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Influence of Organic Acids Excipients: A Comparative Analysis Study of Aspirin in Analgesic Drug Formulation

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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 hyphenated chromatographic methods (HPLC). Results indicates that titrimetric assays required complex stoichiometric corrections, manipulations and produced inconsistent values in formulations containing interfering acidic excipients. Spectrophotometric methods offered rapid screening but frequently yielded results at or below the lower pharmacopeia specification limits, reflecting limited robustness in complex matrices. By contrast, the chromatographic techniques 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; Quality control; Titrimetric; UV–Vis spectrophotometry; HPLC; Pharmacopeias limits; Organic Acids Excipients

1. Introduction

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 [18]. 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.

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. The most common cause of mortality in such cases is cardiopulmonary arrest, often due to pulmonary edema [15]. These risks emphasize the need for precise quantification and monitoring of aspirin content in pharmaceutical preparations.

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. 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. Even minor variations in formulation composition may lead to substantial differences in therapeutic outcomes. Therefore, reliable analytical techniques must be capable of quantifying aspirin accurately in the presence of formulation additives. Hence, accurate analytical methods are essential for evaluating aspirin content, ensuring dosage accuracy, and maintaining stability in both single and multi-drug formulations.

Analytical Methods for Aspirin Quantification

Numerous analytical methods have been developed for the determination of aspirin in bulk drugs and commercial dosage formulations. Classical titrimetric and UV spectrophotometric methods remain popular in routine quality control laboratories due to their simplicity, low cost, and accessibility [1, 6, and 14]. 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) [13] and hyphenated techniques such as LC–MS [11], offer superior specificity and sensitivity, while stability-indicating methods including high performance thin layer chromatography HPTLC [7] and sensor-based UV spectrophotometry [19] have further broadened analytical possibilities. Nonetheless, challenges remain in standardizing methodologies that account for excipient interactions and ensure compliance with pharmacopeias limits.

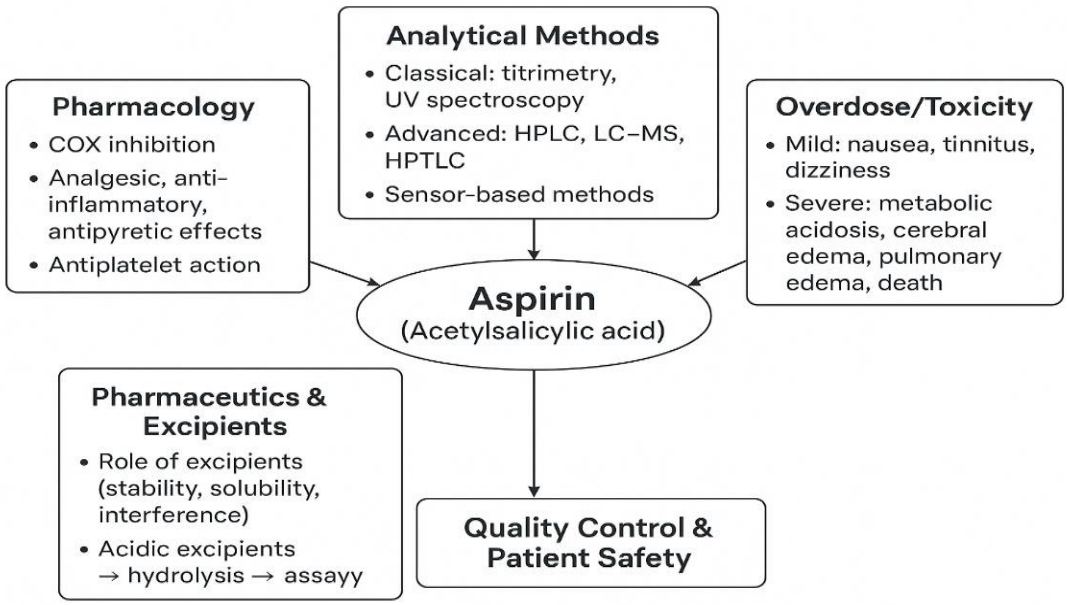
Scope of the Review

This review 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.

