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Direct HPLC enantioseparation of omeprazole and its chiral impurities: Application to the determination of enantiomeric purity of esomeprazole magnesium trihydrate

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Dedicated to Dr. Francesco La Torre for his retirement.

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ABSTRACT

Analytical and semipreparative high-performance liquid chromatography (HPLC) enantioseparation of the proton-pump inhibitor omeprazole (OME) and its potential organic chiral impurities were accomplished on the immobilised-type Chiralpak IA chiral stationary phase (CSP) under both polar organic and normal-phase conditions. The (S)-enantiomers were isolated with a purity of >99% ee and their absolute configuration was empirically assigned by circular dichroism (CD) spectroscopy. A chemo- and enantioselective HPLC method was validated to control the enantiomeric purity of the (S)-enantiomer of OME (ESO), an active ingredient contained in drug products, in the presence of chiral and achiral related substances. The precision, linearity and accuracy of the determination of the (R)-impurity as well as the recovery of ESO from a pharmaceutical preparation were determined.

The proposed method uses the mixture methyl tert-butylether (M*t*BE)–ethyl acetate (EA)–ethanol (EtOH)–diethylamine (DEA) 60:40:5:0.1 (v/v/v/v) as a mobile phase. In these conditions, linearity over the concentration range 0.5–25 μg/ml for (R)-enantiomer was obtained. The limits of detection and quantification were 99 and 333 ng/ml, respectively. The intra and inter-day assay precision was less than 2% (RSD%).

1. Introduction

Owing to the evidences of pharmacodynamic, pharmacokinetic and toxicological differences between enantiomers, stereochemistry has now become a topic issue in the drug research and development [1]. In recent years the production of enantiopure pharmaceuticals has rapidly increased and a significant number of established drugs marketed as racemates have been authorised from regulatory agencies as single enantiomers [2].

Among them, esomeprazole magnesium trihydrate [bis(5-methoxy-2-[(*S*)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulfinyl]-1H-benzimidazole-1yl) magnesium trihydrate] (ESO) is a well-known gastric proton-pump inhibitor (PPI) used in treatment of gastric-acid related diseases. AstraZeneca's Nexium brand of ESO was approved for marketing in 2000. Compared to the corresponding racemic drug omeprazole (OME) (Fig. 1), the (*S*)-enantiomer has more favourable metabolic properties leading to a more pronounced effect on gastrointestinal disorders such as gastric and duodenal ulcers [3,4].

Direct HPLC on chiral stationary phase has been widely used in the enantioselective analysis of OME and other PPI drugs [5–12]. In particular, analytical methods based on polysaccharide-based CSPs seem to be efficient routes for checking their enantiomeric purity [5,13–15,20].

According to the regulatory authorities, an enantioselective HPLC method should be able to separate the optically active drug substance from the enantiomeric impurity and other potential organic impurities. Potential organic impurities include chiral and/or achiral starting materials, intermediates and by-products from the drug substance manufacturing process. The structure of the related substances described in the European Pharmacopoeia (EP) monograph for ESO is reported in Fig. 1. Most of these impurities are strictly similar in structure to the active product ingredient (API). So, a chemo- and enantioselective HPLC assay appears a critical step in the development of high-quality manufacturing processes and quality-control methods.

In a recent paper [5], we reported on the direct enantioseparation of PPIs on the immobilised Chiralpak IA CSP in multimodal conditions. Here a chemo- and enantioselective HPLC method capa-

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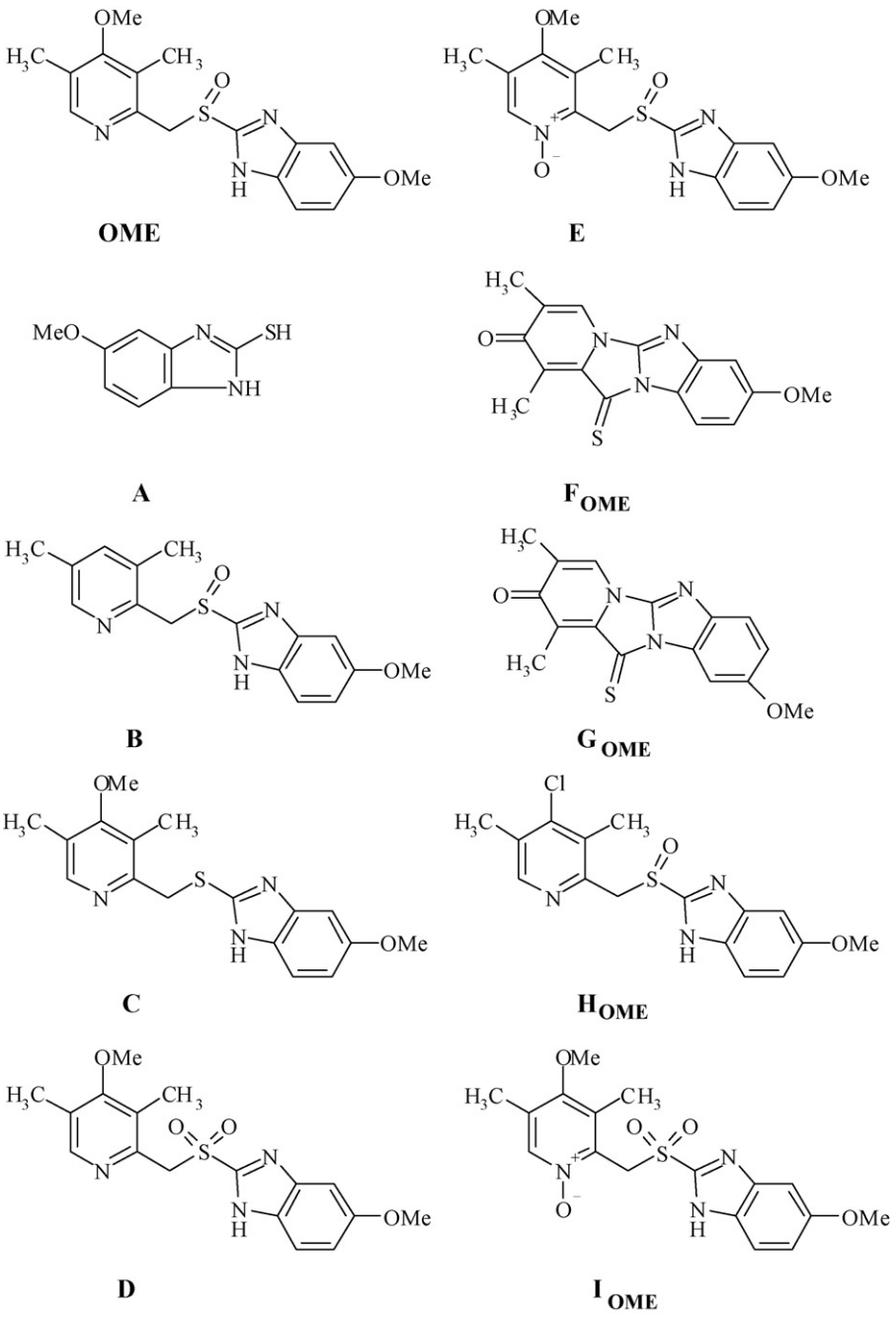

