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Note

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

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Mebeverine is a substance with a spasmolytic activity of the muscolotropic type [1—3]. It has been claimed to have a potent and selective activity on gastrointestinal hypermotility and has frequently been used, mainly in the treatment of the irritable colon syndrome. Although its introduction in pharmacotherapy is far from recent, the literature relative to this drug is very scarce. Little or nothing has been published concerning, for instance, the stability and pharmacokinetics of mebeverine. In an attempt to elucidate some of these aspects, it was our assignment first to construct an analytical method for the determination of mebeverine and its degradation products. The present paper reports on the development of an assay method for mebeverine, which emanates from a standardized analysis strategy for basic drugs using ion-pair extraction and high-performance liquid chromatography (HPLC). The philosophy and advantages of this strategy [4, 5], its application to pharmaceutical dosage forms [6, 7] and cosmetics [8], and its use for drug level determinations in saliva [9] and plasma [10] have been published elsewhere.

EXPERIMENTAL

Chemicals and reagents

Mebeverine hydrochloride, veratric acid and 4-[ethyl(*p*-methoxy-α-methylphenethyl)amino]-1-butanol hydrochloride were gifts from Trenker Pharma-

*For parts I—VII, see refs. 4—10.

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ceutical Labs. (Brussels, Belgium). Imipramine hydrochloride was kindly supplied by Ciba-Geigy (Brussels, Belgium). Acetonitrile (Merck, Darmstadt, F.R.G.) was HPLC-grade, propylamine (Fluka, Buchs, Switzerland) was technical grade and water was demineralized, double-distilled and passed through a water purification system (Gelman Sciences Water-I system) before use. All other reagents were analytical grade and purchased from Merck.

Apparatus

The liquid chromatographic equipment consisted of a three-solvent delivery pump (Varian Model 5060), a syringe-loading sample injector (Rheodyne Model 7125 injection valve) with a sample loop of 10 μl or 100 μl, a variable-wavelength detector (Varian Model UV-100) and a recorder (BD-8, Kipp & Zonen, The Netherlands). The chromatograph was interfaced to a chromatographic data system (Varian Vista CDS 401).

Chromatographic parameters

Chromatography was performed on a 250 × 4 mm I.D. stainless-steel column packed with 10 μm (particle diameter) CN-bonded phase (LiChrosorb CN Hibar column, Merck). When running biological samples, the analytical column was protected with a 30 × 4 mm pre-column dry-packed with the same packing material (LiChrosorb CN with 10 μm particle diameter packing material), and the columns were back-flushed overnight with acetonitrile, dichloromethane and acetonitrile, respectively, using a slow gradient and a very low flow-rate.

The mobile phase used throughout this study consisted of acetonitrile—water—propylamine (80:20:0.01) and the flow-rate was 2 ml/min. Detection was carried out at 227 nm and at a sensitivity of 0.005 a.u.f.s. (plasma) or 0.02 a.u.f.s. (urine) unless stated otherwise.

Procedure for tablet analysis

Ten randomly taken tablets of the same lot were pulverized in a mortar. An aliquot of the homogenized powder, corresponding to one tenth of the mean weight of a tablet, was accurately weighed, brought into a 50-ml volume flask and suspended in ca. 40 ml of the eluent by ultrasonication for ca. 15 min. The volume flask was brought to volume, the contents were thoroughly homogenized and then centrifuged for ca. 10 min. A 10-μl portion of the clear supernatant was injected onto the HPLC column. Calibration curves were constructed by plotting the peak area of the analyte versus concentration.

Procedures for biological fluids

Extraction of urine. To 1 ml of urine, 100 μl of internal standard solution were added in centrifuge tubes equipped with PTFE-covered screw caps. After vortexing, 10 ml of the counter-ion solution (0.05 *M* sodium-*n*-octylsulphate in phosphate buffer, pH 3.0, I = 0.4) and 5 ml of chloroform were added. Partitioning was performed by gently shaking the tubes longitudinally in a shaking bath for 30 min (vortexing for a few minutes might be equally efficient but was not investigated). After centrifugation for 5—10 min, the aqueous phase was discarded and 4 ml of the chloroform phase were trans-

ferred to a clean vial with a conical bottom and evaporated to dryness under a gentle nitrogen stream at ca. 40°C. Each extract was reconstituted immediately prior to chromatography with 200 μl of dichloromethane; half of it was injected into the chromatograph.

