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Validation of an automated liquid chromatographic method for omeprazole in human plasma using on-line solid-phase extraction

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Abstract

An automated system using on-line solid-phase extraction and HPLC with UV detection has been validated in order to determine omeprazole in human plasma. The extraction was carried out using C_{18} cartridges. After washing, omeprazole was eluted from the cartridge with mobile phase onto an Inertsil ODS-2 column. The developed method was selective and linear for drug concentrations ranging between 5 and 500 ng ml^{-1}. The recovery of omeprazole ranged from 88.1 to 101.5%, and the limit of quantitation (LOQ) was 5 ng ml^{-1}. The intraday accuracy ranged from 93.1 to 106.2% and the interday accuracy varied from 95.4 to 105.1%. For the LOQ, good values of precision (8.7 and 17.5% for intraday and interday, respectively) were also obtained. This automated system has been applied to determine omeprazole in human plasma samples from bioequivalence studies.

Keywords: Omeprazole; On-line solid-phase extraction; HPLC; Validation; Prospekt

1. Introduction

Omeprazole is a substituted benzimidazole with a powerful and selective inhibiting activity on gastric acid secretion [1–3]. Compared with ranitidine and cimetidine, omeprazole has been reported to be more effective in the treatment of a gastric ulcer [4,5].

The mechanism of action of this substance is based on an irreversible binding to the proton of the (H^+, K^+)-ATPase pump of the parietal gastric cells [6,7]. Effective control of acid secretion is produced faster at a pH lower than 6, i.e. in diseases that show low pH values. However, omeprazole breaks down rapidly in an acidic medium and therefore, must be administered in the form of enteric preparations [8–10].

After oral administration, omeprazole is rapidly absorbed and concentrations in plasma can be observed within the first half an hour. Elimination is also rapid, being characterised by an elimination half-life lower than 1 h [2,11]. Omeprazole is

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metabolised by the liver and the amount metabolised varies considerably among individuals. The main metabolites present in plasma are hydroxyomeprazole and omeprazole sulphone [12].

Several liquid chromatography methods have been described in the literature [13–20] to determine omeprazole and its metabolites in biological fluids. They are based on liquid–liquid or off-line solid-phase extraction. The application of these methods in bioequivalence studies is very laborious if the large number of samples required is taken into account. In some cases, in particular those that report a limit of quantitation in plasma of 5 ng ml^{-1}, the corresponding relative standard deviation (RSD) value is not shown. These facts and the drawback of large sample volumes required to perform such methods (1 ml), highlight the need to develop and validate a quick and simple automatic method for determining omeprazole in bioequivalence studies.

This report describes the validation of a method to analyse omeprazole in human plasma by means of a completely automatic system that uses a solid-phase extraction that is connected on-line with a liquid chromatograph. In addition, the results of a bioequivalence study are shown.

2. Experimental

2.1. Chemicals and reagents

The omeprazole standard (Fig. 1a), (5-methoxy-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl]sulphinyl}-1H-benzimidazole), was provided by Esteve Química, S.A. (Girona, Spain).

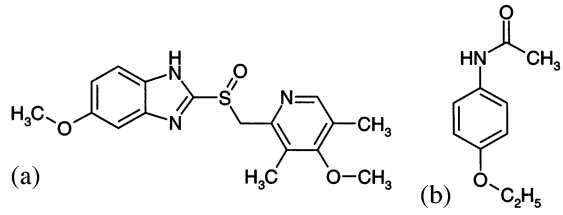

Fig. 1. Chemical structure of (a) omeprazole and (b) phenacetin (internal standard).

The internal standard used was phenacetin (Fig. 1b), (*N*-[4-ethoxyphenyl]acetamide), purchased from Sigma (St. Louis, MO). Omeprazole sulphone (5-methoxy-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulphonyl} - 1H - benzimidazole), omeprazole sulphide (5-methoxy-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]-thio}-1H-benzimidazole) and hydroxyomeprazole (5-methoxy-2{[(4 - methoxy - 3 - methyl - 5 - hydroxymethyl - 2 - pyridinyl)methyl]sulphinyl} - 1H - benzimidazole) were provided by Esteve Química, S.A. (Girona, Spain). Sodium phosphate mono-basic dihydrate supplied by Panreac (Barcelona, Spain) and sodium hydroxide supplied by Merck (Darmstadt, Germany) were of analytical grade. Acetonitrile and methanol were purchased from Scharlau (Barcelona, Spain). Demineralized water was purified in a Milli-Q filtration system (Millipore Corporation, Bedford, MA) to obtain water of HPLC grade. Drug-free human plasma used in this study was supplied by Hospital Clínic (Barcelona, Spain) and stored at −80°C until the assay.

