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ORIGINAL RESEARCH

Validated HPLC method for the estimation of Aspirin and Omeprazole in dosage form

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Abstract

A new simple, rapid, selective, precise and accurate high-performance liquid chromatographic method has been developed for the estimation of aspirin and omeprazole in marketed formulation. The RP-HPLC method was developed for estimation of aspirin and omeprazole in bulk and tablet dosage form by isocratically using 20 mM KH_2PO_4: acetonitrile (pH 3.5) in the ratio of 20:80 v/v as mobile phase, Thermo C-18 column (4.6 x 250 mm, 5µ particle size) column as stationary phase and chromatogram was recorded at 275 nm. Then developed method was validated by using various parameters. The parameters linearity, precision, accuracy, robustness, limit of detection and limit of quantitation were studied according to International Conference on Harmonization guidelines. The proposed method was found to be accurate, repeatability and consistent. It was successfully applied for the analysis of the drug in marketed formulation and could be effectively used for the routine analysis of formulation containing the drug without any alteration in the chromatography conditions.

Keywords: Aspirin, Omeprazole, HPLC, Chromatography, Spectrophotometry

Introduction

Analytical method development plays an important role in the discovery, development, and manufacture of pharmaceuticals. The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities, development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs. Various analytical techniques are used by quality control laboratories to ensure the identity, purity, potency and performance of drug products [1]. Most of the drugs in multicomponent dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method eliminates tedious extraction and isolation procedures. Optimization parameters in HPLC are mainly partition coefficient, pKa and solubility of drug. Column selection is most important part of HPLC method development. Column selection depends upon polarity, pH range of buffer and amount of compound eluting out. It also depends upon the functional groups present in drugs. Aspirin (ASP) is chemically 2-(acetyloxy)-benzoic acid. It is non-selective cyclo-oxygenase inhibitor used as an antipyretic, analgesic, anti-inflammatory and antithrombotic agent. It reduces non-fatal myocardial infarction. It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP), United States pharmacopoeia (USP) and European Pharmacopoeia (EP). It is estimated by acid-base titration method as per IP, BP, and USP & EP. Literature review reveals that HPLC, UV spectrophotometric methods has been reported for estimation of ASP in pharmaceutical dosage forms [2]. Omeprazole (OMP) is chemically known as 5-methoxy-2-[[(4- methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl] benzimidazole. It is officially listed in BP 2011 and U.S.P.XXXII. It is a proton pump inhibitor, used in treatment of peptic ulcer disease and NSAID-associated ulceration, in gastro-esophageal reflux

disease and the Zollinger-Ellison syndrome [3].

In the present work a simple, selective, rapid, precise and economical reverse phase HPLC method has been developed and validated for the estimation of aspirin and omeprazole in marketed formulation.

Materials and methods

Chemicals Acetonitrile (HPLC grade) from Merck, methanol (HPLC grade) from Merck, potassium dihydrogen phosphate from Rankem, water (HPLC grade) from Milli-Q, triethanolamine from Thomas Baker and orthophosphoric acid from Hi Media was obtained. Working standard aspirin and omeprazole was of 99.9% and 99.8% potency. Commercial formulations of strength aspririn (81 mg) and omeprazole (40 mg) were obtained from Aralez pharmaceuticals (Yosprala). Instrument specification HPLC used was of Waters, pump was 5151 binary pump, Injector was Rheodyne injector with a 20-microlitre loop, detector was UV vis detector, software was data ace software, column was Thermo C-18 column (4.6 x 250 mm, 5µ particle size). Milipore water was used throughout the study.

Selection of mobile phase

Initially to estimate aspirin and omeprazole in fix dosage form number of mobile phase in different ratio were tried taking into consideration the system suitability parameter like RT, tailing factor, number of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 20 mM KH_2PO_4: acetonitrile (pH 3.5) in the ratio of 20:80 v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Selection of separation variable

The selection of variables and, strategy for optimization is discussed in table 1. Evaluation of elution conditions used for the separations reveals that the presence of acid in the mobile phase is essential for both the complete resolution of the compounds.

