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Letter to the Editor

Comparison of solid-phase extraction and liquid–liquid extraction for high-performance liquid chromatographic analysis of antipyrine in plasma

Sir,

Antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one; phenazone) has been used to study the influence of drugs, diseases, environmental and genetic factors on drug metabolism in man. High-performance liquid chromatographic (HPLC) methods currently published for the analysis of antipyrine in plasma involve liquid–liquid extraction (LLE) [1–6]. We have developed a solid-phase extraction (SPE) technique in an effort to improve the sensitivity, reproducibility and analysis time over existing methods and have compared it to a published LLE method.

EXPERIMENTAL

Antipyrine and 4-dimethylaminopyrine (internal standard) were obtained from Aldrich (Milwaukee, WI, U.S.A.). Organic solvents (acetonitrile and methanol) were purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.). All other solvents and reagents were HPLC grade from Fisher Scientific.

The liquid chromatograph was a Gilson System 42 equipped with a Hewlett-Packard integrator. An Alltech 10 cm×0.46 cm I.D. (5 μm particle diameter) Spherisorb C_{18} cartridge column was used at a flow-rate of 1.3 ml/min. The mobile phase consisted of 0.05 *M* phosphate buffer (pH 6.5)–acetonitrile (70:30) and the absorbance detector was set at 254 nm (0.005 a.u.f.s.). Extraction cassettes (Analytichem) were processed by conditioning with methanol–Tris buffer (pH 9.0). Plasma samples were applied (500 μl) along with 50 μl of internal standard and the cartridges were washed with two 1.0-ml portions of phosphate buffer (pH 6.5). Elution was accomplished with 1.0 ml of methylene chloride and 10 μl of the eluent were injected after evaporation and reconstitution into 100 μl of mobile phase. The LLE method was modified from Eichelbaum and Spanbrucker [1]. The SPE method was optimized using Tris buffer with regard to pH, extraction solvent volume, eluent volume and cartridge reconditioning procedure.

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RESULTS AND DISCUSSION

Standards diluted in plasma were prepared freshly each day and found to be linear over the concentration range 0.05–2.5 μg/ml for both extraction methods. Three standard curves demonstrated correlation coefficients (r) of 0.9962, 0.998 and 0.9989 for LLE and 0.996, 1.0000 and 0.9969 for SPE.

Mean absolute recovery evaluated at six concentrations and expressed as a percentage of direct injection was 91.0% for SPE compared to 48.7% for LLE. The accuracy (percentage error of assayed samples relative to their spiked concentrations) of these methods are listed in Table I. The precision of the SPE method is somewhat better than that of the LLE method at all concentrations studied. This contradicts what the authors believe to be a popularly held bias that SPE methods are generally less reproducible.

TABLE I

ACCURACY AND PRECISION OF ANTIPYRINE METHODS

Spiked concentration (ng/ml)	n	Liquid–liquid extraction		Solid-phase extraction	
		R.S.D. (%)	Percent error	R.S.D. (%)	Percent error
75	10	9.8	−7.9	5.7	+0.1
500	10	4.7	−2.8	2.5	−1.5
2000	10	4.3	−0.8	3.6	+4.0

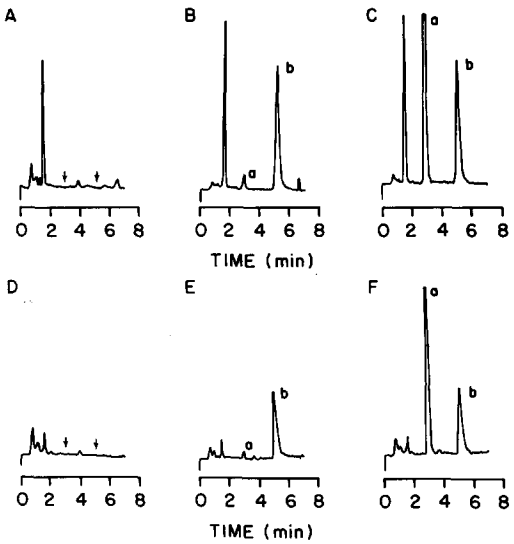

Fig. 1. Chromatograms of (A) blank plasma from LLE method, (B) plasma spiked at 50 ng/ml from LLE method, (C) plasma spiked at 3000 ng/ml from LLE method, (D) blank plasma from SPE method, (E) plasma spiked at 50 ng/ml from SPE method and (F) plasma spiked at 2000 ng/ml from SPE method. Arrows indicate the peak positions in blank chromatograms. Peaks: a = antipyrine; b = internal standard.

The limits of detection (defined as the concentration of antipyrine that would provide a signal equivalent to twice the noise level) was found to be 20 ng/ml in plasma for SPE and 50 ng/ml for LLE. The lower detection limit for SPE is most likely due to the improved recovery of the method. Adequate selectivity was demonstrated for both methods by the lack of interfering peaks in blank plasma chromatograms (Fig. 1). We conclude that the sorbent extraction method is more convenient, accurate, precise and sensitive than conventional LLE for the HPLC analysis of antipyrine.

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