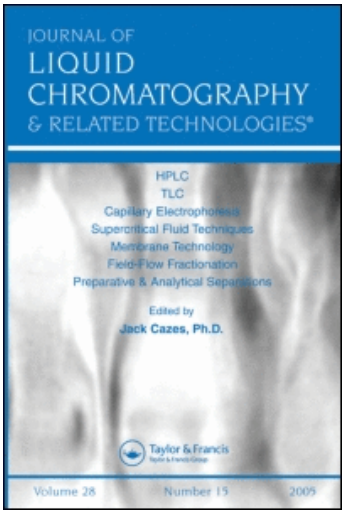

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Rapid Method for Cimetidine and Ranitidine Determination in Human and Rat Plasma by HPLC

J. S. Kaka[a]
[a] Department of Clinical Pharmacy, College of Pharmacy King Saud University, Saudi, Arabia

To cite this Article Kaka, J. S.(1988) 'Rapid Method for Cimetidine and Ranitidine Determination in Human and Rat Plasma by HPLC', Journal of Liquid Chromatography & Related Technologies, 11: 16, 3447 — 3456
To link to this Article: DOI: 10.1080/01483918808082267
URL: http://dx.doi.org/10.1080/01483918808082267

JOURNAL OF LIQUID CHROMATOGRAPHY, 11(16), 3447-3456 (1988)

RAPID METHOD FOR CIMETIDINE AND RANITIDINE DETERMINATION IN HUMAN AND RAT PLASMA BY HPLC

J.S. KAKA
Department of Clinical Pharmacy
College of Pharmacy
King Saud University
P.O. Box 2457
Riyadh 11451
Saudi Arabia

ABSTRACT

A rapid method for determination of cimetidine and rantidine in 100 ul human or rat plasma by isocratic reverse phase HPLC is described. Ranitidine is used as an internal standard for cimetidine assay and vice versa. The procedure consists of one step extraction of alkalinized plasma containing internal standard with 4% v/v isopropranolol in ethyl acetate by vortex for 1 min. The organic phase is transferred and evaporated under a stream of nitrogen. The residue was redissolved with methonal and subsequently eluted on u-Bondapack C_{18} column at 225 nm. The mobile phase consisted of 8% v/v acetonitrile in 5.0 mM potassium phosphate buffer at pH 5.1. The sample was completed eluted in 9 min. with a detection limit of 0.05 ug/ml for cimetidine and 0.1 ug/ml for ranitidine.

INTRODUCTION

Cimetidine and ranitidine have been primarily used to treat gastro-duodenal ulcers, the Zollinger-Ellison syndrome and other

gastric hypersecretory states [1-4]. Experience acquired throughout the years of usage uncovered potential adverse drug interactions, especially for cimetidine [5].

Several high-performance liquid chromatographic (HPLC) methods have been reported for determining plasma cimetidine and ranitidine in the literature [6-16]. Methods of Larson et al. [6], Kunitani et al. [7] and Mihaly et al. [8] extract cimetidine and internal standard from alkalinized plasma but the analytical recoveries were low (65%). Published procedures [9-12] had cimetidine analytical recoveries ranging from 85 to 100% but require atleast 250 ul or more of plasma for the assay. Also analytical recoveries of ranitidine were high (90% or more) but these methods required atleast 200 ul or more of human plasma for analysis [13-15].

In contrast, the method described determines cimetidine as well as rantidine which requires only 100 ul plasma (human or rat) with rapid one-step extraction procedure and analytical recoveries averaging 90% for both drugs.

MATERIALS

Reagents:

Cimetidine hydrochloride (Smith Kline & French Laboratories Ltd., Herts, U.K.), and ranitidine hydrochloride (Glaxo Ltd., Herts, U.K.) were kindly gifted.Acetonitrile, methanol and ethyl acetate (E. Merck, Darmstadt, Germany), di-potassium hydrogen phosphate (BDH Chemicals Ltd., Poole, U.K.) and isopropranolol (Riedel-deHaenag, Seelze-Hannover, Germany) were used.

METHODS

Stock Solutions:

Cimetidine and ranitidine were separately prepared by dissolving 25 mg each in 25ml of methanol. Potassium phosphate buffer (5.0 mM) was prepared and adjusted to pH 5.1 with phosphoric acid. All stock solutions were stored at 4°C.

