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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF POLMACOXIB AND PARACETAMOL IN TABLET BY RP-HPLC

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ABSTRACT

A simple, rapid, and reliable reverse-phase high-performance liquid chromatographic method was developed and validated for the simultaneous estimation of polmacoxib and paracetamol in combined tablet dosage form. No official pharmacopoeial method is available for this combination, highlighting the need for a validated analytical procedure. Chromatographic separation was achieved on a C18 column under optimized isocratic conditions with UV detection at a selected wavelength. The developed method provided good resolution with symmetrical peaks and acceptable retention times. Linearity was observed over the selected concentration ranges with correlation coefficients close to unity. Accuracy and precision studies showed satisfactory recovery and low percentage Relative Standard Deviation values. The method was found specific, robust, economical, and suitable for routine quality control analysis of the combined tablet formulation.

Keywords:

Method Validation, Method Development, Paracetamol, Polmacoxib, RP HPLC.

1. Introduction

Analytical chemistry is used in quality assurance and control to identify and quantify components; techniques are created and approved in accordance with International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human (ICH) principles to guarantee accurate results.[1] It plays an essential role in regulatory compliance and directly supports product quality, patient safety, and data integrity.[2]

The goal of analytical method development is to establish and improve processes in the absence of appropriate formal techniques.[3]Such procedures must adhere to Good Laboratory Practice /Good Manufacturing Practice and meet ICH Q2 (R1) in order to produce accurate and compliant results.[4] New approaches are needed when excipients interfere, patents restrict access, biological matrices require alternatives, monographs are lacking, or current methodologies are expensive or complex.[5] Regulatory alignment, creation, validation, and assistance with routine and stability studies are among the goals.[6]Analyte characterization, requirement setting, and appropriate documentation are the first steps.[7], literature review[8], method selection[9], optimization[7], performance evaluation [10], and evaluation of recovery in actual samples.[7]

Validation under current Good Manufacturing Practice, which is usually broken down into qualification, process validation, and maintenance stages, guarantees that process consistently produce reliable results and is a fundamental component component of quality control.[3] It validates suitability for use across labs and analysts.[11,12] Predetermined procedures, acceptance standards, qualification standards for materials and equipment, revalidation guidelines, SOPs, and documentation are among the requirements.[13]

Key parameters of method validation are system suitability, specificity, linearity, range, precision (repeatability and intermediate precision), accuracy, Limit of Detection, Limit of Quantification, robustness, and stability.[14] Effective validation enhances confidence, efficiency, and cost control while preventing redundant testing.[15]

Complex mixtures are separated under pressure using high-performance liquid chromatography based on interactions between the analyte and stationary phase.[16] chromatograms are produced by detection in order to identify and quantify[17,18]the methods are normal phase and reversed phase.[19]ion exchange, size exclusion, and bioaffinity chromatography, enhanced resolution by

using gradient elution or isocratic elution. Solvent reservoirs are important components. Injectors, pumps, detectors, columns, and data systems[20]pumps can be either syringe, reciprocating piston, constant-pressure.[21]

A literature review is part of HPLC development.[8] recognizing the sample and choosing the additives, solvents, and columns[22]chromatographic modes, buffers, mobile phases, and detectors[23]Retention behavior is used to evaluate performance, efficiency, resolution, and tailing and selectivity.[24]HPLC offers high resolution, sensitivity, speed, and versatility.[25] Clinical analysis, environmental and forensic testing, pharmaceutical quality control, and food evaluation are some examples of applications.[16]

Polmacoxib

Polmacoxib (POL) is an oral Non-Steroidal Anti-inflammatory Drug that binds carbonic anhydrase and specifically inhibits Cyclooxygenase-2 (COX-2); although there is not much solid evidence, this dual action may improve safety and maybe lower cardiovascular risk. [26,27]It delivers anti-inflammatory benefit with intended GI sparing, but class CV concerns persist.[28] The medication is mostly metabolized by CYP3A4, exhibits low plasma exposure, accumulates in red blood cells and inflammatory joints, and is mostly eliminated in feces with a lengthy half-life (~127–131 hours).[29]COX-2 suppression in CV tissue may be limited by preferential CA binding, whereas low CA in joints allows activity.[30]It is taken orally at a dose of 2 mg once daily after meals and is indicated for osteoarthritis.[31]Anasarca, headache, edema, GI distress, and elevated creatinine are among the adverse effects. Minimal interactions occur with ketoconazole; some antidiabetic medications may be amplified; some anti hypertensives may increase the risk of renal/hyperkalemia; and the effectiveness of levamlodipine may be diminished.[32]

Paracetamol

For mild-to-moderate pain and fever, paracetamol (PCM) is a commonly used non-opioid analgesic and antipyretic; NSAIDs are among the most regularly used medications worldwide.[33]and both names denote the same drug [34]Its main mechanism of action is central COX regulation, which has minimal anti-inflammatory effects. A modest CYP route produces NAPQI, which glutathione neutralizes.[35]In addition to lowering central PGE_2, it also affects serotonergic/cannabinoid and AM404 pathways. The BBB is included in the broad distribution, absorption is quick (peaking in 30 to 60 minutes), metabolism mostly takes place in the liver, and metabolites are

excreted in urine.[36]Given orally, IV, or rectally, it is used for various pain states and fever[37]Hepatotoxicity from overdose, gastrointestinal distress, rash, and appetite loss are the main issues; alcohol and enzyme inducers increase liver risk; warfarin may cause more bleeding with prolonged usage; cholestyramine slows absorption; and metoclopramide accelerates it. [36]

For the purpose of simultaneously estimating paracetamol and polmacoxib in their combination pharmaceutical dosage formulation, this method is a recently developed and novel analytical approach.

2. Materials And Methods

The chemicals and instruments used in this study are mentioned in table no. 1.

