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NEW COST EFFECTIVE STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF ASPIRIN AND OMEPRAZOLE IN THE BULK DRUG AND SYNTHETIC MIXTURE

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ABSTRACT

A novel stability-indicating RP-HPLC method has been developed and validated for the simultaneous estimation of Aspirin and Omeprazole in bulk and synthetic mixture. An isocratic separation of Aspirin and Omeprazole was achieved on Thermo C_{18} column (4.6 x 250mm, 5µ particle size) as the stationary phase with a flow rate of 1.0 ml/min and using a UV detector to monitor the eluate at 295nm. The mobile phase consisted of acetonitrile: phosphate buffer pH-6 (70:30v/v) enabled separation of the drug from its degradation products. The method was validated for linearity, accuracy (recovery), precision and specificity. The linearity of the method was satisfactory over the range 5–25 µg/ml (correlation coefficient 0.9997) for both the drugs. The limits of detection and quantification for Aspirin and Omeprazole were 0.89, 2.41 and 0.45, 1.25 µg/ml respectively. The recoveries of Omeprazole and Aspirin from synthetic mixture were found to be 100.07 and 100.06% respectively. Aspirin and Omeprazole was subjected to stress conditions (hydrolysis (acid, base), oxidation, photolysis, and thermal degradation) and the samples were analyzed by this method. Both substances were unstable in basic conditions. The drug was stable under the other stress conditions investigated. The degradation products were well resolved from main peak. The forced degradation study prove the stability indicating power of the method and therefore, the validated method may be useful for routine analysis of Aspirin and Omeprazole as bulk drug, in respective dosage forms, for dissolution studies and as stability indicating assay method in pharmaceutical laboratories and industries.

Keywords: Aspirin, Omeprazole, RP-HPLC, forced degradation, method validation.

INTRODUCTION

Aspirin (ASP) is chemically 2-(acetyloxy)-benzoic acid (Fig.1A). It is nonselective cyclooxygenase inhibitor used as an antipyretic, analgesic, anti-inflammatory and antithrombotic agent. Omeprazole Sodium (OMP) is proton pump inhibitor. It is 5-methoxy-2-[[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl] benzimidazole (Fig. 1B). It is used in treatment of peptic ulcer disease, NSAIDS-associated ulceration and Zollinger-Ellison syndrome, used as antiulcerative. ASP and OMP in combined dosage form are used in cardiovascular disorder and cerebrovascular disorders.[1–2] Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, light and enables storage conditions, retest periods and shelf life to be recommended[3,4]. The two main aspects of study of the stability of a

drug product that play an important role in shelf life determinations are assay of the active drug and the degradation products generated during stability studies. Assay of a drug product in a stability test sample must be performed with stability-indicating method, as recommended by the international Conference on Harmonization (ICH)[5]. The review of literature revealed that various analytical methods involving spectrophotometry[6-8], HPLC[9-13] have been reported for ASP in single form and in combination with other drugs. Several analytical methods have been reported for OMP in single form and in combination with other drugs including spectrophotometry, HPLC and HPTLC[14-20]. This manuscript describes the development and validation, in accordance with ICH guidelines [21], of a rapid, economical, precise and accurate stability-indicating isocratic reversed phase HPLC method for analysis of Aspirin and Omeprazole in the presence of its degradation products. This paper mainly deals with the forced degradation of Aspirin and Omeprazole under the stress conditions such as acidic and basic hydrolysis, oxidation, heat and light and validation of the method for accurate quantification of Aspirin and Omeprazole in the bulk drug and solid dosage form.

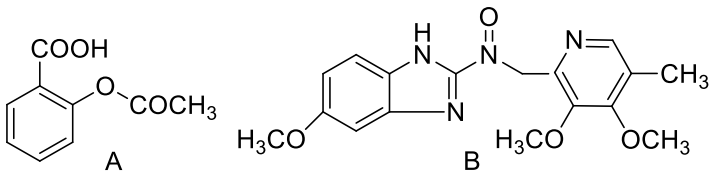


Fig.1. Chemical Structure of Aspirin (A) and Omeperazole (B)

EXPERIMENTAL

Chemicals and Reagents

Pure drug sample of Aspirin and Omeprazole was obtained from Unichem Pharma Ltd. Goa India as a gift sample. Acetonitrile (HPLC grade), Methanol (HPLC grade) were obtained from Merck Fine Chemicals Mumbai, India. Potassium dihydrogen phosphate and Orthophosphoric acid were obtained from Hi Media. Double HPLC grade water was used throughout the experiment. Other chemicals used were of analytical or HPLC grade.

