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Development and validation of methods for the trace determination of phthalates in sludge and vegetables

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Abstract

A routine method which is simple, quick and precise has been set up and validated for phthalate analysis in environmental samples (tomato plants and sewage sludges). Six phthalates have been studied simultaneously: dimethylphthalate, diethylphthalate, di-*n*-butylphthalate, *n*-butylbenzylphthalate, di-2-ethyl-hexyl phthalate (DEHP) and di-*n*-octylphthalate. Optimization of sample, solvent extraction uses a Soxtec apparatus and extract purification with an a solid-phase extraction cartridge allows between 90 and 110% recovery of phthalates. Precise, sensitive and selective identification and quantifying of analytes is by GC–MS in the single ion monitoring mode. This protocol allows analytes with concentrations as low as 10 μg/kg dry matter (DM) to be determined from small (1–2 g DM) samples. This analytical method has been applied to the phthalate transfer study for agricultural recycling of sludges, where phthalate bioavailability has been studied in aquiculture using two types of experiments. Tomatoes have been grown in containers where the trace organics have been directly introduced as pure substances, and in a second experiment under the same growth conditions, sewage sludge has replaced the pure substances. Transfer of these trace organics has been followed into the various parts of the tomato plant and in general only the DEHP is worthy of note although its percentage transfer remains very low even in an experiment designed to maximize this.

Keywords: Phthalates; Extraction methods; Environmental analysis; Sewage sludge

1. Introduction

Each year millions of tons of phthalates are produced in the world for the manufacture of a wide variety of common consumer goods. Their increasing presence in the environment has prompted several countries to investigate population exposure.

Phthalates are esters of phthalic acid based on the structure in Fig. 1.

Although a large number of phthalates exist, only a few are commonly used and will be considered for this analysis (Table 1).

Due to man's activities they are present in the environment in quite large quantities, since they are a group of chemicals which has been used for about the last 50 years as plastifying agents, mainly to make polyvinyl chloride (PVC) supple and flexible. However, not all the phthalates are used to for this, some are used to stop nail varnish flaking, to make perfumes last longer, or to make tool handles stronger and more resistant. Others reinforce or increase the effect of adhesives, paint pigments, caulking and many other materials. They can be found in many industrial sectors: paint, petrochemical, packing, cosmetics, etc. and in view of this widespread use, phthalates have been the subject of intensive research concerning effects on health and the environment.

These substances have been chosen and give great cause for concern because they bioaccumulate (accumulate in liv-

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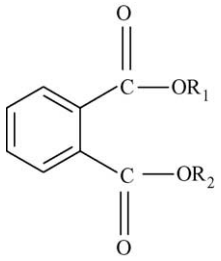

Fig. 1. General formula for phthalates.

ing tissues and in the food chain), are poorly biodegradable and are potentially toxic. The latter can be short-term effects (allergies, asthma, etc.) or longer term (disruptions in nervous and endocrine systems, increased risk of cancer, decrease in fertility, disruptions in child development, etc.) [1]. It should be noted however that these effects are derived from animal studies and very few toxicity analyses on man have been carried out to date.

Europe, by actively pursuing a policy favouring wastewater collection and treatment has ensured the production of clean water but also increased the production of sludges. For the moment, 40% of these sludges are recycled biologically via land application, and this principal method must abide by strict norms concerning levels of various undesirable elements that they could contain. New European legislation is currently being drafted to fix in particular, the level of phthalate esters in sludges [2], and a land application limit value of 100 mg/kg DM for di(2-ethyl-hexyl) phthalate (DEHP) is envisaged.

There would appear to be two potential sources for phthalates in sludges: compounds produced and/or used industrially and abnormally present in effluents, or compounds from plastics in manufactured goods released back into wastewater. Phthalate levels in sludge residues vary according to wastewater treatment plant and particular compound, due to their different physico-chemical properties.

DEHP in particular is present in quite high concentrations in sewage sludges [3] and it would appear necessary to monitor this compound in order to avoid any accumulation in the soil after land application (Table 2).

Different analytical methods have been developed to answer these questions and this article concerns phthalate analysis in the plants and the soil. For the plant analyses, we have used tomato plants grown without sludge to provide a good hydrophilic model for the research.

Table 1
Empirical formulae for the alkyl groups in the six phthalates

Name	Abbreviation	R_1	R_2
Dimethyl phthalate	DMP	CH_3	CH_3
Diethyl phthalate	DEP	C_2H_5	C_2H_5
Di-*n*-butyl phthalate	DBP	C_4H_9	C_4H_9
Benzylbutyl phthalate	BBP	$C_6H_5CH_2$	C_4H_9
Di(2-ethyl-hexyl) phthalate	DEHP	C_8H_{17}	C_8H_{17}
Di-*n*-octyl phthalate	DOP	C_8H_{17}	C_8H_{17}

Table 2
Levels (European mean) of phthalates in sludges expressed in milligrams per kilogram of dry matter (mg/kg DM) [2]

Compound	Level (mg/kg DM)
DMP	10
DEP	30
DBP	10
BBP	20
DEHP	100
DOP	4

The object of this study is the development and optimisation of a method for quantitative determination of phthalate esters to show their presence in sludges and the various parts of plants.

2. Experimental

2.1. Sample collection

To test the different stages of the analytical protocol, two types of sample have been used: sewage sludge from the drier outlet of the Ginestous treatment plant (Toulouse, France) and tomato plants grown under glass on a sludge enriched medium (ENSAT, Toulouse, France).

2.2. Reference material

No certified reference material (CRM) for phthalate analysis exists.

2.3. Solvents, reagents and adsorbents

The solvents used for the whole analytical process are Suprasolv *n*-hexane (VWR Merck) and Chromanorm HPLC acetone (VWR Prolabo, France).

