Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(3):2349-2353

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Development and validation of HPLC method for the simultaneous determination of aspirin

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ABSTRACT

A sensitive, specific, precise and cost effective High Performance Liquid Chromatographic method of analysis for aspirin in presence of its degradation products is developed and validated.

Keywords: Aspirin, HPLC, UV detector.

INTRODUCTION

Aspirin1 (2-acetoxybenzoic acid) is analgesic and antipyretic. Aspirin also inhibits platelet aggregation. Its mode of action as an anti inflammatory and antirheumatic agent may be due to inhibition of synthesis and release of prostaglandin. Aspirin appears to produce analgesia by virtue of both peripheral and CNS effect. Aspirin inhibit platelet aggregation by irreversible inhibition of platelet cyclooxygenase and thus inhibits the generation of thrombooxygenase A2 powerful inducer of platelet aggregation and vasoconstriction.

There are many methods reported for determination of aspirin in individual and combined dosage form, viz. HPLC 2,3,4,5,6, spectrophotometric 7, RP-HPLC 8,9, RP sequential injection chromatography(RP-SIC)10.Literature survey suggest that there are analytical method by HPLC for the estimation of aspirin, but the reported method for aspirin estimation have some disadvantages such as more retention time peak tailing. In present study, a HPLC method has been developed and validated with advantage of the retention time, cost reduction, sharp peaks and low solvent consumption.

EXPERIMENTAL SECTION

Instrumentation:
Waters 2487 gradient HPLC system with auto-sampler and column oven (Water Alliance) was used. Separation and quantization was done on Hypersil BDSC18 (100 x 4.6 mm 5µ) column.

Chromatographic Condition:
The mobile phase was prepared by mixing sodium chlorate buffer (pH 2.5), acetonitrile and isopropyl alcohol in the ratio of 85:1:14 %.The mobile phase was filtered using 0.45 µm Nylon filter and degassed in a sonicator for 10 minutes. The flow rate was 1.5 ml.min−1.Column was maintained at 25 ºC. The injection volume to carry out the chromatography was set at 20µl. Under these conditions aspirin eluted at 4.6 minute. The total run time was 30 minutes.

Method Development:
Chromatographic separation of the active, related Substances and its degraded products was achieved using a BDS Hypersil C18column (100 mm × 4. 6mm) 5µm stainless steel column. The mobile phase was prepared by mixing buffer pH (2.5), acetonitrile and isopropyl alcohol in the ratio of 85:1:14 %.

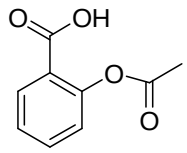
Standard and working solution:
Standard solution of aspirin was prepared at the concentration of 50µg ml-1 dissolving appropriated amount of standard in the mobile phase. This standard solution was used to quantify active and final product. For the preparation of sample solution, 20 tablets were taken and weighed individually. Average weight was calculated and finely powdered. Appropriate portion of this powder equivalent to 50 mg of aspirin was weighed and transferred to a 100 ml volumetric flask. This was dissolved in 70 ml 0.1% orthophosphoric acid by sonicating for 20 min and made up to the volume. 5 ml of above solution was pipetted into 50 ml volumetric flask and volume made by 0.1%orthophosphoric acid and acetonitrile (50:50 % v/v).Filtered through a 0.45µm membrane lter.

Method Optimization
Effect of pH:
The effect of pH on the chromatographic behavior of the drug was studied by varying pH of sodium per chlorate buffer to 2.3, 2.5, and 2.7. 15% acetonitrile was used in respective buffer at flow rate of1.5ml/min.

Effect of stationary phase:
The chromatogram was recorded using following column.· BDS C18 (250 x 4.6 mm) 5µm BDS C18 (100 x 4.6 mm) 5µm

Effect of solvent rate:
Different solvent namely methanol, tetrahydrofuran and mixture of tetrahydrofuran and methanol (1:1) in60% of sodium per chlorate buffer were used. Flow rate was 1.5 ml/min.

Effect of mobile phase ratio:
The chromatogram was recorded by using mobile phase containing 35%, 25%, and 15% of acetonitrile in sodium per chlorate buffer.



Structure of Aspirin

Method Validation
Method validation was done as per ICH guidelines11, 12and accordingly the parameter evaluated were,1. Linearity 2. Precision 3. Reproducibility 4. Specificity 5. Accuracy 6. System suitability

Linearity:
The linearity of analytical procedure is its ability (within given range) to obtain test results which are directly proportional to concentration in sample. This was studies by analyzing ten concentrations within the range of 12.5 µg/ml to 75 µg/ml solutions of aspirin corresponding to about 25% to 150% of target concentration. A graph was plotted in µg/ml on X axis versus response on Y axis.

Precision:
The precision of analytical procedure express the closeness of agreement (Degree of scatter) between series of measurement obtained from multiple sampling of the same homogeneous sample under prescribed condition.

Specificity:
A study to establish the interface of tablet recipients (placebo) was conducted and assay was performed on placebo in triplicate equivalent to about the weights of the placebo in portion of the test method as per the method.

Chromatogram shows no peaks at the retention time of aspirin, this indicates that recipients used in the formulation do not interfere in the estimation of aspirin.

Accuracy:
The accuracy of the analytical procedure express the closeness of the agreement between the value which accepted either as conventional true value or accepted reference value and the value found. A study recovery of aspirin from spiked placebo was conducted. Samples were prepared by mixing placebo, with aspirin raw materials equivalent to 50%, 75%, 100%, 125% and 150% of target concentration. Sample solution were prepared in triplicate for each spike level and assayed per method.

