Determination of ebrotidine and its metabolites by capillary electrophoresis with UV and mass spectrometry detection

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Abstract

This study describes the application of capillary electrophoresis (CE) to the analysis of ebrotidine and its metabolites as an alternative analytical technique to liquid chromatography. Comparison between UV–diode array spectroscopy and mass spectrometry (MS) using an ion-trap system with electrospray ionization as detection systems has been performed. The quality parameters of the UV detection method were established, obtaining linear calibration curves over the range studied (8–200 mg ml^{-1}), limits of detection between 3.4 and 4.3 μg ml^{-1}, and run-to-run and day-to-day precision lower than 14%. For these compounds the protonated species [M+H]$^+$ and, in some cases, sodium adducts were observed in the MS spectra. Using MS coupled to CE, limits of detection were between 0.5 and 2.6 μg ml^{-1}. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Ebrotidine, synthesized and developed by the Centre d'Investigació Grupo Ferrer, is a new H$_2$-receptor antagonist that shows antisecretory activity comparable to that of ranitidine [1,2]. Ebrotidine, unlike other H$_2$-receptor antagonists, shows cytoprotective activity in animals [3,4] and humans [5,6] and acts as a potent inhibitor of protease and lipase produced by *Helicobacter pylori* [7–9]. Ebrotidine is mainly metabolized by oxidation to the sulfoxide and sulfone as well as to the sulfoxide and sulfone resulting from the removal of the thiazolyl rings. These compounds, and also a metabolite formed by

hydrolysis, 4-bromobenzenesulfonamide, have been found in human urine after oral ebrotidine administration [10,11].

The analysis of ebrotidine and its metabolites is based on liquid chromatography (LC). Reversed-phase LC with a C$_{18}$ column, a phosphate buffer–acetonitrile as mobile phase and UV detection is used for the separation of these compounds [10]. A method based on reversed-phase ion-pair LC using 1-hexanesulfonic acid has been used by Rozman et al. for the determination of ebrotidine and its metabolites in human urine [12]. In order to obtain structural information and unequivocally identify ebrotidine metabolites, LC has been coupled to mass spectrometry (MS) [11,13].

Capillary electrophoresis (CE) has been used for the determination of several H$_2$-receptor antagonists, such as cimetidine [14–16], ranitidine [17,18] and

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famotidine [19], as an alternative to LC, because of its high separation efficiency, short analysis time and relatively low cost. In spite of its lack of selectivity, UV detection is widely used in CE, but neither UV detection nor induced fluorescence detection or electrochemical detection can provide sufficient structural information to identify the analytes unequivocally. This kind of information can be obtained by coupling the capillary electrophoresis to mass spectrometry. It is thus possible to combine the high resolution provided by CE with the structural information from MS using an electrospray ionization (ESI) interface. When this interface is used the flow-rate of the carrier electrolyte from the electrophoresis must be increased. A coaxial sheath-flow [20] can be used not only to achieve a high flow appropriate to the ESI source but also to close the electrical circuit in the CE system, and to minimize the surface tension of the carrier electrolyte.

This paper reports the study of two methods, based on CE, for the determination of ebrotidine and its potential metabolites. Experimental conditions to determine these compounds by CZE (capillary zone electrophoresis) with UV detection have been established. Furthermore, different sheath liquid compositions have been tested to couple CE to MS using an electrospray interface. Finally, a spiked human urine sample was extracted using the procedure proposed by Rozman et al. [12] and analyzed by CE–UV using the coupling CE–ESI-MS for peak confirmation. Figures of merit such as run-to-run and day-to-day precision and detection limits were established.

2. Experimental

2.1. Chemicals

Ebrotidine (E) {N-[(4-bromophenyl)sulfonyl]-N'-[2-([{2-[(diaminomethylene)amino]-4-thiazolyl}methyl]thio)ethyl]formamide} and the related compounds ebrotidine sulfoxide (ESO), ebrotidine sulfone (ESO$_2$), 2-N[4-{[(2-aminoethyl)thio]methyl}-2-thiazolyl]guanidine (ATG) and its sulfoxide (ATGSO), N-[2({[2-[(diaminomethylene)amino]-4-thiazolyl}-methyl]thio)ethyl]formamide (DTF), 4-bromobenzenesulfonamide (BrBzSA), N-[(4-bromophenyl)sulfonyl]acetamide (PSA), N-(2-methylsulfinylethyl-

amino-methylene) - 4 - bromobenzenesulfonamide (DTESO) and N-(2-methylsulfonylethylaminomethylene)-4- bromobenzenesulfonamide (DTESO$_2$) were a gift from Grupo Ferrer (Spain) and their structures are shown in Fig. 1. Reagents used for the preparation of buffer solution were analytical grade: 85% (w/w) formic acid (Carlo Erba, Milan, Italy), 85% (w/w) phosphoric acid (Probus, Barcelona, Spain), 98% diethylmalonic acid (Aldrich, Milwaukee, WI, USA), acetic acid, methanol (MeOH) and acetonitrile (ACN) (Merck, Darmstadt, Germany).

