ISSN: 1674-0815

cjhmonline.com

DoI-10.564220/1674-0815

Chinese Journal of Health Management

Chinese Medical Association

Simultaneous Quantification of Colchicine and Aspirin in a Binary Mixture Using an ICH Q2 (R2)-Compliant First-Order Derivative UV Spectrophotometric Method

Mantasha W. Ansari[1], Nusrat K. Shaikh[2], Pallavi U. Barabde[3], Jitendra O. Bhangale[4]
[1]Student, Smt. N. M. Padalia Pharmacy College, Navapura, Ahmedabad, Gujarat, India 382210
[2]Associate Professor, Smt. N. M. Padalia Pharmacy College, Ahmedabad, Gujarat, 382210, India
[3]Assistant Professor, Smt. N. M. Padalia Pharmacy College, Ahmedabad, Gujarat, 382210, India
[4]Professor and Principal, Smt. N. M. Padalia Pharmacy College, Ahmedabad, Gujarat, 382210, India
Corresponding Author Email id: **jitu2586@gmail.com**

Article Information
Received: 23-10-2025
Revised: 07-11-2025
Accepted: 22-11-2025
Published: 26-12-2025

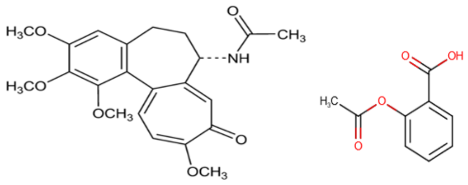
Keywords
Bisoprolol fumarate, Trimetazidine hydrochloride, RP-HPLC, Synthetic mixture, Validation

ABSTRACT:

Aim and Objective: Objective of the work was to develop and validate an accurate and precise first-order derivative UV spectrophotometric method for the estimation of Colchicine (COL) and Aspirin (ASP) in a synthetic mixture. **Method:** Water was used as the solvent for analysis. The wavelength for each drug was determined in the range of 200-400 nm in spectrum mode. The method was validated by evaluating parameters such as linearity, precision, accuracy, limit of detection, limit of quantification, and assay. **Results:** Colchicine showed prominent UV absorbance at λmax 247 nm and 350 nm, while Aspirin exhibited maximum absorbance at 286 nm, enabling their identification by UV-visible spectrophotometry. By application of first-order derivative UV spectra, COL showed absorbance at 260 nm, where ASP exhibited a zero-crossing point, whereas ASP showed absorbance at 286 nm, where COL had a zero-crossing point. Method validation was performed in accordance with ICH Q2 (R2) guideline and demonstrated excellent linearity ($R^2 > 0.99$ for both drugs), high precision (%RSD < 2%), acceptable accuracy with recovery in the standard range of 98.5%-101.2%, and adequate sensitivity as confirmed by limit of detection (LOD) and limit of quantification (LOQ) studies. The method showed linearity in the concentration range of 0.5-2.5 μg/ml for COL at 260 nm and 75-375 μg/mL for ASP at 286 nm. Upon derivative assay, the amounts obtained were 99.90% for COL and 99.94% for ASP. **Conclusion:** The proposed method was recommended for routine quality control analysis of both drugs in a synthetic mixture due to its good precision, high recovery, and low percentage error.

1. INTRODUCTION:

COVID-19 and cardiovascular disease (CVD) share overlapping pathophysiological mechanisms, including systemic inflammation, endothelial dysfunction, and thrombotic dysregulation. In COVID-19, a dysregulated immune response promotes cytokine-mediated inflammation, endothelial injury, and microvascular thrombosis, leading to increased morbidity and mortality, particularly in patients with cardiovascular comorbidities [1]. Similarly, CVD progression and adverse cardiovascular events are driven by chronic vascular inflammation and platelet activation. Despite therapeutic advances, a critical unmet need persists for safe, cost-effective interventions that concurrently target inflammation and thrombosis without impairing immune function [2]. The combination of Colchicine and Aspirin was studied under clinical trial [3] and it was proved that this combination is safe, effective, and well tolerable in patients with moderate COVID-19 [4]. The combined administration of Colchicine and Acetylsalicylic acid (Aspirin) represents a dual-pathway strategy targeting both vascular inflammation and platelet-mediated thrombosis, key drivers of adverse cardiovascular events [5-6]. Colchicine suppresses NLRP3 inflammasome-dependent cytokine signalling, while aspirin inhibits cyclooxygenase-mediated platelet aggregation. This complementary pharmacologic profile supports a synergistic approach to reducing endothelial dysfunction, thrombotic burden, and cardiovascular risk [7-8]. Colchicine [COL; Figure 1 (a)] is a tricyclic alkaloid chemically designated as N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl) acetamide [9-10]. Clinically, it is used for the treatment and prevention of gout and related inflammatory arthropathies. Colchicine exerts its anti-inflammatory effects by inhibiting tubulin polymerization, thereby suppressing leukocyte migration and NLRP3 inflammasome–mediated cytokine release. Through these mechanisms, colchicine attenuates IL-1β–driven vascular inflammation, a central process implicated in COVID-19–associated cytokine storm and inflammation-mediated cardiovascular disease progression [9-10].