Fig. 1. Structure of OME and its potential organic impurities.

Table 1
Chromatographic conditions for the semipreparative resolution of OME, B, E, H_{OME} and the isomeric mixture F_{OME} + G_{OME}.

Compound	Mobile phase	k_1[a]	α[b]	SR[c]	V[d]
OME	M*t*BE–EA–EtOH–DEA 60–40–5–0.1	2.72	1.71	20	0.70
B	Methanol/DEA 100–0.1	0.64	4.78	20	1.00
E	EtOH–DEA 100–0.1	2.20	1.40	2	0.30
H_{OME}	EA–methanol–DEA 95–5–0.1	0.91	1.94	20	1.00
F_{OME} + G_{OME}	Chloroform–acetonitrile 70–30	0.60	1.50	5	0.12

Column: Chiralpak IA (250 × 10 mm I.D.); flow rate: 3 (E), 3.5 (F_{OME} + G_{OME}), 4 (H_{OME}, OME) 5.5 (B) ml/min; detector: UV at 340 nm; temperature: 25 and 35 °C (B).
[a] Retention factor of the first eluted enantiomer, defined as $(t_1 - t_0)/t_0$ where t_0 is the void time of the column.
[b] Enantioselectivity factor defined as k_2/k_1.
[c] Amount of sample (in mg) resolved in a single semipreparative run.
[d] Sample volume injection (ml).

ble of determining the enantiomeric purity of ESO both in raw material and in finished product is presented.

In another step, we focused our efforts on developing of highly enantioselective semipreparative HPLC systems which could be applied to productive separations of OME and its chiral impurities. The absolute configuration of all the isolated enantiomers was empirically established by circular dichroism (CD) spectroscopy and the (*S*)-enantiomers were selected as reference compounds in the HPLC analysis of ESO.

2. Experimental

2.1. Chemical and reagents

OME and the impurities showed in Fig. 1 were obtained by the European Directorate for the Quality of Medicines & Healthcare (EDQM) (France). Commercially available tablets containing 40 mg of esomeprazole magnesium trihydrate were purchased at a local drugstore. HPLC-grade solvents were supplied by Carlo Erba (Milan, Italy). HPLC enantioseparations were performed by using stainless-steel Chiralpak IA (250 mm × 4.6 mm I.D. and 250 mm × 10 mm I.D.) columns (Chiral Technologies Europe, Illkirch, France).

2.2. Instruments and chromatographic conditions

Analytical HPLC apparatus consisted on a Dionex P580 LPG pump, an ASI-100 T autosampler, a STH 585 column oven, a PDA-100 UV detector or a Jasco (Jasco, Tokyo, Japan) Model CD 2095 Plus UV/CD detector; data were acquired and processed by a Chromeleon Datasystem (Dionex Corporation, Sunnyvale, CA). For semipreparative separation a Perkin-Elmer (Norwalk, CT, USA) 200 LC pump equipped with a Rheodyne (Cotati, CA, USA) injector, a 1000 μL sample loop, a Perkin-Elmer LC 101 oven and Waters 484 detector (Waters Corporation, Milford, MA, USA) were used. The signal was acquired and processed by Clarity software (DataApex, Prague, The Czech Republic).

Experimental conditions for analytical and semipreparative enantioseparations are indicated in Table 1.

In analytical separations, fresh standard solutions of OME and single impurities were prepared shortly before using by dissolving 1–3 mg of each analyte in the mobile phase. The injection volume was 20 μl.

In semipreparative enantioseparations of OME and its chiral impurities, standard solutions were prepared by dissolving the racemic sample in the mobile phase. The semipreparative operating parameters (sample concentration, injection volumes, flow rate and column temperature) are reported in Table 1. After semipreparative separation, the collected fractions were pooled, evaporated and analyzed by a chiral analytical column to determine their enantiomeric excess (ee).

