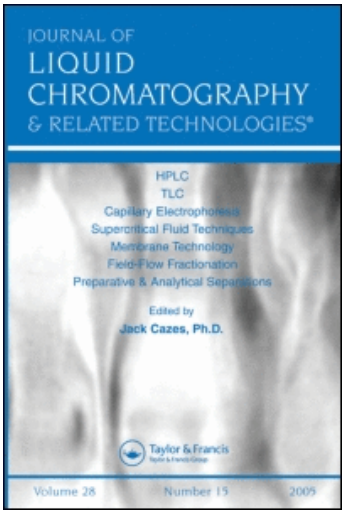

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Boutagy, J. , More, D. G. , Munro, I. A. and Shenfield, G. M.(1984) 'Simultaneous Analysis of Cimetidine and Ranitidine in Human Plasma by HPLC', Journal of Liquid Chromatography & Related Technologies, 7: 8, 1651 — 1664
To link to this Article: DOI: 10.1080/01483918408074073
URL: http://dx.doi.org/10.1080/01483918408074073

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(8), 1651-1664 (1984)

SIMULTANEOUS ANALYSIS OF CIMETIDINE AND RANITIDINE IN HUMAN PLASMA BY HPLC

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ABSTRACT

A rapid method for the simultaneous quantitation of the H_2-receptor antagonist drugs cimetidine and ranitidine in human plasma by isocratic ion-pair reverse-phase HPLC is described. The method involves a simple organic extraction step of the alkalinized plasma containing added internal standard followed by back extraction of the extract with dilute acetic acid and subsequent analysis of the aqueous acidic phase on a reverse-phase (C18) column. The eluting solvent was acetonitrile-water (20:80 v/v) containing 0.005 mole/litre octanesulphonic acid and was monitored at 229 nm. The run time for the assay was 12.5 minutes, with a detection limit for cimetidine of 50 ng/ml/(0.2 μmole/1) and that for ranitidine was 20 ng/ml (0.06 umole/1).

INTRODUCTION

The H_2-receptor antagonists cimetidine and ranitidine are widely used to treat gastric and duodenal ulcers. It has been shown that certain minimal plasma drug concentrations must be attained to achieve adequate inhibition of gastric acid secretion (1). Routine plasma drug assay is not indicated for these

 0148-3919/84/0708-1651$3.50/0

compounds but is of value when compliance is in doubt or for clinical trials. Patients who fail to respond to one of the agents are commonly changed to the other and it is therefore convenient to have an assay that will detect either or both drugs. We developed a sensitive liquid chromatographic assay for a trial in which patients were changing from one H_2-receptor antagonist to the other and we describe it here. There have been several reports for analysis in biological fluids of cimetidine (2-11) and ranitidine (12-15) as individual drugs using HPLC with either normal phase or reverse phase separation. Mobile phases have varied and sample preparation in each case have varied from multistep extraction procedures employing prior salting or protein precipitation of the biological matrix to a single solvent extraction.

Whilst there are significant structural differences between cimetidine and ranitidine they are both weakly basic compounds and our approach for their simultaneous analysis was to use a single solvent extraction of the alkalinised plasma followed by back extraction into dilute aqueous acid solution. No further sample preparation was necessary except for removal of traces of volatile solvent from the aqueous extract. Two internal standards were investigated for quantitating each drug. These were SKF 92373 and AH 20480, analogues of cimetidine and ranitidine respectively. The chromatography was performed on a reverse phase C18 column using an isocratic mobile phase containing paired-ion reagent. No pH adjustment was necessary. The known metabolites of cimetidine and

ranitidine were also investigated for potential interference with analysis of the parent compounds.

EXPERIMENTAL

Materials:

All solvents and reagents used were analytical and LC grade. Cimetidine, cimetidine sulphoxide, guanyl urea cimetidine and SKF 92373 were supplied by Smith, Kline and French, Herts., England. Ranitidine hydrochloride, desmethylranitidine, ranitidine S-oxide, ranitidine N-oxide and AH 20480 were supplied by Glaxo (Australia) Pty. Ltd. Standard stock solutions of cimetidine (mol. wt. = 252) and ranitidine (mol. wt. = 351) were made in methanol at concentrations of 5 mg/100ml and 1.3 mg/100ml respectively. Similarly stock solutions of SKF 92373 and AH 20480 in methanol were made at concentrations of 50 and 40 mg/100ml respectively. All these were diluted appropriately in drug free plasma to calibrate the assay.

