Standard Operating Procedure

for the

Determination of Phthalates in Wine and Spirits

in-house validated by the EC-JRC-IRMM
April 2013
Determination of phthalates in wine and spirits by liquid/liquid partitioning followed by gas chromatography mass spectrometry (GC-MS)

1. SCOPE AND APPLICATION

The method is suitable to determine dimethyl phthalate (DMP), diethyl phthalate (DEP) dipropyl phthalate (DPrP), dibutyl phthalate (DBP) diisobutyl phthalate (DIBP), dipentyl phthalate (DPeP), diheptyl phthalate (DHpP), di-n-octyl phthalate (DNOP), di-n-decyl phthalate (DDP), diallyl phthalate (DAP), bis(2-ethylhexyl) phthalate (DEHP), dicyclohexyl phthalate (DCHP), diphenyl phthalate (DPhP), and benzyl butyl phthalate (BBP) in wine and spirits at concentrations between 0.03 mg/L and 2.50 mg/L. Diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP) can be determined at concentration levels between 0.15 mg/L and 5.00 mg/L.

2. SAFETY

Phthalates are harmful to humans

Protective equipment as laboratory coat, and safety glasses have to be used. All handlings of phthalates and organic solvents should be performed in a fume hood with adequate air flow.

3. PRINCIPLE

A test portion is pipetted into 16 mL screw cap vials and stable isotope labelled phthalates are added. The sample is then extracted with n-hexane by vigorously shaking. Measurement of the analyte is performed by gas chromatography mass spectrometry in single ion monitoring mode. Quantification is done by using the isotopically labelled internal standards.

Note: Some phthalates are ubiquitous in the environment. They are contained in at least small amounts in solvents, water, air, and many kinds of laboratory consumables, especially when made of plastic. Recommendations on how to deal with blank problems are specified in the course of this document.
4. INSTRUMENTS AND GLASSWARE

4.1 GC-MS SYSTEM

4.1.1 Autosampler
Capable of injecting 1 µL of sample

4.1.2 Injection port
Split/splitless injection port with low bleed septum and glass insert suitable for splitless injection

4.1.2 GC column
DB 5 MS, 30 m x 0.25 mm internal diameter and 0.25 µm film thickness, or equivalent

4.1.3 Mass spectrometer
Single quadrupole mass spectrometer operating in electron ionisation mode at 70 eV and capable of performing single ion monitoring (SIM);

4.1.4 Data acquisition and analysis system
Suitable data collection and evaluation software.

4.2 Calibrated microbalance with a readability of 0.001 mg

4.3 Calibrated analytical balance with a readability of 0.01 mg

4.4 Calibrated positive displacement variable-volume pipettes
Capacity 1 mL, 5 mL and 10 mL

Note: The pipette tips applied during the development of this study did not release significant amounts of phthalates. However, the release of phthalates has to be checked prior to their application.

4.5 Glass microliter syringes with glass or metal plungers
Volumes of 10 µL, 25 µL, 50 µL, 100 µL, 500 µL and 1000 µL

Note: The precision of analysis depends very much on the precision of pipetting, e.g. the stable isotope labelled standards. Therefore, preference shall be given to calibrated syringes.

4.6 Wrist arm shaker
Eight position, with adjustable shaking frequency and timer

4.7 Glassware
4.7.1 Volumetric flasks with glass stoppers
Volume 25 mL, 50 mL, 100 mL etc., according to ISO 1042

4.7.2 Single use glass vials
Volume of 16 mL with screw caps and PTFE lined septa

4.7.3 Single use 2 mL autosampler vials

4.7.4 Glass beakers of different size

4.7.5 Large desiccators for the storage of cleaned glass ware

With the exemption of autosampler vials, which are applied as supplied, all reusable glassware is firstly cleaned in a laboratory dish washer and then thoroughly rinsed with methanol and \( n \)-hexane. The 16 mL screw cap vials are heated for 24 hours to at least 300°C and cooled down in a desiccator over aluminium oxide (0). With the exemption of autosampler vials, all glassware is stored in a desiccator over aluminium oxide (0). Glass ware for standard preparation and sample extraction is rinsed twice with a small amount of, depending on the use, either methanol or \( n \)-hexane.
5. REAGENTS AND STANDARDS

Chemicals should be of high purity. All chemicals have to be checked prior to their application for contamination with phthalates.