1 (a) **1 (b)**

Figure 1: Chemical Structures: 1 (a) Colchicine and 1 (b) Aspirin

Aspirin [ASP; Acetylsalicylic acid *2-(acetyloxy) benzoic acid*; Figure 1 (b)] [10] exerts its pharmacological effects through irreversible inhibition of cyclooxygenase (COX)-1 and COX-2, thereby suppressing prostaglandin synthesis. Acetylation of a serine residue on COX-1 results in sustained inhibition of platelet aggregation for the platelet lifespan (7-10 days), preventing the conversion of arachidonic acid to thromboxane A_2 (TXA_2), a key mediator of platelet activation and thrombosis. By reducing platelet aggregation and thromboembolic risk, ASP plays a critical role in limiting arterial and venous thrombotic events, mechanisms directly relevant to COVID-19 - associated coagulopathy and cardiovascular disease [11].

A variety of analytical methods have been developed for the estimation of Colchicine (COL) and Aspirin (ASP), either individually or in combination with other active ingredients. These included HPLC method for Colchicine in pharmaceuticals and biological fluids [12], RP-HPLC for Pantoprazole and Aspirin in Combined Tablet Dosage Form [13], UV-visible spectrophotometry for Colchicine and Rosuvastatin calcium in synthetic mixture [14] and Aspirin and Ticlopidine HCl in bulk and tablet formulation [15], stability-indicating chromatographic methods for Probenecid and Colchicine in their combined tablet [16], HPTLC for simultaneous analysis of Colchicine and Gallic acid n polyherbal formulation [17], Liquid chromatography tandem mass spectrometry for Colchicine in human plasma [18], and gradient UPLC-ESI-MS/MS for Colchicine in human plasma [19]. Despite this extensive literature work, no any UV spectrophotometric method had been reported for the simultaneous estimation of Colchicine and Aspirin in synthetic mixture. This highlights a critical gap and underscores the need for a rapid, cost-effective, sensitive, and precise analytical approach that can facilitate routine quantification of this clinically relevant combination. To address this gap, a first-order derivative UV spectrophotometric method was employed, offering a rapid and economical alternative to chromatographic techniques [20]. This approach avoids expensive columns, large volumes of organic solvents, and extensive sample preparation, while enabling reliable quantification of compounds with overlapping absorption profiles. The objectives of the present work were to develop and validate a linear, precise, and sensitive first-order derivative UV method for the simultaneous estimation of Colchicine and Aspirin in synthetic mixtures, providing a practical and robust solution for routine laboratory applications.

2. MATERIALS AND METHODS:

2.1 Chemicals and reagents

Colchicine and Aspirin were received as gift samples from Zydus Pharmaceuticals Ltd., Ahmedabad, India. HPLC-grade water was procured from Finar Chemicals Pvt. Ltd., India.

2.2 Instruments and apparatus

The analysis was carried out using a Shimadzu UV-1900 ultraviolet visible spectrophotometer, and absorbance measurements were recorded using matched quartz cells with a path length of 1 cm. A Scale-Tec digital analytical balance was used for accurate weighing. Sonication was performed using a Digital Pro$^+$ PS-10AS sonicator (Broleo). All glassware was thoroughly cleaned with HPLC-grade water and dried prior to use.

2.3 Preparation of Solutions

2.3.1 Preparation of standard stock and working standard solutions

Standard stock solutions of Colchicine and Aspirin were prepared separately by accurately weighing 10 mg and 100 mg of each drug and dissolving them in 100 mL of water in volumetric flasks to obtain concentrations of 100 µg/mL and 1000 µg/mL, respectively. The solutions were sonicated for 5 min to ensure complete dissolution. 0.1 ml standard stock of Colchicine (100 µg/ml) and 1.5 ml standard stock solution of Aspirin (1000 µg/ml) were pipetted out into different 10 ml volumetric flask and diluted up to mark with water to get the 1 µg/ml of Colchicine and 150 µg/ml of Aspirin. Each solution was scanned in the range of 200-400 nm.

2.3.2 Preparation of calibration standards

From the respective standard stock solutions, appropriate aliquots of Colchicine (0.05, 0.10, 0.15, 0.20, and 0.25 ml) and Aspirin (0.75, 1.50, 2.25, 3.00, and 3.75 ml) were transferred into five separate 10 mL volumetric flasks and diluted to volume with water to obtain working standard solutions in the concentration range of 0.5-2.5 µg/ml for Colchicine and 75-375 µg/ml for Aspirin.

2.4 UV SEPCTROPHOTOMETRIC METHOD DEVELOPMENT

First Order Derivative Method was selected for simultaneous estimation of Colchicine and Aspirin in Synthetic Mixture.

2.4.1 Spectral measurement and calibration function:

Each working standard solution was scanned individually over the wavelength range of 200-400 nm. The zero-order absorption UV spectra were converted to first-order derivative UV spectra. Calibration functions were established by plotting first-order derivative absorbance against corresponding concentrations for each analyte.

2.4.2 Procedure of selection of wavelength for first order derivative UV Method

0.1 ml standard stock from Colchicine (100 µg/ml) and 1.5 ml standard stock solution of Aspirin (1000 µg/ml) were pipetted out into different 10 ml volumetric flask and diluted up to mark with water to get the 1 µg/ml of Colchicine and 150 µg/ml of Aspirin. Each solution was scanned in the range of 200-400 nm. Colchicine (1 µg/ml) exhibited characteristic absorbance maxima at 247 and 350 nm showed in Figure 2 (a), whereas Aspirin (150 µg/ml) showed a maximum at 286 nm showed in Figure 2 (b). All zero-order spectrum (D^0) were converted to first derivative spectrum (D^1) using delta lambda 4 and scaling factor 2. The overlain first derivative spectrums Colchicine and Aspirin at different concentration were recorded. The zero-crossing point (ZCP) of Colchicine was found to be 286 nm and ZCP of Aspirin was found to be 260 nm showed in Figure 3 (b). Absorbance of each solution was measured at 260 nm for Colchicine (0.5-2.5 µg/ml) (Figure 4) and at 286 nm for Aspirin (75-375 µg/ml) (Figure 5) using water as blank.

