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Note

Rapid determination of paracetamol in plasma by reversed-phase high-performance liquid chromatography

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High-performance liquid chromatography (HPLC) is now widely used for the analysis of drugs in biological fluids, mainly because of improvements in instrumentation. Methods of assays for paracetamol using HPLC in urine[1,2] and in plasma and urine after overdoses[3] have been published. However, these methods require either derivatization or large plasma samples for the analysis following a standard dose. This paper reports a rapid, sensitive and simple HPLC method for the routine determination of paracetamol in plasma.

EXPERIMENTAL

Reagents

Acetonitrile (HPLC grade), methanol (HPLC grade) and ethyl acetate (AnalaR) were purchased from Fisons (Loughborough, Great Britain).

Paracetamol and the internal standard, 3-acetamidophenol, were obtained from Aldrich (Gillingham, Great Britain). Phosphate buffer (pH 7.4, 1 *M*) was prepared using sodium dihydrogen orthophosphate dihydrate (AnalaR) and anhydrous disodium hydrogen orthophosphate (AnalaR), which were obtained from BDH (Poole, Great Britain).

Deionized water was further purified for HPLC by passing through two Whatman mini-filters, firstly grade 80 (8 μm) and secondly grade 10 (0.9 μm).

Instrumentation

A Spectra-Physics (St. Albans, Great Britain) Model SP8000 high-performance liquid chromatograph was used. The stainless-steel column (25 cm × 4.6 mm I.D.) was pre-packed with LiChrosorb RP-8, particle size 10 μm (Spectra-Physics). A 10-μl injection loop was used. Detection was made with a fixed-wavelength UV detector set at 254 nm. The mobile phase was water–acetonitrile (94:6, v/v) delivered at a constant flow-rate of 2 ml/min. The column temperature was maintained at 40° and the column inlet pressure was about 54 bar (5.4 MN/m^2).

The peak areas and retention times of paracetamol and 3-acetamidophenol were calculated by the data system of the SP8000. A calibration graph was obtained by plotting the peak-area ratios of paracetamol to 3-acetamidophenol against the

concentrations of paracetamol and the best straight line was found using the least-squares linear regression method (Texas Instruments SR-51-II calculator).

Sample preparation

A standard graph was prepared by adding a solution of paracetamol in methanol (5 mg/ml) to "blank" (no drug) human plasma so that the final concentrations of paracetamol in plasma were 0.2, 0.5, 2, 5, 10 and 20 μg/ml.

Duplicate samples (200 μl) of the plasma standards or unknowns were each pipetted into a Sovirel (Levalloix-Perret, France) 20-ml (16-mm O.D.) extraction tube to which were added 100 μl of a 50 μg/ml solution of 3-acetamidophenol in water, 1 ml of phosphate buffer (pH 7.4, 1 *M*) and 10 ml of ethyl acetate. The extraction tubes were sealed using screw-caps and mixed for 15 min along their long axes at 25 oscillations/min and then centrifuged at 1000 *g* for 10 min. The upper, ethyl acetate layer (9 ml) from each tube was transferred into a 10-ml BC24/C14T conical centrifuge tube (Quickfit & Quartz, Corning Ltd., Stone, Great Britain) and evaporated to dryness at 60° under nitrogen. Each residue was then taken up in 200 μl of methanol and 10 μl of the solution obtained was injected into the chromatograph.

Validation

An estimate of the reproducibility of the assay was obtained for concentrations of paracetamol of 0.5 and 20 μg/ml by carrying out six replicate analyses of each plasma standard.

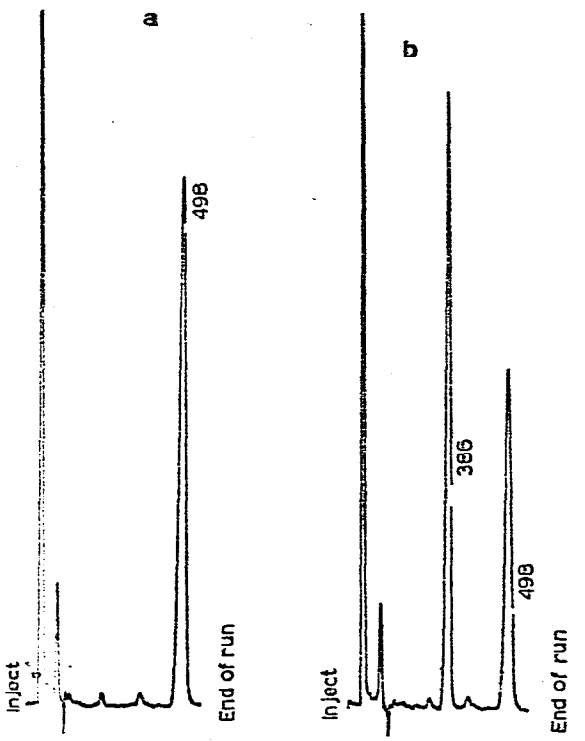

Fig. . HPLC determination of paracetamol in plasma. (a) Blank plasma containing internal standard only; (b) plasma standard containing paracetamol (20 μg/ml) and internal standard. Time in sec.

