Original article

Simultaneous determination of aspirin and esomeprazole magnesium in combined tablets by validated UPLC method

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ABSTRACT

Aim and background: A stability-indicating ultra performance liquid chromatography (UPLC) method has been developed and validated for the simultaneous determination of aspirin and esomeprazole magnesium in pharmaceutical preparations.
Materials and methods: An Agilent Zorbax XDB column (50 × 4.6 mm i.d., 1.8 μm particle size) was used. The mobile phase consisted of a mixture of 0.2% orthophosphoric acid, methanol, and acetonitrile in simple gradient elution. Ultraviolet (UV) detection was performed at 210 nm. Total run time was 6 min; the two drugs were eluted at the retention times of 2.4 and 2.8 min for esomeprazole and aspirin, respectively.
Results: The linearity for both the drugs was found in the range of 32–98 μg/ml for aspirin and 4–12 μg/ml for esomeprazole magnesium. The percentage recoveries of aspirin and esomeprazole magnesium were found to be 99.1–100.5 and 99.2–100.1, respectively. The method distinctly separated the drug and degradation products even in actual samples.
Conclusion: The method was validated in terms of linearity, range, specificity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ).

1. Introduction

Aspirin (acetylsalicylic acid; AS) [Fig. 1a] occurs as white crystals, commonly tabular or needle-like, or white, crystalline powder. It is odorless or has a faint odor. Its chemical name is 2-(acetyloxy) benzoic acid.

Esomeprazole magnesium (ES) [Fig. 1b] is a proton pump inhibitor. Chemically it is bis(5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl]sulfinyl]-lH-benzimidazole-1-yl) magnesium trihydrate. Esomeprazole is the S-isomer of omeprazole. The empirical formula is $(C_{17}H_{18}N_3O_3S)_2$ Mg $\times$ $3H_2O$, representing a molecular weight of 767.2 as a trihydrate and 713.1 on an anhydrous basis. It is available in 81/20 mg of AS and ES, respectively.

So far, some liquid chromatography procedures have been described for the determination of AS and ES.[1–18] But these procedures were developed to estimate either AS or ES individually from formulation or plasma, whereas no single method has been reported for their simultaneous estimation from formulation. Hence, it is necessary to develop a rapid, accurate, and validated method for the simultaneous determination of AS and ES from combined dosage form.

2. Materials and methods

2.1. Instrumentation and chromatographic conditions

The Waters UPLC Acquity system we used consists of a binary solvent manager, a sample manager, and a UV detector. Zorbax XDB C_{18} column, 50 mm × 4.6 mm i.d. with 1.8 μm particles, was used as stationary phase. 0.2% orthophosphoric acid as solvent A and acetonitrile and methanol in the ratio (50:50 v/v) as solvent B were used for mobile phase. The mobile phase was prepared and degassed. Mobile phase was pumped at 0.7 ml/min. The eluants were monitored at 210 nm. The injection volume for samples and standards was 2 μl. Acetonitrile and 0.1 N sodium hydroxide in the ratio 50:50 v/v was used as diluent.

2.2. Reagents

Standards were supplied by Hospira Healthcare (P) Ltd., India. HPLC grade acetonitrile and analytical grade orthophosphoric acid

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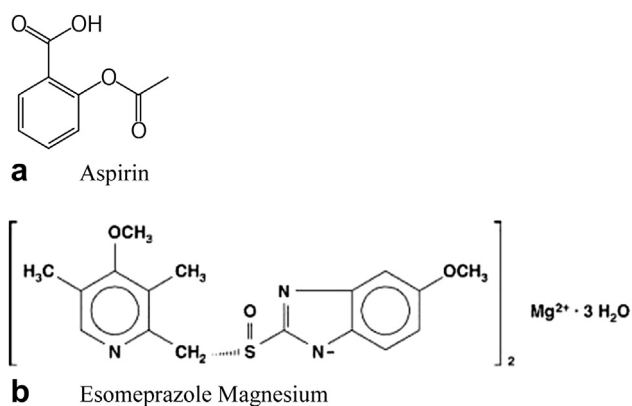E-mail address: Shravankumar.Malisetty@hospira.com (S.K. Malisetty).

