Method OIV-MA-BS-33 : R2013

Type IV method

Method of determination of phthalates in spirituous beverages by gas chromatography/ mass spectrometry

OIV-OENO 521-2013

1. Scope of application

This method applies to the detection and assay of some phthalates in spirit drinks.

2. Principle

The sample is extracted using a non-polar solvent. The extract is then analysed by gas chromatography/mass spectrometry (GC/MS) with an internal standard.

Analysis may also be carried out directly but with higher detection limits. In this case, the internal standard is added directly before injection in GC/MS.

3. Reagents and products

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

3.1. DBP (Dibutyl phthalate) [CAS N°: 84-74-2];

3.2. DEHP (Di-(2-ethylhexyl) phthalate) [CAS N°: 117-81-7];

3.3. BBP (Butyl benzyl phthalate) [CAS N°: 85-68-7];

3.4. DIBP (Diisobutyl phthalate) [CAS N°: 84-69-5];

3.5. other phthalates if necessary (note: diisodecyl and diisononyl phthalate are each a mixture of compounds, some of which are common to both);

3.6. internal standard (for example: dipentyl phthalate [CAS N° 131-18-0]);

3.7. absolute ethanol;

3.8. Milli-Q water;

3.9. non-polar extraction solvent, free from phthalates, such as toluene.

Standard solutions

The concentrations provided in this method are for indicative purposes:

3.10. Internal standard stock solution

- 500 mg/L in ethanol,

3.11. Internal standard working solution

- 50 mg/L in ethanol,

3.12. Phthalates stock solution

- 500 mg/L of different phthalates in ethanol:
- Diisobutyl phthalate,
- Dibutyl phthalate,

- Diethylhexyl phthalate,
- Butylbenzyl phthalate,

Others if required

3.13. Phthalates working solution

The solution is prepared using a 1:5 dilution of the stock solution in the ethanol.

3.14. Calibration range

Prepare a 40% vol. aqueous-alcoholic solution: pour 80 mL of ethanol into a 200 mL flask then make up to volume with water. A multi-point range is prepared according to Table 1:

Table 1: Preparation of standards

40% vol. aqueous-alcoholic solution (mL)	Concentration level (mg/L)	Volume of working solution (µL) [3.13.]	Concentration of working solution (mg/L)
25	0 (blank)	0	100
25	0.2	50	100
25	0.4	100	100
25	0.8	200	100
25	1.2	300	100
25	1.6	400	100
25	2.0	500	100

4. Equipment

- Glassware and volumetric laboratory equipment
- Analytical balance
- GC-MS system

5. Procedure

5.1. Precautions

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

- avoid any contact with plastic equipment,
- test the solvents used,
- use glassware rinsed with an appropriate solvent,
- avoid contamination from the septum of the injection vials.

5.2. Preparing the samples

Samples with an ABV of 40% vol. or which have been adjusted to 40% vol. (+/- 5% vol.) are extracted.

Extraction: in a glass test tube

- 25 mL of sample or calibration solution,
- 50 µL of internal standard solution,
- 2 mL of non-polar solvent.

Extract with a Vortex mixer for 3 minutes.

Recover the organic phase in the automatic injector vials.

Prepare a blank in the same way (with a 40% vol. aqueous-alcoholic solution).

5.3. Chromatography conditions (as an example)

- non-polar type column (DB5 MS: 30 m x 0.25 mm x 0.25 µm),
- injector at 250°C,
- splitless mode of injection,
- in the case of direct analysis, choose the split mode with a ratio of 1:10,
- programming of oven temperature (example): 80°C (0.7 min) at 20°C/min up to 110°C then 6°C/min up to 245°C (30 min isothermal),
- Helium: 1mL/min

