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Determination of omeprazole, hydroxyomeprazole and omeprazole sulfone using automated solid phase extraction and micellar electrokinetic capillary chromatography

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Abstract

A sensitive method for the determination of omeprazole and its metabolites has been developed. It involves an automated solid phase extraction (SPE) procedure and capillary electrophoresis with UV detection. Omeprazole, hydroxyomeprazole and omeprazole sulfone could be separated by micellar electrokinetic capillary chromatography using a background electrolyte composed of 20 mM borate buffer and 30 mM sodium dodecyl sulfate, pH 9.5. The isolation of omeprazole and its metabolites from plasma was automatically accomplished with an original SPE procedure using surface-modified styrene–divinylbenzene polymer cartridges. Good recovery data and satisfactory precision values were obtained. Responses were linear for the three analytes, from 0.08 to 2.0 μg/mL of plasma. Intra- and inter-day precision values of about 1.6% R.S.D. ($n = 10$) and 2.5% R.S.D. ($n = 36$), respectively, were obtained. The method is highly robust and no breakdown of the current or capillary blockages were observed during several weeks of operation. The validated method was applied to the determination of omeprazole in pharmaceutical preparations and for the analysis of plasma samples obtained from three volunteers who received oral doses of omeprazole.

Keywords: Omeprazole; Hydroxyomeprazole; Omeprazole sulfone; Micellar electrokinetic capillary chromatography; Solid phase extraction

1. Introduction

Omeprazole (OME, Fig. 1), a substituted α-pyridymethylsulfinyl benzimidazole, is a gastric H^+-, K^+-ATPase inhibitor. Its therapeutic potential has been described as a potent long-acting inhibitor of gastric acid secretion for the treatment of peptic ulcer, refractory gastroesophageal reflux disease, Zollinger–Ellison syndrome and infection caused by Helicobacter pylori [1]. OME undergoes extensive hepatic metabolism by the cytochrome P450 system (particularly CYP2C19 and CYP3A4) [1–3] and its main metabolites are omeprazole sulfone (OME-S) and 5-hydroxyomeprazole (H-OME) [1–4]. OME has a half-life of about 2 h, but it continues to act for much longer [1].

Several high-performance liquid chromatographic (HPLC) methods with tandem mass spectrometry, ICP-mass spectrometry or photometric detection systems have been described in the literature to determine OME and its metabolites in biological fluids [5–10]. These assays use liquid–liquid or off-line and on-line solid phase extraction for the analytes isolation. Some of these methods suffer from inadequate sensitivity, long analysis times and the use of toxic halogenated solvents.

Capillary electrophoresis (CE) has gained significant acceptance in the analytical laboratory owing to its many advantageous features, such as extremely high efficiency, high resolution, rapid analysis and small consumption of sample and reagents. However, reports on OME are very few [11,15]. Capillary zone electrophoresis (CZE) has been proposed for the determination of OME in human plasma using a running buffer composed of 50 mM phosphate–12.5 mM borate at pH 10.1 [11] and for the simultaneous analysis of OME and lansoprazole in capsules [12], a phosphate buffer (50 mM; pH 9) was used as electrophoretic electrolyte. The separation of the OME enantiomers has been reported using either 20 mM phosphate buffer (pH 4) and sulfated β-cyclodextrin [13] or 40 mM phosphate buffer (pH 2.2) and β-cyclodextrin [14]. For the separation of OME and related sulfoxide drugs, non-aqueous conditions have been recommended [15].

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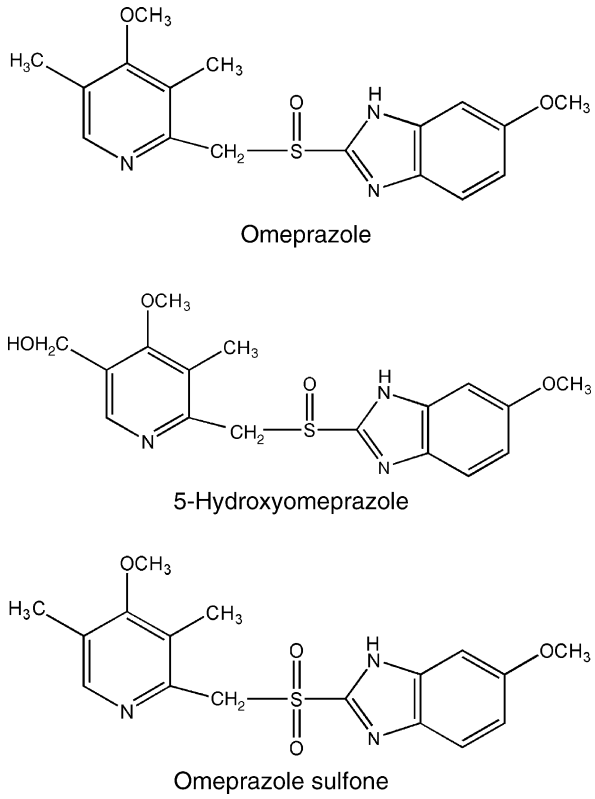

