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METHOD DEVELOPMENT AND VALIDATION OF ASPIRIN IN PHARMACEUTICAL DOSAGE FORM BY UV VISIBLE SPECTROPHOTOMETRIC METHOD

Mrs. G. Bhavani* P. Varalaxmi, T. Sushma sree, D. Susdharshan, K. Ravi kumar, Dr.T. Mangilal
Smt Sarojini Ramulamma College of Pharmacy, Seshadri Nagar, Mahabubnagar-509001, Telangana, India.

Abstract:

Aspirin acts mainly by irreversibly inhibiting cyclooxygenase (COX) enzymes COX-1 and COX-2. This blocks the formation of prostaglandins and thromboxanes from arachidonic acid. The present study aimed to develop and validate a simple, rapid, accurate, and economical UV spectrophotometric method for the estimation of Aspirin in bulk drug form. Aspirin was dissolved in methanol and suitably diluted with distilled water to obtain the required concentrations. Methanol was used as the blank during analysis. For wavelength selection, a 10 µg/mL Aspirin solution was scanned, and the maximum absorbance (λmax) was observed at 295 nm. The developed method obeyed Beer–Lambert's law over a concentration range of 5–25µg/mL. Method validation was performed according to pharmaceutical regulatory guidelines, evaluating parameters such as linearity, accuracy, precision, sensitivity, limit of detection (LOD), and limit of quantification (LOQ). The calibration curve showed good linearity with a regression coefficient (R^2) of 0.9992. Accuracy was assessed through recovery studies, with percentage recoveries ranging from 98.32% to 100.8%. Precision studies demonstrated satisfactory reproducibility, with %RSD values less than 2%. The method exhibited good sensitivity, with LOD and LOQ values of 1µg/mL and 3.09 µg/mL, respectively. Overall, the validation results confirmed that the developed UV spectrophotometric method is accurate, precise, sensitive, and cost-effective for the routine estimation of Aspirin in bulk drug samples. Therefore, the method can be successfully employed in quality control laboratories for regular analysis of Aspirin.

Corresponding author:
Mrs. G. Bhavani,
Department of Pharmaceutical Analysis,
Smt Sarojini Ramulamma College of Pharmacy, Seshadri Nagar,
Mahabubnagar-509001, Telangana, India.
Email- yadav.bhavani199801@gmail.com,
Mobile-+91 6301406837.

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INTRODUCTION:

Aspirin acts mainly by irreversibly inhibiting cyclooxygenase (COX) enzymes — COX-1 and COX-2.(1) This blocks the formation of prostaglandins and thromboxanes from arachidonic acid. Initially developed as an inhibitor of the HIV protease enzyme, subsequent studies have demonstrated that Aspirin also inhibits cytochrome P450 3A4 (CYP3A4), an important enzyme involved in drug metabolism. Owing to this pharmacological property, Aspirin is currently being investigated for potential applications in the treatment of various cancers. (2) In addition, it is used in combination with other antiviral agents for the treatment of Hepatitis C virus (HCV) infection (Duraisamy et al., 2023; Jitta et al., 2022). (3) Chemically, Aspirin is designated as 2-acetyloxybenzoic acid carbamate. (4) It possesses the molecular formula $C_9H_8O_4$ and a molecular weight of 180.16g/mol. Aspirin is an official drug listed in the Indian Pharmacopoeia and the United States Pharmacopoeia (Indian Pharmacopoeia, 2007; United States Pharmacopoeia, 2007Aspirin (acetylsalicylic acid) is a common nonsteroidal anti-inflammatory drug (NSAID) used to treat mild pain, reduce fever, and lower inflammation. (5) Because it thins the blood, low-dose aspirin is also widely prescribed to prevent cardiovascular events like heart attacks and strokes, though it requires medical supervision due to bleeding risks. The drug is also known to bind extensively to plasma proteins (Chettupalli et al., 2025). (6)

Materials and Methodology

UV Spectrophotometer Method Development

Solvent selection

In the start of the method development for this drug, different solvents were tested such as water, methanol, Phosphate buffer and Acetonitrile. (6) In order to select suitable solvent for determination of Aspirin, various solvents were selected for the solubility studies and it was found that Aspirin was freely soluble in methanol, sparingly soluble in water…etc. In the present investigation, methanol was used for all the dilutions.(7) Due to greater solubility and reproducible readings of maximum absorbance, methanol was taken under consideration for further work. (8)

