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Determination of ebrotidine and its metabolites in human urine by reversed-phase ion-pair high-performance liquid chromatography

Elena Rozman[a,*], M.Teresa Galcerán[b], Carlos Albet[a]

[a]*Centro de Investigación Grupo Ferrer, Juan de Sada 32, 08028 Barcelona, Spain*
[b]*Departament de Quimica Analitica, Universitat de Barcelona, Av. Diagonal 647, 08028 Barcelona, Spain*

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Abstract

Ebrotidine is a new H_2-receptor antagonist with powerful antisecretory activity, demonstrated gastroprotection and the ability to inhibit protease and lipase activities of *Helicobacter pylori*. As a tool in the clinical pharmacokinetic study of ebrotidine, an analytical method for the simultaneous determination of ebrotidine an its metabolites in human urine was developed. An ion-pair reversed-phase HPLC separation using 1-hexanesulfonic acid and acetonitrile as mobile phase with gradient elution was optimized. In addition, several procedures of preconcentration and clean-up were tested, including solid-phase and liquid–liquid extraction, the mixture dichloromethane–2-propanol (9:1, v/v) at pH 11 being the most efficient. The quality parameters of the whole analytical method were established, the calibration curves were linear over the range studied (1–200 μg/ml) and the reproducibility of the method was high (inter-day R.S.D. values lower than 4.4%).The limits of detection were between 26 and 110 ng/ml of urine for ebrotidine and its metabolites. The method was applied to the analysis of urine collected from two volunteers during 96 h following oral administration of ebrotidine at a dose of 400 mg.

Keywords: Ebrotidine; Ebrotidine sulfoxide; Ebrotidine sulfone; Desthiazolylebrotidine sulfoxide; Desthiazolylebrotidine sulfone; 4-Bromobenzenesulfonamide

1. Introduction

Ebrotidine is a novel H_2-receptor antagonist with potent antisecretory activity similar to that of ranitidine and about 10 times greater than that of cimetidine [1,2]. Besides its powerful antisecretory activity, ebrotidine shows unique cytoprotective action against mucosal damage in experimental animal models [2–4] and in humans [5,6]. In addition, ebrotidine can inhibit the protease and lipase activities of *Helicobacter pylori* [7] and inhibit the binding of the lipopolysaccharide of this bacterium to gastric mucosal laminin receptors [8].

The pharmacokinetics of ebrotidine shows some similar characteristics to that of the other H_2-receptor antagonists [9]. The product is rapidly absorbed, the peak plasma concentrations being attained within 2 to 2.5 h after dosing. In addition, the pharmacokinetic behavior of ebrotidine is linear with the dose, and the elimination of the drug can be mainly attributed to renal excretion [10]. However, ebrotidine shows a slower elimination than the other H_2-receptor antagonists, probably due to its high lipophilicity.

*Corresponding author.

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Like all structurally related H_2-receptor antagonists [11–14], ebrotidine (E) is mainly metabolized by oxidation of its thioether moiety, and the sulfoxide metabolite (ESO) was found in human urine. Other oxidized metabolites present in the urine samples are ebrotidine sulfone (ESO_2) and two derivatives lacking the thiazolyl ring: N-(2-methylsulfinilethylaminomethylene) - 4 - bromobenzenesulfonamide (desthiazolylebrotidine sulfoxide, DTESO) and N-(2-methyl-sulfonilethylaminomethylene)-4-bromobenzenesulfonamide (desthiazolylebrotidine sulfone, $DTESO_2$). Finally, a metabolite formed by hydrolysis, 4-bromobenzenesulfonamide (BSA) has been identified in human urine [15,16].

Within the family of H_2-receptor antagonists, various types of extraction procedures have been described for the analysis of the main drug and its metabolites in urine samples. The methods include direct injection of the urine for ranitidine [11,17], liquid–liquid extraction for oxmetidine [14], and solid–liquid extraction for cimetidine [18]. Some of the procedures reported are long and tedious, and contain various steps of extraction with different solvents at different pHs [19]. Direct urine injection is always preferred because of its simplicity and quickness of sample preparation, and it has been succesfully used to determine the urinary levels of ebrotidine and its major metabolite (ESO) in pharmacokinetic studies [10]. Nevertheless, the low concentrations of ESO_2, BSA, DTESO and $DTESO_2$ present in human urine did not allow the use of this method for the determination of these compounds in unprocessed urine samples, and a preconcentration and clean-up of urine prior the chromatographic analysis is necessary.

