

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

Article 1	(1) Oils, the characteristics of which comply with those set...
Article 2	(1) The characteristics of oils laid down in Annex I...
Article 2a	(1) For the purpose of this Article, ' olive oil...
Article 3	.....
Article 3a	.....
Article 3	Where it is found that an oil does not correspond...
Article 4	(1) The Member States may approve assessment panels so that...
Article 5	.....
Article 6	(1) The oil content of oil cake and other residues...
Article 7	The Community provisions concerning the presence of contaminants shall apply....
Article 7a	Natural or legal persons and groups of persons who hold...
Article 8	(1) Member States shall notify the Commission of the measures...
Article 9	Regulation (EEC) No 1058/77 is hereby repealed.
Article 10	(1) This Regulation shall enter into force on the third...
	Signature

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ANNEXES  
SUMMARY

ANNEX I  
OLIVE OIL CHARACTERISTICS

Quality characteristics

Purity characteristics

Notes:

- (a) The results of the analyses must be expressed to the...
- (b) If just a single characteristic does not match the values...
- (c) For lampante olive oil, both quality characteristics marked with an...
- (d) If a characteristic is marked with two asterisks (\*\*), this...

Appendix

Decision trees

**Changes to legislation:** There are outstanding changes not yet made to Commission Regulation (EEC) No 2568/91. Any changes that have already been made to the legislation appear in the content and are referenced with annotations. (See end of Document for details) [View outstanding changes](#)

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## ANNEX Ia

### SAMPLING OF OLIVE OIL OR OLIVE-POMACE OIL DELIVERED IN IMMEDIATE PACKAGING

This method of sampling is applied to batches of olive...

‘Batch’ shall mean a set of sales units which are...

‘Increment’ shall mean the quantity of oil contained...

1. CONTENT OF PRIMARY SAMPLE
  - 1.1. Immediate packaging not exceeding 5 litres
  - 1.2. Immediate packaging exceeding 5 litres
2. ANALYSES AND RESULTS
  - 2.1. Each primary sample must be subdivided into laboratory samples, in...
  - 2.2. Where all the results of the analyses comply with the...
3. VERIFICATION OF THE CATEGORY OF BATCH
  - 3.1. In order to verify the batch category, the competent authority...
  - 3.2. When one of the results of the analyses referred to...

## ANNEX Ib

### FLOW-CHART FOR VERIFYING WHETHER AN OLIVE OIL SAMPLE IS CONSISTENT WITH THE CATEGORY DECLARED

#### General table

Table 1 — Extra Virgin Olive Oil — Quality criteria...

Table 2 — Virgin Olive Oil — Quality criteria

Table 3 — Extra Virgin Olive Oil and Virgin Olive...

Table 4 — Lampante Olive Oil — Purity criteria

Table 5 — Refined Olive Oil — Quality criteria

Table 6 — Olive Oil (composed of refined olive oil...

Table 7 — Refined Olive Oil and olive oil composed...

Table 8 — Crude Olive-Pomace Oil — Purity criteria

Table 9 — Refined Olive-Pomace Oil — Quality criteria

Table 10 — Olive Pomace Oil — Quality criteria

Table 11 — Refined Olive-Pomace Oil and Olive-Pomace Oil —...

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## ANNEX II

### DETERMINATION OF FREE FATTY ACIDS, COLD METHOD

1. SCOPE AND FIELD OF APPLICATION
2. PRINCIPLE
3. REAGENTS
  - 3.1 Diethyl ether; 95 % ethanol (v/v), mixture of equal parts...  
Note 1: Diethyl ether is highly inflammable and may form explosive peroxides....  
Note 2: If it is not possible to use diethyl ether, a...
  - 3.2 Potassium hydroxide or sodium hydroxide, titrated ethanolic or aqueous solution,...  
Note 3: A stable colourless solution of potassium hydroxide (or sodium hydroxide)...
  - 3.3 Phenolphthalein, 10 g/l solution in 95 to 96 % ethanol...
4. APPARATUS
5. PROCEDURE
  - 5.1 Preparation of the test sample
  - 5.2 Test portion
  - 5.3 Determination  
Note 4: If the quantity of 0,1 mol/l potassium hydroxide (or sodium...  
Note 5: If the solution becomes cloudy during titration, add enough of...
6. EXPRESSION OF RESULTS

## ANNEX III

### DETERMINATION OF PEROXIDE VALUE

1. Scope
2. Definition
3. Principle
4. Apparatus
  - 4.1. 3 ml glass scoop.
  - 4.2. Flasks, with ground necks and stoppers, of about 250 ml...
  - 4.3. Burette of 5-ml, 10-ml or 25-ml capacity, graduated in at...
  - 4.4. Analytical balance.
5. Reagents
  - 5.1. Chloroform, analytical reagent quality, freed from oxygen by bubbling a...
  - 5.2. Glacial acetic acid, analytical reagent quality, freed from oxygen by...
  - 5.3. Potassium iodide, saturated aqueous solution, recently prepared, free from iodine...
  - 5.4. Sodium thiosulphate, 0,01 mol/l (equivalent to 0,01 N) accurately standardised...

