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Determination of two mebeverine metabolites, mebeverine alcohol and desmethylmebeverine alcohol, in human plasma by a dual stable isotope-based gas chromatographic–mass spectrometric method

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Abstract

A dual stable isotope-based GC–MS method was developed for the simultaneous determination of two metabolites of mebeverine, mebeverine alcohol and desmethylmebeverine alcohol, in human plasma. Plasma samples were treated with β-glucuronidase to cleave the glucuronide conjugates of both compounds prior to analysis. The treated plasma was prepared for analysis by solid-phase extraction using octadecylsilane cartridges. The isolated metabolites were derivatized and analyzed by GC–MS using selected-ion monitoring. Plots of peak-area ratio were linear with metabolite concentration from 2 to 200 ng/ml and the limit of detection for both metabolites was 0.5 ng/ml. The GC–MS methodology was applied to the analysis of plasma from human subjects following peroral administration of mebeverine. Pharmacokinetic parameters for both metabolites were determined and suggest that relative systemic mebeverine exposure may potentially be assessed using metabolite kinetics, if the latter subsequently are demonstrated to be linear with mebeverine dose.

Keywords: Mebeverine; Mebeverine alcohol; Desmethylmebeverine alcohol

1. Introduction

Mebeverine (MEB; see Fig. 1) is a musculotropic antispasmodic agent that is prescribed in Europe for the treatment of irritable bowel syndrome (IBS), a condition generally supposed to involve a disorder of colonic motility [1]. IBS is characterized by irregular bowel habit, abdominal pain and a feeling of incomplete defecation [2]. Peroral (p.o.) administration of MEB has been demonstrated to decrease intestinal motility by a direct action on the gastrointestinal smooth muscles [3] and MEB has been shown to produce therapeutic effects in IBS patients [4–6]. Although MEB has been widely used in Europe, very little is known about the pharmacokinetics or metabolism of this compound in any species. It is known that MEB is rapidly metabolized to mebeverine alcohol and veratric acid in vitro by rat and human plasma and in vivo in the pig following intravenous (i.v.) administration [7,8]. Parent MEB levels have been reported in the pig and rat following i.v. administration and in the rabbit and human following p.o. dosing [7–10]. In general, plasma

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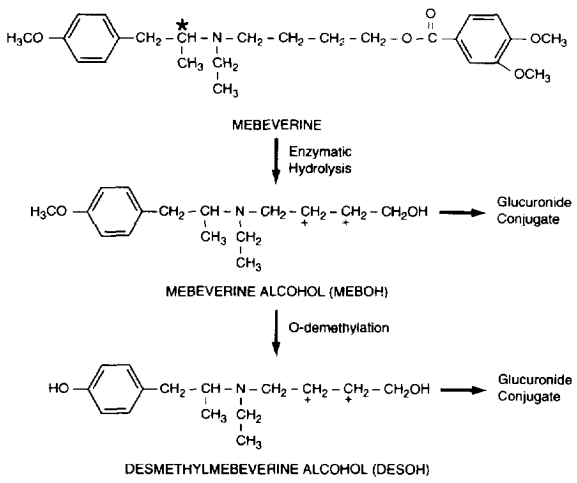

Fig. 1. Metabolic pathway for mebeverine in humans. The position of the ^{14}C-label is indicated by the * symbol in the mebeverine structure. The position of the deuterium isotope labels are denoted by the + symbols in the structure of mebeverine alcohol and desmethylmebeverine alcohol.

levels of MEB are not usually detectable following p.o. dosing due to a high first pass metabolism. In order to measure systemic exposure it is therefore necessary to measure a metabolite of MEB in plasma. We report on the development of GC–MS methodology for the determination of two metabolites of MEB, mebeverine alcohol (MEBOH) and desmethylmebeverine alcohol (DESOH), in human plasma following p.o. administration of MEB. MEBOH and DESOH and their glucuronide conjugates (see Fig. 1) were found to be present in human plasma following p.o. administration of MEB (Procter & Gamble Pharmaceuticals, unpublished data). The analysis methodology involved glucuronidase treatment of the plasma followed by isolation of the analytes on solid-phase extraction cartridges. For GC–MS analysis, dual stable-isotope internal standards (deuterium-label) of MEBOH and DESOH were employed (see Fig. 1) in conjunction with selected-ion monitoring (SIM) for quantification of MEBOH and DESOH levels. Plasma levels of MEBOH and DESOH were determined in eight male subjects following p.o. administration of a commercial MEB product. The plasma levels of the two metabolites were used to calculate their respective pharmacokinetic parameters.

