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Research Article

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Aspirin and Esomeprazole Magnesium in Tablet Dosage Form

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A simple, specific, precise, and accurate reversed-phase HPLC method was developed and validated for simultaneous estimation of aspirin and esomeprazole magnesium in tablet dosage forms. The separation was achieved by HyperChrom ODS-BP C_{18} column (200 mm × 4.6 mm; 5.0 μm) using acetonitrile: methanol: 0.05 M phosphate buffer at pH 3 adjusted with orthophosphoric acid (25 : 25 : 50, v/v) as eluent, at a flow rate of 1 mL/min. Detection was carried out at wavelength 230 nm. The retention times of aspirin and esomeprazole magnesium were 4.29 min and 6.09 min, respectively. The linearity was established over the concentration ranges of 10–70 μg/mL and 10–30 μg/mL with correlation coefficients (r^2) 0.9986 and 0.9973 for aspirin and esomeprazole magnesium, respectively. The mean recoveries were found to be in the ranges of 99.80–100.57% and 99.70–100.83% for aspirin and esomeprazole magnesium, respectively. The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of aspirin and esomeprazole magnesium in their combined tablet dosage form.

1. Introduction

Aspirin (ASP) is chemically 2-(acetyloxy)-benzoic acid (Figure 1). It is nonselective cyclooxygenase inhibitor used as an antipyretic, analgesic, anti-inflammatory, and antithrombotic agent. Esomeprazole magnesium (ESO) is S-isomer of omeprazole and proton pump inhibitor. It is magnesium, bis [5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazolato] (Figure 2). It is used in treatment of peptic ulcer disease, NSAIDS-associated ulceration and Zollinger-Ellison syndrome, used as antiulcerative. ASP and ESO in combined dosage form are used in cardiovascular disorder and cerebrovascular disorders [1–3].

The review of literature revealed that various analytical methods involving spectrophotometry [4–6], HPLC [7–10] and HPTLC [11] have been reported for ASP in single form and in combination with other drugs. Several analytical methods have been reported for ESO in single form and in combination with other drugs including spectrophotometry [12, 13], HPLC [14, 15], and HPTLC [16].

The present work describes the development of a simple, precise, accurate, and reproducible HPLC method for the simultaneous estimation of ASP and ESO in combined dosage form. The developed method was validated in accordance with ICH Guidelines [17] and successfully employed for the assay of ASP and ESO combine dosage form.

2. Experimental Condition

2.1. Materials and Reagents. Analytically pure ASP and ESO were kindly provided by Baroque Pharmaceuticals, Khambhat, Gujarat, India and Osaka Pharmaceuticals, Sakarda, Vadodara, Gujarat, India, respectively, as gift samples. Analytical grade methanol, chloroform, acetonitrile, glacial acetic acid, and ethyl acetate were purchased from SD Fines Chemicals, Bombay, India. Tablet of ASP and ESO in combined dosage form, AXANUM, with a 20 mg ESO and 81 mg ASP label claim, manufactured by Astrazeneca Pharmaceuticals.

2.2. Instrumentation. An isocratic HPLC system (Analytical Technologies Limited) consisted of P2230 plus HPLC

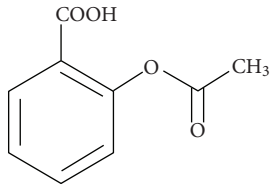

Figure 1: Structure of aspirin.

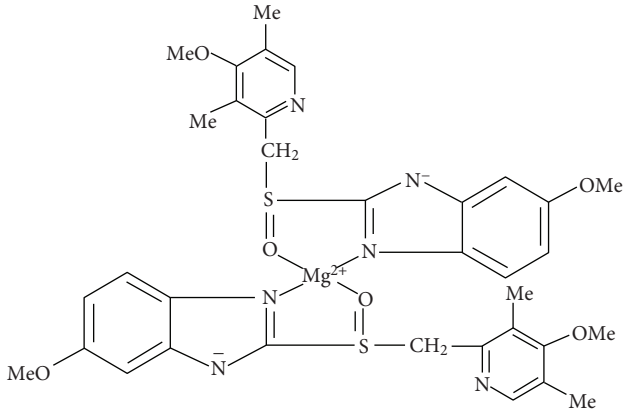

Figure 2: Structure of esomeprazole magnesium.

pump, variable wavelength programmable UV 2230 plus detector system, Rhenodyne valve with 20 μL fixed loop and Analchrom 2006 as operating software. The chromatographic column used was HyperChrom ODS-BP C_{18} column (200 mm × 4.6 mm i.d, particle size 5 μm). Analytical balance K-EA 210 (K-Roy Instrument Pvt. Ltd) was used for weighing purpose.

2.3. Chromatographic Condition. A mixture of Acetonitrile : Methanol : 0.05 M Phosphate Buffer at pH 3 adjusted with orthophosphoric acid (25 : 25 : 50 v/v) was used as mobile phase and was filtered through 0.45 μ membrane filter prior to use. The flow rate of mobile phase was maintained at 1 mL/min. Detection was carried out at 230 nm at the ambient temperature. The total run time 10 min was used with injection volume of 20 μL.

