Soils, sludges and treated bio-wastes – Organic constituents - Determination of selected phthalates by using capillary gas chromatography with mass spectrometric detection

Böden, Schlämme und behandelte Bioabfälle – Organische Bestandteile - Bestimmung ausgewählter Phthalate mittels Gaschromatographie/Massenspektroskopie

ICS:

Descriptors:

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Foreword

This document has been prepared in the framework of the project Horizontal, processed by TF 151 and supported by the Commission of CEN. The following TC`s have been involved in the preparation of the standard: ISO TC 190 (Soil Quality); CEN TC 292 (Waste), CEN TC 308 (Sludge characerization). This standard is applicable and validated for several types of matrices. The table below indicates which ones.

Material	Validated	Document
Soil	□	reference
Sludge	□	reference
Treated bio-waste	□	reference
Soil improvers	□	reference
Waste	□	reference

Introduction (informative)

Project Horizontal is commissioned to develop standards in the field of sludges, soils and treated bio-wastes that are common to several matrices, are consistent with regard to EU regulation, and which leas to equivalent results as far as is technically feasible – so called Horizontal Standards. These draft standards will have been prepared by experts involved in ISO and CEN technical committees in the field of soils, sludges and treated bio-wastes. The standards will be applicable only to matrices for which they have been validated

in accordance with ISO 5725-5. In the course of the work of the project Horizontal, three development levels of draft are produced, combined with consultation of relevant CEN bodies (see annex F). The final draft standards produced by project Horizontal will be further processed by BT/TF 151.

Soils, sludges and treated bio-wastes – Organic constituents - Determination of selected phthalates by using capillary gas chromatography with mass spectrometric detection

1 Scope

This European Standard specifies a method for the determination of selected phthalates in soil, sludges and treted bio-waste after extraction and gas chromatography – mass spectrometry.
The method is applicable to the determination of phthalates (see table 1) soil, sediment, sludge, waste and at the lowest mass content up to 0,1 mg/kg to 0,5mg/kg, depending on the individual substance and the laboratory blank.
The applicability of the method to other phthalates not specified in table 1 is not excluded except the isomeric mixtures ~~f.e~~e.g. DiNP (Di-isononylphthalate), but shall be verified in each case.

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.

ISO 10381-1 Soil Quality – Sampling – Part 1: Guidance on the design of sampling programmes.

~~DIN~~ ISO 10381-2 Soil quality — Sampling — Part 2: Guidance on sampling techniques.

ISO 10381-1 Soil Quality – Sampling – Part 3: Guidance on the design of sampling of stockpiles.

ISO/FDIS 22982:2004: Soil Quality – Guidelines for identification of target compounds by gas chromatography and mass spectrometry
~~DIN~~ ISO 5667-13. Water quality — Sampling— Part 13: Guidance on sampling of sludge from sewage and water-treatment works; February 1998.

ISO 11465 Soil Quality – Determination of dry matter and water content on mass bases – Gravimetric method

ISO/DIS 14507 Soil Quality – Guidance for sample pre-treatment for the determination of organic contaminants in soil.

ISO//DIS 16720:2003: Soil Quality – Pre-treatment of samples for organic characterisation

prEN 14346 – Characterisation of waste – Calculation of dry matter by determination of dry residue and water content.

ISO 8466-1 Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function

~~DIN~~ EN 25667—Water quality—Sampling; Part 2: Guidance on sampling techniques; July 1993

~~DIN~~ EN 25667 — Water quality— Sampling Part 1: Guidance on the design of sampling programmes, November 1993

~~DIN~~ EN 12880—Characterisation of sludge — Determination of dry residue and water content.

Horizontal standard 2 – 2: Sampling of sludges and treated bio-waste.

Horizontal standard 3 – 3: Sampling of soils.

Horizontal standard 17 – 1(draft): Soil materials – Determination of dry matter and water content on a mass basis – Gravimetric method.

Horizontal standard 33 – 2: Solid materials – Pre-treatment for organic characterisation.

3 Terms and definitions

3.1 Analytes
In the context of this standard, the analytes are the in table3 described phthalates.

3.2 Calibration standard
A solution prepared from stock solutions of phthalates and used to calibrate the response of the instrument with respect to analyte concentration.

3.3 Internal standard
The internal standard is added to the test sample before extraction. The internal standard is used to correct for losses during the analysis and is used for calculating the concentration of the analytes.