2. Experimental

2.1. Chemicals

Distilled, deionized water was from a Barnstead NANOpure II system (Dubuque, IA, USA). Methanol (HPLC grade), hexane (HPLC grade), formic acid (reagent grade) and potassium phosphate dibasic (reagent grade) were from J.T. Baker (Phillipsburg, NJ, USA). β-D-Glucuronidase (Type VII-A, *E. coli*) and phenolphthalein glucuronide were from Sigma (St. Louis, MO, USA). Triethylamine (99%) was from Aldrich (Milwaukee, WI, USA). Phosphoric acid (Chempure brand, 85%) was from Curtin Matheson Scientific (Houston, TX, USA). The derivatizing reagent, N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), was from Pierce (Rockford, IL, USA). Blank human plasma was obtained from Hoxworth Blood Center (Cincinnati, OH, USA). MEBOH, DESOH, deuterium-labeled mebeverine alcohol-d_4 (d-MEBOH), deuterium-labeled desmethylmebeverine alcohol-d_4 (d-DESOH) and ^{14}C-Labeled mebeverine alcohol [^{14}C]MEBOH, 109 μCi/mg) were prepared at Procter & Gamble Pharmaceuticals. ^{14}C-Labeled mebeverine [^{14}C]MEB; 109 μCi/mg) was synthesized by Amersham (Arlington Heights, IL, USA). Radiolabeled [^{14}C]DESOH (0.27 μCi/mg), desmethylmebeverine alcohol glucuronide [^{14}C]DESOH-GLUC; 0.27 μCi/mg) and mebeverine alcohol glucuronide [^{14}C]MEBOH-GLUC; 6 μCi/mg) were isolated from urine obtained from dogs and rats, respectively, dosed with [^{14}C]MEB. The position of the deuterium and ^{14}C labels are shown in Fig. 1.

2.2. Glucuronidase treatment optimization

Blank human plasma was spiked with [^{14}C]DESOH-GLUC (1500 ng/ml) and [^{14}C]MEBOH-GLUC (250 ng/ml) and treated with 1000 or 2500 U β-D-glucuronidase per ml plasma. The samples were then incubated at 37°C in a water bath for 1 h. Following incubation, the samples were treated with one volume of acetonitrile and centrifuged to remove protein. The supernatant was analyzed by gradient reversed-phase HPLC with on-line radiochemical detection (HPLC-RAD), as described below.

2.3. Sample preparation by solid-phase extraction

Plasma samples were stored frozen at −80°C. On the day of analysis, plasma samples were thawed, allowed to come to room temperature and mixed by gentle inversion. An aliquot (1.0 ml) of each plasma sample was placed into screw-top test tubes (16× 100 mm) containing 50 ng of both internal standards, d-MEBOH and d-DESOH, in 0.025 ml of methanol. Then 2500 U of β-D-glucuronidase (0.05 ml of a 50 000 U/ml solution) was added to each plasma sample. The plasma samples were sealed with a Teflon-lined cap and incubated at 37°C for 1 h. Following incubation, the test tubes were removed from the water bath and allowed to cool to room temperature. After cooling, an aliquot (1.0 ml) of 1.0 *M* potassium phosphate buffer (pH 9.0) was added to each test tube. The contents of the test tubes were mixed by inversion and then applied directly to octadecylsilane (ODS) Sep-Pak cartridges (Waters) that were previously conditioned with 10 ml of methanol followed by 10 ml of water–methanol (97:3, v/v). Once the samples were applied, the cartridges were successively washed with 5 ml of water–methanol (97:3, v/v) and 5 ml of water–methanol (60:40, v/v). The analytes were then eluted with 5 ml of water–methanol–TEA–formic acid (10:90:0.5:0.3, v/v). The eluent from each cartridge was collected into glass screw-top test tubes and taken to dryness under vacuum on a Speed Vac system (Savant, Farmingdale, NY, USA). The residue in each test tube was derivatized with 0.2 ml of BSTFA for 15 min at 60°C and then the BSTFA was removed under filtered nitrogen. The residue was reconstituted in 0.1 ml of hexane prior to analysis.

