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Determination of cimetidine in human plasma by high-performance liquid chromatography following liquid–liquid extraction

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Abstract

A new method is described for the determination of cimetidine in human plasma. The drug and internal standard (ranitidine) were separated on a Nucleosil C_{18} 5 μm (25 × 4.6 mm I.D.) column using a mobile phase of acetonitrile–phosphate buffer, pH 6.2 (25:75, v/v) containing 2.5 g/l heptane sulphonic acid. The mobile phase was delivered at a flow-rate of 0.9 ml/min, detection was by ultraviolet absorption at 228 nm and concentrations were calculated on the basis of peak areas. The drugs were extracted from alkaline plasma into ethyl acetate using a salting out procedure which involved the addition of 100 ml of a saturated solution of K_2CO_3 to each 250-μl plasma aliquot. The method was validated over the concentration ranges 50–3000 ng/ml and 100–7000 ng/ml for two separate studies. Mean coefficients of variation were less than 6% for both intra- and inter-assay in both studies and recoveries varied between 71 and 81%. The method was successfully applied to the determination of cimetidine in plasma for a pharmacokinetic study.

1. Introduction

Cimetidine is a histamine H2-receptor antagonist which inhibits the secretion of gastric acid. It is extensively used in the treatment of peptic ulcers, in the management of reflux oesophagitis and for the inhibition of gastric acid secretion associated with Zollinger–Ellison syndrome. It is rapidly absorbed following oral administration, peak plasma levels being attained after approximately two hours when taken with food, and after one hour when taken without food. It is about 15–20% protein-bound, with a plasma half-life of about two hours and about two thirds of the oral dose is excreted within 24 h. It is excreted as the unchanged drug (56–85%) and as hydroxymethyl, sulfoxide or guanylurea metabolites. Cimetidine does not cross the blood–brain barrier, but does cross the placental barrier and is excreted in milk. It is usually administered in divided doses of up to 1600 mg daily.

Due to its highly polar nature, cimetidine is normally determined by reversed-phase HPLC using either a C_{18} [1–7], C_8 [8] or polymeric [9] column and in some cases the mobile phase may

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contain an ion-pairing reagent [7]. It has less frequently been determined by normal-phase chromatography [10]. Also, due to its polarity, liquid extraction methods involve partition into a relatively polar organic mixture such as 4% propan-2-ol in ethyl acetate [5]. Early methods for the analysis of cimetidine involved triple extraction which were consequently time-consuming in their execution [11,12]. A later method by Kunitani et al. [1] used protein precipitation with acetonitrile, but also involved a solvent evaporation step and was therefore more time-consuming than double-phase solvent extraction. A method involving protein precipitation with salting out has been described by Rustum and Hoffman [9]. Cimetidine has previously been extracted into ethyl acetate using Extralut columns [10]. This procedure required the use of a relatively large volume of solvent (5 ml) for each sample but offered a limit of quantitation of 30 ng/ml based on a 0.8-ml sample size. The extraction of cimetidine into dichloromethane has also been described [3] but this required 5 ml of the organic solvent for each 200-μl sample size. The analysis time was 17 min and the chromatographic characteristics of both the drug and internal standard were quite poor. Solid-phase extraction (SPE) procedures for the determination of cimetidine in plasma using C_{18} cartridges have been described [2,13] but Chiou et al. [4] found that the isolation of cimetidine from plasma was most effectively accomplished on a C_2 cartridge. More recently, methods have been described for the analysis of cimetidine in tablets using capillary electrophoresis [14] and in serum by micellar electrokinetic capillary chromatography following electrochromatographic solid-phase extraction [15].

In this paper we describe an HPLC method for the determination of cimetidine in plasma. The chromatographic separation was developed with relative ease, though considerably more effort was required to obtain a satisfactory extraction procedure giving selective isolation and reasonable recovery of the drug. The method uses only 250 μl of sample and a one-step extraction into 1 ml ethyl acetate. The procedure is therefore rapid and simple to perform, is cost-effective and involves low consumption of organic solvent. The method was subsequently employed to compare the bioavailablility of two cimetidine formulations, one of which was the standard marketed product and the other was a generic equivalent. The bioavailabilities were compared at two different dose levels, 200 and 800 mg, which necessitated the implementation of two different calibration ranges.

