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Development and Validation of High Performance Liquid Chromatographic Method for The Determination of Esomeprazole in Tablets

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ABSTRACT

A simple, selective and accurate high performance liquid chromatographic (HPLC) method was developed and validated for the analysis of esomeprazole magnesium trihydrate (ES) in tablets. Chromatographic separation was achieved isocratically on a C18 column utilizing a mobile phase of acetonitrile/phosphate buffer (60:40, v/v, pH 7) at a flow rate of 1.0 mL/min with UV detection at 205 nm. Lansoprazole was used as an internal standard (IS). The calibration curve of ES was linear in the range of 100~1000 ng/mL (r = 0.9992, n = 4). The RSD values for intra- and inter-day precision were 0.66~0.86% and 0.84~1.11%, respectively. The proposed method was successfully applied to the determination of ES in tablets. The mean recovery for ES from the tablets ranged between 97.82~98.22%. ES was subjected to neutral, acid and alkali hydrolysis as well as oxidation, dry heat treatment and photodegradation. Being simple, accurate and selective, the method can be used for routine quality control analysis.

Key words: esomeprazole, HPLC, stability-indicating, validation, tablet assay

INTRODUCTION

Different methods including UV-[3], visible-[4] and derivative[5-7] spectrophotometry, differential scanning calorimetry[8], HPLC[9] and capillary electrophoresis[10] have been described for the determination of omeprazole or its sodium salt in pharmaceutical preparations. Some of these methods are stability-indicating[5,6,8,9]. Official methods in USP 27[11] and BP 2004[12] are based on HPLC analysis. In biological fluids, omeprazole was mainly determined by stereoselective HPLC techniques[13-15].

Esomeprazole magnesium trihydrate (ES), bis (5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl-1H-benzimidazole-1-yl) magnesium trihydrate (Figure 1) is the S isomer of racemic omeprazole approved in February 2001 for use as a new pharmacological entity designed to improve the clinical outcome of available proton pump inhibitors in the management of acid-related disorders[1,2]. Yet up to now, there is no method available for the determination of ES.

In the present study, we developed an HPLC method for the determination of ES in tablets. Other analytical methods[3-10] used to quantify omeprazole in pharmaceutical preparations are limited in terms of sensitivity and do not have the full stability indicating properties. Hence HPLC could be a useful tool in the analysis of the ES. Compared to other analytical techniques described in this literature, HPLC would afford advantages in terms of speed and accuracy of analysis and its chromatographic run time of 4.5 min allows the analysis of a large number of samples in a short period of time. Therefore, this HPLC-UV is validated and can be used for the rapid quantitation of ES in the presence of its degradation products.

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MATERIALS AND METHODS

I. *Materials*

Esomeprazole magnesium trihydrate (ES) and its tablets (20 mg per tablet) were generous gifts from Astra Zeneca (Istanbul, Turkey). Lansoprazole (internal standard) was obtained from Sanovel Pharmaceutical Industries (Istanbul, Turkey). All solvents and reagents were of analytical or HPLC grade. HPLC-grade water was prepared by using AquaMAX-ultra water purification system from Young Lin Inst (Korea).

II. *HPLC Instrumentation*

The analyses were performed on a Thermo Separation Products Liquid Chromatograph (TX, USA) which consisted of a P4000 solvent delivery system equipped with a Rheodyne injection valve with a 20-μL loop, an UV-3000 detector set at 205 nm and a SN-4000 automation system

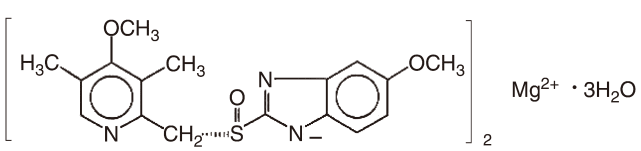

Figure 1. Structure of esomeprazole magnesium trihydrate.

software. Separations were carried out at room temperature on a Phenomenex C18 column (250 × 4.6 mm I.D., 5 μm; Therma Separation, Texas, USA), with a guard column (4 × 3 mm I.D., Phenomenex) packed with the same material. The mobile phase consists of acetonitrile/phosphate buffer (60:40, v/v, pH 7) at a flow rate of 1.0 mL/min. Before used, the mobile phase was degassed by an ultrasonic bath and filtered by a Millipore vacuum filter system equipped with a 0.45 mm HV filter.