To insure complete cleavage of MEBOH and DESOH glucuronide conjugates occurred, a single plasma sample (1.0 ml) from each human subject was spiked with [^{14}C]MEBOH-GLUC and incubated at 37°C for 1 h. Following incubation, the samples were treated with one volume of acetonitrile and centrifuged to remove protein. The supernatant was analyzed by gradient reversed-phase HPLC–RAD, as described below.

2.4. HPLC-RAD conditions

The HPLC system was composed of two Milton Roy Constametric III pumps (Rivera Beach, FL, USA), an Axxiom Model 712 gradient controller (Calabasas, CA, USA), a Perkin-Elmer Model ISS-100 autosampler (Norwalk, CN, USA) and a Flo-One Beta on-line radiochemical detector (Radiomatics, Tampa, FL, USA) with a 0.5-ml flow cell. Mobile phase A was water–TEA–formic acid (100:0.5:0.3, v/v) and mobile phase B was water–methanol–TEA–formic acid (10:90:0.5:0.3, v/v) and the HPLC column was a Waters Associates ODS Radial-Pak cartridge column (100×8 mm, 5 μm). A gradient composed of 100% mobile phase A for 2 min, followed by a linear ramp to 70% mobile phase B over a 13-min period with a hold at 70% mobile phase B for 2 min and a final linear ramp to 100% mobile phase B over a 1-min period with a final hold at 100% mobile phase B for 7 min was used for the HPLC analysis. The HPLC flow-rate was 2 ml/min and liquid scintillation cocktail (Flow Scint II, Radiomatics) was added to the column effluent at a flow-rate of 3 ml/min.

2.5. Absolute recovery of MEBOH and DESOH from SPE

The recovery of MEBOH and DESOH from the ODS cartridges was determined using human plasma obtained from individual donors. The individual plasma samples were treated with glucuronidase and then spiked with [^{14}C]MEBOH (9.2, 92 and 920 ng/ml) or [^{14}C]DESOH (1580 ng/ml). The spiked plasma samples were applied to the ODS cartridges and eluted as described above for SPE sample preparation. The collected eluent was counted on a Packard Model 2000CA liquid scintillation analyzer (Packard Tri-Carb, Downers Grove, IL, USA). Plasma from three individual donors was examined and the recovery at each spiked level was determined in triplicate. The absolute recovery of DESOH was only examined at the high level due to limitations imposed by the specific activity of the isolated [^{14}C]DESOH.

2.6. Preparation of standards for GC–MS analysis

Stock standard solutions containing 2 to 200 ng/ml each of MEBOH and DESOH were prepared in methanol. Working standards were prepared by adding 1.0 ml of the appropriate stock standard solutions into a screw-top glass test tube containing 50 ng each

of both internal standards, d-MEBOH and d-DESOH, in 0.025 ml of methanol. The solvent was then removed using filtered nitrogen, 0.2 ml of BSTFA was added to each tube, the tubes capped with a PTFE-lined cap and heated for 15 min at 60°C. Following heating, the BSTFA was removed with filtered nitrogen and the residue was reconstituted in 0.1 ml of hexane prior to analysis.

2.7. Accuracy and precision

Pooled blank plasma was spiked with MEBOH and DESOH to give metabolite levels of 10, 20, 50, 100 and 200 ng/ml. Aliquots of each spiked sample were treated with glucuronidase and prepared for analysis as described above. For each spiked level, samples were prepared in triplicate.

