac{9{,}204\ \% * 1{,}153\ \% * 16{,}004\ \% * 2}{10\,000} = 0{,}03397$$

0,06907 mol PLLn

$$\text{mol \% PoOLn} = \frac{1{,}015\ \% * 85{,}296\ \% * 0{,}927\ \% * 2}{10\,000} = 0{,}01605$$

$$\text{mol \% LnPoO} = \frac{0{,}927\ \% * 1{,}262\ \% * 68{,}526\ \% * 2}{10\,000} = 0{,}01603$$

$$\text{mol \% OLnPo} = \frac{68{,}526\ \% * 1{,}153\ \% * 1{,}015\ \% * 2}{10\,000} = 0{,}01604$$

0,04812 mol PoOLn

ECN42 = 0,69512 mol TAGs

Note 1: The elution order can be determined by calculating the equivalent carbon numbers, often defined by the relation

$$\text{ECN} = \text{CN} - 2\text{n}$$

, where CN is the carbon number and n is the number of double bonds; it can be calculated more precisely by taking into account the origin of the double bond. If n_o, n_l and n_{ln} are the numbers of double bonds attributed to oleic, linoleic and linolenic acids respectively, the equivalent carbon number can be calculated by means of the relation of the formula:

$$EN = CN - d_o n_o - d_l n_l - d_{ln} n_{ln}$$

where the coefficient d_o, d_l and d_{ln} can be calculated by means of the reference triglycerides. Under the conditions specified in this method, the relation obtained will be close to:

$$ECN = CN - (2{,}60\ n_o) - (2{,}35\ n_l) - (2{,}17\ n_{ln})$$

Note 2: With several reference triglycerides, it is also possible to calculate the resolution with respect to triolein:

$$\alpha = RT^1/RT$$

triolein

by use of the reduced retention time

$$RT^1 = RT - RT\ \text{solvent}$$

The graph of log α against f (number of double bonds) enables the retention values to be determined for all the triglycerides of fatty acids contained in the reference triglycerides — see Figure 1.

Note 3: The efficiency of the column should permit clear separation of the peak of trilinolein from the peaks of the triglycerides with an adjacent RT. The elution is carried out up to ECN 52 peak.

Note 4: A correct measure of the areas of all peaks of interest for the present determination is ensured if the second peak corresponding to ECN 50 is 50 % of full scale of the recorder.

Figure 1

Graph of log α against f (number of double bonds)

