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ORIGINAL ARTICLE **OPEN ACCESS**

HPLC Method Validation for The Estimation of Aspirin in Bulk and Tablet Dosage Form as Per IP

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ABSTRACT

A new HPLC method has been validated with different parameters for Aspirin in Bulk and Tablet dosage form. The chromatograms were developed using a mobile phase of Methanol: Glacial acetic acid: Water (28:3:69) with a flow rate of 2 ml/min. C18 Column of 4.6 x 10 cm dimension was used as a stationary phase, particle size 5µm. The detection was carried out at 275 nm. The method was validated according to ICH guidelines for linearity, Accuracy, precision (Intraday & Interday), Repeatability and Robustness. The response was found to be linear in concentration range of 87.5-262.5 mcg/ml for Aspirin. The validated method was simple, precise, accurate and reproducible and therefore suitable for routine analysis of drugs in tablet dosage form.

***Keywords**: HPLC; Aspirin; IP; ICH; Validation.*

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INTRODUCTION

High performance liquid chromatography is a type of liquid chromatography used for analytical study of hydrophilic and lipophilic chemicals. HPLC is of 2 types i.e. Normal and Reverse phase HPLC. In Normal phase HPLC, Mobile phase is polar(in which Drug is dissolved) and Stationary phase is Non-polar. In case of Reverse phase HPLC, Mobile phase is Non-polar (in which Drug is dissolved) and Stationary phase is polar. Determination of Aspirin was carried out by RP-HPLC method. Aspirin [Chemical Name- 2-acetoxybenzoic acid] is also known as Acetyl Salicylic acid. It is a medication used to treat pain, fever, or inflammation. Different inflammatory conditions like pericarditis, Kawasaki disease and rheumatic fever are treated by Aspirin. Aspirin is also used to prevent ischemic, further heart attacks and blood clots in people at high risk. It may also decrease the risk of certain types of Carcinoma, particularly colorectal cancer. Aspirin is a non-steroidal anti-inflammatory drug (NSAID) and works similarly to other NSAIDs but also suppresses the normal functioning of platelets. It can be given by oral and rectal route. Lysine acetylsalicylate is given by IV and IM.

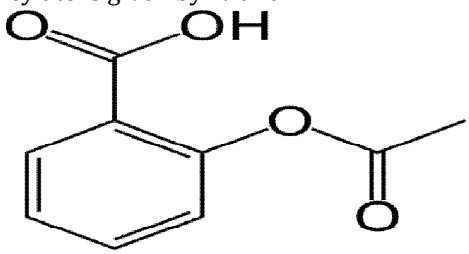

Figure 1: Structure of Aspirin

MATERIAL AND METHODS

Chromatographic conditions:

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation-

Table No-1: Chromatographic Condition

Column	id 4.6 x 10 cm length
Detector	SPD-M20A PDA Detector
Particle size packing	5 µm
Stationary phase	C18
Mobile Phase	Methanol: Glacial acetic acid: Water (28:3:69)
Detection Wavelength	275 nm
Flow rate	2 ml/min
Temperature	Ambient
Degasser	DGU-20 A5 Prominence

Table No-2: List of Reagents and Chemicals

Sr. no	Name of Chemical	Supplied by
1	Methanol, HPLC Grade	Research Lab Fine Chem Industry, Mumbai
2	Water, HPLC Grade	Rankem Industry
3	Glacial Acetic Acid, AR Grade	Research Lab Fine Chem Industry, Mumbai
4	Benzoic Acid, AR Grade	Research Lab Fine Chem Industry, Mumbai

Assay of Aspirin Tabletas perIP:

Apparatus and Instruments

Table No. 3: Instruments Used

Sr. no.	Instruments	Specification
1	UV-1800 Spectrophotometer	Shimadzu Corporation, Japan
2	Weight Balance	Shimadzu Corporation, Japan
3	Sonicator	Leela Electronics, India
4	Pipettes	Borosilicate
5	Measuring Cylinder	Borosilicate
6	Burette	Borosilicate

Reagents and Chemicals

Table No. 4: List of Reagents and Chemicals

Sr. no.	List of Chemicals	Supplied by
1	Distilled Water	Prepared by DistillationProcess
2	Sodium Hydroxide, AR Grade	Research Lab Fine Chem Industry, Mumbai
3	Sodium Citrate, AR Grade	Research Lab Fine Chem Industry, Mumbai
4	Chloroform, AR Grade	Research Lab Fine Chem Industry, Mumbai