Extraction of Samples and Assay Calibration:

Plasma (500 µl) containing the internal standards SKF 92373 and AH 20480 at respective concentrations of 0.2 µg/ml and 0.16 µg/ml was made alkaline with 2M sodium hydroxide (50 µl). The plasma was then extracted with a mixture of ether, chloroform and isopropanol (2:1:1)(4 ml) by vortexing for 30 sec. After centrifugation at 3000 rpm for 10 min. the organic top layer was transferred to a second tube containing 100 µl of dilute acetic

acid (2% v/v) and the mixture again vortexed for 30 sec. After centrifugation the organic (top) layer was removed and discarded. A gentle stream of nitrogen was blown over the aqueous extract to remove traces of volatile solvent following which 20 µl aliquots were used for subsequent analysis. The assay was calibrated from drug free plasma containing added cimetidine over a concentration range of 0.2 to 2.0 µg/ml (0.79 to 7.9 µmole/l) and added ranitidine over a concentration range 0.05 to 0.5 g/ml (0.14 to 1.4 µmole/l). Each sample contained the internal standards as above. Recovery of each compound was calculated by measurement of peak area after extraction from plasma and compared to that of the same amount of compound from standard stock solution after chromatography.

Chromatography and Assay Conditions:

The analyses were carried out on a Varian Series 5000 liquid chromatograph coupled to a Vista CDS 401 controller. Detection was with a Waters Model 441 U/V detector operated at 229 nm (0.01 to 0.02 auf). Sample injection (20 µl) was made by Wisp Model 710B (Waters Associates) automated injector. The chromatography was performed on a 30 cm µ-Bondapak C18 (Waters Associates) reverse phase column (in line with a Bondapak C18/Corasil pre-column) using an isocratic mobile phase consisting of acetonitrile-water (20:80) containing 0.005 mole/litre octanesulphonic acid (as PIC-B8, low U/V reagent) (Waters Associates). The flow rate was 1.5 ml/min.

Quantitation was by ratio of peak areas of cimetidine and ranitidine to the internal standards.

RESULTS AND DISCUSSION

Using the chromatography procedure described the separation of cimetidine, ranitidine, internal standards and some potential metabolites is shown in Figure 1. The retention time under these conditions for cimetidine was 5.8 min. and that for ranitidine was 7.0 min. with adequate separation from the internal standards (retention times 4.3 and 9.7 min. for SKF 92374 and AH 20480 respectively). The monitoring at 229 nm gave adequate response for both parent compounds and standards (each compound has absorption maxima in that portion of the spectrum).

The chromatograms obtained following plasma extraction are shown in Figure 2. Chromatograms from drug-free plasma containing no added drug (Figure 2A) and from the same plasma containing added cimetidine and ranitidine in the amounts indicated (Figure 2B) are shown. Figure 2C and Figure 2D show sample chromatograms obtained from patients undergoing treatment with cimetidine or with ranitidine respectively. The total run time for the analysis is 12.5 min.

Specificity:

The analysis was specific for the parent compounds and known metabolites. The relative retention times (relative to cimetidine)

