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AUTOMATED SOLID-PHASE EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF RANITIDINE FROM URINE, PLASMA AND PERITONEAL DIALYSATE

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SUMMARY

Ranitidine is an H_2-receptor antagonist primarily used to treat peptic ulcer. The present automated solid-phase extraction technique involves sorbent conditioning of a cyano (CN) cartridge with 0.5 ml of methanol and 1.0 ml of extraction buffer (0.005 *M* phosphate, pH 9). Plasma samples were applied by passing 1.0 ml of plasma through the cartridge and subsequently washing with 2 ml of the extraction buffer. Appropriate larger volumes of dialysate were used to concentrate ranitidine onto the cartridge so that the amount eluted was increased to within detectable limits. Urine samples were deluted with distilled water to decrease the ranitidine concentration to within the range of the standard curve. The high-performance liquid chromatographic method (mobile phase 88–89% of 0.02 *M* phosphate buffer pH 3 and 11–12% of methanol; Spherisorb phenyl cartridge column, 10 cm×0.46 cm I.D., 5 μm particle diameter, flow-rate 1.1 ml/min; detection at 228 nm) is sensitive to 2 ng/ml in 1 ml of sample. The internal standard of choice was determined to be *n*-propionylprocainamide as compared to cimetidine and lidocaine. The method was cost-efficient, rapid and simple due to the automated sample processing. The coefficient of variation on replicate assays was less than 10% over all concentrations studied. Recoveries were between 97 and 110%, and the method was linear over the range 1.90–687.20 with a mean correlation coefficient of 0.999.

INTRODUCTION

Ranitidine (N-[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]-thio]ethyl]-N′-methyl-2-nitro-1,1-ethenediamine), like cimetidine, is an H_2-receptor antagonist which is used to treat peptic ulcer [1]. Fig. 1 shows the chemical structure of ranitidine.

The analysis of ranitidine from biological fluids (plasma and urine) has been accomplished using various analytical procedures including high-performance liquid chromatography (HPLC) [1–7], radioimmunoassay [8] and liquid chro-

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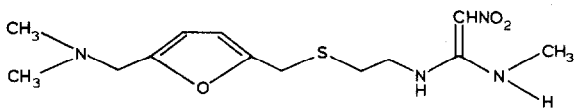

Fig. 1. Chemical structure of ranitidine.

matography–mass spectrometry [9]. Previous extraction techniques for ranitidine have been time-consuming and labor-intensive. The previous procedures were generally accomplished by liquid–liquid extraction of an aqueous sample into an organic solvent, followed by back-extraction of the organic layer into another aqueous phase. The organic solvent is then evaporated to dryness before reconstitution in the appropriate solvent.

In the current (Advanced Automated Sample Preparation, AASP™) method, an automated solid-phase extraction system is used. The solid-phase sorbent is conditioned with methanol and the extraction buffer. Samples are then passed through the sorbent followed by the addition of internal standard. Analytes are bound onto the sorbent, and interfering substances are washed with an appropriate solvent system. Analytes are then eluted on-line and automatically from the sorbent with the mobile phase. The current method was found to reduce errors due to sample manipulation, which provided improved reproducibility as well as superior recovery. Centrifugation and evaporation steps typically used in liquid–liquid extraction schemes can be eliminated. The sorbent extraction is therefore more convenient, and much less time is required for sample processing.

The determination of ranitidine in a large number of plasma, urine and peritoneal dialysate samples was required as part of a pharmacokinetic study of ranitidine. It was important, therefore, to develop a rapid and simple extraction scheme for ranitidine from plasma, urine and dialysate.

EXPERIMENTAL

Chemicals

Ranitidine hydrochloride was a gift from Glaxo Group Research (Ware, U.K.). Cimetidine was obtained from Smith, Kline and French Labs. (Philadelphia, PA, U.S.A.). *n*-Propionylprocainamide and procainamide were obtained from Aldrich Chemicals (Milwaukee, WI, U.S.A.). Lidocaine was purchased from Astra Pharmaceutical Products (Worcester, MA, U.S.A.). Organic solvent (acetonitrile and methanol) were purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.). All other solvents and reagents were HPLC grade from Fisher Scientific unless otherwise specified.

