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Enantioselective high-performance liquid chromatographic determination of omeprazole in human plasma

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Abstract

A new stereoselective HPLC assay was developed to isolate omeprazole enantiomers from human plasma using C_2 solid-phase extraction cartridges and an analogue was used as internal standard. Recoveries of the (+)-isomer were 83.4 and 89.7% at 100 and 250 ng/ml, respectively. Recoveries of the (−)-isomer were 78.4 and 82.8%, respectively. Recovery of the internal standard averaged 77.2%. Direct chiral separation of the enantiomers is achieved on a Resolvosil BSA-7 chiral column (15 cm × 4 mm I.D.) and a matching guard column. The mobile phase is a variable amount of *n*-propanol (0.05–1.0%) in 0.05 *M* ammonium phosphate buffer (pH 7.0) and the flow-rate is 1.5 ml/min. Drug absorbance is monitored at 302 nm. Standard curves are linear from 15 to 250 ng/ml for each enantiomer. The coefficients of variation for intra-day precision at each concentration over the range of the standard curve were between 0.98 and 10.87%. The coefficients of variation for inter-day precision for the analyses of omeprazole enantiomers in plasma (30 and 175 ng/ml) were less than 10% over a four month interval.

1. Introduction

The membranes of the parietal cells of the gastric mucosa secrete gastric acid into the stomach. Current therapy for ulcers is based on one of several approaches: neutralize excess acid in the stomach, inhibit acid production or combat the bacteria that may promote ulcer formation. Omeprazole, a substituted benzimidazole, is a potent anti-ulcer agent that suppresses the secretion of gastric acid by the inhibition of the H^+/K^+ ATPase system [1]. The structure of omeprazole is shown in Fig. 1.

The differences in disposition of enantiomers after administration of racemic compounds is increasingly important. As more questions are answered about the pharmacological activity of each enantiomer and the possible mechanisms involved, the more precisely one can devise a drug design strategy. The goal is to isolate and eliminate the compound responsible for any adverse side effects while maintaining the therapeutic value of the drug.

Much work has been done previously to separate the enantiomers of omeprazole and other racemic compounds of similar structure using a variety of columns and mobile phases. The columns most commonly used are proteins immobilized on a stationary support since when used in reversed-phase mode with polar analytes, the retention can be markedly affected by the mobile phase composition [2–4]. Although the

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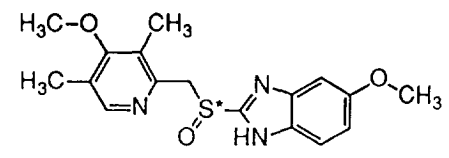
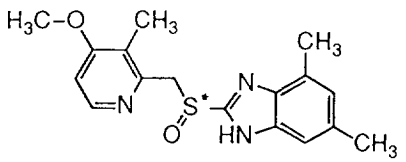


Omeprazole

$C_{17}H_{19}N_3O_3S$ Mol wt. = 345.4



Internal Standard

$C_{16}H_{17}N_3O_2S$ Mol wt. = 315.4

*Chiral center of interest

Fig. 1. Chemical structure of omeprazole and the internal standard.

exact mechanism of action is not clear, work done with chiral sulfur compounds on cellulose-based chiral stationary phases [5] suggest that hydrogen bonding and polar interaction between the solute and the stationary phase may be important. Initial chromatographic parameters for omeprazole were established by Allenmark et al. [6]. However, the ratio of enantiomers of omeprazole after oral or i.v. administration has not been reported. A stereoselective HPLC procedure was developed in human plasma to determine the concentrations of omeprazole enantiomers and support a clinical study in healthy male subjects. A graph of representative plasma concentration–time profiles after administration of omeprazole enantiomers is shown in Fig. 3.

2. Experimental

2.1. Chemicals

HPLC-grade methanol, acetonitrile and *n*-propanol and ACS-grade ammonium phosphate, dibasic sodium carbonate and sodium bicarbonate were purchased from Fisher Scientific (Pittsburgh, PA, USA). Heparinized human control plasma was purchased from Sera-tec Biologicals (New Brunswick, NJ, USA). All solutions were prepared with deionized water.

Solid-phase C_2 (100 mg) extraction (SPE) cartridges were obtained from Varian Analytical Instruments (Sunnyvale, CA, USA).

Omeprazole standard was obtained from the Chemical Data Department of Merck Research Laboratories (Rahway, NJ, USA) while the internal standard, 2-([(4-methoxy-2-pyridinyl)-methyl]sulfinyl)-4,6-dimethyl-1h-benzimidazoled (Fig. 1) was obtained from Astra (Hassle AB, Molndal, Sweden). Omeprazole standard solutions and the internal standard solution (5 μg/ml) were prepared daily and monthly, respectively. All standard solutions were protected from direct light.

