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HPLC Determination of Esomeprazole Magnesium in Tablets

X.H. TANG*†, J.P. HOU‡, A. LIU, Y.M. XIE‡, X. HU and X. SONG†
Department of Pharmaceutical and Biological Engineering,
School of Chemical Engineering, Sichuan University, Chengdu 610065, P.R. China
Fax: (86)(28)85997667; Tel: (86)(28)85997668; E-mail: pharmmateceo@yahoo.com.cn

A simple, rapid and precise HPLC method was developed and validated in the determination of esomeprazole magnesium in tablets. The method employed the Phenomenex Luna C_{18} column with a mobile phase of ammonium dihydrogen phosphate buffer and methanol at a flow rate of 0.8 mL/min and UV detection at 302 nm. Validation parameters, such as linearity, limit of detection (LOD), limit of quantification (LOQ), selectivity, precision, accuracy and robustness were determined. A linear response ($r^2 > 0.999$) was observed in the range of 10-400 µg/mL equivalent to esomeprazole magnesium. The method shows good recoveries, repeatability and intermediate precision. Relative standard deviations of all these three parameters were less than 1.1 %. Finally, the established HPLC method was successfully applied for the quantitative determination of esomeprazole magnesium in tablets.

Key Words: HPLC, Esomeprazole magnesium, Tablets, Validation, Quality control.

INTRODUCTION

Esomeprazole magnesium trihydrate (EMT), chemically, *bis*(5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole-1-yl) magnesium trihydrate, is a compound which highly inhibits gastric acid secretion[1]. Esomeprazole, the S-isomer of omeprazole, is the first proton pump inhibitor to be developed as a single optical isomer, generally provides better acid control than current racemic proton pump inhibitors and has a favourable pharmacokinetic profile relative to omeprazole[2-4]. Erlandsson *et al.*[5] report that the both enantiomers of omeprazole have the same capacity to decrease gastric acid formation *in vitro*, but stereoselective metabolism by CYP2C19 results in different plasma concentrations[6]. Two major enzymes involved in omeprazole metabolism are CYP3A4, which catalyses the substrate to be sulfone and CYP2C19, which catalyzes the substrate to be the major part of the 5-O-desmethyl and hydroxy metabolites[7-9]. Recently, Nexium® (esomeprazole magnesium enteric-coated tablets) has been marketed by

†Chongqing Lummy Pharmaceutical Co., Ltd., Chongqing 401336, P.R. China.
‡Department of Pharmaceutical and Biological Engineering, Sichuan University, Chengdu 610065, P.R. China.

Astra Zeneca. This single isomer is subjected to less first pass metabolism by CYP2C19 and lower plasma clearance than racemic omeprazole, resulting in an AUC almost two times more potent than omeprazole, when equivalent doses are administered[10].

A LC-MS has been used for determination of the major metabolites of esomeprazole magnesium in human blood and plasma[11]. Recently, two UV spectrophotometric methods have been developed for simultaneous estimation of esomeprazole magnesium and domperidone in fixed dose combination capsules[12,13]. However, no references have been found for determination of esomeprazole magnesium in Nexium. Quality control has become a stringent tool in pharmaceuticals in order to minimize batch-to-batch variation and assure quality[14]. Consequently, there is an immense need to develop a sensitive, specific and validated analytical method for the routine analysis of the drug in pharmaceutical dosage forms.

This study describes a rapid, sensitive, accurate and precise method for the determination of esomeprazole magnesium in Nexium using HPLC. The method has been validated with respect to linearity, LOD and LOQ, selectivity, precision, accuracy and robustness.

EXPERIMENTAL

Nexium® (declared amount per tablet 44.5 mg EMT, equivalent to 40 mg esomeprazole) was obtained from AstraZeneca. EMT reference standard (100 % chromatographically pure) was self-made. Methanol in HPLC level was purchased from Merk (Darmstadt, Germany). Deionized water was obtained in the laboratory, using ionic interchanged columns Milli-Q (Millipore). All reagents were purchased from Bodi Chemical Co., Ltd. (Tianjin, China) and were of analytical grade unless indicated otherwise.

Apparatus and chromatographic conditions: Chromatographic measurements were performed on an Agilent1100 series liquid chromatography system (Agilent Technologies, Palo Alto, USA), equipped with G1310A isopump, G1314A UV-Visible detector and an AT-1302 model HPLC column heater (Automatic Science, Tianjin, China). The chromatographic column used was a Luna C_{18} column (4.6 mm × 250 mm, 5 μm particle size, Phenomenex, USA). The mobile phase consisted of 35:65 (v/v) 0.025 M ammonium dihydrogen phosphate buffers-methanol (pH 6.1). The flow rate was 0.8 mL/min. The UV detector was 302 nm and the column temperature was 25 °C. The injection volume was 5 μL. Data was acquired and processed with Chem Station® software (Agilent Tenchologies). The mobile phase was degassed using a KH2200B ultrasonic cleaner (Kunshan Hechuang Ultrasonic Instruments Co., Ltd, China).