3. METHOD VALIDATION

The ICH Q2 (R2) guideline [21] was adopted in the validation of the outlined processes. Linearity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and assay were among the validation aspects assessed [22-23].

3.1 Linearity and Range: (n=6)

Linearity was studied by preparing standard solution at 5 different concentrations. The linearity range for Colchicine and Aspirin were found to be 0.5-2.5 µg/ml and 75-375 µg/ml, respectively. Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

3.2 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Intermediate (Intraday) precision, reproducibility (Interday precision), repeatability.

1) Intraday Precision (n=3): Solutions containing 0.5, 1.0, 1.5 µg/ml of Colchicine and 75, 150, 225 µg/ml of Aspirin were analyzed three times on the same day and % R. S. D. was calculated.
2) Interday Precision (n=3): Solutions containing 0.5, 1.0, 1.5 µg/ml of Colchicine and 75, 150, 225 µg/ml of Aspirin were analyzed three successive days and % R. S. D. was calculated.
3) Repeatability (n=6): Solutions containing 1 µg/ml of Colchicine and 150 µg/ml of Aspirin were analyzed for six times and %R.S.D. was calculated. % R.S.D was not more than 2%.

3.3 Limit of Detection (LOD)

Limit of detection can be calculated using following equation as per ICH Q2 (R2) guideline.

$$\mathrm{LOD} = 3.3 * \frac{\sigma}{S}$$

where, **σ** = Standard deviation of the Y intercept of calibration curve
S = Mean slope of the corresponding calibration curve.

3.4 Limit of Quantification (LOQ)

Limit of quantification can be calculated using following equation using the standard deviation of the Y-intercept (σ) and the mean slope (S) of the calibration curve according to ICH Q2 (R2) guideline.

$$\mathrm{LOQ} = 10 * \frac{\sigma}{S}$$

3.5 Accuracy (Recovery study) (n=3):

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH guideline at three different concentration levels 50%, 100%, 150% and the values were measured for Colchicine (1 µg/ml) and Aspirin (150 µg/ml). This performance was done in triplicate.

3.6 Assay as Analysis of Binary mixture

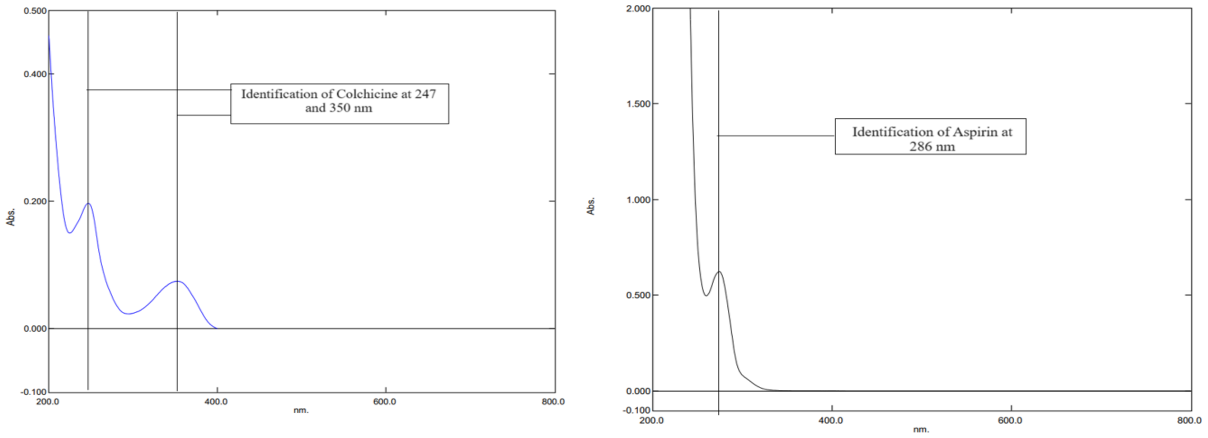
The Binary mixture of Colchicine and Aspirin was prepared in the ratio of 1:150. Accurately weighed equivalently weight of Colchicine (0.5 mg) and Aspirin (75 mg) and transferred in 100 ml volumetric flask and allowed to sonicate and made up to mark with Water. Common excipients such as Micro crystalline cellulose (3.5 mg), Lactose (15 mg), Magnesium stearate (0.5 mg), Talc (0.5 mg), and Croscarmellose sodium (5 mg) were added in the motor pestle along with the drug Colchicine (0.5 mg) and Aspirin (75 mg). This solution was filtered through Whatmann filter paper. The filtrate was diluted to the mark with Water. The mixture obtained 5 µg/ml of Colchicine and 750 µg/ml of Aspirin.

3.6.1 Preparation of sample solution: Accurately 2 ml from the above solution [mixture of Colchicine (5 µg/ml) and Aspirin (750 µg/ml)] was pipetted out into 10 ml volumetric flask and the volume was adjusted up to the mark with Water. Final concentration of Colchicine was 1 µg/ml and Aspirin 150 µg/ml.

