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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF POLMACOXIB AND PARACETAMOL IN TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, precise, and robust reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Polmacoxib and Paracetamol in combined tablet dosage form. Chromatographic separation was achieved using a Shim-pack NT-ODS C18 column (250 × 4.6 mm, 5 µm). The mobile phase consisted of phosphate buffer (pH 5.5) and acetonitrile in the ratio of 40:60 (v/v), with the addition of 1 mL ortho-phosphoric acid, delivered at a flow rate of 1.0 mL/min. Detection was carried out at 238 nm using a PDA detector. The retention times for Paracetamol and Polmacoxib were found to be 4.16 min and 10.61 min, respectively, indicating efficient separation and good resolution between the two analytes. The developed method was validated according to ICH guidelines, evaluating parameters such as specificity, linearity, precision, accuracy, robustness, LOD and LOQ. The method showed excellent linearity over the concentration range of 325–975 µg/mL for Paracetamol and 2–6 µg/mL for Polmacoxib, with correlation coefficients approaching 0.999. Accuracy was confirmed through recovery studies, yielding results within the acceptable range of 98–102%. Precision studies demonstrated %RSD values below 2%, indicating good reproducibility. Forced

degradation studies under acidic, basic, oxidative, and thermal conditions established the stability-indicating nature of the method, with Polmacoxib exhibiting greater stability than Paracetamol.

The method was successfully applied to the assay of marketed formulations, producing results within acceptable limits. Therefore, the proposed RP-HPLC method is suitable for routine quality control analysis.

KEYWORDS: RP-HPLC, Method Development, Method Validation, Forced Degradation, Stability-Indicating Method.

INTRODUCTION

Osteoarthritis is a chronic degenerative joint disorder characterized by progressive cartilage degradation, inflammation, pain, and reduced joint mobility, significantly affecting the quality of life of patients. The disease progression involves a complex interplay of mechanical stress and biochemical mediators such as prostaglandins, cytokines (IL-1, TNF-α), and matrix metalloproteinases, which accelerate cartilage breakdown.

Management of osteoarthritis primarily involves the use of non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics. The combination of Polmacoxib, a selective COX-2 inhibitor, and Paracetamol, a centrally acting analgesic, offers a synergistic therapeutic approach. This combination enhances pain relief while minimizing adverse effects associated with high-dose monotherapy.[1]

In pharmaceutical analysis, accurate and reliable analytical methods are essential for ensuring drug quality, safety, and efficacy. Analytical method development is required when no official method is available for a drug or its combination. These methods are designed to provide specificity, precision, accuracy, and reproducibility while reducing time and cost. High Performance Liquid Chromatography (HPLC) is widely used for quantitative drug analysis due to its high resolution, sensitivity, and reproducibility. However, no suitable method was available for the simultaneous estimation of Polmacoxib and Paracetamol in combined dosage form. Therefore, the present study aims to develop and validate a simple, precise, and robust RP-HPLC method for simultaneous estimation of these drugs in tablet formulation as per ICH guidelines.[2,3]

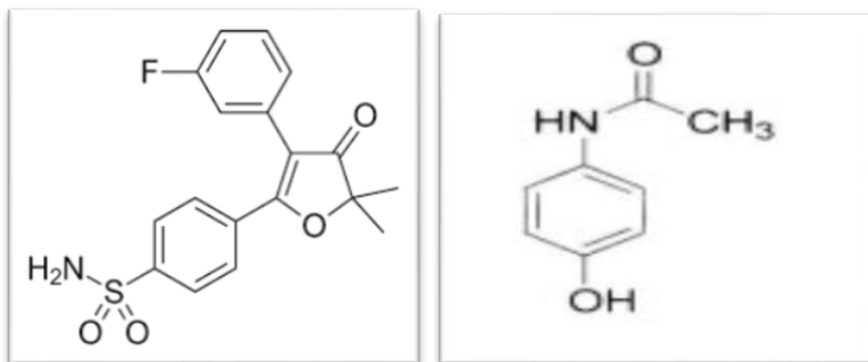

Fig. 1 Structure of Polmacoxib and Paracetamol.

MATERIALS AND METHODS

1. Chemicals and Reagents

- Polmacoxib (POL) – API
- Paracetamol (PCM) – API
- Methyl Alcohol (AR grade)
- Acetonitrile (HPLC grade)
- Water (HPLC Grade)
- Ortho Phosphoric Acid
- Hydrogen Peroxide (AR Grade)
- Hydrochloric Acid (AR Grade)
- Sodium Hydroxide (AR Grade)

2. Instrumentation

- RP-HPLC system with PDA detector
- Column: Shim-pack NT-ODS C18 (250*4.6 mm, 5 µm)
- UV Spectrophotometer
- Analytical balance
- FT-IR spectrometer.