Chromatographic conditions:

The HPLC unit consisted of a Waters Assoc. Model M-45 solvent delivery system, a Waters Assoc. Model 450 variable wavelength detector, a Waters Assoc. Model 46K universal liquid chromatograph injector and Bausch & Lomb D5000 recorder. The column used was a Waters Assoc. radical-pak u-Bondapak C_{18} column (10cm x 8mm I.D., 10 um particle size) inserted in Z-module radial compression separation system. The mobile phase was 5.0 mM potassium phosphate buffer (pH 5.1) - acetonitrile mixture with a final acetonitrile concentration of 8% v/v. The flow-rate was set at 3.0 ml/min. The effluent was monitored at 225 nm with a detection scale of 0.005 or higher as needed.

Sample Preparation:

To a 100 ul of aliquot of plasma, 10 ul of internal standard and 15 ul of 6N NaOH were added. The mixture was extracted with 500 ul of 4% v/v isopropranolol in ethyl acetate by vortexing for 1 min. After centrifugation the organic layer was transferred and evaporated to dryness at 80°C

under a stream of nitrogen. The residue was reconstituted with 50 ul of methanol and 40 ul was injected onto the column.

Standard Calibration Curve:

Blank human and rat plasma aliquots were spiked with cimetidine or ranitidine standard solution to get concentrations ranging from 0.2 to 3.0 ug/ml and subjected to sample procedure. Ranitidine solution (50 ug/ml) in methanol was used as an internal standard for cimetidine assay while cimetidine (10ug/ml) in methanol was used as an internal standard for ranitidine assay. Each point was an average of four replicates.

Recoveries of cimetidine and rantidine from plasma were estimated by comparing peak height of cimetidine obtained after extraction, against that when the same amount of the drug from an aqueous aliquot solution was chromatographed.

Precision Studies:

A single human and rat plasma samples were analysed on five separate days in order to determine the between-day coefficient variation. For elucidation of the within-day variability, five specimens of the same sample were analyzed on each day.

Animal Studies:

Cimetidine and rantidine disposition was assessed in a pilot study, where single fasted rat received cimetidine (40 mg/kg) and second rat received rantidine (50 mg/kg) orally by gastric intubation. A polyethylene cannula (1mm O.D.) was implanted in the abdominal aorta via left femoral artery and

exteriorized from the back of the neck with enough length for safe sampling. Approximately 0.25 ml of blood was taken before and 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.5 and 6h after administration. Heparinized blood was centrifuged, plasma seperated and aliquots were kept at $-20^{o}C$ until analysis.

RESULTS AND DISCUSSION

For all clinical purposes, either cimetidine or rantidine is prescribed and concurrent administrtation is not recommneded, since both are H_2-receptor antagonists. Thus, ranitidine may be safely used as an internal standard for cimetidine and vice versa.

Using the chromatographic procedure described, the separation of cimetidine and rantidine in human and rat plasma is shown in Fig.1. The retention times for cimetidine and rantidine were 6 and 8 min. respectively with no interfering peaks from human and rat plasma in this region. There was a good separation between cimetidine and ranitidine. Each sample was completely eluted in 9 min.

The calibration curves for cimetidine and ranitidine were linear over the range investigated with correlation coefficient 0.998 or greater. The limit of sensitivity of cimetidine and ranitidine by this procedure were atleast 0.05 and 0.1 ug/ml,respectively. Similar limits of sensitivity were reported by other authors [15,17]. Analytical recoveries of