2.2. Instrumentation and chromatographic conditions

The chromatographic system consisted of a Waters Model 6000A pump, a WISP 712 automatic injector (both from Millipore Corporation, Waters Chromatography Division, Milford, MA, USA) and a Kratos Spectroflow 783 variable-wavelength detector (Applied Biosystems, Foster City, CA, USA). Omeprazole and the internal standard were monitored at 302 nm, with 0.001 AUFS. The detector output was exported to a Hewlett-Packard 3357 Laboratory Automation System via a Hewlett-Packard Model 18652A A/D interface.

Drug separations were achieved on a 15×0.4 cm I.D. bovine serum albumin (5 μm) column (Resolvosil BSA-7, Macherey-Nagel, obtained from ES Industries, Marlton, NJ, USA). The concentration of *n*-propanol in the mobile phase was optimized for each column because of variable efficiency in separations. The mobile phase was 0.05 *M* ammonium phosphate solution (pH 7.0) and passed through a nylon (0.2 μm) filter prior to use. After equilibrating the column with buffer solution, a test injection was made to determine the column efficiency for the sepa-

ration of the enantiomers. Starting with 0.05%, *n*-propanol was added to the mobile phase until good separation and peak shape were achieved. The flow-rate was 1.5 ml/min. Lowering the pH to 6.0 would often increase the separation of the enantiomers, however, it also caused an increase in peak width and subsequent loss of sensitivity.

2.3. Preparation of standards

A stock solution of racemic omeprazole (0.1 mg/ml) was prepared by transferring 1.0 mg of reference material into a 10-ml volumetric flask, dissolving the compound in 0.5 ml of methanol and diluting to volume with carbonate buffer pH 9.3. A 0.01 mg/ml dilute stock solution was prepared by diluting 1.0 ml to 10.0 ml with carbonate buffer pH 9.3.

Working standard solutions containing 2.5, 2.0, and 1.0 μg of each enantiomer/ml were prepared by dilution of the 0.1 mg/ml omeprazole stock solution with the carbonate buffer while working standard solutions of 0.5, 0.25, 0.20 and 0.15 μg of each enantiomer were prepared by dilution of the 0.01 mg/ml dilute stock solution with the buffer.

Plasma standards were prepared by adding 100 μl of each working standard to 1-ml aliquots of drug-free plasma. The plasma standards ranged in concentration from 15 to 250 ng/ml of each enantiomer.

2.4. Sample preparation procedure

C_2 solid-phase extraction cartridges were conditioned with 1 ml of methanol and 1 ml of water. A 1.0-ml aliquot of plasma sample was transferred to the column and spiked with 50 μl of the internal standard solution. The samples were slowly aspirated (about 0.5 ml/min) through the packing. The cartridges were then washed with 1.0 ml of water. After drying under vacuum for 10 min, a 0.45-μm nylon filter was placed between the cartridge and the vacuum manifold. The omeprazole and internal standard were eluted from the column with two 1.0-ml volumes of acetonitrile making sure that the column did not run dry between additions. The eluent was evaporated under a stream of nitrogen at room temperature. The samples were reconstituted in 50 μl of methanol, vortex-mixed for 2 min and further diluted with 200 μl of pH 9.3 carbonate buffer. A 190-μl volume was injected onto the chromatographic system for all samples and standards with concentrations less than 20 ng/ml of plasma while a 100-μl volume was injected for all samples and standards with concentrations greater than or equal to 20 ng/ml of plasma.

Peak-height ratios of drug to internal standard were calculated. Sample concentrations were calculated from the equation $y = mx + b$ determined from a linear regression analysis of the daily standard curve. If the enantiomer concentration in a sample exceeded the calibration range, an aliquot of the sample was diluted with human control plasma and reassayed.

3. Results and discussion

3.1. Sample preparation

Liquid–liquid extraction using methylene chloride [7] was unsuccessful because of the presence of interferences in the chromatograms of drug-free plasma. Also, endogenous components in the plasma extracts caused rapid column degradation. In order to extend column life, an improved sample clean-up was necessary. The addition of the nylon filter during the final elution of omeprazole and the internal standard from the SPE cartridge eliminated particles.