Table 1: List of the Chemicals and Instruments used in this Study

S.NO.	CHEMICALS/INSTRUMENTS	MANUFACTURER/SUPPILER
1	PARACETAMOL	Precise Chemipharma Pvt Ltd
2	POLMACOXIB	Precise Chemipharma Pvt Ltd
3	Potassium dihydrogen orthophosphate	Thermo Fisher Scientific India Pvt Ltd
4	Acetonitrile (HPLC Grade)	Advent Chembio Pvt Ltd
5	Methanol (HPLC Grade)	Advent Chembio Pvt Ltd
6	Distilled Water	Bioage
7	Milli-Q Water System	Bioage Model-ZIQ7003TOC
8	Orthophosphoric acid	Advent Chembio Pvt Ltd
9	High Performance Liquid Chromatography (HPLC)	Agilent 1200
10	UV Spectroscopy	Perkin Elmer Model Lambda 35
11	Differential Scanning Calorimetry	Perkin Elmer Model 8500
13	Analytical Balance	Mitter Toledo XP 205
14	Filter Assembly	Millipore
15	Sonicator	York sales Model ATS-1
16	Analytical Column	Inertsil C18, 4.6x150 mm, 5 micron
17	HPLC vials	Agilent
18	0.45 µ PVDF filter paper	Axiva

2.1 Identification Test of Drugs:

2.1.1 UV Spectroscopy:

Standard stock Solution-1: 20 mg of polmacoxib was dissolved in 50 mL of diluent to create stock solution-1. (400 µg/mL)

Standard stock solution-2: 48.75 mg of the paracetamol were dissolved in 25 mL of diluent to create a stock solution-2 (1950 µg/mL).

Final standard solution: 2.5 ml of stock solution-1 (400 µg/mL) and stock solution-2 (1950 µg/mL) diluted with diluent in a 100 ml volumetric flask.

UV spectra of polmacoxib and paracetamol are shown in Figures 1 and 2, respectively.

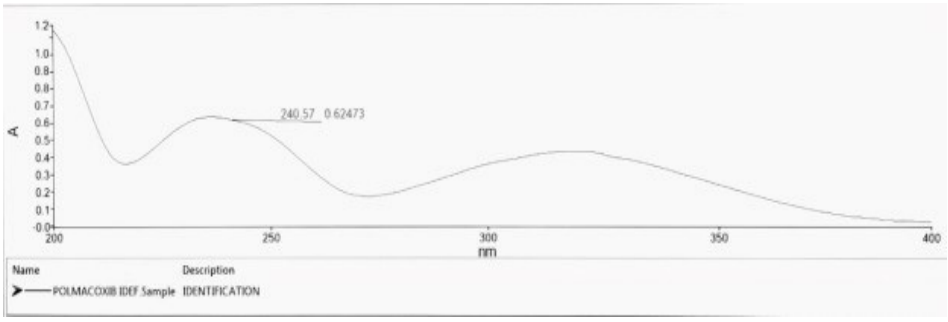


Figure 1. UV Spectra of Polmacoxib

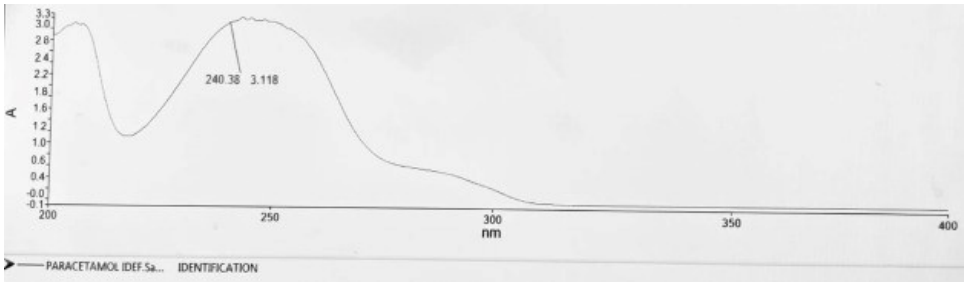


Figure 2. UV Spectra of Paracetamol

2.1.2 Differential Scanning Calorimetry (DSC) analysis:

For paracetamol: DSC was used to examine 1-2 mg of paracetamol under nitrogen (20 ml/min), heated to 30 to 400 °C at a rate of 10 °C/min, and the thermogram was used to determine the melting point.

For polmacoxib: DSC analysis of polmacoxib (1-2 mg) was performed under nitrogen flow (20 ml/min), heated from 30 to 400 °C at 10 °C/min, and the heat flow thermogram was used to determine the drug melting point.

DSC analysis curves for Paracetamol and Polmacoxib are shown in Figures 3 and 4, respectively.