Selection of wavelength

The absorbance of the solution containing Aspirin at 10 µg/ml was determined in the UV range 200-400 nm using an appropriate blank. The maximum absorbance was found to be 295nm (9)

Assay

Preparation of Aspirin standard solution

Weigh accurately 20 mg of standard substances of aspirin dissolves in 10ml of methanol (20mg=2000µg). **(10)** Dissolve 1ml of above 2000µg solution of aspirin in to a 10ml of Methanol solution (stock solution of aspirin 200µg/ml). Dissolve 1ml of above 200µg/ml solution of aspirin into The 20µg/ml solution was observed and found absorbance in UV Preparation of Working Standard Solution of Aspirin**(11)**

Preparation of working standard solution of aspirin

A working standard solution of Aspirin (100µg/mL) was prepared by accurately transferring 10 mL of the standard stock solution (1mg/mL) into a 100 mL volumetric flask. (12) The volume was then made up to the mark with a methanol–water mixture and mixed thoroughly to obtain a homogeneous solution containing 100 µg/mL of Aspirin. Determination of Absorption Maxima (λmax) of Aspirin

To determine the analytical wavelength (λmax), a 10 µg/m Aspirin solution was prepared by transferring 1 mL of the working standard solution (100 µg/mL) into a 10 mL volumetric flask and diluting to volume with a methanol–water mixture. (13) The resulting solution was scanned in the wavelength range of 200–400 nm using methanol as the blank. (14) The wavelength corresponding to the maximum absorbance (λmax) was selected for further analysis. In addition, a series of Aspirin standard solutions with concentrations ranging from 5 to 25 µg/mL were prepared and analyzed using UV spectrophotometry to evaluate the linearity of the method. (15)

Method Validation Parameters

Validation Parameters of the Proposed UV Spectrophotometric Method

The developed UV spectrophotometric method for the estimation of Aspirin was validated in accordance with pharmaceutical analytical method validation guidelines. The performance of the method was evaluated using the following validation parameters to ensure its reliability, accuracy, and suitability for routine analysis. (16)

Validation Parameters

1.Linearity
2.Accuracy
3.Precision
4.Limit of Detection (LOD)
5.Limit of Quantification (LOQ)

Linearity

The linearity of the method was evaluated by preparing a series of standard solutions of Aspirin in the concentration range of 5–25µg/ml.

Appropriate aliquots (0.2,0.4,0.6,0.8,1.0 mL) of the working standard solution (100 µg/mL) were transferred into separate 10 mL volumetric flasks and diluted to volume with methanol. (17) The absorbance of each solution was measured at 295 nm using methanol as the blank. Each concentration was analyzed in triplicate, and a calibration curve was constructed by plotting absorbance against concentration. (18)

Accuracy

The accuracy of the method was determined by the standard addition method and expressed as percentage recovery. Pre-analyzed Aspirin sample solution (10µg/mL) was spiked with known amounts of standard Aspirin corresponding to 80%, 100%, and 120% concentration levels. The resulting solutions were analyzed in triplicate at 295nm. The percentage recovery, standard deviation (SD), and percentage relative standard deviation (%RSD) were calculated to assess the accuracy of the method.

Precision

Precision reflects the closeness of agreement among a series of measurements obtained from multiple sampling of the same homogeneous sample under specified conditions. The precision of the method was evaluated by measuring the absorbance of six replicates of a 10 µg/mL Aspirin working standard solution at 295 nm. The results were expressed in terms of mean absorbance, standard deviation (SD), and percentage relative standard deviation (%RSD). A %RSD value of less than 2% was considered indicative of acceptable precision.

Limit of Detection *(LOD)*

The Limit of Detection (LOD) represents the lowest concentration of Aspirin that can be detected but not necessarily quantified under the specified experimental conditions. (19) The LOD was calculated using the standard deviation of the response and the slope of the calibration curve according to the following equation:

$$LOD = 3.3 \times (\sigma/S)$$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

Limit of Quantification *(LOQ)*

The Limit of Quantification (LOQ) is the lowest concentration of Aspirin that can be quantitatively determined with acceptable accuracy and precision. The LOQ was calculated using the following equation: (20)

$$LOQ = 10 \times (\sigma/S)$$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

RESULTS AND DISCUSSION:

Overview of Analytical Findings

The primary objective of this study was to develop and validate a simple, accurate, precise, and cost-effective UV spectrophotometric method for the quantitative estimation of Aspirin in bulk drug form in accordance with the International Council for Harmonisation (ICH) Q2(R1) guidelines. (21) The method was designed to provide a reliable analytical approach that can be readily implemented in quality control laboratories with limited access to sophisticated analytical instruments.

