BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **20**: 211–216 (2006)
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/bmc.556

ORIGINAL RESEARCH

A validated chiral HPLC method for the determination of mebeverine HCl enantiomers in pharmaceutical dosage forms and spiked rat plasma

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Received 24 May 2005; accepted 22 June 2005

ABSTRACT: A new, precise, simple and accurate HPLC method was developed for the first time to separate and determine mebeverine enantiomers. Enantiomeric resolution was achieved on a cellulose Tris (3,5-dimethylphenyl carbamate) column known as Chiralcel OD, with UV detection at 263 nm. The mobile phase consisted of *n*-hexane, isopropyl alcohol and triethylamine (90:9.9:0.1 v/v/v). Sample run time was 18 min. On using the chromatographic conditions described, mebeverine enantiomers were well resolved with mean retention times of about 11 and 14 min. A linear response ($r > 0.999$) was observed over the concentration range 0.5–20 μg/mL racemic mebeverine. Precision, accuracy and stability were studied according to ICH guidelines. The limit of detection was found to be 0.05 μg/mL for each enantiomer of mebeverine. The proposed method was applied for analysis of mebeverine in commercially available tablets dosage formulations. Examples of application to biological samples are also given. Reanalysis of samples several weeks after the initial analysis showed no degradation of mebeverine.

KEYWORDS: Mebeverine; enantiomers; chiral HPLC assay; dosage forms; cellulose tris (3,5-dimethylphenyl carbamate) chiral stationary phase; plasma

INTRODUCTION

Meberverine hydrochloride, chemically known as 3,4-dimethoxybenzoic acid 4-[ethyl-2-(4-methoxyphenl)-1-methylethyl] aminobutylveratrate hydrochloride, has a direct, nonspecific antispasmodic action on smooth muscle. It is used in the treatment of abdominal pain and spasm associated with gastrointestinal disorders such as mucous colitis (Ritchie and Truelove, 1980; Evans *et al*., 1996; Sweetman, 1999).

Mebeverine has been used clinically for the treatment of irritable bowel syndrome for many years. The drug is official in *The British Pharmacopoeia* (2000), which describes a nonaqueous titration procedure for its assay in bulk, and a spectrophotometric method for its commercially available tablets. The analytical profile of the drug has been reviewed (Radwan *et al*., 1998). The reported methods for its determination are chromatographic, characterized by their good sensitivities. They include high performance liquid chromatography (Hoogewijs and Massart, 1986; Stockis *et al*., 2002), on-line micellar electrokinetic chromatography–mass spectrometry (Somsen *et al*., 2003), high-performance thin-layer chromatography (De Schutter *et al*., 1985; El-Walily *et al*., 1991), capillary supercritical fluid chromatography–mass spectrometry (Pinkston *et al*., 1993), gas chromatography–mass spectrometry, and online reversed-phase liquid chromatography–gas chromatography (Kristinsson *et al*., 1994; Tulich *et al*., 1996; Kraemer *et al*., 2000). Other alternatives include spectrophotometry (El-Walily *et al*., 1991; Shama and Amin, 2004) and potentiometric flow injection analysis (Ibrahim *et al*., 2005).

Enantioselective analysis has become increasingly important in the analysis of drugs. It is well known that many drugs are marketed as racemic mixtures despite the significant differences in the pharmacological, pharmacodynamics and pharmacokinetics of the individual enantiomers. It must be taken into account that one of the enantiomers can be more active or toxic than its antipode, or totally inactive (Aboul-Enein and Wainer, 1997). Although mebeverine has a chiral center as shown in Fig. 1, it is clinically administered as a racemic mixture. Mebeverine itself shows clear antispasmodic activity, but its main metabolites, mebeverine alcohol and veratric acid, do not seem to possess any action on smooth muscles (Stockis *et al*., 2002). Mebeverine administered i.m. exhibits a stronger effect on colon motility in man than when given via

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Abbreviations used: AUP, area under the peak.

