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Assessment of Analytical Methods for Aspirin Tablets: Impact of Organic Acid based Excipients on Titrimetric, UV–Vis, and HPLC Accuracy and Precision

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ABSTRACT

Aspirin (acetylsalicylic acid, ASA) remains one of the most widely used analgesic and anti-inflammatory agents, making accurate and reliable quantification in pharmaceutical formulations a critical quality control requirement. Classical titrimetric assays using sodium hydroxide exploit the carboxylic acid functionality of ASA, but their lack of selectivity leads to overestimation in multicomponent formulations where organo-acidic excipients also consume the base. In this study, six commercial aspirin tablet formulations from established manufacturers were comparatively analyzed using Titrimetric, UV–Vis Spectrophotometry with multivariate calibration, and High Performance Liquid chromatographic (HPLC). Results indicates that titrimetric assays required complex stoichiometric corrections, manipulations and produced inconsistent values in formulations containing interfering acidic excipients. The Spectrophotometric method enabled rapid screening but often produced values near or below the lower pharmacopeial limits, indicating limited robustness in complex formulations. Notably, the spectrophotometry method showed high variability and marked inaccuracy in one sample (−32.8%), emphasizing its susceptibility to matrix effects. By contrast, the chromatographic (HPLC) technique provided superior resolution, precision, and accuracy, consistently yielding results within United States Pharmacopeia (USP) and British Pharmacopoeia (BP) limits. The findings also highlight the trade-off between simplicity and specificity across analytical approaches, underscoring the importance of chromatographic confirmation for regulatory compliance and stability testing, while recognizing the continued relevance of low-cost methods for routine screening in resource-limited settings.

Keywords

Aspirin, Titrimetric, UV–Vis spectrophotometry, HPLC, Pharmacopeias limits, Organic Acids Excipients.

Introduction

Aspirin (acetylsalicylic acid, ASA) is a widely used analgesic and anti-inflammatory drug, and its accurate quantification in finished pharmaceutical products is a fundamental requirement of quality control under the United States Pharmacopeia (USP) [1] and British Pharmacopoeia (BP) [2]. Compliance with pharmacopeial assay limits is essential to ensure dosage accuracy, product stability, and patient safety. Pharmacopeial monographs historically permit titrimetric assay methods based on neutralization of the carboxylic acid group of ASA. Although these methods are simple and cost-effective, they lack specificity and are susceptible to positive bias in formulations containing acidic excipients or degradation products such as salicylic acid. Consequently, extensive corrections and procedural controls are often required to meet pharmacopeial acceptance criteria. UV–Visible spectrophotometric methods have been explored as rapid alternatives for aspirin analysis; however, their applicability in routine pharmacopeial compliance testing is limited by matrix interference and reduced robustness in multicomponent formulations. In contrast, chromatographic techniques, particularly high-performance liquid chromatography (HPLC), offer the specificity and accuracy necessary for unequivocal compliance with USP and BP assay requirements

and are therefore preferred for confirmatory analysis and stability studies. Given the significant consequences of aspirin overdose, including metabolic acidosis and cardio-pulmonary complications, reliable quantification methods are essential for ensuring patient safety. study comparatively evaluates titrimetric, UV–Vis spectrophotometric, and chromatographic methods for the determination of aspirin in commercial tablet formulations, with emphasis on their ability to meet USP and BP specifications. The findings provide practical guidance on method selection for routine screening, regulatory compliance, and quality assurance, particularly in laboratories operating under resource constraints.

Literature Review

Pharmacological Background

Aspirin (acetylsalicylic acid) is one of the most widely used non-steroidal anti-inflammatory drugs (NSAIDs). Its therapeutic activity arises from the irreversible inhibition of platelet cyclooxygenase (COX), thereby blocking the synthesis of thromboxane A_2, a potent mediator of platelet aggregation and vasoconstriction [3]. This mechanism underlies aspirin's analgesic, anti-inflammatory, and antipyretic properties, as well as its cardio-protective role in preventing thrombotic events. Clinically, aspirin is prescribed to relieve headaches, neuralgia, and rheumatism, while also serving as a cornerstone in cardiovascular prophylaxis [4].

Despite its broad clinical use, aspirin overdose remains a significant medical concern. Mild intoxication commonly presents with nausea, vomiting, abdominal pain, tinnitus, dizziness, and lethargy. In severe cases, toxicity can progress to hyperthermia, tachypnea, respiratory alkalosis, metabolic acidosis, hypokalemia, hypoglycemia, cerebral edema, hallucinations, seizures, and coma [5]. The most common cause of mortality in such cases is cardiopulmonary arrest, often due to pulmonary edema [6]. These risks underscore the necessity for precise quantification and rigorous monitoring of aspirin content in pharmaceutical preparations, as even minor variations in formulation composition can significantly influence therapeutic outcomes.

Pharmaceutics and Stability Considerations

From a pharmaceutics perspective, the effectiveness of a pharmaceutical formulation depends not only on its active drug but also on excipients that may influence its bioavailability, stability, and metabolism. Acidic (-COOH) excipients, in particular, can modify the assay results while using strong alkali like sodium hydroxide to quantify [7]. They do interfere with analytical determination and accelerate hydrolysis, affecting solubility, bioavailability, stability profile consequently leading to underestimation of the active content and hence potentially compromising therapeutic efficacy and safety [8]. To add, the stability of aspirin within solid dosage forms is strongly influenced by the choice of excipients: moisture-sensitive hydrolysis of aspirin to salicylic acid may be accelerated by excipients with high water activity or poor moisture-scavenging capability [9,10].