4 Symbols and abbreviations

Abbreviations of the respective phthalates see table 3 and equation (1 – 5).

5 Principle

The dried sample, dried by freeze-drying or with sodium ~~sulph~~sulfate is extracted with ethyl acetate on the shaking machine. An aliquot of the extract is cleaned with aluminium oxide Al_2O_3 (if necessary) followed by gas chromatographic separation using capillary columns and identification and quantification of the phthalates by mass spectrometry.

6 Contaminations

Due to their use as plasticizer agents, phthalates are ubiquitous and the main problem in the analysis of phthalates. The sources of phthalates are multiplex and must be checked and reduced by every laboratory itself. Therefore, special attention shall be paid to avoid contaminations.

6.1 Interferences during sampling

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

6.2 Cross contamination

Chemicals and analytical equipment can have varying quality. 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.

6.3 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~~4.) may as well contain phthalates.

7 Reagents

7.1 General

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

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

7.3 Helium, He, of high purity, at least a volume fraction of 99,~~999~~ 999 %

7.4 Ethyl acetate (EA), high purity, $C_4H_8O_2$, phthalatefree

7.5 Methanol, CH_4O.

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

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

7.8 Aluminium oxide, alumina, Al_2O_3, neutral, ~~-~~50 µm-200 µm, heated to 400 °C for at least 4 h. ~~-~~Store in covered flask or dessicator. Use within five days after heat-treatment.

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.

7.9 Internal standards, for example: deuterated Di-n-butylphthalate, “D4-ring-DBP”, deuterated D4-$C_{16}H_{22}O_4$ deuterated Di-(2-ethylhexyl)phthalate “D4-ring-DEHP”, deuterated D4-$C_{24}H_{38}O_4$; Di-n-octylphthalate, “D4-ring-DOP”, D4-$C_{24}H_{38}O_4$,
^{13}C ~~X~~ labelled standards can also be used, as far as available.

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

7.11 Solutions of the single substances

In a 10 ml volumetric flask (6.~~15~~12), transfer for example 10 mg of each of the reference substances in ethyl acetate (5.~~4~~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.

7.12 Stock solution

In one 10 ml volumetric flask (6.~~15~~12), dissolve between 100 µl and 500 µl of the single substance solutions (5.11) and bring to volume with ethyl acetate (5.~~4~~4) (concentration 10 mg/l to 50 mg/l).
Store the solution in a glass bottle at –~~18~~ 18 °C, protected from light, and check the concentration at least every 3 months.

7.13 Reference solutions for multipoint calibration (Annex A)

Prepare solutions by adequate dilution of the stock solution (5.12) and internal standards (5.14.~~5~~3) with ethyl acetate (5.~~4~~4).
Store the solutions in a glass bottle at –~~18~~ 18 °C, protected from light and check the concentration at least every 3 weeks (Annex A)

7.14 Solution of the internal standards (Annex A)

7.14.1 Internal standard solution of D4-phthalates

Weigh for example 0,1 g of an internal standard phthalate (D4) (5.9) 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). Store the solutions in glass bottles at – 18°C.

~~**5.14.1 Internal standard solution of di-n-octylphthalate (D4)**~~

~~Weigh for example 0,1 g of di-n-octylphthalate (D4-ring) (5.9) 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-butylphthalate (D4)**~~

~~Weigh for example 0,1 g of di-n-butylphthalate (D4-ring) (5.9) 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 Internal standard solution of di-(2-ethylhexyl)phthalate (D4)**~~

~~Weigh for example 0,1 g of di-2-ethylhexylphthalate (D4-ring) (5.9) 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).~~

7.14.~~4~~ 2 Solution I internal standard mix

Combine the ~~three~~ solutions of the single internal standard phthalates (5.14.1) for example by dilution 1:100 as follows: ~~bring~~ transfer with a syringe 0,1 ml of each solution into a 10 ml volumetric flask (6.12) filled with about 5 ml of ethyl acetate (5.~~4~~4). Bring to volume with ethyl acetate. The final concentration of di-n-octylphthalate (D4) di-n-butylphthalate (D4) and di-(2-ethylhexylphthalate) will be 100 mg/l in ethyl acetate.

