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Simultaneous determination of cimetidine, famotidine, nizatidine, and ranitidine in tablets by capillary zone electrophoresis

A simple capillary zone electrophoresis (CZE) method is described for the simultaneous determination of cimetidine (CIM), famotidine (FAM), nizatidine (NIZ), and ranitidine (RAN). The analysis of these drugs was performed in a 100 mM phosphate buffer, pH 3.5. Several parameters were studied, including wavelength for detection, concentration and pH of phosphate buffer, and separation voltage. The quantitative ranges were 100–1000 μM for each analyte. The intra- and interday relative standard deviations ($n = 5$) were all less than 4%. The detection limits were found to be about 10 μM for CIM, 20 μM for RAN, 20 μM for NIZ, and 10 μM for FAM ($S/N = 3$, injection 1 s) at 214 nm. All recoveries were greater than 92%. Applications of the method to the assay of these drugs in tablets proved to be feasible.

Keywords: Capillary zone electrophoresis / H_2 -antagonists / Tablets

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1 Introduction

The histamine₂ (H_2)-antagonists are drugs of choice for the treatment of gastric and duodenal ulcers [1]. These agents benefit ulcer patients by suppressing secretion of gastric acid. Four H_2 -antagonists are available: cimetidine (CIM), famotidine (FAM), nizatidine (NIZ), and ranitidine (RAN) (see Fig. 1 for their structures). The assay methods of the Pharmacopoeia [2] for these analytes in pharmaceuticals are different modes of HPLC methods. The mobile phases include methanol-phosphoric acid for CIM, phosphate buffer-methanol-water for FAM, ammonium acetate buffer-diethylamine-methanol for NIZ, and ammonium acetate buffer-methanol for RAN, respectively. We have to use the tedious procedures to determine the individual content of the pharmaceuticals. In order to simplify the process of quality control, it is necessary to establish a simple and specific method for the simultaneous analysis of H_2 -antagonists.

Some methods have been reported for the determination of the H_2 -antagonists, including HPLC [3–9] and capillary electrophoresis (CE) [10–12]. Only one HPLC method [8], but no available CE method, can offer simultaneous analysis of these four analytes, and its separation time is 20 min. CE is a separation technique that has become popular in many areas. Ease of operation and rapid analy-

sis time are two attractive features of CE. The majority of drugs are either acidic and/or basic water-soluble compounds. The basis for separation in CZE relies upon an exploitation of differences between the analytes' electrophoretic mobilities, which are related to the solutes' charge and size. We are interested in providing a systematic approach to separating compounds that are closely related in mass and structure. Recently, CE has been developed to perform these tests and shown to be a complementary and attractive alternative to the more established methods. In this study, a simple and specific CZE method is developed for the simultaneous separation and quantitation of these four H_2 -antagonists. They were analyzed on an uncoated capillary with 100 mM phosphate buffer; pH 3.0, within 5 min. The quantitation range of each drug is 100–1000 μM . Applications of CIM, FAM, NIZ, and RAN in tablets were demonstrated and proved to be satisfactory.

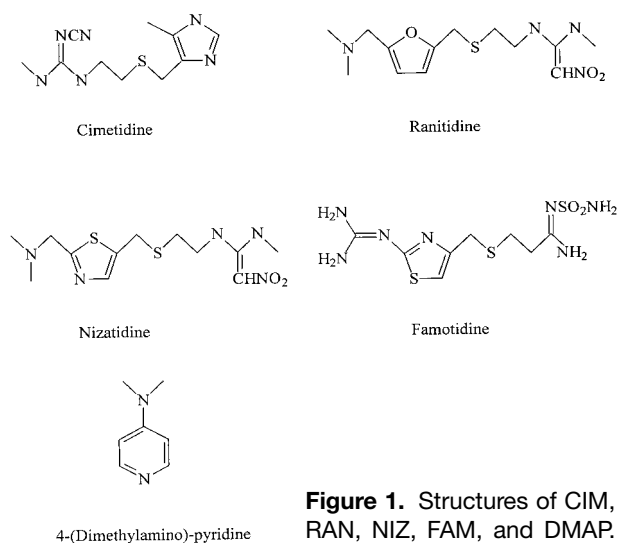


Figure 1. Structures of CIM, RAN, NIZ, FAM, and DMAP.

