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Short communication

Simultaneous determination of amoxicillin and ranitidine in rat plasma by high-performance liquid chromatography

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Abstract

A high-performance liquid chromatography method using ultraviolet detection at 230 nm for the simultaneous determination of amoxicillin and ranitidine in rat plasma has been validated. Plasma samples after pretreatment with acetonitrile to effect deproteinization were dried under N_2 and reconstituted with water. The standard calibration curves for amoxicillin and ranitidine were linear ($r^2 = 0.9999$) over the concentration range of 0.2–20 μg ml^{-1} and 0.03–6 μg ml^{-1} in rat plasma, respectively. The intra- and inter-day assay variability range for amoxicillin was 2.4–8.5% and 3.2–11.7%, and for ranitidine was 1.7–9.0% and 4.5–10.1%, respectively. This method has been successfully applied to a pharmacokinetic study after oral coadministration of amoxicillin and ranitidine to rats.

Keywords: Amoxicillin; Ranitidine; Rat plasma; High-performance liquid chromatography

1. Introduction

Peptic ulcer (PU) is a common disease worldwide, with an estimated 10% of the population effected. Research has shown that the infection of *Helicobacter pylori* (*H. pylori*) is a key factor in the occurrence and reoccurrence of PU [1–3]. *H. pylori* is a gram-negative and microaerophilic organism which can wreck the mucosa, disturb the secretion of gastric acid and induce inflammation [4]. The current treatments for PU are most frequently concerned with the eradication of *H. pylori*.

Amoxicillin is a semi-synthetic penicillin with activity against both gram-positive and gram-negative bacteria. It has a very low minimal inhibitory concentration (MIC) against *H. pylori* in vitro; however, the monotherapy of amoxicillin has proven to be ineffective. Since amoxicillin is an amphoteric substance with a biological activity in a pH range of 5.5–7.5 [5], the low pH value in the stomach (about 1–2) significantly affects the antimicrobial activity of amoxicillin.

Ranitidine, a histamine H_2-receptor antagonist (H_2RA), is frequently described as an anti-ulcer drug. It competitively inhibits the binding of histamine to the histamine H_2-receptors of parietal cells leading to a decrease in the secretion of gastric acid. As well as reducing sensitivity of parietal cells to other stimulators such as gastrin and acetylcholine.

The coapplication of these two drugs has proven effective as a therapy to eradicate *H. pylori* [6]. A potential reason for this phenomenon is that ranitidine raises the stomach pH that allows amoxicillin to achieve the better antimicrobial activity. Advanced pharmacokinetics research is needed to fully understand the mechanism of this phenomenon. Such a study requires an analytical method that can determine concentrations of both drugs simultaneously.

The analytical methods reported for the quantification of amoxicillin include spectrophotometry [7,8], capillary electrophoresis [9,10] and liquid chromatography (LC); detection methods include amperometry [11], fluorometry [12], mass spectrometry [13,14] and UV [15–20].

Several high-performance liquid chromatographic (HPLC) techniques have also been reported for the determination of ranitidine in plasma or serum [21–25].

Most of the reported HPLC methods require liquid–liquid extraction or solid-phase extraction, which are not economically feasible for routine use in pharmacokinetic studies with numerous samples to be analyzed. Furthermore, there is no evidence indicating that any of these methods could be used to determine amoxicillin and ranitidine simultaneously.

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We have developed and validated a sensitive method for the simultaneous determination of amoxicillin and ranitidine in rat plasma by HPLC with UV detection that can be used in pharmacokinetic studies of the coadministered drugs. This method employs a micro volume of plasma (150 μl) and a simple sample preparation without organic solvent extraction which ensure the applicability of the method when only small volume of plasma is available and the reproducibility of the method. The simple sample preparation without organic solvent extraction also ensures the consistent recovery, the low relative standard deviation and the good linearity which promise the elimination of the internal standard.

2. Experimental

2.1. Chemicals and reagents

Amoxicillin trihydrate was supplied by Livzon Syntpharm Co. Ltd. (99.6%, China). Ranitidine·HCl was supplied by Changzhou Longcheng Medicine Raw Material Factory (99.3%, China). HPLC grade acetonitrile and analytical grade triethylamine (TEA), potassium dihydrogen phosphate and concentrated phosphoric acid were obtained from Kewei Agents Company (Tianjin, China).