Assay Procedure forAspirin:

Accurately weighed a quantity of powder containing about 0.7 mg of Aspirin; add 20 ml of water and 2 gm of sodium citrate and heat under reflux condenser for 30 minutes. Cooled and washed the condenser with 30 ml of warm water and titrated with 0.5M sodium hydroxide using phenolphthalein solution as indicator.1 ml of 0.5M sodium hydroxide is equivalent to 0.04504 gm of $C_9H_8O_4$.

Preparation of 0.5M NaOH:

Accurately weighed quantity of sodium hydroxide about 2.0 gm and dissolved it in 100 ml of distilledwater.

Preparation of 80 µg/ml solution of Aspirin:-

Aspirin 0.56 mg was weighed accurately and add 20 ml of distilled water containing 2 gm of sodium citrate to obtain the solution having concentration of 80µg/ml.

Preparation of 100 µg/ml solution of Aspirin:-

Aspirin 0.7 mg was weighed accurately and add 20 ml of distilled water containing 2 gm of sodium citrate to obtain the solution having concentration of 100µg/ml.

Preparation of 120 µg/ml solution of Aspirin:-

Aspirin 0.84 mg was weighed accurately and add 20 ml of distilled water containing 2 gm of sodium citrate to obtain the solution having concentration of 120µg/ml.

Preparation of stock solution:
Dissolve 13.14 mg of Aspirin sample in 100 ml of diluent. This solution was of 100 ppm.
Preparation of solution:
Pipette out 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml from above stock solution in different volumetric flask and volume made upto 10 ml, to get 50-150% solution of Aspirin.
ANALYTICAL METHOD VALIDATION OF ASPIRIN TABLET AS PERIP:
Linearity:
Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte insamples within a given range.
Linearity of the method was studied by preparing concentration of drugs in linear range. Appropriate dilutions of standard stock solutions were analysed as per the developed method. Calibration curve was plotted in the concentration range of 5-25 µg/ml for Aspirin. The acceptance criteria of linearity should not be less than 0.999.
Accuracy:
Accuracy of an analytical procedure is the closeness of agreement between the conventional true value or an accepted reference value and the value found.
In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. The accuracy of the method was performed by conducting the recovery studies (80, 100% and 120%) of pure drugs from marketed formulation, by standard addition method.
Precision:
The repeatability was evaluated by 3 replicates of 3 concentrations of standard solutions of Aspirin on the same day respectively.
The intraday precision of the standard method was evaluated by analyzing samples of different concentrations of Aspirin three times on the same day and % RSD was calculated.The inter day precision was evaluated from the same concentration of Aspirin on five different days and % RSD was calculated.
Range:
The range of an analytical method is the interval from the upper to the lower level that has been demonstrates to be determined with precision, accuracy and linearity.

RESULTS AND DISCUSSION
Linearity Study:
The study of linearity of Aspirin was carried out, and it was found to be linear in a concentration range of 50 to 150 µg/ml. It is mentioned in Table No.5 and Figure 2.

Table No. 5: Linearity Data of Aspirin

Sr. No.	Conc. Level of Sample	Burette Reading
1	50	8.5
2	75	12.9
3	100	16.9
4	125	20.7
5	150	25

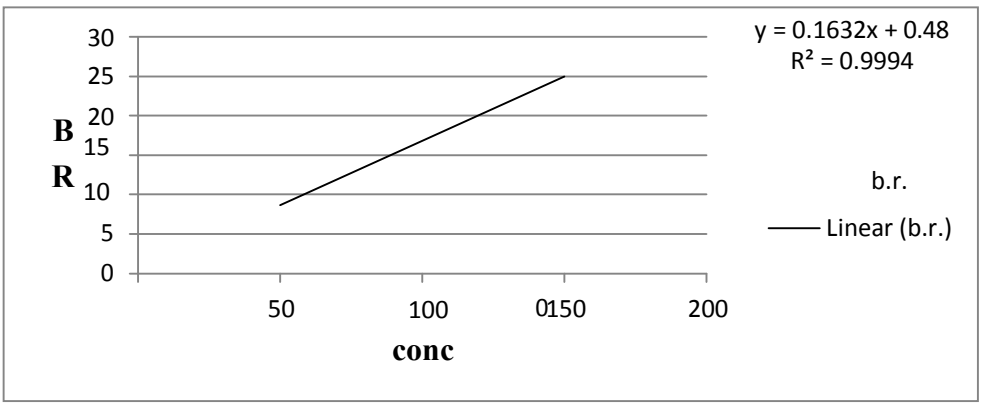

Figure 2: Calibration curve of Aspirin

Accuracy: (% Recovery Study)