Extraction of plasma. To 4 ml of plasma, 100 μl of internal standard solution were added in centrifuge tubes equipped with PTFE-covered screw caps. After vortexing, 8 ml of acetonitrile were added dropwise during continuous vortexing. The tube was allowed to stand for 15 min prior to centrifugation for 10—15 min. The supernatant was decanted into a clear centrifuge tube and the protein pellet and the walls of the tube containing it were gently washed with water, which was also added to the new centrifuge tube. After evaporation of the acetonitrile at ca. 45°C using a gentle nitrogen stream, 10 ml of the counter-ion solution and 5 ml of chloroform were added and the extraction was further carried out as for urine.

Internal standard solution. The internal standard solution contained 800 ng imipramine hydrochloride per 100 μl water.

Calibration. Calibration of the assay was performed using spiked blank samples over the range 20—3500 ng/ml for both mebeverine and its alcohol derivative and in both plasma and urine. The calibration curves were constructed by plotting the analyte/internal standard peak-area ratio versus analyte concentration. The data were fitted by least-squares linear regression analysis.

Extraction recoveries. The extraction yields were determined by intrapolation on a calibration curve (peak area versus concentration) of aqueous nonextracted standards.

RESULTS AND DISCUSSION

Stability of mebeverine

Preliminary experiments using HPLC, thin-layer chromatography (TLC) and nuclear magnetic resonance (NMR) showed decomposition of mebeverine to proceed according to the scheme presented in Fig. 1. The main degradation products of mebeverine to be expected in the bulk powder and in tablets are hence the hydrolysis products veratric acid and 4-ethyl(*p*-methoxy-α-methylphenethyl)amino-1-butanol, henceforth called the alcohol derivative. A chromatogram of a mixture of mebeverine and these two hydrolysis products using the selected HPLC conditions is shown in Fig. 2A. It can be seen that excellent resolution is obtained within a short analysis time. Injection of a mebeverine solution subjected to drastic alkaline conditions showed total breakdown of the molecule and the absence of a peak at 3.2 min in the chromatogram. This demonstrates that no degradation product elutes with the same retention time as mebeverine. Consequently, the chromatographic conditions are perfectly suitable for stability-indicating tests.

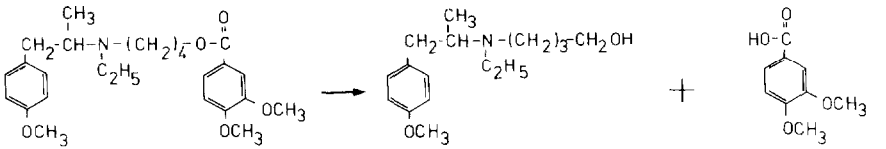

Fig. 1. Decomposition scheme.