Solution preparations

Stock drug solution: EMT (55.7 mg) was dissolved and diluted in methanol to 50 mL, to obtain a final concentration of 1 mg/mL equivalent to esomeprazole, stored at 4 °C and protected from light.

Working solution: An aliquot of the stock solution was taken and diluted to 10 mL with mobile phase. All the solutions were protected from light by using amber glass material.

Assay sample preparation: Twenty tablets were weighed and finely powdered. The portions equivalent to 40 mg esomeprazole were suspended in 50 mL methanol with sonication to assure the complete dissolution of the drug and diluted to a final volume of 100 mL with methanol. The mixture was centrifuged at 4500 rpm for 15 min. 5 mL of supernatant was taken and diluted to a 10 mL volume with mobile phase to obtain a final concentration around 200 μg/mL esomeprazole. The solution was filtered through a 0.22 μm membrane filter (Millipore, cellulose acetate) and 5 μL was injected into the HPLC column.

Content sample preparation: Ten tablets were used to determine the drug content uniformity in each tablet. Each tablet was separately crushed and suspended in 50 mL methanol with sonication to assure the complete dissolution of the drug and diluted to a final volume of 100 mL with methanol. 5 mL of supernatant was taken and diluted to a 10 mL volume with mobile phase to obtain a final concentration around 200 μg/mL esomeprazole. The solution was filtered through a 0.22 μm membrane filter and 5 μL was injected into the HPLC column.

Validation: The validation procedure was followed the International Conference on Harmonization guideline and United States Pharmacopoeia for the analysis of esomeprazole magnesium by HPLC methods[15,16]. The performance parameters evaluated these methods were: linearity, LOD and LOQ, selectivity, precision, accuracy and robustness.

Linearity: Aliquots of EMT stock solution were diluted in mobile phase to obtain seven different concentrations, corresponding to 10, 25, 50, 100, 150, 250 and 400 μg/mL of esomeprazole. The solutions were injected and chromatographed according to the working conditions previously given.

Detection and quantitation limits: LOD and LOQ were determined by serial dilution so as to obtain signal to noise ratios of 3 and 10, respectively.

Selectivity studies

Hydrolysis: Individually 4 mg EMT was dissolved in 5 mL methanol in a 10 mL distillation flask and boiled for 1 h at reflux after adding: (a) 5 mL 0.1 M HCl for acid hydrolysis, (b) 5 mL 0.1 M NaOH for basic hydrolysis.

Photolysis: EMT (4 mg) was dissolved in 10 mL methanol. The solution was transferred to a black box and irradiated with UV light ($\lambda = 254$ nm) at a distance of 15 cm for 1 h.

Thermolysis: EMT (4 mg) was dissolved in 10 mL methanol and heated at 100 °C for 1 h.

Chemical oxidation: EMT (4 mg) was dissolved in 5 mL methanol in a 10 mL distillation flask and boiled for 1 h at reflux after adding 5 mL 3 % H_2O_2 solution. Each obtained solution from the degradation trials was diluted to 10 mL with mobile

phase. The mixture was filtered through a 0.22 μm membrane filter. Samples from these studies were stored at -20 °C and protected from light prior to HPLC analysis. All determinations were performed in triplicate.

Precision: Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time that was evaluated by assaying six sample solutions (n = 6), at the final concentration corresponding to 150 μg/mL of esomeprazole during the same day. Intermediate precision was assessed by comparing the assays on different days (3 days). The esomeprazole concentrations were determined and the relative standard deviations (RSD) were calculated.

Accuracy: The accuracy of the methods was determined through the recovery test, using the equation proposed[17,18]:

$$R\,\% = \frac{[(C_{S+STD}) - C_S]}{C_{STD}} \times 100\,\%$$

where C_{S+STD} is recovery solution (Nexium + EMT reference standard); C_S is concentration solution of Nexium and C_{STD} is concentration solution of EMT reference standard.

Robustness: The robustness of the HPLC method was determined by analysis of samples under a variety of conditions such as small changes in the percentage of mobile phase methanol (63, 65 and 67 %), in the pH (5.9, 6.1 and 6.3), in the mobile phase flow rate (0.5, 0.8 and 1.0 mL/min) and in the temperature (20, 25 and 30 °C).

