RESOLUTION OIV-OENO 477-2013

METHOD OF DETERMINATION OF PHTHALATES BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY IN WINES

THE GENERAL ASSEMBLY,

IN VIEW of article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

FOLLOWING a proposal made by the "Methods of Analysis" Sub-commission,

DECIDES, following a proposal made by Commission II "Oenology", to add the following type IV method to the "Compendium of International Methods of Analysis of Wines and Musts":

METHOD OF DETERMINATION OF PHTHALATES BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY IN WINES

Type of method: IV

1. SCOPE

This method applies to the detection and assay of phthalates in wines.

2. PRINCIPLE

The sample is extracted using isohexane. The extract is concentrated by evaporation. The concentrated extract is analysed by gas chromatography/mass spectrometry (GC/MS) with deuterated internal standards.

3. REAGENTS AND MATERIALS

Unless otherwise specified, all the reagents used are of recognised analytical quality.

3.1. DMP (dimethyl phthalate) [CAS N°: 131-11-3]

3.2. DnBP (dibutyl phthalate) [CAS N°: 84-74-2]

3.3. DEHP (bis (2-ethylhexyl) phthalate) [CAS N°: 117-81-7]

3.4. BBP (butyl benzyl phthalate) [CAS N°: 85-68-7]

3.5. DINP (di-isononyl phthalate) [CAS N°: 068515-48-0/028553-12-0]

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3.6. DIDP (di-isodecyl phthalate) [CAS N°: 068515-49-1/026761-40-0]
3.7. DCHP (dicyclohexyl phthalate) [CAS N°: 84-61-7]
3.8. DEP (diethyl phthalate) [CAS N°: 84-66-2]
3.9. DiBP (di-isobutyl phthalate) [CAS N°: 84-74-2]
3.10. DnOP (di-n-octyl phthalate) [CAS N°: 117-84-0]
3.11. DMP-d4: internal standard [CAS N°: 93951-89-4]
3.12. DEP-d4: internal standard [CAS N°: 93952-12-6]
3.13. DiBP-d4: internal standard [CAS N°: 358730-88-8]
3.14. DnBP-d4: internal standard [CAS N°: 93952-11-5]
3.15. BBP-d4: internal standard [CAS N°: 93951-88-3]
3.16. DCHP-d4: internal standard [CAS N°: 358731-25-6]
3.17. DEHP–d4: internal standard [CAS N°: 93951-87-2]
3.18. DnOP-d4: internal standard [CAS N°: 93952-13-7]
3.19. Isohexane [CAS N°: 107-83-5] and Acetone [CAS N°: 67-64-1]
3.20.Standard solutions

All the volumetric flasks used to prepare the calibration solutions are to be rinsed with acetone then isohexane to avoid any contamination.

3.20.1. Stock solutions

- Phthalate - 1 g/L individual solution: for each phthalate weigh 100 mg into a 100 mL flask, dissolve in the isohexane and make up to 100 mL.
- DINP-DIDP - 5 g/L individual solution: for each phthalate weigh 500 mg into a 100 mL flask, dissolve in the isohexane and make up to 100 mL
- Internal standard - 0.5 g/L individual solution: deuterated standards are packaged in sealed 25 mg ampoules; for each internal standard, all the contents of the bulb are transferred into a 50 mL volumetric flask; make up to 50 mL with isohexane.

3.20.2. Working solutions

- Phthalate 1 mg/L working solution (S1)

Take 100 µL of each 1 g/L and 5g/L stock solution (3.20.1), add the samples to a 100 mL flask, and make up to 100 mL with isohexane.

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- Phthalate 10 mg/L working solution (S2)

Take 1 mL of each 1 g/L and 5g/L stock solution (3.20.1), add the samples to a 100 mL flask, and make up to 100 mL with isohexane.

- Internal standard 10 mg/L working solution (IS)

Take 1 mL of each deuterated standard 0.5 g/L stock solution (3.20.1), add the samples to a 50 mL flask, and make up to 50 mL with isohexane.