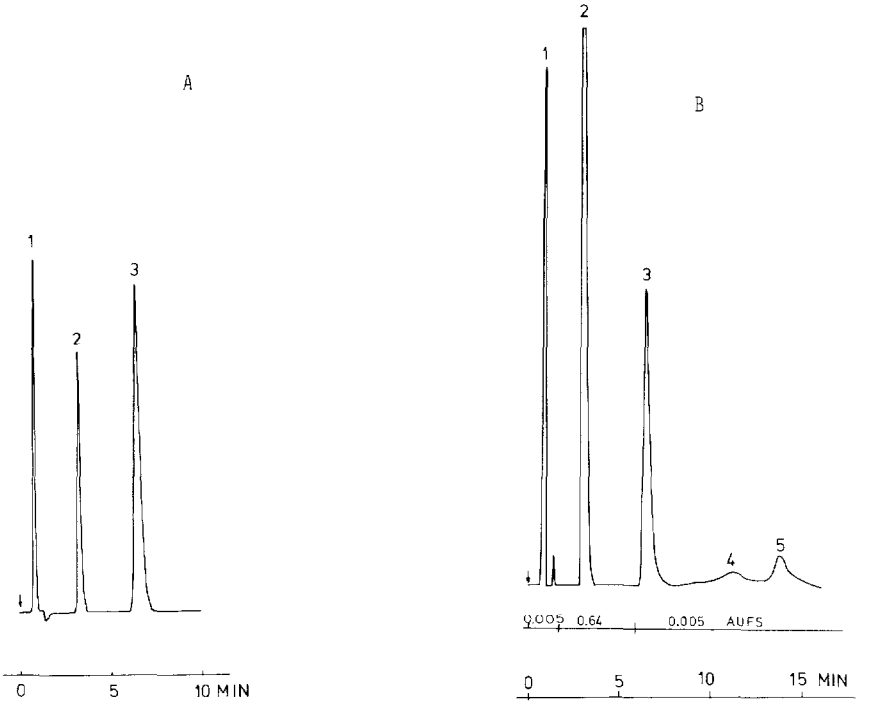

Fig. 2. (A) Chromatogram of mebeverine and its hydrolysis products. For chromatographic conditions, see text. (B) Chromatogram of a tablet. For chromatographic conditions, see text. Peaks: 1 = veratric acid; 2 = mebeverine; 3 = alcohol derivative; 4 = impurity; 5 = impurity.

The detection limits on the column at 227 nm, at a signal-to-noise ratio of 3 and at a detector sensitivity of 0.005 a.u.f.s. were 2 ng for mebeverine and 7 ng for the alcohol derivative.

The HPLC method was used to qualitatively study the stability of mebeverine hydrochloride in solution. Neutral and acidic aqueous solutions, stored at ambient temperature, can be kept for several days before degradation starts to show. Alkaline solutions, however, degrade extremely rapidly and total breakdown can be achieved even at ambient temperature. Solutions of mebeverine hydrochloride in chloroform and dichloromethane are stable. Even evaporation of the solvent at temperatures up to 150°C and reconstitution of the residue causes no deterioration of the molecule. This knowledge is important since it allows enrichment of plasma or urine extracts by evaporation (see below). It can be concluded that mebeverine is relatively stable except in alkaline aqueous solution.

Different lots of bulk powder and different lots of tablets were investigated for the presence of decomposition products. In order to detect the lowest amount possible, relatively concentrated solutions were injected. The recovery and precision of the method were evaluated by performing six replicate assays on a recent lot of tablets. The recovery was found to be 98.2 ± 0.8%. No degradation products or impurities were detected.

This was also the case for tablets and powders kept at ambient temperature for one year and at 50°C for three months. The method was also applied to

a batch of bulk powder and the corresponding tablets, produced in an early stage of the optimization process of the mebeverine synthesis on laboratory scale. Several impurities could be detected. A representative chromatogram of such a tablet is shown in Fig. 2B. The percentages of impurities relative to the amount of mebeverine were found to be 0.2% (veratric acid), 0.2% (alcohol derivative), 0.005% (peak 4) and 0.02% (peak 5).

Determination in plasma and urine

The advantages of the ion-pair extraction technique in comparison to a classical extraction at high pH have been demonstrated previously [1, 3, 8]. In the present application, an additional advantage can be found in the possibility of extracting a basic compound at low pH. A classical extraction at high pH indeed results in hydrolysis of mebeverine. At pH 3.0, however, no

TABLE I

EXTRACTION RECOVERIES

Sample	Concentration (ng/ml)	Extraction recovery (%)	
		Mebeverine	Alcohol derivative
Plasma	100	98.4 ± 2.6 (n = 8)	97.1 ± 3.2 (n = 6)
	300	96.2 ± 3.7 (n = 6)	95.7 ± 4.1 (n = 6)
Urine	600	95.6 ± 1.7 (n = 6)	92.4 ± 1.1 (n = 6)