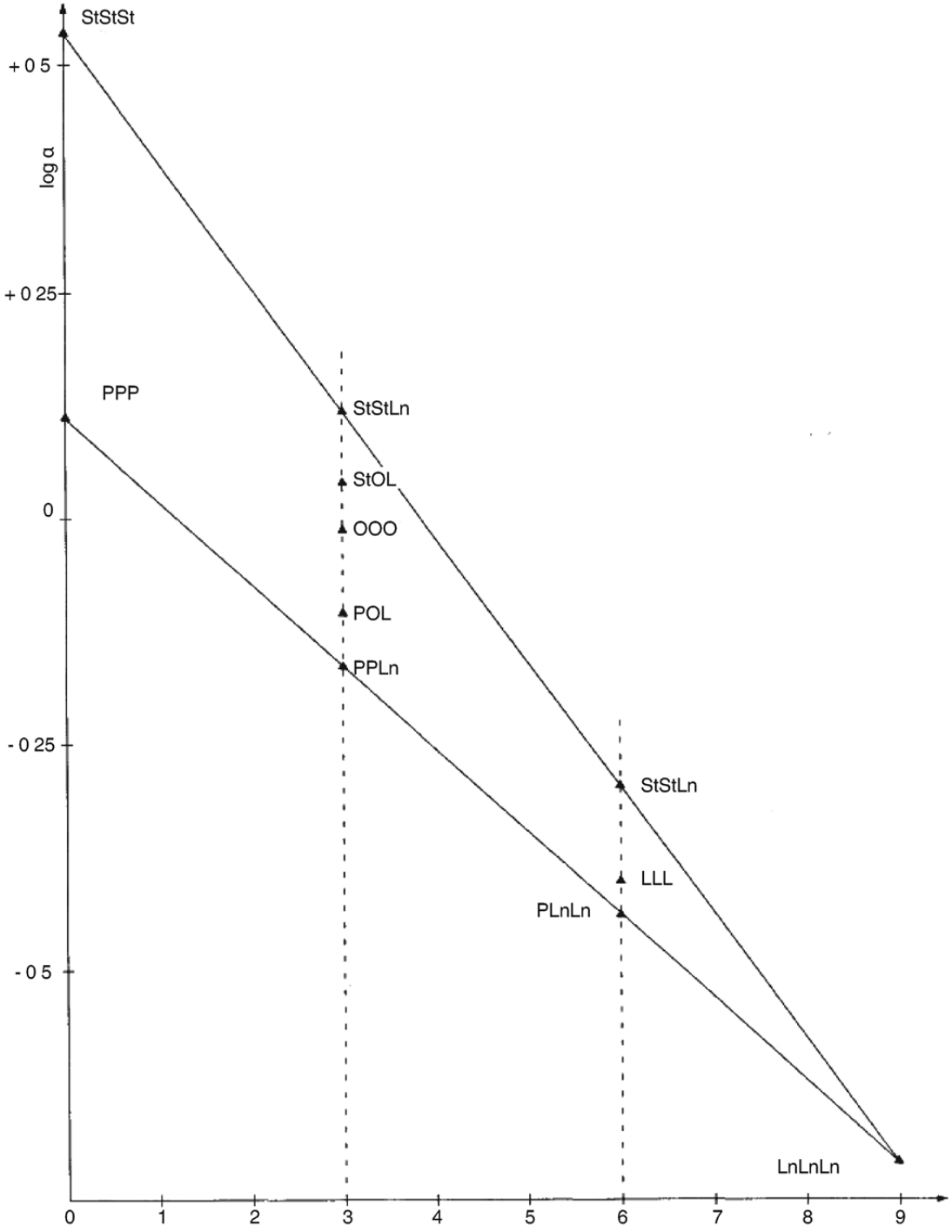
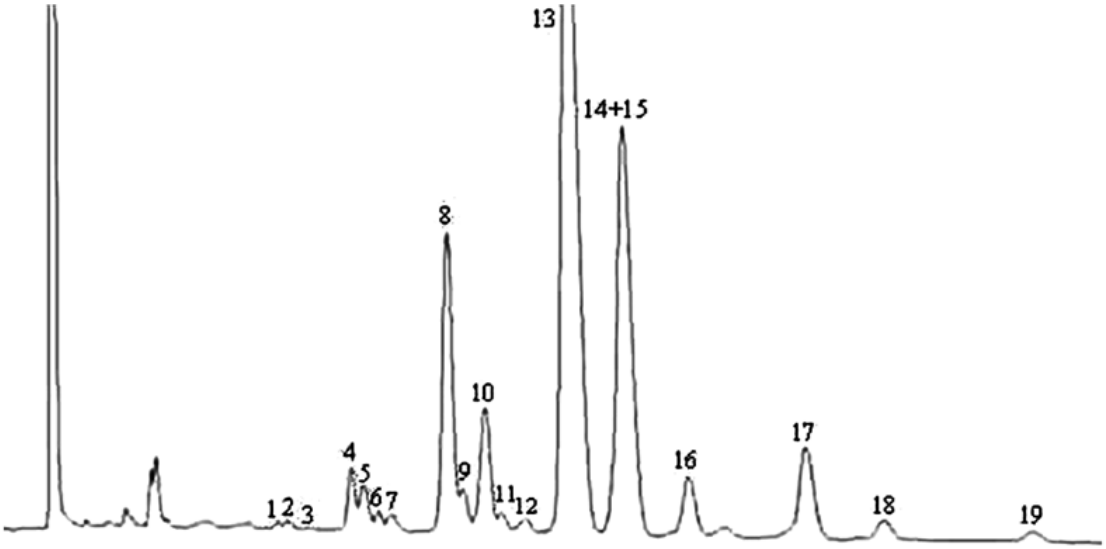


Figure 2

Low linoleic olive oil

(a)



(b)

