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Short communication

Ion-pair solid-phase extraction of cimetidine from plasma and subsequent analysis by high-performance liquid chromatography

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Abstract

An improved method is described for the solid-phase extraction of cimetidine from plasma or serum with subsequent analysis by HPLC. New aspects of the method include protein precipitation with metaphosphoric acid (5%, w/v), followed by selective adsorption of cimetidine and the internal standard ranitidine on the surface of a solid-phase phenyl (PH Bond Elut) column, using octanesulfonate as an ion-pairing agent. Separation was achieved on a LiChrosorb RP-18 column with a mobile phase consisting of acetonitrile–0.01 *M* phosphate buffer pH 3.0 containing 0.005 *M* octanesulfonate (22:78, v/v). The intra-assay coefficient of variation varied between 0.7 and 4.0%. The procedure provides cleaner and more stable samples and a better recovery (90 ± 2.3%) and sensitivity (limit of detection 5 ng/ml and limit of quantitation 25 ng/ml) as compared with previous methods.

1. Introduction

A large number of high-performance liquid chromatographic methods for the quantitation in biological fluids of cimetidine, an H_2-receptor antagonist widely used for the treatment of gastric and duodenal ulcers, has been published. For the sample clean-up of plasma or serum one study used acetonitrile for the precipitation of protein [1]. Others employed single/multiple liquid–liquid extraction procedures [2–9] or solid-phase extraction [10–15]. None of the authors who employed solid-phase extraction for the sample clean-up of plasma or serum took account of the protein binding of cimetidine, which may amount to ca. 13–26% [16]. This is probably one of the reasons why some authors have reported absolute recoveries as low as 59% [12] or 73% [14]. Another important reason for the low recoveries, accompanied in most papers by high coefficient of variations, may be heterogeneity and channeling in the solid-phase bed. However, when the solid-phase extraction technique is used under optimal conditions, an absolute recovery of 90% with negligible interferences should always be achievable. The mean

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particle size of the commonly used bonded silica sorbents is 40 μm [17]. The nominal porosity of most of the sorbents is 60 Å, adequate for compounds with molecular masses up to approximately 15 000. Molecules larger than this (molecular mass of human serum albumin = 69 000) are excluded from the 60 Å pores and are exposed to too little of the sorbent surface area to sufficiently interact with the sorbent functional groups. As a consequence, they pass through these sorbents without being retained. Cimetidine bound to albumin will be lost in this manner.

Therefore, we incorporated a precipitation step in our solid-phase extraction method using metaphosphoric acid. This does not add much time to the procedure, since precipitation of the protein can be carried out simultaneously in large series of samples. A second new element introduced in our procedure is ion-pairing solid-phase extraction. After deproteinization the pH of the resulting supernatant is acidic. Under such conditions cimetidine and ranitidine (internal standard) are in principle not retained by non-polar solid-phase extraction columns. In order to effect retention of both compounds on PH (phenyl) columns we added octanesulfonate, an ion-pairing agent, to the supernatant. Both protein precipitation and ion-pairing solid-phase extraction result in cleaner samples and a better recovery and sensitivity as compared with previously published methods.

2. Experimental

2.1. Reagents and materials

Cimetidine and ranitidine hydrochloride were purchased from Sigma (St. Louis, MO, USA). Potassium dihydrogen phosphate, disodium hydrogen phosphate 2-hydrate, orthophosphoric acid, and metaphosphoric acid were all of analytical grade (Merck, Darmstadt, Germany). Methanol and acetonitrile were HPLC grade (Merck), and 1-octanesulfonic acid sodium salt was from Janssen Chimica (Beerse, Belgium). Vac Elut manifold and Bond Elut PH (Phenyl) columns (1 ml capacity, to which a 4-ml Bond Elut reservoir was attached using a Bond Elut adaptor) were manufactured by Analytichem International (Habor City, CA, USA). Available reverse osmosis water (Millipore) was further purified with a MilliQ system (Millipore) before use. Human blood samples, stored at −20°C, were obtained from the local blood bank.

2.2. Standard solutions

Stock solutions of cimetidine (1 mg/ml) and ranitidine (1 mg/ml) were made with 0.01 *M* phosphate buffer pH 3.0. Working standard solutions were prepared daily by diluting the stock solutions with the same buffer. A 100-μl aliquot of the working standard solutions was used. Stock solutions of cimetidine and ranitidine in 0.01 *M* phosphate buffer pH 3.0 were stable for at least 6 months when stored in the dark at 4°C.