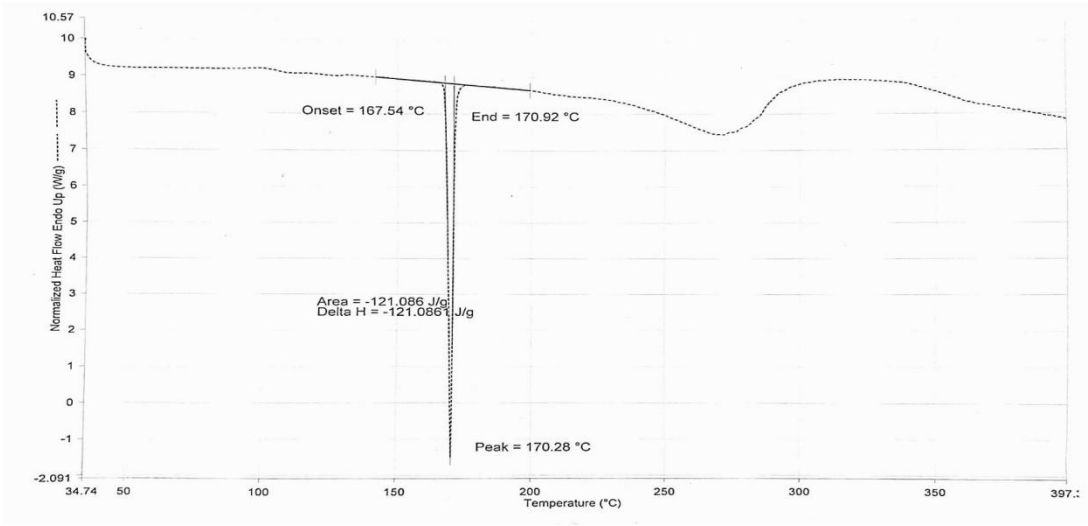


Figure 3. DSC analysis curves for Paracetamol

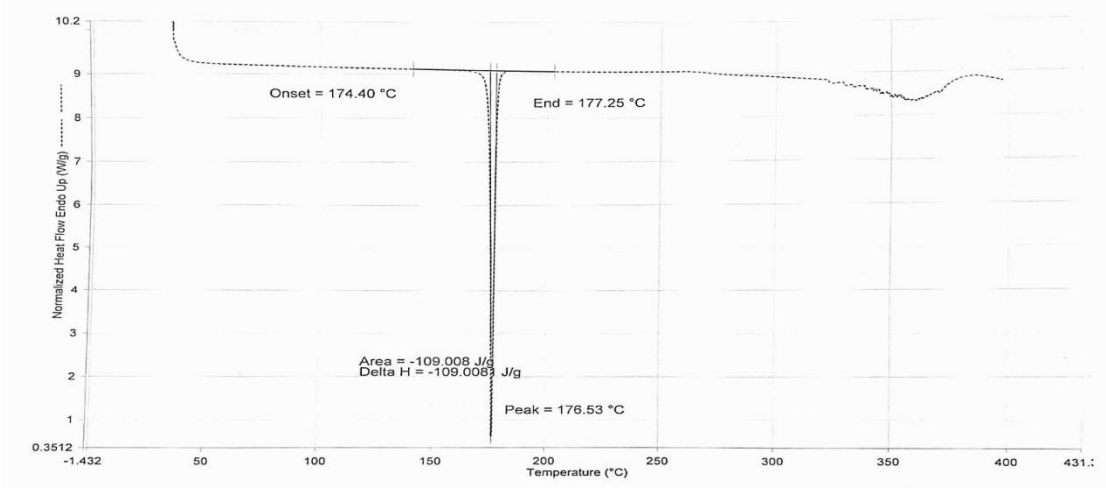


Figure 4. DSC analysis curve for Polmacoxib

2.2 Methodology:

Selection of Wavelength: we examine the working wavelength of paracetamol and polmacoxib in method development using UV spectroscopy.

Selection of Buffers: we experiment with several buffers, but potasium dihydrogen phosphate buffer yields the best results.

Selction of Mobile Phase Ratio: Although we test the approach with varying mobile phase ratios, the 60:40 ratio yields the best results.

Selection of Flow rate: The sample is run at various flow rates, although 1.0 ml/min yields superior results.

Selection of Column: we run the sample at various columns, but the best results are obtained at C18,150x4.5 mm,5 micron.

Selection of column oven and sampler temperature: we kept the temperature of the

sampler and column oven at 25 ˚C, which produced a good response, and these conditions are appropriate for routine analysis since they produced stable chromatographic performance.

2.3 Optimized Method:

The final conditions selected after method development are shown in Table 2.

Table 2: Final Conditions Selected after Method Development

Parameters	Conditions
Instrument	Agilent Technologies
Column	Agilent C18,150X4.6 mm, 5 MICRON
Detector	PDA
Detection wavelength	UV at 240 nm
FLow rate	1.0 ml/min
Mobile phase	BUFFER : ACN (60:40% v/v)
Injection volume	5 µL
Run time	25 min
Sampler temperature	25 ℃
Column temperature	25 ℃

2.4 Preparation of Samples and Solvents Used:

Making a 0.01M potassium dihydrogen phosphate (KH_2PO_4) Buffer:

1.36 grams of KH_2PO_4 are transferred into a 1000 milliliter volumetric flask. 700–800 milliliters of water are added and stirred until the solution is entirely dissolved, and then the volume is adjusted to 1000 milliliters using Milli-Q water and sonicated.

Preparation of Mobile Phase:

0.01M potassium dihydrogen phosphate (buffer) and Acetonitrile in a 60:40 ratio were shaken, sonicated, and then filtered through 0.45 µm filter paper.

Diluent Preparation:

A 40:50:10 water, acetonitrile, and methanol mixture was agitated, sonicated, and then filtered through 0.45 µm filter paper.

Making the Paracetamol drug assay standard solution:

In a 25 mL volumetric flask, 48.75 mg of paracetamol was dissolved with the diluent to create a stock solution with a concentration of 1950 ppm. To create a working standard solution of 48.75 ppm, 2.5 mL of this stock solution was then diluted to 100 mL using the same diluent.

Making the Polmacoxib drug assay standard solution:

To make the Polmacoxib standard stock solution, 20 mg of polmacoxib were precisely weighed and then transferred into a 50 mL volumetric flask. To create a solution with a concentration of 400 ppm, the diluent was added to the volume. To achieve a working standard concentration of 10 ppm, 2.5 mL of this stock was pipetted and diluted to 100 mL with the diluent.

Preparation of Sample Solution of Paracetamol and Polmacoxib Drug for Assay:

The test material for one tablet was carefully weighed and put into a 100 mL volumetric flask. It was dissolved by sonicating it in the diluent. 5.0 mL of this solution was diluted with 10 mL of the diluent to create the final Polmacoxib test solution.

In order to analyze paracetamol, 3.0 mL of the prepared test solution was pipetted into a different 100 mL volumetric flask, and the diluent was used to adjust the volume to the desired level. Prior to being used in chromatographic determination, all solutions were well mixed.

THEORY/CALCULATION:

Assay calculation of polmacoxib and paracetamol in tablet dosage form:

The assay of the polmacoxib 2 mg and paracetamol 325 mg tablets was carried out to quantify the drug content and to evaluate compliance with the labeled claim.

