[F1ANNEX X

DETERMINATION OF FATTY ACID METHYL ESTERS BY GAS CHROMATOGRAPHY

Textual Amendments

F1 Substituted by 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.

PART A

PREPARATION OF THE FATTY ACID METHYL ESTERS FROM OLIVE OIL AND OLIVE-POMACE OIL

1. 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. METHODOLOGY

3.1. **Trans-esterification with methanolic solution of potassium hydroxide at room temperature**

3.1.1. *Principle*

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

3.1.2. *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.

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. *Apparatus*

3.1.3.1. 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.**]**

Status:
Point in time view as at 31/12/2020.

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.