4. RESULTS

4.1 Selection of wavelength for Colchicine and Aspirin

The remarkable absorbance of Colchicine was identified at 247 nm and 350 nm showed in Figure 2 (a) whereas Aspirin at 286 nm showed in Figure 2 (b).



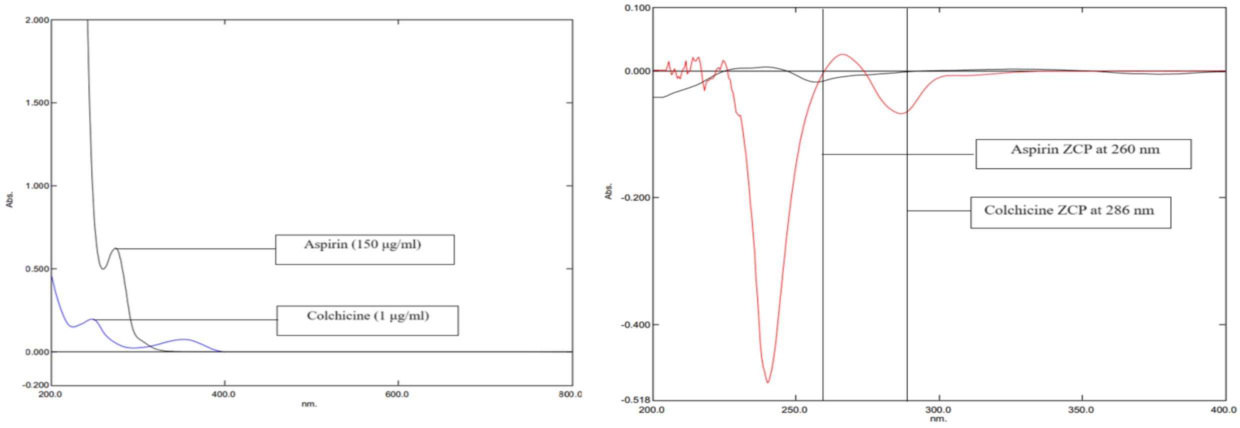
2 (a) **2 (b)**

Figure 2: UV Spectrum of 2 (a) Colchicine (1 µg/ml) at 247 nm and 350 nm, and 2 (b) Aspirin (150 µg/ml) at 286 nm

4.1.1 First order derivative UV method:

All zero-order spectrum (D^0) were converted to first derivative spectrum (D^1) using delta lambda 4 and scaling factor 2. The overlain first derivative UV spectrums of Colchicine and Aspirin at different concentration were recorded. The zero-crossing point (ZCP) of Colchicine was found to be 286 nm and ZCP of Aspirin was found to be 260 nm.

Overlain UV Spectra of Colchicine (1 µg/ml) and Aspirin (150 µg/ml) in Water for Zero order showed in figure 3 (a) and First order derivative showed in figure 3 (b).



3 (a) **3 (b)**

Figure 3: Overlain ultraviolet spectra of Colchicine (1 µg/ml) and Aspirin (150 µg/ml) in Water: 3 (a) zero-order absorption spectra and 3 (b) first-order derivative spectra

4.2 Validation parameters

4.2.1 Linearity and Range:

The absorbance was measured at 260 nm for Colchicine (0.5-2.5 µg/ml) and at 286 nm for Aspirin (75-375 µg/ml), using water as the blank. The corresponding UV spectra over the linearity range are shown in Figures 4 and 5, respectively.