The column hold-up time (t_0 = 3.0 min for 250 mm × 4.6 mm I.D. column) was determined from the elution of an unretained marker (toluene), using methanol as eluent, delivered at a flow rate of 1.0 ml/min.

The circular dichroism (CD) spectra of enantiomers OME and chiral impurities B, E and G_{OME} (Fig. 1), dissolved in ethanol (concentration about 0.1 mg/ml), in a quartz cell (0.1-cm path length) at 25 °C, were measured by using a Jasco Model J-700 spectropolarimeter. The spectra are average computed over three instrumental scans and the intensities are presented in terms of ellipticity values (mdeg).

The ultraviolet (UV) spectrum of OME and its related substances, dissolved in the mobile phase M*t*BE–EA–EtOH–DEA 60:40:5:0.1 (v/v/v/v) (concentration about 10 μg/ml) in a quartz cell, was recorded by using a Unicam UV4 spectrophotometer.

2.3. Absolute configuration and enantiomeric elution order determination

The absolute configuration of the collected enantiomers was assigned by comparing their CD spectra with those reported in literature [5]. The enantiomeric elution order on the Chiralpak IA CSP was established by analysing non-racemic samples enriched by the (*S*)-enantiomers.

2.4. Method validation

2.4.1. HPLC operating conditions

Analytical chromatographic separations were carried out on a Chiralpak IA column (250 mm × 4.6 mm I.D.) with a mobile phase consisting of M*t*BE–EA–EtOH–DEA in the ratio 60:40:5:0.1 (v/v/v/v) at a flow rate of 1 ml/min and maintaining the column temperature at 25 °C. The injection volume was 20 μl, sampler temperature was set at 5 °C, and the detection wavelength was set at 299 nm.

2.4.2. Specificity

The selectivity of the analytical method was evaluated by the analysis of a solution containing (*S*)-OME enantiomer and its main related substances.

2.4.3. Preparation of stock and standard solutions

Standard solutions of (*S*)-OME, carefully protected from light, were prepared and used daily for calibration purpose from about 1.5% to about 150% relative to the working concentration of about 500 μg/ml (100%) of (*S*)-enantiomer of omeprazole. Stock solutions of the single enantiomer of omeprazole were prepared by dissolving about 20 mg in 20 ml volumetric flasks with the mobile phase and kept at −20 °C. Aliquots of 1, 2.5 and 7.5 ml of these solutions were transferred into 5, 10 and 100 ml volumetric flasks and diluted with the mobile phase. One of them was diluted twice. The final concentration of standard solutions was 7.5, 75, 250, 500 and 750 μg/ml. The vials containing the solutions for the injections were put in the autosampler set at 5 °C before chromatographic analysis.

Standard solutions of (*R*)-OME were prepared from about 0.1% to about 5% relative to the working concentration of about 500 μg/ml (100%) of (*S*)-enantiomer of omeprazole.

2.4.4. Recovery

Ten tablets of a commercially available drug containing esomeprazole magnesium trihydrate (40 mg as free base) were ground and homogenized. An amount accurately weighed of about 25 mg was transferred to a 50 ml volumetric flask. After the addition of 40 ml methanol–DEA 100:1 (v/v) mixture, the flask was placed in an ultrasonic bath for 15 min and the volume was completed to 50 ml. An aliquot was filtered through a 0.45 mm membrane (Millipore Co., Bedford, USA) and transferred into a vial and the solvent was evaporated under nitrogen flow. The residue was dissolved in the mobile phase (M*t*BE–EA–EtOH–DEA 60:40:5:0.1, v/v/v/v) and analysed by HPLC on the Chiralpak IA CSP.

2.4.5. Linearity

The linearity evaluation was performed with the standard solutions of (*S*)-OME at the concentrations ranging from 7.5 to 750 μg/ml with a working concentration of about 500 μg/ml. Three injections of each solution were made under the chromatographic conditions described above, using an injection volume of 20 μl. The peak areas response of (*S*)-OME was plotted against the corresponding concentration and the linear regression equations were computed.