RESULTS AND DISCUSSION

Method development and optimization: EMT is hydrophobic and is almost insoluble in aqueous solutions, whereas it is soluble in organic solvents like methanol and ethanol. During the development mobile phase, the use of water to methanol (30:70, v/v) as the mobile phase resulted in asymmetric peak with a greater tailing factor (> 2). When the mixture of 0.025 M ammonium dihydrogen phosphate buffer and methanol (35:65, v/v) was used as mobile phase, the tailing factor was within the acceptable limit (1.2) resulting in good peak symmetry. Increasing the flow rate to 1 mL/min resulted in poor resolution between the drug and degradation product. A flow rate of 0.5 mL/min resulted in drug retention time beyond 15 min that was more time consuming. Hence, the mobile phase was optimized at 0.8 mL/min with the retention time of the drug around 8.0 min. Also, the low flow rate and less run time consumes comparatively less mobile phase solvents which prove cost-effective during routine analysis of drug samples. The peak shape and symmetry were found to be good when a mobile phase composition of 35:65 (v/v, 0.025 M ammonium dihydrogen phosphate buffer: methanol) was used with better resolution of the drug and degradation product.

Method validation

Linearity: The calibration curve constructed was evaluated by its correlation coefficient. The concentration to the peak area of the drug was linear in the range of 10-400 µg/mL. Standard deviations of the slope and intercept for the calibration curves generated on 6 different days were 0.0022 and 0.0058, respectively. The correlation coefficient (r^2) of all the calibration curves were consistently higher than 0.999 (Table-1).

TABLE-1
RESULTS OF REGRESSION ANALYSIS OF THE LINEARITY DATA OF ESOMEPRAZOLE MAGNESIUM

	Mean ± SD (n = 6)
Slope	0.0677 ± 0.0022
Intercept	0.4031 ± 0.0058
Correlation coefficient (r^2)	0.9998 ± 0.0002

Detection and quantitation limits: The limit of detection was 2.1 ng/mL (S/N = 3), while the limit of quantitation was 8.4 ng/mL (S/N = 10).

Selectivity studies: In order to check the proposed method for selectivity, different degradation pathways for EMT were carried out. Fig. 1 shows the chromatograms of selectivity studies. During the study of stress with 0.1 M HCl and thermolysis, hardly any EMT was detected. When 0.1 M NaOH was used as a stress media and the solution was kept at boiling temperature, no unknown peak appeared. When the samples were subjected to an oxidation treatment with H_2O_2 3% and UV light, partial EMT was degraded and several unknown peak appeared. Thus, it can be stated that none of the peaks that could be generated by the stress treatment interfere with the peak corresponding to the active, therefore showing it was a selective method and suitable for routine work[19].

Precision: The results obtained from repeatability studies and for intermediate precision are presented in Table-2. Method precision has RSD values of 0.34-1.03 % for repeatability and 0.62 % for intermediate precision. RSD values lower than 2.0 % assure the precision of the method.

Accuracy: Accuracy is the exactness of the analytical method or the closeness of the agreement between the true value (accepted either as a conventional true value or an accepted reference value) and the tested value[20]. Table-3 summarizes the accuracy results expressed as per cent recovery and RSD. The percentage recovery of esomeprazole was in the range of 99.56-99.90 %. The RSD values in all the cases were < 0.5 %, which implies the accuracy of the method.

Robustness: Method robustness checked after deliberate alterations of mobile phase composition, pH, flow and temperature shows that the changes of the operational parameters do not lead to essential changes of the performance of the chromatographic system. It would be concluded that the method conditions are robust.