3.20.3. Calibration range

The calibration range in isohexane is prepared from the various working solutions (3.20.2), directly into the injection vials that have been heat-treated, rinsed (see § 5.1) and dried under a hood beforehand, according to the following table:

Calibration points	Phthalate concn. (mg/L)*	Vol. of S1 surrogate soln. (µL)	Vol. of S2 surrogate soln. (µL)	Vol. of IS surrogate soln. (µL)	Vol. of isohexane (µL)
C1	0	0	0	50	1000
C2	0,05	50	0	50	950
C3	0,10	100	0	50	900
C4	0,20	200	0	50	800
C5	0,50	0	50	50	950
C6	0,80	0	80	50	920
C7	1,00	0	100	50	900

* to be multiplied by 5 for DINP and DIDP concentrations

4. EQUIPMENT

4.1. Glassware and volumetric laboratory equipment:

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4.1.1. 50 mL and 100 mL class A volumetric flasks

4.1.2. 50 mL glass centrifuge tubes with stopper

4.1.3. 10 mL glass test tubes with stopper

4.1.4. Micropipettes with variable volumes ranging from 25 µl to 1,000 µl, checked in accordance with ISO 8655-6

4.1.5. Nitrogen flow evaporator

4.2.Analytical balance

4.3. GC-MS System (e.g. Varian 450GC-300MS)

5. PROCEDURE

5.1. Precautions

Due to the presence of phthalates in the laboratory environment, precautions must be taken throughout the analysis of these compounds:

- Avoid any contact with plastic equipment (especially flexible PVC) as much as possible. If this is not possible, make sure there is no contamination.
- Test the solvents used and dedicate bottles of solvent to these analyses.
- Heat-treat all non-volumetric glassware (400°C for at least 2 hours). Rinse all the equipment carefully (with acetone then isohexane).
- Make sure the septums of the injection vials are phthalate-free.
- Before and after each injection, rinse the injection syringe several times.
- If possible, work in a clean room or in a room reserved for these analyses.

5.2. Preparing the samples

Place 12.5 mL of the sample in a 50 mL centrifuge tube. Add 10 mL of isohexane.

Shake vigorously (Vortex mixer) for at least one minute.

Let the mixture decant until the 2 phases have separated (30 minutes in a 50°C ultrasound bath will accelerate the separation). Recover 8 mL of the organic phase and transfer it into a 10 mL test tube. Evaporate under a flow of nitrogen (0.3 bar) at 35°C and avoid continuing to dryness (warning: the temperature must not exceed 40°C)

Resume with 1 mL of isohexane.

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Add 50 µl of the 0.01 g/L internal standard solution to each extract.

Transfer into an injection vial.

NOTE: to minimise matrix effects during analysis by GC-MS, a “protective” agent can be added, such as methyl undecanoate [CAS N°: 1731-86-8].

Add 20µL of this compound is added to each calibration solution and to the extracts from the samples prior to evaporation under a flow of nitrogen.

5.3. Blank test

Prepare a “blank” test by following the procedure described in 5.2 without adding the sample.

5.4. GC/MS analysis

Depending on the apparatus available and its performance, choose between SIM and MRM modes for the mass spectrometry.

For information purposes, analysis conditions are provided in Appendix I and a typical chromatogram is provided in Appendix II.

5.4.1. Calibration

First, carry out several solvent injections (at least 2). Next, inject the standard solutions (3.20.3) in duplicate in increasing order of concentration and end with at least two solvent injections.

Establish a calibration curve for each phthalate:

$$A_{analyste}/A_{IS}) = f(C_{analyte}/C_{IS})$$

- A: peak area
- C: concentration
- IS: internal standard

Each phthalate is quantified using to the corresponding deuterated standard, with the exception of DINP and DIDP which are quantified using to DnOP-d4.

5.4.2. Analysing the samples

Start the analysis sequence by analysing the "blank" test (5.3).

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Then inject the samples prepared (5.2) in duplicate.

Plan solvent injections after potentially highly contaminated samples.

End the series by injecting one or more calibration standards to check any signal drift during the analysis series and to check several solvent injections..

For each injection, measure the area of the identified peaks and internal standards, and use the calibration curve equation (5.4.1) to determine the concentration in the extract analysed.

5.4.3. Expressing the results

For each sample, calculate the average of the results obtained (5.4.2) for both injections.

The results are expressed in mg/L.

6. QUALITY CONTROL

During each analysis series, quality control is provided by the analysis of a wine sample supplemented with phthalates at a concentration level of 0.020 mg/L.

The extract of the sample prepared as per 5.2 is analysed at the beginning of the series, and the results obtained, given in terms of recovery rate, are reflected on a control chart.

