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DETERMINATION BY ELECTRON-CAPTURE GAS CHROMATOGRAPHY OF MONO- AND DI(2-ETHYLHEXYL) PHTHALATE IN INTRAVENOUS SOLUTIONS STORED IN POLY(VINYL CHLORIDE) BAGS

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SUMMARY

A gas chromatographic method has been developed for the simultaneous determination of mono- and di(2-ethylhexyl) phthalate (MEHP and DEHP). The compounds were extracted with methylene chloride at pH 2.5 and the monoester was alkylated to the hexyl derivative by solid–liquid phase-transfer catalysis in methyl ethyl ketone. Quantitation was effected by electron-capture gas chromatography. The sensitivity limits of the method are 4 μg/l of DEHP and 1 μg/l of MEHP. The relative standard deviations are 4.2% for DEHP (31 μg/l) and 3.7% for MEHP (27 μg/l) ($n = 10$).

INTRODUCTION

Di(2-ethylhexyl) phthalate (DEHP) is used as a plasticizer in poly(vinyl chloride) (PVC), which is frequently used in medical items such as bags for blood transfusions and intravenous solutions. Many investigations have been published during the last decade about the migration of DEHP from PVC bags into blood products, and levels from 16 to 120 mg/l have been reported[1–6]. The content of DEHP in intravenous solutions stored in PVC bags is about 1000 times lower than in blood products[4,7]. DEHP has been determined by gas chromatography with electron-capture[3,4,8–11] or flame-ionization detection[1,2,5,6,8,9,12–14] and by liquid chromatography[15–18].

Mono(2-ethylhexyl) phthalate (MEHP), a hydrolysis product of DEHP, has been determined in biological material by gas chromatography after methylation with diazomethane[19,20] and trimethylanilinium hydroxide[21]. The reported levels[19,20] in blood products stored in PVC bags are 4–56 mg/l. DEHP and MEHP have been determined simultaneously in intravenous solutions by gas chromatography–mass spectrometry after ethylation with diazoethane[7].

This paper describes a method for the simultaneous determination of DEHP and MEHP in low concentrations by electron-capture gas chromatography after alkylation of MEHP to the hexyl derivative by solid–liquid phase-transfer catalysis.

EXPERIMENTAL

Gas chromatography

A Varian 1400 gas chromatograph with a direct-current ^3H electron-capture detector was used. The glass column (180 × 0.2 cm) was filled with 3% XE-60 on Gas-Chrom Q (80–100 mesh). The column was operated at 190 °C and the injector and detector at 210 °C. The flow-rate of the nitrogen carrier gas was 30 ml/min.

Reagents and chemicals

Methylene chloride, methyl ethyl ketone and toluene (Merck, Darmstadt, G.F.R.; analytical grade) (all distilled in a Büchi Rotavapor R), tetrabutylammonium (TBA) hydrogen sulphate (Lab Kemi, Stockholm, Sweden), sodium hydrogen carbonate (Merck; analytical grade), hexyl iodide (Fluka, Buchs, Switzerland), di(2-ethylhexyl) phthalate (DEHP) (Fluka), mono(2-ethylhexyl) phthalate (MEHP) (a gift from Hässle, Mölndal, Sweden), di(3,5,5-trimethylhexyl) phthalate (dinonyl phthalate, DNP) (Fluka), used as an internal standard, sulphuric acid (1 mol/l) (Merck; analytical grade), hydrochloric acid (1 mol/l) (Merck; Suprapur) and phosphate buffers (ionic strength 0.1) (sodium phosphates; Merck; analytical grade) were used.

Pre-dissolved base. A 40.00-ml volume of methylene chloride or methyl ethyl ketone containing TBA hydrogen sulphate (10^{-3} mol/l) was shaken for 20 min with 6.00 g of sodium hydrogen carbonate and filtered[22,23].

