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Journal of Chromatography B, 689 (1997) 239–243

JOURNAL OF CHROMATOGRAPHY B

Determination of omeprazole in human plasma by high-performance liquid chromatography

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Abstract

A new method for the determination of omeprazole in human plasma was developed. Omeprazole was extracted from plasma with toluene–isoamylalcohol (95:5, v/v), the organic phase was evaporated, dissolved in the mobile phase and injected into a reversed-phase C_{18} column. Flunitrazepam was used as an internal standard. The mobile phase consisted of 47% methanol and 53% of 0.1 *M* dipotassium hydrogenphosphate, pH 7.8. The spectrophotometric detection was performed at 302 nm. Limit of quantitation was 9.7 ng/ml and the calibration curve was linear up to 1240 ng/ml.

Keywords: Omeprazole

1. Introduction

Omeprazole, a gastric acid pump inhibitor, dose-dependently controls gastric acid secretion; the drug has greater antisecretory activity than histamine H_2-receptor antagonists. It has been widely used in the treatment of reflux oesophagitis, Zollinger–Ellison syndrome and peptic ulcer disease [1].

A few HPLC procedures have been reported for determination of omeprazole in biological fluids [2–5]. In all these methods the sample pretreatment consists of liquid–liquid extraction with dichloromethane; the extracts are chromatographed on reversed-phase columns and omeprazole is detected using spectrophotometric detection at 302 nm (absorbance maximum of omeprazole). In the paper by Lagerström and Persson [3] both reversed-phase and normal-phase systems were investigated. The published procedures use internal standards which are commercially unavailable [2–4] or have very low absorbance at 302 nm [5].

The purpose of this paper is to describe a method for the determination of omeprazole in plasma using an alternative solvent toluene–isoamylalcohol which yields cleaner extracts and flunitrazepam as an internal standard.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical grade quality. Omeprazole was obtained from Union Quimico Farmaceutica (Barcelona, Spain). Flunitrazepam was a gift from Léčiva (Prague, Czech Republic). Dipotassium hydrogenphosphate was purchased from Merck (Darmstadt, Germany). *ortho*-Phosphoric acid, methanol and toluene were manufactured by Lachema (Brno, Czech Republic). Iso-

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PII S0378-4347(96)00283-6

amylalcohol was obtained from Fluka (Buchs, Switzerland).

2.2. Apparatus

All HPLC instruments were obtained from Thermo Separation Products (Riviera Beach, FL, USA). The system consisted of the membrane degasser, pump ConstaMetric 4100, automatic sample injector AS 3000, spectrophotometric detector UV2000 and datastation with PC1000 software, version 2.5. The separation was performed on a Nucleosil 120-5 5 μm C_{18}, 250×4 mm I.D. analytical column (Watrex, Prague, Czech Republic) protected by a Nucleosil 120-5 5 μm C_{18}, 10×4.6 mm I.D. precolumn (Watrex). A silica presaturator column 75×2 mm I.D. was inserted between the pump and the injector.

The mobile phase consisted of 47% methanol and 53% of 0.1 *M* dipotassium hydrogenphosphate, pH 7.8 adjusted with ortho-phosphoric acid. Sodium azide (40 mg/l) was added to the buffer to prevent the bacterial growth. The flow-rate was 1.2 ml/min and column temperature was 37°C. The spectrophotometric detector was operated at 302 nm. The time constant was set to 2 s.

2.3. Preparation of standard solutions

Omeprazole (5.83 mg) was dissolved in 10 ml of methanol and solutions for preparation of calibration curve and quality control samples were obtained by serial dilutions with methanol. These solutions were added to drug-free human plasma containing heparin as an anticoagulant in volumes not exceeding 4% of the plasma volume. Flunitrazepam (7.2 mg) was dissolved in 10 ml of methanol and diluted ten times with methanol. All solutions were stored at −20°C and protected from light.

2.4. Preparation of the sample

The samples were stored in the freezer at −20°C. The thawing was allowed at room temperature before processing of the sample. A 10-μl volume of internal standard solution (720 ng) was added to 1 ml of plasma, the tube was briefly shaken and 3 ml of toluene–isoamylalcohol (95:5, v:v) were added. The tube was vortex-mixed for 90 s at 1000 rpm and centrifuged for 10 min at 2600 *g*. The upper organic layer (2.5 ml) was transferred to another tube and evaporated to dryness under nitrogen at 40°C. The sample was reconstituted in 100 μl of the mobile phase before analysis, transferred to a 100-μl polypropylene autosampler vial and 25 μl were injected into the chromatographic system.

