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Short communication

Simple and universal HPLC-UV method to determine cimetidine, ranitidine, famotidine and nizatidine in urine: Application to the analysis of ranitidine and its metabolites in human volunteers

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Abstract

A validated, simple and universal HPLC-UV method for the determination of cimetidine, famotidine, nizatidine and ranitidine in human urine is presented. This is the first single HPLC method reported for the analysis of all four H_2 antagonists in human biological samples. This method was also utilized for the analysis of ranitidine and its metabolites in human urine. All calibration curves showed good linear regression ($r^2 > 0.9960$) within test ranges. The method showed good precision and accuracy with overall intra- and inter-day variations of 0.2–13.6% and 0.2–12.1%, respectively. Separation of ranitidine and its metabolites using this assay provided significantly improved resolution, precision and accuracy compared to previously reported methods. The assay was successfully applied to a human volunteer study using ranitidine as the model compound.

Keywords: H_2 antagonists; High performance liquid chromatography; Bioavailability; Metabolism; Ranitidine; Famotidine; Cimetidine; Nizatidine

1. Introduction

Histamine (H_2) antagonists—ranitidine, cimetidine, nizatidine and famotidine are established drugs used in the treatment of gastro-oesophageal reflux disease and gastric and duodenal ulceration [1].

All four H_2 antagonists are classified as class III drugs (high solubility, low permeability) according to the Biopharmaceutics Classification System (BCS) [2–4]. The BCS classification developed by Amidon et al. in 1995 [5] is used as a means of waiving *in vivo* bioequivalence testing for new or reformulated generic immediate release drug products. Currently, the BCS is only applicable for use with class I (high solubility–high permeability) oral immediate-release dosage forms which exhibit rapid *in vitro* dissolution using recommended test procedures [6–8]. In the case of class III drugs such as the H_2 antagonists, no such waiver is possible and *in vivo* bioavailability studies are mandatory. It is therefore imperative that simple and reliable methods are available for the analysis of these compounds in biological fluids.

A number of HPLC-UV methods have been developed for the analysis of the individual H_2 antagonists in biological samples comprising urine or urine and plasma; cimetidine [9–11], famotidine [12–15], ranitidine [16], nizatidine [17,18]. More complex or sophisticated liquid chromatography methods have also been reported for the individual analysis of H_2 antagonists in urine, including HPLC–MS [19–21], paired-ion HPLC-UV [22–23] post-column florescence derivatisation [24], HPLC-TLC [25] and supercritical chromatography [26]. Most of these methods require either solid-phase or liquid-phase extraction procedures which are time consuming. Moreover, these methods are only capable of analysing one of the four H_2 antagonists. Two groups have described methods for the determination of all four H_2 antagonists in tablet dosage forms [27,28]. However, there has been no report of a single universal assay capable of analysing all four H_2 antagonists in biological samples such as human urine. Analysis from urine is a very useful means of determining the bioavailability of these drugs in human subjects. Urine is a more readily available biological medium compared to plasma, is easier to obtain and is less intrusive.

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We report for the first time a simple and universal HPLC-UV method for the simultaneous analysis of all four H_2 antagonists in human urine. This method was applied for the analysis of ranitidine and its metabolites (ranitidine *N*-oxide, ranitidine *S*-oxide and desmethyl ranitidine) in a study involving healthy human volunteers.

2. Experimental

2.1. Reagents and chemicals

Ranitidine hydrochloride was obtained from Zhongnuo Pharmaceutical Co., Ltd. (Shijiahzuang, China). The metabolites of ranitidine (ranitidine *N*-oxide, desmethyl ranitidine and ranitidine *S*-oxide) were obtained from GlaxoSmithKline (Harlow, UK). Cimetidine, nizatidine and famotidine were purchased from Sigma–Aldrich (UK).

HPLC grade acetonitrile and water were obtained from Fisher Scientific (Loughborough, UK). Water used for sample preparation was obtained from an Elga Purelab option purification system. Sodium acetate (Sigma–Aldrich) and glacial acetic acid (VWR International, Poole, UK) were of analytical-reagent grade. Blank urine was obtained from laboratory personnel.