2.2. Instrumentation

2.2.1. Capillary electrophoresis instrumentation

A Beckman (Fullerton, CA, USA) P/ACE System 5500 with diode array detection–capillary electrophoresis was used; electrophoretic data were processed using the P/ACE Station software. A fused-silica capillary (Supelco, Bellefonte, PA, USA) of 50 cm effective length×75 μm I.D. was used. The temperature was held at 25°C. Hydrodynamic (3.4 kPa) injection mode was applied for 4 s. Direct detection was performed at 235 nm. CE potential was set at 20 kV and the optimum electrolyte was 50 mM acetic acid, pH 5.7 [adjusted with 25% NH$_3$ (1:10)] with 30% of methanol.

2.2.2. Mass spectrometry instrumentation

An LCQ-Finnigan (San Jose, CA, USA) ion trap mass spectrometer equipped with a pneumatically-assisted electrospray interface with a coaxial coupling (Finnigan) was used. The CE capillary was inserted into the atmospheric region of the electrospray ionization source through a sheath liquid flow device. The sheath liquid was methanol–trichloracetic acid (TCA) (7.5 mM) (85:15) and it was introduced at a flow-rate of 5 μl min^{-1} by a Unimetrics syringe pump (500 μl). Additionally, a third concentric tube delivers a gas flow (11 l h^{-1}) which assists spray formation via nebulization. A voltage of 4.0 kV was applied to the ESI needle. Full scan mode, from m/z 150 to 600 was used with 1 microscan and 1000 ms as maximum injection time. MS spectra were obtained by infusion of each analyte, i.e., the CE capillary was filled with the analyte, diluted in the carrier electrolyte, and then a

ebrotidine

ESO

ESO$_2$
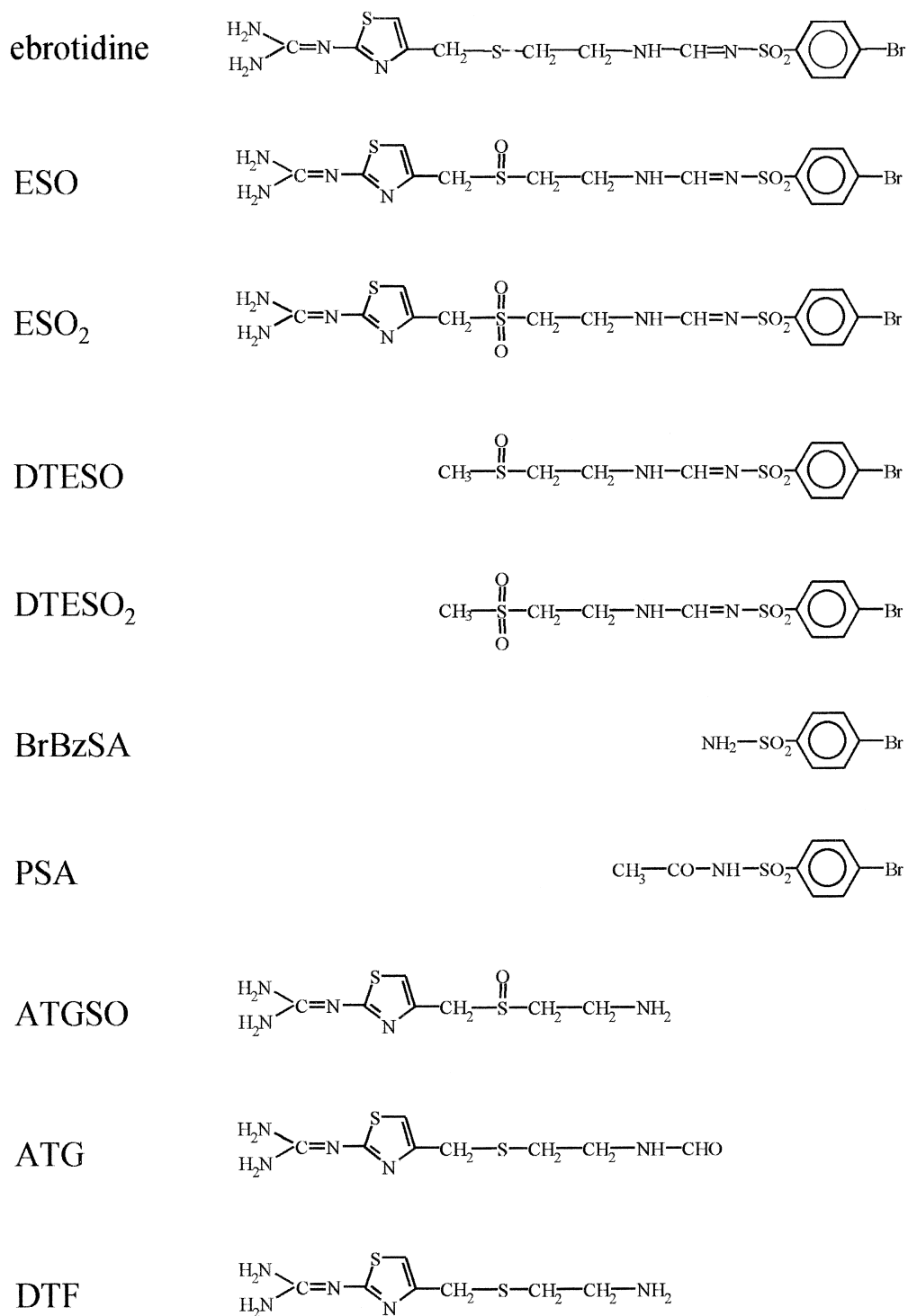
DTESO

DTESO$_2$

BrBzSA

PSA

ATGSO

ATG

DTF

Fig. 1. Structures of ebrotidine and its related substances.

voltage of 20 kV and a pressure of 3.4 kPa were applied.