7.14.~~5~~ 3 Solution II internal standard mix
Take from this 1:100 dilution (5.14.~~4~~2) for example 250 µl, transfer in a volumetric flask, 250 ml (6.12), filled with 250 ml of ethyl acetate (5.~~4.~~4).
The final concentration of di-n-octylphthalate (D4), di-n-butylphthalate (D4) and di-(2-ethylhexylphthalate) will be 0,1 mg/l in ethyl acetate.

7.14.~~6~~4 Solution III internal standard mix

Dilute the solution I internal standard (5.14.~~4~~2.) 1:10: pipette 1ml of the solution (5.14.~~4~~2.) in a 10 ml volumetric flask (6.12) filled with about 5 ml of ethyl acetate (5.~~4~~4). Bring to volume with ethyl acetate. The final concentration of di-n-octylphthalate (D4), di-n-butylphthalate (D4) and di-(2-ethylhexylphthalate) will be 10 mg/l in ethyl acetate.

7.15 Sodium ~~sulph~~sulfate, Na_2SO_4, heated to 400°C for at least 4h

8 Apparatus

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

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

8.2 Drying oven, capable of being maintained at a temperature of 105°C (±5°C)

8.3 Muffle furnace, adjustable, up to temperatures of 400 °C (± 10°C), with capacity for example at least 60 l.

8.4 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

8.5 Vacuum device for clean up (vacubox, extraction box)

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

8.7 Glass cartridges, with Luer- cone

8.8 PTFE (Poly-tetra-fluoro-ethylene, Teflon)- frits for cartridges, 6 ml.

8.9 Aluminium foil, heated to ~~400~~ 400 °C.
8.10 Stainless steel reservoir, for storage of smaller glass apparatus.
8.11 Measuring cylinders, 50 ml, ~~100~~ 100 ml.
8.12 Volumetric flasks, 10 ml, 25 ml and 250 ml.
8.13 Pasteur-pipettes, **e.g. 2 ml.**
8.14 Syringes, ~~2~~ 2 µl, ~~5~~ 5 µl, ~~10~~ 10 µl ~~50~~ 50 µl, 100 µl and 500 µl maximum permitted ~~error~~ error ± ~~2~~ 2 %.

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

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

8.17 Fused silica columns, with non polar stationary phase (see examples annex B); check the quality of the column e.g. by injecting the reference solution (5.13) and ensure that the separation is satisfactory.

8.18 Glass tubes, graduated 5 or 10 ml.

8.19 Nitrogen device for drying the glass cartridges (6.7)

8.20 Beaker, 50 ml, 100 ml

8.21 Erlenmeyer flask, 250 ml

8.22 Shaking machine, horizontal shaking movement

8.23 Freeze drying apparatus

8.24 Metal spoon

8.25 Agate mortar

8.26 Metallic clamp, for stopper

8.27 Balance, for example: range 0,001g→100g

8.28 Pipette; 20 ml, 25 ml, 50 ml

9 Sampling and sample pre-treatment

Collect, preserve and handle samples in accordance with ISO 5667-13, ISO 10381-2
Use for sampling pre-treated sampling bottles (6.1 and 8.1) and make sure that the stoppers are pre-treated 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).
Dry the sample as soon as possible after sample collection. If storage is unavoidable, store the samples in the dark at 4°C. Dried samples are found to be stable for a longer period.
Dry matter content has to be determined in accordance with ISO 11465 and prEN 14346, respectively.

10 Procedure

10.1 Pre-treatment of glass apparatus

Clean all glass apparatus, except the syringes, 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) for at least 4hours at 400°C
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 on the walls rinse the walls with isooctane (5.~~6~~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.

10.2 Pre-treatment

Before starting the analysis~~,~~ homogenate the sample. In the case of sludge, homogenate by shaking, in the case of sediments by stirring or shaking and~~-~~ in the case of soil and waste homogenate by stirring with a metal spoon (6.24), rearrange and crush. Soil and sediments should be sieved to 2 mm and sludge to 0,2 mm.

10.2.1 Drying of the sample

Depending on the water content and the sample themselves dry the sample either with Na_2SO_4 or by freeze drying (see Annex D).
Samples (soil, waste) with a dry matter > 80 % can be dried with Na_2SO_4. In particular sludges and sediments with ~~a high~~high water content shall be dried by the freeze drying.

10.2.1.1 Freeze drying

Freeze at –18°C a part of the homogenated sample or a representative part of the sample. Afterwards carry out a freeze drying at about 0,05 bar, until the constant weight is achieved.
Homogenate the freeze dried sample with the aid of a agate mortar.

