RESEARCH PAPER

RP-HPLC Method Development and Validation for Simultaneous Estimation of Paracetamol and Prochlorperazine Maleate

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ABSTRACT :
A simple, precise, accurate, robust, and stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Paracetamol (PCM) and Prochlorperazine Maleate (PCZM) in pharmaceutical dosage forms. Chromatographic separation was achieved on a Cosmosil C18 column (250 mm× 4.6 mm, 5 µm) using Methanol:Water (60:40, v/v) adjusted to pH 3.0 with orthophosphoric acid as the optimized mobile phase. The mobile phase was delivered at a flow rate of 0.9 mL/min, and detection was carried out at 251 nm. Under the optimized chromatographic conditions, Paracetamol and Prochlorperazine Maleate were eluted at retention times of 4.5 min and 7.0 min, respectively. The method was validated in accordance with ICH Q2(R1) guidelines for linearity, accuracy, precision, robustness, sensitivity, and specificity. Excellent linearity was obtained over the concentration range of 10–50 µg/mL with correlation coefficients (R^2) of 0.9998 for PCM and 0.9995 for PCZM. Accuracy studies performed at 50%, 100%, and 150% concentration levels yielded mean recoveries of 100.15%, 99.84%, and 100.08% for PCM, while recoveries of 100.70%, 99.49%, and 100.13% were obtained for PCZM. Precision studies demonstrated excellent repeatability with %RSD values of 0.08% for PCM and 0.31% for PCZM.Furthermore, forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic stress conditions confirmed the stability-indicating capability of the developed method. The proposed RP-HPLC method was found to be reliable, sensitive, accurate, and suitable for routine quality control analysis and stability assessment of Paracetamol and Prochlorperazine Maleate in pharmaceutical formulations.

Keywords: Paracetamol, Prochlorperazine Maleate, RP-HPLC, Method Validation, Stability-Indicating Method, ICH Q2(R1), Simultaneous Estimation.
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INTRODUCTION :

Paracetamol (PCM), also known as acetaminophen, is one of the most commonly used analgesic and antipyretic drugs worldwide. Chemically, it is designated as N-(4-hydroxyphenyl) acetamide. It is widely prescribed for the treatment of mild to moderate pain and fever owing to its efficacy, safety, and favorable therapeutic profile. The drug exerts its pharmacological action primarily through inhibition of prostaglandin synthesis within the central nervous system, thereby producing analgesic and antipyretic effects[1].Prochlorperazine Maleate (PCZM) is a phenothiazine derivative possessing antiemetic, antipsychotic, and antivertigo properties. It is chemically designated as 2-chloro-10-[3-(4-methylpiperazin-1-yl) propyl] phenothiazine maleate. The drug is extensively used in the management of nausea, vomiting, migraine-associated symptoms, and various vestibular disorders. Its therapeutic action is mainly attributed to dopamine receptor antagonism in the chemoreceptor trigger zone[2].The combination of Paracetamol and Prochlorperazine Maleate is widely employed in the treatment of migraine and associated symptoms. Paracetamol provides effective relief from headache and pain[3], while Prochlorperazine Maleate controls nausea and vomiting frequently associated with migraine attacks. Owing to their combined therapeutic benefits, pharmaceutical formulations containing both drugs have gained considerable clinical importance[2,5].Several analytical methods including UV spectrophotometry, HPLC, RP-HPLC and FT-IR have been reported for the estimation of Paracetamol and Prochlorperazine Maleate either individually or in combination with other drugs. However, the simultaneous estimation of both drugs in combined

pharmaceutical dosage forms requires a simple, accurate, precise, and reproducible analytical method suitable for routine quality control analysis[4,5].Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is one of the most widely accepted analytical techniques due to its high sensitivity, selectivity, accuracy, and reproducibilaity[6]. Therefore, the present study was undertaken to develop and validate a simple, rapid, precise, accurate, and robust RP-HPLC method for the simultaneous estimation of Paracetamol and Prochlorperazine Maleate in pharmaceutical dosage forms in accordance with International Council for Harmonisation (ICH) guidelines.

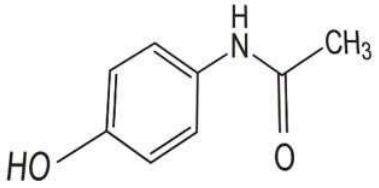

Fig.1 Paracetamol

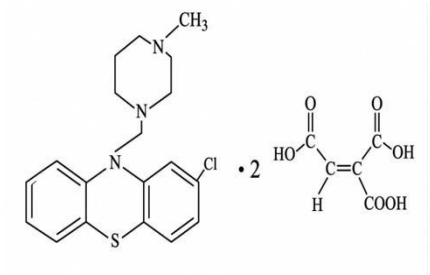

Fig .2 Prochlorperazine Maleate

LITERATURE REVIEW :

Over the past two decades, several liquid chromatographic and spectrophotometric methods have been documented for the quantification of Paracetamol (PAR) and Prochlorperazine Maleate (PRO), either individually, in combination with each other, or alongside other active pharmaceutical ingredients (APIs).

To resolve complex multi-component matrices containing Paracetamol, multi-channel and advanced stationary phases have been extensively explored. Bhosale (2012) developed a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of prochlorperazine maleate, ergotamine tartrate, paracetamol, and caffeine monohydrate in tablet forms using a Purospher star RP-18e column with an ion-pairing gradient system containing sodium octane sulphonate and acetonitrile within an 18-minute runtime[7]. Similarly, Dewani (2013) established a rapid multi-drug separation protocol using a Kinetex-C18 column and an acetonitrile-phosphate buffer (pH 3.3) mobile phase under UV detection at 230 nm to resolve multi-component analgesic and anti-inflammatory mixtures containing paracetamol[8].

In terms of simpler multi-component combinations, Acheampong (2016) achieved successful isocratic separation of chlorpheniramine maleate, paracetamol, and caffeine using a Phenomenex C18 column and a methanol-phosphate buffer (pH 4.0) mobile phase matrix[9]. Furthermore, focused chemometric and binary mixture profiles have also progressed; Borahan (2018) verified an accurate binary RP-HPLC estimation for ibuprofen and paracetamol using a phosphate buffer (pH 7.5) and methanol system[10], while Archakam (2020) utilized chemometric-assisted UV spectrophotometric protocols along with automated RP-HPLC to estimate paracetamol in combinations featuring multi-active cough and cold formulations[11].