Conceptual framework for aspirin analysis:

Aspirin pharmacological action (COX inhibition → analgesic, anti-inflammatory, cardio-protective effects) is counterbalanced by overdose risks (gastrointestinal toxicity, metabolic acidosis,

cardiopulmonary failure). In pharmaceutical formulations, excipients—particularly acidic additives—affect stability and analytical accuracy. Various analytical approaches (titrimetry, spectrophotometry, chromatography, hyphenated methods) are applied to quantify aspirin content and ensure quality control. Figure 1 below shows the framework.



2. Materials and Methods

2.1 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.

2.2 Preparation of Standard Aspirin

Pure aspirin was characterized using established methods, including infrared (IR) spectroscopy, ferric chloride test for phenolic groups, and esterification reaction (Sethi, 2008). A stock solution of pure aspirin was then prepared by dissolving 50 mg of aspirin in 100 mL of 0.1 N NaOH, 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 and 0.1–0.6 mg/mL for HPLC calibration.

2.2 Titrimetric Analysis

The titrimetric assay was carried out following the official USP protocol [17]. 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 standardized 0.055 N HCl using phenolphthalein as indicator. Calculations were done according to[3].The assay was performed in triplicate, and results were expressed as percentage of labeled content.

2.3 UV–Vis Spectrophotometric 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). Sample solutions prepared similarly to titrimetric assays were analyzed in triplicate, and aspirin content was quantified from the regression equation. Estimation of Aspirin from samples (Weight of the samples taken: X gm; Concentration of Aspirin in the solution: Y mg/ml; Concentration of Aspirin from absorbance: Z mg/ml; Percentage error: [(Y–Z)/Y] × 100).This method was adapted from previous validated spectrophotometric assays [2, 4].

2.4 High-Performance Liquid Chromatography (HPLC)

Quantitative analysis of aspirin was carried out using reversed-phase HPLC following modified USP guidelines (USP, 2021). Separation was achieved on a C18 column with a mobile phase of acetonitrile: water (150:850 v/v) containing 0.2% sodium heptanesulfonate, adjusted to pH 3.4 with acetic acid. The flow rate was maintained at 1.9 mL/min, injection volume was 20 µL, and detection wavelength was 297.4 nm. Standard aspirin calibration solutions (0.1–0.6 mg/mL) were used to construct calibration curves. Each sample was analyzed in triplicate, and data were processed using Knauer ChromGate software in-built.

2.5 Quality Control and Data Analysis

All assays were performed in compliance with pharmacopeias limits [5, 17]. Validation parameters, including linearity, accuracy, precision, and limit of detection (LOD), were considered based on ICH Q2 (R1) guidelines [9]. 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

3. Results and Discussion

3.1 Titrimetric Assay

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%

Quantification of aspirin by titrimetric analysis revealed considerable variability among the six formulations tested (Table 1). Percentage errors ranged from –8.26% for Aspro to +5.20% for Micropirin. Notably, Disprin 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.

3.2 UV–VIS Spectroscopic Assay

Table 2: Absorbance data for pure aspirin for calibration curve

Vol. of stock Sol.Pipt d (ml)	Total volum e (ml)	Conc × 10^{-3} mg/ml	Absorb ance 1	Absorb ance 2	Absorb ance 3	Ycal	Y corr	Deviatio n
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

Table 3: Quantitative estimates of Aspirin based on Absorbance

Trade Name	Wt. of drug taken	Cal. Conc. of Aspirin × 10^{-3}mg/ml	Absorbanc e	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%

UV–VIS spectroscopic measurements improved accuracy compared to titrimetry but remained subject to matrix effects (Tables 3). Hedapen (+6.0%) and Dynasprin (+4.8%) showed moderate deviations, while Disprin exhibited a marked negative error (–32.8%), attributable to overlapping chromophores within the excipient matrix. Although calibration with pure aspirin standards produced linear absorbance–concentration curves, excipient interference introduced significant estimation errors in some formulations.

3.3 HPLC Analysis

Table 4 below shows the area under the peaks of dilutions from pure Aspirin stock solution at different concentrations

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 5 below shows the area under peaks, conc (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	7824011	1.71	342	97.7	2.3
Dynasprin	145870.5	0.3	59.2	98.6	1.4

HPLC analysis provided the most consistent and reliable results across all samples (Tables 5). Recovery values ranged from 97.1% (Micropirin) to 99.6% (Aspro), with percentage errors between –0.4% and –2.9%. Chromatographic profiles 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.

