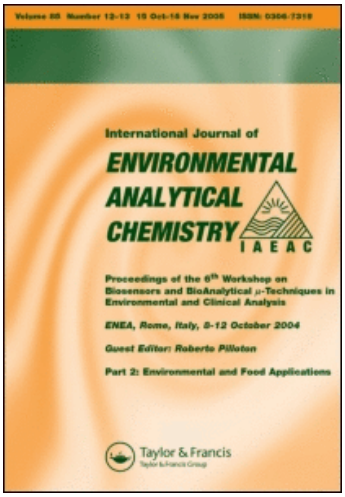

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James C. Peterson[a]; David H. Freeman[a]
[a] Department of Chemistry and Chesapeake Biological Laboratory, University of Maryland College Park, Maryland, U.S.A.

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Method Validation of GC-MS-SIM Analysis of Phthalate Esters in Sediment

JAMES C. PETERSON† and DAVID H. FREEMAN

Department of Chemistry and Chesapeake Biological Laboratory, University of Maryland College Park, Maryland 20742, U.S.A.

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A comprehensive quality assurance program was applied to the analysis of phthalate esters in sediment. By utilizing the rapid ultrasonic extraction of dried sediment and the detection capabilities of selected ion monitoring mass spectrometry, the analytical scheme has been simplified to reduce systematic errors due to contamination and to improve recoveries. Matrix blank levels were 2.5 ng in 5 g of dry samples, and spiked field sample recoveries were greater than 90%. Identification of different phthalate species was accomplished by multiple ion monitoring of characteristic ion fragments and retention time comparisons. Eleven phthalate species have been measured in representative samples from various sites in the Chesapeake bay and the Chester river, Maryland.

INTRODUCTION

The analysis of phthalate esters in environmental matrices is particularly prone to laboratory contamination.[1,2] The assessment of the true environmental levels of phthalate ester pollution has been confused by reports of questionable validity caused by difficulties in achieving blanks significantly lower than the environmental concentrations. This problem is of increased significance in light of a recent report that di(2-ethylhexyl) phthalate causes cancer in mice and rats.[3]

Several authors have approached this problem by simplifying the analytical methodology to reduce the opportunity for laboratory contamination. The selectivity of high pressure liquid chromatography has been exploited to analyze sediment and water extracts without prior clean-up steps.[4,5] However, the reported DEHP detection limit for this technique was 500 ppb for 10 g of dry sediment. This is significantly worse than the detection limits reported for the more conventional gas

†Present address: Laboratoire de Chimie Analytique Physique, Ecole Polytechnique, Route de Saclay, 91128 Palaiseau cedex, France.

chromatography-electron capture detector (GC-ECD) method[6] (2 ppb for 100 g of wet sediment) which, in turn, has the disadvantage of requiring several clean-up steps to eliminate chlorinated hydrocarbon interferences. Selective gas chromatographic detection of phthalate esters with the mass spectrometer in the selected ion monitoring mode (GC-MS-SIM) has also allowed the analysis of phthalate esters in water[7,8] and air particulate extracts[9] without prior clean-up.

This paper describes the extension of a "no clean-up" GC-MS-SIM technique to the more complex matrix of estuarine sediment extracts with the use of glass capillary columns. Sediment which was dried in a controlled atmosphere, was extracted ultrasonically in a closed system to avoid contamination frequently encountered with conventional soxhlet extraction. The entire procedure is outlined in Fig. 1 together with the quality assurance checks on certain steps of the protocol. Sample definition and validation procedures are described below. The matrix

