Soil Quality – Determination of selected phthalates by chromatography / mass spectrometry

1 Scope

This standard specifies a method for the determination of phthalates in sediment, sludge and soil after extraction and gas chromatography – mass spectrometry.
The method is applicable to the determination of phthalates (see table 1) sediment, sludge, waste and soil in mass concentrations between 10 µg/kg and 50µg/kg, depending on the individual substance.
The applicability of the method to other phthalates not specified in table 1 is not excluded, but shall be verified in each case (list of phthalates see Annex E).
WARNING – Persons using this standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

Table 1 — Phthalates determined by this method

No	Name	Formula	Abbreviation	Molar mass g/mol	CAS [1])- No
1	Dimethylphthalate	$C_{10}H_{10}O_4$	DMP	194,2	00131-11-3
2	Diethylphthalate	$C_{12}H_{14}O_4$	DEP	222,24	00084-66-2
3	Dipropylphthalate	$C_{14}H_{18}O_4$	DPP	250,3	00131-16-8
4	Di-(2-methyl-propyl)phthalate	$C_{16}H_{22}O_4$	DiBP	278,4	00084-69-5
5	Dibutylphthalate	$C_{16}H_{22}O_4$	DBP	278,4	00084-74-2
6	Butylbenzylphthalate	$C_{19}H_{20}O_4$	BBzP	312,4	00085-68-7
7	Dicyclohexylphthalate	$C_{20}H_{26}O_4$	DCHP	330,4	00084-61-7
8	Di-(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	DEHP	390,6	00117-81-7
9	Dioctylphthalate	$C_{24}H_{38}O_4$	DOP	390,6	00117-84-0
10	Didecylphthalate	$C_{28}H_{46}O_4$	DDcP	446,7	00084-77-5
11	Diundecylphthalate	$C_{30}H_{50}O_4$	DUP	474,4	03648-20-2

[1]) CAS: Chemical Abstracts System

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.
ISO
ISO hier müssen noch entspr. Normen eingegeben werden.
ISO . *Bestimmung der Trockenmasse*
8466-1 : 1990, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function.*
Hier weiß ich nicht, ob das auch außerhalb ISO benutzt wird

3 Principle

The died sample ,dried by freeze-drying or with sodium chloride, is extracted with ethyl acetate on the shaking machine. An aliquot of the extract is cleaned with Al_2O_3. Gas chromatographic separation using capillary columns. Identification and quantification of the phthalates by mass spectrometry.

4 Interferences

Due to their use as plasticizer agents, phthalates are ubiquitous. Therefore, special attention shall be paid to avoid contaminations.

4.1. Interferences during sampling

In order to avoid interferences and cross contaminations, do not use plastic materials (pipes etc).

4.2. Interferences during enrichment

Solvents and analytical material can have varying quality. Considerable batch-to-batch differences in quality and selectivity of the materials are possible. The recovery of single substances may vary with the concentration. Therefore, check the recovery regularly at different concentrations and whenever new batches are used. Perform calibration and analysis with material from the same batch.
Cross contamination is likely to occur with laboratory air. Therefore, remove, as far as possible, plastic materials from the laboratory. Cleaning agents often contain phthalates and may severely contaminate the laboratory air if in use regularly. Therefore, refrain from using these agents during application of this procedure. Using plastic gloves during pre-treatment may increase the contamination.

4.3. Interferences in gas chromatography

Phthalates may bleed from the septa of the injector into the gas chromatograph, therefore use septa that are not likely to contaminate the system.
Fittings for example of syringes or equipment and septa of the sampling bottles (see clause 6.7.) may as well contain phthalates. Therefore make sure that uncontaminated septa are used.

5 Reagents

5.1. General

Use, as far as available, reagents of analytical quality, or better. Use only reagents with negligibly low concentration of phthalates and verify by blank determinations and, if necessary, apply additional cleaning steps.

5.2. Nitrogen, N_2 of high purity, at least a volume fraction of 99,9% for drying and eventually for concentration by evaporation.

5.3. Helium, He, of high purity, at least a volume fraction of 99,999 %

5.4. Ethyl acetate, highest purity, $C_4H_8O_2$

5.5. Methanol, CH_4O.

5.6. Isooctane, C_8H_{18} (2,2,4-trimethylpentane)

5.7. Quartz wool, heated to 400 °C for at least 4 h.

5.8. Aluminium oxide, alumina, Al_2O_3, neutral, 50 µm-200 µm, heated to 400 °C for at least 4 h. Bring the aluminium oxid to ambient temperature within 6 hours. Store in covered flask. Use within five days after baking.

Alternative materials, like Florisil* or silica may be used, provided their properties and capacity to separate are similar to aluminium oxide and their properties are checked according to 4.2.

NOTE Florisil is a trade name of prepared magnesium silicate.

5.9. Internal standards, for example: Diallylphthalate, DAlP, $C_{14}H_{14}O_4$; Di-n-butylphthalate, "D4-ring-DBP", D4-$C_{16}H_{22}O_4$; Di-n-octylphthalate, "D4-ring-DOP", D4-$C_{24}H_{38}O_4$, $^{13}C_{(6\text{-}12)}$ labelled standard (as far as available).

5.10 Reference substances of the phthalates mentioned in table 1, with defined mass concentrations, for the preparation of reference solutions for the gas chromatographic procedure.

5.11. Solutions of the single substances

In a 10 ml volumetric flask (6.15), dissolve for example 10 mg of each of the reference substances in ethyl acetate (5.4) and bring to volume with ethyl acetate (concentration: 1 g/l).
Store the solutions in glass bottles at –18 °C, protected from light, and check the concentration at least every 3 months.