The percentage assay of the test sample was calculated using the formula:

$$\textbf{Test sample potency} = \frac{\text{Test area}}{\text{Standard area}} \text{ X } \frac{\text{Standard dilution}}{\text{Test dilution}} \text{ X } \frac{\text{Standard potency}}{\text{Label claim}} \text{X} \frac{\text{average weight}}{100}$$

Assay calculation of polmacoxib 2 mg tablet

Claim - 2 mg

Obtained - 1.99 mg/tab

%Assay (Potency of test sample) - 99.74 %

Assay calculation of paracetamol 325 mg tablet

Claim - 325 mg/tab

Obtained - 329.87 mg/tab

%Assay (Potency of test sample) - 101.5 %

By using a developed technique for drug testing in tablet formulation, the results within pharmacopoeial limits, the HPLC assay's low RSD values for polmacoxib (1.99 mg, or 99.74 %) and paracetamol (329.87 mg, or 101.5 %) per tablet confirmed good potency and technique reliability.

RESULT AND DISCUSSION

The developed HPLC method's validation parameters.

4.1 System Suitability:

System suitability testing confirms that an analytical system is operating correctly and producing accurate findings, particularly in chromatographic techniques where steady performance is essential.[38]

The results of the system suitability of polmacoxib and paracetamol are shown in tables no. 3.

The chromatogram of System Suitability is shown in Figure 5.

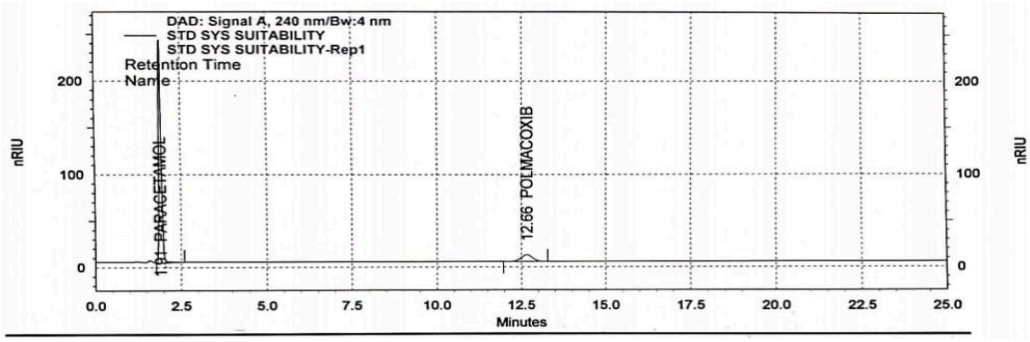


Figure 5. Chromatogram obtained of system suitability

Table 3: System Suitability Parameters for Polmacoxib and Paracetamol

DRUG	Parameter	Mean	SD	%RSD
Polmacoxib	Retention time (min)	12.66	0.01	0.04
	Peak area	359064.8	1765.75	0.49
	Asymmetry	1.03	0.01	0.69
	Theoretical plates	6287.8	39.68	0.63
Paracetamol	Retention time (min)	1.91	0	0
	Peak area	2550596.2	11828.2	0.46
	Asymmetry	1.22	0.01	0.81

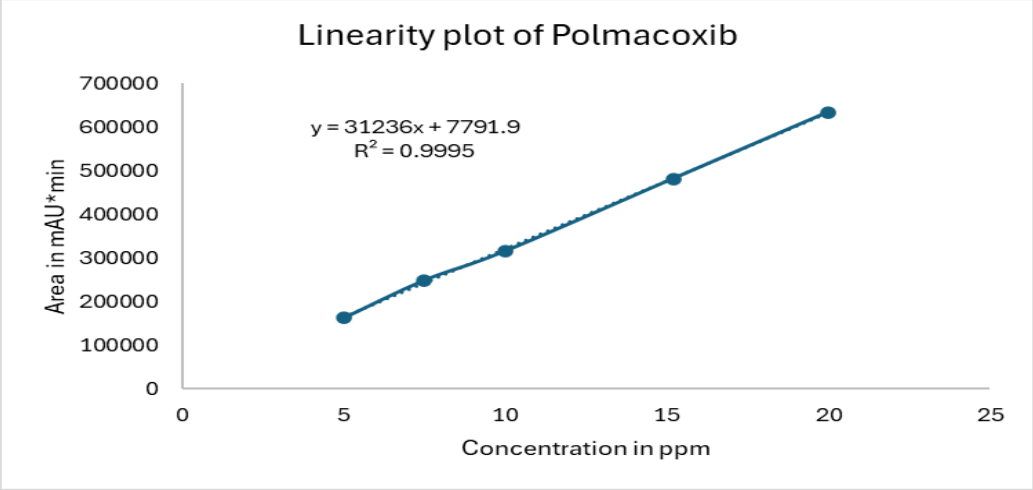
	Theoretical plates	3304.6	27.51	0.83

4.2 Linearity of Polmacoxib and Paracetamol:

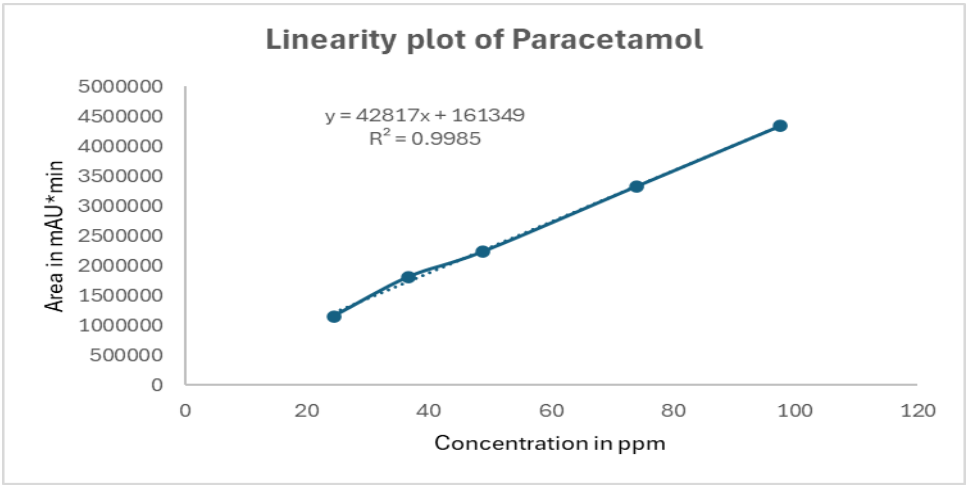
According to the 2005 International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use, linearity is the capacity of a technique to yield results that are proportionate to the analyte's concentration. This is demonstrated by a calibration curve with an R^2 value close to 1, which is created with at least five distinct concentration levels.[39]