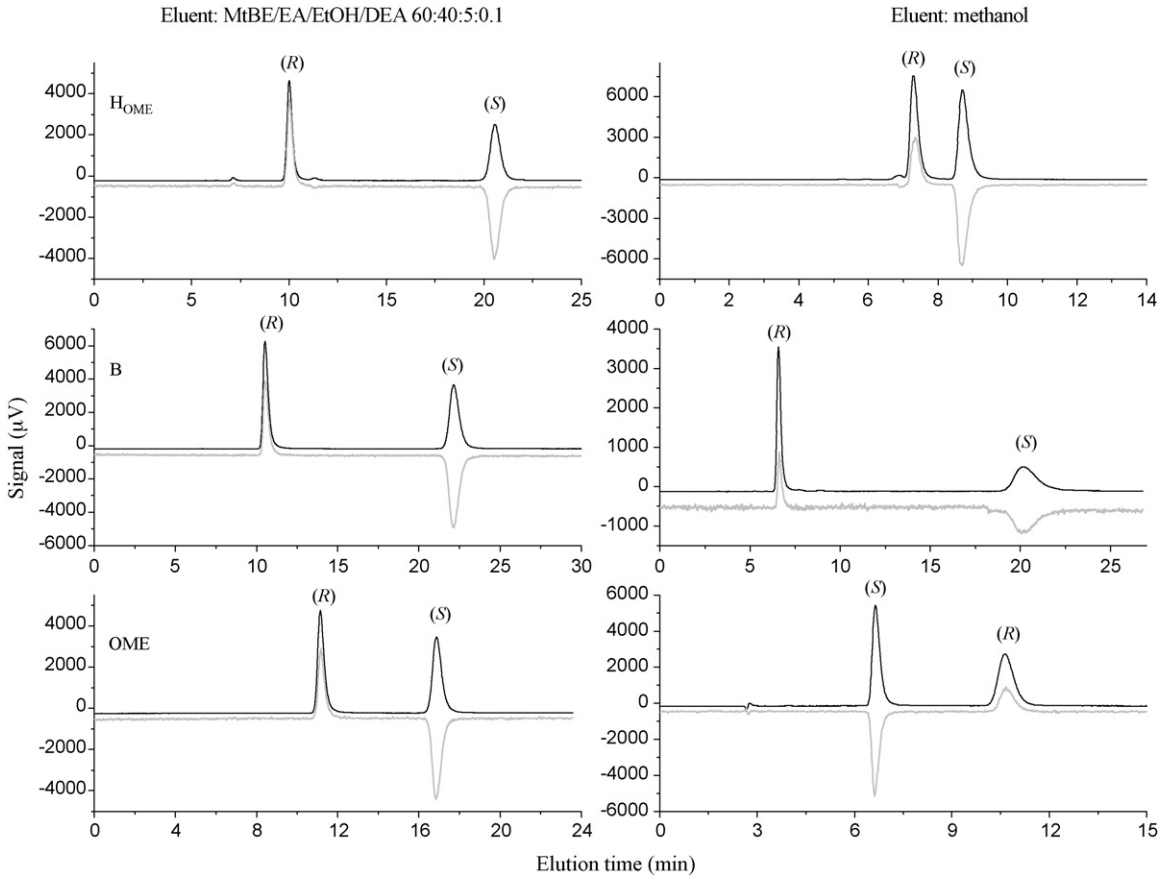

Fig. 2. HPLC chromatograms of OME, B and H_{OME} with UV (black) and CD (grey) detection at 299 nm. Column: Chiralpak IA (250 mm × 4.6 mm I.D.); detection: UV; flow rate: 1.0 ml/min; column temperature: 25 °C.

2.4.6. LOD and LOQ

The limit of detection (LOD) and the limit of quantization (LOQ) represent the concentration of the analyte that would yield a *S*/*N* of 3 and 10, respectively, following the United States Pharmacopoeia [16]. The LOD and LOQ of (*S*)-OME were determined by injecting a series of diluted solutions.

2.4.7. Precision and repeatability

Method precision was determined by measuring the repeatability (intra-day precision) and intermediate precision (inter-day precision) of retention times and peak areas for (*S*)-OME enantiomer. The intra-day variability was performed by the same analyst over one day, while inter-day precision was carried out by another independent analyst over three days. In order to determine the repeatability of the method, replicate injections ($n = 6$) of 75 μg/ml of (*S*)-OME were carried out. The intermediate precision was evaluated over three days by performing six consecutive injections each day. Precision was reported as percentage of relative standard deviation (RSD%).

2.4.8. Accuracy

The accuracy of the method was tested by analyzing samples of (*S*)-OME form at three various concentration levels. Solutions of esomeprazole magnesium trihydrate extracted from commercially available tables were also analyzed. In this case the accuracy was evaluated by testing three samples at the nominal concentration of about 500 μg/ml.

3. Results and discussion

3.1. Analytical and semipreparative HPLC and absolute configuration

The HPLC analytical method described in the EP monograph for checking the enantiomeric purity of ESO is based on the use

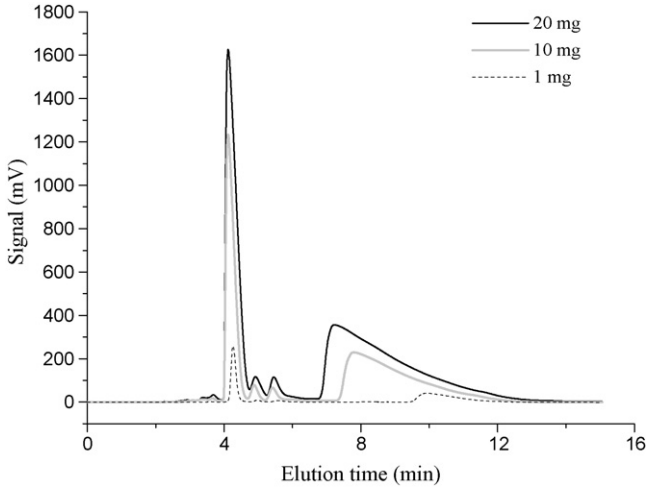

Fig. 3. Typical chromatograms illustrating the resolution of 1, 10 and 20 mg of the chiral impurity B. Column: Chiralpak IA (250 mm × 10 mm I.D.); detection: UV at 340 nm; eluent: methanol–DEA 100:0.1 (v/v); flow rate: 5.5 ml/min; column temperature: 35 °C.