3. Chromatographic Conditions

- Mobile Phase: Phosphate buffer (pH 5.5) : Acetonitrile (40:60 v/v) + 1 mL OPA
- Flow rate: 1.0 mL/min
- Detection wavelength: 238 nm
- Injection volume: 50 µL
- Run time: 20 min

- Retention time: PCM (4.16 min), POL (10.61 min)

4. Preparation of Standard Stock Solutions

Paracetamol (PCM)

- Accurately weigh 100 mg PCM and dissolve in 100 mL methanol. Final concentration: **1000 µg/mL**

Polmacoxib (POL)

- Accurately weigh 20 mg POL and dissolve in 100 mL methanol. Final concentration: **200 µg/mL**

5. Preparation of Working Standard Solutions

PCM

- Take 65 mL of stock solution and dilute to 100 mL with methanol. Final concentration: **650 µg/mL**

POL

- Take 2 mL of stock solution and dilute to 100 mL with methanol. Final concentration: **4 µg/mL**

6. Preparation of Sample Solution

- Crush 10 tablets. Take powder equivalent to 325 mg PCM + 2 mg POL. Dissolve in 100 mL methanol. Dilute 2 mL to 10 mL. Final concentration: 650 µg/mL (PCM) and 4 µg/mL (POL)

7. Preparation of Mobile Phase

- Prepare phosphate buffer (pH 5.5). Mix with acetonitrile in ratio **40:60 (v/v)**. Add 1 mL OPA. Filter and degas before use.

Forced degradation studies

Forced degradation studies were performed to evaluate the stability-indicating capability of Paracetamol and Polmacoxib under various stress conditions in accordance with Forced degradation requirements.

1. Acid Degradation

Acid degradation of Paracetamol (PCM) and Polmacoxib (POL) was performed using 0.01 N hydrochloric acid at room temperature for 30 minutes. The samples were neutralized with

0.01 N sodium hydroxide and diluted with methanol, while a blank solution was similarly prepared to ensure absence of interference. The solutions were analyzed by the optimized RP-HPLC method, and chromatograms were examined for changes in peak area and formation of degradation products. Percentage degradation was calculated by comparison with unstressed samples, confirming the stability-indicating nature of the method.

2. Alkali Degradation

Alkali degradation was performed using 0.1 N sodium hydroxide at room temperature for 30 minutes, followed by neutralization with 0.1 N hydrochloric acid and dilution with methanol. A blank solution was similarly prepared. The stock solution was treated under the same conditions, filtered if required, and injected into the chromatographic system. Chromatograms were examined for degradation peaks, changes in retention time, and reduction in peak area, and percentage degradation was calculated by comparison with the unstressed standard solution.

3. Oxidative Degradation

Oxidative degradation was performed using 1% hydrogen peroxide at room temperature for 30 minutes, followed by dilution with methanol. A blank solution was similarly prepared.

The stock solution was treated under the same conditions, and 20 µL was injected into the chromatographic system. Chromatograms were examined for degradation products, and percentage degradation of PCM and POL was determined by comparison with the unstressed solution.

4. Thermal Degradation

Thermal degradation was performed by exposing tablet powder containing PCM and POL to dry heat at 70 °C for 3 hours. The stressed sample was cooled, and a stock solution was prepared in methanol followed by suitable dilution. A 20 µL aliquot was injected into the chromatographic system, and chromatograms were compared with the unstressed sample to evaluate changes in peak area and detect degradation products.

METHOD VALIDATION[4-7]

1. Specificity

Peak identification of Paracetamol (PCM) and Polmacoxib (POL) was carried out by comparing the retention times of sample peaks with those of individual and mixed standard

solutions analyzed under optimized chromatographic conditions. The consistency of retention time, peak shape, and resolution confirmed the identity of both analytes.

2. Linearity

Linearity of the HPLC method was assessed by preparing standard solutions of Paracetamol (PCM) and Polmacoxib (POL) at different concentration levels using methanol as diluent. Stock solutions of 1000 µg/mL (PCM) and 200 µg/mL (POL) were prepared, and five concentration levels were obtained by appropriate dilution. Each solution was injected three times under optimized chromatographic conditions, and calibration curves were constructed by plotting concentration against peak area. The method showed acceptable linearity over the concentration range of 325–975 µg/mL for PCM and 2–6 µg/mL for POL.

3. Precision

Repeatability

Repeatability was evaluated using a standard mixture solution of 650 µg/mL PCM and 4 µg/mL POL, injected six times under the same chromatographic conditions.

Peak areas were recorded, and precision was assessed by calculating mean, standard deviation (SD), and %RSD.

Intraday Precision

Intraday precision was determined by analyzing standard solutions at three concentration levels (325:2, 650:4, and 975:6 µg/mL of PCM: POL) in triplicate within the same day. Peak areas were measured, and mean, SD, and %RSD were calculated.