Note: Standard solutions may be prepared from neat materials or from commercial solutions in appropriate solvents.

5.1 Neat phthalates
supplied in screw cap vials with minimum 100 mg analyte content

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Acronym</th>
<th>CAS</th>
<th>Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzyl butyl phthalate</td>
<td>BBP</td>
<td>85-68-7</td>
<td>98.0±0.4*</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>DEHP</td>
<td>117-81-7</td>
<td>99.6±0.2*</td>
</tr>
<tr>
<td>Diallyl phthalate</td>
<td>DAP</td>
<td>131-17-9</td>
<td>99.2</td>
</tr>
<tr>
<td>Dibutyl phthalate</td>
<td>DBP</td>
<td>84-74-2</td>
<td>99.6±0.1*</td>
</tr>
<tr>
<td>Dicyclohexyl phthalate</td>
<td>DCHP</td>
<td>84-61-7</td>
<td>99.6</td>
</tr>
<tr>
<td>Didecyl phthalate</td>
<td>DDP</td>
<td>84-77-5</td>
<td>99.8</td>
</tr>
<tr>
<td>Diethyl phthalate</td>
<td>DEP</td>
<td>84-66-2</td>
<td>99.5</td>
</tr>
<tr>
<td>Diheptyl phthalate</td>
<td>DHpP</td>
<td>3648-21-3</td>
<td>97</td>
</tr>
<tr>
<td>Diisobutyl phthalate</td>
<td>DIBP</td>
<td>84-69-5</td>
<td>99.5±0.2*</td>
</tr>
<tr>
<td>Disodecyl phthalate</td>
<td>DIDP</td>
<td>26761-40-0</td>
<td>&gt;99.0, mixture of isomers</td>
</tr>
<tr>
<td>Disononyl phthalate</td>
<td>DINP</td>
<td>28553-12-0</td>
<td>&gt;99.0, mixture of isomers</td>
</tr>
<tr>
<td>Dimethyl phthalate</td>
<td>DMP</td>
<td>131-11-3</td>
<td>99.4</td>
</tr>
<tr>
<td>Di-n-octyl phthalate</td>
<td>DNOP</td>
<td>117-84-0</td>
<td>99.4</td>
</tr>
<tr>
<td>Dipentyl phthalate</td>
<td>DPeP</td>
<td>131-18-0</td>
<td>&gt;99.0</td>
</tr>
<tr>
<td>Diphenyl phthalate</td>
<td>DPnP</td>
<td>84-62-8</td>
<td>99.9</td>
</tr>
<tr>
<td>Dipropyl phthalate</td>
<td>DPPrP</td>
<td>131-16-8</td>
<td>99.5</td>
</tr>
</tbody>
</table>

* certified values with expanded uncertainty (k=2)

5.2 Stable isotope labelled phthalates
supplied in screw cap vials with minimum 10 mg analyte content

<table>
<thead>
<tr>
<th>Analyte</th>
<th>CAS</th>
<th>Purity</th>
<th>Isotope purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis(2-ethylhexyl) phthalate-3,4,5,6-D4</td>
<td>93951-87-2</td>
<td>99.3</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Dibenzyl phthalate-3,4,5,6-D4</td>
<td>1015854-62-2</td>
<td>99.4</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Dibutyl phthalate-3,4,5,6-D4</td>
<td>93952-11-5</td>
<td>99.4</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Dicyclohexyl phthalate-3,4,5,6-D4</td>
<td>358731-25-6</td>
<td>99.3</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Diethyl phthalate-3,4,5,6-D4</td>
<td>93952-12-6</td>
<td>99.3</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Dihexyl phthalate-3,4,5,6-D4</td>
<td>1015854-55-3</td>
<td>99.4</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Diisobutyl phthalate-3,4,5,6-D4</td>
<td>358730-88-8</td>
<td>99.1</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Dimethyl phthalate-3,4,5,6-D4</td>
<td>93951-89-4</td>
<td>99.8</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Dioctyl phthalate-3,4,5,6-D4</td>
<td>93952-13-7</td>
<td>99.2</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>
5.3 Methanol
LC-MS grade

