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Development and Validation of LC Method for the Assay of Omeprazole Enantiomers in Pharmaceutical Formulations

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ABSTRACT

A simple and sensitive RP-HPLC method for the determination of omeprazole R-enantiomer (R-OME) and omeprazole S-enantiomer (S-OME) in bulk drug samples and pharmaceutical formulations has been developed and validated. The separation of R-OME and S-OME was achieved on a chiral AGP column using UV detector at 301 nm. The mobile phase consisted of 0.025 mol L^{-1} di sodium hydrogen phosphate/ acetonitrile, (90:10, V/V) (pH 7.0)–. The linear range of detection was found to be 0.01–150 µg/ml (R^2=0.9993) and 0.015–152 µg/ml (R^2=0.9999) for R-OME and S-OME respectively. The method has been applied successfully for the determination of S-OME in pharmaceutical preparations. The excipients commonly present in formulations did not interfere in the assay of S-OME.

Keywords: Omeprazole, RP-HPLC, validation, formulation, enantiomers.

INTRODUCTION

Omeprazole (OME) is a substituted benzimidazole, 5-methoxy-2-(((4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl) sulphinyl)-1H-benzimidazole. It is a proton pump inhibitor. OME is a potent anti ulcer agent that suppresses the secretion of gastric acid by the inhibition of the H^+/K^+ ATPase in the gastric parietal cell. It is an effective drug used in the treatment of acid peptic disorders and has found worldwide popularity over the past decade [1]. None of the subsequently developed and produced antisecretory drugs, including those in the proton-pump inhibitor class, have been shown to be significantly superior to OME in clinical practice [2]. The drug is a racemate and contains a tricoordinated sulphur atom in the pyramidal structure, which gives two optical active isomers (enantiomers), (S) and (R)-OME (Fig. 1) [3].

Analytical methods reported for the assay of OME in human blood plasma include HPLC [4-7], derivative spectrophotometry [8], LCMS [9-12], fluorescence spectrometry [13], HPLC method (only for chromatographic purity) -United states pharmacopia (USP) [14] and capillary electrophoresis [15] are available in the literature. However, validated reverse phase HPLC method for the determination of S-OME and its separation from Rs-OME is not reported so far. Hence, it was felt necessary to develop a chromatographic method for quantitative determination of S-OME and R-OME enantiomer in bulk drug and pharmaceutical formulations.

MATERIALS AND METHODS

Chemicals and reagents:

R-OME and S-OME were obtained as gift samples from Cipla Ltd., India and its formulations Prilosec, Nexium, Nexium® i.v and Zegerid® were obtained commercially. HPLC grade acetonitrile was purchased from Spectrochem Ltd, India. Disodium hydrogen phosphate and orthophosphoric acid were obtained from Merck (Germany). HPLC grade water obtained from a Milli-Q water purification system (Millipore, USA) was used throughout the study.

Operating conditions:

The HPLC system consisted of a waters alliance 2695 separation module (Milford, USA) equipped with a 2996 photo diode array detector (PDA) module with data processing on Empower 2 (version 6.10.00.00) software was employed. The separation was performed on a Chiral AGP (100 mm x 4.6 mm id, 5µm particle size) column using UV detector at 301 nm. The mobile phase consisted of 0.025 M disodium hydrogen phosphate/acetonitrile, (90:10, V/V) (pH 7.0) and diluted ortho phosphoric acid was used for pH adjustment. An Inertsil ODS (4 cm x 4.6 mm, i.d.) was used as a guard column. A flow rate of 1.0 mL/min was maintained throughout the analysis. The mobile phase was filtered through 0.45-µm Millipore membrane filter and degassed. 20 µL of blank, sample and standard solutions were injected by the auto sampler of 2695 separation module. Measuring the absorbance at 301 nm effected quantification. A chromatographic run time of 15 min was maintained throughout. The separation was carried out at ambient temperature.

