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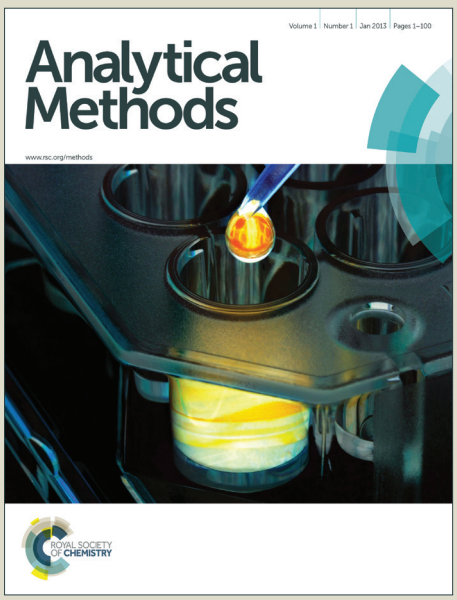

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Determination of Histamine H2 Receptor Antagonists in Pharmaceutical Formulations by CE-MS

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In memoriam of professor Juan José Berzas Nevado

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Abstract

A simple, fast and new method for the qualitative and quantitative determination of the histamine H2 receptor antagonists by using capillary zone electrophoresis (CZE) coupled to electrospray ionization (ESI) mass spectrometry (MS) has been developed. The optimized electrolyte for CZE separation includes 32 mM formic acid–ammonium formate buffer solution at pH 4.5. The high selectivity of the proposed method due to the MS detector allows the simultaneous determination of the nistamine H2 receptor antagonists cimetidine, ranitidine, roxatidine, famotidine and nizatidine in pharmaceutical formulations and also in biological fluids. The method has been applied for the analysis of seven different pharmaceutical preparations with recoveries obtained between 90.0 and 115.0 %.

1. INTRUDUCTION

Histamine H2 receptor antagonists inhibit interactions of histamine with H2 receptors via a competitive mechanism. These substances are highly selective and have little or no effect on receptors other than those of the H2 type. Although H2 receptors occur in many tissues including smooth vascular and bronchial muscle, H2 antagonists hinder to minimal extent physiological functions other than gastric acid secretion, their principal effect is to inhibit such secretion, either basal, diurnal, nocturnal or meal-stimulated. Peptic (gastric or duodenal) ulcers constitute losses of tissue or erosion in local areas of the stomach or duodenum caused by exposure to acid-pepsin gastric juice. Although stress, alcohol, and diet have been deemed major causal agents for ulcers, no definitive evidence of their being the actual origin of ulcers exists at present.

A literature search retrieved a large number of references including analytical methods for determining histamine H2 receptor antagonists, their impurities and metabolites and applications in pharmaceutical preparations and biological fluids. These compounds are usually separated by high-performance liquid chromatography (HPLC) [1-5] or capillary electrophoresis (CE) [6–12], and detected by UV–visible spectrophotometry. However, an increasing number of methods are relying on mass spectrometry for detection in this context. For example, cimetidine (CIM) in human plasma [13] was determined by liquid chromatography coupled with atmospheric-pressure chemical ionization and tandem mass spectrometry (LC/APCI-MS/MS). Also, CIM and structurally related impurities accompanying it were separated and the drug identified by liquid chromatography coupled to sector field inductively coupled plasma mass spectrometry (SF-ICP-MS)[14]. Ranitidine (RAN), its metabolites and impurities in pharmaceutical preparations and urine were determined by LC–MS [15–20]. The same hyphenated technique was used to quantify famotidine (FAM) in plasma and urine [21–23]. Similar compounds such as roxatidine (ROX) and nizatidine

(NIZ, as internal standard) have also been determined by mass spectrometry [24, 25]. Some methods allow the simultaneous separation of these compounds from others with similar properties. This is the case of the screening of 75 basic drugs containing CIN, FAN, RAM and NIZ by LC/MS-MS [26]. However, our literature search retrieved no reference to the simultaneous determination of several histamin H2 receptors antagonists by capillary electrophoresis - mass spectrometry (CE–MS) even though this hyphenated technique offers great selectivity and lower and lower limits of detection every day

