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Research Article

Analytical Method Development and Validation for Analysis of Aspirin: A Component of Anti-hypertensive Drugs by using RP-HPLC Method

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ABSTRACT

For the estimation of aspirin in bulk form, a straightforward, sensitive, and focused liquid chromatographic approach has been established. Chromatographic conditions included Column C-18 (250 x 4.6 mm, 5 m particle size), water at pH 3.0, 0.1% orthophosphoric acid (v/v), and acetonitrile (45:55), flow rate of 1 ml, and a running time of 20 minutes. The wavelength of interest was 237 nm. The average recovery percentage was found to be 99.9%, while the retention duration was 4.01 minutes. ICH guidelines were determined to be followed by the suggested procedure and can be used for the analysis of aspirin as a bulk drug and also in combination with other drugs.

INTRODUCTION

Numerous new medications are released on the market each day. The time frame between the date a medication is introduced to the market and the date it is included in pharmaceutical protocols is frequently longer. This is due to possible imperfections in the continuous and extensive use of these medications, reports of new side effects, an increase in patient resistance, and the introduction of superior drugs by competitors. In such circumstances, probably, Pharmacopoeias do not provide access to established scientific strategies for these pharmaceuticals.

In this approach, developing a new explanatory mechanism for such treatments becomes necessary. Additionally, since medications contain life, quality is important in every service or product. investigation that reveals the spatial organization of an ion as a particle and the location or proximity to a particular natural beneficial gathering in a specific compound. Additionally, surface analysis plays a crucial role in material studies to obtain surface-related physical features, such as geography, profundity profiling, atom introduction, and so forth. Concoction examination involves some key steps, such as system selection, testing, preliminary specimen

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preparation, partitioning, last estimation, and outcomes evaluation.

In the first step, particularly involves choosing a system, care should be taken to select the ideal instrument for a fruitful investigation. Making an incorrect choice right now will result in a pointless investigation. The three main categories of analytical techniques are physical, chemical, and instrumental analysis. Physical observations include compound descriptions, measurements of its dimensions (size, form), color, and odor, among other things. Titrimetric analysis of compounds, such as potentiometric, audiometric, argentometric, permagnometric, etc., is part of chemical analysis. Experimental chemistry now relies heavily on instrumental techniques for chemical analysis.

Both new and current products undergo method development. It is more difficult to implement an adequate optimization lesson and improve on the level of familiarity obtained via such a lesson when there are no immediate lit equilibriums between what is desired and what has already been understood. To evaluate the characterized normal for a medicine substance or medication item, an official analytical method is used. The candidate suggests logical technique as an alternative to administrative systematic method. Security testing is a crucial step in the development of pharmaceutical products.

The purpose of protection testing is to demonstrate how the nature of a medication substance or medication item changes over time as a result of a variety of environmental factors, including temperature, moisture, and light. It also enables the suggestion of capacity conditions, retest intervals, and timeframes for realistic usability to be established. Tests of active medications and degradations produced during soundness studies are the two main components of medication items that play a significant role in the timeline of realistic usability determination.

Modern methods for quantitative examination decision-making include:

Reversed-phase: The most used HPLC arrangement, known as RP-HPLC, is the same as NP-HPLC in reverse, with the stationary phase being more nonpolar than the eluting solvent.

A nonpolar stationary phase, such as C-18 silica, and a moderately polar aqueous mobile phase are typical components of RP-HPLC. Surface-modified silica, also known as RMe_2SiCl, is a typical stationary phase for RP-HPLC, where R is an alkyl group with a straight chain, such as $C_{18}H_{37}$ or C_8H_{17}.

Nowadays, HPTLC is becoming a standard investigative technique due to its advantages of inexpensive working costs, high specimen throughput, and minimal example cleaning requirements. A key advantage of HPTLC is that, unlike HPLC, a limited number of samples may be run simultaneously using a small amount of a flexible stage, reducing the amount of time and money required for each research.