10.2.1.2 Drying with Sodium ~~sulph~~sulfate (Na_2SO_4)

Depending on the expected phthalate content of the sample weigh between 1-10g of the wet sample into a beaker (6.20) and give as much sodium ~~sulph~~sulfate (5.15) into the wet sample until a trickle mixture is achieved. Mortar the mixture in a agate mortar (6.25). When a free-flowing mixture is obtained the humidity is bound. In parallel determine the dry matter content of the sample (see section 2).

10.2.2 Extraction

Transfer between 1-10g , referred to the dry matter content and the expected phthalate concentration, of the mixture of the sample and Na_2SO_4 (8.2.1.2) or the freeze dried sample (8.2.1.1) into a 250 ml Erlenmeyer flask (6.21) and give for example 20 ml ethyl acetate with internal standard (5.14.43) to the sample. If a high amount of phthalates is expected, the extraction solvent (5.14.43) can be doubled or multiplied (see table 2). Because of the unknown amount of the blank dilutions of the sample extract shall be avoided. Close the Erlenmeyer flask (6.21) with a stopper and fix the stopper with a metallic clamp (6.26)
Extract the sample at least 30 minutes on the shaking machine (6.22). Make sure that a good through mixing of the sample and the solvent is obtained. After the extraction take approximately 1 ml with a pipette (6.13) and transfer the extract into a GC vial (6.4), or approximately 3 ml is required if a clean up (8.3.) is necessary. Place the heated aluminium foil (6.9) between vial and caps in order to avoid a contamination by phthalates from the septa. The extract can be analysed by GC-MS directly.
If a clean up is necessary e.g. due to interferences of the target analyte in the GC-MS chromatography, see 8.3

Table 2: Examples of sample intake and ratio dry matter/ solvent volume

Matrices	Sample intake [g]	Ratio dry matter/ solvent	Remark
Sludge (sewage)	1- 10	< 1: 80	A high amount of DEHP is expected
Sediment / suspended solid	2 -10	< 2:20	DEHP is expected
Compost	2 – 10	< 2:20	Low- high concentration of DEHP
Soil	2 – 10	< 2:20	Low- high concentration of DEHP

~~Note: Because of the unknown amount of the blanc. dilutions of the sample extract shall be avoided~~
Note 1: If a sample intake lower than 1g is unavoidable, an appropriate balance shall be used.
Note 2: Take care that the amount of solvent is sufficient for collecting the extract (at least 3 ml)

Note 3: The described method of extraction (shaking) is recommended due to the small contamination potential. Using soxhlet extraction or ASE (accelerated solvent extraction) comparable amount of phthalates can be achieved, but the contamination risk is higher. Moreover the extraction relation (solvent and sample intake) should be adjusted to the respective extraction method.

10.3 Clean- up

A clean - up is only necessary, if interferences in the GC-MS-chromatogram, coming from matrices, are expected, otherwise it should be avoided due to the additional risk of contamination.

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

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 cartridge volume of ethyl acetate (5.44).
Dry with nitrogen (5.2) for 1 min.
Fix the cleaned cartridge with stainless steel cock (6.6) and place it on the vacuum device (6.5).
Let the extract run through the cartridge and collect it in a glass tube (6.18.
Transfer the extract to GC vials (6.4). Attach heated aluminium foil (6.9) between vial and cap in order to avoid a contamination by phthalates from the septa.

10.4 Gas chromatography

Optimise the GC-apparatus (6.15) according to the instrument manufacturer's manual.
Use capillary columns (6.17, annex B) for separation.
In order to clean the inlet system free from phthalates, inject ethyl acetate (5.44) at least 5 times from various GC-vials (see clause 6) before measuring the sample extracts or calibration solutions.

10.5 Blank monitoring

Check the proper conditions of instruments and reagents by blank monitoring at regular intervals.
For the blank measurements, treat sodium ~~sulph~~sulfate (5.15) in the same weight as the sample (8.2, 8.3~~.~~).
Weigh nearly as much sodium ~~sulph~~sulfate as it is needed to dry the samples. DEP, DiBP, DBP and DEHP are the most ubiquitous phthalates. The blank limit of each of the phthalates should not be greater than 5pg/µl.
With each sample series determine two blanks. The difference of the two ~~blanc~~blanks shall not be greater than 30%, otherwise the determination shall be repeated. The results of the blank monitoring will be used for blank correction as described in section 10.

