COMMISSION REGULATION (EC) No 2091/2002

of 26 November 2002

amending Regulation (EC) No 2870/2000 laying down Community reference methods for the analysis of spirits drinks

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Regulation (EEC) No 1576/89 of 29 May 1989 laying down general rules on the definition, description and presentation of spirit drinks [1], as amended by the Act of Accession of Austria, Finland and Sweden, and in particular Article 4(8) thereof,

Whereas:

(1) Commission Regulation (EC) No 2870/2000 of 19 December 2000 laying down Community reference methods for the analysis of spirits drinks [2] describes these methods in an Annex.

(2) Four methods of analysis for the determination of trans-anethole in aniseed-flavoured spirit drinks, glycyrrhizic acid and chalcones in pastis and egg yolk in egg liqueur and liqueur with egg have been validated according to internationally recognised procedures as part of a research project supported by the Commission.

(3) These four methods may be recognised as Community reference methods and must be added to the Annex to Regulation (EC) No 2870/2000.

(4) The measures provided for in this Regulation are in accordance with the opinion of the Implementation Committee for Spirit Drinks,

HAS ADOPTED THIS REGULATION:

Article 1

The Annex to Regulation (EC) No 2870/2000 is hereby amended as follows:

1. In the summary of the Annex, the term '(p.m.)' under points V, VI, VII and IX shall be deleted.

2. Chapters V, VI, VII and IX in the Annex to this Regulation shall be added after Chapter III.

Article 2

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

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

Done at Brussels, 26 November 2002.

For the Commission
Franz FISCHLER
Member of the Commission

[1] OJ L 160, 12.6.1989, p. 1.
[2] OJ L 333, 29.12.2000, p. 20.

ANNEX

V. ANETHOLE. GAS CHROMATOGRAPHIC DETERMINATION OF TRANS-ANETHOLE IN SPIRIT DRINKS

1. Scope

This method is suitable for the determination of trans-anethole in aniseed-flavoured spirit drinks using capillary gas chromatography.

2. Normative references

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

3. Principle

The trans-anethole concentration of the spirit is determined by gas chromatography (GC). The same quantity of an internal standard, e.g. 4-allylanisole (estragole) when estragole is not naturally present in the sample, is added to the test sample and to a trans-anethole reference solution of known concentration, both of which are then diluted with a 45 % ethanol solution and injected directly into the GC system. An extraction is necessary before sample preparation and analysis for liqueurs that contain large amounts of sugars.

4. Reagents and materials

During the analysis, use only reagents of a purity of at least 98 %. Water of at least grade 3 as defined by ISO 3696 should be used.

Reference chemicals should be stored cold (at 4 °C), away from light, in aluminium containers or in tinted (amber) glass reagent bottles. The stoppers should preferably be fitted with an aluminium seal. Trans-anethole will need to be 'thawed' from its crystalline state before use, but in this case its temperature should never exceed 35 °C.

4.1. Ethanol 96 % vol. (CAS 64-17-5)

4.2. 1-methoxy-4-(1-propenyl) benzene; (trans-anethole) (CAS 4180-23-8)

4.3. 4-allylanisole, (estragole) (CAS 140-67-0), suggested internal standard (IS)

4.4. Ethanol 45 % vol.

Add 560 g of distilled water to 378 g of ethanol 96 % vol.

4.5. Preparation of standard solutions

All standard solutions should be stored at room temperature (15 to 35 °C) away from light in aluminium containers or in tinted (amber) glass reagent bottles. The stopper should preferably be fitted with an aluminium seal.

Trans-anethole and 4-allylanisole are practically insoluble in water, and it is therefore necessary to dissolve the trans-anethole and 4-allylanisole in some 96 % ethanol (4.1) before the addition of 45 % ethanol (4.4).

The stock solutions must be freshly prepared each week.