Chromatography

Today's chromatographic procedures mostly involve the separation of substances in a sample mixture rather than the separation of color (although the technique names originated from the initial work of separating dyes or plant pigments on paper). To accommodate the many physical and chemical states of sample mixtures that one may be interested in separating from and studying, a variety of separation methods have evolved. Chromatography differs from the majority of other physical and chemical separation techniques in that it brings two mutually immiscible phases into contact, one of which is stationary and the other mobile. While the stationary phase can only be a liquid or a solid, the mobile phase can also be a gas. Liquid-liquid chromatography is the term used when the separation consists mostly of a straightforward portioning between two immiscible liquid phases, one stationary and the

other mobile. Liquid solid chromatography is the term used to describe the procedure when physical surface forces are primarily responsible for the stationary phase's capacity to retain samples. In a column or on an open bed, liquid chromatography has been carried out.

MATERIALS AND METHODS

Acetylsalicylic acid(>=99.0%) was purchased from merck, orthophosphoric acid, acetonitrile, HPLC grade water.

Description

Acetylsalicylic acid (ASA), also known as aspirin, is a frequently prescribed medication for the treatment of pain and fever resulting from a variety of reasons. Acetylsalicylic acid possesses antipyretic and anti-inflammatory properties. Additionally, this medication prevents platelet aggregation and is used to treat myocardial infarction (MI), stroke, and blood clots.

Molecular formula: C9H8O4

Molecular weight: 180.1574 g/mol

Chemical Name: 2-(acetyloxy) benzoic acid

Synonym: Acid acetyl salicylique

Physical description: Solid

IDENTIFICATION AND CHARACTERISATION OF DRUGS

Determination of λ_{max} of Aspirin

By running the spectrum of the drug solution in a double-beam UV spectrophotometer the λ_{max} of aspirin was determined.

METHOD DEVELOPMENT FOR ANALYSIS OF ASPIRIN

Development and verification of a method for estimating aspirin using RP-HPLC.

Selection and procedure for preparation of mobile phase

Initially, various mobile phase ratios were tested to estimate aspirin in a fixed dosage form.

The mobile phase that was determined to be the most acceptable for analysis is as follows:

pH of HPLC Water 3.00 ± 0.05 with 0.1 % (v/v) orthophosphoric acid (v/v) and mix with Acetonitrile in the ratio of 45:55.

Selection of diluent

The mobile phase is used as a diluent for the preparation of Standards and samples.

Use the Diluent/mobile phase as the blank solution.

Standard Solution Preparation

Standard Stock Solution: Weigh 37.5 mg of Aspirin, in 50 mL volumetric flask and 25 mL of diluent and mix well. Sonicate for five minutes. Cool the solution to room temperature and make up the volume with diluent

Standard Working Solution: Pipet 5 ml of the Standard stock solution and dilute to 50 mL with diluent.

Concentration of working standard - Aspirin – 75 ppm (75 µl/mL),

Filter the working standard solution using the 5-10 mL disposable syringe and PTFE filters into a 2-ml Autosampler vial and seal with the caps.

SAMPLE SOLUTION PREPARATION

Sample Preparation

1. Transfer the 75/150 mg Aspirin dosage form to the 100/200 mL volumetric flask. 50% of the volumetric flask should be filled, and then thoroughly mixed. Until the sample is completely dissolved, sonicate it. Shake ferociously to guarantee complete sample dissolution. Once the solution has reached room temperature, dilute it to create volume.
2. Pipet 5 ml of the above solution and dilute to 50 mL with diluent.
3. Concentration of sample standard - Aspirin – 75 ppm (75 µl/mL),
4. Use the 3-ml disposable syringe and PTFE filters to filter the sample working solution

before transferring it to a 2-ml Autosampler vial and capping it.

OPERATING CONDITIONS

Typical Instrument Settings:

Detector	237nm
Flow rate	1.0 mL/min
Injection volume	20 µL
Mobile phase	Water at pH 3.00 with 0.1 % (v/v) orthophosphoric acid (v/v) and Acetonitrile (45:55)
Running time	20 min
System suitability	Retention Time: Aspirin- 4 min
Tailing factor	Not More than 1.2 for analyte in standard solution
Theoretical plates	Not Less than 2,500 for all analyses in standard solution
Resolution	Not less than 2.0