2.2. Instrumentation and chromatographic conditions

The HPLC system consisted of a Hewlett–Packard 1050 Series high-performance LC (HPLC) system, equipped with UV detector set at 230 nm for the determination of the four parent H_2 antagonists. The wavelength was adjusted to 320 nm for the separate analysis of ranitidine and its metabolites. The chromatographic data was collected using PC/Chrom software (H&A Scientific Co., UK). Separation of all molecules was achieved with a Phenomenex Luna SCX column (250 mm × 4.6 mm I.D.) packed with 5 μm strong cation-exchange resin (VWR International) at 50 °C. The mobile phase used for analysis consisted of acetonitrile:0.1 M sodium acetate buffer acidified with glacial acetic acid (pH 5.0; 0.1 M) (20:80, v/v). Analysis was complete within 15 min with a flow rate of 2.0 ml/min.

2.3. Preparation of standard solutions, quality control samples and volunteer samples

2.3.1. H_2 antagonists—cimetidine, famotidine, nizatidine and ranitidine

Standard solution of each drug was individually prepared in mobile phase and urine and injected onto the HPLC column to determine the individual retention times of the molecules. Then a stock solution containing 500 μg/ml of each standard in the form of free base was prepared in diluted blank urine (blank human urine diluted in a 50:50 ratio with a solution of 20:80 acetonitrile:water). Working standard solutions were prepared by serial dilutions of the stock solution with urine over the range of 0–500 μg/ml. A 10 μl volume from each solution was injected in the chromatographic system under the conditions detailed in Section 2.2.

2.3.2. Ranitidine and its metabolites

A stock solution containing 500 μg/ml ranitidine and 50 μg/ml of each metabolite of ranitidine (ranitidine *N*-oxide, ranitidine *S*-oxide and desmethyl ranitidine) was prepared using blank human urine diluted in a 50:50 ratio with a solution of 20:80 acetonitrile:water (control urine). A series of standards over the range of 0–500 μg/ml ranitidine and 0–50 μg/ml of each metabolite were prepared by serial dilution using control urine. For the human study, each volunteer sample was also diluted in a 50:50 ratio with a solution of 20:80 acetonitrile:water. A 10 μl volume from each solution was injected in the chromatographic system under the conditions detailed in Section 2.2.

2.4. Method validation

Quality control standards of low, medium and high concentrations of the molecules were prepared in control urine to evaluate the precision and accuracy of the method. Separate standards of low concentrations were prepared to investigate the limit of detection and quantification.

2.4.1. Linearity

The linearity of the method was determined at five different concentrations that ranged from 0.5 to 500 μg/ml for cimetidine, famotidine, nizatidine and ranitidine and from 0.1 to 50 μg/ml for the metabolites of ranitidine.

2.4.2. Accuracy, precision and reproducibility

The accuracy and precision of the assay were evaluated by calculating the intra- and inter-day coefficient of variation. The quality control samples were investigated for accuracy and precision using five determinations for each quality control concentration at three different time points. The inter-day variation was also evaluated at the three different concentrations on four different non-consecutive days.

2.4.3. Sensitivity

The limit of detection (LOD) was determined as the lowest concentration of analyte that produced at least twice the baseline noise level and the limit of quantification (LOQ) was determined as the lowest concentration of analyte that could be determined with adequate precision of 20% and accuracy of 80–120%.

2.5. Volunteer study

2.5.1. Sample collection

Blank urine was provided by healthy volunteers free of ranitidine and stored at −20 °C. Control human urine was prepared by mixing blank samples from volunteers in a 50:50 ratio with a solution of 20:80 acetonitrile:water.

Six volunteers participated in the study after giving informed written consent. All subjects were non-smokers, declared themselves healthy and had no history of gastrointestinal disease. The experimental protocol was approved by The Joint UCL/UCLH Committees on the Ethics of Human Research. The study was conducted in accordance to the Helsinki guidelines for ethics in research (1965) and its subsequent revisions.