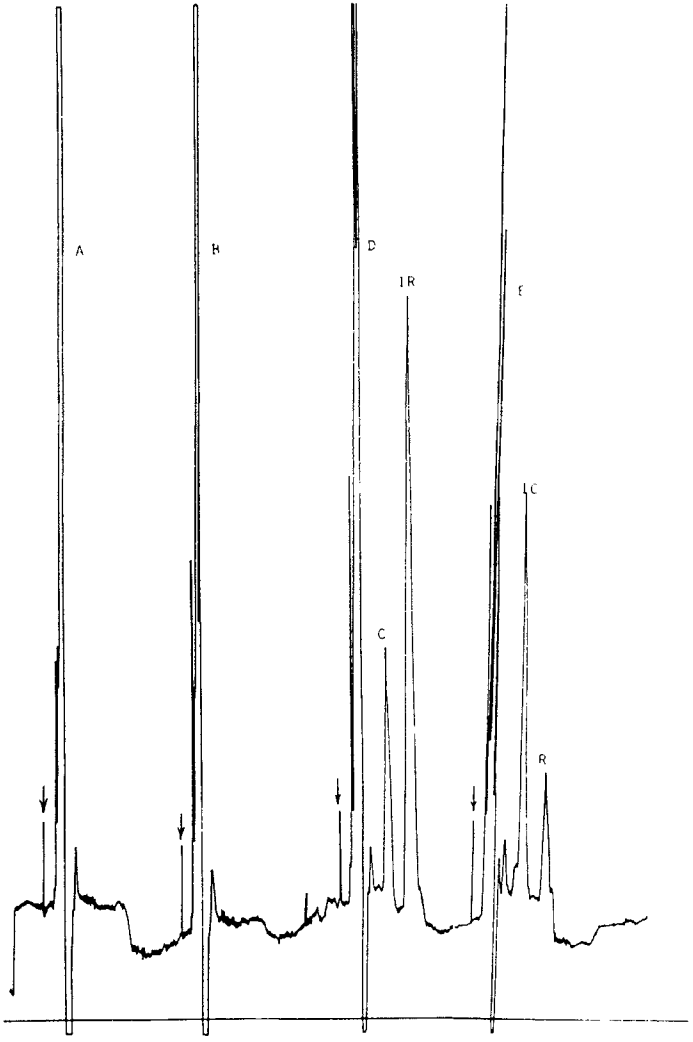

Fig.1:High-pressure liquid chromatograms for a blank human plasma extract (A), a blank rat plasma extract (B), human plasma extract containing cimetidine (C) with ranitidine (IR) as internal standard (D) and rat plasma extract containing ranitidine (R) with cimetidine (IC) as internal standard (E).

cimetidine and ranitidine from rat and human plasma ranged from 85 to 95% respectively. These recoveries were independent of the concentration range investigated. The absolute recovery for cimetidine averaged 65% which compared favourably with those reported by other workers[11,16]. Similarly, absolute of ranitidine recovery averaged 60% [14].

The precision of cimetidine assay was assessed by five replicate of aliquots of human and rat plasma spiked with pure cimetidine. The coefficient of variation for within-day assays in human and rat plasma at 1 ug/ml were 6 and 3%, respectively. The coefficients of variation for day-to-day assay over a week at lug/ml were approximately 5% for both plasma. The precision of rantidine assays was similar to that obtained for cimetidine. Within-day assays in human and rat plasma at 1 ug/ml varied 4 and 9%, respectively. Also, coefficient of variaton for day-to-day assays over a week were 8 and 12% at 1 ug/ml for human and rat plasma.

The assay was applied to the measurement of cimetidine and rantidine plasma obtained from a pilot pharmacokinetic rat study. Fig. 2 shows the semilogarithmic plots of cimetidine and rantidine plasma concentration following oral doses.

The advantages of this assay over other earlier published methods are one step extraction process, only 8% v/v acetonitrile in the mobile phase and requires 100 ul of rat or human plasma without compromizing the minimum detectable limits for cimetidine and rantidine.

