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Soils, sludges and treated bio-wastes — Determination of selected phthalates — Method using capillary gas chromatography with mass spectrometric detection

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Contents

Page

Foreword

This document (BT/TF151 WI CSS99042) has been prepared by Technical Committee CEN/TC BT "Horizontal", the secretariat of which is held by DS.

This document is a working document.

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	X	reference
Sludge	X	reference
Treated bio-waste	X	reference
Soil improvers	□	reference
Waste	□	reference

Introduction

This document is developed in the project 'Horizontal'. It is the result of a desk study "Horizontal European standard for determination of phtalates in sludge, soil and biowaste" and aims at evaluation of the latest developments in assessing phthalates in sludge, soil, treated biowaste and neighbouring fields. After discussion with all parties concerned in CEN at the three phases of a standard development (desk study, ruggedness evaluation and validation), the standard will be developed further as an modular horizontal test method.

Until now test methods determining properties of materials were often prepared in Technical Committees (TCs) working on specific products or specific sectors. In those test methods often steps as sampling, extraction, release or other processing, analyses, etc were included. In this approach it was necessary to develop, edit and validate similar procedural steps over and over again for every material or product. Consequently this has resulted in a lot of duplication of work. To avoid such duplication of work for parts of a testing procedure often was referred to parts of test methods from other TCs. However the following problems are often encountered while using references in this way: 1) The referenced parts are often not edited in a way that they could easily be referred to, 2) the referenced parts are often not validated for the other type of material and 3) the updates of such test standards on products might lead to inadequate references.

In the growing amount of product and sector oriented test methods it was recognised that many steps in test procedures are or could be used in test procedures for many products, materials and sectors. It was supposed that, by careful determination of these steps and selection of specific questions within these steps, elements of the test procedure could be described in a way that can be used for all materials and products or for all materials and products with certain specifications.

Based on this hypothesis a horizontal modular approach is being investigated and developed in the project 'Horizontal'. 'Horizontal' means that the methods can be used for a wide range of materials and products with certain properties. 'Modular' means that a test standard developed in this approach concerns a specific step in a assessing a property and not the whole test procedure (from sampling to analyses).

The use of modular horizontal standards implies the drawing of test schemes as well. Before executing a test on a certain material or product to determine certain characteristics it is necessary to draw up a protocol in which the adequate modules are selected and together form the basis for the test procedure.

The other horizontal modules that will be available in due time are to be found in the informative annex [*xxx*] which contains a brief overview of the modules that are or will be worked out in the project 'Horizontal.'

1 Scope

This European Standard specifies a method for the determination of selected phthalates in soil, sludges and treated 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 0.5 mg/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 e.g. DiNP (Di-isononylphthalate), but shall be verified in each case.

WARNING – Persons using this European Standard shall be familiar with normal laboratory practice. This European 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 [a] - No
1	Dimethylphthalate	$C_{10}H_{10}O_4$	DMP	194.2	00131-11-3
2	Diethylphthalate	$C_{12}H_{14}O_4$	DEP	222.2	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

[a] CAS: Chemical Abstracts System

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

CSS99031 Sludge, treated biowaste, and soils in the landscape – Sampling – Framework for the preparation and application of a sampling plan

CSS99058 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 1: Guidance on selection and application of criteria for sampling under various conditions

CSS99057 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 2: Guidance on sampling techniques

CSS99032 Sludge, treated biowaste, and soils in the landscape – Sampling - Part 3: Guidance on sub-sampling in the field

CSS99059 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 4: Guidance on procedures for sample packaging, storage, preservation, transport and delivery

CSS99060 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 5: Guidance on the process of defining the sampling plan

CSS99035 Soil, sludge and treated biowaste – Pre-treatment for organic characterisation

CSS99022 Soil, sludge and treated biowaste – Determination of dry matter – Gravimetric method

ISO/FDIS 22982:2004[1)] *Soil Quality – Guidelines for identification of target compounds by gas chromatography and mass spectrometry*

3 Terms and definitions

3.1
Analytes
In the context of this international standard, the analyte is one or more of the o-phthalic acid esters mentioned in table 1.

3.2
Calibration standard
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
D4-Dibutylphthalate, D4-di(2-ethylhxyl)phthalate, and D4-di-n-octylphthalate were added to the extraction solvent before the extraction. The internal standard is used to correct for losses during the analysis and is used for calculating the concentration of the analytes.

4 Principle

The dried sample, dried by freeze-drying or with sodium 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.

5 Contaminations

5.1 General

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 shall be checked and reduced by every laboratory itself. Therefore, special attention shall be paid to avoid contaminations.