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Abbreviations: CIM, cimetidine; DMAP, 4-(dimethylamino)-pyridine; FAM, famotidine; H_2 , histamine₂; NIZ, nizatidine; RAN, ranitidine

2 Materials and methods

2.1 CZE system

A Beckman P/ACE System 2200 (Fullerton, CA, USA) equipped with a filter UV detector and a liquid-cooling device was used. CZE was performed in an uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 37 cm (effective length 30 cm) \times 50 μ m ID. The detector was set at 214 nm. The running buffer was 100 mM phosphate buffer; pH 3.5. Capillary conditioning before startup is water (10 min), 1 N NaOH (20 min), water (10 min), 1 N HCl (20 min), water (10 min), and running buffer (30 min) in regular sequence. The conditioning between runs was effected by rinsing with water (3 min) and running buffer (3 min), under positive pressure applied at the injection end. This conditioning procedure can keep the capillary stable more than 24 h. At 25°C, a constant voltage of 20 kV (anode at injection end) was applied throughout the run. The current gradually increased to about 85 μ A during the first 15 s after power application. All operation and electropherograms were computer controlled using GOLD software Version 7.1.

2.2 Chemicals and reagents

CIM, RAN-HCl, NIZ (Sigma, St. Louis, MO, USA), FAM, Gaster (Yamanouchi, Japan), Tagamet (China Chemical, Taiwan), Zantac (GlaxoWellcome, Taiwan), Tazac (Lilly, Taiwan), NaH₂PO₄ · 2H₂O, 4-(dimethylamino)-pyridine (DMAP), NaOH, and H₃PO₄ (85%) (Merck, Darmstadt, Germany) were used without further treatment. Milli-Q water (Millipore, Bedford, MA, USA) was used for the preparation of buffer and related aqueous solutions. Millex-SR filter membranes (Merck) were used for the filtration of tablets. Solutions of various phosphate buffers at different pHs were prepared by neutralizing the related NaH₂PO₄ solution with H₃PO₄.

2.3 Reference and sample solutions

Stock solutions of four H₂-antagonists at 1.0 mM were prepared in water and suitably diluted as reference solutions. For the assay of these analytes, sample solutions were prepared as followed: 20 tablets of CIM (Tagamet), 10 tablets of RAN (Zactac), 10 tablets of NIZ (Tazac) or 20 tablets of FAM (Gaster) were weighed and finely powdered. We weighed an accurate portion of the powder, equivalent to about 3.0 mg of CIM, RAN, NIZ, or FAM, by dissolving each tablet in water in 50-mL volumetric flask with the aid of sonication for 5 min. A suitable amount of the resulting water extract was centrifuged at 1000 \times g

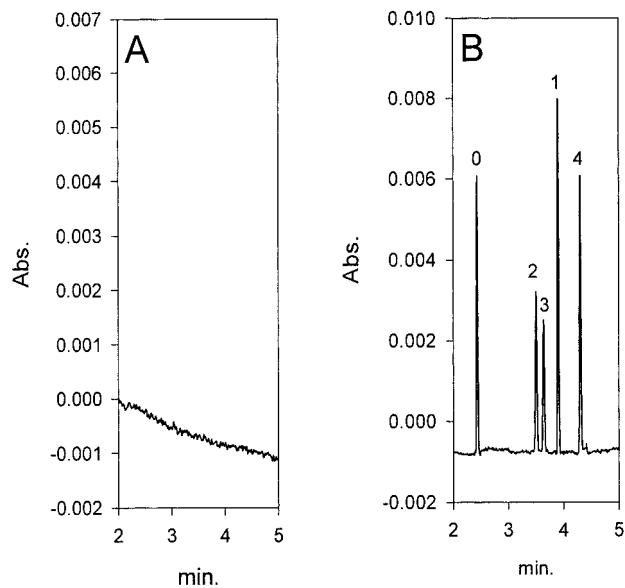


Figure 2. Electropherograms of (A) blank, (B) H₂-receptor antagonists each at 100 μ M. Peaks: 0, 1, 2, 3, and 4 for DMAP, CIM, RAN, NIZ and FAM, respectively. CZE conditions: buffer, 100 mM phosphate (pH 3.5); applied voltage, 20 kV (detector at cathode side); uncoated fused-silica capillary, 30 cm (effective length) \times 50 μ m ID; sample size, 1 s by pressure; wavelength, 214 nm.

for 10 min. The supernatant was filtered with Millex-SR filter membrane (0.5 μ m pore size) and the resulting filtrate was used for the CZE analysis.