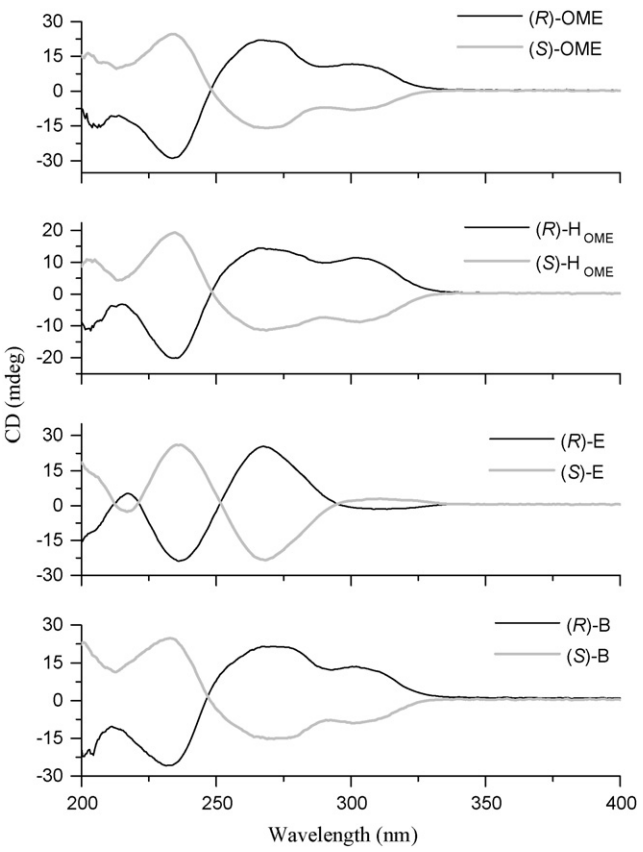

Fig. 4. CD spectra of the enantiomers of OME, B, E and H_{OME} in ethanol at 25 °C.

of the AGP chiral stationary phase in reversed-phase conditions. The aim of the approved method is to determinate the content of the (*R*)-enantiomer as related substance (named impurity F). The monograph does not provide any information about the selectivity of the chiral HPLC system towards other related substances (impurities A–E). The impurities A–E of ESO are the same to those reported in the EP monograph for the racemic form whereas the other related substances showed in Fig. 1, here named F_{OME}–I_{OME}, appear exclusively in the monograph of the racemic OME. The chiral impurities B and E are reported as racemic mixtures. In our opinion, this is a critical point. In fact, as the AstraZeneca production of ESO has been achieved via asymmetric oxidation of the prochiral sulfide intermediate [17], the chiral impurities of ESO whatever their origin (from incomplete reaction in previous steps or degradation reactions) must have the same (*S*)-configuration of the API. So, before developing a chemo- and enantioselective method to be applied to analysis of ESO, we focused our efforts on enantioseparation and stereochemical characterization of OME and its chiral impurities. On the basis of our previous experience on the enantioseparation of PPIs [5,13], we evaluated the chiral discrimination ability of the immobilised Chiralpak IA CSP under polar organic and normal phase conditions. To prevent the acid degradation of OME and their chiral related substances a small percentage (0.1%) of diethylamine (DEA) was added to the mobile phase. The chiral impurities under study include also the compound H_{OME} described only in the EP monograph for racemic OME but potentially present in pharmaceutical formulations of ESO. Table 1 summarises the best chromatographic results in terms of enantioselectivities obtained in the screening of the elution conditions. It can be seen, the Chiralpak IA shows a high selectivity towards structures related to OME. The enantioseparation factor values ranged from 1.40 (compound E, eluent: EtOH–DEA 100:0.1) to 4.78 (compound B, eluent:

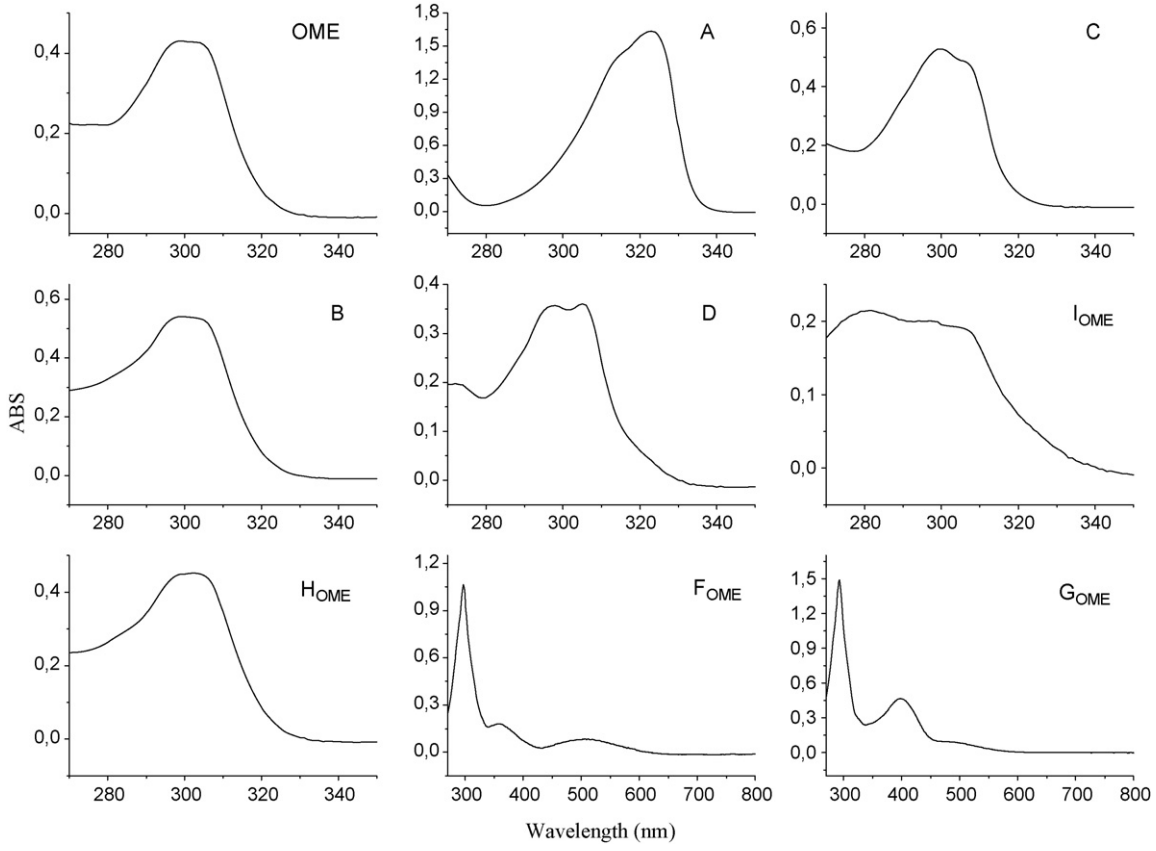

Fig. 5. UV spectra of about equimolar solutions of compounds OME and its potential organic related substances in M*t*BE–EA–EtOH–DEA 60:40:5:0.1 (v/v/v/v) at 25 °C.

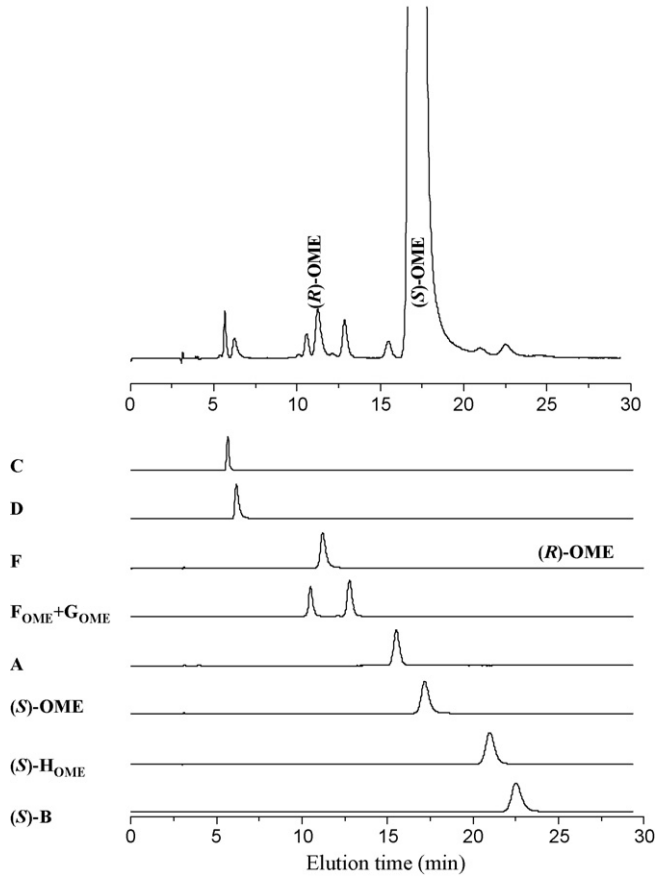

Fig. 6. Typical HPLC chromatograms of (*S*)-OME spiked with impurities (top) and single impurities. Column: Chiralpak IA (250 mm × 4.6 mm I.D.); detection: UV at 299 nm; flow rate: 1.0 ml/min; column temperature: 25 °C.

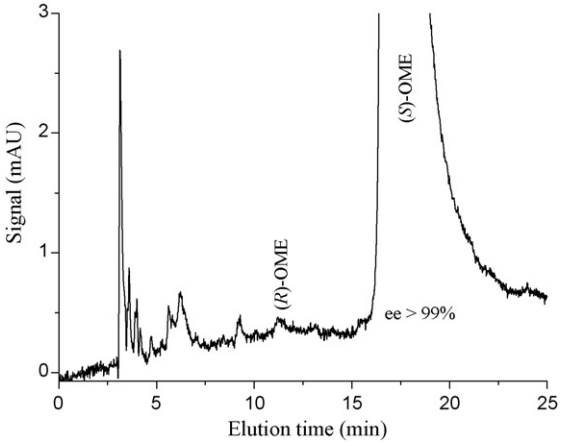

Fig. 7. Check of the enantiomeric purity of ESO in a pharmaceutical preparation. Column: Chiralpak IA (250 mm × 4.6 mm I.D.); detection: UV at 299 nm; flow rate: 1.0 ml/min; column temperature: 25 °C.

methanol–DEA 100:0.1). However, whereas for the racemates B, E and H_{OME} the enantiomeric elution order was the same in each investigated condition, OME showed a reversed order passing from pure methanol or ethanol to eluents containing ethyl acetate or chlorurate solvents (Fig. 2).

Due to the high efficiency, enantioselectivity and loading capacity of the immobilised Chiralpak IA CSP [15,18–24], the analytical enantioseparations were easily scaled-up for semipreparative productions of the (*S*)-form of API and impurities (Table 1). The successful transfer of the enantioseparation from a 4.6 to a 10 mm I.D. Chiralpak IA column is demonstrated in Fig. 3. Despite the presence of two impurities, the complete separation of the two enantiomers could be observed at the load as high as 20 mg.

The CD spectra of the isolated enantiomers are shown in Fig. 4. By comparing the position and sign of cotton effects of two enantiomers of OME of known chirality with those of the structurally related impurities was possible to empirically assign the absolute configuration of the stereogenic centre. HPLC analysis of non-racemic chiral impurities enriched by the (*S*)-enantiomers made it possible to determine the enantiomer elution order on the Chiralpak IA CSP: by using the mixture M*t*BE–EA–EtOH–DEA 60:40:5:0.1 (v/v/v/v) as a mobile phase the (*R*)-enantiomers of the chiral impurities and OME were eluted before their (*S*)-counterparts.