Instrumentation and chromatographic conditions

The programmable gradient liquid chromatograph was assembled from Gilson Model 302 pumps, Model 116 UV detector, 802 B manometric module, 811 dynamic mixer and 620 data module. The AASP Vac-Elut™ system for processing extraction cassettes was purchased from Analytichem International (Harbor City,

CA, U.S.A.) and was used as received. An Apple Model D computer was used to fully automate sample injection from the Varian AASP (Varian Instruments, Sugarland, TX, U.S.A.) onto the column. The AASP was programmed to inject the processed sample, and the computer was programmed to autozero the baseline for each chromatogram during the period necessary to analyze all samples. An Alltech 10 cm×0.46 cm I.D. (5 μm particle diameter) Spherisorb phenyl cartridge column was used at a flow-rate of 1.0 ml/min. The mobile phase for plasma samples consisted of 0.02 *M* phosphate buffer pH 3–acetonitrile (89:11). The UV detector was set at 228 nm and 0.0001 a.u.f.s. for absorption measurements.

The mobile phase was modified to separate interfering peaks from urine and dialysate by altering the solvent strength of the mobile phase to 0.02 *M* phosphate buffer–acetonitrile (88:12). The total time of analysis of ten plasma samples (including chromatographic and sample preparation time) was about 125 min.

Standard curve

Ranitidine hydrochloride (26.75 mg) was weighed out exactly and dissolved in a 25-ml volumetric flask with deionized, distilled water. Dilutions were made from the stock solution fresh each day for various concentration levels (0–687.2 ng/ml) of ranitidine in the appropriate fluid (plasma, urine and dialysate) . Direct injection (unextracted) of standards diluted in water were analyzed for each standard point concentration in recovery studies. Spiked biological fluid solutions were aliquoted, stored frozen and run as controls for precision studies.

Plasma analysis

Nitrogen was used as a source of pressure to push solutions through the cartridge using the Vac-Elut System. The cartridge sorbent material was conditioned by passing 0.5 ml of methanol through the CN cartridge followed by 1 ml of 0.005 *M* phosphate buffer solution, pH 9 (extraction buffer). The samples were prepared by passing 50 μl of 5 μg/ml internal standard (*n*-propionylprocainamide) through the cartridge, followed by 1.0 ml of plasma from drug-free normal volunteers and 1.0 ml of extraction buffer. The AASP conditions used for analysis included a pre- and post-injection purge of 250 μl with deionized distilled water and a valve reset time of 3.0 min. The prepared cassettes (containing ten sorbent cartridges per cassette) were then loaded onto the AASP for on-line elution and analysis. The selectivity of the method was assessed by assaying blank biological fluids for interfering peaks. Replicate spiked biological fluid samples were analyzed to determine the reproducibility of the method.

Dialysate analysis

The sorbent was again conditioned by passing 0.5 ml of methanol through the cartridge followed by 1.0 ml of pH 9 extraction buffer. Unused blank dialysate fluid (1 ml) for peritoneal dialysis was passed through the CN cartridge followed by 1.0 ml of extraction buffer. This sample application step can be repeated in order to concentrate the sample before washing the sorbent with an additional 2.0 ml of extraction buffer. The dialysate was then eluted and analyzed using the same conditions as for the plasma assay although no internal standard was used.

Urine analysis

After conditioning the sorbent with 0.5 ml of methanol and 1.0 ml of extraction buffer, 50 μl of *n*-propionylprocainamide (internal standard) was passed through the cartridge. Typically one volume of urine was diluted with four volumes of extraction buffer to provide ranitidine concentrations that were within the range at the calibration curve although two-fold dilutions and undiluted urines were also used. A 1-ml volume of urine dilution was added to the cartridge and pushed through the cartridge under nitrogen pressure. The cartridge was then washed twice with 1.0 ml of extraction buffer. The same conditions for plasma analysis were used to analyze urine samples.

RESULTS AND DISCUSSION

HPLC method

UV absorption scans were collected and demonstrated absorption maxima for ranitidine at 228 and 330 nm. The 228-nm wavelength was used for detection because cimetidine did not absorb significantly at 330 nm and the other potential internal standards, *n*-propionylprocainamide and procainamide, demonstrated peaks that were unresolved from interferences at 330 nm. Duplication of the HPLC conditions of Vandenberghe et al. [2] produced inadequate resolution of the ranitidine peak from plasma contaminants with our lot of pooled plasma. Both a 25 cm $\times$ 4.6 mm I.D. phenyl column (5 μm particle diameter) and a 25 cm $\times$ 4.6 mm I.D. ODS (10 μm particle diameter) column demonstrated retention and peak shape characteristics that were inferior to those produced with the 10-cm phenyl column.