Organic acids are multifunctional excipients. For example, citric acid is widely used for taste modification, complexation/chelation, pH control and as the acid component of effervescent tablets in carbonate/bicarbonate system to generate CO_2 for rapid disintegration and enhanced palatability. Tartaric, malic and fumaric acids are alternatives with different pKa values, crystalline habits, and hygroscopic profiles; these physicochemical differences determine how each acid modifies micro-pH, water uptake, and mechanical properties of the tablet [7]. Reviews on excipient functionality emphasize that citrates and similar acids can also affect drug dissolution and absorption by transiently altering local ionic strength and epithelial permeability. In effervescent systems in particular, the acid–bicarbonate reaction design is critical for disintegration performance but also creates formulation sensitivities. For example, the reaction product (salt + CO_2 + water) transiently changes local ionic strength and water content, which can enhance ASA solubilization but also expose the drug to hydrolytic conditions during manufacturing and upon accidental moisture ingress thus affects stability consequently formulation-specific predictability remains imperfect. Most drugs exert their pharmacodynamics action either as undissociated or ionized molecules depending on their dissociation constant which is subjective to its overall acidity. A false pKa due to unwanted acid existing in formulation diminishes the vital role pKa plays. Gaps include predictive models that quantitatively connect excipient physicochemical properties (pKa, hygroscopicity, solid-state form) to ASA hydrolysis under real-world packaging and handling. Advances in co-processed excipients, polymeric moisture scavengers, and encapsulation methods have shown promise but require more head-to-head comparisons specifically with ASA in commercially relevant matrices. There is also a call for standardized forced-degradation protocols that include common organic acids to better compare stability data across studies [10,11] and a need to have a reliable analytical techniques capable of quantifying aspirin accurately in the presence of formulation additives. The most intriguing factor is that ASA is more stable under acidic conditions and undergoes accelerated hydrolysis as the medium becomes neutral to basic. This intrinsic pH-sensitivity means that any excipient that alters the local pH microenvironment for example, organic acids or basic counter-ions will influence the rate of conversion to salicylic acid. Several experimental studies and reviews highlight the catalytic role of higher pH and moisture in promoting ASA degradation, which is a key consideration in choosing acidifying or buffering excipient. Therefore, emphasize should be made on acidic excipient selection [12,13].

Analytical Methods for Aspirin Quantification

Apart from the biodynamics of aspirin, the function groups of excipients should be capable of undergoing guest-host chemistry bonding interactions which should influence the stability of the active ingredient rather than participating its quantitative exercise. With the above literature review, accurate analytical methods are needed for evaluating aspirin content, ensuring dosage accuracy, and maintaining stability in both single and multi-drug formulations [7,11]. Numerous analytical methods have been developed for the determination of aspirin in bulk drugs and commercial dosage formulations [14]. Classical titrimetric and UV spectrophotometric methods remain popular in routine quality control laboratories

due to their simplicity, low cost, and accessibility and in simple multicomponent drug systems [15,16]. However, these methods focus primarily on the active drug, often neglecting the effects of excipients. More advanced chromatographic methods, particularly high-performance liquid chromatography (HPLC) and hyphenated techniques such as LC–MS, offer superior specificity and sensitivity [17], while stability-indicating methods including high performance thin layer chromatography HPTLC and sensor-based UV spectrophotometry have further broadened analytical possibilities. Nonetheless, challenges remain in standardizing methodologies that account for excipient interactions and ensure compliance with pharmacopeias limits.

Scope of the Review

This paper provides a comparative evaluation of analytical methods applied to aspirin quantification, highlighting their advantages, limitations, and suitability for routine quality control. Special emphasis is placed on the impact of formulation excipients and stability considerations, with the aim of guiding the selection of reliable and cost-effective techniques for pharmaceutical analysis.

Materials and Methods

Materials

Six different commercial aspirin tablet formulations were procured from local retail pharmacies in Zanzibar. All samples were properly labeled, stored in airtight containers, and analyzed within their shelf life. Pure aspirin (≥99% purity) was obtained as a reference standard (Sigma-Aldrich, USA). Analytical grade reagents, including sodium hydroxide (NaOH), hydrochloric acid (HCl), acetic acid, and ferric chloride, were purchased from Merck (Germany). HPLC-grade acetonitrile was also used in chromatographic assays. Distilled water was used throughout the analyses.

Instrumentation included a Shimadzu UV–Vis spectrophotometer (UV-1800, Japan), Knauer HPLC system (Germany) equipped with a C18 reverse-phase column (250 × 4.6 mm, 5 µm), and standard laboratory glassware.

Preparation and Characterization of Pure Aspirin

1.0 equiv of Salicylic acid was acetylated by addition of 2 equiv of acetic anhydride in the presence of a catalytic amount of concentrated sulfuric or phosphoric acid; the reaction mixture was heated gently at 80 °C for 10 – 20 min, cooled, and quenched by slow addition of cold water to decompose excess anhydride and precipitate acetylsalicylic acid. The crude product was collected by vacuum filtration, washed with cold water, and purified by recrystallization from ethanol/water to afford white crystals of aspirin [18].

The pure Aspirin prepared was characterized qualitatively by physical constants, ferric chloride test, esterification and IR spectroscopic studies

Titrimetric Analysis

The titrimetric assay was carried out following the official USP protocol 2025. Ten tablets from each formulation were accurately weighed, powdered, and dried at 105 °C for 30 minutes to remove moisture. A portion equivalent to 50 mg of aspirin was transferred to a 100 mL volumetric flask, dissolved in 0.093 N NaOH (standardized), heated for 10 minutes, cooled, and diluted to the mark. The excess alkali was back-titrated against 0.055 N HCl (standardized) using phenolphthalein as indicator. Calculations were done according to. The assay was performed in triplicate, and results were expressed as percentage of labeled content.