When CE is coupled to MS, because of physical limitations, the capillary must be longer than in UV detection (86.5 cm total length). Moreover, in order to prevent hydrodynamic flow, a difference in height between the CE electrode vial and the electrospray was avoided. The sensitivity is highly affected by position of the capillary in the needle of the electro-spray, and a distance between 0.1 and 0.2 mm is needed. The position was varied until the maximum sensitivity was obtained and was then maintained throughout the study.

With MS the injection time can be increased because the difference in the molecular masses of the compounds removes the resolution problems observed with UV detection. So, hydrodynamic injection mode was applied for 10 s.

2.3. Capillary conditioning

For UV detection, new capillaries were pre-treated with 1 M NaOH for 30 min and, at the beginning of each session, the capillary was treated with 0.1 M NaOH for 10 min, then rinsed with ultrapure water for 10 min and the selected buffer was passed for 15 min. For CE–MS, capillaries were also treated with 1 M HCl to remove sodium ion traces, then capillaries were activated for 15 min with NH_3 (1:10) and rinsed with the buffer for 15 min. In both CE–UV and CE–MS the capillary was rinsed with the buffer for 2 min before each run.

2.4. Procedure

Carrier electrolytes were prepared by diluting a stock solution of the corresponding acid (acetic acid, formic acid, phosphoric acid and diethylmalonic acid) to 50 mM and setting the appropriate pH with 1 M NaOH, 1 M KOH or 25% NH_3 (1:10). Then other additives, such as organic solvents (methanol or acetonitrile), were added in the appropriate proportion.

Stock solutions (1000 μg ml^{-1}) of each compound were prepared in methanol (ebrotidine, ATGSO, DTF and ATG), acetonitrile (ESO_2, DTESO, $DTESO_2$, PSA and BrBzSA) and acetonitrile–dimethyl sulfoxide (90:10, v/v) (ESO). For the

study of the separation, standard solutions of 10 μg ml^{-1} of ESO and PSA and 100 μg ml^{-1} of the other compounds, prepared by dilution of the stock solutions with Milli-Q ultrapure water, were used and the signal of the organic solvent was used as electro-osmotic flow (EOF) marker. For quantification, standard solutions between 8 and 200 μg ml^{-1} of each compound were prepared diluting stock solutions with Milli-Q ultrapure water.

A liquid–liquid extraction procedure [12] was used for the separation of ebrotidine, ESO, ESO_2, DTESO and $DTESO_2$ in urine spiked at 5 μg ml^{-1} concentration level of each analyte. Briefly, after addition of 100 μl of 1 M NaOH to 1 ml of urine, the sample was shaken for a few seconds. Then, 5 ml of CH_2Cl_2–2-PrOH (9:1) was added to the mixture and mechanically shaken for 15 min; finally centrifugation at 4500 rpm (2000 g) was applied for 5 min. An aliquot of 3 ml of the organic layer was evaporated to dryness at 40°C using nitrogen and the residue was dissolved with 150 μl of Milli-Q ultrapure water.

3. Results and discussion

3.1. CE–UV

3.1.1. Influence of carrier electrolyte

In a preliminary study, four buffers were tested in order to separate the ebrotidine and nine of its expected metabolites. These buffers were chosen to cover a wide pH range (between 3 and 8) allowing a different degree of ionization for the compounds and thus a variety of effective mobilities. The selected buffers were based on phosphoric acid (pH: 3.0, 6.1 and 8.1), formic acid (pH: 3.0, 3.8 and 4.8), acetic acid (pH: 3.8, 4.7 and 5.7), and diethylmalonic acid (pH: 6.3, 7.3 and 8.2).