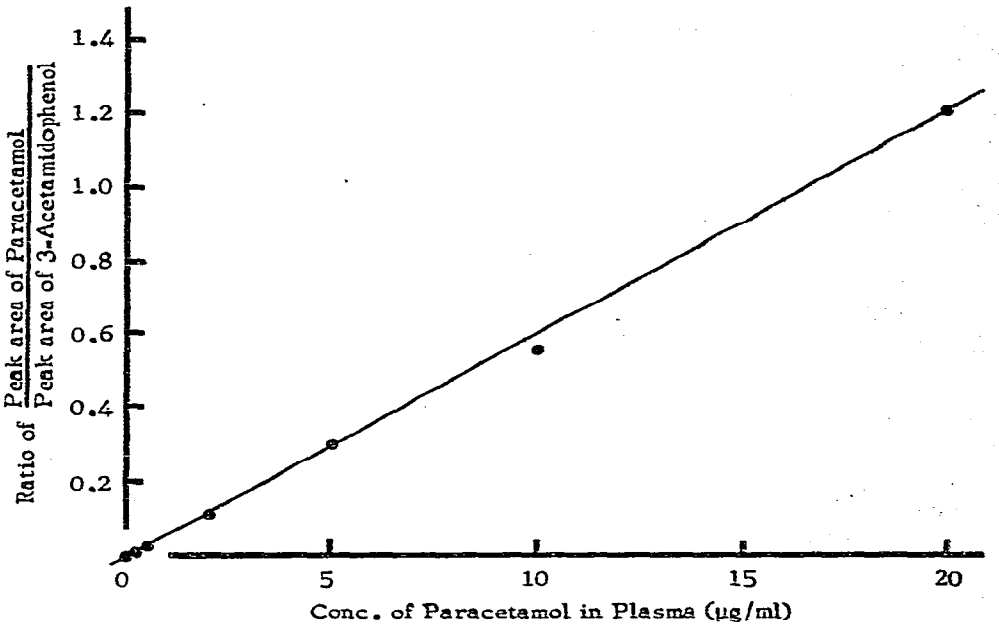

Fig. 2. Calibration graph of paracetamol in plasma.

RESULTS

Chromatograms resulting from the analysis of "blank" plasma to which the internal standard only was added and similarly treated plasma with paracetamol and the internal standard added are shown in Fig. 1. Paracetamol and 3-acetamidophenol eluted from the column as symmetrical peaks with retention times of 5.1 and 8.2 min, respectively. No interfering peaks were observed at the retention times of the compounds of interest. The total time required for one analysis was 9 min.

The standard graph (Fig. 2) for paracetamol in plasma was linear ($y = 0.6116x - 0.0069$, correlation coefficient $= 0.9995$, $2P < 0.001$) over the range of plasma concentrations from 0 to 20 μg/ml.

The precision of the method was determined by six replicate assays at concentrations of 0.5 and 20 μg/ml. The coefficients of variation for paracetamol were 6.2% and 1.7%, respectively. The readings for each of six replicates are shown in Table I.

TABLE I

ASSAY PRECISION

Concentration of paracetamol in plasma standard (µg/ml)	*Peak area of paracetamol/peak area of 3-acetamidophenol*								
	1	*2*	*3*	*4*	*5*	*6*	*Mean*	*Standard deviation*	*Coefficient of variation (%)*
0.5	0.026	0.028	0.024	0.027	0.026	0.024	0.0258	0.0016	6.2
20	1.217	1.220	1.188	1.233	1.252	1.223	1.222	0.0210	1.7

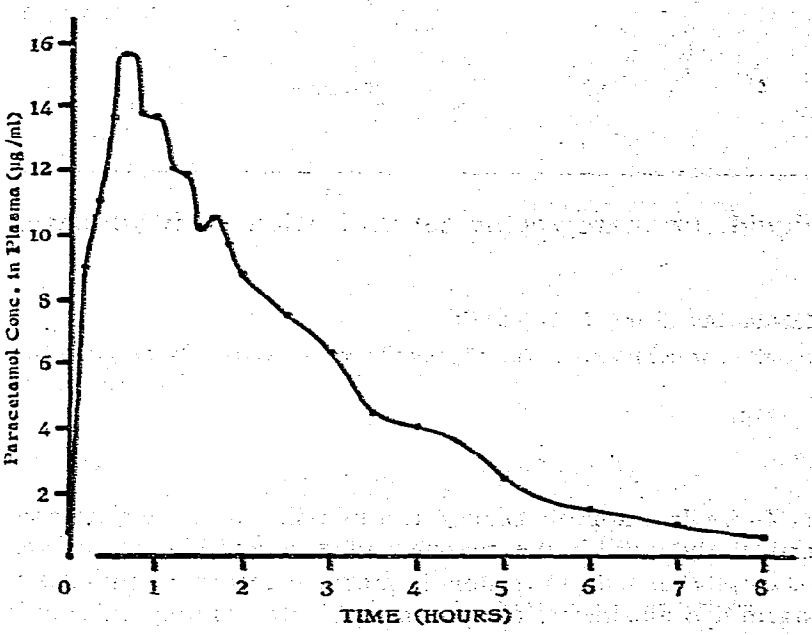

Fig. 3. A typical plasma profile from a healthy female after taking 500 mg of paracetamol as a tablet.

An example of the plasma concentration graph obtained from a healthy female volunteer after taking 500 mg of paracetamol as a tablet is shown in Fig. 3.

CONCLUSION

The HPLC method described permits the rapid determination of paracetamol in plasma down to 0.2 µg/ml. The preparation of plasma samples prior to chromatography is simple as only a single-step extraction is required. No derivatization procedure is necessary. Both the sensitivity and precision of the method are good and no interfering peaks are seen in plasma. Only a small sample size is needed for analysis, and therefore this method is suitable for routine clinical monitoring of plasma levels in patients and for use in research studies involving pharmacokinetics and bioavailability.

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