2. Experimental

2.1. Instrumentation and operating conditions

The Shimadzu (Kyoto, Japan) HPLC system used in this study consisted of a liquid chromatographic pump Model LC-9A which was used to deliver the mobile phase at a flow-rate of 0.9 ml/min. The UV absorbance of the eluent stream was monitored at 228 nm with an SPD-6A ultraviolet detector which was connected to a CR6A integrator for the collection of data. Sample introduction was via an SIL-9A autoinjector. The drugs and internal standard were separated on a 250×4.6 mm I.D. column, packed with Nucleosil C_{18} (5 μm) packed by Chromex, UK. The mobile phase consisted of acetonitrile–0.01 *M* phosphate buffer pH 6.2 (25:75, v/v) containing 2.5 g/l heptanesulphonic acid.

2.2. Reagents and solvents

Cimetidine (BN 89D3185) and the internal standard (I.S.) (BN CK249) were obtained from SK&F (UK) and Glaxo (UK), respectively. Heptane sulphonic acid was supplied by Waters (Milford, MA, USA) and HPLC grade water, acetonitrile, ethyl acetate and methanol were supplied by Labscan Analytical Sciences (Dublin, Ireland). All other reagents were of AnalaR grade, obtained from BDH (Dorset, UK).

2.3. Standards and solutions

Stock solutions containing 1 mg/ml cimetidine and I.S. were prepared in methanol and stored at 4°C. Working standards, used to spike drug-free plasma (with drug and I.S.) or subject

plasma samples (with I.S. only) were prepared by serial dilution of the stock solutions in methanol–water (1:1, v/v).

Aliquots of drug free plasma (5 ml) were spiked to contain between 50 and 3000 ng/ml cimetidine (200 mg dose study) or between 100 and 7000 ng/ml (800 mg dose study). These were then divided into 250-μl aliquots and stored in polypropylene microcentrifuge tubes at −20°C. To prepare the calibration curves, a set of standards was thawed and spiked with 25 μl I.S. to give plasma concentrations of 250 ng/ml I.S.

2.4. Extraction procedure

Plasma standards and samples were treated in exactly the same fashion: following addition of the I.S., the aliquots were mixed with 20 μl of 2.5 *M* NaOH and 100 μl of a saturated solution of potassium carbonate. They were vortex-mixed for one minute after which 1 ml of ethyl acetate was added followed by vortex-mixing for another 90 s. The aliquots were centrifuged at 9500 *g* after which the organic layers were transferred to autosampler vials and evaporated to dryness at 37°C under a stream of nitrogen. The residues were reconstituted in 500 μl water and 50 μl was injected onto the column. The concentration of cimetidine in subject samples was determined from peak-area ratio (drug:I.S.) calibration curves constructed from duplicate sets of standards.

2.5. Assay validation

Recovery of cimetidine was determined by comparing the peak areas obtained after injection of extracted standards with peak areas obtained following direct injection of unextracted authentic standards. Recovery was estimated by calculating the percentage difference between the individual peak areas of extracted and authentic standards. The mean recovery (%) was given as the mean of the recoveries at each of the calibration points.

The precision of the method was evaluated in terms of the variability both between (inter-assay) and within (intra-assay) batches of replicate analyses over the calibration range. Intra-assay variability was determined by analysing four replicates at each concentration in the range 50–3000 ng/ml (200 mg dose study) and 100–7000 ng/ml (800 mg dose study). For each range, a calibration curve based on mean peak-height ratios was constructed and individual peak-height ratios were interpolated as unknowns on the curve to yield four new values of concentration ("amount found") at each calibration point. The intra-assay precision was then expressed as the mean % coefficient of variation (%C.V.) of the amounts found at each calibration point. Inter-assay variability was assessed by analysing four replicates at each concentration on four consecutive days. Peak-height ratios were interpolated on the four individual regression lines to yield four "amounts found" at each calibration point.

Linearity was defined by the correlation coefficient of the intra-assay regression line and accuracy was assessed on the basis of the difference between the amount added and the amount found by interpolation of standards as unknowns on the regression lines.