In a previous work [27], we assessed the ability of the CE technique in combination with diode-array detection to resolve five histamin H2 receptor antagonists, and reported on the advantages and disadvantages of using aqueous and non-aqueous media for this purpose. Based on the results, we developed and validated a new method for the simultaneous detection and quantitation of the five analytes in pharmaceutical products and concluded that non-aqueous media showed better selectivity than aqueous media. Coupling capillary electrophoresis to mass spectrometry for detection (CE–MS) boosts selectivity and facilitates the use of an aqueous medium, and it is also a valuable technique for the unambiguous identification and/or confirmation of the analytes.

Thus, in the present work, asimple and rapid method has been developed for the determination of the histamine H2 receptor antagonists CIM, RAN, ROX, FAM and NIZ using CE-ESI-MS. Moreover, thanks to its simple sample preparation, this methodology could also be implemented as a screening tool.This method has been applied to the analysis of pharmaceutical formulations containing only one of the analytes in the presence of other compounds (i.e. excipients, impurities or degradation products) as matrix.

2. EXPERIMENTAL

2.1 Reagents and chemicals

RAN (ranitidine hydrochloride), FAM and NIZ were supplied by Sigma (Madrid, Spain); CIM was obtained from Tocris (Biogen Cientifica S.L., Madrid, Spain) and ROX (roxatidine acetate hydrochloride) from Zambón (Barcelona, Spain). Ammonium formate, formic acid and methanol were purchased from Panreac (Madrid, Spain). Ultrapure water (Milli-Q^{TM}, Millipore) was used throughout the work

2.2 Apparatus and methods

A capillary electrophoresis apparatus model P/ACE MDQ (Beckman, Fullerton, USA) equipped with a diode-array detection system and modified for its coupling to a mass spectrometer was used. Electrophoretic separations were carried out in coated fused-silica capillaries with a total length of 80 cm and 75 μm of i.d. at a voltage of 25 kV. Injection was done by using a pressure of 0.5 psi for 10 s (43 nL). The running buffer was an aqueous solution of 32 mM ammonium formate adjusted at pH 4.5 with formic acid. The capillary temperature was set at 25 ºC. Under these conditions, the capillary current was 62 μA. Prior to first use, the capillary was conditioned by rinsing 0.1 M NaOH for 10 min, water for 5 min and separation electrolyte for 10 min. At the beginning of each analytical sequence, the capillary was flushed with running electrolyte for 3 min in order to restore and re-equilibrate its wall surface.

The sheath liquid used was a methanol:water mixture (80:20; v:v) containing 0.05 % acetic acid at a flow rate of 5 μL/min. The ESI voltage was set at 3.5 kV. Other electrospray conditions were set at their optimum values (15 arbitrary units for the nebulization gas flow and 250 ºC for temperature). Mass spectrometry was implemented on a Thermo LCQ™ DECA XP spectrometer equipped with an ion-trap analyser. CE–MS data were acquired over the range of 50–400 m/z in the full scan mode, using the centroid mode, positive polarity and a maximum injection time of 80 ms. Other

acquisition conditions were 3 microscans per scan, an isolation width of 1 Th, q-value of 0.25 and an activation time (AT) of 80 ms. The chemical structures, precursor ions, product ions and normalized collision energies (%) used in the MS^2 and MS^3 tests are shown in Table 1. The data were subsequently processed with the Xcalibur 1.4 software.

2.3 Sample preparation

The solutions used to develop the proposed method were prepared by dissolving the analytes in an appropriate solvent which was selected on the grounds of recovery with respect to the nominal value for each drug in its pharmaceutical product. Thus, ROX was dissolved in ethanol, RAN and CIM in water, and FAM and NIZ in 50:50 ethanol/water.

The contents of ten tablets or capsules of each pharmaceutical preparation were weighed and ground. An amount of solid corresponding to the weight of one tablet or capsule was dissolved in 100 mL of the previously selected solvent (ethanol for roxatidine, water for ranitidine and cimetidine and ethanol/water for famotidine) and sonicated for 5 min. An aliquot of each solution was diluted with water to obtain a final concentration of 5 mg/L of the corresponding analyte.