Fig. 1. Chemical structures of aspirin and esomeprazole magnesium.

were purchased from Merck (Mumbai, India). Water was prepared by Millipore Milli Q Plus water purification system. Commercial pharmaceutical preparations of combined tablets were purchased from the market. The declared content of tablets was AS 81 mg and ES 20 mg per tablet.

2.3. Preparation of standard solutions

A standard solution containing 65 μg/ml of AS and 8 μg/ml of ES was prepared by dissolving appropriate amount of AS and ES in diluent. All the solutions were covered with aluminum foil to prevent photolytic reaction until the time of analysis.

2.4. Sample preparation

Ten tablets, each containing 81 mg of AS and 20 mg of ES, were dissolved in 500 ml diluents to get 162 μg/ml of AS and 40 μg/ml of ES. Five milliliters of the above solution was diluted to 20 ml to get 40 μg/ml of AS and 10 μg/ml of ES. The solution was filtered through 0.45 μm Millipore PVDF filter. Then, 2 μl of these solutions were injected in the column. The retention times of ES and AS were found to be 2.4 min and 2.8 min, respectively.

2.5. System suitability solution criteria

The system suitability was assessed by five replicate analyses of the drugs at concentrations of 40 μg/ml of AS and 10 μg/ml of ES. The acceptance criteria were not more than 2.0% for the relative standard deviation (RSD) for the peak areas and not more than 2.0 for tailing factor for the peaks of the both the drugs.

2.6. Method validation

Method validation was performed as per ICH guidance for simultaneous determination of AS and ES in the formulations. The following validation characteristics were addressed: linearity, detection limit, quantification limit, precision, accuracy, and specificity.

2.7. System suitability criteria

The system suitability test solution was injected and the chromatographic parameters like RSD for replicate injections of both AS and ES and the tailing factor for AS and ES peaks were evaluated for proving the system suitability.

2.7.1. Specificity − forced degradation studies

Forced degradation studies were performed on AS and ES combined tablets to prove the stability-indicating property of the method. The stress conditions employed for degradation study of AS and ES include acid hydrolysis (1 N HCl, 50 °C), base hydrolysis (1 N NaOH, 50 °C), water hydrolysis (50 °C), and oxidation (3% H_2O_2, 30 °C). For light studies, the monitoring period was 10 days, whereas for heat, acid, base, and water hydrolysis, it was 48 h. Oxidation was carried out for 24 h. Peak purity of the principal peak in the chromatogram of stressed samples of AS and ES tablets was checked using photodiode array detector (PDA).

2.7.2. Linearity of response

Linearity solutions were prepared from stock solution at three concentration levels from 20 to 60 μg/ml for AS and from 5 to 15 μg/ml for ES. The slope, Y-intercept, and correlation coefficient were calculated.

2.7.3. Precision

Repeatability (intra-day): The precision of the assay method was evaluated by carrying out six independent assays of AS and ES (0.162 mg/ml of AS and 0.040 mg/ml of ES) test samples against qualified reference standard. The percentage of RSD of six assay values was calculated.

Intermediate precision (inter-day): Different analysts from the same laboratory evaluated the intermediate precision of the method on different days. This was performed by assaying the six samples of AS and ES tablets against qualified reference standard. The percentage of RSD of six assay values was calculated.

2.7.4. Accuracy (recovery study)

The accuracy of the method was evaluated in triplicate at six concentration levels, i.e. 50%, 100%, and 150% of target test concentration (0.162 mg/ml of AS and 0.04 mg/ml of ES) in combined tablets. The percentages of recoveries were calculated.

2.7.5. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for AS and NS were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentration.

2.8. Robustness

To determine the robustness of the method, the experimental conditions were deliberately changed and the resolution of AS and ES, tailing factor, and % RSD for five replicate injections was evaluated. The mobile phase flow rate was 0.7 ml/min; to study the effect of flow rate on resolution, it was changed to 0.6 and 0.8 ml/min. The effect of column temperature was studied at 35 °C and 45 °C (instead of 40 °C). In all these experiments, the mobile phase components were not changed.