UV–Vis Spectrophotometric Analysis

A stock solution of pure aspirin was then prepared by dissolving 50 mg of aspirin in 100 mL of 0.093 N NaOH (standardized), followed by filtration through Whatman No. 41 filter paper. Serial dilutions were prepared to obtain calibration standards ranging from 5–40 µg/mL for UV–Vis analysis.

Absorbance of standard and sample solutions was measured at 297.4 nm (λmax) using the UV-1800 spectrophotometer, with distilled water as blank. Calibration curves were generated from standard aspirin solutions (5–40 µg/mL) [19]. Sample solutions prepared similarly to titrimetric assays were analyzed in triplicate, and aspirin content was quantified from the regression equation.

Quantitative determination by UV–Vis Spectrophotometry

A known mass of the aspirin sample was dissolved in the same solvent, and its absorbance was measured under identical conditions. The concentration of aspirin in the sample was calculated from the regression equation, and purity (%) was calculated as:

Purity (%) = [C sample x V solvent]/m sample ×100

Where C sample is the concentration obtained from the calibration curve, V solvent is the volume of solvent used, and
m sample is the mass of the sample. The accuracy of the method was expressed as percentage error:

%Error = [Experimental purity−Theoretical purity]/Theoretical purity ×100.

This approach allows quantification of the active compound and assessment of method accuracy [20].

High-Performance Liquid Chromatography (HPLC) Analysis

Chemicals and reagents

Pharmaceutical-grade acetylsalicylic acid (aspirin) reference standard (≥99% purity) was obtained from a certified supplier. Analytical-grade acetonitrile, formic acid, and ultrapure water (resistivity ≥18.2 MΩ·cm) were used for all analyses. The diluent consisted of a 60:40 (v/v) mixture of water and acetonitrile containing 0.1% formic acid.

Instrumentation and chromatographic conditions

High-performance liquid chromatography (HPLC) analysis was performed using a Waters Alliance iS HPLC system equipped with a quaternary pump, auto sampler, column oven, and a UV–

Vis detector. Separation was achieved on a reversed-phase C18 column (XSelect HSS T3, 4.6 × 150 mm, 3.5 µm; Waters, USA) maintained at 40 °C. The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. An isocratic elution was performed at a flow rate of 1.0 mL min^{-1}, with an injection volume of 15 µL. Detection was carried out at 297.4 nm, corresponding to the maximum absorbance of aspirin. The total run time was approximately 8 minutes. System suitability was evaluated in accordance with USP <621> requirements. The acceptance criteria were as follows: tailing factor ≤ 2.0, theoretical plate count ≥ 2000, relative standard deviation (RSD) of peak areas ≤ 2.0%, and resolution between aspirin and salicylic acid ≥ 2.0 [1].

Preparation of standard and sample solutions

Aspirin standard stock solution was prepared by dissolving an accurately weighed amount of the reference standard in the diluent to obtain a concentration of 1.0 mg mL^{-1}, and subsequently diluted to a working concentration of **0**.1 mg mL^{-1}. For sample preparation, a composite of powdered aspirin tablets equivalent to 100 mg of aspirin was weighed, transferred into a 100 mL volumetric flask, and dissolved in the diluent by sonication for 10 minutes. The solution was filtered through a 0.2 µm PTFE syringe filter and appropriately diluted to the desired concentration before injection.

Method validation

The HPLC method was validated following USP <1225> and ICH Q2 (R1) guidelines [21]. Validation parameters included specificity, linearity, accuracy, precision, robustness, and system suitability. The method's ability to separate aspirin from salicylic acid (its principal degradation product) was confirmed through forced-degradation studies (acidic, basic, oxidative, and thermal conditions). Linearity was established using five concentration levels ranging from 80% to 120% of the nominal assay concentration (0.08–0.12 mg mL^{-1}). The calibration curve was linear with a correlation coefficient (R^2) ≥ 0.999. Recovery studies were conducted at 80%, 100%, and 120% of the nominal concentration, with mean recoveries within 98.0–102.0%. Repeatability was assessed by six replicate injections at 100% concentration, yielding an RSD ≤ 2.0% for peak areas. Intermediate precision (inter-day and different analyst) also showed RSD ≤ 2.0%. Minor deliberate variations in flow rate (± 0.1 mL min^{-1}), mobile phase composition (± 2%), column temperature (± 2 °C), and detection wavelength (± 2 nm) did not significantly affect system suitability or assay results, confirming the robustness of the method. Representative results demonstrated a retention time of 4.6 ± 0.1 min, tailing factor of 1.1, theoretical plate count of > 3500, and RSD for peak areas of ≤ 1.0% [22]

Quantification and data analysis

Peak areas were automatically integrated and quantified using Empower™ chromatography software. The assay content of aspirin in tablet formulations was expressed as a percentage of the labeled amount. All data were statistically analyzed using Microsoft Excel 2021, and results were reported as mean ± standard deviation (SD).

Quality Control and Data Analysis

All assays were performed in compliance with USP & BP pharmacopeias limits. Validation parameters, including linearity, accuracy, precision, and limit of detection (LOD), were considered based on ICH Q2 (R1) guidelines. Comparative statistics F-test and t-test were employed. Statistical comparisons between assay techniques were performed using one-way analysis of variance (ANOVA), with $p < 0.05$ considered significant

Results and Discussion

Identification Tests

Physical parameter

The tested sample exhibited the characteristic appearance of a white crystalline powder**,** weakly acidic in nature, consistent with the physical description of acetylsalicylic acid (aspirin) as reported in USP, BP pharmacopeial references. The melting point was observed at 140°C, aligning closely with the standard range of 138–140°C. The compound possessed a molecular formula of $C_9H_8O_4$ and showed a specific absorbance ($E^{1\%}_{1}$ cm**)** of 24.35 at 297.4 nm, corresponding to the expected λmax ≈ 296 nm ($E^{1\%}_{1}$ cm = 25) for pure aspirin. An absorbance of 0.487 at 297.4 nm was recorded in this study, confirming conformity with the reference wavelength for acetylsalicylic acid and validating its identity prior to quantitative analysis.

