I. DETERMINATION OF VOLATILE SUBSTANCES AND METHANOL OF SPIRIT

I.1. GENERAL REMARKS

1. Definitions

Regulation (EEC) No 1576/89 sets minimum levels of volatile compounds other than ethanol and methanol for a series of spirit drinks (rum, spirits of viticultural origin, fruit spirits, etc.). For this series of drinks only, these levels are conventionally considered to be equivalent to the sum of the concentrations of:

1. volatile acids expressed as acetic acid;
2. aldehydes expressed as ethanal by the sum of ethanal (acetaldehyde) and the ethanal fraction contained in 1,1-diethoxyethane (acetal);
3. the following higher alcohols: propan-1-ol, butan-1-ol, butan-2-ol, 2-methylpropan-1-ol, assayed by individual alcohol and 2-methylbutan-1-ol, and 3-methylbutan-1-ol assayed as individual alcohol or the sum of the two;
4. ethyl acetate.

The following are the conventional methods for measuring volatile compounds:

- the volatile acids by means of volatile acidity,
- the aldehydes (ethanal and acetal), ethyl acetate and the alcohols by means of gas chromatography (GPC).

2. Gas chromatographic analysis of volatile compounds

Gas chromatographic assays of volatile compounds other than those set out above may prove particularly interesting as a means of determining both the origin of the raw material used in the distillation and the actual conditions of distillation.

Some spirits contain other volatile components, such as aromatic compounds, which are characteristic of the raw materials used to obtain the alcohol, of the aroma of the spirit drink and of the special features of the preparation of the spirit. These compounds are important for evaluating the requirements set out in Regulation (EEC) No 1576/89.

I.2. GAS CHROMATOGRAPHIC DETERMINATION OF VOLATILE CONGENERS: ALDEHYDES, HIGHER ALCOHOLS, ETHYL ACETATE AND METHANOL

1. Scope

This method is suitable for use for the determination of 1,1-diethoxyethane (acetal), 2-methylbutan-1-ol (active amyl alcohol), 3-methylbutan-1-ol (isoamyl alcohol), methanol (methyl alcohol), ethyl ethanoate (ethyl acetate), butan-1-ol (n-butanol), butan-2-ol (sec-butanol), 2-methylpropan-1-ol (isobutyl alcohol), propan-1-ol (n-propanol) and ethanal (acetaldehyde) in spirit drinks using gas chromatography. The method uses an internal standard, for example pentan-3-ol. The concentrations of the analytes are expressed as grams per 100 litres of absolute alcohol; the alcoholic strength of the product must be determined prior to analysis. The spirit drinks that can be analysed using this method include whisky, brandy, rum, wine spirit, fruit spirit and grape marc spirit.

2. Normative References

ISO 3696:1987: Water for analytical laboratory use — Specifications and test methods.

3. Definition

Congeners are volatile substances formed along with ethanol during fermentation, distillation and maturation of spirit drinks.

4. Principle

Congeners in spirit drinks are determined by direct injection of the spirit drink, or appropriately diluted spirit drink, into a gas chromato-

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graphy (GC) system. ~~A suitable internal standard is added to the spirit drink prior to injection~~. The ethanol contained in the analyzed alcoholic product is used as an internal standard.

The congeners are separated by temperature programming on a suitable column and are detected using a flame ionisation detector (FID). The concentration of each congener is determined with respect to the internal standard from response factors, which are obtained during calibration under the same chromatographic conditions as those of the spirit drink analysis.

5. Reagents and materials

Unless otherwise stated, use only reagents of a purity greater than 97 %, purchased from an ISO-accredited supplier with a certificate of purity, free from other congeners at test dilution (this may be confirmed by injection of individual congener standards at the test dilution using GC conditions as in 6.4) and only water of at least grade 3 as defined in ISO 3696. Acetal and acetaldehyde must be stored in the dark at < 5 ºC, all other reagents may be stored at room temperature.

5.1. Ethanol absolute (CAS 64-17-5).

5.2. Methanol (CAS 67-56-1).

5.3. Propan-1-ol (CAS 71-23-8).

5.4. 2-methylpropan-1-ol (CAS 78-33-1).

~~5.5.~~ ~~Acceptable internal standards: pentan-3-ol (CAS 584-02-1), pentan-1-ol (CAS 71-41-0), 4-methylpentan-1-ol (CAS 626-89-1) or methyl nonanoate (CAS 1731-84-6).~~

5.6. 2-methylbutan-1-ol (CAS 137-32-6).

5.7. 3-methylbutan-1-ol (CAS 123-51-3).

