OIV-MA-AS315-16 Determination of releasable 2,4,6-trichloroanisole in wine by cork stoppers

Type IV method

1. Scope

The method of determination of releasable 2,4,6-trichloroanisole (TCA) by cork stoppers measures the quantity of TCA released by a sample of cork stoppers macerated in a aqueous-alcoholic solution. The aim of this method is to evaluate the risk of releasing by the lot of analyzed cork stoppers and to provide a method for controlling the quality of cork stoppers.

2. Principle

The method aims to simulate 2,4,6-trichloroanisole migration phenomena susceptible of being produced between the cork stopper and wine in bottles. Cork stoppers are macerated in a wine or a aqueous-alcoholic solution, until a balance is obtained. The TCA of the head space is sampled from an appropriate part of the macerate by the solid-phase micro-extraction technique (SPME), then analyzed by gas chromatography, with detection by mass spectrometer (or by electron-capture detector).

3. Reagents and products

3.1. White wine with an alcoholic strength ranging between 10 and 12 % vol. (It can be replaced by an aqueous-alcoholic solution with an alcoholic strength of 12 % vol). The wine and/or the aqueous-alcoholic solution must be free of TCA.

3.2. Sodium chloride ≥ 99.5 %

3.3. Internal standard for GC/MS analysis: 2,4,6-trichloroanisole (TCA)-d5 purity ≥ 98% or 2,3,6-trichloroanisole purity ≥ 99%.

Internal standard for GC/ECD analysis; 2,6-dibromoanisole purity ▫ 99% or 2,3,6-trichloroanisole purity ≥ 99%.

3.4. 2,4,6-trichloroanisole (TCA) purity ≥ 99.0%

3.5. Absolute ethanol

3.6. Pure de-ionised water void of TCA (Standard EN ISO 3696)

3.7. Aqueous-alcoholic solution at 12 % vol.

Prepared using absolute ethanol (3.5) and de-ionised water void of TCA (3.6).

3.8. Internal standard stock solution (500 mg/L)

Add either 0.050 g of 2,4,6-trichloroanisole-d_5 (or 2,6-dibromoanisole or 2,3,6-trichloroanisole (3.3) to approximately 60 ml of absolute ethanol (3.5). After dissolution, adjust the volume to 100 mL with absolute ethanol (3.5). It can be kept in a glass bottle with a metallic or glasscover.

3.9. Intermediate solution of internal standard (5.0 mg/L)

Add 1 mL of a solution of either 2,4,6-trichloroanisole-d_5 (or 2,6-dibromoanisole or 2,3,6-trichloroanisole) at 500 mg/L (3.8) to approximately 60 mL of absolute ethanol (3.5). Adjust the volume to 100 mL with absolute ethanol (3.5). It can be kept in a glass bottle with a metallic or glass cover.

3.10. Internal standard solution (2.0 µg/L)

Add 40 µL of a solution of either 2,4,6-trichloroanisole-d_5 (or 2,6-dibromoanisole or 2,3,6 trichloroanisole) at 5.0 mg/L (3.9) to approximately 60 mL of absolute ethanol (3.5). Adjust the volume to 100 ml with absolute ethanol (3.5). It can be kept at an ambient temperature in a glass bottle with a metallic or glass cover.

3.11. Stock solution of TCA standard (40 mg/L)

Add 0.020g of 2,4,6-trichloroanisole to approximately 400 ml of absolute ethanol (3.5). Following dissolution, adjust volume to 500 mL with absolute ethanol (3.5).

3.12. Intermediate solution A of TCA standard (80 µg/L)

Add 1 mL of 2,4,6-trichloroanisole solution at 40 mg/L (3.11) to approximately 400 mL of absolute ethanol (3.5). Following dissolution, adjust volume to 500 mL with absolute ethanol (3.5).

3.13. Intermediate solution B of TCA standard (160 ng/L)

Add 1 mL of solution 2,4,6-trichloroanisole at 80 µg/L (3.12) to approximately 400 mL of pure de-ionised water (3.6). Following dissolution, adjust the volume to 500 mL with pure de-ionised water (3.6)

3.14. Use the standard-addition technique to make up a range of standard solutions of TCA. Standard solutions in the range from 0.5 ng/L to 50 ng/L can be used, by making additions with a solution of 2,4,6-trichloroanisole at 160 ng/L (3.13) to 6 ml of absolute ethanol (3.5). Following dissolution, adjust volume to 50 mL with pure de-ionised water (3.6)

The calibration curve obtained should be evaluated regularly and in any case whenever there is a major change in the GC/MS or GC/ECD systems.

3.15. Carrier gas: Helium, chromatographic purity (≥ 99.9990 %)

4. Apparatus

4.1. Laboratory glassware

4.1.1. Graduated 100-mL flask

4.1.2. 100- µL microsyringe

4.1.3. Wide-neck glass jar of a capacity adapted to the sample size, closed with a glass or metallic stopper or a material which does not bind TCA.

4.1.4. 20-mL glass sample bottle closed with a perforated capsule and a liner with one side Teflon-coated.