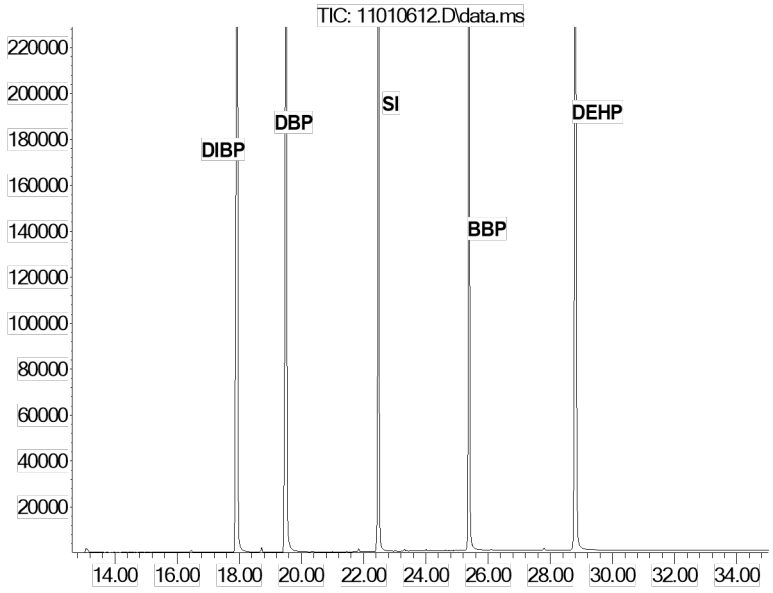
Injection volume: 1 µL

MS conditions:

Ionisation in EI Mode

SIM mode on ion m/z 149 or SIM/SCAN mode

Figure 1 – GC/MS Chromatogram of a phthalates solution and the internal standard



Temps

5.4. Injection sequence

Start the sequence by analysing the “blank”, then inject the calibration solutions and the samples. Regular injection of the blanks is recommended.

5.5. Expressing the results

Identification is carried out using the retention time.

The results are expressed in µg/L or in mg/L.

Use the multiplication factor corresponding to the dilution performed to adjust the sample to 40% vol.

The results of the blanks (average and dispersion) should be considered, with or without correction of the blank, to evaluate:

the limits of quantification and of detection,

the uncertainty of the measurement.

The average of the blanks and their dispersion should be estimated based on the repetitions carried out with different calibrations. The consistency of the results of the blanks with these references should be verified.

6. Method characteristics

The method characteristics are presented in relation to the described procedure with liquid/liquid extraction.

6.1. Linearity

The linearity (Table 2) was evaluated at 7 levels (see Table 1). For the higher concentration levels, a curve was observed.

Table 2: Linearity

Compound	Working Range (mg/L)	Linear R^2
DIBP	0.0-2.5	1.000
DBP	0.0-2.5	1.000
BBP	0.0-2.5	1.000
DEHP	0.0-3.5	1.000

6.2. Recoveries

Table 3 shows the observed recoveries.

Table 3: Recoveries					
		DIBP	DBP	BBP	DEHP
	Average recovery (%)	91%	93%	101%	98%
	Min. recovery (%)	86%	85%	96%	91%
	Max. recovery (%)	95%	101%	104%	101%

6.3. Repeatability and intermediate precision (intralaboratory)

The analyses to determine the repeatability and intermediate precision have been carried out on a spiked sample (Tables 4 and 5). The intermediate precision corresponds to 2 months of results based on this sample. used as an internal control.

Table 4: Repeatability (mg/L)

	N° of degrees of freedom	Level	Standard deviation	CV (%)
DIBP	9	0.3135	0.0026	0.8%
DBP	9	0.3290	0.0024	0.7%
BBP	9	0.5141	0.0034	0.7%
DEHP	9	1.4887	0.0159	1.1%

Table 5: Intermediate precision (mg/L)

	N° of degrees of freedom	Level	Standard deviation	CV (%)
DIBP	28	0.3075	0.0088	2.8%
DBP	28	0.3230	0.0076	2.3%
BBP	28	0.5211	0.0111	2.1%
DEHP	28	1.5213	0.0663	4.4%

6.4. Limits of detection and of quantification

Table 6 shows the evaluated limits of detection and of quantification.

Table 6: limits of detection and of quantification (mg/L)

	Direction injection Calculated detection limit *	Extraction Calculated detection limit*	Extraction. Quantification limit **
DIBP	0.019	<0.001	0.010
DBP	0.059	<0.001	0.010
BBP	0.100	<0.001	0.010
DEHP	0.033	<0.001	0.050

* according to the calculation based on the signal: noise (S:N) ratio
** limit of quantification taking the blanks into consideration

7. References

1. WENZL T.. 2009. Methods for the determination of phthalates in food. Outcome of a survey conducted among European food control laboratories. EUR 23682 EN – 2009.