1) Under preparation

5.2 Interferences during sampling

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

5.3 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.

5.4 Using plastic gloves during pre-treatment may increase the contamination. 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 clause7.5) may as well contain phthalates.

6 Reagents

6.1 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.

6.2 Nitrogen, N2 of high purity, at least a volume fraction of 99.9 % for drying and eventually for concentration by evaporation.

6.3 Helium, He, of high purity, at least a volume fraction of 99.999 %

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

6.5 Methanol, CH_4O.

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

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

6.8 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[2)] or silica may be used, provided their properties and capacity to separate are similar to aluminium oxide and their properties are checked according to 5.3.

6.9 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-

2) Florisil is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

DOP", D4-$C_{24}H_{38}O_4$,
^{13}C - labelled standards can also be used, as far as available.

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

6.11 Solutions of the single substances

In a 10 ml volumetric flask (7.13), transfer for example 10 mg of each of the reference substances in ethyl acetate (6.4) 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 three months.

6.12 Stock solution

In one 10 ml volumetric flask (7.13), dissolve between 100 µl and 500 µl of the single substance solutions (6.11) and bring to volume with ethyl acetate (6.4) (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 three months.

6.13 Reference solutions for multipoint calibration (Annex A)

Prepare solutions by adequate dilution of the stock solution (6.12) and internal standards (6.9) with ethyl acetate (6.4).

Store the solutions in a glass bottle at –18 °C, protected from light and check the concentration at least every three weeks (see Annex A)

6.14 Solution of the internal standards (Annex A)

6.14.1 Internal standard solution of D4-phthalates

Weigh for example 0.1 g of an internal standard phthalate (D4) (6.9) in a 10 ml volumetric flask (7.13) filled with about 5 ml of ethyl acetate (6.4) and bring to volume with ethyl acetate (6.4). Store the solutions in glass bottles at – 18°C.

6.14.2 Solution I internal standard mix

Combine the solutions of the single internal standard phthalates (6.9) for example by dilution 1:100 as follows: transfer with a syringe 0.1 ml (7.15) of each solution into a 10 ml volumetric flask (7.13) filled with about 5 ml of ethyl acetate (6.4). 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.

6.14.3 Solution II internal standard mix

Take from this 1:100 dilution (6.14.2) for example 250 µl, transfer into a volumetric flask, 250 ml (7.13), filled with 250 ml of ethyl acetate (6.4).

The final concentration of di-n-octylphthalate (D4), di-n-butylphthalate (D4) and di-(2-ethylhexylphthalate) is 0.1 mg/l in ethyl acetate.

6.14.4 Solution III internal standard mix

Dilute the solution I internal standard (6.14.2.) 1:10: pipette 1ml of the solution (6.14.2.) in a 10 ml volumetric flask (7.13) filled with about 5 ml of ethyl acetate (6.4). Bring to volume with ethyl acetate. The final concentration of di-n-octylphthalate (D4), di-n-butylphthalate (D4) and di-(2-ethylhexylphthalate) is 10 mg/l in ethyl acetate.

6.15 Sodium sulfate, Na_2SO_4, heated to 400°C for at least 4h

7 Apparatus

7.1 General

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 (7.2) and checked by the blank determination.

7.2 Wide-neck flat bottomed flasks with glass stoppers, preferably brown glass, 500 ml and 1 000 ml

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

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

7.5 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

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

7.7 Stainless steel cock, with stainless steel cone or PTFE cock with Luer[3)] connection for separate vacuum connection

3) Luer is an example of a suitable product available commercially. This information is given for the convenience of this European Standard and does not constitute an endorsement by CEN of this product.

7.8 Glass cartridges, with Luer- cone

7.9 PTFE (Polytetrafluoro-ethylene, Teflon®[4] - frits for cartridges, 6 ml.

7.10 Aluminium foil, heated to 400 °C.

7.11 Stainless steel reservoir, for storage of smaller glass apparatus**.**

7.12 Measuring cylinders, 50 ml, 100 ml.

7.13 Volumetric flasks, 10 ml, 25 ml and 250 ml.

7.14 Pasteur-pipettes, e.g. 2 ml.

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

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

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

7.18 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 (6.13) and ensure that the separation is satisfactory.

7.19 Glass tubes, graduated 5 ml or 10 ml.

7.20 Nitrogen device for drying the glass cartridges (7.8)

7.21 Beaker, 50 ml, 100 ml

7.22 Erlenmeyer flask, 250 ml

7.23 Shaking machine, horizontal shaking movement

7.24 Freeze drying apparatus

7.25 Metal spoon

7.26 Agate mortar

7.27 Metallic clamp, for stopper

7.28 Balance, for example: range 0.001g **à** 100g

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4) Teflon is an example of a suitable product available commercially. This information is given for the convenience of this European Standard and does not constitute an endorsement by CEN of this product.