2.5. Calibration curves

The calibration curve was constructed in the range 9.70–1240 ng/ml to encompass the expected concentrations in measured samples. The calibration curves were obtained by weighted linear regression (weighing factor $1/y^2$): the ratio of omeprazole peak height to flunitrazepam peak height was plotted vs. ratio of omeprazole concentration to that of internal standard in ng/ml.

3. Results and discussion

3.1. Chromatography

Under the chromatographic conditions described the retention times of omeprazole and flunitrazepam were 10.1 and 11.4 min, respectively. The column efficiency expressed by the number of theoretical plates was 7500 and 9000 for omeprazole and flunitrazepam, respectively. The resolution of both the peaks was better than 2.5 at pH 7.8 while at lower pH values the resolution was worse. A pH >7 was also preferable due to instability of omeprazole under acidic conditions [5]. Although the solubility of silica gel is limited at a pH <8, a silica gel saturator column was placed before the injector for the protection of the analytical column.

Flunitrazepam is commercially available, has excellent peak shape and can be added to plasma in concentrations comparable to omeprazole. Phenacetin, the only commercially available compound which was used as an internal standard in another procedure [5], had to be added in concentration 10 μg/ml, because its absorbance at 302 nm is very low. In such a high concentration its

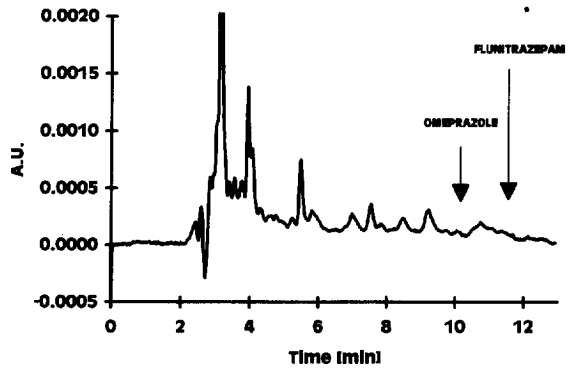

Fig. 1. Chromatogram of drug-free human plasma. The arrows indicate the retention times of omeprazole and flunitrazepam (internal standard).

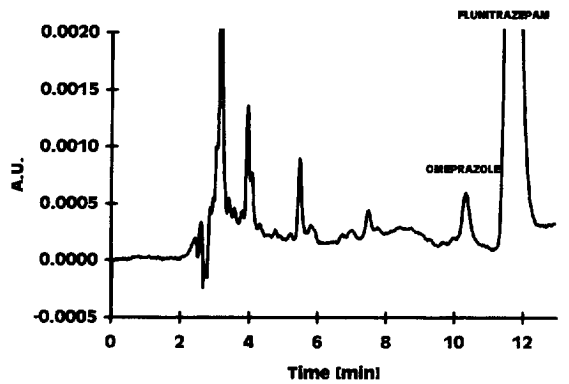

Fig. 3. Chromatogram of a plasma sample spiked with omeprazole (9.7 ng/ml, limit of quantitation).

possibility to compensate for e.g. minor adsorption losses is limited.

The blank sample chromatogram (Fig. 1) showed no interfering peaks. The chromatogram of a plasma sample 3.5 h after administration of 40 mg omeprazole to a volunteer is shown in Fig. 2. The concentration of omeprazole was 269 ng/ml. The peaks with retention times 5.2 and 6.6 min are omeprazole metabolites. However, it was not the aim of the present study to quantitate them. The chromatogram of plasma spiked with omeprazole at concentration 9.7 ng/ml (limit of quantitation) is shown in Fig. 3.

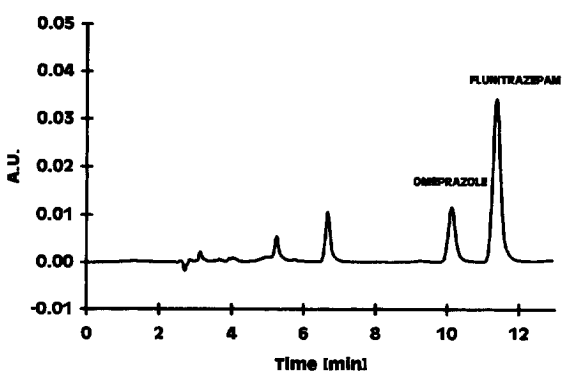

Fig. 2. Chromatogram of a plasma sample from a volunteer 3.5 h after administration of 40 mg of omeprazole. The measured concentration of omeprazole was 269 ng/ml.