5.8. Ethyl acetate (CAS 141-78-6).

5.9. Butan-1-ol (CAS 71-36-3).

5.10. Butan-2-ol (CAS 78-92-2).

5.11. Acetaldehyde (CAS 75-07-0).

5.12. Acetal (CAS 105-57-7).

5.13. 40 % v/v ethanol solution

To prepare 400 ml/l ethanol solution pour 400 ml ethanol (5.1) into a 1-litre volumetric flask, make up to volume with distilled water and mix.

5.14. Preparation and storage of standard solutions (procedure used for the validated method).

All standard solutions must be stored at < 5 ºC and be prepared freshly on a monthly basis. Masses of components and solutions should be recorded to the nearest 0,1 mg.

5.14.1. Standard solution — A

Pipette the following reagents into a 100-ml volumetric flask, containing approximately 60-ml ethanol solution (5.13) to minimise component evaporation, make up to volume with ethanol solution (5.13) and mix thoroughly. Record the weight of the flask, each component added and the total final weight of contents.

Component	Volume (ml)
Methanol (5.2)	3,0
Propan-1-ol (5.3)	3,0
2-methylpropan-1-ol (5.4)	3,0
2-methylbutan-1-ol (5.6)	3,0
3-methylbutan-1-ol (5.7)	3,0
Ethyl acetate (5.8)	3,0
Butan-1-ol (5.9)	3,0
Butan-2-ol (5.10)	3,0
Acetaldehyde (5.11)	3,0
Acetal (5.12)	3,0

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Note 1: It is preferable to add acetal and acetaldehyde last in order to minimise losses through evaporation.

~~5.14.2. Standard solution — B~~

~~Pipette 3 ml of pentan-3-ol, or other suitable internal standard, (5.5) into a 100-ml volumetric flask, containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.~~

~~Record the weight of the flask, the weight of pentan-3-ol or other internal standard added and the total final weight of contents.~~

5.14.3. Standard solution — C

Pipette 1 ml solution A (5.14.1) ~~and 1 ml solution B (5.14.2)~~ into a 100-ml volumetric flask containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.

Record the weight of the flask, each component added and the total final weight of contents.

5.14.4. Standard solution — D

In order to maintain analytical continuity, prepare a quality control standard using the previously prepared standard A (5.14.1). Pipette 1 ml solution A (5.14.1) into a 100-ml volumetric flask containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.

Record the weight of the flask, each component added and the total final weight of contents.

~~5.14.5. Standard solution — E~~

~~Pipette 10 ml solution B (5.14.2) into a 100-ml volumetric flask containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.~~

Record the weight of the flask, each component added and the total final weight of contents.

5.14.6. Standard solutions used to check the linearity of response of FID

Into separate 100-ml volumetric flasks, containing approximately 80 ml ethanol (5.13), pipette 0, 0,1, 0,5, 1,0, 2,0 ml solution A (5.14.1) ~~and 1 ml solution B (5.14.2)~~, make up to volume with ethanol solution (5.13) and mix thoroughly.

Record the weight of the flask, each component added and the total final weight of contents.

5.14.7. QC standard solution

Pipette 9 ml standard solution D (5.14.4) and 1 ml ~~of standard solution E (5.14.5)~~ *40% v/v ethanol solution (5.13)* into a weighing vessel and mix thoroughly.

Record the weight of the flask, each component added and the total final weight of contents.

6. Apparatus and equipment

6.1. Apparatus capable of measuring the density and alcoholic strength.

6.2. Analytical balance, capable of measuring to four decimal places.

6.3. A temperature programmed gas chromatograph fitted with a flame ionisation detector and integrator or other data handling system capable of measuring peak areas or peak heights.

6.4. Gas chromatographic column(s), capable of separating the analytes such that the minimum resolution between the individual components (other than 2-methylbutan-1-ol and 3-methylbutan-1-ol) is at least 1.3.

Note 2: The following columns and GC conditions are suitable examples:

1. A retention gap 1 m × 0,32 mm i.d. connected to a CP-WAX 57 CB column 50 m × 0,32 mm i.d. 0,2 μm film thickness (stabilised polyethylene glycol) followed by a Carbowax 400 column 50 m × 0,32 mm i.d. 0,2 μm film thickness. (Columns are connected using press-fit connectors.)

Carrier gas and pressure: Helium (135 kPa)

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Column temperature: 35 °C for 17 min., 35 to 70 °C at 12 °C/min., hold at 70 °C for 25 min.

Injector temperature: 150 °C

Detector temperature: 250 °C

Injection volume: 1 μl, split 20 to 100:1

2. A retention gap 1 m × 0,32 mm i.d. connected to a CP-WAX 57 CB column 50 m × 0,32 mm i.d. 0,2 μm film thickness (stabilised polyethylene glycol). (Retention gap is connected using a press-fit connector.)