5.12. Stock solution

In one 10 ml volumetric flask (6.15), dissolve between 100 µl and 500 µl of the single substance solutions (5.11) and bring to volume with ethyl acetate (5.4) (concentration 10 mg/l to 50 mg/l).
Store the solution in a glass bottle at –18 °C, protected from light, and check the concentration at least every 3 months.

5.13. Reference solutions for multipoint calibration

Prepare solutions by adequate dilution of the stock solution (5.12) and internal standards (5.14.5) with ethyl acetate (5.4.).
Store the solutions in a glass bottle at –18 °C, protected from light and check the concentration at least every 3 weeks (Annex A)

5.14. Solution of the internal standards (Annex A)

5.14.1. Internal standard solution of di-n-octylphthalat (D4)

Weigh for example 0,1 g of di-n-octylphthalate (D4-ring) (5.10) in a 10 ml volumetric flask (6.12) filled with about 5 ml of ethyl acetate (5.4), and bring to volume with ethyl acetate (5.4).

5.14.2. Internal standard solution of di-n-butylphthalat (D4)

Weigh for example 0,1 g of di-n-butylphthalate (D4-ring) (5.10) in a 10 ml volumetric flask (6.12) filled with about 5 ml of ethyl acetate (5.4) and bring to volume with ethyl acetate (5.4).

5.14.3. Solution I internal standard

Combine both solutions for example by dilution 1:100 as follows: pipette 0,1 ml of each solution in a 10 ml volumetric flask (6.12) filled with about 5 ml of ethyl acetate (5.4). Bring to volume with ethyl acetate. The final concentration of di-n-octylphthalat (D4) and di-n-butylphthalat (D4) will be 100 mg/l in ethyl acetate.

5.14.4. Solution II internal standard

Take from this 1:100 dilution (5.14.3) for example 250 µl, transfer in a volumetric flask, 250 ml (6.12), filled with 250 ml of ethyl acetate (5.4.).
The final concentration of di-n-octylphthalat (D4) and di-n-butylphthalat (D4) will be 0,1 mg/l in ethyl acetate.

5.14.5. Solution III internal standard

Dilute the solution I internal standard (5.14.3) 1:10: pipette 1ml of the solution (5.14.3) in a 10 ml volumetric flask (6.12) filled with about 5 ml of ethyl acetate (5.4). Bring to volume with ethyl acetate. The final concentration of di-n-octylphthalat (D4) and di-n-butylphthalat (D4) will be 10 mg/l in ethyl acetate.

5.15. Standard solution for the determination of the retention times

Dilute the solutions of the single substances (5.12) for example 1:1000 with ethyl acetate.

5.16. Sodium sulphate, Na_2SO_4, heated to 400°C for at least 4h

6 Apparatus

Equipment or parts of it which are likely to come into contact with the soil sample or its extract shall be free from phthalates. This may be achieved by thorough cleaning of all glass apparatus (8.1).

6.1. Wide -neck flat bottomed flasks with glass stoppers, preferably brown glass, 500 ml and 1000 ml

6.2. Drying oven, capable of being maintained at a temperature of 105°C (+10°C)

6.3. Muffle furnace, adjustable, up to temperatures of 400 °C, with capacity at least 60 l.

6.4. Vacuum device for clean up (vacubox, extraction box)

6.5. Stainless steel cock, with stainless steel cone or PTFE cock with and Luer*-connection for separate vacuum connection.

6.6. Sampling vial, glass, with inert stopper, e.g. septum, lined with polytetrafluoroethene (PTFE) for storage of the extracts, and sampling bottles, glass, with inert septum, 2 ml, for storage of the extracts for auto sampler operation**.**

6.7. **Glass cartridges, with Luer* cone**

6.8. PTFE- frits for cartridges, 6 ml.

6.9. Aluminium foil, heated to 400 °C.

6.10. Stainless steel reservoir, for storage of smaller glass apparatus.

6.11. **Measuring cylinders,** 50 ml, 100 ml.

6.12. **Volumetric flasks,** 1 ml, 2 ml, 10 ml, 25 ml and 250 ml.

6.13. **Pasteur-pipettes,** e.g. 2 ml.

6.14. **Syringes,** 2 µl, 5 µl, 10 µl 50 µl, 100µl and 500µl maximum permitted error ± 2 %.

6.15. **Gas chromatograph,** with capillary column, temperature controlled, with mass spectrometric detection.

6.16. **Operating gases for gas chromatography/mass spectrometer,** of high purity and in accordance with manufacturer's specifications.

6.17. **Fused silica columns,** with non polar stationary phase (see annex B); inner diameter $\leq$ 0,32 mm, length about 30 m, film thickness 0,10µm to 0,50 µm. Check the quality of the column e.g. by injecting the reference solution (5.14) and ensure that the separation is satisfactory.

6.18. **Glass tubes,** graduated 10 ml.

6.19. **Nitrogen device for drying**

6.20. Beaker, 50ml, 100ml

6.21. Erlenmeyer flask, 250 ml

6.22. Shaking machine,

6.23. Freeze drying apparatus

6.24. Metal spoon

6.25. Achat mortar

6.26. Metallic clamp, for stopper

6.27. Balance, range 0,001g---100g

6.28. Pipette; 20ml, 25ml 50ml

7. Sampling and sample pre-treatment

Collect, preserve and handle samples in accordance with ISO 5667-1, ISO 5667-2 and ISO 5667-3.
Use for sampling pre-treated sampling bottles (6.1 and 8.1) and make sure that the stoppers are pretreated as well.

In general, sampling should be carried out using stainless steel containers or glass vessels. In order to avoid contaminations, do not use any plastics material (tubes and other). If plastic parts in the sampling apparatus are unavoidable, flush the apparatus with at least 5 times the volume of the sample. If applicable, state this step in the test report.
Analyse the sample as soon as possible after sample collection. If storage is unavoidable, store the samples in the dark at 4°C.