Extraction procedure

Three types of AASP cartridges were investigated for use in the study. The CN cartridge was found to give the best recovery under the chromatographic conditions and was therefore used. Each initial recovery experiment was done in triplicate by injecting 50 ng of ranitidine in plasma via the current procedure onto the HPLC column. The peak areas from these injections were compared to peak areas from direct injection, and the C_2, CN and C_8 cartridges demonstrated recoveries (mean $\pm$ R.S.D.) of 5 ± 7.3, 93 ± 2.5 and 7 ± 5.6%, respectively. Phenyl cartridges were not tried because the CN cartridges were adequate and our laboratory had a large supply.

Choice of extraction buffer and wash solvent

Preliminary studies were carried out to evaluate the possibility of extracting ranitidine at low pH because of a cleaner extract that was provided. This resulted in recoveries below 5% using C_2, CN and C_8 cartridges. A 0.02 *M* phosphate buffer, pH 3, was used to condition the cartridges for this evaluation, and 200 μl of the same buffer were then used as the extraction solvent for every 500-μl plasma sample. Increasing the volume of extracting solution from 200 to 800 μl did not improve the amount of ranitidine recovered above 5%. The CN AASP cartridge extraction method given under *Plasma analysis* was optimized with respect to

extraction solvent volume and cartridge conditioning procedure. This method was found to show the best recoveries while maintaining good selectivity.

The selection of internal standard was partly based on the availability of four similar compounds. Cimetidine and procainamide co-eluted with interfering peaks from plasma. Lidocaine was retained 20 min longer than *n*-propionylprocainamide and was therefore considered unacceptable. *n*-Propionylprocainamide was therefore selected as the internal standard on the basis of both its chromatographic and extraction behavior.

The volume of dialysate containing ranitidine that can be applied to the CN cartridge without loss of the drug was evaluated. It was found that a total of 4.0 ml of ranitidine solution may be concentrated onto the CN cartridge under assay conditions. Volumes greater than 4.0 ml resulted in a sharp drop in the amount recovered relative to the amount added. The amount of *n*-propionylprocainamide decreased with increasing volumes of dialysate solution, however, and it was decided to eliminate the internal standard from the dialysate procedure. This may be due to a partial wash of the internal standard by the dialysate solution. The ionic strength and pH of the dialysate are very important for binding of ranitidine and *n*-propionylprocainamide to the sorbent. Making the dialysates alkaline and adding salt did not solve the problem, however.

Method validation

Standards diluted in plasma were linear over the concentration range 1.90–687.20 ng/ml. The normal linear regression line was $y = 0.013x + 0.082$, where y was ranitidine concentration and x the peak-area ratio (i.e. ranitidine/internal standard). Back calculated values of the standards using the linear regression line showed less than 10% error. Mean values (ng/ml) of ten standard samples back-calculated from the regression curve were 691.21, 532.07, 379.47, 230.81, 77.03, 37.14, 19.28, 7.65, 4.00 and 2.08 for the 687.20, 534.30, 382.20, 229.30, 76.40, 38.20, 19.10, 7.60, 3.80 and 1.90 standards, respectively. The mean correlation coefficient for calibration curves extracted from plasma was 0.997 ($n = 6$). The mean correlation coefficient of urine and dialysate curves was 0.999 ($n = 3$).

Absolute recovery was defined as the peak area of the extracted sample divided by the peak area produced from an equivalent amount of drug injected directly onto the column from aqueous solution. This ratio was expressed as percent, and the results of this study are presented in Table I. All recoveries were between 96 and 116% indicating adequate recovery of the method. Recovery studies were not carried out in depth for urine and dialysate matrices although the recovery from these fluids was apparently equivalent to that from plasma.

The accuracy of the method is indicated by the error of assayed samples relative to their spiked concentrations. These values are listed in Table II along with the concentration at which the study was carried out in the three fluids. The method demonstrated superior accuracy in that all errors were less than 10%, with many points showing less than 1% error.

