WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 19, 686-695. Research Article ISSN 2277–7105

DEVELOPMENT AND VALIDATION OF A SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF ASPIRIN USING FERRIC NITRATE

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Article Received on
10 August 2025,

Revised on 02 Sept. 2025,
Accepted on 23 Sept. 2025

https://doi.org/10.5281/zenodo.17221951

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ABSTRACT

Aspirin (Acetylsalicylic acid) is a widely used analgesic, antipyretic, and anti-inflammatory drug. It acts by inhibiting cyclooxygenase enzymes and thereby reducing prostaglandin synthesis. A novel, simple, and rapid spectrophotometric method was developed and validated for the estimation of Aspirin in pure form and pharmaceutical formulations using ferric nitrate as a complexing agent. Shimadzu UV-1800 Model UV-VIS double beam spectrophotometer was employed for the study. The method is based on the reaction between the hydrolyzed product of Aspirin (salicylic acid) and ferric nitrate to form a violet-coloured complex exhibiting maximum absorbance at 528 nm within the wavelength range of 300–800 nm. Aspirin linearity was observed over the concentration range 10–120 µg/ml. The linear regression equation was found to be $y = 0.0087x – 0.0827$ with correlation coefficient $R^2 = 0.9957$. The method showed a Limit of Detection (LOD) of 3.5 µg/ml and a Limit of Quantification (LOQ) of 10.6 µg/ml, indicating good sensitivity. The method was validated by precision and accuracy studies and was found suitable for routine analysis of Aspirin content in pharmaceutical preparations.

KEYWORDS: Aspirin, Spectrophotometric method, Ferric Nitrate, Salicylic acid, Absorbance, Validation, Analytical method.

INTRODUCTION

Aspirin, chemically known as acetylsalicylic acid, is a widely used non-steroidal anti-inflammatory drug (NSAID) known for its analgesic, antipyretic, anti-inflammatory, and

antiplatelet properties.[1] The preparation procedure involves treating acetylsalicylic with acetic anhydride.[2,3] It is commonly employed in the treatment of mild to moderate pain, fever, inflammation, and in the prevention of thrombotic cardiovascular events due to its ability to inhibit platelet aggregation.[4,5,6] The pharmacological action of aspirin primarily arises from its irreversible inhibition of the cyclooxygenase (COX) enzymes, thereby suppressing the synthesis of prostaglandins and thromboxanes.[7,8] Due to its widespread therapeutic applications and narrow therapeutic index in some cases, the accurate estimation of aspirin content in pharmaceutical formulations is essential to ensure quality, safety, and efficacy. Various analytical techniques are available for this purpose, including titrimetric analysis, high-performance liquid chromatography (HPLC), gas chromatography (GC), and UV-visible spectrophotometry.[9,10,11] Among these, UV-visible spectrophotometry stands out as a cost-effective, simple, and reliable method, particularly suitable for routine quality control in settings with limited access to advanced instrumentation.[7,12] In this study, a colorimetric method is focused on, in which ferric nitrate is added to an aspirin solution, resulting in the formation of a violet-colored complex, the intensity of which is determined by the concentration of aspirin present.

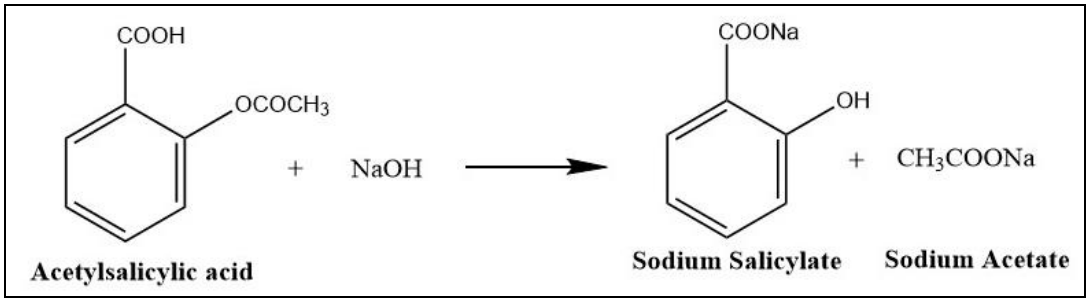

Fig. 1: Decompose Aspirin to Sodium Salicylate.