Standard stock solution (1mg/ml) of both drug were prepared by dissolving the working standard in HPLC grade ACN and diluting with the same solvent. Standard calibration solutions (5-25 µg/ml) for assessment of linearity were prepared from this stock solution by dilution with diluent.

Chromatography

A high performance liquid chromatographic system from Waters comprising of manual injector, waters 715 pump for constant flow and constant pressure delivery and U.V. vis. Detector connected to software

data Ace for controlling the instrumentation as well as processing the data generated was used. The chromatographic analysis was performed by using a mobile phase of acetonitrile: phosphate buffer pH-6 (70:30v/v). These were filtered through 0.45µ membrane filter and degassed by sonication before use. The mobile phase was pumped isocratically at a flow rate of 1.0ml/min during analysis at ambient temperature. The run time was set at 10 min and the volume of injection was 20µl and eluent was detected at 295 nm on a Thermo C_{18} column (4.6 x 250mm, 5µ particle size).

Analysis of Laboratory Sample

Twenty tablets were weighed and their mean weight was determined. Weight equivalent to 81 mg of ASP and 40mg of OMP were dissolved in 50ml of diluent in 100ml of volumetric flask and shaken thoroughly for about 10 minutes, then the volume was made up to the mark with the diluents, mixed well and filtered. Further dilutions were made and the assay of injections was completed according to general procedure.

Forced Degradation Study

To study the effect of acid, accurately weighed about 50 mg drug samples was dissolved in 40 ml 0.1 M HCl and volume was made upto 50 ml with 0.1 M HCl to gets a concentration of 1000 µg /ml and kept on water bath at 80°C for 8h. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve.

To study the effect of alkali, accurately weighed about 50 mg drug samples was dissolved in 40 ml 0.1 M NaOH and volume was made upto 50 ml with 0.1 M NaOH to gets a concentration of 1000 µg /ml and kept on water bath at 80°C for 8h. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve.

To study the effect of oxidizing conditions, 50 mg of pure drugs sample was taken into a 50 ml round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve.

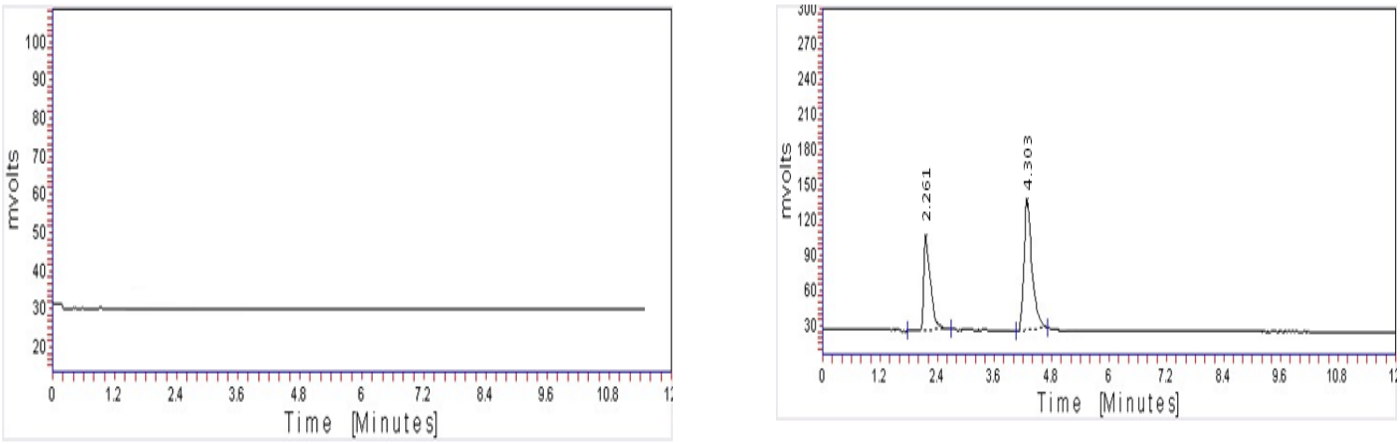
METHOD VALIDATION

The method was validated for specificity, linearity, limits of detection (LOD) and quantification (LOQ), system suitability, precision, accuracy, robustness and stability in accordance with ICH guidelines. To assess specificity, peak purity was determined by use of U.V. vis detector.