The method was also shown to be precise in studies of replicate assays (Table II). Studies carried out in the three biologic fluids of interest all demonstrated

TABLE I

RECOVERY OF RANITIDINE

Concentration added (ng/ml)	Mean concentration found (ng/ml)	Recovery (%)	Coefficient of variation (%)
Plasma (n=3)			
1.9	2.1	109.6	8.0
3.8	4.0	105.3	9.1
7.6	7.6	100.6	3.0
19.1	19.3	100.9	5.0
38.2	37.1	97.2	1.3
76.4	77.0	100.8	0.9
229.3	230.8	100.7	1.4
Urine (n=2)			
2.0	2.2	107.5	18.4
10.0	9.9	99.9	3.3
50.0	49.1	98.1	1.6
400.0	397.5	99.4	0.2
Dialysate (n=2)			
2.0	2.3	115.8	10.8
10.0	10.7	107.0	6.5
50.0	47.9	95.8	3.2
400.0	392.5	98.1	1.6

TABLE II

ACCURACY AND PRECISION OF RANITIDINE METHOD

Spiked concentration (ng/ml)	Assayed concentration (mean ± S.D.) (ng/ml)	Coefficient of variation (%)	Relative error (%)
Plasma (n=10)			
26.7	25.2 ± 1.3	5.1	5.5
134.1	135.0 ± 5.4	4.0	0.7
402.1	401.9 ± 9.6	2.4	0.1
536.8	560.2 ± 18.4	3.3	4.4
Urine (n=4)			
3000 (1:5 dilution)	3007.3 ± 17.0	0.6	0.2
375 (1:5 dilution)	377.5 ± 17.1	4.5	0.7
25 (1:5 dilution)	26.0 ± 3.1	11.9	4.0
Dialysate (n=4)			
600	615.0 ± 19.8	3.2	2.5
75	81.8 ± 4.4	5.4	9.1
5	5.6 ± 0.4	7.9	12.0

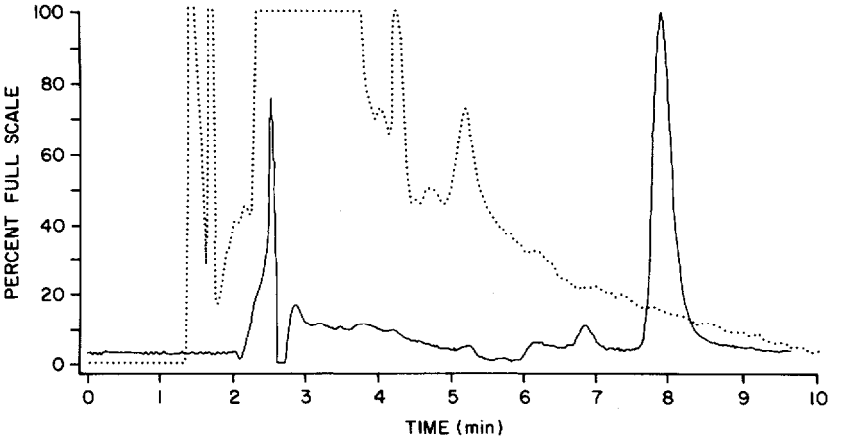

Fig. 2. Chromatograms of blank plasma (broken line; normalized at $4.3 \cdot 10^{-5}$ a.u.f.s.) and plasma spiked with 10 ng/ml ranitidine with internal standard (solid line; normalized at $7.6 \cdot 10^{-4}$ a.u.f.s.); retention times: 6.9 min for ranitidine and 8.0 min for *n*-propionylprocainamide.