Fontainebleau sand (particle size 150–300 μm) (VWR Prolabo) to control boiling and powdered Florisil (Florisil PR particle size 60–100 mech, magnesium silicate) (VWR Prolabo, France) to adsorb grease, are added to the matrix in the cellulose extraction cartridge (30 cm × 100 cm) (Schleicher & Schuell) (VWR Prolabo). Clean up is by 1 g SPE Florisil cartridges (Supelco, France).

2.4. Preparation of standards

Six phthalate congeners have been used as standards (DMP, DEP, DBP, DEHP, DOP), and the standard 2000 μg/mL mixture of these six phthalates in isooctane is from Cluzeau Info Labo (France). Deuterated [3,4,5,6-$^2H_4$] diethyl-hexylphthalate (DEHP-d_4) is used as the internal standard, chosen because it is ideal for gas chromatography coupled to mass spectrometry since it has the same physico-chemical behaviour (same chromatograph behaviour and same ionisation yield) under electron impact, as the com-

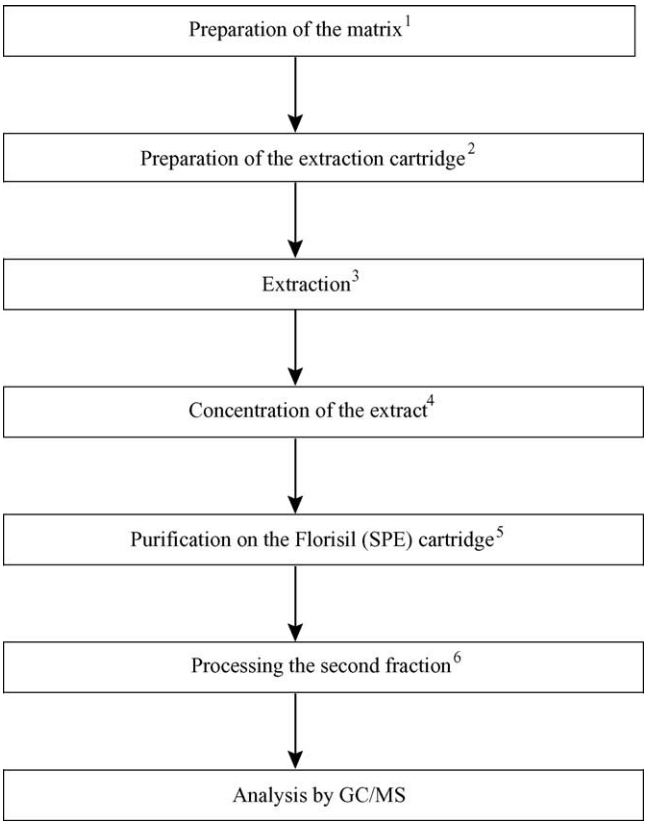

Fig. 2. Description of the different treatment stages of the solid matrixes for phthalate assessment. *Notes*: (1) freezing, lyophilization and grinding up (<0.2 mm); (2) loading the cartridge with two spatulas full of Fontainebleau sand, one spatula of Florisil, 2*g* of matrix and 10 μL of benzyl benzoate (5000 μg/mL) and homogenisation; (3) one 100 mL measure of *n*-hexane using the special beaker for the Soxtec. extraction in boiling mode and in rinsing mode; (4) concentration of the extract to 10 mL with a rotary evaporator. Evaporation with a stream of nitrogen to 1 mL; (5) conditioning of the cartridge with 10 mL of *n*-hexane. Extract deposited, with change of collection vessel tube rinsed with 1 mL *n*-hexane and deposited on cartridge. Elution with 10 mL *n*-hexane and recuperation of this 1st fraction. Elution with 5 ml of a *n*-hexane/acetone (90:10, v:v) mixture and recuperation of this second fraction; (6) concentration with a stream of nitrogen Addition of internal standard: 10 μL of DEHP-d_4 (5000 μg/mL).

pounds being assessed. In addition, the 4 mass unit difference means there can be no confusion between the ions of the "normal" molecule and those of the standard. It is added to the purified extract just before the gas chromatography analysis.

Benzylbenzoate (1 mL at 5000 mg/L in *n*-hexane) is the internal extraction standard and is added to extraction cartridge just before the extraction. Both these standards were supplied by Cluzeau (France).

3. Analytical procedure

Determining the phthalates in environmental samples is carried out according to a protocol of several determinative steps, i.e. extraction, purification, analysis (Fig. 2).

Table 3
Summary of the methods, cited in the bibliography, used to extract and analyze phthalates

Reference	Matrix	Extraction		Analysis
		Method	Solvent	
[4]	Soil	Ultrasonic	*n*-Hexane/ethanol	GC/MS EI
[5]	Soil	Soxhlet	*n*-Hexane or acetone/*n*-hexane	GC/MS EI
[5]	Plant matter	Soxhlet	Methanol	GC/MS EI
[6]	Sludge	Soxhlet	*n*-Hexane/methyl ethyl cetone/methanol	GC/MS EI
[7]	Plant matter	Contact	Acetone/petroleum ether	GC/FID
[8]	River water	Soxhlet	Dichloromethane	GC/MS EI
[8]	Suspended matter	Ultrasonic	Methanol	GC/MS EI

3.1. Extraction

Quantifying organic pollutants such as phthalate esters in environmental samples necessitates an extraction stage aimed at isolating these compounds from the actual matrix of the sample.

Several techniques can be used in order to extract the phthalates from the various matrixes (sewage sludge, soil or plant matrixes), and certain authors advocate the use of ultrasonic extraction [4]. However, this method is still less effective than Soxhlet [5,6].