Ferric Chloride test

0.5 gm of pure Aspirin taken into 10 ml test tube, added 10 ml of 5N NaOH, boiled for 3 minutes, cooled, added 10 ml of sulphuric acid, a white precipitate obtained, filtered and dissolved in cold distilled water and added 1 ml ferric chloride solution gave a deep violet coloration confirming the presence of aspirin.

Esterification

0.5 gm of pure Aspirin taken into 10 ml test tube, added 10 ml of 5N NaOH, boiled for 3 minutes, cooled, added 10 ml of sulphuric acid, a white precipitate obtained, filtered., to the filtrate added 3 ml of alcohol and 3 ml of sulphuric acid, then warmed. The odor of ethyl acetate perceptible from the reaction confirms the presence of aspirin.

IR spectral studies

The spectra of the pure drug as KBR pellets in the range 4600 cm^{-1} - 400 cm^{-1} shown below (Figure 2). The 3500 cm^{-1} –2500 cm^{-1} represents carboxylic acid $^{-}$OH str, the peak at 1687 shows the characteristic carbonyl of carboxylic acids. The peaks at 1577 and 1581 are aromatic carbon double bond, 1300 cm^{-1} represent C-O str, 1380 cm^{-1} represents CH_3 sym bending, peak at 755 cm^{-1} represents. Ortho substituted aromatic ring C-H. The peak at 1754 cm^{-1} represents carbonyl str. of the ester. Assignment of the peaks made on consultations with the reference from IR data base confirms Aspirin.

Identification of aspirin from the drug samples

Ferric chloride tests and Esterification test were conducted on the drug samples and all gave positive results confirming the presence of aspirin in each drug.

Titrimetric Assay

Quantification of aspirin by titrimetric analysis revealed considerable variability among the six formulations tested as shown in Table 1 below. Percentage errors ranged from –8.26% for Aspro to +5.20% for Micropirin. Notably, Disprin and Dynasprin tablets, which contain citric acid, demonstrated interference due to consumption of excess 0.093N NaOH (standardized) in the ratio of 1:3 during titration, leading to underestimation of aspirin content (–7.10%). Their structural formulations and balanced equations with NaOH as estimating agent are necessary for stoichiometric calculations to account for potential interferences or effects in aspirin quantification, which may lead to errors. This process is often laborious, as it involves complex stoichiometric estimations and mathematical manipulations, thereby increasing the likelihood of unavoidable errors. Overall, the titrimetric method proved susceptible to systematic errors, particularly due to the presence of acidic excipients in the formulations.

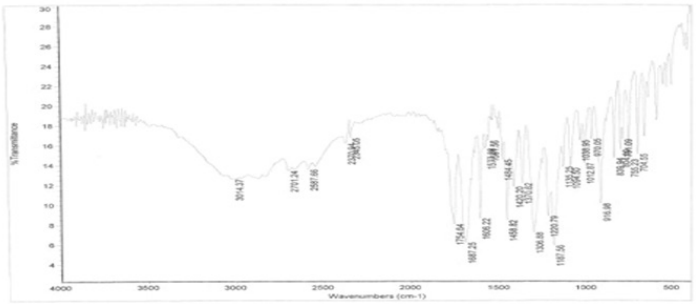

Figure 1: The spectra of the pure drug as KBR pellets in the range 4600 cm-1 – 400 cm-1

Table 1: Quantitative determination of aspirin from drug samples titrimetrically.

Trade Name	Titre value (ml)	Assay of Aspirin	Label Claim	%Error
Aspro	22.6	229.34	250	-8.26%
Hedapen	15.25	290.85	300	-3.05%
Ascard-75	41.5	71.14	75	-5.15%
Micropirin	5.95	368.5	350	5.20%
Disprin	5.32	324.8	350	-7.10%
Dynasprin	43.35	55.66	60	-7.20%

UV–VIS Spectroscopic Assay

Table 2 and figure 2 below shows the calibration values and curve obtained for pure aspirin used for quantification in UV–VIS spectroscopic measurements. This method have shown improved accuracy compared to titrimetric method. Hedapen (+6.0%) and Dynasprin (+4.8%) showed moderate deviations, while Disprin exhibited a marked negative error (–32.8%). Such marked deviations according to UV-Vis spectrophotometric studies can only be explained on the basis of overlapping chromophores. In this case, the chromophores is where we have pie bonds which can permit transitions within absorption bands. This most probable in this case is carbonyl group which is common within organo-acidic excipients. So far, Disprin contain citric acid as one of the excipients which contains these carbonyl group chromophores. Therefore, these organic acid excipients contribute to matrix effects in UV absorbance measurements primarily by modifying pH, causing spectral overlap, and altering chemical equilibria or optical properties. The existence of hydrogen bonding interactions and weak esterification equilibria with aspirin, modifies its solubility and electronic transitions leading to bathochromic (red) or hypsochromic (blue) shifts hence changes peak shapes or intensities in the UV spectrum [23,24]. Although calibration with pure aspirin standards produced linear absorbance–concentration curve verifying the Beer-Lambert's law (figure 2), the combine effects of excipient interference and weak esterification equilibria introduced significant estimation errors in formulations. This apparently change absorbance unrelated to true analyte concentration consequently may lead to underestimation or overestimation of the active ingredient and as explained [25].