III. *Preparation of Stock and Standard Solutions*

Stock solutions of esomeprazole magnesium trihydrate (ES) (1 mg/mL, calculated as free base) and internal standard (1 mg/mL) were prepared in methanol and diluted further with the mobile phase to obtain standard solutions of 10 μg/mL.

IV. *Linearity*

By appropriate dilution of the ES standard solution with the mobile phase, ten working solutions ranging between 100 and 1000 ng/mL were prepared. The concentration of IS in the samples was 800 ng/mL. The solutions (20 μL) were injected and chromatographed (n = 4) according to the chromatographic conditions previously given. For ES quantitation, the chromatographic signals were evaluated on the basis of peak area ratios of ES to IS.

V. *Precision*

The intra-day and inter-day precision were determined by analyzing the samples of ES at concentrations of 200, 600 and 1000 ng/mL. Determinations were performed with five replicates on the same day as well as on four separated days.

VI. *Assay Procedure for Tablets*

Twenty tablets were individually weighed to get the average weight of the tablets. A sample of the powdered tablets, claimed to contain 100 mg of ES was transferred to 100-mL calibrated flask. About 75 mL of methanol was added and then extraction was performed mechanically for 20 min followed by sonication for 20 more min. The volume was brought to 100 mL with methanol. The content was centrifuged for 10 min at 3000 ×g, and then a 0.1-mL aliquot of the supernatant was diluted to 10 mL with the mobile phase. One milliliter of this solution and 0.8 mL of IS standard solution were transferred into a 10-mL calibrated flask and diluted to the volume with the mobile phase. A 20 μL of its aliquot was injected and chromatographed (n = 5).

VII. *Accuracy/Recovery Studies*

Recovery studies were carried out with the assay samples to which known amounts of ES corresponding to 50 and 150% of label claim were added. The mixtures were then analyzed by the proposed method. The experiments were conducted five times.

VIII. *Robustness*

Assay procedure was repeated using columns from two different manufacturers and solvent (acetonitrile) of two different lots. Besides, separation studies were also performed by two different analysts.

IX. *Forced Degradation of Standard ES*

A stock solution prepared as 1 mg/mL ES (calculated as base) in methanol was used for forced degradation studies.

(I) *Hydrolysis*

Individually, 5 mL of the standard solution was transferred to a 10-mL distillation flask and boiled for 1 hr at 80°C after adding: (a) 5 mL of water for neutral hydrolysis (b) 5 mL of 1 N HCl for acid hydrolysis (c) 5 mL of 1 N NaOH for basic hydrolysis. Before the analysis, (b) and (c) solutions were neutralized.

(II) *Chemical Oxidation*

To 5 mL of the standard solution, 100 μL of 30% H_2O_2 solution (v/v) were added and mixed. The solution was left at room temperature for 1 hr in the dark.

(III) *Photochemical Degradation*

The photochemical stability of the ES was studied by exposing the methanolic stock solution to direct sunlight for 8 hr (from 9 AM to 5 PM at ≅ 20°C).

(IV) *Thermal Stress*

Bulk drug was subjected to dry heat at 105°C for 5 hr.

To each of the stressed solutions, IS was added and then it was diluted with the mobile phase to obtain a theoretical concentration of 1000 ng/mL for ES. The resulting concentration of IS was 800 ng/mL. Each solution was analyzed in duplicate.

