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Note

Determination of cimetidine in plasma and urine by high-performance liquid chromatography

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The analysis of cimetidine in biological samples by high-performance liquid chromatography (HPLC) has been reported by several workers [1–15]. Most of the HPLC analyses were done on normal-phase silica columns. They usually required a long equilibration time with the mobile phase and often resulted in long analysis times. Poor peak shape was a common problem encountered and high flow-rates were used in an attempt to overcome this problem [1–6, 11]. Sample clean-up prior to HPLC analysis often involved tedious solvent extraction techniques [12]. Apffel et al. [13], Bartlett and Segelman [14] and Nitsche and Mascher [15] used a C_{18} Sep-Pak® cartridge in the sample clean-up. In our experience a C_2 cartridge gave higher recovery of drug and internal standard. Procaine hydrochloride has been used as the internal standard [13] in the analysis of cimetidine using Sep-Pak C_{18} cartridges for sample clean-up. The recovery from biological fluids, however, is low at neutral and acidic pH values. At basic pH values the recovery is higher, but procaine hydrochloride is base-labile, making it a poor choice for an internal standard. Codeine phosphate is a much better internal standard. It gives an excellent peak shape, does not degrade during the sample clean-up and has a high recovery.

This paper describes a fast reversed-phase HPLC procedure that gives excellent chromatography for both cimetidine and codeine phosphate, the internal standard. The analysis time is short (less than 4 min) and there is minimal interference from endogenous compounds in both plasma and urine. The sample clean-up procedure is very simple and no stability problems have been encountered. The assay is very rugged and has been successfully used for the assay of biological samples following therapeutic doses of cimetidine.

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EXPERIMENTAL

Reagents

Acetonitrile, methanol, sodium perchlorate and phosphoric acid (85%) were all HPLC grade and obtained from Fisher Scientific. All other chemicals were reagent grade and supplied by Fisher Scientific. Milli-Q water was used throughout. Cimetidine and codeine phosphate, the internal standard, were supplied by the stockroom of Merck Sharp & Dohme Research Labs. (Rahway, NJ, U.S.A.). Human control plasma was purchased from Sera-Tec Biologicals (North Brunswick, NJ, U.S.A.). C_2 Bond-Elut cartridges (100 mg) were supplied by Analytichem International (Harbor City, CA, U.S.A.).

Instrumentation

A Varian Model 5000 liquid chromatograph with a built-in column heater and a UV-100 variable-wavelength ultraviolet detector were used for this analysis. The autosampler was a Waters WISP 710B, and a Spectra-Physics SP4270 computing integrator was used for the calculation of the results.

Standard solutions

A stock solution of cimetidine (20 mg) was prepared in methanol (0.5 ml) and water (dilute to 10 ml). Working standards for plasma were prepared in water at concentrations of 2, 5, 10, 15 and 20 μg/ml cimetidine. Corresponding working standards for urine were prepared at 62.5, 125, 250, 500, 750 and 1000 μg/ml cimetidine (these working standard solutions will produce equivalent concentrations of 0.2—2 μg/ml for plasma and 62.5—1000 μg/ml for urine). A stock solution of codeine phosphate (2 mg/ml), the internal standard, was prepared in the same way as that of cimetidine. Working standards of codeine phosphate for plasma (10 μg/ml) and urine (500 μg/ml) were prepared in water.

HPLC conditions

The mobile phase used for this analysis consisted of aqueous buffer—organic modifier (92:8). The aqueous buffer used was 0.1 *M* sodium perchlorate and 0.01 *M* phosphoric acid. The organic modifier was acetonitrile. The analytical column was a C_{18} reversed-phase Sepralyte (5 cm $\times$ 4.6 mm, 3-μm packing) supplied by Analytichem. The column temperature was maintained at 50°C and the flow-rate was 1.5 ml/min. The detector was set at 228 nm at a sensitivity of 0.005 a.u.f.s. The injection volume was 15 μl for plasma and 5 μl for urine samples. The integrator attenuation was 2 for plasma and 16 for urine.