7. METHOD CHARACTERISTICS

The analyses performed in the laboratory, under repeatability and intermediate precision conditions, on a red wine and a white wine supplemented with phthalates at two concentration levels (0.040 mg/L and 0.080 mg/L), gave the following repeatability (CV_r%), intermediate reproducibility (CV_{IP}%), and recovery values:

Phthalates	Recovery %	CV_r%	CV_{IP}%
DMP (dimethyl phthalate)	67	5	8
DEP (diethyl phthalate)	84	8	11

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DiBP (di-isobutyl phthalate)	93	7	10
DnBP (dibutyl phthalate)	95	5	7
BBP (butyl benzyl phthalate)	98	5	6
DCHP (dicyclohexyl phthalate)	97	5	7
DEHP (bis(2-ethylhexyl) phthalate)	98	6	7
DnOP (dioctyl phthalate)	98	6	7
DINP (di-isononyl phthalate)	104	7	8
DIDP (di-isodecyl phthalate)	96	8	11

i.e. the following average values for all the phthalates:

Repeatability (given in CV_r%): 6%

Intermediate precision (given in CV_{IP}%): 8%

8. DETECTION AND QUANTIFICATION LIMITS

For each phthalate being analysed for, the detection and quantification limits are provided in the following table:

Phthalates	Quantification limit (mg/L)	Detection limit (mg/L)

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DMP (dimethyl phthalate)	0.010	0.004
DEP (diethyl phthalate)	0.010	0.004
DiBP (di-isobutyl phthalate)	0.010	0.004
DnBP (dibutyl phthalate)	0.010	0.004
BBP (butyl benzyl phthalate)	0.010	0.004
DCHP (dicyclohexyl phthalate)	0.010	0.004
DEHP (bis(2-ethylhexyl) phthalate)	0.010	0.004
DnOP (dioctyl phthalate)	0.010	0.004
DINP (di-isononyl phthalate)	0.050	0.020

9. REFERENCES

1. FV 1371. Detection and assay of phthalates in alcoholic beverages. 2011

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2. FV 1234. Questions about phthalates. 2006

APPENDIX I

(for information)

Gas chromatography conditions

VF-5ms type capillary column: 30 m x 0.25 mm internal diameter, film thickness 0.25 µm

Temperature programming:

For detection in SIM mode:

Oven maintained at 100°C for 1 min; increase to 230°C at a rate of 10°C/min; increase to 270°C at a rate of 10°C/min; maintain for 2 min, increase to 300°C at a rate of 25°C/min; maintain for 8 min.

Note: this programming separates the DEHP and DCHP peaks (which cannot be done with the MRM mode programming)

For detection in MRM mode:

Oven maintained at 80°C for 1 min; increase to 200°C at a rate of 20°C/min; increase to 300°C at a rate of 10°C/min; maintain for 8 min.

Injector: maintained at 150°C for 0.5 min; increase to 280°C at a rate of 200°C/min, in splitless mode at injection

Helium: 1 mL/min at a constant flow rate

Volume injected: 1 µL

Mass spectrometry (MS) conditions

Ionisation in EI mode at 70 eV

Source temperature: 250°C

Transfer line temperature: 300°C

Manifold: 40°C

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Phthalate quantification and identification parameters

For an analysis in SIM mode, table 1 provides the quantification ion and the two qualifier ions for each phthalate and its deuterated homologue.

For an analysis in MRM mode, table 2 reflects the quantifying and qualifying transitions for each phthalate and its deuterated homologue.

Note: DIDP and DINP are each a mixture of compounds. Chromatography cannot separate them completely. They are therefore assayed as a "group".

APPENDIX I

(for information)

Table 1

		Quantification ion m/z	Qualifier ions m/z 1 - 2	
DMP	(dimethyl phthalate)	163	77	194
DMP-d4		167	81	198
DEP	(diethyl phthalate)	149	177	222
DEP-d4		153	181	226
DiBP	(di-isobutyl phthalate)	149	167	223
DiBP-d4		153	171	227
DnBP	(dibutyl phthalate)	149	205	223
DnBP-d4		153	209	227
BBP	(butyl benzyl phthalate)	149	91	206
BBP-d4		153	95	210

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DCHP	(dicyclohexyl phthalate)	149	167	249
DCHP-d4		153	171	253
DEHP	(bis(2-ethylhexyl) phthalate)	149	167	279
DEHP-d4		153	171	283
DnOP	(dioctyl phthalate)	149	167	279
DnOP-d4		153	171	283
DINP	(di-isononyl phthalate)	149	293	
DIDP	(di-isodecyl phthalate)	149	307	

Table 2

		Quantifying transition	Qualifying transition
DMP	(dimethyl phthalate)	194>163	194>77
DMP-d4		198>167	198>81
DEP	(diethyl phthalate)	177>149	177>93
DEP-d4		181>153	181>97
DiBP	(di-isobutyl phthalate)	223>149	205>149
DiBP-d4		227>153	209>153
DnBP	(dibutyl phthalate)	223>149	205>149
DnBP-d4		227>153	209>153
BBP	(butyl benzyl phthalate)	206>149	149>121
BBP-d4		210>153	153>125
DCHP	(dicyclohexyl phthalate)	249>149	249>93
DCHP-d4		253>153	253>97