Published online 6 September 2005

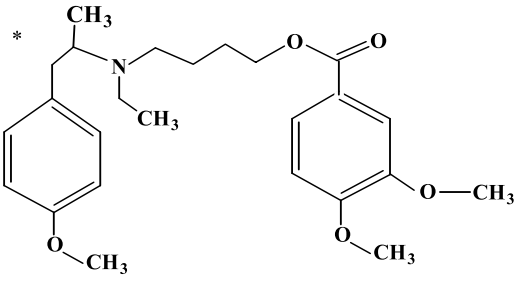

Figure 1. The chemical structure of mebeverine (the asterisk indicates the position of the chiral center).

the oral route. Therefore, the possibility that the mebeverine enantiomers are enantioselectively absorbed in patients cannot be excluded, and accordingly its effect could be due to one of the enantiomers. To our knowledge, no study has been published describing the stereoselectivity of mebeverine. Therefore, this investigation was undertaken to develop and validate chiral HPLC assay to separate mebeverine enantiomers. The utility of the proposed method to determine both enantiomers in two pharmaceutical dosage forms and in spiked rat plasma was also demonstrated.

EXPERIMENTAL

Chemicals and reagents. Racemic mebeverine HCl powder was a generous gift from SAJA Pharmaceuticals, Jeddah, Saudi Arabia. All other reagents and chemicals were analytical grade, and used as received.

Chromatographic system and conditions. A Waters HPLC system was used in this study. It was equipped with a Waters 484 variable UV absorbance detector (set at 263 nm), and a Waters 717 Plus auto-sampler. Waters 515 solvent delivery system was used to operate the isocratic flow. The data were collected with an Empower Pro Chromatography Manager Data Collection System, utilizing a Pentium 4 computer connected to Inkjet HP 895 Cxi printer.

The analytical column (250 mm × 4.6 i.d., 10 µm particle size) used was a Chiralcel OD column (Daicel Industries, Tokyo, Japan). The mobile phase consisted of *n*-hexane, isopropyl alcohol and triethylamine (90:9.9:0.1, v/v/v), filtered through a 0.22 µm Millipore filter and degassed by sonication. Separation was carried out isocratically, at ambient temperature (22 ± 1°C), and a flow rate of 1.0 mL/min, with ultraviolet (UV) detection at 263 nm. The injection volume was 100 µL.

Preparation of standard solutions. Stock solution of mebeverine was prepared by dissolving 1 mg/mL of racemic mebeverine HCl in methanol and stored in 4 mL amber glass vials at –20°C until used. Calibration standards were prepared daily by dilution with the mobile phase to give mebeverine concentrations in the range 0.5–20 µg/mL.

Determination of accuracy, precision and reproducibility. On a single day, the accuracy and precision of the within-day assay were studied at concentrations of 0.5, 5 and 10 µg/mL by measuring their relative standard deviation (RSD%). The reproducibility of the assay (within-day and between-day) was evaluated by comparing the linear regression analysis of three standard plots obtained from the results of six replicates on three different days within 3 weeks.

Determination of mebeverine in the pharmaceutical dosage forms. Duspatalin tablets (SAJA Pharmaceuticals, Jeddah, Saudi Arabia, under license from Solvay Pharmaceuticals BV, Weesp, Holland) and Spasmotalin tablets (Amriya Pharmaceutical Industry, Alexandria, Egypt), labeled as containing 135 and 100 mg of mebeverine HCl, respectively, were analyzed according to the proposed conditions. One tablet was grinded and the content was suspended in methanol and transferred to a 100 mL volumetric flask with methanol. The contents of the flask were vortexed for 2 min and sonicated for 15 min; the flask was then made up to volume with methanol. An aliquot of the contents was filtered and a specified volume of the filtrate was further diluted with the mobile phase to obtain final sample solutions which contained about 10 µg/mL of mebeverine HCl. Six tablets were analyzed for each dosage form. The content of each enantiomer was determined using external standard working solutions from pure reference compound run simultaneously.

Determination of mebeverine in spiked rat plasma. Two hundred microliters of rat plasma samples were spiked with 20–50 µL mebeverine stock solution in 1.8 mL disposable polypropylene microcentrifuge tube. The tube was vortexed for 30 s. The solution was mixed with 600 µL acetonitrile, vortexed at high speed for 1 min, and centrifuged at 20,000 rpm for 15 min. The supernatant was transferred to a 5 mL centrifuge tube and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 150 µL of the mobile phase. Each concentration was prepared at least in triplicate.

The recovery of each enantiomer was assessed by extracting plasma specimens spiked to contain drug concentrations from 0.5 to 10 µg/mL. The area under the peak (AUP) was then compared with the AUP for mobile phase standards containing equivalent amounts of the drug without extraction.