For the dedicated assessment of Prochlorperazine Maleate, analytical methods have historically emphasized overcoming challenges related to matrix retention and peak tailing. Wate (2012) reported an economical and reproducible dual-wavelength spectrophotometric method utilizing isosbestic points at 267.5 nm and 290.5 nm for the simultaneous estimation of prochlorperazine maleate and pyridoxine hydrochloride[12]. In liquid chromatography, Patel (2014) achieved a robust reverse-phase separation of betahistine dihydrochloride and prochlorperazine maleate in tablet dosage forms using an X-Terra C18 column with a mobile phase consisting of acetonitrile, methanol, and a phosphate buffer (pH 4.0) pumped at 0.8 mL/min[13]. Highly specific bioanalytical assays have also been reported; Sundaram (2009) designed an advanced bioassay utilizing Solid Phase Extraction (SPE) followed by isocratic RP-HPLC on a Phenomenex C18 column to quantify sustained-release prochlorperazine formulations in human plasma[14], whereas Miao (2009) implemented a highly sensitive and specific liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) technique utilizing single-step liquid-liquid extraction with dichloromethane to trace prochlorperazine levels in clinical plasma profiles[15]. More recently, alternative planar chromatographic techniques, such as the high-performance thin-layer chromatography (HPTLC) densitometric method proposed by Ali (2019), have been introduced to achieve simultaneous determination of paracetamol in unique drug pairings using dual-wavelength tracking[16].

Despite these reported methodologies, there remains an analytical gap for a ultra-rapid, cost-effective, and highly eco-friendly isocratic RP-HPLC method specifically optimized for binary combinations of Paracetamol and Prochlorperazine Maleate using modern volatile buffers. Hence, the current study was conceptualized.

MATERIALS AND METHOD:

Chemicals and reagents :

Paracetamol (PCM) and Prochlorperazine Maleate (PCZM) working standards were obtained as gift

samples from Zydus Cadila. HPLC-grade methanol and acetonitrile were procured from Thomas Baker Chemicals Pvt. Ltd., India. Potassium dihydrogen phosphate, orthophosphoric acid, sodium hydroxide, and hydrochloric acid of analytical reagent grade were used throughout the study. Ultrapure water was used for the preparation of all solutions.

Instrumentation :

Chromatographic analysis was performed using a Jasco HPLC system (Series 2000, Japan) equipped with a UV detector. A UV-visible double beam spectrophotometer (Jasco V-630, Japan) was used for wavelength selection. pH measurements were carried out using an EQ-610 pH meter. Samples were filtered through a 0.45 µm membrane filter and sonicated using a UCB-40 sonicator.

Chromatographic Conditions:

Chromatographic separation was achieved on a C18 column using a mobile phase consisting of Methanol:Water (60:40, v/v). The pH of the mobile phase was adjusted to 3.0 using orthophosphoric acid. The mobile phase was filtered through a 0.45 µm membrane filter and degassed before use. The flow rate was maintained at 0.9 mL/min and detection was carried out at 251 nm.The injection volume was 20 µL and analysis was performed at ambient temperature[17].

Preparation of Standard Stock Solutions :

Standard stock solutions of Paracetamol and Prochlorperazine Maleate were prepared separately by accurately weighing 10 mg of each drug and transferring into separate 10 mL volumetric flasks. The drugs were dissolved in methanol and the volume was made up to the mark with the same solvent to obtain stock solutions containing 1000 µg/mL. Further dilutions were prepared using the mobile phase to obtain the required working concentrations.

Preparation of Standard Working Solutions

Working standard solutions of Paracetamol and Prochlorperazine Maleate were prepared by suitable dilution of the respective stock solutions (1000 µg/mL) using the mobile phase. The resulting solutions were used for calibration and validation studies over the selected linear concentration range.

Method Validation :

The developed RP-HPLC method was validated according to ICH Q2(R1) guidelines for parameters including linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantification (LOQ), and system suitability[18].

Accuracy (% Recovery)

Accuracy is defined as the measure of exactness of an analytical procedure, expressing the closeness between the conventional true value and the value found. In accordance with the ICH guidelines, accuracy was evaluated using the standard addition method at three distinct concentration levels covering the specified range. For this study, the recovery experiments were performed by spiking known amounts of pure standard drugs (PCM and PCZM) into pre-analyzed sample solutions at 50%, 100%, and 150% levels. The samples were analyzed in triplicate (n=3) at each level, and the percentage recovery was calculated to ensure compliance with the standard acceptance criteria of 98.0–102.0%[19].

Precision

Method precision was verified through the assessment of both intra-day and inter-day variations. Repeatability (intra-day precision) was executed by performing replicate injections (n=3) of three different standard concentrations within a single operational day. Intermediate precision (inter-day variation) was established by analyzing the identical concentration strength on three consecutive days. The precision profiles were documented in terms of percentage relative standard deviation (% RSD) to ensure the method's reproducibility[20].

Linearity and Range

Linearity of the developed RP-HPLC method was determined over a concentration range of 10 -50µg/mL for both Paracetamol (PCM) and Prochlorperazine Maleate (PCZM). Standard calibration curves were constructed by plotting the nominal analyte concentrations (µg/mL) on the X-axis against their respective obtained peak areas on the Y-axis at a detection wavelength of 251 nm.

The method's linearity was validated based on the acceptable coefficient of correlation (R^2), where the regression equations were found to be y=12050x + 3500 (R^2 = 0.9998) for PCM and y = 2603.5x + 758.1 (R^2 = 0.9995) for PCZM. The obtained correlation coefficients (R^2 > 0.999) strictly satisfy the regulatory acceptance criteria under ICH Q2(R1) guidelines, demonstrating an excellent proportional relationship between the detector response and analyte concentration within the specified analytical range[21].

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

The sensitivity of the proposed method was evaluated by determining the Limit of Detection (LOD) and Limit of Quantification (LOQ). These values were calculated based on the standard deviation (\sigma) of the response (y-intercept) and the slope (S) of the calibration curve using the following specific formulas[20] :

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve

Robustness

The robustness of the developed RP-HPLC method was verified by introducing deliberate, minor variations in the optimized chromatographic conditions. The parameters modified included small changes in the detection wavelength and the mobile

phase pH .
A standard solution of 10µg/mL was analyzed under each robust condition. The method's robustness was evaluated based on its capacity to remain unaffected, ensuring that the percentage relative standard deviation (%RSD) for peak areas and retention times (Rt) remained strictly within the acceptable limit of < 2.0%[22].