3. RESULTS AND DISCUSSION

3.1 Optimization of CE separation conditions

Ammonium acetate and ammonium formate buffers were used to study the effect of pH on the separation efficiency; tests were conducted over the pH range 4–7.5, where the analytes are positively charged. Although resolution was poor in all cases, we chose pH 4.5 because short migration times and more efficient ionization of the samples were obtained. Poor resolution may be circumvented thanks to the selectivity or even specificity of the MS detector, which enables the unambiguous identification and quantitation of individual compounds based on their m/z ratio with virtually no

interferences. Buffer concentrations from 15 to 65 mM were studied and 32 mM was chosen as an effective compromise between peak shape, electrical current intensity and separation time. Ammonium formate was selected as separation electrolyte over ammonium acetate because it provided a lower baseline noise. The optimum separation voltage and capillary temperature were 25 kV and 25 ºC, respectively.

3.2 Optimization of ESI source conditions

The CE-ESI-MS interface conditions were optimized to obtain an efficient electrospray signal for detection. The paraemeters studied were sheath liquid composition and flow rate, sheath gas flow rate, electrospray voltage, temperature and CE capillary length protruding from the electrospray needle.

A sheath liquid is typically used to stabilize and enhance ESI signals. In order to assess the effect of its composition on the MS signal (abundance and stability), methanol:water mixtures in variable ratios and containing 0.05 % formic or acetic acid were used. The most stable conditions were obtained by using an 80:20 (v:v) methanol:water mixture. Increasing the flow rate reduced the response of the the studied histamin H2 receptor antagonists through dilution; however, a too low flow rate resulted in spray instability. A flow rate of 5 μL min^{-1} was thus selected as the best compromise. The flow rate of sheath gas was investigated over the range of 5–20 arbitrary units and the best response was obtained for 15 a.u., which was selected as optimal.

The electrospray voltage was studied from 3.0 to 4.5 kV. A voltage of 3.5 kV was adopted because voltages above 4.5 kV might cause discharges in the electrospray source.

The temperature of the MS heated capillary on signal intensity was investigated from 150 to 300 ºC. A temperature of 250 ºC provided the best results in terms of S/N ratios and reproducibility in peak areas.

The length of CE capillary protruding from the electrospray needle was examined

over the range of 0 to 0.5 mm. The best signal intensity was obtained with a length of 0.1 mm.

The distance between the CE capillary and MS heated capillary was also optimized in order to maximize transmission of ions, obtaining the best response when 0.8 cm was used.

Finally an injection time of 80 ms was selected on grounds of sensitivity and resolution between peaks.

Figure 1 shows the extracted ion electropherograms (MS^1) of the studied histamine H2 receptor antagonists obtained in the optimized conditions.

3.3 Study of parameters of validation for CE-ESI-MS method

Capillary Electrophoresis-Mass Spectrometry method was validated in terms of quality parameters such as precision, limits of detection (LODs), limits of quantitation (LOQs), and linearity.

The precision was measured as repeatability. Seven replicate injections of a standard solution of the five analytes at ¿concentración? under identical operating conditions over a short interval were carried out.). The relative standard deviations of corrected peak areas (Peak Area/t_m) ranged from 3.9 to 6.5 %, and those of migration times were all less than 0.6 % (see table 2).

LODs and LOQs were obtained as 3 and 10 times, respectively, the standard deviation of the blank signal divided by the slope of the calibration curve (see table 2).

The relationship between concentration and corrected peak area for the histamine H2 receptor antagonists was studied by measuring the corrected peak areas of the extracted ion electropherograms of each analyte at eight concentrations (LOQ, 0.5, 1, 5, 10, 15, 20, 40 and 60 mg L^{-1}). This relationship was linear from LOD to 20 mg L^{-1}. Table 3 shows the corresponding regression equations of corrected peak areas for

concentrations between the LOQ and 20 mg L^{-1} and their coefficients of determination.