This effect is observed because aspirin is known to undergo hydrolysis in the presence of moisture or alkaline conditions, leading to the formation of salicylic acid and acetic acid. The salicylic acid produced, containing a phenolic group, is reacted with ferric ions to form the violet complex. In alkaline medium such as sodium hydroxide, especially when heat is applied, hydrolysis is accelerated, and sodium salicylate along with sodium acetate is formed. The violet-colored complex is then immediately formed when ferric nitrate is added to sodium salicylate.[13]

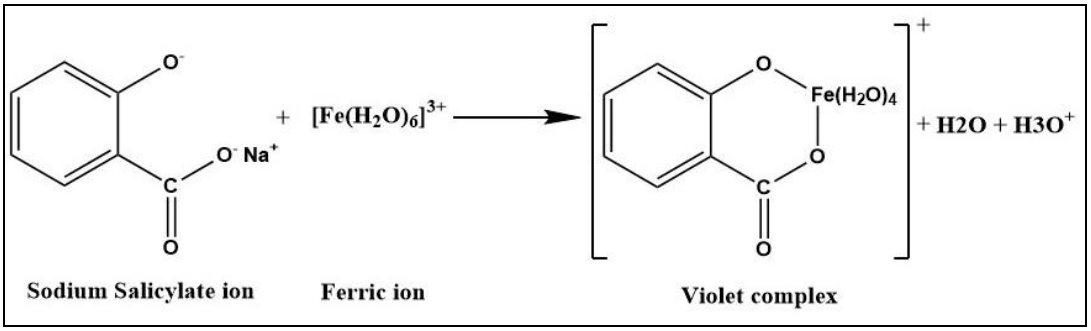

Fig. 2: Formation of violet complex from Sodium salicylate ion and ferric ion.

Since this reaction is dependent on the interaction between ferric nitrate and the salicylate ion, sodium salicylate is used as a standard solution to allow for a comparative analysis with aspirin. To determine the concentration of aspirin, a calibration curve is prepared using standard solutions of known concentration, allowing the concentration of unknown samples to be estimated. Alternatively, the standard addition method is employed, where a known amount of standard is added to the unknown, and the resulting response is measured for comparison.[14,15,16] The method development includes optimization of critical parameters such as reaction conditions, reagent concentrations, and wavelength of maximum absorbance. The proposed method was validated in accordance with International Council for Harmonisation (ICH) guidelines by evaluating its linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness and ruggedness. This study aims to develop and validate a simple, rapid, and sensitive UV visible spectrophotometric method for the quantitative estimation of aspirin using ferric nitrate as the chromogenic reagent. The proposed method provides a practical alternative to more sophisticated techniques, making it especially valuable for use in academic, industrial, and quality control laboratories with limited resources. Furthermore, the underlying principle of this method can be extended to the analysis of other phenolic compounds, thereby enhancing its potential utility in broader pharmaceutical applications.

MATERIALS AND METHODS

Equipments

A UV–Visible Spectrophotometer (Shimadzu UV-1800) was employed for absorbance measurements using matched quartz cuvettes of 10 mm path length, a digital weighing balance (Accurate Weighing System AWT 300) was used for weighing, Heating mantle of Indosati make, volumetric flask and a pipette were utilized for sample preparation.

Materials and Reagents

All the chemicals used were of analytical reagent grade. Aspirin standard was obtained from Oxford Lab Fine Chem LLP and commercial aspirin tablets were procured from USV Pvt. Ltd. Ethanol was supplied by Loba Chemie Pvt. Ltd., ferric nitrate solution by Isochem Laboratories, sodium hydroxide by Nice Chemical (P) Ltd., and distilled water was used throughout the experiment.

Assay of Aspirin

An accurately weighed quantity of 1.5 g of aspirin was dissolved in 15 mL of ethanol, since aspirin is only slightly soluble in water. To this solution, 50 mL of 0.5 M sodium hydroxide was added, and the mixture was gently boiled for 10 minutes. After cooling, the excess alkali was titrated against 0.5 M hydrochloric acid using phenol red as an indicator, with the endpoint indicated by the appearance of a reddish violet color. A blank determination was carried out under identical conditions using 15 mL of ethanol and 50 mL of 0.5 M sodium hydroxide without aspirin, followed by boiling, cooling, and titration with 0.5 M hydrochloric acid. The difference in the volume of hydrochloric acid consumed in the blank and sample titrations corresponded to the amount of sodium hydroxide that had reacted with aspirin.

Preparation of 1% $Fe(NO_3)_3$ solution

A weighed quantity of 10 g of ferric nitrate was accurately taken and dissolved in approximately 800 ml of deionized water. The solution was then transferred to a 1000 ml volumetric flask and the volume was made up to the mark with deionized water. The contents were mixed thoroughly to ensure uniformity.

