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COMMUNICATION

Simultaneous High-Performance Liquid Chromatographic Analysis for Famotidine, Ranitidine HCl, Cimetidine, and Nizatidine in Commercial Products

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ABSTRACT

A high-performance liquid chromatographic (HPLC) procedure for the simultaneous determination of famotidine (FMT), ranitidine HCl (RNT), cimetidine (CMT), and nizatidine (NZT) was developed using a two-level, full-factorial design with three variables (volume of methanol, percentage of triethylamine, and concentration of phosphate buffer) to select an acceptable mobile phase. A column (15 cm × 4.6 mm ID) of Inertsil ODS-2 (5 μm) was used, and 0.04M aqueous sodium dihydrogen phosphate/acetonitrile/methanol/TEA at a proportion of 345/20/35/0.7 (v/v/v/v) was the selected mobile phase (1 ml/min). The detection wavelength was set at 230 nm, and procaine HCl was used as the internal standard. Precision and linearity of the method were assessed. None of the commercial samples was found to be outside the compendial limits of 90.0% to 110.0% of the claim amount.

INTRODUCTION

Famotidine (FMT), ranitidine HCl (RNT), cimetidine (CMT), and nizatidine (NZT) are histamine H_2-receptor antagonists used in conditions for which inhibition of gastric acid secretion may be beneficial, such as duodenal and gastric ulcers (1). Dosage forms of these four drugs are commercially available in Taiwan.

A search of the literature revealed that a number of high-performance liquid chromatography (HPLC) methods have been developed to determine the individual drug in dosage forms or in biological materials. Nevertheless, no HPLC method has been reported for the simultaneous separation of these four compounds. For a government laboratory to analyze large numbers of commercial samples containing any of the four drugs, time, energy, and

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cost are involved. We attempted to develop a simultaneous HPLC procedure that can separate, identify, and quantitate the four H_2 blockers from a single chromatogram.

Various HPLC parameters, such as pH, flow rate, temperature, solvent composition, and buffer concentration can affect the retention times of these drugs (2). Ordinarily, pharmaceutical analysts who deal with the development of HPLC methods use experimental designs based on the variation of one factor at a time. This approach, however, is time consuming and may fail due to the many variables and interactions involved. Accordingly, formal optimization methods are generally preferred. Factorial designs have been employed to optimize HPLC separations systematically (3,4). The major advantage of factorial designs is to bring a simplified representation of a phenomenon from very few experiments.

Therefore, in this study, a two-level, full-factorial design and computer program were used to select an acceptable HPLC condition rapidly for the simultaneous separation of FMT, RNT, CMT, and NZT. Then, the chromatographic procedure was validated in accordance with the USP guidelines on validation of analytical procedures for drug substances (5).

EXPERIMENTAL

Reagents

Methanol and acetonitrile of liquid chromatography (LC) grade were purchased from Labscan Limited (Dublin, Ireland) and used without further purification. Triethylamine (TEA) was obtained from Riedel-de Haen (Hannover, F. R. Germany), and sodium dihydrogen phosphate was from Wako Pure Chemical Industries, Limited (Osaka, Japan). Ultrapure water was drawn from a Milli-Q water purification system (Millipore Corp., Bedford, MA). The RNT and NZT were kindly supplied by Glaxo Taiwan and Eli Lilly and Company Incorporated (Taiwan), respectively. The CMT and FMT were purchased from USPC, Incorporated (Rockville, MD), while procaine HCl (as an internal standard, IS) was purchased from Sigma Chemical Companies, (St. Louis, MO). Standard materials for the preparations of reproducibility, linearity, and recovery studies were all used as received.

Chromatographic Apparatus

Two LC systems were employed in our work. System 1 was comprised of an LC pump (Waters 510, USA), which was connected to an ultraviolet-visible (UV-Vis) diode array spectrophotometer (Waters 991), with a wavelength range of 190–800 nm, and a printer plotter (Waters 5200) and was monitored by a computer (PowerMate SX/16, NEC, Japan) with software (Waters PDA). System 2 consisted of the same pump as System 1, Waters 600 E System Controller, Waters 717 Auto Sampler, Waters 486 Tunable Absorbance Detector, and an LEO microcomputer (Intel 486DX-33) with Chem-Lab chromatographic software system (Taipei, Republic of China).

An Inertsil ODS-2 (GL Sciences, Inc., Japan) 5-μm column (150 mm × 4.6 mm ID) was used at ambient temperature. Prior to any analysis, mobile phases were degassed and filtered using a Milli-Q system and a nylon filter unit 25 mm × 0.45 μm (Sun Brokers, Inc., NC). The system was equilibrated for 30 min before making an injection.