Fig. 1. Chemical structures of omeprazole and its metabolites.

Micellar electrokinetic capillary chromatography (MEKC), one of the most important modifications of CE, has proved to be an excellent alternative to HPLC for drug analysis. MEKC is faster and less costly than HPLC and for some analyses, it has replaced HPLC as the method of choice.

To summarize, to the best of our knowledge, no successful CE method has been described for the simultaneous determination of OME and its metabolites. The purpose of the work described in this study was to develop a rapid and straightforward CE method for the direct determination of OME, H-OME and OME-S (Fig. 1). The method also involves an automated and efficient SPE procedure for the pretreatment of the plasma samples and MEKC analysis. The assay was validated by determining its accuracy, precision, linearity and selectivity.

2. Materials and methods

2.1. Reagents and solutions

Demineralized water was obtained using a Milli-Q system (Millipore-Ibérica, Madrid, Spain). Methanol (HPLC-grade) was purchased from Romil (Leoughborough, Leicestershire, UK). OME was obtained from Sigma–Aldrich Quimica (Madrid, Spain) and H-OME and OME-S were generous gifts from Astra Zeneka (Mölndal, Sweden). Sodium dodecyl sulfate was obtained from Sigma; other chemicals were of the highest grade commercially available and were used without further purification.

Stock solutions (200 μg/mL) of OME, H-OME and OME-S were prepared by weighing 10 mg of each compound into separate 50 mL volumetric flasks, dissolving the materials in 10 mL of methanol and a few drops of 0.1 M sodium hydroxide and filling the flask to volume with ultrapure water. The solutions were stable for at least 3 months when stored at 5 °C and protected against direct sunshine and no evidence of the degradation of the analytes was observed in the electropherograms during this period. Working standard solutions were freshly prepared by dilution of the stock solution with ultrapure water.

Buffer solutions were prepared from sodium tetraborate or sodium dihydrogen phosphate and adjusting with 1 mol/L sodium hydroxide or hydrochloric acid to an appropriate pH.

All solutions were degassed by sonication and were filtered through 0.45 μm membrane filters (Millipore) prior to use.

2.2. Apparatus and running conditions

A P/ACE System 5500 (Beckman Coulter, Fullerton, CA, USA) equipped with a diode-array detector, an automatic injector, a fluid-cooled cartridge and a System Gold data station were used in this study. Electrophoresis was performed in an uncoated fused-silica capillary (75 μm i.d., 375 μm o.d.) of 57 cm total length (50 cm effective length) thermostated at 25 °C. Except for some buffers used for optimizing of analyte separation, the running buffer consisted of 20 mM borate adjusted to pH 9.5 containing 30 mM SDS. Sample introduction was made at the anode side using the pressure option (0.5 psi ≈ 3.45 kPa) for 5 s. Analyses were performed with a normal electrode polarity. The voltage applied was 18 kV and the current was ca. 35 μA with the running buffer. The capillary was conditioned every morning before starting a sequence of runs by flushing for 5 min with 0.1 M sodium hydroxide and 5 min with the running buffer. It was then equilibrated with the buffer for 5 min applying the separation voltage of 18 kV. Between experiments, the capillary was rinsed with buffer for 2 min. The capillary inlet and outlet vials were replenished after every 15 injections. When long automated sequences of samples were being run, different wash and run buffer vials were used to avoid lowering the fluid level in the run buffer vial and to prevent consequent hydrodynamic siphoning.