The solid/liquid extraction is derived from the classic Soxhlet approach and is carried out with a Soxtec System HT2 (Tecator, France). This is a semi-automated apparatus working on the Soxhlet principle, while allowing extractions which are more rapid, economical (better solvent recuperation) and safe (dissociation of the extraction and heating units). The apparatus is composed of two parts: an oil bath plus a unit with two plates heated by the oil, and above, systems for fixing the cartridge and for cooling.

Solvents, extraction times and eluants have been set from bibliographical and experimental investigations (Table 3).

About 2 g (exact weight recorded) of the lyophilised sample have been extracted in two stages with 100 mL of *n*-hexane for 45 min.

The sample is first placed in a cellulose cartridge immersed in the solvent (boiling mode) for 30 min to give a rapid, total contact. Next, the cartridge is lifted up above the still boiling solvent (rinsing mode) allowing the condensing solvent to rinse the sample. Then, a rotary evaporator (Rotavapor, Büchi) and 30 °C temperature controlled bath is used to concentrate the solvent down to 10 mL. Concentration of the *n*-hexane extract to 1 mL before purification, is by a stream of nitrogen.

3.2. Clean-up

The aim of the clean up stage is to eliminate the unwanted compounds such as lipids, co-extracted with the ph-

thalates, which could interfere with the latter's final determination and quantification. The protocol chosen for purification of the extract comes from a previously published paper [6].

First, the 6 g Florisil SPE cartridge is placed on a vacuum manifold and rinsed with 10 mL of *n*-hexane. A 10 mL graduated tube is placed in the manifold to collect the extract from the cartridge. The 1 mL *n*-hexane extract is placed at the top of the cartridge, and a first elution with a 1–2 drops/s flow rate is carried out with 8 mL of *n*-hexane. This fraction is collected and put aside and a second tube is placed in the manifold. Two types of eluants have been tested, *n*-hexane, and a *n*-hexane/acetone mixture (90/10, v/v). Once the 5 mL of eluants have passed through the SPE cartridge, a second fraction is collected, and this is concentrated down to 1 mL under nitrogen.

3.3. Chromatographic analysis by GC–MS

The extracts are analysed using GC–MS on electron impact mode: the most widely used technique for these compounds [4–8]. Ionisation by this technique is the most sensitive and reproducible.

A Finnigan Trace 2000 Series (Ecole Nationale de Formation Agronomique, Complexe Agricole, Auzeville, France) apparatus is used, which is a gas phase chromatograph coupled to a mass spectrometer with a quadruple type analyzer. The chromatograph is fitted with a Restek RTX-5MS capillary column (5% diphenyl; 95% dimethylpolysiloxane) 30 m long, 0.25 mm in diameter and with a 0.25 μm film thickness. A helium Alpha 2 (Air Liquide) gas vector is used, flow rate 1.2 mL/min. A 1 μL sample is injected into the split/splitless inlet in splitless mode (splitless for 1 min, split flow: 50 mL/min) at 280 °C. The temperature of the GC–MS interface is 250 °C and at the end, the oven temperature program chosen started at 50 °C for 1 min, followed by an increase of 20 °C/min to 310 °C which is maintained for 6 min.

The full scan electron impact data is obtained as follows: solvent delay 5 min, electron impact energy 70 eV, source temperature 200 °C, emission current 150 μA, scan rate 4 scan/s, detector voltage 350 V.

The internal standard quantification method has been chosen because it overcomes the non-reproducibility problems of injections and detector response. The compounds are quantified using the relation between the analyte response and that of the internal standard (peak area), and this standard (DEHP-d_4) is added to the extract just prior to the analysis. The calibration curves for the six phthalates, showing the relative peak areas as a function of the concentration injected, are obtained by linear regression. In each case the regression coefficient is greater than 0.90 and each calibration solution or extract has been injected three times.

4. Results and discussion

4.1. Extraction optimisation

Lyophilised urban sludge has been used to test the effectiveness of the extraction procedure and this sludge has been used without any added phthalates.

The solvent used for the phthalate extraction, *n*-hexane, is the one most often cited in publications [4].

The influence of extraction time on the phthalate ester concentrations (in mg/kg DM) have been studied. Two extraction procedures have been tested:

Procedure 1: Soxtec extraction with 100 mL *n*-hexane for 45 min.
Procedure 2: Soxtec extraction with 100 mL *n*-hexane for 3 h.

Three replicates have been made for each extraction procedure and the results are shown in Table 4.

There is no significant difference between the two extraction times and so the total extraction time has been set at 45 min (30 min in boiling mode and 15 min in rinsing mode).

4.2. Purification optimisation

At this stage of the protocol, the extract contains a large amount of co-extract compounds such as lipids, pigments and organic macromolecules which could interfere with the final GC–MS level of identification.

In order to test the effectiveness of the purification stage, 1 mL of a 150 μg/mL solution of phthalates has been placed at the top of the cartridge. Florisil has been chosen as the purification solid phase, a manifold has been used, and two types of eluants have been selected, based on the premise that the initial cartridge eluant is 10 mL of *n*-hexane to pick up the polychlorinated biphenyls (PCB).

Procedure 1: Elution with *n*-hexane.
Procedure 2: Elution with a *n*-hexane/acetone mixture (90/10, v/v).

The results are shown in Tables 5 and 6.

The two successive fractions of 10 mL of *n*-hexane are devoid of phthalates: they did not elute them.

