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Rapid determination of omeprazole in human plasma by protein precipitation and liquid chromatography–tandem mass spectrometry

J. Macek *, J. Klíma, P. Ptáček

Pharmakl s.r.o., Seydlerova 2451, CZ-15800 Prague 13, Czech Republic

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Abstract

A rapid, sensitive and reliable method was developed to quantitate omeprazole in human plasma using liquid chromatography–tandem mass spectrometry. The assay is based on protein precipitation with acetonitrile and reversed-phase liquid chromatography performed on an octadecylsilica column (55 mm × 2 mm, 3 μm particles), the mobile phase consisted of methanol–10 mM ammonium acetate (60:40, v/v). Omeprazole and flunitrazepam, the internal standard, elute at 0.80 ± 0.10 min with a total run time 1.35 min. Quantification was through positive ion mode and selected reaction monitoring mode at *m/z* 346.1 → 197.9 for omeprazole and *m/z* 314.0 → 268.0 for flunitrazepam, respectively. The lower limit of quantitation was 1.2 ng/ml using 0.25 ml of plasma and linearity was observed from 1.2 to 1200 ng/ml. Within-day and between-day precision expressed by relative standard deviation was less than 5% and inaccuracy did not exceed 12%. The assay was applied to the analysis of samples from a pharmacokinetic study.

Keywords: Omeprazole; Protein precipitation; Liquid chromatography; Mass spectrometry

1. Introduction

Omeprazole (Fig. 1a) is a well studied proton pump inhibitor that reduces gastric acid secretion. Omeprazole inhibits the gastric parietal cell proton pump (H^+/K^+-ATPase), dose-dependently reducing basal and stimulated gastric acid secretion and raising intragastric pH [1].

Omeprazole is an acidolabile compound; it is absorbed within 3–6 h from the small intestine following oral administration of enteric coated granules. The bioavailability of omeprazole after single oral dose is about 35%. Following single oral 40 mg dose the mean peak plasma levels are 300–600 ng/ml, but C_{max} and AUC values show large interindividual variations due to the genetically different activities of *S*-mephenytoin hydroxylase metabolizing omeprazole. The rate of omeprazole absorption is decreased by concomitant food intake. The elimination half-life in plasma is reported to be about 40–60 min [2,3].

To date, several analytical methods have been reported for determination of omeprazole in plasma. Many assays employed high-performance liquid chromatography (HPLC) with spectrophotometric detection at 302 nm (absorbance maximum of omeprazole) [4–10]. In all these methods the sample pretreatment consisted of liquid–liquid extraction, the limit of quantitation (LOQ) was in the range 5–10 ng/ml and the chromatographic run time was between 8 and 22 min. The assay of Jia et al. [11] is based on the same detection principle to quantitate omeprazole in rat plasma, but protein precipitation was selected as a sample preparation technique. However, the limit of quantitation was too high (20 ng/ml) and the analysis time was long (20 min). The column-switching and on-line solid-phase extraction methods reported by Shimizu et al. [12] and García-Encina et al. [13] have LOQ sensitivity 3–5 ng/ml, but are too lengthy for routine applications (20–25 min).

Several assays are based on liquid chromatography–mass spectrometry (LC–MS) [14–18]. The sample preparation employs liquid–liquid extraction, LOQ is in the range 0.4–5 ng/ml and chromatographic run time within 2.75–14 min. Two LC–MS methods using solid-phase extraction were also published, but the run time was too long, 11–25 min [19,20].

* Corresponding author. Tel.: +420 251 081 772; fax: +420 235 524 176.
E-mail address: macek@pharmakl.cz (J. Macek).

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Liquid–liquid and solid-phase extraction protocols frequently include an evaporation and reconstitution steps and are time consuming. Column switching approaches avoid these problems but lead to extended chromatography times. The method reported by Song and Naidong [15] eliminates evaporation after robotic liquid–liquid extraction by direct injection of the extract onto silica column using hydrophilic interaction chromatography. Total run time in a gradient elution system was 2.75 min. This is an elegant approach, nevertheless, the liquid–liquid extraction still includes many pipetting steps which may be time consuming if performed manually.