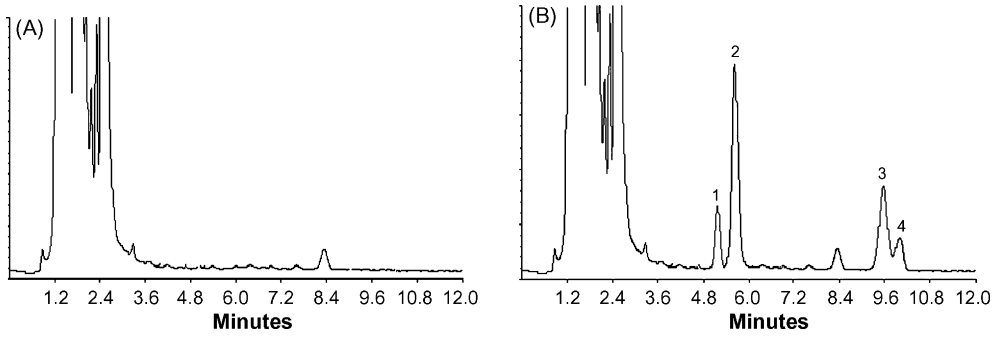

Fig. 1. Representative chromatograms of (A) blank human urine, (B) blank human urine spiked with H_2 antagonists at lower limits of quantification: (1) famotidine, (2) cimetidine, (3) ranitidine and (4) nizatidine.

The volunteers reported to the study centre after an overnight fast and each received a single dose of 168 mg ranitidine hydrochloride (equivalent to 150 mg ranitidine) in 150 ml water. A standardised sandwich lunch was provided 4 h post-dose.

Cumulative urine samples were collected from each volunteer and involved the collection and measurement of bladder output over the following time periods: 0 (pre-dose), 0–2, 2–4, 4–6, 6–12 and 12–24 h. For each collection period, 20 ml aliquot was retained and stored at −20 °C.

2.5.2. Analysis of urine samples

Prior to the analysis of volunteer samples, the stability of ranitidine and its metabolites in urine under different storage conditions was investigated. Spiked samples at two different concentrations of ranitidine (267 and 80 μg/ml) were prepared in duplicate. The spiked samples were analysed after different storage conditions; immediately after being placed on the auto sampler, 4, 9 and 24 h, after one, two and three freeze/thaw cycles and after 3 months storage at −20 °C. Three freeze/thaw cycles and 48 h room temperature stability was also investigated for the metabolites of ranitidine at two different concentrations (9 and 30 μg/ml).

The frozen urine aliquots from volunteers were thawed out at room temperature and 0.65 ml mixture of 20:80 acetonitrile:water was added to 0.65 ml of each sample in duplicates. After vortex-mixing for a few seconds, 10 μl aliquot of each solution was injected onto the HPLC column using the method described in Section 2.2.

3. Results and discussion

3.1. Method validation

3.1.1. Retentions times

A representative chromatogram of blank urine is shown in Fig. 1A. A typical chromatogram produced by the developed HPLC method at 230 nm for the standard solutions of cimetidine, famotidine, nizatidine and ranitidine at their limit of quantification is shown in Fig. 1B. The retention times for famotidine, cimetidine, ranitidine and nizatidine were 5.1, 5.6, 9.5 and 9.9 min respectively.

A typical chromatogram using the method at 320 nm for blank urine and spiked standard solutions of ranitidine and its metabolites (ranitidine *N*-oxide, desmethyl ranitidine, ranitidine *S*-oxide) are shown in Fig. 2A and B, respectively. The retention times of ranitidine *N*-oxide, desmethyl ranitidine, ranitidine and ranitidine *S*-oxide were 4.1, 7.8, 10.1 and 11.6 min respectively.

3.1.2. Linearity and correlation coefficient

The standard curves had linear response up to 500 μg/ml for cimetidine, ranitidine, famotidine, nizatidine and 50 μg/ml for each of the metabolites of ranitidine. Good linearity ($r^2 > 0.9960$) was obtained for all molecules; cimetidine (0.9999), famotidine (0.9995), ranitidine (0.9987), nizatidine (0.9995), ranitidine *N*-oxide (0.9983), desmethyl ranitidine (0.9971) and ranitidine *S*-oxide (0.9966).