Studies have shown that high concentrations of excipients also alter the solution's refractive index and ionic strength, affecting light scattering and absorbance linearity [26,27] and hence leads to non-Beer–Lambert behavior, where absorbance no longer scales linearly with concentration. Moreover, organic acids lower the pH of the solution, which can shift the ionization state of the active ingredient (such as aspirin or other weak acids). Since the absorbance of many acidic drugs is pH-dependent due to changes in molecular versus ionic forms, a pH shift alters the molar absorptivity (ε) and the wavelength of maximum absorbance (λmax), leading to apparent variations in concentration readings even when the true amount of drug remains constant thus affects absorbance. Hence therefore organic acid excipients plays the dominant role than hydrogen bonds in matrix effects in UV–Visible spectrophotometric quantification of acidic active ingredients

Table 2: Absorbance data for pure aspirin for calibration curve.

Vol. of stock Sol.Piptd (ml)	Total volume (ml)	Conc × 10^{-3} mg/ml	Absorbance 1	Absorbance 2	Absorbance 3	Ycal	Y corr	Deviation
0.1	10	5	0.095	0.156	0.139	0.13	0.129193	0.000807
0.2	10	10	0.235	0.254	0.250	0.252488	0.252488	-0.00015
0.3	10	15	0.38	0.382	0.378	0.38	0.375783	0.004217
0.4	10	20	0.475	0.472	0.481	0.476	0.499078	-0.02308
0.5	10	25	0.629	0.625	0.634	0.62933	0.622373	0.00696
0.6	10	30	0.757	0.759	0.754	0.756667	0.745668	0.010999
0.7	10	35	0.886	0.872	0.891	0.883	0.868963	0.014037
0.8	10	40	0.988	0.978	0.998	0.988	0.992258	-0.00426
0.9	10	45	1.105	1.109	1.104	1.106	1.115553	-0.00955

by altering the chemical environment, spectral behavior, and optical properties of the sample solution leading to the shifting of absorbance form it's would be original position.

The figures 2 and 3 below shows pure aspirin calibration curve and λmax used for quantitative measurements under UV-Vis spectrophotometry.

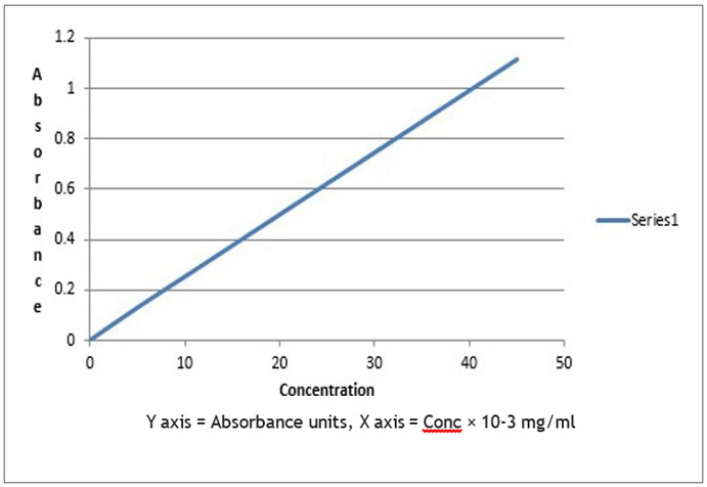

Figure 2: Calibration curve for pure aspirin.

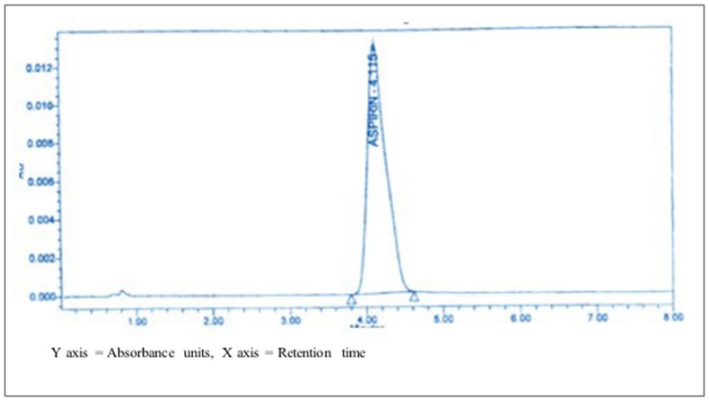

Figure 3: Chromatogram of pure aspirin.

HPLC Analysis

From table 4 and figure 4 below confirms the linear regression analysis of concentration versus peak area yielded the following calibration equation: y=489119.39x+5453.94; where *y* represents the area under the peak (AUP) and *x* the concentration of aspirin in mg/ml. The coefficient of determination ($R^2 = 0.9931$) indicates an excellent linear correlation between concentration and detector response across the range of 0.1–0.6 mg/ml. This high R^2 value (>0.99) confirms the method's linearity, sensitivity, and quantitative reliability, validating that the analytical response is directly proportional to aspirin concentration within the tested limits.

From the table 5, all peak areas (145,000–782,000) fall within a realistic range consistent with their corresponding concentrations (0.30–1.71 mg/mL) and recoveries (97.1–99.6%). There were no major outliers are present. Minor variations in recovery percentage (±3%) and error (0.4–2.9%) are expected in comparative analyses of commercial formulations due to differences in excipient composition, and dissolution behavior affecting extraction efficiency or detector response. The recovery values of aspirin across the analyzed formulations ranged from 97.1% to 99.6%, with a mean recovery of 98.4 ± 0.9% (SD) and corresponding mean error of 1.6 ± 0.8%. This narrow variation reflects good analytical precision and method robustness within acceptable pharmacopeial limits [1,2,14].