2.2. Instrumentation

Chromatographic separations were performed using a Hewlett Packard equipment (Waldbronn, Germany), consisting of a model HP-1050 quaternary pump and a model 79853C ultraviolet detector. The software used to acquire the chromatograms was Access*Chrom supplied by Perkin Elmer (Cupertino, CA). The chromatograms were kept as data processing files.

The automated sample handling system consisted of a Prospekt (Programmable On-Line Solid Phase Extraction Technique), a refrigerated autosampler (Triathlon) and a solvent delivery unit. All of them were manufactured by Spark Holland (Emmen, The Netherlands).

2.3. Chromatographic conditions

The chromatographic separation was performed on an Inertsil ODS-2 analytical column (150 mm × 4.6 mm i.d.; 5 μm particle size) pur-

chased from GL Sciences (Tokyo, Japan). To protect the analytical column, a Tracer ODS cartridge (Kromasil; 10 mm × 3 mm i.d.; 5 μm particle size) supplied by Tecnokroma (Barcelona, Spain) was also used. This precolumn was replaced daily. The mobile phase used was sodium phosphate mono-basic (pH 7.2; 20 mM)-acetonitrile (70:30, v/v). The mobile phase was degassed prior to use under vacuum by filtration through a 0.2 μm Millipore membrane and during the chromatographic process with helium. The flow-rate was set at 0.5 ml min^{-1}. The UV detection was done at 302 nm (0.004 AUFS).

2.4. Preparation of stock solutions and working standard solutions

A stock solution of omeprazole (100 μg ml^{-1}) was prepared by dissolving 10 mg of omeprazole in 100 ml of methanol. Drug concentrations in the working standard solutions chosen for the calibration curve were 0.05, 0.1, 0.2, 0.4, 1, 2, 4 and 5 μg ml^{-1}. These working solutions were made by further dilution of the stock solution with water.

A stock solution of the internal standard (100 μg ml^{-1}) was prepared by dissolving 10 mg of phenacetin in 100 ml of methanol, from which a working standard solution of 10 μg ml^{-1} in water was made. All the solutions were prepared daily and protected from direct light.

2.5. Preparation of plasma standards and samples

The frozen drug-free human plasma was thawed at room temperature, vortexed and centrifuged at $2000 \times g$ for 10 min prior to use. Plasma standards and calibration standards for validation were prepared by adding 30 μl of each working standard solution to 270 μl aliquots of plasma. The vials were vortexed vigorously and placed in the Triathlon autosampler (10°C). The internal standard (30 μl; 10 μg ml^{-1}) was added automatically by the autosampler. The injection volume of the resultant mixture was 100 μl. Omeprazole concentrations in plasma standards ranged from 5 to 500 ng ml^{-1}.

In the bioequivalence studies, aliquots of 300 μl of plasma were pipetted into vials. Afterwards, the samples were treated as described above. Quality control samples were prepared by spiking drug-free human plasma with the different working standard solutions of omeprazole.

2.6. Solid-phase extraction

Solid-phase extraction of the samples was made on disposable C_{18} cartridges of Analytichem (10 mm × 2 mm i.d.) supplied by Spark Holland (Emmen). The solid-phase extraction procedure is summarised in Table 1.

2.7. Validation

The following parameters were determined for the validation of the analytical method developed for omeprazole in human plasma: selectivity, linearity, range, precision, accuracy, limit of quantitation, recovery, stability and ruggedness [21].

2.8. Application of the method

The developed method has been applied to bioequivalence studies in which the concentration of omeprazole was measured in more than 10 000 plasma samples.

As an example, we show the results obtained in a randomized crossover study. In this study, single oral doses (20 mg) of an omeprazole formulation developed by Laboratorios Dr. Esteve, S.A. (Test) or Mopral® (Astra; Reference) were administered in capsules to 36 volunteers of both sexes. The medication was administered under fasting conditions with 125 ml of water. Blood samples were collected at 0, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12 and 24 h after drug administration. After each blood sampling, plasma was separated by centrifugation at 3000 rpm for 5 min and stored at −80°C until assay.