4.5.1. Standard solution A

Stock solution of trans-anethole (concentration: 2 g/l)

Weigh 40 mg of trans-anethole (4.2) in a 20 ml volumetric flask (or 400 mg in 200 ml, etc.). Add some 96 % ethanol (4.1) and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

4.5.2. Internal standard solution B

Stock solution of internal standard, e.g. estragole (concentration: 2 g/l)

Weigh 40 mg of estragole (4.3) in a 20 ml volumetric flask (400 mg in 200 ml etc.). Add some 96 % vol. ethanol (4.1) make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

4.5.3. Solutions used to check the linearity response of the flame ionisation detector (FID)

The linearity response of the FID must be checked for the analysis taking into account a range of concentrations of trans-anethole in spirits from 0 g/l up to 2.5 g/l. In the procedure of analysis, the unknown samples of spirits to be analysed are diluted 10 times (8.3). For the conditions of the analysis described in the method, stock solutions corresponding to concentrations of 0, 0,05, 0,1, 0,15, 0,2, and 0,25 g/l of trans-anethole in the sample to be analysed are prepared as follows: take 0,5, 1, 1,5, 2, and 2,5 ml of stock solution A (4.5.1) and pipette in separate 20 ml volumetric flasks; pipette into each flask 2 ml of internal standard solution B (4.5.2) and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

The blank solutions (8.4) is used as the 0 g/l solution.

4.5.4. Standard solution C

Take 2 ml of standard solution A (4.5.1) and pipette into a 20 ml volumetric flask, then add 2 ml of internal standard solution B (4.5.2) and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

5. **Apparatus and equipment**

5.1. A capillary gas chromatograph fitted with a flame ionisation detector (FID) and integrator or other data handling system capable of measuring peak heights or areas, and with an automatic sampler or the necessary equipment for manual sample injection.

5.2. Split/splitless injector

5.3. Capillary column, for example:

Length: 50 m

Internal diameter: 0,32 mm

Film thickness: 0,2 µm

Stationary phase: FFAP — modified TPA polyethylene glycol cross-linked porous polymer.

5.4. Common laboratory equipment: A grade volumetric glassware, analytical balance (precision: ± 0,1 mg).

6. **Chromatography conditions**

The column type and dimensions, and the GC conditions, should be such that anethole and the internal standard are separated from each other and from any interfering substances. Typical conditions for the column given as an example in 5.3 are:

6.1. Carrier gas: analytical helium

6.2. Flow rate: 2 ml/min

6.3. Injector temperature: 250 °C

6.4. Detector temperature: 250 °C

6.5. Oven temperature conditions: isothermal, 180 °C, run time 10 minutes

6.6. Injection volume: 1 µl, split 1:40.

7. **Samples**

Samples should be stored at room temperature, away from light and cold.

8. **Procedure**

8.1. Sample screening for estragole

To ensure that there is no estragole naturally present in the sample, a blank analysis should be carried out without the addition of any internal standard. If estragole is naturally present then another internal standard must be chosen (for instance menthol).

Pipette 2 ml sample into a 20 ml volumetric flask and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

8.2. Preparation of unknown samples

Pipette 2 ml sample into a 20 ml volumetric flask then add 2 ml of internal standard solution B (4.5.2) and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

8.3. Blank

Pipette 2 ml of internal standard solution B (4.5.2) into a 20 ml volumetric flask and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

8.4. Linearity test

Prior to the commencement of the analysis the linearity of the response of the FID should be checked by successively analysing in triplicate each of the linearity standard solutions (4.5.3).

From the integrator peak areas or peak heights for each injection plot a graph of their mother solution concentration in g/l versus the ratio R for each.

R = trans-anethole peak height or area divided by the estragole peak height or area.

A linear plot should be obtained.

8.5. Determination

Inject the blank solution (8.3), followed by standard solution C (4.5.4), followed by one of the linearity standards (4.5.3) which will act as a quality control sample (this may be chosen with reference to the probable concentration of trans-anethole in the unknown), followed by five unknowns (8.2); insert a linearity (quality control) sample after every five unknown samples, to ensure analytical stability.

9. **Calculation of response factor**

Measure either peak areas (using an integrator or other data system) or peak heights (manual integration) for trans-anethole and internal standard peaks.

9.1. Response factor (RF_i) calculation

The response factor is calculated as follows

$RF_i = (C_i/\text{area or height}_i)*(\text{area or height}_{is}/C_{is})$

where:

C_i	is the concentration of trans-anethole in the standard solution A (4.5.1)
C_{is}	is the concentration of internal standard in the standard solution B (4.5.2)
area_i	is the area (or height) of the trans-anethole peak
area_{is}	the area (or height) of the internal standard peak

RF_i is calculated from the five samples of solution C (4.5.4).