RESULTS AND DISCUSSION

Method Development :

UV spectroscopic analysis of the drugs showed maximum absorbance at 251 nm. An appropriate and precise HPLC technique for the simultaneous analysis of Paracetamol (PCM) and Prochlorperazine Maleate (PCZM) was employed after several trials with different mobile phase ratios were done.
The 1st trial started with Methanol : Water (70:30 v/v) adjusted to pH 3.0 with Orthophosphoric acid using a Cosmosil C18 column for PCM. But this trial was not selected as rapid elution with poor resolution was observed (Rt = 3.8min). 2nd trial was done by increasing the organic phase ratio to Methanol : Water (80:20 v/v) for PCM under the same conditions. The trial was not selected as no improvement in retention and broad peak shape was observed (Rt = 3.8 min). 3rd trial was done by decreasing the organic modifier ratio to Methanol : Water (60:40 v/v) for PCM, which showed an extended retention behavior (Rt = 4.4 min).
Concurrently, for the highly hydrophobic drug PCZM, the 4th trial was conducted using Methanol : Water (20:80 v/v). This trial was not selected as a significantly prolonged elution time was observed (Rt = 11.0 min). The 5th trial was carried out by drastically increasing the organic phase to Methanol : Water (80:20v/v) for PCZM. However, this trial was not selected as it resulted in sharp but premature elution (Rt = 3.1 min). The 6th trial was performed using Methanol : Water (60:40v/v) for PCZM alone, which yielded an optimum and stable retention time of 7.0 min.
Finally, the 7th trial was executed for the combination mixture of PCM and PCZM using the optimized mobile phase composition of Methanol : Water (60:40v/v), adjusted to pH 3.0 with Orthophosphoric acid at a flow rate of 0.9 mL/min. The obtained chromatogram was found to be in good shape with baseline separation and symmetric peaks showing well-resolved retention times of 4.5 min for PCM and 7.0 min for PCZM[23].

System Suitability

System suitability parameters were evaluated by performing six replicate injections of the standard working solution containing Paracetamol (PCM) and Prochlorperazine Maleate (PCZM). Chromatographic parameters, including peak retention time, theoretical plate count, tailing factor, and the percentage relative standard deviation (%RSD) of the peak areas, were calculated to monitor the system's performance. The obtained values were found to be within the strictly acceptable regulatory limits, demonstrating adequate resolution and reproducibility. This system verification confirms the performance and precision of the developed RP-HPLC method for routine pharmaceutical quality control analysis[24].

Table No.1: System suitability data of Paracetamol and Prochlorperazine maleate

Sr.no.	Parameters	Paracetamol(PCM)	Prochlorperazine Maleate (PCZM)	Acceptance Criteria
1	Retention time(min)	4.509	7.063	System specific
2	Peak area	625104	37486	System specific
3	Theoretical plates (N)	8160	7856	>2000
4	Tailing factor/ Asymmetry(T)	1.28	1.31	<2.00
5	Resolution (R_s)	8.17	-	>2.00

Accuracy (% Recovery) :

The accuracy of the method was evaluated by the recovery of PCM and PCZM at three different percentage concentrations (50%, 100%, and 150%). The results of the accuracy studies are summarized in Table 2. The recoveries of PCM and PCZM were calculated to be 99.84%,100.15% and 99.49 %, 100.70%, respectively. The percentage relative standard deviation (% RSD) values were found to be 0.08% for PCM and 0.31 % for PCZM. As all the
% RSD values are strictly less than 2%, the proposed method is confirmed to be highly accurate within the desired analytical range[25].

Table No.2: Accuracy of PCM &PCZM

Drug name	Spiked Level(%)	Standard area	Sample area	Recovery	Mean Peak area ±SD(n=3)	%RSD
Paracetamol(PCM)	50%	3164917	3169702	100.15	1047874.66±1268.79	0.08
	100%	4300781	4294080	99.84	3163413.33±1891.30	0.08
	150%	5336382	5340943	100.08	5340484.00±4262.61	0.08

Prochlorperazine Maleate(PCZM)	50%	178354	78904	100.70	78904.33±527.75	0.31
	100%	105979	105444	99.49	105444.66±410.68	0.31
	150%	130667	130846	100.13	130846.33±484.77	0.31

Precision

The precision of the proposed RP-HPLC method was rigorously evaluated by conducting both intraday (repeatability) and interday (intermediate precision) profiling. To establish the method's reliability across its operational linear range, precision studies were executed at three distinct concentration levels representing low, medium, and high concentrations (50µg/mL, 100µg/mL, and 150µg/mL) for both Paracetamol (PCM) and its co-formulated component (PCZM). The system variability was quantitatively measured and expressed through the statistical parameters of Mean Peak Area and percentage Relative Standard Deviation (%RSD).

A) Precision Profiling for PCM

The method precision for PCM was evaluated by analyzing the designated concentrations (50 µg/mL, 100 µg/mL and 150 µg /mL). The intermediate precision (interday validation), assessed by tracking injections across two separate, consecutive operational sequences (Day 1 and Day 2), yielded a mean peak area of 315,675 with an exceptionally low %RSD of 0.10%.

Concurrently, the intra-sequence repeatability (intraday validation) was conducted by monitoring the response variation at distinct diurnal intervals (morning and evening sessions) within a single 24-hour cycle. The intraday analysis exhibited a mean peak area of 3,166,139 with a corresponding %RSD of 0.16%. The comprehensive statistical breakdown for PCM is tabulated in Table 3.

B) Precision Profiling for PCZM

Under identical experimental conditions, the system precision for PCZM was verified at the corresponding target concentrations of(50µg/mL,100µg/mL,and150µg/mL). The interday validation matrix calculated over a two-day testing window generated a mean chromatographic peak response of 78,788 with a %RSD of 0.42%.

The intraday precision study, capturing variations between morning and evening analytical batches, demonstrated a mean peak area of 78,443 and a %RSD of 0.35%. These findings are detailed in Table 4.