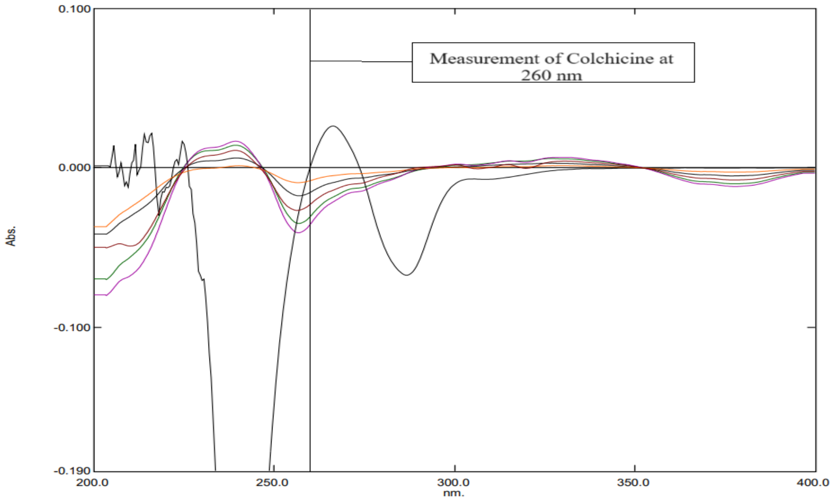


Figure 4: Overlain UV Spectra of Colchicine (0.5-2.5 µg/ml) at 260 nm

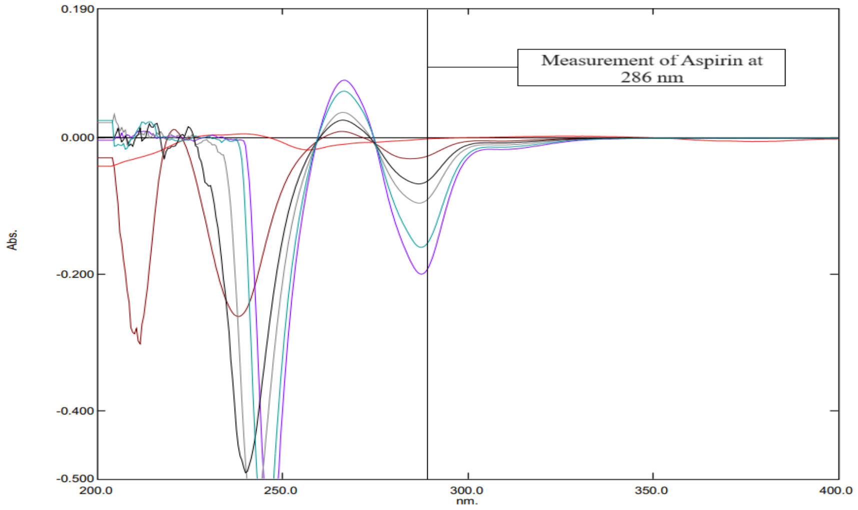


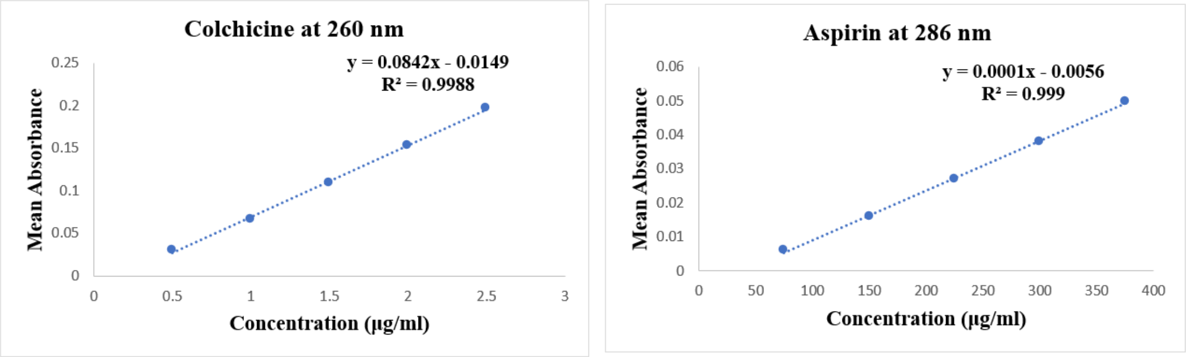
Figure 5: Overlain UV Spectra of Aspirin (75-375 µg/ml) at 286 nm

***4.2.1.1 Colchicine**:* It was demonstrated a proportional increase in absorbance with increasing concentration (linear) over the range of 0.5-2.5 µg/mL at both 260 nm. Correlation Coefficient for Colchicine 0.9988 at 260 nm were obtained [Figure 6 (a)]. The mean absorbance values obtained from six replicate measurements showed low standard deviation, and the %RSD values at both wavelengths were below 1.5%, indicating satisfactory precision and repeatability of the proposed UV spectrophotometric method. Linearity data of Colchicine had been showed in Table 1.

Table 1: Linearity data of Colchicine

Colchicine (260 nm)		
Conc. (µg/ml)	**Mean Absorbance ± SD (n=6)**	**% RSD**
0.5	|-0.030| ± 0.000336	1.12
1.0	|-0.068| ± 0.000652	0.96
1.5	|-0.109| ± 0.000795	0.73
2.0	|-0.154| ± 0.000877	0.57
2.5	|-0.197| ± 0.000709	0.36

Calibration curve with Regression equation for Colchicine at 260 nm showed in figure 6 (a).



6 (a) 6 (b)

Figure 6: Calibration curves: 6 (a) Colchicine (0.5-2.5 µg/ml) at 260 nm 6 (b) Aspirin (75-375 µg/ml) at 286 nm

4.2.1.2 Aspirin: A linear absorbance response was observed for Aspirin in the concentration range of 75-375 µg/mL at 286 nm with Correlation Coefficient 0.999. Calibration curve of Aspirin at 286 nm had been showed in Figure 6 (b). The mean absorbance values (n = 6) were associated with low standard deviation, and %RSD values less than 1.5% confirmed the precision and reproducibility of the developed analytical method. Linearity data of Aspirin showed in Table 2.

Table 2: Linearity data of Aspirin

Aspirin (286 nm)		
Conc. (µg/ml)	**Mean Absorbance ± SD (n=6)**	**% RSD**
75	|-0.006| ± 0.000075	1.25
150	|-0.016| ± 0.000163	1.02
225	|-0.027| ± 0.000221	0.82
300	|-0.038| ± 0.000239	0.63
375	|-0.050| ± 0.000240	0.48

4.2.2 Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling. The precision of an analytical method is the closeness of a series of individual analyte measurements applied repeatedly to multiple aliquots of the same sample. It is calculated as a Relative Standard Deviation are homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility**:**

4.2.2.1 Colchicine: The precision of the proposed method for Colchicine was evaluated by intraday, interday, and repeatability studies, and the results are summarized in **Table 3.** Intraday and interday precision were assessed at three concentration levels by analyzing samples three times within the same day and on three consecutive days, respectively. Repeatability was determined by multiple measurements of a single concentration. The %RSD values at 260 nm were found to be less than 2.0%, indicating good precision and repeatability of the method.

