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Short communication

Solid-phase extraction and determination of ranitidine in human plasma by a high-performance liquid chromatographic method utilizing midbore chromatography

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Abstract

An improved high-performance liquid chromatographic (HPLC) method utilizing solid-phase extraction (SPE) and midbore chromatography was developed for the determination of ranitidine in human plasma. A mobile phase of 20 m*M* K_2HPO_4–acetonitrile–triethylamine (87.9:12.0:0.1, v/v) pH 6.0 was used with a phenyl analytical column and ultraviolet detection (UV). The method demonstrated linearity from 25 to 1000 ng/ml in 500 μl of plasma with a detection limit of 10 ng/ml. The method was utilized in a pharmacokinetic study evaluating the effects of pancreatico–biliary secretions on ranitidine absorption.

Keywords: Ranitidine

1. Introduction

Ranitidine, N,N-dimethyl-5-[2-(1-methylamino-2-nitrovinylamino)ethylthiomethyl]furfurylamine, is a H_2 receptor antagonist commonly used in the treatment of acid-peptic disease [1]. Analysis of ranitidine in human plasma is commonly performed using HPLC [2–5]. A HPLC method for determination of ranitidine was previously developed [6] and employed to characterize the pharmacokinetic disposition of ranitidine in patients with renal failure [7,8]; however, it was necessary to modify the method when the vendor for the advanced automated sample preparation (AASP, Varian Instruments, Sugarland, TX, USA) equipment discontinued replacement parts for this product. The method detailed in the present communication utilizes a simple solid-phase extraction process, employs current HPLC midbore column technology, and provides good sensitivity and reduced mobile phase requirements.

2. Experimental

2.1. Chemicals, extraction buffer, and sorbent

Ranitidine hydrochloride (CAS 66357-35-5) and *n*-propionylprocainamide (CAS 67635-46-5) were purchased from Sigma (St. Louis, MO, USA). Dibasic potassium phosphate, orthophosphoric acid,

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potassium hydroxide, and triethylamine were purchased from Fisher Scientific (Fair Lawn, NJ, USA) and were HPLC grade. Acetonitrile (Burdick and Jackson UV grade) was purchased from Baxter (Columbia, MD, USA). Ultrapure distilled and deionized water was prepared in-house and filtered prior to use. The Bond Elute cyano sorbent (50 mg tube) was purchased from Varian (Harbor City, CA, USA). The extraction buffer was 100 m*M* dibasic potassium phosphate (K_2HPO_4) with pH adjustment to 10.0 using 5 *M* potassium hydroxide solution.

2.2. HPLC equipment and mobile phase

The HPLC equipment consisted of a LKB Model 2150 HPLC pump (Gaithersburg, MD, USA) and LKB Model 2152 LC controller. The analytical column was a Hypersil phenyl, 150 mm×3.2 mm I.D., 3 μm packing (Phenomenex, Torrance, CA, USA). The C_{18} guard column, 30 mm×4.6 mm I.D., 40–50 μm pellicular packing (Alltech, Deerfield, IL, USA) was replaced prior to each analytical run which typically consisted of approximately 50 samples. The mobile phase consisted of 20 m*M* K_2HPO_4–acetonitrile–triethylamine (87.9:12.0:0.1, v/v) adjusted to pH 6.0 using concentrated phosphoric acid. The mobile phase was degassed daily using helium sparging and the flow-rate was maintained at 0.6 ml/min. Typical operating pressure was 14 000 kPa at ambient temperature. An injection volume of 25 μl of the extracted plasma sample was accomplished using a WISP Model 712 (Waters, Milford, MA, USA) autosampler. Compound detection was achieved with a Shimadzu SPD-6A UV detector (Tokyo, Japan) operating at 228 nm with a 1-s response time. A 345 kPa back-pressure regulator (SSI, State College, PA, USA) was coupled to the detector outlet to prevent outgassing. Data acquisition and component computations were performed using Turbochrom (PE Nelson, Norwalk, CT, USA) chromatography software on a Hewlett-Packard (Palo Alto, CA, USA) 486 DX-33 personal computer.