2.9. Solution stability and mobile phase stability

The stability of AS and ES in solution was determined by leaving test solutions of the sample and reference standard in tightly capped volumetric flasks at room temperature for 48 h during which they were assayed at 24 h intervals. Stability in the mobile phase was determined by analysis of freshly prepared sample solutions at 24 h intervals for 48 h and comparing the results with those obtained from freshly prepared reference standard solutions. The mobile phase was prepared at the beginning of the study period and not changed during the experiment. The RSD (%) of the results was calculated for both the mobile phase and solution-stability experiments.

2.10. Method development and optimization of stability-indicating assay method

The method was optimized to separate major degradation products formed under varies stress conditions from AS and ES. The main target of the chromatographic method is to get the separation for closely eluting degradation products, mainly for the degradation product at 2.5 RT, which is eluting very closely to the ES. The degradation samples were run using different stationary phases like C18 and C8 and mobile phases containing buffers like phosphate and acetate with different pH values (2–7), and using organic modifiers like acetonitrile and methanol in the mobile phase. But the separation was satisfactory in the adopted chromatographic conditions only. It indicated that the gradient elution with 0.2% orthophosphoric acid in water as solvent A and acetonitrile and methanol in the ratio 50:50 v/v as mobile phase B was successful in separating drugs and all chromatographic degradation products [Fig. 2].

3. Results and discussion

3.1. Method validation

Validation of an analytical procedure is the process by which it is established, by laboratories studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications.

3.2. System suitability

The system suitability test solution was injected and the chromatographic parameters like RSD for replicate injections of AS and ES and the tailing factor for both AS and ES peaks were evaluated. The RSD for replicate injections of both AS and ES was 0.2% and 0.3%, respectively. The tailing factors for both AS and NS peaks were 1.1% and 1.3%, respectively. This indicates the suitability of the system.

3.3. Specificity – forced degradation studies

Degradation was not observed in AS and ES stressed samples that were subjected to light, heat, water, and oxidation. However, the degradation was observed under base hydrolysis and acid hydrolysis. The peak purity test results derived from PDA confirmed that the AS and ES peaks were pure and homogeneous in all the analyzed stress conditions. This indicates that the method is specific and stability indicating [Fig. 2].

3.4. Linearity of response

Calibration curve obtained by least square regression analysis between average peak area and the concentration showed linear relationship with a regression coefficient of 0.999. Analysis of residuals indicated that the residuals were normally distributed around the mean with uniform variance across all concentrations, suggesting the homoscedastic nature of data. Selected linear model with univariant regression showed minimum percentage bias indicating goodness of fit which was further supported by the low standard error of estimate and mean sum of residual squares.