Interday Precision

Interday precision was evaluated by analyzing the same concentration levels in triplicate over three different days under identical conditions. Peak areas were recorded, and mean, SD, and %RSD were calculated to assess consistency.

4. Accuracy

Accuracy of the HPLC method was evaluated by recovery studies using the standard addition technique by spiking pre-analyzed tablet formulation with PCM and POL standards at 50%, 100%, and 150% levels. Samples were prepared and analyzed in triplicate under optimized conditions, and peak areas were recorded to calculate percentage recovery.

Tablet and standard stock solutions were prepared in methanol, filtered, and diluted to obtain working solutions. Different volumes of standard solution were added to the tablet solution to achieve required concentration levels, and total concentrations corresponding to unspiked, 50%, 100%, and 150% levels were analyzed.

5. Robustness

Robustness was evaluated by introducing small variations in chromatographic parameters, including flow rate (±0.2 mL/min) and mobile phase composition (±2 mL), while keeping other conditions constant. Standard solutions were analyzed in triplicate, and chromatograms were examined for changes in peak area. The %RSD was calculated to confirm that the method remained unaffected by these variations.

6. LOD & LOQ

LOD and LOQ were determined using the standard deviation of response and slope of the calibration curve obtained from linearity studies. These parameters represent the minimum detectable and quantifiable concentrations of PCM and POL and were calculated using the equations

$LOD = 3.3 \times (\sigma/S)$ and $LOQ = 10 \times (\sigma/S)$.

7. Assay

Standard solution was prepared by dissolving PCM and POL in methanol, followed by dilution to obtain working concentrations. Sample solution was prepared from tablet powder, sonicated, diluted, and filtered to remove excipients.

Both standard and sample solutions were injected into the HPLC system, and peak areas were recorded. The assay was calculated by comparing peak areas of sample and standard using the formula:

$\%\ Assay = (AT/AS) \times (CS/CT) \times 100$

RESULTS AND DISCUSSION

Optimized Chromatographic Conditions

The chromatographic separation was carried out using a Shim-pack NT-ODS C18 column (250 × 4.6 mm, 5 µm) as the stationary phase, with a mobile phase consisting of phosphate buffer (pH 5.5) and acetonitrile in the ratio of 40:60 (v/v) along with 1 mL OPA. Methanol was used as the diluent, and the elution was performed in isocratic mode at a flow rate of 1

mL/min. Detection was carried out at 238 nm with an injection volume of 50 µL and a run time of 20 minutes. The retention times were observed at 4.16 minutes for PCM and 10.61 minutes for POL.

Forced degradation studies

1) Acid degradation study

Fig. 2: Blank for Acid degradation.

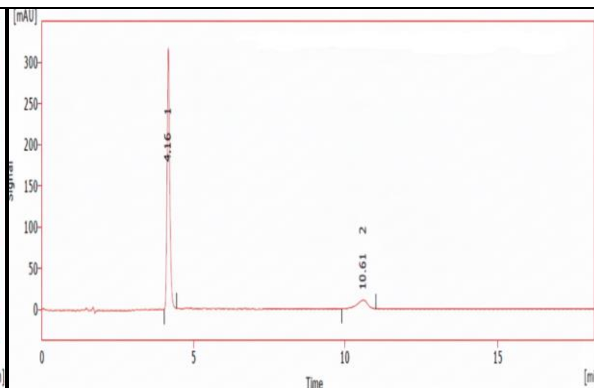

Fig. 3: API as such for Acid degradation.

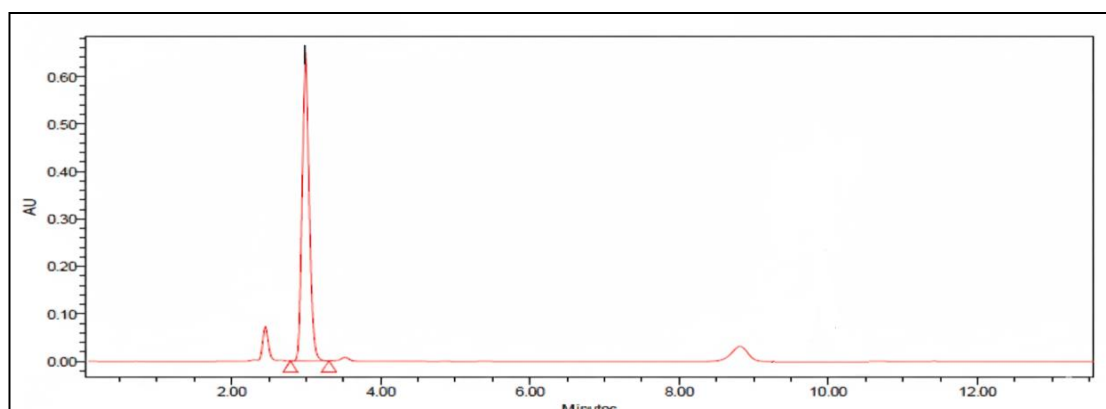

Fig. 4: Acid degradation for combination.