10.6 Identification of individual compounds

10.6.1 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.
Conditions see also ISO 22892.

~~Table~~ Table 3 — Example of typical 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 (100)	194 (7,8)	135 (4,5)
2	Diethylphthalate	DEP	149 (100)	177 (23)	222 (1,6)
3	Dipropylphthalate	DPP	149 (100)	209 (5,9)	191 (6,9)
4	Di (2-methyl-propyl)phthalate	DiBP	149 (100)	223 (7,4)	205 (1,9)
5	Dibutylphthalate	DBP	149 (100)	223 (5,6)	278 (1,0)
6	Butylbenzylphthalate	BBzP	149 (100)	206 (22)	312 (1,0)
7	Dicyclohexylphthalate	DCHP	149 (100)	167 (32)	249 (5,5)
8	Di (2-ethylhexyl)phthalate	DEHP	149 (100)	167 (34)	279 (8,8)
9	Dioctylphthalate	DOP	149 (100)	279 (6,6)	207 (4,4)
10	Didecylphthalate	DDcP	149 (100)	307 (6,4)	---
11	Diundecylphthalate	DUP	149 (100)	321 (5,4)	---
12	D4-ring- Dibutylphthalate	D4-DBP	153 (100)	227 (5,7)	
13	D4-ring-Di(2-ethylhexyl)phthalat	D4-DEHP	153 (100)	171 (31)	283 (14)
14	D4-ring-Dioctylphtalate	D4-DOP	153 (100)	283 (17)	
~~14~~	~~Diallylphthalate~~	~~DalP~~	~~149 (100)~~	~~189~~	

NOTE 1: The relations of the masses can vary, depending on the used tune.
NOTE 2: Depending on the concentration of the phthalates, the qualifier can not be seen always.(Small amount)
NOTE 3 M_1 is used for quantification, M_2 and M_3 may be used for identification

11 Calibration

11.1 General

Establish for each compound a calibration function and graph using single or, for practical reasons, multicomponent reference solutions
and make sure to obtain a linear relation of measuring signal to concentration.
The linear working range should be determined 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 also on the operating conditions of the gas chromatograph. It needs regular checking. For routine purposes, a two-point calibration is sufficient (see 9.3).
A procedure is given for the setup of a calibration function and the working range has to be adjusted to the working conditions (preparation of the reference solution according to 5.13).

11.2 Calibration with internal standard

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 of the internal standard $\boldsymbol{\rho_{i,is}}$ shall be the same for calibration and sample measurement.

Plot the rational value $y_{i,std}/y_{i,is}$ (peak areas, peaks heights or integration units) for each substance i on the ordinate and the associated rational mass concentration $\rho_{i,std}/\rho_{i,is}$ on the abscissa.
Establish the linear regression function using the pairs of value $y_{i,std}/y_{i,is}$ and $\rho_{i,std}/\rho_{i,is}$ of the measured series in the following equation:

$$\frac{y_{\mathrm{i,std}}}{y_{\mathrm{i,is}}} = a_i \frac{\rho_{\mathrm{i,std}}}{\rho_{\mathrm{i,is}}} + b_{\mathrm{i}} \qquad (1)$$

$y_{\mathrm{i,std}}$ Is the measured value, for example expressed as area values, for the substance I (subscript i) in the calibration (subscript e) depending on $\rho_{\mathrm{i,std}}$, the unit of which depends on the type of evaluation performed

$Y_{\mathrm{i,is}}$ is the measured value of the internal standard (subscript is) i in the calibration, depending on $\rho_{\mathrm{i,std}}$, the unit depends on the evaluation, for example, area value, for the total procedure

$\rho_{\mathrm{l,std}}$ is the (independent variable) mass concentration of the substance *i* in the calibration solution for the total procedure, expressed in nanogram per millilitre [ng/ml]

$\rho_{\mathrm{l,is}}$ is the (independent variable) mass concentration of the internal standard, expressed in nanogram per millilitre [ng/ml]

a_{i} is the slope of the calibration curve from $y_{i,std} / y_{i,is}$ as a function of the mass concentration ratio $\rho_{l,stde} / \rho_{l,is}$

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

11.3 Recalibration

Inject at least two calibration standards with concentrations of 20 ± 10% and 80 ± 10% of the established linear range and calculate the straight line from these measurements. If the straight line falls within the 95% confidence limits of the initial calibration line, the initial calibration line is assumed to be valid. If not, a new calibration line has to be established according to 9.2.