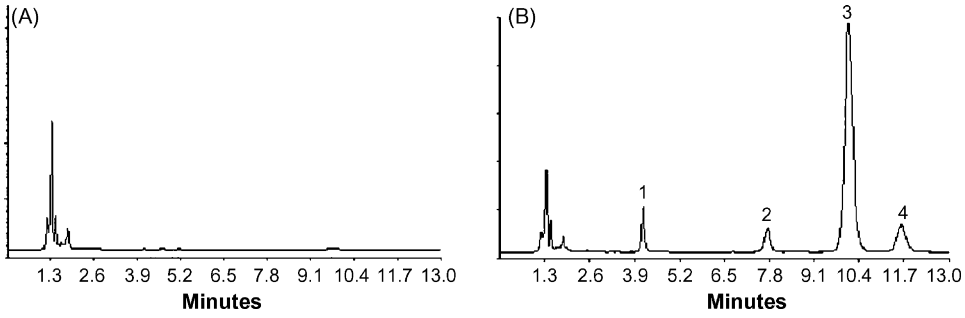

Fig. 2. Representative chromatograms of (A) blank human urine, (B) blank human urine spiked with ranitidine and its metabolites: (1) ranitidine *N*-oxide (0.6 μg/ml), (2) desmethly ranitidine (0.6 μg/ml), (3) ranitidine (6 μg/ml) and (4) ranitidine *S*-oxide (0.6 μg/ml).

Table 1
Accuracy, intra- and inter-day precision, limit of detection and limit of quantification of H_2 antagonists and the metabolites of ranitidine in human urine

QC sample (μg/ml)	Calculated concentration (μg/ml)	Accuracy	Intra-assay C.V. (%)	Inter-assay C.V. (%)	LOD (μg/ml)	LOQ (μg/ml)
Cimetidine						
9	7.99 ± 0.28	88.8	8.6	9.5		
30	34.8 ± 0.13	115.9	1.9	3.7		
300	300.2 ± 0.55	100.1	3.1	2.0	0.5	3
Famotidine						
5	4.48 ± 0.49	89.7	3.4	4.0		
30	31.5 ± 0.11	96.4	2.5	4.5		
300	292.6 ± 0.47	97.3	3.0	3.5	0.3	3
Nizatidine						
5	5.27 ± 0.05	89.6	13.6	12.0		
30	33.2 ± 0.19	110.5	13.6	12.1		
300	295.3 ± 1.0	98.4	1.8	2.1	0.3	3
Ranitidine						
3	2.9 ± 0.18	97.9	2.7	4.3		
30	34.3 ± 0.05	114.4	0.5	0.8		
300	300.2 ± 0.17	102.4	0.1	0.5	0.25	2.0
Ranitidine *N*-oxide						
3	3.34 ± 0.02	111.4	0.8	0.2		
9	9.08 ± 0.01	100.9	0.5	0.8		
30	29.6 ± 0.01	98.7	0.3	0.6	0.1	0.6
Desmethyl ranitidine						
3	3.06 ± 0.04	102.1	4.5	1.3		
9	8.87 ± 0.06	98.6	1.6	4.8		
30	29.7 ± 0.04	99.1	0.2	1	0.1	0.6
Ranitidine *S*-oxide						
3	3.02 ± 0.05	100.8	4.2	5.5		
9	9.51 ± 0.06	105.7	0.5	2.7		
30	30.2 ± 0.08	100.7	3.1	3.7	0.5	3

3.1.3. Precision, accuracy, limit of detection and quantification

The intra- and inter-day precision, accuracy, limit of detection and limit of quantification are reported in Table 1.

The precision for all compounds ranged from 0.2 to 13.6%. In the analysis of ranitidine and its metabolites, the coefficients of variation for intra- and inter-day variability by this method is lower than in previously reported assays [16].

The separation of ranitidine and its metabolites obtained by this method is significantly better than that obtained by other reported methods [16,25]. The resolution obtained by this method for ranitidine and its metabolites was greater than 2.5 for all peaks and tailing was minimal, mean ± S.D. was 1.01 ± 0.11. The resolution between cimetidine and famotidine was greater than 1.8 whilst the resolution between ranitidine and nizatidine was low (1.1). Such low resolution between ranitidine and nizatidine has previously been reported [27,28]. In the study by Gyeresi et al. [27], using United States Pharmacopeia (USP 23) HPLC method, nizatidine and ranitidine could not be separated as the retention times for both molecules was the same. In our study, whilst the retention times for ranitidine and nizatidine were close, two clear peaks were observed for the individual drugs. In reality, it is unlikely that all four drugs will be administered concurrently to human subjects and hence there is little need to separate all four molecules simultaneously. The retention times obtained with this new method are comparable to individual assays, but as would be expected, the sensitivity and intra- and inter-day variability are lower than individual assays and other more sophisticated methods such as HPLC–MS. The new method described in this paper provides a generic, simple and universal approach for the identification and quantification of any of the four H_2 antagonists. The method utilizes a simplified urine sample processing involving dilution of urine which is cheaper and more time efficient than liquid/solid-phase extractions methods used in many of the previous reports.