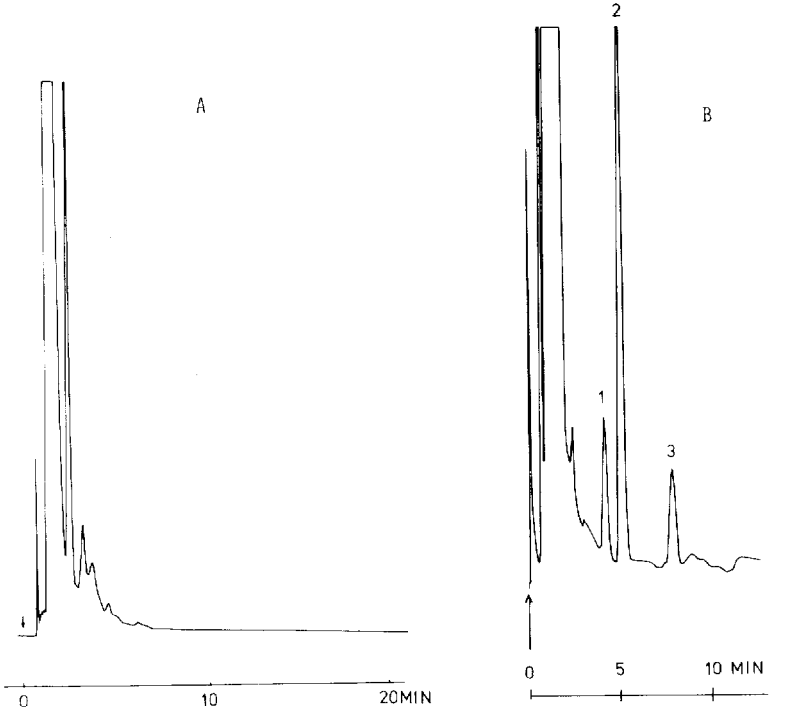

Fig. 3. (A) Chromatogram of a blank urine sample. For chromatographic conditions, see text. (B) Chromatogram of a plasma standard. For chromatographic conditions, see text. Peaks: 1 = mebeverine (24 ng/ml); 2 = internal standard; 3 = alcohol derivative (26 ng/ml).

decomposition of the analyte occurs during the extraction step and the use of ion-pair formation with NaOS results in high extraction recoveries, as can be seen from Table I. The purity of the extracts was evaluated using blank samples of plasma (4 ml) and urine (1 ml) from different individuals and from two laboratory animals (dog, pig). The resulting chromatograms all showed a similar pattern. An example is shown in Fig. 3A and it can be seen that endogenous compounds do not interfere.

Although the assay method is intended for studying the pharmacokinetics of mebeverine in laboratory animals and selected human volunteers, so that interferences from other drugs are not to be expected, some other drugs were chromatographed in order to evaluate the selectivity of the method. Their retention times, relative to mebeverine, are tabulated in Table II, and it can be seen that none of these substances interferes. Imipramine elutes inbetween mebeverine and its alcohol derivative and exhibits an acceptable absorbance at 227 nm. Consequently, it was selected as internal standard.

TABLE II

RETENTION TIME OF VARIOUS DRUGS RELATIVE TO MEBEVERINE

Drug	Relative retention time	Drug	Relative retention time
Lorazepam	0.42	Tiapride	1.20
Trimethoprim	0.47	Pholcodine	1.26
Pyrimethamine	0.47	Melperone	1.37
Loxapine	0.49	Imipramine	1.48
Bromperidon	0.54	Piperocaine	1.49
Haloperidol	0.54	Chlorpheniramine	1.58
Propoxyphene	0.65	Metoclopramide	1.64
Mexiletine	0.81	Triprolidine	1.72
Bromazine	0.87	Acebutolol	1.93
Doxepine	0.94	Mebeverine alcohol derivative	2.13
Mebeverine	1.00	Nortriptyline	3.80
Thioridazine	1.09	Desipramine	5.28

The linearity of the calibration curves (peak area/peak area internal standard versus concentration) for mebeverine and its alcohol derivative was evaluated in both plasma and urine in concentrations up to 3.5 μg/ml. The calibration curves passed through the origin and were linear [e.g. mebeverine, $r^2 = 0.998$ (plasma, $n = 6$) and 0.998 (urine, $n = 3$); alcohol derivative, $r^2 = 0.999$ (plasma, $n = 6$) and 0.998 (urine, $n = 6$)] up to at least 3.5 μg/ml, hence providing a wide dynamic range. A chromatogram of a plasma standard containing 24 ng/ml mebeverine and 26 ng/ml alcohol derivative is shown in Fig. 3B. The day-to-day precision at the 100 ng/ml plasma level was evaluated to be 4.2 and 5.6% ($n = 6$), respectively. In order to demonstrate the usefulness of the assay method for analysing real samples, some preliminary results of a pharmacokinetic study in a laboratory animal are reported: 50 mg of mebeverine hydrochloride were administered intravenously to a pig (20 kg); blood samples were withdrawn at various times and analysed using the method described.

