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Direct determination of ranitidine and famotidine by CE in serum, urine and pharmaceutical formulations

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Abstract

A simple and sensitive capillary electrophoresis method using UV detection has been developed for the direct determination of ranitidine (RANT) and famotidine (FAMT) in serum, urine and pharmaceutical formulations. A buffer consisting of 60 mM phosphate buffer adjusted to pH 6.5 was found to provide a very efficient and stable electrophoretic system for the analysis of both drugs. The detection limits obtained were 0.088 μg ml^{-1} for RANT and 0.16 μg ml^{-1} for FAMT.

Keywords: Capillary electrophoresis; Ranitidine; Famotidine; Serum; Urine; Pharmaceutical formulations

1. Introduction

Ranitidine (RANT) and famotidine (FAMT) are h_2-receptor antagonists that are used in the treatment of gastric and duodenal ulcers and other related disorders; they work by inhibiting the secretion of gastric acid. Both drugs are hydrophilic molecules containing a substituted furano ring (RANT) or a substituted thiazol ring (FAMT) Fig. 1

Methods used for determining RANT and FAMT include ultraviolet spectrophotometry [1], visible spectrophotometry after reaction with different reagents [1–3], polarography [4–6] and flow injection analysis with spectrophotometric, potentiometric, fluorimetric and chemiluminimetric detection [7–9]. These methods, however, are not adaptable for use in pharmokinetic studies because of their lack of selectivity.

High-performance liquid chromatography with UV or fluorescence detection has been widely used for the determination of both drugs in serum, urine and pharmaceutical formulations [10–14]. Most of these methods use considerable amounts of expensive and environmentally hazardous organic solvents. Safe disposal or recycling results in substantial additional cost. Over the past few decades, microseparation techniques such as capillary electrophoresis (CE) have offered a substantial advantage over other separation techniques. CE is increasingly regarded as an attractive separation method because it combines

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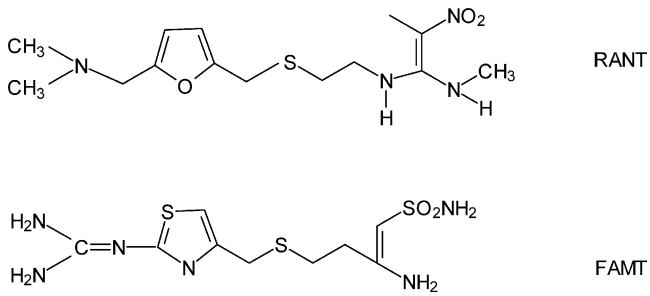

Fig. 1. Structures of RANT and FAMT.

high resolution and ease of automation with modest sample requirements and low solvent consumption [15,16]. However, the CE methods proposed for the determination of RANT and FAMT are scarce. Altria et al. [17–19] and Morris et al. [20] have developed assays for the separation of RANT of its degradation products. Yet, for the determination of RANT and FADT the main improvement in CE was reported by Wu et al. [21,22] while the present work was in progress. The use of either a phosphate buffer of pH 3.5 or a binary buffer comprising ethylene glycol and NaH_2PO_4 (pH 5.0) was proposed. Thus, despite the major progress in CE separations of these two compounds, there is still room for improvement as far as the optimization of CE separation is concerned. Accordingly, the aim of the present investigation was to optimize the CE conditions for the determination of the analytes. Effects of pH, type of buffer and its concentration and applied voltage on mobility, resolution, sensitivity and speed were carefully evaluated. The developed method was useful for the individual and simultaneous determination of both drugs in serum, urine and pharmaceutical formulations. The method was validated by determining the accuracy, precision, linearity, specificity and robustness of the assay.

2. Experimental

2.1. Capillary electrophoresis system

Separations were performed on a P/ACE 5500 automated CE system (Beckman Instruments, Palo Alto, CA) equipped with a diode array detector, fluid-cooled column cartridge and automatic injector. Fused silica capillaries (Beckman) of i.d 75 μm, o.d 375 μm and lengths 57 cm were used. New capillaries were first rinsed with 1.0 M sodium hydroxide for 5 min under high pressure (3.45 kPa), followed by rinsing with the separation electrolyte for 10 min. The capillary was then left to equilibrate in the separation electrolyte for 10 min by applying a separation voltage of 10 kV. Each separation was preceded by a 1 min high pressure rinse with the separation electrolyte. The samples were introduced using a 10 s low pressure injection (0.5 psi) and the separation was carried out for 8 min at 10 kV and 25 °C. The electroosmotic breakthrough time was measured with benzyl alcohol or acetone.