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- 5.5. Starch solution, 10 g/l aqueous dispersion, recently prepared from natural...
6. Sample
7. Procedure
8. Expression of results

## ANNEX IV

### DETERMINATION OF WAX CONTENT BY CAPILLARY COLUMN GAS CHROMATOGRAPHY

1. SUBJECT
2. PRINCIPLE
3. EQUIPMENT
  - 3.1. 25 ml Erlenmeyer flask.
  - 3.2. Glass column for gas chromatography, internal diameter 15,0 mm, length...
  - 3.3. Suitable gas chromatograph with a capillary column, equipped with a...
    - 3.3.1. Thermostatic chamber for the columns, equipped with a temperature programmer...
    - 3.3.2. Cold injector for direct introduction into the column.
    - 3.3.3. Flame ionisation detector and converter-amplifier.
    - 3.3.4. Recorder-integrator capable of working with the converter-amplifier (3.3.3), rate of...
    - 3.3.5. Glass or fused silica capillary column 8 to 12 m...
  - 3.4. 10 µl microsyringe for on-column injection, equipped with a hardened...
  - 3.5. Electro vibrator.
  - 3.6. Rotary evaporator.
  - 3.7. Muffle furnace.
  - 3.8. Analytical balance with guaranteed precision of  $\pm 0,1$  mg.
  - 3.9. Normal laboratory glassware.
4. REAGENTS
  - 4.1. Silica gel with a granule size of between 60 and...
  - 4.2. n-hexane, for chromatography.
  - 4.3. Ethyl ether, for chromatography.
  - 4.4. n-heptane, for chromatography.
  - 4.5. Standard solution of lauryl arachidate, at 0,1 % (m/v) in...
    - 4.5.1. Sudan 1 (1-phenyl-azo-2-naphthol).
  - 4.6. Carrier gas: hydrogen or helium, gas-chromatographic purity.
  - 4.7. Auxiliary gases:
5. PROCEDURE
  - 5.1. Preparation of the chromatographic column.  
NB:
  - 5.2. Analysis by gas chromatography
    - 5.2.1. Preparatory work
    - 5.2.2. Choice of operating conditions
  - 5.3. Performance of the analysis

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- 5.4. Identification of peaks
  - 5.5. Evaluation of quantity
6. EXPRESSION OF RESULTS
- Figure Chromatogram of the waxes of an olive oil

## Appendix

Determination of the linear velocity of the gas

## ANNEX V

### DETERMINATION OF THE COMPOSITION AND CONTENT OF STEROLS AND TRITERPENES DIOLCOHOLS BY CAPILLARY-COLUMN GAS CHROMATOGRAPHY

- 1. SCOPE
- 2. PRINCIPLE
- 3. APPARATUS
- 4. REAGENTS
  - 4.1. ....
  - 4.2. Potassium hydroxide ethanolic solution, approximately 2 N.
  - 4.3. ....
  - 4.4. Potassium hydroxide ethanolic solution, approximately 0,2 N.
  - 4.5. ....
  - 4.6. ....
  - 4.7. ....
  - 4.8. ....
  - 4.9. ....
  - 4.10. ....
  - 4.11. ....
  - 4.12. ....
  - 4.13. ....
  - 4.14. ....
  - 4.15. ....
  - 4.16. ....
  - 4.17. ....
  - 4.18. Sample solutions of sterol trimethylsilyl ethers.
  - 4.19. ....
  - 4.20. ....
  - 4.21. ....
  - 4.22. ....
  - 4.23. ....
  - 4.24. ....
  - 4.25. ....
- 5. PROCEDURE
  - 5.1. ....
    - 5.1.1. ....

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- Note 1: . . . . .
  - 5.1.2. . . . .
  - 5.1.3. . . . .
  - 5.1.4. . . . .
  - 5.1.5. . . . .
  - 5.2. Separation of the sterol and triterpene dialcohols fraction (erythrodiol +...
    - 5.2.1. . . . .
    - Note 3: . . . . .
    - 5.2.2. . . . .
    - Note 4: . . . . .
    - 5.2.3. . . . .
    - 5.2.4. . . . .
    - 5.2.5. . . . .
    - 5.2.6. . . . .
  - 5.3. . . . . .
    - 5.3.1. . . . .
    - Note 6: . . . . .
    - 5.3.2. . . . .
    - Note 7: . . . . .
  - 5.4. . . . . .
    - 5.4.1. . . . .
      - 5.4.1.1. Fit the column (point 3.11) in the gas chromatograph, by...
      - 5.4.1.2. If the column is being used for the first time,...
      - Note 8: . . . . .
    - 5.4.2. . . . .
      - 5.4.2.1. The operating conditions are as follows:
    - 5.4.3. Analytical procedure
      - 5.4.3.1. By using the 10 µl microsyringe, take 1 µl of...
      - 5.4.3.2. . . . .
    - 5.4.4. Peak identification
    - 5.4.5. . . . .
      - 5.4.5.1. . . . .
      - 5.4.5.2. Calculate the concentration of each individual sterol, in mg/kg of...
6. EXPRESSION OF THE RESULTS
- 6.1. Report individual sterol concentrations as mg/kg of fatty material and...
  - 6.2. Calculate the percentage of each individual sterol from the ratio...
  - 6.3. . . . . .
  - 6.4. Calculate the percentage of erythrodiol and uvaol:

## Appendix

Determination of the linear speed of the gas

## ANNEX VI

### DETERMINATION OF ERYTHRODIOL AND UVAOL

#### INTRODUCTION

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1. SCOPE
2. PRINCIPLE OF THE METHOD
3. APPARATUS
  - 3.1. ....
4. REAGENTS
  - 4.1. ....
  - 4.2. ....
5. PROCEDURE
  - 5.1. Preparation of the unsaponifiables.
  - 5.2. Separation of erythrodiol and the sterols.
    - 5.2.1. ....
    - 5.2.2. ....
    - 5.2.3. ....
    - 5.2.4. ....
    - 5.2.5. ....
    - 5.2.6. ....
  - 5.3. Preparation of the trimethylsilyl esters
  - 5.4. Gas chromatographic analysis
6. EXPRESSION OF THE RESULTS

## ANNEX VII

### DETERMINATION OF THE PERCENTAGE OF 2-GLYCERYL MONOPALMITATE

1. PURPOSE AND SCOPE
2. PRINCIPLE
3. APPARATUS AND MATERIALS
  - 3.1. 25 ml Erlenmeyer flask
  - 3.2. 100, 250 and 300 ml beakers
  - 3.3. Glass chromatograph column, internal diameter 21-23 mm, length 400 mm,...
  - 3.4. 10, 50, 100 and 200 ml measuring cylinders
  - 3.5. 100 and 250 ml flasks
  - 3.6. Rotary evaporator
  - 3.7. 10 ml conical-bottomed centrifuge tubes with groundglass stopper
  - 3.8. Centrifuge for 10 and 100 ml tubes
  - 3.9. Thermostat permitting a stable temperature of  $40 \pm 0,5$  °C...
  - 3.10. 1 and 2 ml graduated pipettes
  - 3.11. 1 ml hypodermic syringe
  - 3.12. 100 µl microsyringe
  - 3.13. 1 000 ml funnel
  - 3.14. Capillary gas chromatograph with an on-column cold injector for direct...
  - 3.15. On-column cold injector for direct injection of the sample into...
  - 3.16. Flame ionisation detector and electrometer
  - 3.17. Recorder-integrator adapted to the electrometer with a response rate no...
  - 3.18. Capillary column made of glass or fused silica 8-12 metres...