Experimental Procedure

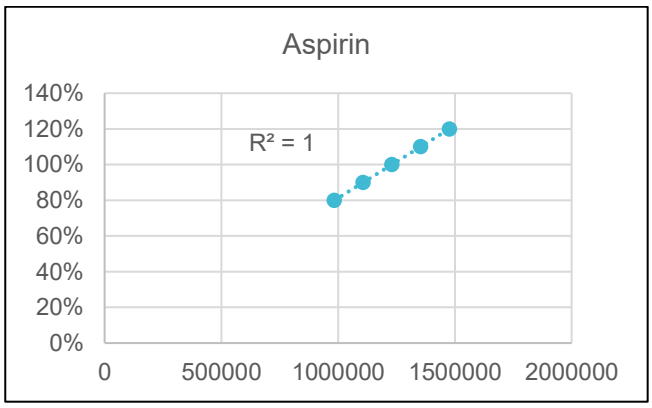
1. Permit the instrument to operate in the chosen conditions.
2. Inject the Blank Solution to verify the system's specificity.
3. Determine the aspirin retention time for system testing by injecting the working standard solution.
4. For system appropriateness, inject the Reference Standard five duplicate. The peak area for the five injections' relative standard deviation (RSD) shouldn't be more than 2.0%.
5. To verify continued system appropriateness, inject the working standard solution after each sequence of ten or fewer injections of samples.
6. For the entire set of Standard injections, the Global RSD shouldn't be more than 2.0%.
7. Use the average of five replicate standard injections to inject each sample twice and calculate the sample size.

RESULTS AND DISCUSSION

LINEARITY

The capacity of an analytical method to produce test findings that are proportional to the concentration of analyte in the sample within a specified range, either directly or through a well stated mathematical transformation, is known as linearity.

Linearity - Aspirin			
Injection	**Area**	**Average**	**Conc.**
1	984640	984560	80%
2	984480		
1	1107720	1107630	90%
2	1107540		
1	1230800	1230700	100%
2	1230600		
1	1353880	1353770	110%
2	1353660		
1	1476960	1476840	120%
2	1476720		
Correlation coefficient		**1.0000**	



Observation & Conclusion: The observed correlation of Co-efficient is 1.000 which meets the acceptance criteria. Hence Analytical method for the determination of Aspirin, in therapeutic dosage meets the Linearity criteria.

SPECIFICITY

Specificity is the ability to access unequivocally an analyte in the presence of an extraneous component that may be expected to be present.

Solutions	Retention Time
Blank	0.2
Standard - Aspirin	4.01
Sample - Aspirin	4.01
Capacity Factor - Aspirin	19.1

Observations & Conclusions: In blank, no peak observed corresponds to the retention time of the peak of Aspirin in standard solution. The analytical method for the determination of Aspirin in therapeutic dosage is specific.

SYSTEM SUITABILITY

The precision of an instrument is the degree of agreement among the replicate injection of the standard.

Injection	Area of Aspirin
1	1230914
2	1229830
3	1231620
4	1230614
5	1230520
Mean	1230700
Standard Deviation	649.79
Relative Standard Deviation (%)	0.05
RT	4.01
RRT	N/A
Theoretical Plates	3347
Tailing Factor	1.01
Resolution	N/A

Observation & Conclusion: The observed RSD of the replicate five standard injections is less than 2.0% which meets the system suitability parameter. Hence Analytical method for the determination of Aspirin in therapeutic dosage meets the system precision criteria.

PRECISION(REPEATIBILITY)

When the procedure is frequently applied to various preparations of a homogenous sample, the precision of an analytical method is measured by the degree of agreement among separate test findings.

Test Preparation	Assays of Aspirin
1	99.2%
2	98.8%
3	97.9%
4	100.3%
5	101.2%
6	100.8%
Mean	99.7%
Standard Deviation	0.013
Relative Standard Deviation (%)	1.28

Observation & Conclusion: The observed Related Standard Deviation of the six different determinations is less than 2.0% which meets the acceptance criteria. Hence Analytical method for the determination of Aspirin, in therapeutic dosage meets the method precision (Repeatability) criteria.

PRECISION(REPRODUCIBILITY)

When an analytical method is used repeatedly on various preparations of a homogenous sample, its precision is measured by the degree of agreement between individual test findings.

Test Preparation	Assays of Aspirin
1	100.0%
2	99.5%
3	98.6%
4	99.9%
5	99.6%
6	99.9%
Mean	99.6%
Standard Deviation	0.005
Relative Standard Deviation (%)	0.52

% Aspirin First Set	% Aspirin Second Set	% Difference between the mean
99.7%	99.6%	0.1%

Observation & Conclusion: The observed Related Standard Deviation of the six different determinations is less than 2.0% which meets the acceptance criteria and also % difference between an average of repeatability and reproductivity is less than 2.0%. Hence Analytical method for the determination of Aspirin, in therapeutic dosage meets the method precision (Reproducibility) criteria.