The results of the linearity of Polmacoxib and Paracetamol are shown in table no. 4.

Linearity plots of polmacoxib and paracetamol between concentration vs. area are shown in Figures 6 and 7, respectively.



Figures 6. Linearity plot of Polmacoxib b/w concentration vs area



Figures 7. Linearity plot of Paracetamol b/w concentration vs area

Table 4: Linearity Range of Polmacoxib and Paracetamol

S.NO.	CONC (ppm)	CONC (ppm)	POLMACOXIB		PARACETAMOL	
			Area	AVERAGE	Area	AVERAGE
1	5	24.37	160943	161973.33	1149366	1151733
	5	24.37	160605		1155496	
	5	24.37	164372		1150337	
2	7.5	36.56	244605	248611.33	1795131	1807422
	7.5	36.56	248796		1807193	
	7.5	36.56	252433		1819942	
3	10	48.75	315537	315515.33	2204393	2230398.33
	10	48.75	312545		2211310	
	10	48.75	318464		2275492	
4	15.2	74.1	480436	481397.33	3329966	3327750.33
	15.2	74.1	484107		3311287	
	15.2	74.1	479649		3341998	
5	20	97.5	647637	633754.33	4502684	4332892.33
	20	97.5	628663		4283335	
	20	97.5	624963		4212658	
Slope	**31235.57**				**42816.6**	
Intercept	**7791.86**				**161348.79**	

Linearity conclusion

Strong linearity was demonstrated by the approach; polmacoxib and paracetamol were linear from 5–20 ppm (R^2 = 0.9995) and 24.37–97.50 ppm (R^2 = 0.9985), respectively, confirming accurate quantification for routine analysis.

4.3 Accuracy

The degree to which a measured result corresponds with the actual value of the analyte is known as accuracy, and it is usually assessed by recovery experiments.[40]

The results of the accuracy of polmacoxib and paracetamol are shown in tables no. 5 and 6, respectively.

Table 5: Accuracy Study for Polmacoxib

S. No.	Level	Initial Amount (µg/mL)	Amount added (µg/mL)	Total Amount (µg/mL)	Area	Total Amount found (µg/mL)	% Recovery	Avg	% RSD
1	80%	100	80	180	584683	179.33	99.16	99.980	1.085
		100	80	180	587898	179.66	99.57		
		100	80	180	593589	180.91	101.21		
2	100%	100	100	200	651613	199.86	99.86	98.750	0.974
		100	100	200	648674	198.29	98.23		
		100	100	200	649990	198.70	98.16		
3	120%	100	120	220	719368	220.64	100.54	100.423	0.927
		100	120	220	717727	219.33	99.44		
		100	120	220	726699	221.48	101.29		
Average								99.718	
SD								0.867	
% RSD								0.869	

Table 6: Accuracy Study for Polmacoxib

S. No.	Level	Initial Amount (µg/mL)	Amount added (µg/mL)	Total Amount (µg/mL)	Area	Total Amount found (µg/mL)	% Recovery	Avg	% RSD
1	80%	100	80	180	3974804	180.05	100.13	100.167	0.366
		100	80	180	3969267	179.80	99.82		
		100	80	180	3987846	180.64	100.55		
2	100%	100	100	200	4420102	200.22	100.29	100.707	0.358

		100	100	200	4433810	200.84	100.91		
		100	100	200	4440429	201.14	100.92		
3	120%	100	120	220	4874254	220.79	100.80	100.270	0.486
		100	120	220	4850890	219.73	99.84		
		100	120	220	4866622	220.45	100.17		
Average								100.381	
SD								0.287	
% RSD								0.286	

4.5 Precision:

Precision indicates the consistency of repeated measurements taken under particular circumstances and is commonly expressed as a percentage of RSD. It is evaluated statistically using the standard deviation, which is calculated from the deviations of the individual values from the mean.[1]

4.5.1 Repeatability:

Repeatability, also known as intra-assay precision, is a metric that evaluates consistency under the same conditions over a brief period of time. Within the designated concentration range, nine determinations are usually used to evaluate it.

The results of the repeatability of polmacoxib and paracetamol are shown in tables no. 7 and 8, respectively.

Table 7: Repeatability Result of Paracetamol

S.NO.	LEVEL	Conc. (ppm)	Area	Mean	SD	% RSD
1	50 %	24.37	1166727	1143131.33	21086.06	1.84
	50 %	24.37	1126132			
	50 %	24.37	1136535			
2	100 %	48.75	2143294	2147616.67	11833.73	0.55
	100 %	48.75	2138552			
	100 %	48.75	2161004			
3	150 %	97.5	4171293	4192947	22991.05	0.55
	150 %	97.5	4190473			
	150 %	97.5	4217075			

Table 8: Repeatability Result of Polmacoxib

S.NO.	LEVEL	Conc. (ppm)	Area	Mean	SD	% RSD
1	50 %	5	158847	157688.33	1048.18	0.66
	50 %	5	156806			
	50 %	5	157412			
2	100 %	10	301211	302563.00	1710.53	0.57
	100 %	10	304486			
	100 %	10	301992			
3	150 %	20	613294	614283.67	4432.15	0.72
	150 %	20	610430			
	150 %	20	619127			

4.5.2 Intermediate Precision:

The variations that take place inside a single laboratory between days, analysts, and equipment are evaluated by intermediate precision. [1]

The results of the intermediate precision study of polmacoxib and paracetamol on day I analyst I and day II analyst II are shown in tables no. 9.