Preparation of solutions:

A standard solution of OME (200 µg/ml) was prepared in the mobile phase. This stock solution was diluted further as and when required. Bulk drug sample solution was also prepared by dissolving a weighed quantity of bulk drug in the mobile phase. For the analysis of OME enantiomers in Prilosec, Nexium (delayed release oral suspension and capsules), Nexium® i.v. (for Injection) and Zegerid® (immediate-release capsules and powder for oral suspension) by the proposed method, appropriate amounts of the samples were dissolved in the mobile phase and then filtered through 0.45-µm Millipore membrane filter. They were then degassed in an ultrasonic bath. Known volumes of solution were used for analysis.

Validation:

HPLC method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. Analytical methods need to be validated or revalidated before their

introduction into routine use. The validity of a specific method should be demonstrated in laboratory experiments using samples or standards that are similar to unknown samples analyzed routinely. HPLC method was validated as per the ICH guidelines.

Calibration curve for RP-HPLC method:

Working solutions of pure R-OME (0.01-150 µg/ml) and S-OME (0.015-152 µg/ml) were prepared separately in mobile phase. 20 µL injections in triplicate were injected to check the reproducibility of the detector response at each concentration level. The peak areas of standard were plotted against the concentration of drug to obtain calibration graph. The results were subjected to regression analysis to obtain calibration equation and the correlation coefficient.

Accuracy:

The accuracy of the assay method was evaluated in triplicate at three concentration levels (50, 100 and 150 µg/ml). It was also evaluated by fortifying a mixture of formulation sample with three known concentrations of the drug. The accuracy of the methods was determined by calculating OME enantiomers recoveries by the standard addition method

Precision:

Intra-day precision and inter-day precision were evaluated by analyzing six replicates of three different concentrations (50, 100, 150 µg/ml) on the same day and on different days, respectively. The respective %Relative Standard Deviation (RSD) values were calculated.

Specificity and selectivity:

Specificity of the method was established through study of resolution factors of the drug peak from the nearest resolving peak and also among all the other peaks. The presence/absence of peaks due to excipients, impurities and degraded products was examined to study the interference from these in the assay of the drug.

Limit of detection and quantification:

The values of LOD and LOQ were determined at a signal to noise ratio of 3: 1 and 10: 1, respectively by injecting a series of test solutions of known concentrations within linearity range. Determination of the signal-to-noise ratio shall be performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably detected and quantified. Precision study was also carried out at the LOQ level by injecting six preparations.

Robustness:

To determine the robustness of the developed method, experimental conditions were deliberately altered to check the reproducibility and quantitative recovery of the drug. This was carried out by varying the flow rate of mobile phase in the range of 0.8-1.2 mL/min, the column temperature in the range of 22-30 °C and the composition of the mobile phase (88-92% buffer and 8-12% acetonitrile). The results obtained were compared with those obtained under the optimum chromatographic conditions mentioned earlier. In all the deliberately varied chromatographic conditions (flow rate, column temperature and mobile phase composition variation) and the system performance parameters satisfied the system suitability criteria.

Solution stability and mobile phase stability:
The solution stability of OME enantiomers in the assay method was carried out individually by leaving both the test solutions of the sample and reference standard in tightly capped amber colored volumetric flasks separately, at room temperature, up to the study period of 72 h. The chromatograms of these solutions were recorded separately at an interval of 1 h up to 72 h and the peak responses were compared.

The mobile phase stability was carried out by assaying the freshly prepared bulk drug and formulation sample solutions against freshly prepared reference/standard solution at an interval of 8 h. The same mobile phase was used throughout the experiment. %Relative Standard Deviation (RSD) values were calculated for mobile phase and solution stability experiments.