Absorbance spectra of OME, H-OME and OME-S dissolved in the background electrolyte were recorded with an Unicam UV–vis spectrophotometer (Unicam Ltd., Cambridge, UK) with a 1 cm path-length quartz cell to identify the peaks obtained. The detection of the analytes was performed at 300 nm.

The pH of the electrophoretic buffers was adjusted by means of a pH-meter 900 Precisa (Dietikon, Switzerland).

All extraction steps were performed automatically using a Gilson ASPEC XLi (Gilson, Middleton, WI, USA). Strata X, surface modified styrene–divinylbenzene polymer (Phenomenex, CA, USA), was used as the solid phase in the extraction.

2.3. Automated extraction of OME, H-OME and OME-S

Strata X cartridges (200 mg) with a 3 mL reservoir were conditioned with 1 mL of methanol followed by 1 mL of ultra-

pure water separated by 1 mL of air, prior to application the sample. A volume of 1 mL of plasma was diluted with 1 mL of ultrapure water and the mixture was then passed through the cartridge at a flow rate of 0.5 mL/min. The cartridge was washed with 3 mL ultrapure water and 3 mL of air. The retained analytes were eluted at 0.3 mL/min with 4 mL of methanol followed by 4 mL of air. The eluate was evaporated to dryness at 60 °C by passing an air stream for 3 min. The residue was dissolved with 10 μL of methanol and 90 μL of ultrapure water and transferred by the ASPEC into the sample microvial of the CE system.

2.4. Preparation of plasma standards and samples

The frozen drug-free human plasma was thawed at room temperature, vortexed and centrifuged at 2000 × *g* for 20 min prior to use. Plasma standards were prepared by adding 25 μL of variable concentrations of OME, H-OME and OME-S working standard solutions to 1000 μL of plasma. The concentration of each drug in plasma standards ranged from 0.08 to 2.0 μg/mL. The samples were submitted to SPE before CE analysis.

Blood samples were taken from healthy adults volunteers 3 h after a single oral dose of 20 mg OME (Omeprazole capsules, Normon, Madrid, Spain) following an overnight fast. The blood was put into vials containing EDTA as the anticoagulant and immediately centrifuged for 20 min at 2000 × g. The supernatant plasma was frozen and maintained at −20 °C until analysis. After the same treatment as described above for plasma standards, the clinical plasma samples were analysed.

2.5. Assay specification

Calibration curves were constructed by plotting the results obtained on a graph representing peak area versus plasma concentration and the best relationship was obtained by linear least-squares regression analysis. No internal standard was used.

The absolute recoveries were determined by comparing the concentration of spiked plasma samples (in the 0.1–1 μg/mL range, $n = 4$ for each concentration) calculated on calibration curves constructed by direct analysis of aqueous standard solutions of each analyte, with the nominal concentration of the samples. Recovery was expressed as percentage of the amount extracted.

Precision was expressed as relative standard deviation (R.S.D.) and accuracy as percent of deviation between the true and the measured value. The selectivity of the method was assured by analysing standard solutions of several types of drugs at a concentration of 500 μg/mL. The drug that was detected at similar migration times to the analytes was added to aliquots of 1 mL plasma at its maximum therapeutic concentration and submitted to the automated SPE process before MEKC analysis. Drug-free plasma samples were also analysed to assess the capacity of sample pretreatment to eliminate endogenous interferents.

3. Results and discussion

3.1. Capillary zone electrophoresis

OME and its metabolites H-OME and OME-S are ampholytes and have two dissociation constants. Two consistent pK_a values of 7.1 and 14.7 were obtained for OME [16]. The pK_a of 7.1 is assigned to the dissociation from the protonated pyridine nitrogen and the pK_a of 14.7 corresponds to the dissociation of the hydrogen from one-position of the benzimidazole ring. At pH < 5, OME decomposes after protonation at the three-position benzimidazole nitrogen atom. Therefore, the separation of these compounds by CZE was investigated at pH > 5 using 20 mM phosphate and/or borate buffer. Fig. 2 shows the pH-dependence of the migration time of OME, H-OME and OME-S. The best pH range for the separation of the three compounds proved to be between 7.5 and 9.5 and this range was studied closely. Relatively good separation of OME-S from OME and H-OME was obtained under electrophoresis buffer conditions of pH 8.5 with 20 mM sodium borate; however, the pair OME/H-OME could not be separated.