The aim of this study was to develop a simple, high throughput LC–MS/MS method for determination of omeprazole in human plasma using a standard laboratory equipment. The overall speed of analysis was improved by the elimination of tedious extraction steps and optimization of chromatographic conditions.

2. Experimental

2.1. Chemicals

Acetonitrile (for liquid chromatography) was Sigma–Aldrich (Prague, Czech Republic) product and methanol (for chromatography) was manufactured by Merck (Darmstadt, Germany). Ammonium acetate (Puriss. p.a., ACS) was obtained from Fluka (Buchs, Switzerland). Omeprazole and flunitrazepam (internal standard; Fig. 1b) were obtained from Zentiva (Prague, Czech Republic).

2.2. Apparatus and conditions

The HPLC/MS–MS system consisted of the P4000 pump, TSQ Quantum Discovery Max triple quadrupole mass spectrometer with electrospray ion source, data station with Xcalibur software, version 1.4 (all from Thermo Electron Corporation, San Jose, CA, USA). The Midas autosampler (Spark Holland BV, The Netherlands) was equipped with a 1 μl sample loop, methanol was used as a washing solution in the autosampler and the injection was performed in a full-loop mode. The temperature of the column oven was 45 °C.

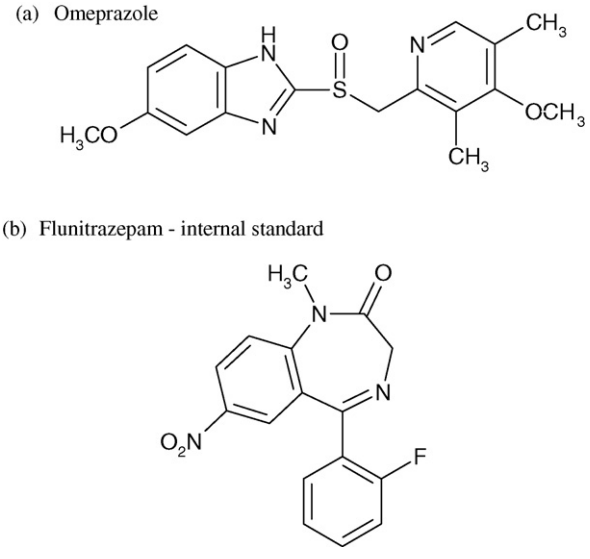

Fig. 1. Chemical structure of: (a) omeprazole and (b) flumitrazepam, internal standard.

The separation was performed on a Purospher STAR C18, 3 μm, 55 mm × 2 mm column (Merck) protected with a C18 4 mm × 3 mm precolumn (Phenomenex, Torrance, CA, USA). The mobile phase consisted of methanol–10 mM ammonium acetate (60:40, v/v), the flow-rate was 0.5 ml/min.

The detection of the analytes was carried out using positive electrospray ionization technique and selected reaction monitoring mode to monitor the transitions (precursor → product) *m/z* 346.1 → 197.9 for omeprazole and *m/z* 314.0 → 268.0 for flunitrazepam, respectively. The dwell time was 0.2 s for both analytes, scan width was set to 0.5 *m/z* and peak width 0.7 for both Q1 and Q3 quadrupole. Ion spray voltage was set to 4500 V, temperature of the ion transfer capillary was 310 °C. Collision energy was 12 and 25 V for omeprazole and flunitrazepam, respectively. The pressure of argon in the collision cell was 1.5 mTorr. The pressure of the sheath gas (sweep gas, auxiliary gas) was 23, 5 and 20 arbitrary units, respectively.

2.3. Standards

Stock standard solutions of omeprazole were made by dissolving of approximately 15 mg of accurately weighed substance in 25 ml of methanol. Separate solutions were prepared for the calibration curve samples and quality control ones. Further standard solutions were obtained by serial dilutions of stock solutions with methanol. The standard solutions were stored at −18 °C and were protected from light; they were stable at least 10 weeks under these conditions.

The calibration and quality control plasma samples were prepared by addition of standard solutions to drug-free plasma in volumes not exceeding 2% of the plasma volume.

