RESOLUTION OIV/OENO 374/2009

DETERMINING THE PRESENCE AND CONTENT OF POLYCHLOROPHENOLS AND POLYCHLOROANISOLS IN WINES, CORK STOPPERS, WOOD AND BENTONITES USED AS ATMOSPHERIC TRAPS

THE GENERAL ASSEMBLY,

CONSIDERING Article 2 paragraph 2 iv of the Agreement of 3 April, 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING resolution OENO 8/2006 on determining the presence and content of polychlorophenols and polychloroanisols in wines, cork stoppers, wood and atmospheric traps

CONSIDERING the works of the Sub-commission of Methods of Analysis and the necessity of reviewing the above-mentioned resolution

DECIDES on proposal of the Oenology Commission to modify the AS315-13-PCAPCP Method as described currently in the Compendium of International Methods of Analysis of Wine and Must by the following type IV method:

Title	Method type
Determining the presence and content of polychlorophenols And polychloroanisols in wines, cork stoppers, wood and bentonites used as atmospheric traps	IV

1. SCOPE

All wines, cork stoppers, bentonites (absorption traps) and wood.

2. PRINCIPLE

Determination of 2,4,6-trichloroanisol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachloroanisol, 2,3,4,6-tetrachlorophenol, pentachloroanisol and pentachlorophenol by gas chromatography, by injecting a hexane extract of the wine and an ether/hexane extract of the solid samples to be analyzed and internal calibration.

3. REAGENTS

Preliminary remark: all the reagents and solvents must be free of the compounds to be determined listed in 2 at the detection limit.

3.1. Purity of hexane > 99 %

3.2. Purity of ethylic ether > 99 %

3.3. Ether/hexane mixture (50/50; v/v)

3.4. or 2,5-dibromophenol purity ≥ 99 %

3.5. Pure ethanol

3.6. Pure deionized water, TCA free, type II in accordance with ISO standard EN 3696

3.7. 50 % vol. aqueous-alcoholic solution. Place 100 ml of absolute ethanol (3.<5) in a graduated 200-ml flask (4.9.9), add 200 ml of deionized water (3.6), and homogenize.

3.8. Internal standard:

3.8.1. 200 mg/l stock solution. Place 20 mg of internal standard (3.4) in a graduated 100-ml flask (4.9.8), add the 50 % volume aqueous-alcoholic solution (3.7) and homogenize.

3.8.2. Internal standard solution (2 mg/l). Place 1 ml of the stock solution of internal standard (3.8.1) in a graduated 100-ml flask (4.9.8), add the 50% vol aqueous-alcoholic solution (3.7) and homogenize.

3.8.3. Internal standard solution (20 µg/l). Place 1 ml of stock solution of internal standard (3.8.2) in a 100 ml graduated flask (4.9.8), add with 50 % vol aqueous-alcoholic solution

3.9. Pure products

3.9.1. 2,4,6-trichloroanisole: ≥ 99 %, case: 87-40-1

3.9.2. 2, 4, 6-trichlorophenol: ≥ 99.8 %, case: 88-06-2

3.9.3. 2,3,5,6-tetrachloroanisole: ≥ 99 %, case: 6936-40-9 (note: the product sought in the samples is 2,3,4,6-tetrachloroanisole but is does not exist on the market)

3.9.4. 2, 3, 4, 6-tetrachlorophenol: ≥ 99 %, case: 58-90-2

3.9.5. pentachloroanisole: ≥ 99 %, case: 1825-21-1

3.9.6. pentachlorophenol: 99 %, case: 87-86-5

3.10. Reagents for derivatisation - Piridine: acetic anydride (1:0,4) vol.

3.10.1. Piridine: ≥ 99 %

3.10.2. Acetic anydride: ≥ 98 %

3.11. Calibration stock solution at 200 mg/l

In a graduated 100-ml flask (4.9.8), place approximately 20 mg of the pure reference products (3.9.1 to 3.9.6) but whose exactly weight is known (4.7), add absolute ethanol (3.5). Homogenize.

3.12. Intermediate calibration solution at 200 µg/l

In a graduated 100-ml flask (4.9.8) filled with absolute ethanol (3.5), add 100 µl of the calibration stock solution at 200 mg/l (3.11) using the 100-µl micro-syringe (4.9.1) and homogenize.

3.13. Calibration surrogate solution at 4 µg/l

In a graduated 50-ml flask (4.9.7) containing 50 % vol aqueous-alcoholic solution (3.7) add 1 ml of the intermediate calibration solution at 200 µg/l (3.11) using a 1-ml pipette (4.9.6). Add to volume 50 ml with pure ethanol (3.5) and homogenize.

3.14. Calibration solutions. It is possible to prepare various standard solutions with various concentrations by adding, using the 100-µl micro-syringe of (4.9.1), for example 50 µl of the surrogate calibration solution at 4 µg/l (3.12) to 50 ml of wine to enrich it with 4 ng/l of the substances to be determined.