2.2. Instrumentation

The apparatus used for this work was an Agilent 1100 quaternary pump, with a variable wavelength detector, thermostatted autosampler and column thermostat. A Hypersil ODS_2 C_{18} column (250 mm × 4.6 mm I.D., 5 μm, Thermo, UK) was fitted with a Phenomenex guard column packed with octadecyl C_{18} (Phenomenex, USA). The mobile phase comprised of 50 mM potassium dihydrogen phosphate buffer, triethylamine, and acetonitrile (1000:7:40, v/v/v), was adjusted with concentrated phosphoric acid to pH 2.6.

Analyses were run at a flow rate of 1.0 ml min^{-1} at 25 °C and the samples were quantified using peak areas.

2.3. Assay standards

Stock solutions of amoxicillin and ranitidine were prepared daily by dissolving the appropriate amount of amoxicillin trihydrate and ranitidine·HCl in water to yield a final solution with an amoxicillin concentration of 300 μg ml^{-1} and ranitidine concentration of 90 μg ml^{-1}. Separate solutions were prepared for the calibration standards. Further solutions were obtained by serial dilutions of stock solutions with water. These solutions were added to drug free rat plasma in volumes not exceeding 8% of the plasma volume.

2.4. Sample preparation

The rat plasma sample (150 μl) was transferred into a 1.5 ml polypropylene micro-centrifuge tube. The sample was deproteinized by the addition of 450 μl acetonitrile, vortexed for 30 s and then centrifuged at 3000 rpm for 10 min. The supernatant fluid was transferred to another 1.5 ml polypropylene micro-centrifuge tube and dried under a steam of N_2 at room temperature. The residue was reconstituted with 150 μl water and 100 μl of the reconstituted solution was injected onto the HPLC column.

2.5. Assay validation

Standard calibration curves were constructed by spiking drug-free plasma with a known amount of amoxicillin and ranitidine in the concentration range of 0.2–20 μg ml^{-1} (amoxicillin) and 0.03–6 μg ml^{-1} (ranitidine). The plasma standards were also used to determine the intra-day and inter-day precision and accuracy of the method. Absolute recoveries of seven different concentrations of amoxicillin and eight concentrations of ranitidine in plasma were determined by assaying the samples as described above and comparing the peak areas of both drugs with those obtained from direct injection of aqueous drug solutions of the corresponding concentrations.

2.6. Plasma collection

Male Wistar rats (250–300 g) were used in this experiment. The rats were fasted overnight before use and were given a single dose of amoxicillin (100 mg kg^{-1}) and ranitidine (50 mg kg^{-1}) simultaneously through oral gavage. Heparinized samples of blood (0.4 ml) were collected at 5, 15, 30, 60, 100, 140, 180, 240, 300, 360 and 480 min after administration. Plasma samples were harvested after centrifugation and stored frozen at −20 °C until analysis.

3. Results and discussion

3.1. Chromatography

The aim of this work, a new, simple, accurate, reproducible and sensitive HPLC method to simultaneously determine amoxicillin and ranitidine in rat plasma has been developed. A satisfactory separation of amoxicillin and ranitidine from endogenous components in rat plasma was obtained.

Representative chromatograms of blank rat plasma, drug-free rat plasma spiked with drugs and rat plasma after the coadministration of amoxicillin and ranitidine are shown in Fig. 1.

The plasma deproteinization by acetonitrile was employed because of its simplicity and high reproducibility. The optimum wavelength for detection was 230 nm at which both of the amoxicillin and ranitidine had good response. The mobile phase comprised of 50 mM potassium dihydrogen phosphate buffer, triethylamine, and acetonitrile (1000:7:40, v/v/v), was adjusted with concentrated phosphoric acid to pH 2.6. The composition of the mobile phase was chosen to provide the best peak resolution and retention times. As such, the added triethylamine in the mobile phase is an important factor, since it permits the ranitidine peak shape. The effect of varying the pH value of the mobile phase was investigated (Fig. 2). A pH of 2.6 was chosen to achieve a satisfactory separation of amoxicillin and ranitidine from endogenous components in rat plasma.

