

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR 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. The method employed Hypersil BDS C₁₈ (100 x 4.6 mm 5 μ) column as stationary phase. The mobile phase consisted of sodium perchlorate buffer (pH 2.5): acetonitrile: isopropyl alcohol (85:14:1 % v/v). It is pumped through the chromatographic system at a flow rate of 1.5 ml min⁻¹. The UV detector is operated at 275 nm. This system was found to give good resolution between aspirin and its degradation products. Method was validated as per ICH guidelines

Keywords: Aspirin, HPLC, UV detector.

INTRODUCTION AND EXPERIMENTAL

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 thromboxygenase A₂ a 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}, spectrophotometric⁷, 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 Data:

The Acetonitrile and Methanol used are of HPLC grade, supplied by Ranbaxy Fine chemicals Ltd. Mohali. Aspirin, Batch No. Lot X-34 is supplied by Alta Laboratories Ltd. Khopoli.

Instrumentation:

Waters 2487 gradient HPLC system with auto-sampler and column oven (Water Alliance) was used. Separation and quantitation was done on Hypersil BDS C₁₈ (100 x 4.6 mm 5 μ) 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 μ m Nylon filter and degassed in a sonicator for 10 minutes. The flow rate was 1.5 ml.min⁻¹. Column was maintained at 25°C. The injection volume to carry out the chromatography was set at 20 μ l. Under these conditions aspirin eluted at 4.6 minute. The total run time was 30 minutes.

Selection of wavelength:

Aspirin shows good absorbance at 275 nm so it was selected as wavelength of detection.

Method Development

Chromatographic separation of the active, related Substances (synthesis impurities) and its degraded products was achieved using a BDS Hypersil C₁₈ column (100 mm × 4.6 mm) 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 filter.

METHOD OPTIMIZATION:**Effect of pH:**

The effect of pH on the chromatographic behavior of the drug was studied by varying pH of sodium perchlorate buffer to 2.3, 2.5, and 2.7. 15% acetonitrile was used in respective buffer at flow rate of 1.5ml/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) in 60% of sodium perchlorate 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 perchlorate buffer.

Effect of Flow rate:

The flow rate 1.3, 1.5, 1.7 ml/min were used and chromatogram was recorded.

METHOD VALIDATION:

Method validation was done as per ICH guidelines^{11, 12} and accordingly the parameter evaluated were,

1. Linearity
2. Precision

3. Reproducibility
4. Specificity
5. Accuracy and
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 studied by analyzing ten concentrations within the range of 12.5 µg/ml to 75 µg/ml solution 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. The precision of the method was demonstrated by

1. System Precision: Standard solution of aspirin was injected in 10 replicate injection and %RSD was calculated.
2. Method Precision: Analyzing six replicate injections of aspirin standards and sample solution. Percentage assay of sample to that of label claim was calculated by comparing the sample solution response to that of standard solution response. %RSD of assay result was calculated.
3. Intermediate Precision: Intermediate precision express within laboratory variation. Two analysts on different HPLC system conducted and analyst to analyst variability study by assaying six different test preparations of aspirin tablets blend of same batch was calculated.

Specificity:**Placebo interface:**

A study to establish the interface of tablet excipients (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 excipients used in the formulation do not interfere in the estimation of aspirin.

Interface from the degradation product

Interface from degradation product was carried to demonstrate the effective separation of degradants from aspirin. Separate portion of the drug product, drug substance (50µg/ml) and placebo were exposed to following stress condition,

1. Refluxed with 1 N Hydrochloric acid solution at 60°C for about 30 min for drug substance.
2. Refluxed with 1 N Sodium Hydroxide solution at 60°C solution for about 30 min for drug substance

3. Treated with 1% hydrogen per oxide for about 30 min on bench top
4. Refluxed with purified water for 30 min.

Stressed sample after appropriate dilution were injected into the HPLC system with diode array detector and degradant peaks were resolved from aspirin peak in the chromatogram of all samples.

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 or 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 a system, before or during analysis of unknowns, to ensure 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 Hypersil C₁₈ 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°C.