ACCURACY(RECOVERY)

The degree to which test results produced using the suggested method are near to the actual value is the accuracy of an analytical procedure. Applying the approach to a Placebo that has had known amounts of Analyte added at three concentration levels—80%, 100%, and 120% of test concentration—will help determine the method's accuracy.

Test ID	Analyte	Wt. standard (gm)	Wt. of Placebo (gm)	Amount of standard added in (ml)	% Added	% Recovered	Recovery %
80%-1	Aspirin	0.0375	0.221	4	27.15	27.25	100.4
80%-2	Aspirin	0.0375	0.222	4	27.03	27.12	100.3
80%-3	Aspirin	0.0375	0.225	4	26.67	26.82	100.6
100%-1	Aspirin	0.0375	0.218	5	34.40	34.39	100.0
100%-2	Aspirin	0.0375	0.220	5	34.49	33.98	99.7
100%-3	Aspirin	0.0375	0.221	5	33.94	33.81	99.6
120%-1	Aspirin	0.0375	0.223	6	40.36	40.31	99.9
120%-2	Aspirin	0.0375	0.218	6	41.28	41.12	99.6
120%-3	Aspirin	0.0375	0.221	6	40.72	40.90	100.4

Observation & Conclusion: The observed individual percentage recovery at all the levels i.e. 80%, 100%, and 120% is in the range of 96-104%, and mean recovery at all levels is in the range of 97-103% which meets the acceptance criteria. Hence Analytical method for the determination of Aspirin in therapeutic dosage meets the Accuracy (Recovery) criteria.

RANGE

The degree to which test findings generated using the advised approach are close to the real value is the analytical procedure's accuracy. It will be possible to assess the method's precision by using Placebo to which known amounts of Analyte have been added at three different concentration levels—80%, 100%, and 120% of test concentration.

Observation & Conclusion: The accuracy, precision, and linearity meet the acceptance criteria. Hence range of 80-120% of test concentration is assigned to the Analytical method for the determination of Aspirin in therapeutic dosage.

ROBUSTNESS

An analytical method's robustness, which measures its ability to remain unaffected by minute but intentional changes in method parameters, reveals its dependability under typical conditions. A change in the mobile phase composition, a change in the number of columns, and a change in the flow rate are all used to assess how robust the approach is.

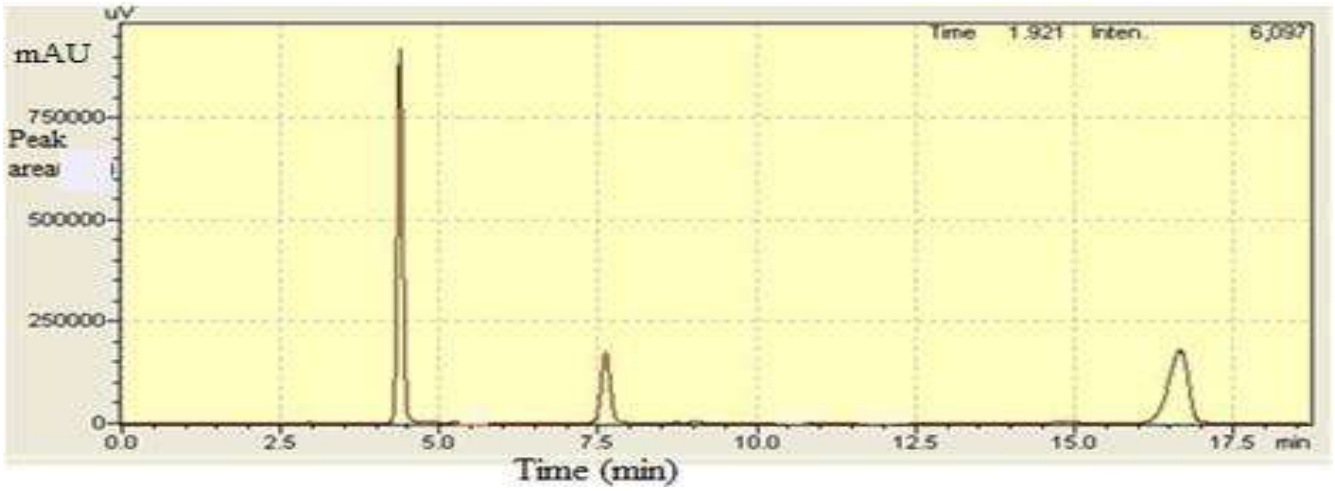
Test ID	Analyte	% Assay First Set - Method Precision	% Assay First Set - Robustness	% Difference
Buffer pH-2.95	Aspirin	99.70	99.60	0.01
Buffer pH-3.05	Aspirin	99.70	99.40	0.30
Mobile phase composition change				
Buffer: CAN (40:60)	Aspirin	99.70	99.10	0.60
Buffer: CAN (50:50)	Aspirin	99.70	98.90	0.80
Change in column lot	Aspirin	99.70	99.10	0.60
Change in column particle size	Aspirin	99.70	99.90	0.30
Change in flow rate 0.9 ml	Aspirin	99.70	98.70	1.00
Change in flow rate 1.1ml	Aspirin	99.70	99.40	0.30