3. Results and discussion

3.1. Optimization of the chromatographic conditions

Several stationary and mobile phases were

Table 1
Solid-phase extraction procedure

Time (min:s)	Solvent	Flow-rate (ml min^{-1})	Comment
0:00	Methanol	1.5	Change of cartridge Activation of cartridge with methanol
1:00	Water	1.5	Activation of cartridge with water
3:00	Water	1.5	The injector adds the IS and shakes the mixture
9:30	Water	1.0	Injection of sample Adjusting flow-rate over cartridge Washing of sample with water
11:00	Water/CH_3CN (90:10 v/v)	1.5	Adjusting flow-rate over cartridge Washing of sample with water/CH_3CN (90:10 v/v)
11:45	Water/CH_3CN (90:10 v/v)	0.1	Start of elution Start of data collection Adjusting flow-rate over cartridge
13:45	Water/CH_3CN (90:10 v/v)	1.5	End of elution Adjusting flow-rate over cartridge Washing of sample with water/CH_3CN (90:10 v/v)
15:00	Water	1.5	Washing of capillaries with water
18:00	Methanol	1.5	Washing of capillaries with methanol
20:00		0.0	End of washing End of method

checked to establish the optimum separation and the highest analytical sensitivity for omeprazole. Such sensitivity was given by the slope of calibration curve obtained for the assayed conditions. The best results were obtained with the conditions reported previously in the experimental section. The pH of the mobile phase played a key role in the stability of the omeprazole during the process. Omeprazole is an ampholyte with pKa values of 3.97 (pyridine) and 8.8 (benzimidazole). It is rapidly degraded in acidic solutions but it has an acceptable stability at neutral and alkaline pH [3]. Consequently, the mobile phase was buffered at a pH of 7.2 in order to ensure the stability of omeprazol. A higher pH was avoided because of the risk of degradation of the silica-based column.

The working solutions were prepared in water so as not to exceed 0.5% organic solvent in plasma and to avoid peak distortion phenomena in HPLC determination of omeprazole [22].

The mean retention times for omeprazole and the internal standard were 14.9 min and 12.2 min respectively. The analysis time was set at 20 min. This time assured the elution of all endogenous compounds and metabolite peaks and it allowed the preparation and extraction of the next sample. The solid-phase extraction of the samples was performed during the run time of the previous analyses.

Different solid-phase extraction cartridges (C_2, C_8, C_{18} and CN) were also tested. C_{18} cartridges were chosen because they showed the highest recovery for omeprazole and the internal standard under the assay conditions.

The use of on-line solid-phase extraction method provided time-saving in the handling of the samples as well as a good response using only 100 μl of sample. The manual sample preparation for the validation of the method was reduced to the dilution of plasma with the working solution containing omeprazole. Unlike omeprazole, the internal standard was added automatically by the autosampler. In the samples, the handling was reduced to the addition of 300 μl of plasma to each vial.

A representative chromatogram of drug-free human plasma is shown in Fig. 2a. A chromatogram corresponding to a plasma standard sample spiked with 100 ng ml^{-1} omeprazole and 1000 ng ml^{-1} internal standard is shown in Fig. 2b. Baseline resolution of the substances was achieved under the chromatographic conditions of the study.