4. Comparative Evaluation

Comparative quantification of Aspirin content in commercial tablet formulations by different analytical techniques w.r.t. label claim was done and shown in tabled 6 below**.** According to USP 2016, the HPLC (assay) method for aspirin should yield a content not less than 99.5 % and not more than 100.5 % on a dried basis though more lenient ranges (e.g. 98–102 %) are also referenced for the “dried basis” assay of USP-grade aspirin bulk material. The British Pharmacopoeia (BP) monograph also defines an **assay** (by

titration) as acceptable when the measured content is very close to the labeled amount; in the BP monograph, the titrimetric method is described (dissolving aspirin in ethanol + NaOH, then back-titrating) with the assumption that the result should correspond to the labeled content within plus or minus 10% .

Table 6: Shows the percentage error of HPLC, UV-VIS, Titrimetry

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

*So a negative %Error means your method underestimated the aspirin content; positive means overestimated**

Statistically, a one-way ANOVA was performed to compare the percentage errors obtained by HPLC, UV-VIS spectrophotometry, and titrimetry across the six aspirin formulations. The analysis yielded an F-value of 0.172 ($p = 0.843$), indicating no statistically significant difference in mean errors among the three methods. Pairwise t-tests (HPLC vs. UV-VIS, HPLC vs. titrimetry, and UV-VIS vs. titrimetry) similarly showed no significant differences ($p > 0.05$ in all cases). However, descriptive statistics revealed notable differences in precision: HPLC showed the lowest variability (SD = 0.88), followed by titrimetry (SD = 4.99), while UV-VIS exhibited the highest variability (SD = 14.47). These findings indicate that, although the average errors across methods were not statistically different, HPLC consistently produced the most reliable results. Among the three analytical techniques, HPLC demonstrated the lowest error margins and the highest recovery rates, confirming it as the most accurate and pharmacopeia-compliant method. By contrast, titrimetry and UV–VIS spectroscopy were more prone to interference from acidic or UV-absorbing excipients, leading to greater variability. Despite being simple and cost-effective, these methods frequently underestimated aspirin content in certain formulations. Thus, these findings demonstrate clear differences in accuracy, reliability, and susceptibility to organo-acid excipients interference among the three methods.

Titrimetric, 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 Thus, while titrimetry remains valuable in resource-limited settings, it lacks the robustness required for precise quantification in multi-component formulations.

UV–VIS spectroscopic assays provided moderately improved accuracy but remained prone to interference from absorbing chromophores in the drug matrix. Disprin, for example, showed an underestimation error of –32.8%, highlighting the limitations of direct absorbance-based methods when excipients absorb near the analyte's λmax. Previous studies have also noted spectral overlap as a major drawback in multi-drug formulations [6]. Although calibration with pure aspirin produced linear regression curves, the inability to

fully resolve excipient interference limits the reliability of UV–VIS for routine analysis in such formulations.

Superiority of HPLC Techniques

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%). Chromatographic separation ensured specificity, with excipients and potential degradation products not interfering at the retention time of aspirin. These findings align with earlier reports where HPLC was validated as a stability-indicating method for aspirin and related compounds [1]. Furthermore, hyphenated chromatographic methods have been widely endorsed for their superior sensitivity and specificity in complex pharmaceutical matrices [10]. 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. MS/GC proves to be comparable with HPLC but rather laborious and time consuming due to extraction and isolation processes

Comparative Evaluation and Implications

The comparative analysis highlights the importance of selecting analytical techniques based on formulation complexity and regulatory requirements. While titrimetry and UV–VIS are practical for routine screening, their inherent limitations compromise accuracy in the presence of excipients. In contrast, HPLC provides a pharmacopeia-compliant, stability-indicating method suitable for quality control and regulatory submission. Moreover, employing placebo studies in triplicate, as suggested in the present work, can further minimize excipient interference and improve accuracy.

5. Recommendations for Quality Control Practice

Given the significant consequences of aspirin overdose, including metabolic acidosis and cardiopulmonary complications, reliable quantification methods are essential for ensuring patient safety. This study underscores the necessity for manufacturers to declare the quantitative and qualitative composition of excipients, as they can significantly impact assay accuracy. The dispensary condition too must adhere to the regulatory standards others the drugs could also decompose affecting purity. Adoption of advanced chromatographic and hyphenated techniques, coupled with multivariate calibration approaches, should be encouraged for regulatory-quality assays.

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