RESOLUTION OENO 8/2006

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 dated April 3, 2001 establishing the International Organization of Vine and Wine,

UPON THE PROPOSAL of the Sub-commission of Methods of Analysis and Appraisal of Wine,

DECIDES: to introduce into 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.

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3.1. Purity of hexane > 99%

3.2. Purity of ethylic ether > 99%

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

3.4. Pure product for internal standard: lindane (Hexachlorocyclohexane), case 55963-79-6 or 2,4-dibromoanisole case 21702-84-1, purity > 99%

3.5. Pure ethanol

3.6. Distilled and/or microfiltered water

3.7. 50% vol. hydroalcoholic solution. Place 50 ml of pure ethanol (3.5) in a graduated 100-ml flask (4.9.7), add 100 ml of distilled water (3.6), and homogenize.

3.8. Internal standard:

3.8.1. 1.5 g/l stock solution. Place 150 mg of lindane or 2,4-dibromoanisole (3.4) in a graduated 100-ml flask (4.9.7), add the 50% volume hydroalcoholic solution (3.7) and homogenize.

3.8.2. Internal standard solution. Place 1 ml of the stock solution of lindane or 2,4-dibromoanisole (3.8.1) in a graduated 100-ml flask (4.9.7), add the 50% volume hydroalcoholic solution (3.7) and homogenize.

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: 98%, 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: 99.9%, case: 58-90-2

3.9.5. pentachloroanisole: 98%, case: 1825-21-1

3.9.6. pentachlorophenol: 98%, case: 87-86-5

3.10. Calibration stock solution at 200 mg/l

In a graduated 100-ml flask (4.9.7), 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 pure ethanol (3.5). Homogenize.

3.11. Intermediate calibration solution at 200 µg/l

In a graduated 50-ml flask (4.9.8) filled with pure ethanol (3.5), add 50 µl of the calibration stock solution at 200 mg/l (3.10) using the 100-µl micro-syringe (4.9.1) and homogenize.

3.12. Calibration surrogate solution at 4 µg/l

In a graduated 50-ml flask (4.9.8) containing pure ethanol (3.5) add 1 ml of the

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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.

3.13. 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 pure hydroalcoholic solutions, or wine, or to enrich an extraction medium with a known quantity of pure products.

3.14. 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 type: 0.32 mm x 50 m, thickness of film 0.12 µm

4.3. Chromatographic conditions:

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 U ® [2] (or argon methane)

4.3.4. Furnace temperature:

- 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 and bars.

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

4.7. Precision balance to within one mg

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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 100-ml flask

4.9.8. Graduated 50-ml flask

4.9.9. 100-ml separating funnel

4.9.10. Pasteur pipettes and suitable propipette pear

4.9.11. Household aluminum foil, roll-form.

5. SAMPLE PREPARATION

5.1. The stopper is grated (4.8) into granules (diameter < 3 mm)

5.2. Wood is cut with a clipper to obtain pieces only a few millimeters long.

5.3. The bentonite (3.14) (30 g for example) is spread out over a strip of aluminum foil (4.9.11) of approximately 30 cm x 20 cm and is exposed to the atmosphere to be analyzed for 72 hours.

6. OPERATING METHOD

6.1. Extraction process

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 (3.14) 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) but 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)

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

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

6.1.9. repeat the operation 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

6.2.1. Sample 50 ml of wine using the graduated flask (4.9.8)

6.2.2. Place them in the 100-ml graduated flask (4.9.7)

6.2.3. Add with the microsyringe (4.9.1) 50 µl of internal standard (3.8.2)

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 the wine in the 100-ml graduated flask (4.9.7)

6.2.8. repeat the extraction of the wine 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 (4.9.9)

6.2.11. Recover the organic phase with the emulsion in a 30-ml flask (4.9.3)

6.2.12. Mix the 2 organic phases and break the emulsion by very slow agitation using a magnetic bar (4.5), periodically elutriating the aqueous phase, i.e. by eliminating the lower aqueous phase using a Pasteur pipette (4.9.10) fitted with a propipette pear.

Note: It is also possible to break the emulsion by centrifugation.

6.2.13. Final wine extract: the residual organic extract after decanting the wine and the emulsion residue

6.3. Inject 2 µl of the final extract (6.1.11 or 6.2.13) into the chromatograph.

7. CALCULATION:

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

Response factor = concentration of calibration solution (3.13) * (Peak area of the internal standard / *(Peak area of the pure product in the calibration solution).

Check the calibration every two months, acceptance limit for the response factors +/-

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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

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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

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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

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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

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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

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