Table 3: Precision study of Colchicine

Precision study of Colchicine (260 nm)		
Intraday precision of Colchicine		
Conc. (µg/ml)	**Mean Absorbance ±SD (n=3)**	**% RSD**
0.5	|-0.030| ± 0.000330	1.10
1.0	|-0.068| ± 0.000640	0.94
1.5	|-0.109| ± 0.000773	0.71
Interday precision of Colchicine		
Conc. (µg/ml)	**Mean Absorbance ±SD (n=3)**	**% RSD**
0.5	|-0.030| ± 0.000336	1.12
1.0	|-0.068| ± 0.000652	0.96
1.5	|-0.109| ± 0.000795	0.73
Repeatability of Colchicine		
Conc. (µg/ml)	**Mean Absorbance ±SD (n=6)**	**% RSD**
1.0	|-0.068| ± 0.000632	0.93

4.2.2.2 Aspirin: The precision study for Aspirin was carried out similarly, and the results are presented in **Table 4**. Intraday and interday precision were evaluated at three different concentration levels, while repeatability was assessed at a single concentration. The %RSD values obtained at 286 nm were below 2.0%, demonstrating satisfactory precision and consistency of the analytical method.

Table 4: Precision study of Aspirin

<table>
<tr><td colspan="3">Precision study of Aspirin (286 nm)</td></tr>
<tr><td colspan="3">Intraday precision of Aspirin</td></tr>
<tr><td>Conc. (µg/ml)</td><td>Mean Absorbance ±SD (n=3)</td><td>% RSD</td></tr>
<tr><td>75</td><td>|-0.006| ± 0.000074</td><td>1.23</td></tr>
<tr><td>150</td><td>|-0.016| ± 0.000161</td><td>1.00</td></tr>
<tr><td>225</td><td>|-0.027| ± 0.000218</td><td>0.81</td></tr>
<tr><td colspan="3">Interday precision of Aspirin</td></tr>
<tr><td>Conc. (µg/ml)</td><td>Mean Absorbance ±SD (n=3)</td><td>% RSD</td></tr>
<tr><td>75</td><td>|-0.006| ± 0.000075</td><td>1.25</td></tr>
<tr><td>150</td><td>|-0.016| ± 0.000163</td><td>1.01</td></tr>
<tr><td>225</td><td>|-0.027| ± 0.000221</td><td>0.81</td></tr>
<tr><td colspan="3">Repeatability of Aspirin</td></tr>
<tr><td>Conc. (µg/ml)</td><td>Mean Absorbance ±SD (n=6)</td><td>% RSD</td></tr>
<tr><td>150</td><td>|-0.016| ± 0.00016</td><td>1.00</td></tr>
</table>

4.2.3 LOD and LOQ:

The limits of detection (LOD) and quantification (LOQ) were calculated in accordance with ICH Q2 (R2) guideline using the standard deviation of the response and the slope of the calibration curve. The LOD and LOQ values for Colchicine and Aspirin showed in Table 5. The low LOD and LOQ values obtained at the selected wavelengths indicated the adequate sensitivity of the proposed UV spectrophotometric method for the estimation of both drugs.

Table 5: LOD and LOQ data

Parameter	**Colchicine**	**Aspirin**
LOD (µg/ml)	0.013	2.47
LOQ (µg/ml)	0.04	7.50

4.2.4 Accuracy:

Accuracy of the proposed method was evaluated by the standard addition method. Known amounts of Colchicine and Aspirin standards were added to pre-analyzed samples at three concentration levels (50%, 100%, and 150%), and the mixtures were analyzed in triplicate. The results of the recovery study for both drugs are presented in Table 6. The mean percentage recovery values for Colchicine and Aspirin were found to be within the ICH-accepted range of 98-102%, with low standard deviation. These results confirm the accuracy, trueness, and reliability of the developed method and indicated that excipients present in the synthetic mixture did not interfere with the estimation of either drug.

Table 6: Recovery study

Name of Drug	**% Level of recovery**	**Test Amount (µg/ml)**	**Amount of drug taken (µg/ml)**	**Total Std Amt. (µg/ml)**	**Total amount Recovered (µg/ml)**	**% Mean Recovery ± SD (n=3)**
Colchicine	50	1	0.5	1.50	1.49	99.34±0.007
	100	1	1.0	2.0	1.99	99.50±0.012
	150	1	1.5	2.5	2.51	100.4±0.014
Aspirin	50	150	75	225	224.9	99.96±0.130
	100	150	150	300	299.9	99.97±0.156
	150	150	225	375	374.8	99.95±0.156

4.2.5 Analysis of Synthetic mixture:

The developed UV spectrophotometric method was applied to the analysis of a synthetic mixture containing Colchicine and Aspirin. The results of the assay were presented in Table 7. The mean percentage assay values for Colchicine and Aspirin were found to be close to 100%, with low standard deviation and %RSD values less than 2.0%, indicating the accuracy and precision of the method for simultaneous estimation of both drugs.

