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Soil quality — Determination of selected phthalates using capillary gas chromatography with mass spectrometric detection (GC/MS)

Qualité du sol — Détermination de phthalates sélectionnés en utilisant la chromatographie gazeuse capillaire avec détection par spectrométrie de masse

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics*.

Introduction

This International Standard is applicable and validated for several types of matrices as indicated in Table 1 (see also Annex A for the results of the validation).

Table 1 — Matrices for which this International Standard is applicable and validated

Matrix	Materials used for validation
Sludge	Municipal sludge
Biowaste	Fresh compost
Soil	Sludge amended soil

Soil quality — Determination of selected phthalates using capillary gas chromatography with mass spectrometric detection (GC/MS)

WARNING — Persons using this International Standard should be familiar with usual laboratory practice. This International 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.

IMPORTANT — It is absolutely essential that tests conducted according to this International Standard be carried out by suitably trained staff.

1 Scope

This International Standard specifies a method for the determination of selected phthalates in sludge, treated biowaste, and soil, after extraction and gas chromatographic analysis with mass spectrometric detection.

The method is applicable for the determination of phthalates (see Table 2) at the lowest mass content of 0,1 mg/kg to 0,5 mg/kg (expressed as dry matter), depending on the individual substance.

The applicability of the method to other phthalates not specified in Table 2 is not excluded except the isomeric mixtures, e.g. DiNP (Di-isononylphthalate), but shall be verified in each case.

Table 2 — Phthalates that can be determined according to this International Standard

No	Name	Formula	Abbreviation	Molar mass g/mol	CAS-RN[a]
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] Chemical Abstracts Service Registry Number.

2 Normative references

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

ISO 5667-13, *Water quality — Sampling — Part 13: Guidance on sampling of sludges*

ISO 5667-15, *Water quality — Sampling — Part 15: Guidance on the preservation and handling of sludge and sediment samples*

ISO 10381-2, *Soil quality — Sampling — Part 2: Guidance on sampling techniques*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 14507, *Soil quality — Pretreatment of samples for determination of organic contaminants*

ISO 22892, *Soil quality — Guidelines for the identification of target compounds by gas chromatography and mass spectrometry*

3 Principle

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

4 Interferences

4.1 General

Due to their use as plasticizer agents, phthalates are ubiquitous. The sources of phthalates are multiple and shall be checked and reduced by every laboratory, itself. Therefore, special attention shall be paid to avoid contaminations.

4.2 Interferences during sampling

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

4.3 Cross contamination

Chemicals and analytical equipment can be of various qualities. 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 can severely contaminate the laboratory air if in use regularly. Therefore, refrain from using these agents during application of this procedure.

Using plastic gloves during pretreatment can increase the contamination.

4.4 Interferences in gas chromatography

Phthalates can bleed from the septa of the injector into the gas chromatograph; therefore, use septa that are not likely to contaminate the system.

Fittings, e.g. of syringes, or equipment and septa of the sampling bottles (6.5) can also contain phthalates.

5 Reagents

5.1 General

All reagents shall be of recognized analytical grade.

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, if necessary, for concentration by evaporation.

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

5.4 Ethyl acetate, $C_4H_8O_2$, phthalate-free, high purity.

5.5 Methanol, CH_3OH.

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, Al_2O_3, neutral, 50 µm to 200 µm particle size, heated to 400 °C for at least 4 h.

Store heat-treated aluminium oxide in covered flask or desiccator. Use within five days after heat-treatment.

NOTE Alternative materials, like Florisil®[1)] or silica can be used, provided their properties and capacity to separate are similar to aluminium oxide and their properties are checked according to 8.6.

5.9 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-labelled standards can also be used, if available.

5.10 Reference substances.

Table 2 gives a list of phthalates 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 (6.13), transfer e.g. 10 mg of each of the reference substances (5.10) in ethyl acetate (5.4) and bring to volume with ethyl acetate (5.4) (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.

5.12 Stock solution.

In a 10-ml volumetric flask (6.13), 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).

1) This information is given for the convenience of users of this document and does not constitute an endorsement by ISO TC 190/SC 3 of the product named. Equivalent products may be used if they can be shown to lead to the same results.

Store the solution in a glass bottle at –18 °C, protected from light, and check the concentration at least every three months.

5.13 Reference solutions for multipoint calibration (see Annex B).

Prepare solutions by adequate dilution of the stock solution (5.12) and internal standards (5.9) with ethyl acetate (5.4).

Store the solutions in glass bottles at –18 °C, protected from light, and check the concentration at least every three weeks.