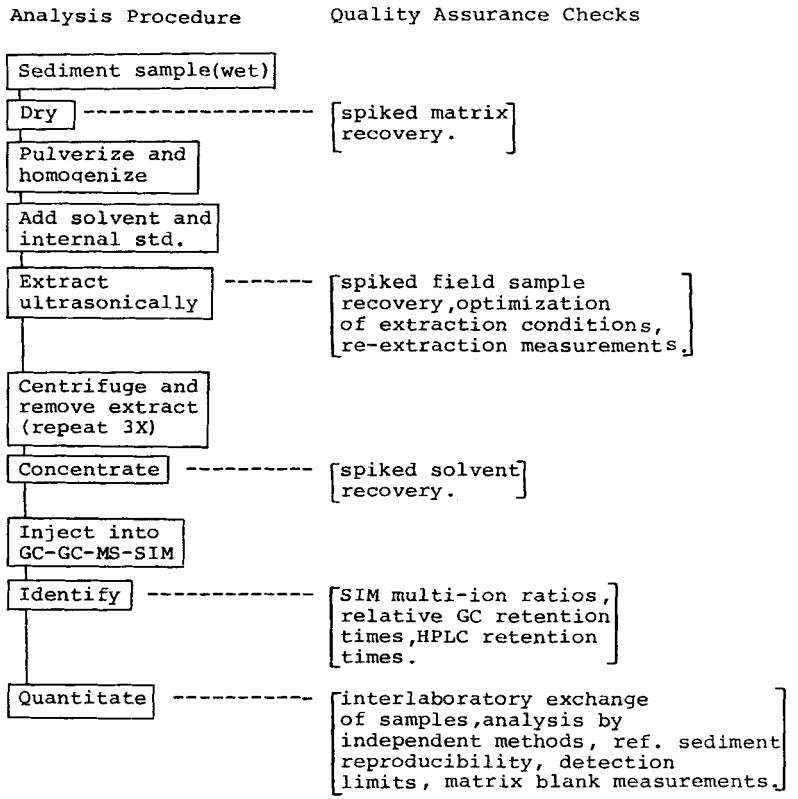

FIGURE 1 Quality assurance checks on the analytical procedure.

blank is an "organic-free" clay which is treated the same as a real sediment and carried through the entire analytical procedure. Since there is no certified reference sediment yet available for trace organics, a field sample was carefully homogenized, measured repeatedly, and used as a reference sample. This sample was divided—both wet and dry—for inter-laboratory exchanges. The reference sample was measured as part of each set of field samples for quality control and as a working standard. This assisted in the detection of errors and in the evaluation of the long term stability of the analytical method.

EXPERIMENTAL

Decontamination of glassware, materials and reagents

All glassware was washed with detergent, rinsed with tap water and distilled water. The washed glassware and aluminum foil was baked at 400°C for at least one hour in a glass fabrication annealing oven. The glassware was slowly cooled (4 h) and exposed areas were covered with baked aluminum foil. Teflon vial cap liners were soxhlet-extracted with methylene chloride for 24 h. XAD-2 organophilic resin (Rohm and Haas, Philadelphia, PA) used in the organic carbon traps was soxhlet-extracted with methanol for 24 h followed by benzene for 24 h.

Technical-grade solvents were distilled twice using a 1 m distillation column fitted with a spray trap and packed with glass beads.

Analytical procedure

Approx. 30 g of wet sediment spread on an aluminum weighing boat were placed in a vacuum oven fitted with an inlet moisture trap (100 g sodium sulfate) and an organic trap (successive sections of 100 g activated charcoal, Applied Science, and 500 g XAD-2 resin, Rohm and Haas, Philadelphia, PA). The purified laboratory air flowed through the oven toward a suction pump at the outlet side. Sediment in 2 cm thick layers was dried at 45°C to 1–2% moisture content in 24–48 h. The dry sediment was pulverized and homogenized with mortar and pestle and stored in glass vials at 4°C.

At the start of the extraction, 160 ng of deuterated anthracene (Supelco, Bellefonte, PA) internal standard were added together with 10 ml of methylene chloride to 5 g of dry sediment in a glass sample vial. After agitation with a vortex mixer, the vial was placed in an ultrasonic water bath (Bransonic 220, 150 W) for 2 min. The suspended particles were settled by centrifugation at 2500 G for 15 min and the supernatant liquid was removed. After repeating the extraction two more times with fresh solvent, the combined extracts were concentrated to 200 μl with a nitrogen

blow-down apparatus which was fitted with an XAD-2 organic carbon trap.[10]

A Hewlett Packard 5992A GC-MS system equipped with a 20 m × 0.27 mm glass capillary column coated with SE-52 methyl silicone stationary phase was used. Splitless injections of 3 μl were analyzed by programming the temperature from 150°C to 275°C at 7.5°C/min. Six individual mass ions were monitored by the mass spectrometer (see Table 3 below) during each run for consecutive intervals of 50 msec each. Quantitative results were based on integrated SIM peaks of phthalate esters (m/z 149, except dimethyl, m/z 163) relative to the peak area of deuterated anthracene (m/z 188) added to the sediment. Relative response factors were obtained by analyzing a series of standard mixtures containing the phthalates and the internal standard.