5.4 n-Hexane
suitable for ECD analysis, stored over 20 g/L preconditioned aluminium oxide (0)
(10 seconds shaking prior to use)

5.5 Aluminium oxide
The aluminium oxide is conditioned at minimum 300 °C for 24 hours prior to use, and stored
in a desiccator

5.6 Deionised water with low phthalate content
e.g. water of LC-MS quality

6. STANDARD SOLUTIONS

The concentrations given below are just indicative. Therefore, both the volumes of the standard
preparation scheme and the concentration levels shall be adapted to the actual situation.

The concentrations of DINP and DIDP are in all standard solutions about five times the concentration
of the other native phthalates. This takes into account that DINP and DIDP are mixtures of many
isomers, which cannot be chromatographically resolved.

Note: All standard solutions are stored refrigerated. Allow standard solutions to get to ambient
temperature before further use. The shelf life of the stock standard solutions is at least 3 months.

6.1 Stock standard solution of native phthalates in n-hexane (1000 µg/mL)
Weigh to the nearest of 0.001 mg about 20 mg of each of the neat phthalates (0) except DINP
and DIDP into a 20 mL volumetric flask, and make up to volume with
n-hexane. For DINP
and DIDP the amount weighed into the 20 mL flask is about 100 mg each. This solution can
be stored at below 10°C for at least 3 months.

Note: The high viscosity of some neat phthalates hampers pipetting exact volumes of the neat liquid.
Therefore, the microliter syringes (separate syringes for each neat phthalate) are only used as a tool
for the transfer of the neat substance onto the inner surface of the volumetric flask. The amount
transferred is determined gravimetrically. It might be necessary to rinse the inner glass wall of the
volumetric flask with a few hundreds microliter of n-hexane, after recording of the transferred amount
of neat phthalate, in order to prevent cross-contamination of the neat phthalates. However, the total
mass of the closed volumetric flask including the transferred amount of neat substance and rinsing
solvent is recorded after each rinsing step.

6.2 Intermediate standard solution of native phthalates in n-hexane (100 µg/mL)
Transfer 5000 µL of the stock solution of DEHP in n-hexane (0) (1000 µg/mL) to a 50 mL
volumetric flask and make up to volume with n-hexane.

6.3 Stock standard solution of stable isotope labelled phthalates in n-hexane
(500 µg/mL)

Note: The indicative standard concentration is only applicable for a packaging sizes of 25 mg of
stable isotope labelled phthalate per original container, and assumes complete transfer of it into the
specified volumetric flask.
Note: The high viscosity of phthalates hampers pipetting of the neat liquid. To circumvent pipetting problems following procedure is applied.

Record the tare weight of a 50 mL volumetric flask (incl. glass stopper) on an analytical balance. Rinse the flask twice with n-hexane, and add finally about 2 mL of n-hexane. Record the actual weight including the n-hexane.

Record the weight of the vials with the stable isotope labelled phthalates on a microbalance. It is advisable to perform repeated weighing of the vials. Open the first vial containing a labelled phthalate and add with a microliter syringe n-hexane to the viscous liquid. Transfer with the microliter syringe the n-hexane solution from the vial into the 50 mL volumetric flask. Rinse the vial incl. screw cap with n-hexane by closing the vial and shaking it for a few seconds. Repeat the rinsing step another two times. Transfer all rinsing solutions into the volumetric flask. Repeat these steps (from opening of the vial till transfer of the rinsing solution) with all other labelled phthalates and fill the 50 mL volumetric flask finally up to the mark with n-hexane. Record the total weight of the flask incl. glass stopper.

Record the tare weight of the vial that contained the labelled phthalates on the microbalance after evaporation of residual rinsing solvent. Weight constancy was usually found after leaving the vial open for several hours. Repeated weighing is advisable. Calculate the amount of labelled phthalate transferred into the 50 mL volumetric flask from the difference of the initial weight (vial containing the phthalate) and the tare weight.