Preparation of stock solution of Aspirin

100mg of aspirin was taken and dissolved it in 15 mL of 0.1 N NaOH. The solution was gently heated to ensure complete dissolution, and then the volume was made up to 100 mL in a volumetric flask. This gave a stock solution with a concentration of 1000 µg/ml.

Preparation of sample solution of Aspirin

From the above stock solution, 8 mL was pipetted into a new flask, and 2 mL of 1% $Fe(NO_3)_3$ solution was added. The volume was then made up to 100 mL with distilled water to prepare a solution of 80µg/ml. Then the % purity was calculated by standard curve method, based on the linear equation $y = mx + c$.

Validation Parameters

The method was validated according to ICH Q2B guidelines for validation of analytical procedures of analytical procedures in order to determine the accuracy, linearity, precision, robustness, ruggedness of solution.[17]

Linearity

Six concentrations (20, 40, 60, 80, 100, and 120 µg/mL) were prepared, and their absorbance was measured using UV spectroscopy at 528 nm. Then, the slope, intercept, co-relation coefficient, standard error of intercept, standard deviation of intercept was calculated by plotting the absorbance values on the linearity curve.

Accuracy

Different concentrations (40, 80, 120 ppm) were prepared from a 1000 ppm stock solution in 10 mL volumetric flasks. The volume was made up to 10 mL for each, and three absorbance readings were taken for each concentration and % of recovery was calculated.

Precision

0.8 mL of 1000 ppm stock solution was taken and the volume was made up to 10 mL in a volumetric flask. The absorbance (528nm) of this solution (same concentration) was measured five times, and the % of RSD was calculated.

Robustness

In this process, the absorbance of a specific concentration (80µg/mL) was measured three times at different wavelengths (527, 528, and 529 nm), different strength of NaOH (0.09, 0.1, and 0.11 N), and different volumes of $Fe(NO_3)_3$ solution (0.9ml, 1ml, and 1.1ml). The % of RSD (Relative Standard Deviation) was then calculated.

Ruggedness

In this process, readings were taken in two different laboratories by two different analysts using the same concentration (80 µg/mL). Absorbance was measured six times for each, and the % of RSD (Relative Standard Deviation) was calculated for each set of readings.

RESULT AND DISCUSSION

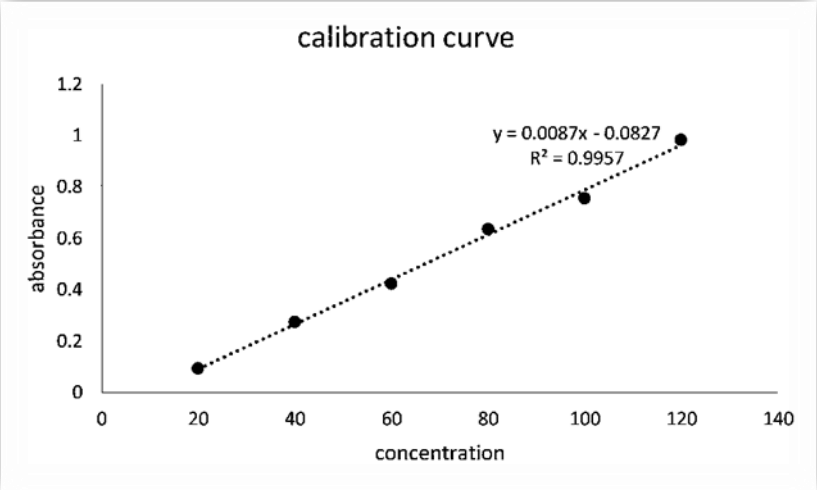

Fig.3: Calibration curve of aspirin.

Standard deviation of intercept = Standard error of intercept x √n = 0.00376486 x √6 = 0.009222.

Table 1: Linearity of Aspirin.

Concentration (µg/ml)	Absorbance
20	0.094
40	0.275
60	0.423
80	0.635
100	0.756
120	0.985

Table 2: System suitable parameters of Aspirin.

Optical character	Results
λmax	528 nm
Beer law limit(µg/ml)	20-120
Slope	0.0087
Correlation coefficient	0.9957

Table 3: LOD and LOQ of Aspirin.

Parameters	Aspirin(µg/ml)
LOD	3.5
LOQ	10.6

LOD and LOQ of proposed UV method was found to be 3.5 and 10.6 µg/ml.