3.2. Micellar electrokinetic capillary chromatography

Analytes are now mainly separated by observing the difference in partitioning between the micellar phase and the aqueous phase and also according to their electrophoretic mobility. This technique has been reported to have advantages over CZE in the separation of both electrically neutral and ionic substances [17].

There are several ways of manipulating the selectivity in MEKC, the most effective is to change the type of surfactant [18]. In this respect, a method was developed in which anionic, cationic and non-ionic surfactants were studied. Triton X-100, sodium cholate and sodium taurocholate were not suitable for

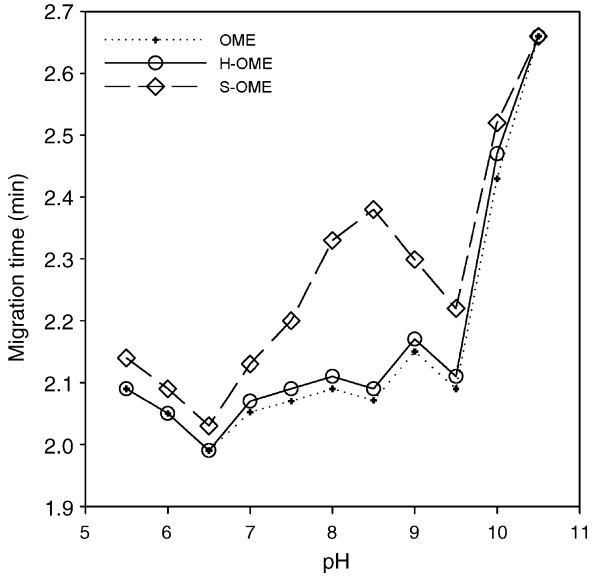

Fig. 2. Effect of pH on the migration in CZE of OME, H-OME and OME-S. Electrophoretic conditions: capillary 57 cm × 75 μm; applied voltage, 18 kV; pressure injection, 5 s at 0.5 psi.

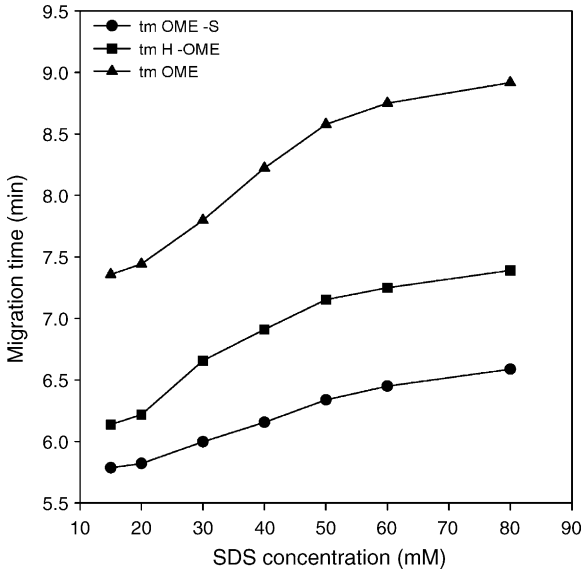

Fig. 3. Effect of the SDS concentration on the migration time in MEKC. BGE: borate buffer (20 mM, pH 9.5) containing 15–80 mM SDS. Other experimental conditions as in Fig. 2.

use as micellar phase because OME and H-OME could not be separated. Cetyltrimethylamonium bromide was also unsuitable because the migration times were very long (more than 40 min) and OME co-eluted with H-OME. When sodium dodecylsulfate (SDS) was added to the running electrolyte, a clear improvement of the separation was observed. The migration time of OME, H-OME and OME-S increased with increasing SDS concentration (Fig. 3) because of stronger interaction with micelles. The SDS concentration selected in the background electrolyte (BGE) was 30 mM, which gave the best base line separation for the three compounds.

The migration times of the OME and its two metabolites obtained using a BGE containing 30 mM SDS and 20 mM borate decreased with increasing pH. At the same time, it was noted that the migration order for the three compounds remained unchanged throughout the range of pH values examined (7.0–10.0). This observation suggests that there are no major changes in the extent to which these compounds are ionized at the pH values investigated. The best results in the terms of separation were achieved at pH 9.5, when the resolutions of the peaks were the greatest.