5.14 Solution of the internal standards (see Annex B).

5.14.1 Internal standard solution of D4-phthalates.

Weigh e.g. 0,1 g of an internal standard phthalate (D4) (5.9) in a 10-ml volumetric flask (6.13) filled with about 5 ml of ethyl acetate (5.4) and bring to volume with ethyl acetate (5.4). Store the solution in a glass bottle at –18 °C.

5.14.2 Solution I internal standard mix.

Combine the solutions of the single internal standard phthalates (5.9) e.g. by dilution 1:100 as follows: Transfer with a syringe 0,1 ml (6.15) of each solution into a 10-ml volumetric flask (6.13) filled with about 5 ml of ethyl acetate (5.4). Bring to volume with ethyl acetate. The final concentration of di-n-octylphthalate (D4), di-n-butylphthalate (D4), and di-(2-ethylhexylphthalate) is 100 mg/l in ethyl acetate (5.4).

5.14.3 Solution II internal standard mix.

Take from this 1:100 dilution (5.14.2) e.g. 250 µl, transfer into a 250-ml volumetric flask (6.13), filled with 250 ml of ethyl acetate (5.4).

The final concentration of di-n-octylphthalate (D4), di-n-butylphthalate (D4), and di-(2-ethylhexylphthalate) is 0,1 mg/l in ethyl acetate (5.4).

5.14.4 Solution III internal standard mix.

Dilute the solution I internal standard (5.14.2.) 1:10. Pipette 1 ml of the solution (5.14.2.) in a 10-ml volumetric flask (6.13) filled with about 5 ml of ethyl acetate (5.4). 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.

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

6 Apparatus

6.1 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 can be achieved by thorough cleaning of all glass apparatus and checked by the blank determination.

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

6.3 Drying oven, capable of maintaining at a temperature of (105 ± 5) °C.

6.4 Muffle furnace, adjustable, up to temperatures of (400 ± 10) °C, with capacity of e.g. at least 60 l.

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

6.6 Vacuum device for clean-up, vacubox, extraction box.

6.7 Stainless steel cock, with stainless steel cone or polytetrafluoroethylene (PTFE) cock with Luer connection for separate vacuum connection.

6.8 Glass cartridges, with Luer cone.

6.9 Polytetrafluoroethylene (PTFE) frits, for cartridges, 6 ml.

6.10 Aluminium foil, heated to 400 °C.

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

6.12 Measuring cylinders, volumes 50 ml and 100 ml.

6.13 Volumetric flasks, volumes 10 ml, 25 ml, and 250 ml.

6.14 Pasteur pipettes, e.g. 2 ml.

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

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

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

6.18 Fused silica columns, with non-polar stationary phase (see Annex C for examples).

Check the quality of the column e.g. by injecting the reference solution (5.13) and ensure that the separation is satisfactory.

6.19 Glass tubes, graduated 5 ml or 10 ml.

6.20 Nitrogen device, for drying the glass cartridges (6.8).

6.21 Beaker, volumes 50 ml and 100 ml.

6.22 Erlenmeyer flask, volume 250 ml.

6.23 Shaking device, horizontal shaking movement.

6.24 Freeze drying apparatus.

6.25 Metal spoon.

6.26 Agate mortar.

6.27 Metallic clamp, for stopper.

6.28 Balance, e.g. range 0,001 g to 100 g.

6.29 Pipette, volumes 20 ml, 25 ml, and 50 ml.

7 Sampling and sample storage

Collect, preserve, and handle samples in accordance with ISO 5667-13, ISO 5667-15, and ISO 10381-2.

Use for sampling pretreated sampling bottles (6.2) 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).

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.

Determine the dry matter content in accordance with ISO 11465.

8 Procedure

8.1 Preparation of glass apparatus

Clean all glass apparatus, except the syringes, used during analysis in the dishwasher with water and subsequently dry in the oven (6.3) at 105 °C.

Heat the pre-rinsed glass apparatus in the muffle furnace (6.4) for at least 4 h at 400 °C.

Subsequently let the apparatus cool to room temperature within 12 h.

NOTE Glassware for volumetric purposes can change its properties due to the heating process.

Close the cooled glass apparatus (bigger vessels) with the respective stoppers or with aluminium foil (6.10). Store smaller glass apparatus in decontaminated (heated) and appropriately closed stainless steel containers (6.11).

In order to avoid losses by adsorption on the walls, rinse the walls with isooctane (5.6) by using Pasteur pipettes (6.14). 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.