2.6. Bioavailability study protocol

The study was a randomised single-dose cross-over study in 12 healthy male and female volunteers. The subjects were aged 18–40, within 15% of their ideal body weight and had no history of either drug abuse or chronic disease. Blood samples were taken from each of the volunteers prior to (predose) and 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0 h following administration. The blood was withdrawn into lithium heparin tubes, plasma was separated by centrifugation and then stored at −20°C.

3. Results and discussion

Cimetidine is not highly lipophilic, even when unionised, and an ion-pairing method using heptane sulphonic acid was employed to enhance its ability to partition into the apolar stationary phase. The pH of the mobile phase was 6.2 and a mixture containing 25% acetonitrile was found to give satisfactory separation of the drug and

I.S. within an acceptable period of time. Under the chromatographic conditions employed, the retention times of cimetidine and I.S. were approximately 6.3 and 7.1 min, respectively.

Various extraction procedures were investigated, for example, pH adjustment with NaOH followed by extraction into ethyl acetate, evaporation of the separated organic phase followed by reconstitution in the mobile phase. It was found, however, that drug recovery was very low ($< 50\%$) and that there were many interfering peaks present in the blank plasma extracts. The first approach adopted to solve this problem was to investigate a variety of solvent mixtures principally in order to improve the selectivity of the method. While reasonable clean blank plasma chromatograms could be obtained using mixtures of varying polarities, recovery of the drug remained low. It was then decided to investigate a salting out approach. Addition of a salt to the plasma lowers the degree of hydration of the analyte and tends to promote its transfer into the organic phase. Such a procedure had previously been used for the determination of xipamide in plasma [16], and since Rustum had already used potassium carbonate in a salting out method in the determination of cimetidine [9], a procedure was adopted here whereby 100 ml of a saturated solution of K_2CO_3 was added to 250 μl of plasma. This was found to be satisfactory in that recovery of both drugs was increased from approx 50% to an average of 75%. A further slight improvement in selectivity was achieved by reconstituting the residues in water rather than in mobile phase. Chromatograms of blank plasma and plasma spiked with cimetidine and I.S. are presented in Fig. 1.

The method was validated using the proce-

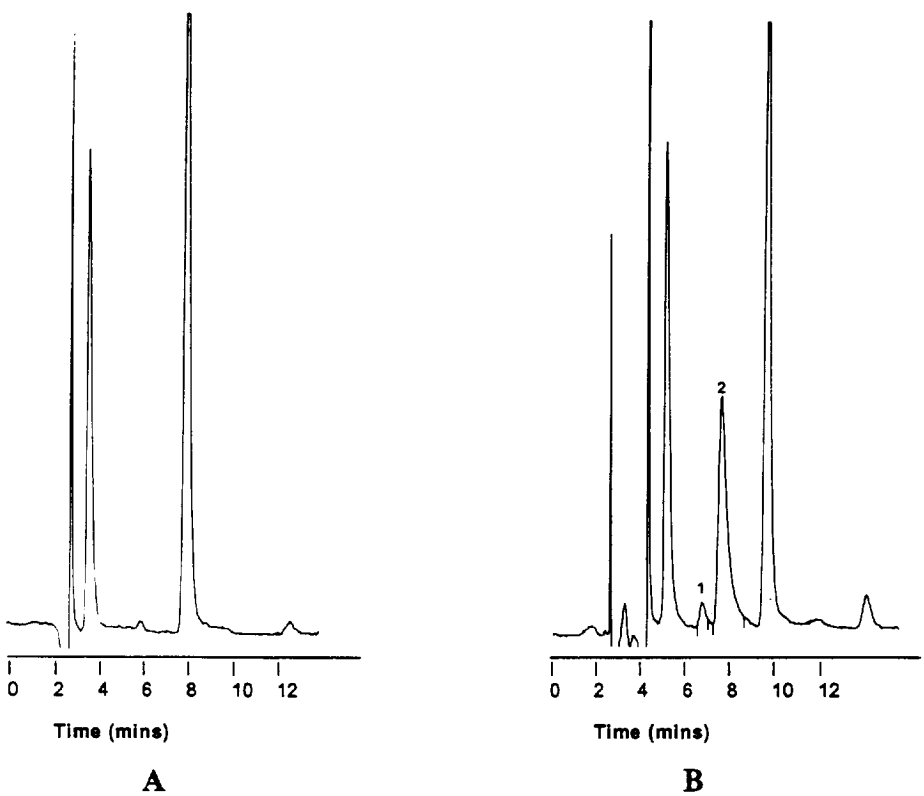

Fig. 1. (A) Drug-free pooled human plasma and (B) plasma spiked with 50 ng/ml cimetidine (1) and 250 ng/ml I.S. (2). Chromatographic conditions as described in the text.

