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Short Communication

Comparison of reversed-phase enantioselective HPLC methods for determining the enantiomeric purity of (*S*)-omeprazole in the presence of its related substances ☆

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ABSTRACT

A simple analytical high-performance liquid chromatography (HPLC) method was applied for the enantiomeric excess determination of esomeprazole ((*S*)-OME), the enantiopure active ingredient contained in drug products, in the presence of its potential organic impurities A-E. The enantioselective separation was accomplished on the immobilized-type Chiralpak ID-3 chiral stationary phase (CSP) under reversed-phase conditions. The results were evaluated and compared with those obtained by the official enantioselective method of European Pharmacopoeia used as the reference for checking the enantiomeric excess of (*S*)-OME. It has been established that the use of the Chiralpak ID-3 CSP allows the determination of the enantiomeric purity of (*S*)-OME without any interference coming from its chiral and achiral related substances. The analytical procedure of the drug regulatory agencies based on the AGP CSP suffered instead from poor specificity due to overlap of the peaks pertinent to the achiral impurity A and the chiral impurity (*R*)-OME (impurity F).

1. Introduction

Omeprazole (OME, bis(5methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl)-1H-benzimidazole-1yl) (Fig. 1) is a well-known gastric proton-pump inhibitor (PPI) used in the treatment of gastric-acid related diseases. In 2000 in Europe and 2001 in USA, AstraZeneca launched the first enantiopure PPI, the magnesium trihydrate salt of the (*S*)-enantiomer of OME, under the Nexium brand. Compared with the corresponding racemic drug, (*S*)-OME is demonstrated to have the same pharmacodynamic properties as the corresponding racemic drug, but it has a more favorable metabolic behavior and a more pronounced effect on duodenal ulcers, erosive esophagitis, and gastroesophageal reflux disease [1,2].

According to the monograph for esomeprazole magnesium trihydrate active substance in European Pharmacopoeia (EP) [3], the enantiomeric impurity of (*S*)-OME, namely (*R*)-OME, is checked by an enantioselective HPLC method based on the use of the AGP (100 mm × 4.0 mm, 5μm) column with a mobile phase containing a mixture of acetonitrile–pH 6 phosphate buffer (13:87, v/v). The content of (*R*)-OME has an acceptance criterion fixed in an upper limit of 0.6%. The monograph does not furnish any data about the selectivity of the chiral HPLC method towards other related substances (impurities A-E) whose structures are very similar to that of the active product ingredient (API) (Fig. 1). In addition, the chiral impurity B (IMP-B) and IMP-E are reported as racemic mixtures, even though the prochiral sulfide intermediate is synthesized via an asymmetric oxidation reaction [4]. As a rule of thumb, the chiral impurities of the (*S*)-OME, whatever their origin (from synthetic or degradation steps) is, should normally have the (*S*)-configuration as the API does. Therefore, the ability of the current official chiral method to accurately measure the enantiomeric excess of (*S*)-OME in the presence of the achiral and the (*S*)-forms of chiral substance correlates, which should be a crucial aspect of the analysis to ensure high-quality API, has yet to be demonstrated.

Although a great number of HPLC methods have been developed for the direct enantioseparation of OME in normal phase, polar organic and reversed-phase conditions [5–10], the quantitative analysis of the enantiomeric purity of (*S*)-OME in the presence of its related substances is extremely challenging to achieve [11].

Our group has published for the first time the separation of (*S*)-OME from its potential organic chiral and achiral impurities on the