2.8. Preparation of plasma check sample

A plasma check sample was prepared by adding 1.0 ml of a methanol solution containing 10 μg/ml each of MEBOH and DESOH into a 200-ml volumetric flask and diluting to volume with blank human plasma pooled from three different donors. The check plasma, containing 50 ng/ml each of DESOH and MEBOH, was aliquoted (5 ml) into polypropylene vials, capped and stored along with the plasma samples from the clinical study. Check plasma samples were prepared and analyzed in triplicate, each day clinical samples were analyzed.

2.9. GC–MS conditions

The GC–MS analyses were done on a Hewlett-Packard (HP, Avondale, PA, USA) Model 5890A gas chromatograph equipped with a HP Model 7673A autosampler and a HP Model 5971A mass-selective detector (MSD). The column was a HP Ultra 2 (30 m×0.20 mm, 0.11 μm film thickness) and helium was used as the carrier gas with a column head pressure of 138 kPa. The thermal program involved an initial temperature of 75°C for 1 min followed by a 20 °C/min linear ramp to 300°C. The injection port and the transfer line were held at 225 and 290°C, respectively. The injection volume was 0.5 μl using a splitless mode for 1.0 min and a 4 mm I.D. straight liner packed with persilylated quartz wool. The MSD conditions involved electron impact (70 eV) with SIM at m/z 216.0 (MEBOH and DESOH) and m/z 220.0 (d-MEBOH and d-DESOH) with a dwell time of 150 ms for both ions and low resolution conditions on the MSD. The MSD was tuned for each run using an automated user tune program which monitored perfluorotributylamine ions of m/z 69, 219 and 264.

2.10. Calculation of unknown sample and check standards

The working standards (2 to 200 ng/ml) were used to construct a standard curve by plotting the peak-area ratio for each analyte and internal standard pair (MEBOH/d-MEBOH and DESOH/d-DESOH) versus the concentration of the analyte. The concentration of MEBOH and DESOH in unknown and plasma check samples were determined by interpolation from their respective linear regression curve.

2.11. Dosing of human subjects

Subjects received a p.o. dose of 135 mg of MEB hydrochloride from a commercial preparation (Colofac tablets) in the fasted state. For two subjects in a pilot group, blood samples were obtained at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48 and 72 h post-dose. The results from the analysis of the pilot subjects were used to determine the sampling times for the subsequent subjects. For six subsequent subjects, blood samples were obtained at 0. 0.17, 0.25, 0.33, 0.5, 0.75, 1, 1.25, 2, 4, 5, 6, 8, 10, 12, 16, and 24 h post-dose. The blood (10 ml) was collected in 10-ml Vacutainer tubes containing lithium heparin as an anticoagulant and were immediately placed on ice. The blood was centrifuged at 1500 g for 10 min at 4°C to separate the plasma. The plasma was isolated, placed in polypropylene tubes and stored at −80°C until analyzed.

2.12. Pharmacokinetic analyses

Plasma concentration data for MEBOH and DESOH were analyzed using a model independent approach. All pharmacokinetic parameter calculations were conducted using Pharm-NCA (Simed, Creteil, France). The terminal rate constant (λz) was

determined via log–linear regression of the terminal time points (selected by inspection of a log–linear plot) of the plasma concentration–time curve. Area under the plasma concentration–time curve (AUC) up to the last time point were calculated using the log–linear trapezoidal rule. AUCs were extrapolated to infinity using the ratio of the plasma concentration at the last time point and the terminal rate constant. Area under the first moment of the plasma concentration–time curve (AUMC) was also calculated using trapezoidal rule and similarly extrapolated to infinity. Peak concentration (C_{max}) and time to peak concentration (T_{max}) were determined as observed maxima from plasma concentration data.