Standard Solutions

Standard materials of CMT USP, FMT, RNT, NZT, and IS were accurately weighted, and all stock standard solutions were prepared in methanol. Appropriate dilution of the stock standard solutions gave suitable concentrations for all compounds before use.

Sample Solutions

For FMT, RNT, and CMT determinations, more than 20 tablets were weighed and finely powdered. A portion of sample composite equivalent to about 10 mg of FMT and 6 mg of RNT and CMT was accurately weighed into a 100 ml volumetric flask. A suitable amount of IS stock solution and methanol was added, and the mixture was sonicated for sufficient time with occasional stirring and diluted to volume with the same solvent. Then, it was mixed, centrifuged, and filtered through 0.45-μm membrane filter.

For NZT determination, a portion of capsule powder containing about 10 mg NZT was transferred to a 100-ml volumetric flask. The remaining steps above from the sonication were performed as described for FMT, RNT, and CMT determinations.

Factorial Experiment

The two-level factorial experiment has been comprehensively described (6–8). In this paper, two values, denoted + (the upper level) and − (the lower level), are given for each factor to define the experimental domain.

Table1

Experimental Conditions in a 2^3 Factorial Design

	Experiment No.							
Factors	1	2	3	4	5	6	7	8
X1	+1	+1	+1	+1	−1	−1	−1	−1
X2	+1	+1	−1	−1	+1	+1	−1	−1
X3	+1	−1	+1	−1	+1	−1	+1	−1

X1 = methanol (ml), +1 = 7.5, −1 = 5; X2 = triethylamine (ml), +1 = 0.25, −1 = 0.15; X3 = NaH_2PO_4(M), +1 = 0.1, −1 = 0.04.

With three factors, 2^3 experiments (8 total) have to be performed for a complete factorial design. The volume of methanol, content of TEA, and concentration of phosphate buffer were chosen for this investigation as variables X1, X2, and X3, respectively (Table 1). These factors and the values to use for the upper and lower levels of each were established on the basis of prior knowledge from the literature and personal experience. The experimentally measured response variable in this study was the retention times for FMT, RNT, CMT, and NZT. In such a design, the response variables are modeled by the following equation:

$$\begin{aligned} tR = {} & \beta 0 + \beta 1 * X1 + \beta 2 * X2 \\ & + \beta 3 * X3 + \beta 4 * X1X2 + \beta 5 * X2X3 \\ & + \beta 6 * X1X3 + \beta 7 * X1X2X3 \end{aligned}$$

where *tR* is the measured retention time in minutes for each compound under a given set of conditions, and X1, X2, and X3, are values of the three variables indicated above.

Calculations

The computer software of Microsoft® Excel for Windows 95 Chinese Version 7.0 (Serial No. 40360-439-0790050-95728, Microsoft Corporation, Redmond, WA) was used for the calculations, including reproducibility, linearity, and the quantitation of model mixtures prepared in the recovery study and commercial samples, as well as the multiple linear regression of the measured response variables to obtain the regression coefficients β0–β7 of the above equation. A computer program developed in house and coded in BASIC language was used to select the acceptable HPLC separation conditions as systematically changing the three variables X1, X2, and X3 within certain ranges.

RESULTS AND DISCUSSION

Method Development

To facilitate the method development activities, such as the selection of mobile phase and detection wavelength, an LC chromatograph connected to a photodiode array detector (System 1) was used first in the preliminary studies. After completing some testing runs, it was decided that the volume of methanol, the concentration of phosphate buffer, and the volume of TEA in the mobile phase were chosen as three variables for the factorial experiment.

Table 2

Experimental Retention Times (min) of H_2 Blockers

	Experiment No.							
Drugs	1	2	3	4	5	6	7	8
FMT	6.53	9.77	5.97	8.59	8.31	12.48	7.31	11.65
RNT	7.53	14.98	6.69	10.25	9.81	18.47	8.53	13.81
CMT	11.02	20.58	8.8	16.08	13.69	26.34	10.59	21.2
NZT	15.02	30.46	10.85	23.62	19.24	40.65	13.25	32.28

Table 3

Regression Coefficients of Mathematical Model for H_2 Blockers

	Regression Coefficients							
Drugs	β0	β1	β2	β3	β4	β5	β6	β7
FMT	8.826	−1.111	0.446	−1.796	−0.011	−0.056	0.331	−0.099
RNT	11.259	−1.396	1.439	−3.119	−0.046	−0.909	0.366	−0.064
CMT	16.038	−1.918	1.87	−5.013	−0.19	−0.54	0.803	−0.03
NZT	23.171	−3.184	3.171	−8.581	−0.419	−0.631	1.529	−0.036

Table 2 shows the experimental retention times of each compound for the eight experiments of the factorial design. The elution order of the four drugs remained the same in all eight experiments. It was noted, however, that peaks of FMT, RNT, and CMT were not well separated in experiment 3.