The results show that the best compromise is with 5 mL of a *n*-hexane/acetone (90/10, v/v) mixture. The first 10 mL of *n*-hexane elute the PCBs if need be, and the following 5 mL

Table 4
The influence of the extraction time on the concentration in phthalate esters (in mg/kg of dry matter DM)

Extraction time	Concentration (mg/kg DM)					
	DMP	DEP	DBP	BBP	DEHP	DOP
45 min ($n = 3$)	Nd	Nd	6 ± 2	Nd	79 ± 4	Nd
3 h ($n = 3$)	Nd	Nd	4 ± 1	Nd	77 ± 2	Nd

Concentrations not determined "Nd". Replicated three "n" times.

Table 5
Elution procedure number 1: two successive *n*-hexane elutions

	DMP	DEP	DBP	BBP	DEHP	DOP
Initial concentration (μg/mL)	150.3	148.2	153.3	152.5	154.6	155.4
Fraction 1: elution with 10 mL of *n*-hexane	0	0	0	0	0	0
Fraction 2: elution with 10 mL of *n*-hexane	0	0	0	0	0	0

Each fraction has been analysed to determine the phthalate concentration.

Table 6
Elution procedure number 2: an elution with *n*-hexane followed by one with a *n*-hexane/acetone (90:10, v/v) mixture

	DMP	DEP	DBP	BBP	DEHP	DOP
Concentration (μg/mL)	150.3	148.2	153.3	152.5	154.6	155.4
Fraction 1: 10 mL *n*-hexane	0	0	0	0	0	0
Fraction 2: 5 mL *n*-hexane/acetone	151.2	149.4	154.6	152.9	156.4	157.3
Recovery (%)	100	101	101	100	101	101

Each fraction has been analysed to determine the phthalate concentration.

of binary mix allows 100% of the target compounds to be recovered.

4.3. Development of the GC–MS analytical method

Separation has been set up using a standard mixture of the six phthalates at 2000 μg/mL in isooctane. Detection is initially in SCAN mode to determine the masses of the characteristic fragments of the compounds, and identification is confirmed at the NIST library.

The six phthalates can be separated in 20 min using the temperature settings given in the experimental method. The characteristic fragment mass and their relative intensity are determined by the width half way up the chromatograph peak (Table 7).

The retention times and the fragments of the phthalates detected by the mass spectrometer can be determined from the chromatogram obtained (Fig. 3).

Having separated the different phthalates being studied, there is then the internal standard, DEHP-d_4 to consider. This is first put through on SCAN mode to determine its retention time and characteristic fragments (Table 7).

While this internal standard has the same retention time as the DEHP, it has different characteristic fragment masses (Fig. 4). Thus calibration can be carried out using identification from these fragments rather than by the retention time.

Benzylbenzoate (BBz) has been chosen as the extraction standard using supplier catalogue data, and it is used to verify optimal extraction. It is injected in the GC–MS in SCAN mode to determine the characteristic fragments (Table 7).

Ten microliters of a 5000 μg/mL solution of this compound in *n*-hexane is introduced directly into the matrix before extraction. Thus, if there is total extraction there should be 50 μg/mL in the final extract.

4.4. Optimisation of the analytical method: SIM method

All the data (Table 7) is used to set up single ion monitoring (SIM) detection, and this technique consisting of focusing detection on particular masses, increases sensitivity and selectivity, and is well adapted to phthalates, which are trace compounds in the matrixes being studied. Three retention time windows have been used for the SIM method, each corresponding to the ions selected per compound (Table 8).

Fig. 5 shows a chromatogram where the concentration in each of the six phthalates is 100 μg/mL, that of the internal standard 50 μg/mL and that of the extraction standard, 50 μg/mL.

Table 7
Phthalate, BBz and DEHP-d_4 molecular masses, empirical formulae, characteristic fragment masses (*m*/*z*) and retention times

Name	Abbreviation	Molecular mass (g/mol)	Empirical formula	Fragment masses (*m*/*z*) (% relative intensity)	Retention times (min)
Dimethyl phthalate	DMP	194	$C_{10}H_{10}O_4$	163 (100%); 135 (19%); 104 (17%)	8.29
Diethyl phthalate	DEP	222	$C_{12}H_{14}O_4$	149 (100%); 177 (62%); 104 (28%)	9.14
Di-*n*-butyl phthalate	DBP	278	$C_{16}H_{22}O_4$	149 (100%); 104 (27%); 205 (16%)	11.13
Benzylbutyl phthalate	BBP	312	$C_{19}H_{20}O_4$	149 (100%); 104 (50%); 206 (25%)	12.98
Di(2-ethyl-hexyl) phthalate	DEHP	390	$C_{24}H_{38}O_4$	149 (100%); 104 (50%); 167 (36%)	13.67
Di-*n*-octyl phthalate	DOP	390	$C_{24}H_{38}O_4$	149 (100%); 104 (17%); 279 (13%)	14.41
Deuterated [3,4,5,6-$^2H_4$ d_4 di-ethyl-hexyl phthalate	DEHP-d_4	394	$C_{24}H_{37}O_4D_1$	153 (100%); 171 (19%); 283 (17%)	13.67
Benzylbenzoate	BBz	212	$C_{14}H_{12}O_2$	105 (100%); 194 (13%); 167 (9%); 213 (4%)	10.20