RESULTS AND DISCUSSION

I. *Development of the HPLC Method*

In order to separate ES, internal standard and degradation products produced under stressed conditions, aqueous buffer-acetonitrile mixtures were used as the mobile phase. Satisfactory resolution was obtained using the mobile phase system of acetonitrile/phosphate buffer (60:40, v/v, pH 7) at a flow rate of 1 mL/min. As ES

showed maximum absorption at 205 nm (Figure 2), the detector was set at 205 nm. Under these conditions, ES was resolved from the IS with the retention times of 3.64 ± 0.07 and 4.31 ± 0.09 min, respectively. In Figure 3, a typical chromatogram obtained under these conditions is shown. Chromatograms of stressed reaction solutions are given in Figures 4 and 6. They indicate that the developed method was successful to separate the drug and its chromophoric degradation products.

II. *Validation of the Developed Method*

The calibration curve was prepared by plotting the peak area ratios of ES to IS against drug concentration and was linear in the range of 100~1000 ng/mL. The data were subjected to least-squares linear regression analysis to calculate the calibration equation and correlation coefficients. The regression equation was found as A = 0.0013C – 0.021 (r = 0.9992, n = 4) (A = aC + b where A is the peak area ratio of ES to the IS, a is the slope, b is the intercept and C is the concentration of the measured solution in ng/mL). The results show that there is an excellent correlation between the peak area ratios and the concentrations of ES in the range tested.

The limit of detection, with a signal to noise ratio of 3:1, was found to be 10 ng/mL. The limit of quantitation was 100 ng with a coefficient of variation 1.40% (n = 4).

The intra-day (n = 5) and inter-day (n = 5, four different days) reproducibilities expressed as relative standard deviation (RSD) were found to be 0.66~0.86% and 0.84~1.11%, respectively, indicating good precision (Table 1). The relative error (R.E.) below 1.55% revealed satisfactory accuracy for the method.

To evaluate the robustness of the method, two analytical columns, one (Phenomenex C18 column and the

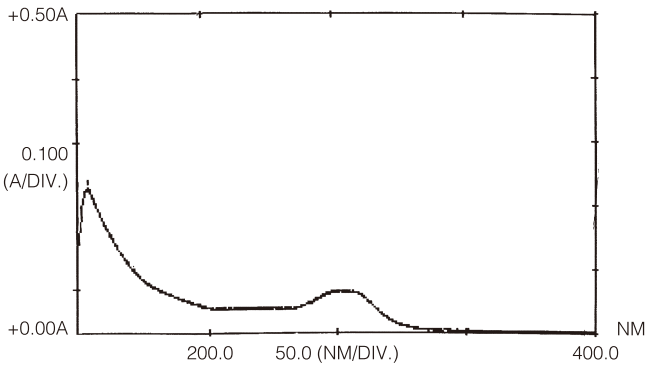

Figure 2. Absorption spectrum of ES in mobile phase.