Table 9: Comparisons between Day 1 and Day 2 Intermediate precision of paracetamol and Polmacoxib

S.No.	Paracetamol		Polmacoxib	
	Day 1's Intermediate Precision	Day 2's Intermediate Precision	Day 1's Intermediate Precision	Day 2's Intermediate Precision
1	99.17	101.4	100.31	100.01
2	100.59	99.78	98.61	99.71
3	100.29	99.15	98.77	101.2
4	99.8	98.75	98.48	99.97
5	98.78	99.25	99.99	99.7
6	98.99	98.68	98.66	100.37
Average	99.6	99.5	99.14	100.16
SD	0.74	1.01	0.8	0.57
% RSD	0.74	1.02	0.8	0.56

4.6 Specificity:

For paracetamol (~2.0 min) and polmacoxib (~12.5 min), specificity testing revealed single, well-resolved peaks with no interference in the blank, demonstrating the

method's ability to precisely detect and quantify both medications at the same time. The chromatogram of specificity is shown in Figure 8.

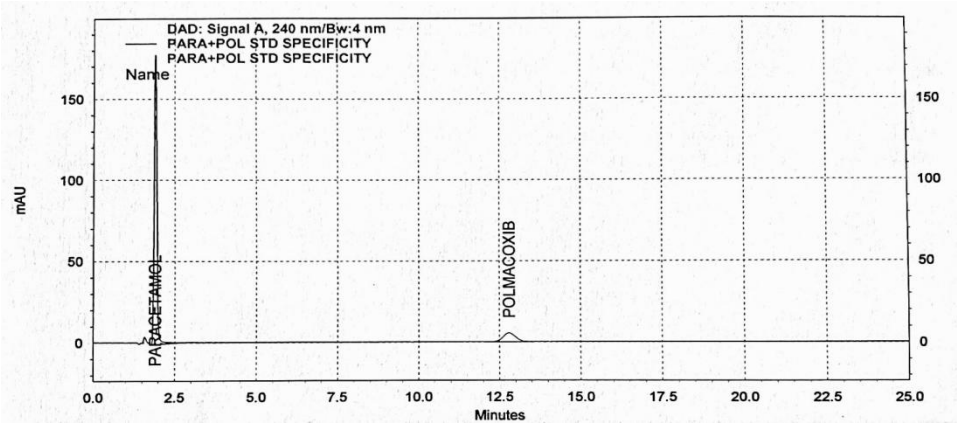


Figure 8. Chromatogram obtained for standard solution

4.7 Robustness:

Robustness testing confirmed the method's dependability by demonstrating that minor intentional adjustments to wavelength, temperature, buffer strength, and flow rate did not significantly alter retention duration or peak area (% RSD within limits).[14]

The results of the robustness of polmacoxib and paracetamol are shown in tables no. 10 and 11, respectively.

Table 10: Robustness Data of Paracetamol

S.NO.	Parameters	Conditions	Average	SD	%RSD
1	Buffer (±2 %)	612	2533062	46015.17	1.82
		600	2314745.8	22054.91	0.95
		588	2692894.2	29416.53	1.09
2	Column Temperature (±5 ˚C)	30	2288752.2	3819.65	0.17
		25	2314745.8	22054.91	0.95
		20	2327326.8	22585.17	0.97
3	Wavelength (±2 nm)	242	2426437.6	9965.05	0.41
		240	2314745.8	22054.91	0.95
		238	2142433.6	12294.35	0.57
4	Flow Rate (mL/min) (±10 %)	1.1	2116445.4	5578.96	0.26
		1	2314745.8	22054.91	0.95
		0.9	2597702.4	10064.6	0.39

Table 11: Robustness Data of Polmacoxib

S.NO.	Parameters	Conditions	Average	SD	%RSD
1	Buffer (±2 %)	612	347949.2	1992.62	0.57
		600	325491.2	2215.7	0.68
		588	364371.4	4245.89	1.17
2	Column Temperature (±5 ˚C)	30	326166.6	404.18	0.12
		25	325491.2	2215.7	0.68
		20	326876	2742.63	0.84
3	Wavelength (±2 nm)	242	318865.6	1971.72	0.62
		240	325491.2	2215.7	0.68
		238	331163	1890.74	0.57
4	Flow Rate (mL/min) (±10 %)	1.1	299862.8	1395.67	0.47
		1	325491.2	2215.7	0.68
		0.9	366001.2	1463.75	0.40

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

The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use recommends that the LOQ be the lowest quantifiable level with acceptable precision and accuracy, usually at 10:1, while the LOD is the lowest detectable analyte concentration (usually 3:1 signal-to-noise).[1]

Limit of Detection (LOD)

The following formula was used to get the detection limit:

$$LOD = 3.3 \times (\sigma/S)$$

Here,

S= slope of the calibration curve

σ = standard deviation of the response

The LOD was 4.38 µg/mL and 0.52 µg/mL for paracetamol and polmacoxib, respectively.

Limit of Quantification (LOQ)

The following formula was used to determine the quantification limit:

$$LOQ = 10 \times (\sigma/S)$$

Here,

σ = standard deviation of the response

S= slope of the calibration curve

The LOD was 13.29 µg/mL and 1.56 µg/mL for paracetamol and polmacoxib, respectively.

4.9 Range

Using this method, it was demonstrated that the concemtration ranges of polmacoxib and paracetamol, which 5 to 20 µg/mL and 24.37

to 97.50 µg/mL, respectively, are precisely, linear, and accurate.