2.3. Standard, internal standard, and control preparation

Stock standards of ranitidine (1 mg/ml) and the internal standard *n*-propionylprocainamide (1 mg/ml) were prepared in methanol and stored at 4°C. Working standards of 25, 50, 100, 250, 500, and 1000 ng/ml ranitidine were prepared using blank plasma as the diluent. The working internal standard solution was 38 μg/ml prepared using deionized water as the diluent. Control samples of 75, 400, and 750 ng/ml ranitidine were prepared using blank plasma as the diluent and stored at −30°C with the study subject's plasma samples.

2.4. Sample conditions

Subject plasma samples were collected using heparin vacutainers, centrifuged to achieve separation, and frozen at −30°C until analysis. For analysis, plasma samples were thawed to ambient temperature, mixed thoroughly by inversion, and centrifuged to eliminate extraneous plasma fibrin.

2.5. Solid-phase extraction

Plasma samples were extracted with a cyano sorbent utilizing the following sequence. A vacuum manifold operating at 5 in.Hg was used for sorbent conditioning, sample application, wash steps, and elution of the analytes. The cyano sorbent was conditioned using 1 ml of acetonitrile followed by two 1-ml extraction buffer washes. Sample pretreatment consisted of pipetting 25 μl of the working internal standard, 500 μl of the plasma sample, and 500 μl of extraction buffer into a 12×75 mm polypropylene culture tube and vortex-mixing for 10 s. The pretreated sample was applied to the conditioned sorbent and subsequently washed by two 0.5-ml extraction buffer washes. The analytes were eluted off the sorbent using 250 μl acetonitrile–water (50:50, v/v) into a 12×75 mm polypropylene tube and vortex-mixed. The eluted analytes were transferred to polypropylene autosampler microvials; 25 μl was injected into the HPLC system.

3. Results and discussion

3.1. Chromatography

The method demonstrated excellent chromatographic selectivity with no endogenous or metabolite interferences at the retention times for ranitidine (5.6

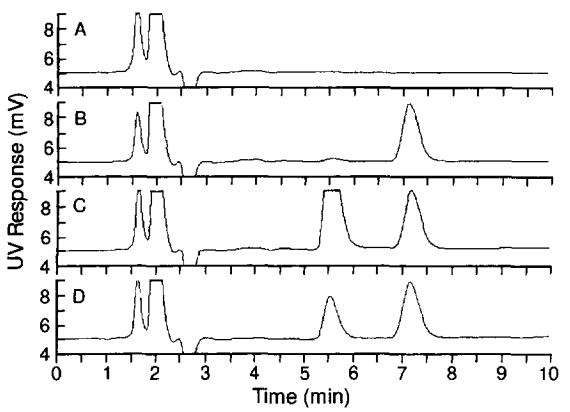

Fig. 1. Chromatograms of (A) extracted blank human plasma, (B) extracted blank human plasma spiked with 25 ng/ml ranitidine, (C) extracted blank human plasma spiked with 1000 ng/ml ranitidine, (D) subject dosed with 300 mg ranitidine (60 min sample-ranitidine concentration 335 ng/ml). Peak at 5.6 min= ranitidine; peak at 7.2 min=*n*-propionyl-procainamide at 38 μg/ml.

min) and *n*-propionylprocainamide (7.2 min) (Fig. 1A). Chromatograms of extracted blank human plasma containing low (25 ng/ml) and high (1000 ng/ml) concentrations of ranitidine (Fig. 1B and 1C, respectively) indicated good detector response and baseline resolution between ranitidine and *n*-propionylprocainamide with an analytical run time of 10 min. A typical chromatogram from one subject dosed with ranitidine (300 mg via oroenteric tube) is shown in Fig. 1D.

3.2. Linearity, limit of detection, and computations

The method was linear throughout the concentration range of 25 to 1000 ng/ml with a mean correlation coefficient of 0.99965 ($n=10$ analytical runs). The limit of detection for the method (approximately 10 ng/ml) was determined by forced integration of the blank plasma baseline at ranitidine's retention time and determining the 3 sigma concentration level ($n=6$). For all component calculations, normal linear regression using Lotus 1-2-3 with internal standardization and peak-area ratios was used.