Quality assurance samples and procedures

1. *Matrix blank*: The material selected was attaclay (Englehard Minerals and Chemicals Corp., Attapulgus, GA). This clay had been treated at 1000°C (and, therefore, was practically organic-free) prior to delivery. A portion was again baked overnight at 400°C before use. Twice distilled water was then added to give a 60% moisture content, resembling a wet sediment sample.

2. *Reference sediment*: Several kilograms of sediment collected at the mouth of the Chester river, MD (39° 02′ 54″N, 76° 16′ 6″ W) were dried to a 1% moisture content and stored at 4°C for use as a working standard for method testing, quality control and interlaboratory exchanges.

3. *Spiked matrix blank*: Equal parts (W/V) of attaclay and 200 μg/l solution of DEP, DBP and DEHP in methylene chloride were mixed and the solvent was evaporated in a stream of nitrogen.

4. *Spiked field sample*: A 250 μl aliquot of a 200 μg/l solution of DEP, DBP and DEHP in methylene chloride was aided to 10.00 g of reference sediment "R".

5. *Soxhlet extraction technique*: After the paper thimble was pre-extracted for 24 h, 90 g of wet sediment were extracted with 500 ml methanol for 24 hours to remove the water followed by 48 hours extraction with methylene chloride. The volumes of methanol and methylene chloride were reduced with a Büchi rotovaporator and the final 20–30 ml of aqueous methanol was extracted with 5 volumes of methylene chloride. The extracts were combined and concentrated to 5 ml. Dry sediment was extracted similarly except the initial methanol drying step was eliminated.

6. *Contamination source patterns determinations:* A finger, a bakelite vial cap, and a plastic and a brass piston core liner were each immersed in methylene chloride and ultra-sonicated for 1 min. The values from the subsequent analyses were reported on the basis of exposed surface area (ng/cm^2). For the air contamination value, a clean TLC plate was exposed to the open laboratory air for one month. The value is reported on the basis of TLC plate area exposed per day (ng/cm^2 day).

RESULTS AND DISCUSSION

Optimization of the ultrasonic extraction techniques

Before comparison to soxhlet extraction, the ultrasonic extraction technique was optimized for the variable of extraction solvent, extraction time, and the number of extractions. The details of these experiments are described elsewhere.[11] Methylene chloride extracted phthalates with equal or greater efficiency than methanol and benzene, while hexane was a poor extractor of all phthalates tested. Extended ultrasonic extraction times of up to 4 h did not increase yields over short extraction times of 30 sec. However, repeated ultrasonication with fresh solvent showed increases which reached a plateau after three extractions. On the basis of these experiments ultra-sonication was performed with methylene chloride for less than 5 min and repeated three times with fresh solvent.

Comparison of ultrasonic and soxhlet extraction techniques

Table I compares the two extraction techniques in terms of extraction efficiency, proneness to contamination, co-extraction of coloured substances, reproducibility and experimental time. Ultrasonication was equal to or better than soxhlet extraction in extraction efficiency. In addition, in soxhlet extraction with large solvent-to-sediment ratios, sample contact with the extraction thimble and difficult to clean apparatus yielded higher blanks than ultrasonication. In fact, two of the five wet soxhlet trials had to be discarded due to gross contamination. Also, the smaller amount of co-extracted coloured materials from dry sediment showed that fewer non-volatile components in the extract are available to foul the GC injection port. The worse reproducibility observed for soxhlet extraction suggests inconsistent contact of the extracting liquid with the sediment sample. Finally, the slow rate and laborious preparation time of soxhlet extraction makes it less cost effective than the rapid ultrasonic technique.