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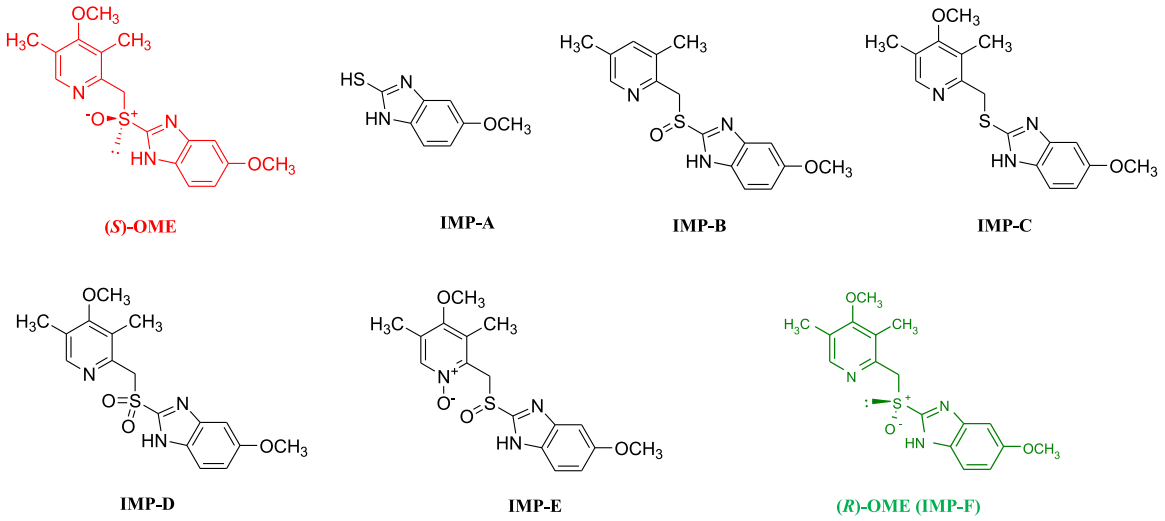

Fig. 1. Structures of (*S*)-OME and its potential organic impurities.

immobilized-type Chiralpak IA CSP [12]. The proposed method was based on a mixture of methyl *tert*-butylether–ethyl acetate–ethanol–diethylamine (60:40:5:0.1, v/v/v/v) as the mobile phase delivered at a flow rate of 1.0 mL/min. Chemo- and enantioselective HPLC separations were simultaneously achieved on an IA (250 mm × 4.6 mm) column in an analysis time of about 25 min. Recently, with the aim to simplify the eluent mixture and get the analytical method more sustainable, we reported an easy way to separate the enantiomers of OME and other three PPIs (lansoprazole, rabeprazole and pantoprazole) on polysaccharide-based CSPs under water-rich conditions [13]. In particular, it was demonstrated that using a Chiralpak ID-3 (100 mm × 4.6 mm, 3 μm) column the baseline enantioseparation of OME within 8 min could be achieved. A volume of only 4 mL of ACN was employed for each eluting step. The aim of this work was to compare the ability of the two commercial available Chiralpak ID-3 and AGP CSPs to separate the enantiomers of OME in the presence of its organic related substances under reversed-phase conditions.

2. Experimental

2.1. Chemicals and reagents

OME and the impurities shown in Fig. 1 were purchased from the European Directorate for the Quality of Medicines and Healthcare (EDQM) (France) and United States Pharmacopoeial Convention, Rockville (MD). HPLC-grade solvents were used as supplied by Aldrich (Milan, Italy). Sodium dihydrogen orthophosphate and sodium phosphate dibasic salts were purchased from Carlo Erba (Milan, Italy). HPLC enantioseparations were performed using stainless-steel Chiralpak ID-3 (100 mm × 4.6 mm, 3 μm) and Chiralpak AGP (100 mm × 4.0 mm, 5 μm) columns (Chiral Technologies Europe, Illkirch, France).

2.2. Instruments and chromatographic conditions

The HPLC apparatus consisted of a Dionex P580 LPG pump, an ASI-100 T autosampler, an 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 a Waters 484 detector (Waters Corporation, Milford, MA, USA) was used. The signal was acquired and processed by Clarity software (DataApex, Prague, the Czech Republic).

Experimental conditions for semipreparative enantioseparations of OME, IMP-B and IMP-E are reported elsewhere [12].

In analytical separations, fresh standard solution of OME and single impurities were prepared shortly before using by dissolving 1–3 mg of each analyte in the mobile phase.

2.3. Preparation of stock and standard solutions

Stock solutions of the single enantiomer of OME were prepared by dissolving 4.97 mg in 10 mL volumetric flasks with the mobile phase and kept at −20 °C protected from light. Suitable dilutions were carried out to obtain the final concentrations of standard solutions of 0.2485, 0.497, 24.85, 99.40, 248.50 and 497 μg/mL. Standard solutions of (*S*)-OME were prepared and used daily for calibration purpose from 0.1% to 200% relative to the working concentration of 248.50 μg/mL (100%) of (*S*)-enantiomer of OME.