The achiral impurities F_{OME} and G_{OME} are important degradation products of OME and they are furnished by chemical manufacturers as an isomeric dark brown coloured (F_{OME}/G_{OME} 35.1:63.2) (w/w) mixture. The two isomers were baseline resolved using chloroform–acetonitrile 70:30 (v/v) as a mobile phase. The isolated major isomer G_{OME}, second eluted on the Chiralpak IA CSP, appeared as a brown-yellow powder whereas the minor isomer F_{OME} was a purple powder. The UV spectra of OME and its impurities are showed in Fig. 5.

3.2. Validation of the method

3.2.1. Specificity and chromatography

As previously mentioned, the control the enantiomeric purity is a critical aspect to produce drug substance and drug product of acceptable quality. Here, we selected a mobile phase consisting in M*t*BE–EA–EtOH–DEA 60:40:5:0.1 (v/v/v/v) to evaluate the discrimination ability of the Chiralpak IA CSP towards ESO and its potential organic impurities. The UV detection was set at 299 nm.

Fig. 6 shows a chromatogram of (*S*)-OME spiked with the 0.5% of (*R*)-OME, 0.2% of the (*S*)-enantiomer of the chiral impurities and 0.2% of the achiral ones (Fig. 1). No interference in the determination of the enantiomeric excess (ee) was observed. The compounds E and I_{OME} are not eluted in these conditions and do not interfere with the analysis of ESO. A typical chromatogram corresponding to a pharmaceutical formulation of ESO is showed in Fig. 7. Thus the developed method was found reliable for determination of the ee of ESO in bulk drugs and pharmaceutical formulations. The optimised chemo- and enantioselective HPLC system based on the immobilised Chiralpak IA CSP was validated and the analytical results obtained are described below.

3.2.2. System suitability

Impurity A and (*S*)-OME were chosen to determine the system suitability parameters. Six consecutive analyses were performed.

3.2.3. Linearity

The linearity of the HPLC method was evaluated by injecting standard concentrations of (*S*)- and (*R*)-OME samples with a concentration ranging from 7.5 to 750 μg/ml (1.50–150%) and from 0.5 to 25 μg/ml (0.1–5%). The peak area response was plotted versus the nominal concentration of the enantiomer. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The obtained calibration curve for the (*S*)-OME showed correlation coefficient greater than 0.999: $y = 924.53x + 14.113$ ($r^2 = 0.9999$) where y is the peak area and x is the concentration. The calibration curve for the (*R*)-OME showed correlation coefficient greater than 0.999: $y = 910.8x + 14.1348$ ($r^2 = 0.9999$).

3.2.4. LOD and LOQ

The LOD and LOQ concentrations were estimated to be 169 and 513 ng/ml for (*S*)-OME enantiomer, and 99 and 333 when the *S*/*N* of 3 and 10 were used as the criteria.

3.2.5. Precision and repeatability

The precision of the HPLC method was determined by repeatability (intra-day) and intermediate precision (inter-day). Method precision had a relative standard deviation (RSD%) below 2.0% for repeatability (0.17% for retention times and 0.27% for area) and for intermediate of precision (0.25% for retention time and 0.5% for area), which comply with the acceptance criteria proposed (RSD%: not more than 2.0%).

3.2.6. Accuracy

Accuracy for the determination of enantiopure (*S*)-OME was determined by preparing three drug substance samples at 10%, 40% and 120% of the target (50–600 μg/ml). Apparent recovery ranged from 98.64% to 99.72%. Overall percent recovery was 99.24 (RSD% 0.55).

Solutions of extracted (*S*)-OME from commercially available tables were also analyzed with accuracy of 99.60% (RSD% 0.10).

4. Conclusions

The applicability of the Chiralpak IA CSP for semipreparative enantioseparations of OME and its chiral related substances was demonstrated. The availability of the (*S*)-enantiomers of all the chiral pyridine-benzimidazole sulfinyl compounds permitted to find and optimize the HPLC conditions useful to determine the ee in the presence of potential organic impurities of ESO. The validated method uses the mixture M*t*BE–EA–EtOH–DEA 60:40:5:0.1 (v/v/v/v) as a mobile phase and it fulfils the EP requirements for the routine check of enantiomeric purity in raw material and pharmaceutical preparations.

Conflict of interest

None declared.