TABLE I

Comparison of methods for extraction of phthalate esters from the reference sediment "R"

	Amount extracted, ppm, standard deviation, (n)		
Extraction method	Ultrasonication	Soxhlet, dry	Soxhlet, wet
DEP	0.19±0.03 (3)	0.10±0.03 (3)	0.05±0.10 (5)
DBP	0.36±0.07 (3)	0.33±0.14 (3)	0.26±0.07 (5)
DEHP	0.40±0.06 (3)	0.34±0.06 (3)	0.21±0.12 (5)
Av. rel. S.D. (%)	16	24	78
Solvent to sediment ratio	4:1	12.5:1	25:1
Co-extractives[a]	++	++	++++
Experimental time (h)	1[b]	48	72
Blank level (DEHP)	0.003±0.001 (3)	0.030 ppm (1)	0.063 ppm (1)

[a]Based on visual estimation of extract color density (+ is clear, ++++ is dark brown).
[b]Includes time for extraction, centrifugation and removal of the extract, repeated three times.
(n) Number of replicates. ppm = ng/g dry sediment.

Recovery testing

The key steps of sediment drying, extraction and concentration in the ultrasonic analytical procedure were tested for possible losses. The results, as reported in Table II, show that only slight losses occurred during sediment drying in the vacuum oven, ultrasonic extraction or concentration of the extract with the nitrogen "blow-down" apparatus.

TABLE II

Experimental recoveries of phthalate esters

		% Recovery[a]		
Procedure step	(n)	DEP	DBP	DEHP
Drying	(5)	90±7	95±6	98±2
Extraction	(3)	96±4	90±4	95±10
Concentration	(3)	ND	ND	100±4
Re-extraction of residual sediment				
Ultrasonication	(3)	<1	<1	<1
Soxhlet	(1)	<1	<1	<1

[a]Based on amount added prior to procedure step.

Other investigators[12,13] have noted that spiked recovery experiments do not simulate natural adsorption conditions. Therefore, residual sediment which had been extracted three times ultrasonically was re-extracted by the ultrasonic and soxhlet extraction techniques. Less than 1% of the original amount extracted was found by either technique.

Due to the high recoveries obtained, calculations of final sediment concentrations did not require a correction factor for partial recovery.

Selective detection of phthalate esters by mass spectrometer

Further fractionation or clean-up of the sediment extract was found to be unnecessary due to the high selectivity provided by the mass spectrometer in the selected ion monitoring mode (SIM). Phthalate esters with characteristically dominant m/z 149 (phthalic anhydride) base peaks are particularly well suited for this technique. This effectiveness is illustrated in Fig. 2 in which the total ion scan TIS and SIM chromatograms of the same Chester river sediment extract are compared. In the SIM chromatogram, interfering peaks of the crude extract present in the TIS scan were dramatically diminished and the phthalate peaks were greatly enhanced.

Normally, m/z 149 was used for quantitation of phthalate esters. Qualitative confirmations were carried out for at least two other ions for each of the phthalate esters. The characteristic ions are listed in Table III

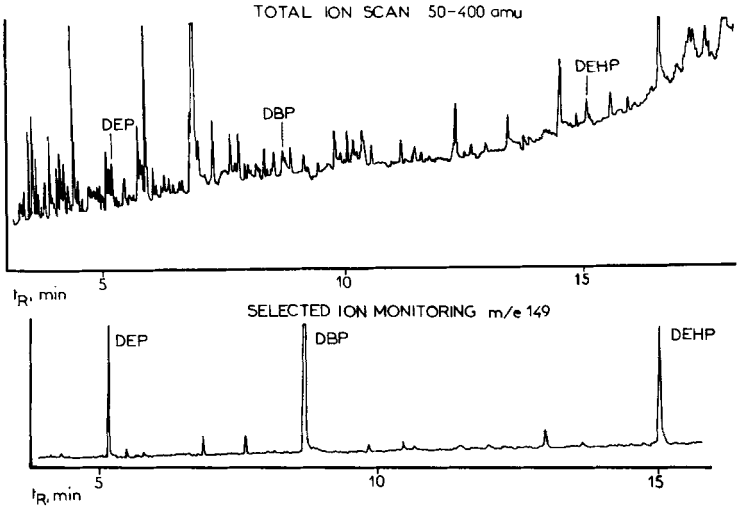

FIGURE 2 Comparison of GC-MS-TIS and GC-MS-SIM chromatograms of a Chester river sediment extract. The amount of DEHP injected is 10 ng.