7.29 Pipette; 20 ml, 25 ml, 50 ml

8 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 (7.2) 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 shall be determined in accordance with ISO 11465 and prEN 14346, respectively.

9 Procedure

9.1 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 (7.3) at 105 °C.

Heat the pre-rinsed glass apparatus in the muffle furnace (7.4) for at least 4 h 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 (7.10.). Store smaller glass apparatus in decontaminated (heated) and appropriately closed stainless steel containers (7.11).

In order to avoid losses by adsorption on the walls rinse the walls with isooctane (6.6) by using Pasteur pipettes (7.14). 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.

9.2 Pre-treatment

Homogenate the sample before starting the analysis . 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 (7.25), rearrange and crush. Soil and sediments should be sieved to 2 mm and sludge to 0.2 mm.

9.2.1 Drying of the sample

Depending on the water content and the kind of sample 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 high water content shall be dried by the freeze drying.

9.2.1.1 Freeze drying

Freeze at –18°C a part of the homogenated sample or a representative part of the sample. Afterwards lyophilise at about 0.05 bar, until the constant weight is achieved.

Homogenate the freeze dried sample with the aid of an agate mortar (7.26).

9.2.1.2 Drying with Sodium sulfate (Na_2SO_4)

Depending on the expected phthalate content of the sample weigh between 1 g – 10 g of the wet sample into a beaker (7.21) and give as much sodium sulfate (6.15) into the wet sample until a trickle mixture is achieved. Mortar the mixture in an agate mortar (7.26). When a free-flowing mixture is obtained the humidity is bound. In parallel determine the dry matter content of the sample (see clause 2).

9.2.1.3 Extraction

Transfer between 1 g – 10 g , referred to the dry matter content and the expected phthalate concentration, of the mixture of the sample and Na_2SO_4 (9.1.1.2) or the freeze dried sample (9.1.1.11) into a 250 ml Erlenmeyer flask (7.22) and give for example 20 ml ethyl acetate with internal standard (6.9) to the sample. If a high amount of phthalates is expected, the extraction solvent (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 (7.22) with a stopper and fix the stopper with a metallic clamp (7.27).

Extract the sample at least 30 min on the shaking machine (7.23). Make sure that a good thorough mixing of the sample and the solvent is obtained. After the extraction take approximately 1 ml with a pipette (7.14) and transfer the extract into a GC vial (7.5), or approximately 3 ml is required if a clean up (9.3) is necessary. Place the heated aluminium foil (7.10) 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 10.3.

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

If a weighted sample of less than 1 g is unavoidable, an appropriate balance shall be used.

NOTE 1 Take care that the amount of solvent is sufficient for collecting the extract (at least 3 ml)

NOTE 2 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.

9.3 Clean-up

A clean-up is only necessary, if interferences in the GC-MS-chromatogram, originating 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 (7.14) and clean the extract with the aid of an Al_2O_3 clean-up (6.8).

Clean the extracts as follows:

Place 1 g of activated aluminium oxide, Al_2O_3 (6.8) in the cartridges (7.8) between two PTFE frits (7.9).

Clean the Al_2O_3 (6.8) with one cartridge volume of ethyl acetate (6.4).

Dry with nitrogen (6.2) for 1 min.

Fix the cleaned cartridge with stainless steel cock (7.7) and place it on the vacuum device (7.6).

Let the extract run through the cartridge and collect it in a glass tube (7.19).

Transfer the extract to GC vials (7.5). Attach heated aluminium foil (6.8) between vial and cap in order to avoid a contamination by phthalates from the septa.

9.4 Gas chromatography

Optimise the GC-apparatus (7.16) according to the instrument manufacturer's manual.

Use capillary columns (7.18, annex B) for separation.

In order to clean the inlet system free from phthalates, inject ethyl acetate (6.4) at least five times from various GC-vials (see clause 5) before measuring the sample extracts or calibration solutions.

9.5 Blank monitoring

Check the proper conditions of instruments and reagents by blank monitoring at regular intervals.

For the blank measurements, treat sodium sulfate (6.15) in the same way as the sample (10.2, 10.3). Weigh nearly as much sodium sulfate as 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 5 pg/µl.

With each sample series determine two blanks. The difference of the two blanks shall not be greater than 30 %, otherwise the determination shall be repeated. The result of the blank monitoring is used for blank correction as described in clause 11.1.