Analysis of plasma samples

Plasma standards were prepared by taking blank plasma (1 ml), cimetidine working standard (100 μl), codeine phosphate working internal standard (100 μl) and potassium hydroxide (1 *M*, 100 μl). A C_2 extraction cartridge was activated with methanol (1 ml) and sodium carbonate (0.1 *M*, 1 ml). The plasma solution was loaded onto this activated cartridge. A vacuum was applied and the eluates were discarded. Each cartridge was subsequently washed with

sodium carbonate (0.1 *M*, 2 × 1 ml) and the eluates were again discarded. Each cartridge was then eluted with methanol (250 μl) and then the aqueous part of the HPLC mobile phase (250 μl) and the combined eluates were collected. An aliquot (15 μl) of this solution was then injected for HPLC analysis. Plasma samples were assayed in the same way as plasma standards, substituting patients' plasma for blank plasma and water for the cimetidine working standards.

Analysis of urine samples

Urine standards were prepared by taking blank urine (100 μl), cimetidine working standard (100 μl), codeine phosphate working internal standard solution (100 μl) and potassium hydroxide (1 *M*, 100 μl). The solutions were then processed using a C_2 extraction cartridge as described for plasma. An aliquot (5 μl) of the final solution was injected for HPLC analysis.

Urine samples were assayed in the same way as urine standards, substituting patients' urine for blank urine and water for the cimetidine working standards.

RESULTS AND DISCUSSION

The standard lines were linear over the concentration range 0.2—2 μg/ml for plasma and 62.5—1000 μg/ml for urine. Manual plots of concentration (x) versus the ratio of drug area/internal standard area (y) confirmed this and correlation coefficients calculated from linear regression equations were higher than 0.999 for plasma and urine standard lines.

The extraction efficiencies of cimetidine and codeine phosphate from plasma and urine using the procedures described were checked. Direct, i.e. non-extracted, standards were prepared in the methanol—aqueous mobile-phase solvent system used in the elution step of the sample clean-up. The absolute recoveries of cimetidine and codeine phosphate were 73 and 79% from plasma

TABLE I

INTER-DAY VARIATION FOR THE ASSAY OF CIMETIDINE IN PLASMA AND URINE

Concentration (μg/ml)	Mean ratio drug/internal standard (n = 5)	S.D.	R.S.D. (%)
Plasma			
0.2	0.0424	0.0004	0.9
0.5	0.1096	0.0045	4.1
1.0	0.2235	0.0071	3.2
1.5	0.3388	0.0084	2.5
2.0	0.4510	0.0119	2.6
Urine			
62.5	0.1438	0.0054	3.8
125	0.2820	0.0099	3.5
250	0.5741	0.0174	3.0
500	1.1790	0.0247	2.1
750	1.8061	0.0481	2.7
1000	2.4352	0.0450	1.8

and 91 and 92% from urine, respectively. The recovery rates were constant over the entire range of both the plasma and urine standard lines (0.2—2 μg/ml for plasma and 62.5—1000 μg/ml for urine).

The addition of potassium hydroxide to the biological solutions prior to extraction was found to be a critical step. It eliminated an endogenous compound in plasma that co-eluted with cimetidine and that could not be resolved chromatographically. It also increased the percentage recovery of internal standard during this procedure from both plasma and urine. In urine, for example, omitting the potassium hydroxide gave 60% recovery of cimetidine and only 10% recovery of the internal standard.

The reproducibility of this assay was checked by running the inter-day variation. The results are given in Table I. All points on the standard lines have less than 4.1% relative standard deviation (R.S.D.).