RESULTS AND DISCUSSION

Method development:
The chiral column chemistry utilizes the α_1-acid glycoprotein as stationary phase and which is a small acute-phase glycoprotein (Mr = 44,000) that is negatively charged at physiological pH (13-14). It consists of a chain of 181 amino acids, and contains 40% carbohydrate by weight and has 16 sialic acid residues (10-14% by weight). Five hetropolysaccharide groups are linked via an N-glycosylic bond to asparaginyl residues of the protein. The protein contains tetrantennary as well as di- and triantennary glycans. The above mentioned glycoprotein was covalently bound to silica gel in stationary phase of column. The mobile phase was chosen after several trials with mixture of buffers at different concentrations with uncharged organic modifiers like methanol, acetonitrile, 2-propanol, 1-propanol and ethanol at different pHs in various proportions. With the mixture of phosphate buffer (pH 5.0) and isopropyl alcohol (85:15, V/V) on reverse phase chiral column, separation factor ($\alpha > 1.5$) was achieved, but tailing factor was more than 3.5 and run time was longer. A mixture containing 0.025 M disodium hydrogen phosphate/acetonitrile (90:10, V/V) (pH 7.0), was employed as the mobile phase on a chiral AGP column to achieve maximum separation, enantioselectivity and sensitivity. Chiral column having 1-acid glycoprotein as AGP stationary phase having isoelectric point at pH 2.7 where the protein has a net charge zero. During the method development, pH of the mobile phase was optimized to achieve enantioselectivity and separation by increasing net negative charge of stationary phase, AGP at higher pH. Using reverse phase column, the retention time of R-OME and S-OME was observed to be 3.65 min and 9.17 min, respectively. The maximum absorption of OME enantiomers was found to be at 301 nm (Fig. 2) and hence, this wavelength was chosen for analysis. Chromatogram of standard drug solution and formulation was shown in Fig. 3 and Fig. 4 respectively

Validation of the method:
The correlation was obtained between the peak area and concentration of the drugs. RP-HPLC method, linearity range for R-OME and S-OME were 0.01-150 µg/ml and 0.015-152 µg/ml respectively. Linearity of the calibration curves was validated by the high value of correlation coefficients of the regression (Table 1) and a validation results are compared statistically with the reported results [15] and shown in Table 2. Precision and accuracy results of proposed method were precise and accurate by reveling lower RSD values than that of reported results.

The model accuracy was studied by the standard addition method. The percent recoveries were ranged from 99.0 to 100.2 and from 99.0 to 99.6 for R-OME and S-OME in within-day analysis and from 98.0 to 100.0 and from 98.8 to 101.0 in between-day analysis for R-OME and S-OME, respectively. These results revealed that the proposed RP-HPLC method is accurate (Table 3).

The RSD values of instrument precision for both R-OME and S-OME were 0.4 and 0.7 respectively. The RSD values of inter-day precision for R-OME, S-OME and OME by RP-HPLC method were ranged from 0.3 to 1.2, 0.6 to 0.9 and 0.3 to 1.1 respectively and intra-day precision RSD values ranged from 0.5 to 0.8, 0.2 to 0.7 and 0.3 to 0.9 for R-OME, S-OME and OME. Low RSD values indicate that the proposed method was precise (Table 4)**.**

The suitability of the method was checked by calculating the chromatographic parameters. The observed values of purity angle of less than purity threshold, the resolution value of 24.9 (between R-OME and S-OME), capacity factor of 0.27 and 0.11 for R-OME and S-OME and the HETP value of greater than 2000 revealed the ideal chromatographic conditions for quantitative determination of R-OME and S-OME. The corresponding results are shown in Table 1.

LOD for R-OME and S-OME was found to be 0.0029 µg/ml and 0.0031 µg/ml respectively. LOQ for R-OME and S-OME was found to be 0.01 µg/ml and 0.015 µg/ml respectively. These statistical results show that both methods are highly sensitive for R-OME and S-OME determination, compare to report one.

Percentage recovery for R-OME was 99.6, 100.2, 100.5 and 100.8 for altered flow rate, column oven temperature, acetonitrile and buffer ratios in mobile phases respectively. Similarly for S-OME recovery was 98.8, 99.6, 99.7 and 100.6 for altered chromatographic conditions as mentioned for R-OME. Hence, the proposed method was found to be robust for the assay of R-OME and S-OME in bulk drug and formulations (Table 5).

The standard drug solution stability and mobile phase stability was evaluated by assying the analyte and the % RSD values obtained were less than 2. These values indicated that the standard and sample solution preparations of OME (R-OME and S-OME) or in its single enantiomeric form are also the mobile phase was stable enough for analysis.