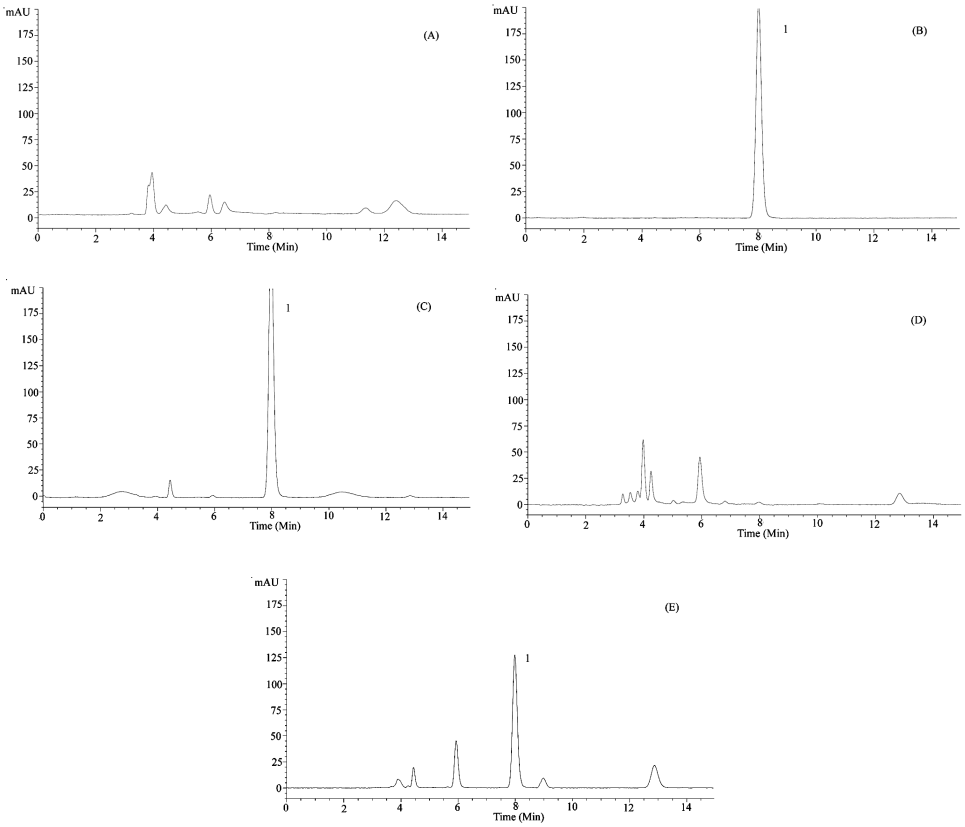

Fig. 1. Chromatograms of selectivity studies (A) treat with 0.1 M HC1 test; (B) treat with 0.1 M NaOH test; (C) photolysis test; (D) high temperature test; (E) oxidation test; (1) esomeprazole magnesium

TABLE-2
RESULTS OF THE REPEATABILITY AND THE INTERMEDIATE PRECISION

	Repeatability			Intermediate precision (n = 18)
	Day 1 (n = 6)	Day 2 (n = 6)	Day 3 (n = 6)	
Mean	149.97	150.11	150.23	150.02
SD	0.52	1.54	1.10	0.93
RSD (%)	0.34	1.03	0.73	0.62

Assay of tablets: Devloped HPLC method is sensitive and specific for the quantitative determination of esomeprazole. Hence, it was applied for the estimation of esomeprazole magnesium in tablets. The results obtained are shown in Table-4. All analyzed batches demonstrated esomeprazole contents close to the labeled amount. The esomeprazole content in the tablet samples varied from 98.72 to 102.38 % and the RSD values varied from 0.63-1.01 %. None of the tablet ingredients interfered with the analyte peak as seen in Fig. 2.

TABLE-3
RECOVERY OF STANDARD SOLUTION ADDED TO COMMERCIALLY AVAILABLE SAMPLES

Amount added (μg/mL)	Amount founded (μg/mL)	Recovery (%)	Mean (%) (n = 5)	RSD (%)
25	24.79	99.15	99.78	0.36
	25.02	100.06		
	24.98	99.92		
	24.96	99.84		
	24.99	99.95		
50	49.73	99.12	99.59	0.35
	49.81	99.35		
	49.75	99.93		
	49.86	99.72		
	49.87	99.85		
100	99.76	99.76	99.90	0.13
	100.05	100.05		
	100.01	100.01		
	99.87	99.87		
	99.79	99.79		
150	149.34	99.50	99.56	0.07
	149.26	99.51		
	149.51	99.67		
	149.42	99.61		
	149.28	99.52		
200	199.56	99.78	99.89	0.08
	199.70	99.85		
	199.74	99.87		
	199.96	99.98		
	199.90	99.95		

TABLE-4
ASSAY OF ESOMEPRAZOLE IN COMMERCIAL TABLETS (n = 6)

Batch	A	B	C
Mean content (%)	98.72	102.38	100.25
SD	1.00	0.78	0.63
RSD (%)	1.01	0.76	0.63

Content uniformity: Results of content uniformity experiment exhibited that esomeprazole magnesium contents of 10 tablets examined were in the range of 98.2-103.6 % and the RSD value was 2.75 %. It indicates uniform distribution of drug in tablets without significant variation. According to USP pharmacopoeia the acceptance limit for drug content uniformity and the RSD is 85-115 % and less than 6 %, respectively.

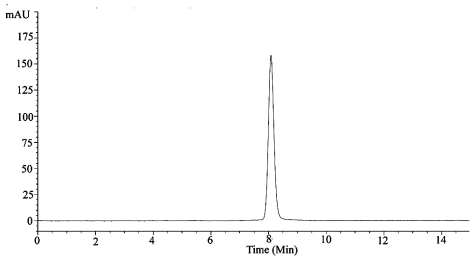

Fig. 2. Chromatogram of esomeprazole magnesium extracted from Nexium

Conclusion

The above described HPLC method applies a simple mobile phase composition and the rapid run time around 8 min and the relatively low flow rate of 0.8 mL/min allows analysis of large number of samples with high resolution and less consumption of mobile phase which has been proved to be cost-effective. Therefore, a simple, rapid and precise HPLC method has been established for routine quantitative determination of esomeprazole magnesium in tablets.

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