Robustness:

The robustness of analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variation in method parameter and provides an indication of its reliability during normal usage and done by changing,

1. Influence of variation of pH in the mobile phase (± 0.2)
2. Influence of variation of flow rates ($\pm 10\%$).

Ruggedness (System to system variation)

Ruggedness of analytical method is degree of reproducibility of test result obtained by the analysis of the same sample under verity of condition such as laboratory, analyst and instruments. System to system variation was conducted on two HPLC systems by using the same column by assaying six different test preparation of aspirin blend (50µg/ml) as per method.

Filter Validation:

A study to establish the suitability of filter was conducted using two different filters namely 0.45µm PVDF filter and 0.45µm Nylon filter. Test preparation were prepared in triplicate were centrifuged and filtered through different filters, were assayed against unfiltered standards (50µg/ml). The difference in % assay values between centrifuged and filtered sample were calculated.

RESULTS

HPLC METHOD DEVELOPMENT AND OPTIMIZATION OF ASPIRIN:

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. Results are given in the table 1. The chromatograms are shown in figure 1, figure 2 and figure 3.

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. The results are given in the table 2. The chromatograms are shown in figure 4 and figure 5.

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) of 70:30, 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 and 35:65 when used gave low retention time with subsequent reduction in capacity factor, from which it is difficult to distinguish the aspirin peak from early eluting impurities. At 14:85:1 % v/v ratio of acetonitrile and sodium perchlorate buffer (pH 2.5), isopropyl alcohol, a symmetrical peak eluted at around 4.0 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 1.5 ml/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 1.7 ml/min. The results are shown in the table 3. The chromatograms are shown in figure 6, figure 7 and figure 8.

Effect of Temperature:

Peak symmetry was not achieved by any combination of column and mobile phase, hence temperature increased to 50°C.

HPLC ASSAY METHOD VALIDATION ASPIRIN:**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 excipients used in formulation do not interfere in the estimation of aspirin. Chromatogram of aspirin is shown in the figure 10.

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. The result was summarized in the figure 11.

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 and 24 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. Results are depicted in the table 4.

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%. Results are given in the table 4.

Intermediate Precision:

Two analyst 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. The limits are summarized in the table 4.

Linearity:

The data obtained in linearity experiments was subject to linear regression analysis. The coefficient of regression (r^2) was found to be 0.997. Linearity data and plot are reported in the table 4 and graph 12 respectively.

Accuracy

The results from recovery study for accuracy determination are depicted in the Table 4. 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. The results are summarized in the table 4.

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. System suitability parameters, assay results are summarized in the table 4.

Filter Validation:

Test preparation in triplicate were centrifuged and filtered through either filters, were assayed against unfiltered standards. The difference in the %assay values between centrifuged and filtered samples within to be within limits. This study indicates that both filters are suitable for filtration. Filter description given in the table 5 and result are summarized in table 6.

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. The results are summarized in the table 7.

DISCUSSION

The ultimate Goal of the validation process is to challenge the method and determine limits of allowed variability for the condition needed to run the method.

The proposed analytical method has been proved to be simple, specific and accurate which fulfill all the parameters of the validation (table 4). Therefore this method has been successfully applied to raw materials and dosage form like tablets.

Table 1: Effect of pH

Parameters	pH 2.3	pH 2.5	pH 2.7
Retention Time	5.2	4.6	4.7
Theoretical Plates	3686	2806	3461
Capacity Factors	6.42	4.8	4.2

Table 2: Effect Of stationary Phases:

Parameters	BDS Hypersil C ₁₈ column (25 cm × 4.6mm i.e., 5μm)	BDS Hypersil C ₁₈ column (5 cm × 4.6mm i.e., 5μm)
Retention Time	9.4	3.8
Asymmetry Factors	1.2	1.0

Table 3: Effect of Flow Rate

Parameters	1.3 ml/min	1.5 ml/min	1.7 ml/min
Retention Time	5.3	4.6	4.1
Theoretical Plates	NA	2806	1246
Capacity Factors	5.6	4.8	4.8

Table 4: validation Parameters:

Parameters			Obtained Results
r² (Representive linearity)			0.997
Accuracy (% RSD)	Recovery levels	50%	97.8 %
		75%	98.0 %
		100%	99.8 %
		125%	100.0 %
		150%	100.0 %
	Assay		101.1 % w/w
Precision (% RSD)	System Precision		0.6 %
	Intermediate Precision	Analyst 1	0.48 %
		Analyst 2	0.32 %
	Method Precision		1.3 %
Robustness (% RSD)	Effect of variation in pH	pH 2.3	0.16 %
		pH 2.5	0.10 %
		pH 2.7	0.20 %
	Effect of variation in flow rate	1.3 ml/min	0.52 %
		1.5 ml/min	0.57 %
		1.7 ml/min	0.65 %
Ruggedness	System to system variability	System 1	0.10 %
		System 2	0.63 %