3. Results and discussion

3.1. Glucuronidase treatment of plasma

MEB is extensively metabolized in rat and dog following p.o. administration to give MEBOH and DESOH respectively, as shown in Fig. 1 (Procter & Gamble Pharmaceuticals, unpublished data). MEB is hydrolyzed to give MEBOH which undergoes subsequent O-demethylation to give DESOH. MEBOH and DESOH are both extensively conjugated with glucuronic acid prior to excretion. DESOH can be conjugated at both the phenolic and alcohol positions, leading to the potential formation of multiple glucuronide metabolites of DESOH. MEB was not measurable in human plasma, at any time point after oral administration of 135 mg of MEB, using an HPLC method with a LOQ of 2 ng/ml (Procter & Gamble, unpublished data). In order to follow systemic exposure following oral administration of MEB both metabolites had to be monitored since in vivo human metabolism of MEB had not been reported. To measure the total levels of these metabolites their glucuronide conjugates had to be cleaved by treatment with glucuronidase prior to analysis. The optimal conditions for cleavage of the glucuronide conjugates was determined using blank human plasma spiked with [^{14}C]DESOH-GLUC and [^{14}C]MEBOH-GLUC. HPLC-RAD analysis was used to monitor the extent of the glucuronidase cleavage reaction under various incubation conditions. A typical HPLC-RAD profile for a spiked plasma sample, before and after glucuronidase incubation, is shown in Fig. 2. A single peak was observed for [^{14}C]MEBOH-GLUC at retention time 12.5 min while multiple peaks were observed for [^{14}C]DESOH-GLUC in the retention time region from 7 to 9 min. The multiple peaks for [^{14}C]DESOH-GLUC are likely due to the conjugation of both the phenolic and alcoholic groups of DESOH with glucuronic acid. Complete cleavage of MEBOH and DESOH conjugates was achieved with 1000 U β-D-glucuronidase per ml plasma using a 1-h incubation at 37°C. To insure complete cleavage however, 2500 U β-D-glucuronidase per ml plasma was employed for the treatment of plasma samples from clinical subjects. Additionally, one sample from each subject was spiked with [^{14}C]MEBOH-GLUC,

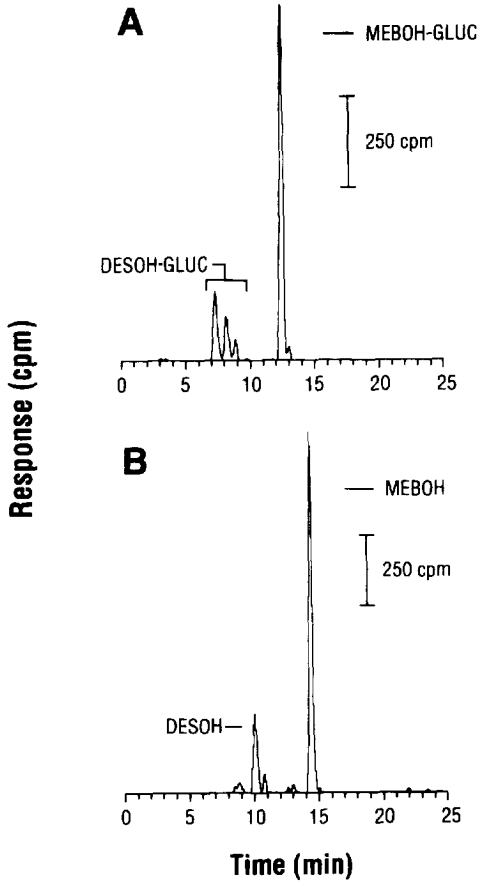

Fig. 2. HPLC–RAD profile for human plasma spiked with [^{14}C]MEBOH-GLUC and [^{14}C]DESOH-GLUC (A) before and (B) after treatment with β-D-glucuronidase.

treated with glucuronidase and profiled by HPLC-RAD. The [^{14}C]MEBOH-GLUC was completely cleaved under these conditions for all subjects (data not shown).

3.2. Full scan mass spectrum

The full scan EI mass spectrum for MEBOH and DESOH derivatized with BSTFA are shown in Fig. 3. A number of ions are formed by the EI process but the major ion generated for the derivatized MEBOH and DESOH was m/z 216, resulting from the cleavage of the carbon bond alpha to the benzyl carbon. A similar fragment was observed in the EI spectra for the deuterated-internal standards but at m/z 220 due to the incorporation of the four deuterium atoms (data not shown). For GC–MS analysis, the m/z 216 and 220 ions were used for monitoring the two analytes and their deuterated-internal standards, respectively.