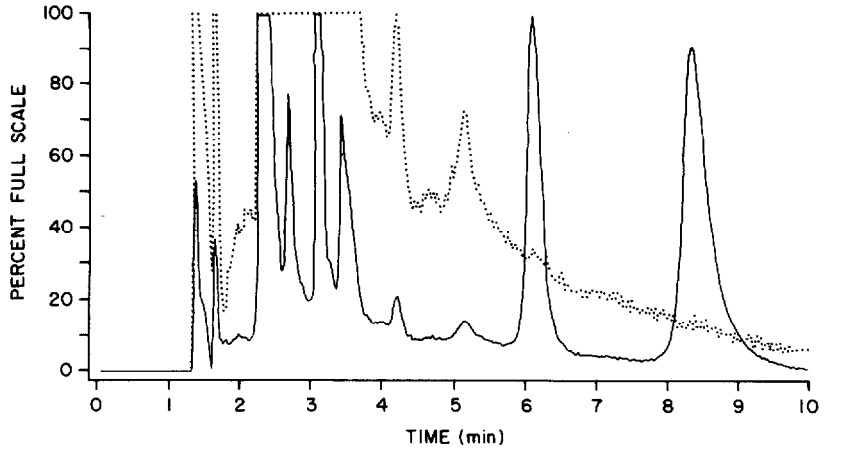

Fig. 3. Chromatogram of blank urine (broken line; normalized at $1.0 \cdot 10^{-8}$ a.u.f.s.) and urine spiked with 50 ng/ml ranitidine with internal standard (solid line, normalized at $4.78 \cdot 10^{-4}$ a.u.f.s.); retention times: 6.2 min for ranitidine and 8.4 min for *n*-propionylprocainamide.

less than 12% (coefficient of variation) which demonstrates good reproducibility of the method.

The limit of detection of the method was defined as the concentration of ranitidine that would provide a signal equivalent to twice the noise level. This was found to be 2 ng/ml in plasma and urine although lower levels may be measured in dialysate (0.7 ng/ml) by concentrating the sample onto the cartridge from larger volumes.

Good selectivity of the method is indicated by the lack of interfering peaks in plasma (Fig. 2), urine (Fig. 3) and dialysate (Fig. 4) when compared to spiked fluids. In each case, the blank fluid is normalized to a more sensitive electronic setting so that a more accurate picture of the baseline can be obtained. Several drug compounds were tested at levels greater than therapeutic for interference

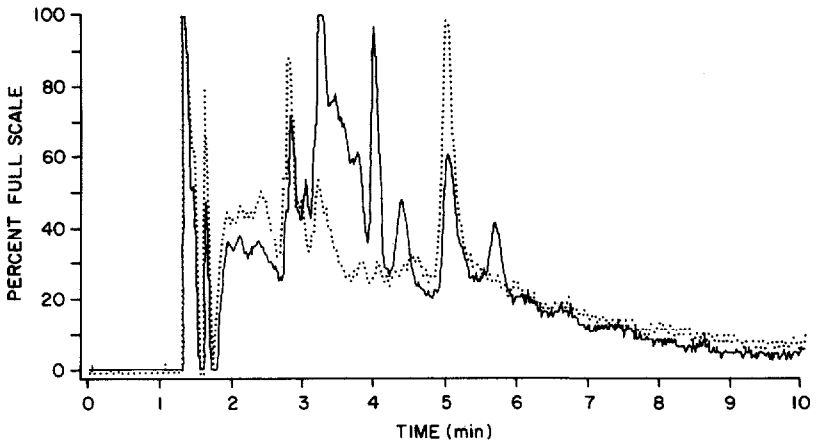

Fig. 4. Chromatogram of blank peritoneal dialysate (broken line; normalized at $1.02 \cdot 10^{-4}$ a.u.f.s.) and dialysate from a patient receiving ranitidine that was analyzed to contain 15.6 ng/ml drug (solid line; normalized at $9.8 \cdot 10^{-5}$ a.u.f.s.); retention time: 5.8 min for ranitidine.

with the method. Procaine and *n*-acetylprocainamide produced peaks that were unresolved from the ranitidine peak, whereas *n*-propionylprocainamide, lidocaine, cimetidine, flurbiprofen, diazepam, oxazepam, phenobarbital, phenytoin and carbamazepine demonstrated no interferences.

CONCLUSION

We conclude that the automated sorbent extraction method is more convenient than existing procedures. The method is accurate and precise and more sensitive than other HPLC methods. The lowest detection limit previously reported for an HPLC method was 5 ng/ml [2]. We believe that our superior sensitivity of 2 ng/ml is due to the fact that the entire sample is injected on to the column. In previous liquid–liquid extractions, the dried extract is reconstituted in an appropriate solvent, then injected. It is difficult to inject the entire extract, however, and this is a decided advantage of the sorbent extraction method. The method is useful for controlled studies and should be more free of interferences than liquid–liquid extraction methods. This is predicted since the sorbent cartridge is in the mobile phase path for a controlled period of time and the cartridge is purged previous to injection. This adds the capability of using the prime purge and valve reset time adjustments to remove both short- and long-eluting interferences.

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