3.2. Sample preparation

Initially we tried liquid–liquid extraction with dichloromethane, but the extracts were not sufficiently clean. Toluene–isoamylalcohol (95:5, v/v) had the same extraction efficiency as dichloromethane and interfering compounds were not co-extracted. The best results were obtained at physiological pH values and therefore the addition of a buffer prior to extraction is not necessary.

3.3. Linearity and limit of quantitation

The calibration curves were linear in the studied range. The calibration curve equation is $y=bx+c$, where y represents ratio of omeprazole peak height to that of flunitrazepam and x represents the ratio of omeprazole concentration to that of internal standard. The mean ($n=6$) typical equation of the calibration curve obtained from 7 points was $y=1.17(\pm 0.12)x-0.0003$ (correlation coefficient $r=0.9984\pm 0.0018$). The residuals (difference between observation and prediction) were centered around zero and the mean relative difference from six calibration curves was lower than 3% for all calibration points.

The limit of quantitation was 9.70 ng/ml. At this concentration the precision, characterised by relative standard deviation, was 12.1% and inaccuracy, defined as the deviation between the true and the measured value expressed in percents, was −3.1% at this concentration ($n=6$).

Table 1
Intra-day precision and accuracy

n	Concentration added (ng/ml)		Bias (%)	R.S.D. (%)
	Added	Measured		
6	19.40	20.49	5.6	7.6
6	124.9	117.8	−5.7	6.6
6	1015	981.2	−3.3	3.0

n= number of samples.

3.4. Precision and accuracy

3.4.1. Intra-day precision and accuracy

Intra-day precision and accuracy of the method is illustrated in Table 1. Three sets of quality control samples (each with six samples) were analysed with calibration samples on one day. Precision was better than 8%, while the inaccuracy was better than 6% at all levels.

3.4.2. Inter-day precision and accuracy

Inter-day precision and accuracy was evaluated by processing a set of calibration and quality control samples (three levels analysed twice, results averaged for statistical evaluation) on six separate days. The samples were prepared in advance and stored at −20°C. The respective data are given in Table 2. The precision was better than 10% and the inaccuracy did not exceed 2% at any level.

3.5. Stability study

3.5.1. Processed sample stability

A set of samples (n=5) was analysed and left in the autosampler at room temperature for 24 h. The samples were then analysed again and the concentration was calculated from calibration curve obtained with freshly prepared calibration samples. The values measured after 24 h were slightly lower than those obtained from freshly prepared samples, but the average bias was only −8.8% . It can be concluded that the samples should be analysed within 24 h after their preparation.

Table 2
Inter-day precision and accuracy

n	Concentration (ng/ml)		Bias (%)	R.S.D. (%)
	Added	Measured		
6	19.40	19.17	−1.2	9.2
6	124.9	123.6	−1.0	2.9
6	1015	1019	0.4	3.0

n= number of days.

Table 3
Long-term stability

n	Concentration (ng/ml)		Bias (%)	R.S.D. (%)
	Added	Measured		
6	20.33	20.06	−1.3	11.8
6	1091	1111	1.8	0.2

n= number of samples.

3.5.2. Storage stability

The results of long-term sample stability are shown in Table 3. The samples are stable at −20°C for at least three weeks.

3.5.3. Freeze–thaw stability

A low concentration sample (10 ml, 20.33 ng/ml) and a high concentration sample (16 ml, 1091 ng/ml) were prepared. The solutions were stored at −20°C for three weeks and then subjected to 3 thaw–freeze cycles. During each cycle duplicate 1-ml aliquots were processed, analysed and the results averaged. The relative differences of measured concentrations measured from the theoretical value were within 15% (low concentration) or within 8% (high concentration), indicating no significant substance loss during repeated thawing and freezing.

4. Conclusion

The results obtained indicate that this simple and rapid method for the assay of omeprazole in human plasma is sufficiently sensitive to follow the pharmacokinetics of this drug.

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