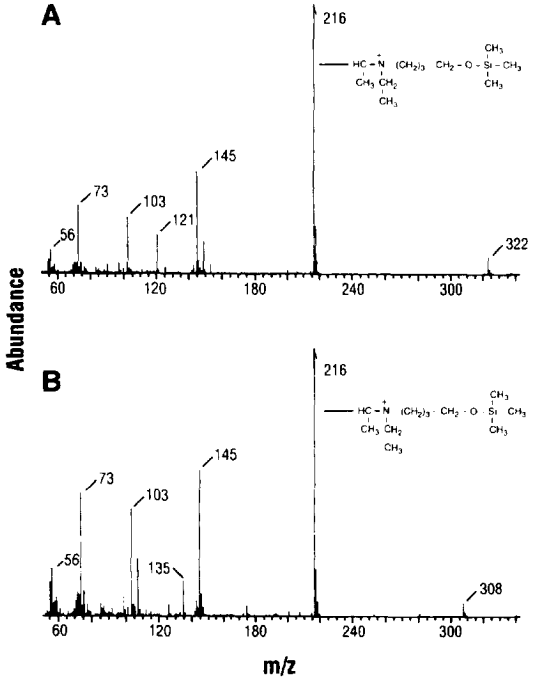

Fig. 3. Electron impact (70 eV) mass spectrum of BSTFA derivatives of (A) MEBOH and (B) DESOH.

3.3. Absolute recovery of MEBOH and DESOH from SPE

Prior to GC–MS analysis, MEBOH and DESOH were isolated from the glucuronidase treated plasma by direct application of the plasma to an SPE cartridge. Although the use of the stable-isotope internal standards should correct for any loss of MEBOH or DESOH during sample preparation, a high absolute recovery of the analytes is still desirable. The absolute recoveries of MEBOH and DESOH were examined using plasma from three separate donors spiked with either [^{14}C]MEBOH or [^{14}C]DESOH. The absolute recovery of MEBOH was generally greater than 83%, with R.S.D. values of less than 5% across a concentration range from 9.2 to 920 ng/ml (Table 1). The absolute recovery of DESOH was only examined at a high concentration (1580 ng/ml) due to limitations in the specific activity of the [^{14}C]DESOH isolated from rat urine. The recovery of DESOH ranged from 72 to 95% in the various plasma samples and exhibited higher R.S.D. values (7–13%) than those obtained for MEBOH.

3.4. Plasma sample analysis: selectivity, accuracy and precision

The combination of SPE sample preparation and GC–MS analysis resulted in a highly selective approach for the quantitation of MEBOH and DESOH. A chromatogram for a 5-h post-dose subject plasma sample is shown in Fig. 4. Excellent chromatographic peak shape was obtained for both

Table 1
Recovery of MEBOH and DESOH from SPE cartridges

Concentration (ng/ml)	Mean recovery (%)		
	Plasma A	Plasma B	Plasma C
MEBOH			
9.2	87.0 (4.1)	87.7 (0.9)	83.6 (2.3)
92	88.0 (2.6)	88.4 (4.8)	93.5 (2.2)
920	94.2 (2.1)	92.6 (4.4)	88.6 (2.7)
DESOH			
1580	95.1 (9.7)	73.4 (7.3)	72.1 (13.2)

Values in parentheses are R.S.D. (%).

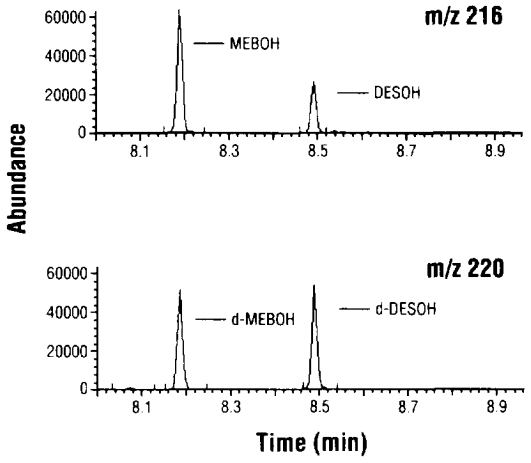

Fig. 4. Selected-ion monitoring profile obtained for a plasma sample from a human subject (5 h post-dose) following a 135-mg mebeverine p.o. dose.