2.4. Preparation of Mobile Phase and Standard Stock Solutions. Accurately weighed potassium dihydrogen phosphate (3.4 g) was dissolved in 500 mL of water. This solution was mixed with 250 mL of acetonitrile and 250 mL of methanol. Finally the pH was adjusted to 3.0 with orthophosphoric acid. The solution was sonicated for 10 minutes and filtered through 0.45 μ membrane filter. 100 mg of standard ASP and ESO were accurately weighed and transferred separately to a 100 mL volumetric flask and dissolved in 50 mL mobile phase. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 1000 μg/mL ASP and ESO, respectively. Appropriate volume of aliquot from ASP and ESO standard stock solution was further diluted with mobile phase to obtain final concentration of 100 μg/mL and 100 μg/mL, respectively.

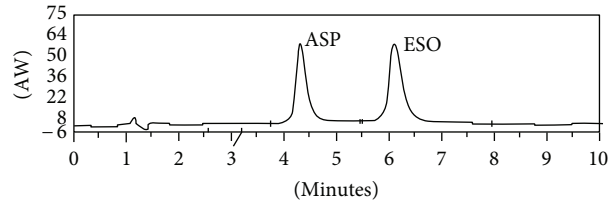

Figure 3: Chromatogram of mixed standard solution containing 10 μg/mL ESO and 10 μg/mL ASP using mobile phase acetonitrile : methanol : 0.05 M phosphate buffer at pH 3 adjusted with orthophosphoric acid (25 : 25 : 50 v/v).

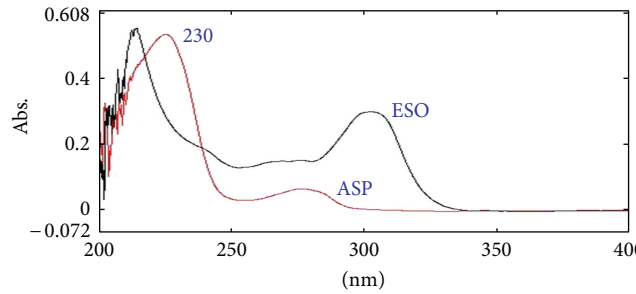

Figure 4: Overlain spectrum of 10 μg/mL ASP and 5 μg/mL ESO in methanol.

Table 1: Summary of validation parameters.

Parameters	ASP	ESO
Recovery %	99.80–100.57	99.70–100.83
Repeatability (C.V., n = 6)	0.205123	0.537209
Precision (% RSD)		
Intra-day (n = 3)	0.14–0.38	0.22–0.49
Inter-day (n = 3)	0.38–0.83	0.22–0.86
Limit of detection (μg/mL)	3.25067	2.09632
Limit of quantitation (μg/mL)	9.85051	6.35250
Specificity	Specific	Specific
Robustness	Robust	Robust
Solvent stability	Solvent stable for 24 hrs.	Solvent stable for 24 hrs.

Table 2: Calibration data for ASP and ESO.

Parameter	ASP	ESO
Linear range (μg/mL)	10–70	10–30
Slope	89.00785	93.47274
Intercept	−237.786	23.3054
Standard deviation of slope	1.93647	2.79913
Standard deviation of intercept	87.67731	59.37857

Table 3: Robustness study.

Condition varied	Changed condition	Area (n = 3)		% Assay	
		ESO ± S.D.	ASP ± S.D.	ESO	ASP
Change in mobile phase ratio (v/v)	28 : 22 : 50	1299.76 ± 4.90	4797.53 ± 12.33	98.01	99.91
	22 : 28 : 50	1310.43 ± 5.60	4782.56 ± 15.12	100.82	99.68

Table 4: System suitability test parameter.

System suitability parameters	Proposed Method	
	ESO	ASP
Retention times (R_T) (min) ± S.D.	6.088 ± 0.10740	4.286 ± 0.03983
Theoretical plates (N) ± S.D.	3063.4 ± 25.8438	2535.4 ± 5.8242
Resolution (R_S)	4.62	
Tailing factor (A_S) ± S.D.	1.364 ± 0.07197	1.412 ± 0.05167
Capacity factor	5.088	3.286

2.5. Determination of ESO and ASP from Combined Dosage Form

2.5.1. Sample Preparation. A powder quantity equivalent to 40 mg ESO and 162 mg ASP was accurately weighed and transferred to volumetric flask of 100 mL capacity. 60 mL of solvent (acetonitrile : methanol : water (25 : 25 : 50)) was transferred to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through membrane filter (0.45 μ). From this solution 3.5 mL was transferred to volumetric flask of 100 mL capacity. Volume was made up to the mark to give a solution containing 14 μg/mL of ESO and 56.7 μg/mL of ASP. The resulting solution was analyzed by proposed method. The prepared sample solution was chromatographed for 10 minutes using mobile phase at a flow rate of 1.0 mL/min. From the peak area obtained in the chromatogram, the amounts of both the drugs were calculated.

3. Method Validation

The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness, and system suitability. The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision (% RSD) was expressed with respect to the repeatability, intraday, and interday variation in the expected drug concentrations. After validation, the developed methods have been applied to pharmaceutical dosage form.