Table 1 shows the influence of pH on the effective mobility of the studied compounds. The effective mobility of ebrotidine and most of its metabolites increased at low pH due to the ionization of the amino group. In contrast, effective mobility of compounds BrBzSA, DTESO and $DTESO_2$ was zero in the pH range studied, probably due to the attractive effect of the substituents near the amine group,

Table 1
Effective mobilities for ebrotidine and related compounds at different pH

Acid	pH	Effective mobility ($cm^2 kV^{-1} s^{-1}$)						
		ATG	ATGSO	DTF	E	ESO	ESO$_2$	PSA
Formic	3.01	0.430	0.419	0.209	0.155	0.155	–	−0.017
Acetic	3.79	0.462	0.450	0.251	0.191	0.191	0.196	−0.060
Formic	3.85	0.400	0.388	0.194	0.161	0.161	0.171	–
Acetic	4.70	0.420	0.402	0.223	0.173	0.173	0.167	−0.174
Formic	4.75	0.385	0.371	0.203	0.158	0.158	0.161	−0.175
Acetic	5.72	0.384	0.357	0.200	0.155	0.138	0.141	−0.234
Phosphoric	6.07	0.275	0.240	0.132	0.111	0.111	–	–
Diethylmalonic	6.27	0.299	0.266	0.134	0.100	0.079	0.078	−0.240
Diethylmalonic	7.28	0.223	0.160	0.030	0.027	0.027	0.000	−0.260

which prevents their ionization. Finally, the effective mobility of PSA increased in the counterelectroosmotic direction until pH 6, when it became practically constant, raising the electrophoretic mobility corresponding to the ionized form. This behavior could be explained by the attractive effect of the substituents linked to the amine group. These groups may provide the low electronic density on the nitrogen necessary for dissociating the proton. Thus, a high effective mobility to the anode occurred as the dissociation degree increased at high pH. pH higher than 7.0 was not used because the increase in electroosmotic flow prevented peak differentiation. The optimum separation was obtained using acetic acid–acetate buffer at pH 5.7 or diethylmalonic–diethylmalonate buffer at pH 6.2 as carrier electrolytes. However, at these conditions the pairs ebrotidine/ESO and ESO/ESO$_2$ were poorly discriminated. Moreover, apparent mobility of BrBzSA, DTESO and DTESPO$_2$ coincided with that of the EOF marker, thus these compounds were masked by the unavoidable EOF signal produced by the sample solvent.

3.1.2. Influence of buffer cation

In this work the effect of using Na$^+$, K$^+$ and NH$_4^+$ as counterions in both (acetic acid–acetate at pH 5.7 and diethylmalonic–diethylmalonate at pH 6.2) carrier electrolytes was studied. Using NH$_4^+$ or K$^+$, the migration times were higher than those obtained with Na$^+$. In addition, buffers containing ammonium or potassium ions provided high resolution allowing the complete separation between ebrotidine and ESO (resolution higher than 2.0), conversely the pair

ESO/ESO$_2$ could not be resolved. Resolutions using K$^+$ or NH$_4^+$ were very similar, but with K$^+$ migration times were longer, for that reason NH$_4^+$ was chosen as optimum counterion. When comparing the electropherograms obtained with acetic acid–acetate buffer at pH 5.7 and diethylmalonic–diethylmalonate buffer at pH 6.2 using ammonia as counterion the best efficiency, in terms of width, shape and migration times, corresponded to the acetic acid–acetate buffer at pH 5.7, so this buffer was used in the remainder of this study.

3.1.3. Influence of the addition of organic solvents

Under the optimum conditions described, an acceptable separation for all the compounds except for the pair ESO/ESO$_2$ and the non-ionised BrBzSA, DTESO and DTESO$_2$ was obtained. In order to improve the separation efficiency, the presence of an organic modifier in the carrier electrolyte such as methanol or acetonitrile at concentrations ranging between 0 and 30% (v/v) was studied.

The migration time of the electroosmotic marker increased with the addition of an organic solvent because this addition causes changes in viscosity and dielectric constant and, consequently, in the ζ potential [21]. Moreover, most of the compounds, except BrBzSA, DTESO and DTESO$_2$ that comigrated in all conditions with the EOF marker, showed lower effective mobility with the addition of an organic solvent. This could be due to the variation of the dielectric constant and to its effect on the ionization equilibrium of the amines.

In Table 2, resolutions between the pairs eb-

Table 2
Resolutions obtained with different contents of methanol and acetonitrile[a]

Organic solvent (%)	E/ESO		ESO/ESO$_2$	
	MeOH	ACN	MeOH	ACN
0	2.1		0.7	
10	2.7	2.4	1.2	1.2
20	3.1	4.1	1.5	1.7
25	4.6	4.2	1.9	2.1
30	5.4	4.7	2.3	2.3

[a] Buffer: acetic acid–acetate at pH 5.7 (NH$_4^+$).

rotidine/ESO and ESO/ESO$_2$ using acetic acid–acetate buffer at pH 5.7 (NH$_4^+$) are shown. As the concentration of the organic solvent increased, the resolution also increased. However, the analysis time was unavoidably longer, so a compromise between resolution and analysis time had to be established. Furthermore, although acetonitrile seemed to provide the best results, a decrease in sensitivity was also observed; therefore, 30% (v/v) methanol was selected as the optimum modifier.

Comigration of compounds BrBzSA, DTESO and DTESO$_2$ with the EOF marker could not be solved by changing pH, organic modifier or counterion of the carrier electrolyte, so a modified-silica capillary, based on bonded polymer containing sulfonic acid as functional group (NaAMPS), was used. Acetic acid (50 mM) adjusted with NH$_3$ (1:10) at three pH values (3.8–5.2) was tested as carrier electrolyte. Unfortunately, none of these conditions allowed the separation between BrBzSA, DTESO and DTESO$_2$ and the EOF signal.