Table 3 shows the calculated regression coefficients of variables X1, X2, and X3 of individual H_2 blockers. Microsoft Excel was used for the calculation of these coefficients. Then, with the use of the in-house-developed computer simulation program, apparently no acceptable HPLC conditions were found between the +1 and −1 range of the experimental variables. Hence, the searched intervals were extended to between +2 and −1. An acceptable HPLC condition was found when X1, X2, and X3 were +2, −0.5, and −1, respectively, representing a composition of mobile phase 0.04 M sodium dihydrogen phosphate–acetonitrile-methanol-TEA (345:20:35:0.7 v/v/v/v). On testing these calculated conditions with the HPLC system, satisfactory separation was obtained.

Method Validation

To be used every day as a quantitative technique, the proposed HPLC method was validated in accordance with the USP guidelines on validation of analytical procedures for drug substances.

Figure 1 shows the chemical structures of the substances studied in this work. A typical chromatogram of a reference preparation is shown in Fig. 2. Table 4 gives some chromatographic parameters of the experiment. When each of properly prepared solutions for the validation study was injected into the chromatograph (9), the resolution (Rs) should be greater than 1.5, the capacity factors k', should be between 2 and 10, and selectivity α should be between 1.05 and 2. Using the reversed-phase conditions described, the internal standard and the four drugs were well separated. Peaks with good baseline separation and minimal tailing thus facilitated accurate measurement of the peak areas of each drug.

The precision of the method was estimated by both interday and intraday reproducibilities. The intraday reproducibility was determined by replicate injections of standard solutions appropriately prepared prior to analysis. The interday reproducibility of the method was determined by six repeated analysis of standard solutions over

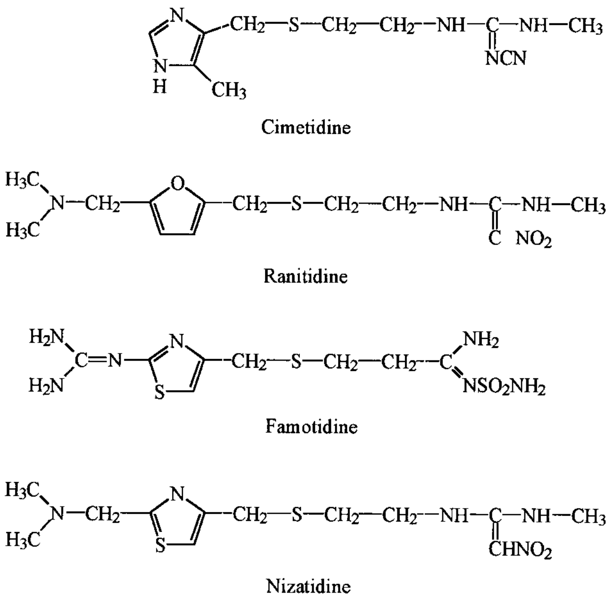

Figure 1. Chemical structures of H_2 receptor blockers.