9.6 Identification of individual compounds

9.6.1 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 22982.

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)phthalate	D4-DEHP	153 (100)	171 (31)	283 (14)
14	D4-ring-Dioctylphtalate	D4-DOP	153 (100)	283 (17)	

NOTE 1 The ratio 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

10 Calibration

10.1 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 10.3).

A procedure is given for the setup of a calibration function and the working range shall be adjusted to the working conditions (preparation of the reference solution according to 6.13).

10.2 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:

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

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

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

$r_{I,std}$ 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]

$r_{I,is}$ 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 $r_{I,stde} / r_{I,is}$

b_i is the axis intercept of the calibration curve on the ordinate.

10.3 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 shall be established according to10.2.

11 Calculation

11.1 Calculation of single results after calibration with internal standard

Calculate the mass concentration $r_{i,tm}$ of the substance using equation (2):

$$r_{i,tm} = \frac{r_{i,std,bl} * V * F_1}{E * T_m * F_2} \qquad (2)$$

Calculation of $r_{i,std,bl}$ by using (3):

$$\frac{\left(\frac{y_{i,std}}{y_{i,is}} - \underline{b_i}\right)}{a_i} * r_{i,is} - \frac{\left(\frac{y_{i,std,bl}}{y_{i,is}} - \underline{b_i}\right)}{a_i} * r_{i,is} = r_{i,std,bl} \qquad (3)$$

Building of the mean of the blank

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

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

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

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

$Y_{i,std,bl1}$ / $y_{I,std,bl2}$ Is the measured value of the first / second 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 10.5

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

n Amount of measurements for the blank determination, see 10.5

$r_{I,is}$ 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 10.5 depending on $\rho_{i,std}$,

$r_{I,std,bl}$ 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)]

$r_{I,tm}$ 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 (m)

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

12 Expression of results

In the case of sludge, sediment, soil, and treated bio-waste report the results in milligrams 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 D_m

13 Precision

The performance characteristics of the method (Annex E) data have been evaluated. Table 4 gives the resulting typical values for repeatability and reproducibility limits as their observed ranges. The typical value is derived from the data in Table E.2 in Annex E by taking the median value and rounding the numbers.

Table 4 — Typical values and observed ranges of the repeatability and reproducibility limits

The reproducibility limit provides a determination of the differences (positive and negative) that can be found (with a 95 % statistical confidence) between a single test result obtained by a laboratory using its own facilities and another test result obtained by another laboratory using its own facilities, both test results being obtained under the following conditions : The tests are performed in accordance with all the requirements of the present standard and the two laboratory samples are obtained from the same primary field sample and prepared under identical procedures. Conversely, the repeatability limit refers to measurements obtained from the same laboratory, all other conditions being identical. The reproducibility limit and the repeatability limit do not cover sampling but cover all activities carried out on the laboratory sample including its preparation from the primary field sample.		
Results of the validation of selected phthalates by capillary gas chromatography with mass spectrometric detection (GC-MS) in soil, sludge and treated biowaste	Typical value %	Observed range %
Repeatability limit, r DBP	30	*25 - 60*
Reproducibility limit, R DBP	200	*120 - 260*
Repeatability limit, r DEHP	26	*26 - 40*
Reproducibility limit, r DEHP	110	*100 - 120*

NOTE 1. The above results refer to the difference that may be found between two test results performed on two laboratory samples obtained under the same conditions. In the case when reference is made to the dispersion of the values that could reasonably be attributed to the parameter being measured, the above typical reproducibility values and observed reproducibility ranges should be divided by √2 to obtain the corresponding typical dispersion limit and its observed range (cf. the detailed note of Section …). In the example of DEHP in Compost 1 the result and its dispersion limit is 0.56 ± 0.47 (2 * s_R = 83.55% of 0.56). This means that with a 95 % statistical confidence, the values reasonably attributable to the measured parameter are larger than 0.09 mg/kg and lower than 1.03 mg/kg.

NOTE 2 The repeatability limit (r) and the reproducibility limit (R) as given in Table 2 (Annex XX) and in this table are indicative values of the attainable precision if the determination of selected phthalates by capillary gas chromatography with mass spectrometric detection (GC-MS) is performed in accordance with this standard [CSS99042].

NOTE 3 A limited number of materials and parameters were tested. Consequently, for other materials and parameters, performance characteristics may fall outside the limits as derived from the validation of the determination of selected phthalates by capillary gas chromatography with mass spectrometric detection (GC-MS) in soil, sludge and treated biowaste.