Absorbance was monitored at 228 nm and data were collected and processed using the System Gold data station.

2.2. Buffer preparation and chemicals

Demineralized water from a Milli-Q system (Millipore Ibérica, Madrid, Spain) was used for the preparation of the solutions. The aqueous background electrolyte used for CE separation consisted of 60 mM phosphate buffer adjusted to pH 6.5. Stock standard solutions of RANT (300 μg ml^{-1}) and FAMT (300 μg ml^{-1}) were prepared by dissolving appropriate amounts of the drugs (Sigma, St. Louis, MO) in water.

Phosphate, ACES (*N*-[carbamoylmethyl-2-aminoethano] sulfonic acid) and MES (2-[*N*-Morpholino] ethanesulfonic acid) buffers were prepared

from the corresponding acids by adjusting to the desired pH with sodium hydroxide.

3. Results and discussion

3.1. Method development

The influence of several parameters was investigated to identify the key variables that affect separation efficiency of RANT and FAMT using standard solutions at the concentration of 5 μg ml^{-1}. These parameters included the nature, pH and concentration of the run buffer, and applied voltage.

3.1.1. Effect of pH

In an attempt to find a suitable pH value for the run buffer and considering that the molecular structures of RANT and FAMT provide information on their acido-base properties, the effect of pH on migration times and base line resolution was investigated. Fig. 2 shows the pH dependance of the apparent mobility of RANT and FAMT. In the pH range 3–7 both drugs are positively charged because their migration times at each pH are shorter than those obtained for the electroosmotic breakthrough time. It was observed that the best defined peaks were obtained at pH values between 5.5 and 6.5. The resolution as a function of pH is shown in Fig. 3. A pH value of 6.5 was selected because this enabled the optimum resolution between RANT and FAMT with the shortest analysis time.

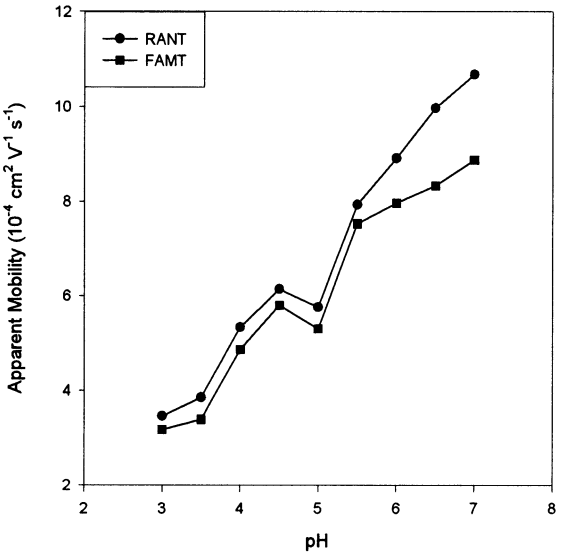

Fig. 2. Apparent mobility as a function of pH. The effective capillary length was 50 cm, the total length 57 cm, and applied voltage 12 kV, Phosphate buffer (50 mM) was used as the electrophoretic buffer.

3.1.2. Buffer nature

The type of electrophoretic buffer affected the degree of separation of two analytes. Three different buffers (phosphate, ACES and MES) were used at the same molar concentration (40 mM) and the pH value was adjusted to 6.5 in all cases. Since phosphate buffer provided the best results as regards peak symmetry and differences in the migration times, this buffer was selected for further studies.