Table 1: Separation variable

Variable	Condition
Column	250mm x 4.60mm
Particle Size	5µ
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
20mM KH_2PO_4	20 parts
Acetonitrile	80 parts
Flow rate	1.0 ml/min
Temperature	Ambient
Sample Size	20 µl
Detection wavelength	275 mm
Retention time	
Aspirin	2.024 ± 0.3 min.
Omeprazole	3.819± 0.3 min.

Preparation of standard solution

Accurately weighed 10 mg of aspirin and omeprazole was transferred into 50 ml volumetric flasks separately and dissolved in 10 ml of acetonitrile, then volume was made up to 50 ml with acetonitrile and vortex it to get complete dissolution of drug. Stand it aside for few minute, Concentration of aspirin and omeprazole was 200 µg/ml (Stock- A). 5ml of solution was taken from Stock-A of aspirin transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent (Acetonitrile) to give concentration of 100 µg/ml (Stock-B). 0.5ml, 1.0 ml, 1.5ml, 2.0ml and 2.5ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with (Acetonitrile). This gives the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml for drug. In same manner 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml of omeprazole also prepared.

Linearity and calibration graph

To establish the linearity of analytical method, a series of dilution ranging from 5-25 µg/ml was prepared. All the solution were filtered through 0.2µm membrane filter and injected, chromatograms were recorded at 275 nm and it was repeated for three times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

System suitability parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of aspirin 10 µg/ml was injected separately. Peak report and column performance report were recorded for all chromatogram.

Laboratory sample analysis

The commercial tablet formulation of aspirin was available in the strength of 81 mg. Based on this different standard solutions were prepared for quantitative analysis, which gives satisfactory results. Stock solution was prepared in the same manner. Further dilutions were made to prepare the mixed standard of desired concentration.

Table 2: Laboratory sample analyses

Standard Number	Concentration of Aspirin (µg/ml)	Concentration of Omeprazole (µg/ml)
1.	5	5
2.	10	10
3.	15	15
4.	20	20
5.	25	25

Validation of developed method

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test,

which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration were recorded thrice, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components.

Precision

Precision of the method was determined in the terms of intraday and inter-day variation (%RSD) was assessed by analyzing standard drug solution within the calibration range, three times on the same day. Inter-day precision (%RSD) was assessed by analyzing drug solution within the calibration range on three different days over a period of 7 days.

Intermediate precision

Intra-day precision was determined by analyzing the standard solution of ASP (20µg/ml) and OMP (10µg/ml) at 8.00am and 4.00pm on same day following the procedure of repeatability.

Range

The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the curve. The correlation coefficient (r2=0.999) of least square linear regression for ASP and OMP was calculated.

Accuracy

20 tablets of ASP were weighed and finely powdered; an accurately weighed powder (15.54mg) equivalent to 10 mg of ASP was dissolved and diluted to 50 ml methanol. 2ml of above solution was transferred in four different 10ml volumetric flask labelled as 0%, 50%, 100%, and 150%. Then 0, 1, 2, 3 ml of 'Std Stock Mix AO' (200ug/ml ASP &100ug/ml OMP), were added and made up to the mark with mobile phase & their chromatogram were obtain under the same chromatographic condition after getting a stable baseline. Peak areas were recorded and percent recoveries were calculated.

Sensitivity

The sensitivity of measurement of ASP and OMP by the use of proposed method was estimated in terms of limit of

detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated by equation. Based on the standard deviation of the response and the slope, LOD and LOQ were estimated using the formulae: LOD= 3.3 σ/S Where, σ = the standard deviation of the response S = the slope of the calibration curve LOQ = 10 σ/S Where, σ = the standard deviation of the response. S = the slope of the calibration curve LOD and LOQ were determined from the standard deviations of the responses for six replicate determinations.

Robustness

Combined standard solution of ASP (20µg/ml), OMP '= (10µg/ml) was prepared and analyzed at different flow rates (0.9, 1.0, 1.1 ml/min) separately.