Flunitrazepam (12.66 mg) was dissolved in 25 ml of methanol. Fifty microliters of this solution was diluted with acetonitrile to final volume of 100 ml (flunitrazepam concentration 253.2 ng/ml) and this solution was used for protein precipitation of plasma samples. This solution was stable for at least 2 days at room temperature.

2.4. Preparation of the sample

The samples were stored in the freezer at −18 °C and allowed to thaw at room temperature before processing. Two hundred and fifty microliters of plasma were pipetted to the polypropylene tube and 0.75 ml of acetonitrile with internal standard (flunitrazepam, 253.2 ng/ml) was added, the tube was vortex-mixed for 30 s at 2000 rpm. The tube was centrifuged 3 min at 2500 × *g* and 500 μl of the supernatant was transferred to an autosampler vial. One microliter was injected into the chromatographic system. A batch of 24 samples can be prepared in 15 min.

2.5. Calibration curves

The calibration curve was constructed in the range 1.187–1229 ng/ml to encompass the expected concentrations in measured samples, the concentrations of individual calibrators were 1.187, 4.471, 19.98, 80.35, 331.0 and 1229 ng/ml. The calibration curves were obtained by weighted linear regression (weighing factor $1/x^2$): the peak area ratio (analyte/internal standard) was plotted versus the analyte concentration. The suitability of the calibration model was confirmed by back-calculating the concentrations of the calibration standards.

3. Results and discussion

3.1. Sample preparation

The absolute limit of detection of omeprazole under the chromatographic and mass spectrometric conditions described in this paper is about 25 fg (signal-to-noise ratio 5:1). This sensitivity enabled us to reach the desired limit of quantitation 1.2 ng/ml using only protein precipitation as the sample preparation technique. The response of omeprazole is so high that only 1/1000 of 0.25 ml plasma sample (1 μl of the final volume 1 ml) can be injected which eliminates undesirable matrix effects, because the absolute amount of co-injected interferences is minimized. The recovery was determined by comparison of peak areas of omeprazole and internal standard in processed spiked plasma sample and directly injected standard solutions. It was in the range 95–105% for both compounds.

In order to study matrix effects on the ratio of omeprazole/internal standard peak areas the following experiment was performed: 500 μl of processed blank plasma samples from six different subjects were spiked with a mixture of omeprazole and internal standard (concentrations 80 and 182 ng/ml, respectively) and the samples were injected into the column. The relative standard deviation of peak area ratios was 1.4% indicating no significant matrix effect on this parameter.

An additional benefit of the small injection volume is that no peak distortion due to injection of sample solvent with higher elution strength than the mobile phase was observed. The samples after protein precipitation with acetonitrile are clear while precipitation of proteins with methanol yields opaque samples. Therefore acetonitrile was selected to precipitate plasma proteins. To minimize pipetting steps, the internal standard was added directly to the precipitation solvent.

3.2. Chromatography

In a high throughput method it is advantageous to use isocratic elution, because an equilibration step after finishing gradient can be omitted. The capacity ratio of the analyte should be in the range 1–2 to avoid ionization suppression by compounds eluting at the dead time. The internal standard should elute in approximately the same time as the analyte; in such case it can compensate for the fluctuations in ionization and it does not prolong the analysis time.

Flunitrazepam was selected as an internal standard; methanol was selected as an organic modifier, because using acetonitrile the retention times of omeprazole and flunitrazepam were significantly different. Column temperature 45 °C was selected to lower the viscosity of the mobile phase; relatively high flow-rate (0.5 ml/min) was used at moderate back-pressure (17 MPa). The use of formic acid in the mobile phase was avoided, because omeprazole very rapidly decomposes at acidic pH. Ten millimolar ammonium acetate provided good chromatographic shape and ionization for both compounds analyzed.

After optimization of chromatographic conditions, the retention time of both compounds was 0.80 ± 0.10 min which corresponds to the capacity factor 1.7. Total chromatographic run time was 1.35 min.