3.1.3. Effect of ionic strength and buffer additives

The ionic strength of the phosphate buffer was tested at a constant pH of 6.5. As the concentration was changed from 10 to 80 mM, an increase in migration times was obtained. It is suggested that

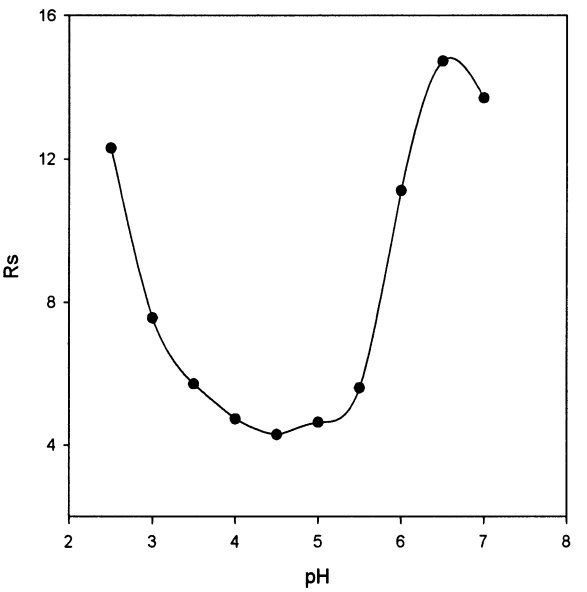

Fig. 3. Influence of pH on resolution. Conditions as for Fig. 2.

such an effect is related to a lower electroosmotic flow, resulting from a decrease of the zeta potential at the capillary wall-solution interface. On increasing the phosphate concentration, the analysis time was longer. The difference of migration times between RANT and FAMT was 26 s when 10 mM phosphate buffer was used and became 42 s with 80 mM phosphate buffer. A good compromise between separation, run time and Joule heat generated inside the capillary was obtained with 60 mM phosphate buffer.

While cyclodextrins (CD) are widely used as buffer additives to obtain chiral separations in CE, they can be also used to adjust the selectivity in non-chiral applications [23]. Indeed, several studies have shown that adding CD can enhance the selectivity of CE because these compounds form inclusion complexes with a wide variety of guest organic molecules. In the present study, the use of α-, β- and γ-CD at the concentration range 1–10 mM does not provide increased separation between RANT and FAMT.

The addition of organic solvents to the electrophoretic buffer was also considered, although this effect is hard to predict because it affects several variables, including viscosity, dielectric constant and zeta potential. The presence of acetonitrile, methanol or ethanol in the run buffer lowered the current and slightly improved the separation at the expense of a longer analysis time.

3.1.4. Effect of applied voltage

The effect of applied voltage on peak efficiency of the analytes was studied over the range 5–25 kV. Using 60 mM phosphate buffer at pH 6.5, the increase of applied voltage led both to shorter migration times and shaper peaks. As expected, on increasing the applied voltage there is an increase in electroosmotic flow, leading to shorter analysis times and higher efficiencies. However, higher applied voltages exhibit also higher currents and increased Joule heating. To limit this heating inside the capillary, the maximum applied voltages were chosen from the Ohm's plot. The best defined peaks were achieved with a voltage of 10 kV (current $\approx$60–62 μA).

3.1.5. Injection time

Sensitivity in CE is limited by the volume injected into the capillary. Sample solutions were hydrodynamic injected at a pressure of 3.45 kPa while the injection time was varied from 1 to 20 s. Peaks areas of RANT and FAMT increased linearly on increasing the injection time up to 15 s. Generally, it is recommended a plug length which should not exceed 1–2% the whole capillary length in order to control efficiency and resolution [15]. Here an injection time of 10 s was chosen, which corresponds to approximately 2% of the capillary length.

3.2. Analytical performance characterisitics

Choosing a 60 mM phosphate at pH 6.5, an applied voltage of 10 kV (corresponding to 175 V/cm), a temperature of 25 °C, and a hydrodynamic injection of 10 s at 3.45 kPa, resulted in a fast, sensitive and complete separation assay in less than 8 min. Satisfactory separation of RANT and FAMT was achieved with symmetrical peaks in the migration time window comprised between 5.3 and 7.8.