To test linearity, test solutions of ASP and OMP were prepared at six concentrations (5–25µg/ml. Each solution was injected in triplicate and calibration graphs were obtained by plotting peak area against concentration. Linearity was checked over the same concentration range on three consecutive days. RSD (%) of the slope and Y-intercept of the calibration plot were also calculated. The limits of detection (LOD) and quantification (LOQ) for ASP and OMP were determined, as the amounts for which signal-to-noise ratios were 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentration. Precision, as RSD (%) was determined by measuring the concentration of drug in the tablets six times. Intermediate (inter-day) precision was evaluated by two analysts on different days in the same laboratory. The accuracy of the method was studied by measurement of recovery after adding known amounts of the drug (80, 100, and 120% of the label claim of 81 mg ASP and 40 mg OMP per tablet) to the placebo. Three samples were prepared for each recovery level and results were calculated by use of the calibration plot. The stability of ASP, OMP and sample solutions (at ambient temperature) were tested by analysis after 24, 48, and 72 h, comparison of the results with those obtained from freshly prepared standard solutions, and calculation of RSD.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions The primary objective in developing this stability indicating HPLC method was to achieve resolution between ASP, OMP and its degradation products. To achieve this, Waters with U.V. vis. detector and C18 column was employed for envisaged work. Combination of acetonitrile: phosphate buffer (70:30) as mobile phase was attempted for quantitation of ASP, OMP with acceptable system suitability parameters (RT, tailing factor, number of theoretical plates and HETP) at 295nm as detection wavelength. Linearity was found 5-25 µg/ml at 5.5 + 0.5 min with correlation coefficient r2 = 0.9997 having equation as: AUC = 105.5x+ 26.69 for ASP and AUC = 161.2x+ 11.28 for OMP. The column temperature was 25°C. The tailing factor for ASP and OMP were <2 and retention times were approximately 2.261 ± 0.5, 4.303± 0.5 min for main peak Fig 1-2 and less than 10 min for the degradation products. This low total runs time resulted in high productivity and low cost of analysis as per sample.



(a) **(b)**

Fig 2 Chromatogram resulting from (a) Mobile Phase Blank (b) Both Drugs

Forced Degradation Study

Singh and Bakshi[22] suggested target degradation of 20–80% when establishing the stability-indicating properties of analytical methods, because even intermediate degradation products should not interfere with any stage of drug analysis. Although conditions used for forced degradation were adjusted to achieve degradation in this range, this could not be achieved for conditions other than exposure to acid, base and oxidising agent, even after long exposure. Peak purity test results confirmed the ASP and OMP peak were homogeneous under all the stress conditions tested. The mass balance of ASP and OMP in stress samples was close to 100% and, moreover, assay of unaffected ASP and OMP in the tablets confirmed the stability-indicating nature of the method. The results from forced degradation studies are summarised in Table 1. Chromatographic peak-purity data were obtained from the spectral analysis report; peak purity greater than 99 is indicative of a homogeneous peak. The peak purity for ASP and OMP from degradation studies was in the range 99.9–100.0, indicating homogeneous peaks and thus establishing the specificity of the method. Chromatograms from the solutions obtained after degradation under acidic, basis and oxidising conditions are shown in Fig 3 respectively. No peaks co-eluted with the ASP and OMP peak, suggesting the method enabled specific analysis of ASP and OMP in the presence of its degradation products.

Table 1: Results of Forced degradation studies

Stress conditions	Drug recovered (Aspirin) (%)	Drug decomposed (%)	Drug recovered (Omeprazole) (%)	Drug decomposed (%)
Standard drug	99.95	0	99.95	0
Acidic hydrolysis	85.65	14.35	90.25	9.75
Alkaline hydrolysis	85.45	14.55	85.65	14.35
Oxidative degradation	90.25	9.75	83.15	16.85