Table 7: Analysis of synthetic mixture

Name of Drug	Amount in synthetic mixture (µg/ml)	Mean Amount found (µg/ml)	% Mean Assay ± SD (n=3)	% RSD
Colchicine	1	0.999	99.90 ± 0.0035	0.0035
Aspirin	150	149.9	99.94 ± 0.0026	0.0026

5. DISCUSSION:

A first-order derivative UV-spectrophotometric method was successfully developed and validated for the simultaneous estimation of Colchicine and Aspirin in a synthetic mixture. The application of derivative spectrophotometry improved spectral resolution and minimized interference between the two drugs, enabling their accurate and selective quantification without prior separation. Colchicine and Aspirin showed distinct zero-crossing points at 260 nm and 286 nm, respectively, which were selected for quantitative analysis. Both drugs obeyed Beer–Lambert's law over the concentration ranges of 0.5-2.5 µg/ml for Colchicine and 75-375 µg/ml for Aspirin. The calibration curves exhibited excellent linearity, with regression coefficients (r^2) of 0.9988 for Colchicine and 0.999 for Aspirin, confirming a strong linear relationship between derivative absorbance and concentration. The precision of the method was demonstrated by intraday and interday studies. The %RSD values for both drugs were found to be less than 2%, indicating acceptable repeatability and intermediate precision in accordance with ICH guidelines. Repeatability studies further supported the reliability of the method, with %RSD values of 0.98% for Colchicine and 1.00% for Aspirin. Accuracy was evaluated using recovery studies, and the results were found to be within the acceptable range. The percent recovery values ranged from 99.34% to 100.4% for Colchicine and from 99.95% to 99.97% for Aspirin, confirming the absence of interference from excipients present in the synthetic mixture and demonstrating the accuracy of the proposed method. The sensitivity of the first-order derivative method was assessed by determining the limits of detection and quantitation. The low LOD and LOQ values obtained for Colchicine (0.013 µg/ml and 0.04 µg/ml) and Aspirin (2.47 µg/ml and 7.50 µg/ml) indicate that the method is sufficiently sensitive for routine analytical applications. The assay values of 99.90% for Colchicine and 99.94% for Aspirin further confirmed the suitability of the method for simultaneous estimation in combined formulations. Overall, the validated first-order derivative UV-spectrophotometric method is simple, precise, accurate, sensitive, and cost-effective, making it suitable for routine quality control analysis of Colchicine and Aspirin in synthetic mixtures.

6. CONCLUSION:

A simple, rapid, and reliable first-order derivative UV-spectrophotometric method was successfully developed and validated for the simultaneous estimation of Colchicine and Aspirin in a synthetic mixture. The application of derivative spectrophotometry effectively resolved spectral overlap and enhanced method selectivity through well-defined zero-crossing points, enabling accurate quantification without prior separation.

The validated method exhibited excellent linearity, precision, accuracy, and sensitivity in compliance with ICH guidelines. Low %RSD values confirmed good reproducibility, while recovery studies demonstrated the absence of interference from excipients. The low limits of detection and quantitation further established the suitability of the method for routine analysis. Due to its simplicity, cost-effectiveness, and minimal solvent consumption, the proposed method is well suited for routine quality control analysis of Colchicine and Aspirin in combined pharmaceutical formulations.

ACKNOWLEDGEMENT:

The authors are grateful to Smt. N. M. Padalia Pharmacy College, Ahmedabad, for encouragement and for providing the necessary facilities to carry out this research work.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

REFERENCES:

1. Nishiga M, Wang DW, Han Y, Lewis DB, Wu JC. COVID-19 and cardiovascular disease: from basic mechanisms to clinical perspectives. *Nature Reviews Cardiology.* 2020;17(9):543–558. doi:10.1038/s41569-020-0413-9.
2. Lawal IO, Kgatle MM, Mokoala KG, Farate A, Sathekge MM. Cardiovascular disturbances in COVID-19: an updated review of the pathophysiology and clinical evidence of cardiovascular damage induced by SARS-CoV-2. *BioMed Central Cardiovascular Disorders.* 2022; 22: 93. doi:10.1186/s12872-022-02534-8.
3. ClinicalTrials.gov. *Colchicine and Non-Enteric Coated Aspirin in the Cardiovascular Outcomes Trial of Patients with Type 2 Diabetes (COLCOT-T2D).* Identifier: **NCT05633810**. *National Library of Medicine*; 2024. Available from:

https://clinicaltrials.gov/study/NCT05633810.