3 Results and discussion

Figure 2 presents an electropherogram of the CZE separation of four H₂-antagonists. This indicates that baseline separation of the four compounds was achieved within 5 min. Simple parameters were studied to determine the optimal separation conditions, including wavelength for detection, concentration and pH of phosphate buffer, and analytical voltage.

3.1 Wavelength for detection

Scanned by a Beckman spectrophotometer (model DU 640B), the spectra of these H₂-antagonists in running buffer were shown in Fig. 3. The wavelengths of maximal absorbance were 219 nm (CIM), 229 and 314 nm (RAN), 254 and 315 nm (NIZ), 207 and 282 nm (FAM). The 214 nm wavelength was chosen for the simultaneous determination. For the individual analysis of NIZ, the 200 nm wavelength could be set, and afforded about a twofold sensitivity.

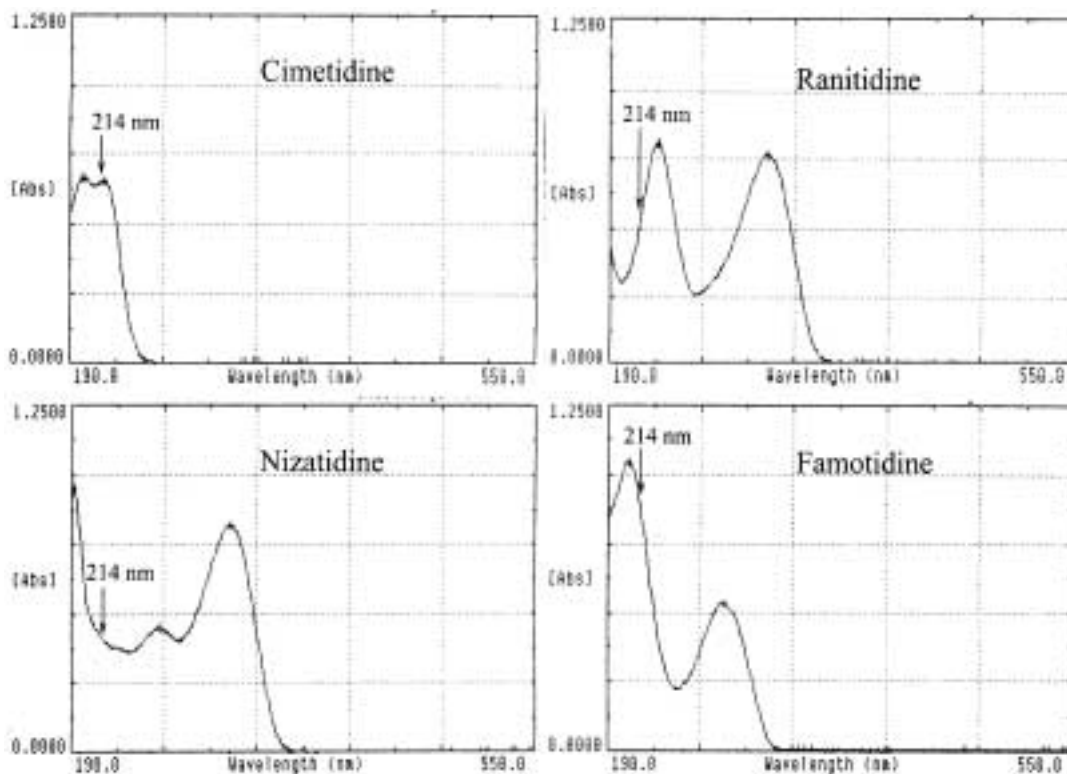


Figure 3. Spectra of H₂-blockers. The conditions: source, deuterium lamp; solvent, 100 mM phosphate buffer (pH 3.5); concentration of each drug, CIM (30 μ M), RAN (50 μ M), NIZ (40 μ M), and FAM (30 μ M).