8. Procedure

8.1. Pre-treatment of glass apparatus

Clean all glass apparatus used during analysis in the dishwasher with water and subsequently dry in the oven (6.2) at 105 °C.
Heat the pre-rinsed glass apparatus in the muffle furnace (6.3) using for example the following temperature programme.
To 100 °C with 2,5 °C/ min; to 250 °C with 10 °C/min; to 400°C in 75 min isothermic.
Subsequently let the apparatus cool to room temperature within 12 h.
NOTE Glassware for volumetric purposes may change its properties due to the heating process.

Close the cooled glass apparatus (bigger vessels) with the respective stoppers or with aluminium foil (6.9.). Store smaller glass apparatus in decontaminated (heated) and appropriately closed stainless steel containers (6.10).
In order to avoid losses by adsorption at the walls rinse the walls with isooctane (5.6) by using Pasteur pipettes. Discard the solvent.
Let residual solvent evaporate under a fume hood.
Carry out this deactivation of the surface after heating and cooling or immediately prior to use.

8.2. Extraction

Before starting the analysis, homogenate the sample with a metal spoon.

Depending on the content of the sample weight exact between 1-10g of the wet sample into a beaker (6.20) and give so much Sodium sulphate (5.16) into the wet sample until a trickle mixture is achieved. Mortar the mixture in a achat mortar (6.25). When a trickle mixture is obtained the humidity is bounded.
Parallel determine the dry content of the sample (2).

Note: Freeze drying can also be used if cross contamination are excluded.

Transfer the mixture or the freeze dried sample into a 250 ml Erlenmeyer flask (6.21) and give for example 20 ml ethyl acetate with internal standard (5.14.4) to the sample. Close the Erlenmeyer flask (6.20)with a stopper and fix the stopper with a metallic clamp (6.26)
Extract the sample at least 30 minutes on the shaking machine. Make sure that a good homogenisation of the sample and the solvent is obtained.

After the extraction take ca. 3 ml of the extract with a pipette (6.13) and clean the extract with the aid of a Al_2O_3 clean-up.

8.3. Extract cleaning

Clean the extracts as follows:
Place 1 g of activated aluminium oxide, Al_2O_3 (5.8) in the cartridges (6.7) between two PTFE frits (6.8)
Clean the Al_2O_3 (5.8) with one bed volume of ethyl acetate (5.4).
Dry with nitrogen (5.2) for 1 min.
Fix the cleaned cartridge with stainless steel cock (6.5) and place it on the vacuum device (6.4).
Let the extract run through the cartridge and collect it in a glass tube (6.18.
Transfer the extract to GC vials (6.6). Attach heated aluminium foil (6.9) between vial and caps in order to avoid a contamination by phthalates from the septa.

8.3. Gas chromatography

Optimise the GC-apparatus (6.16) according to the instrument manufacturer's manual.
Use capillary columns (6.16, annex B) for separation.
In order to clean the inlet system free from phthalates, inject ethyl acetate (5.4) at least 5 times from various GC-vials (see clause 6) before measuring the sample extracts or calibration solutions.

8.4. Blank monitoring

Check the proper condition of instruments and reagents by blank monitoring at regular intervals.
For the blank measurements, treat Sodium sulphate (5.16) in the same way as the sample (8.2, 8.3.). Weight nearly as much Sodium sulphate as it is needed to dry the samples. With each sample series determine two blanks.

8.5. Identification of individual compounds

8.5.1. General

Individual compounds are identified by comparison of the retention times of the respective peaks in the sample chromatogram with the substance peaks of a reference solution measured under the same conditions.
The compound is classified as not detected if the chromatogram of the sample extract does not contain a peak at the substance specific retention time.
The presence of a distinct compound is classified as possible if a peak occurs at the substance specific retention time. If necessary, the identity of the compound shall be verified by additional investigations.

8.5.2. Identification of individual compounds with mass spectrometric detection

Consider individual compounds in the sample to be identified if:

— the retention time (t_R) of the respective peaks in the total ion-current chromatograms or in the individual mass chromatograms lie within a tolerance of t_R = 0,1 min, compared with the retention times of the peaks of the substances in the total ion current chromatograms or individual mass chromatograms of a reference solution, measured under identical conditions, and

— complete, background-corrected mass spectra of the reference compounds agree with the background corrected mass spectra obtained at the respective retention time in the total ion-current chromatogram of the sample; or

— at least the characteristic molecular ions or fragment ions of the reference compounds (see table 2) agree with specified tolerances which should not be greater than 20%, with those of the compounds to be identified as to the relative peak intensities.

Table 2 — Mass fragments of the reference compounds

	Compound	Abbreviation	Specific monitored ions		
			Target ion M_1	Qualifier ion M_2	Qualifier ion M_3
1	Dimethylphthalate	DMP	163	194	135
2	Diethylphthalate	DEP	149	177	222
3	Dipropylphthalate	DPP	149	209	191
4	Di (2-methyl-propyl)phthalate	DiBP	149	223	---
5	Dibutylphthalate	DBP	149	223	278
6	Butylbenzylphthalate	BBzP	149	206	312
7	Dicyclohexylphthalate	DCHP	149	167	249
8	Di (2-ethylhexyl)phthalate	DEHP	149	167	279
9	Dioctylphthalate	DOP	149	279	207

10	Didecylphthalate	DDcP	149	307	---
11	Diundecylphthalate	DUP	149	321	---
12	D4-ring- Dibutylphthalate	D4-DBP	153	---	---
13	D4-ring-Dioctylphtalate	D4-DOP	153	---	---
14	Diallylphthalate	DalP	149	189	132

NOTE M_1 is used for quantification, M_2 and M_3 may be used for identification

9 Calibration

9.1. General

Establish for each compound a calibration function and graph using single, or, for practical reasons, multicomponent reference solutions.
Make sure to obtain a linear relation of measuring signal to concentration.
Determine the linear working range by at least five points from five different concentrations.
The calibration function determined for a single component is valid only for the respective concentration range and depends as well from the operating conditions of the gas chromatograph and needs regular checking. For routine purposes, a two-point calibration is sufficient.
A procedure is given for the setup of a calibration function and the working range is adjusted to the working conditions (preparation of the reference solution according to 5.14).
Calibration of the gas chromatographic step (calibration with external standard, not using the overall procedure and including an internal standard).
Subscripts used in the following text see table 3.