HPLC has been the technique generally used for the quantitative determination of H_2-receptor antagonists and their metabolites in human urine. The procedures described include normal-phase [14,19], reversed-phase [14,18] ion-pairing reversed-phase [11] and cation-exchange chromatography [17]. The separation of ebrotidine and its potential metabolites in human urine has also been performed using HPLC. Reversed-phase HPLC using a C_{18} column and phosphate buffer–acetonitrile as mobile phase provided a useful method for the analytical and semipreparative separation of these compounds [15], whereas the same type of column and ammonium acetate–acetonitrile as mobile phase was used for the mass spectrometric identification of the urinary metabolites of ebrotidine by both APCI [16] and electrospray [20] ionization techniques. The methods developed showed a long chromatographic time (40–60 min) and were not suitable for the quantitative analysis of these compounds in a clinical pharmacokinetic study, where a large number of samples must be processed.

In this paper we report a quantitative method for the simultaneous determination of ebrotidine and its metabolites in human urine. First, an ion-pair reversed-phase HPLC separation using 1-hexanesulfonic acid and acetonitrile has been developed in order to achieve a rapid chromatographic analysis of all the compounds, and interferences with some potentially co-administered drugs have been studied. Next, several procedures have been tested for the preconcentration and clean-up of ebrotidine and its metabolites from urine, including solid-phase extraction using different adsorbent materials and liquid–liquid extraction with different solvents and conditions. Quality parameters for the optimized procedure have been established and the quantitative method has been applied to the analysis of human urine following oral administration of ebrotidine.

2. Experimental

2.1. Chemicals and reagents

Ethyl acetate (Carlo Erba, Milan, Italy), 1 *M* sodium hydroxide, glacial acetic acid (Panreac, Barcelona, Spain), methanol, acetonitrile, methylene chloride, 2-propanol, dimethylsulfoxide sodium chloride and sodiumdihydrogenphosphate (Merck, Darmstadt, Germany) were of analytical grade. Acetonitrile for HPLC was gradient grade (Merck) and water for HPLC was purified using a Milli-Q plus system (Millipore, Milford, MA, USA) and filtered through a 0.45-μm membrane filter (Millipore). All HPLC solvents were degassed with helium for 30 min before use. The ion-pairing reagent 0.1 *M* 1-hexanesulfonic acid was supplied by Scharlau (Barcelona, Spain).

Ebrotidine (E), ESO, ESO_2, BSA, DTESO,

$DTESO_2$, 2-[4-[[(2-aminoethyl)thio]methyl]-2-thiazolyl]guanidine (ATG), its sulfoxide (ATGSO), N-[2-[[[2-[(diaminomethylene)amino]-4- thiazolyl]methyl]thio]ethyl]formamide (DTF) and N-[(4-bromophenyl-sulfonyl]acetamide (PSA) were synthesized by Centro de Investigación Grupo Ferrer, as reported previously [15,16]. Stock solutions (2 mg/ml) of each compound in methanol (ebrotidine, ATG, ATGSO and DTF), acetonitrile (ESO_2, BSA, DTESO, $DTESO_2$ and PSA) and acetonitrile–dimethylsulfoxide (90:10, v/v) (ESO) were prepared. A standard solution containing 0.1 mg/ml of ebrotidine, ESO, ESO_2, BSA, DTESO and $DTESO_2$ was used to spike blank urine samples for extraction experiments, and standard solutions (0.001 to 0.2 mg/ml) of each compound were prepared in methanol for the standard calibration curves and the recovery experiments, and stored at −25°C until analysis.

Aspirin, paracetamol, codeine phosphate, ranitidine, cimetidine, amoxicillin, theophylline, imipramine hydrochloride and caffeine were provided by Sigma (Alcobendas, Spain). Omeprazole and diazepam were extracted from commercial tablets. Individual solutions of each compound were prepared in water (except diazepam which was dissolved in methanol) at the concentration of 0.1 mg/ml, and injected onto the chromatographic system for the drug-assay interference study.