TABLE III

Retention times and characteristic ions of selected phthalate esters

Phthalate ester	Abbr.	Relative retention time[a]	R.R.' (#C's)	m/z (relative abundances) $(M-R-OR-H)^+$	$(M-OR)^+$	$(M-2R+3H)^+$	$(M-R+2H)^+$	M^+
Dimethyl	DMP	0.46	1, 1	—	163(100)			194(12)
Diethyl	DEP	0.68	2, 2	149(100)	177(22)			222(3.1)
Dipropyl	DPP	0.81	3, 3	149(100)	191(5.0)		209(2.0)	
Diallyl	DAP	0.94	3, 3	149(100)	189(10)			
Diisobutyl	DIBP	1.15	4, 4	149(100)	205(2.0)	167(3.1)	223(6.0)	
Di-n-butyl	DBP	1.35	4, 4	149(100)	205(3.1)	—	233(4.1)	
Dihexyl	DHP	2.13	6, 6	149(100)		167(1.5)	251(3.0)	
Diheptyl[b]	7, 7P	2.35–2.50	7, 7	149(100)		167(2.7–6.7)	265(4.5–5.4)	
Di(2-ethylhexyl)	DEHP	2.53	8, 8	149(100)		167(38)	279(10)	
Heptyl nonyl[b]	7, 9P	2.70–2.85	7, 9	149(100)		167(2.6–8.3)	265(1.4–4.8)	
							293(1.3–3.6)	
Di-n-octyl	DnOP	2.89	8, 8	149(100)		167(2.4)	279(3.6)	
Octyl decyl	8, 10P	3.25	8, 10	149(100)		167(2.5)	279(3.1)	
							307(1.7)	
Diisodecyl[b]	DIDP	3.0–3.4	10, 10	149(100)		167(2.5–6.0)	307(1.5–3.5)	
Di-n-decyl	DnDP	3.61	10, 10	149(100)		167(3.0)	307(3.0)	
Heptyl undecyl[b]	7, 11P	3.07–3.19	7, 11	149(100)		167(3.3–8.1)	265(3.0–3.5)	

[a]Relative to the internal standard, 10-[^{2}H]-anthracene.

[b]Mixtures of branched isomers.

with corresponding relative GC retention times. Some standards listed are mixtures of isomers that were occasionally difficult to distinguish. It was found empirically that increased branching leads to increased abundance of the fragment ions above m/z 149, especially m/z 167 which corresponds to the phthalic acid ion. Also the branched isomers elute chromatographically prior to the straight chain isomer. The identity of a phthalate ester was confirmed if the retention time of the three monitored ions coincided with that of an authentic reference standard and if the ratios of integrated peaks of corresponding ion chromatograms were equivalent (within 20%) to standards.

Procedural blanks and limits of detection and determination

A primary challenge in the analysis of phthalate esters is to obtain low procedural blanks. A matrix of organic-free clay defined the total background level. The range of matrix blank measurements is reported in Table IV. Matrix blank levels of less than 2.5 ng of DEHP in 5 g of dry sediment is equivalent to or better than those reported of other workers[1,4,6] based on their reported reagent blanks. The levels for our own reagent blanks were 2 to 20 times lower than the matrix blanks. This suggests the inadequacy of the reagent blank as a measure of the overall background contamination in sediment analysis.

TABLE IV

Blank levels and limits of detection and quantitation

Phthalate ester	Sb[a] (blank)	Sb + 3σ LOD (limit of detection)	Sb + 10σ LOQ (limit of quantitation)
DEP	2.0 ± 0.8 ppb	5.3 ppb	13 ppb
DBP	1.0 ± 0.4	2.7	7.6
DEHP	0.5 ± 0.2	1.3	3.1

[a]Matrix blanks for DEP, DBP and DEHP ranged from 1–16 ppb, 0.5–4 ppb, and 0.5–1 ppb, respectively, during an experimental period of one year. Values are based on 5 g of dry sediment.

The potential sources of phthalate contamination must be known in order to reduce their effect on the measurement. Our approach to this problem was based on pattern analysis. Each source of contamination has a characteristic group of phthalate esters each consisting of different species and concentrations. The contamination patterns of the materials that came into direct or indirect contact with sediment samples are presented

in Fig. 3. The blank patterns of earlier experiments showed a predominance of DEP. Based on these source patterns, the solvent and the drying oven were identified as major sources of phthalate contamination in the blank. The matrix blank level was subsequently lowered by distilling the solvent twice instead of once, and by adding charcoal to the vacuum oven air purification trap. Knowledge of source patterns also can be used to recognize spurious contamination which is not reflected in the blank measurements.