The optimum conditions using a silica capillary were: 50 mM acetic acid–acetate buffer at pH 5.7 with a 30% (v/v) of MeOH and an applied voltage of 20 kV. Under these conditions ebrotidine and six metabolites were well resolved, but, because of the addition of methanol, analysis times increased, so it was necessary to apply a pressure gradient (3.4 kPa) to detect the anionic compound PSA. Fig. 2 shows the electropherogram obtained for the separation of a standard mixture. Compounds BrBzSA, DTESO and DTESO$_2$ comigrated with the EOF marker, so these compounds could not be determined using the method proposed.
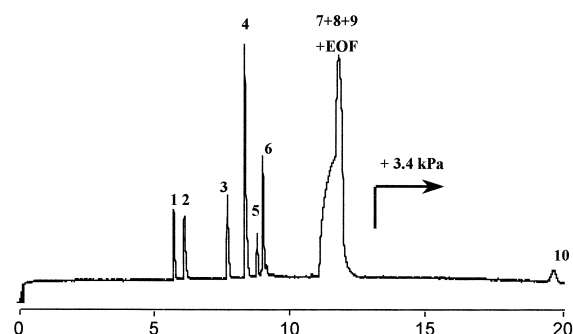

Fig. 2. Electropherogram obtained for ebrotidine and charged metabolites under the optimum conditions: 50 mM acetic acid–acetate, pH 5.7, 30% (v/v) MeOH, 20 kV. Capillary: fused-silica, 57 cm (effective length 50 cm)×75 μm I.D. Peaks: 1=ATG, 2=ATGSO, 3=DTF, 4=E, 5=ESO, 6=ESO$_2$, 7=BrBzSA, 8=DTESO, 9=DTESO$_2$, 10=PSA and EOF=electroosmotic flow marker. Time scale in min.

3.2. CE–MS

3.2.1. CE–ESI-MS

The CE system was coupled to the LCQ mass spectrometer with an ion trap analyzer to confirm the identity of the compounds. Some changes to the UV method are necessary when coupling CE to MS. First the capillary must be longer than in UV detection because of physical limitations, which causes longer analysis times. Electrophoretic currents above 20–25 μA cause an important Joule effect and, when coupling CE–MS, part of the capillary is outside of the thermostated section, so a radial temperature gradient in the capillary can occur, causing losses of efficiency. For the CE–UV optimum conditions, the current was about 40 μA, so it was necessary to modify buffer conditions to lower it. To decrease the electrophoretic current, electrolyte concentration had to be decreased to 10–15 mM. Although the addition of methanol improved the resolution, in our case, for CE–MS, this was prevented since analysis time increased greatly. The best conditions for the coupling were 15 mM acetic acid–acetate buffer (pH 5.7) as electrolyte and a voltage of 20 kV. The optimum sheath liquid was, as indicated in the Experimental section, methanol–7.5 mM TCA (85:15) and was introduced at a flow-rate of 5 μl min^{-1}. Acetic acid, formic acid and TCA were tested as sheath liquid and the best responses were obtained with TCA. This behavior is related to the acidity of

these acids, that helps the protonation of amine groups.

Fig. 3 shows the CE–MS electropherogram obtained under the optimum conditions for ebrotidine and compounds that give a signal in positive mode ESO, ESO_2, DTESO, $DTESO_2$, DTF, ATG and ATGSO. It was possible to solve all these compounds because of their different masses.

3.2.2. MS spectra

Mass spectra were obtained for ebrotidine (Fig. 4)