The effect of the ionic strength of the BGE was tested at a constant pH of 9.5 and SDS concentration of 30 mM. As the borate buffer concentration in the BGE increased from 10 to 60 mM, an increase in the migration times was observed. The resolution of the peaks improved with increasing borate buffer concentration up to 20 mM, above which it remained virtually constant for the pair H-OME/OME and decreased for OME-S/H-OME. The concentration selected was 20 mM.

The effect of applied voltage on peak efficiency was studied at a constant temperature of 25 °C using a solution containing 30 mM SDS and 20 mM borate buffer (pH 9.5) as BGE. An increase in applied voltage led to shorter migration times, sharper peaks and higher efficiencies. To limit the heating inside the capillary, the maximum applied voltage was chosen from an Ohm's plot. The best separation was achieved with a voltage of 18 kV.

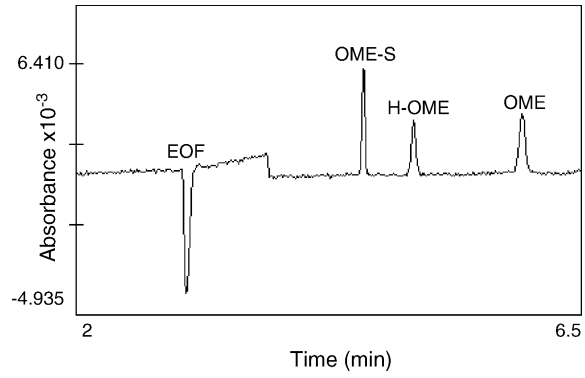

Fig. 4. MEKC separation of a standard solution containing OME, H-OME and OME-S (8 μg/mL, each one). BGE: borate buffer (20 mM, pH 9.5) containing 30 mM SDS. Detector wavelength 300 nm. Other electrophoretic conditions as in Fig. 2.

The length of the sample injection time was examined in an attempt to improve sensitivity. The sample solutions were hydrodynamically injected by applying a small pressure (0.5 psi, 3.45 kPa) at the inlet of the capillary, while the injection time was varied from 1 to 10 s. Satisfactory linearity between the peak area and injection time (analyte quantity) was achieved for the three compounds. The injection time selected was 5 s, which corresponded to a plug length of ca. 1.2% of the whole capillary length.

The result of the optimization of the BGE composition and the electrophoretic parameters can be seen from Fig. 4: baseline separation of all analytes is obtained within 6.5 min in an uncoated fused-silica capillary. This system was then applied to the determination of the analytes in standard solutions and in human plasma.

3.3. MEKC analysis of standard solutions

The optimized procedure was applied to the analysis of aqueous solution of OME, H-OME and OME-S. Calibration graphs were obtained by injection standard solutions of the analytes in the concentration range 0.6–50 μg/mL (at least 12 samples covering whole range of concentrations were used). External calibration was used because no improvement was observed when an internal standard was used. The regression equations obtained by means of the least square method were: $Y = 0.002 + 0.016X$ ($r = 0.9992$) for OME, $Y = 0.005 + 0.010X$ ($r = 0.9992$) for H-OME and $Y = 0.006 + 0.012X$ ($r = 0.9995$) for OME-S, where Y is the peak area and X is the analyte concentration expressed in μg/mL. The detection limits, calculated on the basis of the minimum analyte concentration that provides a signal-to-noise ratio of 3, were within the range 0.04–0.07 μg/mL.

The reproducibility of the method was assessed by analysing 10 replicates of the same standard solution (containing 0.9 μg/mL of each analyte) on the same day, R.S.D.s for the peak area were 0.74%, 0.90% and 0.83% for OME, H-OME and OME-S, respectively. The between-day precision was studied by analysing on six consecutive days, the same standard solution

Table 1
Recovery data of spiked blank plasma samples

Compound	Amount added (ng/mL)	Amount found (ng/mL)	Reproducibility within-day (%)	Precision between-day (%)
OME	100	99	1.8	2.8
	300	295	1.4	2.0
	500	498	1.1	1.9
	1000	997	1.0	1.9
H-OME	100	98	1.6	2.7
	200	196	1.2	2.5
	300	294	1.2	2.4
	500	491	1.1	2.4
OME-S	100	99	1.8	2.9
	200	197	1.6	2.6
	300	294	1.2	2.6
	500	495	1.1	2.5