Accuracy

Table 4: Accuracy of Aspirin.

Sample level %	Amount taken (µg/ml)	Absorbance	% Recovery	Range
50	40	0.260	98.55 %	98.5-102.8 %
50	40	0.267	100.5 %	
50	40	0.275	102.8 %	
100	80	0.632	102.36 %	98.78-102.36 %
100	80	0.629	101.92 %	
100	80	0.614	98.78 %	
150	120	0.990	102.3 %	98.7-102.3 %
150	120	0.974	100.77 %	
150	120	0.963	98.73 %	

The accuracy of an analytical method is the closeness of the test result to the a true value. A standard quantity equivalent to 80%, 100%, 120%. Result within the range of 98-101%.

Precision

Table 5: Precision of Aspirin.

Concentration (µg/ml)	Absorbance	% Recovery	% RSD
80	0.621	100.78 %	0.0345
80	0.620	100.65 %	
80	0.628	101.78 %	
80	0.612	99.50 %	
80	0.628	101.78 %	

Precision were found to be by %RSD.

Robustness

The robustness of the method was tested in terms of variation in wavelength, amount of 0.1N NaOH added, $FeCl_3$ added.

Table 6: Evaluation of Aspirin Robustness at various wavelengths.

Wavelength	Concentration (µg/ml)	Absorbance	% Recovery	% RSD
527	80	0.635	102.801 %	0.815 %
		0.635	102.801%	
		0.634	102.730 %	
528	80	0.643	104.011 %	
		0.635	102.801 %	
		0.629	102.033 %	
529	80	0.622	101.011 %	
		0.635	102.801 %	
		0.639	103.402 %	

The %RSD of variations across three different wavelengths was found to be **0.815%.**

Table 7: Evaluation of Aspirin Robustness at varying strength of NaOH.

NaOH	Concentration (µg/ml)	Absorbance	% Recovery	% RSD
0.09	80	0.622 0.639 0.635	101.011 % 102.402% 103.801 %	1.002 %
0.1	80	0.633 0.635 0.629	102.604 % 103.801 % 102.030 %	
0.11	80	0.635 0.643 0.634	103.403 % 104.011 % 102.733 %	

The %RSD of variations across three different normalities of NaOH was found to be **1.002%.**

Table 8: Evaluation of Aspirin Robustness at varying volumes of $Fe(NO_3)_3$ solution.

$Fe(NO_3)_3$	Concentration (µg/ml)	Absorbance	% Recovery	% RSD
0.9	80	0.633 0.635 0.629	102.604 % 103.801% 102.030 %	0.745 %
1	80	0.622 0.635 0.639	101.011 % 102.801 % 103.404 %	
11	80	0.635 0.635 0.634	102.802 % 102.806 % 102.730 %	

The %RSD of variations across three different volume of $Fe(NO_3)_3$ was found to be **0.745%.**

Ruggedness

Table 9: Ruggedness of Aspirin.

Parameter		Concentration (µg/ml)	Absorbance	% Recovery	% RSD
Different lab	1	80	0.622 0.635 0.639	101.011 % 102.802% 103.404 %	0.013741
	2	80	0.628 0.612 0.628	101.780 % 99.501 % 101.780 %	
Different analyst	1	80	0.632 0.629 0.614	102.361 % 101.922 % 98.784 %	0.020276
	2	80	0.635 0.643 0.634	103.403 % 104.011 % 102.733 %	

The results of the method development and validation show that the aspirin assay using Ferric Nitrate reagent is accurate, precise, and robust. The standard curve is linear over the range of concentrations tested, and the accuracy is within acceptable limits that is determined by

%Recovery. The precision of the method is also within acceptable limits as determined by the %RSD that is less than 2. The LOD and LOQ values indicate that the method was sensitive.

CONCLUSION

Based on the experimental results, it can be concluded that the newly proposed spectrophotometric method for the determination of aspirin is rapid, accurate, economical, and a viable alternative to HPLC methods. The method utilizes the reaction of sodium salicylate with ferric ions to form a violet-colored complex that absorbs in the visible region, thereby eliminating the need for costly UV detection. Owing to its simplicity, sensitivity, selectivity, and cost-effectiveness, the proposed method can be used for quality control purposes in the pharmaceutical industry.

ACKNOWLEDGEMENT

The authors are grateful to Haldia Institute of Pharmacy management for providing the facilities such as equipment and chemicals to complete the work. There is no conflict of interest.

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