2) Base degradation study

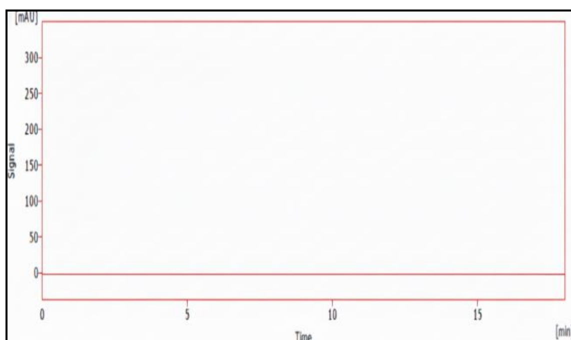

Fig. 5: API as such for Base degradation.

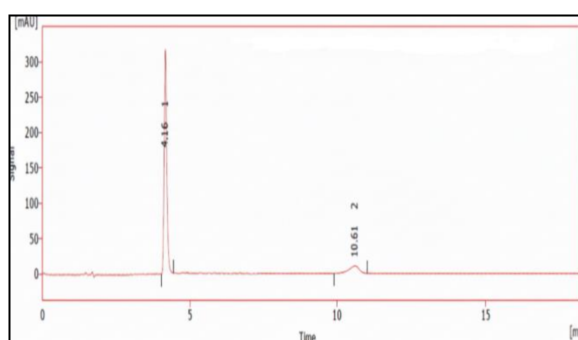

Fig. 6 Blank for Base degradation.

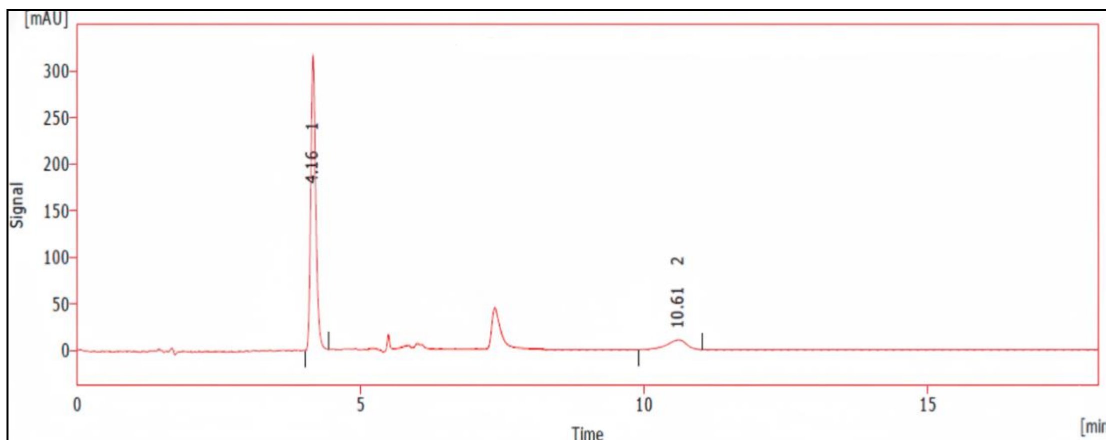

Fig. 7: Base degradation for combination.

3) Oxidative degradation studies

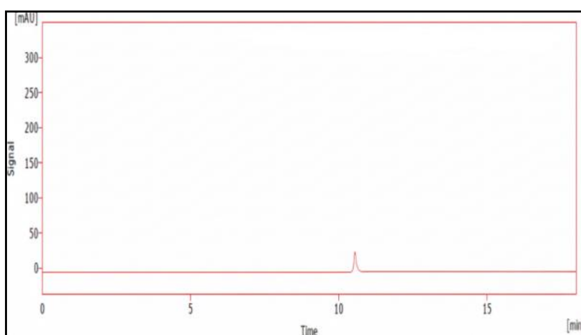

Fig. 8 Blank for Oxidative hydrolysis.

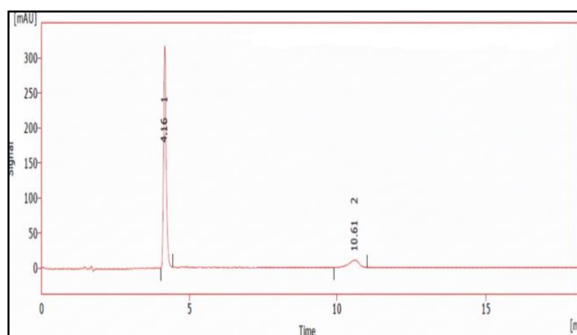

Fig. 9: API as such for Oxidative hydrolysis.