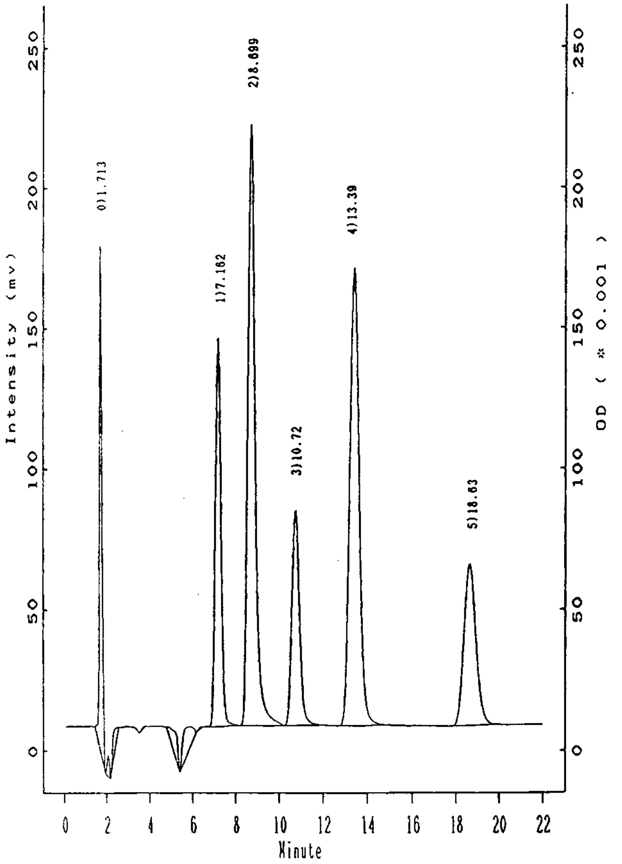

Figure 2. The HPLC chromatogram of four kinds of H_2 blockers and internal standard (IS): (1) famotidine; (2) ranitidine HCl; (3) procaine HCl (IS); (4) cimetidine; (5) nizatidine. Column was an Inertsil ODS-2, 5 μm (4.6 mm × 150 mm) with a mobile phase of 0.04 M KH_2PO_4/ACN/MeOH/TEA (345:20:35:0.7 v/v/v/v). Detector used was UV at 230 nm, and flow rate was 1.0 ml/min.

three different days. An analysis of variance (ANOVA) test was performed with a confidence interval of 95% to compare the difference of the mean peak area ratios. It was found that the relative standard deviations (RSDs) (or coefficient of variation, CV) calculated from the peak area ratios of each drug versus the IS for actual determinations, both intraday and interday, were all less than 1.5% for the four drugs (Table 5).

Calibration curves, shown in Figs. 3–6, were obtained by plotting the peak area ratios of FMT, RNT, CMT, and NZT to the IS against the respective concentrations of these drugs. The linear ranges tested were 5–75 μg/ml for RNT and CMT and 10–150 μg/mL for FMT and NZT (Table 6). The correlation coefficients for each range were all greater than 0.999.

Recovery studies were performed by spiking various amounts of standard powders to accurately weighted portions of powdered placebo mixture. After extraction, an appropriate amount of stock internal standard solution was added into the extracted solutions. Percentage recoveries were 100.72, 100.98, 101.53, and 101.76 for FMT, RNT, CMT and NZT, respectively (Table 7).

Four formulations of commercial products were analyzed by this method. Assay results obtained by six replicate injections of respective sample solutions were in agreement with the declared amounts (Table 8).

CONCLUSIONS

The application of factorial design to the rapid selection of an acceptable HPLC condition for the simultaneous separation, identification and quantitation of famoti-

Table 4

Chromatographic Parameters of System Suitability Test

Drugs	k′ (Capacity Factor)	α (Selectivity)	Rs (Resolution)
FMT	3.181		
		1.282 (FMT-RNT)	1.50 (FMT-RNT)
RNT	4.078		
		1.289 (RNT-IS)	1.82 (RNT-IS)
IS	5.258		
		1.297 (IS-CMT)	3.23 (IS-CMT)
CMT	6.817		
		1.639 (CMT-NZT)	4.85 (CMT-NZT)
NZT	9.876		

Table 5

Calculation Results of Reproducibility Studies

	Intraday					Interday		
	Mean (Area Ratios)							
Drug	Day 1	Day 2	Day 3	CV (%)	95% CI	Mean	CV (%)	95% CI
FMT	.682	.685	.678	.58	(.677, .685)	.681	1.23	(.661, .702)
RNT	.623	.624	.625	.33	(.622, .626)	.624	.33	(.619, .629)
CMT	.574	.576	.572	.33	(.572, .576)	.574	.74	(.563, .584)
NZT	.634	.635	.631	.45	(.630, .636)	.633	.78	(.621, .646)

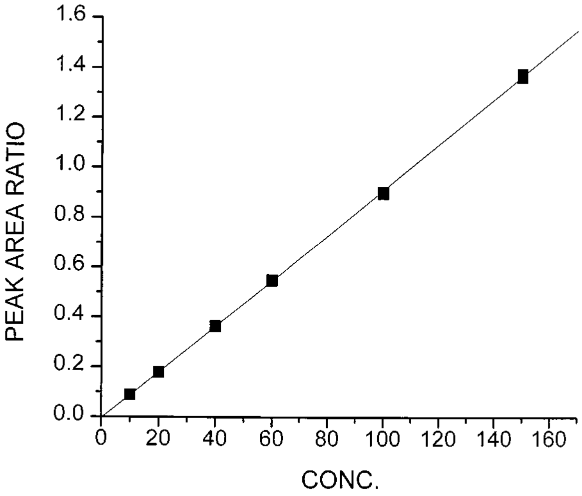

Figure 3. Calibration curve of famotidine.