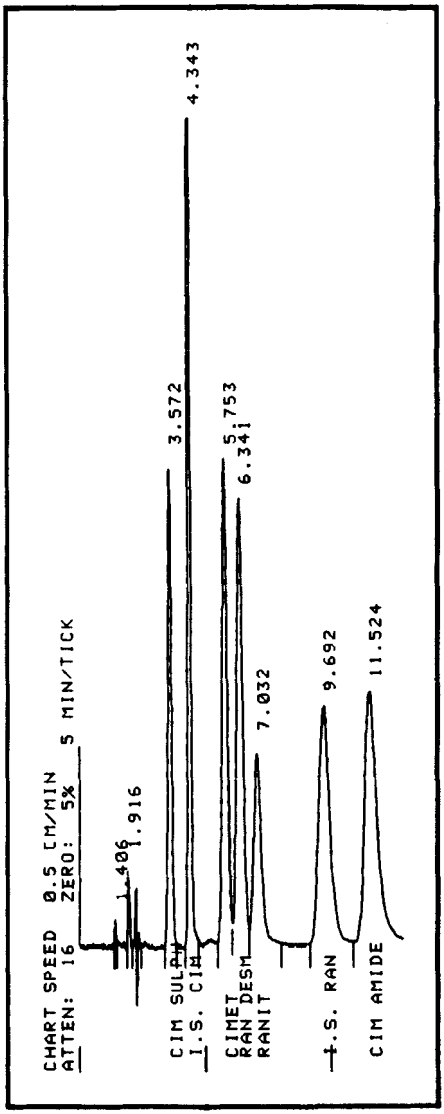

FIGURE 1. Chromatogram of standard compounds showing separation of cimetidine, ranitidine, internal standards SKF 92373 and AH 20480 and major metabolites.

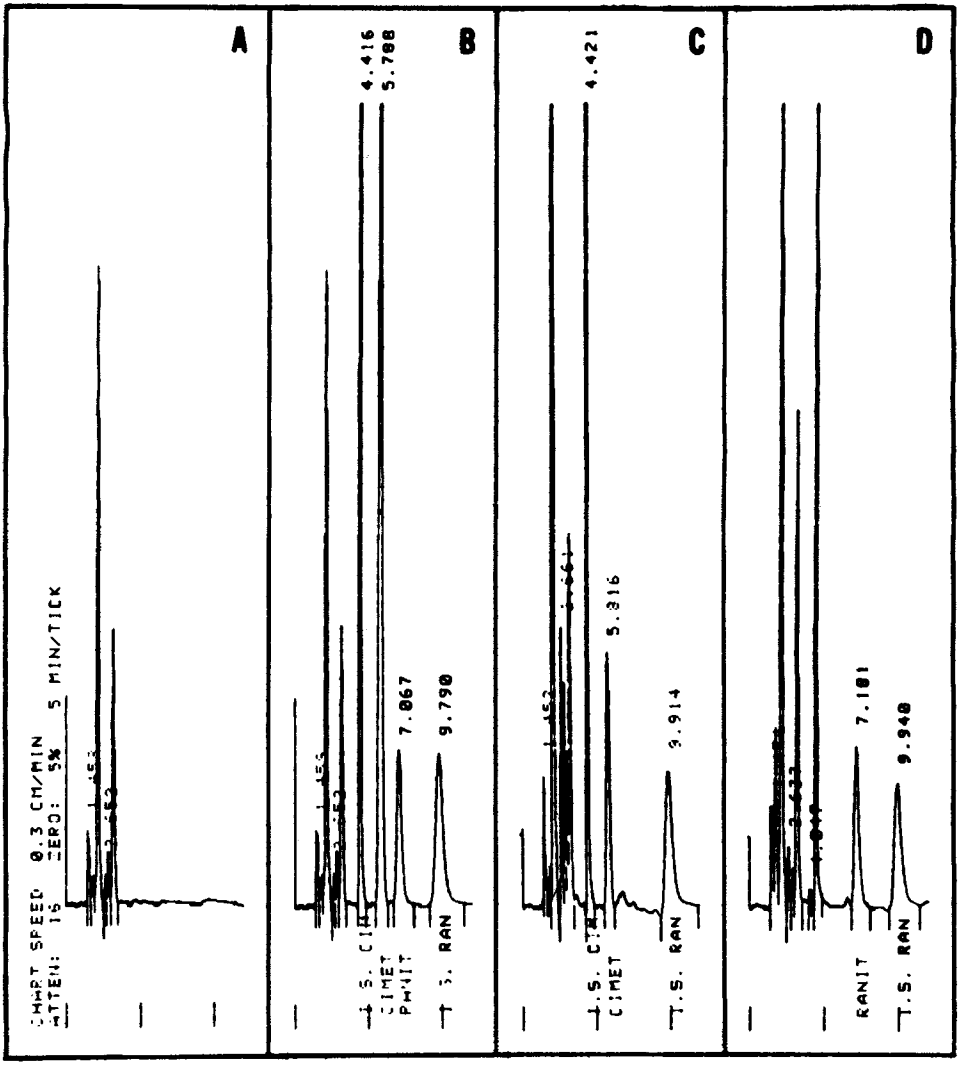

FIGURE 2. Chromatogram following plasma extraction. **A.** Drug free plasma. **B.** Drug free plasma containing added cimetidine (6.58 µmole/l), ranitidine (0.81 µmole/l) and internal standards. **C.** Plasma from patient receiving cimetidine. **D.** Plasma from patient receiving ranitidine.