In order to assess the accuracy and precision of the within-day assay, six extractions of plasma samples of mebeverine, at concentrations 0.5, 5 and 10 µg/mL, were performed on a single day by measuring their RSD%. The reproducibility of the assay (within-day and between-day) was also evaluated by comparing the linear regression analysis of three standard plots obtained from the results of six replicate assays on three different days within 3 weeks.

Stability. The freeze–thaw stabiltity of mebeverine HCl enantiomers was evaluated at three concentrations 5.0, 10.0 and 20.0 µg/mL. These samples were analyzed in triplicate without being frozen first, and then stored at −20°C and thawed at room temperature (22°C).

The stability of mebeverine in reconstituted extracts during run-time in an HPLC auto-injector was investigated. Samples were kept in the sample rack of the auto-injector and injected into HPLC system every 2 h from 0 to 24 h.

Data and statistical analysis. All results were expressed as the mean ± SD of six replicates. The results were calculated by linear regression without weighing, using the formula $Y = a + bX$, where Y is the AUP of the drug, a is the intercept, b is the slope and X is the concentration of mebeverine. The RSD% was calculated for all values. Since no internal standard was available for the above-mentioned conditions, the AUP was used for each enantiomer. Student's t-test was used to examine the concentration difference at each day, and one-way analysis of variance (ANOVA) was employed to evaluate the reproducibility of the assay. The level of confidence was 95%.

RESULTS AND DISCUSSION

Chromatography

In recent years, the direct separation of enantiomers by chiral chromatography has been the target of intense research. Enantiomeric selectivity is usually achieved through the appropriate choice of a chiral stationary phase and the mobile phase conditions. In this study a stereoselective HPLC method was developed and validated for the rapid quantitative determination of mebeverine enantiomers. Figure 2 shows a representative chromatogram, which indicates that mebeverine peaks are free from matrix interference [Fig. 2(A)] and baseline resolution of the two enantiomers was achieved under the current HPLC conditions [Fig. 2(B)]. The mean retention times of the two enantiomers were 11 and 14 min, respectively.

The performance of the HPLC assay was assessed using the parameters linearity, limit of quantitation (LOQ), limit of detection (LOD), specificity, stability, precision, accuracy and recovery. Various percentages of *n*-hexane, isopropyl alcohol and triethylamine in the mobile phase were tested to achieve the optimum resolution between mebeverine enantiomers. Increasing the concentration of isopropyl alcohol resulted in poor separation of the two enantiomers. Lower isopropyl alcohol contents resulted in better resolution, but under such conditions the retention times drastically increased. Table 1 represents the results of the system suitability test. Accordingly, the selected conditions showed an optimum resolution and detection of mebeverine enantiomers.

Table 1. System suitability ($n = 3$)

Analyte	k'[a]	R_s[b]	N[c]	T[d]	α[e]
Enantiomer I	2.56	1.78	1820	1.44	1.35
Enantiomer II	3.45		1521	1.58	

[a] Capacity factor; [b] USP resolution; [c] number of theoretical plates; [d] tailing factor; [e] enantioselectivity.