The empirical results demonstrate that all calculated %RSD values for both intra-diurnal and inter-diurnal precision blocks fall significantly below the maximum threshold of 2.0% mandated by current ICH guidelines. This minimal dispersion among replicate injections confirms that the proposed method possesses a high level of analytical ruggedness, repeatability, and system reproducibility[26].

Table 3: Intraday and Interday Method Precision Evaluation Data for PCM

Validation Parameter	(Concentration µg/mL)	Replicate Injection	Peak Area	Statistical Evaluation
Interday Precision	50	Day 1	3,164,917	**Mean Peak Area**:315,675
	100	Day1	3,165,064	
	150	Day1	3,160,259	**%RSD**:0.10%
	50	Day 2	3,168,591	
	100	Day2	3,169,724	
	150	Day2	3,165,675	
Intraday Precision	50	Morning	3,164,971	**Mean Peak Area**:3,166,139
	100	Morning	3,165,064	
	150	Morning	3,160,259	**%RSD**:0.16%
	50	Evening	3,163,604	
	100	Evening	3,167,495	
	150	Evening	3,175,442	

Table 4: Intraday and Interday Method Precision Evaluation Data for PCZM

Valiadtion Parameter	(Concentration µg/mL)	Replicate Injection	Peak Area	Statistical Evaluation
Interday Precision	50	Day1	78,354	**Mean Peak Area**:78,788
	100	Day2	78,308	
	150	Day3	78,241	**%RSD**:0.42%
	50	Day1	78,891	

	100	Day2	78,971	
	150	Day3	78,788	
Intraday Precision	50	Morning	78,354	**Mean Peak Area**:78,443
	100	Morning	78,908	
	150	Morning	78,241	**%RSD**:0.35%
	50	Evening	78,392	
	100	Evening	78,609	
	150	Evening	78,154	

Linearity and Range:
The proposed RP-HPLC method was shown a good linearity in the concentration range of (10 to 50 µg/mL and 10 to 50µg/mL) for PCM, PCZM respectively. The linear equations of the straight lines are y=12050x + 3500 (R^2 = 0.9998) for PCM and y = 2603.5x + 758.1 (R^2 = 0.9995) for PCZM. The results are satisfactory, because there is a significant correlation between concentration of drugs and response factor within the concentration range[27].

Table 5:Linearity

Parameter	Paracetamol(PCM)	Prochlorperazine Maleate (PCZM)
Linearity Range (µg/mL)	10-50	10-50
Regression Equation(y=mx+c)	y=12050x+3500	y=2603.5x+758.1
Correlation Coefficient (R^2)	0.9998	0.9995

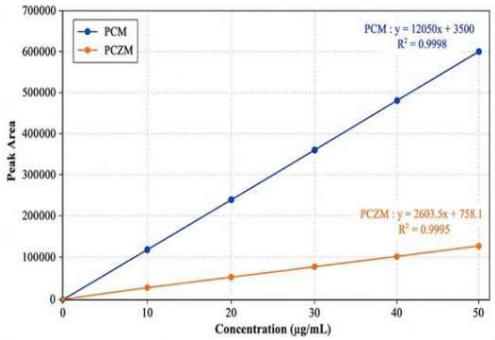

Fig 3: Calibration curve for PCM & PCZM

LOD and LOQ :
The analytical sensitivity of the developed RP-HPLC method was evaluated based on signal-to-noise (S/N) ratios of 3:1 and 10:1 for the Limit of Detection (LOD) and Limit of Quantitation (LOQ), respectively.
In the proposed method, the LOD and LOQ were determined to be 0.25 µg/mL and 0.76µg/mL for Paracetamol (PCM), and 0.24µg/mL and 0.75µg/mL for Prochlorperazine maleate (PCZM), respectively. These minimal values confirm the high sensitivity of the method for the trace-level quantification of both active pharmaceutical ingredients. The results are summarized in Table 6[28].

Table 6: LOD and LOQ Results

Sr. no	Drug Name	LOD(µg/mL)	LOQ(µg/mL)
1	Paracetamol (PCM)	0.25	0.76
2	Prochlorperazine maleate (PCZM)	0.24	0.75

Robustness
The analytical robustness of the developed RP-HPLC method was verified by introducing deliberate, minor deviations into the critical chromatographic parameters using a 10µg/mL sample solution. Operational variations were restricted to one parameter at a time to systematically track variations in retention profile and peak characteristics. The altered parameters included detection wavelength (249, 251, and 253nm) and mobile phase buffer pH (2.8, 3.0,and 3.2).
The resulting system suitability responses exhibited negligible variance, confirming that small operational alterations do not compromise the performance of the assay. For both target analytes, Paracetamol (PCM) and Prochlorperazine maleate (PCZM), the calculated percentage Relative Standard Deviation (%RSD)

remained strictly under the acceptable threshold of 2.0%, achieving maximum values of 0.11% and 0.40%, respectively. This low variance demonstrates the excellent ruggedness and reliable stability of the proposed method for routine quality control applications. The consolidated statistical attributes are summarized in Table 7[27].

Table 7: Robustness result for PCM and PCZM

Parameter	Replicate	PCM Peak Area	PCM %RSD	PCZM Peak Area	PCZM %RSD	Inference
Wavelength249nm	1	2119495	-	51648	-	-
Wavelength 251nm	2	2123141	0.11 %	51632	0.40 %	Pass
Wavelength 253nm	3	2124326	-	51275	-	-
Buffer pH 2.8	1	2119495	-	51648	-	-
Buffer pH 3.0	2	2118249	0.07 %	51532	0.11 %	Pass
Buffer pH3.2	3	2121289	-	51573	-	-

Assay

The assay sample was prepared by calculating the average weight of 10 tablets. A quantity of tablet powder equivalent to the required formulation dosage (containing Paracetamol and Prochlorperazine Maleate) was accurately weighed and transferred to a 100 mL volumetric flask. The volume was made up with the mobile phase solvent to prepare the stock solution. After 10 minutes of sonication and subsequent filtration through a 0.45 µm Whatman filter paper, an aliquot of this filtrate was appropriately diluted to prepare the working sample solution. Three 20 µL injections of this solution were made into the column under optimized chromatographic conditions, and the peak areas were recorded. The content of Paracetamol and Prochlorperazine Maleate in the pharmaceutical tablet dosage form was found by using the developed method. The percentage purity of Paracetamol and Prochlorperazine Maleate were found to be 100.09% and 100.25%, respectively[29] .

