# High-performance Liquid Chromatographic Determination of Di(2-ethylhexyl) Phthalate in Blood Stored in PVC Blood Bags

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Poly(vinyl chloride) (PVC) materials in medical applications have produced remarkable therapeutic benefits to patients.[1,2] However, it was recognised by Trimble *et al.*[3] and Guess *et al.*[4] that the plasticiser di(2-ethylhexyl) phthalate (DEHP) is leached from PVC by blood and various injection solutions. The leachability of phthalate plasticisers from PVC containers and their subsequent presence in intravenous fluids, blood and blood products have been extensively documented[1,5–8] and the toxic hazard associated with phthalate plasticisers has been a matter of serious concern.[2,6,9,10] Transfusion of blood stored in PVC containers might lead to risks due to the plasticiser[5] used.

As part of a development programme with disposable PVC* blood bags at this Institute, it was necessary to monitor the extent of plasticiser leachability from our Chitra bags into blood and to compare it with the data obtained for similar blood bags currently in use (Tuta, Fenwal and Russian). Squirrell[11] has reported that high-performance liquid chromatography (HPLC) has now become a valuable tool for plastics analysis, particularly in the additive field. We report here the results of an HPLC procedure for determining plasticiser leachability into blood stored in disposable blood bags.

## Experimental

### Apparatus

The HPLC system was a Waters Model 6000 A solvent delivery pump equipped with a U6K injector, a μStyragel column (30 × 0.78 cm i.d., pore size 100 Å) and a Model 440 absorbance detector. Chromatograms were obtained on a Houston Instruments Omni-Scribe recorder and peak absorbance values were read directly from the digital display of the Model 440 system. Linearity of the absorbance scale was checked using chloroform solutions with known concentrations of DEHP.

### HPLC Conditions

A flow-rate of 0.2 ml $min^{-1}$ of the mobile phase was used and the absorbance was monitored at 254 nm.

### Reagents

DEHP (Indo-Nippon) was used for preparing plasma standards and Hatcol 200 (Hatco Chemical Company, Fords, NJ) (an ester of a branched oxo alcohol and 1,3-dihydro-1,3-dioxo-5-isobenzofurancarboxylic acid) was used as the internal standard. Spectroscopic-grade methanol (S.D. Chemicals, Bombay) and freshly distilled analytical-reagent grade chloroform (Glaxo Laboratories, Bombay) were used, the latter being the mobile phase.

### Plasma Samples

Plasma samples were obtained from calf blood stored at 4 °C in indigenous (Chitra) and imported PVC blood bags for 1, 7 and 21 d. The blood from the same animal was simultaneously stored in a glass bottle to serve as a control in each individual set of experiments.

### Sample Preparation

The concentration of DEHP in the standard plasma samples ranged from 10 to 100 μg $ml^{-1}$. These standards were prepared by evaporating to dryness aliquots from a 200 μg $ml^{-1}$ chloroform stock solution of DEHP and dissolving the residue in 5 ml of control plasma.

DEHP was extracted from the plasma standards and unknown samples by the following procedure. A 10-ml volume of methanol was added to 5 ml of plasma to precipitate the plasma proteins and the supernatant was collected after centrifuging at 1 000 *g* for 15 min. Methanol was evaporated by heating the supernatant at 60 °C and the residue was extracted with three batches of 5 ml of chloroform in a separating funnel. To 5 ml of concentrated chloroform extracts a 1.5-μl aliquot of a 5.47 mg $ml^{-1}$ chloroform solution of Hatcol 200 (internal standard) was added. A 100-μl volume of the chloroform extract was then injected on to the column.

Independent checks confirmed that the evaporation steps did not lead to contamination from the solvents and there was no detectable loss of DEHP.

## Results and Discussion

Under the experimental conditions used, DEHP and Hatcol 200 had retention times of 23.2 ± 0.1 and 20.9 ±0.1 min, respectively. Fig. 1 is a chromatogram of DEHP (77.5 μg $ml^{-1}$) and Hatcol 200 (15.6 μg $ml^{-1}$) in the mobile phase. Fig. 2(*a*) is a representative chromatogram of a control plasma with the internal standard added. All controls revealed the presence of DEHP. This contamination comes from tapping of the blood into a plastic (PVC) set before transferring into glass bottles to serve as controls. Identical blood tapping

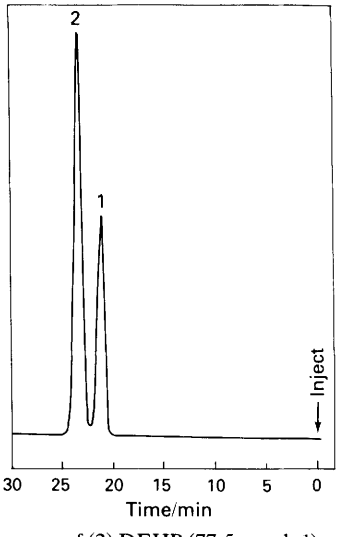


**Fig. 1.** Chromatogram of (2) DEHP (77.5 μg $ml^{-1}$) and (1) Hatcol 200 (15.6 μg $ml^{-1}$) in the mobile phase

* Compound No. 42, Polymer Technology Division.