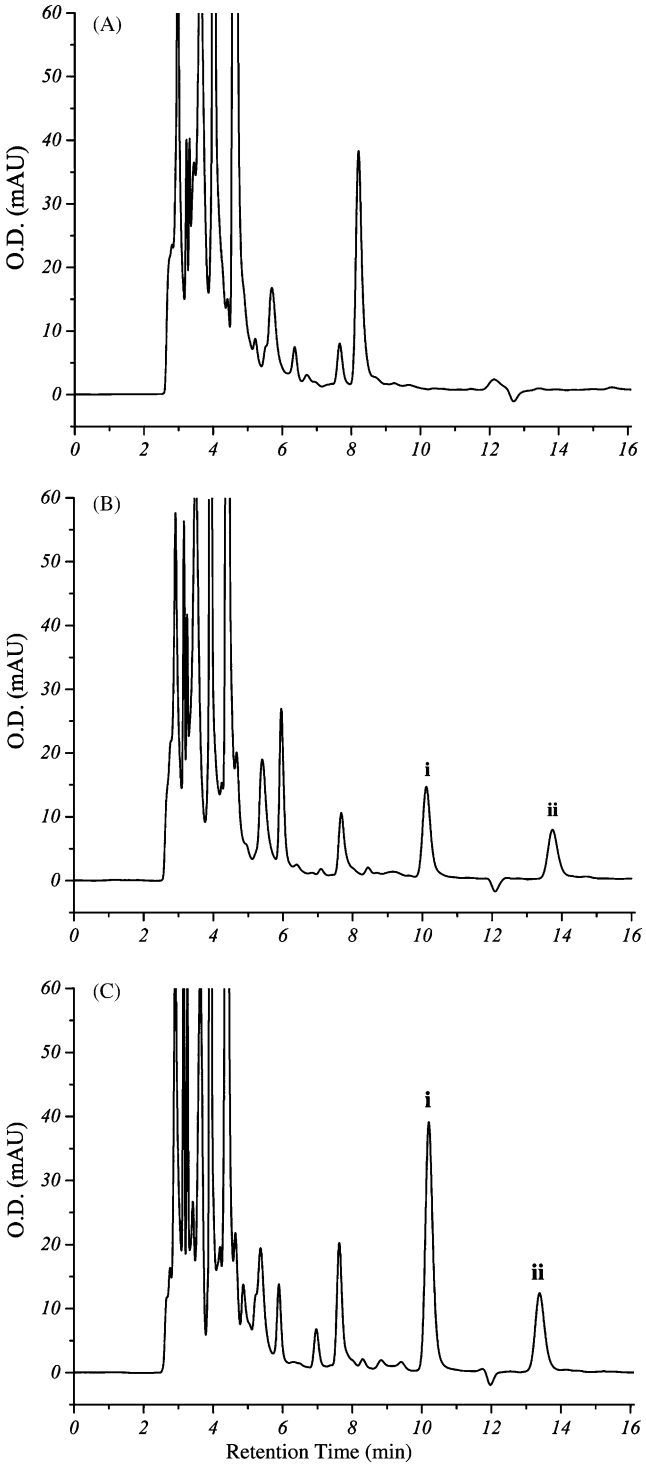

Fig. 1. (A) HPLC chromatogram of drug-free rat plasma. (B) HPLC chromatogram of rat plasma containing amoxicillin (2 μg ml^{-1}, i) and ranitidine (0.6 μg ml^{-1}, ii). (C) Rat plasma sample after oral coadministration of amoxicillin (100 mg kg^{-1}) and ranitidine (50 mg kg^{-1}).