Table 2
Recovery of DESOH and MEBOH from spiked human plasma samples

Concentration added (ng/ml)	Concentration found (mean±S.D.) (ng/ml)	Recovery (mean) (%)
DESOH		
10	9.9±0.5	99
20	17.3±1.0	87
50	56.9±1.7	114
101	95.6±3.5	95
201	191±3.4	95
MEBOH		
10	9.9±0.1	99
20	18.3±0.4	92
50	49.7±1.6	97
102	96.1±3.0	94
204	197±5.0	97

compounds and MEBOH, DESOH and the deuterated-internal standards were quantitated without interference from matrix components. The LOQ for MEBOH and DESOH was 2 ng/ml with a *S/N* ratio of at least 12 and the LOD was 0.5 ng/ml. Accuracy and precision were examined for blank plasma samples spiked with MEBOH and DESOH over a concentration range from 10 to 200 ng/ml. The accuracy of the MEBOH analysis ranged from 92 to 99%, with R.S.D. values of less than 3.5% for all spiked concentrations (Table 2). The accuracy of the DESOH analysis ranged from 88 to 114% with R.S.D. values of less than 6% for all spiked concentrations. Plasma check samples, spiked with 50 ng/ml each of MEBOH and DESOH, were analyzed in triplicate each day that subject samples were run (Table 3). The average recovery for MEBOH and DESOH ranged from 93 to 105%, with R.S.D. values of less than 5%.

3.5. Pharmacokinetic analysis of human clinical samples

Graphs of MEBOH and DESOH plasma concentrations appear in Fig. 5. The plasma concentrations of DESOH were higher and persisted longer than those of MEBOH. Control plasma sample analyses are shown in Table 3. The pharmacokinetic parameters for MEBOH and DESOH are shown in Table 4 and Table 5, respectively. Peak MEBOH concentrations averaged about 250 pmol/ml with a mean time to peak concentration of 1.2 h. Peak DESOH averaged 550 pmol/ml with a mean time to peak concentration of 1.4 h. The mean MEBOH terminal half-life was about 1.8 h with a large

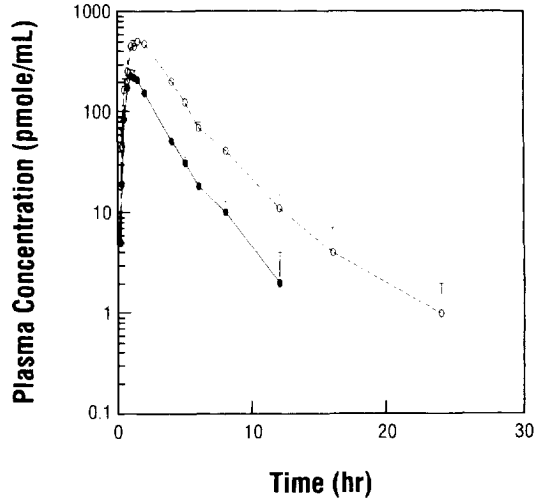

Fig. 5. Mean plasma concentrations (+S.E.M.) of MEBOH (●) and DESOH (○) following p.o. administration of 135 mg mebeverine to healthy male subjects.

Table 3
Control plasma sample analysis

Analysis day	Recovery (mean) (%)	
	MEBOH	DESOH
1	94.7 (2.5)	96.9 (2.3)
2	96.5 (1.4)	100.7 (1.2)
3	97.7 (2.0)	92.7 (1.8)
4	96.5 (5.4)	96.5 (5.4)
5	100.1 (4.4)	103.0 (1.2)
6	98.4 (1.0)	99.7 (2.1)
7	104.9 (2.1)	100.7 (2.1)
8	101.3 (1.2)	101.3 (1.2)

Values in parentheses are R.S.D. (%).