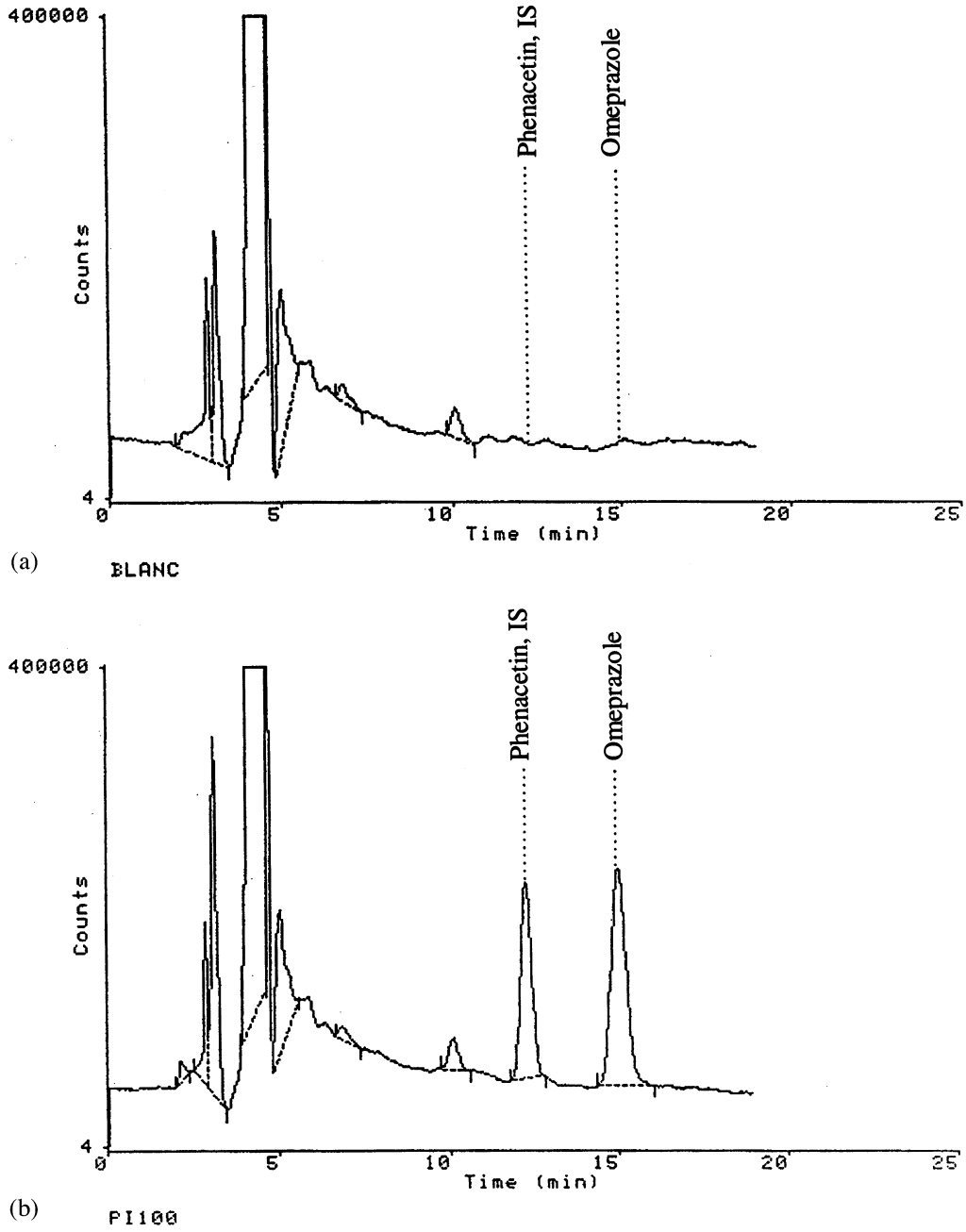

Fig. 2. Representative chromatograms obtained from (a) drug-free human plasma and (b) human plasma spiked with omeprazole (100 ng ml^{-1}) and internal standard (1000 ng ml^{-1}).

The suitability of the chromatographic system was checked daily before analysis by evaluating the tailing factor, the resolution and the system repeatability of three injections of a solution of omeprazole (100 ng ml^{-1}) and internal standard in mobile phase. The mean experimental concentration (% ± SD) obtained for these unextracted samples was 99.5 ± 4.5%. This value demonstrates the good performance of the chromatographic system throughout the study.

3.2. Selectivity

The selectivity of the method was determined by injecting drug-free human plasma from six different sources [23]. These chromatograms were free of interferences at the retention times of omeprazole and the internal standard (Fig. 2a–b). Moreover, the pre-dose samples of the volunteers included in the bioequivalence study did not show any relevant interference.

Three metabolites of omeprazole namely omeprazole sulphide, hydroxyomeprazole and omeprazole sulphone, which might be present in samples, were tested for possible interferences with omeprazole analysis. Omeprazole sulphide, a minor plasma metabolite, eluted with a relative retention time (t_{RR}) of 3.75. The relative retention times for hydroxyomeprazole and sulphone were 0.53 and 1.46, respectively. None of these compounds had relative retention times that could interfere with the measurement of omeprazole ($t_{RR} = 1.22$) or the internal standard ($t_{RR} = 1.00$). The chromatograms shown in Fig. 3 are from a volunteer who was dosed with 20 mg of omeprazole (Fig. 3a is the pre-dose sample and Fig. 3b is the 2.5 h post-administration sample).