4. Naik KS, Andhalkar N, Pendse S. Effect of colchicine and aspirin given together in patients with moderate COVID-19. *Contemporary Clinical Trials Communications.* 2023; 32: 101070. doi: 10.1016/j.conctc.2023.101070.
5. Eikelboom JW, Jolly SS, Belley-Cote EP, Whitlock RP, Rangarajan S, Xu L, Heenan L, Bangdiwala SI, Tarhuni WM, Hassany M, Kontsevaya A, Harper W, Sharma SK, Lopez-Jaramillo P, Dans AL, Palileo-Villanueva LM, Avezum A, Pais P, Xavier D, Felix C, Yusufali A, Lopes RD, Berwanger O, Ali Z, Wasserman S, Anand SS, Bosch J, Choudhri S, Farkouh ME, Loeb M, Yusuf S. Colchicine and aspirin in community patients with COVID-19 (ACT): an open-label, factorial, randomised, controlled trial. *Lancet Respiratory Medicine.* 2022; 10 (12):1160-1168. doi:10.1016/S2213-2600(22)00299-5.
6. Costa F, Micari A. Aspirin-free strategy after ACS implementing colchicine: looking for a substitution. *Journal of the American College of Cardiology: Cardiovascular Interventions.* 2023;16(15):1856-1859. doi: 10.1016/j.jcin.2023.06.033.
7. Inokuchi T, Homma T, Kitasato Y, Akiyama M, Chikasue A, Nishii Y, Ban S, Adachi T, Sonezaki A, Masuda H, Kamei H, Takenaka M, Tanaka M, Okamoto M, Hoshino T, K-Cocoa. Oral colchicine and low-dose aspirin combination therapy for non-elderly, non-severe, early-onset adult outpatients with COVID-19 during the fifth pandemic wave in Japan. *Kurume Medical Journal*, 2023, 70 (1.2), 39-45. doi:10.2739/kurumemedj.MS7012003.
8. American College of Cardiology. The role of colchicine in recent clinical trials. Published August 13, 2020. Available from: https://www.acc.org/latest-in-cardiology/articles/2020/08/13/13/11/the-role-of-colchicine-in-recent-clinical-trials.
9. Alkadi H, Khubeiz MJ, Jbeily R. Colchicine: a review on chemical structure and clinical usage. *Infect Disord Drug Targets.* 2018;18(2):105–121. doi:10.2174/1871526517666171017114901.
10. O'Neil MJ, ed. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th ed. Whitehouse Station, NJ: Merck & Co., Inc.; 2001:147, 976.
11. Alegbeley J, Mohammad AA. Pharmacology, pharmaceutics, and clinical use of Aspirin: a narrative review. *Journal of Drug Delivery and Therapeutics.* 2020; 10(5-S): 1-8. doi:10.22270/jddt. v10i5-s.4351.
12. Samanidou V.F, Sarantis G.A, Papadoyannis I.N, "Development and validation of a rapid HPLC method for the direct determination of colchicine in pharmaceuticals and biological fluids", *Journal of liquid chromatography & related technologies*, **2006**, *29*(1), pp 1-13. DOI: 10.1080/10826070500357755.
13. Shahid A, Brajesh K, Mrs. Sheenam, Mr. Rahim and Jalaluddin Mo, "Analytical Method Development and Validation for the Determination of Pantoprazole and Aspirin Using Reverse Phase HPLC Method in Combined Tablet Dosage Form" *World Journal of Pharmaceutical Research.* **2022**; *11(3)*: pp 1575-1589.
14. Shaikh FAV, Shaikh NK, Bhangale JO. Development and validation of UV-spectrophotometric method for simultaneous estimation of Colchicine and Rosuvastatin calcium in synthetic mixture, *Journal of Applied Bioanalysis*, 2025, 11(12): 213-224. http://doi.org/10.53555/jab.v11si12.1676.
15. Bhalerao SV, Bachhav RS, Adhav NM. Method development and validation for simultaneous estimation of Aspirin and Ticlopidine HCl in bulk and tablet formulation by UV spectrophotometry. *International Journal of Pharmaceutical Sciences*. 2024, 2 (7), 563-578. DOI: 10.5281/zenodo.12699350.
16. Tantawy M.A, Wahba I.A, Saad S.S, and Ramadan N.K, Stability-indicating chromatographic methods for the simultaneous determination of probenecid and colchicine in their combined tablet. *Journal of Chromatographic Science, 59(10),* **2021** pp 956-963. DOI: 10.1093/chromsci/bmab050.
17. Ali A, Qadir A, Khan M.A, Al-Otaibi F.O, Khan S, and Abdin M.Z, A simple validated HPTLC method for simultaneous analysis of colchicine and gallic acid in polyherbal formulation: Habb-E-Irqun-Nisha. *Food Analytical Methods*, *10*, **2017** pp 2934-2942**.** DOI: 10.1007/s12161-017-0835-0
18. Jiang Y, Wang J, Wang Y Li H, Fawcett J.P, and Gu J, Rapid and sensitive liquid chromatography–tandem mass spectrometry method for the quantitation of colchicine in human plasma. *Journal of Chromatography B, 850(1-2),* **2007** pp 564-568. DOI: https://doi.org/10.22159/ijpps.2017v9i7.18336
19. Puram S.R, and Nithya G, Sensitive method for the determination of colchicine in human plasma by gradient UPLC-ESI-MS/MS: Separation of endogenous interference. *Research Journal of Pharmacy and Technology*, *13*(7), **2020,** 3274-3278. DOI:10.5958/0974-360X.2020.00580.6.
20. Shaikh NK, Bhangale JO. Analytical methods for detecting and quantifying Vildagliptin and Remogliflozin in bulk and combined dosage form and their potentiality, *Journal of Molecular Science*, **2025**, 35(3): 1100-1107. https://doi.org/10.004687/1000-9035.2025.147.
21. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), *ICH Q2(R2): Validation of Analytical Procedures*, Geneva: ICH; 2023, pp. 1-38.
22. Shaikh NK, Jat R, Bhangale JO. Development and validation of stability-indicating RP-HPLC and UV method for simultaneous quantitation of Repaglinide and Sitagliptin phosphate in combination, *American Journal of PharmTech Research*, **2020**, 10(6): 95-114. https://doi.org/10.46624/ajptr.2020.v10.i6.007.
23. Shaikh NK, Jat R, Bhangale JO. Analysis of Vildagliptin and Nateglinide for simultaneous estimation using spectro-chromatographic methods, *European Journal of Molecular and Clinical Medicine*, **2020**, 7(8): 741-755. https://ejmcm.com/article_3194.html.