2.2. HPLC instrumentation and operating conditions

HPLC was conducted using a Merck–Hitachi (Tokyo, Japan) Model L-6200 gradient pump, a Merck–Hitachi Model AS-2000A autosampler and a Merck–Hitachi Model L-4000 UV detector, set at 235 nm. Chromatograms were recorded using a Merck–Hitachi Model D-2500 chromato-integrator.

The chromatographic system was a C_{18} 10 μm μBondapack column (30×0.39 cm I.D.) (Waters, Millipore, Mildford, MA, USA) eluted with 5 m*M* 1-hexanesulfonic acid acidified with glacial acetic acid (pH 3) and acetonitrile at a flow-rate of 1.5 ml/min, using the following gradient: initial composition (80:20, v/v) and linear program to (65:35, v/v) in 30 min. All experiments were carried out at ambient temperature.

2.3. Extraction methods for the urine sample

In Table 1 the reagents and solvents used in each particular extraction method are given. The general operation procedures were performed as follows:

2.3.1. Solid-phase extraction

The Sep-Pack cartridges (Millipore) were equilibrated with 2 ml of each conditioning solvent prior to operation.

Table 1
Reagents and solvents used in the different urine extraction procedures

(a) *Solid-phase extraction*

Method	S1	S2	S3
Sep-Pack cartridge	CN	C_{18}	C_{18}
Cartridge conditioning solvents	MeOH, NaH_2PO_4 10 m*M*	MeOH, NaOH 1 *M*	MeOH, HSFA[a]
Reagent added	1 ml NaH_2PO_4 10 m*M*	100 μl NaOH 1 *M*	1 ml HSFA[a]
Extraction pH	5.5	11.0	3.0
Washing solvent	NaH_2PO_4 10 m*M*	H_2O	HSFA[a]

(b) *Liquid–liquid extraction*

Method	L1	L2	L3	L4
Reagent added	100 μl NaOH 1 *M*	100 μl NaOH 1 *M* + excess NaCl	1 ml HSFA[a]	100 μl NaOH 1 *M*
Extraction pH	11.0	11.0	3.0	
Organic solvent	AcOEt	AcOEt	AcOEt	CH_2Cl_2–2-propanol (9:1)

[a] 5 m*M* 1-hexanesulfonic acid (pH 3).

The different reagents given in Table 1 were added to 1 ml of urine spiked with 10 μg of each compound and the sample was vortex-mixed for a few seconds and loaded onto a Sep-Pack cartridge, washed with 1 ml of the washing solvent and eluted with 1 ml of methanol. The eluate was evaporated to dryness using nitrogen at 40°C and the residue was dissolved with 0.2 ml of methanol in a vortex-mixer. After filtration through a 0.45-μm filter (Millipore), 20 μl were injected onto the chromatographic system.

2.3.2. Liquid–liquid extraction

Different reagents given in Table 1 were added to 1 ml of urine spiked with 10 μg of each compound and the sample was vortex-mixed for a few seconds. After addition of 5 ml of the organic solvent, the sample was mechanically shaken for 15 min and centrifuged at 2700 *g* for 5 min. A 4-ml aliquot of the organic layer was evaporated to dryness using nitrogen at 40°C and the residue was dissolved with 0.2 ml of methanol in a vortex-mixer. After filtration through a 0.45-μm filter (Millipore), 25 μl were injected onto the chromatographic system.

2.4. Volunteer study

Ebrotidine tablets (400 mg each) were orally administered to two healthy male subjects in a single dose. Urine samples corresponding to 0–4, 4–12, 12–24, 24–48, 48–72 and 72–96 h after dosing were collected and immediately frozen to −25°C until analysis.

3. Results and discussion

3.1. HPLC separation and drug-assay interference

In a previous paper [15] the HPLC separation of ebrotidine and some of its potential metabolites was described. The chromatographic system consisted of a C_{18} column and a pH 7.5 phosphate buffer–acetonitrile mobile phase, using a gradient program for the elution of the compounds. Nevertheless, some of the analytes showed peak tailing and also the time of analysis was too long (40 min).