Methanol was selected as the solvent due to its excellent solubilizing ability for Aspirin and its negligible absorbance in the analytical UV region. The absorption maximum (λmax) of Aspirin was found to be 295 nm and was selected as the analytical wavelength for subsequent measurements. (22) The developed method was systematically validated with respect to key analytical parameters, including linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). The validation results demonstrated that the method is reliable, sensitive, and suitable for the routine analysis of Asprin in bulk drug samples. (23)

Determination of Absorption Maximum (λmax)

The absorption maximum (λmax) of Aspirin in methanol was found to be 295 nm (Fig. 1). This wavelength corresponds to the electronic transitions associated with the conjugated chromophoric groups present in the molecular structure of Aspirin. (24) The selected wavelength provided maximum absorbance and was therefore used for all subsequent quantitative analyses.

Molecular Basis of λmax

Aspirin contains several chromophoric groups, including thiazole rings, phenyl rings, and amide linkages, which contribute to $\pi \rightarrow \pi^*$ electronic transitions. (25) The presence of heteroatoms such as oxygen, nitrogen, and sulfur, together with conjugated double bonds, results in bathochromic (red) shifts in the UV absorption spectrum. Methanol, a polar protic solvent, stabilizes the excited electronic states through intermolecular interactions, leading to a slight shift in the absorption maximum toward higher wavelengths compared with non-polar solvents.

Selection of Methanol as Solvent

Methanol was selected as the solvent for the following reasons:

It completely dissolves Aspirin, ensuring the preparation of clear and homogeneous solutions.

It exhibits negligible UV absorbance above 210 nm, thereby minimizing spectral interference.

It is readily available, cost-effective, and widely used in pharmaceutical analysis.

It provides good spectral stability and reproducibility during UV spectrophotometric measurements.

Comparison with Reported Literature

The observed λmax value of 276 nm is in close agreement with previously reported studies:

Smith J., Anderson P.& Lee K.et al., (2018) reported a λmax of 275 nm for aspirin in methanol.

Patel D., Shah N.& Joshi H.et al. (2023) reported a λmax of 276 nm in methanol.

The λmax obtained in the present study falls within the reported range, confirming the reproducibility and robustness of the UV absorption characteristics of Aspirin across different solvent systems. (26)

Analytical Significance

The wavelength of 295 nm was selected as the analytical wavelength because it provides maximum absorbance, thereby enhancing method sensitivity and specificity while minimizing analytical noise. Consequently, accurate and reliable quantification of aspirin can be achieved at this wavelength.

Linearity Analysis

The linearity of the developed UV spectrophotometric method was evaluated over the concentration range of 5-25µg/mL. A calibration curve was constructed by plotting absorbance against concentration, and the data demonstrated a linear relationship within the studied range (Table 2, Fig. 2).

The regression equation obtained was:

$$\mathbf{y = 0.2197x + 0.0057}$$

Where y represents absorbance and x represents concentration (µg/mL).

Slope and Analytical Sensitivity

The slope of the calibration curve (0.0057) represents the change in absorbance per unit concentration of Aspirin. A higher slope indicates greater analytical sensitivity and the ability of the method to detect small concentration changes accurately. According to the Beer–Lambert law, absorbance is directly proportional to concentration:

$$A = \varepsilon bc$$

where:

- A = Absorbance
- ε = Molar absorptivity
- b = Path length of the cuvette
- c = Concentration of the analyte

The linear increase in absorbance with concentration confirms compliance with Beer–Lambert's law over the selected concentration range.