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3.19. 10 µl microsyringe fitted with a hardened needle, at least...

#### 4. REAGENTS

- 4.1. Silica gel with a grain size of between 0,063 and...
- 4.2. n-hexane (chromatography grade). Hexane may be replaced by iso-octane (2,2,4-...
- 4.3. Isopropanol
- 4.4. Isopropanol, 1/1 (v/v) aqueous solution
- 4.5. Pancreatic lipase. It must have an activity of between 2,0...
- 4.6. Buffer solution of trishydroxymethylaminomethane: 1 M aqueous solution adjusted to...
- 4.7. Enzyme-quality sodium cholate, 0,1 % aqueous solution (this solution must...
- 4.8. Calcium chloride, 22 % aqueous solution
- 4.9. Diethyl ether for chromatography
- 4.10. Developer solvent: mixture of n-hexane/diethyl ether (87:13 v:v)
- 4.11. Sodium hydroxide, 12 % by weight solution
- 4.12. Phenolphthalein, 1 % solution in ethanol
- 4.13. Carrier gas: hydrogen or helium, for gas chromatography
- 4.14. Auxiliary gases: hydrogen, 99 % minimum purity, free from moisture...
- 4.15. Silanisation reagent: mixture of pyridine/hexamethyldisilazane, trimethylchlorosilane 9/3/1 (v/v/v). (Ready-to-use solutions...
- 4.16. Reference samples: pure monoglycerides or monoglyceride mixtures with a known...

#### 5. METHOD

- 5.1. Sample preparation
  - 5.1.1. Oils with a free acidity of less than 3 %...
    - 5.1.1.1. Pour 50 g of oil and 200 ml n-hexane into...
  - 5.1.2. Put 1,0 g of the oil prepared as above into...
  - 5.1.3. Preparation of the chromatography column
  - 5.1.4. Column chromatography
- 5.2. Hydrolysis by pancreatic lipase
  - 5.2.1. Weigh into the centrifuge tube 0.1 g of the oil...
  - 5.2.2. Add 20 mg of lipase, shake carefully (avoid wetting the...
  - 5.2.3. Add 1 ml of diethyl ether, stopper and shake vigorously,...
- 5.3. Preparation of the silanised derivatives and gas chromatography
  - 5.3.1. With a microsyringe insert 100 µl of solution (5.2.3) into...
  - 5.3.2. Remove the solvent under a slight nitrogen current, add 200...
  - 5.3.3. After 20 minutes, add 1 to 5 ml of n-hexane...
- 5.4. Gas chromatography
  - 5.4.1. Identification of the peaks
  - 5.4.2. Quantitative evaluation

#### 6. EXPRESSION OF RESULTS

#### 7. ANALYSIS REPORT

Figure 2

- (A) unesterified olive oil, after lipase; after silanisation; under these conditions...
- (B) unesterified oil after lipase; after silanisation; under these conditions (8-12...

#### 8. NOTES



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- Note 1. PREPARATION OF THE LIPASE
- Note 2. MONITORING LIPASE ACTIVITY

## ANNEX VIII

### DETERMINATION OF TRILINOLEIN CONTENT

- 1. SCOPE
- 2. FIELD OF APPLICATION
- 3. PRINCIPLE
- 4. APPARATUS
  - 4.1. ....
  - 4.2. ....
  - 4.3. ....
  - 4.4. ....
  - 4.5. ....
- 5. REAGENTS
  - 5.1. ....
  - 5.2. ....
  - 5.3. ....
  - 5.4. ....
  - 5.5. ....
  - 5.6. ....
- 6. PREPARATION OF SAMPLES
- 7. PROCEDURE
  - 7.1. ....
- 8. CALCULATION AND EXPRESSION OF RESULTS
  - Note 1.
  - Note 2. Examples:
  - Note 3.
  - Note 4.
  - Note 5:
    - .....
    - .....

## ANNEX IX

### SPECTROPHOTOMETRIC INVESTIGATION IN THE ULTRAVIOLET

#### FOREWORD

- 1. SCOPE

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2. PRINCIPLE OF THE METHOD
3. EQUIPMENT
  - 3.1. A spectrophotometer suitable for measurements at ultraviolet wavelengths (220 nm...
    - 3.1.1. Wavelength scale: This may be checked using a reference material...
    - 3.1.2. Absorbance scale: This may be checked using commercially available sealed...
  - 3.2. Rectangular quartz cuvettes, with covers, suitable for measurements at the...
  - 3.3. One- mark volumetric flasks, capacity 25 ml, class A.
  - 3.4. Analytical balance, capable of being read to the nearest 0,0001...
4. REAGENTS
5. PROCEDURE
  - 5.1. The sample must be perfectly homogeneous and without suspended impurities....
  - 5.2. Weigh accurately approximately 0,25 g (to the nearest 1 mg)...
  - 5.3. If necessary, correct the baseline (220-290 nm) with solvent in...
  - 5.4. After measuring the absorbance at 268 or 270 nm, measure...
6. EXPRESSION OF THE RESULTS
  - 6.1. Record the specific extinctions (extinction coefficients) at the various wavelengths...
  - 6.2. Variation of the specific extinction ( $\Delta K$ )

## ANNEX X

### DETERMINATION OF FATTY ACID METHYL ESTERS BY GAS CHROMATOGRAPHY

1. SCOPE
2. PRINCIPLE

#### PART A

#### PREPARATION OF THE FATTY ACID METHYL ESTERS FROM OLIVE OIL...

1. SCOPE
2. FIELD OF APPLICATION
3. METHODOLOGY
  - 3.1. Trans-esterification with methanolic solution of potassium hydroxide at room temperature...
    - 3.1.1. Principle
    - 3.1.2. Reagents
      - 3.1.2.1. 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.