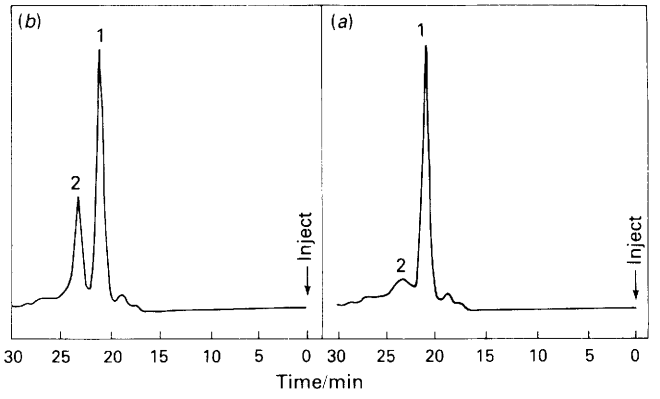


**Fig. 2.** Chromatograms obtained from calf plasma samples. (*a*) Control plasma sample stored in a glass bottle with (1) the internal standard Hatcol 200 added. Peak 2 corresponds to DEHP contamination of the control, giving an upper limit of 6.4 μg ml$^{-1}$. (*b*) Plasma sample from blood stored in a PVC bag. The DEHP peak (2) corresponds to a plasma concentration of 36.6 μg ml$^{-1}$

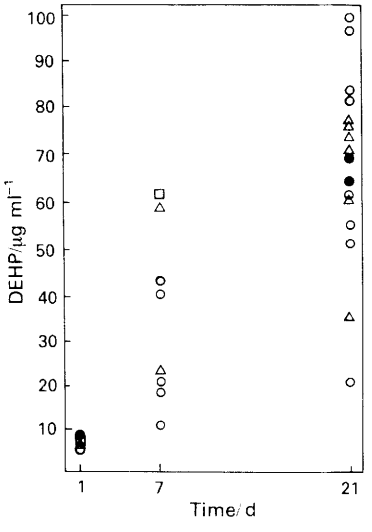


**Fig. 3.** Extent of leaching of DEHP by blood stored at 4 °C for 1, 7 and 21 d in different containers, showing individual variations. Blood bags: ○, Chitra; △, Tuta; □, Fenwal; and ●, Russian

procedures ensured that the extent of contamination due to this source, prior to storage, was the same for the sample and its control. The DEHP concentrations in the controls generally varied from 2 to 12 μg ml$^{-1}$ (in two controls, however, values as high as 17 and 24 μg ml$^{-1}$ were detected). These values are significant, as prior to storage contamination due to this source also occurs in the usage of blood bags. Vessman and Rietz,[12] for instance, in a study with Fenwal bags, found high levels of DEHP in blank samples corresponding to zero storage time. The limit of detection was about 2 μg ml$^{-1}$ and was essentially imposed by the blood tapping procedure employed. This was satisfactory for the present purpose as the expected values for the unknown samples were much higher. Moreover, the determinations of DEHP in the unknown samples were made against their respective controls as reference blanks.

Fig. 2(*b*) shows a representative chromatogram of an unknown sample with the internal standard added. The extraction and detection procedures used show a relatively clean plasma background (the most intense absorbance peak in the control still being due to DEHP contamination occurring prior to storage) in the chromatogram obtained.

Fig. 3 shows the extent of leaching of DEHP by blood stored at 4 °C for 1, 7 and 21 d in different containers. The values obtained in this study are of the same order of magnitude as those found by Marcel and Noel,[13] Piechoki and Purdy[14] and Vessman and Rietz.[12] It should be noted that there is a significant bag-to-bag variation for both indigenous and imported bags for the same storage duration. The values ranged from 21 to 95.5 μg ml$^{-1}$ of plasma in blood samples stored in eight indigenous bags for 21 d. This, to a certain extent, may be the result of possible inhomogeneities in the viscous sample. However, differences between individual bags (the DEHP content in ten different bags varied from 16 to 120 μg ml$^{-1}$) were also observed by Vessman and Rietz in their study of Fenwal blood bags.[12]

Hatacol 200 has been reported to show much less leachability than DEHP[15] and might replace the latter in PVC formulations used in medical applications (the present procedure can be easily adapted for the determination of Hatcol 200 in plasma with DEHP as the internal standard).

While much concern has been shown for the contamination of blood with plasticiser during storage and the better plasticisers are being sought, it should be noted that significant contamination of blood might also occur during tapping (body temperature) and prior to storage at 4 °C. Some attention should also be paid to this aspect.

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