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Note

Liquid chromatographic method for the determination of phthalate esters

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Phthalate esters are probably one of the most common plasticizers employed in the plastics industry. They are added frequently to paints, lacquers and plastic materials to reduce rigidity and impart flexibility to solids. Particular attention has been paid recently to their role as plasticizers in plastics used for packaging food and storing blood. It has been reported that in some of these applications, phthalates have found their way into the human body by the leaching of plastic materials, notably polyvinyl chloride (PVC). Jaeger and Rubin[1] have shown that blood packaged in PVC units can, in a few months time, accumulate plasticizer levels of up to 5–7 mg per 100 ml blood. An editorial article in the British Medical Journal[2] has also reviewed the biological effects of phthalate plasticizers. Recently Vessman and Rietz[3] have developed a method to determine nanogram levels of phthalates in plasma and fractionated plasma proteins. The increasingly widespread use of these compounds has resulted in the development of IR, gas chromatographic and, more recently, liquid chromatographic (LC) methods for their determination.

IR methods of analysis are not specific enough to distinguish between closely related members of a phthalate series. Furthermore, these methods cannot be used for the analyses of mixtures and lack the sensitivity necessary for trace analysis[4].

Gas–liquid chromatography has been described by Esposito[5] and also by Krishen[6] in their determination of plasticizers. Temperature-programmed gas chromatography was used after methylation and hydrolysis of some of the plasticizers found in polymeric materials. Vessman and Rietz[3] used an electron capture detector for the trace analysis of phthalates.

Lately, LC has received a great deal of attention in the analysis of relatively high-molecular-weight compounds. This technique, however, cannot easily resolve neighboring members of the homologous series of both high- and low-molecular-weight phthalates without using gradient elution in a reversed-phase system or employing a series of different solvents in a normal phase separation[7].

This note reports a LC procedure for the determination of phthalates found in industrial and biological samples. The method is simple, rapid and does not require gradient elution or solvent change during separation in the analyses of mixtures containing both high- and low-molecular-weight phthalates.