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- 3.1.2.6. Elution solvent for purifying the oil by column/SPE chromatography, mixture...
- 3.1.2.7. Potassium hydroxide, approximately 2M methanolic solution: dissolve 11,2 g of...
- 3.1.2.8. Silica gel cartridges, 1 g (6 ml), for solid phase...
- 3.1.3. Apparatus
  - 3.1.3.1. Screw-top test tubes (5 ml volume) with cap fitted with...
  - 3.1.3.2. Graduated or automatic pipettes, 2 ml and 0,2 ml.
- 3.1.4. Purification of oil samples
- 3.1.5. Procedure

## PART B

### ANALYSIS OF FATTY ACID METHYL ESTERS BY GAS CHROMATOGRAPHY

1. SCOPE
2. REAGENTS
  - 2.1. Carrier gas
    - Note 1: Hydrogen can double the speed of analysis but is hazardous....
  - 2.2. Auxiliary gases
    - 2.2.1. Hydrogen (purity  $\geq 99,9\%$ ), free from organic impurities.
    - 2.2.2. Air or oxygen, free from organic impurities.
    - 2.2.3. Nitrogen (purity  $> 99\%$ ).
  - 2.3. Reference standard
3. APPARATUS
  - 3.1. Gas chromatograph
    - 3.1.1. Injection system
    - 3.1.2. Oven
    - 3.1.3. Capillary column
      - 3.1.3.1. Tube, made of a material inert to the substances to...
      - 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...
      - 3.1.3.3. Assembly and conditioning of the column
        - Note 3: Suitably pre-conditioned columns are available commercially.
    - 3.1.4. Flame ionisation detector and converter-amplifier
  - 3.2. Syringe
  - 3.3. Data acquisition system
4. PROCEDURE
  - 4.1. Test conditions
    - 4.1.1. Selection of optimum operating conditions for capillary columns
    - 4.1.2. Determination of the resolution (see Appendix A)
5. EXPRESSION OF RESULTS
  - 5.1. Qualitative analysis
  - 5.2. Quantitative analysis
    - 5.2.1. Determination of the composition
    - 5.2.2. Method of calculation
      - 5.2.2.1. General case
        - Note 4: For fats and oils, the mass fraction of the fatty...

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#### 5.2.2.2. Use of correction factors

Note 5: These correction factors are not identical to the theoretical FID...

Note 6: The calculated value corresponds to the percentage of mass of...

#### 5.2.2.3. Use of an internal standard

### 6. TEST REPORT

### 7. PRECISION

- 7.1. Results of interlaboratory test
- 7.2. Repeatability
- 7.3. Reproducibility

## Appendix A

### Figure 1

ω 0,5 width at half height of the triangle (ABC)...

## Appendix B

Figure 1 Gas chromatographic profile obtained by the cold methylation...

The chromatographic peaks correspond to the methyl and ethyl esters...

## ANNEX XI

### DETERMINATION OF VOLATILE HALOGENATED SOLVENTS CONTENT OF OLIVE OIL

#### 1. METHOD

#### 2. EQUIPMENT

- 2.1. Gas chromatography apparatus fitted with an electron capture detector (ECD)....
- 2.2. Head space apparatus.
- 2.3. Gas chromatography column, of glass, 2 m long and 2...
- 2.4. Carrier and auxiliary gas: nitrogen for gas chromatography, suitable for...
- 2.5. Glass flasks, 10 to 15 ml, with teflon coating and...
- 2.6. Hermetically sealing clamps.
- 2.7. Gas syringe 0,5 to 2 ml.

#### 3. REAGENTS

#### 4. PROCEDURE

- 4.1. Exactly weigh around 3 g of oil in a glass...
- 4.2. Reference solutions: prepare standard solutions using refined olive oil with...
- 4.3. Quantitative assessment: correlate the surfaces or the elevations of the...

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- 4.4. Expression of results: in ppm (mg/kg). The detection limit for...

## ANNEX XII

### THE INTERNATIONAL OLIVE COUNCIL'S METHOD FOR THE ORGANOLEPTIC ASSESSMENT OF VIRGIN OLIVE OIL

1. PURPOSE AND SCOPE
2. GENERAL BASIC VOCABULARY FOR SENSORY ANALYSIS
3. SPECIFIC VOCABULARY
  - 3.1. Negative attributes
    - 3.1.1. Other negative attributes
  - 3.2. Positive attributes
  - 3.3. Optional terminology for labelling purposes
4. GLASS FOR OIL TASTING
5. TEST ROOM
6. ACCESSORIES
7. PANEL LEADER AND TASTERS
  - 7.1. Panel leader
    - 7.1.1. Deputy panel leader
  - 7.2. Tasters
8. TEST CONDITIONS
  - 8.1. Presentation of the sample
  - 8.2. Test and sample temperature
  - 8.3. Test times
  - 8.4. Tasters: general rules of conduct
9. PROCEDURE FOR THE ORGANOLEPTIC ASSESSMENT AND CLASSIFICATION OF VIRGIN OLIVE...
  - 9.1. Tasting technique
    - 9.1.1. The tasters shall pick up the glass, keeping it covered...
    - 9.1.2. When organoleptically assessing a virgin olive oil, it is recommended...
  - 9.2. Use of the profile sheet by tasters
  - 9.3. Use of the data by the panel leaders
  - 9.4. Classification of the oil  
Note 1: When the median of the bitter and/or pungent attribute is...
  - 9.5. Criteria for the acceptance and rejection of duplicates

