Comparison of methods: Results obtained by the proposed method (y), compared with those of radial immunodiffusion and laser immunonephelometry (x), agreed well and yielded the respective regression equations: 

\[ y = 0.91x + 1.33; \quad n = 40, \quad r = 0.96, \quad S_{xy} = 82.19, \quad \bar{y} = 22.98 \text{ and } y = 0.88x + 0.42; \quad n = 27, \quad r = 0.97, \quad S_{xy} = 71.83, \quad \bar{y} = 27.62, \quad y = 24.52. \]

In summary, we suggest that inaccurate dye-binding methods should be prohibited and, at the least, every abnormal sera with hypoalbuminemia risk should be analyzed by an immunological method. Radial immunodiffusion is simple but slow, and consequently is convenient only for routine use. For a rapid determination, particularly for the indirect assessment of oncotic pressure and thus for monitoring intravascular fluid in nephrotic syndrome, fast methods are required. Laser immunonephelometry is more rapid than radial immunodiffusion but requires special apparatus. The proposed immunoturbidimetric method is easier and less time consuming (15 min for the overall determination), and results correlate well with those by radical immunodiffusion and laser immunonephelometry. Moreover, because the immunoturbidimetric assay requires a more diluted antibody, less antiserum is used.

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References

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Liquid-Chromatographic Assay of Cimetidine in Plasma and Gastric Fluid
Maged Abdel-Rahim, David Ezra, Carl Peck, and Jeffrey Lazar

In this micro-scale method for liquid-chromatographic measurement of cimetidine in 250-μL volumes of plasma or gastric fluid, a combination of organic- and aqueous-phase extractions, and protonation of the internal standard and cimetidine, enabled us to detect 2.0 ng of cimetidine on the column. We also used a radial compression module, which reduced the retention times for cimetidine and the internal standard to only 2.78 and 2.00 min. The speed and sensitivity of this method facilitates analysis of large numbers of samples.

Additional Keyphrases: peptic ulcers, radial compression, reversed-phase

Cimetidine, a histamine H2-receptor antagonist, is widely used for treating peptic ulcers. Several methods of analysis have been reported for this compound in plasma and gastric fluid, but each has substantial shortcomings. For example, in the methods of Larsen et al. (1) and Kunitani et al. (2), ethyl acetate is used to extract cimetidine and the internal standard, metiamide, from alkalinized serum, but the extraction efficiency is low (65%). The methods of Bartlett and Segelman (3), and Randolph et al. (4), require 3 to 5 mL of blood, more than can reasonably be obtained in multisample pharmacokinetic or pharmacodynamic studies. Improved sensitivity and reproducibility are claimed for the method of Lorenzo and Drayer (5), but nearly 10 min are required between injections—13 min in the method of Guay et al. (6), which precludes efficient assay of large numbers of samples.

The present method was developed to overcome these limitations.

Materials and Methods
Apparatus
A "high-pressure" liquid chromatograph equipped with a U-6K injector, a guard column, a radial compression module (RCM-100 cartridge, reversed-phase), all from Waters Associates, Milford, MA, and a variable-wavelength ultraviolet detector (Schoeffel Monochromator GM 970 and Spectroflow Monitor SF 770; Kratos, Inc., Westwood, NJ) set at 228 nm were used throughout.
Reagents

Cimetidine and the internal standard (N-cyano-N'-methyl-N'-[3-(4-imidazolyl)propyl]-guanidine) were supplied by Ms. Donna Love, Medical Affairs, Smith Kline & French Laboratories, Philadelphia, PA. Methanol and acetonitrile (HPLC grade) were purchased from Fisher Scientific. Other chemicals were of the highest purity available.

The mobile phase consisted of phosphate buffer (per liter, 8.7 mmol of KH2PO4 and 3.04 mmol of Na2HPO4, pH 7.41) containing 340 g of methanol per liter, and was prepared freshly daily.

Procedures

Preparation of standard curves for plasma. Place 250 μL of blood bank plasma into 12 × 75 mm glass culture tubes, and add 150 μL of methanol containing 1200 ng of the internal standard plus various amounts of cimetidine (0.36, 0.72, 1.45, 2.71, or 3.62 μg). This will result in cimetidine concentrations ranging from 1.45 to 14.48 μg of cimetidine per milliliter of plasma. Add 25 μL of NaOH (5 mol/L), vortex-mix briefly, and then add 2 mL of acetonitrile. Shake the tubes for 5 min, then centrifuge (4000 × g, 10 min).

Transfer the organic layer to a clean 12 × 75 mm tube, add 1 mL of HCl (20 mmol/L) saturated with NaCl (300 g/L), shake for 10 min, and centrifuge as above. Transfer the aqueous phase to another clean tube, add 100 μL of 5 mol/L NaOH and 2 mL of acetonitrile, and shake for 5 min. Transfer the organic layer into a clean culture tube, and evaporate under dry nitrogen in a water bath at 40°C. Dissolve the residue in 250 μL of methanol containing 1 mmol of HCl per liter, and inject 15 μL onto the chromatographic column.

For the lower concentrations for the standard curve, add 150 μL of methanol containing 918 ng of the internal standard plus various amounts of cimetidine (69.6, 435.9, 1740 ng) to the plasma, and process the tubes as above. These final concentrations of cimetidine range from 0.28 to 6.96 μg/mL of plasma.