2.4. Method validation

2.4.1. HPLC operating conditions

Analytical chromatographic separations were carried out on a Chiralpak ID-3 column (100 mm × 4.6 mm, 3 μm) with a mobile phase consisting of ACN-H_2O in the ratio 50:50 (v/v) at a flow rate of 1 mL/min and maintaining the column temperature at 40 °C. The injection volume was 20 μL, sampler temperature was set at 5 °C, and the detection wavelength was set at 280 nm or 300 nm for validation purposes.

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.

Standard solutions of (*R*)-OME were prepared from 0.05% to 5% relative to the working concentration of 248.50 μg/mL (100%) of (*S*)-enantiomer of OME.

2.4.3. Linearity

The linearity evaluation was performed with the standard solutions of (*S*)-OME at the concentrations ranging from 0.2485 to 497 μg/mL with a working concentration of 248.50 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.

2.4.4. Limit of determination (LOD) and limit of quantitation (LOQ)

LOD and LOQ represent the concentration of the analyte that would yield a signal-to-noise (S/N) of 3 and 10, respectively, following the EP. The LOD and LOQ of (*S*)-OME were determined by injecting a series of diluted solutions.

2.4.5. Precision

The precision was determined by measuring the repeatability of retention time and peak areas on replicate injections ($n=6$) of 248.50 μg/mL of (S)-OME solution. Precision was reported as percentage of relative standard deviation (RSD%).

3. Results and discussion

3.1. Analytical HPLC enantioseparation and enantiomer elution order on the ID-3 and AGP CSPs

Because the enantiomeric elution order of the chiral IMP-B and IMP-E of OME on the AGP CSP has not been established, we started our research by analyzing the chiral samples in the racemic form under the HPLC analytical method described in the EP monograph for checking the enantiomeric purity of (*S*)-OME [chiral column: AGP (100 mm × 4.0 mm, 5μm) column; mobile phase: acetonitrile–pH 6 phosphate buffer (13:87, v/v); flow rate: 0.6 mL/min; temperature: 25 °C]. The analytical results were compared with those obtained from the enantioselective analysis of only OME on the Chiralpak ID-3 (100 mm × 4.6 mm, 3 μm) column in the chromatographic conditions optimized in an our previous work [column temperature: 40 °C; flow rate: 1.0 mL/min; mobile phase: acetonitrile–water (50:50, v/v)] [13].

The typical on-line CD traces and the chromatographic results obtained using the experimental conditions described above are shown in Fig. 2. Chromatographic results reveal that the chiral discrimination ability of the ID-3 CSP was generally superior to that of AGP CSP. The enantioselectivity factors values were high on both CSPs just in case of OME ($\alpha=3.11$ and $\alpha=2.97$) while the resolving power of the AGP CSP significantly lowered for the IMP-B ($\alpha=1.44$). The enantiomers of the compound IMP-E were not separated at all on the AGP CSP and weakly resolved on the ID-3 CSP ($\alpha=1.10$).

The enantiomer elution order of the investigated chiral compounds was easily and unambiguously established by evaluating the on-line CD signal monitored at 280 nm during the enantioselective analysis (Fig. 2). As demonstrated previously [13], at the diagnostic wavelength of 280 nm and under reversed-phase conditions, there is an univocal correlation between CD properties and absolute configuration of the eluting enantiomer. The first eluting enantiomer on the ID-3 CSP exhibited negative CD signal at 280 nm (Fig. 2). On the basis of the aforementioned strategy, its absolute configuration was determined as (*S*). Consequently, the (*R*)-configuration was assigned to the more retained enantiomer, with positive CD signal at the same wavelength.

It is interesting to note that the (*S*)-enantiomer of OME was found to be eluted later than (*R*)-counterpart on the AGP CSP. In the case of the structurally related IMP-B, the enantiomer elution order was reversed with respect to OME on the ID-3 CSP ((*S*)-IMP-B was eluted later than (*R*)-IMP-B) whereas it was the same as that observed on the AGP CSP. Finally, the (*R*)-form of IMP-E was first eluted on the ID-3 CSP. The availability of pure enantiomeric forms of OME, IMP-B and IMP-E and the knowledge of their absolute configuration [11] allowed us to confirm the enantiomer elution order established by the on-line CD signal recorded at 280 nm.