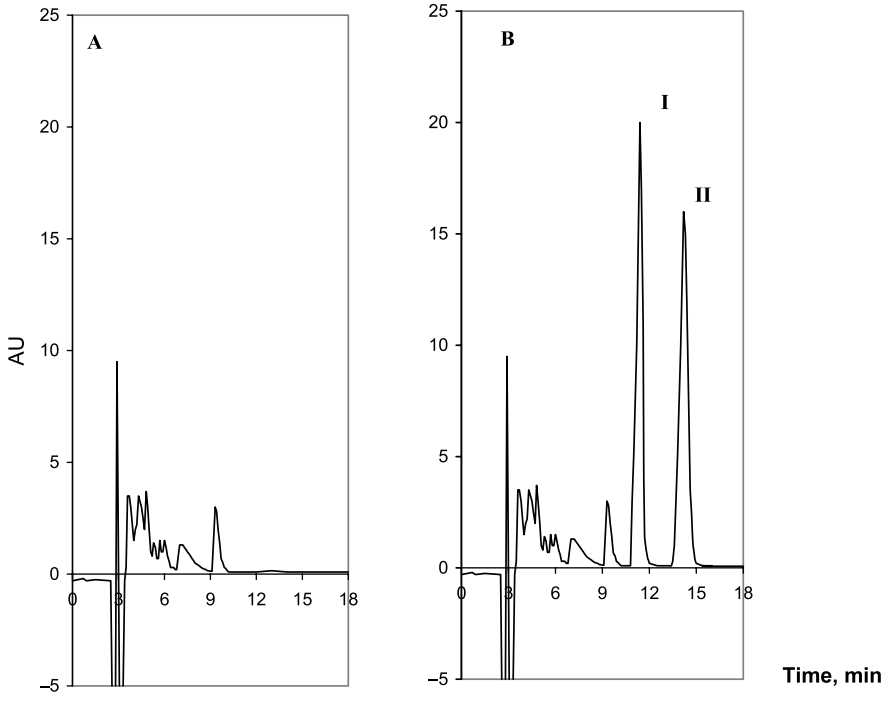

Figure 2. Representative chromatograms, analyzed on Chiralcel OD column, of extracted drug-free plasma (A), and plasma samples spiked with 5 µg/mL of Mebeverine HCl (B). **I**, Enantiomer I and **II**, Enantiomer II.

Table 2. Mean calibration curves of mebeverine enantiomers (n = 18)

	Linearity ($Y = a + bX$)[a]		
Analyte	$a \pm$ SD	$b \pm$ SD	Correlation coefficient
Enantiomer I	32484 ± 6106	66970 ± 1565	0.9994 ± 0.001
Enantiomer II	29575 ± 6200	67023 ± 2104	0.9991 ± 0.0007

[a] $Y = a + bX$, where Y is the area under the peak and X is the enantiomer concentration in μg/mL.

Linearity

The regression plots showed that there was a linear dependence of the AUP on the concentration range (0.5–20.0 μg/mL). Table 2 shows the mean values of the standard deviation of the slope and intercepts. The good linearity of the calibration curve and the negligible scatter of the experimental points are clearly evident by the values of the correlation coefficients and the standard deviations.

Limit of detection and quantitation

The minimum detectable concentration (LOD) of each enantiomer of mebeverine was 0.05 μg/mL (signal-to-noise ration, S/N = 3). The lowest quantitation level (LOQ) was found to be 0.1 μg/mL (S/N = 10) for each enantiomer.

Specificity

The specificity of the method was investigated by observing any interference encountered from either common tablet excipients or endogenous plasma components. No interference was encountered from common tablet excipients or endogenous plasma components as shown in Fig. 2(A) for plasma. Veratric acid, a direct product of enzymatic hydrolysis of mebeverine and a major metabolite which is found readily *in vivo* and in *vitro* (Stockis *et al*., 2002), did not interfere with the assay and so the method could be used as a stability-indicating assay. The interference of the other metabolites, namely mebeverine alcohol and mebeverine acid, will be the scope of further communication.

Precision, accuracy and reproducibility

The within- and between-day precision (RSD%) calculated following replicate analysis (n = 6) of mebeverine enantiomers was <4% over a wide range of concentrations. The reproducibility of the assay was evaluated by comparing the linear regressions of three standard plots prepared at three different days over a 3 week period. The results of this evaluation are summarized in Table 3. The mean correlation coefficient was >0.998 with the RSD% of the slopes of the three lines 2.3 and 3.1% for enantiomer I and II, respectively. Analysis of variance (Table 3) of the data indicated no significant difference ($p > 0.05$) in the slopes, within-day and between-day, of the calibration curves. The results confirmed the reproducibility of the assay method.

Analytical application

The applicability of the proposed method in chiral analysis was evaluated by the determination of mebeverine enantiomers in tablets and spiked rat plasma. Table 4 summarizes the results obtained in the two formulations assayed, namely Duspatalin and Spasmotalin tablets. Compared with the content of mebeverine declared by the manufacturer, the recoveries were in the ranges 95.5–97.5%. All the analytical values fell within labeled amount of 95–105% required by the *The British Pharmacopoeia* (2000).