The stability of cimetidine and codeine phosphate in plasma and urine was confirmed by spiking known amounts of each into blank biological fluid and freezing the samples. The samples were thawed and carried through the clean-up procedure together with freshly prepared solutions. No degradation of either compound was observed in the samples that had been frozen. The day-to-day accuracy of the assay was checked by spiking known amounts of cimetidine into blank plasma and urine at low and high values on the standard lines. These samples were frozen and assayed alongside patient samples. These quality control samples were always within ±5% of their target values and showed no change in assay over the four weeks that they were stored frozen.

The selectivity of the assay was confirmed by taking blank plasma through the sample clean-up procedure. A further check was to run patient pre-dose samples. For both plasma and urine no endogenous interference was encountered. Typical chromatograms for the plasma are shown in Fig. 1 and for urine in Fig. 2. The retention times were 2.7 and 3.8 min for cimetidine and codeine, respectively. The limits of detection for cimetidine using this procedure were 0.1 μg/ml for plasma and 10 μg/ml for urine. This was sufficient to measure drug levels following therapeutic doses of cimetidine.

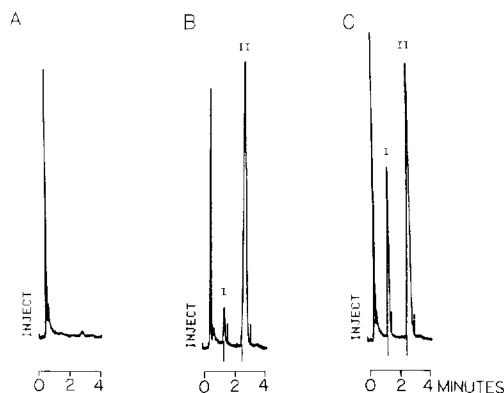

Fig. 1. Typical chromatograms for cimetidine (I) and codeine (II), the internal standard, in plasma. (A) Blank human plasma; (B) blank human plasma containing 0.2 μg/ml cimetidine and 1 μg/ml codeine; (C) patient plasma containing 1 μg/ml cimetidine and 1 μg/ml codeine.

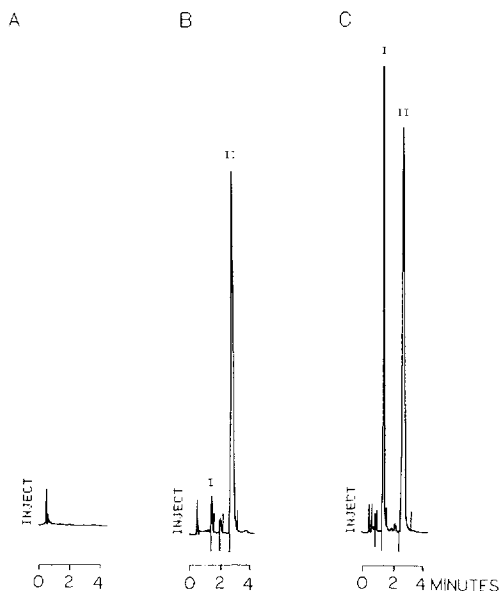

Fig. 2. Typical chromatograms for cimetidine (I) and codeine (II), the internal standard, in urine. (A) Blank human urine; (B) blank human urine containing 62.5 μg/ml cimetidine and 500 μg/ml codeine; (C) patient urine containing 780 μg/ml cimetidine and 500 μg/ml codeine.

The stability of both cimetidine and codeine phosphate, the internal standard, were checked at two stages during the standard and sample clean-up procedure. The solutions of plasma and urine containing the drug, internal standard and potassium hydroxide (i.e. the solution prepared prior to the C_2 cartridge extraction scheme) were stable for at least 3 h at room temperature. This allowed plenty of time for the sample clean-up to be carried out. The final solutions from this procedure (i.e. after clean-up) were stable for at least 40 h at room temperature. Overnight and weekend runs were therefore possible. The procedure described is therefore particularly suitable for the routine analysis of cimetidine clinical samples where large numbers of samples are submitted for assay and long HPLC runs can be set up.

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