During % Recovery Study of Aspirin, three samples of each concentration of 80%, 100% and 120% were taken. The amount of Aspirin was calculated at each level and % recoveries were computed. The % recoveries obtained were 99.09%, 100.18% and 99.69% respectively for 80%, 100% and 120%. It is shown in Table No.6.

Table No. 6: Result of % Accuracy for Aspirin

Conc. % of spiked level of sample	Amount of Drug Added (µg/ml)		Burette Reading Found	Amount of pure drug found (ppm)	% Recovery	Statistical Analysis of % Recovery
	Pure	Formulation				
80 1	140	175	14.7	139.0540	99.324	Mean: 99.099 %RSD: 1.7158
80 2	140	175	14.9	140.9459	100.675	
80 3	140	175	14.4	136.2162	97.297	
100 1	175	175	18.2	172.1621	98.378	Mean: 100.180 %RSD: 1.6484
100 2	175	175	18.6	175.9459	100.540	
100 3	175	175	18.8	177.8378	100.621	
120 1	210	175	21.9	207.1621	98.648	Mean: 99.699 %RSD: 1.1370
120 2	210	175	22.4	211.8918	100.900	
120 3	210	175	22.1	214.7297	99.549	

Inter Day and Intra Day Precision:

The Inter Day and Intra Day Precision study were carried for Aspirin. Three samples of 50%, 100% and 150% were taken. The average % relative standard deviation (RSD) for aspirin was found to be 0.88 and 1.25 for Inter Day and Intra Day Precision respectively. It is mentioned in Table No.7.

Table No. 7: Inter Day and Intra Day Precision Data for Aspirin

	Conc. Level of Sample		Burette Reading	%RSD
Inter Day	50	DAY 1	8.133	0.7098
	100	DAY 2	16.433	0.9295
	150	DAY 3	24.233	1.0384
Intra Day	50	1	8.233	1.8552
	100	2	16.233	1.2823
	150	3	24.266	0.6294

Ruggedness:

Ruggedness of Aspirin was carried by 2 different analysts. The % drug obtained was 98.37% and 99.12% respectively by analyst-1 and analyst-2. It is shown in Table No.8.

Table No. 8: Ruggedness Data for Aspirin

Sr. No.	Ruggedness Parameter	% Assay Result
		ASP
1	Analyst - 1	98.37
2	Analyst - 2	99.12
Mean		98.745
SD		0.5303
%RSD		0.5370

Study of Range:

The Aspirin was tested at 50% and 100% concentration. The 6 samples of each concentration were taken and the result is shown in Table No.9.

Table No. 9: Range for Aspirin

Sr. No.	50% ASPIRIN	150% ASPIRIN
1	8.4	24.0
2	8.2	24.2
3	8.3	24.1
4	8.1	24.6
5	8.2	25.0
6	8.1	24.8
Mean	8.2166	24.45
SD	0.1169	0.4086
%RSD	1.4227	1.6713

Assay of Marketed Formulation:

The assay of marketed formulation (Tablet) was carried out. It contains Aspirin in a concentration of 350 mg. The % amount of drug found was 98.37%. The result is show in Table No.10.

Table No. 10: Assay Result for Tablets

Formulation	Amount of Drug in Tablet (mg)	Amount of Drug taken (mg)	Amount of Drug found(mg)	%Amount of Drug found(mg)
Tablet	350	175	172.16	98.37

CONCLUSION

The main goal of this work is based on to selects the more suitable, accurate, precise, validated and reliable method for the determination of Aspirin tablet which are given in Indian Pharmacopoeia.

From the chromatographic study, I concluded that IP method is more linear, accurate, precise, reliable and reproducible for routine analysis of Aspirin in a dosage form. So one can perform validation and assay as per IP.

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