Preparation of patients' plasma samples. Draw 0.5-ml blood samples into plastic syringes containing one volume of sodium citrate (0.1 mol/L) to eight volumes of blood. Store the samples on ice, then centrifuge (800 × g, 10 min) and either freeze the plasma at −30°C or process the samples as described above.

Preparation of standard curves for gastric fluid. We altered the range of the standard curve (61.5–768.75 μg/mL) because cimetidine concentrations in gastric fluid are anticipated to be considerably higher than plasma concentrations.

Place 250 μL of gastric fluid (we used fluid from monkeys) in 12 × 75 mm glass culture tubes, and add 150 μL of methanol containing 6400 ng of the internal standard and various amounts of cimetidine (15.40, 30.75, 61.50, 115.30, or 192.20 μg). Add 2 mL of HCl (20 mmol/L) saturated with NaCl), vortex-mix briefly, add 2 mL of acetonitrile, then shake and centrifuge as above. Discard the organic phase, and repeat the extraction with 2 mL of acetonitrile. Add 100 μL of 5 mol/L NaOH and 2 mL of acetonitrile to the aqueous phase, shake for 10 min, centrifuge, and evaporate the organic phase under nitrogen, as described above. Dissolve the residue in 250 μL of methanol containing 1 mmol of HCl per liter, and inject 15 μL onto the column.

Precision studies. Intra-assay variability was assessed by six replicate determinations of cimetidine/internal standard peak-height ratios in each of two different plasma samples to which known amounts of cimetidine had been added (3594 and 359.4 ng). Similarly, we assessed the precision at the low end of the standard curve with the following amounts of cimetidine: 1740, 870, 435, and 69.6 ng. The values chosen represented the extremes of the usual standard curve. In addition, we determined three samples of intermediate concentration (2695.5, 1437.6, and 718.8 ng) in duplicate on the same day.

Interassay performance was determined on seven different days, by the construction of complete standard curves and by the analysis on each day of two plasma samples containing high (6.9 μg/mL) and low (2.2 μg/mL) concentrations of cimetidine.

Recovery studies. The recovery of cimetidine from plasma was determined by comparing the peak-height ratios of pure cimetidine standard with those of plasma samples of known initial and final volumes, to which four different amounts (1740, 870, 435, and 69.6 ng) of cimetidine had been added.

Statistical methods. We used standard methods to calculate the mean, standard deviation, standard error of the mean, coefficient of variation, and linear regression parameters (7). One-way analysis of variance was used to test for differences among the recovery rates of the four different cimetidine concentrations.

Results

Figure 1B shows a typical chromatogram obtained from plasma to which both cimetidine and the internal standard had been added. Comparison with the chromatogram in Figure 1A demonstrates the enhanced resolution achieved by protonating both cimetidine and the internal standard with dilute acid, which allows the detection of as little as 2.0 ng of cimetidine on-column. At a flow rate of 3 mL/min (pressure of 6900 kPa), the retention times for cimetidine and the internal standard were 2.78 and 2.00 min, respectively.

Analytical recovery of cimetidine from plasma ranged from 85.5% (SEM 1.4%) to 91.6% (SEM 3.7%), and was independent of concentration over the range 0.28 to 6.96 μg/mL of plasma.

Intra-assay CVs ranged from 4.04% to 9.41% and were independent of the amount of cimetidine analyzed. Interassay CVs varied from 6.39% to 6.58%, and were also unrelated to the amount of analyte. Day-to-day variation in standard curve parameters were minimal and not statistically significant. Coefficients of determination (R²) were 0.994, 0.985, and 0.999 for the standard curve for cimetidine in plasma, for the lower end of the plasma.

Fig. 1. Typical chromatographic tracing of cimetidine and internal standard

A. Cimetidine and the internal standard were added to plasma, extracted, and chromatographed as described in the text. The internal standard and cimetidine eluted at 2.00 and 2.78 min, respectively. B. Same as A, but omitting the protonation step from the extraction procedure.

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standard curve, and for the gastric fluid standard curve, respectively; intercepts were -0.021, 0.1194, and 0.007, respectively.

Discussion

Studies of the pharmacokinetics and pharmacodynamics of cimetidine demand analytical methods that not only are sensitive and specific, but also allow for the efficient throughput of samples. We developed the method described here because the existing methods have low extraction efficiency or do not allow for the rapid determination of small samples with low concentrations of cimetidine.

The extraction procedure utilized was modified from that described by Lorenzo and Drayer (5), and simplified by the elimination of a complex solvent mixture for extraction. We used the back-extraction of protonated cimetidine and internal standard to get the compounds into an organic phase suitable for rapid evaporation. We believe that the enhanced resolution and sensitivity justify the additional steps of protonation and back-extraction.

The high on-column sensitivity of this method should allow the determination of cimetidine in the amounts of plasma that could be obtained from a small laboratory animal, while the steepness of the lower portion of the standard curve suggests acceptable results for samples with very low concentrations of cimetidine.

Figure 2 demonstrates the clinical use of the method. In a study approved by our institutional review board, an apparently healthy woman voluntarily received an intravenous bolus of 400 mg of cimetidine (Tagamet; Smith Kline & French). Blood samples (0.5 mL) were drawn at the times indicated, and analyzed as described. Seven hours after the cimetidine administration, the lowest cimetidine concentration measurable was reached, 0.2 μg/mL.

The ability to easily and reproducibly assay cimetidine in both plasma and gastric fluid will facilitate studies of the pharmacokinetic-pharmacodynamic interrelationships of this drug and should aid in the investigation of patients with so-called cimetidine resistance.

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References