Clinical pharmacological studies indicated that about 95% of orally administered mebeverine in humans is metabolized via five partially overlapping pathways (Stockis *et al*., 2002). A simple method was required to determine mebeverine in the biological fluids following

Table 3. Reproducibility of data from standard plots of mebeverine enantiomers (enantiomers I and II)

Standard plot[a]	Slope[b]		Intercept[b]		Correlation coefficient[b]	
	I	II	I	II	I	II
1	66848	67075	57449	64617	0.9988	0.9983
2	68255	68666	21755	15948	0.9997	0.9996
3	65807	65329	18248	8161	0.9997	0.9995

No significant difference between slopes or correlation coefficient of the three curves at $p > 0.05$.
[a] Obtained from assays on three different days.
[b] The mean of six determinations.

Table 4. Determination of mebeverine HCl in pharmaceutical dosage forms ($n = 5$)

Preparation	Strength, mg	Enantiomer I		Enantiomer II	
		Content (%)	RSD (%)	Content (%)	RSD (%)
Duspatalin (SAJA)	135	95.5	9.3	96.1	9.8
Spasmotalin (Amriya)	100	96.6	8.2	97.5	7.3

Table 5. Within-day precision and accuracy of mebeverine enantiomers (enantiomers I and II) recovery from spiked rat plasma samples ($n = 6$)

Spiked concentration (μg/mL)	Mean ± SD measured concentration (μg/mL)		Recovery, % ± SD		Relative standard deviation, %	
	Enat I	Enat II	Enat I	Enat II	Enat I	Enat II
0.5	0.43 ± 0.033	0.49 ± 0.008	85.9 ± 7.8	97.8 ± 1.7	7.8	1.7
5.0	4.98 ± 0.231	4.72 ± 0.450	99.5 ± 4.6	94.3 ± 9.6	4.6	9.6
10.0	8.05 ± 0.916	8.21 ± 0.832	80.5 ± 11.4	82.1 ± 10.1	11.4	10.1

its administration at normal therapeutic dose levels in order to carry out bioavailability studies on different dosage forms of mebeverine. Plasma spiked with known amounts of mebeverine HCl was analyzed. Figure 2(A) and (B) shows the chromatograms obtained for blank plasma and plasma spiked with 5 μg/mL mebeverine HCl. No interference was observed in blank plasma at the retention times of both mebeverine enantiomer peaks.

The least-squares regression calibration curve was found to be linear at plasma concentrations between 0.5 and 10 μg/mL of mebeverine. The mean linear regression equation of the peak area ratios (Y) vs drug concentrations (X) of mebeverine in μg/mL was typically of the form $Y = (a \pm SD) + (b \pm SD)\ X$ and it was $Y = (11439 \pm 5236) + (51442 \pm 9182)\ X$ for enantiomer I and $Y = (4686 \pm 878) + (56924 \pm 9243)\ X$ for enantiomer II with mean correlations of 0.998 ± 0.0012 and 0.997 ± 0.0046, respectively. The detection limit of the assay was 50 ng/mL for each enantiomer at S/N > 3.

Within-day precision and accuracy of the method were determined from replicate analysis ($n = 6$) of three spiked plasma test standards at concentrations within the linear range of the assay for each drug (Table 5). The mean percentage recovery of 0.5–10 μg/mL of mebeverine was 88.6 ± 9.8 for enantiomer I and 91.4 ± 8.2 for enantiomer II with RSD% of ≤11.4 for both enantiomers. Table 5 shows the recoveries of three different concentrations of mebeverine. Extraction efficacy was found not to significantly vary among different concentrations of mebeverine enantiomers.

The reproducibility of the assay was evaluated by comparing the linear regressions of three standard plots prepared at three different days over a 3 week period. The mean correlation coefficient was >0.992 with the RSD% of the slopes of the three lines <12%. Analysis of variance of the data indicated no significant difference ($p > 0.05$) in the slopes, within-day and between-day, of the calibration curves. The results confirmed the reproducibility of the assay method.

Stability

No significant degradation was detected in the samples of racemic mebeverine following three freeze–thaw cycles. There was no significant decomposition observed after the reconstituted extracts of racemic mebeverine were stored in the auto-injector at room temperature for 24 h.

CONCLUSION

For the first time a chiral HPLC method was developed for the stereoselective determination of mebeverine HCl enantiomers. The method was completely validated, and the obtained results demonstrated that the proposed method is useful for the application to mebeverine pharmaceutical formulation quality control. Further, the results obtained from spiked rat plasma will be followed by a pharmacokinetic study of mebeverine in rats and human in the near future.