6.4 Stock standard solution of native phthalates in methanol (1000 µg/mL)
Weigh to the nearest 0.001 mg about 20 mg of each native phthalate (0) into a 20 mL volumetric flasks, and make up to volume with methanol. See 0 for further explanation.

6.5 Working standard solution of native phthalates in methanol (100 µg/mL)
Transfer 2000 µL of the stock standard solution of native phthalates in methanol (0) (1000 µg/mL) to a 20 mL volumetric flask and make up to volume with methanol.

6.6 Stock standard solution of stable isotope labelled phthalates in methanol (1000 µg/mL)
Repeat the standard preparation as detailed above for the stock standard solution of stable isotope labelled phthalates in n-hexane (in 0) with methanol as solvent.

6.7 Working standard solution of stable isotope labelled phthalates in methanol (60 µg/mL)
Transfer 3000 µL of the stock standard solution of stable isotope labelled phthalates in methanol (0) (1000 µg/mL) to a 50 mL volumetric flask and make up to volume with methanol.

6.8 Calibration standards

Note: The given concentration levels are only indicative and refer to the concentration of the native phthalates in the n-hexane solution. The real concentrations have to be calculated from the actual concentrations of the stock standard solutions and intermediate standard solutions.

6.8.1 Standard 0 ng/mL: Add with an appropriate syringe or pipette 100 µL of stock standard solution of stable isotope labelled phthalates in n-hexane (0; 500 µg/mL) to a 25 mL volumetric flask and make up to volume with n-hexane.
6.8.2 **Standard 160 ng/mL:** Add with an appropriate syringe or pipette 100 µL of stock standard solution of stable isotope labelled phthalates in n-hexane (0; 500 µg/mL) and 40 µL of intermediate standard solution of native phthalates in n-hexane (0; 100 µg/mL) to a 25 mL volumetric flask and make up to volume with n-hexane.

6.8.3 **Standard 400 ng/mL:** Add with an appropriate syringe or pipette 100 µL of stock standard solution of stable isotope labelled phthalates in n-hexane (0; 500 µg/mL) and 100 µL of intermediate standard solution of native phthalates in n-hexane (0; 100 µg/mL) to a 25 mL volumetric flask and make up to volume with n-hexane.

6.8.4 **Standard 800 ng/mL:** Add with an appropriate syringe or pipette 100 µL of stock standard solution of stable isotope labelled phthalates in n-hexane (0; 500 µg/mL) and 200 µL of stock standard solution of native phthalates in n-hexane (0; 100 µg/mL) to a 25 mL volumetric flask and make up to volume with n-hexane.

6.8.5 **Standard 2000 ng/mL:** Add with an appropriate syringe or pipette 100 µL of stock standard solution of stable isotope labelled phthalates in n-hexane (0; 500 µg/mL) and 500 µL of stock standard solution of native phthalates in n-hexane (0; 100 µg/mL) to a 25 mL volumetric flask and make up to volume with n-hexane.

6.8.6 **Standard 4000 ng/mL:** Add with an appropriate syringe or pipette 100 µL of stock standard solution of stable isotope labelled phthalates in n-hexane (0; 500 µg/mL) and 1000 µL of stock standard solution of native phthalates in n-hexane (0; 100 µg/mL) to a 25 mL volumetric flask and make up to volume with n-hexane.

6.8.7 **Standard 6000 ng/mL:** Add with an appropriate syringe or pipette 100 µL of stock standard solution of stable isotope labelled phthalates in n-hexane (0; 500 µg/mL) and 1500 µL of stock standard solution of native phthalates in n-hexane (0; 100 µg/mL) to a 25 mL volumetric flask and make up to volume with n-hexane.

6.8.8 **Standard 10000 ng/mL:** Add with an appropriate syringe or pipette 100 µL of stock standard solution of stable isotope labelled phthalates in n-hexane (0; 500 µg/mL) and 2500 µL of stock standard solution of native phthalates in n-hexane (0; 100 µg/mL) to a 25 mL volumetric flask and make up to volume with n-hexane.