12 Calculation

12.1 Calculation of single results after calibration with internal standard

Calculate the mass concentration $\rho_{i,tm}$ of the substance using equation (3):

$$\frac{\left(\frac{y_{i,std}}{y_{i,is}} - \underline{b_i}\right)}{a_i} * \rho_{i,is} - \frac{\left(\frac{y_{i,std,bl}}{y_{i,is}} - \underline{b_i}\right)}{a_i} * \rho_{i,is} = \rho_{i,std,bl} \qquad (2)$$

$$\rho_{i,tm} = \frac{\rho_{i,std,bl} * V * F_1}{E * T_m * F_2} \qquad (3)$$

Building of the mean of the ~~blanc~~blank

$$y_{i,std,bl} = \frac{y_{i,std,bl1} + y_{i,std,bl2}}{n} \qquad (4)$$

The simplification of the formular (2) and (3) is shown in formular (5)

$$\rho_{i,tm} = \frac{\frac{\left(\frac{y_{i,std} - y_{i,std,bl}}{y_{i,is}} - \underline{b_i}\right)}{a_i} * \rho_{i,is} * V * F_1}{E * T_m * F_2} \qquad (5)$$

$Y_{i,std}$ See equation (1)

$Y_{i,std,bl1}$ / $y_{I,std,bl2}$ Is the measured value of the first / second ~~blanc~~blank, for example expressed as area values, for the substance I (subscript i) in the calibration (subscript e) depending on $\rho_{i,std}$, the unit of which depends on the type of evaluation performed, see 8.5

$Y_{i,is}$ See equation (1)

n Amount of measurements for the ~~blanc~~blank determination, see 8.5

$\rho_{I,is}$ See equation (1)

a_i See equation (1)

b_i See equation (1)

$y_{i,std,Bl}$ Is the measured value, for example expressed as area values, for the substance i (subscript i) in the blank sample (subscript bl) in accordance to ~~chapter~~ clause 8.5 depending on $\rho_{i,std}$,

$\rho_{I,std,bl}$ is the (independent variable) mass concentration of the substance *i* ,corrected with the blank amount for the total procedure, expressed in nanogram per millilitre [ng/ml]

$\rho_{I,tm}$ Is the mass concentration of the substance i in the sample based on dry matter in microgram per kilogram [µg/kg]

V Is the volume of the extraction solvent (mainly 20 ml) in millilitre [ml]

T_m Is the dry matter content of the sample in percent [%]

E Is the weight mass of the sample in gram [g]
F_1 Is the conversion factor for percent (mainly 100) [%]
F_2 Is the conversion factor for the units; F_2=1: µg/Kg; F_2=1000: mg/Kg

For the phthalates DMP to BBzP ~~-~~use as internal standard D4- ring-DBP, for the phthalates DEHP, DCHP use as internal standard D4- ring-DEHP and for the phthalates DOP to DUP as internal standard D4-ring-DOP

13 Expression of results

In the case of sludge, sediment, ~~and~~ soil, and treated bio-waste report the results in milligram per kilogram mg/kg, with two significant digits.
EXAMPLES

Dioctyl-phthalate	0,65	mg/kg D_m
Didecyl-phthalate	1,5	mg/kg D_m
Dimethyl-phthalate	12	mg/kg Dm

14 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 pre-treatment, 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
(informative)

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~~1	**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~~1	**Internal standard solution of D4-DEHP** Weight 0,1g of D4-DBP in 10 ml ethyl acetate	c=10g/L EA
4	5.14.~~4~~2	**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 c D4-DEHP=100 mg/L EA
5	5.14.~~5~~3	**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 c D4-DEHP= 100 µg/L EA
6	5.14.~~6~~4.	**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 c D4-DEHP= 10 mg/L EA

2. Solution of the single phthalates (5.11)

In a 10 ml volumetric flask transfer for example 10 mg of each reference substance separately 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 transfer between 100 and 500µl of the single solutions of the phthalates and fill up with ethyl acetate (5.44)

Phthalate- solutions of the single phthalates	**µl** of the single phthalates solutions	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 (5.14.4) in a 10 ml volumetric flask and fill up with ethyl acetate.

