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

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

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 87, *Cork*.

This second edition cancels and replaces the first edition (ISO 20752:2007), which has been technically revised.

Introduction

This International Standard intends to simulate migration phenomena that might occur when cork stoppers are used to close wine bottles.

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

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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 and all their cork constituents.

2 Normative references

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

ISO 633, *Cork — Vocabulary*

ISO 17727, *Cork — Cork stoppers for still wine — Sampling plan for the quality control of cork stoppers*

3 Terms and definitions

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

3.1

simulant

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

PDMS	polydimethylsiloxane
GC/ECD	gas chromatography/electron capture detector
GC-MS	gas chromatography/mass spectrometry
SPME	solid phase microextraction
TCA	2,4,6-trichloroanisol

5 Principle

Determination of releasable TCA from cork stoppers, previously subjected to maceration in a wine simulant, 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.

6.1 Hydro-alcoholic solution, 12 % (v/v) (wine simulant).

6.2 Ethanol, with a minimum purity of 95 %.

6.3 Distilled water.

6.4 Sodium chloride (NaCl) p.a.

6.5 Internal standard, (GC-MS) 2,4,6-trichloroanisole (TCA) d_5 , purity ≥ 98 % or (GC/ECD) 2,3,6-trichloroanisole, purity ≥ 99 %.

6.6 2,4,6-trichloroanisole (TCA), purity ≥ 99 %.

7 Apparatus

The usual laboratory apparatus and, in particular, the following:

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

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

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

7.4 SPME fibre.

EXAMPLE 100 μm PDMS.

7.5 Heating source, for the vial ([7.3](#)), set between 30 °C and 50 °C.

7.6 Automatic stirring system, for the SPME.

7.7 Appropriate gas, of chromatographic purity.

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

7.9 Low-polarity fused silica capillary column.

EXAMPLE Length of 30 m, internal diameter of 0,25 mm, film thickness of 0,25 μm ; stationary phase: copolymer of 5 % diphenyl and 95 % dimethyl polysiloxane.

8 Sampling

The size of the sample to be tested shall be in accordance with the sampling standard (ISO 17727) or agreed between customer and supplier.

9 Procedure

9.1 Calibration

The set of calibration solutions for TCA is obtained by adding known concentrations of analyte to the wine simulant. Standard solutions from 0,5 ng/L to 10 ng/L can be used.

The calibration curve obtained shall be evaluated regularly and whenever any large change is recorded by the GC-MS or GC/ECD.

9.2 Sample preparation

Intact straight cork stoppers are subjected to maceration for $24 \text{ h} \pm 2 \text{ h}$ at room temperature, using enough wine simulant, to keep the stoppers entirely immersed, and then analyse the macerate.

EXAMPLE 1 20 corks (45×24) mm, in a 1 L flask.

EXAMPLE 2 50 stoppers (45×24) mm, in a 2 L flask.

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

For granulated cork, 40 g of granulated cork are subjected to maceration for $24 \text{ h} \pm 2 \text{ h}$ at room temperature in a 2 L flask, using enough wine simulant to keep the granulated cork entirely immersed, and then analyse the macerate.

For cork discs, 20 cork discs are subjected to maceration for $24 \text{ h} \pm 2 \text{ h}$ at room temperature in a 0,5 L flask, using enough wine simulant to keep the granulated cork entirely immersed, and then analyse the macerate.

For cork stoppers for sparkling wines, immerse in the wine simulant only the discs of natural cork and 1 cm of the agglomerate without cutting the stoppers, for $24 \text{ h} \pm 2 \text{ h}$ at room temperature, and then analyse the macerate.

9.3 SPME

9.3.1 Test portion

Take a volume of the solution to be tested not greater than 50 % of the vial capacity (7.3).

EXAMPLE 1 For a vial (7.3) of 20 mL, take 10 mL, in such a way that the fibre does not touch the liquid.

The test portion should be saturated with NaCl (approximately 0,30 g/mL).

Add the internal standard solution, 12 %, v/v ethanol/water, to the test portion in the vial (7.3) in such a way that the ethanol content and the total volume of the liquid do not change significantly.

EXAMPLE 2 100 μL internal standard solution/10 mL total liquid.

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

Adjust the time and temperature in the headspace, according to the fibre used.

EXAMPLE Fibre PDMS 100 μm , 15 min in the headspace, stirring at a temperature of $35 \text{ }^{\circ}\text{C} \pm 2 \text{ }^{\circ}\text{C}$.

9.4 GC

9.4.1 Desorption

Splitless injection from 1 min to 2 min at 250 °C. The fibre shall be conditioned/cleaned 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.

An example of a temperature program is shown below.

Temperature	Time	Increment
50 °C	2 min	—
From 50 °C to 150 °C	—	9 °C/min
150 °C	0,5 min	—
From 150 °C to 260 °C	—	20 °C/min
260 °C	5 min	—

9.5 Detection

9.5.1 Determination by MS

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

Ions are the following:

- for 2,4,6 TCA: m/z 195, 210, 212, quantification with m/z 195;
- for 2,4,6 TCA d₅: 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	171
2,4,6 TCA	212	197	169

9.5.2 Determination by ECD

Identify the peaks corresponding to analyte and to the internal standard by comparing, on the chromatogram, the retention time in the sample with the retention time in the standard peak.

9.6 Blank test

Carry out a parallel test, without the stoppers mentioned in [9.2](#).

10 Expression of results

The results shall be expressed in nanogram per litre and shall be rounded to the nearest to 0,5.

11 Test report

The test report shall include the following information:

- a) number of stoppers tested and capacity of the maceration flask used;
- b) reference to this International Standard (i.e. 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 releasable 2,4,6 TCA, in nanogram 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.

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