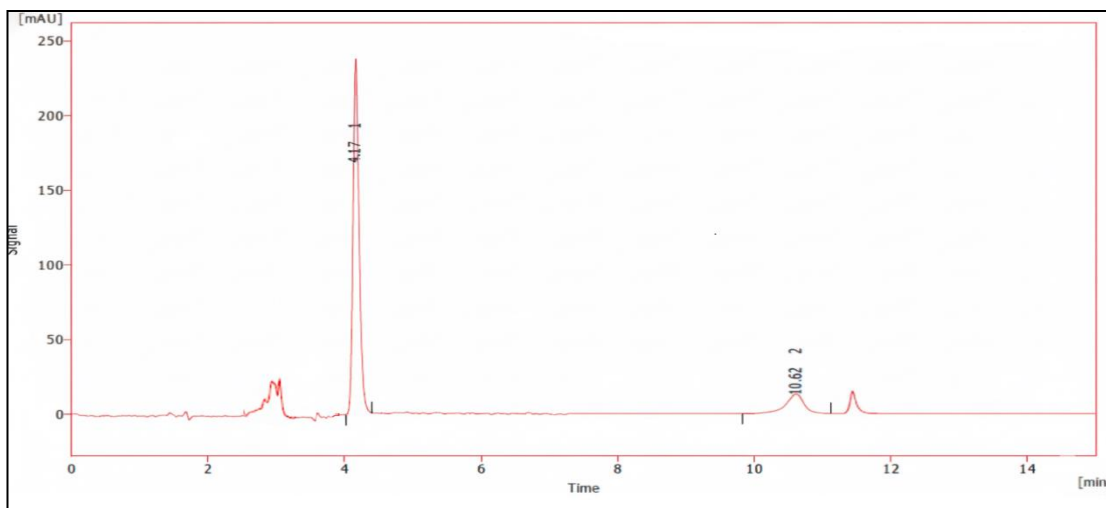

Fig. 10: Oxidative hydrolysis for combination.

4) Thermal Degradation Study

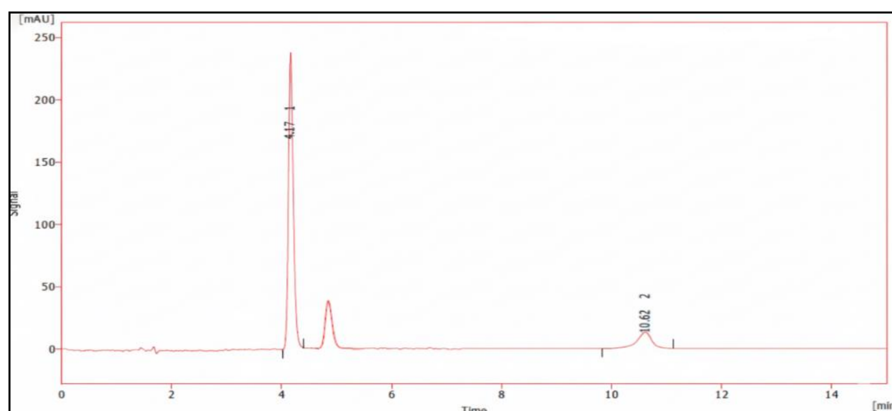

Fig. 11: Thermal degradation for combination.

Table 1: Summary of forced degradation studies.

Stress Conditions	% Degradation (PCM)	% Degradation (POL)	Remarks
Acid	7.36%	1.12%	Polmacoxib is comparatively more stable in acidic environment.
Base	19.18%	1.96%	Polmacoxib is comparatively more stable in alkali environment.
Oxidative	25.66 %	5.50 %	Polmacoxib is comparatively more stable in oxidative media.
Thermal	16.36 %	3.50 %	Polmacoxib is comparatively more resistant to heat.

Validation of Developed HPLC Method

1) Specificity

The specificity of the developed HPLC method was confirmed by analyzing individual standard solutions of Paracetamol (PCM) and Polmacoxib (POL). Each drug exhibited distinct and well-defined peaks at their respective retention times.

Peak identification was performed by comparing these retention times, and no interfering peaks were observed. The chromatograms showed well-resolved peaks, demonstrating that the method is specific and suitable for accurate identification of both analytes.

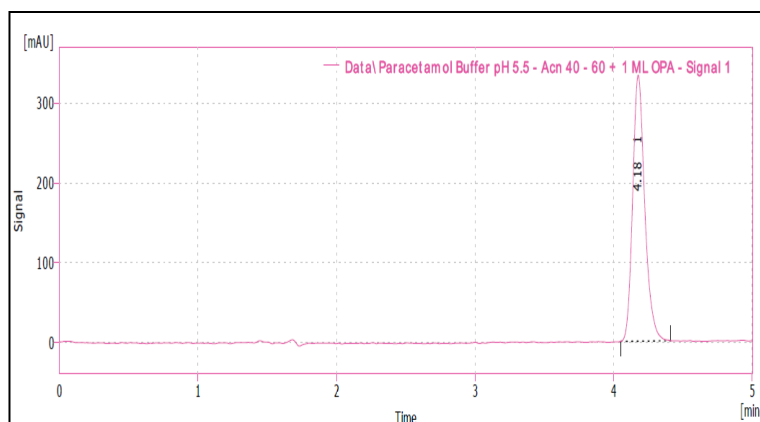

Fig. 12: Chromatogram of Paracetamol.