8.2 Drying of the sample

8.2.1 General

Pretreat the samples according to ISO 14507, if not otherwise specified.

Depending on the water content and the matrix, dry the sample either with sodium sulfate (5.15) or by freeze drying (see also Annex D).

Samples (i. e. soil, waste) with a dry matter > 80 % can be dried with sodium sulfate (see 8.2.3).

Sludge with high water content shall be dried by freeze drying (see 8.2.2).

8.2.2 Freeze drying

Freeze a part of the homogenized sample or a representative part of the sample at –18 °C. Afterwards, lyophilize at about 5 kPa until the constant mass is achieved.

Homogenize the freeze-dried sample with the aid of an agate mortar (6.26).

8.2.3 Drying with sodium sulfate (Na_2SO_4)

Depending on the expected phthalate content of the sample, weigh between 1 g to 10 g of the wet sample into a beaker (6.21) and give as much sodium sulfate (5.15) into the wet sample until a trickle mixture is achieved. Mortar the mixture in an agate mortar (6.26). When a free-flowing mixture is obtained, the humidity is bound. In parallel, determine the dry matter content of the sample (see Clause 7).

8.3 Extraction

Transfer between 1 g to 10 g, referred to the dry matter content and the expected phthalate concentration, of the mixture of the sample and sodium sulfate (prepared according to 8.2.3) or the freeze-dried sample (prepared according to 8.2.2) into a 250-ml Erlenmeyer flask (6.22) and give e.g. 20 ml ethyl acetate (5.4) with internal standard (5.9) to the sample. If a high amount of phthalates is expected, the extraction solvent can be doubled or multiplied (see Table 3). Since the concentration of phthalate in a blank is unknown, dilutions of the sample extract shall be avoided. Close the Erlenmeyer flask (6.22) with a stopper and fix the stopper with a metallic clamp (6.27).

Extract the sample for at least 30 min on the shaking device (6.23). 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 (6.14) and transfer the extract into a GC vial (6.5), or approximately 3 ml are required if a clean-up (see 8.4) is necessary. Place the heated aluminium foil (6.10) 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 8.4.

Table 3 — Examples of sample intake and ratio dry matter/solvent volume

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

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

The described method of extraction (shaking) is recommended due to the small contamination potential. Using Soxhlet extraction or accelerated solvent extraction (ASE), 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. For blank criteria, see 8.6.

8.4 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 approximately 3 ml of the extract with a pipette (6.14) and clean the extract with aluminium oxide (5.8).

Clean the extracts as follows.

— Place 1 g of activated aluminium oxide (5.8) in the cartridges (6.8) between two PTFE frits (6.9).

— Clean the aluminium oxide (5.8) with one cartridge volume of ethyl acetate (5.4).

— Dry with nitrogen (5.2) for 1 min.

— Fix the cleaned cartridge with stainless steel cock (6.7) and place it on the vacuum device (6.6).

— Let the extract run through the cartridge and collect it in a glass tube (6.19).

— Transfer the extract to GC vials (6.5). Attach heated aluminium foil (6.10) between vial and cap in order to avoid a contamination by phthalates from the septa.

8.5 Gas chromatography

Optimize the GC apparatus (6.16) according to the instrument manufacturer's manual.

Use capillary columns (see Annex C and 6.18) for separation.

In order to clean the inlet system free from phthalates, inject ethyl acetate (5.4) at least five times from various GC vials (see Clause 4) before measuring the sample extracts or calibration solutions.

8.6 Blank monitoring

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

For the blank measurements, treat sodium sulfate (5.15) in the same way as the sample (see 8.2 and 8.3). 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 lower than 50 % of the lowest reporting limit.

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 10.

8.7 Identification of individual compounds

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.

Refer to ISO 22892 for the identification of analytes.

Table 4 — Examples of typical mass fragments of the reference analytes

Analyte		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 tune used.

NOTE 2 Depending on the concentration of the phthalates, the qualifier cannot always be seen (small amount).

NOTE 3 M_1 is used for quantification; M_2 and M_3 can be used for identification.

9 Calibration

9.1 General

Establish a calibration function and graph for each compound 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 only valid 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 shall be adjusted to the working conditions (preparation of the reference solution according to 5.13).

9.2 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 pretreatment can be avoided. Additionally, the concentration determination is independent from matrix effects in the sample, provided the recoveries of the substances analysed and the internal standard are about the same.