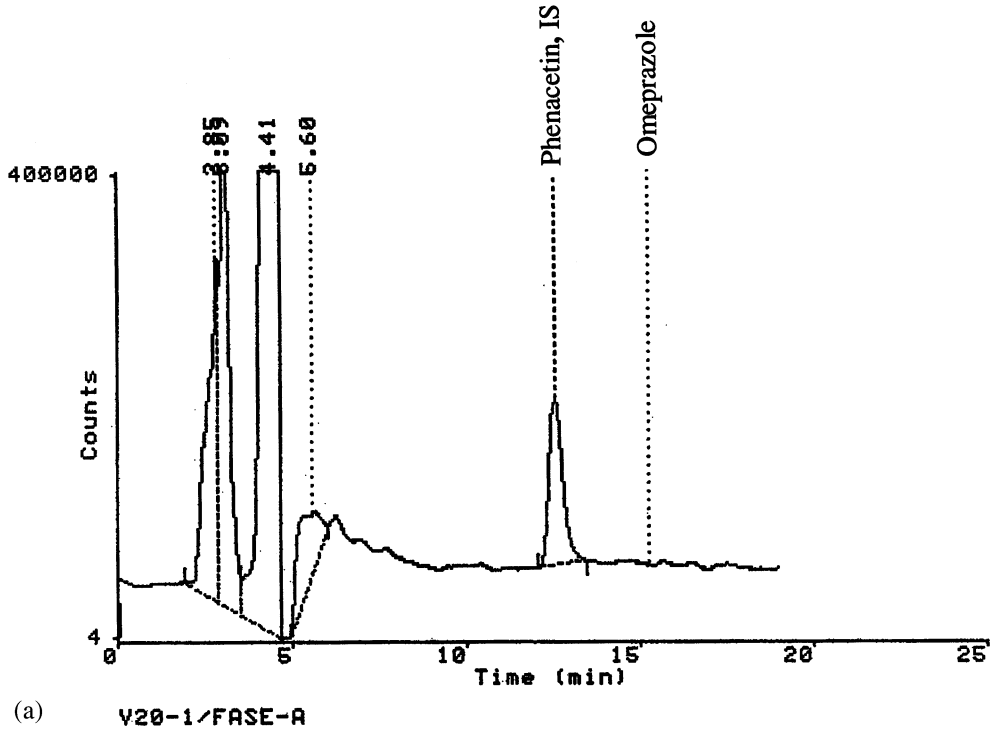

Fig. 3. Chromatograms of plasma samples from a volunteer. Column: Inertsil ODS-2 (150 mm × 4.6 mm i.d.; 5 μm); mobile phase: sodium phosphate mono-basic (pH 7.2; 20 mM)-acetonitrile (70:30, v/v); flow rate: 0.5 ml min^{-1}; detection: UV at 302 nm; The samples were extracted with C_{18} cartridges. (a) pre-dose and (b) 2.5 h after a 20 mg oral dose of omeprazole. (Omeprazole concentration = 132.0 ng ml^{-1}).

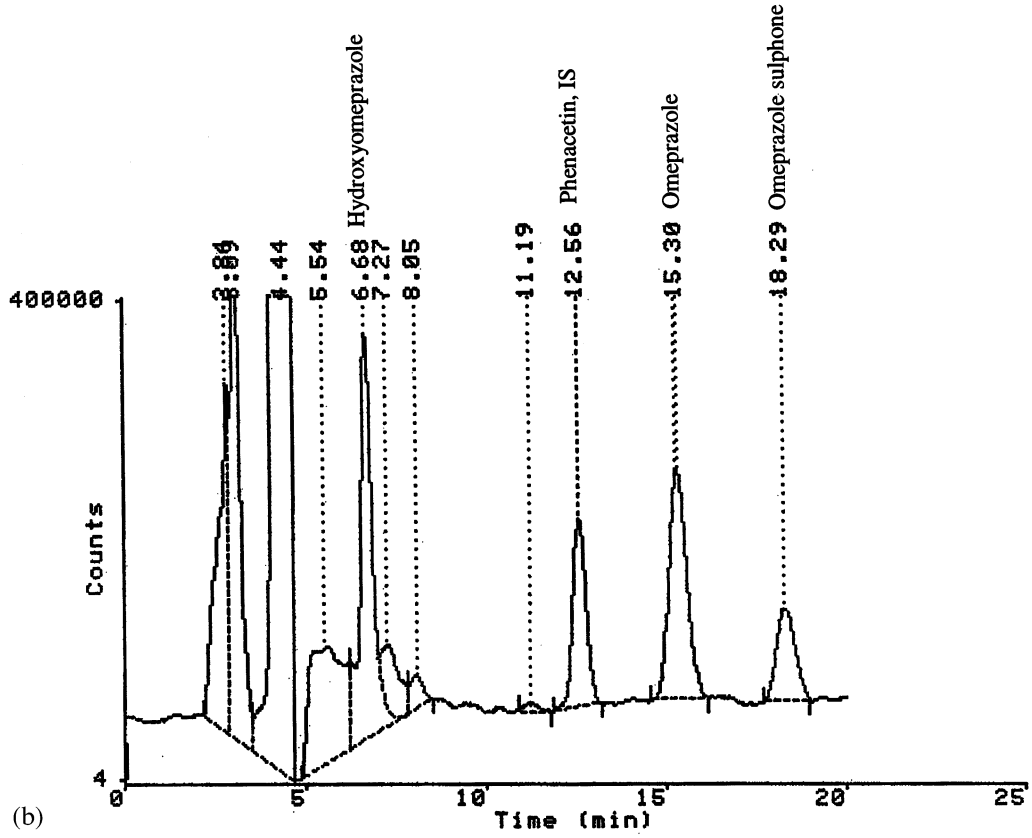

Fig. 3. (*Continued*)

3.3. Linearity and range

The linear range for omeprazole in human plasma was validated using eight standards covering the range from 5 to 500 ng ml^{-1}.