NOTE 4 In particular for relatively heterogeneous materials, the repeatability and the reproducibility limits may be larger than the values given in Table E.2 (Annex E) and this table.

14 Test report

The report shall refer to this European 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 12

g) Method and degree of confirmation of the result

Annex A
(informative)

Examples

A.1 Solutions of the internal standards

Solution	Chapter	Name and Preparing	Concentration
1	6.14.1	**Internal standard solution of D4-DOP** Dissolve 0.1 g of D4-DOP in 10 ml ethyl acetate	c=10 g/L EA
2	6.14.1	**Internal standard solution of D4-DBP** Dissolve 0.1 g of D4-DBP in 10 ml ethyl acetate	c=10 g/L EA
3	6.14.1	**Internal standard solution of D4-DEHP** Dissolve 0.1 g of D4-DBP in 10 ml ethyl acetate	c=10 g/L EA
4	6.14.2	**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= 100 mg/L EA c D4-DBP= 100 mg/L EA c D4-DEHP=100 mg/L EA
5	6.14.3	**Solution II internal standard** (Dilution 1:1000 of solution I internal standard) Take 250 µl of the solution I internal standard, transfer into a 250 ml volumetric flask, filled with ca. 200 ml ethyl acetate, and fill up to volume with ethyl acetate	c D4-DOP= 100 µg/L EA c D4-DBP= 100 µg/L EA c D4-DEHP= 100 µg/L EA
6	6.14.4.	**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

A.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

A.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 (5.4)

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

A.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)

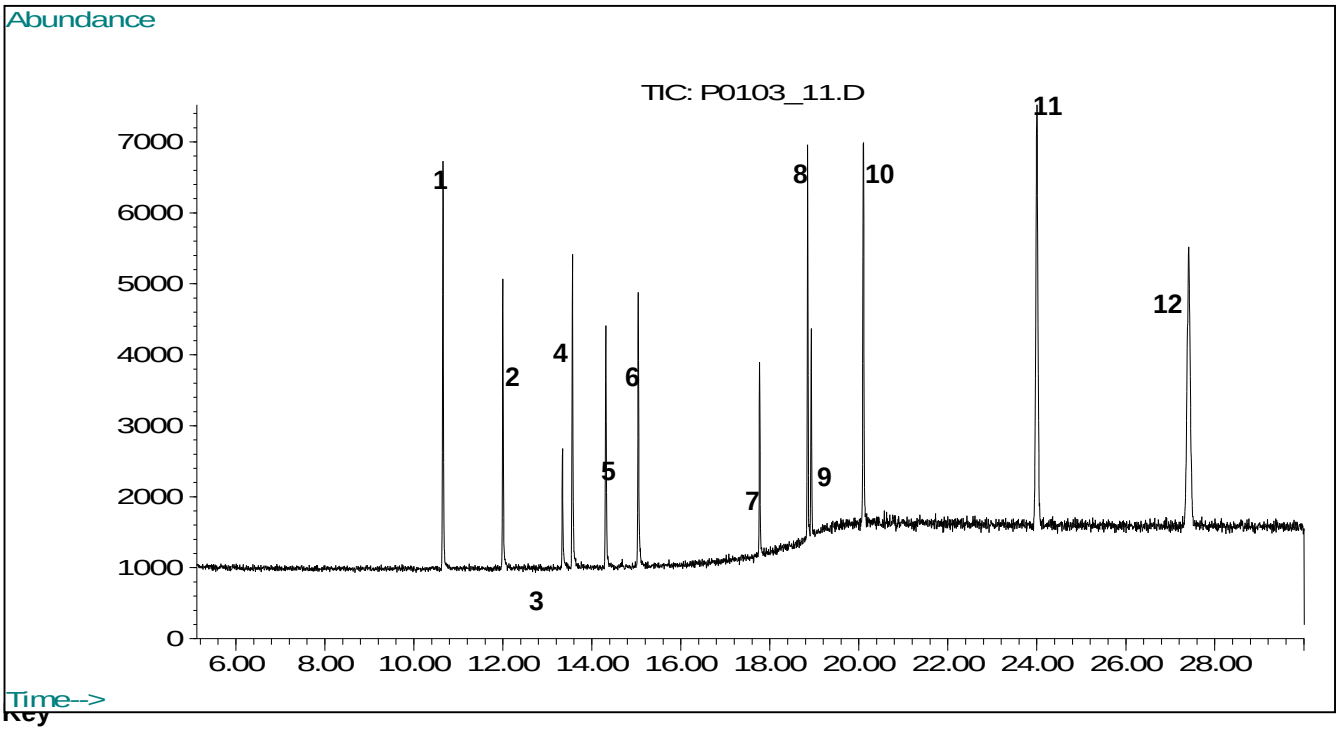
Example of gas chromatographic conditions