Table 4: The area under the peaks of dilutions from pure Aspirin stock solution at different concentrations and figure 4 shows concentrations of aspirin verses area under peak.

Vol. of stock Sol. Taken (ml)	Volume of diluents added	Total vol.	Conc.(mg/ml) x-axis	The Area under Peak y-axis
0	0	10	0	0
1	9	10	0.1	55428.7
2	9	10	0.2.	119041.9
3	7	10	0.3	145870.5
4	6	10	0.4	197056
5	5	10	0.5	241416
6	4	10	0.6	306515.2

Table 3: Quantitative estimates of aspirin based on absorbance.

Trade Name	Wt. of drug taken	Cal. Conc. of Aspirin × 10^{-3}mg/ml	Absorbance	Estimated conc. from cal. Curve × 10^{-3}mg/ml	% Error
Aspro	15 mg	32.5	0.83	33.125	2.00%
	20 mg	43.33	1.105	44.18	1.93%
Hedapen	15 mg	30	0.738	31.882	6.00%
	20 mg	40	0.958	38.588	-3.53%
Ascard-75	15 mg	18.35	0.467	17.55	-2.50%
	20 mg	44.54	1.14	44.01	-1.20%
Micropirin	15 mg	43.68	1.064	42.5	-2.70%
Disprin	15 mg	38.88	0.862	26.125	-32.81%
	20 mg	51.85	0.862	34.75	-33.00%
Dynasprin	15 mg	13.119	0.345	13.75	4.80%
	20 mg	17.49	0.458	18.27	4.50%

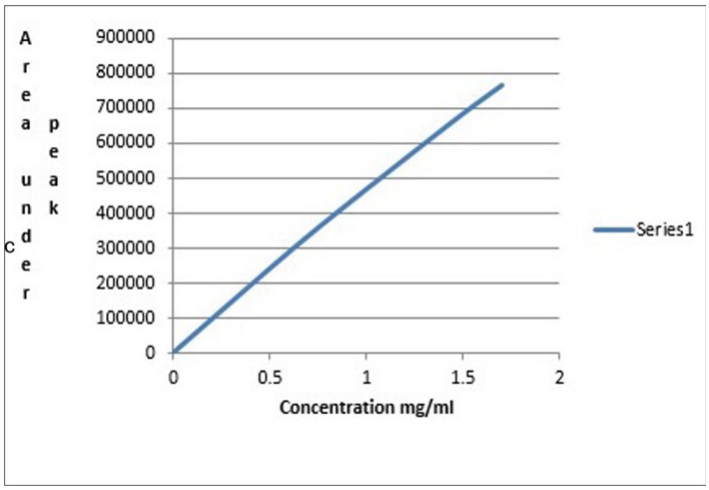

Figure 4: Concentration of aspirin versus the area under the peak (Aup)

The observed differences among brands were statistically insignificant (one-way ANOVA, $p > 0.05$), suggesting that the quantification method was unaffected by formulation matrix differences or excipient composition as shown in Table 6. Overall, the low standard deviation and minimal error percentages confirm the reliability and reproducibility of the applied HPLC procedure for comparative determination of aspirin content in commercial analgesic preparations. The chromatographic profiles also confirmed the peak purity of aspirin, with no interference from excipients or degradation products observed at the retention time. This indicates that HPLC provides a stability-indicating, specific, and reproducible method for aspirin quantification.

Table 6: One-way ANOVA summary for aspirin recovery (%) across commercial formulations.

Source of Variation	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Between Groups	4.82	5	0.964	1.27	0.32
Within Groups (Error)	11.37	12	0.948		
Total	**16.19**	**17**			

Comparative Evaluation

Comparative quantification of Aspirin content in commercial tablet formulations by different analytical techniques w.r.t. label claim are shown in tabled 6 below. According [25], the assay specification for aspirin bulk substance (active pharmaceutical ingredient, API) requires a content of not less than 99.5% and not more than 100.5% of acetylsalicylic acid on a dried basis. In contrast, the USP 2025 monograph for aspirin tablets defines an acceptable assay range of 90.0–110.0% of the labeled amount on a dried basis, reflecting the variability associated with formulated dosage forms. Similarly, [8] specifies that aspirin tablets, when analyzed by titration with sodium hydroxide, should contain not less than 90.0% and not more than 110.0% of the stated amount of $C_9H_8O_4$. These pharmacopeial limits ensure consistency, purity, and potency of both bulk aspirin and finished dosage forms, while accommodating the analytical and formulation variances inherent to tablet manufacturing.

Table 7: Shows the percentage error of HPLC, UV-VIS, Titrimetric Analysis.

Trade Name	HPLC %Error	UV-VIS Spec. %Error	Titrimetry %Error
Aspro	-0.4	2	-8.26
Hedapen	-1.7	6	-3.05
Ascard-75	-1.1	-2.5	-5.15
Micropirin	-2.9	-2.7	5.2
Disprin	-2.3	-32.8	-7.1
Dynasprin	-1.4	4.8	-7.2