## Appendix

Method for calculating the median and the confidence intervals

Median  
Robust standard deviation  
Robust coefficient of variation (%)

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#### Confidence intervals of the median at 95%

##### References

- (1) Wilkinson, L. 1990. Systat: The system for statistics. Evanston, IL.SYSTAT...
- (2) Cicchitelli, G. 1984. Probabilità e Statistica. Maggioli Editore, Rimini.
- (3) Massart, D.L.; Vandeginste, B.G.M.; Deming, Y.; Michotte, L. 1988. Chemometrics....
- (4) Kendall, M.G.; Stuart, A. 1967. The advanced theory of statistics....
- (5) McGill, R.; Tukey, J.W.; Larsen, W.A. 1978. Variation of Box...
- (6) IOC/T.28/Doc. No 1 September 2007, Guidelines for the accreditation of...
- (7) IOC/T.20/Doc. No 14.
- (8) IOC/T.20/Doc. No 15.
- (9) ISO/IEC 17025:05.

### ANNEX XIII

#### NEUTRALIZATION AND DECOLORIZATION OF OLIVE OIL IN THE LABORATORY

1. NEUTRALIZATION AND DECOLORIZATION OF OLIVE OIL IN THE LABORATORY
  - 1.1. Neutralization of the oil
    - 1.1.1. Apparatus
    - 1.1.2. Reagents
    - 1.1.3. Procedure
      - (a) Oils with a free fatty acid content, expressed as oleic...
      - (b) Oils with a free fatty acid content expressed as oleic...
  - 1.2. Decolorization of neutralized oil
    - 1.2.1. Apparatus
    - 1.2.2. Procedure

### ANNEX XIV

#### ADDITIONAL NOTES 2, 3 AND 4 TO CHAPTER 15 OF THE COMBINED NOMENCLATURE

2. A. ....
- A. ....
- B. ....
- I. ....
- II. ....
- C. ....
- D. ....
- E. ....
3. ....
4. ....

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## ANNEX XV

1. OIL CONTENT OF OLIVE RESIDUE
  - 1.1. Apparatus
  - 1.2. Reagent
2. PROCEDURE
  - 2.1. Preparation of the test sample
  - 2.2. Test portion
  - 2.3. Preparation of the extraction thimble
  - 2.4. Preliminary drying
  - 2.5. Preparation of the round-bottomed flask
  - 2.6. Initial extraction
  - 2.7. Second extraction
  - 2.8. Removal of solvent and weighing of extract
3. EXPRESSION OF RESULTS
  - 3.1. Method of calculation and formula
  - 3.2. Repeatability

## ANNEX XVI

### DETERMINATION OF IODINE VALUE

1. SCOPE
2. DEFINITION
  - 2.1. iodine value. The mass of iodine absorbed by the sample...
3. PRINCIPLE
4. REAGENTS
  - 4.1. water, complying with the requirements of ISO 3696, Grade 3...
  - 4.2. potassium iodide, 100 g/l solution, not containing iodate or free...
  - 4.3. starch, solution.
  - 4.4. sodium thiosulfate, standard volumetric solution  $c(\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}) = 0,1$  mol/l,...
  - 4.5. solvent, prepared by mixing equal volumes of cyclohexane and acetic...
  - 4.6. Wijs reagent, containing iodine monochloride in acetic acid. Commercially available...
5. APPARATUS
  - 5.1. glass weighing scoops, suitable for the test portion and for...
  - 5.2. conical flasks, of 500 ml capacity, fitted with ground glass...
6. PREPARATION OF THE TEST SAMPLE
7. PROCEDURE
  - 7.1. Test portion
  - 7.2. Determination
    - Note:
  - 7.3. Number of determinations

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## 8. EXPRESSION OF RESULTS

### ANNEX XVII

#### METHOD FOR THE DETERMINATION OF STIGMASTADIENES IN VEGETABLE OILS

##### 1. PURPOSE

##### 2. SCOPE

##### 3. PRINCIPLE

##### 4. APPARATUS

- 4.1. 250 ml flasks suitable for use with a reflux condenser....
- 4.2. Separating funnels of 500 ml capacity.
- 4.3. 100 ml round-bottom flasks.
- 4.4. Rotary evaporator.
- 4.5. Glass chromatography column (1,5 to 2,0 cm internal diameter by...
- 4.6. Gas chromatograph with flame ionization detector, split or cold on-column...
- 4.7. Fused silica capillary column for gas chromatography (0,25 or 0,32...  
Note 1:
- 4.8. Integrator-recorder with possibility of valley-valley integration mode.
- 4.9. 5 to 10 ml microsyringe for gas chromatography with cemented...
- 4.10. Electrical heating mantle or hot place.

##### 5. REAGENTS

- 5.1. Hexane or mixture of alkanes of b.p. interval 65 to 70 °C,...
- 5.2. 96 v/v ethanol.
- 5.3. Anhydrous sodium sulphate.
- 5.4. Alcoholic potassium hydroxide solution at 10 %. Add 10 ml...  
Note 3:
- 5.5. Silica gel 60 for column chromatography, 70 to 230 mesh, ...  
Note 4:
- 5.6. Stock solution (200 ppm) of cholesta-3,5-diene (Sigma, 99 % purity)...
- 5.7. Standard solution of cholesta-3,5-diene hexane at concentration of 20 ppm, ...  
Note 5:
- 5.8. Solution of n-nonacosane in hexane at concentration of approximately 100...
- 5.9. Carrier gas for chromatography: helium or hydrogen of 99,9990 %...
- 5.10. Auxiliary gases for flame ionization detector: hydrogen of 99,9990 %...