3.5. Precision

The precision of an analytical method gives information on the random error. It is an expression of agreement between a series of measurements obtained from multiple sampling of the same
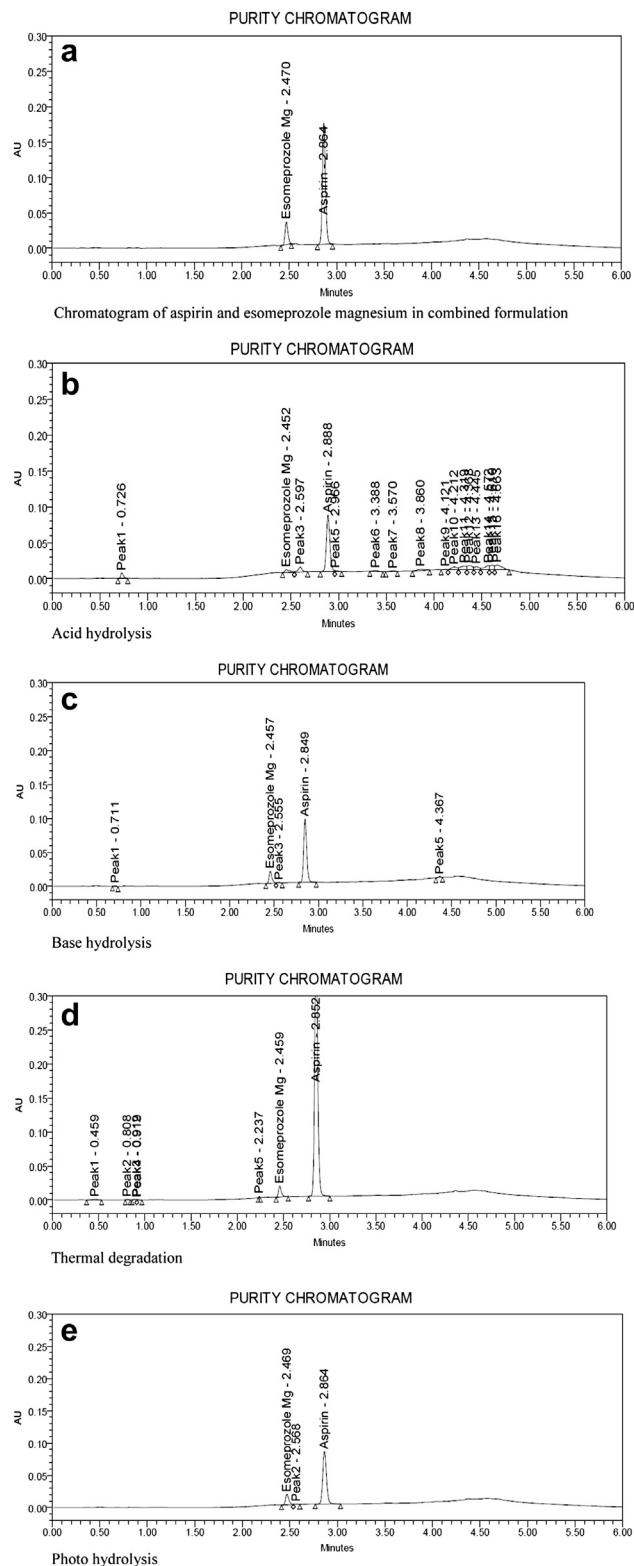

Fig. 2. A typical chromatogram obtained from aspirin and esomeprazole magnesium tablets and from stressed samples.

homogeneous sample under prescribed conditions. The percentage RSD values for the precision study were 0.8%, 0.5% (inter-day precision) and 0.6%, 1.3% (intra-day precision) for AS and ES, respectively. This confirms good precision of the method [Table 1].

Table 1
Precision results.

S. No.	Parameter	Variation	% RSD for assay	
			Aspirin	Esomeprazole magnesium
1.	Repeatability (inter-day)	(a) Analyst 1 (b) Waters Acquity UPLC system with PDA detector (c) Day 1	0.3	0.9
2.	Intermediate precision (intra-day)	(a) Analyst 2 (b) Waters Acquity UPLC system with TUV detector (c) Day 2	0.5	0.4

Table 2
Accuracy.

S. No.	Concentration (%)	Mean recovery (%) ($n = 3$)		% RSD	
		AS	ES	AS	ES
1.	50	99.1	99.9	0.3	0.4
2.	100	99.4	100.1	0.4	0.2
3.	150	100.5	99.2	0.2	0.3

3.6. Accuracy (recovery test)

The percentage recovery of AS ranged from 98.8 to 100.2 and for ES ranged from 99.1 to 100.6. Excellent recoveries were made at each added concentration [Table 2].

3.7. LOD and LOQ

The LOD of AS and ES was 2.9 and 1.4 µg/ml, respectively, for 2 µl injection volume. The LOQ of AS and ES was 9.6 and 4.4 µg/ml, respectively, for 2 µl injection volume.

3.8. Robustness

When mobile phase flow rate and column temperature were deliberately varied, resolution between AS and ES was greater than 3.0, and tailing factor and % RSD for five replicate injections of AS and ES was less than 1.5, illustrating the robustness of the method.

3.9. Stability in solution and in the mobile phase

RSD (%) for assay of AS and ES during solution stability and mobile phase stability experiments was within 0.9%. No significant changes in the amounts of the two drugs were observed during solution stability and mobile phase experiments. The results from solution stability and mobile phase stability experiments confirmed that standard solutions and mobile phase were stable for up to 48 h during assay determination.

4. Conclusion

A simple specific stability-indicating liquid chromatographic method is developed for the quantification of AS and ES simultaneously in combined dosage forms. This method was validated and was found to be specific, precise, accurate, robust, and linear for the detection and quantification of AS and ES.

Conflicts of interest

All authors have none to declare.

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