Closely related to the blank level are the limits of detection and determination (Table IV). These limits, as defined by the recent ACS guidelines for environmental analysis[14] were calculated using the standard deviation, σ, of the blank measurements, instead of simply the electronic

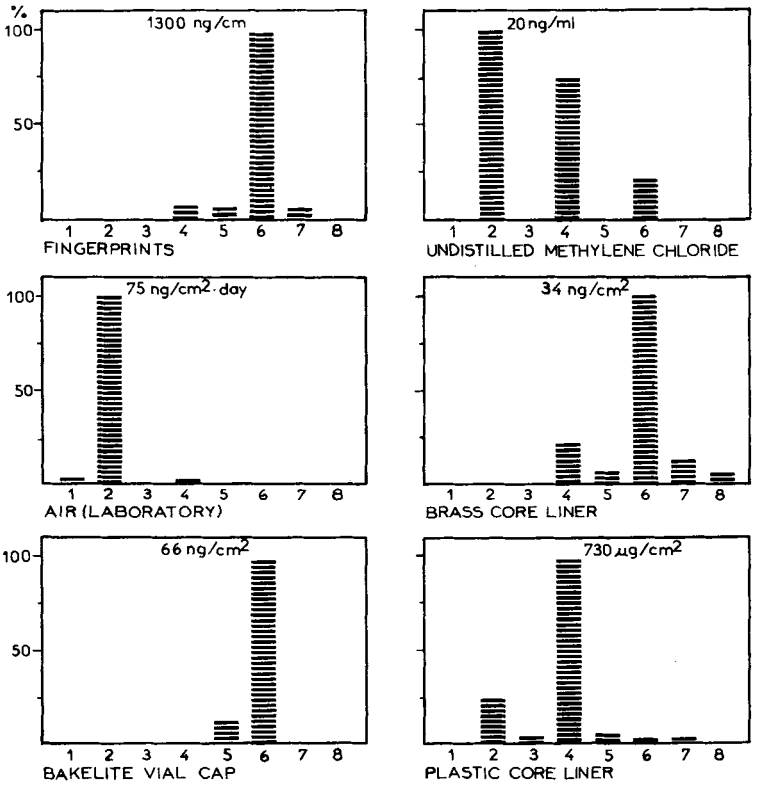

FIGURE 3 Phthalate ester contamination source patterns. The concentration of the dominant is indicated. The phthalate ester species are: 1 = DMP, 2 = DEP, 3 = DIBP, 4 = DBP, 5 = DHP, 6 = DEHP, 7 = DnOP, 8 = 8, 10 P.

noise of the instrument. Thus, while DEP and DBP have lower "instrumental" detection limits than DEHP, the corresponding limits for sediment analysis are higher than that of DEHP due to higher blank levels.

Analysis of Chesapeake Bay and Chester River sediments

Table V presents results of representative samples taken from sites in the Chesapeake bay and the Chester river, Maryland; the sample sites are in Fig. 4. The arrays of phthalates from each of the sites varies considerably indicating different phthalate sources and their varied behaviour in the environment. Site TP is a wastewater-holding pond adjacent to a plasticizer manufacturing plant outfall. This explains the extraordinarily high concentration level. Sites R and 55 reflect higher pollution levels in the upper Chesapeake bay compared to those in the middle to lower bay regions as indicated by Site 62. Site 5, in the Chester river is dominated by DBP as a possible result of an unreported spill in this generally non-industrialized area.