Omeprazole-to-standard internal peak area ratios were plotted against the corresponding concentrations. Data were fitted to the equation $y = mx + b$, where y is the peak area ratio, x is the drug concentration and m and b are the slope and y-intercept of the calibration curve, respectively.

To construct a calibration curve in bioanalysis, a linear regression analysis is generally used. This analysis assumes univariant regression, implying that the residuals are minimised around the dependent variable (or response) and that the independent variable (or concentration) is errorless. Other assumptions involved are the independence and normal distribution of residuals as well as their homocedasticity (equal variances).

Homocedasticity was not observed in our data, as usually occurs in bioanalysis [24–26]. In these cases, a weighted regression can be applied. The inverse of the variance ($1/\sigma^2$) is the most appropriate weighting factor to be used. Other factors commonly used ($1/x$, $1/x^2$, etc.) are approximations to $1/\sigma^2$ [25].

Accordingly, in the current study, weighting factors of $1/x$, $1/x^2$ and $1/\sigma^2$ were investigated. The best fit was obtained using the reciprocal of σ^2 and therefore, this was the weighting used for subsequent analyses.

The variance of each point was calculated by linear fitting of the standard deviation ($n = 10$) of omeprazole-to-internal standard peak area ratio versus omeprazole concentration. The variance was obtained from the square of the predicted standard deviation associated to each value of concentration.

Table 2
Back-calculated concentration (mean ± SD) of calibration samples ($n = 10$)

Concentration added (ng ml^{-1})	Concentration found (ng ml^{-1})	RSD (%)	RE (%)
5	5.1 ± 0.1	2.3	2.4
10	9.6 ± 0.8	8.5	−4.4
20	18.8 ± 0.9	4.9	−6.2
40	39.9 ± 2.5	6.3	−0.4
100	103.9 ± 4.0	3.8	3.9
200	199.2 ± 12.2	6.1	−0.4
400	404.9 ± 20.2	5.0	1.2
500	520.6 ± 30.5	5.9	4.1

The calibration curves obtained during 10 days showed a linear relationship with a mean determination coefficient of 0.993 for the range of concentrations used (5–500 ng ml^{-1}). The mean y-intercept value represented the 6% of the lowest calibration standard of the curve indicating negligible interference [27].

The linearity of the calibration curves was demonstrated by fitting the data comprising each curve to the equation $y = mx^N + b$ [28,29], and checking that the value of N was not different from 1. The confidence interval (CI) associated to N was calculated according to the following equation:

$$\mathrm{CI} = N \pm t_{\alpha/2,\,\mathrm{df}} \times \mathrm{SE}$$

where N is the exponent of the equation; $t_{\alpha/2,\,\mathrm{df}}$ is the Student t distribution for the one-tailed probability level of 95% ($\alpha/2 = 0.025$) with $n - 3$ degrees of freedom from error; SE is the standard error corresponding to the exponent and n is the number of points included in the calibration curve.

The confidence intervals obtained for each calibration curve included the unity (N: 1.05 ± 0.12; mean ± SD) suggesting that the method proposed to determine omeprazole in human plasma is linear in the concentration range studied (5–500 ng ml^{-1}).

Back-calculated values for the calibration standards of the method in human plasma are presented in Table 2. The RSD (RSD = SD/mean × 100) ranged between 2.3 and 8.5%. On the other hand, the relative error of the mean measurement (RE = (calculated value-nominal value/nominal value) × 100) ranged from −6.2 to 4.1%. Based on the RSD and RE values of calibration standards, the method demonstrates sufficient adherence to a linear model over the concentration range from 5 to 500 ng ml^{-1}.

3.4. Precision and accuracy

The precision of the assay for omeprazole was evaluated by determining the intraday and interday RSD of the measured peak area ratios for different concentrations. The intraday precision of the method was determined by measuring eight plasma standards at six concentrations (5, 10, 20, 100, 200 and 500 ng ml^{-1}). These plasma standards were different from the calibration standards to avoid the influence of the calibration curve [30,31]. The results are presented in Table 3. The values obtained were in all cases lower than 8.7%. The interday precision was evaluated at six different concentrations (5, 10, 20, 100, 200 and 500 ng ml^{-1}) during 8 days. The values ranged from 5.5 to 17.5%. As expected, the RSD increases as the concentration levels of omeprazole decrease. Both intraday and interday precision values fell within the limits considered as acceptable [23].