Table 2
Calibration fits of plasma samples after SPE pretreatment

Compound	Intercept ($\times 10^{-3}$ area units)	Slope ($\times 10^{-2}$ area units)	Correlation coefficient	DL (ng/mL)
OME	1.43 ± 1.17	14.1 ± 0.3	0.9993	3.6
H-OME	−7.73 ± 1.25	12.7 ± 0.4	0.9991	5.8
OME-S	4.19 ± 3.64	20.3 ± 0.6	0.9991	5.2

(containing 2.0 μg/mL of each analyte), which was injected six times every day. The R.S.D.s were 1.29%, 1.93% and 1.41% for OME, H-OME and OME-S, respectively. The migration times were very reproducible (R.S.D. less than 0.85%).

The selectivity was quite good. There was no interference in the electropherograms from H_2-receptor antagonist, cimetidine, ranitidine or famotidine, which might by chance be present in real samples.

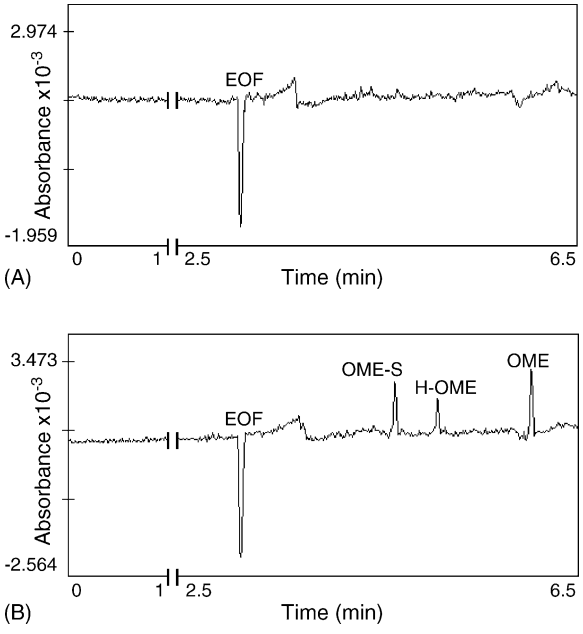

Fig. 5. Typical electropherograms of (A) plasma-free drug after the SPE procedure and (B) spiked plasma with 0.3 μg/mL of OME, H-OME and OME-S. The analytical conditions were as in Fig. 4.

3.4. MEKC analysis of plasma samples

The present method was designed to examine the content of OME and its metabolites (H-OME and OME-S) in human plasma.

Standard samples of drug-free human plasma spiked with different amounts of OME, H-OME and OME-S were used to check the automated on line pretreatment system described in Section 2.3. The adsorption of the analytes on the cartridges was tested using Strata X (Phenomenex), Oasis HLB (Waters), active carbon and C_{18} (Sigma). As Strata X gave the highest recoveries, a cartridge containing 200 mg of this solid with a 3 mL reservoir was selected. For analyte desorption from the cartridge, the solvents acetone and methanol were studied. Methanol was the most suitable solvent. The amount of solvent required for optimum elution was determined by eluting with different volumes of methanol and measuring the recoveries. The results demonstrated that 4 mL was the minimum volume of elution solvent that could be used since no significant difference was achieved for higher volumes. The extraction yield or absolute recovery of the automated SPE procedure was evaluated on blank plasma

Table 3
Concentrations of OME, H-OME and OME-S in plasma samples obtained 3 h after the oral administration of 20 mg of OME

Subject	OME concentration (ng/mL)	H-OME concentration (ng/mL)	OME-S concentration (ng/mL)
1	99	489	98.6
2	292	95	495
3	498	285	197

Table 4
Determination of OME in pharmaceutical formulations

Pharmaceutical formulation[a] (supplier)	Stated concentration (mg unit^{-1})	Found concentration[b] (mg unit^{-1})	
		CZE method	Reference method [16]
Ulceral (Tedec-Meji Farma)	20	19.95 ± 0.03	19.97
Omeprazol Normon (Lab. Normon)	20	19.97 ± 0.03	19.98
Omeprazol Esteve (Lab. Dr. Esteve)	20	19.98 ± 0.01	19.96