Table 4
Pharmacokinetic parameters for MEBOH

Subject	C_{max} (pmol/ml)	T_{max} (h)	Terminal half-life (h)	AUC (pmol/ml per h)
1	282	1.0	1.1	590
2	174	1.0	1.2	411
3	241	2.0	1.7	738
4	160	1.5	5.2	522
5	295	1.3	1.2	678
6	364	1.0	1.1	714
7	371	1.0	1.7	764
8	145	1.0	1.6	412
Mean	254	1.2	1.8	604
S.D.	89	0.4	1.4	142

Table 5
Pharmacokinetic parameters for DESOH

Subject	C_{max} (pmol/ml)	T_{max} (h)	Terminal half-life (h)	AUC (pmol/ml per h)
1	627	1.0	2.2	1740
2	670	2.0	1.5	2355
3	325	2.0	2.1	1361
4	612	1.5	3.7	2174
5	601	1.5	1.9	1980
6	630	1.3	17.3	2236
7	469	1.0	2.5	1500
8	466	1.0	2.2	1586
Mean	550	1.4	4.2	1866
S.D.	118	0.4	5.4	372

standard deviation due to subject 4 who had a half-life of 5.2 h. Excluding this subject, the average MEBOH half-life was about 1.4 h. The average terminal half-life for DESOH was 4.2 h with a large standard deviation. The later was due primarily to subject 6 who had a terminal half-life of 17.3 h and to a lesser extent to subject 4 who had a terminal half-life of 3.2 h. The longer observed half-lives in these subjects are the result of the detection of the metabolite up to 24 and 16 h, respectively. This permitted detection of a more slowly eliminating phase in these subjects. However, the shape of the plasma-concentration curves of these subjects did not differ substantially from those of the other six subjects. This observation suggests that this slowly eliminating phase may also be present in other subjects but was not detected due to analytical detection limits. Excluding subjects 4 and 6, the average DESOH terminal half-life was about 2.1 h. The AUCs for MEBOH averaged 600 pmol/ml per h, whereas the AUCs for DESOH averaged about 1900 pmol/ml per h. The mean ratio of the AUCs for DESOH to MEBOH was 3.1.

4. Conclusions

MEB was metabolized in humans, following p.o. administration, to give MEBOH and DESOH. Solid-phase extraction on ODS cartridges in conjunction with GC–MS analysis allowed MEBOH and DESOH to be quantitated in human plasma without interference from matrix components. The methodology allowed the pharmacokinetic parameters of both MEBOH and DESOH to be determined in humans following p.o. administration of MEB. These results suggest that systemic MEB exposure may be assessed from the plasma pharmacokinetic behavior of MEBOH and DESOH, however, subsequent work would be required to demonstrate linear metabolite pharmacokinetics with MEB dose before this conclusion could be validated.

References

[1] H. Haddad and G. Devroede-Bertrand, Med. Clin. N. Am., 65 (1981) 1377.

[2] K.J. Ivey, Gastroenterology, 68 (1975) 1300.
[3] A.D. Hertog and J. Van den Akker, Eur. J. Pharmacol., 139 (1973) 353.
[4] K. Luttecke, Curr. Med. Res. Opin., 6 (1980) 437.
[5] C. Tasman-Jones, N.Z. Med. J., 77 (1973) 232.
[6] L. Capurso, M. Koch, M. Tarquini, A. Dezi, C. Papi and P. Fracasso, Clin. Trial J., 21 (1984) 285.
[7] G. Hoogewijs and D. L. Massart, J. Chromatogr., 377 (1986) 391.
[8] R.G. Dickinson, P.V. Baker, M.E. Frankilin and W.D. Hooper, J. Pharm. Sci., 80 (1991) 952.
[9] A.A. Al-Angary, S.H. Khidr, S.S. Abd-Elhady, M.A. Bayomi, G.M. Mahrous, Anal. Lett., 25 (1992) 1251.
[10] A. Eshra, Alex. J. Pharm. Sci., 6 (1992) 198.