Table 5: Filter Description:

Filter Description	Filters	
	NYLON66	PVDF
Manufacturers Name	Micro Devices	Milex
Size	0.45µm	0.45µm

Table 6: Filter Validation:

Centrifuged			PVDF			NYLON 66		
% Assay			% Assay			% Assay		
1	2	3	1	2	3	1	2	3
100.8	100.8	101	101	101.7	100.7	101.5	100	99.17

Table 7: System Suitability Parameters:

Parameters	Aspirin
USP plate Count	3254
USP tailing	1.0
% RSD of six Replicate Injection	0.6

Effect of the pH

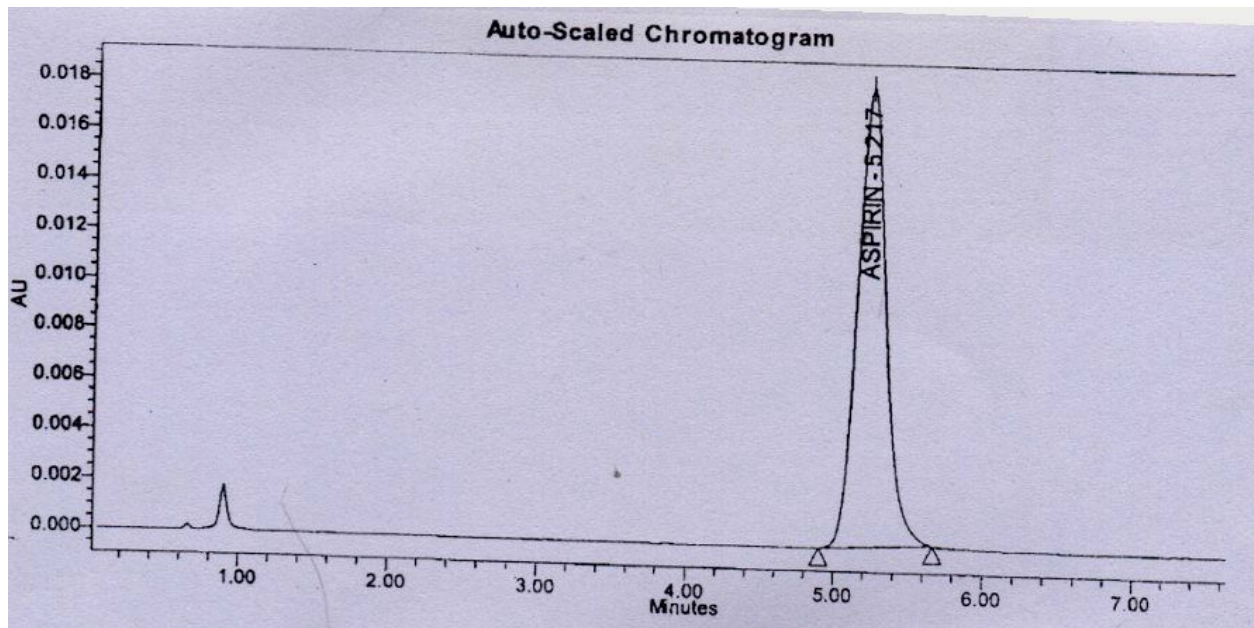


Fig 1: Mobile Phase pH 2.3

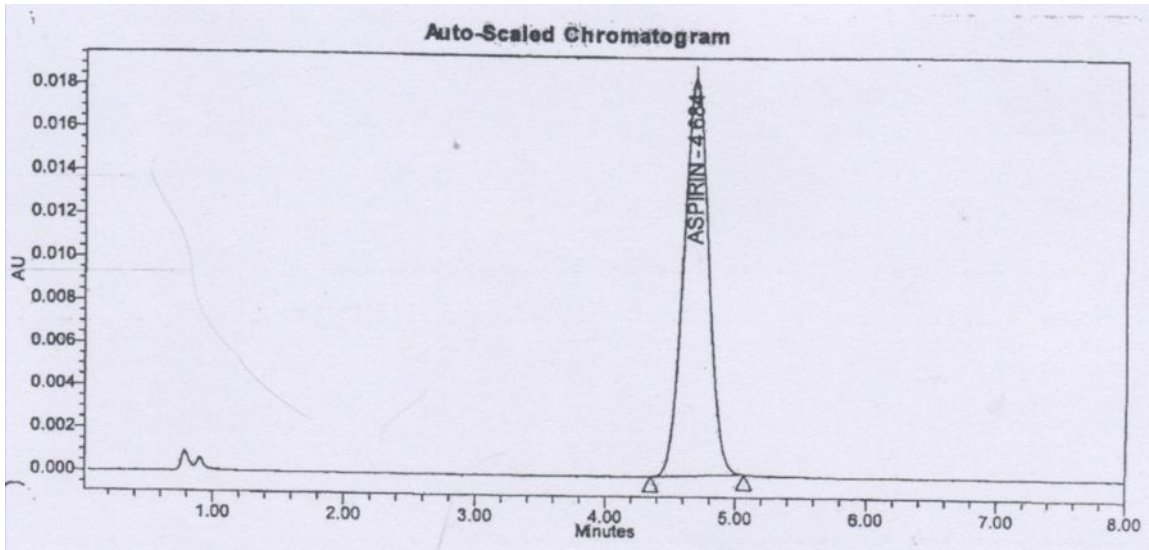


Fig 2: Mobile Phase pH 2.5

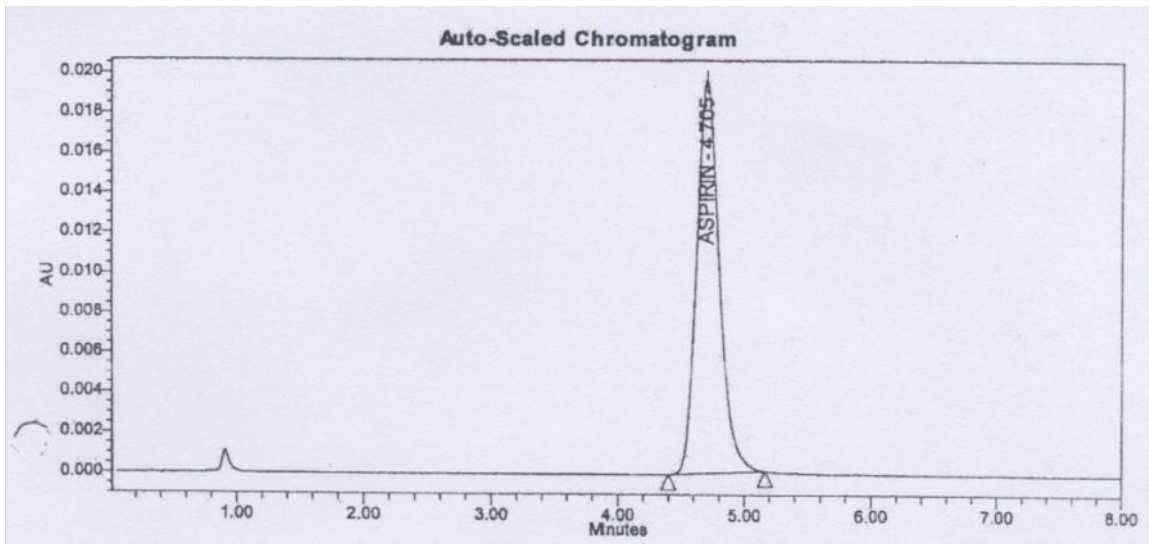


Fig 3: Mobile pH 2.7

Chromatograms of Effect of stationary Phases:

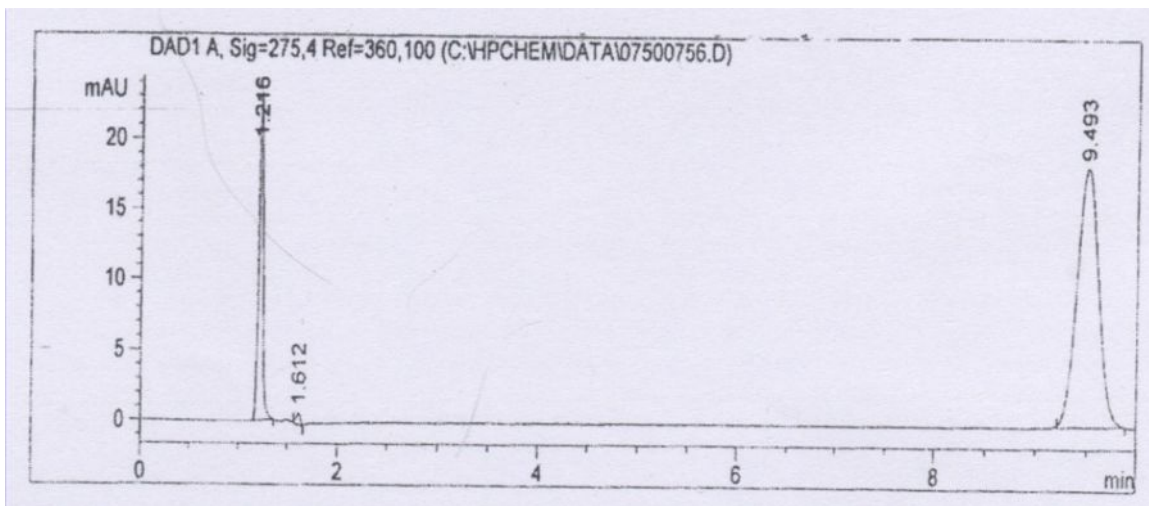


Fig 4: Using the Column Hypersil BDS C₁₈ (250 x 4.6mm 5µm)

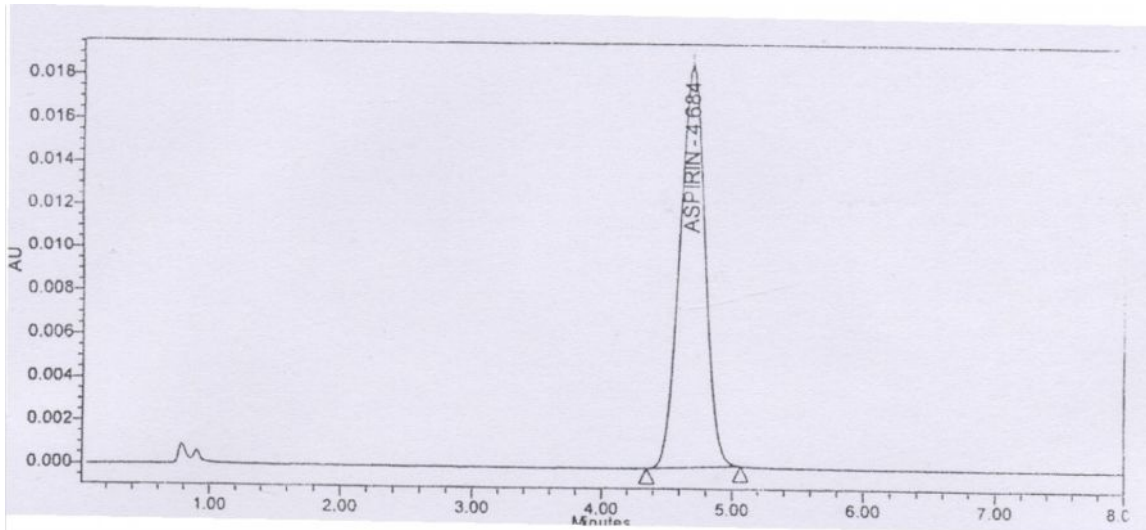


Fig 5: Using the Column Hypersil BDS C₁₈ (100 x 4.6 mm 5 μ m)

Chromatograms of Effect of flow rate:

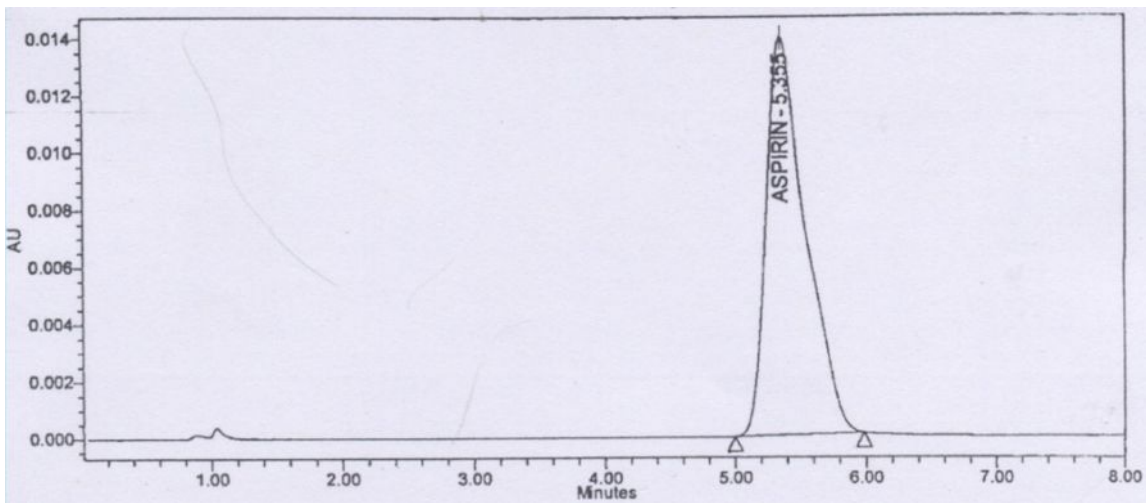


Fig 6: Flow rate 1.3 ml/min

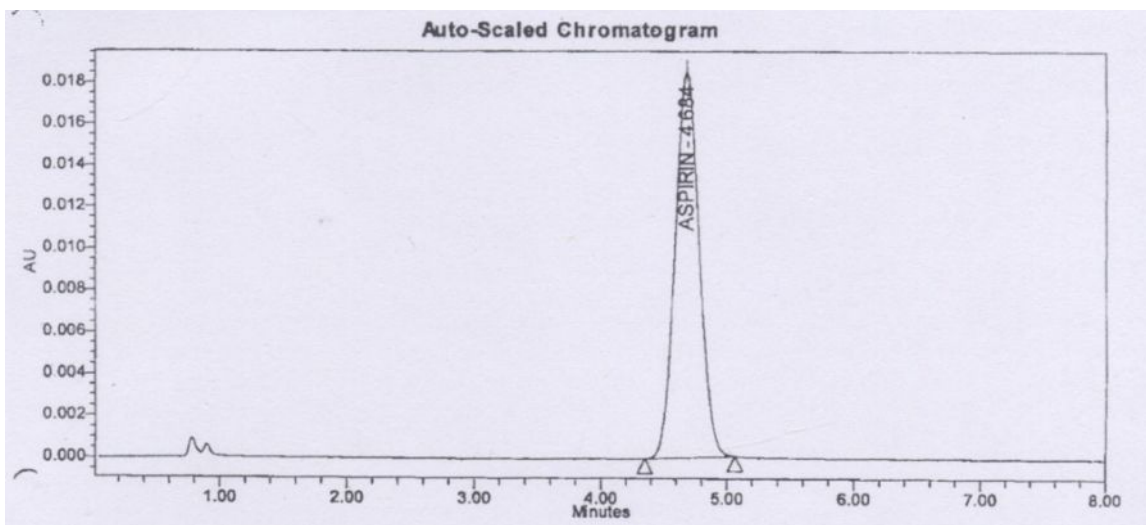


Fig 7: Flow rate 1.5 ml/min

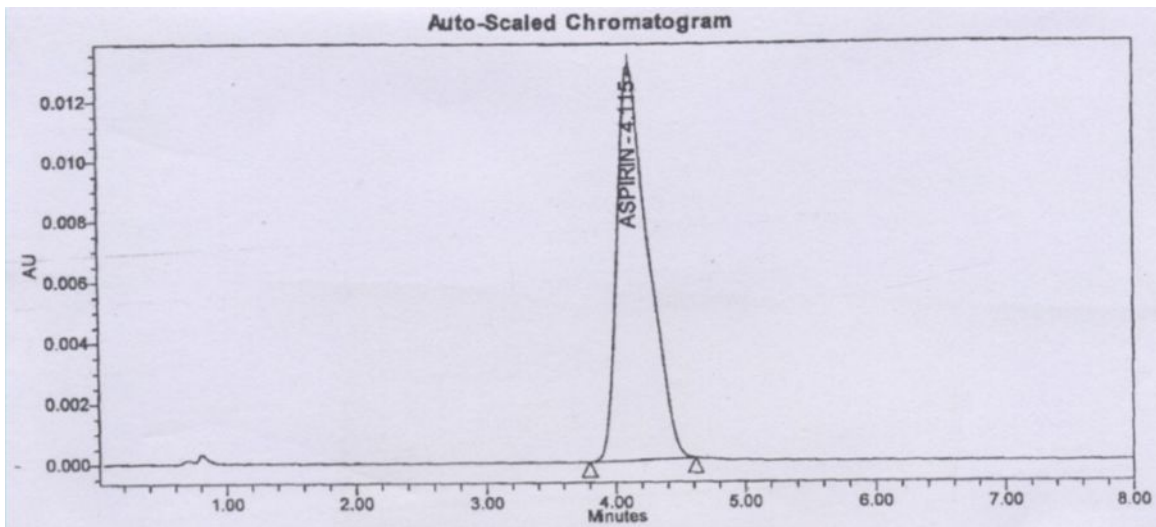


Fig 8: Flow rate 1.7 ml/min