Table 8 : Assay Results

Sr. no.	Drug Name	Area of Standard	Area of Sample	% Assay
1	Paracetamol	3164917	3168010	100.09
2	Prochlorperazine Maleate	78354	78555	100.25

Forced Degradation:

Forced degradation studies were performed to evaluate the stability-indicating capability of the developed RP-HPLC method for Paracetamol (PCM) and Prochlorperazine Maleate (PCZM). The drugs were subjected to various stress conditions such as acidic, alkaline, oxidative, photolytic, and thermal degradation in accordance with ICH guidelines.

The results indicated that both drugs exhibited varying degrees of degradation under different stress conditions. Higher degradation was observed under acidic and oxidative conditions, suggesting that both PCM and PCZM are more susceptible to hydrolytic and oxidative pathways. Moderate degradation was observed under alkaline conditions, while comparatively lower degradation was seen under photolytic and thermal stress, indicating better stability in light and heat exposure.

The method successfully separated the intact drug peaks from their degradation products without any interference, confirming its specificity and stability-indicating nature. Hence, the developed RP-HPLC method can be effectively used for stability studies and routine analysis of PCM and PCZM in pharmaceutical formulations.

Acid Degradation

Under acidic stress conditions (0.1 N HCl, 60°C for 60 min), both PCM and PCZM underwent acid hydrolysis, leading to the formation of degradation products. PCM exhibited 7.41% degradation, whereas PCZM showed 6.14% degradation. The observed degradation indicates the susceptibility of both drugs to acidic conditions. Furthermore, the degradation products were adequately resolved from the principal drug peaks, confirming the specificity and stability-indicating capability of the developed RP-HPLC method.

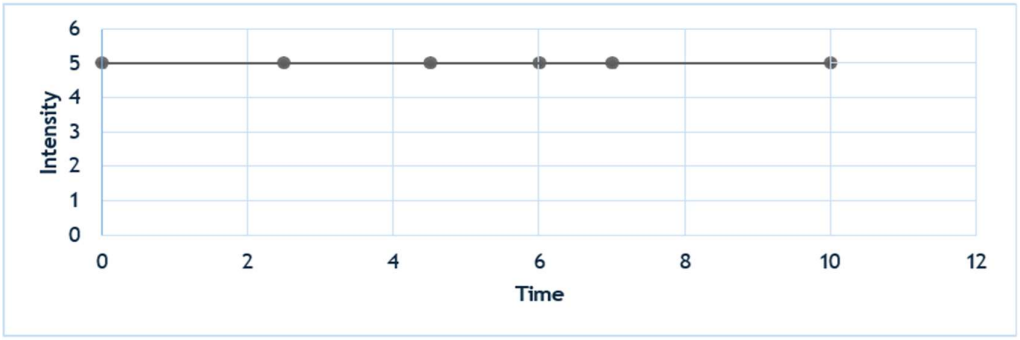

Figure 4: Chromatogram of Acid Hydrolysis [Blank]

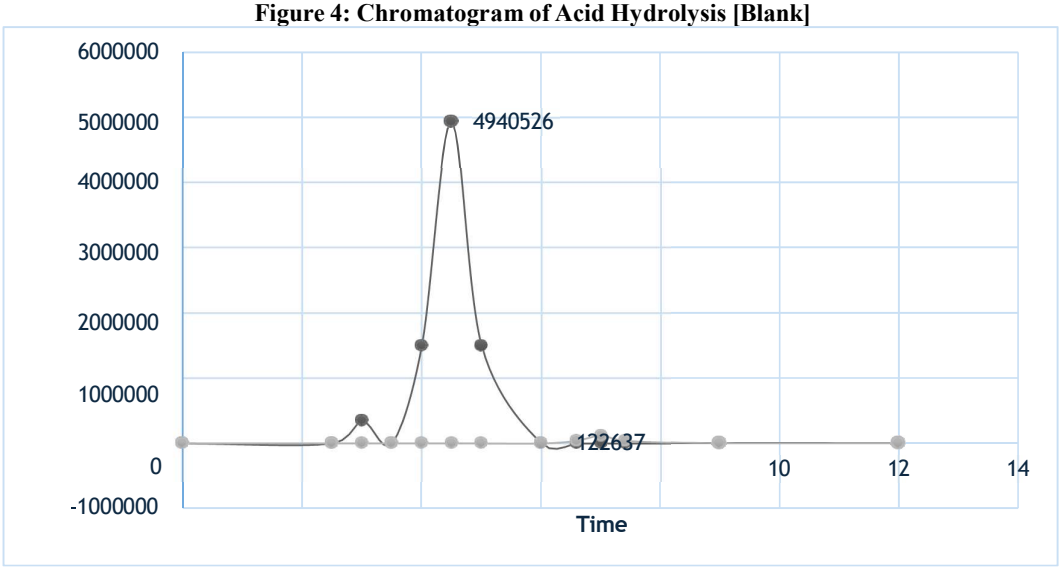

Figure 5: Chromatogram of Acid Hydrolysis [Treated][0.1 N HCL,60ºC for 60 min] Base Degradation

Under alkaline stress conditions (0.1 N NaOH, 60°C for 60 min), both PCM and PCZM underwent base-catalyzed hydrolysis, resulting in the formation of degradation products. PCM exhibited 4.29% degradation, whereas PCZM showed 4.49% degradation. The relatively low extent of degradation indicates moderate stability of both drugs under alkaline conditions. Furthermore, the degradation products were adequately resolved from the principal drug peaks, confirming the specificity and stability-indicating capability of the developed RP-HPLC method.