The same reasoning can be used to prepare calibration solutions of various concentrations, either using aqueous-alcoholic solutions, or wine, or to enrich an extraction medium with a known quantity of pure products.

3.15. Commercially available Bentonite.

4. APPARATUS

4.1. Gas phase chromatograph with Split-splitless injector coupled to an electron capture detector. (It is likewise possible to use a mass spectrometer)

4.2. Capillary tube of non-polar steady-state phénylmethylpolysiloxane type: (0.32 mm x 50 m, thickness of film 0.12 µm or the equivalent

4.3. Chromatographic conditions, as an example:

4.3.1. Injection in "split-splitless" mode (valve closing time 30 seconds)

4.3.2. Carrier gas flow rate: 30 ml/min including 1 ml in the column Hydrogen U ®2 (It is likewise possible to use helium)

4.3.3. Auxiliary gas flow rate: 60 ml/min – Nitrogen with chromatographic purity (≥ 99,9990 %). It is also possible to use argon methane.

4.3.4. Furnace gradient temperature for information purposes:

- from 40 °C to 160 °C at a rate of 2 °C/min
- from 160 °C to 200 °C at a rate of 5 °C/min
- step at 220 °C for 10 min

4.3.5. Injector temperature: 250 °C

4.3.6. Detector temperature: 250 °C

4.4. Acquisition and integration: acquisition is by computer. The peaks of the various compounds identified by comparison with the reference are then integrated.

4.5. Magnetic agitator.

4.6. Vortex with adaptation for 30-ml flask (4.9.3)

4.7. Precision balance to within 0.1 mg

4.8. Manual or electric household grate

4.9. Laboratory equipment:

4.9.1. 100-µl micro-syringe

4.9.2. 10-µl micro-syringe

4.9.3. 30-ml flask closing with a screwed plug and cover with one side Teflon-coated

4.9.4. 10-ml stick pipette graduated 1/10 ml

4.9.5. 5-ml stick pipette graduated 1/10 ml

4.9.6. 1-ml precision pipette

4.9.7. Graduated 50-ml flask

4.9.8. Graduated 100-ml flask

4.9.9. Graduated 200-ml flask

4.9.10. 100-ml separating funnel

4.9.11. Pasteur pipettes and suitable propipette pear

4.9.12. Household aluminum foil, roll-form.

4.9.13. Centrifuge

5. SAMPLE PREPARATION

5.1. The stopper is grated (4.8) or cut into pieces (dimension < 3 mm)

5.2. Wood is cut with a clipper to obtain pieces (dimension < 3 mm)

5.3. The bentonite (3.15) (30 g for example) is spread out over a strip of aluminum foil (4.9.12) of approximately 30 cm x 20 cm and is exposed to the atmosphere to be analyzed for at least 5 days.

6. OPERATING METHOD

6.1. Extraction process for solid samples:

6.1.1. Stopper: in a 30-ml flask (4.9.3), place approximately 1 g of grated stopper (5.1) but of a precisely known weight (4.7)

6.1.2. Wood: in a 30-ml flask (4.9.3), place approximately 2 g of wood chips (5.2) but of a precisely known weight (4.7)

6.1.3. Control Bentonite: in a 30-ml flask (4.9.3), place approximately 5 g of bentonite (3.15) but of a precisely known weight (4.7)

6.1.4. Sample bentonite: in a 30-ml flask (4.9.3), place approximately 5 g of bentonite (5.3) of a precisely known weight (4.7)

6.1.5. Add 10 ml (4.9.4) of ether/hexane mixture (3.3)

6.1.6. Add with the micro-syringe (4.9.1) 50 µl of the internal standard solution (3.8.2)

6.1.7. Agitate with the vortex (4.6) for 3 min

6.1.8. Recover the ether/hexane liquid phase in a 30-ml flask (4.9.3)

6.1.9. Repeat the extraction operation on the sample with 2 times 5 ml of ether/hexane mixture (3.3)

6.1.10. Final extract: mix the 3 phases of ether/hexane.

6.2. Extraction of the wine and calibration solution

6.2.1. Sample 50 ml of wine or calibration solution (using the graduated flask (4.9.7)

6.2.2. Place them in the 100-ml graduated flask (4.9.8)

6.2.3. Add with the microsyringe (4.9.1) 50 µl of internal standard (3.8.3)

6.2.4. Add 4 ml (4.9.5) of hexane (3.1)

6.2.5. Carry out the extraction using the magnetic stirrer (4.5) for 5 min.

6.2.6. Elutriate into the funnel (4.9.10)

6.2.7. Recover the organic phase with the emulsion in a 30-ml flask (4.9.3) and aqueous phase in the 100-ml graduated flask (4.9.8)

6.2.8. Repeat the extraction of the wine or calibration solution using 2 ml of hexane (3.1)

6.2.9. Carry out the extraction using the magnetic stirrer (4.5) for 5 min.

6.2.10. Elutriate into the funnel (4.9.10)

6.2.11. Recover the organic phase with the emulsion in the same 30-ml flask mentioned in 6.2.7 (containing the organic phase obtained upon the first extraction)

6.2.12. Break the emulsion of the organic phase by centrifugation (4.9.13) by eliminating the lower aqueous phase using a Pasteur pipette (4.9.11) fitted with a propipette pear.