System Suitability:
System suitability is defined as, the checking of system, before or during analysis of unknowns, tonsure system performance. A data from five injection of system precision (50µg/ml) were utilized for calculating system suitability parameter like %RSD, Tailing factor, and theoretical plates. BDS HypersilC18 column (250 mm × 4. 6mm) 5µm was used as stationary phase. The mobile phase consist of acetonitrile, 2.5 pH buffer solution and isopropyl alcohol in the ratio of 14:85:1 respectively.275nm was detection wavelength. Flow rate was 1.5ml/min.Column run for 10 min at temperature of 50 ºC.

RESULTS AND DISCUSSION

Effect of pH:
The retention time of aspirin was decreased as the pH of mobile phase increased. This may be due to ionized state of aspirin at higher pH. From the table 1 it can be noted that pH 2.5 buffer gave less retention time when compared to pH 2.7.Although pH 2.3 buffer gave optimum retention time, it was not selected as this pH the efficiency and column life are adversely affected.

Effect of the stationary Phase:
On BDS Hypersil C18 column (10 cm × 4. 6mm i.e., 5µm), aspirin eluted with desirable retention and symmetrical peak. For the study Hypersil C18 column (10 cm × 4. 6mm i.e., 5µm) was selected because of its lower asymmetric factor when compared to other column. Also C18 columns are hydrophobic in nature enhances the retention time with added advantages of more column stability.

Effect of solvent strength:
Different solvent like methanol acetonitrile, in buffer (pH 2.5) were used at flow rate of 1.5 ml/min. When methanol was used, peak broadening was observed along with the high back pressure. With the methanol and water peak tailing was observed. For the present study, 14% acetonitrile and 1% isopropyl alcohol in buffer pH 2.5 was selected because it gave good separation.

Effect of Ratio of mobile phase:
The proportion of acetonitrile and buffer (pH 2.5) of70:30, 45:55, 35:65 and acetonitrile, buffer, isopropyl alcohol in 14:85:1% v/v ratio was used as mobile phase. The mobile phase ratio of 70:30, 45:55 and35:65 when used gave low retention time with subsequent reduction in capacity factor, from which it's difficult to distinguish the aspirin peak from early eluting impurities. At 14:85:1 % v/v ratio of acetonitrile and sodium per chlorate buffer (pH 2.5), isopropyl alcohol, a symmetrical peak eluted at around4.0 min with good capacity factor and it was selected as for further studies.

Effect of Flow rate:
1.5 ml/min flow rate gave symmetrical peak with acceptable capacity factor. For the present study 1.5ml/min was selected on the basis of less retention time, good peak shape, Acceptable back pressure and better separation of impurities from drug. At flow rate of 1.3 ml/min peak broadening was observed and peak shape was irregular with peak broadening at flow rate 1.7ml/min.

HPLC Assay method validation

Specificity:

Assay was performed on placebo in triplicate equivalent to about the weights of placebo in portion of test preparation as per test methods. Chromatograms of placebo solution showed no peaks at the retention time of aspirin and its degradation product. This indicates the recipients used in formulation do not interfere in the estimation of aspirin.

Interference with the degradation products

The aspirin peak was well resolved from the degraded impurities. The peak purity test of aspirin at the stress condition had revealed that the method was stability indicating and specific.

Solution stability:

A solution of aspirin (50µg/ml) was prepared and stored at room temperature for 24 hrs. The sample solution withdrawn at intervals of 0, 2, 4, 6, 8, 12 and24 hrs and analyzed. No additional peak was observed in the solution that was kept for 24 Hrs.

Precision:

System Precision:

The % RSD of repeated injection was found to 0.6% it was found to be within the acceptable value of 1.0%hence proposed method was precised.

Method Precision:

The precision of test method was evaluated by assaying six sample of aspirin tablet blend (50µg/ml). The mean % assay was found to be 100.2%and %RSD of assay was found to be 1.3%.

Intermediate Precision:

Two analysts on different HPLC system conducted analyst to analyst variability study by assaying six different test preparation of aspirin tablet blend. The average %assay obtained by both analysts was found to be 101.0 and 101.1 with RSD of 0.48% and 0.32% respectively .The system suitability parameter were evaluated as per method by both analyst and found to be within limits.

Linearity:

The data obtained in linearity experiments was subject to linear regression analysis. The coefficient of regression (r2) was found to be 0.997.

Accuracy

The results from recovery study for accuracy determination are depicted. Recovery of aspirin from spiked placebo was conducted. Sample solution was analyzed in triplicate for each concentration level and assayed as per method. The percentage recovery was found to be within the limits (97.7-100.2%). The mean recovery of aspirin tablet should not be less than 97%and not more than 103%.

Robustness:

No significant change in the chromatographic parameters were observed when change in the optimized condition like change in the pH and flow rates.

Ruggedness:

System to System variability: System to system variability was conducted by two HPLC systems by using same column by assaying six different test preparation of aspirin blend in same condition. The system suitability parameter found to be within limits. The average assay for system was found to be 100.8 and 100.9% with %RSD of 0.3% and 0.4% respectively. Comparison of the result obtained on two system shows that the assay method is rugged for system to system variability.

Filter Validation:

Test preparation in triplicate was centrifuged and filtered through either filters, were assayed against unfiltered standards. The difference in the %assay values between centrifuged and filtered samples with into is within limits.

System Suitability:
The result of system suitability throughout the validation studies are given in the table 6.All the values of system suitability were found to be within in the acceptable limits. It concluded that the method and systems are adequate for the analysis to be performed.

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