Analysis of pharmaceutical preparations:

The proposed method was successfully applied to the analysis of OME and its (R) and (S) enantiomers; R-OME and (S)-OME in different formulations and the results are shown in (Table 6). For recimic formulation the recovery was ranged from 99.2 to 101.3 and for S-OME 99.6 to 101.2 respectively. High percent recovery values indicated that the commonly employed excipients consisting of croscarmellose sodium and crospovidone (disintegrant), sodium stearyl fumarate and magnesium stearate (lubricant), xylitol, sugar spheres, sucralose, xanthan gum and sucrose (sweetener), citric acid (preservative), iron oxide (colorant), hydroxypropyl cellulose, glyceryl monostearate and hypromellose (binder), methacrylic acid copolymer C, polysorbate (emulsifier), and talc (filler) did not interfere at the levels generally present in pharmaceutical formulations containing OME. The low values of RSD indicated high precision of the method.

Fig No.1: Strcture of R-enantiomer (a) and S-enantiomer (b) of omeprazole

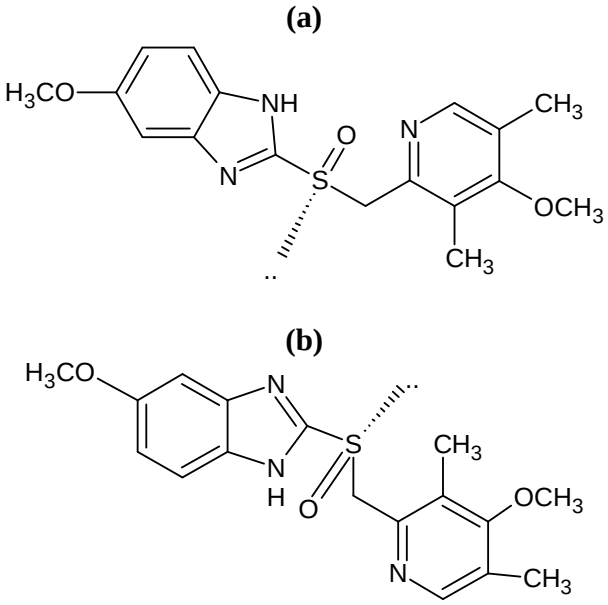


Fig No.2: Overlaid UV spectrum of R and S-enantiomers of OME

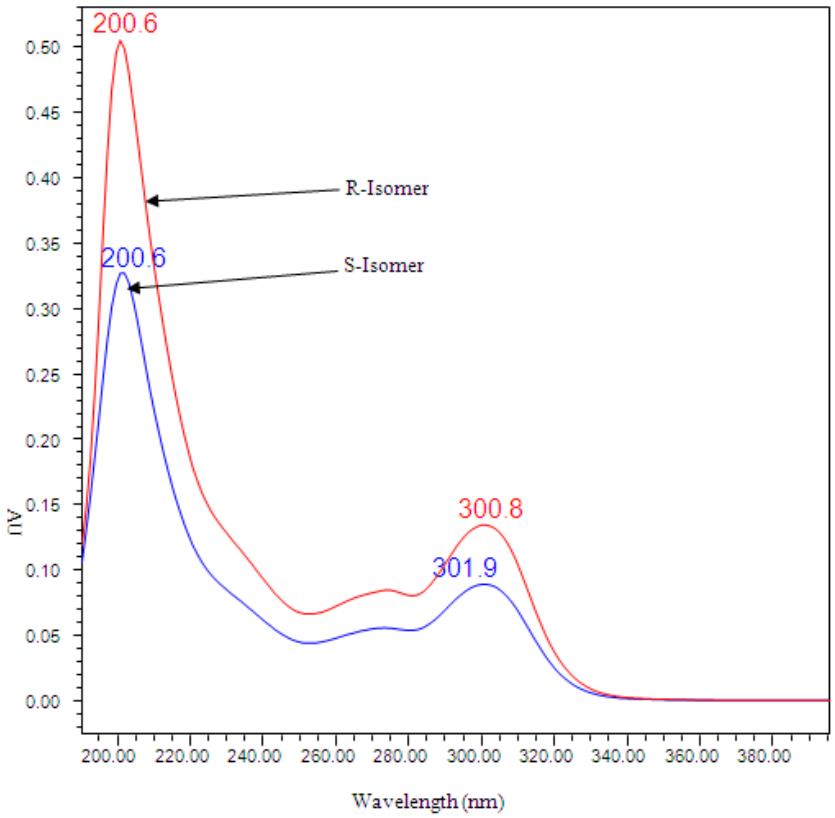


Fig No.3: Chromatogram of standard drug solution: R and S-enantiomers of OME at LOD level