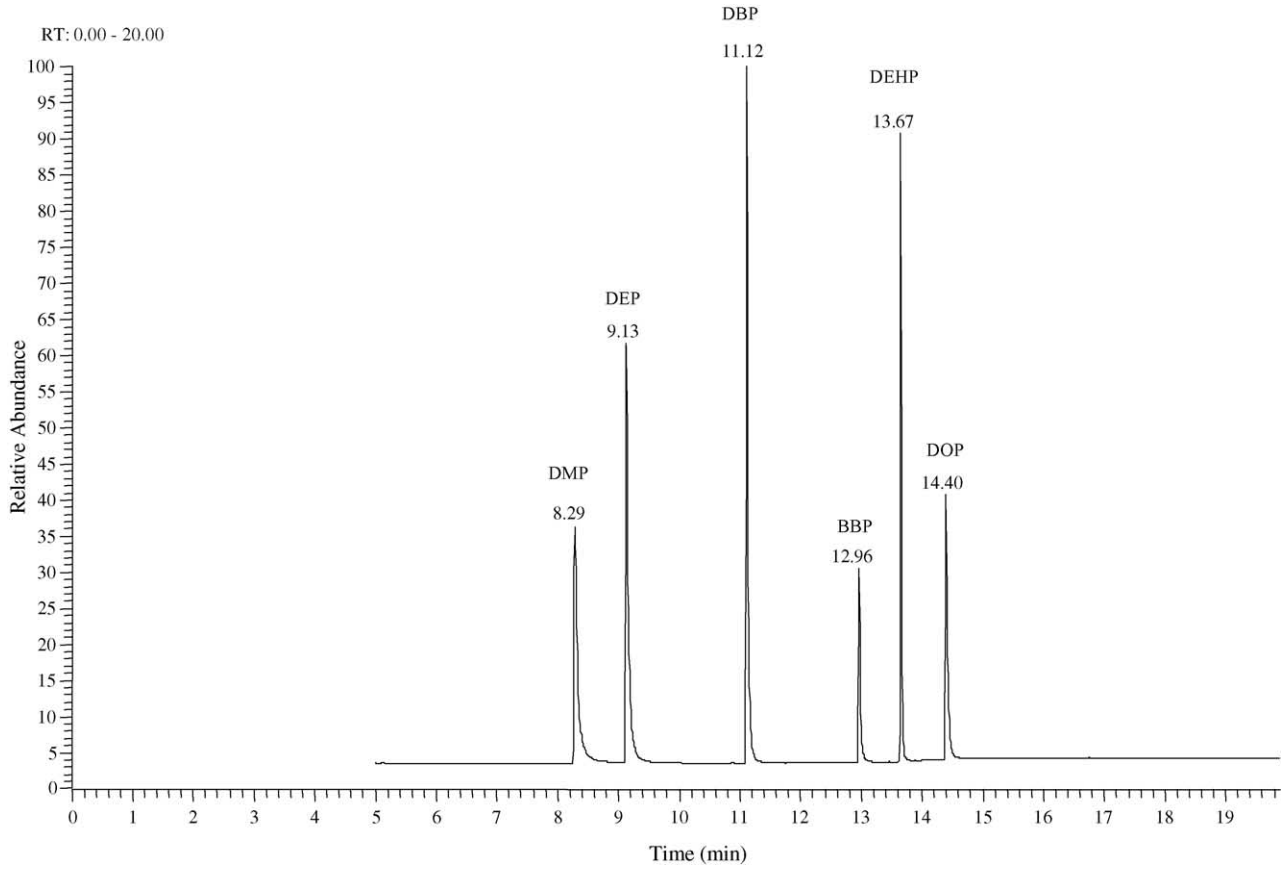

Fig. 3. Chromatogram of a mixture of the six phthalates (10 mg/L).

4.5. Method validation

The method was validated according to the AFNOR regulation XP T 90–210 [9].

Calibration is by internal standard with several points. The compounds are quantified using the relation between the analyte response and that of the internal standard (chromatogram peak area). The internal standard (DEHP-d_4) is added to the extract to be analysed just before the analysis. Quantification of the target compounds had to be done within the linearity zone of the calibration curve and in order to determine this zone for the detector, several standard solutions have been prepared and injected at different concentrations. The calibration range is from 1 to 10 μg/mL for low concentrations and from 20 to 300 μg/mL for higher concentrations. The extraction standard concentration in this range varies from 1 to 150 μg/mL in order to cover the expected (50 μg/mL) value (Table 9). Calibration curves have been obtained for each compound by linear regression of the peak area against the concentration injected. The regression coefficient in each case is greater than 0.90 (Table 10).

Table 8
Acquisition program with windows, range of retention times, the compounds and characteristic ions

RT windows	Time (min)	Compounds	SIM ions (*m*/*z*)
1	5.0–9.8	DMP DEP	149; 163; 177
2	9.8–10.8	BBz	105; 167; 194; 213
3	10.8–20.0	DBP BBP DEHP DEHP-d4 DOP	149; 153; 167; 171; 206; 279; 283

The reproducibility of the analysis is expressed as the relative standard deviation (in %) of a check calibration standard and should be under 20%. The whole process has been repeated 10 times using sludge containing a low phthalate concentration, and the results shown in Table 11 are expressed in mg/kg DM.

The repeatability of the whole analytical procedure is expressed as the relative standard deviation (in %), and is an evaluation of the overall extraction–purification–analysis procedure. It is calculated on the basis of five replications of five different sludge samples and must be less than 20%. The results are shown in Table 12. The repeatability for DEHP is 4% i.e. 162 ± 6 mg/kg DM.