3.3. Accuracy, precision, and recovery

The accuracy and precision of this HPLC method was determined by evaluation of replicate control samples ($n\geq 20$ each) over the course of all analytical runs at concentrations of 75, 400, and 750 ng/ml. The accuracy of the method was reported as the percentage error from the theoretical versus measured ranitidine concentrations and was less than 1.5% for all control samples (Table 1). The precision of the method was reported as percent relative standard deviation and was less than 6.7% for all control levels (Table 1). Absolute recovery for the method was performed by evaluating unextracted standards prepared in acetonitrile and deionized water (50:50, v/v) versus extracted plasma samples containing 75, 400, and 750 ng/ml ranitidine ($n=3$). The mean absolute recovery for ranitidine was 90%.

3.4. Stability

Stability of control samples containing 75, 400, and 750 ng/ml ranitidine was demonstrated as the calculated concentrations for the controls did not significantly decrease over the course of the study (two months). The controls were prepared in bulk at the onset of the study and stored at −20°C with the patient samples. A freeze–thaw study was performed by freezing and thawing the control samples. Three freeze–thaw evaluations demonstrated no significant decrease in ranitidine concentrations which indicates freeze–thaw stability of ranitidine.

3.5. Pharmacokinetics study

In the course of assessing the influence of pancreatico–biliary secretions on ranitidine's absorption, a 300-mg dose was administered via oroenteric tube to each study subject ($n=4$). Fig. 2 shows one study

Table 1
Combined intra- and inter-day accuracy and precision

Theoretical concentration (ng/ml)	Measured concentration (ng/ml)	*n*	R.S.D (%)	Error (%)
75.0	73.9	22	6.7	−1.5
400.0	398.1	22	3.3	−0.5
750.0	750.6	20	2.6	0.1

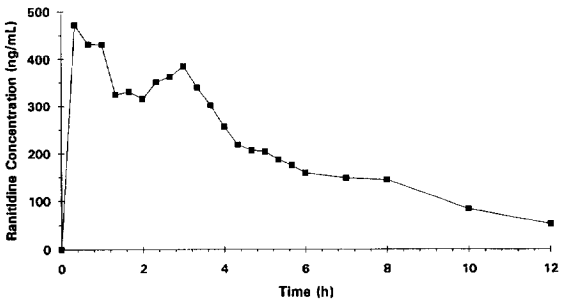

Fig. 2. One study subject's pharmacokinetic profile for ranitidine in plasma with time (h) versus concentration (ng/ml).

subject's pharmacokinetic profile for ranitidine in plasma.

4. Conclusion

We found it necessary to modify a previously published method for ranitidine [6] due to mechanical problems with the AASP and discontinued support for this technology by the vendor. The authors chose to use the same phase of extraction sorbent (cyano) as it offered excellent selectivity and good recovery. This method was further advanced by employing current midbore HPLC column technology which offered good sensitivity and reduced mobile phase requirements. Since acetonitrile is the expensive component of the mobile phase utilized for this method, the cost of analysis (solvent procurement and subsequent hazardous waste disposal) was reduced significantly. It is noteworthy that using the midbore column did not necessitate modifications to the HPLC system (e.g. injection volume, tubing size, and detector cell volume). The method offers a fast analytical run time of 10 min to achieve baseline resolution between endogenous substances, ranitidine, and the internal standard. The method was employed in the evaluation of over 500 plasma samples from a pharmacokinetic study without methodological problems.

References

[1] P.F. Carey, L.E. Martin and P.E. Owen, J. Chromatogr., 225 (1981) 161–168.
[2] S.T. Bauer, A. Grabow, H.G. Trautsch-Forster and H. Huller, Pharmazie, 46 (1991) 533–534.
[3] T.L. Lloyd, T.B. Perschy, A.E. Gooding and J.J. Tomlinson, Biomed. Chromatogr., 6(6) (1992) 311–316.
[4] P. Ballard and B. Law, J. Pharm. Biomed. Anal., 8(8–12) (1990) 877–880.
[5] A.C. Blum et al., Pharm. Res., 10 (1993) 1688–1692.
[6] H.T. Karnes, K. Opong-Mensah, D. Farthing and L.A. Beightol, J. Chromatogr., 422 (1987) 165–173.
[7] D.A. Sica, T. Comstock, A.M. Harford and F. Eschelman, Eur. J. Clin. Pharmacol., 32 (1987) 587–591.
[8] T.J. Comstock, D.A. Sica, S. Stone, J. Davis, W.R. Garnett and H.T. Karnes, J. Clinical Pharmacol., 28 (1988) 1081–1085.