TABLE V

Results of Chesapeake bay and Chester river, Maryland, sediment samples

	Concentrations (ppb at site ± standard deviation), (n)				
ester	R	55	62	5	TP($\times 10^3$)
DEP	11 ± 2(6)	42 ± 8(3)	22(2)	26 ± 5(5)	< 0.1
DPB	1.8 ± 0.6(4)	13(2)	5.9(2)	3.5 ± 1.1(5)	0.2 ± 0.1(3)
DIBP	2.6 ± 0.9(4)	5.6(2)	< 1	2.9 ± 0.7(4)	< 0.1
DBP	28 ± 4(6)	89 ± 7(3)	27(2)	560 ± 230(5)	< 0.1
DHP	< 3	5.6(1)	< 3	2.4 ± 0.5(5)	$< 0.1 \times 10^3$
DEHP	110 ± 9(6)	180 ± 24(3)	12(1)	25 ± 3.9(5)	1200 ± 100(5)
DnOP	< 5	< 5	< 5	< 5	12 ± 3(3)
7, 9P	< 5	< 5	< 5	< 5	33 ± 7(3)
8, 10P	< 5	< 5	< 5	< 5	24 ± 4(3)
DIDP	< 25	< 25	< 25	< 25	690 ± 200(3)
DnDP	< 10	< 10	< 10	< 10	11(2)

(n) = number of replicates.
ppb = ng/g dry sediment.

The variability of the method during $2\frac{1}{2}$ years of implementation is illustrated in the quality control charts in Fig. 5. While the first measurements of reference sediment "R" were erratic, the measurements during the final eleven months became quite stable. This achievement coincided with the change of internal standard from dimethoxyethyl phthalate to deuterated anthracene.

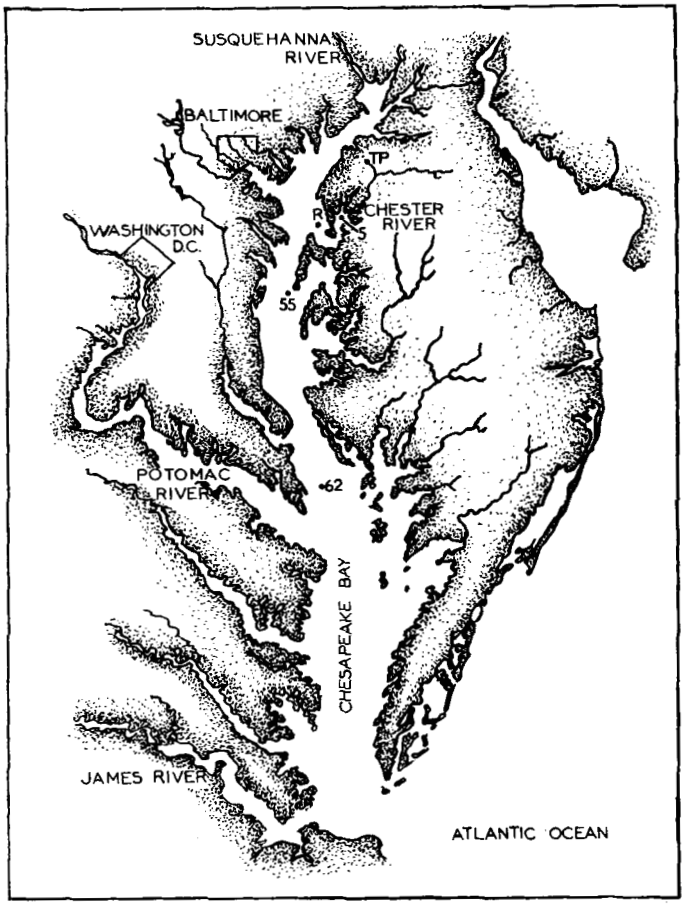

FIGURE 4 Map of the Chesapeake Bay illustrating sample sites 55, 62, R, 5, and TP.

Tests of quantitative accuracy

With no certified reference available for trace organics, quantitative accuracy was tested by comparisons of results on split samples with a U.S. government environmental research laboratory, results from an independent analytical method (HPLC), and results from an alternate quantitation technique (standard addition). These are reported in Table VI.