6.8.9 **Standard 14000 ng/mL:** Add with an appropriate syringe or pipette 100 µL of stock standard solution of stable isotope labelled phthalates in n-hexane (0; 500 µg/mL) and 3500 µL of stock standard solution of native phthalates in n-hexane (0; 100 µg/mL) to a 25 mL volumetric flask and make up to volume with n-hexane.

7. **PROCEDURE**

7.1 **Test sample preparation**
Transfer with a calibrated pipette (0) 8 mL of the alcoholic beverage sample into a 16 ml glass vial with screw cap and PTFE lined septum. Add with a microliter syringe (0) 50 µL of the working standard solution of stable isotope labelled phthalates in methanol (0).
Shake the vial shortly by hand and add afterwards with a suitable calibrated pipette 1.5 mL of n-hexane to the sample. Put a piece of aluminium foil over the opening of the vial and close the vial with the screw cap. Shake the sample firstly 30 seconds on a Vortex shaker and then for 3 hours on a wrist arm shaker (0) at highest shaking frequency. Other instruments that provide good mixing of sample and extractant may be applied alternatively.
Centrifugate the 16 mL vials for 5 min at 4000 rpm in order to speed up phase separation. Transfer after phase separation an aliquot of the \textit{n}-hexane phase into a suitable GC autosampler vial and analyse by GC-MS in selected ion monitoring mode. Prevent contact of \textit{n}-hexane with the PTFE lined septum by covering the vial's opening with aluminium foil prior to capping.

Test samples shall be prepared in duplicate.

Note: Extraction time was not optimised.

\textbf{7.2 Procedural blank sample}

The procedural blank sample consists of 50 µL of working standard solution of stable isotope phthalates in methanol (0) and 100 µL of deionised water (0). A microliter syringe is used (0) for the transfer of the working standard solution of stable isotope phthalates in methanol (0). This sample is then extracted with 1.5 mL of \textit{n}-hexane (0) as described before (see 0) and analysed by GC-MS. The phthalate content of the procedural blank sample is subtracted from the phthalate content of the test samples. However, it shall not exceed 30 \% of the phthalate content of "Standard 160". If this is the case, then root-cause-analysis has to be performed and the source of contamination has to be eliminated

Procedural blank samples shall be prepared for the proper establishment of background contamination at least in triplicate

\textbf{7.3 GC-MS determination}

\textbf{7.3.1 GC-MS conditions}

The following GC parameters were successfully applied for the determination of phthalates in wine and spirits.

<table>
<thead>
<tr>
<th>GC parameters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GC Column</td>
<td>DB 5 MS, 30 m x 0.25 mm i.d., 0.25 µm d.f.</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>He, 1.0 mL/min constant flow</td>
</tr>
<tr>
<td>Temperature programme</td>
<td>60.0 °C/0 min - 20.0 °C/min - 200.0 °C/1.5 min - 4.0 °C/min - 260.0 °C - 3.0 °C/min - 280.0 °C/0.0 min - 10 °C/min - 300.0 °C/5.0 min</td>
</tr>
<tr>
<td>GC-inlet</td>
<td>Split/splitless injection port with low bleed septum; aluminium foil was placed between the injection port and the septum</td>
</tr>
<tr>
<td>Inlet temperature</td>
<td>300 °C, constant</td>
</tr>
<tr>
<td>Injection volume</td>
<td>1.0 µL</td>
</tr>
<tr>
<td>Injection mode</td>
<td>Splitless for 1.5 min</td>
</tr>
<tr>
<td>Total run time</td>
<td>37 min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MS parameters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass filter</td>
<td>Quadrupole</td>
</tr>
<tr>
<td>Ionisation</td>
<td>Electron Ionisation, 70 eV</td>
</tr>
<tr>
<td>Operation mode</td>
<td>Selected ion monitoring (SIM)</td>
</tr>
<tr>
<td>Solvent delay</td>
<td>6 min</td>
</tr>
<tr>
<td>Recorded ions (dwell time in seconds)</td>
<td></td>
</tr>
<tr>
<td>6.0 min to 7.0 min</td>
<td>m/z = 77 (0.1), 163 (0.1), 167 (0.1)</td>
</tr>
<tr>
<td>7.0 min to 8.0 min</td>
<td>m/z = 149 (0.1), 153 (0.1), 177 (0.2)</td>
</tr>
<tr>
<td>8.0 min to 12.0 min</td>
<td>m/z = 149 (0.1), 153 (0.1), 177 (0.2)</td>
</tr>
</tbody>
</table>
Correspondence between stable isotope labelled phthalates and native phthalates, retention times of native phthalates, and m/z-ratios of native phthalates used for quantification (confirmation of identity).