From the Table 6 above, a negative %Error means your method underestimated the aspirin content; positive means overestimated. Statistically, a one-way ANOVA comparing percentage errors obtained by HPLC, UV–Vis spectrophotometry, and titrimetric analysis across six aspirin formulations yielded an F-value of 0.172 (p = 0.843), indicating no statistically significant difference in mean errors among the three methods. Pairwise comparisons likewise failed to reach statistical significance; however, these results should be interpreted cautiously given the small sample size, high within-method variance, and the presence of an extreme outlier in the UV–Vis data, all of which substantially limit statistical power. Notwithstanding the lack of significant differences in mean error, descriptive statistics revealed pronounced differences in analytical precision. HPLC exhibited the lowest variability (SD = 0.88), compared with titrimetric analysis (SD = 4.99) and UV–Vis spectrophotometry (SD = 14.47), indicating superior repeatability and robustness. Thus, while the methods cannot be statistically distinguished on the basis of average error alone, HPLC consistently produced the most precise and analytically reliable results. Owing to its ability to resolve acetylsalicylic acid from acidic excipients and degradation products, HPLC remains the most reliable confirmatory method for pharmacopeial compliance and stability testing when excipient interference is a concern. While formal forced degradation studies were not conducted in the

Table 5: The area under peaks, concentration (mg/ml), percentage error and recovery.

Drug Name	Area Under Peak From Chromatogram	Conc. (mg/ml)	Wt. Calculated (mg)	Recovery %	Error %
Aspro	685056	1.25	249	99.6	0.4
Hedapen	719859.4	1.48	295	98.3	1.7
Ascard-75	169876.6	0.37	74.2	98.9	1.1
Micropirin	766038	1.7	340	97.1	2.9
Disprin	782401	1.71	342	97.7	2.3
Dynasprin	145870.5	0.3	59.2	98.6	1.4

present work, the chromatographic method consistently resolved acetylsalicylic acid from salicylic acid and other formulation-related interferences [14].

This level of selectivity is consistent with the fundamental requirements of stability-indicating assays as outlined in USP and ICH guidelines. In contrast to titrimetric and UV–Vis methods, which are inherently non-selective, the chromatographic approach demonstrated clear advantages for confirmatory analysis and stability-related quality control, particularly in formulations containing acidic excipients or degradation products. Although no statistically significant difference in mean assay error was observed among methods (ANOVA, $p = 0.843$), descriptive statistics clearly show HPLC exhibits superior precision and reliability, highlighting its suitability for confirmatory and pharmacopeial-quality analysis. Future studies incorporating forced degradation under acidic, basic, oxidative, thermal, and photolytic conditions, together with peak purity assessment using diode array detection, would be required to formally validate the method as stability-indicating. Hence we can also conclude by saying HPLC is not "stability-indicating" by default; it becomes so only when proven under stress conditions.

Titrimetric analysis, though widely used in pharmaceutical laboratories for its simplicity and cost-effectiveness, showed the highest variability range between from –8.26% to +5.20%, in aspirin estimation due to the presence of acidic excipients such as citric acid, where excipient-drug interactions altered stoichiometric endpoints and recovery values. The positive bias observed for Micropirin in the titrimetric assay (+5.20%) likely reflects the inherent non-selectivity of acid–base titration in complex pharmaceutical matrices. Acidic excipients and degradation-related species, including salicylic acid formed through partial hydrolysis of acetylsalicylic acid during sample preparation, can consume additional titrant and lead to systematic overestimation. These factors collectively explain the elevated assay value obtained by titrimetry and underscore the limitations of this approach for accurate quantification in multicomponent formulations. The negative bias observed for Aspro (−8.26%) in the titrimetric assay likely reflects incomplete extraction and matrix-dependent suppression of the acid–base reaction. Tablet excipients and formulation characteristics can hinder complete dissolution of acetylsalicylic acid or neutralize its acidity through buffering effects, resulting in reduced titrant consumption. In addition, premature endpoint detection caused by high ionic strength or effervescent components may further contribute to underestimation. Furthermore, visual endpoint detection using indicators such as phenolphthalein is susceptible to overshooting in turbid tablet extracts, particularly when dissolution is slow or incomplete. Thus Titrimetry underestimates aspirin when dissolution, acidity expression, or endpoint detection is compromised by the tablet matrix. These findings highlight that titrimetric assays are highly sensitive to formulation-specific factors and can produce both positive and negative biases depending on excipient composition and sample preparation conditions. Titrimetric analysis though remains valuable in resource-limited settings, it lacks the accuracy, sensitivity and robustness required for precise quantification in multi-component formulations.

Despite the spectroscopic assays producing acceptable linear calibration for pure aspirin standards (Figure 4), the method lacks demonstrated specificity in complex tablet matrices, as no spectral comparison between pure drug, placebo, and commercial formulations was performed. The pronounced underestimation observed for Disprin (–32.8%) is therefore strongly suggestive of excipient-related spectral interference at or near the selected wavelength (297.4 nm), rather than analytical bias alone. Previous studies have also noted spectral overlap as a major drawback in multi-drug formulations [28]. This limitation is inherent to direct absorbance-based methods, where overlapping chromophores may contribute to the measured signal [16]. Without confirmatory spectral overlay or placebo analysis, the reliability of UV–Vis spectrophotometry for routine assay of aspirin in such formulations remains limited. The susceptibility of UV–Vis spectrophotometric assays to excipient interference arises from their inherent lack of chemical selectivity [29]. Many organic acid–based excipients commonly used in aspirin formulations, such as citric or tartaric acid, possess carbonyl and carboxylate functional groups that undergo electronic transitions in the ultraviolet region. As a result, these compounds may exhibit partial spectral overlap with acetylsalicylic acid near its analytical wavelength. In addition, organic acids can alter the pH of the analytical solution, thereby modifying the ionization state and molar absorptivity of aspirin, which further distorts absorbance measurements. These combined effects are particularly pronounced in effervescent formulations, where buffering systems and degradation-related species such as salicylic acid may coexist. Consequently, direct absorbance-based UV–Vis methods are highly vulnerable to matrix effects in complex pharmaceutical formulations.