The intraday and interday accuracy of the assay were calculated from the comparison of omeprazole concentrations determined in plasma standards with the corresponding nominal values. The accuracy was expressed as mean percentage of analyte recovered in the assay (Accuracy = (calculated value/nominal value) × 100). Table 3 shows the intraday accuracy ($n = 8$) evaluated at six

Table 3
Precision and accuracy of the method for the determination of omeprazole in human plasma ($n = 8$)

Concentration (ng ml^{-1})	Mean found ± SD (ng ml^{-1})	Precision RSD (%)	Accuracy (%)
Intraday			
5	5.3 ± 0.5	8.7	106.2
10	9.3 ± 0.8	8.0	93.1
20	19.8 ± 1.3	8.3	98.9
100	103.1 ± 5.4	5.2	103.1
200	197.2 ± 6.1	3.2	98.6
500	491.4 ± 31.8	6.5	98.3
Interday			
5	4.8 ± 0.5	17.5	95.4
10*	9.9 ± 1.1	11.3	99.2
20	20.2 ± 1.5	14.9	101.0
100	101.8 ± 7.3	13.9	101.8
200	210.1 ± 15.1	5.5	105.1
500	477.0 ± 19.3	9.2	95.4

* $n = 6$.

concentrations (5, 10, 20, 100, 200 and 500 ng ml^{-1}). The values obtained ranged from 93.1 to 106.2%. The interday accuracy was evaluated during 8 days at the same concentrations used to calculate the intraday accuracy. Values varying from 95.4 to 105.1% were found. All the values obtained for accuracy were within the limits considered as acceptable for bioanalysis [23].

3.5. Limit of quantitation (LOQ)

The limit of quantitation, defined in the presented study as the lowest plasma concentration in the calibration curve that can be measured routinely with acceptable precision (RSD < 20%) and accuracy (80–120%), was 5 ng ml^{-1} (Table 3).

3.6. Recovery

The percentage of omeprazole recovered from plasma using the proposed procedure, was calculated by comparison of the drug peak area in the extracted plasma samples ($n = 8$) with the mean peak area obtained from direct injection of the corresponding unextracted standard solutions. The recovery was measured at six different concentrations (5, 10, 20, 100, 200 and 500 ng ml^{-1}) over the calibration range used. Regarding the internal standard, recovery was only calculated at the working concentration (1000 ng ml^{-1}).

Table 4 shows the recovery, expressed as percentage, obtained for both omeprazole and internal standard. Regardless of the drug concentration, the recovery found ranged from 88.1 to 101.5%. No clear relationship between concentration and recovery was found. For the internal standard ($n = 40$), a recovery of 71.9% was obtained. The low recovery of phenacetin could be explained by the fact that this compound is unrelated chemically with omeprazole.

In on-line solid-phase extraction, the variability associated with the recovery can be accounted for the impossibility of adding to the matrix the

Table 4
Recovery of omeprazole and its internal standard (phenacetin) from human plasma samples ($n = 8$)

Concentration (ng ml^{-1})	Recovery ± SD (%)	
	Omeprazole	IS
5	89.4 ± 14.0	–
10	89.2 ± 9.2	–
100	101.5 ± 6.1	–
200	88.1 ± 2.7	–
500	94.2 ± 1.5	–
1000	–	71.9 ± 9.1

Table 5
Relative standard deviation values obtained for the quality control samples assayed in three studies during 9 months[a]

Concentration (ng ml^{-1})	Duration of the study		
	3 months	2 months	1 month
10	7.0 (21)	8.2 (32)	–
20	7.0 (26)	8.5 (78)	7.6 (33)
40	6.9 (34)	8.1 (51)	6.5 (26)
80	9.8 (28)	–	–
100	8.7 (31)	8.0 (92)	7.0 (31)
200	7.1 (25)	8.5 (93)	7.3 (32)
400	9.1 (24)	8.5 (60)	4.9 (37)
500	8.9 (24)	7.4 (64)	5.1 (27)

[a] Values in parenthesis represent the number of replicates.

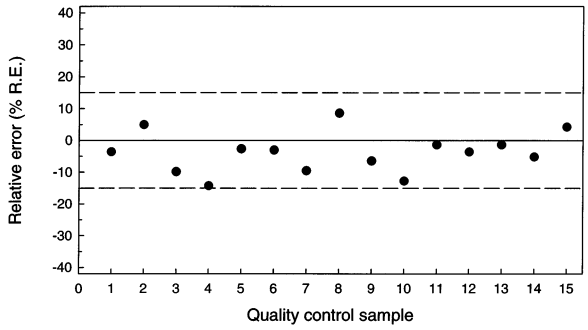

Fig. 4. Graphical representation of relative error from quality control samples assayed during a batch.

unextracted internal standard. However, in off-line extraction, the addition of internal standard helps to minimize any error related with the injection.