9.2. Analysis of the linearity response test solutions

Inject the linearity response test solutions (4.5.3).

9.3. Analysis of the sample

Inject the unknown sample solution (8.2).

10. **Calculation of results**

The formula for the calculation of the concentration of trans-anethole is the following:

$c_i = C_{is} * (\text{area or height}_i/\text{area or height}_{is})*RF_i$

where:

c_i	is the unknown trans-anethole concentration
C_{is}	is the concentration of internal standard in the unknown (4.5.2)
Area or height_i	is the area or height of the trans-anethole peak
$\text{Area or height}_{is}$	the area or height of the internal standard peak
RF_i	is the response coefficient (calculated as in 9.1)

The trans-anethole concentration is expressed as grams per litre, to one decimal place.

11. **Quality assurance and control**

The chromatograms should be such that anethole and the internal standard are separated from each other and from any interfering substances. The RF_i value is calculated from the results for the five injections of solution C (4.5.4). If the coefficient of variation (CV % = (standard deviation/mean)*100)) is within plus or minus 1 %, the RF_i average value is acceptable.

The calculation above should be used to calculate the concentration of trans-anethole in the sample selected for the quality control from the linearity control solutions (4.5.3).

If the mean calculated results from analysis of the linearity solution selected for internal quality control sample (IQC) are within plus or minus 2,5 % of their theoretical value, then the results for the unknown samples can be accepted.

12. **Treatment of spirits sample containing large amount of sugar and of liqueur sample prior to GC analysis**

Extraction of alcohol from spirit drink containing a large amount of sugar, in order to be able to determine the trans-anethole concentration using capillary gas chromatography.

12.1. Principle

An aliquot of the liqueur sample is taken and to this is added the internal standard, at a concentration similar to that of the analyte (trans-anethole) in the liqueur. To this are added sodium phosphate dodecahydrate and anhydrous ammonium sulphate. The resulting mixture is well shaken and chilled, two layers develop, and the upper alcohol layer is removed. An aliquot of this alcohol layer is taken and diluted with 45 % ethanol solution (4.4) (Note: no internal standard is added at this stage, because it has already been added). The resulting solution is analysed in gas chromatography.

12.2. Reagents and materials

During the extraction use only reagents of a purity greater than 99 %.

12.2.1. Ammonium sulphate, anhydrous, (CAS 7783-20-2).

12.2.2. Sodium phosphate, dibasic, dodecahydrate, (CAS 10039-32-4).

12.3. Apparatus and equipment

Conical flasks, separating flasks, refrigerator.

12.4. Procedure

12.4.1. Sample screening for estragole

To ensure that there is no estragole naturally present in the sample, a blank extraction (12.6.2) and analysis should be carried out without the addition of any internal standard. If estragole is naturally present then another internal standard must be chosen.

12.4.2. Extraction

Pipette 5 ml of 96 % ethanol (4.1) into a conical flask, weigh into this flask 50 mg of internal standard (4.3), and add 50 ml of the sample. Add 12 g of ammonium sulphate, anhydrous (12.2.1), and 8.6 g of dibasic sodium phosphate, dodecahydrate (12.2.2). Stopper the conical flask.

Shake the flask for at least 30 minutes. A mechanical shaking device may be used, but not a Teflon coated magnetic stirring bar, as the Teflon will absorb some of the analyte. Note that the added salts will not dissolve completely.

Place the stoppered flask in a refrigerator (T < 5 C) for at least two hours.

After this time, there should be two distinct liquid layers and a solid residue. The alcohol layer should be clear; if not, replace in the refrigerator until a clear separation is achieved.

When the alcohol layer is clear, carefully take an aliquot (e.g. 10 ml), without disturbing the aqueous layer, place in an amber vial and close securely.

12.4.3. Preparation of the extracted sample to be analysed

Allow extract (12.4.2) to reach room temperature.

Take 2 ml of the alcohol layer of the attemperated extracted sample and pipette into a 20 ml volumetric flask, make up to volume with 45 % ethanol (4.4), mix thoroughly.