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DETERMINATION OF THE METHANOL AND ETHANOL CONTENT IN VIRGIN OLIVE OILS

1. Purpose and scope

This method is applicable to the determination of the methanol (MeOH) and ethanol (EtOH) content in virgin olive oils.

2. Principle

The sample is heated at 110 ºC in a hermetically sealed vial until complete desorption of the MeOH and EtOH into the gas phase. When equilibrium is reached, a specific portion of the gas phase is injected into the gas chromatography column through which MeOH and EtOH are separated and then detected with the aid of a flame ionization detector (FID). MeOH and EtOH are the main peaks in the chromatogram and are quantified using 1-propanol (PrOH) as the internal standard (IS).

3. Laboratory equipment and reagents

3.1 Vials, 20 mL (75 mm high x 22 mm id, approx.)
3.2 Aluminium crimp cap with PTFE/silicone septum (20 mm id)
3.3 Crimping pliers
3.4 Graduated pipette for automatic dosage, 500 µL
3.5 Screw-cap glass bottle, 50 mL
3.6 Analytical balance (± 0.0001 g)
3.7 1-Propanol (PrOH), analytical grade (99.8 % purity, 0.803 g/mL density)
3.8 Chemically refined olive pomace oil or another volatile-free refined oil.

4. Apparatus and testing conditions

The instructions given are for the usual equipment used for gas chromatography employing head space sampler, capillary columns, and FID.

4.1 Head space sampler

Oven temperature: 110 ºC
Loop temperature: 115 ºC
Transfer line temperature: 120 ºC
Vial equilibration: 60 min
Injection duration: 0.5 min
Vial shaking off

4.2 Gas chromatograph

4.2.1 Inlet H_2

Heater: 150 ºC
Pressure: 8.9 psi
Total flow: 79.5 mL/min
Split ratio: 50:1
Split flow: 75 mL/min

4.2.2 Capillary column

HP-88 or equivalent: 30 m x 250 µm x 0.2 µm
Pressure: 8.9 psi
Flow: 1.5 mL/min
Average velocity: 41.8 cm/sec

4.2.3 Oven: The oven should be capable of heating the capillary column to at least 260 °C and of maintaining the desired temperature to within 0.1 °C. This last requirement is important when using fused silica columns. The use of temperature-programmed heating is recommended:

Initial temperature: 50 ºC, hold for 4 mins
Rate: 30 ºC/min till 150 ºC, hold for 2.5 mins

4.2.4 FID

Heater: 150 ºC

H_2 flow: 30 mL/min

Air flow: 400 mL/min

Makeup flow (combined): 25 mL/min

4.2.5 Data acquisition system suitable for peak integration and normalization.

5. Procedure

5.1 Preparation of the internal standard solution

A working solution of PrOH (3.7), prepared as follows, is used as the IS for the quantitative determination of MeOH and EtOH:

A) Prepare a stock solution from a volatile-free refined oil (3.8): Using a graduated automatic pipette (3.4), add 1200 µL of PrOH (3.7) cooled at 4 ºC to 96 g of volatile-free refined oil (3.8) in a screw-cap glass bottle (3.5) and homogenize. Keep at 4 ºC.

B) Prepare the IS working solution by diluting 1 g of the stock solution in 24 g of the volatile-free refined oil (3.8) in a screw-cap glass bottle (3.5) and homogenize. Keep at 4 ºC.

5.2 Sample preparation

Weigh approximately 3 g of oil in a clear glass vial (3.1). Add 300 mg of the cooled IS working solution (B), quickly close the vial with an aluminium crimp cap (3.2) and seal hermetically with crimping pliers (3.3)

6. Expression of results

The concentration of MeOH and EtOH is calculated by reference to the IS. Hence, the content of a compound i (W_i), expressed in mg/kg, is obtained according to the following formula:

$W_i = 1000 \times A_i \times m_{IS} / m \times A_{IS}$

Where:

A_i is the area of the compound i divided by the corresponding response factor (see Annex)

A_{IS} is the area of PrOH;

m is the mass of the sample, in g;

m_{IS} is the mass of PrOH added to the sample, in mg.

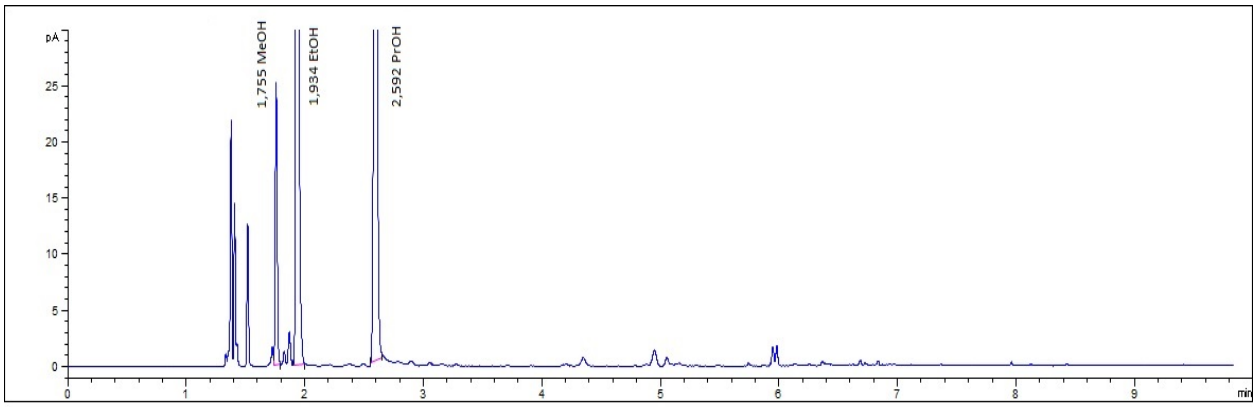
The results are expressed in mg/kg to two decimal places.