3.2. Linearity and limit of quantification

The linearity of the method was evaluated with calibration curves made in rat plasma ranging from 0.2 to 20.0 μg ml^{-1} (amoxicillin) and from 0.03 to 6.0 μg ml^{-1}(ranitidine). For

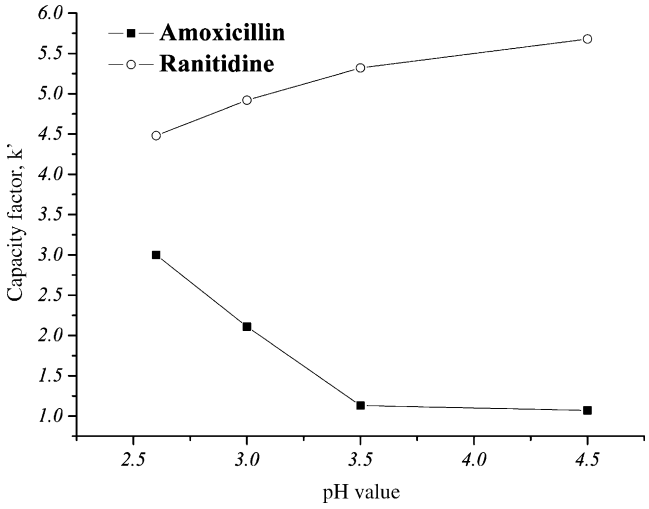

Fig. 2. Influence of pH value on the capacity factors of amoxicillin and ranitidine. Mobile phase: 50 mM potassium dihydrogen phosphate buffer–triethylamine–acetonitrile (1000:7:40, v/v/v).

both compounds, a good linear relationship was found, as described by the following linear regression equations: $y = 0.0087x - 0.0016$ ($r^2 = 0.9999$) for amoxicillin and $y = 0.0039x + 0.0009$ ($r^2 = 0.9999$) for ranitidine, where y is the drug concentration (μg ml^{-1}) and x is the peak area. Values for the coefficients of determination are all satisfactory.

Detection limits were determined as the concentration of components giving a ratio of signal to noise = 3:1. The limits of detection for amoxicillin and ranitidine in rat plasma were found to be 0.05 and 0.01 μg ml^{-1}, respectively. The limits of quantitation (LOQ) for amoxicillin and ranitidine in rat plasma were chosen as the concentrations used for the lowest concentration level on the calibration curves and for which the R.S.D. was <15% (i.e. 0.2 and 0.03 μg ml^{-1} for amoxicillin and ranitidine, respectively).

3.3. Analytical recovery

The percentage recoveries ($n = 6$) of amoxicillin and ranitidine over the entire concentration range are given in Table 1. These results indicate that the simple protein precipitation procedure with acetonitrile is sufficient to ensure stable and high extraction recovery from plasma.

3.4. Intra-day and inter-day variations and accuracy

The intra- and inter-day variations and accuracy of the amoxicillin and ranitidine determined in rat plasma are summarized in Table 2. All results are within acceptable ranges for bioanalytical purposes.

3.5. Application

The developed method has been successfully applied to an analysis of plasma samples collected from rats coadministered

Table 1
Absolute recovery of amoxillin and ranitidine ($n = 6$)

Amoxicillin			Ranitidine		
Concentration ($\mu g\,ml^{-1}$)	Recovery (Mean%)	R.S.D. (%)	Concentration ($\mu g\,ml^{-1}$)	Recovery (Mean%)	R.S.D. (%)
–	–	–	0.03	106.1	3.5
0.2	80.8	5.4	0.06	98.3	8.2
0.5	88.0	9.4	0.15	99.7	4.9
1.0	85.4	3.4	0.3	97.2	7.7
2.0	86.0	5.2	0.6	97.4	2.4
5.0	87.4	6.7	1.5	97.9	1.8
10.0	86.5	1.4	3.0	98.5	2.9
20.0	89.6	5.4	6.0	100.5	1.7

Table 2
The intra- and inter-day variations and accuracy of amoxicillin and ranitidine ($n = 6$)