Typical chromatograms of drug-free plasma (a), spiked plasma at limit of quantitation 1.2 ng/ml (b) and plasma from a pharmacokinetic study containing 243.5 ng/ml omeprazole (c) are shown in Fig. 2. The method selectivity was demonstrated on six blank plasma samples obtained from healthy volunteers: the chromatograms were found to be free of interfering peaks.

The range of the calibration curve was over three orders of magnitude which means that carry-over may represent a significant problem. Indeed, with other autosampler and/or different injection technique the carry-over up to 0.2% was observed, which was not acceptable. This problem was solved by using full-loop injection instead of partial-loop injection, because in this injection technique the injection valve is washed more thoroughly by the sample and wash solution [21]. In this way, the carry-over after injection of the highest calibrator was typically not more than 10% of the peak area of the lowest calibrator.

3.3. Method validation

3.3.1. 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 omeprazole/internal standard peak area ratio and x represents concentration of omeprazole in ng/ml. The mean equation (curve coefficients ± standard deviation) of the calibration curve ($N = 6$) obtained from six points was $y = 0.00539(\pm 0.00037)x - 0.00100(\pm 0.00074)$ (correlation coefficient $r = 0.999$).

The limit of quantitation was 1.187 ng/ml ($N = 6$). The precision, characterised by the relative standard deviation, was 7.3% and accuracy, defined as the deviation between the true and the measured value expressed in percents, was −13.0% at this concentration ($N = 6$).

3.3.2. Intra-assay precision

Intra-assay precision of the method is illustrated in Table 1. It was estimated by assaying the quality control samples (low, medium and high concentration) six times in the same analytical

Table 1
Intra-assay precision and accuracy

N	Concentration (ng/ml)			
	Added	Measured	Bias (%)	R.S.D. (%)
6	2.260	2.431	7.0	4.6
6	40.15	42.07	4.5	1.7
6	1004	951.2	−5.5	2.2

Table 2
Inter-assay precision and accuracy

N	Concentration (ng/ml)			
	Added	Measured	Bias (%)	R.S.D. (%)
6	2.260	2.521	11.5	3.5
6	40.15	43.46	8.2	1.5
6	1004	990.7	−1.3	3.6

run. The precision was better than 5% and the bias did not exceed 7% at all levels.

3.3.3. Inter-assay precision and accuracy

Inter-assay 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 runs. The samples were prepared in advance and stored at −18 °C. The respective data are given in Table 2. The precision was better than 4% and the inaccuracy did not exceed 12% at all levels.

3.3.4. Sample dilution

The possibility of dilution of samples with analyte concentration above upper limit of quantitation with blank plasma was demonstrated. Three spiked samples with concentration 2786 ng/ml were diluted 1:2 with blank plasma and analysed. The bias was −12.7% and relative standard deviation was 0.6%.