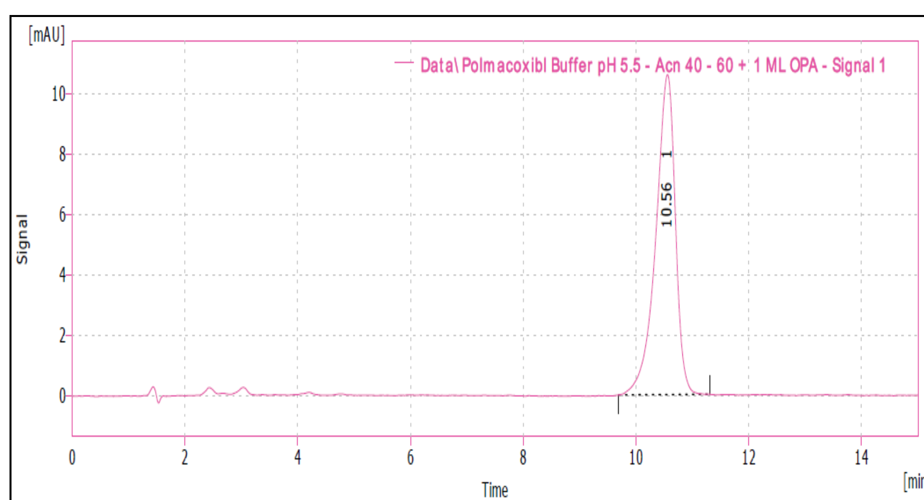

Fig. 13: Chromatogram of Polmacoxib.

2) Linearity

The developed HPLC method showed good linearity over the concentration range of 325–975 µg/mL for Paracetamol (PCM) and 2–6 µg/mL for Polmacoxib (POL). Calibration curves were constructed by plotting peak area versus concentration, with correlation coefficients (R^2) close to 1 for both analytes, indicating excellent linear relationships. The chromatograms at different levels showed consistent and proportional responses, and the linearity range encompasses the accuracy study levels, confirming the method range.

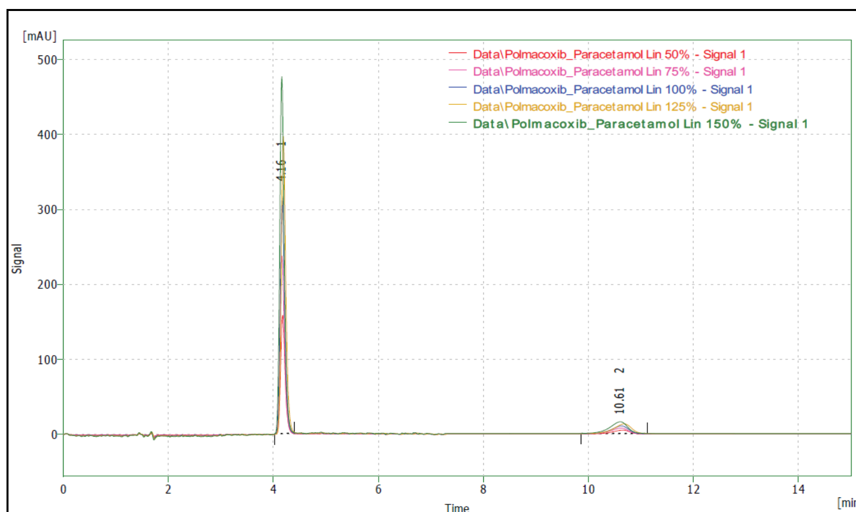

Fig. 14: Linearity Overlay.

Table 2: Linearity Data for PCM and POL.