   Carrier gas and pressure: Helium (65 kPa)

   Column temperature: 35 °C for 10 min., 35 to 110 °C at 5 °C/min., 110 to 190 °C at 30 °C/min., hold at 190 °C for 2 min.

   Injector temperature: 260 °C

   Detector temperature: 300 °C

   Injection volume: 1 μl, split 55:1

3. A packed column (5 % CW 20M, Carbopak B), 2 m × 2 mm i.d.

   Column temperature: 65 °C for 4 min., 65 to 140 °C at 10 °C/min., hold at 140 °C for 5 min., 140 to 150 °C at 5 °C/min., hold at 150 °C for 3 min.

   Injector temperature: 65 °C

   Detector temperature: 200 °C

   Injection volume: 1 μl

7. Sampling and samples.

7.1. Laboratory sample

On receipt, the alcoholic strength of each sample is measured (6.1).

8. Procedure (used for the validated method)

8.1. Test portion

~~8.1.1. Weigh an appropriate sealed weighing vessel and record the weight.~~

~~8.1.2. Pipette 9 ml laboratory sample into the vessel and record the weight (M_{SAMPLE}).~~

~~8.1.3. Add 1 ml of standard solution E (5.14.5) and record the weight (M_{IS}).~~

~~8.1.4. Shake the test material vigorously (at least 20 inversions). Samples must be stored at less than 5 °C prior to analysis in order to minimise any volatile losses.~~

8.1.1 Put laboratory sample into 2 mL chromatographic vial for analysis.

8.2. Blank test

~~8.2.1. Using a four decimal place balance (6.2), weigh an appropriate sealed weighing vessel and record the weight.~~

~~8.2.2. Pipette 9 ml 400 ml/l ethanol solution (5.13) into the vessel and record the weight.~~

~~8.2.3. Add 1 ml of standard solution E (5.14.5) and record the weight.~~

~~8.2.4. Shake the test material vigorously (at least 20 inversions). Samples must be stored at less than 5 °C prior to analysis in order to minimise any volatile losses.~~

8.2.1 Put ethanol solution (5.13) into 2 mL chromatographic vial for analysis.

8.3. Preliminary test

Inject standard solution C (5.14.3) to ensure that all of the analytes are separated with a minimum resolution of 1.3 (except 2-methylbutan-1-ol and 3-methylbutan-1-ol).

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8.4. Calibration

The calibration should be checked using the following procedure. Ensure that the response is linear by successively analysing in triplicate each of the linearity standard solutions (5.14.6) containing internal standard (*ethanol*). From the integrator peak areas or peak heights for each injection calculate the ratio R for each congener and plot a graph of R versus the concentration ratio of congener to internal standard (*ethanol*), C. A linear plot should be obtained, with a correlation coefficient of at least 0,99.

$$R = \frac{\text{Peak area or height of congener}}{\text{Peak area or height of IS}}$$

$$C = \frac{\text{Concentration of congener } (\mu g / g)}{\text{Concentration of IS } (\mu g / g)}$$

$$R = \frac{\text{Peak area of congener}}{\text{Peak area of ethanol}}$$

$$C = \frac{\text{Concentration of congener (g/100 L of anhydrous ethanol)}}{\text{Concentration of ethanol (78927 g/100 L)}}$$

8.5. Determination

Inject standard solution C (5.14.3) and 2 QC standard solutions (5.14.7). Follow with unknown samples (prepared according to 8.1 and 8.2) inserting one QC standard every 10 samples to ensure analytical stability. Inject one standard solution C (5.14.3) after every 5 samples.

9. Calculation

An automated system of data handling can be used, provided the data can be checked using the principles described in the method below.

Measure either peak areas or peak heights for congener and internal standard (*ethanol*) peaks.

9.1. Response factor calculation.

From the chromatogram of the injection of standard solution C (5.14.3), calculate response factors for each congener using equation (1).

$$(1)\ \text{Response factor} = \frac{\text{Peak area or height IS}}{\text{Peak area or height congener}} \times \frac{\text{Conc. congener } (\mu g / g)}{\text{Conc. IS } (\mu g / g)}$$

~~where:~~

~~IS = Internal Standard~~

~~Conc. congener = concentration of congener in solution C (5.14.3)~~

~~Conc. IS = concentration of internal standard in solution C (5.14.3).~~

$$(1)\ \text{Response factor} = \frac{\text{Peak area of ethanol}}{\text{Peak area of congener}} \times \frac{\text{Concentration of congener (g/100 L of anhydrous ethanol)}}{\text{Concentration of ethanol (78927 g/100 L)}}$$

9.1.2. Sample analysis

Using equation (2) below, calculate the concentration of each congener in the samples.