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

Plot the rational value $y_{i,\mathrm{std}} / y_{i,\mathrm{is}}$ (peak areas, peaks heights, or integration units) for each substance *i* on the ordinate and the associated rational mass concentration $\rho_{i,\mathrm{std}} / \rho_{i,\mathrm{is}}$ on the abscissa.

Establish the linear regression function using the pairs of value $y_{i,\mathrm{std}} / y_{i,\mathrm{is}}$ and $\rho_{i,\mathrm{std}} / \rho_{i,\mathrm{is}}$ of the measured series in Formula (1):

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

where

$y_{i,\mathrm{std}}$ is the measured value, for example, expressed as area values, for the substance *i* in the calibration depending on $\rho_{i,\mathrm{std}}$, the unit of which depends on the type of evaluation performed;

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

$\rho_{i,\mathrm{std}}$ is the mass concentration of the substance *i* in the calibration solution for the total procedure, in nanograms per millilitre (ng/ml);

$\rho_{i,\mathrm{is}}$ is the mass concentration of the internal standard, in nanograms per millilitre (ng/ml);

a_i is the slope of the calibration curve from $y_{i,\mathrm{std}} / y_{i,\mathrm{is}}$ as a function of the mass concentration ratio $\rho_{i,\mathrm{std}} / \rho_{i,\mathrm{is}}$;

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

9.3 Verification of calibration

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 to 9.2.

10 Calculation

Calculate the mass concentration $\rho_{i,\mathrm{tm}}$ of the substance using Formula (2):

$$\rho_{i,\mathrm{tm}} = \frac{\rho_{i,\mathrm{std,bl}} \cdot V \cdot F_1}{E \cdot d_\mathrm{s} \cdot F_2} \tag{2}$$

Calculation of $\rho_{i,\mathrm{st,bl}}$ using Formula (3):

$$\frac{\left(\frac{y_{i,\mathrm{std}}}{y_{i,\mathrm{is}}} - b_i\right)}{a_i} \cdot \rho_{i,\mathrm{is}} - \frac{\left(\frac{y_{i,\mathrm{std,bl}}}{y_{i,\mathrm{is}}} - b_i\right)}{a_i} \cdot \rho_{i,\mathrm{is}} = \rho_{i,\mathrm{st,bl}} \tag{3}$$

Building of the mean of the blank using Formula (4):

$$y_{i,\mathrm{std,bl}} = \frac{y_{i,\mathrm{std,bl1}} + y_{i,\mathrm{std,bl2}}}{n} \tag{4}$$

The simplification of Formulae (2) and (3) is given in Formula (5):

$$\rho_{i,\mathrm{tm}} = \frac{\frac{\left(\frac{y_{i,\mathrm{std}} - y_{i,\mathrm{std,bl}}}{y_{i,\mathrm{is}}} - b_\mathrm{i}\right)}{a_i} \cdot \rho_{i,\mathrm{is}} \cdot V \cdot F_1}{E \cdot d_\mathrm{s} \cdot F_2} \tag{5}$$

where

$y_{i,\,\mathrm{std}}$ see Formula (1);

$y_{i,\,\mathrm{std,\,bl1}}$ is the measured value of the first blank, e.g. expressed as area values, for the substance *i* in the calibration depending on $\rho_{i,\mathrm{std}}$, the unit of which depends on the type of evaluation performed (see 8.6);

$y_{i,\,\mathrm{std,\,bl2}}$ is the measured value of the second blank, e.g. expressed as area values, for the substance *i* in the calibration depending on $\rho_{i,\mathrm{std}}$, the unit of which depends on the type of evaluation performed (see 8.6);

$y_{i,\,\mathrm{is}}$ see Formula (1);

n is the number of measurements for the blank determination, see 8.6;

$\rho_{i,\mathrm{is}}$ see Formula (1);

a_i see Formula (1);

b_i see Formula (1);

$y_{i,\,\mathrm{std,\,bl}}$ is the measured value, e.g. expressed as area values, for the substance *i* in the blank sample in accordance with 8.6 depending on $\rho_{i,\mathrm{std}}$;

$\rho_{i,\,\mathrm{std,\,bl}}$ is the mass concentration of the substance *i*, corrected with the blank amount for the total procedure, in nanograms per millilitre (ng/ml);

$\rho_{i,\mathrm{tm}}$ is the mass concentration of the substance *i* in the sample based on dry matter, in micrograms per kilogram (µg/kg);

V is the volume of the extraction solvent (mainly 20 ml), in millilitres (ml);

d_s is the dry matter content of the sample, in percent (%);

E is the mass of the sample, in grams (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 = 1 000:mg/kg.