The detection limits in this study were higher than obtained by previous studies for ranitidine, and the *N*-oxide and desmethyl metabolites but lower for the *S*-oxide metabolite [16]. The limits of quantification were not reported in the previous HPLC assays for ranitidine and its metabolites [16,19,23].

3.2. Volunteer study

The results from the stability study showed that relative errors at the two different concentrations studied (267 and 80 μg/ml) were less than 6% for ranitidine. This indicates that ranitidine, when added to urine is stable at different storage conditions. Relative errors at the different concentrations investigated for the metabolites for three freeze/thaw cycles and 48 h room temperature were less than 10% for the *N*-oxide and desmethyl ranitidine

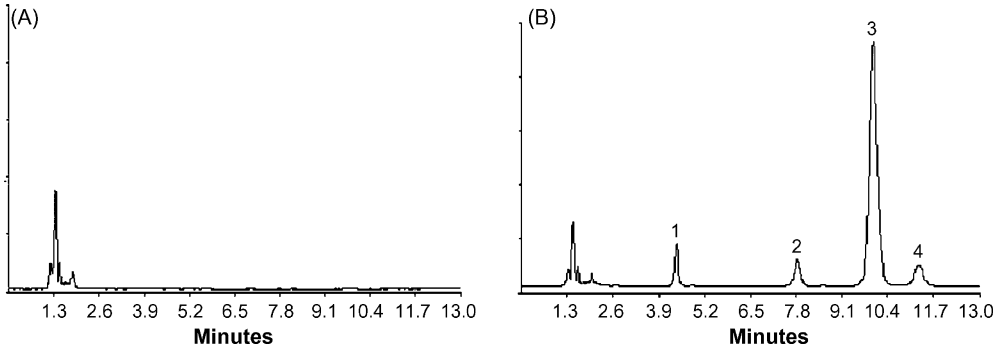

Fig. 3. Representative chromatograms of urine from a volunteer (A) pre-dose, (B) post-administration of 150 mg ranitidine: (1) ranitidine *N*-oxide (2 μg/ml), (2) desmethyl ranitidine (0.5 μg/ml), (3) ranitidine (10 μg/ml) and (4) ranitidine *S*-oxide (0.9 μg/ml).

Table 2
Excretion of ranitidine and its metabolites in human volunteers

Volunteer	Cumulative amount excreted in 24 h (mg)			
	Ranitidine	Ranitidine *N*-oxide	Desmethyl ranitidine	Ranitidine *S*-oxide
1	42	6.1	3.3	3.7
2	31	3.8	0.9	1.4
3	20	2.4	0.3	0.8
4	47	4.3	1.5	4.1
5	45	14.0	4.8	6.4
6	29	7.1	7.0	2.1
Mean	36	6.3	3.0	3.1
S.D.	12	4.1	2.6	2.1

metabolites, however the *S*-oxide metabolite was less stable with a relative error of approximately 20% after the freeze/thaw cycles.

Representative chromatograms obtained pre-dose and post-dose ranitidine administration (150 mg) in one individual volunteer are shown in Fig. 3A and B. The bioavailability of ranitidine, assessed by the cumulative amounts of unchanged ranitidine and its metabolites excreted in urine over 24 h is shown in Table 2. The average excretion of ranitidine was 36 mg with a range of 20–47 mg between volunteers. The average amount of ranitidine excreted is equivalent to 24% of the administered dose. This is in good agreement with literature values [25,29,30]. The mean amount of metabolites excreted during the 24 h after administration of ranitidine was 6.3 mg (ranitidine *N*-oxide), 3.1 mg (desmethyl ranitidine) and 3.1 mg (ranitidine *S*-oxide). These are equivalent to 4.2% for ranitidine *N*-oxide and 2% each of desmethyl ranitidine and ranitidine *S*-oxide of the administered ranitidine dose. These values for the metabolites are also similar to those previously published [16,25,26].

4. Conclusion

The HPLC-UV method described is a simple, universal, convenient and reproducible method that can be used to determine and quantify any of the four H_2 receptor antagonists. The method was successfully applied to the analysis of ranitidine and its metabolites in the urine of healthy human volunteers following administration of ranitidine. This method is useful for bioavailability studies and has the potential of being useful in studying drug interactions in clinical pharmacology trials.

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