6.2.13. Final wine extract and calibration solutions: the residual organic extract

6.3. Analyze:

6.3.1. Add final extract (6.1.11 or 6.2.13) 100 µl (4.9.1) of the pyridine acetic anhydride reagent mixture (3.10) for the derivatisation.

6.3.2. Mix using a magnetic stirrer (4.5) for 10 min.

6.3.3. Inject 2 µl of derivatised final extract (6.3.2) into the chromatograph

7. CALCULATION:

$$Concentration\ of\ product = \frac{Product\ peak\ area}{Peak\ area\ of\ internal\ standard} \times Response\ factor$$

$$Response\ factor = \frac{concentration\ of\ calibration\ solution\ (3.13) \times Peak\ area\ of\ the\ internal\ standard}{Peak\ area\ of\ the\ pure\ product\ in\ the\ calibration\ solution}$$

Check the calibration by ensuring the response factors +/- 10 %.

8. RESULTS

The results are expressed in ng/l for the wine and ng/g for the cork stoppers, bentonites and wood.

9. CHARACERISTICS OF THE METHOD

9.1. Coverage rate

The coverage rate calculated in relation to the quantities added in terms of wood chips, polychloroanisols and polychlorophenols of 115 ng/g is:

- 2,4,6-trichloroanisol: 96 %
- 2,4,6-trichlorophenol: 96 %
- 2,3,4,6-tetrachloroanisol: 96 %
- 2,3,4,6-tetrachlorophenol: 97 %
- pentachloroanisol: 96 %
- pentachlorophenol: 97 %

9.2. Measurement repeatability

Calculated for each product, the uncertainties are as follows:

In a stopper ng/g	Mean	Standard deviation	Repeatability
2,4,6-trichloroanisol	1.2	0.1	0.28
2,4,6-trichlorophenol	26	3.3	9.24
2,3,4,6-tetrachloroanisol	1.77	0.44	1.23
2,3,4,6-tetrachlorophenol	2.59	0.33	0.92
pentachloroanisol	23.3	2.9	8.12
pentachlorophenol	7.39	1.91	5.35

In wood with 23 ng/g	Standard deviation	Repeatability
2,4,6-trichloroanisol	1.9	5.3
2,4,6-trichlorophenol	1.9	5.3
2,3,4,6-tetrachloroanisol	2.6	7.4
2,3,4,6-tetrachlorophenol	3.3	9.3
pentachloroanisol	2.7	7.5
pentachlorophenol	3.6	10.1

In wine with 10 ng/l	Standard deviation	Repeatability
2,4,6-trichloroanisol	0,4	1,1
2,4,6-trichlorophenol	2,1	5,9

2,3,4,6-tetrachloroanisol	0,6	1,7
2,3,4,6-tetrachlorophenol	4	11,2
pentachloroanisol	1,2	3,4
pentachlorophenol	6,5	18,2

In bentonite with15ng/g	Standard deviation	Repeatability
2,4,6-trichloroanisol	0,9	2,5
2,4,6-trichlorophenol	4	11,2
2,3,4,6-tetrachloroanisol	1,2	3,4
2,3,4,6-tetrachlorophenol	5,2	14,6
pentachloroanisol	4,3	12,0
pentachlorophenol	12,1	33,9

9.3. Detection limits (DL) and quantification limits (QL) calculated according to the OIV method:

9.3.1. Wood

	DL in ng/g	QL in ng/g
2,4,6-trichloroanisol	0.72	2.4
2,4,6-trichlorophenol	0.62	2.0
2,3,4,6-tetrachloroanisol	0.59	2.0
2,3,4,6-tetrachlorophenol	1.12	3.74

pentachloroanisol	0.41	1.4
pentachlorophenol	0.91	3.1

9.3.2. Bentonite

	DL in ng/g	QL in ng/g
2,4,6-trichloroanisol	0.5	1
2,4,6-trichlorophenol	1	3
2,3,4,6-tetrachloroanisol	0.5	1
2,3,4,6-tetrachlorophenol	1	3
pentachloroanisol	0.5	1
pentachlorophenol	Not det.	Not det.

9.3.3. Stopper

	DL in ng/g	QL in ng/g
2,4,6-trichloroanisol	0.5	1.5
2,4,6-trichlorophenol	1	2
2,3,4,6-tetrachloroanisol	0.5	1.5
2,3,4,6-tetrachlorophenol	1	2

pentachloroanisol	0.5	1.5
pentachlorophenol	1	2

9.3.4. Wine

	DL in ng/l	QL in ng/l
2,4,6-trichloroanisol	0.3	1
2,4,6-trichlorophenol	1	3
2,3,4,6-tetrachloroanisol	0.3	1
2,3,4,6-tetrachlorophenol	0.3	1
pentachloroanisol	0.5	3
pentachlorophenol	1	3

®[2] Air Liquide