Table 3 — Subscripts

Subscript	Meaning
i	Identity of the substance
e	Calibrations
g	Total procedure
j	Consecutive number of pairs of values

9.2 Calibration with external standard, not using the overall procedure

Set up a calibration function from at least five reference solutions (5.13.), for practical reasons, determine all phthalates mentioned in table 1 within one procedure.
The knowledge of the retention times of the single components is prerequisite. The retention times are determined using the solutions of the single components (5.15).
Inject aliquots from the reference solutions (5.13). Make sure that the injection volume is the same during calibration and measurement of the sample.

For a graphical presentation of the calibration curve, plot the respective measured values y_{iej} (peak area, peak height or integration units) for each substance i on the ordinate against the respective mass concentration r_{iej} on the abscissa.
Use the series of measured values thus obtained to establish the linear regression function as follows:

$$y_{ie} = m_i \cdot \mathrm{r}_{iej} + b_i \qquad (1)$$

where

y_{ie} Is the measured value for the substance *i* during calibration, depending on r_{iej}, the unit depends on the evaluation, for example expressed as area values;

m_i Is the slope of the calibration function of the substance i (corresponds to the substance-specific response factor) expressed for example as (peak area x micro liters per picogram)

r_{iej} Is the mass concentration of the substance i (external standard in the reference solution), in pico gram per mikrolitre ;

b_i Is the ordinate intercept of the calibration function of the substance *i*., the unit depends on the evaluation,

e.g. area value x micro liters per picogram

9.3. Calibration with internal Standard

When using the internal standard calibration, the determination is independent from possible errors made during injection. Apart from this, errors caused by sample losses during distinct steps of sample pre-treatment may be avoided. Additionally, the concentration determination is independent from matrix effects in the sample, provided the recoveries of the substances analyse and the internal standard are about the same.
The mass concentration c_{is} shall be the same for calibration and sample measurement.

Plot the rational value y_i/y_{is} (peak areas, peaks heights or integration units) for each substance I on the ordinate and the associated rational mass concentration c_i/c_{ISi} on the abscissa.
Establish the linear regression function using the pairs of value y_i/y_{is} and c_i/c_{Isi} of the measured series in the following equation:

$$\frac{y_{ieg}}{y_{Ieg}} = m_{igI}\frac{r_{ieg}}{r_{leg}} + b_{igl} \qquad (2)$$

y_{ie} is the (dependent variable) measured response of the substance *i* in the calibration, depending on r_{ie}; the unit depends on the evaluation, for example, area value;

y_{leg} is the measured response of the internal standard *i* in the calibration, depending on r_{ie}; the unit depends on the evaluation, for example, area value;

r_{ieg} is the (independent variable) mass concentration of the substance *i* in the calibration solution, in pico grams per litre;

r_{leg} is the (independent variable) mass concentration of the internal standard *I*, in pico grams per litre;

m_{igl} is the slope of the calibration curve from y_{ie} / y_{le} as a function of the mass concentration ratio r_{ie} / r_{le}, often called the response factor;

b_{igl} is the axis intercept of the calibration curve on the ordinate.

10 Calculation

10.1 Calculation of single results

Calculation of single results during calibration with external standard.
Calculate the mass concentration r_{iej} of the substance i in the sample according to equation (1)

$$\rho_{iT} = \frac{(r_{iej} - r_{ieBlj})* V * 100(\%)}{T_M(\%) * E_{wori}} \qquad (3)$$

r_{iej} :Is the mass concentration of the substance i (external standard in the reference solution), in pico gram per mikrolitre ;

$r_{iejBL:}$ Is the mass concentration of the substance i in the blank sample, analysed in accordance to chapter 8.4. (external standard in the reference solution), in pico gram per mikrolitre ;

V: Is Volume of the extraction solvent (ethyl acetate) in ml

T_M: Is the dry matter content of the sample in %

E_{wori}: Is the mass of the sample in g

10.2. Calculation of single results after calibration with internal standard

Calculate the mass concentration ρ_{iT} of the substance using equation (2):

$$\rho_{iT} = \frac{(((((y_{ie}/y_{ieg})-b_{iel})/m_{iel}))* r_{ileg})- ((((y_{ieBl}/y_{iebl}-b_{ie})/m_{ieg}))* r_{iBlj}))* V *100}{TM * E_{wori}} \quad (4)$$

ρ_{iT} : Is the mass concentration of the substance I in the sample (external standard in the reference solution), in µg/kg on basis of dry matter

y_{ie} is the (dependent variable) measured response of the substance *i* in the calibration, depending on r_{ie}; the unit depends on the evaluation, for example, area value;

y_{leg} is the measured response of the internal standard *i* in the calibration, depending on r_{ie}; the unit depends on the evaluation, for example, area value;

r_{ieg} is the (independent variable) mass concentration of the substance *i* in the calibration solution, in pico grams per microlitre;

r_{leg} is the (independent variable) mass concentration of the internal standard *I*, in pico grams per microlitre;

m_{igl} is the slope of the calibration curve from y_{ie} / y_{le} as a function of the mass concentration ratio r_{ie} / r_{le}, often called the response factor;

b_{igl} is the axis intercept of the calibration curve on the ordinate.