The laboratory exchange samples consisted of two collected sediment samples, one heavily contaminated (TP) and the other with trace contamination (R). Also, one attaclay sample (A) spiked with DEHP was included. The interlaboratory exchange resulted in reasonable agreement

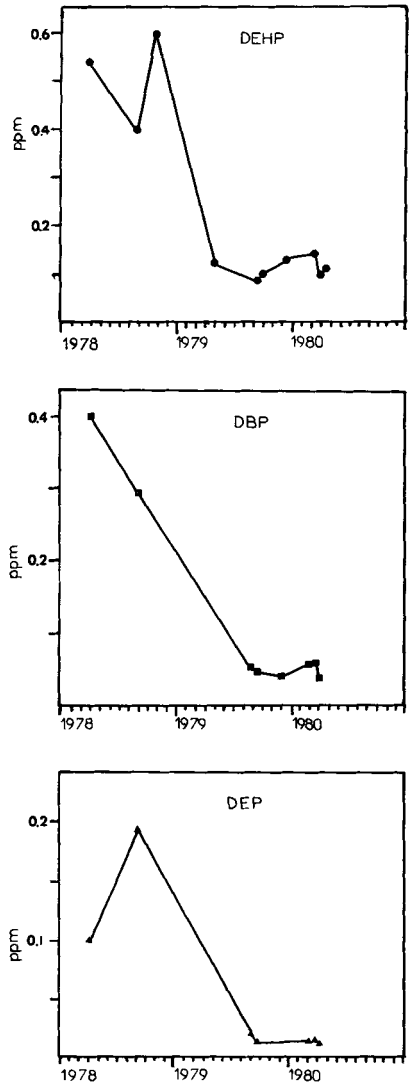

FIGURE 5 Quality control charts of analyses of the reference sediment R.

for samples with higher concentrations, TP and A. However, on the first round of exchanges, the values for site R differed by two orders of magnitude. Six months later, the exchange was repeated after our own laboratory completed detailed tests to evaluate the methodology. The results of the second exchange showed closer agreement, but the difference

TABLE VI

Comparison of results by two laboratories, two methods and two calibration techniques

Type of comparison	Sample	Compound	Results	
Interlaboratory:			U. of Md.	Laboratory A[a]
	Pond sediment (TP)	DEHP	1200 ± 100(5)	1700(1)
	Reference sediment (R)	DEHP	0.60 ± 0.10(3)	58(1)
			0.12 ± 0.10(6)	0.3(1)
	Attaclay, spiked (A)	DEHP	200 ± 10(5)	121(1)
Method:			GC-MS-SIM	HPLC[b]
	Pond sediment (TP)	DEHP	1200 ± 100(5)	1300 ± 200(4)
Calibration techniques			Internal standard	Standard addition
	Chester river sediment, site 4		0.092(1)	0.035(1)
			0.540(1)	0.46(1)
			0.072(1)	0.087(1)

[a]Ultrasonic extraction. Florisil clean-up, quantitation by GC-MS-SIM with external standards.
[b]Reversed-phase HPLC with Bondapak-C_{18} column.

remained substantial. The participating laboratory commented at the time of the first exchange that their laboratory had been experiencing contamination problems which were traced to their ultrasonic disrupter probe. It is not possible to determine which laboratory was closer to the true values on the second exchange, but the higher value of the participating laboratory could be due to continued ultrasonic probe contamination.

DEHP in sample TP was measured by reversed-phase HPLC. These results (1300 ± 200 ppm) showed close agreement with the GC-MS-SIM technique (1200 ± 200 ppm). The comparison of the internal standard calibration technique to the standard addition technique also showed good agreement for DBP (0.54 ppm/0.46 ppm) and DEHP (0.072 ppm/0.087 ppm). The reason for a discrepancy in the DEP concentration (0.092 ppm/0.035 ppm) is not known. Losses due to the higher volatility of DEP do not explain the difference since such an occurrence would result in lower values for the internal standard technique.

Conclusion

Phthalate esters can be extracted quickly and reproducibly by ultrasonication after sediment has been dried in a controlled atmosphere.

Cumbersome error-prone clean-up steps can be eliminated from the sample preparation scheme by using the selective detection capabilities of GC-MS-SIM. It was found possible to detect quantities as low as 100 pg of DEHP in an injected crude estuarine sediment extract.

A program of quality assurance was applied to validate the results by this method. Results of reference sediment analyses remained stable over an extended period and have compared closely to results from other analytical techniques and independent laboratories.

These methods have since been applied to the extraction and analysis of other neutral, nonpolar compounds such as normal hydrocarbons and polynuclear aromatic hydrocarbons with equal success as with phthalate esters. It is therefore possible to analyze various compound classes in a single unfractionated sediment extract quickly and accurately.

Acknowledgements

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