The limit of detection (LOD) is defined as the smallest amount of an analyte in a sample which can be detected and considered as different from the blank value but not quantified with certainty. The limit of quantification (LOQ) is defined as the smallest amount of an analyte in a sample which can be detected and quantified with certainty. Ten

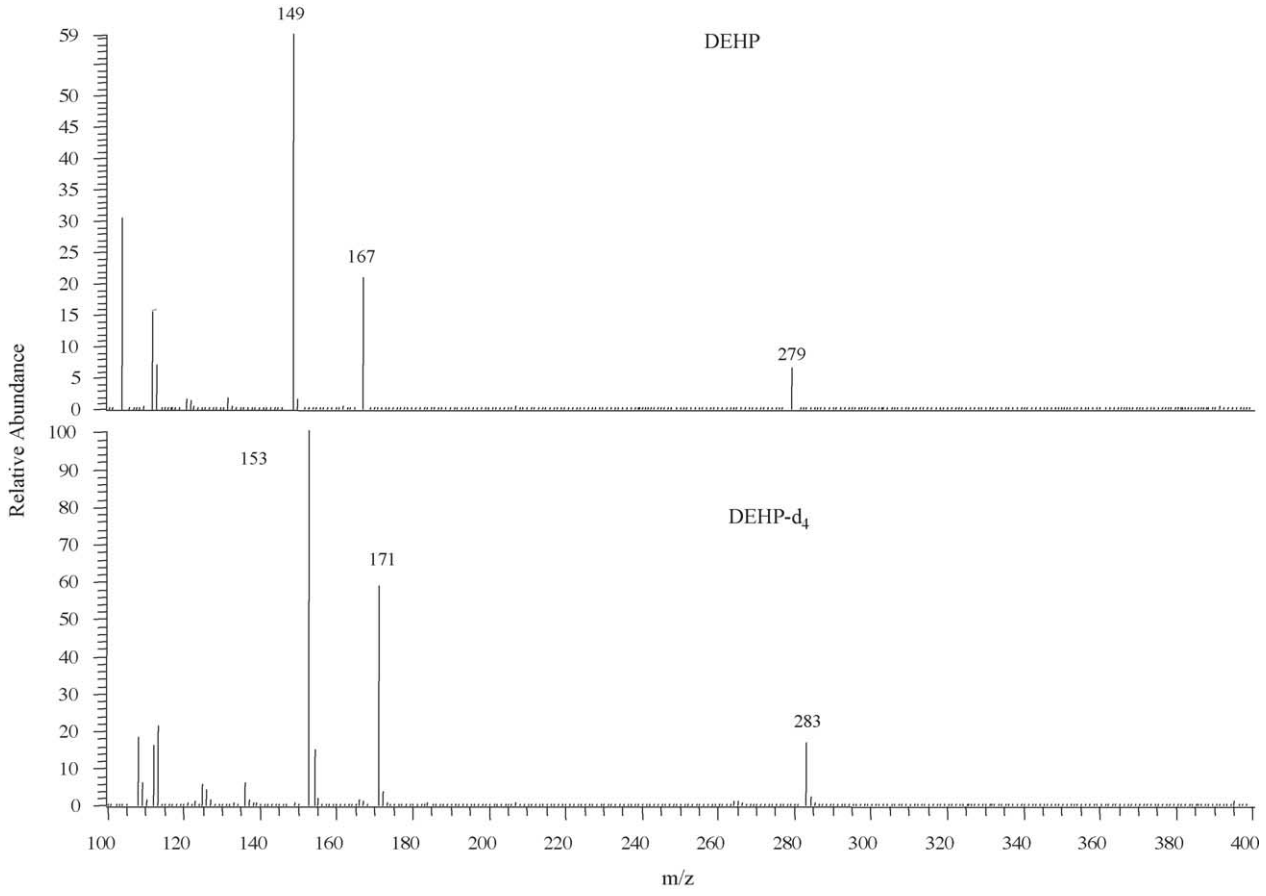

Fig. 4. Mass spectra on electron impact for DEHP and DEHP-d_4.

Table 9
Phthalate calibration range data

Number of vials	Vials									
	1	2	3	4	5	6	7	8	9	10
Concentration of phthalates (μg/mL)	1	3	5	8	10	20	50	100	200	300
Concentration of extraction standard (μg/mL)	1	3	5	8	5	10	25	50	100	150
Concentration of internal standard (μg/mL)	50	50	50	50	50	50	50	50	50	50

measurements are made for a sample with a very low concentration of the chosen LOQ. The latter is validated when the relative standard deviation is less than 20% for these 10 readings. The limit of detection (3 × standard deviations) is 0.003 μg/mL for the phthalates studied. The limit of quantification for these phthalates (10 × standard deviations) is 0.01 μg/mL.

The purpose of blank analysis is to verify the absence of any contamination that could lead to quantification errors. This must be thoroughly carried out to determine any trace

Table 10
Equations and correlation coefficients for linearity of phthalates for the two concentration ranges

	Between 1 and 10 μg/mL		Between 20 and 300 μg/mL	
	Equation	Correlation coefficient	Equation	Correlation coefficient
DEP	$y = 0.0175x - 0.0161$	$R^2 = 0.9242$	$y = 0.0072x + 0.6137$	$R^2 = 0.8840$
DMP	$y = 0.0199x - 0.0190$	$R^2 = 0.9648$	$y = 0.0067x + 0.5658$	$R^2 = 0.9165$
BB	$y = 0.013x - 0.0110$	$R^2 = 0.9697$	$y = 0.015x + 0.0365$	$R^2 = 0.9849$
DBP	$y = 0.0321x - 0.0290$	$R^2 = 0.9296$	$y = 0.0066x + 0.8289$	$R^2 = 0.9145$
BBP	$y = 0.0141x - 0.0358$	$R^2 = 0.7247$	$y = 0.0111x + 0.2526$	$R^2 = 0.9852$
DEHP	$y = 0.0228x + 0.0401$	$R^2 = 0.9742$	$y = 0.014x + 0.5216$	$R^2 = 0.9793$
DOP	$y = 0.0209x - 0.0387$	$R^2 = 0.7809$	$y = 0.0099x + 0.5452$	$R^2 = 0.9473$