y_{ieBl} is the (dependent variable) measured response of the substance *i* in the calibration and in the blank sample, in accordance to chapter 8.4, depending on r_{ie}; the unit depends on the evaluation, for example, area value;

y_{leBl} is the measured response of the internal standard *i* in the calibration and in the blank sample in accordance to chapter 8.4, depending on r_{ie}; the unit depends on the evaluation, for example, area value;

V: Is Volume of the extraction solvent (ethyl acetate) in ml

T_M: Is the dry matter content of the sample in %

E_{wori}: Is the mass of the sample in g

For the phthalates DMP to DEHP use as internal standard D4- ring-DBP and for the phthalates DOP to DUP as internal standard D4-ring-DOP ;

11 Expression of results

In the case of sludge, sediment, and soil report the results in micrograms per kilogram µg/kg, with two significant digits.
EXAMPLES

Dioctyl-phthalate	65	µg/kg
Dodecyl-phthalate	150	µg/kg
Dimethyl-phthalate	1 200	µg/kg

12 Test report

The report shall refer to this International Standard and contain the following information

a) Identity of the sample including all information concerning sampling and sampling technique;

b) Sample pretreatment, if applicable;

c) Storage prior to analysis and time between sampling and analysis, if applicable;

d) Sample preservation;

e) Any deviation from this procedure and all circumstances which may have affected the results;

f) Expression of results, according to clause 11;

g) Method and degree of confirmation of the result.

Annex A

Examples

1. Solutions of the internal standards

Solution	Chapter	Name and Preparing	Concentration
1	5.14.1.	**Internal standard solution of D4-DOP** Weight 0,1g of D4-DOP in 10 ml ethyl acetate	c=10g/L EA
2	5.14.2	**Internal standard solution of D4-DBP** Weight 0,1g of D4-DBP in 10 ml ethyl acetate	c=10g/L EA
3	5.14.3	**Solution I internal standard** Take 0,1 ml of solution 1(5.14.1) and 0,1 ml of the solution 2 (5.14.2) in a 10 ml volumetric flask, filled with 5 ml of ethyl acetate and bring it to a volume of 10 ml with ethyl acetate.	c D4-DOP= 100mg/L EA c D4-DBP= 100 mg/L EA
4	5.14.4	**Solution II internal standard** (Dilution 1:1000 of solution I internal standard) Take 250µl of the solution I internal standard, transfer in a 250 ml volumetric flask, filled with ca. 200 ml ethyl acetate, and fill it with ethyl acetate	c D4-DOP= 100µg/L EA c D4-DBP= 100 µg/L EA
5	5.14.5.	**Solution III internal standard** (Dilution 1:10 of the solution I internal standard) Take 1 ml of the solution I internal standard in a 10 ml volumetric flask, filled with 5 ml of ethyl acetate and bring it to a volume of 10 ml with ethyl acetate.	c D4-DOP= 10 mg/L EA c D4-DBP= 10 mg/L EA

2. Solution of the single phthalates (5.11.)

In a 10 ml volumetric flask dissolve for example 10 mg of each reference substance seperate in ethyl acetate and bring to a volume of 10 ml

Phthalate- solutions of the single phthalates	Mass of the single phthalates in 10 ml ethyl acetate **(mg)**	Concentration of each single phthalate solution **(g/L EA)**
DMP	10	1
DEP	10	1
DPP	10	1
DiBP	10	1
DBP	10	1
BBzP	10	1
DCHP	10	1
DEHP	10	1
DOP	10	1
DDcP	10	1
DUP	10	1

3. Stock solution of the phthalates (5.12)

In a 10 ml volumetric flask dissolve between 100 and 500µl of the single solutions of the phthalates and fill up with ethyl acetate (5.4)

Phthalate- solutions of the single phthalates	**µl** of the single phthalates solutions (2)	Concentration of each phthalate in the solution (mg/L EA)
DMP	100	10
DEP	100	10
DPP	100	10
DiBP	100	10
DBP	100	10
BBzP	200	20
DCHP	100	10
DEHP	100	10
DOP	200	20
DDcP	500	50
DUP	500	50

4. Reference solution for multipoint calibration

Prepare solutions by adequate dilution of the stock solution (5.12) and the solution III internal standard (5.14.5) in a 10 ml volumetric flask and fill up with ethyl acetate

Level	**µl** stock solution (5.12)	**µl** solution III internal standard (5.14.6)
L1	2,5 µl	100
L2	5 µl	100
L3	10µl	100
L4	20 µl	100
L5	40 µl	100
L6	50µl	100
L7	100µl	100
L8	150µl	100
L9	300µl	100
L10	450µl	100

The obtained concentrations from L1- L10 are as followed:

Phthalate	**L1 (pg/µl)**	**L2 (pg/µl)**	**L3 (pg/µl)**	**L4 (pg/µl)**	**L5 (pg/µl)**	**L6 (pg/µl)**	**L7 (pg/µl)**	**L8 (pg/µl)**	**L9 (pg/µl)**	**L10 (pg/µl)**
DMP	2,5	5,0	10	20	40	50	100	150	300	450
DEP	2,5	5,0	10	20	40	50	100	150	300	450
DPP	2,5	5,0	10	20	40	50	100	150	300	450
DiBP	2,5	5,0	10	20	40	50	100	150	300	450
DBP	2,5	5,0	10	20	40	50	100	150	300	450
BBzP	5,0	10,0	20	40	80	100	200	300	600	900
DCHP	2,5	5,0	10	20	40	50	100	150	300	450
DEHP	2,5	5,0	10	20	40	50	100	150	300	450
DOP	5,0	10,0	20	40	80	100	200	300	600	900
DDcP	12,5	25,0	50	100	200	250	500	750	1500	2250
DUP	12,5	25,0	50	100	200	250	500	750	1500	2250
D4-DBP	100	100	100	100	100	100	100	100	100	100
D4-DOP	100	100	100	100	100	100	100	100	100	100

