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Cork stoppers — Determination of releasable 2, 4, 6-trichloroanisol (TCA)

Bouchons en liège — Dosage du 2, 4, 6-trichloroanisol (TCA) relargable

Reference number
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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 20752 was prepared by Technical Committee ISO/TC 87, *Cork*.

Introduction

This method intends to simulate migration phenomena that may occur when cork stoppers are used to close wine bottles.

It consists of determining the releasable 2,4,6-trichloroanisol (TCA) based on an equilibrium between the solid (cork) and the liquid (hydro-alcoholic simulator) matrices, after submitting a sample of cork stoppers to a period of maceration in a hydro-alcoholic solution.

Cork stoppers — Determination of releasable 2, 4, 6-trichloroanisol (TCA)

1 Scope

This International Standard specifies a test method to determine releasable 2,4,6-trichloroanisol (TCA) from cork stoppers.

This International Standard is applicable to all types of cork stoppers.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 633 [1)], *Cork — Vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 633, and the following, apply.

3.1
simulator
solution that intends to simulate the wine

3.2
internal standard
compound of known concentration added to a sample to facilitate the qualitative identification and/or quantitative determination of the sample components

4 Symbols and abbreviated terms

GC/ECD	Gas chromatography/electron-capture detector
GC/MS	Gas chromatography/mass spectrometry
SPME	Solid phase microextraction
TCA	2,4,6-Trichloroanisol

1) Under revision.

5 Principle

Determination of releasable 2,4,6-trichloroanisol (TCA) from cork stoppers, previously subjected to maceration in a wine simulator, using solid-phase microextraction followed by the detection and quantification of this compound by GC/MS or GC/ECD.

6 Reagents

Use only reagents of recognized analytical grade and distilled water.

6.1 Hydro-alcoholic solution, 12 % (volume fraction).

6.2 Absolute ethanol.

6.4 Sodium chloride (NaCl).

6.5 2,4,6-Trichloroanisol (TCA) d_5, purity $\geqslant$ 98 % (for GC/MS).

6.6 2,4,6-Trichloroanisol (TCA), purity $\geqslant$ 99 %.

6.7 Selected internal standard, purity $\geqslant$ 99 % (for GC/ECD), of 2.3.6-T.C.A, for example.

7 Apparatus

Standard laboratory apparatus and the following.

7.1 Balance, with a resolution of at least 0,1 mg.

7.2 Glass maceration flasks, with a stopper of glass or metal or any other material not absorbing TCA, and with a capacity appropriate to the sample size.

7.3 Glass flasks (vial), of 10 ml minimum capacity (solution shall occupy at least 50 % of the vial capacity), with a silicone/PTFE septum and an appropriate stopper.

7.4 SPME fibre, 100 µm polydimethlysiloxane PDMS, with either a manual support or a support suitable for automatic operation.

7.5 Heating source for the vial, set at a temperature of 35 °C.

7.6 Automatic stirring system, for the SPME.

7.7 Appropriated gas, of chromatographic purity.

7.8 Gas chromatograph, with a mass detector (MS) or an electron-capture detector (ECD).

7.9 Capillary column of light polarity silica glass.

EXAMPLE

Operating conditions: length 30 m, inside diameter 0,25 mm, film thickness 0,25 µm, stationary phase: diphenyl copolymer (5 %) and dimethylsiloxane (95 %).

8 Sampling

The size of the sample to be tested will be agreed upon by the customer and supplier.

9 Procedure

9.1 Calibration

Add known concentrations to the wine simulator to obtain the set of calibration solutions for TCA. Standard solutions from 0,5 ng/l to 50 ng/l can be used.

Evaluate regularly the calibration curve obtained, re-evaluate it whenever any large change is recorded by the GC/MS or GC/ECD.

9.2 Sample preparation

Subject straight cork stoppers, "*ras-de-bague*", to maceration for 24 h ± 2 h at room temperature, using enough simulator to keep the stoppers totally immersed.

For bar-top stoppers, cut off the flange before macerating.

EXAMPLE

50 stoppers of 45 mm × 24 mm, in a 2 l flask.

For cork stoppers for sparkling wines, immerse only the discs of natural cork and 1 cm of the agglomerate without cutting the stoppers.

9.3 SPME

9.3.1 Test portion

Take a volume of the solution to be analysed not greater than 50 % of the vial capacity (7.3); for example; 10 ml of the solution to be analysed for a vial of 20 ml, in such a way that the fibre does not touch the liquid. Saturate the test portion with NaCl (approximately 0,30 g/ml) (6.3).

Add the Internal standard solution, 12 % (by volume) ethanol/water (6.1), to the test portion in the vial in such a way that the ethanol content and the total volume of the liquid do not change significantly.

EXAMPLE

Add 100 µl of internal standard solution for a total liquid volume of 10 ml.

The concentration of the internal standard solution shall be in the calibration curve range and shall be as close as possible to the specification.

9.3.2 Adsorption

Stir at a temperature of 35 °C ± 2 °C for a minimum of 15 min in the vial open space.

9.4 GC

9.4.1 Desorption

Splitless injection for 2 min, at 250 °C (conditioning temperature of the 100 µm PDMS fibre). The fibre shall be conditioned between two extractions.

9.4.2 Chromatographic analysis

The resolution grade and the time needed for the chromatographic separation shall be optimized by using a temperature program.

EXAMPLE

Temperature program: 50 °C for 2 min, from 50 °C to 150 °C at 9 °C/min, 0,5 min at 150 °C, from 150 °C to 260 °C at 20 °C/min, 260 °C for 5 min.

9.5 Detection

9.5.1 Determination by MS

Detection either in selected ion monitoring (SIM) or in MS/MS mode with detection of 3 ions, and quantification through the most abundant ion.

Ions are the following:

For TCA: m/z 195, 210, 212, quantification with m/z 195.

For TCA d_5: m/z 199, 215, 217, quantification with m/z 215.

The operating conditions in MS/MS mode are shown in Table 1

Table 1 — Operating conditions in MS/MS mode

Compound	Precursor ion	Product ion
2,4,6 TCA-d_5	217	199
2,4,6 TCA	212	197

9.5.2 Determination by ECD

Identify the analyte by comparing, on the chromatogram, the time retention of the sample peak to that of the standard peak.

9.6 Blank test

Carry out a parallel test, without the stoppers in 9.2.

10 Expression of results

Express concentration in nanograms per litre (ng/l).

2,4,6 TCA concentrations shall be rounded to the nearest whole number.

11 Test report

The test report shall include the following information:

a) number of stoppers tested and volume of simulator used;

b) a reference to this International Standard (ISO 20752);

c) all data needed to identify completely the tested sample;

d) capillary column used, gas chromatography conditions applied and type of detection used;

e) internal standard used;

f) concentration of 2,4,6 TCA released, in nanograms per litre;

g) detection limit and quantification limit;

h) any incident likely to have affected the results, noted during the analysis;

i) all optional operations or those not specified in this International Standard.