Distribution studies

The distribution experiments were performed in centrifuge tubes by mechanical shaking at 25 °C using an equilibrium time of 20 min. MEHP was dissolved in the organic phase. Equal phase volumes were used except at pH < 4 for *n*-heptane and pH < 7 for methylene chloride, when an organic phase of 4.00 ml and an aqueous phase of 30.00 ml were used. The concentration of MEHP in the organic phase was determined by photometric measurements at 274 nm. The concentration in the aqueous phase was calculated as the difference between the initial concentration in the organic phase and the concentration in the organic phase found at the equilibrium stage.

Evaluation of reaction conditions for the alkylation of MEHP

The reaction was studied with methylene chloride or methyl ethyl ketone as solvent in a thermostated water-bath at 20.0 ± 0.1 °C. A 20.00-ml volume of a solution of pre-dissolved base was mixed with 10.00 ml of methylene chloride or methyl ethyl ketone containing MEHP (2×10^{-4} or 4×10^{-3} mol/l) and the internal standard. A 10.00-ml volume of a solution of hexyl iodide (4 or 0.4 mol/l) was added and 1.00 ml of the mixture was removed at various times up to 24 h and shaken with 0.2 ml of sulphuric acid (1 mol/l) to stop the reaction. The organic phase was analysed by GC to determine the hexyl ester.

Procedure for the determination of DEHP and MEHP in intravenous solutions stored in PVC bags

A 200.0-ml volume of the intravenous solution was transferred to a separating funnel and hydrochloric acid (1 mol/l) was added to pH 2.5. The mixture was

extracted with 10.0 ml of methylene chloride containing the internal standard. The methylene chloride extract was filtered through glass-wool, collected in a centrifuge tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 1.0 ml of methyl ethyl ketone containing pre-dissolved base after which 1.0 ml of methyl ethyl ketone containing hexyl iodide (0.2 mol/l) was added. After 30 min at room temperature the reaction mixture was shaken with 1.0 ml of sulphuric acid (1 mol/l) and centrifuged. The organic phase was transferred to another tube and evaporated in a stream of nitrogen. Toluene (1.0 ml) was added, and the solution was shaken with saturated silver sulphate in water. After centrifugation, 1 μl of the organic phase was analysed by GC.

A calibration graph was constructed using five standard samples containing 200.0 ml of distilled water and increasing amounts of MEHP and DEHP. These standard samples were treated according to the method described above.

RESULTS AND DISCUSSION

The general contamination of solvents and laboratory equipment with DEHP and other phthalate esters has been noted by many investigators[3,10,14]. This contamination was a problem in the analysis of DEHP at low concentrations. The content of DEHP in the organic solvents could be reduced by distillation. All glassware was carefully washed with a detergent, thoroughly rinsed with glass-distilled water and ethanol, and the chemicals were tested prior to use so as to be able to choose the qualities and batches with the lowest possible content of DEHP.

Extraction conditions

Methylene chloride was chosen for the extraction of DEHP and MEHP as it gave a very high distribution of the compounds. This was needed as a large volume of the intravenous solution had to be extracted, and the volume of the organic phase had to be as small as possible owing to the contamination of phthalate esters in the organic solvents. Fig. 1 shows the distribution ratio of MEHP as a function of

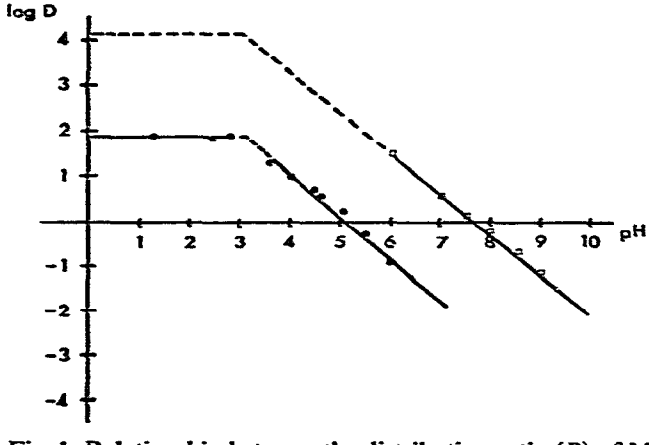

Fig. 1. Relationship between the distribution ratio (D) of MEHP and the pH of the aqueous phase. Organic phase: □, methylene chloride; ●, n-heptane.