GC:	Gaschromatograph with autosampler and mass spectrometric detector
column	see above
Carrier gas:	Helium 5.0 ; pressure : 4.5 bar
Injector:	Pulsed splitless ; split: 20 ml / min ; splitless period: 1.5 min
	Septum: leak free quality
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

Figure B.1 — Chromatogram

Example of chromatogram obtained with GC/MS detection



Key

1 Dimethylphthalate

2 Diethylphthalate

3 Diallylphthalate

4 Dipropylphthalate

5 Di (2-methylpropyl)phthalate

6 Dibutylphthalate

7 Butylbenzylphthalate

8 Dicyclohexylphthalate

9 Di (2-ethylhexylphthalate)

10 Dioctylphthalate

11 Didecylphthalate

12 Diundecylphthalate

Annex C

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

Table C1 — Precision data

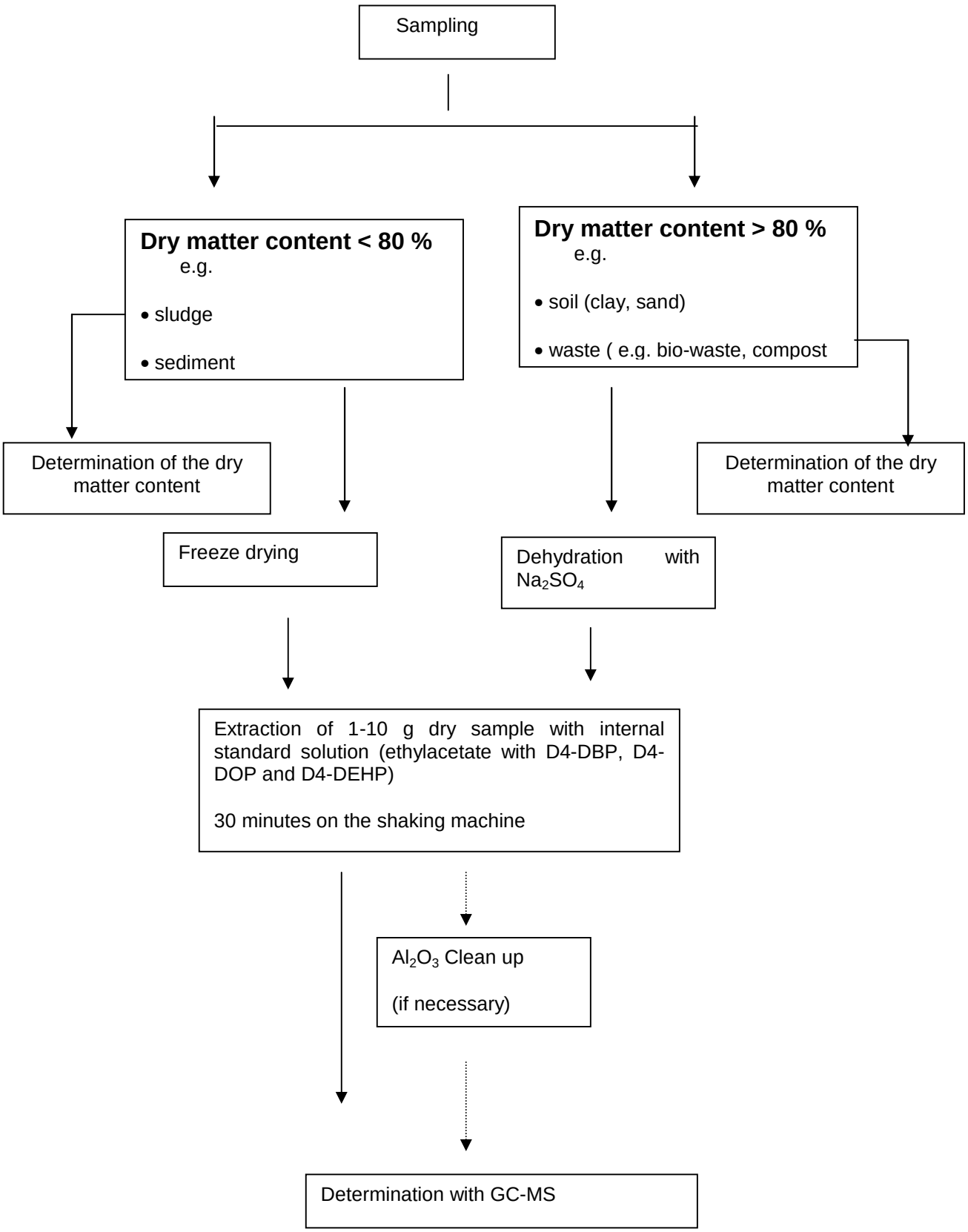
Compound	*L*	*N*	*NAP* %	*r* ng/l	r_t ng/l	*A* %	s_R ng/l	CV_R %	s_r ng/l	CV_r %
Dimethylphthalate										
Diethylphthalate										
Dipropylphthalate										
Di(2-methylpropyl)phthalate										
Dibutylphthalate										
Butylbenzylphthalate										
Dicyclohexylphthalate										
Di(2ethylhexyl)-phthalate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