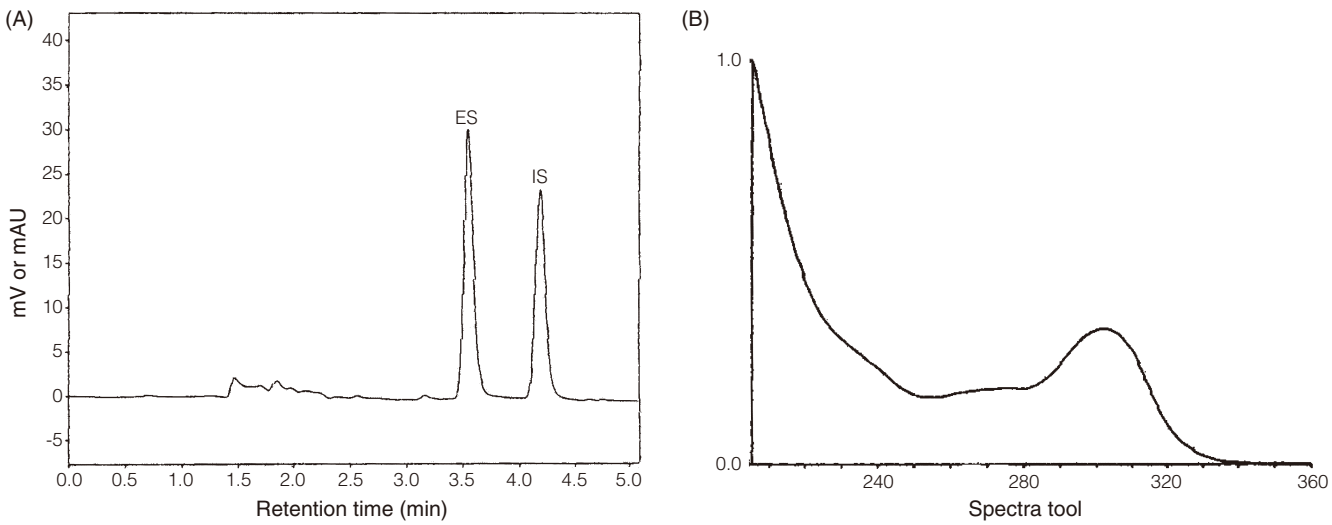

Figure 3. (A) A typical chromatogram of 1000 ng/mL ES standard and 800 ng/mL IS, 20 mL injection, (B) UV spectrum of ES standard.

Table 1. Intra-day and inter-day precision and accuracy of ES

Actual concentration (ng/mL)	Intra-day (n = 5)			Inter-day[a] (n = 5)		
	Found concentration (ng/mL)	RSD (%)	R.E.[b] (%)	Found concentration (ng/mL)	RSD (%)	R.E. (%)
200	200.31	0.80	0.16	198.77	1.11	0.61
600	601.54	0.66	0.26	594.11	0.84	0.98
1000	984.46	0.86	1.55	984.99	0.95	1.50

[a]Results of four different days.
[b]Relative error.

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other Shim-Pack CLS-ODS analytical column were used. The assay results (mean ± RSD) found as 101.03 ± 0.67% and 101.41 ± 1.97%, respectively, indicated that different columns do not lead to significantly different results. Two different lots of acetonitrile from the same manufacturer or two different manufacturers of acetonitrile were also tested for the robustness and only insignificant differences in peak areas and in retention time were observed (99.62 ± 0.55% and 99.71 ± 1.23% for the two lots as well as, 98.37 ± 0.41% and 98.65 ± 1.10% for the two manufacturers). The proposed method was carried out by two analysts and no considerable difference was observed (99.66 ± 0.75% vs. 99.81 ± 0.41%). The RSD values of less than 2.0% for peak areas and retention times indicated that the developed method was capable of producing results with high precision.