7. Test report

The test report will specify the methods used to prepare the sample and for the gas chromatographic analysis. It should also mention all operating details not specified in this method or regarded as optional, together with details of any incidents that may have influenced the results.

The test report should include all the information necessary for complete identification of the sample.

8. Sample chromatogram



ANNEX

Supplementary information

1. Calculation of the response factor

The response factor of MeOH and EtOH to PrOH (IS) can be calculated analysing a set of samples of different concentrations in duplicate. The ratio between the areas will result in the response factor. An example of the calculations is given below.

Sample	ppm MeOH	ppm EtOH	ppm PrOH	Area PrOH	Area MeOH	Area EtOH	EtOH/ PrOH	MeOH/ PrOH
1A	5.015	5.015	5.015	19.356	27.536	31.266	1.62	1.42
1B	5.015	5.015	5.015	19.557	27.371	31.471	1.61	1.40
1C	5.015	5.015	5.015	20.703	27.479	31.479	1.52	1.33
2A	10.093	10.093	10.093	40.429	54.741	62.698	1.55	1.35
2B	10.093	10.093	10.093	39.573	53.812	61.821	1.56	1.36
2C	10.093	10.093	10.093	38.836	52.854	61.149	1.57	1.36
3A	30.135	30.135	30.135	122.370	167.874	189.646	1.55	1.37
3B	30.135	30.135	30.135	120.586	160.794	184.109	1.53	1.33
3C	30.135	30.135	30.135	121.207	156.693	182.022	1.50	1.29

Response factor EtOH = 1.56 (SD = 0.04; 3SD = 0.12; RSD % = 7)

Response factor MeOH = 1.36 (SD = 0.04; 3SD = 0.12; RSD % = 9)

2. Method validation

The precision of the method was established in 2019 by an interlaboratory test carried out in accordance with ISO 5725-1 [7] and ISO 5725-2 [8]. Some 11 laboratories took part in this test from Australia, France, Germany, Italy, Morocco, Spain, Tunisia and the USA.

Table 2.1 Summary of statistical results for MeOH

Sample	**A**	**B**	**C**	**D**	**E**
Number of participating laboratories (P)	11	11	11	11	11
Number of laboratories retained after eliminating outliers (p)	9	9	10	8	9
Number of individual test results of all laboratories on each sample (z)	18	18	20	16	18
Mean value (m), (mg/kg)	1.93	8.87	9.94	6.27	6.36
Repeatability standard deviation (sr), (mg/kg)	0.164	0.503	0.518	0.321	0.407
Repeatability coefficient of variation (CVsr), %	8.45	5.66	5.21	5.11	6.38
Repeatability limit (r), (sr* 2.8), (mg/kg)	0.46	1.41	1.45	0.90	1.14
Reproducibility standard deviation (sR), (mg/kg)	0.371	2.192	1.293	1.571	1.207
Reproducibility coefficient of variation (CVsR), %	19.31	24.70	13.01	25.07	18.99
Reproducibility limit (R) (sR* 2.8), (mg/kg)	1.04	6.14	3.62	4.40	3.38
HoR	1.33	2.14	1.15	2.07	1.57

Table 2.2 Summary of statistical results for EtOH

Sample	**A**	**B**	**C**	**D**	**E**
Number of participating laboratories (P)	11	11	11	11	11
Number of laboratories retained after eliminating outliers (p)	10	9	9	9	9
Number of individual test results of all laboratories on each sample (z)	20	18	18	18	18
Mean value (m), (mg/kg)	10.17	2.13	44.21	6.34	27.28
Repeatability standard deviation (sr), (mg/kg)	0.961	0.225	2.343	0.279	1.714
Repeatability coefficient of variation (CVsr), %	10.15	10.56	5.30	4.37	6.29
Repeatability limit (r), (sr* 2.8), (mg/kg)	2.69	0.63	6.56	0.78	4.80
Reproducibility standard deviation (sR), (mg/kg)	2.879	0.557	6.721	0.632	4.286
Reproducibility coefficient of variation (CVsR), %	28.31	26.06	15.20	9.97	15.71
Reproducibility limit (R) (sR* 2.8), (mg/kg)	8.06	1.56	18.82	1.77	12.00
HoR	2.51	1.83	1.68	0.82	1.61