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LITERATURE CORNER

SEP-PAK® CARTRIDGE ION-PAIR EXTRACTION OF PARAQUAT AND DIQUAT FROM URINE

Paraquat (P) and diquat (D) quaternary salts are used extensively throughout the world as non-selective herbicides, with most commercial preparations consisting of a mixture of the two. These compounds exhibit extreme toxicity in man and have been encountered in biological fluids and agricultural products.

Many of the reported HPLC methods for the analysis of these herbicides in biological fluids used ion-exchange columns or ion-pair extraction into organic solvents with the resulting chromatograms showing poorly shaped peaks (2,3).

A method developed by researchers in Great Britain has reported the use of pre-treated SEP-PAK^R C_{18} cartridges for the rapid extraction of the quaternary herbicides from urine followed by reversed-phase, paired-ion chromatography. SEP-PAK^R C_{18} cartridges were pre-treated according to Scheme I.

SCHEME I

(i) Wash C_{18} SEP-PAK^R cartridge with 5 ml water, 5 ml methanol, then 5 ml water.

(ii) Wash with 5 ml of 0.05% cetyltrimethylammonium bromide (solution is prepared in 0.5% ammonia).

(iii) Wash with 5 ml water, 5 ml methanol (2X), then 5 ml water.

(iv) Wash with 10 ml of 2% sodium heptanesulfonate (solution is prepared in 2% ammonia).

Following the pre-treatment of the SEP-PAK^R cartridge, the urine extraction is described in Scheme II.

SCHEME II

(i) 100 µl of internal standard 1,1'diethyl-4,4'-bipyridyldiylium diiodide (100 µg/ml in water) is added to 1 ml of urine; the solution is made alkaline with 200 µl of concentrated ammonia.

(ii) Pass through pre-treated C_{18} SEP-PAK^R cartridge.

(iii) Wash cartridge with 3 ml water, then 3 ml of methanol. Discard effluents.

(iv) Wash cartridge with 5 ml acidic methanol (1% HCl in methanol) to elute quaternary herbicides. Evaporate to dryness, dissolve residue in 500 µl of mobile phase and inject.

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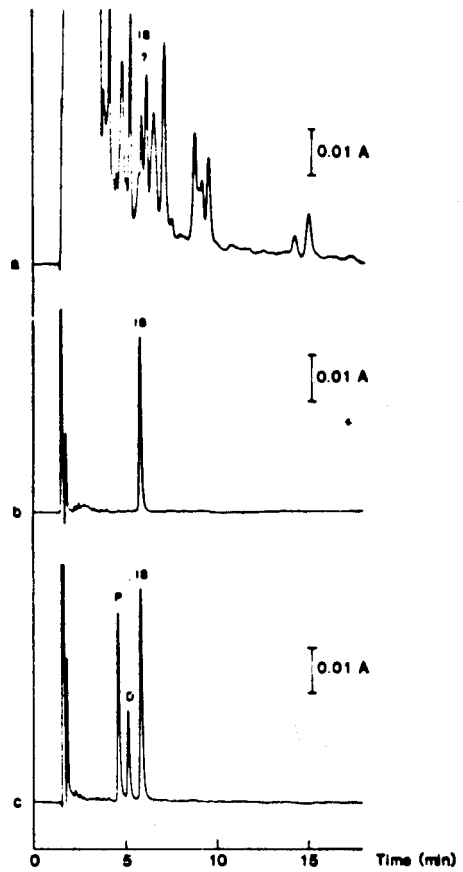
FIGURE 1: ANALYSIS OF URINE BY HPLC



CHROMATOGRAPHIC CONDITIONS:

Column:	C_{18}
Mobile Phase:	13.5 ml of o-phosphoric acid, 10.3 ml of diethylamine and 2.022 gr of sodium heptanesulfonate diluted to 1000 ml with 25% (v/v) aqueous methanol.
Flow Rate:	1.0 ml/min.
Detector:	UV at 290 nm.

(a) Blank urine spiked with internal standard (IS, 10 µg/ml), direct injection (20 µl); (b) Blank urine spiked with internal standard (10 µg/ml) following extraction procedure on SEP-PAK^R cartridge; (c) Blank urine spiked with paraquat dichloride (P, 4.96 µg/ml), diquat dibromide (D, 4.83 µg/ml) and internal standard (IS, 10.0 µg/ml) following extraction procedure on SEP-PAK^R C_{18} cartridge.

Figure 1 shows the results of the extraction procedure, along with chromatographic conditions. Recoveries of the paraquat, diquat and internal standard from the SEP-PAK^R extraction procedure were measured at four different concentrations and all results exceed 90%.

This method may prove to be useful for cleanup of other sample matrices not only for HPLC but for other analytical techniques as well (colorimetric reactions). In addition, other types of quaternary ammonium compounds (anti-cholinesterase drugs) could also be extracted by a similar approach.

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3. D. C. Paschal, L.H.Needham, Z. J. Rollen and J. A. Liddle, J. Chromatogr. 177, 85 (1979).