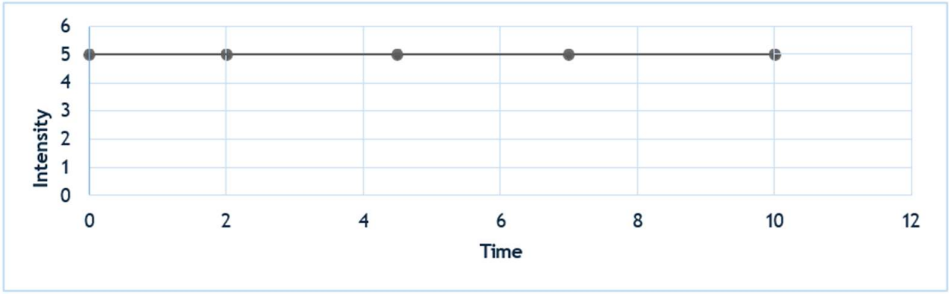

Figure 6: Chromatogram of Base Hydrolysis [Blank]

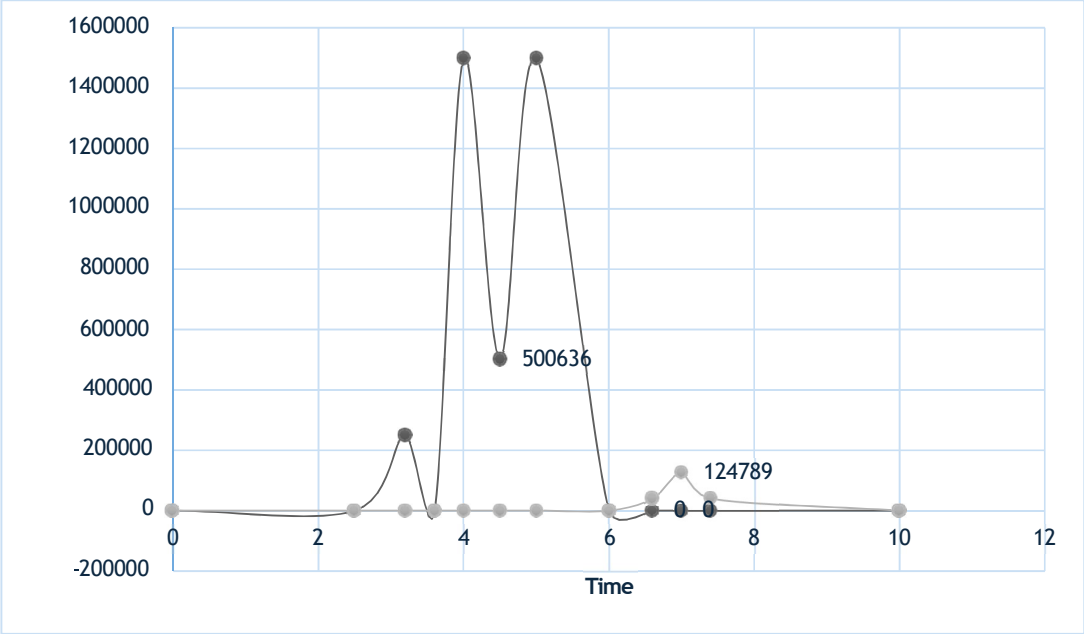

Figure 7: Chromatogram of Base Hydrolysis [Treated] [0.1N HCL,60ºC for 60 min] Oxidative Degradation

Under oxidative stress conditions (3% H_2O_2, 60°C for 60 min), both PCM and PCZM underwent oxidative degradation due to the oxidation of susceptible functional groups present in the drug molecules. PCM exhibited 6.42% degradation, whereas PCZM showed 5.30% degradation. The results indicate moderate susceptibility of both drugs to oxidative stress. Furthermore, the degradation products were adequately resolved from the principal drug peaks, confirming the specificity and stability-indicating capability of the developed RP-HPLC method.

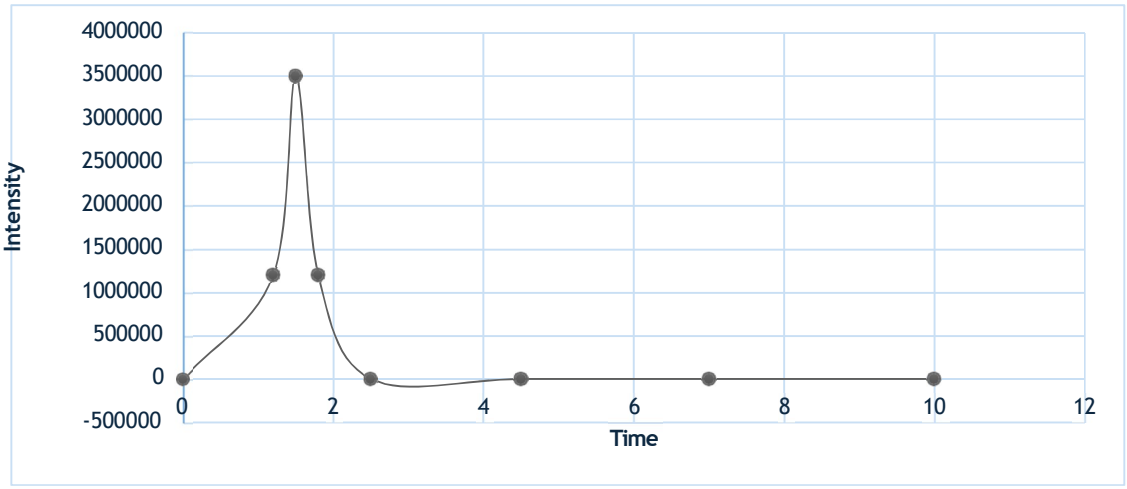

Figure 8: Chromatogram of Oxidative Hydrolysis [Blank]