TABLE 1

Relative Retention Times of Cimetidine, Ranitidine, Known Metabolites and Some Basic Drugs

Compound	Relative retention time (cimetidine = 1.0)
Cimetidine	1.0
SKF 92374	0.76
Ranitidine	1.22
AH 20480	1.69
Ranitidine N-Oxide	1.31
Ranitidine S-Oxide	0.65
Procainamide	1.16
N-Acetylprocainamide	1.29
Lignocaine	> 3
Mexiletine	> 3
Quinidine	> 3
Cimetidine sulphoxide	0.62
Desmethylranitidine	1.11
Cimetidine amide	2.02

for these compounds and other potentially administered basic drugs are shown in Table 1. There was adequate resolution for each of the compounds shown. The metabolites of cimetidine and ranitidine did not interfere and furthermore the S-oxide and the N-oxide of ranitidine when added to drug-free plasma did not extract adequately to be detected.

Linearity:

The standard curve for cimetidine and ranitidine was constructed from drug free plasma with constant amount of internal standards added per sample. Cimetidine was calibrated with SKF 92374 as the internal standard and produced a linear plot (r=0.999) over the concentration range of 0.2 to 2.0 µg/ml (0.79 to 7.9

μmole/1) (Figure 3). For ranitidine, curves were constructed with either AH 20480 or with SKF 92374 as standards. There was no significant difference as to which standard was used and all ranitidine assays were subsequently calibrated using SKF 92374 as the standard. The plot obtained was linear (r=0.999) from 0.05 to 0.5 μg/ml (0.14 to 1.4 μmole/1) (Figure 4). For both cimetidine and ranitidine the curve was still linear at values greater than twice the concentration shown for each plot. The lower limit of detection was 0.05 μg/ml (0.2 μmole/1) for cimetidine and 0.02 μg/ml (0.06 μmole/1) for ranitidine. The plasma concentrations known to cause 50% inhibition of food-stimulated gastric acid secretion are 0.37 to 0.45 μg/ml (1.5 to 1.8 μmole/1) for cimetidine and 0.07 to 0.09 μg/ml (0.20 to 0.26 μmole/1) for ranitidine (1).

Recovery:

Known amounts of cimetidine and ranitidine were added to drug free plasma to make high and low concentrations of each drug. After extraction and adjustment of the final volume of the acidic extract the peak area after chromatography of each compound was measured and compared to the peak area of the same amount of compound added directly to the equal volume of the aqueous extract. The recovery or extraction efficiencies are shown in Table 2 and ranged from 73-81%. In the same way the recovery for the internal standards were also determined at the concentrations used in the assay.

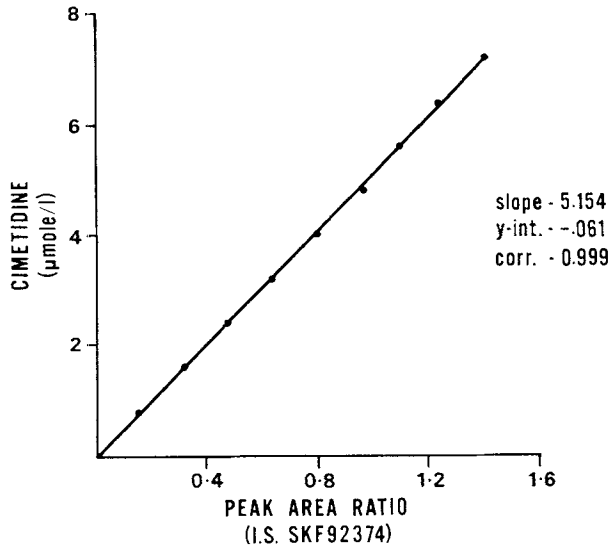

FIGURE 3. Calibration curve for cimetidine from plasma using SKF 92374 as standard.