Sr. No.	PCM			POL		
	Conc. (µg/mL)	**Peak Area (Mean ± S. D.)**	**% RSD**	**Conc. (µg/mL)**	**Peak Area (Mean ± S.D.)**	**% RSD**
1.	325	960.55 ± 2.08	0.22	2	125.38 ± 1.15	0.92
2.	487.5	1438.05 ± 1.47	0.10	3	185.14 ± 2.27	1.23
3.	650	1916.82 ± 3.91	0.20	4	249.57 ± 1.58	0.63
4.	812.5	2415.16 ± 3.36	0.14	5	315.62 ± 2.62	0.83
5.	975	2875.37 ± 4.65	0.16	6	377.71 ± 3.76	0.99
Regression equation	y=2.958x - 1.5134				y=63.514x - 3.3691	
Regression Coefficient	R^2 =0.9999				R^2 =0.9997	

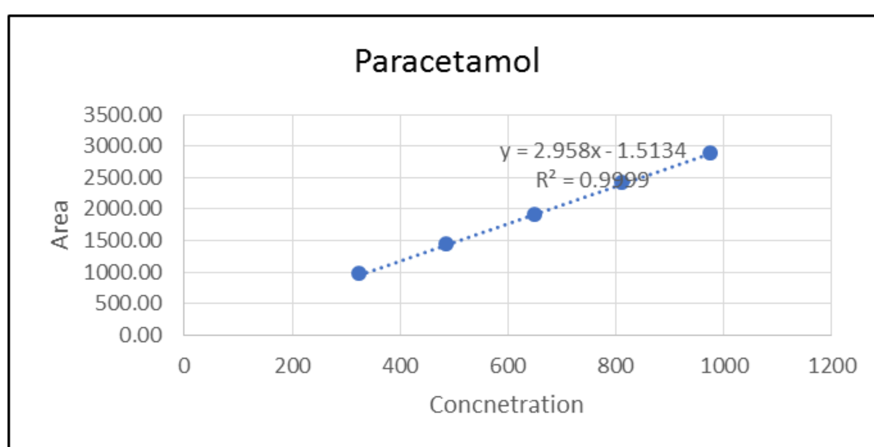

Fig. 15. Calibration curve for linearity of PCM.

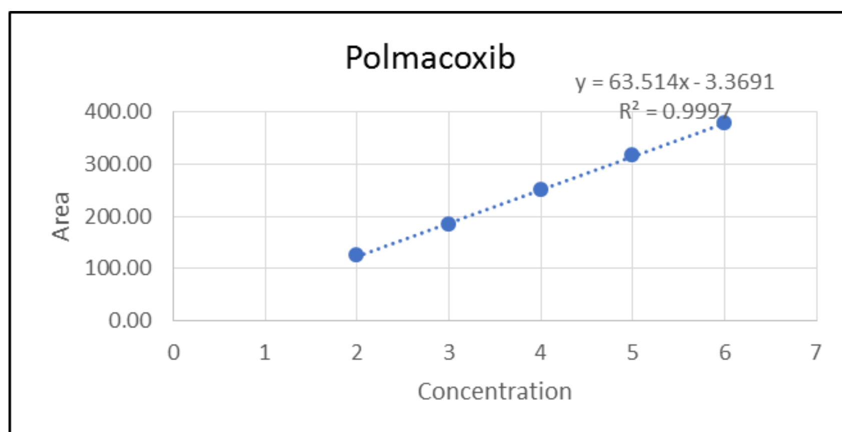

Fig. 16: Calibration curve for linearity of POL.

3) Precision

Repeatability

Repeatability of the developed HPLC method was evaluated by analyzing samples at 650 µg/mL for Paracetamol (PCM) and 4 µg/mL for Polmacoxib (POL) using six replicate injections under the same conditions. The %RSD of the responses was found to be less than 2% for both analytes, indicating good precision. The results demonstrate that the method is repeatable and provides consistent results for both analytes.

Table 3: Repeatability Data for PCM and POL.

Area	Concentration of PCM (650 µg/mL)	Concentration of POL (4 µg/mL)
1.	1917.07	251.30
2.	1912.82	248.25
3.	1920.65	249.23
4.	1923.21	252.38
5.	1919.26	251.84
6.	1918.38	246.54
Mean	1918.56	249.92
SD	3.51	2.29
% RSD	0.18	0.92

Intraday Precision

The intra-day precision of the developed HPLC method was evaluated at 325, 650, and 975 µg/mL for Paracetamol (PCM) and 2, 4, and 6 µg/mL for Polmacoxib (POL). The analysis was performed in triplicate at different time intervals within a single day under identical chromatographic conditions. The %RSD values of peak areas were less than 2% at all levels, indicating acceptable precision and demonstrating the method's reproducibility and suitability for routine analytical applications.

Table 4: Data of Intraday for PCM.

Concentration (µg. mL^{-1})	Intraday (Mean Area ± SD)	% RSD
325	959.01 ± 3.28	0.34
650	1916.33 ± 4.74	0.25
975	2872.80 ± 3.71	0.13

Table 5: Data of Intraday for POL.

Concentration (µg. mL^{-1})	Intraday (Mean Area ± SD)	% RSD
2	125.48 ± 1.25	0.99
4	249.02 ± 2.38	0.96
6	375.04 ± 1.43	0.38

Inter-day precision

The inter-day precision of the developed HPLC method was evaluated at 325, 650, and 975 µg/mL for Paracetamol (PCM) and 2, 4, and 6 µg/mL for Polmacoxib (POL). The analysis was performed in triplicate over non-consecutive days under uniform chromatographic conditions. The %RSD values were found to be less than 2% at all levels, indicating acceptable precision and confirming the method's reproducibility and reliability for routine analysis.