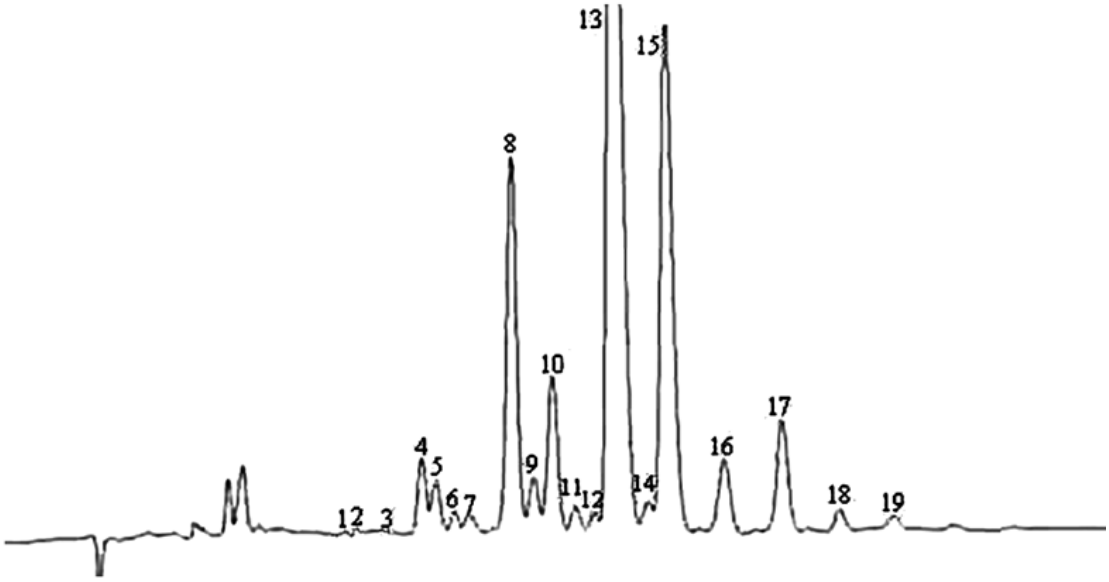
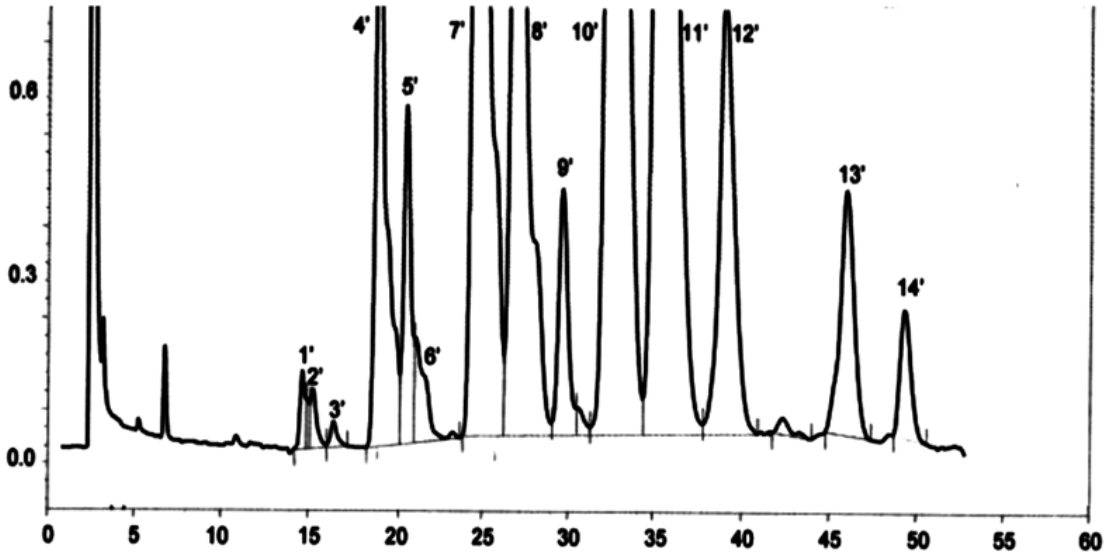


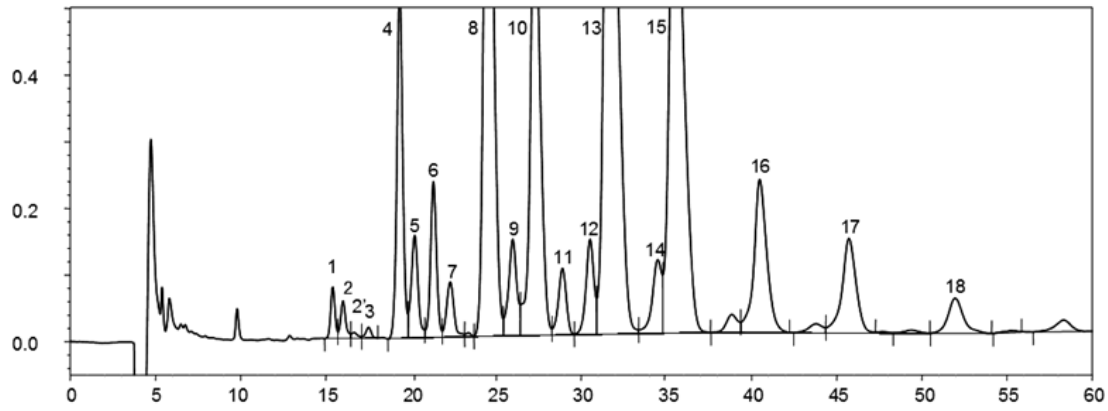
Figure 3

High linoleic olive oil

(a)



(b)



ANNEX III

‘ANNEX	Results of conformity checks carried out on olive oils referred to in Article 8(2)Internal market (mill, bottlers, retail stage), export, import.Each characteristic of olive oil set out in Annex I shall have a code.Conform/ not conform.Not required for olive oil and pomace-oil.’LabellingChemical parametersOrganoleptic characteristicsFinal conclusionSampleCategoryCountry of originPlace of inspectionLegal nameDesignation of originStorage conditionsErroneous informationLegibilityC/NCParameters out of limitY/NIf so, please indicate which one(s)C/NCMedian defectFruity MedianC/NCRequired actionSanction
XXI	

(**1**) OJ L 299, 16.11.2007, p. 1.

(**2**) OJ L 248, 5.9.1991, p. 1.

(**3**) OJ L 228, 1.9.2009, p. 3.

(**4**) OJ L 228, 1.9.2009, p. 3.’;