INTERNATIONAL STANDARD

ISO 20752

Third edition
2023-12

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

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

Reference number
ISO 20752:2023(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 document 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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO TC 87, *Cork*.

This third edition cancels and replaces the second edition (ISO 20752:2014), which has been technically revised.

The main changes are as follow:

— Clauses 1, 2, 3, 4, 5, 10, 11 and 12 were modified.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

INTERNATIONAL STANDARD **ISO 20752:2023(E)**

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

1 Scope

This document specifies a test method to determine releasable 2,4,6-trichloroanisole (TCA) from all types of cork stoppers and their constituents.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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, *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.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at https://www.iso.org/obp
- IEC Electropedia: available at https://www.electropedia.org/

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 Test conditions

The test shall be carried out in an environment free of odours at an ambient temperature of (21 ± 4) ºC

7 Reagents

Use only reagents of recognized analytical grade.

7.1 Hydro-alcoholic solution, 12 % (volume fraction) (wine simulant) previously analysed.

7.2 Ethanol, with a minimum purity of 95 % (volume fraction).

7.3 Pure desionized water, filtered water type III or of higher quality.

7.4 Sodium chloride (NaCl) p.a.

7.5 Internal standard for **GC-MS analysis**, 2,4,6-trichloroanisole (TCA)-d5 purity 98 % (volume fraction) or 2,3,6-trichloroanisole purity 99 % (volume fraction).

7.6 Internal standard for GC-ECD analysis, 2,6-dibromoanisole purity 99 % (volume fraction) or 2,3,6-trichloroanisole purity 99 % (volume fraction).

8 Apparatus

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

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

8.2 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.

8.3 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 suitable for SPME.

8.4 SPME fibre.

EXAMPLE 100 µm PDMS.

8.5 Heating source, for the vial (8.3), set between 30 °C and 50 °C.

8.6 Automatic stirring system, for the SPME.

8.7 Appropriate gas, of chromatographic purity.

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

8.9 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.

9 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.

10 Procedure

10.1 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 50 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.

10.2 Sample preparation

Macerate the sample for 24 h ± 2 h at room temperature (as defined in Clause 6), using enough wine simulant to keep the whole contact surface of the sample entirely immersed, and then analyse the macerate, see Table 1.

Table 1 — Sampling size — Examples for each type of sample

Type of sample	Quantity	Flask volume
Completely inserted natural, colmated, agglomerated, microagglomerated cork stoppers, and cork bodies	20 (45 × 24) cork stoppers/bodies	1 000 ml
	50 (45 × 24) cork stoppers/bodies	2 000 ml
Granulated cork	40 g	2 000 ml
Bar-top stoppers with natural cork bodies and agglomerated cork bodies [a]	20 (27 × 20) cork bodies	500 ml
	60 (27 × 20) cork bodies	1 000 ml
Cork discs	20 cork discs	500 ml

[a] For bar-top stoppers, cut off the flanges before maceration. However, it's recommended to carry out the test before capsulating.

For partially inserted cork stoppers for sparkling wines, immerse the stoppers to a height of 22 mm in the wine simulant (corresponding to the height of two 6 mm discs + 1 cm of agglomerated cork body) without cutting the stoppers.

The number of stoppers/discs to be used shall assure a correct proportion between calibre/exposed surface. In all other cases, a correct calibre/exposed surface ratio shall be assured.

10.3 SPME

10.3.1 Test portion

To increase extraction efficiency and subsequent sensitivity of the method, a quantity of approximately 3 g of sodium chloride (7.4) can be added in order to saturate the solution. 50 µl of the internal standard solution at 2,0 µg/l (see 3.2) are immediately added, then the glass flask (vial) (8.3) is closed using a perforated metal capsule fitted with a silicone/polytetrafluoroethylene coated liner.

Take a volume of the solution to be tested not greater than 50 % of the vial capacity (8.3).

EXAMPLE 1 For a vial (8.3) of 20 ml, take 10 ml, in such a way that the fibre does not touch the liquid.

Add the internal standard solution, 12 %, volume fraction ethanol/water, to the test portion in the vial (8.3) 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 middle of calibration curve range as close as possible to the specification.

10.3.2 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 °C ± 2 °C.

10.4 GC

10.4.1 Desorption

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

10.4.2 Chromatographic analysis

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

Table 2 — Example of temperature program

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	—

10.5 Detection

10.5.1 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:

Table 3 — Ions for MS

Compound	Quantifier ion (m/z)	Qualifier ion (m/z)		
2,4,6-TCA d_5	215	199	215	217
2,4,6-TCA	195	195	210	212
2,3,6-TCA	212	195	210	212

Table 4 — Ions for MS/MS

Compound	Precursor ion (m/z)	Product ion (m/z)	
2,4,6-TCA d_5	217	199	171
2,4,6-TCA	212	197	169

10.5.2 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.

10.6 Blank test

Carry out a parallel test, without the stoppers mentioned in 10.2.

11 Expression of results

The results shall be expressed in nanogram per litre and shall be rounded off to the nearest 0,5. Whenever the measurement of the uncertainty is reported, the results shall be rounded off to the nearest 0,1 ng/l.

12 Test report

The test report shall include the following information:

a) quantity and type of sample tested and capacity of the maceration flask used;

b) a reference to this document, i.e. ISO 20752:2023;

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

d) internal standard used;

e) concentration of releasable 2,4,6-TCA, in nanograms per litre;

f) measurement uncertainty, if the expression of the results is rounded off to the nearest 0,1 ng/l;

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 document.