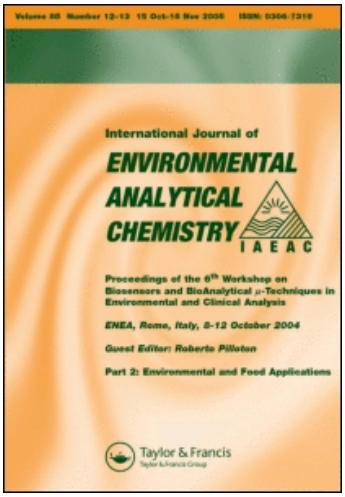

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Clean-up with Sulphuric Acid Prior to the Gas Chromatographic Determination of Phthalate Esters

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A clean-up procedure facilitating the determination of phthalate esters in water, sediment, and fish tissues is reported. The method is based upon the differential solubility of phthalate esters in sulphuric acid, hexane, and hydrated sulphuric acid. About 20 times less solvent was needed for the described method compared with that needed for the Florisil clean-up. Background levels of phthalates were reduced by a factor of about 10. The recovery of DBP and DEHP ranged from 56 to 113%; a fatty extract was as easy to handle as a water sample and they resulted in comparable recoveries. The method allowed a complete separation of PCBs from the phthalates.

KEY WORDS: Clean-up, phthalate ester, gas chromatography, di-2-ethylhexyl-phthalate, dibutylphthalate.

INTRODUCTION

Phthalates show properties similar to those shown by many of the "traditional" fat-soluble, persistent pollutants, such as DDT, BHC, lindane, and PCBs. Consequently, the distributional patterns of phthalates in the environment and within various organisms are also similar to the patterns exhibited by these other fat-soluble compounds.[1–3]

A number of methods are available for separating phthalates from the sample matrix and from other pollutants interfering in the final identification and quantification. The majority of these methods are based on adsorption of the sample onto silica gel, aluminium oxide, Florisil, etc., followed by elution with a combination of different solvents.[4–7] These clean-up procedures are both time-consuming and require large amounts of solvents, which may result in high blank levels. It is well known that most chemicals and solvents generally contain trace amounts of various phthalates.[8,9]

To reduce the time needed for clean-up, the amount of solvent required, and to simultaneously eliminate the sample matrix, a procedure was developed based on the differential solubility of phthalates in hexane and sulphuric acid.

EXPERIMENTAL

The phthalates were extracted as described previously.[10]

APPARATUS

A Varian model 3700 gas chromatograph with a flame ionization detector (FID) and an electron capture detector (ECD), equipped with a two 25 m × 0.25 mm (i.d.) fused silica columns (SE-54), was used for the separation and quantification. The samples were injected on-column.[11] The chromatographic conditions were: injector temperature, 50°C, detector temperature, 260°C. The oven was programmed from 50 to 150°C at a rate of 40°C/min, held isothermal at 150°C for 10 min, and from 150 to 250°C at a rate of 20°C/min for the phthalates. The minimum detectable quantity (MDQ) on the FID (signal to noise ratio 2:1) for DEHP and DBP was 0.05 ng. The quantification of PCBs was made on the ECD and the conditions were: injector and detector temperature as described above, and the oven was programmed from 50 to 240°C at a rate of 15°C/min. Hydrogen was used as carrier gas (1.5 ml/min) and nitrogen as make-up gas (50 ml/min).

CLEAN-UP

Chemicals All solvents were of pesticide quality (Merck). Fuming

concentrated sulphuric acid (5% SO_3) was prepared by mixing sulphuric acid with fuming sulphuric acid (30%). Hydrated sulphuric acid ($H_2SO_4 \cdot n\,H_2O$) was obtained by adding distilled water to cooled concentrated sulphuric acid. Standard solutions of PCBs (Clophen A 50) and phthalate esters (dibutylphthalate (DBP), di-2-ethylhexylphthalate (DEHP)) were prepared by dissolving appropriate amounts of these compounds in hexane.