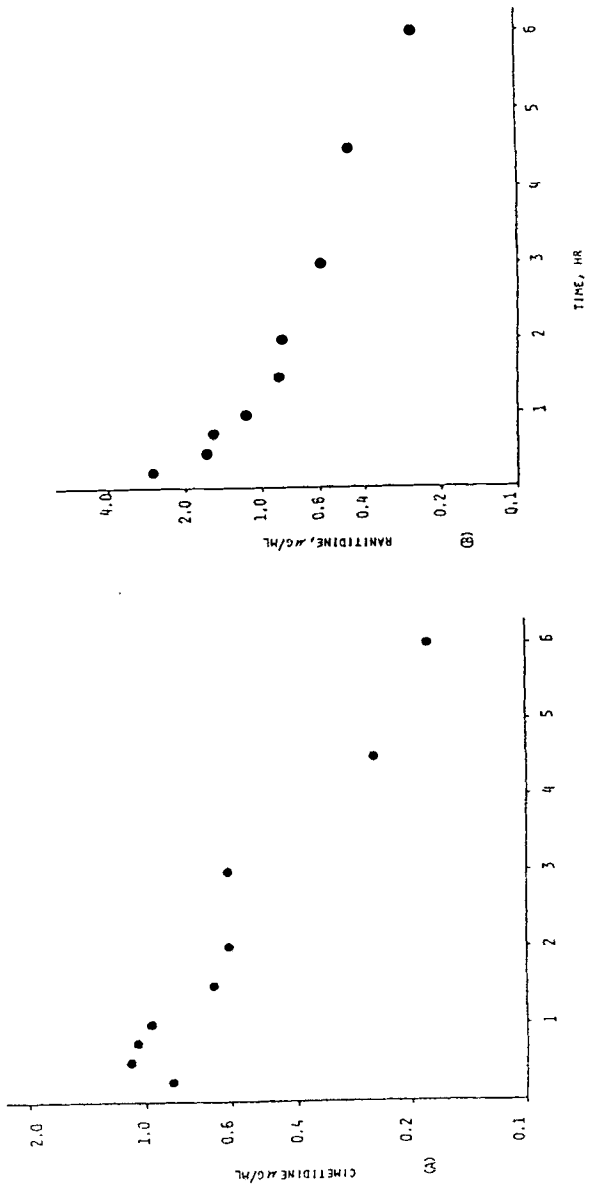

Fig.2:Semilogarithmic plots of plasma cimetidine (A) and ranitidine (B) following oral administration of cimetidine (40 mg/kg) and ranitidine (50 mg/kg) respectively to rats.

ACKNOWLEDGEMENTS

Special gratitude is directed to Mr. A.R. Qureishi for technical assistance.

REFERENCES

1. Freston, J.W., Cimetidine I. Development pharmacology and efficacy. Ann. Intern Med. 97 573 1982.

2. Powell J.R. and Donn, K.H. Pharmacokinetic basis for H_2-receptor antagonist drug interaction: concepts and implications. J. Clin. Gastroenterol. 5 (Suppl.1.) 95 1983.

3. Moshal, M.G., Spitaels, J.M. and Khan, F. A double blind endoscopically controlled trail of ranitidine in a high incidence area. Scand. J. Gastroenterol. 16 (Suppl. 69) 129 1981.

4. Bohman, t., Myren, J. and Larsen, S. Inhibition of the histamine-stimulated gastric secretion in healthy subjects by H_2-receptor antagonist ranitidine. Scand. J. Gastroenterol 15 183 1980.

5. Narario, M. The hepatic and renal mechanisms of drug interactions with cimetidine. Drug Intell. Clin. Pharm. 20 342 1986.

6. Larsen, N.E., Hesselfeldt, D. Rune, S.J. and Hvidberg, E.F., Cimetidine assay in human plasma by liquid chromatography. J. Chromatogr. 163 57 (1979).

7. Kunitani, M.G., Johnson, D.A., Upton,R.A. and Rieglman, S. Convenient and sensitive high-performance liquid chromatography assay for cimetidine in plasma and urine. J. Chromatogr. 224 156 1983.

8. Mihaly, G.w., Cockbain, S., Jones, D.B., Hanson, R.G. and Smallwood, R.A. High-pressure liquid chromatgraphic determination of cimetidine in plasma and urine. J. Pharm. Sci. 71 590 1982.

9. Fleitman, J., Torosian, G. and Perrin, J.H. Improved high-performance liquid chromatographic assay for cimetidine using ranitidine as an internal standard. J. Chromatogr. 229 255 1982.

10. Larsen, N.E. and Hvidberg, E.F. Cimetidine assay in human plasma by liquid chromatography. J. Chromatogr. 163 57 1979.

11. Bartlett, J.M. and Segelman, A.B. Bioanalysis of cimetidine by high-performance liquid chromatography. J. Chromatogr. 225 239 1983.

12. Abdelrehim, M., Ezra, D., Deck, C. and Lazar, J. Liquid-chromatographic assay of cimetidine in plasma and gastric fluid. Clin. Chem. 31 621 1985.

13. Mihaly, G.W., Drummer, O.H., Marshall, A., Smallwood, R.A. and Laris, W.J. High-pressure liquid chromatographic determination of ranitidine, a new H_2-receptor antagonist, in plasma and urine. J. Pharm. Sci. 69 1155 1980.

14. Vandenbuerg, H.M., Macleod, S.M., Mahon, W. A., Lebert, P.A. and Soldin, S.J. Analysis of rantitidine in serum by high performance liquid chromatography. Ther. Drug. Monit. 2 379 1980.

15. Boutogy, J., More, D.G., Munro, I.A. and Shenfield, G.M. Simultaneous analysis of cimetidine and ranitidine in human plasma by HPLC. J. Liq. Chromatogr. 7 1671 1984.

16. Chiarmoate, D.A. and Schentag, J.J. A specific and sensitive high pressure liquid chromatographic procedure for cimetidine and creatinine. Ther. Drug Monit. 1 545 1979.

17. Ziemniak, J.A., Chiarmonte, D.A. and Schentag, J.J. Liquid chromatographic determination of cimetidine, its known metabolities and creatinine in serum and urine. Clin Chem 27 272 1981.