(informative)

Flow scheme



Annex E (informative)
Repeatability and reproducibility data

E.1 Performance characteristics

E.1.1 Objective of the interlaboratory comparison

In a European wide interlaboratory comparison study according to ISO 5725-2, the performance characteristics of the standard “Determination of selected phthalates by capillary gas chromatography with mass spectrometric detection (GC-MS) in soil, sludge and treated biowaste” were established.

E.1.2 Materials used in the interlaboratory comparison study

The interlaboratory comparison of selected phthalates by capillary gas chromatography with mass spectrometric detection (GC-MS) in soil, sludge and treated biowaste was carried out with 7 - 9 European laboratories on 3 materials. The materials selected for the interlaboratory comparison were chosen to represent soil, sludge and biowaste as broad as possible, because the standard will find general application across different types of soil and soil related materials. (Detailed information can be found in the final report on the interlaboratory comparison study mentioned in the Bibliography).

In the interlaboratory comparison study the following starting points were used:

The laboratory samples were all taken from one large batch of the different materials according to the normal practice. The normal size reduction and the normal repeated mixing were carried out as needed to obtain representative laboratory samples from the large batch sample (ref JRC).

Note: the samples provided for the validation should not be confused with reference samples provided for certification purposes, as the performance results obtained have to be directly applicable to daily practice (less rigorous sample preparation than for a reference material).

The experimental plan was designed by project HORIZONTAL on the basis of each laboratory being given two laboratory samples of each material to be tested. This is in accordance with ISO 5725-2.

The materials examined cover all the grain size classes to which the determination of selected phthalates by capillary gas chromatography with mass spectrometric detection (GC-MS) in soil, sludge and treated

biowaste applies: very fine grained materials (like sludge: 0 µm to about 125 µm) and fine-grained materials (soil and compost: 0 mm to 4 mm).

Table E.1 provides a list of the types of materials chosen for testing and the selected components.

Table E.B.2 — Material types tested and components analysed in the interlaboratory comparison of the method for the determination of selected phthalates by capillary gas chromatography with mass spectrometric detection (GC-MS) in soil, sludge and treated biowaste.

Grain size class	Sample code	Material type tested
Sludge (<0.5 mm) Fine grained (< 2 mm)	Sludge 1	Sewage sludge 1: Mix 1 of municipal WWTP sludges from North Rhine Westphalia, Germany
	Compost 1	Compost 1: Fresh compost from Vienna, Austria
	Soil 3	Soil 3: A sludge amended soil from Barcelona, Spain

E.1.3 Interlaboratory comparison results

The statistical evaluation was conducted according to ISO 5725-2. The average values, the repeatability standard deviation (s_r) and the reproducibility standard deviation (s_R) were obtained (Table e.2).

The repeatability is determined as an interval around a measurement result (i.e. "repeatability limit"). This interval corresponds to the maximum difference that can be expected (with a 95% statistical confidence) between one test result and another, both test results being obtained under the following conditions: The tests are performed in accordance with all the requirements of the present standard by the same laboratory using

its own facilities and testing laboratory samples obtained from the same primary field sample and prepared under identical procedures.

The repeatability limit was calculated using the relationship: $r_{test} = f \cdot \sqrt{2} \cdot s_{r,test}$ with the critical range factor f = 2.

For instance, for the first line of Table E.2, the repeatability limit around a measurement result of 0.50 mg DEHP/kg is ± 0.13mg DEHP/kg (i.e ± 26 % of 0.5)

NOTE The above relationship refers to the difference that may be found between two measurement results performed each on two laboratory samples obtained under the same conditions. The value f = 2 used in the factor $f \cdot \sqrt{2}$ corresponds to the theoretical factor of 1,96 for a pure normal distribution at 95 % statistical confidence. Also, this value f = 2 corresponds to the usual value k = 2 of the coverage factor recommended in the "Guide to the Expression of Uncertainty in Measurement (GUM)". However it may be necessary to use a larger value for f in situation as described in clause 13.