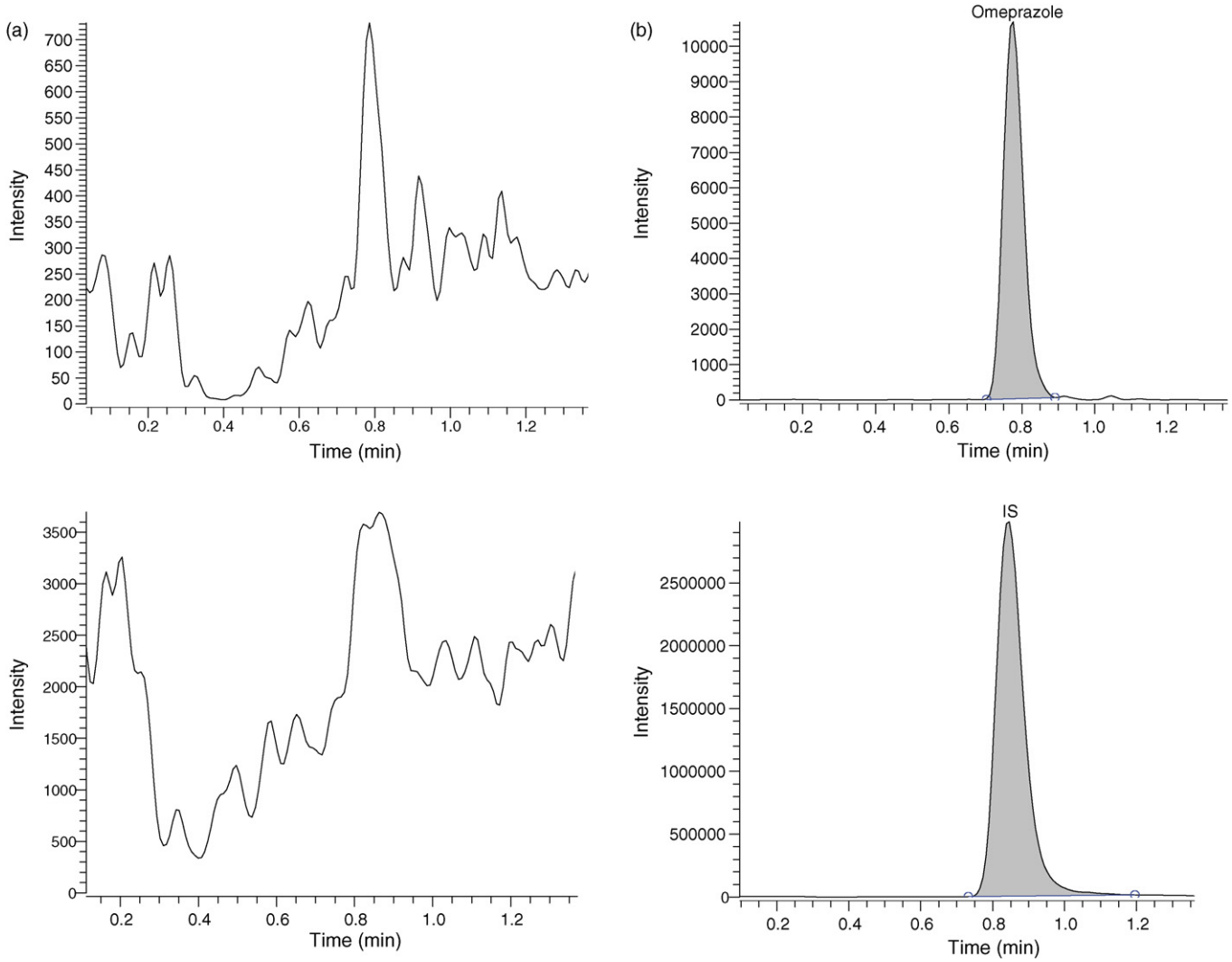

Fig. 2. Chromatograms of: (a) drug-free human plasma, (b) spiked plasma at limit of quantitation (1.2 ng/ml) and (c) a plasma samples from a subject 2 h after administration of 40 mg omeprazole, the measured concentration was 243.5 ng/ml. The upper panel shows selected reaction monitoring of the transition *m/z* 346.1 → 197.9 (omeprazole); the lower one shows the transition *m/z* 314.0 → 268.0 (flunitrazepam, internal standard).