2.3. Apparatus and chromatographic conditions

The chromatographic system consisted of a Spectra Physics (Eindhoven, Netherlands) P2000 binary gradient pump, a Hewlett-Packard (Amsterdam, Netherlands) 5 μm ODS Hypersil guard column (20 × 2.1 mm I.D.), a Hewlett-Packard 5 μm LiChrosorb RP-18 analytical column (200 × 4.6 mm I.D.) and a Spectra Physics AS 3000 autosampler with a built-in column heater. The mobile phase, acetonitrile–0.01 *M* phosphate buffer pH 3.0 containing 0.005 *M* octanesulfonate (22:78, v/v) maintained under a helium sparge during use, was delivered at a flow-rate of 1 ml/min, the resulting pressure being 8.0 MPa. The column effluent was monitored with a Spectra Physics UV1000 variable-wavelength detector set at 228 nm. The signal was processed by a Spectra Physics SP4400 integrator. The column heater was set at 40°C and the injection volume was 10 μl (50 μl at concentrations lower than 0.05 μg/ml).

2.4. Sample pretreatment

Into a plastic tube were pipetted successively, 0.5 ml plasma, 100 μl internal standard (5 mg

ranitidine per 100 ml of 0.01 *M* phosphate buffer pH 3.0) and 2 ml of a 5% (w/v) metaphosphoric acid solution. The tube was vortex-mixed for 5 s. After standing for 5 min at room temperature, the tube was again vortex-mixed for 5 s. Subsequently, the tube was centrifuged at 2000 *g* and 20°C for 10 min. The supernatant was transferred into another plastic tube, and 0.8 ml of 0.3 *M* Na_2HPO_4 solution containing 0.02 *M* octanesulfonate was added. The tube was then vortex-mixed for 5 s. The sample was now ready to be loaded onto the PH (phenyl) solid-phase extraction column.

2.5. Extraction procedure

Extraction of cimetidine and ranitidine (internal standard) from plasma was performed with bonded silica solid-phase extraction columns (Bond Elut PH, 1 ml capacity). The columns were conditioned prior to use by drawing through the column three column volumes (approx. 3 ml) of methanol followed by a similar volume of 0.01 *M* phosphate buffer pH 3.0 containing 0.005 *M* octanesulfonate. The sample was loaded onto the column and subsequently drawn through. The column was then washed with four column volumes of 0.01 *M* phosphate buffer pH 3.0 containing 0.005 *M* octanesulfonate. The cover of the manifold was then removed and the blunt-nose stainless-steel needle of the Vac Elut cover was wiped with a tissue to remove drops of washing solution. The Vac Elut rack holding a 2-ml glass sampling tube, was placed under the column. Cimetidine and ranitidine were eluted from the column with 0.75 ml of acetonitrile–0.01 *M* phosphate buffer pH 3.0 containing 0.005 *M* octanesulfonate (30:70, v/v). To adjust the elution strength of the collected eluate to that of the mobile phase, 275 μl of 0.01 *M* phosphate buffer pH 3.0 containing 0.005 *M* octanesulfonate, was added. This was done to prevent peak broadening whenever larger volumes (50 μl) had to be injected onto the column. The eluate was then analyzed as described. The eluate was found to be stable for more than two weeks at room temperature and for more than four weeks at 4°C.

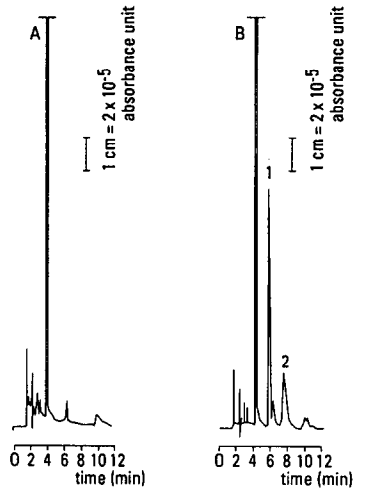

Fig. 1. Chromatograms obtained for (A) blank plasma and (B) blank plasma spiked with 1.25 μg/ml cimetidine (1) and 1.25 μg/ml ranitidine (2). Injection volume = 10 μl.

3. Results and discussion

3.1. Chromatography

Fig. 1 shows typical chromatograms for blank plasma and for blank plasma spiked with cimetidine and the internal standard (I.S.) ranitidine. The retention times of cimetidine and ranitidine were 5.85 and 7.57 min, respectively, while also an endogenous peak with a retention time of 6.37 min appeared. Without the presence of the ion-pairing agent octanesulfonate the retention times of cimetidine and ranitidine were 2.87 and 3.28 min.