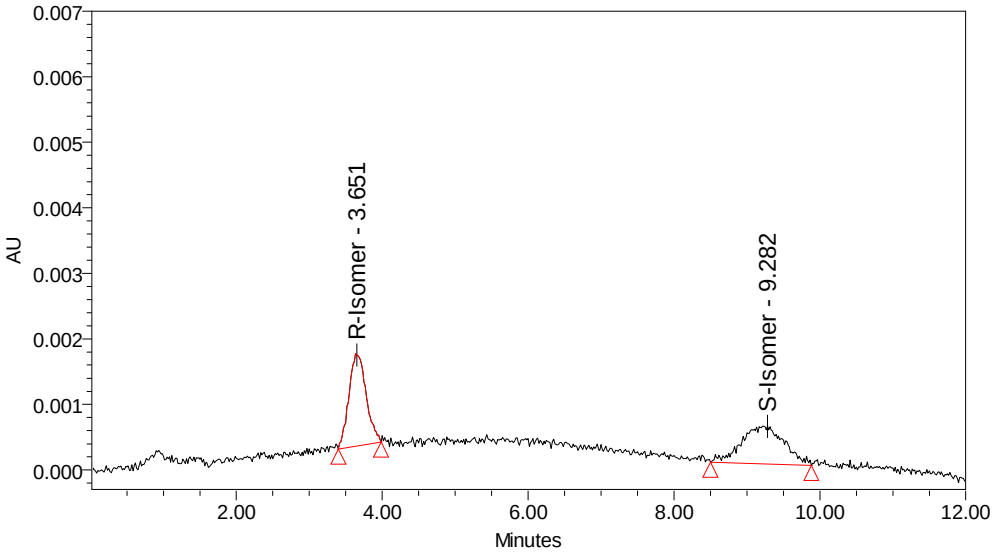
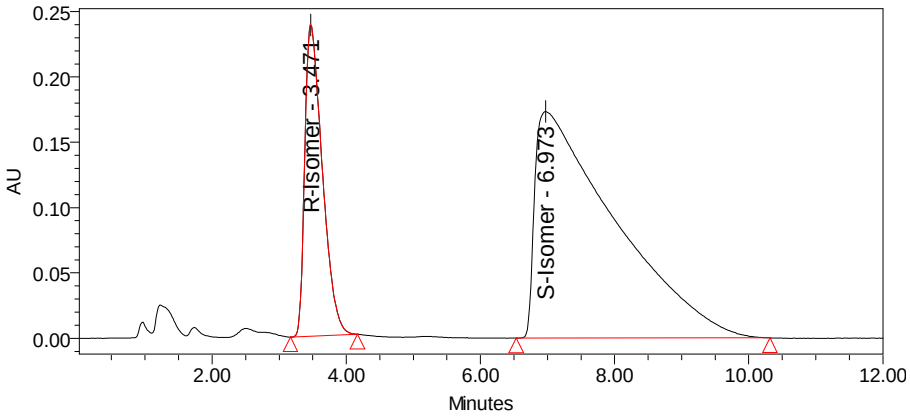


Fig No.4: Chromatogram of formulation: (A) Prilosec (100.0 µg/ml) and (B) Nexium (100.0 µg/ml)

(A)



(B)

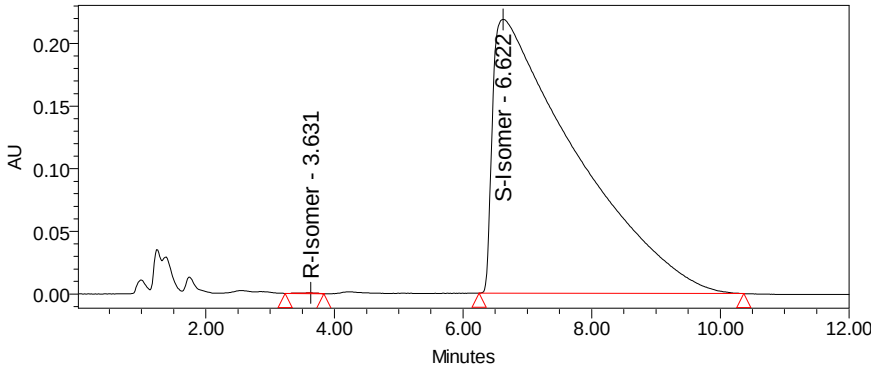


Table 1. Regression analysis of the calibration curve for R-OME and S-OME and system suitability parameters