3.4 Analysis of pharmaceutical preparations

To demonstrate the usefulness of this capillary electrophoretic procedure the method was used to determine the concentrations of the analytes in pharmaceutical preparations commercially available in Spain, which contain one of studied histamine H2 receptor antagonists and other components (excipients, impurities or degradation products...) in the pharmaceutical matrix. The pharmaceutical formulations analyzed were: Zarocs (ROX), Ratiopharm (RAN), Mabo and Ratiopharm (FAM), Tamaget and Fremet (CIM) and Distaxid (NIZ).

Each and every one of the active ingredients was successfully detected and quantified in the corresponding commercial formulations (Fig. 2). The recoveries of each analyte were between 90.0 % to 108.0 % so there seemed to be virtually no interference with the matrices. Moreover, the results were quite consistent with the nominal contents of the pharmaceutical formulations. Only roxatidine exhibited somewhat recoveries over 108 %.

The ion trap mass spectrometer was employed to generate tandem mass spectrometric (MS^n) data of the compounds under investigation. MS^2 and MS^3 tests (Table 1) were used to confirm the identities of the analytes in the presence of the different matrices. As an example, figure 3 shows the multistep fragmentation ion trap spectra used for the analysis of the commercial preparation Mabo (FAM). These fragmentation studies could be then utilized to identify the anlaytes in different biological fluids, thus providing relevant profiling information.

4. CONCLUSIONS

The experimental results obtained indicate that the procedure proposed is highly specific and enables the sensitive and accurate determination of the histamine H2

receptor antagonists in pharmaceutical formulations and it could be a valuable alternative to current official methods established by the European Pharmacopoeia.

Moroever, the information extracted from in the ion trap could be used for the unambiguous identification of the analytes in biological fluids such as urine, serum or plasma.

Acknowledgements

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Dedications

In memoriam of Professor Juan José Berzas Nevado

References

1. N. Helali, F. Darghouth, L.Monser, *Chromatograhia* 2004, **60**, 455.

2. K.M. Kalani, M. Aziz, M.A. Hegazt, L.A. Fattah, *J. AOAC.Int.,* 2002, **85***,* 1015.

3. T. Iqbal, Ch.S. Karyekar, M. Kinjo, G.C. Ngan, T.C. Dowling, *J. Chromatogr. B.,* 2004**, 799**, 337.

4. D.A.I. Ashiru, R. Patel, A.W. Basit, *J. Chromatogr. B*., 2007*,* **860**, 235.

5. D. Zendelovska, T. Stafilov, *J. Pharmac. and Biomed. Anal.,* 2003, **33***,* 165.

6. T. Perez-Ruiz, C. Martinez-Lozano, V. Tomas, E. Bravo, R. Galera, *J. Pharmac. and Biomed. Anal.,* 2002, **30**, 1055.