The use of ion-pairing reagents in the mobile phase is a suitable technique for the HPLC separation of samples that are prone to tailing in other HPLC systems [21]. Sulfonic acids are normally used as ion-pairing reagents for the reversed-phase HPLC analysis of basic ionizable compounds, such as amines [22]. Thus, in the case of ebrotidine and its derivatives, the use of 1-hexanesulfonic acid improved the peak width and the time of analysis in comparison with the phosphate buffer–acetonitrile system described before [15]. In Fig. 1a the chromatogram obtained with ebrotidine and all its potential metabolites using the system 1-hexanesulfonic acid–acetonitrile with a gradient program is given, allowing the separation of the ten compounds in 30 min. Of these ten compounds, only six were found in human urine [15,16], and the gradient program was subsequently modified and adapted to those. The chromatogram obtained is shown in Fig. 1b, where the six analytes are eluted with high resolution and short analysis time (24 min). This method was chosen as the best for the analysis of ebrotidine and its metabolites in human urine.

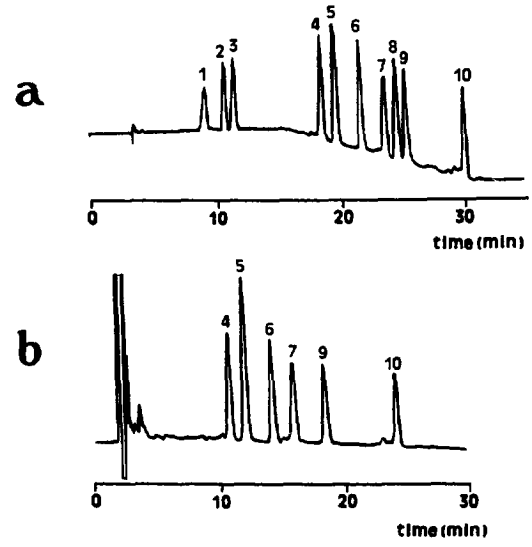

Fig. 1. Chromatograms of two gradient programs used for the separation of ebrotidine and its derivatives. Mobile phase: 5 m*M* 1-hexanesulfonic acid pH 3–acetonitrile; (a) 85:15 initial composition and linear program to 60:40 in 30 min; flow-rate, 1 ml/min; (b) 80:20 initial composition and linear program to 65:35 in 30 min; flow-rate, 1.5 ml/min. Peaks: 1=ATGSO, 2=DTF, 3=ATG, 4=DTESO, 5= BSA, 6=ESO, 7=$DTESO_2$, 8=PSA, 9=ESO_2, 10=E. Detector sensitivity, 0.001 AUFS.

Table 2
Drug-assay interference for the HPLC separation of ebrotidine and its metabolites

Compound	Therapeutic family	Retention time (min)
Aspirin	Analgesic	7.9
Paracetamol	Analgesic	2.0
Codeine	Analgesic	4.9
Ranitidine	Anti-ulcer	3.8
Cimetidine	Anti-ulcer	3.7
Omeprazole	Anti-ulcer	13.2
Amoxicillin	Antibacterial	2.0
Theophylline	Vasodilator	2.3
Diazepam	Tranquilizer	2.3
Caffeine	Central stimulant	3.1
Imipramine	Antidepressant	N.D.[a]

[a] Not detected.

In order to apply the method to clinical studies, in which ebrotidine could be administered simultaneously with other drugs, a set of 11 pharmaceutical compounds in common use was evaluated for potential assay interference. Some drugs belonging to different therapeutic families than ebrotidine were chosen, such as common analgesics, antibacterial or tranquilizer drugs, and in addition three compounds of the same class as ebrotidine (anti-ulcer drugs) were tested: two H_2-receptor antagonists, cimetidine and ranitidine, and the proton-pump inhibitor omeprazole. The results of the chromatographic study are given in Table 2. Most of the drugs were eluted with low retention times, and their presence in the urine sample would not interfere in the analysis of ebrotidine and its metabolites. Only aspirine and omeprazole were substantially retained in this chromatographic system, but aspirine would not interfere because it would elute 3 min before the ebrotidine derivatives. Omeprazole would appear in between the six compounds; nevertheless, considering its retention time (13.2 min) and the high resolution of the chromatographic system, omeprazole would elute between peaks 5 and 6 (Fig. 1b), and therefore it would not cause interference.