Amoxicillin			Ranitidine		
Concentration ($\mu g\,ml^{-1}$)	Precision (R.S.D.%)	Accuracy (%)	Concentration ($\mu g\,ml^{-1}$)	Precision (R.S.D.%)	Accuracy (%)
Intra-day					
–	–	–	0.03	9.0	114.2
0.2	8.5	90.2	0.06	8.7	102.2
0.5	8.4	93.8	0.15	8.5	102.1
1.0	7.2	106.0	0.3	6.7	101.5
2.0	7.0	100.4	0.6	4.0	99.4
5.0	6.7	99.2	1.5	2.2	101.0
10.0	3.0	100.1	3.0	3.2	99.2
20.0	2.4	99.9	6.0	1.7	100.5
Inter-day					
–	–	–	0.03	10.1	104.1
0.2	3.2	106.7	0.06	5.7	100.4
0.5	11.7	96.5	0.15	9.4	96.5
1.0	6.1	100.9	0.3	8.9	96.7
2.0	3.2	97.2	0.6	4.5	99.1
5.0	4.2	101.4	1.5	5.6	97.2
10.0	6.2	98.9	3.0	7.7	97.5
20.0	5.3	102.4	6.0	4.5	97.5

with amoxicillin and ranitidine. The concentration–time profiles of amoxicillin and ranitidine after oral coadministration of amoxicillin ($100\,mg\,kg^{-1}$) and ranitidine ($50\,mg\,kg^{-1}$) are shown in Figs. 3 and 4.

The double-peak profiles in the case of ranitidine are caused by the nonhomogeneous distribution and absorption of ranitidine along the gastrointestinal tract (GI tract) after its administration [26–28]. This nonhomogeneous distribution of ranitidine is

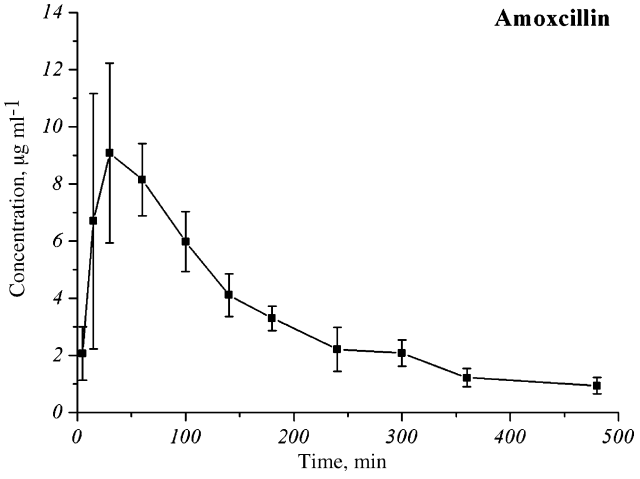

Fig. 3. The concentration–time profiles (mean ± S.D.) of amoxicillin after coadministration with ranitidine to rats ($n = 4$).

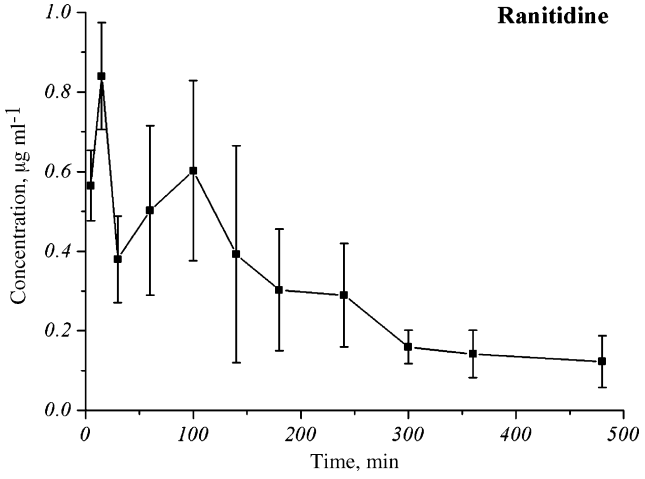

Fig. 4. The concentration–time profiles (mean ± S.D.) of ranitidine after coadministration with amoxicillin to rats ($n = 4$).

influenced by the motility of the GI tract which varies greatly among different animals, and leads to highly variable ranitidine levels found among animals.

4. Conclusions

The method described in this paper is a new, specific and sensitive HPLC-UV method to simultaneously quantify both amoxicillin and ranitidine in rat plasma. According to our knowledge, no similar method has been reported. The method has been successfully applied to quantify amoxicillin and ranitidine simultaneously in rat plasma after oral coadministration.

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