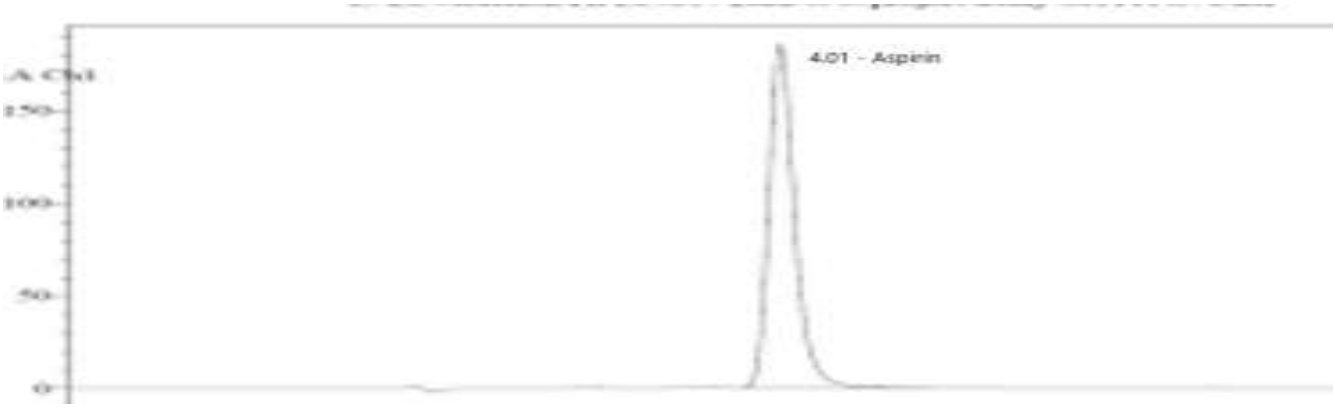
Observation & Conclusion: With all the changes system suitability parameter was achieved and the observed % variation in the result obtained with deliberate change is less than ± 2% which meets the acceptance criteria. Hence Analytical method for the determination of Aspirin in therapeutic dosage meets the Robustness criteria.

SOLUTION STABILITY

According to the stability of the sample and standard solution, it should be stored properly at room temperature or in a refrigerator. The solution stability is the stability of the standard and extracted sample solution (ready to inject) from the sample or matrix and analyzed in accordance with the specified method.

Test id	Analyte	Initial	6 Hours	12 Hours	18 Hours	24 Hours	36 Hours
Solution Stability	Aspirin	99.70%	99.50%	99.20%	99.90%	100.00%	100.20%
% Difference	Aspirin	N/A	0.20%	0.50%	-0.20%	-0.30%	0.32%





CONCLUSION

For Aspirin the RP-HPLC method was created. Column C-18 (250 x 4.6 mm), particle size 5 um, mobile phase water at pH 3.00 with 0.1%(v/v) orthophosphoric acid (v/v) and acetonitrile (45:55) were discovered to be the chromatographic requirements for the optimized procedure. For Aspirin the retention time was determined to be 4.01 min, with an average percentage recovery of 99.9%. It was determined that the suggested approaches complied with ICH criteria. These techniques can be used in the future to regularly determine the presence of Aspirin in bulk medications along with other medicines in combination.

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