Acknowledgment

The first author wishes to express her gratitude to Dr Ahmed Mitwalli Awad for his endless support during this study. One of the author (Professor Aboul-Enein) wishes to express his gratitude to the administration of King Faisal Specialist Hospital and Research Centre for its support.

REFERENCES

Aboul-Enein HY and Wainer IW. *The Impact of Stereochemistry on Drug Development and Use*. Wiley: New York, 1997.

De Schutter JA, Van der Weken G, Van den Bossche W and De Moerloose P. Reversed-phase high-performance thin-layer chromatography of mebeverine hydrochlordie and related compounds. *Journal of Chromatography A* 1985; **350**: 135.

El-Walily AFM, El-GindyA and Bedair MF. Application of first-derivative UV-spectrophotometry, TLC-densitometry and liquid chromatography for the simultaneous determination of mebeverine hydrochloride and sulpiride. *Journal of Pharmaceutical and Biomedical Analysis* 1999; **21**: 535.

Evans PR, Bak YT and Kellow JE. Mebeverine alters small bowel motility in irritable bowel syndrome. *Aliment Pharmacology and Therapeutics* 1996; **10**: 787.

Hoogewijs G and Massart DL. Development of a standardized analysis strategy for basic drugs using ion-pair extraction and high-performance liquid chromatography. VIII. Method construction for the determination of mebeverine in tablets and biological fluids. *Journal of Chromatography B: Biomedical. Science and Applications* 1986; **377**: 391.

Ibrahim H, Issa YM and Abu-Shawish HM. Potentiometric flow injection analysis of mebeverine hydrochloride in serum and urine. *Journal of Pharmaceutical and Biomedical Analysis* 2005; **36**: 1053.

Kraemer T, Bickeboeller-Friedrich J and Maurer HH. On the metabolism of the amphetamine-derived antispasmodic drug mebeverine: gas chromatography–mass spectrometry studies on rat liver microsomes and on human urine. *Drug Metabolism and Disposition* 2000; **28**: 339.

Kristinsson J, Snorradottir I and Johannsson M. The metabolism of mebeverine in man: Identification of urinary metabolites by gas chromatography/mass spectrometry. *Pharmacology and Toxicology* 1994; **74**: 174.

Pinkston JD, Venkatramani CJ, Tulich LJ, Bowling DJ and Wehmeyer KR. Evaluation of capillary supercritical fluid chromatography with mass spectrometric detection for the analysis of a drug (mebeverine) in a dog plasma matrix. *Journal of Chromatography A* 1993; **622**: 209.

Radwan MA, Foda NH and Al Deeb OA. *Analytical Profile of Drug Substances and Excipients*, Vol. 25, Brittain HG (ed). Academic Press: New York, 1998; 165.

Ritchie JA and Truelove SC. Comparison of various treatments for irritable bowel syndrome. *British Medical Journal* 1980; **281**: 1317.

Shama SA and Amin AS. Spectrophotometric microdetermination of efopam, mebeverine and phenylpropanolamine hydrochloride in pharmaceutical formulations using alizarins. *Spectrochimica Acta Part A* 2004; **160**: 769.

Somsen GW, Mol R and de Jong GJ. On-line micellar electrokinetic chromatography–mass spectrometry: feasibility of direct introduction of non-volatile buffer and surfactant into the electrospray interface. *Journal of Chromatography A* 2003; **1000**: 953.

Stockis A, Guelen PJ and Vos Dde. Identification of mebeverine acid as the main circulating metabolite of mebeverine in man. *Journal of Pharmaceutical and Biomedical Analysis* 2002; **29**: 335.

Sweetman SC (ed.). *Martindale, The Complete Drug Reference*, 33rd edn. Pharmaceutical Press: Massachusetts, 1999; 1233.

The Britsh Pharmacopoeia. HMSO: London, 2000; 2076.

Tulich LJ Randall JL, Kelm GR and Wehmeyer KR. Determination of two mebeverine metabolites, mebeverine alcohol and desmethylmebeverine alcohol, in human plasma by a dual stable isotope-based gas chromatographic–mass spectrometric method. *Journal of Chromatography B: Biomedical Applications* 1996; **682**: 273.