7. J.W. Luo, H.W. Chen, Q.H. He, *Chromatographia,* 2001, **53***,* 295.

8. S.M. Wu, Y.H. Ho, H.L. Wu, S.H. Chen, H.S. Ko, *Electrophoresi,s* 2001, **22***,* 2717.

9. S.M. Wu, Y.H. Ho, H.L.Wu, S.H. Chen, H.S. Ko, *Electrophoresis,* 2001, **22***,* 2758.

10. Y.S. Chang, Y.R. Ku, K.C. Wen, L.K. Ho*, J. Liq. Chromatogr. Relat. Technol.,* 2000, **23** (13), 2009.

11. M.A. Nelly, K.D. Altria, C. Grace, B.J. Clark, *J. Chromatogr. A.,* 1998, **798**, 297.

12. D.R. Ellis, M.E. Palmer, L.W. Tetler, C. Eckers, , *J. Chromatogr. A.,* 1998, **808**, 269.

13. K. Xu, V.K. Arora, A.K. Chaudhary, R.B. Cotton, I.A. Blair, *Biomed. Chromatogr*., 1999, **13**, 455.

14. E.H. Evans, J.C. Wolff, C. Eckers, *Anal. Chem.,* 2001, **73**, 4722.

15. L.E. Martin, J. Oxford, RJN. Tanner, *Xenobiotica,* 1981, **11***,* 831.

16. D.M. Jones, S.R. Plumb*, J. Sep. Sci ,* 2006, **29**, 2409.

17. E.W. Chung, E.N.M. Ho, D.K.K. Leung, F.P.W. Tang, K.C.H. Yiu, T.S.M. Wan, *Chromatographia,* 2004, **59 (1),** S29.

18. M.E. Abdel- Hamid, D. Sharma, *J. Liq. Chromatogr. Relat. Technol.* 2004, **27**, 641.

19. H. Kataoka, H.L. Lord, J. Pawliszyn, *J. Chromatogr. B. Biomed. Appl.* 1999, **731**, 353.

20. M.S. Lant, L.E. Martin, J. Oxford, *J. Chromatogr. A*., 1985, **323**, 143.

21. L. Zhong, R. Eisenhandler, K.C. Yeh, *J. Mass. Spectrom*., 2001, **36,** 736.

22. M..A. Campanero; I. Bueno; M.A. Arangoa, M. Escolar; E.G. Quetglas; A. Lopez-Ocariz; J.R. Azanza, *J. Chromatogr. B. Biomed. Appl.* 2001, **763,** 21.

23. B.S. Shin, J.W. Choi, J.P. Balthasar, D.K. Hong, J.J. Kim, S.D. Yoo, *Rapid Commun. Mass Spectrom.,* 2007, **21**, 329.

24. R. Ju-Hee, Ch. Sang-Jun, L. Heon-Woo, Ch. Seung-Ki, L. Kyung-Tae, *J. Chromatogr. B.*, 2008, **876**, 143.

25. S.A. Parekh, A. Pudage, S.S. Joshi, V.V. Vaidya, N.A. Gomes, *J. Chromatogr. B.,* 2008, **867***,* 172.

26. G.N.W. Leung, D.K.K. Leung, T.S.M. Wan, C.H.F. Wong, *J. Chromatogr. A.,* 2007, **1156**, 271.

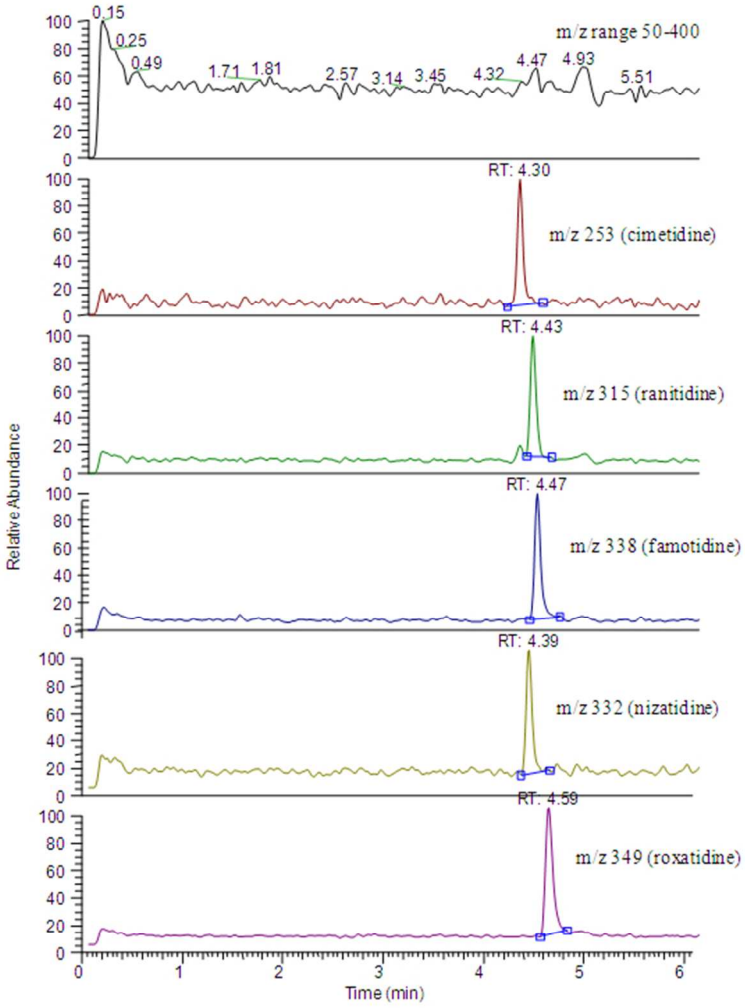
27. J.J. Berzas, G. Castañeda, R.M. Rodriguez, *Analytical Sciences*, **2011**, 27, 427.