Annex B

(informative)

Example for capillary columns

EXAMPLE 1

Phase: 5 % phenyl methyl siloxane

Length: 30 m, inner diameter : 0,25 mm, film thickness : 0,25 µm

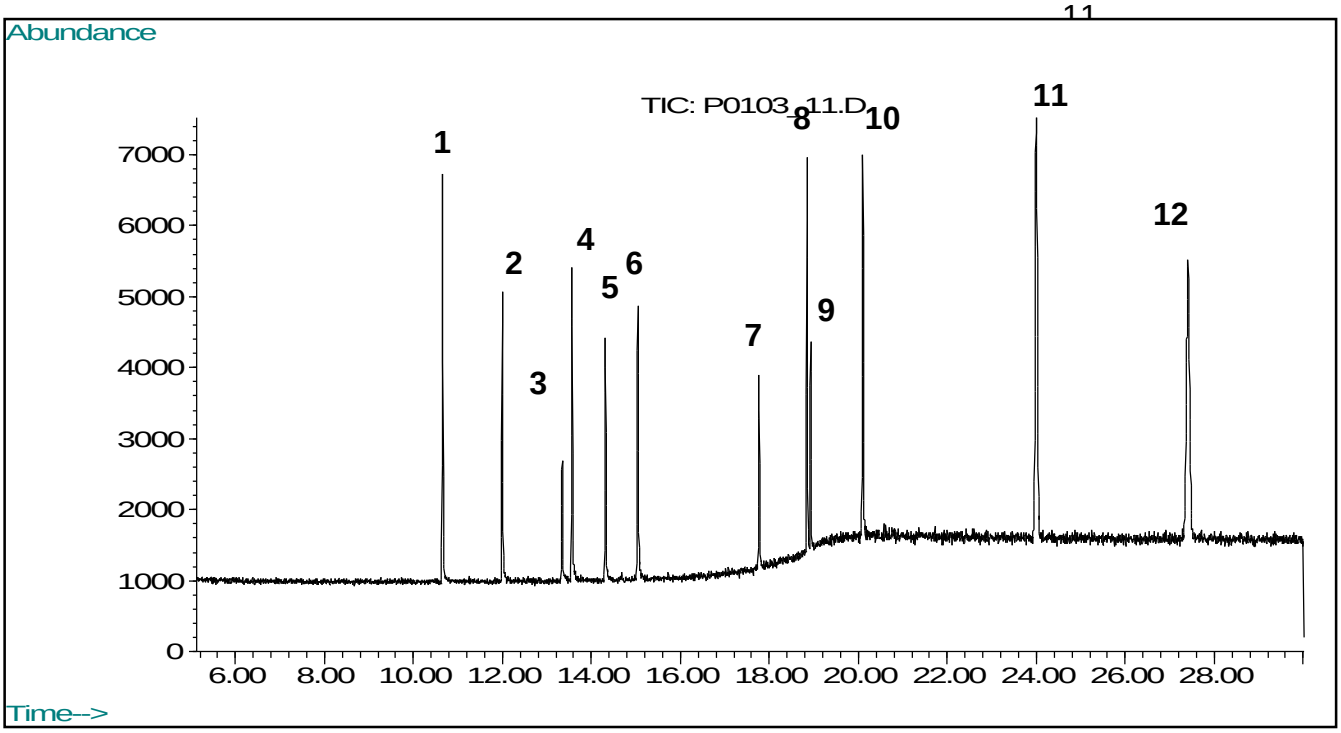
EXAMPLE 2

Phase: 34 % / 64 % / 2% phenyl- / methyl- / vinyl- silicone
Length: 30 m, inner diameter : 0,32 mm, film thickness : 0,25 µm

(informative)

Example of gas chromatographic conditions

GC:	HP 6890 Series with autosampler HP 6890 series; detector : mass spectrometric detector, quadrupol
column	HP - 5MS ; 30 m; inner diameter 0,25 mm ; film thickness 0,25 µm
Carrier gas:	Helium 5,0 ; pressure : 4,5 bar
Injector:	Pulsed splitless ; split: 20 ml / min ; splitless period: 1,5 min
Injector temperature:	250 °C
Detector temperature:	290 °C
Injection volume:	1 µl (automatic)
Temperature programme :	70°C, 3 min isotherm, 13°C-/-min to 280°C, 20 min isotherm
Source temperature:	230 °C
Ionisation mode:	EI
Concentration of standard solution:	between 25 pg / µl and 190 pg / µl



Key

1	Dimethylphthalate
2	Diethylphthalate
3	Diallylphthalate
4	Dipropylphthalate
5	Di (2-methyl-propyl)phthalate
6	Dibuthylphthalate
7	Butylbenzylphthalate
8	Dicyclohexylphthalate
9	Di (2-ethylhexylphthalate)
10	Dioctylphthalate
11	Didecylphthalate
12	Diundecylphthalate

Figure B.1 — Chromatogram

Annex C

An interlaboratory trial, carried out in xxx, delivered the data given in table 4.