4.10 Stability Study of Paracetamol and Polmacoxib

It was confirmed that both paracetamol and polmacoxib were stable under test settings since their peak areas showed very little variation over a 24-hour period (%RSD 0.59 % and 0.42 %).

The stability data of polmacoxib and paracetamol is shown in Table no. 12.

Chromatograms of stability study are shown in figure 9,10,11,12,13, and 14.

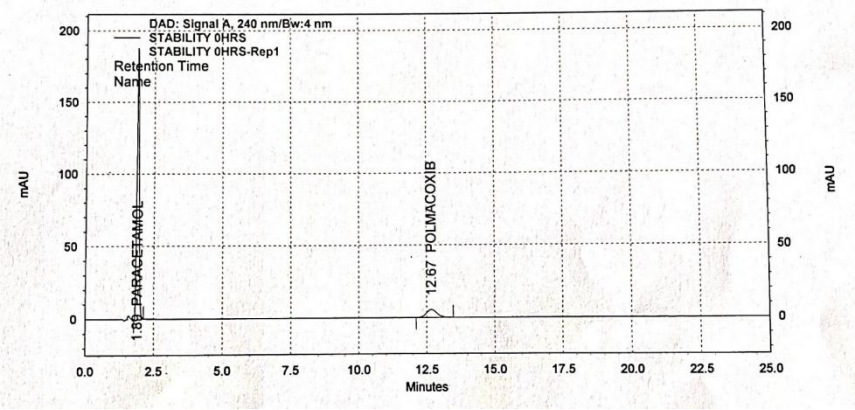


Figure 9. *Chromatogram of Paracetamol and Polmacoxib at 0 hr*

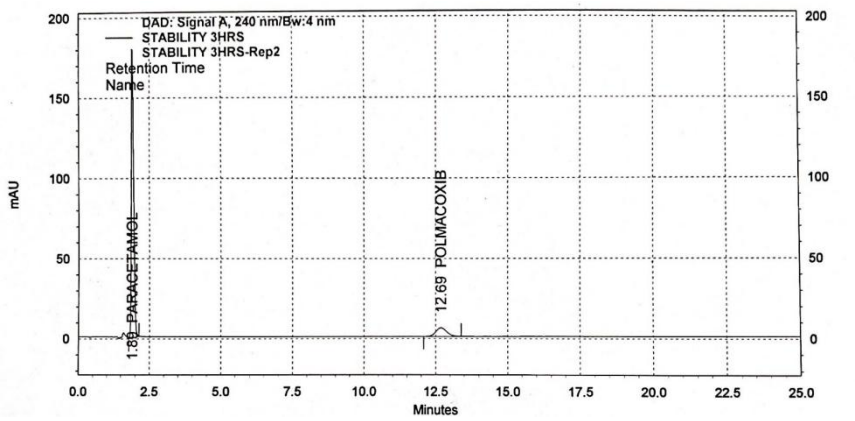


Figure 10. *Chromatogram of Paracetamol and Polmacoxib at 3 hr*

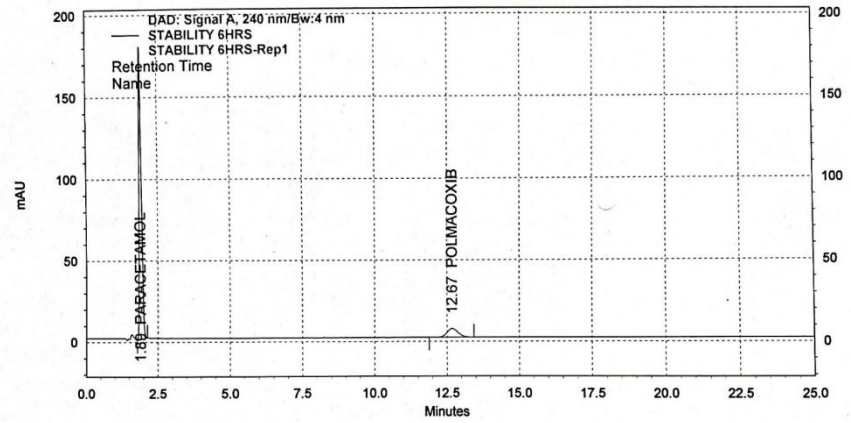


Figure 11. *Chromatogram of Paracetamol and Polmacoxib at 6 hr*

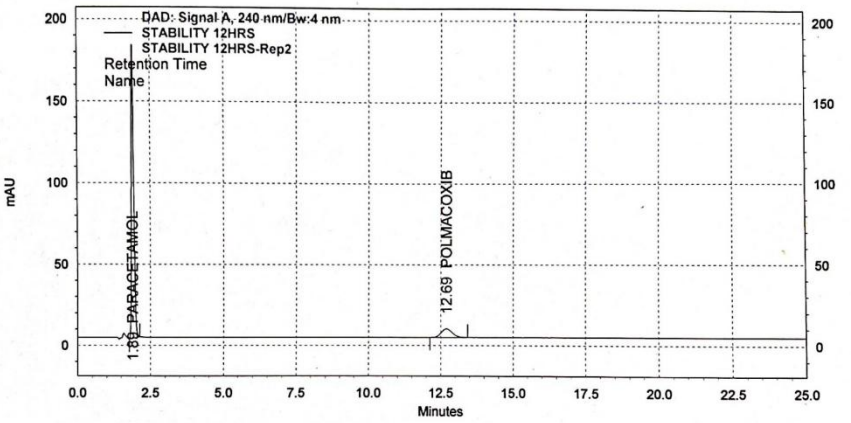


Figure 12. *Chromatogram of Paracetamol and Polmacoxib at 12 hr*

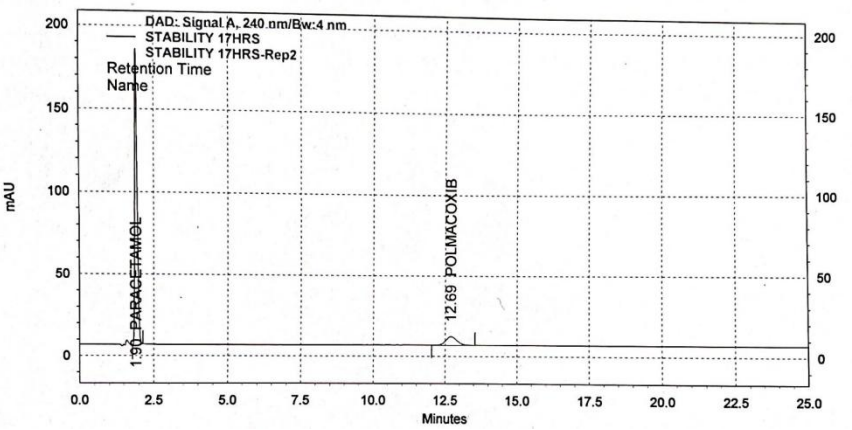


Figure 13. *Chromatogram of Paracetamol and Polmacoxib at 17 hr*

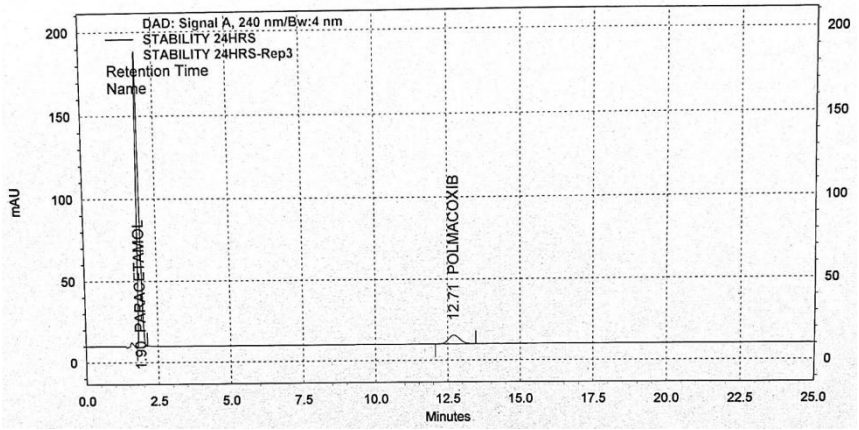


***Figure 14**. Chromatogram of Paracetamol and Polmacoxib at 24 hr*

Table 12: Stability result for Paracetamol and Polmacoxib

S.NO.	STORAGE CONDITION	TIME (HRS)	PCM AREA	POL AREA
1.	25°C ± 5°C/ 60% RH	0	2193165.34	304415.67
2.		3	2169060.67	304072.00
3.		6	2169684.34	304521.34
4.		12	2173101.34	303914.00
5.		17	2182103.67	306799.34
6.		24	2199286.00	306583.34
MEAN			2181066.89	305050.95
SD			12779.24	1291.52
% RSD			0.59	0.42

Table 13: Results of Validation

S.NO.	Parameter	Acceptance Criteria	Result	
			Paracetamol	Polmacoxib
1	System Suitability	TP should be NLT 2000	3305	6288
		NMT 2.0 should be the TF	1.16	1.03
		NMT 2.0 % should be the RSD of five replicate injections	0.83	0.63
2	Linearity	NLT 0.99 should be the correlation coefficient	0.998	0.999
3	Accuracy	Mean recovery should be between	100.37	99.71

		98 % and 102 %		
4	Precision	Repeatability: The RSD of the area for paracetamol and polmacoxib should be NMT 2.0 %	0.98	0.65