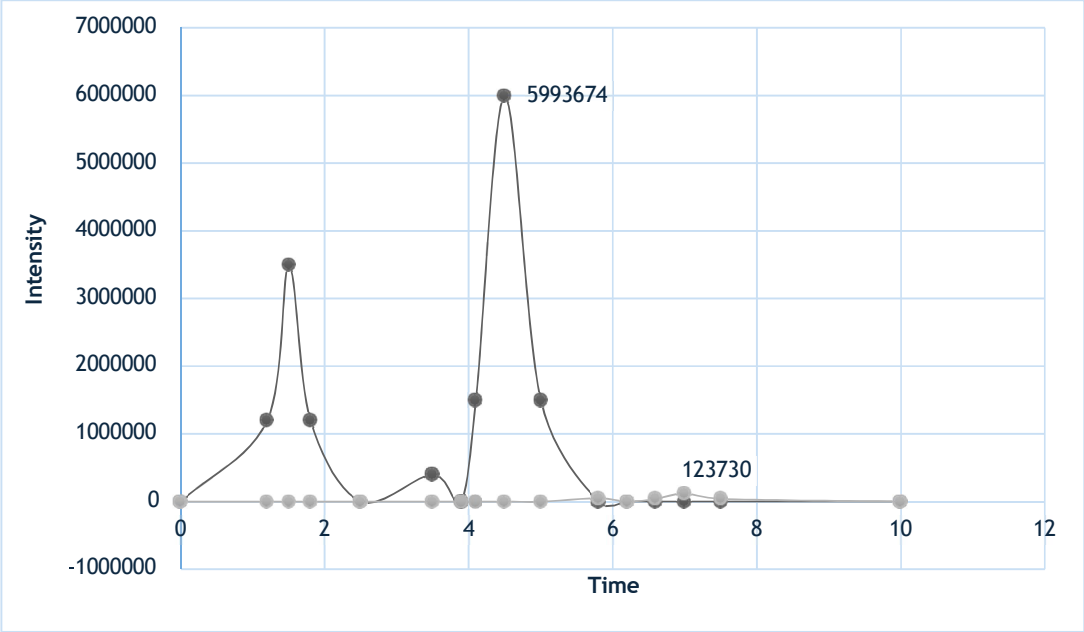

Figure 9: Chromatogram of Oxidative Hydrolysis [Treated] [3 % v/v ,60ºC for 60 min] Photolytic Degradation

Under photolytic stress conditions (UV light exposure), both PCM and PCZM underwent slight photodegradation due to light-induced chemical changes in the drug molecules. PCM exhibited 1.43% degradation, whereas PCZM showed 2.11% degradation. The low percentage degradation indicates good photostability of both drugs under the applied conditions. Furthermore, the degradation products were adequately resolved from the principal drug peaks, confirming the specificity and stability-indicating capability of the developed RP-HPLC method.

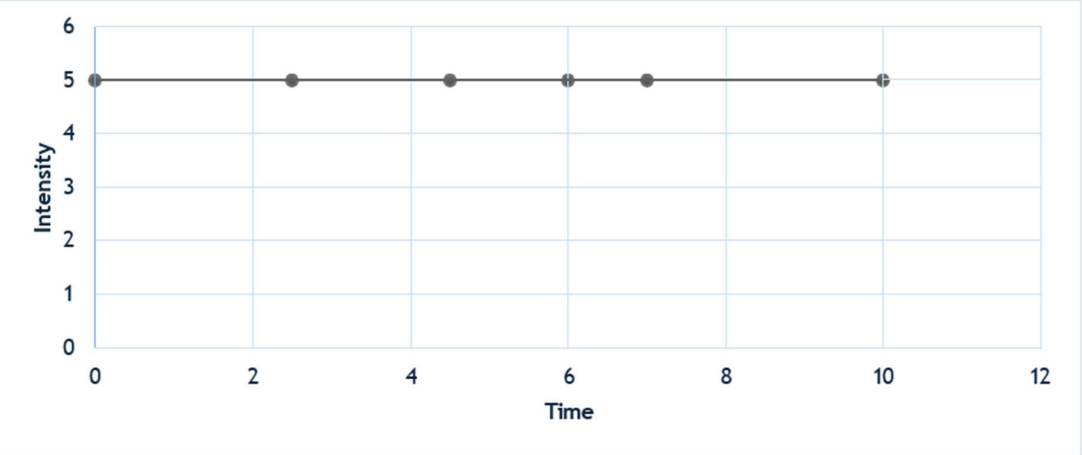

Figure 10: Chromatogram of Photolytic Hydrolysis [Blank]

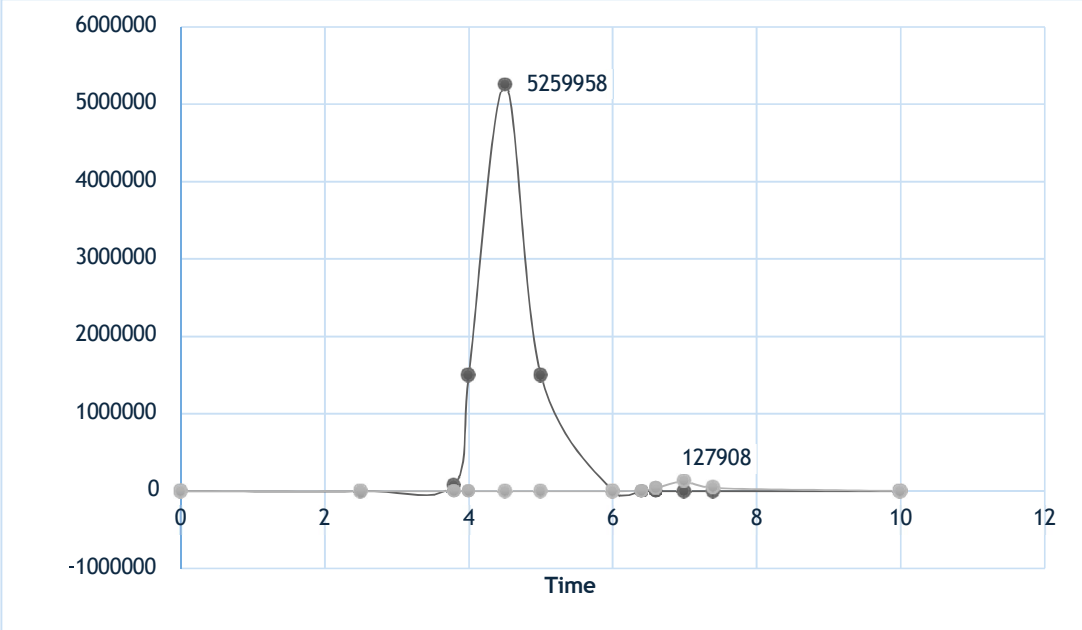

Figure 11: Chromatogram of Photolytic Hydrolysis [Treated] [UV light exposure for 24h]

Thermal Degradation

Under thermal stress conditions (60°C), both PCM and PCZM underwent thermal degradation due to heat-induced chemical changes in the drug molecules. PCM exhibited 0.24% degradation, whereas PCZM showed 1.27% degradation. The low percentage degradation indicates that both drugs are relatively stable under thermal stress conditions. Furthermore, the degradation products were adequately resolved from the principal drug peaks, confirming the specificity and stability-indicating capability of the developed RP-HPLC method.[29–30].