dures outlined in the Experimental section. In the 200 mg dose study (Table 1), the intra-and inter-assay variabilities were found to be 5.35% and 4.26%, respectively. The method was linear over the concentration range (50–3000 ng/ml) with a coefficient of variation of 0.998 based on the intra-assay calibration curve. Recovery was found to be 81.41%

In the 800 mg dose study (Table 2) the intra- and inter-assay variabilities were calculated to be 3.88% and 3.28%, respectively. The correlation coefficient was 0.999 over the calibration range (100–7000 ng/ml) and the mean % recovery was determined to be 79.54%. The method is selective and, as shown by the chromatograms in Fig. 1, there are no peaks interfering with the analysis at the lowest calibration point. The limit of determination was deemed to be 50 ng/ml, the level at which reliable quantitation could consistently be made, and the limit of detection where the signal-to-noise ratio was 3:1 was 15 ng/ml.

3.1. Application of method

The method was applied to the determination of cimetidine following oral administration of 200 mg and 800 mg of both the market leader and a generic equivalent. Fig. 2 shows a typical plasma concentration–time curve for both formulations at the 800 mg dosage level, indicating that the limit of detection was adequate for the determination of plasma levels post dose. Fig. 3 shows a typical chromatogram of extracted subject plasma predose and following oral administration.

Table 1
Validation data 200 mg dose study

Amount added (ng/ml)	Amount found (ng/ml)	S.D.	C.V. (%)	Added-found (%)
Intra-assay				
50	38.0	4.79	12.62	−23.98
100	87.44	5.56	6.36	−12.56
200	204.98	12.13	5.92	2.49
400	386.94	17.07	4.41	− 3.26
1000	963.17	54.73	5.68	− 3.68
2000	2165.46	17.44	0.81	8.27
3000	2904.0	48.59	1.67	− 3.20
Mean C.V. (%)	5.35%			
Regression line	$y = 0.0013x + 2.7173 \cdot 10^{-3}$			
Correlation coefficient (r)	0.998			
Inter-assay				
50	40.01	3.03	7.58	−19.79
100	104.65	4.17	3.99	4.65
200	200.46	14.59	7.28	0.23
400	438.57	15.49	3.53	− 9.64
1000	984.51	4.23	0.43	− 1.55
2000	2116.37	52.00	2.46	5.82
3000	3037.86	137.82	1.67	− 3.20
Mean C.V.	4.26%			
Recovery				
Mean peak-area ratio (extracted/authentic): 81.41%				

Table 2
Validation data 800 mg dose study

Amount added (ng/ml)	Amount found (ng/ml)	S.D.	C.V. (%)	Added-found (%)
Intra-assay				
100	98.60	5.55	5.63	−12.56
200	216.06	12.13	5.61	8.03
400	397.90	17.06	4.29	0.52
1000	973.76	54.69	5.62	− 2.62
2000	2175.26	17.43	0.80	8.76
3000	2924.98	40.32	1.38	− 2.50
4000	3831.84	168.26	4.39	− 4.20
7000	7081.60	235.90	3.33	1.17
Mean C.V.	3.88%			
Regression line	$y = 0.0013x + 1.2753 \cdot 10^{?}$			
Correlation coefficient (r)	0.999			
Inter-assay				
100	108.33	3.59	3.31	8.33
200	199.38	12.84	6.44	− 0.31
400	425.56	17.04	4.00	6.39
1000	943.75	15.18	1.61	− 5.62
2000	2022.66	9.89	0.49	1.13
3000	2890.61	88.89	3.08	− 3.65
4000	3852.88	123.71	3.21	− 3.68
7000	8066.26	328.36	4.07	15.23
Mean C.V.	3.28%			
Recovery				
Mean peak-area ratio (extracted/authentic): 79.54%				