##### 6. PROCEDURE

- 6.1. Preparation of unsaponifiable matter
  - 6.1.1. Weigh  $20 \pm 0,1$  g of oil into a 250-ml...  
Note 6:
  - 6.1.2. Transfer the aqueous phase beneath to a second separating funnel...
  - 6.1.3. Pass the hexane solution through anhydrous sodium sulphate (50 g),...
- 6.2. Separation of steroidal hydrocarbon fraction
  - 6.2.1. Take the residue to the fractioning column with the aid...  
Note 7:
  - 6.2.2. Evaporate the second fraction in a rotary evaporator at 30...  
Note 8:



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- 6.3. Gas chromatography
  - 6.3.1. Working conditions for split injection:
  - 6.3.2. Peak identification
    - Note 9:
  - 6.3.3. Quantitative analysis

Figure 1  
Gas chromatogram obtained from a refined olive oil sample analysed...

## ANNEX XVIII

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

- 1. SCOPE
- 2. FIELD OF APPLICATION
- 3. PRINCIPLE
- 4. METHOD
  - 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,...
    - 4.1.4. Separating funnels, 250 ml, with normalised cone (male) at the...
    - 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...
    - 4.1.13. Column: stainless steel tube 250 mm length x 4,5 mm...
    - 4.1.14. Data processing software.
    - 4.1.15. Vials, of about 2 ml volumes, with Teflon-layered septa and...
  - 4.2. Reagents
    - 4.2.1. Petroleum ether 40-60 °C chromatographic grade or hexane. Hexane may be...
    - 4.2.2. Ethyl ether, peroxide-free, freshly distilled.
    - 4.2.3. Elution solvent for purifying the oil by column chromatography mixture...
    - 4.2.4. Silica gel, 70-230 mesh, type Merck 7734, with water content...
    - 4.2.5. Glass wool.
    - 4.2.6. Acetone for HPLC.
    - 4.2.7. Acetonitrile or propionitrile for HPLC.

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- 
- 4.2.8. HPLC elution solvent: acetonitrile + acetone (proportions to be adjusted...
  - 4.2.9. Solubilisation solvent: acetone.
  - 4.2.10. Reference triglycerides: commercial triglycerides (tripalmitin, triolein, etc.) may be used...
  - 4.2.11. Solid phase extraction column with silica phase 1 g, 6...
  - 4.2.12. Heptane, chromatographic quality. Heptane may be replaced by iso-octane (2,2,4-trimethyl...
  - 4.3. Sample preparation
    - 4.3.1. Chromatographic column preparation
    - 4.3.2. Column chromatography
    - 4.3.3. SPE purification
  - 4.4. HPLC analysis
    - 4.4.1. Preparation of the samples for chromatographic analysis
    - 4.4.2. Procedure
    - 4.4.3. Calculation and expression of results
  - 4.5. Calculation of triacylglycerols composition (moles %) from fatty acid composition...
    - 4.5.1. Determination of fatty acid composition
    - 4.5.2. Fatty acids for calculation
    - 4.5.3. Conversion of area % into moles for all fatty acids...
    - 4.5.4. Normalisation of fatty acid moles to 100 % (2)
    - 4.5.5. Calculation of the fatty acid composition in 2- and 1,...
      - 4.5.5.1. Saturated fatty acids in 2-position [P(2) and S(2)] (4):
      - 4.5.5.2. Unsaturated fatty acids in 2-position [Po(2), O(2), L(2) and Ln(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...
    - 4.5.6. Calculation of triacylglycerols
      - 4.5.6.1. TAGs with one fatty acid (AAA, here LLL, PoPoPo) (7)...
      - 4.5.6.2. TAGs with two fatty acids (AAB, here PoPoL, PoLL) (8)...
      - 4.5.6.3. TAGs with three different fatty acids (ABC, here OLLn, PLLn,...
      - 4.5.6.4. Triacylglycerols with ECN42
  - 5. EVALUATION OF THE RESULTS
  - 6. EXAMPLE (THE NUMBERS REFER TO THE SECTIONS IN THE TEXT...
    - 4.5.1 Calculation of moles % fatty acids from GLC data (normalised...
    - 4.5.3 Conversion of area % into moles for all fatty acids...
    - 4.5.4 Normalisation of fatty acid moles to 100 % (see formula...
    - 4.5.5 Calculation of the fatty acid composition in 2- and 1,3-positions...
      - 4.5.5.1 Saturated fatty acids in 2-position [P(2) and S(2)] (see formula...
      - 4.5.5.2 Unsaturated fatty acids in 2-position [Po(1,3), O(1,3), L(1,3) and Ln(1,3)]...
      - 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...
    - 4.5.6 Calculation of triacylglycerols
      - 4.5.6.1 TAGs with one fatty acid (LLL, PoPoPo) (see formula (7))...
      - 4.5.6.2 TAGs with two fatty acids (PoLL, SLnLn, PoPoL) (see formula...
      - 4.5.6.3 TAGs with three different fatty acids (PoPLn, OLLn, PLLn, PoOLn)...

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(b)  
(b)

## ANNEX XIX

### DETERMINATION OF THE STEROL COMPOSITION AND CONTENT AND ALCOHOLIC COMPOUNDS BY...

1. SCOPE
2. PRINCIPLE

#### PART 1

##### PREPARATION OF THE UNSAPONIFIABLE MATTER

1. SCOPE
2. PRINCIPLE
3. APPARATUS
4. REAGENTS
5. PROCEDURE  
Note 1: Animal or vegetable oils and fats containing appreciable quantities of...  
Note 2: Any emulsion can be destroyed by adding small quantities of...

#### PART 2

##### SEPARATION OF THE ALCOHOLIC COMPOUNDS FRACTIONS

1. SCOPE
2. PRINCIPLE
3. APPARATUS
4. REAGENTS
5. REFERENCE METHOD: SEPARATION OF THE ALCOHOLIC COMPOUNDS BY BASIC THIN-LAYER...  
Note 3: The developing mixture should be replaced for every test, in...  
Note 4: Higher temperature could worsen the separation.
6. SEPARATION OF THE ALCOHOLIC FRACTION BY HPLC  
Note 5: Carefully control the pressure of the HPLC pump, the ethyl...