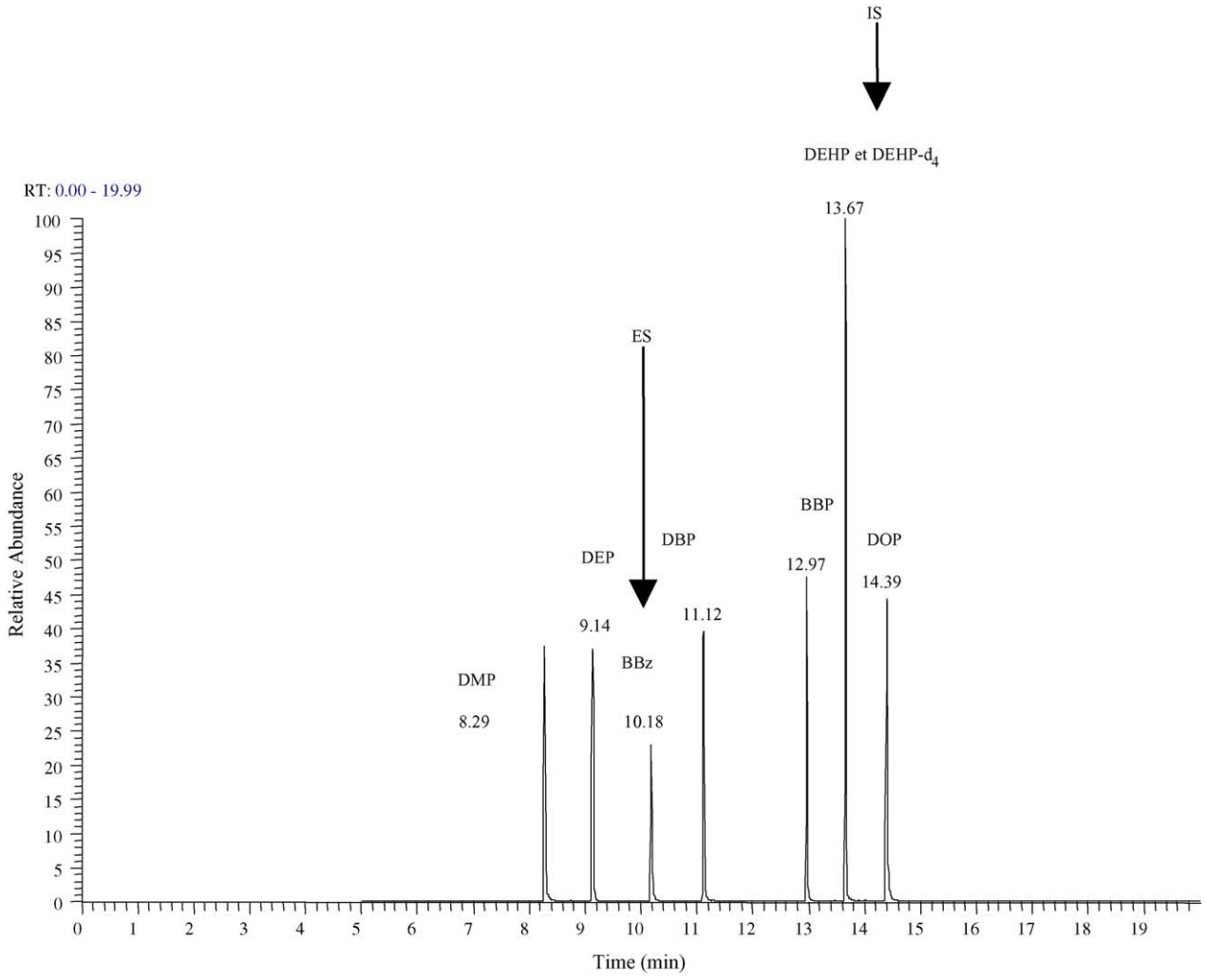

Fig. 5. Chromatogram, with the concentration levels of the six phthalates at 100 μg/mL, the internal standard (IS) at 50 μg/mL and the extraction standard (ES) at 50 μg/mL in SIM mode.

Table 11
Analysis reproducibility study: 10 injections of the same low concentration sample

	Concentration (mg/kg DM)					
	DMP	DEP	DBP	BBP	DEHP	DOP
Trial 1	0.830	5.780	17.252	5.356	170.763	2.160
Trial 2	0.831	6.456	18.466	5.278	173.954	1.965
Trial 3	0.831	6.253	18.169	5.337	172.233	2.136
Trial 4	0.831	6.400	18.434	5.333	172.669	2.219
Trial 5	0.831	6.205	18.259	5.438	172.107	2.297
Trial 6	0.831	6.006	17.635	5.407	168.554	2.258
Trial 7	0.830	6.090	17.592	5.435	171.064	2.347
Trial 8	0.830	6.120	17.679	5.423	171.662	2.380
Trial 9	0.830	6.280	18.066	5.532	172.540	2.229
Trial 10	0.830	6.030	17.491	5.601	170.745	2.317
Mean	0.830	6.162	17.904	5.414	171.629	2.231
Standard deviation	0.001	0.200	0.426	0.097	1.461	0.115
RSD (%)	0.12	3.25	2.38	1.80	0.85	5.15

Table 12
Evaluation of the repeatability of the analytical protocol by injecting five successive extractions of the same sample

	Concentration (mg/kg DM)					
	DMP	DEP	DBP	BBP	DEHP	DOP
Trial 1	0.83	8.00	15.99	4.79	160.93	2.85
Trial 2	0.84	8.43	15.50	4.57	155.37	2.50
Trial 3	0.69	9.85	17.18	5.00	159.42	2.72
Trial 4	0.68	9.97	14.81	5.01	163.22	2.76
Trial 5	0.83	6.16	17.90	5.41	171.63	2.23
Mean	0.77	8.48	16.28	4.96	162.11	2.61
Standard deviation	0.08	1.56	1.26	0.31	6.04	0.25
RSD (%)	11	18	8	6	4	10