<table>
<thead>
<tr>
<th>Stable isotope labelled phthalate</th>
<th>Native phthalate</th>
<th>Retention time (min)</th>
<th>Quantifier (qualifier ions) (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl phthalate-3,4,5,6-D4</td>
<td>DMP</td>
<td>6.5</td>
<td>163 (77)</td>
</tr>
<tr>
<td>Diethyl phthalate-3,4,5,6-D4</td>
<td>DEP</td>
<td>7.4</td>
<td>149 (177)</td>
</tr>
<tr>
<td></td>
<td>DAP</td>
<td>8.6</td>
<td>149 (189)</td>
</tr>
<tr>
<td>Diisobutyl phthalate-3,4,5,6-D4</td>
<td>DiHP</td>
<td>8.8</td>
<td>149 (191)</td>
</tr>
<tr>
<td></td>
<td>DiBP</td>
<td>9.7</td>
<td>149 (223)</td>
</tr>
<tr>
<td>Dibutyl phthalate-3,4,5,6-D4</td>
<td>DBP</td>
<td>10.9</td>
<td>149 (223)</td>
</tr>
<tr>
<td></td>
<td>DFp</td>
<td>13.5</td>
<td>149 (237)</td>
</tr>
<tr>
<td>Dihexyl phthalate-3,4,5,6-D4</td>
<td>BBP</td>
<td>16.5</td>
<td>149 (91)</td>
</tr>
<tr>
<td>Dicyclohexyl phthalate-3,4,5,6-D4</td>
<td>DCHP</td>
<td>19.5</td>
<td>149 (167)</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl) phthalate-3,4,5,6-D4</td>
<td>DEHP</td>
<td>19.2</td>
<td>149 (167)</td>
</tr>
<tr>
<td></td>
<td>DFhP</td>
<td>20.0</td>
<td>225 (77)</td>
</tr>
<tr>
<td>Dioctyl phthalate-3,4,5,6-D4</td>
<td>DNOP</td>
<td>23.2</td>
<td>149 (279)</td>
</tr>
<tr>
<td>Dibenzyl phthalate-3,4,5,6-D4</td>
<td>DINP</td>
<td>22.0-27.0</td>
<td>293 (149)</td>
</tr>
<tr>
<td></td>
<td>D1D</td>
<td>23.0-29.0</td>
<td>307 (149)</td>
</tr>
<tr>
<td></td>
<td>DDPA</td>
<td>30.0</td>
<td>149 (55)</td>
</tr>
</tbody>
</table>

Note: Signals of the stable isotope labelled phthalates are recorded at m/z=153, except dimethyl phthalate, which is recorded at m/z=167.

Note: DINP and DIDP cannot be chromatographically separated and consequently quantified based on their base peak ion (m/z= 149). Therefore, the m/z values of 293 (corresponding to fragment [M-C_{10}H_{17}]^{+} of DINP) and 307 (corresponding to the fragment [M-C_{10}H_{19}]^{+} of DIDP) are applied for quantification.

7.3.2 Analysis sequence

Inject at the beginning of each sequence at least twice n-hexane (0), in order to clean the system. Inject then the calibration standards "Standard 0", followed by "Standard 160" for the
evaluation of the system suitability. Afterwards the test samples, procedural blank samples, quality control samples, and calibration standards are injected in random order. Inject after maximum 10 samples n-hexane (0) to identify potential carry over.