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## PART 3

### GAS CHROMATOGRAPHIC ANALYSIS OF THE ALCOHOLIC COMPOUNDS FRACTIONS

1. SCOPE
2. PRINCIPLE
3. APPARATUS
4. REAGENTS
5. PREPARATION OF THE TRIMETHYLSILYL ETHERS  
Note 6: Ready for use solutions are available commercially. Other silylation reagents, ...  
Note 7: The slight opalescence, which may form, is normal and does...
6. GAS CHROMATOGRAPHIC ANALYSIS
  - 6.1. Preliminary operations, capillary column conditioning  
Note 8: The conditioning temperature must always be at least 20 °C less...
  - 6.2. Operating conditions
    - 6.2.1. Aliphatic alcohols
    - 6.2.2. Sterol and triterpenic dialcohols
  - 6.3. Analytical procedure
  - 6.4. Peak identification
  - 6.5. Quantitative evaluation
7. EXPRESSION OF THE RESULTS

#### Appendix

- Figure 1 — TLC of the unsaponifiable fraction from olive...
- Figure 2 — GC-FID chromatographic profile of the sterol and...
- Figure 3 — GC-FID chromatographic profile of the sterol and...
- Figure 4 — GC-FID chromatographic profile of aliphatic alcohols and...
- Figure 5 — GC-FID chromatographic profile of aliphatic alcohols and...
- Figure 6 — HPLC Chromatogram of an olive oil unsaponifiable...

#### ANNEX XX

Method for the determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography

1. PURPOSE
2. PRINCIPLE
3. APPARATUS
  - 3.1. Erlenmeyer flask, 25 ml .

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- 3.2. Glass column for liquid chromatography, internal diameter 15 mm, length...
  - 3.3. Gas chromatograph suitable for use with a capillary column, equipped...
    - 3.3.1. Thermostat-controlled oven with temperature programming .
    - 3.3.2. Cold injector for direct on-column injection
    - 3.3.3. Flame ionisation detector and converter-amplifier .
    - 3.3.4. Recorder-integrator (Note 1) for use with the converter-amplifier (point 3.3.3),...  
Note 1: Computerised systems may also be used where the gas chromatography...
    - 3.3.5. Capillary column, fused silica (for analysis of the waxes and...  
Note 2: Suitable commercial liquid phases are available for this purpose such...
  - 3.4. Microsyringe , 10 µl, with hardened needle, for direct on-column...
  - 3.5. Electric shaker .
  - 3.6. Rotary evaporator .
  - 3.7. Muffle oven .
  - 3.8. Analytical balance for weighing to an accuracy of  $\pm 0,1$ ...
  - 3.9. Usual laboratory glassware.
4. REAGENTS
- 4.1. Silica gel , 60-200 µm mesh. Place the silica gel...
  - 4.2. n-hexane, chromatography grade or residue grade. Hexane may be replaced...
  - 4.3. Ethyl ether, chromatography grade
  - 4.4. n-heptane , chromatography grade, or iso-octane
  - 4.5. Standard solution of lauryl arachidate ( Note 3 ), at...  
Note 3: Palmityl palmitate, myristyl stearate or arachidyl laureate may also be...
  - 4.6. Standard solution of methyl heptadecanoate, at 0,02 % (m/V) in...
  - 4.7. Sudan 1 (1-phenylazo-2-naphthol) .
  - 4.8. Carrier gas: hydrogen or helium, pure, gas chromatography grade ....  
WARNING
  - 4.9. Auxiliary gases :  
WARNING
5. PROCEDURE
- 5.1. Preparation of the chromatography column  
Note 4: The n-hexane/ethyl ether (99:1) mixture should be freshly prepared every...  
Note 5: 100 µl of Sudan I dye at 1 % in...
  - 5.2. Gas chromatography analysis
    - 5.2.1. Preliminary procedure
    - 5.2.2. Choice of operating conditions for waxes and methyl and ethyl...  
Note 6: Due to the high final temperature, positive drift is allowed...
  - 5.3. Performance of the analysis
  - 5.4. Peak identification
  - 5.5. Quantitative analysis of the waxes
    - 5.5.1. Quantitative analysis of the methyl and ethyl esters
6. EXPRESSION OF RESULTS
- Note 7: The components for quantification refer to the peaks with even...

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## Appendix A

### Determination of linear gas speed

#### ANNEX XXa

#### METHOD FOR THE DETECTION OF EXTRANEEOUS OILS IN OLIVE OILS

1. SCOPE
2. PRINCIPLE
3. MATERIAL AND REAGENTS
  - 3.1. Oil purification
    - 3.1.1. ....
    - 3.1.2. ....
    - 3.1.3. ....
    - 3.1.4. ....
    - 3.1.5. ....
    - 3.1.6. ....
    - 3.1.7. ....
  - 3.2. HPLC analysis of triacylglycerols
    - 3.2.1. ....
    - 3.2.2. ....
    - 3.2.3. ....
  - 3.3. Preparation of fatty acid methyl esters
    - 3.3.1. ....
    - 3.3.2. ....
    - 3.3.3. ....
    - 3.3.4. ....
  - 3.4. GC analysis of FAMES
    - 3.4.1. ....
    - 3.4.2. ....
    - 3.4.3. ....
    - 3.4.4. ....
    - 3.4.5. ....
4. APPARATUS
  - 4.1. ....
  - 4.2. ....
  - 4.3. HPLC equipment composed of:
  - 4.4. Capillary gas chromatography equipment described in Annex X A, provided...
  - 4.5. ....
5. ANALYTICAL PROCEDURE
  - 5.1. Oil purification
  - 5.2. HPLC analysis of triacylglycerols
  - 5.3. Preparation of fatty acid methyl esters
  - 5.4. GC analysis of fatty acid methyl esters
6. INTEGRATION OF CHROMATOGRAPHIC PEAKS
  - 6.1. HPLC chromatogram

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6.2. GC chromatogram

7. DETECTION OF EXTRANEEOUS OILS IN OLIVE OILS

ANNEX XXI

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- (1) OJ No 172, 30.9.1966, p. 3025/66.
- (2) OJ No L 353, 17.12.1990, p. 23.
- (3) OJ No L 128, 24.5.1977, p. 6.
- (4) OJ No L 166, 1.7.1988, p. 10.