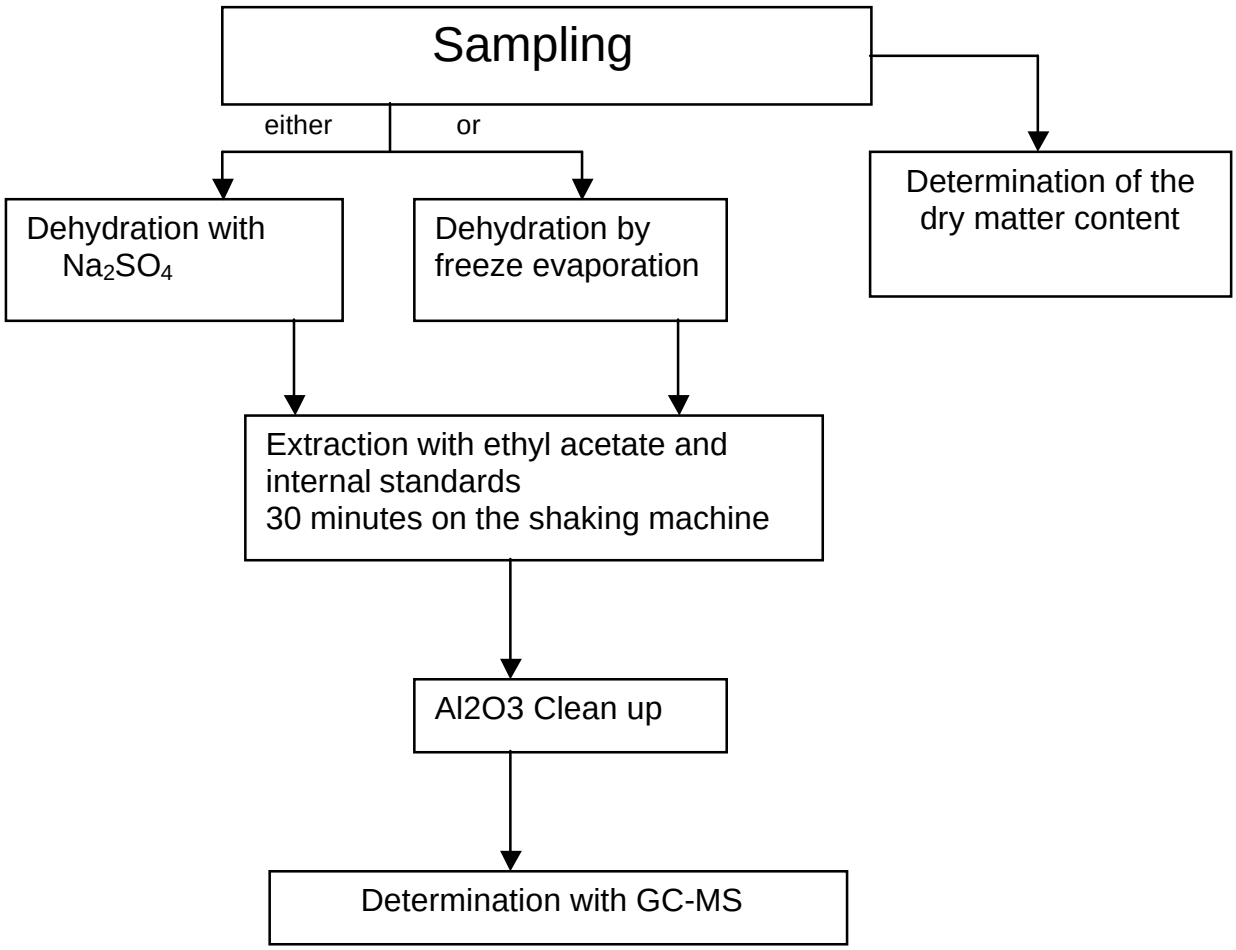
Table B.1 — Precision data

Compound	*L*	*N*	*NAP* %	r ng/l	r_t ng/l	*A* %	s_R ng/l	CV_R %	s_r ng/l	CV_r %
Dimethylphthalate Diethylphthalate Dipropylphthalate Di(2-methyl-propyl)phthalate Dibutylphthalate Butylbenzylphthalate Dicyclohexylphthalate Di(2ethylhexyl)-phthalate Dioctylphthalate Didecylphthalate Diundecylphthalate										

L	is the number of laboratories;
N	is the number of values;
NAP	is the number of the outlier percentage;
r	is the total mean;
r_t	is the true value (by convention);
A	is the recovery rate.
s_R	is the reproducibility standard deviation;
CV_R	is the reproducibility variation coefficient;
s_r	is the repeatability standard deviation;
CV_r	is the repeatability variation coefficient;

Annex D

Flow sheme



Annex E

(informative)

List of phthalates

The following list gives an outline on various phthalates. The method may not be applicable to isomeric mixtures of phthalates causing peak patterns in gas chromatography.