3.2. Extraction methods

In Table 3, the percentage recoveries of ebrotidine and its metabolites for the different extraction procedures assayed are given. Every extraction method was repeated three times, and the percentage recovery was approximately the same (±5%) on each occasion.

Solid-phase extraction is a modern and attractive method of sample preparation, mainly due to its speed and easy automation and, consequently, it is very useful in pharmakokinetic studies where a large number of samples must be processed. In this paper adsorbents of different polarity C_{18} and CN were used and also an ion-pair procedure was assayed. Nevertheless, the solid-phase extraction procedures did not produce acceptable results for the extraction of ebrotidine and its metabolites from urine samples. As can be seen in Table 3, the use of C_{18} cartridges in alkaline medium (S2) gave poor recoveries for the two oxidized metabolites DTESO (44%) and $DTESO_2$ (2%), and also ebrotidine sulfone (ESO_2)

Table 3
Comparison of different extraction procedures: percentage recovery of ebrotidine and its metabolites from human urine

Compound	Solid-phase extraction			Liquid–liquid extraction			
	S1 Sep-Pack CN pH 5.5	S2 Sep-Pack C_{18} pH 11.0	S3 Sep-Pack C_{18} pH 3.0 (ion-pair)	L1 AcOEt pH 11.0	L2 AcOEt (+NaCl) pH 11.0	L3 AcOEt (ion-pair) pH 3.0	L4 CH_2Cl_2/2-PrOH (9:1) pH 11.0
E	81	96	100	98	92	89	87
ESO	93	99	100	97	89	19	98
ESO_2	[a]	[b]	[a]	85	76	73	97
BSA	61	97	91	99	100	100	99
DTESO	72	44	100	67[c]	66[c]	62	86
$DTESO_2$	62	2	100	100	99	100	92

[a] The compound coelutes with a urine interference.
[b] The compound decomposes during the extraction procedure.
[c] The chromatographic peak has a shoulder corresponding to a urine interference.

was hydrolyzed during operation. On the other hand, the use of CN cartridges (S1) gave higher recoveries for DTESO and $DTESO_2$ but a chromatographic urine interference for ESO_2 was observed. Using ion-pair C_{18} solid-phase extraction (S3) high recoveries were obtained for all the compounds, but an interference with ESO_2 and the presence of a large number of urine endogenous compounds were observed. This last fact is due to the similar behaviour of the ebrotidine derivatives and the urine endogenous compounds against the ion-pairing agent, which prevented an adequate clean-up of the sample.

The liquid–liquid extraction methods assayed in this study mainly used ethyl acetate as the organic solvent. Extraction at pH 11.0 (L1) gave high recoveries (≥75%) for all the compounds except for DTESO, which could only be extracted at 67% of efficiency. The addition of sodium chloride to the alkalinized urine sample prior the extraction (salting-out) (L2) did not improve the recovery of DTESO. In both cases (L1 and L2) a chromatographic peak shoulder corresponding to a urine peak was observed for this compound. When an ion-pair extraction was performed at pH 3 (L3) the recovery of DTESO was 62% (lower than in L1 and L2) and in addition the compound ESO was poorly recovered (19%). Another solvent assayed to carry out the extraction was the mixture CH_2Cl_2–2-propanol (9:1, v/v), which has been described for the extraction of ranitidine from plasma samples [23]. Alkaline extraction with this solvent (L4) gave high recoveries (≥85%) for all the compounds. In addition, a low extraction of urine endogenous materials was observed, and a clean chromatogram without interferences was obtained (Fig. 2a and b). Therefore, the method L4 was chosen as the best to be applied for the quantitative determination of ebrotidine and its metabolites in human urine.

3.3. Linearity, precision and sensitivity

Standard curves were constructed for each compound after the injection of standard solutions, using peak area as the response. The curves were linear over the range studied (10 standards from 1 to 200 μg/ml for each compound) with correlation coefficients higher than 0.9995, as can be seen in Table 4 where the linearity data obtained for all the compounds are given.