The reproducibility, like repeatability is also determined as an interval around a measurement result (i.e. "reproducibility limit"). This interval corresponds to the maximum difference that can be expected (with a 95% statistical confidence) between one test result and another test result obtained by another laboratory, both test results being obtained under the following conditions : The tests are performed in accordance with all the requirements of the present standard by two different laboratories using their own facilities and testing laboratory samples obtained from the same primary field sample and prepared under identical procedures.

The reproducibility limit was calculated using the relationship: $R = f \cdot \sqrt{2} \cdot s_R$ with the critical range factor f = 2.

For instance the first line of Table E.2, the reproducibility limit around a measurement result 0.50 mg DEHP/kg is ± 0.56 mg DEHP /kg (i.e ± 113 % of 0.5)

NOTE The above relationship refers to the difference that may be found between two measurement results performed each on two laboratory samples obtained under the same conditions. The value f = 2 used in the factor $f \cdot \sqrt{2}$ corresponds to the theoretical factor of 1.96 for a pure normal distribution at 95 % statistical confidence. Also, this value f = 2 corresponds to the usual value k = 2 of the coverage factor recommended in the "Guide to the Expression of Uncertainty in Measurement (GUM"). In the case when reference is made to the dispersion of the values that could reasonably be attributed to the parameter being measured, the dispersion limit is equal to $k \cdot s_R$ with the usual value k = 2, resulting in a

dispersion limit lower than the reproducibility limit (i.e. a ratio of √2). However it may be necessary to use a larger value f · √2 (or k) in situation as described in clause 13.

In case of relatively heterogeneous materials, the repeatability and the reproducibility limits may be larger than the values given in Tables E.2 (this means that the value chosen for the critical range factor f is larger than 2 as well as for the coverage factor k for dispersion). This is because the extreme results may have been obtained in accordance with the present standard and/or be caused by the variability within, or in between, the laboratory samples.

Table E.B.3 — Results of the interlaboratory comparison studies of the method for determination of selected phthalates by capillary gas chromatography with mass spectrometric detection (GC-MS) in soil, sludge and treated biowaste.

Matrix	Parameter	Mean	sr	sR	r	R	p	Outliers	Total number of data
Sludge 1	DBP	0.0849	10.67%	44.3%	0.025	0.105	5	2	16
Compost 1	DBP	0.0457	8.47%	73.4%	0.0108	0.094	5	1	16
Soil 3	DBP	0.0156 mg/kg	20.80%	94.7%	0.0091 mg/kg	0.041 mg/kg	4	1	11
Sludge 1	DEHP	23.1	9.33%	40.3%	6.05	26.1	9	0	36
Compost 1	DEHP	0.559	14.41%	41.8%	0.225	0.65	7	0	22
Soil 3	DEHP	0.528	9.42%	38.9%	0.139	0.57	7	1	28

Abbreviations: sr Repeatability standard deviation; SR Reproducibility standard deviation; r Repeatability limit (comparing two measurements); R Reproducibility limit (comparing two measurements); p Number of labs; N Number of data in statistical evaluation.

Note 1. In judging the results it is important to consider the concentration levels, at which measurements have been carried out. The choice was made to avoid spiking of samples. This implies that particularly in soil and compost low concentrations have been observed for some congeners and results below detection for other congeners. If measurement results are well below a possible critical level (regulation), between lab variabilities of up to 70 % may prove fit for purpose.

Note 2. The experience of labs with some of the new emerging contaminants may be limited adding to the between lab variability.

Note 3. Repeatability and reproducibility results are available from a German intercomparison study using the matrices sludge, compost and sediment. Concentration of DEHP, relative repeatability and relative reproducibility, calculated by the programme “Prolab” according to ISO 5725.

matrix	no. of labs	no. of values	mean conc.[mg/kg]	rel. repeatablity	rel. reproducibility
sediment	7	27	4.44	6.2 %	13.7 %
sewage sludge	8	30	39.23	6.2 %	34.5 %
without Lab 1*	7	26	43.70	5.8 %	14.3 %
compost	7	26	1.938	20.6 %	25.1 %

Sediment: the results of one lab were outliers according to Grubbs

Compost: one lab with <-value

*s.s. without Lab 1: this lab had produced very low values which lay well below of the results of the other labs, but which were not recognized by the programme as outliers.

These results complement the data from the Horizontal validation nicely.

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