Calibration graphs were obtained by injecting standard solutions of the analytes in the concentration range 0.5–50 μg ml^{-1} (at least 15 samples covering the whole range of concentrations were used). External calibration was used because no improvement was observed when an internal standard was used. Each point of the calibration graph corresponded to the mean value from three independent peak area measurements. The corresponding regression equations and other characteristic parameters for the determination of RANT and FAMT are shown in Table 1. The limits of detection were determinated at a signal to noise ratio of 3 and found to be 0.088 μg ml^{-1} for RANT and 0.16 μg ml^{-1} for FAMT. The noise in the base line was determinated using the mean peak-to-peak noise. The within-day precision of the method was studied with eleven repeated injections of one standard solution containing 1.5 μg ml^{-1} of each analyte. The peaks of the two analytes were completely separated (Fig. 4), and the relative standard deviation (RSD) of the peak area of each drugs was 1.1% for RANT and 1.5%

Table 1
Figures of merit of CE method for determination of RANT and FAMT

Compound	$Y = A + BX$	r^2	D.L.	N/m
RANT	$A = 6.4 \times 10^{-3} \pm 6 \times 10^{-3}$ $B = 4.2 \times 10^{-2} \pm 2 \times 10^{-4}$	0.9996	0.088	280 000
FAMT	$A = -2.9 \times 10^{-3} \pm 4 \times 10^{-}$ $^{3}B = 3.1 \times 10^{-2} \pm 1 \times 10^{-4}$	0.9994	0.16	220 000

X, analyte concentration in μg ml^{-1}; A, intercept of the regression lines fitted to the calibration data set ±S.D.; B, slope of the regression lines fitted to the calibration data set ±S.D.; D.L., detection limit in μg ml^{-1} (signal-to-noise ratio = 3); N/m, number of theoretical plater per meter.

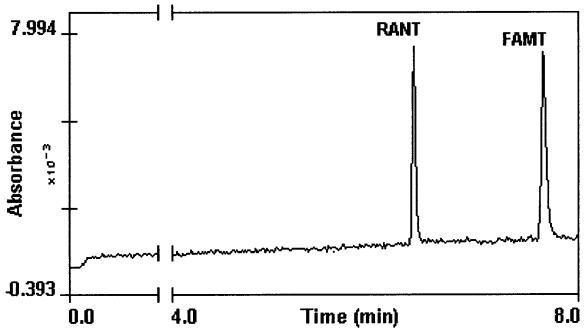

Fig. 4. CZE separation of RANT (7.0 μg ml^{-1}) and FAMT (7.0 μg ml^{-1}). Running buffer: 60 mM phosphate (pH 6.5). Effective capillary length, 50 cm; total length, 57 cm. Applied voltage, 10 kV. Hydrodynamic injection was performed for 10 s at 0.5 psi.

for FAMT. The between-day precision was studied by analysing on 5 consecutive days, three identical samples (containing 1.5 μg ml^{-1} of each analyte) which were injected six times every day. The RSD was 1.9% for RANT and 2.4% for FAMT.

3.3. Robustness

The influence of significant changes of the buffer pH (6.3–6.7), electrolyte concentration (50–70 mM) and applied voltage (9–11 kV) were investigated in the presence of imipramine as internal standard.

Although the absolute migration times and the peak area of RANT and FAMT varied when each variable was altered in turn while keeping the other constants, the relative migration times and the area ratios of the drugs versus imipramine did not change significantly. Deviations less than 3.4% relative to the optimum value of each variable (used as reference) were always found.

3.4. Applications

The CE assay is characterized by long-term stability and reproducibility. More than 1000 analysis could be performed without the need to replace the capillary. To demonstrate the usefulness of the method for the determination of RANT and/or FAMT, pharmaceutical formulations and human serum and urine were analysed for the presence of these drugs.

Analysis of drugs in urine samples by CE is always a delicate problem. If urine is directly injected into the capillary, proteins and the other biomolecules in the urine matrix are absorbed to the wall of the capillary. By experimenting with different urine dilution ratios we showed that a 10-fold dilution of urine was suitable for the analysis of RANT and FAMT because this avoided adverse matrix effects.