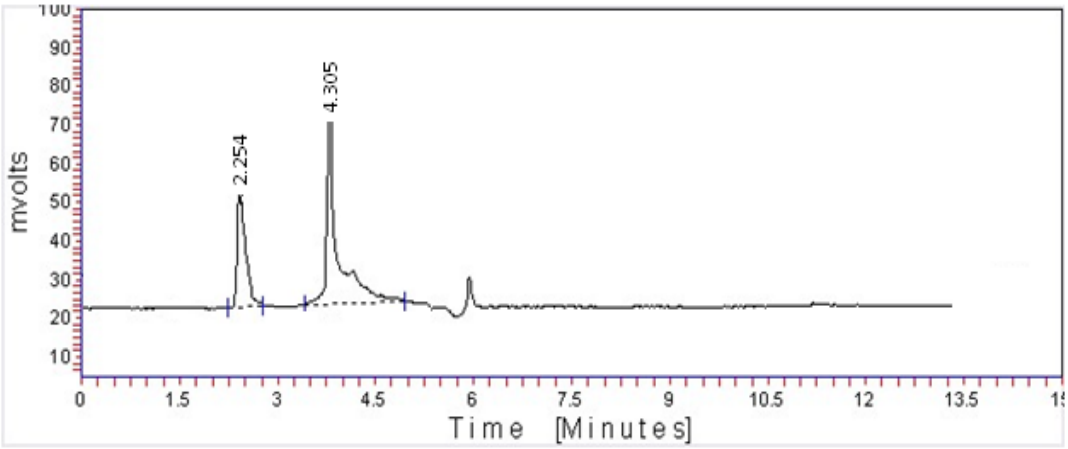


Fig 3 Chromatogram resulting from force degradation study

METHOD VALIDATION

Peak purity was >99.9% for drug substances and drug degradation products at 295 nm, which shows the analyte peaks were pure and that formulation excipients and degradation products were not interfering with analyte peaks. LOD and LOQ for ASP and OMP were 00.89, 2.41 and 0.45, 1.25 µg/ml respectively, for 20 µL injection volume. Results from regression analysis are listed in Table 2, with system suitability data. When precision was determined by six fold analysis of drug tablets, the RSD of ASP and OMP peak area was less than 2%, indicating that the method is reliable. Results from assessment of precision are listed in Table 3and 3a . Results obtained from determination of recovery are listed in Table 4.

Table 2: Results from regression analysis and system suitability data

Parameters	Aspirin	Omeprazole
Retention Time*	2.261 ± 0.5 min	4.303 ± 0.5 min
Tailing Factor*	1.11	1.42
Theoretical Plate*	3232	3048
Linear range (µg/ml)	5-25	5-25
Limits of detection (µg/ml)	0.89	0.45
Limits of quantification (µg/ml)	2.41	1.25
Linear Equation	105.5x+ 26.69	161.2x+ 11.28
Slope	105.5	161.2
Intercept	26.69	11.28
Correlation coefficient	0.9997	0.9997

**Mean of six readings*

Table 3a: Intermediate Precision of Aspirin

Intra-day Precision		Inter-day Precision	
	% Label Claim		**% Label Claim**
After 1hr	99.98	First day	98.98
After 2hr	99.81	Second day	98.12
After 3hr	99.55	Third day	98.00
After 4hr	99.45		
After 5hr	99.32		
After 6hr	99.05		
Mean	99.527	**Mean**	98.367
SD	0.335	**SD**	0.535
% RSD	0.337	**% RSD**	0.543

Table 3b: Intermediate Precision of Omeprazole

Intra-day Precision		Inter-day Precision	
	% Label Claim		% Label Claim
After 1hr	99.12	First day	98.00
After 2hr	99.05	Second day	97.98
After 3hr	99.01	Third day	97.50
After 4hr	98.75		
After 5hr	98.21		
After 6hr	98.05		
Mean	98.698	**Mean**	97.827
SD	0.460	**SD**	0.283
% RSD	0.467	**% RSD**	0.289

Table 4: Recovery of Aspirin and Omeperazole

Level of addition	Std. Drug sol. Added (µg/ml)	% Mean* recovered	
		ASP	**OMP**
80	**8**	100.04	100.08
100	**10**	100.03	100.26
120	**12**	99.98	99.94

**Average of five determination*

CONCLUSION

The method developed for quantitative analysis of Aspirin and Omeprazole is rapid, precise, accurate and selective. Peak purity studies under all the stress conditions showed the drug peak to be pure and hence the method is stability indicating. In other words it can be mentioned that the method developed can be utilized for the successful quantification of the drug in presence of its degradation product and excipients. The method was completely validated and satisfactory results were obtained for all the characteristics tested. The methods stability-indicating and can be used to assess the stability of ASP and OMP in the bulk drug. The method can be conveniently used for routine analysis of ASP and OMP as bulk drug, in respective dosage forms, for dissolution studies and as stability indicating assay method in pharmaceutical laboratories and industries.

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