Fig. 2 shows representative chromatograms of omeprazole enantiomers and internal standard in plasma. No extraneous interferences were found in either the human control plasma (Fig. 2B) or clinical study subjects plasma prior to dosing with omeprazole. Baseline resolution of the two enantiomers was achieved with this chromatographic system (Fig. 2A,C). Under similar chromatographic conditions (see legend for Fig. 2D for description of conditions), the primary sulfone metabolite [7] was retained on the column far longer than the parent compounds with a

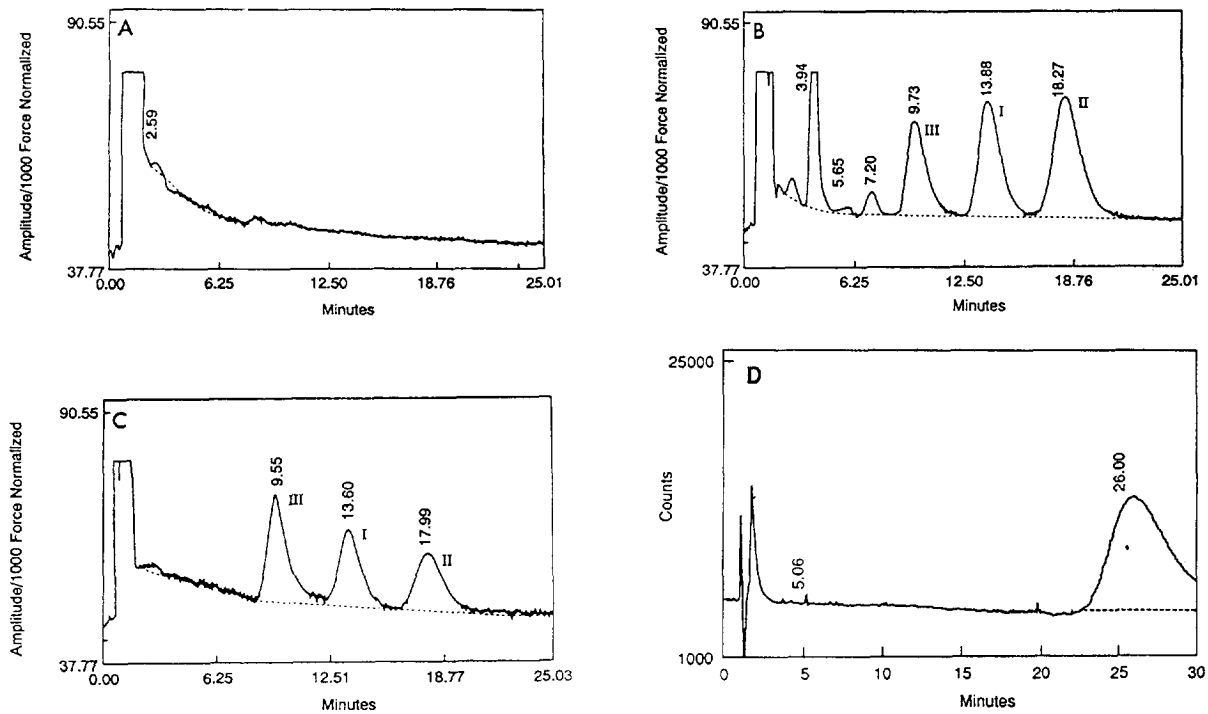


Fig. 2. (A) Chromatogram of an extracted pre-dose human plasma sample. The column was a 15 cm × 4 mm I.D. Resolvosil column. The mobile phase consisted of 0.75% *n*-propanol in 0.05 *M* ammonium phosphate, dibasic, pH adjusted to 7.0. The solvent flow-rate was 1.5 ml/min. (B) Chromatogram of an extracted clinical sample after the oral administration of omeprazole. Peaks: I = (+)-omeprazole, II = (−)-omeprazole, III = internal standard. (C) Chromatogram of an extracted control plasma sample, spiked with 100 ng of each enantiomer. Peaks: I = (+)-omeprazole, II = (−)-omeprazole, III = internal standard. (Identity of other peaks is unknown.) (D) Chromatogram of primary metabolite, sulfone. Chromatographic conditions were similar to those described for Figs. 2A–C, the difference being the 0.05 *M* ammonium phosphate, dibasic, was adjusted to pH 6.0.

retention time of 26 min (Fig. 2D). No metabolite was observed in the clinical samples assayed.

Elution order of the omeprazole enantiomers was determined by collecting fractions from a phenylcarbamate cellulose column with isopropanol–*n*-hexane–diethylamine (20:80:0.1, v/v) as the mobile phase and evaporating the solvent at room temperature. The two fractions were then dissolved in a small amount of methanol, diluted with pH 9.3 carbonate buffer and injected onto our HPLC system. The optical rotation of each fraction was confirmed with polarimetry. The enantiomers isolated from this procedure were not pure but sufficient to produce a positive or negative optical rotation in carbonate buffer solution and thus allow the elution order to be assigned.