(2) Congener concentrations, ~~(µg/g)~~ ***(g/100 L)***=

$$\frac{\text{Peak area or height congener}}{\text{Peak area or height IS}} \times \frac{M_{IS}\ (g)}{M_{SAMPLE}\ (g)} \times \text{Conc. IS } (\mu g / g) \times RF$$

where:

M_{SAMPLE} = weight of sample (8.1.2);

M_{IS} = weight of internal standard (8.1.3);

Conc. IS = concentration of internal standard in solution E (5.14.5);

$$\frac{\text{Peak area of congener}}{\text{Peak area of ethanol}} \times \text{Concentration of ethanol (78927 g/100 L)} \times RF$$

RF = response factor calculated using equation 1.

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9.1.3. Quality control standard solution analysis

Using equation (3) below, calculate the percentage recovery of the target value for each congener in the quality control standards (5.14.7):

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$$(3)\quad \%\ \text{recovery of QC sample} = \frac{\text{concentration of analyte in QC standard}}{\text{concentration of analyte in solution D}} \times 100$$

The concentration of the analyte in the QC standard is calculated using equations (1) and (2) above.

~~9.2. Final presentation of results~~

~~Results are converted from μ/g to g per 100 litres absolute alcohol for samples using equation (4):~~

$$(4)\quad \text{Concentration in g per 100 literes absolute alcohol} = \text{Conc } (\mu g\ /\ g) \times \rho \times 10/(\text{strengh } (\%\ \text{vol}) \times 1\ 000)$$

~~where~~

~~ρ = density in kg/m^3.~~

~~Results are quoted to 3 significant figures and a maximum of one decimal place e.g. 11,4 g per 100 l absolute alcohol.~~

10. Quality assurance and control (used for the validated method)

Using equation (2) above, calculate the concentration of each congener in the quality control standard solutions prepared by following the procedure as in 8.1.1 to 8.1.4. Using equation (3), calculate the percentage recovery of the target value. If the analysed results are within ± 10 % of their theoretical values for each congener, analysis may proceed. If not, an investigation should be made to find the cause of the inaccuracy and remedial action taken as appropriate.

11. Method performance characteristics (precision)

Statistical results of the interlaboratory test: the following tables give the values for the following compounds: ethanal, ethyl acetate, acetal, total ethanal, methanol, butan-2-ol, propan-1-ol, butan-1-ol, 2-methyl-propan-1-ol, 2 methyl-butan-1-ol, 3 methyl-butan-1-ol.

The following data were obtained from an international method performance study carried out to internationally agreed procedures.

Year of interlaboratory test	2023
Number of laboratories	32
Number of samples	5
Analyte	ethanal

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	28	26	27	27	28
Number of outliers (laboratories)	2	4	3	3	2
Number of accepted results	56	52	54	54	56
Mean value ($\bar{x}$) μg/g	63,4	71,67	130,4	38,4 13,8 (*)	28,6 52,2 (*)
Repeatability standard deviation (S_r) μg/g	3,3	1,9	6,8	4,1	3,6
Repeatability relative standard deviation (RSD_r) (%)	5,2	2,6	5,2	15,8	8,9
Repeatability limit (r) μg/g	9,3	5,3	19,1	11,6	10,1
Reproducibility standard deviation (S_R) μg/g	12	14	22	6,8	8,9
Reproducibility relative standard deviation (RSD_R) (%)	18,9	19,4	17,1	26,2	22,2

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Samples	A	B	C	D	E
Reproducibility limit (R) µg/g	33,5	38,9	62,4	19,1	25,1

Sample types
A Brandy; blind duplicates.
B Kirsch; blind duplicates.
C Grappa; blind duplicates.
D Whisky; split levels (*).
E Rum; split levels (*).

Year of interlaboratory test	2023
Number of laboratories	32
Number of samples	5
Analyte	ethyl acetate

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	24	24	25	24	24
Number of outliers (laboratories)	2	2	1	2	2
Number of accepted results	48	48	50	48	48
Mean value ($\bar{x}$) µg/g	96,8	1 046	120,3	112,5 91,8 (*)	99,1 117,0 (*)
Repeatability standard deviation (S_r) µg/g	2,2	15	2,6	2,1	2,6
Repeatability relative standard deviation (RSD_r) (%)	2,3	1,4	2,1	2,0	2,4
Repeatability limit (r) µg/g	6,2	40,7	7,2	5,8	7,3
Reproducibility standard deviation (S_R) µg/g	6,4	79	8,2	6,2	7,1
Reproducibility relative standard deviation (RSD_R) (%)	6,6	7,6	6,8	6,2	6,6
Reproducibility limit (R) µg/g	17,9	221,9	22,9	17,5	20,0

Sample types
A Brandy; blind duplicates.
B Kirsch; blind duplicates.
C Grappa; blind duplicates.
D Whisky; split levels (*).
E Rum; split levels (*).