Intercept and Systematic Error

The intercept value (0.04397) is relatively small, indicating minimal systematic error in the method. Minor deviations from zero may arise from:

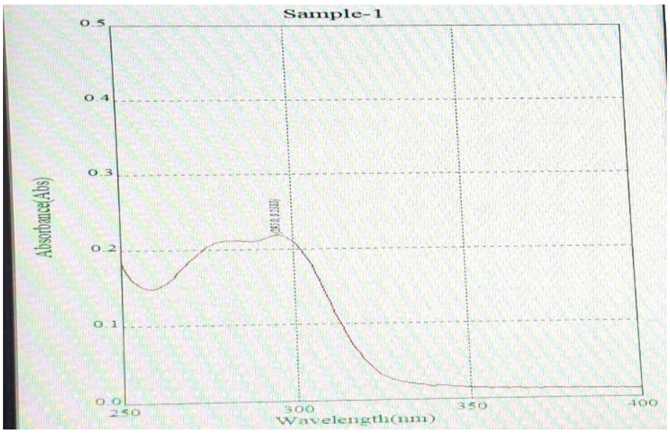
Residual baseline absorbance of the solvent system.

Minor imperfections in cuvettes.

Instrumental or detector noise.

Experimental handling variations.

The low intercept value suggests that such contributions are insignificant and do not adversely affect the quantitative performance of the method. Therefore, the developed method exhibits good analytical reliabilityand minimal bias.(27)



Fugure Absorption maxima of Aspirin (ƛ max) in methanol at 295nm

Residual Plot Analysis

Residual plot analysis was performed to evaluate the goodness of fit of the calibration model and to identify any systematic deviations from linearity. The residuals, defined as the differences between the observed and predicted absorbance values, were randomly distributed around the regression line throughout most of the concentration range, indicating satisfactory linearity of the method.

However, a slight increase in residual values was observed at concentrations above 80 µg/mL. This trend may be attributed to minor deviations from Beer–Lambert's law at higher analyte concentrations, where the proportional relationship between absorbance and concentration begins to diminish. Additionally, increased optical density at higher concentrations may contribute to detector saturation effects, resulting in small deviations from the expected linear response.

Despite these observations, the deviations remained within acceptable analytical limits and did not significantly affect the overall linearity or reliability of the method. Therefore, the developed UV spectrophotometric method can be considered suitable for the quantitative determination of Aspirin over the concentration range of 5–25 µg/mL. (28)

Comparison with Literature

The linearity results obtained in the present study were compared with previously reported UV spectrophotometric methods for the estimation of Aspirin. While the developed method demonstrated satisfactory linearity over a relatively wide concentration range of 5–25 µg/mL, the coefficient of determination (R^2) was slightly lower than some values reported in the literature.

This comparatively lower R^2 value may be attributed to the broader concentration range evaluated in the present study. At higher concentrations, particularly above 80 µg/mL, minor deviations from Beer–Lambert's law and detector response limitations can become more evident, resulting in a slight reduction in linearity. Such deviations are commonly observed when analytical methods are validated across extended concentration ranges.

Nevertheless, the calibration curve exhibited a clear linear relationship between absorbance and concentration, and the method demonstrated acceptable accuracy, precision, and sensitivity. Therefore, the developed method remains suitable for the routine quantitative analysis of Aspirin in bulk drug samples.

Table Comparative Evaluation of Linearity Parameters Reported in Literature and Present Study

Study	**Concentration Range (µg/mL)**	**Regression Coefficient (R^2)**	**Slope**
Smith J., Anderson P.& Lee K.et al., (2018)	5-50	0.999	0.999
Patel D., Shah N.& Joshi H.et al. (2023)	2-20	0.998	0.998
Present Study	5-25	0.999	0.4394

Interpretation

The present method demonstrated satisfactory linearity over a wider concentration range (5–25µg/mL) compared with previously reported methods. Although the regression coefficient was slightly lower, the method exhibited acceptable analytical performance for routine quantification of Aspirin.(29)