Known amounts of the analytes (between 0.8 and 25 μg ml^{-1}) were spiked in 10-fold diluted urine to establish the calibration curve. In the concentration ranges studied the calibration curves were linear and the migration times were reproducible (RSD < 2.2% were always obtained). The results obtained in the analysis of three different samples are indicated in Table 2.

The serum samples were spiked with different quantities of both drugs, so that their concentrations were similar to those used in clinical applications. The serum sample (100 μl) was treated with perchloric acid (0.5 M, 50 μl) to separate the proteins. After centrifugation (5 min at 3000 × g), The liquid supernatant was adjusted to a pH of about the 6.5 with 0.5 mol^{-1} sodium hydroxide, filtered through a 0.45-μm filter and diluted with demineralized water to an appropriate volume. In

Table 2
Recovery of RANT and FAMT in real samples

Sample	RANT			FAMT		
	Added μg ml^{-1}	Mean recovery %	RSD%	Added μg ml^{-1}	Mean recovery %	RSD%
Urine 1	4.0 (6)[a]	98.7	1.1	4.0 (6)	102.5	1.1
Urine 2	8.0 (4)	101.7	1.4	8.0 (5)	100.1	2.2
Urine 3	15.0 (4)	99.6	2.1	15.0 (5)	99.4	1.8
Serum 1	0.9 (5)	97.7	3.2	0.9 (5)	90.6	3.7
Serum 2	1.8 (4)	98.3	3.4	1.8 (4)	91.1	3.6
Serum 3	3.6(4)	96.6	2.1	3.6 (4)	90.2	2.8

[a] Number of samples is in parenthesis.

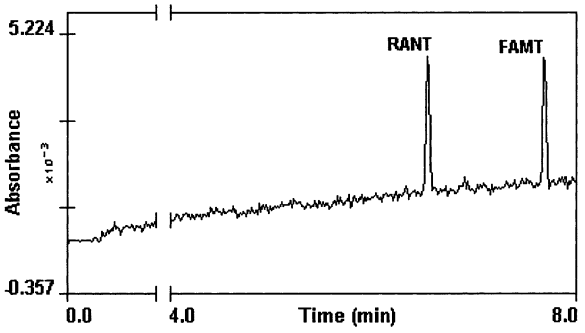

Fig. 5. Electropherogram of serum-sample. Hydrodynamic injection 10 s. Other conditions as for Fig. 3.

the concentration range studied (0.8–40 μg ml^{-1} for each analyte), the calibration curves were linear and the migration times were reproducible. Table 2 summarizes the results obtained and Fig. 5 shows the separation of RANT and FAMT for a serum sample. The method developed was also used to quantify medicaments and the results obtained can be seen in Table 3.

4. Conclusions

The results of this study suggest that a 60 mM phosphate buffer of pH 6.5 is a very efficient electrophoretic electrolyte for separating RANT and FAMT. Considering the data related to the quantitation (reproducibility in peak-area) short (<8 min) and reproducible migration times, it is apparent that the proposed CE method is a very promising alternative to the determination of these drugs. In addition, the results obtained in the analysis of RANT and FAMT in serum and urine samples and in pharmaceutical formulations demonstrate the applicability of the method.

Table 3
Determination of RANT and FAMT in pharmaceutical preparations

Preparation[a]	Supplier	Amount certified mg per tablet	Amount found[b] mg per tablet	
			CE method	HPLC method
Ranuber (RANT)	ICN Ibérica	150	149.1±1.8	149.4±1.1
Zantac (RANT)	Glaxo Wellcome	300	302.7±1.2	299.6±1.6
Famotidina	Ratiopharm	20	19.8±2.0	19.9±1.9
Fagastril (FAMT)	Quimifar	40	40.5±1.5	40.6±2.1

[a] Composition: Ranuber: RANT, 150 mg; silica; titanium dioxide; cellulose; magnesium stereate; methylhydroxypropyl cellulose; sodium lauryl sulphate and talc. Zantac: RANT, 300 mg; cellulose; magnesium stereate; metylhydroxypropyl cellulose; titanium dioxide and triacetin. FAMT ratiopharm: FAMT, 20 mg; silica; cellulose; talc and magnesium stereate. Fagastril: FAMT, 40 mg; excipient.
[b] Mean of five determinations.

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