Intra-day precision was evaluated by spiking human blank urine with the six compounds and carrying five replicate samples through the entire

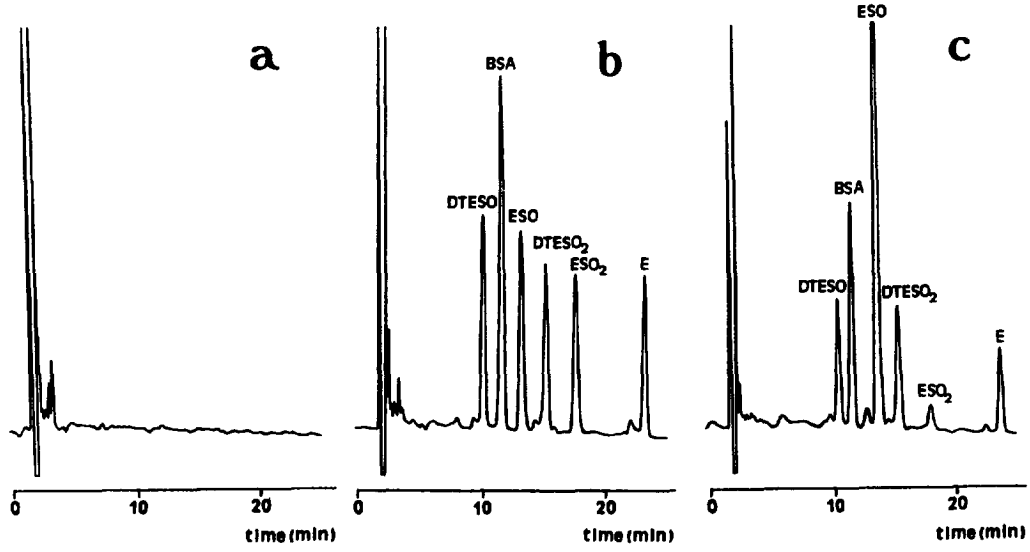

Fig. 2. Chromatograms of different urine samples extracted with CH_2Cl_2–2-propanol (9:1, v/v) at pH 11. (a) Blank urine; (b) blank urine spiked with 10 μg of E, ESO, ESO_2, BSA, DTESO and $DTESO_2$; (c) urine collected from subject 1 during 4–12 h following oral administration of 400 mg of ebrotidine. Detector sensitivity: 0.001 AUFS.

Table 4
Linearity data for ebrotidine and its metabolites

Compound	Slope (area counts/μg)	Intercept (area counts)	Correlation coefficient (r)
E	256 463.66	6666.67	0.9999
ESO	293 928.66	5619.22	0.9999
ESO_2	259 703.66	8224.97	0.9999
BSA	431 840.41	10 629.56	0.9999
DTESO	348 607.66	6196.39	0.9997
$DTESO_2$	277 967.22	7036.15	0.9999

analytical procedure in one day. The samples were injected in duplicate and the assay was performed at three different concentrations: 1, 5 and 10 μg/ml of urine. The peak area obtained for each compound in each analysis was introduced in the corresponding standard curve to evaluate the recovery. The mean recoveries for each set of replicates were calculated, and the precision was estimated as the relative standard deviation values.

Inter-day precision was similarly evaluated, performing five replicate samples at the spiked concentration of 5 μg/ml of urine in three different days.

In Table 5, the results of intra-day and inter-day precision are given. The maximum values of R.S.D. obtained in the intra-day assay are over 3%, except for the ESO at the concentration of 1 μg/ml, which shows a slightly higher value (4.4%). The R.S.D. values obtained in the inter-day assay are also low, ranging from 1.5 to 3.9. These results demonstrate the high reproducibility of the whole analytical method, which makes the use of an internal standard unnecessary. The limits of detection of the present method, based on a signal-to-noise ratio of 3, were 47, 26, 110, 92, 70 and 97 ng/ml of urine for DTESO, BSA, ESO, $DTESO_2$, ESO_2 and ebrotidine (E), respectively. These detection limits are adequate for the determination of the drug levels usually found in the clinical pharmacokinetic study of ebrotidine.

