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Development and validation of HPLC method for simultaneous determination of omeprazole and domperidone

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ABSTRACT

A simple selective high performance liquid chromatographic (HPLC) method was developed and validation for the analysis of omprazole magnesium trihydrate (ES) in tablets. ES was subjected to neutral, acid and alkali hydrolysis as well as oxidation, dry heat treatment and photodegration. Being simple, accurate and selective, the method can be used for routine quality analysis.

Keywords: Omeprazole, domeperidone, HPLC, validation, haloperidol, stability-indicating.

INTRODUCTION

Omeprazole is a proton pump inhibitor used in the treatment of dyspepsia, peptic ulcer disease, gastro esophageal reflux disease, laryngopharyngeal reflux and syndrome. In peptic ulcers, it suppresses gastric acid secretion by specific inhibition of the H^+/K^+-ATP as in the gastric parietal cell. By acting specifically on the proton pump, omeprazole blocks the final step in acid production, thus reducing gastric acidity. It is an official drug in IP and BP. Few bioanalytical methods by HPLC using human plasma and also spectrophotometric methods using pharmaceutical dosage forms have been reported for the estimation of Omeprazole.

Domperidone is an Antiemetic drug used to treat nausea and vomiting and to stimulate lactation in women. It is used in the inhibition of receptive relaxation, causes enhancement of coordinated antral-duodenal motility and results acceleration of transit in the small intestine. It is an official drug in IP and BP. There are very few analytical methods reported for the estimation of Domperidone which includes RP-HPLC, Spectrophotometer.

The combination of Omeprazole and Domperidone is very useful in the treatment of gastric related disorders. On literature survey, it was found that no UV method have been reported for

the simultaneous estimation of Omeprazole and Domperidone in combined dosage forms and no method is available in the pharmacopoeias. In view of the need for suitable methods for routine analysis in combined formulations, attempts are being made to develop simple, precise and accurate analytical methods for simultaneous estimation of titled drugs and extend it for their determination in formulations. As chromatographic methods of analysis is a pre-requisite for the marketing of most of the formulations, one HPLC and HPTLC along with Spectrophotometric methods such as simultaneous equation method (Vierodt's method), First order derivative method, Area under the curve method and Q-ratio method are planned to develop and validate for the simultaneous estimation of titled drugs, using water as a solvent.

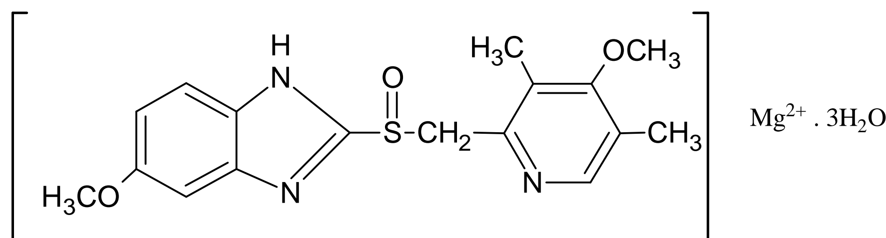
MATERIALS AND METHODS

I. Materials

Omeprazole sodium trihydrate (ES) and its tablets (20 mg per tablet) were generous gifts from Astra Zeneca. Lansoprazole (internal standard) was obtained from Sanovel Pharmaceutical Industries. All solvents and reagents were of analytical or HPLC grade. HPLC-grade water was prepared by using Aqua MAX-ultra water purification system.

II. HPLC Instrumentation

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



III. Preparation of Stock and Standard Solutions.

Stock solutions of omeprazole 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 of10 μg/mL. Linearity By appropriate dilution of the ES standard solution with the mobile phase, ten working solutions ranging between100 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 quantization, the chromatographic signals were evaluated on the basis of peak area ratios of ES to IS. 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 20µL 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 to50 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.

(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 for8 hr.

(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. ES as resolved from the IS with the retention times of 3.64 ± 0.07and 4.31 ± 0.09 min, respectively.

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 = a C + b where A is the peak area ratio of ES to the IS, 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 quantization was100 ng with a coefficient of variation 1.40% (n = 4).

The intra-day (n = 5) and inter-day (n = 5, four different days) reproducibility's expressed as relative standard deviation (RSD) were found to be 0.66~0.86%and 0.84~1.11%, respectively, indicating good precision. The relative error (R.E.) below 1.55% revealed satisfactory accuracy for the method. To evaluate the robustness of the method, two analytical columns,

Detector	Internal standard	Column	Mobilephase	Exctraction	LOQ/LOD
HPLC-UV	Diphenylamine 250nm	C18 Nucleosil 250 x 4 mm	Methanol-0.2M	LLE	5ng/ml,LOD

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. 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.

The peak purity of the parent drug was checked by its UV spectrum. On the other hand, after the acidic hydrolysis, the peak corresponding to the parent drug substantially disappeared. 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, 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

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