No.	Name	Formula	Abbreviation	Molar mass	CAS- No
1	Butylbutoxyethylphthalate	$C_{18}H_{26}O_5$	BboEP	322,4	33374-28-6
2	Butylcyclohexylphthalate	$C_{18}H_{24}O_4$	BCHP	304,4	84-64-0
3	Butyl 2-butoxy-2-exoethylphthalate	$C_{18}H_{24}O_6$	BboOeP	336,4	85-70-1
4	Butyldecylphthalate	$C_{22}H_{34}O_4$	BDcP	362,6	89-19-0
5	*Butylbenzylphthalate*	*$C_{19}H_{20}O_4$*	*BBzP*	*312,4*	*85-68-7*
6	Butyl 2-ethylhexylphthalate	$C_{20}H_{30}O_4$	BEHP	334,5	85-69-8
7	Butyl 8-methylnonylester	$C_{22}H_{34}O_4$	BMNP	362,6	89-18-9
8	Benzyl 2-ethylhexylphthalate	$C_{23}H_{28}O_4$	BzEHP	368,6	18750-05-5
9	Butyl-2-methylpropylphthalate	$C_{16}H_{22}O_4$	BMPP	278,4	17851-53-5
10	*Diallylphthalate*	*$C_{14}H_{14}O_4$*	*DalP*	*246,3*	*131-17-9*
11	Butyloctylphthalate	$C_{20}H_{30}O_4$	BOP	334,5	84-78-6
12	Di (2-butoxyethyl)phthalate	$C_{20}H_{30}O_6$	DboEP	366,5	117-83-9
13	*Dibutylphthalate*	*$C_{16}H_{22}O_4$*	*DBP*	*278,4*	*84-74-2*
14	Dicyclopentylphthalate	$C_{18}H_{22}O_4$	DCPeP	302,4	18699-38-2
15	Dibenzylphthalate	$C_{22}H_{18}O_4$	DBzP	346,3	523-31-9
16	*Didecylphthalate*	*$C_{28}H_{46}O_4$*	*DDcP*	*446,7*	*84-77-5*
17	*Dicyclohexylphthalate*	*$C_{20}H_{26}O_4$*	*DCHP*	*330,4*	*84-61-7*
18	Didodecylphthalate	$C_{32}H_{54}O_4$	DDdP	502,8	2438-90-8
19	Di (2-ethylbutyl)phthalate	$C_{20}H_{30}O_4$	DEBP	334,5	7299-89-0
20	*Diethylphthalate*	*$C_{12}H_{14}O_4$*	*DEP*	*222,4*	*84-66-2*
21	*Di (2-ethylhexyl)phthalate*	*$C_{24}H_{38}O_4$*	*DEHP*	*390,6*	*117-81-7*
22	Dihexylphtalate	$C_{20}H_{30}O_4$	DHP	334,5	84-75-3
23	Di (2-ethoxyethyl)phthalate	$C_{16}H_{22}O_6$	DeoEP	310,4	605-54-9
24	Diheptylphthalate	$C_{22}H_{34}O_4$	DHpP	362,5	3648-21-3
25	Di(3-methylbutyl)phthalate	$C_{18}H_{26}O_4$	DMBP	306,4	605-50-5
26	Di (1-methylethyl)phthalate	$C_{14}H_{18}O_4$	DMEP	250,3	605-45-8
27	Dimethyl cyclohexylphthalate	$C_{22}H_{30}O_4$	DMCHP	358,5	27987-25-3
28	Di (5-methylhexyl)phthalate	$C_{22}H_{34}O_4$	DMHP	362,5	41451-28-9
29	Di (11-methyldodecyl)phthalate	$C_{34}H_{58}O_4$	DMDdP	530,8	27253-26-5
30	Di (6-methylheptyl)phthalate	$C_{24}H_{38}O_4$	DMHpP	390,6	131-15-7
31	Di (8-methylnonyl)phthalate	$C_{28}H_{46}O_4$	DMNP	446,7	89-16-17
32	*Dimethylphthalate*	*$C_{10}H_{10}O_4$*	*DMP*	*194,2*	*131-11-3*
33	Di (2-methoxyethyl)phthalate	$C_{14}H_{18}O_6$	DmoEP	282,3	117-82-8
34	Di (4-methylpentyl)phthalate	$C_{20}H_{30}O_4$	DMPeP	334,5	146-50-9
35	Di (7-methyloctyl)phthalate	$C_{26}H_{42}O_4$	DMOP	418,6	28553-12-0
36	Di (2-methylpropyl)phthalate	$C_{16}H_{22}O_4$	DMPP (DiBP)	278,4	84-69-5
37	Dinonylphthalate	$C_{26}H_{42}O_4$	DNP	418,6	84-76-4
38	Diphenylphthalate	$C_{20}H_{14}O_4$	DPhP	318,3	84-62-8
39	Dioctylphthalate	$C_{24}H_{38}O_4$	DOP	390,6	117-84-0
40	*Dipropylphthalate*	*$C_{14}H_{18}O_4$*	*DPP*	*250,3*	*131-16-8*

41	Dipentylphthalate	$C_{18}H_{26}O_4$	DpeP	306,4	131-18-0
42	Ditridecylphthalate	$C_{34}H_{58}O_4$	DTdP	530,9	119-06-2
43	Di (3,3,5-trimethylhexyl)phthalate	$C_{26}H_{42}O_4$	DTMHP	418,6	4628-60-8
44	2-Ethylhexyl 8-methylnonylphthalate	$C_{26}H_{42}O_4$	EHMNP	418,6	89-13-4
45	*Diundecylphthalate*	*$C_{30}H_{50}O_4$*	*DUP*	*474,7*	*3648-20-2*
46	Methyl2-ethoxy-2-oxoethylphthalate	$C_{13}H_{14}O_6$	MeoOeP	266,3	85-71-2
47	Ethyl 2-ethoxy-2-oxoethylphthalate	$C_{14}H_{16}O_6$	EeoOeP	280,3	84-72-0
48	Hexyldecylphthalate	$C_{24}H_{38}O_4$	HDcP	390,6	25724-58-7
49	Hexyl 8-methylnonylphthalate	$C_{24}H_{38}O_4$	HMNP	390,6	61702-81-6
50	Methylmethoxyoxoethylphthalate	$C_{12}H_{12}O_6$	MmoOeP	252,2	53161-30-1
51	Methylbutylphthalate	$C_{13}H_{16}O_4$	MBP	236,2	34006-76-3
52	Octyldecylphthalate	$C_{26}H_{26}O_4$	OdcP	418,6	119-07-3
53	6-Methylhepthyl-8-methylnonyl-phthalate	$C_{26}H_{42}O_4$	MHpMNP	418,6	119-05-1
54	Octyl 8-methylnonylphthalate	$C_{26}H_{42}O_4$	OMNP	418,6	1330-96-7
55	Monobutylphthalate	$C_{12}H_{14}O_4$	SBP	222,2	131-70-4
56	Monomethylphthalate	$C_9H_8O_4$	SMP	180,2	4376-18-5
57	Mono (2-ethylhexyl)phthalate	$C_{16}H_{22}O_4$	SEHP	278,4	4376-20-9
58	Monopentylphthalate	$C_{13}H_{16}O_4$	SpeP	236,2	24539-56-8
59	Monoethylphthalate	$C_{10}H_{10}O_4$	SEP	194,2	2306-33-4

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