3.2. Validation of the method

3.2.1. Specificity

Having established the chiral discrimination ability of ID-3 and AGP CSPs towards the racemic samples, OME, IMP-B and IMP-E, and identified the enantiomers of each chiral substance in the pertinent HPLC elution profile, the next step of our work was to check the specificity of two analytical enantioselective methods based on the ID-3 and AGP CSPs. The specificity of the analytical methods was checked by injecting the (*S*)-form of OME, the enantiomeric impurity (*R*)-OME, the (*S*)-forms of IMP-B and IMP-E and the achiral related substances A, C and D individually. The obtained chromatograms are presented in Figs. 3 and 4. As can be seen in the case of the ID-3 CSP, the retention times of the two enantiomers of OME were clearly different from those of the related substances. Therefore, the proposed method based on the polysaccharide-type CSP can separate the target (*S*)-OME from its

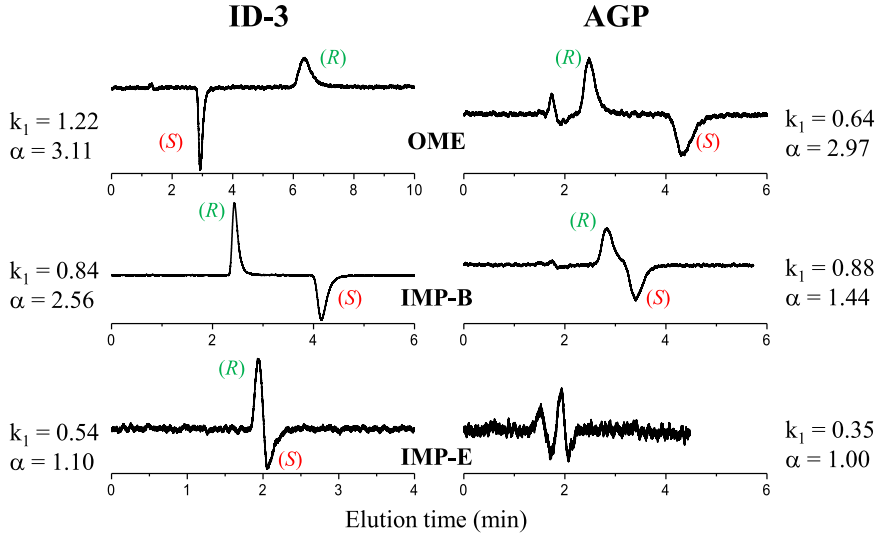

Fig. 2. Typical CD traces recorded during the enantioselective HPLC of OME, IMP-B and IMP-E. Columns: Chiralpak ID-3 (100 mm × 4.6 mm, 3μm) (left side) and AGP (100 mm × 4.0 mm, 5μm) (right side); detection: CD at 280 nm; flow rate: 1.0 (left side) and 0.6 (right side) mL/min; column temperature: 40 °C (left side) and 25 °C (right side).

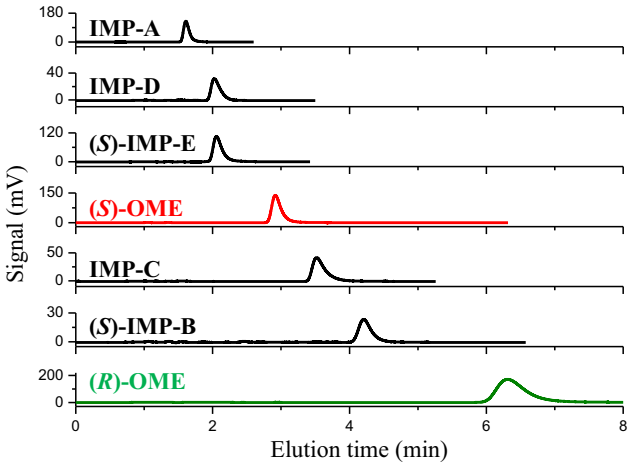

Fig. 3. Typical HPLC chromatograms of (*S*)-OME and its impurities A-F on the ID-3 CSP. Column: Chiralpak ID-3 (100 mm × 4.6 mm, 3μm); detection: UV at 280 nm; flow rate: 1.0 mL/min; column temperature: 40 °C.