Procedures One millilitre of fuming concentrated sulphuric acid was added to a test tube containing 1.0 ml of a phthalate extract dissolved in hexane. The mixture was shaken vigourously for 3 min and then centrifuged to separate the two phases. The upper hexane layer, containing the nonpolar material (e.g. PCBs), was decanted. To completely remove interfering substances the sulphuric acid (containing the phthalates) was washed with 2×0.5 ml portions of hexane. The portions were combined and analysed for PCBs.

Two millilitres of hydrated sulphuric acid followed by 1.0 ml hexane were then added to the cooled sulphuric acid. The mixture was shaken for 3 min and the hexane phase (containing the phthalates) was transferred to a test tube. The procedure was repeated and the solvents were combined, dried over sodium sulphate, and evaporated under nitrogen to about 100 μl.

To examine the variables affecting the efficiency by which the phthalates are re-extracted from the sulphuric acid into the hexane, the following parameters were varied:

1) Time: the phthalates were extracted from the sulphuric acid after 10 min, 1 h, and 12 h.

2) Temperature: the extraction was performed at 0°C and 20°C.

3) Hydration: the sulphuric acid used was either mono-, di-, tri- or tetrahydrated.

Recovery To study the partitioning of phthalates and other fat-soluble, persistent compounds in sulphuric acid/hexane mixtures, tap water (1 l), sediment (20 g), and cod muscle (0.5–2.0 g) were spiked with PCBs (Clophen A50), DBP, and DEHP. The samples were homogenized[10] and cleaned up as described above. The mean level of extractable fat in the cod muscle was 2.0%.

RESULTS

The recovery rates of the phthalates added to sediment, water, and fish tissue ranged from 56 to 94% for DBP and from 77 to 113% for DEHP. The method allowed a complete separation of PCBSs from the phthalates; from cod muscle the recovery of PCBs that had been added along with the phthalates was 92% (Table I).

Table I Recovery (%) of phthalates and PCBs added to the various samples and determined by the proposed method ($n=6$). Numbers within brackets denote standard deviation.
(n.a. = not analyzed)

Substance	Sediment		Water		Fish tissue	
	Added (ug)	Recovery (%)	Added (ug)	Recovery (%)	Added (ug)	Recovery (%)
DBP	53.9	77 (12)	10.8	94 (17)	53.9	56 (11)
DEHP	48.8	113 (10)	9.4	97 (18)	48.8	77 (12)
PCB	n.a.	n.a.	n.a.	n.a.	1.2	92 (17)

The recovery rates of DBP and DEHP were influenced neither by the reaction temperature nor by the time of exposure of hexane to the sulphuric acid (Table II). However, the proportion of phthalates dissolved in the hexane fraction was positively related with the level of hydration of the sulphuric acid (Table III). The effect was most pronounced for DBP.

Table II Recovery (%) of phthalates added to hexane solutions at different reaction temperatures and with different duration of the sulphuric acid treatment. Means of three measurements.

Substance	Temperature		Time		
	0°C	20°C	10 min	1 h	12 h
DBP	99	98	99	98	98
DEHP	100	97	100	100	100

Table III Recovery (%) of phthalates after treatment with differential hydrated sulphuric acid. Means of three measurements.

Substance	Sulphuric acid			
	Monohydrate	Dihydrate	Trihydrate	Tetrahydrate
DBP	2	27	99	102
DEHP	17	97	100	100

Using the above method, and evaporating the final hexane fraction to 100 μl, the background levels of DBP and DEHP were kept below 10 ng.

DISCUSSION

The mechanism by which phthalates are differentially distributed between the acid and hexane phases is not fully understood. However, we assume that after contact with the concentrated sulphuric acid, DBP and DEHP undergo acidic hydrolysis, resulting in a shift of the phthalate equilibrium towards the acid. The ester linkage is probably protonized; as a consequence, the phthalates, which are ionized weak bases, should dissolve in the sulphuric acid. The hydrolysis separates the phthalates from the fat-soluble, non-ionized, persistent residues which are dissolved in the hexane phase. A similar observation was reported by Tanaka and Takeshita.[12] Hydration of the acid will, however, deionize the phthalates and restore the original equilibrium; consequently, the compounds return to the hexane free from the sample matrix and from substances that may interfere in the final determination.