3.7. Stability

The stability was studied under three storage conditions: stability in methanolic solutions (100 μg ml^{-1}) of omeprazole and phenacetin (internal standard) at 4°C for 1 week, stability in plasma samples (100 ng ml^{-1}) frozen at −80°C for 6 months and stability after three freeze/thaw cycles at −80°C.

The results of the stability studies of omeprazole and its internal standard in methanolic solution showed that this solution could be stored at 4°C for 7 days (101.8 ± 1.3% for omeprazole and 101.5 ± 1.8% for phenacetin, expressed both as percentage found ± SD).

The stability of omeprazole in plasma was demonstrated by analysing ten aliquots (100 ng ml^{-1}) stored at −80°C. After 6 months in storage, the mean percentage of initial concentration was 99.9 ± 4.3%. The mean percentage found after three freeze/thaw cycles was 98.9 ± 5.1%.

The results of the stability studies showed that omeprazole was stable in plasma under the storage conditions assayed. These results agree with those obtained by other authors [14,19].

3.8. Ruggedness

Peak shape and resolution of omeprazole from other peaks in the matrix remained visually acceptable throughout the assay. The limit of quantitation demonstrated a reproducible response readily distinguishable from the noise level.

The quality control samples of three bioequivalence studies were used to evaluate the ruggedness of the method [25]. The method was applied during 9 months using 15 analytical columns, two apparatus, two analysts and several batches of chemical reagents. Table 5 shows the RSD obtained in the three studies. The values were all lower than 9.8%, suggesting that the method does not change with time or study conditions.

3.9. Application of the method

This procedure has been applied successfully to the analysis of samples from several bioequivalence studies.

In a study with 36 volunteers, human plasma samples together with calibration standards and quality control samples were assayed for omeprazole content.

Drug concentrations determined in the quality control samples were in good agreement with the concentrations added. Fig. 4 shows the relative error of the 15 quality control samples analysed during one batch. All the values obtained were within the interval of ± 15%. Although the results were dependent on the extraction repeatability, this figure gives us information about the in-process stability of samples during the run.

Fig. 5 shows the mean relative errors of quality controls (accepted and rejected) obtained during all the batches prepared in the study. The value obtained was lower than 10.9%.

Fig. 6 illustrates mean plasma concentrations ± SE of omeprazole following single oral doses of 20 mg of Esteve omeprazole (Test) and 20 mg of Mopral® (Astra; Reference).

4. Conclusions

The presented study describes and validates an HPLC method for the determination of omeprazole in human plasma. The method proved to be lineal in the concentration range studied (5–500 ng ml^{-1}) as well as accurate, precise, sensitive and selective. The usefulness of this method in the routine analysis was successfully demonstrated by the analysis of a large number of samples from bioequivalence studies. The ruggedness of the method was confirmed during its application.

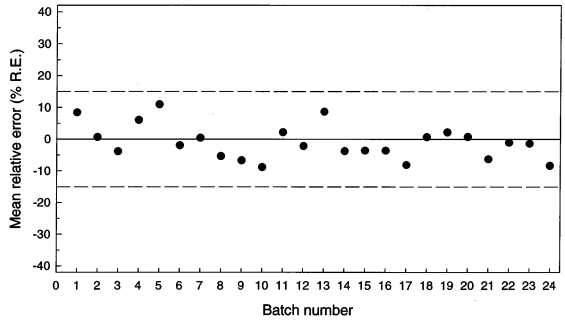

Fig. 5. Graphical representation of mean relative error from quality control samples assayed during the study.

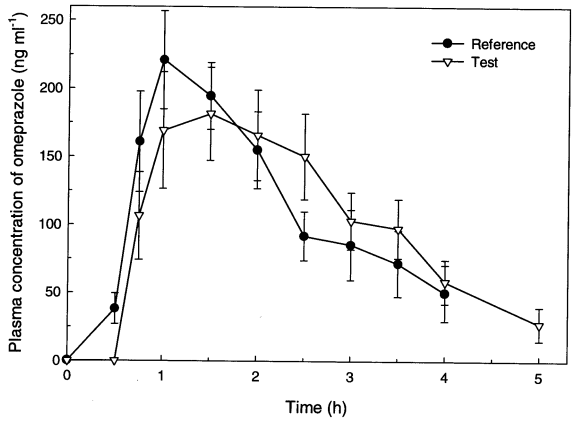

Fig. 6. Mean (± SE) time-concentration profiles of omeprazole in human plasma samples of a bioequivalence study. The volunteers ($n = 36$) were administered 20 mg omeprazole orally.

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