Table 6: Data of Inter-day for PCM.

Concentration (µg. mL^{-1})	Intraday (Mean Area ± SD)	% RSD
325	955.99 ± 3.97	0.42
650	1915.18 ± 5.18	0.27
975	2874.49 ± 4.38	0.15

Table 7: Data of Inter-day for POL.

Concentration (µg. mL^{-1})	Intraday (Mean Area ± SD)	% RSD
2	121.65 ± 2.26	1.86
4	244.61 ± 4.27	1.75
6	375.84 ± 3.86	1.03

4) Accuracy

The accuracy of the developed HPLC method was evaluated by recovery studies using the standard addition method at 50%, 100%, and 150% levels, each analyzed in triplicate. The percentage recovery for Paracetamol (PCM) and Polmacoxib (POL) was within 98–102% at all levels, indicating accuracy and absence of interference from excipients. The consistent recovery values demonstrate the reliability of the method for determination of both analytes in the formulation.

Table 8: Accuracy data for PCM and POL.

Level of Spiking	Concentration of formulation	Amount of drug Added (µg/mL)	Amount of drug Recovered (µg/mL)	% Mean recovery ± SD
Accuracy data for PCM				
Un-spiked	325 µg/ml + 2 µg/ml	-	-	-
50%	325 µg/ml + 2 µg/ml	325	324.68	99.90 ± 0.07
100%	325 µg/ml + 2 µg/ml	650	650.78	100.12 ± 0.06
150%	325 µg/ml + 2 µg/ml	975	974.79	99.98 ± 0.04
Accuracy data for POL				
Un-spiked	325 µg/ml + 2 µg/ml	-	-	-
50%	325 µg/ml + 2 µg/ml	2	2.00	100.03 ± 0.99
100%	325 µg/ml + 2 µg/ml	4	3.99	99.79 ± 0.74
150%	325 µg/ml + 2 µg/ml	6	5.99	99.75 ± 0.13

5) Robustness

The robustness of the developed HPLC method was evaluated by introducing small deliberate variations in flow rate and mobile phase composition. The %RSD values for Paracetamol (PCM) and Polmacoxib (POL) were less than 2%, with no significant changes in peak area or chromatographic behavior. These results indicate that the method is robust and reliable for routine analysis of both analytes.

Table 9: Robustness data for PCM and POL.

Parameter	Level of Change	Observation			
		PCM		POL	
		Area ± SD	% RSD	Area ± SD	% RSD
Flowrate 1 mL/min	0.8 mL/min	1922.36 ± 6.32	0.33	248.11 ± 3.87	1.56
	1.2 mL/min	1920.02 ± 13.29	0.69	249.56 ± 0.57	0.23
Mobile phase composition (Buffer: ACN) (40:60)	42: 58	1922.18 ± 7.89	0.41	259.02 ± 2.38	0.92
	38: 62	1929.46 ± 8.27	0.43	249.02 ± 2.38	0.96

6) LOQ and LOQ

The LOD & LOQ were calculated on basis of formula

LOD = $3.3 \times \sigma / S$

LOQ = $10 \times \sigma / S$

Table 10: LOD-LOQ data for PCM and POL.

PCM	POL
LOD = 3.3 x (σ/S) = 3.3 x (0.534417 / 2.954987) = 0.60 μg. mL^{-1}	LOD = 3.3 x (σ/S) = 3.3 x (2.803701/ 63.51367) = 0.15 μg. mL^{-1}
PCM	**POL**
LOQ = 10 x (σ/S) = 10 x (0.534417 / 2.954987) = 1.81 μg. mL^{-1}	LOQ = 10 x (σ/S) = 10 x 3.3 x (2.803701/ 63.51367) = 0.44 μg. mL^{-1}

7) Assay of marketed formulation

Table 11: Assay result for PCM and POL.

%Assay ± SD		%RSD	
PCM	POL	PCM	POL
99.95 ± 0.03	99.84 ± 0.39	0.03	0.39

CONCLUSION

A novel, robust, and precise RP-HPLC method was developed and validated for the simultaneous quantification of Paracetamol (PCM) and Polmacoxib (POL) in pharmaceutical dosage forms. The method showed good specificity with well-resolved peaks and no interference. Linearity exhibited a strong correlation between concentration and peak area, while precision studies showed %RSD values within acceptable limits. Accuracy was confirmed through recovery studies within acceptance criteria. The method remained unaffected by small variations in parameters, indicating robustness. LOD and LOQ demonstrated sensitivity, and stability studies confirmed no interference from degradation products. The method is suitable for routine quality control and stability assessment of both analytes.

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