and its metabolites by infusing a solution of each one (ca. 30 ppm) in the optimum buffer (15 mM acetic acid–acetate buffer, pH 5.7) and by applying 20 kV. Mass spectral data for these compounds are given in Table 3. In all the compounds, the base peak (in positive mode) was the ion $[M+H]^+$, except in ATG, where the base peak was the ion at m/z 155, which corresponded to the rupture of the thioether (in Fig. 4, fragment a). The spectrum of the compounds ESO_2, DTESO and $DTESO_2$ only shows peaks corresponding to $[M+H]^+$ and/or $[M+Na]^+$,
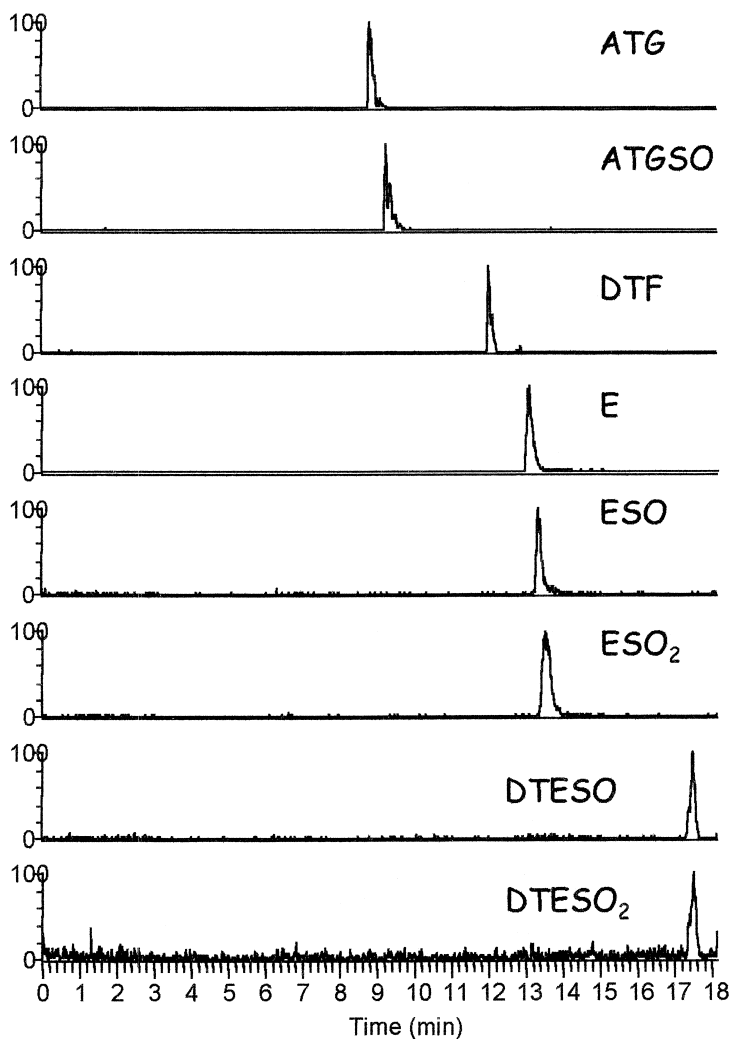

Fig. 3. CE–MS ion electropherograms of a standard solution containing ebrotidine and metabolites ATG, ATGSO, DTF, ESO, ESO_2, DTESO and $DTESO_2$. Sheath liquid: MeOH–7.5 mM TCA (85:15), 5 μl min^{-1}. Sheath gas: 11 l h^{-1}. Electrolyte running solution: 15 mM acetic acid, pH 5.7. Applied voltage: 20 kV.
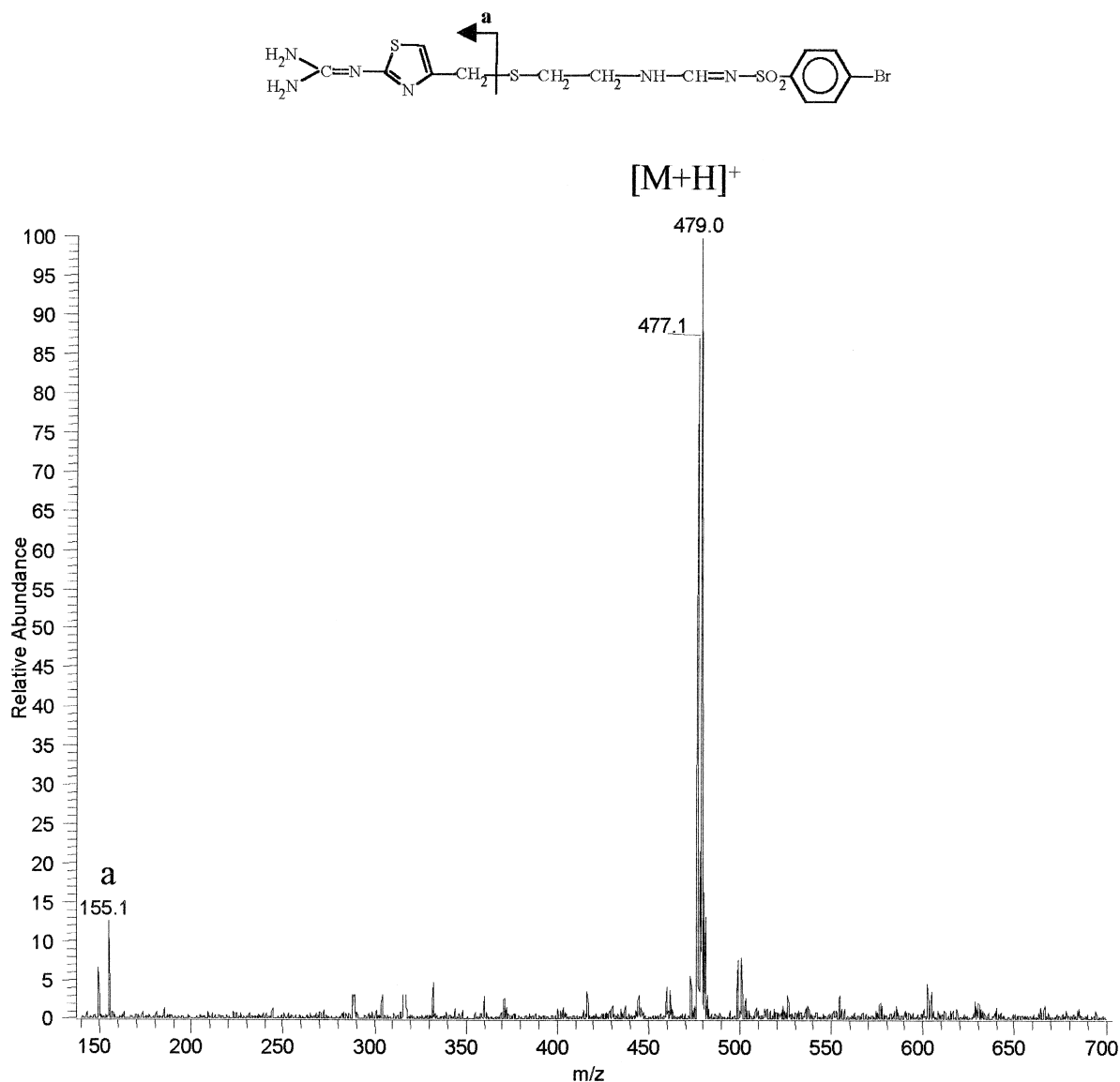
Fig. 4. CE–MS spectra of ebrotidine. Electrolyte running solution: 15 mM acetic acid–acetate buffer (pH 5.7). Sheath liquid: methanol–7.5 mM TCA (85:15).