Least squares regression calibration curves were constructed by plotting the omeprazole concentration versus peak-height ratios and were linear from 15 to 250 ng/ml plasma. A representative standard line for the (+)-isomer is described by the equation, $y = 0.0078x + 0.0032$, and for the (−)-isomer by the equation, $y = 0.0061x + 0.011$. In each case, the square of the correlation coefficient was greater than 0.995.

The recovery of the analytes was determined by comparing the peak heights of the unextracted standards with appropriate dilutions with those from extracted standards from plasma. Recoveries at 100 and 250 ng/ml were 83.4 and 89.7% for the (+)-isomer and 78.4 and 82.8% respectively for the (−)-isomer. The recovery of the internal standard was 77.2%.

Table 1
Within-day variability of the assay

Nominal concentration (ng/ml)	Accuracy (mean, $n = 6$) (%)		Precision (C.V., %; $n = 6$)	
	(+)	(−)	(+)	(−)
15	15.8	17.1	10.87	5.22
20	19.7	18.9	8.97	4.12
25	24.9	25.0	5.10	10.24
50	45.5	45.0	0.98	3.17
100	102.8	98.2	2.44	5.79
200	213.6	212.2	4.90	5.29
250	255.0	275.0	4.80	1.68

Six replicates of each standard were prepared and analyzed to assess intra-day precision. Table 1 lists the mean calculated concentrations and coefficients of variation for plasma analysis.

Between-day reproducibility was assessed through the daily analysis of quality control (QC) standards, 30 and 175 ng/ml of each enantiomer. Large volumes of QC standard solutions were prepared, assayed, divided into aliquots and stored at -20 ± 5°C. Two pairs of quality control samples were analyzed over a period of four months. The coefficients of variation for 30 and 175 ng/ml of 17 runs were 9.94 and 6.96% respectively for the (+)-enantiomer and 9.18 and 7.12% for the (−)-enantiomer. The inter-day results listed in Table 2 were obtained over a four-month interval and show that omeprazole is stable for at least 4 months in plasma stored at -20 ± 5°C.

Subsequent reanalysis of the QC samples after storage at -20 ± 5°C for 21 months showed a 28% loss. This amplifies previous stability data of Lagerstrom and Persson [7].

Fig. 3 shows a representative graph of plasma concentrations of omeprazole enantiomers following administration of a 20-mg omeprazole i.v. dose. It shows that when a racemic dose of omeprazole is administered, a similar ratio of enantiomers is observed in the clinical samples.

4. Conclusions

The ruggedness of the described procedure was successfully demonstrated by the analysis of approximately 500 clinical samples. The concentrations of (+)-omeprazole and (−)-omeprazole were essentially equal in the plasma samples assayed. The improved extraction procedures have resulted in an extended column life from about 50 to about 250 sample injections.

Acknowledgement

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Table 2
Between-day variability as assessed by coefficients of variation of quality control samples

Concentration (ng/ml)	Accuracy (mean) (%)		Precision (C.V., %)	
	(+)	(−)	(+) ($n = 31$)	(−) ($n = 33$)
30	31.67	30.11	9.94	9.18
175	177.46	169.90	6.96	7.12

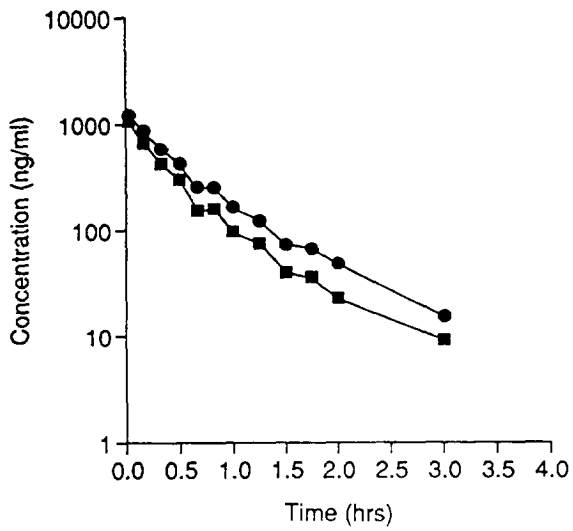

Fig. 3. Plasma concentrations of omeprazole enantiomers following omeprazole 20 mg i.v. dose. (○) is the (−)-enantiomer of omeprazole and (□) is the (+)-enantiomer of omeprazole.

enantiomers from the phenylcarbamate cellulose column.

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