TABLE I

ALKYLATION OF MEHP

Conditions as described under Experimental.

Solvent	*MEHP concentration (mol/l)*	*$TBAHSO_4$ concentration in the pre-dissolution step (mol/l)*	*Hexyl iodide concentration (mol/l)*	*$k' \cdot 10^3$ (min^{-1})* (refs. 22 and 23)*	*Calculated time for 99% alkylation(min)*
Methylene chloride	$0.5 \cdot 10^{-4}$	10^{-3}	1.00	4.4	105
Methyl ethyl ketone	$0.5 \cdot 10^{-4}$	10^{-3}	1.00	–	<2
Methyl ethyl ketone	$0.5 \cdot 10^{-4}$	10^{-3}	0.10	44	11
Methyl ethyl ketone added to th · evaporation residue from the extraction	$0.5 \cdot 10^{-4}$	10^{-3}	0.10	24	19
Methyl ethyl ketone	10^{-3}	10^{-2}	0.10	42**	11
Methyl ethyl ketone, undistilled	10^{-3}	10^{-2}	0.10	27**	17

* k' = Observed rate constant obtained from the slope of the pseudo-first-order plot[22,23].

** Determined by GC with flame-ionization detection.

pH with *n*-heptane and methylene chloride as organic solvents[24]. The study with *n*-heptane as the organic phase was performed in order to be able to evaluate the acid dissociation constant (K_{HA}^*) of MEHP. This could not be done with methylene chloride for experimental reasons, owing to the very high distribution to this solvent. The study with *n*-heptane as the organic phase indicates a pK_{HA}^* value of about 3.1 for MEHP. The curve for methylene chloride was plotted from that value which gave a distribution constant of *ca.* 10,000. The distribution of DEHP was even higher as it contains an additional lipophilic 2-ethylhexyl chain. The conditions described in the procedure resulted in > 99% extraction of DEHP, MEHP and the internal standard (DNP).

Preparation of the hexyl ester of MEHP

The alkylation of MEHP has been effected previously with diazomethane[19,20], diazoethane[7] and trimethylanilinium hydroxide[21]. Both the methyl and ethyl derivatives of MEHP have much shorter retention times than DEHP and DNP on the gas chromatographic column. The hexyl ester derivative of MEHP was chosen so as to be able to determine the three components simultaneously. The alkylation was effected by a solid–liquid phase-transfer catalysed process which has been investigated previously in this laboratory[22,23]. This process resulted in quantitative alkylation of benzoic acid, and the reaction rate could be controlled by the choice of solvent and the concentration of the alkyl iodide[22,23]. Table I shows that 0.1 mol/l of hexyl iodide in methyl ethyl ketone resulted in quantitative alkylation within 10 min; a much higher concentration of the hexyl iodide was needed in methylene chloride. There were two reasons for keeping the concentration of the alkylating agent as low as possible: (1) the hexyl iodide was contaminated with rather high concentrations of DEHP and (2) the reagent had to be removed by evaporation prior to the GC analysis as it had a high electron-capture response which caused disturbances in the chromatogram.

Previous experiments have shown that the alkylation reaction was slower in the presence of water[23]. For this reason, the influence of the initial extraction step on the alkylation rate was investigated. Table I shows that the reaction rate was slower after extraction and the subsequent evaporation of a methylene chloride phase compared with the direct reaction in methyl ethyl ketone. Most of the co-extracted water was probably distilled azotropically when methylene chloride was evaporated[25,26].

Distillation of methyl ethyl ketone resulted in higher reaction rate although methyl ethyl ketone of analytical quality was used.

The reaction yield was more than 99%. This was checked by analysing the underivatized MEHP in the last two alkylation experiments shown in Table I (10^{-3} mol/l of MEHP). The analyses were carried out by high-performance liquid chromatography[18].