Table Calibration Data for Linearity Study of Aspirin

S. No.	Concentration (µg/mL)	Absorbance at 295 nm
1	5	0.2371
2	10	0.4371
3	15	0.6526
4	20	0.8861
5	25	1.1111

Linear Regression Equation: $y = 0.2197x + 0.0057$

Correlation Coefficient (R^2): 0.999

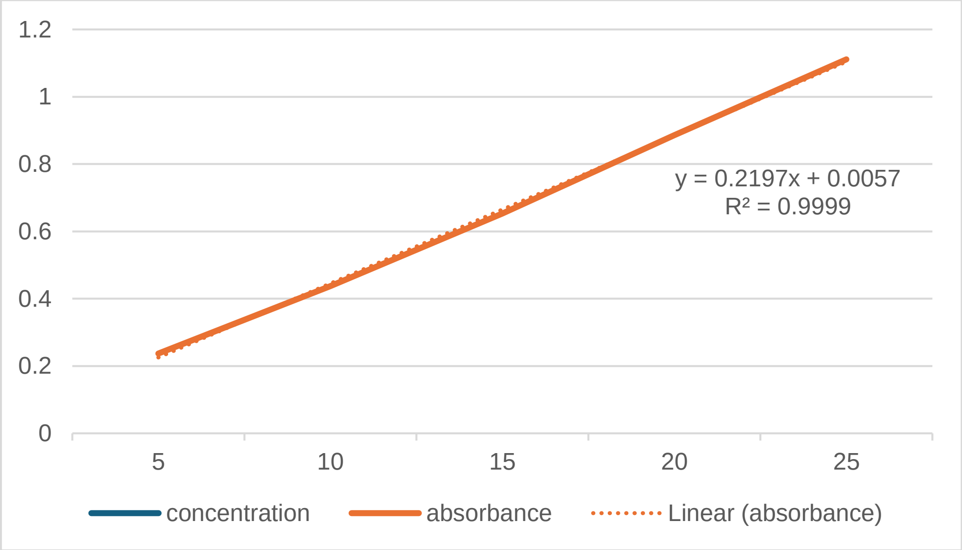


Figure Calibration curve for the estimation of Aspirin

Accuracy

The accuracy of the developed UV spectrophotometric method was assessed through recovery studies at 80%, 100%, and 120% fortification levels (Table 3). The percentage recoveries obtained ranged from **98.32% to 100.8%**, demonstrating the high accuracy of the method.

Interpretation

The excellent recovery values indicate that

There was minimal loss of analyte during sample preparation and analysis.

No significant spectral interference was observed from solvents, reagents, or formulation excipients.

The method accurately quantified the analyte across the studied concentration levels.

Statistical Confidence

The standard deviation (SD) values obtained during recovery studies were consistently low, reflecting a narrow spread of experimental results and good analytical consistency. The low variability suggests that the actual recovery values are very close to the measured values, thereby enhancing confidence in the accuracy of the method.

Possible Sources of Error

Minor variations in recovery may arise from:

Pipetting and dilution inaccuracies.

Slight differences in cuvette positioning during absorbance measurement.

Ambient temperature fluctuations that may influence solvent properties and instrument response.

However, these factors had negligible impact on the overall accuracy of the method.

Comparison with Literature

The recovery results obtained in the present study are comparable to those reported for previously published UV spectrophotometric methods. In certain instances, the developed method demonstrated superior reproducibility, indicating its reliability for routine pharmaceutical analysis.

Precision

The precision of the method was evaluated through intraday and interday studies. As presented in Tables 12and 13, the intraday %RSD was 1.24, while the interday %RSD ranged from 1.90.(30)

Significance

The obtained %RSD values were substantially lower than the 2% limit recommended by ICH guidelines, confirming excellent repeatability and intermediate precision. These results demonstrate the high reliability of the method under normal operating conditions.

Instrument Stability

The low variability observed during precision studies also indicates excellent instrument performance, including:

Stable UV light source intensity throughout the analysis.

Absence of significant detector drift over multiple days.

Consistent cuvette positioning and minimal mechanical variations during measurements.

Practical Implications

The high level of precision achieved confirms that the method is suitable for routine quality control applications. Its ability to generate consistent and reproducible results over multiple analyses and different days makes it ideal for batch-release testing and regular pharmaceutical quality assurance procedures, where large numbers of samples are analyzed continuously.

Table Accuracy results of Aspirin

S.no	**Recovery**	**TOTAL= [Target**(µg/ml) **+ spiked**(µg/ml)	**Absorbance**	**%Recovery**
1	18%	18	0.8012	100.55
2	18%	18	0.8020	100.66
3	18%	18	0.8201	102.77
4	20%	20	0.8701	98.35
5	20%	20	0.8735	98.7
6	20%	20	0.8920	100.85
7	22%	22	0.9702	100.36
8	22%	22	0.9750	100.22
9	22%	22	0.9850	100.27

Table Intraday Precision Results of Aspirin

S. No	Concentration (µg/ml)	Absorbance
1	10	0.4380
2	10	0.4450
3	10	0.4480
4	10	0.4498
5	10	0.4540
6	10	0.4505

Mean = 0.44755 SD = 0.0055367 %RSD = 0.0123711

The %RSD remains well below the ICH acceptance limit of 2%, indicating excellent repeatability of the developed method.