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References

[1] P.A. Patil, M.A. Kothekar, Development of safer molecules through chirality, Indian J. Med. Sci. 60 (2006) 427–437.
[2] I. Agranat, H. Caner, J. Caldwell, Putting chirality to work: the strategy of chiral switches, Nat. Rev. Drug Discov. 1 (2002) 753–768.
[3] P.B. Kale-Pradham, H.K. Landry, W.T. Sypula, Esomeprazole for acid peptic disorders, Ann. Pharmacother. 36 (2002) 655–663.
[4] T. Andersson, Single-isomer drugs: true therapeutic advances, Clin. Pharmacokinet. 43 (2004) 279–285.
[5] R. Cirilli, R. Ferretti, B. Gallinella, E. De Santis, L. Zanitti, F. La Torre, High-performance liquid chromatography enantioseparation of proton pump inhibitors using the immobilized amylose-based Chiralpak IA chiral stationary phase in normal-phase, polar organic and reversed-phase conditions, J. Chromatogr. A 1177 (2008) 105–113.
[6] H. Stenhoff, Å. Blomqvist, P.O. Lagerström, Determination of the enantiomers of omeprazole in blood plasma by normal phase liquid chromatography and detection by atmospheric pressure ionization tandem mass spectrometry, J. Chromatogr. B 734 (1999) 191–201.
[7] T. Yoshida, M. Kito, M. Tsujii, T. Nagasawa, Microbial synthesis of a proton pump inhibitor by enantioselective oxidation of a sulfide into its corresponding sulfoxide by Cunninghamella echinulata MK40, Biotechnol. Lett. 23 (2001) 1217–1222.
[8] S. Allenmark, B. Bomgren, H. Boren, P.O. Lagerström, Direct optical resolution of a series of pharmacologically active racemic sulfoxides by high-performance liquid affinity chromatography, Anal. Biochem. 136 (1984) 293–297.
[9] P. Erlandsson, R. Isaksson, P. Lorentzon, P. Lindberg, Resolution of the enantiomers of omeprazole and some of its analogues by liquid chromatography on a trisphenylcarbamoylcellulose-based stationary phase: the effect of the enantiomers of omeprazole on gastric glands, J. Chromatogr. 532 (1990) 305–319.
[10] L. Toribio, M.J. del Nozal, J.L. Bernal, C. Alonso, J.J. Jimenez, Comparative study of the enantioselective separation of several antiulcer drugs by high-performance liquid chromatography and supercritical fluid chromatography, J. Chromatogr. A 1091 (2005) 118–123.
[11] K. Balmer, B.A. Persson, P.O. Lagerström, Stereoselective effects in the separation of enantiomers of omeprazole and other substituted benzimidazoles on different chiral stationary phases, J. Chromatogr. A 660 (1994) 269–273.
[12] M. Miura, H. Kagaya, H. Tada, T. Uno, N. Yasui-Furukori, T. Tateishi, T. Suzuki, Enantioselective disposition of rabeprazole in relation to CYP2C19 genotypes, Br. J. Clin. Pharmacol. 61 (2006) 315–320.
[13] R. Cirilli, R. Ferretti, B. Gallinella, L. Turchetto, L. Zanitti, F. La Torre, Development and validation of an enantioselective and chemoselective HPLC method using a Chiralpak IA column to simultaneously quantify (R)-(+)- and (S)-(−)-lansoprazole enantiomers and related impurities, J. Pharm. Biomed. Anal. 50 (2009) 9–14.
[14] R. Nageswara Rao, A. Narasa rajua, D. Nagaraju, Enantiospecific resolution of rabeprazole by liquid chromatography on amylose-derived chiral stationary phase using photo diode array and polarimetric detectors in series, Talanta 70 (2006) 805–810.
[15] H. Stenhoff, A. Blomqvist, P. Lagerström, Determination of the enantiomers of omeprazole in blood plasma by normal-phase liquid chromatography and detection by atmospheric pressure ionization tandem mass spectrometry, J. Chromatogr. B 734 (2005) 191–201.
[16] United States Pharmacopoeia, 24th ed., United States Pharmacopoeial Convention, Rockville, MD, 2000, p. 2150.
[17] L. Olbe, E. Carlsson, P. Lindberg, A proton-pump inhibitor expedition: the case histories of omeprazole and esomeprazole, Nat. Rev. Drug Discov. 2 (2003) 132–139.
[18] T. Zhang, M. Schaeffer, P. Franco, Optimization of the chiral separation of a Ca-sensitizing drug on an immobilized polysaccharide-based chiral stationary phase: case study with a preparative perspective, J. Chromatogr. A 1083 (2005) 96–101.
[19] R. Cirilli, A. Simonelli, R. Ferretti, A. Bolasco, P. Chimenti, D. Secci, E. Maccioni, F. La Torre, Analytical and semipreparative high performance liquid chromatography enantioseparation of new substituted 1-thiocarbamoyl-3,5-diaryl-4,5-dihydro-(1H)-pyrazoles on polysaccharide-based chiral stationary phases in normal-phase, polar organic and reversed-phase conditions, J. Chromatogr. A 1101 (2006) 198–203.
[20] M. Biava, R. Cirilli, V. Fares, R. Ferretti, B. Gallinella, F. La Torre, G. Poce, G.C. Porretta, S. Supino, C. Villani, HPLC enantioseparation and absolute configuration of novel anti-inflammatory pyrrole derivatives, Chirality 20 (2008) 775–780.
[21] P. Franco, T. Zhang, Common approaches for efficient method development with immobilised polysaccharide-derived chiral stationary phases, J. Chromatogr. B 875 (2008) 48–56.
[22] R. Ferretti, B. Gallinella, F. La Torre, L. Zanitti, L. Turchetto, A. Mosca, R. Cirilli, Direct high-performance liquid chromatography enantioseparation of terazosin on an immobilised polysaccharide-based chiral stationary phase under polar organic and reversed-phase conditions, J Chromatogr. A 1216 (2009) 5385–5390.
[23] R. Cirilli, S. Fiore, F. La Torre, E. Maccioni, D. Secci, M.L. Sanna, C. Faggi, Semipreparative HPLC enantioseparation, chiroptical properties, and absolute configuration of two novel cyclooxygenase-2 inhibitors, Chirality 22 (2010) 56–62.
[24] R. Cirilli, R. Ferretti, F. La Torre, A. Borioni, V. Fares, M. Camalli, C. Faggi, D. Rotili, A. Mai, Chiral HPLC separation and sbsolute configuration of novel S-DABO derivatives, Chirality 21 (2009) 604–612.