7.3.3 System suitability
The complete elimination of phthalate background is difficult. Phthalates might be released from some GC parts (e.g. inlet septa), or might enter the GC via the carrier gas supply. Therefore the GC-MS system is checked at the beginning of each analysis sequence for its suitability to analyse the test samples. The system is considered suitable when the phthalate peak abundances of "Standard 0" does not exceed 20% of the phthalate peak abundance of "Standard 160". If this is the case then root-cause-analysis has to be performed and the source of contamination has to be eliminated.

8. IDENTIFICATION AND CALCULATION OF RESULTS

The peak identity is confirmed by comparison of the peak ratios of quantifier ion and qualifier ion from sample extracts and standard solutions. The ratios should not differ more than ±20% from those obtained for standard solutions.

Calibration by internal standardisation is applied for the determination of native phthalates. A calibration graph is constructed in which the ratio of the areas of the peaks of native phthalates and the areas of the peaks of m/z=153 of the corresponding stable isotope labelled phthalates (see above) is plotted against the ratio of the concentrations of native phthalates and stable isotope labelled phthalates in the respective calibration solution. The calibration function is determined by linear regression.

\[
\frac{A_{\text{native}}}{A_{\text{labelled}}} = a \cdot \frac{C_{\text{native}}}{C_{\text{labelled}}} + b \quad \text{Equation 1}
\]

where

- \( A_{\text{native}} \) is the area of the quantifier ion of the native phthalate peaks
- \( A_{\text{labelled}} \) is the area of the corresponding stable isotope labelled phthalate peaks (of m/z 153)
- \( a \) is the slope of the calibration function
- \( C_{\text{native}} \) is the concentration of native phthalates
- \( C_{\text{labelled}} \) is the concentration of corresponding stable isotope labelled phthalates
- \( b \) is the intercept of the calibration function
Calculate for each sample the amount of native phthalate that was extracted from the sample \( X_{\text{native}} \) using the following equation:

\[
X_{\text{native}} = \frac{\frac{A_{\text{native}}}{V_{\text{labelled}}} - b}{a} \times X_{\text{labelled}} \times V_{\text{labelled}}
\]

Equation 2

where

- \( X_{\text{native}} \) is the concentration of native phthalates (in mg/L) in the sample.
- \( A_{\text{native}} \) is the area of the native phthalate peak of the test sample.
- \( A_{\text{labelled}} \) is the area of the corresponding stable isotope labelled phthalate peak (peak of m/z=153 of the test sample).
- \( X_{\text{labelled}} \) is the concentration (in µg/mL) of the working standard solution of stable isotope labelled phthalates in methanol (6.7).
- \( V_{\text{labelled}} \) is the volume (in mL) of the working standard solution of stable isotope labelled phthalates in methanol (6.7).
- \( a \) is the slope of the calibration function.
- \( b \) is the intercept of the calibration function.
- \( V_{\text{sample}} \) is the volume of extracted sample (in mL).

Calculate according to equation 2 the phthalate content in the procedural blank samples and subtract the average content of the procedural blank samples from the results of the test samples.

The results of the test samples are reported corrected for the background contamination to three significant figures. The reporting unit is mg/L.

9. QUALITY CONTROL

For each batch of samples the following controls are used:

9.1 Laboratory reference materials

Proper spiked wine/spirit sample or other suitable control samples are recommended for use as laboratory internal reference materials. The phthalate content of the laboratory reference material should be between 0.05 mg/L and 0.50 mg/L.

For this reason add 500 µL of the working standard solution of native phthalates in methanol (0) into a 100 mL volumetric flask and make up to volume with a blank or at least only low contaminated wine/spirit sample.

9.2 Control chart.

The results for the laboratory reference materials should be monitored in control charts. Acceptable results should be within the limits of 3 times the intermediate precision standard deviation of the method.
Annex

Annex 1: Example of GC-MS chromatogram of a wine sample, a) spiked to about 0.03 mg/L, scaled to 100000 arbitrary abundance units; b) system blank sample scaled to 100000 arbitrary abundance units

a)

b)
Annex 2: Analysis scheme

In 16 mL Screw cap-Vial:
8 ml wine or spirit

Addition of stable isotope labelled phthalates

Addition of 1.5 ml n-hexane

Extraction, 30 sec on Vortex shaker, 180 min on wrist arm shaker, followed by centrifugation

GC-MS analysis