A 5-point calibration will be sufficient (9.1.). The appropriate concentration levels for calibration depend on the expected phthalate concentration in the sample. The levels mentioned in the table may serve as examples.

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-DEHP	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

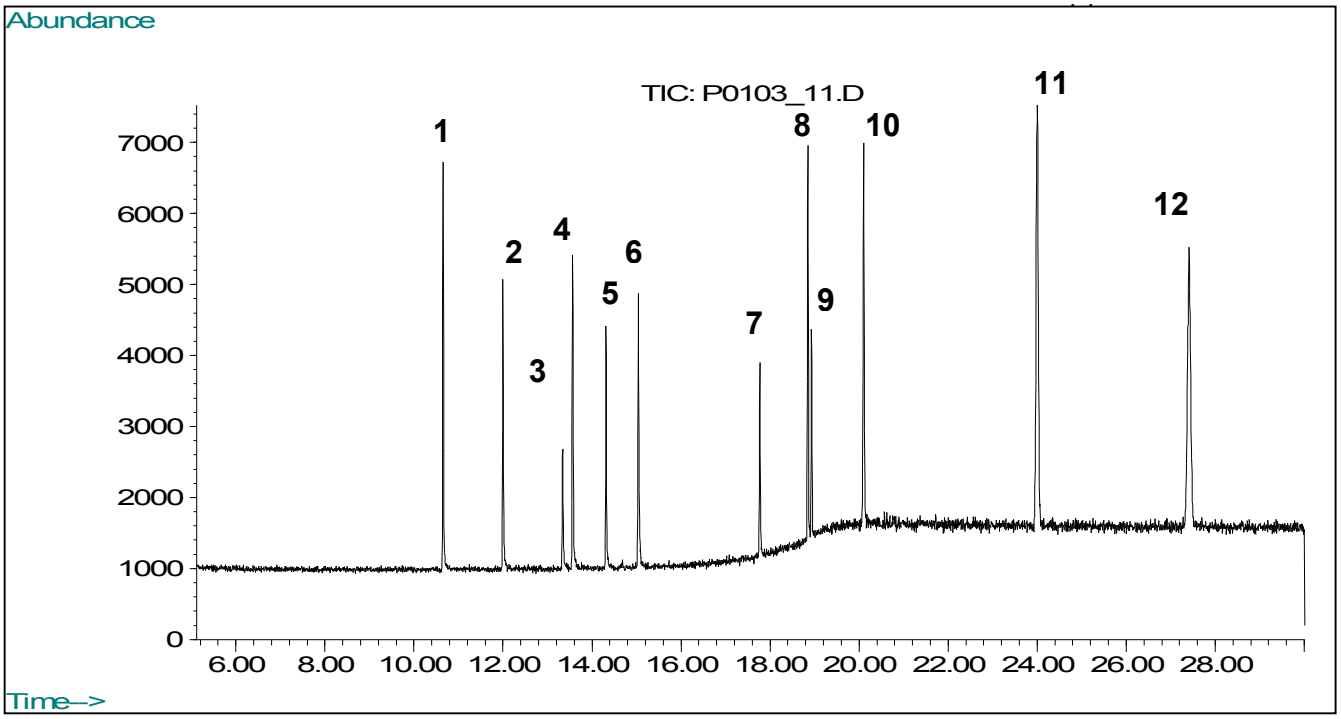
Phase: 34 % / 64 % / 2% phenyl- / methyl- / vinyl- silicone
Length: 30 m, inner diameter : 0,32 mm, film thickness : 0,25 µm

(informative)

Example of gas chromatographic conditions

GC:	~~HP 6890 Series~~Gaschromatograph with autosampler ~~HP 6890 series; detector :~~ and mass spectrometric detector~~, quadrupol~~
column	~~HP - 5MS ; 30 m; inner diameter 0,25 mm ; film thickness 0,25 µm~~see above
Carrier gas:	Helium 5,0 ; pressure : 4,5 bar
Injector:	Pulsed splitless ; split: 20 ml / min ; splitless period: 1,5 min
	Septum: ~~Merlin~~ leak free quality~~Septum~~
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