Parameter	R-OME	S-OME
Concentration Range	0.01- 150 µg/ml	0.015- 152 µg/ml
Slope [a]	5.925 ± 0.16	5.750 ± 0.13
Coefficient of Determination (R^2)	0.9993	0.9999
Capacity factor	0.27	0.11
Resolution	Not applicable	24.9
HETP	3217	3051
Purity angle	0.251	0.201
Purity threshold	0.307	0.285

[a] *Mean ± SD, n = 5*

Table 2. Comparison of validation parameters of the proposed method with the published method

Parameter	Proposed method		Reported method [15]	
	R-OME	S-OME	R-OME	S-OME
Precision [a] CV (%)				
Within-day	0.7	1.1	1.7	1.7
Between-day	0.5	0.9	1.9	1.8
Accuracy [a]				
Within-day (%)	-0.4	-0.7	-1.0	1.0
Between-day (%)	-0.7	0	0	-2.0
Detection limit (µg/ml)	0.0029	0.0031	0.0028	0.0061

Mean, (n = 3)

Table 3. Accuracy assessment of proposed method for R-OME and S-OME; standard addition method, each R-OME and S-OME at 50 µg/ml concentration

Added concentration (µg/ml)		Measured concentration (µg/ml)[a]		Recovery (%) [b]	
R-OME	S-OME	R-OME	S-OME	R-OME	S-OME
Within-day					
25.0	25.0	49.8	49.3	99.6 ± 0.4	99.0 ± 0.3
25.0	25.0	49.5	49.7	99.0 ± 0.1	99.4 ± 0.5
25.0	25.0	50.1	49.8	100.2 ± 0.7	99.6 ± 0.2
Between-day					
25.0	25.0	49.0	50.5	98.0 ± 1.1	101.0 ± 0.9
25.0	25.0	49.9	50.0	99.8 ± 0.8	100.0 ± 0.3
25.0	25.0	50.0	49.4	100.0 ± 0.5	98.8 ± 1.2

[a] Mean, n = 6; [b] Mean ± Standard deviation (S.D)

Table 4. Precision of the method

		RSD (%)[a]		CV (%)[b]	
	Taken (µg/ml)	Intra-day (n = 5)	Inter-day (n = 5)	Intra-day	Inter-day
R-OME	50.0	0.5	0.3	0.8	1.1
	100.0	0.7	0.8	0.7	0.9
	150.0	0.8	1.3	1.2	1.5
S-OME	50.0	0.2	0.6	0.6	1.5
	100.0	0.5	0.9	0.9	1.7
	150.0	0.7	1.1	0.7	1.8
OME (R- and S-OME)	50.0	0.6	0.4	0.3	1.3
	100.0	0.3	0.7	0.9	1.7
	150.0	0.9	1.5	1.1	1.8

[a] Relative standard deviation; [b] Coefficient of variation

Table 5. Evaluation of robustness at Lower, central and upper values in terms of accuracy of the proposed method in OME(R- and S-OME) bulk samples (50 µg/ml).

Parameters	Values	Amount of drug added (µg/ml)		Recovered [a] (µg/ml)		Recovery (%) [b]	
		R-OME	S-OME	R-OME	S-OME	R-OME	S-OME
Flow rate (mL min^{-1})	0.8 1.0 1.2	25.0	25.0	24.9 ± 0.2	24.7 ± 0.1	99.6 ± 0.3	98.8 ± 0.1
Temperature (°C)	22 25 30	50.0	50.0	50.1 ± 0.5	49.8 ± 0.4	100.2 ±0.1	99.6 ± 0.9
Acetonitrile ratio (V/V) in mobile phase	8 10 12	75.0	75.0	75.1 ± 0.5	74.8 ± 0.3	100.5 ±0.5	99.7 ± 0.6
Buffer ratio (V/V) in mobile phase	92 90 88	100.0	100.0	100.8 ± 0.8	100.6 ±0.9	100.8± 0.7	100.6 ± 0.4

[a] Mean ± RSD, n = 3
[b] Mean ± RSD,

Table 6. Analysis of pharmaceutical formulations; recovery study, OME (R- and S-OME) standard addition method (50 µg/ml).