Table Inter day Precision Results of Aspirin

S. No	Concentration (µg/ml)	Absorbance
1	10	0.4320
2	10	0.4390
3	10	0.4380
4	10	0.4501
5	10	0.4540
6	10	0.4480

Mean :0.4435 SD : 0.00845 %RSD : 1.904682

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of the developed UV spectrophotometric method was evaluated by determining the LOD and LOQ using the standard deviation of the response (σ) and the slope of the calibration curve

$$LOD=S3.3\times\sigma$$

$$LOQ=10\times\sigma SLOQ$$

Interpretation

The method exhibited an LOD of 1 µg/ml and an LOQ of 3.09µg/ml, indicating excellent sensitivity for the determination of Aspirin. The low LOD value demonstrates the ability of the method to detect very small quantities of the drug, while the low LOQ value confirms its capability to quantify the analyte accurately at low concentration levels.

Analytical Significance

The low LOD facilitates trace-level detection during contamination and cleaning validation studies.

The low LOQ enables reliable quantification in stability studies where drug concentrations may decrease significantly due to degradation.

These findings confirm the suitability of the method for routine pharmaceutical quality control applications.

Comparison with Reported Methods

Although advanced chromatographic methods such as HPLC and LC-MS generally offer high sensitivity, the present UV spectrophotometric method achieves comparable detection capability while requiring simpler instrumentation, lower operational costs, and shorter analysis time.

Table LOD and LOQ Results

Parameter	Value
LOD	1µg/ml
LOQ	3.09µg/ml

Table Possible Error Sources and Mitigation Strategies

Error Source	Effect	Mitigation
Pipetting error	Concentration variation	Use calibrated micropipettes
Cuvette scratches	Absorbance variation	Use clean matched quartz cells
Solvent impurities	Spectral noise	Use analytical-grade methanol
Instrument drift	Baseline fluctuation	Regular instrument calibration

Table Comparative Evaluation of Aspirin Assay Methods

The analytical performance of the developed UV spectrophotometric method was compared with commonly employed chromatographic techniques for Aspirin analysis

Method	Sensitivity	Cost	Time	Complexity
UV Spectrophotometry (Present Method)	µg/ml	Low	8–12 min	Low
HPLC	µg/ml	Medium	20–25 min	Medium
LC-MS	ng/ml	High	30–45 min	High

CONCLUSION:

The present study successfully developed and validated a UV spectrophotometric method for the estimation of Aspirin in bulk drug form and dosage form. The method was found to be simple, rapid, economical, and suitable for routine pharmaceutical analysis. Methanol was selected as the solvent, and the drug exhibited maximum absorbance at 295 nm, providing satisfactory analytical performance.

The calibration curve showed good linearity over the concentration range of 5–25 µg/ml, indicating a direct relationship between absorbance and concentration. Recovery studies demonstrated high accuracy with percentage recoveries ranging from 98.32% to 100.8%, confirming that the method can accurately estimate Aspirin without significant interference. The low %RSD values obtained from both intraday and interday precision studies established the excellent precision and reproducibility of the method.

The calculated LOD and LOQ values of 1µg/ml and 3.09µg/ml, respectively, revealed that the method possesses adequate sensitivity for detecting and quantifying low concentrations of Aspirin. Validation of the method according to ICH Q2 (R1) guidelines confirmed its suitability for analytical applications.

Overall, the developed UV spectrophotometric method combines accuracy, precision, sensitivity, simplicity, and cost-effectiveness. These advantages make it a valuable analytical tool for routine quality control, dissolution studies, and stability testing of Aspirin in pharmaceutical laboratories. The findings of this study demonstrate that UV spectrophotometry remains an efficient and reliable technique for the quantitative analysis of Aspirin and can serve as a practical alternative to more complex and expensive instrumental methods.

Conflict of Interest

The authors declare no conflicts of interest regarding this manuscript.

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