### Changes to legislation:

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### Changes and effects yet to be applied to :

- Art. 1(1)-(7) words substituted by [S.I. 2019/1422 reg. 6\(2\)\(a\)](#)
- Art. 2(2) words omitted by [S.I. 2019/1422 reg. 6\(3\)\(a\)\(i\)\(aa\)](#)
- Art. 2(2) words substituted by [S.I. 2019/1422 reg. 6\(3\)\(a\)\(i\)\(bb\)](#)
- Art. 2(2) words substituted by [S.I. 2019/1422 reg. 6\(3\)\(a\)\(ii\)](#)
- Art. 2(2) words substituted by [S.I. 2019/1422 reg. 6\(3\)\(a\)\(iii\)](#)
- Art. 2(2) words substituted in earlier amending provision [S.I. 2019/1422, reg. 6\(3\)\(a\)\(i\)\(bb\)](#) by [S.I. 2020/1453 reg. 14\(16\)\(b\)](#)
- Art. 2(3) words substituted by [S.I. 2019/1422 reg. 6\(3\)\(b\)\(i\)](#)
- Art. 2(3) words substituted by [S.I. 2019/1422 reg. 6\(3\)\(b\)\(ii\)](#)
- Art. 2a(1) words substituted by [S.I. 2019/1422 reg. 6\(4\)\(a\)](#) (This amendment not applied to [legislation.gov.uk](#). [Reg. 6\(4\)\(a\)](#) substituted immediately before IP completion day by [S.I. 2020/1453, regs. 1\(2\)\(b\), 14\(16\)\(c\)\(i\)](#))
- Art. 2a(1) words substituted by [S.I. 2019/1422, reg. 6\(4\)\(a\)](#) (as substituted) by [S.I. 2020/1453 reg. 14\(16\)\(c\)\(i\)](#)
- Art. 2a(2) words substituted by [S.I. 2019/1422 reg. 6\(4\)\(b\)](#)
- Art. 2a(4) words substituted by [S.I. 2019/1422 reg. 6\(4\)\(d\)\(i\)](#)
- Art. 2a(4) words substituted by [S.I. 2019/1422 reg. 6\(4\)\(d\)\(ii\)](#)
- Art. 2a(4) words substituted in earlier amending provision [S.I. 2019/1422, reg. 6\(4\)\(d\)\(ii\)](#) by [S.I. 2020/1453 reg. 14\(16\)\(c\)\(iii\)](#)
- Art. 2a(5) words substituted by [S.I. 2019/1422 reg. 6\(4\)\(e\)](#)
- Art. 4 substituted by [S.I. 2019/1422 reg. 6\(6\)](#)
- Art. 4 words substituted in earlier amending provision [S.I. 2019/1422, reg. 6\(6\)](#) by [S.I. 2020/1453 reg. 14\(16\)\(d\)\(i\)](#)
- Art. 4 words substituted in earlier amending provision [S.I. 2019/1422, reg. 6\(6\)](#) by [S.I. 2020/1453 reg. 14\(16\)\(d\)\(ii\)](#)
- Art. 7 words substituted by [S.I. 2019/1422 reg. 6\(7\)](#)
- Art. 7a words omitted by [S.I. 2019/1422 reg. 6\(8\)](#)
- Art. 8 omitted by [S.I. 2019/1422 reg. 6\(9\)](#)
- Art. 10 omitted by [S.I. 2019/1422 reg. 6\(9\)](#)

### Changes and effects yet to be applied to the whole legislation item and associated provisions

- Signature words omitted by [S.I. 2019/1422 reg. 6\(10\)](#)
- Art. 1(8) inserted by [S.I. 2019/1422 reg. 6\(2\)\(b\)](#)
- Art. 1(8)(a)(ii)(bb) omitted in earlier amending provision [S.I. 2019/1422, reg. 6\(2\)\(b\)](#) by [S.I. 2020/1453 reg. 14\(16\)\(a\)\(i\)](#)
- Art. 1(8)(b) words substituted in earlier amending provision [S.I. 2019/1422, reg. 6\(2\)\(b\)](#) by [S.I. 2020/1453 reg. 14\(16\)\(a\)\(ii\)](#)
- Art. 1(8)(c)(ii) omitted in earlier amending provision [S.I. 2019/1422, reg. 6\(2\)\(b\)](#) by [S.I. 2020/1453 reg. 14\(16\)\(a\)\(iii\)](#)
- Annex 1a para. 1.1 words substituted by [S.I. 2019/1422 reg. 6\(11\)\(a\)](#)
- Annex 1a para. 1.2 words substituted by [S.I. 2019/1422 reg. 6\(11\)\(b\)](#)
- Art. 2a(3)(e) words substituted by [S.I. 2019/1422 reg. 6\(4\)\(c\)](#) (This amendment not applied to [legislation.gov.uk](#). [Reg. 6\(4\)\(c\)](#) substituted immediately before IP completion day by [S.I. 2020/1453, regs. 1\(2\)\(b\), 14\(16\)\(c\)\(ii\)](#))
- Art. 2a(3)(e) words substituted by [S.I. 2019/1422, reg. 6\(4\)\(c\)](#) (as substituted) by [S.I. 2020/1453 reg. 14\(16\)\(c\)\(ii\)](#)
- Art. 3 words substituted by [S.I. 2019/1422 reg. 6\(5\)\(a\)](#)

– Art. 3 words substituted by [S.I. 2019/1422 reg. 6\(5\)\(b\)](#)