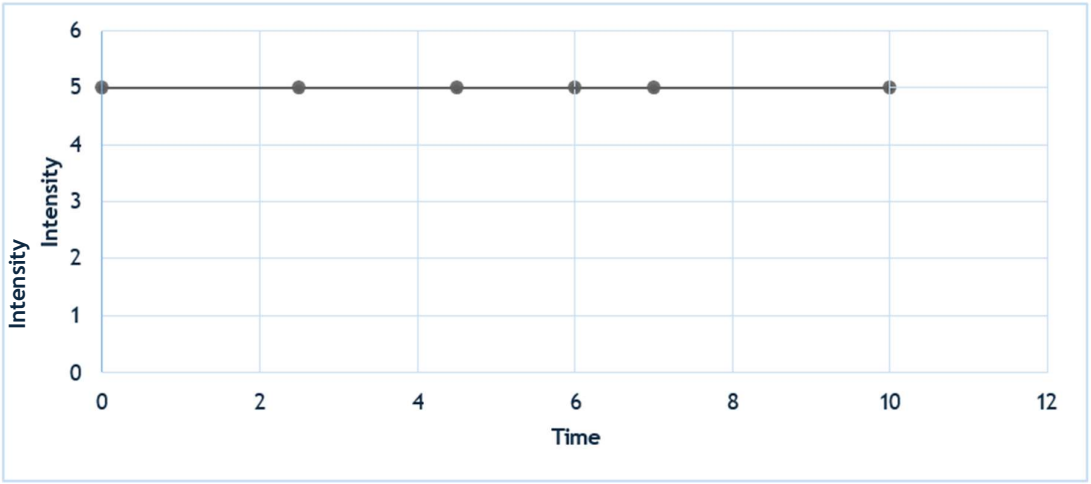

Figure 12: Chromatogram of Thermal Hydrolysis [Blank]

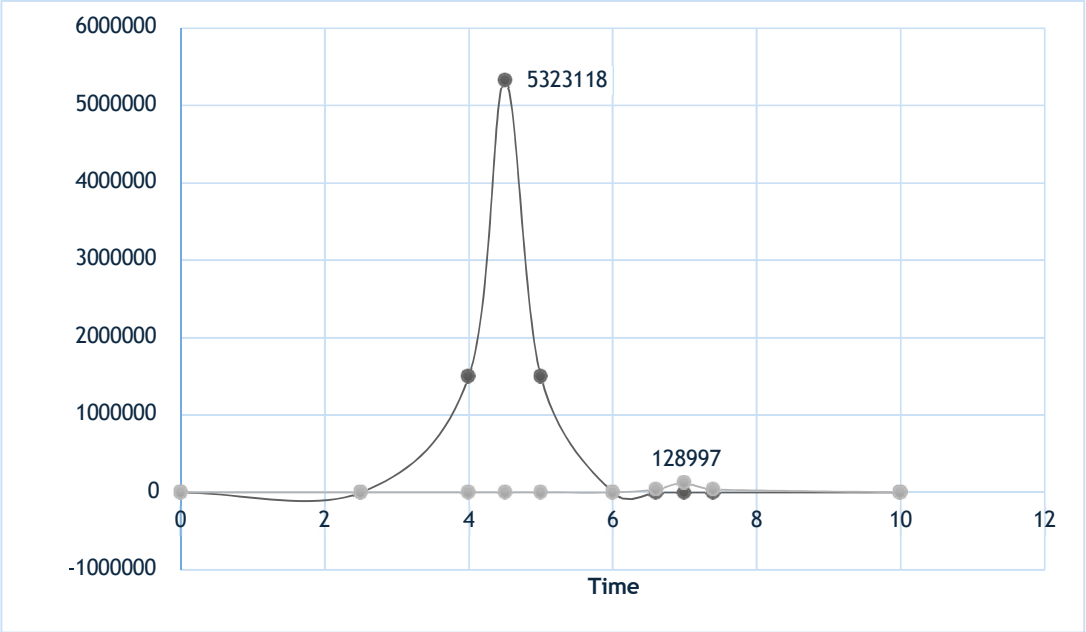

Figure 13: Chromatogram of Thermal Hydrolysis [Treated] [Dry heat at 60ºC for 24h]

Table 8 :Forced Degradation Results of Paracetamol (PCM)

Sr. no.	Degradation Condition	Area of Standard	Area of Degraded Sample	%Degradation
1	Acid Degradation	5336382	4940526	7.41
2	Base Degradation	5336382	5000636	4.29
3	H_2O_2 Degradation	5336382	5993674	6.42
4	Photolytic Degradation	5336382	5259958	1.43
5	Thermal Degradation	5336382	5323118	0.24

Table 9 :Forced Degradation Results of Prochlorperazine Maleate (PCZM)

Sr. no.	Degradation Condition	Area of Standard	Area of Degraded Sample	%Degradation
1	Acid Degradation	130667	122637	6.14
2	Base Degradation	130667	124789	4.49
3	H_2O_2	130667	123730	5.30

	Degradation			
4	Photolytic Degradation	130667	127908	2.11
5	Thermal Degradation	130667	128997	1.27

CONCLUSION

A simple, precise, and reproducible RP-HPLC method was successfully developed and validated for the simultaneous estimation of Paracetamol and Prochlorperazine Maleate. The method demonstrated acceptable chromatographic performance with good resolution, specificity, accuracy, and precision in accordance with ICH guidelines. The developed method is rapid, reliable, and suitable for routine quality control analysis of combined pharmaceutical dosage forms. Hence, it can be effectively applied for simultaneous quantification of both drugs in bulk and formulation analysis.

Future Scope

The developed RP-HPLC method for the simultaneous estimation of Paracetamol and Prochlorperazine Maleate can be further extended to include comprehensive forced degradation studies under ICH-recommended stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic environments to evaluate the stability-indicating capability of the method. The method also holds potential for application in bioanalytical studies, including pharmacokinetic and bioavailability assessments in biological matrices.

Further improvements may focus on adapting the method for routine quality control analysis in pharmaceutical industries with enhanced robustness and higher sample throughput. Integration with advanced analytical techniques such as LC-MS/MS may be explored to achieve greater sensitivity and selectivity for trace-level quantification. In addition, future research may emphasize the development of green analytical methodologies by minimizing solvent usage and employing environmentally sustainable mobile phase systems to align with modern regulatory and ecological requirements.

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