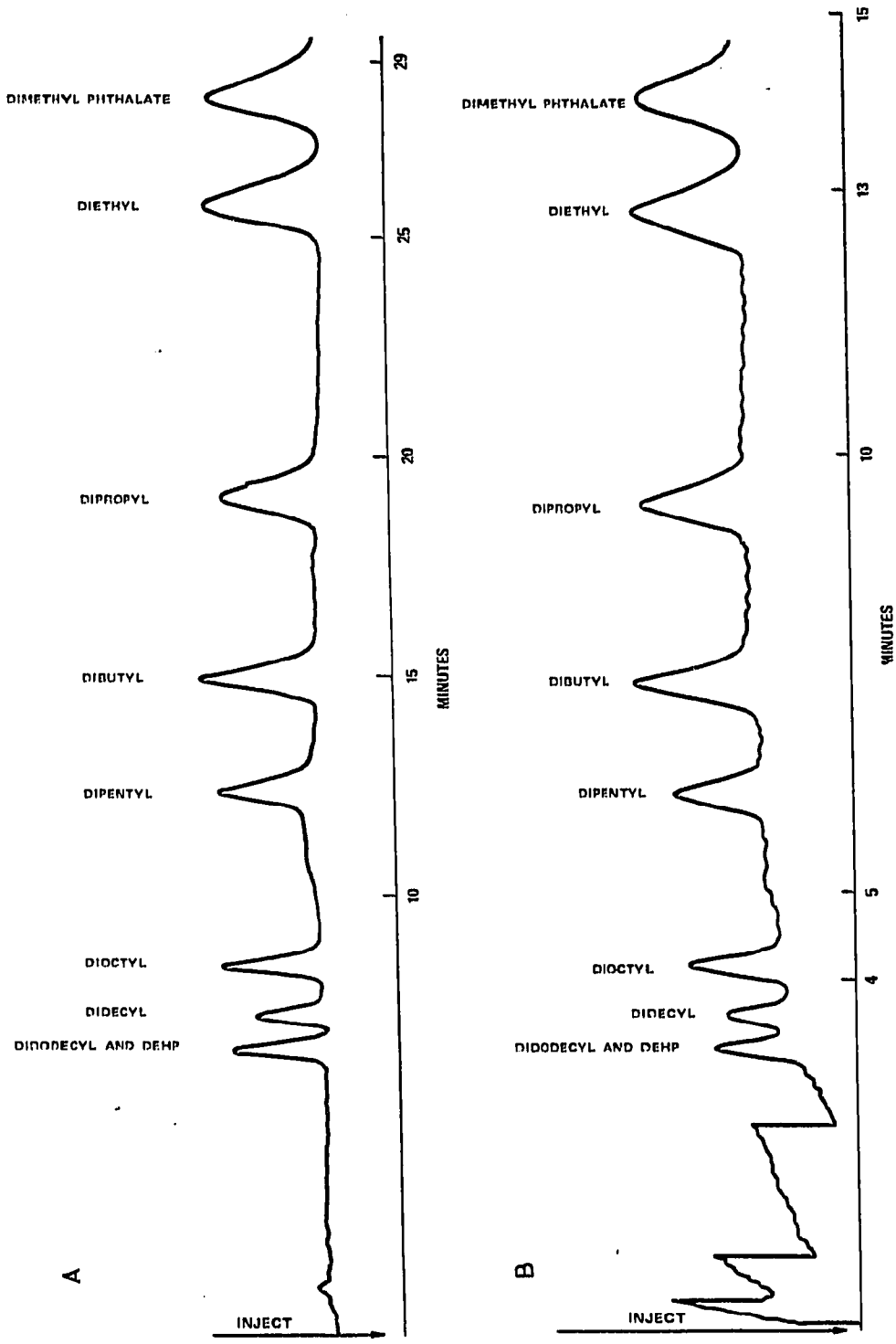

Fig. 1. Typical chromatograms of a mixture of phthalates. Flow-rates: (A) 3 ml/min; (B) 6 ml/min.

EXPERIMENTAL

Apparatus and reagents

A Waters Associates Model 202 liquid chromatograph equipped with a Model 6000 high-pressure pulseless liquid pump and a UV detector absorbing at 254 nm was used. Analyses were carried out on a 1-ft.-long, 10-μm Porasil polar column. Normal phase separation was used with 50% methylene chloride–50% isooctane mobile phase. The solvents were glass-distilled reagents obtained from Burdick and Jackson Labs. (Muskegon, Mich., U.S.A.). A flow-rate of 3 ml/min was maintained with a column pressure up to 1500 lb./sq.in. Reagent-grade phthalates (Eastman-Kodak, Rochester, N.Y., U.S.A., and Fisher Scientific, Pittsburgh, Pa., U.S.A.) were used as standards. Septum injections of 1 μl were made with a high-pressure injection syringe manufactured by Scientific Glass Engineering Ltd. Standards were made up to 0.1% concentration using eluent solvent. Three-millilitre fractions of LC effluent were collected in a 5-ml vial containing 25 mg of KBr. The solvent content of the vial was evaporated using dry nitrogen. The residue consisting of KBr and sample was pressed into a micropellet. Spectra were recorded with a Perkin-Elmer 621 IR spectrophotometer using a Perkin-Elmer Refractive Beam Condenser.

Sample preparation

Lacquers consisting of a cellulose-type polymer (nitrocellulose or ethylcellulose), a low-molecular-weight phthalate and a mixture of solvents were analyzed. Excess methanol was first added to the lacquer solution to precipitate the cellulose material. After centrifugation, the supernatant liquid was separated and its solvent content evaporated. The residue was redissolved in the methylene chloride–isooctane solvent mixture which had been drawn from the reservoir of the liquid chromatograph and brought up to 10-ml volume.

Solid plastics were extracted with methanol at room temperature for several hours. The mixture was centrifuged and the supernatant liquid evaporated with a stream of dry nitrogen. The residue was redissolved in the eluent solvent.

Blood samples of 10 ml were taken from a standard PVC blood packaging unit and extracted with 20 ml of toluene three times. The organic layer was separated by centrifugation and the supernatant toluene layer removed and evaporated to dryness under a dry nitrogen stream. The residue was redissolved in 1 ml of the methylene chloride–isooctane eluent.

The buffer solution present in the blood packaging bags was also examined for phthalate contamination using a similar extraction and analysis procedure.

RESULTS AND DISCUSSION

A typical chromatogram shown in Fig. 1A, was run at a flow-rate of 3 ml/min and required about 30 min to complete. The same separation, using a flow-rate of 6 ml/min, took only 14 min and is shown in Fig. 1B. The solvent system used provides good resolution from C_1–C_{12} without resorting to gradient elution or to the use of a series of solvents. Of the two lacquer solutions analyzed, one was found to contain 3% dimethyl phthalate, the other 1% dibutyl phthalate. These results were also checked by IR analyses and confirmed gravimetrically. The calibration curve constructed