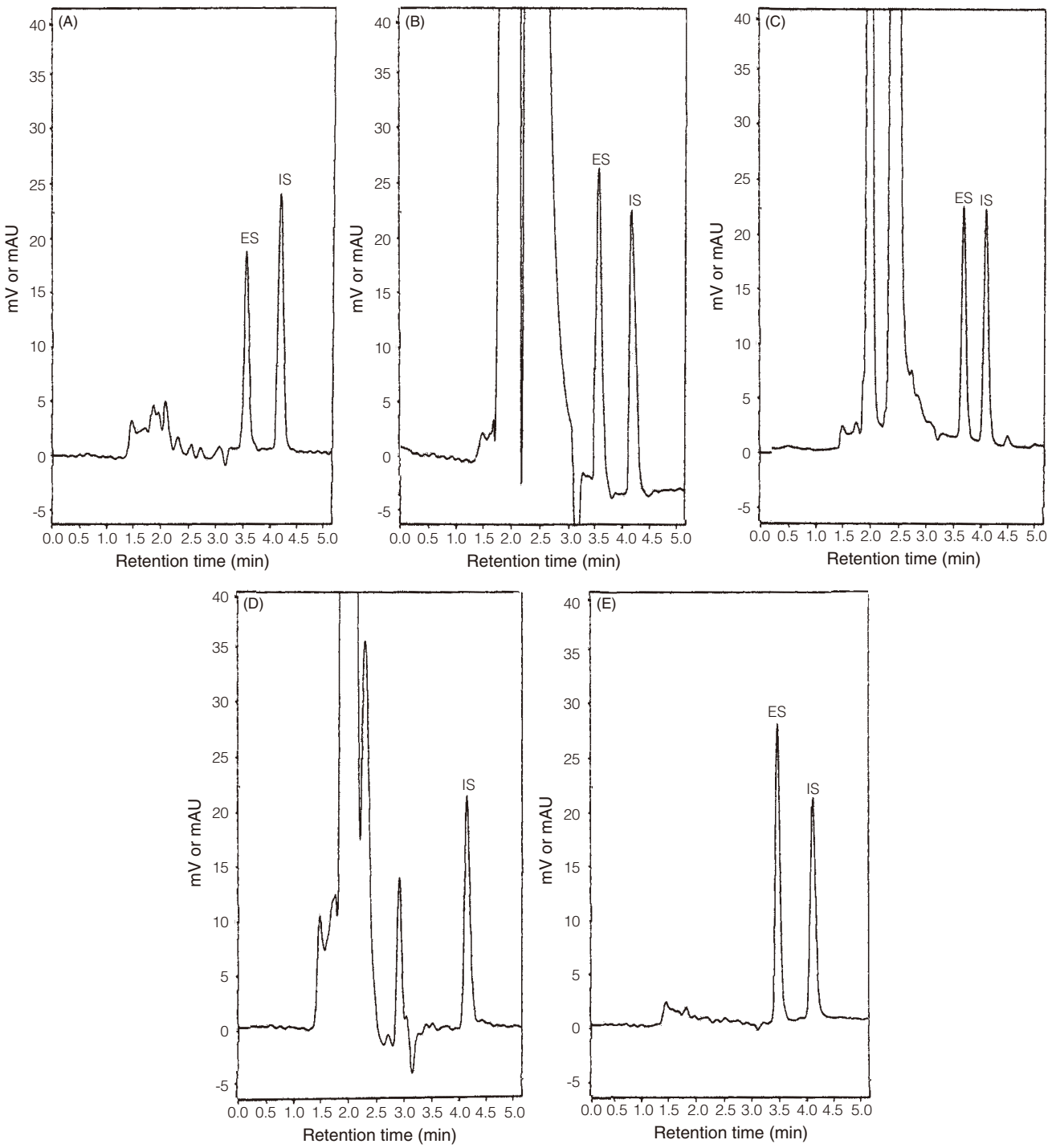

Figure 4. Chromatograms corresponding to ES solution subjected to (A) neutral and (B) alkaline hydrolysis, (C) chemical oxidation, (D) acid hyrolysis and (E) thermal stress.

III. *Assay Procedure for Tablets*

The proposed method was applied to the analysis of marketed product (Nexium tablet) and the results obtained are given in Table 2. The blank solution was prepared containing the components indicated in tablets except the active principle. No interference was observed from the tablet excipients.

To examine the accuracy of the method, recovery studies were carried out by standard addition method. The percent recovery of the added standard to the assay samples was calculated from:

Recovery % = $[(C_t - C_u) / C_a] \times 100$

where C_t is the total concentration of the analyte found; C_u is the concentration of the analyte present in the formulation; and C_a is the concentration of the pure analyte added to the formulation. The results are shown in Table 3. The average percent recoveries obtained as 97.82~98.22% indicate good accuracy of the method.

IV. *Selectivity*

Stability-indicating methods have received considerable attention for the determination of a vast number of drugs[16-20]. The International Conference on Harmonization (ICH) guideline entitled "Stability Testing of New Drug Substances and Products" requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substances[21]. Susceptibility to oxidation is one of the required tests. The hydrolytic and photolytic stabilities are also required. An ideal stability-indicating method is one that quantifies the drug per se and also resolves its degradation products.

In order to check the proposed method for selectivity, different degradation pathways for ES were performed, due to that its degradation products were not available. This study was carried out by employing the following tests: hydrolysis (neutral, acidic and basic), chemical oxidation, photolysis and thermolysis.