3.2 Concentration and pH of phosphate buffer

CZE of the drugs in phosphate buffer (pH 3.5) in the concentration range of 50–150 mM can give good separation efficiency. From the results of current generated, we have to notice the problem of heat generation/dissipation. When the concentration of phosphate buffer is above 125 mM, the current is higher than 100 μ A. To prevent the generation of too much Joule heating, 100 mM of phosphate buffer was chosen. The EOF is greatly reduced in acidic buffer, the separation mechanism is mainly based on differences in the charge-to-mass ratio. Based on the pK_a values of CIM at 6.8, RAN at 2.7 and 8.2, FAM at 6.45, and NIZ at 2.1 and 6.8 [13], these four drugs can be fully protonated in the pH < 5 buffers. Phosphate buffers (100 mM) with different pHs (2.5, 3.0, 3.5, 4.0) were studied. The results (Fig. 4) indicate that a baseline resolution was obtained at the lower pH range (pH 2.5–3.5). In order to shorten the separation time, the optimal pH was set at pH 3.5. Based on the good buffer capacity of phosphate buffer (pK_a 2.5), we achieved short analytical times (about 5 min), sharp peak shapes, and good resolution of the drugs under the present conditions, other buffer systems were not studied.

3.3 Analytical voltage

Both the electroosmotic and electrophoretic velocities are directly proportional to the field strength, so the use of the highest voltages possible will result in the shortest times for the separation. The short separation times will give the

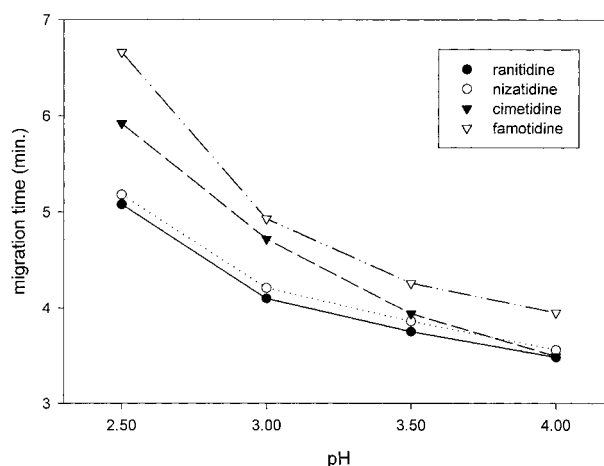


Figure 4. Effects of buffer pH on the migration of H₂-receptor antagonists each at 100 μ M. See Fig. 2 for other CE conditions.

highest efficiencies since diffusion is the most important feature contributing to bandbroadening. The limiting factor here is Joule heating. Four voltages (10, 15, 20, 25 kV) were studied. The optimal voltage was set at 20 kV, it can afford shortest migration time and acceptable current generated.

3.4 Analytical calibration

For evaluating the quantitative applicability of the method, five different concentrations of CIM, RAN, FAM, and NIZ each in the range of 100–1000 μM were analyzed using DMAP (600 μM) as an internal standard (IS). The linearity between the normalized peak-area ratios (Y) of the related analyte to IS and the concentration (X , μM) of analyte was investigated. The results of the linear regression equations ($n = 5$) indicate that high linearity ($r > 0.993$) between Y and X is attainable over the range studied. When the extension of linearity range reaches 4000 μM , all the correlation coefficients can be higher than 0.960. At 214 nm, the detection limits ($S/N = 3$, injection 1 s) are 10 μM for CIM, 20 μM for RAN, 20 μM for NIZ, and 10 μM for FAM. The relative standard deviations (RSDs) of the method based on the peak-area ratio for replicate determinations of these H_2 -antagonists each at 130, 220, 280 μM were studied. As shown in Table 1, the intraday's RSDs ($n = 5$) of the four analytes were all below 3.7% at 130 μM , 220 μM , and 280 μM ; in parallel, the interday's RSDs ($n = 5$) were all below 3.05% at 130 μM , 220 μM , and 280 μM . The RSDs ($n = 5$) of the area and migration for each drug at 130 μM were all below 4.57 and 0.14 for intraday assay, and 7.31 and 1.37 for interday assay, respectively. In this method, there is no interference with roxatidine (extracted from the roxatidine tablet). Roxatidine is also one kind of H_2 -antagonists, to our deep regret we can not get its reference standard.

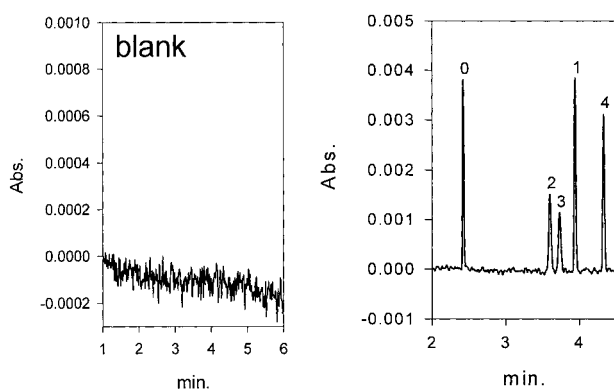


Figure 5. Typical electropherogram for the analysis of CIM, RAN, NIZ and FAM in tablets. See Fig. 2 for CZE conditions. Peaks: 0, 1, 2, 3, and 4 for DMAP, CIM, RAN, NIZ and FAM, respectively.