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25 µg/ml for aspirin indicates the precision under the same operating condition over short interval time. Results of repeatability are reported in table respectively.

Analysis of tablet sample

Twenty tablets were taken and their average weight was determined. They are crushed to fine powder; amount equal to 10 mg of aspirin was taken in 100-ml volumetric flask. The volume is made up to the mark by mobile phase and filtered by whatman filter paper (no.41) and the filtrate was used to prepare samples of different concentration.

Method validation is done as according to International Conference on Harmonization guideline [4].

Results and discussion

The developed UV spectrophotometric method was found to be rapid, simple, inexpensive, reproducible, and applicable over a wide concentration range with high precision and accuracy.

The RP-HPLC method was developed for estimation of aspirin and omeprazole in bulk and tablet dosage form by isocratically using 20 mM KH_2PO_4: acetonitrile (pH 3.5) in the ratio of 20:80 v/v as mobile phase, Thermo C-18 column (4.6 x 250mm, 5µparticle size) column as stationary phase and chromatogram was recorded at 275 nm. Then developed method was validated by using various parameters.

System suitability

The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 10 µg/ml of aspirin and omeprazole were injected separately and chromatogram was

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recorded. The result of system suitability parameter is reported in table.

Table 3: Results of system suitability parameters

Parameters	Aspirin	Omeprazole
No. of Theoretical Plates	3124.1667	3232.833
Tailing Factor	3.7639	1.163
Retention time	2.024 ± 0.3	3.819± 0.3

Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded. The results of linearity are reported in table.

Table 4: Results of linearity of aspirin and omeprazole

Parameter	Aspirin	Omeprazole
Conentration(µg/ml)	5-25	5-25
Correlation Coefficient (r^2)*	0.999	0.998
Slope (m)*	80.77	121.333
Intercept (c)*	13.27	16.913

*value of five replicate

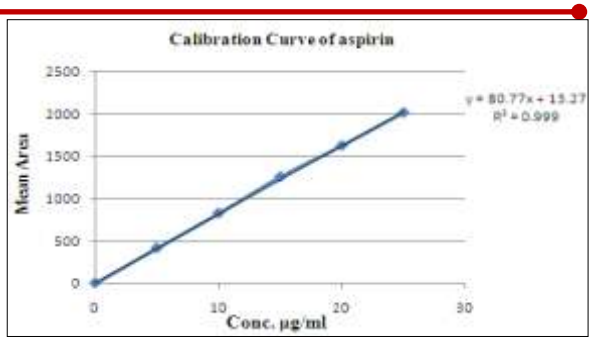

Figure 1: Calibration curve of aspirin

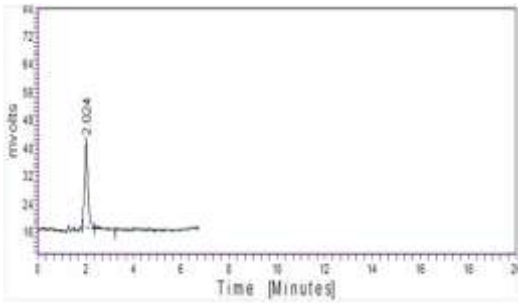

Figure 2: Chromatogram of aspirin

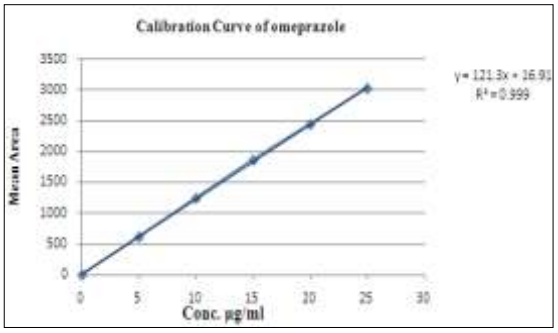

Figure 3: Calibration Curve of omeprazole

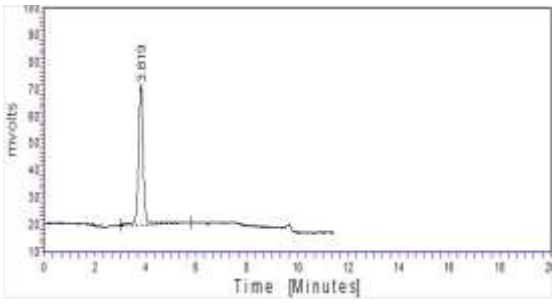

Figure 4: Chromatogram of omeprazole

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Specificity

Specificity of the method was determined and the peaks of diluent, mobile phase and excipient of tablets did not interfere with standard peaks aspirin and omeprazole.