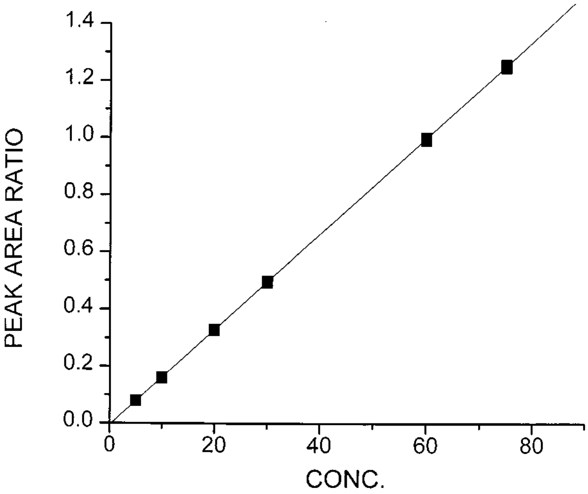

Figure 5. Calibration curve of cimetidine.

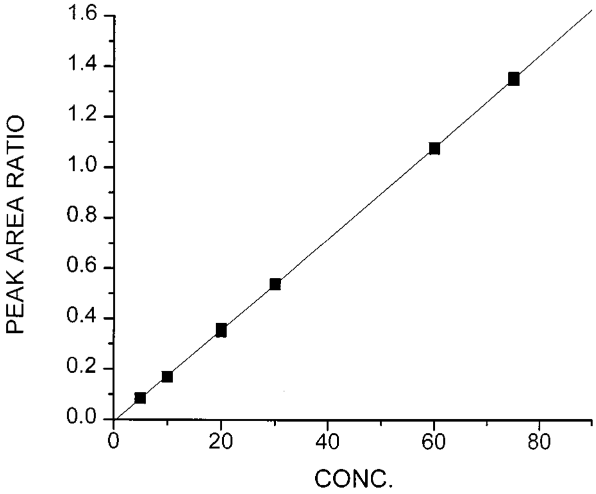

Figure 4. Calibration curve of ranitidine HCl.

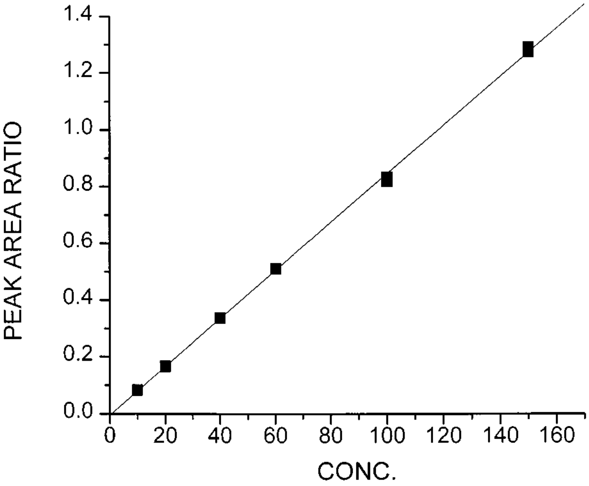

Figure 6. Calibration curve of nizatidine.

dine, ranitidine HCl, cimetidine, and nizatidine in commercial products is very useful. The selected HPLC method in this study proved to be fast, simple, and reliable, and its precision is sufficient for routine analysis in our laboratory.

Table 6

Linear Regression and Correlation of H_2 Blockers

Drugs	Range of Linearity (μg/ml)	Equations of Linear Regression	r
FMT	10 ~ 150	$Y = 0.00914X - 0.00397$	.99994
RNT	5 ~ 75	$Y = 0.01811X - 0.00734$	.99997
CMT	5 ~ 75	$Y = 0.01669X - 0.00539$	.99999
NZT	10 ~ 150	$Y = 0.00851X - 0.00495$	.99971

Table 7

Percentage Recoveries of H_2 Blockers in Synthetic Samples

Drugs	% Found (Mean ± SD; CV)
FMT	100.72 ± 2.27; 2.25
RNT	100.98 ± 2.36; 2.34
CMT	101.53 ± 1.15; 1.14
NZT	101.76 ± 1.65; 1.62

Table 8

Assay Results of Some Commercial Products

Samples	Formulation	% Found (Mean ± SD; CV)
1	FMT (20 mg/tab)	94.88 ± 1.32; 1.39
2	RNT (150 mg/tab)	104.44 ± 2.14; 2.05
3	CMT (200 mg/tab)	100.45 ± 3.64; 3.63
4	NZT (150 mg/cap)	94.49 ± 1.52; 1.62

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