

# COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS FOR SPIRITUOUS BEVERAGES AND ALCOHOLS

Method of determination of phthalates in spirituous beverages by gas-chromatography/mass  
spectrometry (Type IV)<sup>m</sup>

## **OIV-MA-BS-33 Method of determination of phthalates in spirituous beverages by gas chromatography/ mass spectrometry**

Type IV method

### **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,
11. Internal standard working solution
  - 50 mg/L in ethanol,
12. Phthalates stock solution

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- 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

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- Analytical balance
  - GC-MS system
- 

### 5. Procedure

#### 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: 1 mL/min

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Injection volume: 1 µL

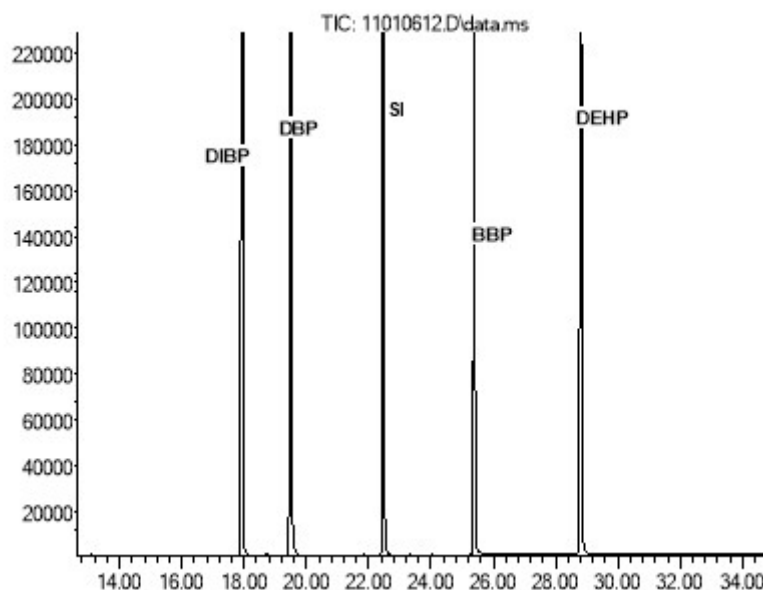
### MS conditions:

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

Abundance :



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

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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 <sup>2</sup>
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

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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)
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	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

- 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.