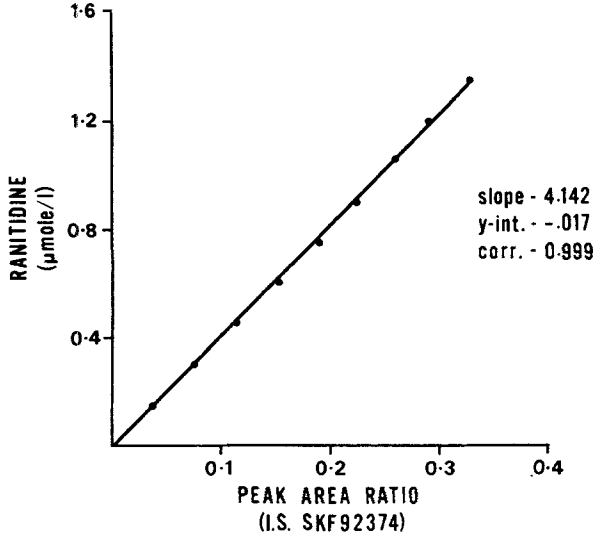

FIGURE 4. Calibration curve for ranitidine from plasma using SKF 92374 as standard.

TABLE 2

Recovery from Plasma. Extraction Efficiency was Calculated for Cimetidine and Ranitidine from High and Low Plasma concentrations for each drug. (N = 5 for each determination).

Compound	Concentration (μmole/l)	Recovery (mean % ± S.D.)
Cimetidine	1.70	74.4 ± 2.0
	6.58	81.1 ± 3.2
Ranitidine	0.21	73.2 ± 3.1
	0.81	73.7 ± 2.2

The extraction efficiency for SKF 92374 and AH 2480 was 65.2 ± 3.1% and 78.3 ± 10.6% respectively at the plasma concentrations in the assay.

TABLE 3

Assay Reproducibility. The Within-Run Precision and the Day-to-Day Precision (Calculated Over a Period of Five Days) were Calculated for Cimetidine and Ranitidine at Two Different Plasma Concentrations. (N = 5 for each determination).

	Mean (μmole/l ± S.D.)	%C.V.
WITHIN-RUN		
Cimetidine	1.70 ± 0.04	2.3
Cimetidine	6.58 ± 0.09	1.4
Ranitidine*	0.21 ± 0.019	4.4
Ranitidine*	0.81 ± 0.044	5.5
Ranitidine#	0.21 ± 0.008	3.9
Ranitidine#	0.81 ± 0.023	2.9
DAY-TO-DAY		
Cimetidine	4.21 ± 0.15	3.5
Cimetidine	8.64 ± 0.32	3.7
Ranitidine#	1.40 ± 0.06	4.3
Ranitidine#	0.36 ± 0.012	3.3

* Ranitidine measured using an AH 20480 as internal standard

Ranitidine measured using SKF 92374 as internal standard

Precision:

The reproducibility and precision of the assay following analysis of five aliquots of plasma pools at concentration shown in Table 3. For within day assays the CV's ranged from 2.3 to 5.5% and for the day-to-day precision ranged from 3.3 to 4.3%.

Background Interference:

Where possible, plasma samples from patients were tested prior to administration of either drug. With those samples and with pooled drug-free plasma processed in this way peak interference with endogenous substances at retention times corresponding to either parent drug or standards was not significant.

Stability:

Storage of plasma at $-20^{o}C$ for a period of at least one month did not alter the amount of either drug recovered. This enabled samples to be batched and assayed at the one time.

CONCLUSION

This assay procedure has proved successful for the repeated analysis of cimetidine and/or ranitidine in plasma samples. In our laboratory to date over 200 patient samples have been assayed by this procedure. The assay is rapid, reproducible and sufficiently sensitive. The use of the paired-ion (as a commercial reagent pack) made the preparation of the mobile phase relatively easy without the need for fine pH adjustment, giving good reproducible control over retention volumes. With the relatively simple

extraction procedure and with the use of the autosampler many samples can be batched and assayed at the one time.

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