The efficiency of the analytical column used to obtain the chromatogram in Fig. 1 was 9000 plates per 20 cm for the cimetidine peak. The theoretical plate number remained practically the same after 700 samples, while the column pressure slightly increased. After renewal of the guard column the system pressure attained its original value.

3.2. Calculation

The cimetidine concentration in a sample was determined from a standard curve of the peak-height ratio versus cimetidine concentration.

Whenever a sample containing cimetidine was measured, a standard curve was generated by adding different amounts of cimetidine to blank plasma. A linear relationship was found between the peak-height ratio of cimetidine to ranitidine (y) and the plasma cimetidine concentration (x), as given by the equation $y = 0.841x + 0.002$ ($r = 0.9999$, $n = 6$) for the plasma cimetidine concentration range 0.25–5 μg/ml (I.S. = 5 μg/ml) and $y = 8.472x + 0.009$ ($r = 0.9997$, $n = 5$) for the plasma cimetidine concentration range 0.025–0.25 μg/ml (I.S. = 0.5 μg/ml).

3.3. Recovery

The overall recovery obtained with the extraction procedure was determined by comparing the peak heights of cimetidine and ranitidine obtained after injection of non-extracted standard solutions with peak heights obtained after injection of extracted plasma containing equal concentrations of both compounds. The absolute recovery of cimetidine and ranitidine is 90% and independent of the concentration. The coefficient of variation (C.V.) is equal or less than 2.3% (Table 1).

3.4. Efficiency of the deproteinization procedure

To investigate the efficiency of the protein precipitation with 5% (w/v) metaphosphoric acid, 0.5-ml aliquots of a series of ten spiked plasma samples were deproteinized. After centrifugation the volume of the supernatant was measured. The volume was found to be 2.43 ml (C.V. = 0.8%, $n = 10$), which is 90% of the initial volume (0.5 ml plasma + 2 ml 5% metaphosphoric acid + 100 μl standard solution + 100 μl internal standard solution). From these results it was concluded that cimetidine and ranitidine existed in a completely free form in the deproteinized sample, 90% in the supernatant and 10% in the pellet.

Table 1
Recovery of cimetidine and ranitidine from plasma

Compound	Concentration (μg/ml)	Recovery (%)	C.V. (%)
Cimetidine	0.25	90	2.3
	2.50	90	2.3
Ranitidine	0.50	89	2.2
	2.50	91	1.2

For all concentrations, $n = 6$; C.V. = coefficient of variation.

3.5. Sensitivity, precision and applicability

The limit of detection (signal-to-noise ratio of 2) of the method described was 5 ng/ml. Table 2 shows the precision and accuracy of cimetidine measurement in plasma. The intra-assay C.V. varied between 4.0 and 0.7% over a cimetidine concentration range of 0.063–2.500 μg/ml. At the limit of quantitation (five times limit of detection) the C.V. was 14.1%.

Several drugs that are often concurrently administered with cimetidine were examined for possible interference with the assay. Plasma samples from patients who were treated with therapeutic doses of acetylsalicylic acid, amiloride, atenolol, azapropazone, diclofenac, furosemide, hydrochlorothiazide, indomethacin, naproxen, omeprazole, oxazepam, paracetamol, phenylbutazone, piroxicam, prednisone, salazopyrine, and salicylic acid were examined. None of the drugs showed interfering peaks at the retention times for cimetidine and ranitidine.

Table 2
Precision and accuracy of cimetidine analysis in spiked plasma samples

Spiked concentration (μg/ml)	Observed concentration (μg/ml)	C.V. (%)	Accuracy (%)
0.025	0.025	14.1	100.0
0.063	0.064	4.0	98.4
0.125	0.128	3.9	97.7
0.188	0.194	2.7	96.9
0.250	0.245	2.3	102.0
0.625	0.638	2.6	98.0
1.250	1.298	0.7	96.3
1.875	1.839	1.1	102.0
2.500	2.503	0.7	99.9

For all concentrations, $n = 5$; C.V. = coefficient of variation.

The H_2-antagonists nizatidine and metiamide did not interfere, whereas the retention time of famotidine coincided with that of cimetidine.

4. Conclusions

The method described here for the determination of cimetidine in plasma or serum is a significant improvement over previously published methods in terms of sensitivity, recovery and stability and cleanliness of the final sample. It should prove to be valuable for clinical monitoring of plasma or serum levels and detailed pharmacokinetic studies.

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