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DEHP	(bis(2-ethylhexyl) phthalate)	279>149	279>93
DEHP-d4		283>153	283>97
DnOP	(dioctyl phthalate)	279>149	279>93
DnOP-d4		283>153	283>93
DINP	(di-isononyl phthalate)	293>149	
DIDP	(di-isodecyl phthalate)	307>149	

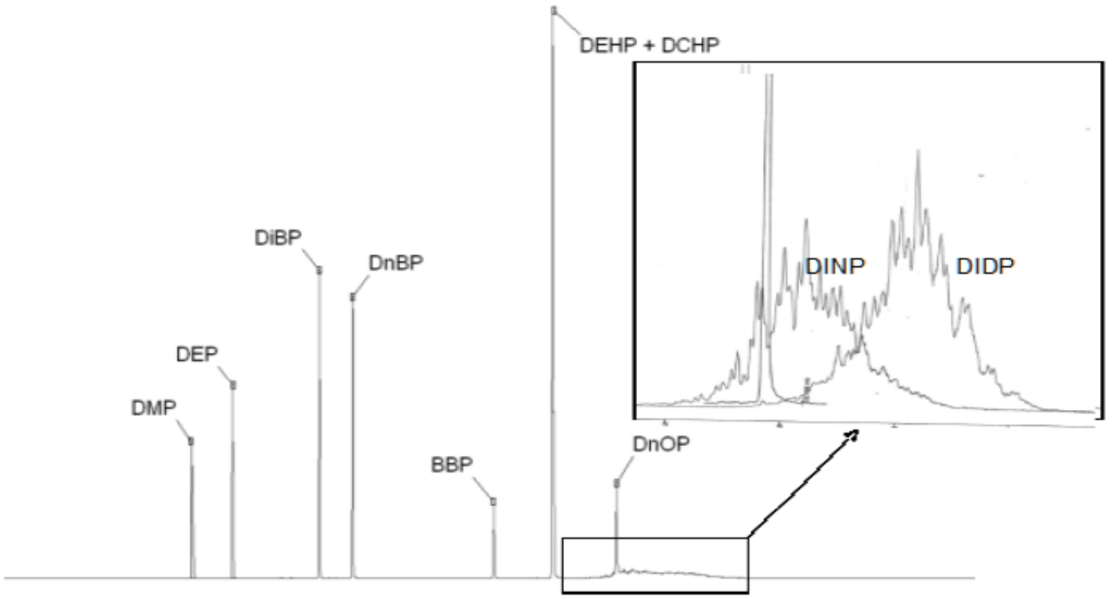
APPENDIX II

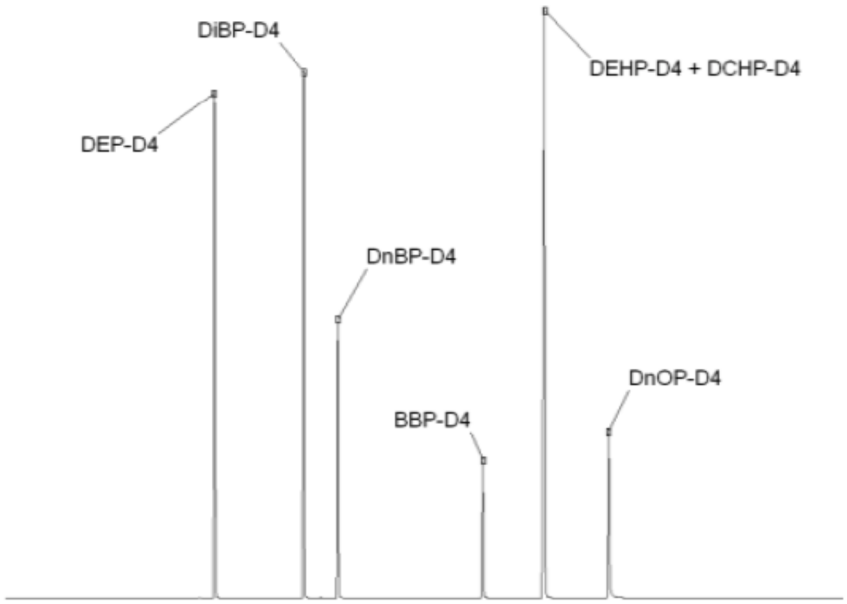
(for information)

GC/MS chromatograms of a phthalate standard solution and deuterated internal standards.

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