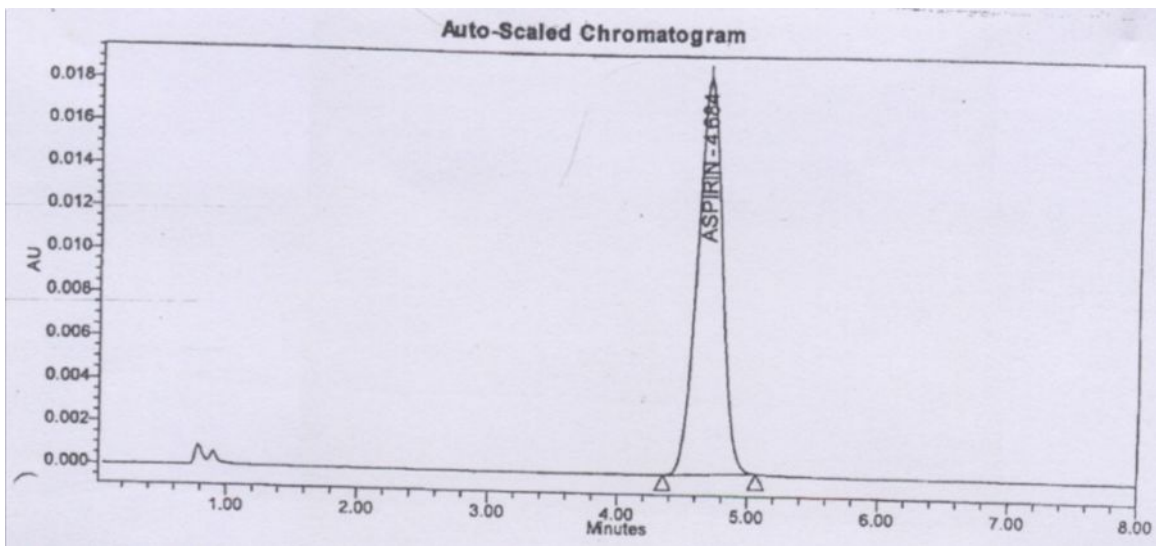


Fig 9: representative chromatogram of the aspirin under optimized condition

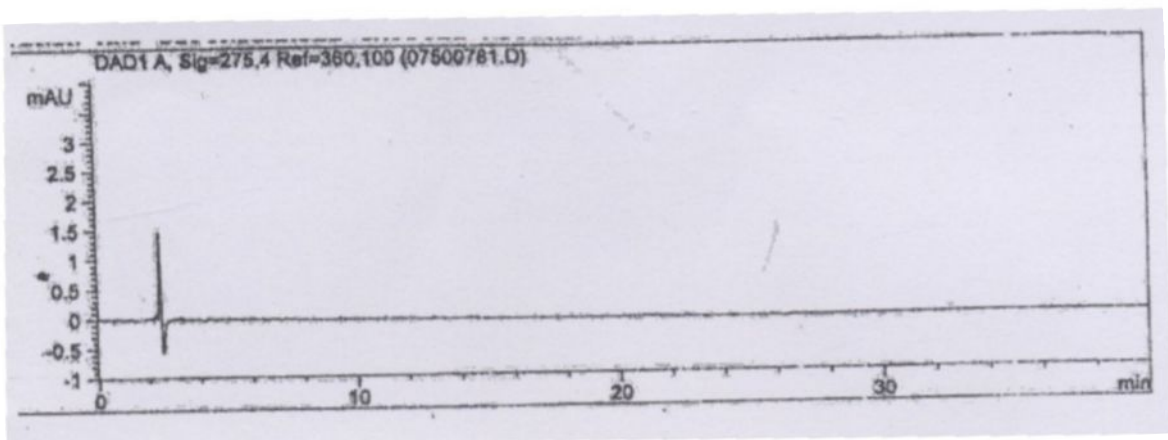


Fig 10: Chromatogram of Aspirin Placebo