Table 13
Data on results of extraction blanks

	Concentration (μg/mL)					
	DMP	DEP	DBP	BBP	DEHP	DOP
Mean	< 0.003	<0.003	0.006	0.004	0.009	0.006
Standard deviation	–	–	0.001	0.002	0.001	0.001

contaminants. A blank (i.e. with no sample added) is analyzed after each batch of 10 measurements, and follows the same analytical procedure as with the samples. The cartridge is simply filled up with the Fontainebleau sand and powdered Florisil and extracted with hexane under the same conditions as for a 'normal' extraction, including clean up and concentration. For the phthalate esters, the concentrations of each congener in the blank extract is always less than the limit of quantification (Table 13).

5. Application to environmental samples

Once the stages of the analytical protocol had been validated and with the reclamation of sludges for agriculture in mind, a study of the behavior of phthalates in a nutrient solution—plant system has been carried out on growing tomato plants. To investigate the transfer potential, tomato plants (*Lycopersicum esculentum* var Rondello F1) have been grown in aquiculture to provide optimal transfer conditions [10], in plant containers inside a temperature and humidity regulated plant house.

The trace organics have been introduced in two ways:

- As pure substances. Four phthalate esters have been chosen: DMP, DEP, DBP and DEHP.
- In the form of a wastewater treatment plant sludge tea.

Samples of tomato roots, leaves, sap and fruits have been analyzed.

5.1. Experiment with the nutrient solution spiked with pure phthalates

Using the values determined from the calibration graphs, the initial concentration in the sample can be found.

$$\text{Concentration (mg/kg DM)} = \frac{\text{Concentration (mg/mL)} \times \text{Initial volume of extract(mL)}}{\text{Extraction yield} \times \text{Mass (kg DM)}} \times \text{Concentration factor}$$

with $0 < \text{Extraction yield} < 1$; Concentration factor = Initial volume of extract/Final volume of extract.

Table 14 shows the average levels of phthalates found in the roots, sap, leaves and fruits of tomato plants grown in the nutrient solutions spiked with pure phthalates. Similarly, Table 15 shows the average levels of phthalates found in the roots, sap, leaves and fruits of tomato plants grown in the nutrient solutions spiked with sludge. The control plants have been used for the blank measurements in the analyses.

The experiments with large quantities of pure substances in the nutrient solutions, correspond to the best conditions possible for transfer into the plant, and here phthalates have not been detected in the sap extracts. Concerning transfer of nutrient solution compounds to other parts of the plant, there would appear to be practically no transfer of DMP, and whereas the DEP and the DBP are present in the sap and the leaves, they are not stored in the roots. DEHP is the only phthalate to be found in large quantities in the roots and the leaves. This result agrees with the work of authors who found the greatest concentrations of phthalates in the roots of their plants [11–13].

5.2. Experiment with the nutrient solution spiked with a sludge tea containing phthalates

For the sludge experiments, the levels of DMP, DEP and DBP are less than the detection limits for all parts of the plant, and this can be explained by the fact that the initial concentrations of these compounds in the sludge tea is very

Table 14
Results for the phthalate analyses in the experiment with pure substances

	Amount in μg/kg dry matter			Amount in μg/kg fresh matter, sap
	Roots	Leaves	Fruits	
DMP	<10	50 ± 4	<10	<10
DEP	<10	3279 ± 254	<10	14 ± 2
DBP	995 ± 10	50 ± 6	<10	<10
DEHP	173238 ± 1000	269 ± 36	<10	<10

Mean values found in the tomato plants after introduction of the pure phthalates.

Table 15
Results for the phthalate analyses in the experiment with the sludge tea

	Amount in μg/kg dry matter			Amount in μg/kg fresh matter, sap
	Roots	Leaves	Fruits	
DMP	<10	<10	<10	<10
DEP	<10	<10	<10	<10
DBP	<10	<10	<10	<10
DEHP	1350 ± 57	234 ± 65	10 ± 2	314 ± 78

Mean values found in the tomato plants after introduction of the sludge tea.

low. The DEHP on the other hand, is found in the roots, leaves and sap. Studies have also demonstrated this transfer into the plant [14–16].

5.3. Percentage transfer

The percentage transfer of DEHP, defined as the ratio of the mass of DEHP in 1 g of tomato plant fresh matter and the initial mass of DEHP in the growth medium multiplied by 100, has been calculated. Less than 0.01% is transferred into 1 g of tomato plant.

A review of published work reveals that no overall study of the six phthalates has been made. Results from this study show that in terms of phthalate transfer, only DEHP is important, however even for this, transfer into the tomato plant remains very low.

6. Conclusion

A routine method which is simple, quick and precise has been set up and validated for phthalate analysis in environmental samples (tomato plants and sewage sludges). This protocol includes solvent extraction of samples with a Soxtec apparatus and extract purification on an SPE cartridge, with final analyte identification and quantification by GC–MS. This protocol allows determination of these compounds at concentrations as low as 10 μg/kg DM with low sample masses.

This analytical method has been applied to the phthalate transfer study whose objective is agricultural recycling of sludges. To this end, phthalate bioavailability has been studied in aquiculture using two types of experiments. Tomatoes have been grown in containers where the trace organics have been directly introduced as pure substances and in a second experiment under the same growth conditions, sewage sludge has been substituted. Transfer of these trace organics has been followed into the various parts of the tomato plant and in general only the DEHP is worthy of note although its percentage transfer remains very low even in an experiment designed to maximise this.

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