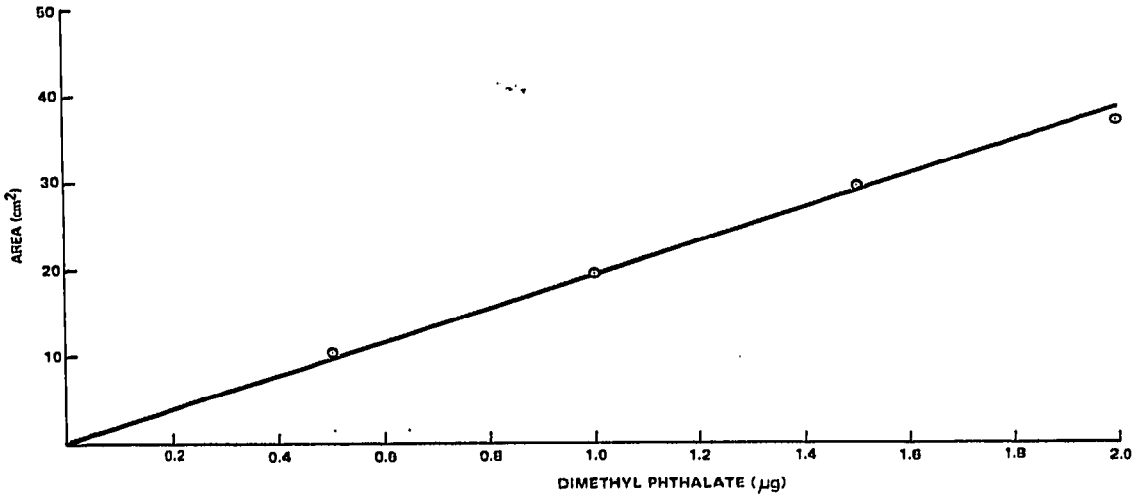

Fig. 2. Calibration curve for the determination of dimethyl phthalate.

for the determination of dimethyl phthalate is shown in Fig. 2. This curve projects a limit of detection of 0.1 μg of dimethyl phthalate. Under the conditions used in this work, the low-molecular-weight phthalates elute last from the column and increasing band broadening is observed. As a result, lowest sensitivity can be expected for low-molecular-weight phthalates (C_1–C_5) while higher-molecular-weight phthalates (C_6–C_{12}) with much shorter retention times will exhibit higher sensitivities, with the limit of detection approaching 0.05 μg of phthalate.

PVC used in the fabrication of a blood packaging unit was analyzed for the qualitative identification of the plasticizer added. Analysis by high resolution LC identified the plasticizer as di-2-ethylhexyl phthalate (DEHP). It was found that since

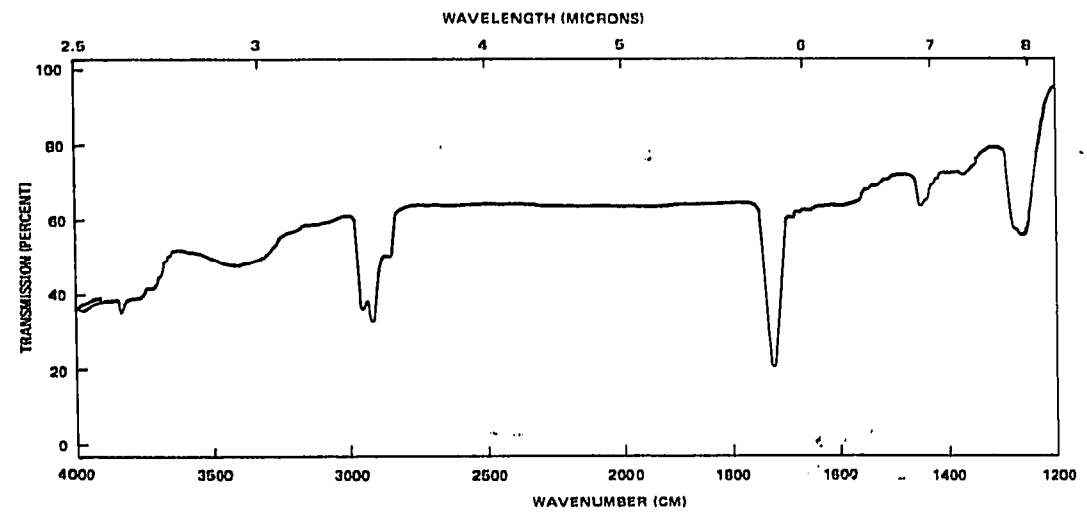

Fig. 3. IR spectrum of an LC fraction (DEHP).

didodecyl phthalate has a retention time close to DEHP, it was necessary to distinguish the plasticizer found in the PVC bag from didodecyl phthalate. This was accomplished by IR spectroscopy.

Eluent fractions from LC were collected and dispersed in KBr. Micropellets were prepared and the spectrum obtained (Fig. 3) showed that the plasticizer was DEHP.

The buffer solution present in the blood packaging unit was analyzed by LC and no phthalate was detected. Blood serum was contacted for 24 h with strips of the plasticized PVC taken from a blood packaging unit. At the end of this period, the test serum was analyzed and found to be contaminated with DEHP. Whole blood was then removed from a packaging unit with a recently expired shelf life (blood in contact with plastic bag for four months) and an extraction performed. Analysis of these samples showed an average of 5 mg of phthalate per 10 ml of whole blood. These results tend to confirm and extend previously reported findings[1,2], namely, the buffer solution does not extract plasticizer from a PVC blood unit, while stored whole blood can become contaminated and accumulate relatively high amounts of phthalate.

In summary, the method described avoids the cumbersome use of gradient elution and variation of types of solvents. It is simple to use and rapid requiring very little sample preparation and instrument time. By using high pressure solvent delivery (*ca.* 3000 lbs./sq. in.), elution times are shortened and analyses can be completed in about 15 min. without a substantial loss of resolution.

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