Table 1. Precision and accuracy for the determination of RAN, CIM, NIZ and FAM

Concentration known (μM)	Concentration found (μM)	RSD (%)	RE ^a (%)	
Intraday analysis ($n = 5$)				
RAN	130.0	129.2 \pm 3.1	2.4	-0.6
	220.0	214.6 \pm 1.3	0.6	-2.4
	280.0	279.4 \pm 3.6	1.3	-0.2
CIM	130.0	127.2 \pm 2.0	1.6	-2.2
	220.0	214.8 \pm 0.9	0.4	-2.4
	280.0	281.5 \pm 2.1	0.8	-0.5
NIZ	130.0	124.5 \pm 4.6	3.7	-4.2
	220.0	217.7 \pm 2.2	1.0	-1.1
	280.0	274.6 \pm 3.2	1.2	-2.0
FAM	130.0	125.7 \pm 3.5	2.8	-3.3
	220.0	220.1 \pm 7.6	3.5	0.04
	280.0	279.9 \pm 7.7	2.8	-0.1
Interday analysis ($n = 5$)				
RAN	130.0	129.3 \pm 2.4	1.8	-0.5
	220.0	216.7 \pm 2.0	0.9	-1.5
	280.0	275.6 \pm 4.1	1.5	-1.6
CIM	130.0	127.9 \pm 3.3	2.6	-1.6
	220.0	217.8 \pm 3.1	1.4	-1.0
	280.0	280.3 \pm 2.2	0.7	0.1
NIZ	130.0	126.6 \pm 3.9	3.1	-2.6
	220.0	213.2 \pm 4.1	2.0	-3.1
	280.0	271.3 \pm 3.9	1.4	-3.1
FAM	130.0	128.2 \pm 3.8	3.0	-1.4
	220.0	216.8 \pm 6.2	2.9	-1.5
	280.0	279.6 \pm 5.3	1.9	-0.1

a) RE (% relative error) = (conc. found – conc. known) \times 100/(conc. known)

3.5 Application

The application of the method to the assay of the H_2 -antagonists in tablets was studied. The results of percentage of claimed content (%) are 92.1–93.8 for CIM, 96.4–104.8 for RAN, 100.2–106.3 for NIZ, and 91.9–95.9 for FAM. All the analytical values fell within labeled amount of 90–110% required by the USP XXIII [2]. A typical electropherogram for the analysis of CIM, RAN, FAM, and NIZ in tablets is shown in Fig. 5. The recoveries of these four drugs were studied, which were based on three known amounts of reference H_2 -antagonists each added to the powder tablet resulting in the preparation of three spiked levels (50, 100, 150 μM). All the recoveries are above 92% (108.1 ~ 92.3%) as shown in Table 2.

In conclusion, a simple and specific CZE method has been successfully demonstrated for the assay of four H_2 -antagonists in tablets. CE offers a completely different

Table 2. Recoveries of CIM, RAN, NIZ and FAM added to commercial formulation

	Concentration spiked (μM)	Concentration ^{a)} found (μM)	Recovery (%)
CIM	–	128.7 \pm 1.6	–
	50.0	178.9 \pm 1.2	100.3
	100.0	231.5 \pm 3.6	102.7
	150.0	289.3 \pm 5.6	107.0
RAN	–	120.5 \pm 6.5	–
	50.0	174.5 \pm 2.7	108.1
	100.0	226.7 \pm 4.3	106.3
	150.0	265.3 \pm 3.5	96.7
NIZ	–	121.6 \pm 3.1	–
	50.0	173.2 \pm 6.0	103.2
	100.0	217.7 \pm 5.6	96.1
	150.0	277.4 \pm 8.2	103.9
FAM	–	124.7 \pm 2.0	–
	50.0	178.1 \pm 1.9	106.9
	100.0	217.0 \pm 2.0	92.3
	150.0	269.8 \pm 4.6	96.8

a) Mean \pm SD ($n = 5$)

selectivity process and is, therefore, truly a complementary and alternative technique to HPLC in pharmaceutical assay.

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