Legends to figures

Figure 1. Base peak electropherogram and extracted ion electropherograms for a mixture of five pharmaceutical preparations containing 1 mg $L^{-1}$of of CIM, RAN, FAM, NIZ and ROX.

Figure 2. Extracted ion electropherograms of the pharmaceutical formulations analyzed: Zarocs (ROX), Ratiopharm (RAN), Mabo and Ratiopharm (FAM), Tamaget and Fremet (CIM) and Distaxid (NIZ).

Figure 3. MS^n fragmentations of FAM in Rathiopharm a) MS^2 and b) MS^3.



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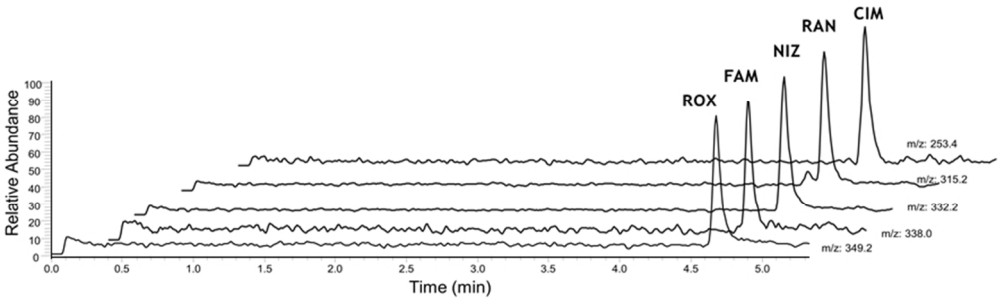

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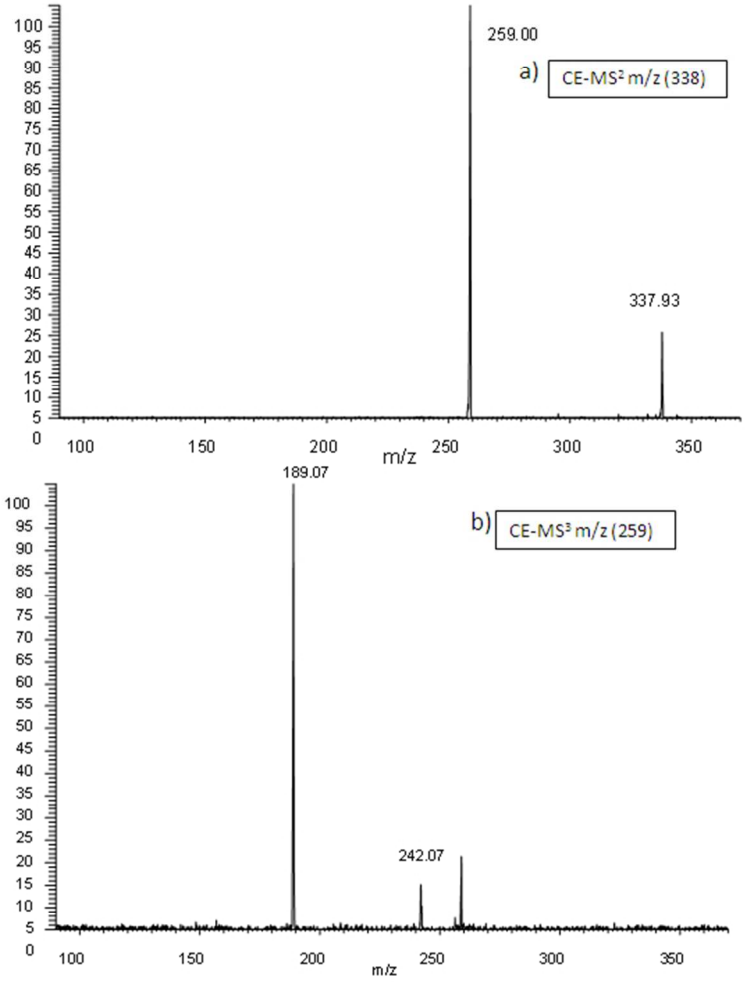