3.4. Volunteer study

The pharmacokinetics of ebrotidine when administered orally to healthy human volunteers has been extensively studied with doses ranging from 150 to 1600 mg of daily administered drug. For the therapeutic doses of 400 and 800 mg/day [10] the profiles observed in most cases were fitted to a two-compartment model, the absorption parameters that define the process being: $K_{01}=1.63\pm0.91$ h^{-1} and 0.99 ± 0.33 h^{-1}, $T_{max}=1.95\pm1.04$ h and 2.60 ± 1.07 h, $C_{max}=872\pm300$ ng/ml and 1168 ± 391 ng/ml, $AUC_{0\rightarrow\infty}=4849\pm2369$ ng h/ml and 9269 ± 2031 ng h/ml (doses of 400 and 800 mg, respectively). The most representative values of distribution and elimination phases were: $t_{1/2\beta}=13.87\pm6.15$ h and 20.34 ± 5.20 h, $Cl/F=98.7\pm41.8$ l/h and 90.9 ± 23.8 l/h, $V_d/F=1770.6\pm676.0$ and 2654.0 ± 937.8 l (doses

Table 5
Calculation of intra- and inter-day precision for the determination of ebrotidine and its metabolites in human urine

Spiked conc. (μg/ml)	DTSEO		BSA		ESO		$DTSEO_2$		ESO_2		E	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Intra-day ($n=5$)												
1	84.1	2.5	98.0	3.2	102.3	4.4	90.2	1.6	95.0	3.3	86.3	2.6
5	88.5	3.0	102.4	0.8	103.8	3.1	87.2	1.2	96.3	1.7	87.2	2.4
10	85.9	2.9	101.5	1.3	101.5	3.2	92.0	3.2	96.1	2.8	87.1	3.1
Inter-day ($n=3$)												
5	87.8	3.3	102.5	1.5	101.0	3.9	89.6	3.2	95.6	2.1	86.9	2.7

Table 6
Urinary recoveries of ebrotidine and its metabolites collected from subjects 1 and 2 during 96 h following oral administration of 400 mg of ebrotidine

Period (h)	Subject	Cumulative urinary excretion (%) of dose					
		DTESO	BSA	ESO	$DTESO_2$	ESO_2	E
0–4	1	0.30	0.11	1.53	0.18	0.08	0.37
	2	0.25	0.04	2.55	0.14	0.10	0.78
4–12	1	1.35	1.48	6.45	1.76	0.35	1.35
	2	0.75	0.64	6.00	0.86	0.24	1.62
12–24	1	1.47	1.68	7.08	2.20	0.39	1.39
	2	0.95	0.67	7.41	1.35	0.27	2.07
24–48	1	1.50	1.78	7.58	2.50	0.44	1.39
	2	1.02	0.73	8.19	1.71	0.27	2.29
48–72	1	1.50	1.79	7.60	2.51	0.44	1.39
	2	1.04	0.78	8.58	1.83	0.27	2.47
72–96	1	1.50	1.79	7.69	2.51	0.44	1.39
	2	1.05	0.78	8.77	1.85	0.27	2.47
TOTAL	1	1.50	1.79	7.69	2.51	0.44	1.39
	2	1.05	0.78	8.77	1.85	0.27	2.47

of 400 and 800 mg, respectively). After a repeated administration for 7 days, no drug accumulation was observed, and the behaviour of ebrotidine was found to be linear at the dose interval between 150 and 800 mg/day.

The high lipophilicity of ebrotidine is a great obstacle for its intravenous administration; for this reason the bioavailability of ebrotidine has to be estimated from the total urinary recovery of the drug, as other authors proposed for roxatidine and etintidine [9] where plasma data from intravenous administration were not available. Urinary recoveries of ebrotidine and its metabolites collected during 96 h following oral administration of ebrotidine to two volunteers are shown in Table 6, and the chromatogram of a urine sample is shown in Fig. 2c. Ebrotidine was predominantly excreted in urine as the sulfoxide metabolite, the percentages recovered being 7.69 and 8.77 of the administered dose for the two subjects. The amounts of the other metabolites and also of unchanged ebrotidine were not higher than 2.51% of the dose. The period of maximum drug elimination was the same for both subjects (4–12 h) and the total urinary recovery of the drug, calculated by adding the individual percentages of all the compounds, was 15.3 and 15.2, respectively.

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