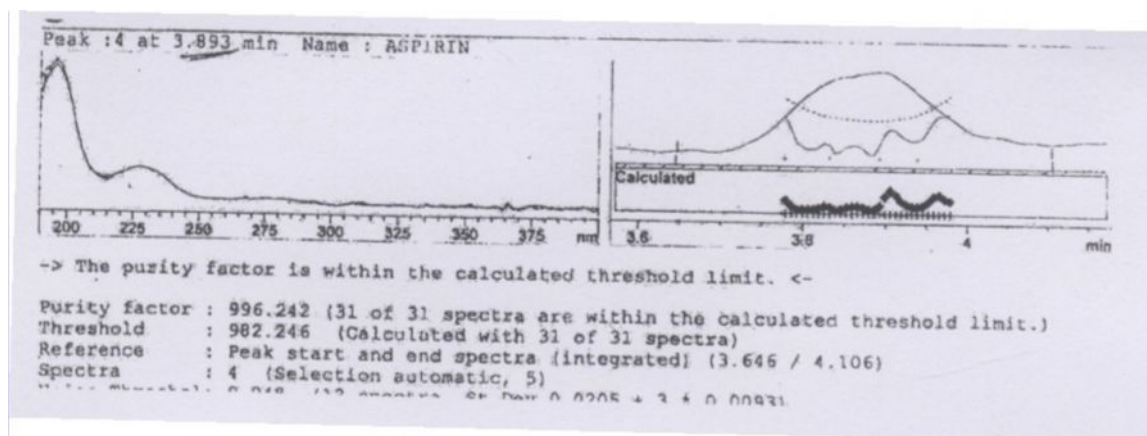


Fig 11: Interference of degradation Product

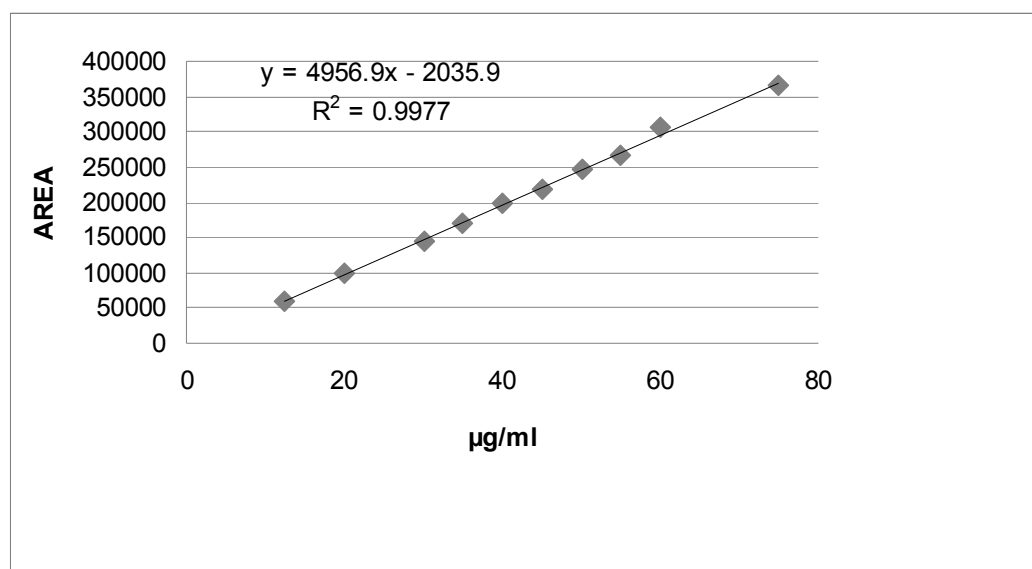


Fig 12: Representative Linearity of Aspirin

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