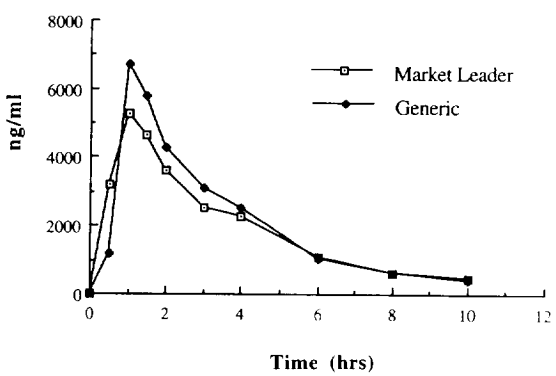

Fig. 2. Plasma concentration-time curve for one subject following administration of 800 mg cimetidine.

4. Conclusions

A rapid method was developed for the determination of cimetidine in plasma. Ion-pairing chromatography was found to provide satisfactory separation of the drug and internal standard from the endogenous plasma components. Isolation from plasma involved a simple, one-step liquid–liquid extraction procedure, recoveries from which were substantially enhanced by the addition of potassium carbonate prior to the organic solvent. By using small sample volumes, the entire procedure may be carried out on a very small scale and thus the consumption of

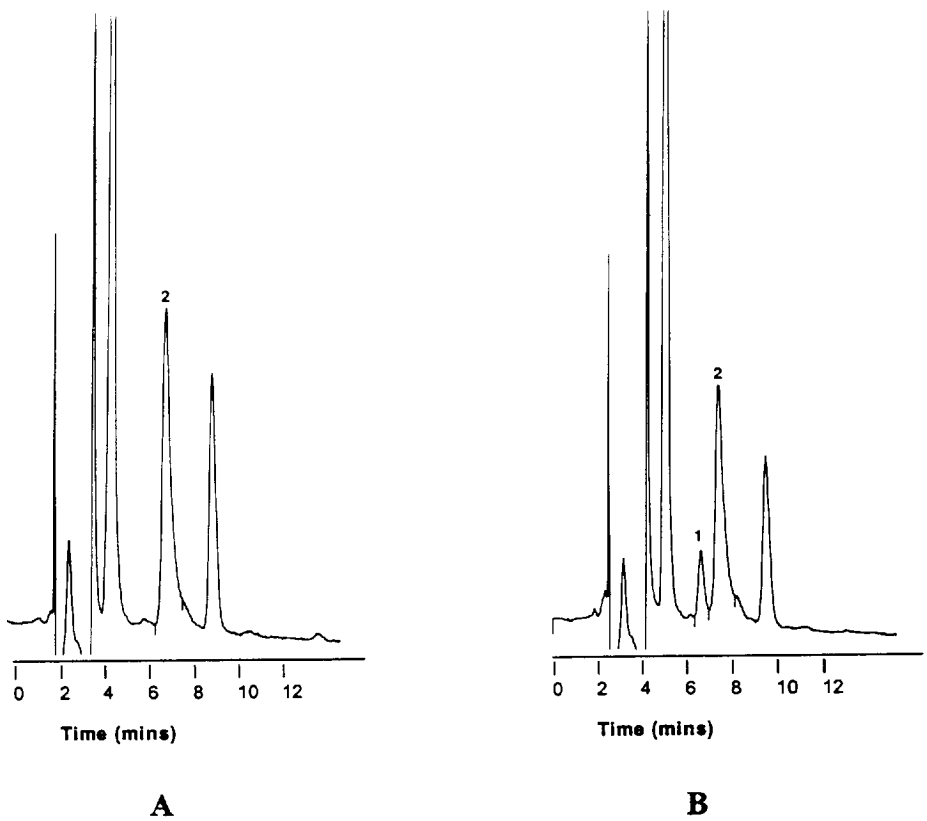

Fig. 3. Plasma chromatograms for one subject. (A) Pre-dose and (B) 4 h post-dose. Cimetidine (1) and I.S. (2). Chromatographic conditions as described in the text.

hazardous organic solvents is comparable with that of solid-phase extraction at considerably less expense. The method was linear over the two concentration ranges used with mean coefficients of variation of less than 6%. The method was successfully applied in the determination of cimetidine in plasma as part of a pharmacokinetic study.

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