Example of chromatogram obtained with GC/MS detection



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

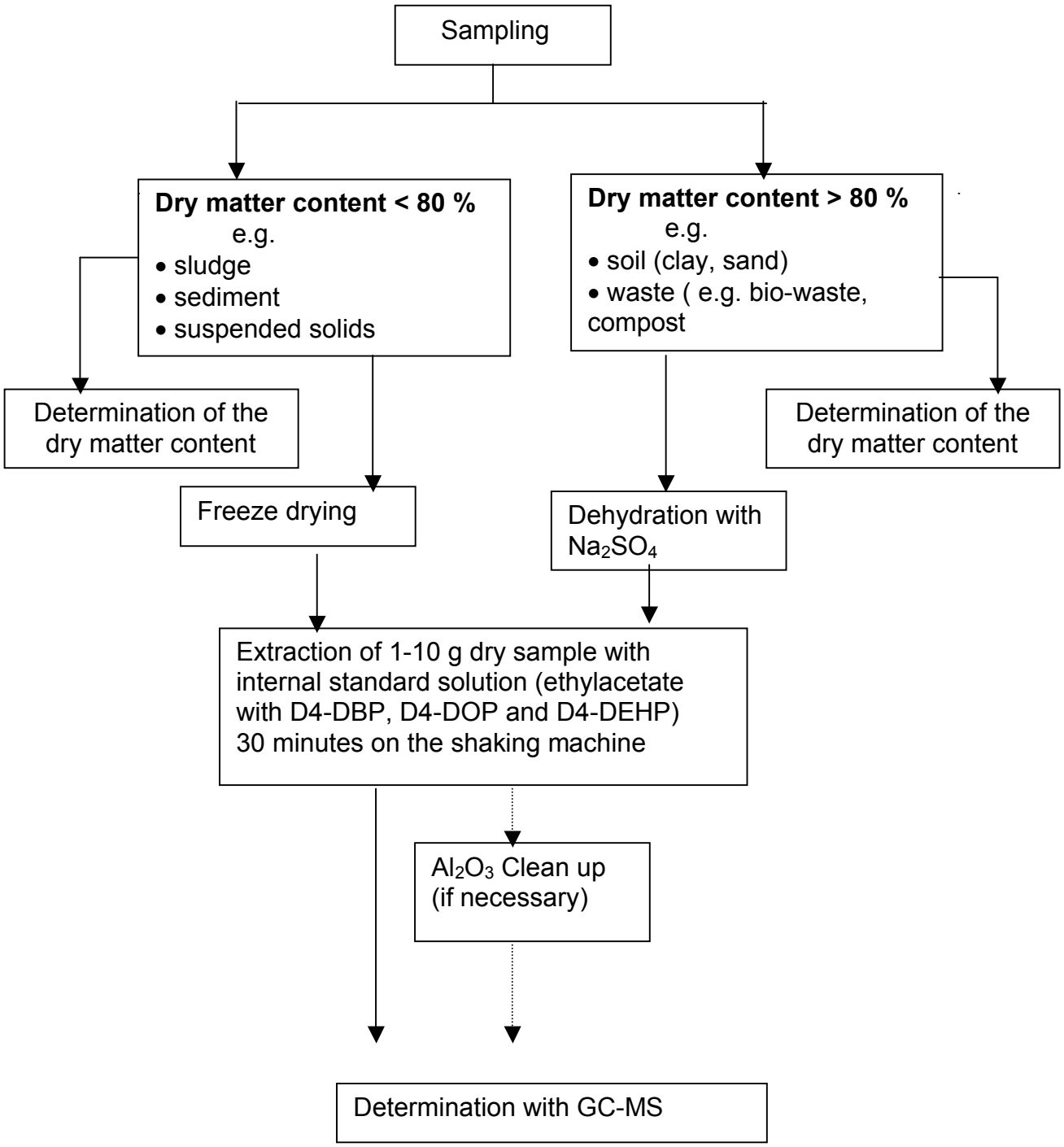
An interlaboratory trial, carried out in xxx, delivered the data given in table 4.

Table C1 — Precision data

Compound	L	N	NAP %	ρ ng/l	ρ_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;
ρ	is the total mean;
ρ_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
(informative)
Flow scheme



Bibliography

[1] FURTMANN K., Phthalates in surface water - a method for routine trace level analysis; Fresenius´ Journal of Analytical Chemistry, 1994, 348: 291-296

[2] ISO 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.

[3] ISO 383. 1976, Laboratory glassware — Interchangeable conical ground joints