Gas chromatography

The stationary phase used was 3% XE-60 as it produced good separations and peak symmetry. This column has been used for several months without any sign of deterioration. Phthalates can be determined with high sensitivity and selectivity by electron-capture detection. The mechanism of electron capture of the phthalate esters

and the MDQ value of DNP ($5.2 \cdot 10^{-14}$ mol/sec) have been reported earlier[3,10]. Prior to the GC analysis the toluene solution was washed with a silver sulphate solution[27–30] to prevent the long tailing front in the chromatogram caused by degradation products from quaternary ammonium halide formed as a by-product in the alkylation reaction.

Fig. 2 shows a gas chromatogram from an analysis of 5% glucose intravenous solution containing 34 μg/l of DEHP and 39 μg/l of MEHP.

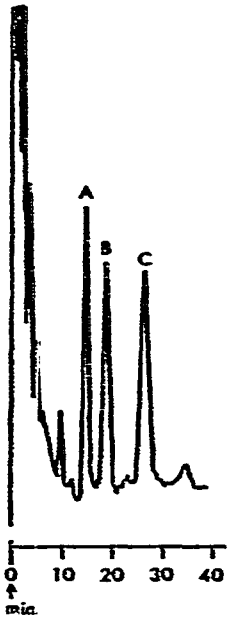

Fig. 2. Gas chromatogram of a 5% glucose intravenous solution containing 34 μg/l of DEHP and 39 μg/l of MEHP. A = Hexyl ester of MEHP; B = DEHP; C = DNP.

Stability

Both DEHP and DNP were stable during the analytical procedure. This was checked by analysis of samples spiked with the two compounds. The hexyl ester of MEHP was stable in the reaction mixture for several days.

Sensitivity and precision of the method

The sensitivity and precision of the analysis of DEHP were dependent on the extent of contamination of the reagents and chemicals by the compound. The reagent blank corresponded to a concentration of about 2 μg/l of DEHP, which means that the lower limit for quantitative determinations was about 4 μg/l. However, DEHP could be determined down to 1 μg/l if the alkylation reaction was omitted, as this step contributed to a great extent to the reagent blank. MEHP could be determined down to 1 μg/l with acceptable precision as the reagent blank was much lower in this instance.

The relative standard deviations of the method for the simultaneous determination of 31 μg/l of DEHP and 27 μg/l of MEHP were 4.2 and 3.7%, respectively

($n = 10$). The relative standard deviation at a concentration of 10 μg/l of DEHP was 8.0% ($n = 10$).

If the alkylation step was omitted, the relative standard deviation for the determination of DEHP was much lower (1.9% at 20 μg/l, $n = 10$).

Concentrations of DEHP and MEHP in intraveous solutions stored in PVC bags

Table II shows some results of analyses of MEHP and DEHP in intravenous solutions of different ages. The identities of DEHP and the hexyl ester of MEHP were confirmed by GC–mass spectrometry on the final toluene extracts from some of the samples. The concentrations were much lower than in blood products stored in PVC bags. The investigated solutions were all sterilized at 120 °C, except one solution of 0.9% saline. This bag was shaken in a shaking apparatus for 3 h before analysis. The concentration of MEHP in the solution was below the detection limit of the method, indicating that the amounts of MEHP in the solutions were probably formed by the hydrolysis of DEHP during heat sterilization.

TABLE II

CONCENTRATIONS OF DEHP AND MEHP IN INTRAVENOUS SOLUTIONS STORED IN PVC BAGS

Solution	*Volume (ml)*	*Age (months)*	*Concentration (μg/l)*	
			DEHP	*MEHP*
5% glucose	1000	5	4	27
		12	4	31
5% glucose	500	0.5	34	39
		3	7	12
0.9% saline	1000	5	7	200
		14	24	110
0.9% saline	500	0.5	13	219
		3	< 4	276
0.9% saline	100	6	8	46
0.9% saline*	1000	—	8	<1

* Not heat sterilized.

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