4.2. Solid-phase microextraction system (SPME) with a fiber coated with a polydimethylsiloxane film 100 µm thick

4.3. Heating system for sample bottle (4.1.4)

4.4. Stirring system for sample bottle (4.1.4)

4.5. Gas chromatograph equipped with a "split-splitless" injector and a mass spectrometer detector (MS) or an electron-capture detector (ECD)

4.6. Data-acquisition system

4.7. If required, an automatic sampling and injection system operating with an SPME system

4.8. Capillary column coated with an apolar stationary phase, of the phenylmethylpolysiloxane type (e.g.: 5 % phenyl methylpolysiloxane, 30 m x 0,25 mm x 0,25 µm film thickness or equivalent.)

5. Sample preparation

The corks are placed whole in a glass closed container. The container capacity (4.1.3), the same as the quantity of wine or aqueous-alcoholic solution (3.1 or 3.7), must be chosen in accordance to the sample size while ensuring that the corks are completely covered and immersed in the maceration container.

Example 1: 20 corks (45x24) mm, in a 1 L container;

Example 2: 50 corks (45x24) mm, in a 2 L container.

Most of the TCA released during maceration of the groups of stoppers is generally derived from a very low percentage of these stoppers. In order to obtain the best representation of a batch of stoppers, a number of appropriate analyses according to sampling rules and risk with regard to wine contamination should be carried out.

6. Operating method

6.1. Extraction

After macerating at ambient temperature for (24 ± 2) hours under laboratory ambient temperature conditions, the maceration is homogenized by inversion. A part of the aliquot of the 10ml maceration solution (5) is transferred to a glass sample bottle (4.1.4)

To increase extraction efficiency and subsequent sensitivity of the method, a quantity sodium chloride (3.2) can be added. The amount of sodium chloride can be adjusted / optimized by the users of this method, depending on the desired level of sensitivity and possible matrix effects that may occur. For example, a quantity of about 3 g of sodium chloride is suggested. 50 µL of the internal standard solution at 2.0 µg/L (3.10) are immediately added, then the bottle is closed using a perforated metal capsule fitted with a silicone / Teflon-coated liner. The capsule is crimped. The contents of the bottle are homogenized for 10 minutes by mixing using a stirring system (4.4) or by using an automatic system (4.7).

The bottle containing the sample is placed in the heating system (4.3) set to 35 °C ± 2 °C, with stirring (4.4). The extraction of the headspace is carried out using the SPME system (4.2) for at least 15 minutes.

6.2. Analysis

The fiber is then desorbed at 260 °C for at least 2 minutes in the injector of a gas chromatograph, in splitless mode (4.5). The separation is carried out using a capillary column with a non-polar stationary phase (4.8). The carrier gas is helium with a constant flow of 1 ml/min. A temperature program from 35 °C (for 3 min) to 265 °C (at 15 °C/min) is given as an example.

6.3. Detection and quantification

Detection and quantification are carried out by mass spectrometry with a selection of specific ions. For example, the following ion ratio is suggested:

	Analyte	*Interesting ions for detection (m/z):*	*Ion Quantification (m/z) :*
Analysis in SIM mode	*2,4,6-TCA*	*195, 210, 212*	*195*
	(2,4,6-TCA)-d_5	*199, 215, 217*	*215*
	2,3,6-TCA	*195, 210, 212*	*212*

	Analyte	*Parent ions (m/z):*	*Daughter ion (m/z) :*
Analysis in tandem mode (MS/MS)	*2,4,6-TCA*	*212*	*169, 197*
		196	*167, 169*
	(2,4,6-TCA)-d_5	*217*	*171, 199*

For the determination of ECD, identify the analyte and internal standard (2,6-dibromoanisole or 2,3,6 trichloroanisole) in the chromatogram, by comparing the retention time of the sample peak corresponding to that of the standard solution peak.

7. Calculations

The area of the chromatographic peak obtained for the 2,4,6-trichloroanisole is corrected by the area obtained for the chromatographic peak of the internal standard. The content in 2,4,6-trichloroanisole of each sample is obtained using a calibration curve. The points on this curve are obtained by tracing the relative responses of the 2,4,6-trichloroanisole/internal standard, obtained for aqueous-alcoholic solutions (3.7) containing known concentrations of 2,4,6-trichloroanisole, as a function of the concentrations of these solutions (3.14).

The results are given in ng/L of TCA present in the maceration, rounded off to the nearest 0.1 ng/L.

8. Characteristics of the method

As an indication, the detection limit of the analysis of the macerations must be lower than 0.5 ng/L, and the quantification limit close to 1 ng/L. The coefficient of variation is lower than 5% for 5 ng/L, when the selected internal standard is the deuterated analogue TCA-d_5.

An interlaboratory trial was carried out in order to validate the method. This interlaboratory trial was not carried out according to the OIV protocol and the validation parameters mentioned in the FV 1224.

9. Bibliography

- HERVÉ E., PRICE S., BURNS G., Chemical analysis of TCA as a quality control tool for natural corks. *ASEV Annual Meeting*. 1999.
- ISO standard 20752:2007 Cork stoppers — Determination of releasable 2, 4, 6-

trichloroanisol (TCA).

- FV 1224 - Résultats de l’analyse collaborative Ring test 3-TCA SPME.