When the ES solution was exposed to neutral and basic hydrolysis, and chemical oxidation with H_2O_2, the chromatographic peaks corresponding to the parent drug reduced indicating that the compound was decomposed about 40%, 15% and 25%, respectively (Figure 4 A~C). The peak purity of the parent drug was checked by its UV spectrum (Figure 5). On the other hand, after the acidic hydrolysis, the peak corresponding to the parent drug substantially disappeared (Figure 4 D). Degradation products could not be distinguished because the peaks observed with retention time between 1~3 min were also present in the chromatograms of the blank studies. As can be seen from Figure 4 (D), these peaks did not interfere with the signal corresponding to the parent drug, which has a retention time of 3.64 min.

No decomposition was observed when the ES as powder was subjected to dry heat at 105°C for 5 hr

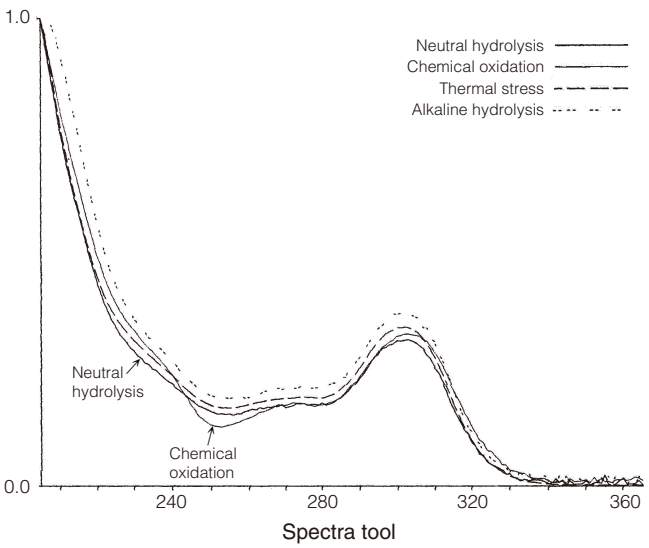

Figure 5. The UV spectrum of the chromatographic peak corresponding to the parent drug after subjected to neutral, alkaline hydrolysis, chemical oxidation and thermal stress.

Table 2. Analysis of ES in tablets

Formulation	Label claim (mg/per tablet)	Amount found ± S.D.[b] (mg, n = 5)	Recovery (%)	RSD (%)
Nexium[a]	20	19.81 ± 0.17	99.05	0.86

[a]Marketed by Astra Zeneca.
[b]Standard deviation.

Table 3. Results of recovery studies by standard addition method

Amount of drug in tablet (mg)[a]	Amount of pure drug added (mg)	Total found (mg)[b] (mean ± S.D.[c])	RSD (%)	Recovery of pure drug added (%)
19.81	10	29.63 ± 0.3	1.02	98.22
19.81	50	68.72 ± 0.6	0.92	97.82

[a]Nexium tablet (20 mg).
[b]Five independent analyses.
[c]Standard deviation

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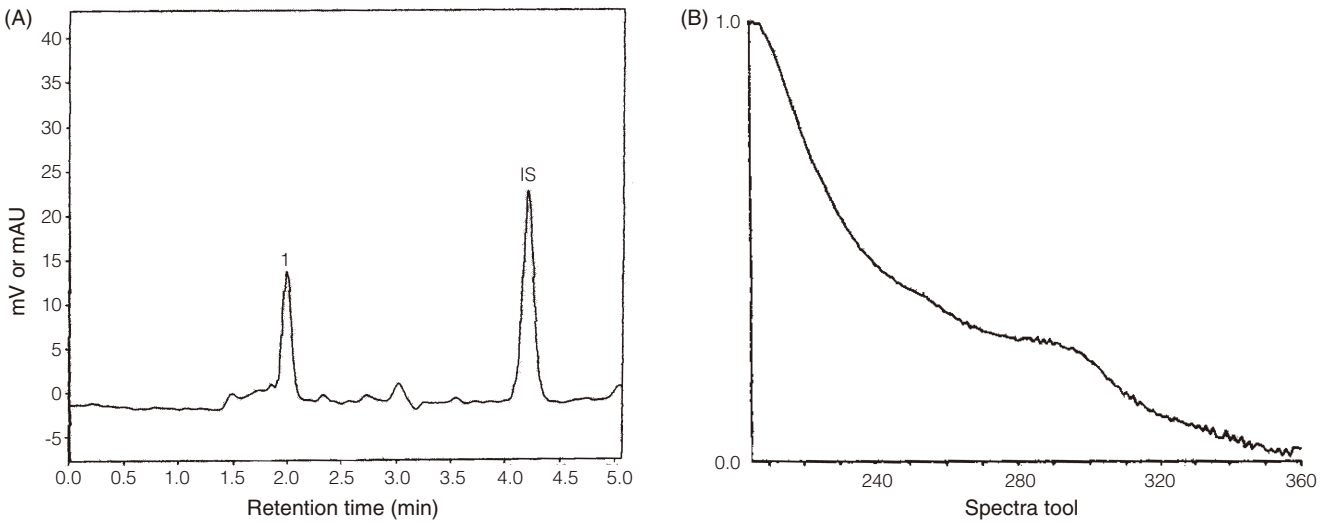