while the remaining compounds showed an extra peak at m/z 155. This spectra agree with those obtained by Rozman et al. [13] using a LC–MS system.

3.3. Quality parameters

Under the optimum conditions, the quality param-

eters for CE–UV and CE–MS methods were determined. Standard curves based on heights were constructed for each compound and linearity over the range (8 to 200 μg ml^{-1} for UV and 8 to 80 μg ml^{-1} for MS) with correlation coefficients around 0.99 was obtained. The limits of detection for each compound, based on a signal-to-noise ratio of 3 are given in Table 4, showing that the limits of detection

Table 3
Mass spectral data of ebrotidine and its metabolites under optimal conditions

Compound	M_r	ESI-MS spectra		
		m/z	Relative abundance (%)	Fragment
Ebrotidine	476	477/479	80/100	$[M+H]^+$
		155	12	$[a]^+$
ESO	492	515/517	11/13	$[M+Na]^+$
		493/495	85/100	$[M+H]^+$
		155	18	$[a]^+$
		156	14	$[a+H]^{+\cdot}$
ESO_2	508	509/511	90/100	$[M+H]^+$
DTESO	352	375/377	34/40	$[M+Na]^+$
		353/355	84/100	$[M+H]^+$
$DTESO_2$	368	369/371	80/100	$[M+H]^+$
ATG	231	232	75	$[M+H]^+$
		155	100	$[a]^+$
DTF	259	260	100	$[M+H]^+$
		155	65	$[a]^+$
ATGSO	247	270	30	$[M+Na]^+$
		248	100	$[M+H]^+$
		155	16	$[a]^+$

obtained in CE–MS are between 4- and 8-times lower than those obtained using UV. Nevertheless, it must be mentioned that injection time in CE–MS was higher (10 s) than in CE–UV (4 s) because of the selectivity of MS detection.

Run-to-run precision was evaluated by injecting 10 replicates of a standard solution of ebrotidine and its metabolites the same day, and day-to-day precision was evaluated by determining five replicates of a standard solution on 3 different days (Table 4). For

Table 4
Precision (RSD) for migration times and for concentration and limits of detection

	RSD (%)							
	Migration time			Concentration				
	Run-to-run		Day-to-day, $n=15$, UV	Run-to-run		Day-to-day, $n=15$, UV	LOD (mg l^{-1})	
	$n=10$ UV	$n=5$ MS		$n=10$ UV	$n=5$ MS		UV	MS
Ebrotidine	7.3	0.4	8.8	4.8	28.4	6.3	4.3	0.7
ESO	8.2	2.3	9.9	5.1	28.9	6.7	3.4	1.1
ESO_2	8.8	2.4	10.5	5.5	29.2	8.0	3.5	0.8
DTESO	–	1.5	–	–	69.5	–	–	0.8
$DTESO_2$	–	1.7	–	–	50.0	–	–	2.6
BrBzSA	–	–	–	–	–	–	–	–
PSA	0.3	–	2.9	7.8	–	12.8	4.0	–
ATGSO	3.7	1.1	5.2	5.9	76.5	10.9	3.9	1.6
DTF	6.1	2.4	7.8	4.3	a	13.9	3.5	0.5
ATG	3.3	2.5	4.6	4.9	65.0	10.4	3.8	0.5