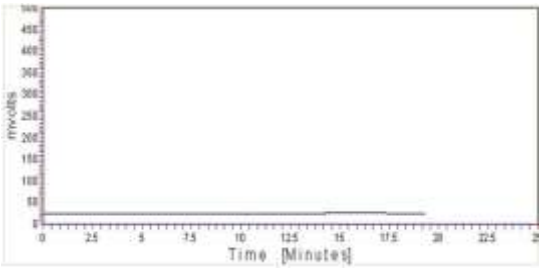

Figure 5: Chromatogram of blank diluent

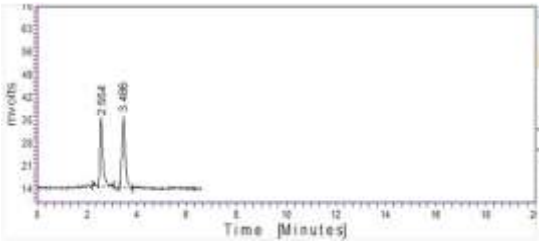

Figure 6: Chromatogram of both the drugs

Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in table 5.

Precision

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision shown in table 6.

Table 5: Results of recovery study

% Level	% MEAN±SD*	
	Aspirin	**Omeprazole**
80%	99.10±0.318	99.48±0.215
100%	99.72±0.235	99.14±0.440
120%	99.56±0.254	98.54±0.553

***** Value of three replicate and three concentrations.

Table 6: Results of precision

Parameter	% MEAN±SD*	
	Aspirin	**omeprazole**
Repeatability	99.447±0.036	98.790±0.162
Intermediate precision		
Day to day precision	99.276 ±0.041	99.179±0.085
Analyst to Analyst	98.833±1.242	99.125±0.215
Reproducibility	99.172±0.026	

***** Value of five replicate and five concentrations

Robustness

The robustness of developed method was checked by changing in the deliberate

variation in solvent. Result of robustness shown in table 7.

Table 7: Results of robustness

Parameter	% MEAN±SD*	
	Aspirin	Omeprazole
Robustness	99.458 ±0.065	99.415±0.065

*Value of five replicate and five concentrations

LOD and LOQ

Detection limit and quantitation limit of described method were observed as 0.570 µg/ml, 0.350 µg/ml and 0.50 µg/ml respectively and quantitation limit 1.54 µg/ml, 0.95µg/ml and 1.58µg/ml respectively based on the SD of response and slope, which meet the requirement of new method.

Assay of tablet formulation

The results of the analysis of tablet formulation were reported. The assay value of drugs was close to 100, SD and % RSD are less than 2 indicate the no interference of excipient in the estimation of drugs.

Table 8: Assay of tablet formulation

S. No.		API	
		Aspirin	Omeprazole
1.	Mean	99.125	99.458
2.	S.D.	0.125	0.147
3.	% RSD	0.214	0.217

This study, therefore describes an isocratic HPLC method using UV detection, which provides adequate sensitivity for routine use and diminishing the time of sampling and chromatographic analysis.

Conclusion

The results obtained by RP-HPLC method for determination of aspirin and omeprazole are reliable, accurate and precise. The method does not require prior separation of one drug from another. Hence, it can be employed for routine quality control analysis of ASP and OMP in marketed formulation. The proposed method is less time consuming so can be successfully applied for the dissolution analysis of the two drugs, estimation from the biological fluids. The method can be used as stability indicating method for the estimation of two drugs in presence of their degradation products.

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