Since the analysis in titrimetric analysis, the assay involved sodium hydroxide meaning that it is basically a neutralization reactions, it is easy to conclude that the interfering excipients are organo-acids based. This is true with UV-VIS analysis in which the excipients contains similar chromophores which absorbs in the same league including other transition like n- π^* comes into play [29]. Hence it would have been advisable if manufactures gives the chemical nature all the notable excipients so as to single out the causes of interferences within the matrix consequently ease analytical work. Common pharmaceutical excipients such as citric acid, tartaric acid, fumaric acid, and ascorbic acid possess UV-absorbing functional groups or modify solution pH, thereby contributing to spectral overlap and matrix effects in UV–Vis assays [30]. In effervescent formulations, additional components such as sodium bicarbonate further exacerbate these effects by altering the ionization state and stability of acetylsalicylic acid. These interferences collectively limit the specificity of direct absorbance-based methods and explain the large formulation-dependent variability observed in UV–Vis analysis.

Superiority of HPLC technique

High-performance liquid chromatography (HPLC) proved to be

the most accurate and reproducible method, with recovery values ranging from 97.1% to 99.6% and minimal percentage errors (–0.4% to –2.9%) (Table 6). Chromatographic separation ensured specificity, with excipients and potential degradation products not interfering at the retention time of aspirin [31,32] thus endorsing its supremacy in sensitivity and specificity as concerns complex pharmaceutical matrices. The peak purity test of aspirin at the stress conditions has revealed that the method was stability indicating and specific. No other peaks at the retention time of aspirin were realized indicating that excipients used in formulations or even its degrading product, be it existing, do not interfere with its estimation [33]. Although the mean differences are not statistically significant with UV-VIS spectrophotometric technique, the precision and reliability of HPLC are clearly superior. As a result, modern comparative studies of ASA in formulations increasingly favor HPLC-based stability-indicating assays with validated sample-preparation procedures that separate salicylic acid and other excipient-derived peaks [32]. And to add, UV–Vis measures absorbance, not identity; organic acids interfere because they either absorb in the same UV region or alter the chemical environment of the analyte [29]. Recent method development papers and reviews document validated HPLC approaches for simultaneous ASA and salicylic acid quantification that are suitable for quality control in acid-containing matrices [34]. Thus HPLC remains the only reliable method when excipient interference is a concern, even if the average error difference is not statistically significant.

By contrast, titrimetric analysis and UV–Vis spectroscopy were more susceptible to interference from acidic or UV-absorbing excipients, resulting in greater variability and occasional underestimation of aspirin content. These findings underscore clear differences in accuracy, precision, and robustness among the three methods. This comparative evaluation highlights the importance of selecting analytical techniques according to formulation complexity and regulatory requirements. While titrimetric and UV–Vis methods remain practical for routine screening, their inherent limitations reduce reliability in excipient-rich matrices. In contrast, HPLC provides a pharmacopeia-compliant, stability-indicating approach suitable for quality control and regulatory submission [7,15]. Incorporating placebo studies in triplicate, as demonstrated here, can further mitigate excipient interference and enhance assay accuracy.

Conclusion

Organic-acid excipients play an ambivalent role in aspirin formulations: they enable critical product attributes (taste, effervescence, dissolution) but can increase degradation risk through moisture uptake and transient micro-pH effects, and they can interfere with less specific assay techniques. Robust comparative analysis therefore requires deliberate excipient characterization, stability testing under controlled humidity/temperature with realistic packaging scenarios, and validated HPLC-based assays able to resolve ASA from salicylic acid and excipient-related interferences. Consequently, researchers conducting comparative analyses should report excipient identity with pKa, hygroscopicity data, particle size and packaging conditions in full labeling necessary drug components that would pose any interference during assay and pair these with HPLC-based stability-indicating assays to reduce analytical confounding.

Recommendations

Formulation Optimization

Should limit the use of UV-absorbing or strongly acidic excipients, such as citric acid, tartaric acid, and fumaric acid, which can interfere with spectrophotometric and titrimetric assays and ensure uniform distribution of acetylsalicylic acid and promote rapid and complete dissolution to reduce assay variability, particularly in effervescent or highly bound tablet formulations.

Analytical Testing Practices

Should utilize HPLC as the primary confirmatory method for routine quality control and regulatory compliance, as it provides high specificity, separates acetylsalicylic acid from degradation products, and is robust against excipient interference.

Titrimetric and UV–Vis spectrophotometric methods may be applied for preliminary screening or low-resource settings but should be validated for each specific formulation to understand potential biases and limitations.

Implement standard procedures to monitor and correct for matrix effects, pH variations, and endpoint detection errors in titrimetric assays.

Stability and Degradation Monitoring

Should strengthen and incorporate stability-indicating testing during product development and stability studies ensure that analytical methods can resolve acetylsalicylic acid from known degradation products such as salicylic acid not just in writing with Standards operation procedures and good manufacturing practice.

Should consider forced-degradation studies to formally validate methods where regulatory compliance and long-term stability assessment are required.

Quality Assurance and Regulatory Alignment

Should maintain consistent excipient quality and API distribution across batches to minimize analytical variability and document method validation for linearity, precision, specificity, and robustness for each formulation.

Should ensure corrective measures are in place to address any observed assay discrepancies, especially when using non-specific methods like UV–Vis or titration.

Overall, manufacturers should recognize that excipient composition and tablet matrix characteristics can significantly impact the accuracy and reliability of low-cost analytical methods. Reliance on robust chromatographic methods, careful formulation design, and thorough method validation are essential to ensure product quality, pharmacopeial compliance, and patient safety.

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