The amount of the phthalates recovered from the acid phase seems to be related to the amount of water added; when the acid is made mono-,di-,tri-, or tetrahydrated the recovery increases accordingly. However, we found that treatment with trihydrated sulphuric acid resulted in a cleaner sample; an increase in the extent of hydration of the sulphuric acid seems to cause the less water-soluble organic

substances of the sample matrix to move into the hexane phase. To regulate the partitioning of the phthalates between the acid and the solvent, it is important to completely remove any traces of water in the phthalate extract.

The recovery of DBP and DEHP added to water, sediment, and fish tissues was similar to those reported for other methods (70 to 100%, Ref. 5; 60 to 90%, Ref. 10; 83 to 90%, Ref. 12). Moreover, a fatty extract was as easy to handle as a water sample and comparable recoveries were obtained provided that the level of fat in the sample did not exceed 5.5%.

The time spent on separation and clean-up and the extraction temperature did not appear to be critical to the outcome. The separation can be accomplished over a wide range of temperatures in just a few minutes, and longer treatment periods do not increase recovery rates. No signs of degradation of DBP or DEHP were observed in any of the material subjected to the various treatment regimes.

For the clean-up procedure, the sulphuric acid treatment requires about twenty times less solvent than required by the Florisil separation method. Accordingly, the limit of detection should be lower for the sulphuric acid treatment. Compared with the Florisil clean-up,[5,10] the blank levels using sulphuric acid are lowered by a factor of about ten.

The acid treatment removed most of the impurities in the sample matrix that normally would have interfered with the final gas chromatographic separation. However, independently of the sample matrix, one saturated fatty acid (heptadecanoic acid) still remained in the hexane phase after the sulphuric acid treatment. Since this fatty acid has a retention time similar to that of DBP on a fused silica column of SE-54 type (Figure 1), care should be taken to select a temperature program that allows these two compounds to elute separately.

A complete separation of PCBs from the phthalates was obtained; thus, in addition to DBP and DEHP, the method facilitates the determination of other persistent, lipid-soluble pollutants in environmental samples. Preliminary results indicate that dimethylphthalate, diethylphthalate, diisononylphthalate, and diisodecylphthalate can be determined by the method described here.

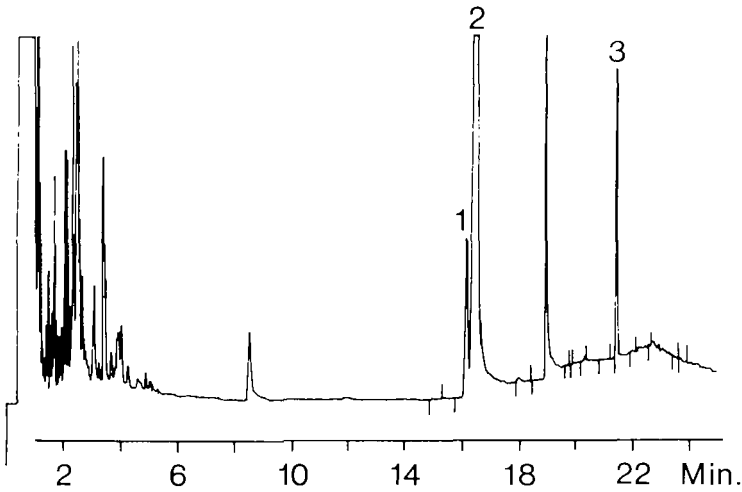

Figure 1 Gas chromatogram (FID) of the final hexane phase from a sample of cod muscle spiked with PCBs, DBP, and DEHP. Peaks 1, 2, and 3 represent DBP (0.5 ng), heptadecanoic acid, and DEHP (2 ng), respectively. Heptadecanoic acid originates from the biological sample matrix. Chromatographic conditions are described in the text.

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