190x200mm (96 x 96 DPI)

Table 1. Mass spectrometry data for CE–MS of the studied antiulcers

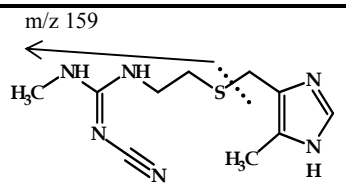
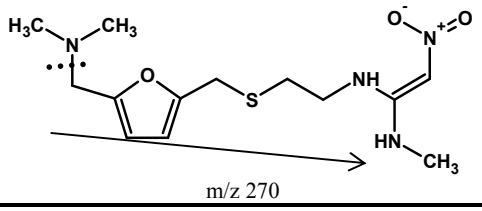
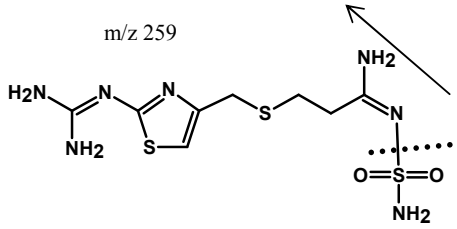
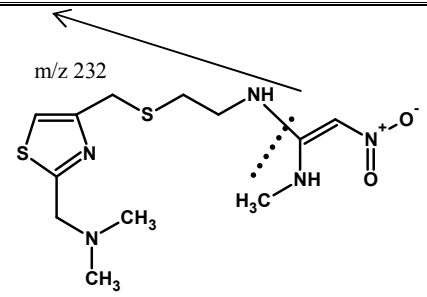
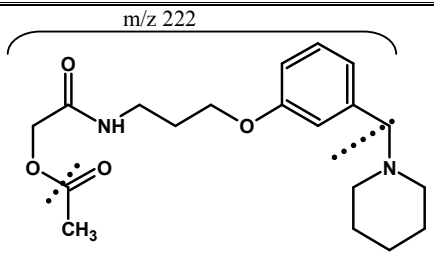
COMPOUNDS	Structure and fragmentation	$[M+H]^+$ m/z	Collision energy[1] %	Product ion MS[2]	Collision energy[2] %	Product ion MS[3]
CIM		253	26	159	27	117
RAN		315	26	270	23	224
FAM		338	20	259	20	189
NIZ		332	27	232	24	215
ROX		349	38	222	24	114

Table 2. LODs, LOQs and precision of the proposed method

Analyte	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)	Peak area RSD (%) ($n = 7$)	Migration time RSD (%) ($n = 7$)
CIMETIDINE	110	370	4.8	0.56
RANITIDINE	60	200	4.5	0.52
NIZATIDINE	40	130	3.9	0.38
FAMOTIDINE	66	220	6.5	0.50
ROXATIDINE	66	220	5.0	0.51

Table 3. Regression equations for the histamine H2 receptor antagonists

Analyte	Regression equation	R^2
CIMETIDINE	$Y = (433\,053.76 \pm 85\,864.16)X + (133\,014.72 \pm 806\,437.83)$	0.980
RANTIDINE	$Y = (903\,647.31 \pm 60\,769.50)X + (242\,368.58 \pm 570\,748.27)$	0.997
NIZATIDINE	$Y = (951\,197.95 \pm 114\,939.06)X + (629\,143.09 \pm 107\,9509.70)$	0.991
FAMOTIDINE	$Y = (212\,002.72 \pm 21\,705.06)X + (–42\,795.79 \pm 203\,854.38)$	0.994
ROXATIDINE	$Y = (269\,0598.81 \pm 195\,592.82)X + (–31\,8768.00 \pm 994\,489.04)$	0.998