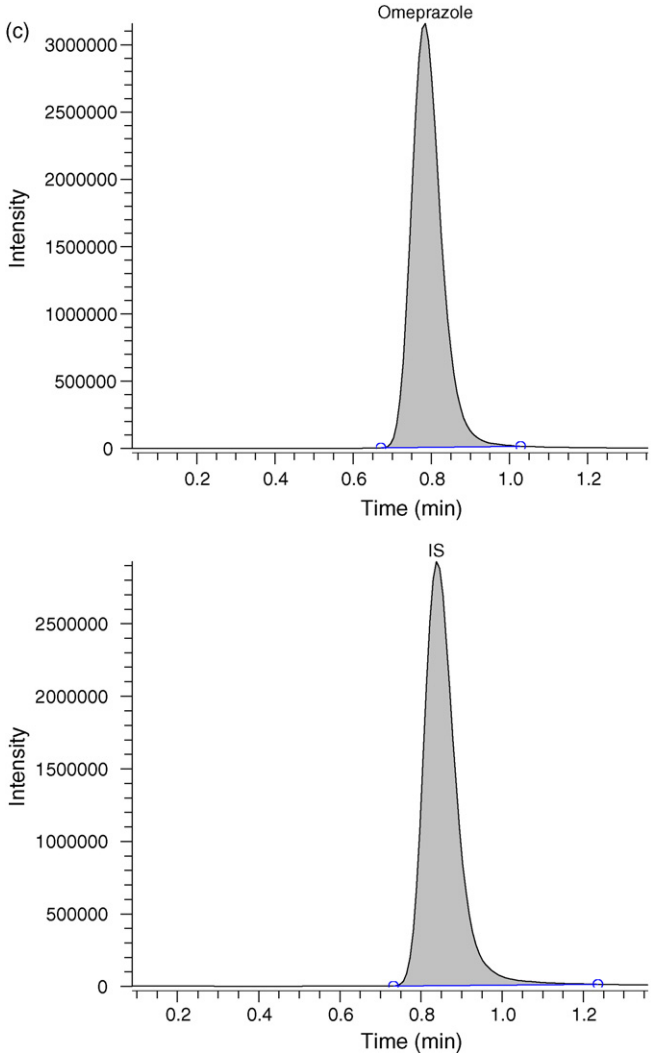

Fig. 2. *(Continued).*

3.3.5. Sample stability

The data on sample stability of plasma were taken from our previous validated HPLC method [8]. No problems with stability were encountered on plasma samples: (1) subjected for three thaw and freeze cycles, (2) stored −18 °C for 10 weeks or (3) stored at +25 °C for 24 h. The stability of omeprazole was proved also in the other LC–MS methods [15–18].

The stability of processed samples was studied by the following procedure: two sets of samples with a low and a high concentration of omeprazole were analysed and left in the autosampler at ambient temperature. The samples were analysed using a freshly prepared calibration samples 4 days later. The results are presented in Table 3. The processed samples are stable at room temperature for 4 days.

3.4. Application to biological samples

The proposed method was applied to the determination of omeprazole in plasma samples from a pharmacokinetic study, which was approved by the local ethics committee. The plasma samples were collected up to 17 h after a single oral dose of 40 mg omeprazole (Losec® 20 capsules, AstraZeneca) administered with high-fat food to 44 healthy male volunteers: mean age of the group was 29 years (range 18–44), mean weight was 77 kg (range 52–97). Fig. 3 shows the mean plasma concentrations of omeprazole; the error bars show standard deviations at individual time points and confirm high inter-subject variability of omeprazole pharmacokinetics (see Section 1).

The plasma levels reached their maximum 4.5 h after the administration and thereafter the plasma level declined with an elimination half-time of 0.74 h. The time of maximum concentration was shifted to higher values after administration of omeprazole with food and many individual concentration–time curves exhibited two peaks. These values agree with previously published reports on the pharmacokinetics of omeprazole. The mean area under concentration–time curve (AUC) measured from 0 to the last non-zero sampling point was 99.4% of the value of AUC extrapolated from 0 to infinity. In all subjects this value was higher than 96% which indicates a suitability of the analytical method for pharmacokinetic studies.

Table 3
Stability of processed samples

Sample	*C* (ng/ml)	*N*	*C* found (ng/ml)	R.S.D. (%)	Difference (%)
New	2.260	6	2.431	4.6	
4 days old	2.260	6	2.347	3.1	−3.5
New	1004	6	951.2	2.2	
4 days old	1004	6	999.5	1.6	5.1