3.1. Specificity. Commonly used excipients (starch, microcrystalline cellulose, and magnesium stearate) were spiked into a preweighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

3.2. Linearity. Appropriate volume of aliquot from ASP and ESO standard stock solution was transferred to same volumetric flask of 10 mL capacity. The volume was adjusted to the mark with mobile phase to give a solution containing ASP (10, 25, 40, 55, and 70 μg/mL) and ESO (10, 15, 20, 25, and 30 μg/mL). The mixed standard solution was chromatographed using above chromatoghraphic condition (n = 6). All solutions were filtered through 0.45 μm filter prior to use. Calibration curves were constructed by plotting average peak area versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

3.3. Accuracy. Accuracy was assessed by determination of the recovery of the method by addition of standard drug to preanalyzed test sample preparation at 3 different concentration levels 80, 100, and 120%, taking into consideration percentage purity of added bulk drug samples. Each concentration was chromatographed 3 times and average recoveries were measured.

3.4. Precision. The repeatability was evaluated by assaying 6 times of test samples prepared for assay determination. The intraday and interday precision study of ASP and ESO was carried out by estimating different concentrations of ASP and ESO, 3 times on the same day and on 3 different days and the results are reported in terms of % RSD.

3.5. Detection Limit and Quantitation Limit. ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio, and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the $3.3\sigma/s$ and $10\sigma/s$ criterions, respectively, where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

3.6. Robustness. The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. For the proposed method it was done by changing the mobile phase composition (acetonitrile : methanol : buffer, 28 : 22 : 50 and 22 : 28 : 50, v/v by observing the stability of the drugs for 24 hr in the mobile phase.

Table 5: Assay results of marketed formulation.

Formulation	Actual concentration μg/mL		Amount obtained μg/mL		% ESO	% ASP
	ESO	ASP	ESO	ASP		
Tablet	14	56.7	13.92	56.55	99.42	99.73

3.7. System Suitability. The suitability of the chromatographic system was tested before each stage of validation. Five replicate injections of standard preparation were injected and resolution, asymmetry, number of theoretical plates, and relative standard deviation of peak area were determined.

3.8. Determination of ASP and ESO from Combined Dosage Form. Sample solution was injected 6 times at above chromatographic conditions. An average peak area was measured from chromatograms. The quantitation was carried out by keeping these values to the straight line equation of calibration curve.

4. Results and Discussion

Optimizations of chromatographic conditions were performed to obtain the best resolution and peak parameter (asymmetry, theoretical plates). For the selection of mobile phase initially methanol-water and acetonitrile-water have been tried in different ratio which gave poor peak shape. Then acetonitrile : methanol : 0.050 M potassium dihydrogen phosphate buffer adjusted to pH 3.0 in different ratio have been tried. Finally, acetonitrile : methanol : 0.05 M phosphate buffer at pH 3 adjusted with orthophosphoric Acid (25 : 25 : 50 v/v) was found to be satisfactory and gave two symmetrical peaks with good resolution (4.62) for ASP and ESO at flow rate of 1 mL/min. The average retention times for ASP and ESO were 4.286 ± 0.03983 and 6.088 ± 0.10740 minutes, respectively. The asymmetric factors for ASP and ESO were 1.412 ± 0.05167 and 1.364 ± 0.07197, respectively. For the selection of detection wavelength overlain UV spectrum of ASP and ESO was taken which revealed that at 230 nm both the drugs possess significant absorbance (Tables 3 and 4; Figures 3 and 4).

Summary of validation parameters for proposed method was given in Table 1.

The developed HPLC method was validated. The linear range, correlation coefficient, detection limit and standard deviation for ASP and ESO by HPLC method are shown in Table 2. Accuracy was determined by calculating the recovery. The method was found to be accurate with % recovery 99.80–100.57% for ASP and 99.70–100.83% for ESO respectively.

Precision was calculated as repeatability and intra and interday variation for both the drugs. The method was precise with % RSD 0.14–0.38 for intraday (n = 3) and % RSD 0.38–0.83 for interday (n = 3) for ASP and 0.22–0.49 for intraday (n = 3) and % RSD 0.22–0.86 for interday (n = 3) for ESO, respectively.

The method was specific as no interference observed when the drugs were estimated in presence of excipients. The method was also robust as there was no change in area up to 24 hours of preparation of solution in acetonitrile : methanol : 0.05 M phosphate buffer at pH 3 adjusted with orthophosphoric acid (25 : 25 : 50 v/v). The LOD for ASP and ESO was found to be 3.25 μg/mL and 2.09 μL/mL, respectively. Summary of validation parameters is tabulated in Table 1. Marketed formulation was analyzed by the proposed method and assay result of marketed formulation was shown in Table 5.

5. Conclusion

The proposed HPLC method provide simple, specific, precise, accurate, and reproducible quantitative analysis for simultaneous analysis of ASP and ESO in combined dosage form. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness, and reproducibility. The proposed method can be used for routine analysis and quality control assay of ESO and ASP in combined dosage form.

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