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

11 Expression of results

Express the results of the determination in milligrams per kilogram dry matter, with two significant digits.

EXAMPLE

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

12 Precision

The performance characteristics of the method data have been evaluated (see Annex A).

13 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard (i.e. ISO 13913);

b) the complete identification of the sample;

c) the expression of results, according to Clause 11;

d) any details not specified in this International Standard or which are optional, as well as any factor which could have affected the results.

Annex A
(informative)

Repeatability and reproducibility data

A.1 Materials used in the interlaboratory comparison study

The interlaboratory comparison of selected phthalates by capillary gas chromatography with mass spectrometric detection (GC-MS) in sludge, treated biowaste, and soil was carried out by up to nine European laboratories on three materials.

Table A.1 lists the types of materials tested.

Table A.1 — Materials tested in the interlaboratory comparison for the determination of selected phthalates by capillary gas chromatography with mass spectrometric detection (GC-MS) in sludge, treated biowaste, and soil

Grain size	Sample	Material tested
Sludge (< 0,5 mm)	Sludge 1	Mix of municipal waste water treatment plant sludges from North Rhine Westphalia, Germany
Fine grained (< 2,0 mm)	Compost 1	Fresh compost from Vienna, Austria
	Soil 3	Sludge amended soil from Barcelona, Spain

A.2 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 A.2).

Table A.2 — Results of the interlaboratory comparison studies for the determination of selected phthalates by capillary gas chromatography with mass spectrometric detection (GC-MS) in sludge, treated biowaste, and soil

Matrix	l	n	n_o	$\bar{\bar{x}}$ mg/kg	s_R mg/kg	$C_{V,R}$ %	s_r mg/kg	$C_{V,r}$ %	BD
DBP									
Sludge 1	7	16	2	0,085	0,038	44	0,009	10,7	10
Compost 1	6	16	1	0,046	0,034	73	0,004	8,5	9
Soil 3	5	11	1	0,016	0,015	95	0,003	20,8	14
DEHP									
Sludge 1	9	36	0	23,1	9,32	40	2,16	9,3	0
Compost 1	7	22	0	0,56	0,23	42	0,08	14,4	0
Soil 3	8	28	1	0,53	0,21	39	0,05	9,4	0

l number of laboratories
n number of analytical results
n_o number of rejected laboratories
$\bar{\bar{x}}$ total mean of analytical results (without outliers)
s_R reproducibility standard deviation
$C_{V,R}$ coefficient of variation of reproducibility
s_r repeatability standard deviation
$C_{V,r}$ coefficient of variation of repeatability
BD number of measurements below detection limit

NOTE The experience of laboratories with some of the phthalates was limited and therefore led to results only for DEHP and DBP.

Table A.3 — Repeatability and reproducibility results obtained from an interlaboratory comparison study carried out in Germany on DEHP

Matrix	l	n	$\bar{\bar{x}}$ mg/kg	$C_{V,r}$ %	$C_{V,R}$ %
Sediment	7	27	4,44	6,2	13,7
Sewage sludge	8	30	39,23	6,2	34,5
Sewage sludge (without Lab 1[a])	7	26	43,70	5,8	14,3
Compost	7	26	1,938	20,6	25,1

l number of laboratories

n number of analytical results

$\bar{\bar{x}}$ total mean of analytical results (without outliers)

$C_{V,r}$ coefficient of variation of repeatability

$C_{V,R}$ coefficient of variation of reproducibility

[a] This laboratory had produced very low values which lay well below of the results of the other laboratories, but which were not recognized by the programme as outliers.

Annex B
(informative)

Example of a 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 (5.4).

A five-point calibration is sufficient (see 9.1). The appropriate concentration levels for calibration depend on the expected phthalate concentration in the sample. The levels mentioned in Table B.1 can serve as examples.

Table B.1 — Recommended concentration levels for calibration

Level	Amount of stock solution[a] µl	Amount of solution III internal standard[b] µl
L 1	2,5	100
L 2	5	100
L 3	10	100
L 4	20	100
L 5	40	100
L 6	50	100
L 7	100	100
L 8	150	100
L 9	300	100
L 10	450	100

[a] See 5.12.

[b] See 5.14.4.

Table B.2 — Resulting concentrations from L 1 to L 10

All concentrations in picograms per microlitre (pg/µl)

Phthalate	L 1	L 2	L 3	L 4	L 5	L 6	L 7	L 8	L 9	L 10
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	1 500	2 250
DUP	12,5	25,0	50	100	200	250	500	750	1 500	2 250