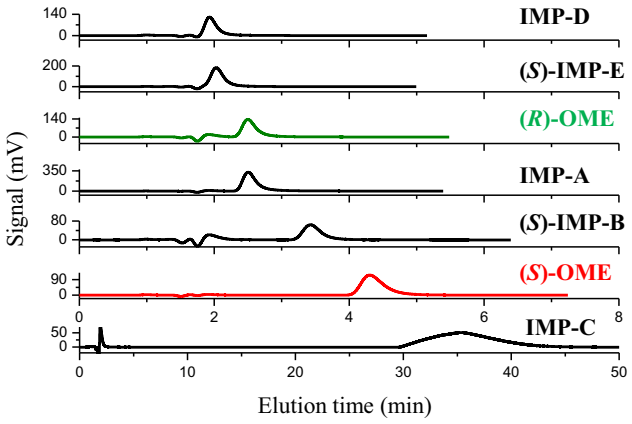

Fig. 4. Typical HPLC chromatograms of (*S*)-OME and its impurities A-F on the AGP CSP. Column: AGP (100 mm × 4.0 mm, 5μm); detection: UV at 280 nm; flow rate: 0.6 mL/min; column temperature: 25 °C.

enantiomer (IMP-F) and such a separation is achieved with no interference of the impurities A-E. Due to its specificity, it can be applied for in-process and quality controls of the enantiomeric purity drugs. In the case of the analytical procedure of the drug regulatory agencies based on the AGP CSP, the peak pertinent to the IMP-A is expected to overlap with that of (*R*)-OME. The peaks interference may be sufficient to corrupt the analysis of the enantiomeric purity of (*S*)-OME and to make the method non-specific. As the last remark, a very broad peak at the retention time of about 35 min was observed for the IMP-C when eluted on the AGP CSP.

The enantioselective HPLC system based on the immobilized Chiralpak ID-3 CSP was partially validated and the analytical results obtained are described below.

3.2.2. *Linearity*

The linearity of the HPLC method was evaluated by injecting standard concentrations of (*S*)- and (*R*)-OME samples with a concentration ranging from 0.2485 to 497 μg/mL (0.1%–200%) and 0.12425–12.425 μg/mL (0.05%–5%), respectively. The peak area response was plotted versus the nominal concentration of the enantiomer. The obtained calibration curves for the (*S*)-OME and (*R*)-OME were as follows: $y=26314x+15171$ ($r^2=1.0$) and $y=26324x-359.15$ ($r^2=1.0$), where x is the concentration and y is the peak area.

3.2.3. *LOD and LOQ*

The LOD and LOQ were estimated to be 9.8 and 32.66 ng/mL for (*S*)-OME and 33.32 and 111.06 ng/mL for (*R*)-OME enantiomers on Chiralpak ID-3 CSP. In the case of the AGP CSP, the LOD and LOQ values were 20.75 and 69.16 ng/mL for (*S*)-OME and 12.45 and 41.5 ng/mL for (*R*)-OME.

3.2.4. *Precision*

The precision of the HPLC method was determined by repeatability for retention times, with an RSD of 0.15%, and for area, with an RSD of 0.30%, which comply with the acceptance criteria proposed (RSD not more than 2.0%).

4. Conclusions

In summary, the presented work is an original extension of previously published studies on HPLC enantioseparation of OME. For the first time, we have established the HPLC separation of OME enantiomers in the presence of its potential organic impurities A-E under reversed-phase mode. In order to achieve that, the following objectives were necessary: (i) to separate the enantiomers of OME and its chiral impurities on the Chiralpak ID-3 CSP; (ii) to determine which of the two eluting enantiomers has (*S*)-configuration; (iii) to evaluate the retention times of (*S*)-OME and all its chiral and achiral impurities under reversed-phase mode [mobile phase: acetonitrile–water (50:50, v/v)].

Finally, the paper demonstrated that the enantioselective method reported in the current Pharmacopoeias is not specific due to the overlapping of IMP-A and the (*R*)-OME form. The proposed enantioselective method is simple, rapid, sensitive and precise, and can be adopted for routine use to establish the enantiomeric purity of (*S*)-OME in raw materials or in working standards.

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