[a] Composition of samples—Ulceral: 20 mg OME, glucose, hipromellose phtalate, diethyl phtalate, lactose, sodium laurylsulfate, sodium phosphate, saccharose, starch, cellulose; Omeprazol Normon: 20 mg OME, saccharose, starch, cellulose, hipromellose, sodium laurylsulfate; Omeprazol Esteve: 20 mg OME, saccharose, talcum, triethylcitrate, cellulose.
[b] Average of five determinations ± S.D.

samples spiked with four different concentrations of the analytes. The amounts found for each analyte are listed in Table 1, which gave average recoveries of 99% for OME, 97% for H-OME and 98% for OME-S. The reproducibility and precision of the assay was good, the mean R.S.D. was less than 2.9% for all analytes.

Calibration curves from spiked blank plasma samples were set up for the three analytes in the 0.08–2.0 μg/mL concentration range. The parameters of the linear regression equations are given in Table 2. Of particular note is the high sensitivity of the method in the analysis of plasma samples, due to the enrichment factor of 10 achieved in the SPE pretreatment (1 mL of plasma is reconstituted in a sample volume of 100 μL before the CE analysis).

The use of the above on-line SPE procedure saved time in sample handling and effectively eliminated matrix interference. Sample preparation for validating the MEKC method was reduced to diluting the plasma with the working standard solution containing the analytes. A representative electropherogram of drug-free human plasma is shown in Fig. 5A and the electropherogram corresponding to the same plasma standard sample spiked with 0.3 μg/mL of each analyte is shown in Fig. 5B. Comparison of the two electropherograms shows that no interference peak appears at the migration times of the analytes, demonstrating the reliable determination of OME, H-OME and OME-S.

The method described here has been used to determine the concentrations of OME, H-OME and OME-S in plasma samples obtained 3 h after the oral administration of 20 mg of OME (Table 3). Based on these results, subjects 1 and 2 would be classified as extensive metabolizers and would be expected to exhibit normal CYP2C19 activity for subject 1, while normal CYP3A4 activity is found for subject 2. Volunteer 3 would be classified as a poor metabolizer. The accuracy of the method was evaluated by means of recovery studies. Known amounts of the standard analyte solutions were added to an aliquot of the above plasma samples and the resulting mixture was analyzed. Recoveries higher than 98% were obtained for the three analytes.

3.5. Determination of OME in pharmaceutical preparations

The proposed method was also used to determine OME in pharmaceutical preparations. Additives and excipients did not interfere. The quantification of OME in pharmaceuticals was carried out from the calibration graph obtained for the standard solutions. The data in Table 4 show that the assay results were in good agreement with the labeled content and the spectrophotometric method [19].

Table 5
Recovery data for OME spiked to pharmaceuticals

Pharmaceutical formulation	Added (mg unit^{-1})	Found[a] (mg unit^{-1})	Recovery (%)
Ulceral	4	3.93 ± 0.04	98.25
	8	8.01 ± 0.16	100.10
	12	11.97 ± 0.06	99.75
Omeprazol Normon	4	3.96 ± 0.04	99.00
	8	8.00 ± 0.04	100.00
	12	12.02 ± 0.10	100.16
Omeprazol Esteve	4	4.01 ± 0.10	100.25
	8	7.98 ± 0.14	99.75
	12	12.02 ± 0.24	100.16

[a] Average of five determinations.

The recoveries were determined by adding various amounts of OME to each pharmaceutical preparation and subtracting the results obtained for pharmaceuticals to which no OME had been added. Each sample was analysed five times; the recovery percentages obtained were in the range 98.2–100.1% with R.S.D. between 0.2% and 1.1% (Table 5).

4. Conclusions

A method based on MEKC with UV detection for the simultaneous determination of OME, H-OME and OME-S in human plasma has been developed. This method is sensitive, precise, accurate and selective. The 6.5 min run-time of the assay is much shorter that described for HPLC assays, thus allowing increased sample throughput. Additionally, the SPE step of the assay is fully automated, permitting the SPE and CE systems to work concurrently, i.e. while plasma sample-1 was being analysed, plasma sample-2 was simultaneously purified from the biological matrix by the ASPEC. The method has proven to be a suitable alternative for the analysis of plasma samples collected during human clinical studies with OME.

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