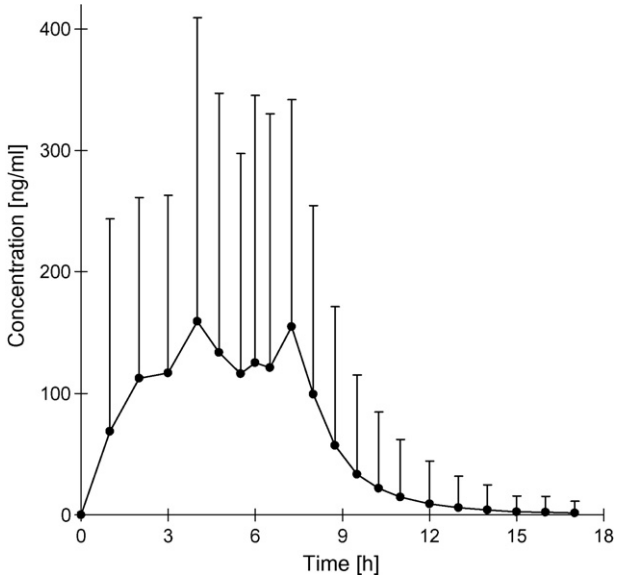

Fig. 3. Mean plasma concentrations (+S.D.) of omeprazole after a single 40 mg oral dose of the drug administered with food to 44 healthy subjects.

4. Conclusions

The validated method allows determination of omeprazole in the 1.2–1200 ng/ml range. The assay is rapid, the analysis time is only 1.35 min. About 300 samples can be easily prepared and analysed in one working day. The precision and accuracy of the method are well within the limits required for bioanalytical assays. The limit of quantification 1.2 ng/ml permits the use of the method for pharmacokinetic studies.

References

[1] H.D. Langtry, M.I. Wilde, Drugs 56 (1998) 447.
[2] M.I. Wilde, D. McTavish, Drugs 48 (1994) 91.
[3] C. Litalien, Y. Theoret, C. Faure, Clin. Pharmacokinet. 44 (2005) 441.
[4] G.W. Mihaly, P.J. Prichard, R.A. Smallwood, N.D. Yeomans, W.J. Louis, J. Chromatogr. 278 (1983) 311.
[5] P.-O. Lagerström, B.-A. Persson, J. Chromatogr. 309 (1984) 347.
[6] M.A. Amantea, P.K. Narang, J. Chromatogr. 426 (1988) 216.
[7] K. Kobayashi, K. Chiba, D.-R. Sohn, Y. Kato, T. Ishizaki, J. Chromatogr. 579 (1992) 299.
[8] J. Macek, P. Ptáček, J. Klíma, J. Chromatogr. B 689 (1997) 239.
[9] A. Zarghi, S.M. Foroutan, A. Shafaati, A. Khoddam, Arzneimittelforschung 56 (2006) 382.
[10] K.H. Yuen, W.P. Choy, H.Y. Tan, J.W. Wong, S.P. Yap, J. Pharm. Biomed. Anal. 24 (2001) 715.
[11] H. Jia, W. Li, K. Zhao, J. Chromatogr. B 837 (2006) 112.
[12] M. Shimizu, T. Uno, T. Niioka, N. Yaui-Furukori, T. Takahata, K. Sugawara, T. Tateishi, J. Chromatogr. B 832 (2006) 241.
[13] G. García-Encina, R. Farran, S. Puig, L. Martinez, J. Pharm. Biomed. Anal. 21 (1999) 371.
[14] U. Hofmann, M. Schwab, G. Treiber, U. Klotz, J. Chromatogr. B 831 (2006) 85.
[15] Q. Song, W. Naidong, J. Chromatogr. B 830 (2006) 135.
[16] V.A. Frerichs, C. Zaranek, C.E. Haas, J. Chromatogr. B 824 (2005) 71.
[17] J. Wang, Y. Wang, J.P. Fawcett, Y. Wang, J. Gu, J. Pharm. Biomed. Anal. 39 (2005) 631.
[18] H. Stenhoff, A. Blomqvist, P.-O. Lagerström, J. Chromatogr. B 734 (1999) 191.
[19] E.J. Woolf, B.K. Matuszewski, J. Chromatogr. A 828 (1998) 229.
[20] H. Kanazawa, A. Okada, Y. Matsushima, H. Yokota, S. Okubo, F. Mashige, K. Nakahara, J. Chromatogr. A 949 (2002) 1.
[21] P.T. Vallano, S.B. Shugarts, E.J. Woolf, B.K. Matuszewski, J. Pharm. Biomed. Anal. 36 (2005) 1073.