Experimental results				
Formulation Brand	Pure drug added (µg/ml)	Total drug found[a], (µg/ml)	Recovery(%) [b]	RE(%) [c]
OME (R- and S-OME)				
Prilosec	50.0	99.2 ± 0.8	99.2 ± 0.5	-0.8
	100.0	151.1 ± 1.0	100.7 ± 0.1	0.7
Zegerid®	50.0	99.4 ± 0.5	99.4 ± 0.3	-0.6
	100.0	150.9 ± 0.9	101.3 ± 0.6	1.3
S-OME enantiomer				
Nexium	50.0	75.91 ± 0.7	101.2 ± 0.2	1.2
	100.0	125.82 ± 0.3	100.7 ± 0.6	0.7
Nexium® i.v	50.0	74.71 ± 0.5	99.6 ± 0.4	-0.4
	100.0	125.93 ± 0.9	100.7 ± 0.8	0.7

[a] Mean ± RSD, n = 9
[b] Mean ± RSD, n = 9
[c] Relative error (%) = [100 x (predicted concentration – nominal concentration)/ nominal concentration].

CONCLUSION

The proposed method is precise, specific, accurate and enantioselective with highest sensitivity. R-OME and S-OME can be determined in bulk powder and pharmaceutical formulation by HPLC method. In view of this, the proposed method could be adopted for quality control and routine analysis.

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REFERENCES

[1] M. Espinosa Bosch, A. J. Ruiz Sánchez, F. Sánchez Rojas and C. Bosch Ojeda, *J. Pharm. Biomed. Anal*. **2007**, 44: 831-844.
[2] W. A. Hoogerwerf and P. J. Pasricha, 11th ed, McGraw-Hill, New York **2006**, p. 967-981.
[3] L. Olbe, E. Carlsson and P. Lindberg, *Nat. Rev. Drug Discov.* **2003,** 2:132-139.
[4] M. G. Héctor, E. M. Romero, J. C. Teresa de, A. A. P. Víctor Quezada and C. Hoyo-Vadillo, *J. Chromatogr. B.* **2002**, 780: 459-465.
[5] Q. B. Cass, V. V. Lima, R. V. Oliveira, N. M. Cassiano, A. L. G. Degani and J. Pedrazzoli Jr., *J. Chromatogr. B.* **2003**, 798: 275-281.
[6] K. Balmer, Bengt-Arne Persson and Per-Olof Lagerstrom, *J. Chromatogr. A.* **1994**, 660: 269-273.
[7] Ann M. Cairns, Rita H.-Y. Chiou, J. D. Rogers and J. L. Demetriades, *J. Chromatogr. B.* **1995,** 666: 323-328.
[8] D. Castro, M. A. Moreno., S. Torrado, and J. L. Lastres., *J. Pharm. Biomed. Anal.* **1999**, 21: 291-298.
[9] M. S. Ute Hofmann., Gerd Treiber., Ulrich Klotz., *J. Chromatogr. B.* **2006**, 831: 85-90.
[10] H. Stenhoff, A. Blomqvist and Per-Olof Lagerstrom, *J. Chromatogr. B.* **1999**, 734: 191-201.
[11] J. Wang, Y. Wang, J. Paul Fawcett, Yan Wang and Jingkai Gu, *J. Pharm. Biomed. Anal.* **2005,** 39: 631-635.
[12] Bryan M. Bunton and Thomas A. walker. *J. chromatogr A.* **1995**, 699: 389-394.
[13] Feng Zhang, Yingxiang Du, Baofen Ye and Ping Li, *J. photochemistry and photobiology B: Biology*, **2007**, 86: 246-251.
[14] United States Pharmacopia, 31-NF-26 33(3) 436.
[15] Pierina S. Bonato and Fernanda O. Paias, *J. Braz. Chem. Soc.* **2004**, 15: 318-323.