[a] Compound DTF is used as internal standard.
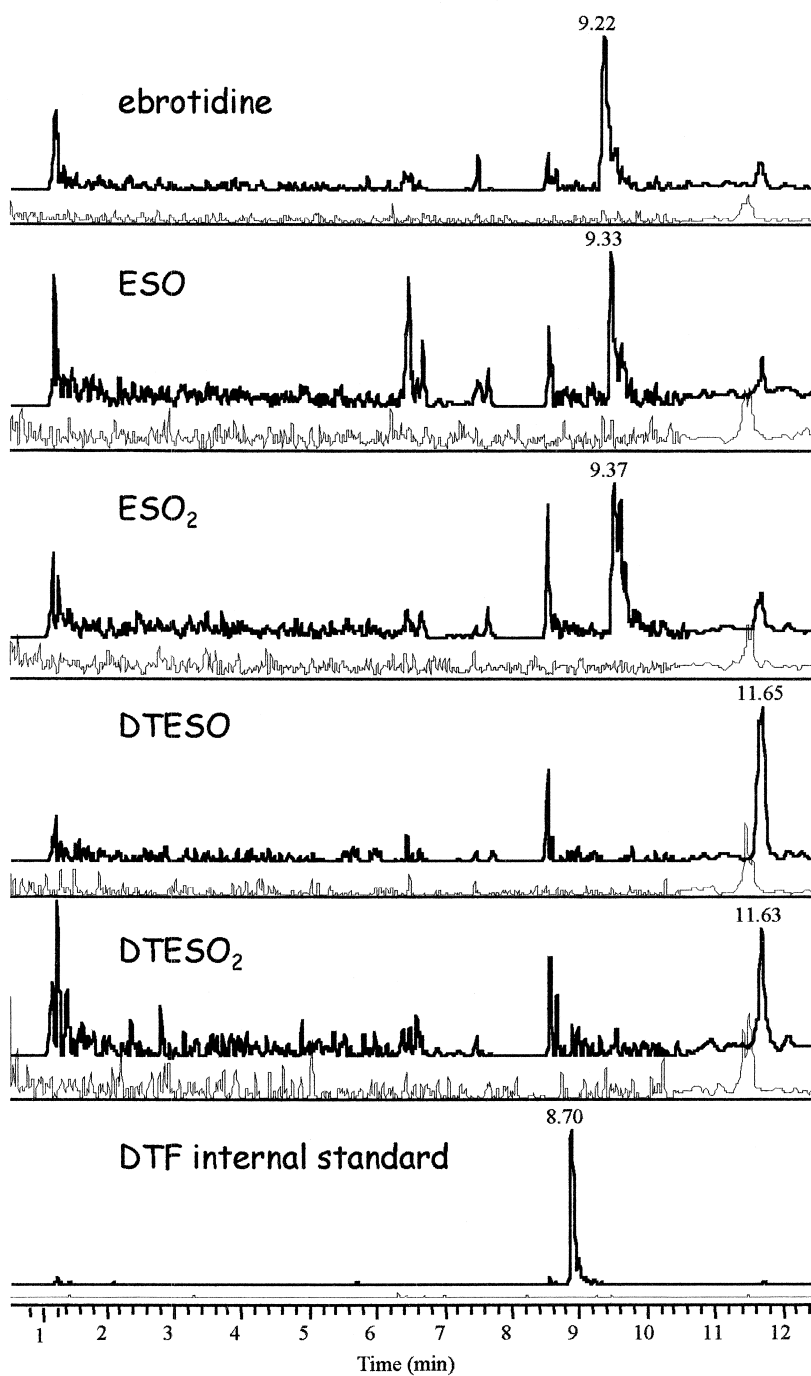
Fig. 5. CE–MS ion electropherogram of spiked human urine samples (5 µg ml^{-1}) (above) and the corresponding blanks (below). Conditions as in Fig. 3.

the CE–UV method run-to-run precision were up to 8.8% and 7.8% for the migration times and the concentration, respectively and day-to-day precision were up to 10.5% for both, migration times and concentrations.

The run-to-run precision obtained by the CE–MS method (five replicates) were up to 2.5% for the migration times and higher than 25% for concentrations. These values are higher than those obtained by CE–UV but they are in agreement some data found in the literature [22].

3.4. Application

To show the applicability of the method, a sample of human urine spiked with ebrotidine, ESO, ESO_2, DTESO and $DTESO_2$ at 5.0 µg ml^{-1} was analyzed. Extraction and pre-concentration of the human urine sample was performed by the method propose by Rozman et al. [12], as described in the Experimental section. Ebrotidine, ESO and ESO_2 were identified by CE–MS and quantified in human urine by CE–UV. Recoveries for Ebrotidine, ESO and ESO_2 were 87%, 98% and 97%, respectively, in agreement with the values previously reported [12]. These values were applied to the quantitation of the analytes and the results were in agreement with the spiked level in the original sample. The internal standard used was DTF, which migrates at 7.7 min where no overlap with matrix peaks was observed.

The CE–MS electropherogram allowed the reliable confirmation of ebrotidine, ESO and ESO_2 in the human urine sample, but quantification of the analytes could not be performed because of the poor run-to-run precision. Fig. 5 shows the CE–MS electropherogram for the urine sample and its blank, where it can be seen that a false positive signal appear at migration time of EOF preventing the analysis of DTESO and $DTESO_2$.

4. Conclusions

Ebrotidine and charged metabolites can be determined by CZE using UV detection. Furthermore, the coupling to mass spectrometry allows the con-

firmation of the structures. Only three of the five compounds found in human urine can be identified with the coupling CE–MS and quantified by UV detection.

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