Figure 6. (A) Chromatogram of ES exposed to sunlight for 8 hr, (B) UV spectrum of the degradation product 1 (R_t = 1.98).

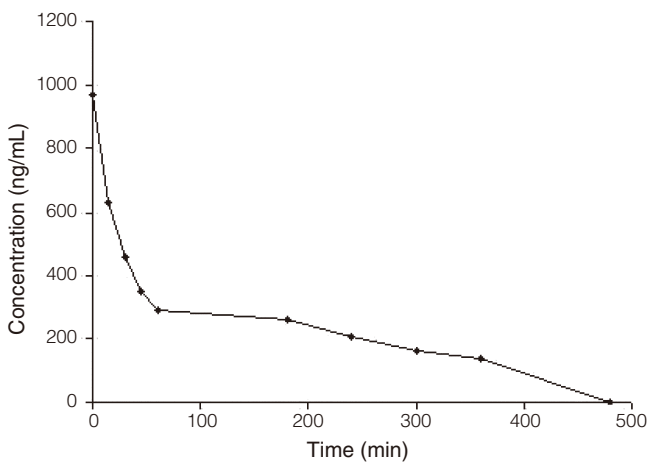

Figure 7. Effect of sunlight on the concentration of ES during 8 exposure.

Table 4. Degradation trial for ES

Condition	Time (hr)	Recovery (%)
Acid, 1 N HCl, 80°C	1	Not detected
Base, 1 N NaOH, 80°C	1	85.08
Neutral hydrolysis, 80°C	1	58.42
H_2O_2, 30%, at room temperature	1	74.23
Dry heat 105°C	5	99.42
Daylight 20°C	8	Not detected

indicating that heating had no effect on the ES (Figure 4 E).

When the ES solution was exposed to the sunlight, almost complete degradation of the drug was observed and a new signal appeared in the chromatogram. As can be seen from Figure 6, resolution of ES from its photodegradation product could be achieved. Effect of sunlight on the concentration of ES during 8 hr is shown in Figure 7. This test showed that ES in methanol solution is very sensitive to sunlight exposure.

Recovery data of the degradation tests for ES are given in Table 4.

In conclusion, forced degradation studies under described conditions showed that ES solution remained stable under thermal stress but degraded partially with neutral and basic hydrolysis, and chemical oxidation, and totally, with acid hydrolysis and exposure to sunlight. These results are in accordance with the ones obtained with omeprazole and omeprazole sodium[8,9]. The proposed method can be used as a stability-indicating one because the peak of the parent drug, ES, is not interfered by any other signal in the chromatogram. The method has one disadvantage of not being stereo-selective.

CONCLUSIONS

This is the first report to describe a simple and stability-indicating HPLC method for the analysis of esomeprazole magnesium trihydrate in tablets. Acquired validation parameters indicate that the proposed method is selective, precise, accurate, robust and hence suitable for routine analysis of ES in tablets.

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