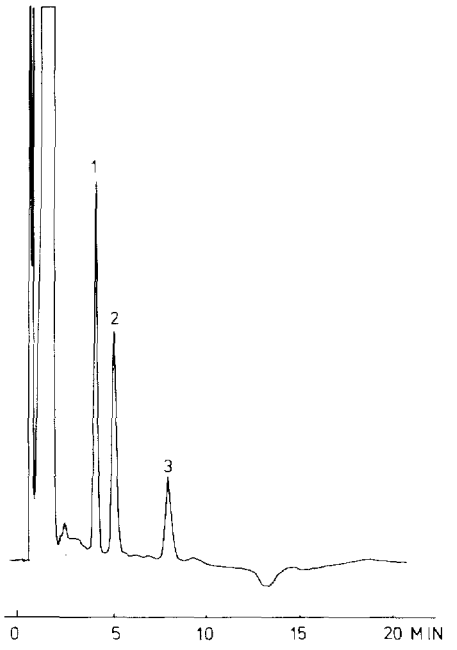

Fig. 4. Chromatogram of a plasma sample from a pig. Sampling was performed 1 h after intravenous administration of 50 mg of mebeverine hydrochloride. For chromatographic conditions, see text. (detector sensitivity: 0.02 a.u.f.s.). Peaks: 1 = mebeverine (126 ng/ml); 2 = internal standard; 3 = alcohol derivative (57 ng/ml).

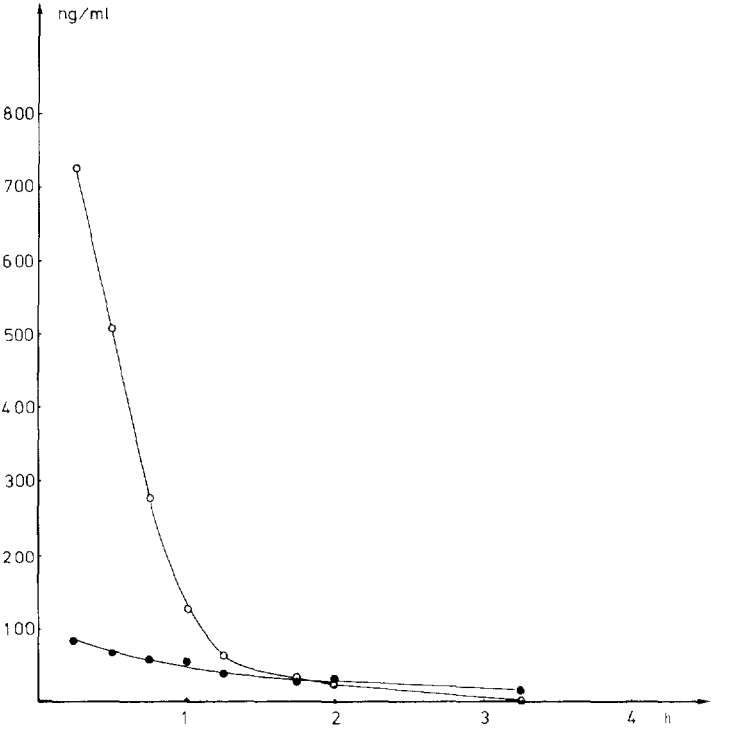

Fig. 5. Plasma–time profile of mebeverine (○) and its alcohol derivative (●) in a pig (20 kg) after intravenous administration of 50 mg of mebeverine hydrochloride.

Fig. 4 shows a typical chromatogram and the plasma–time profile is shown in Fig. 5. It can be seen that the plasma levels of mebeverine and the alcohol derivative are rather low and that elimination occurs quite rapidly. The method is currently applied for studying the pharmacokinetics of mebeverine in the dog and in humans [11].

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