		Intermediate Precision (Day l): The area's for Paracetamol and Polmacoxib should be NMT 2.0 %	0.54	1.67
		Intermediate Precision (Day 2): The area's for Paracetamol and Polmacoxib should be NMT 2.0 %	0.37	0.54
5	specificity	Other peaks should not interfering with the main peak	Complied	Complied
		The peak purity should be 1	1	1
6	Robustness	Change in Buffer (±2 %): The area of peaks should have an RSD of NMT 2.0 %	1.28	0.80
		Change in Flow Rate (±10 %): The area of peaks should have an RSD of NMT 2.0 %	0.53	0.51
		Change in Wavelength (±2 nm): The area of peaks should have an RSD of NMT 2.0 %	0.64	0.62
		Change in Column Temperature (±5 ˚C): The area of peaks should have an RSD of NMT 2.0 %	0.69	0.54
7	LOD	-	4.38 µg/mL	0.52 µg/mL
8	LOQ	-	13.29 µg/mL	1.56 µg/mL
9	Range	-	24.37 to 97.50 µg/mL	5 to 20 µg/mL
10	Drug Assay	As per I.P, acceptance limit 95 % - 105 %	101.50 %	99.74 %

CONFLICT OF INTEREST: The authors declare that there is no conflicts of interest related to this work.

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AUTHOR'S CONTRIBUTIONS

Akash Prajapati carried out the conception, design, execution of the study, and drafting of the manuscript. **Avishek Mukherjee** performed the data analysis and interpretation. **Meenakshi Dahiya, Nidhi Jain and Babita Kumar** contributed by reviewing and critically revising the manuscript and have approved the final version. All authors agree to be accountable for all aspects of the work.

REFERENCES

1. More SS, Madam SP, Redasani VK. A review on analytical method development and validation. International Journal of Pharmaceutical Research. 2025;10(1):1177-95.
2. Mali C, Sonawane B, Mali R. Recent advances in analytical method validation as per ICH Q2(R2): a comparative review with ICH Q2(R1). Int J Pharm Sci.2025;3(7):1560– 1567. doi:10.5281/zenodo.15863307.
3. Sharma S, Goyal S, Chauhan K. A review on analytical method development and validation. International Journal of Applied Pharmaceutics. 2018 Nov 7;10(6):8-15.
4. Söğüt Ertaş Ö. and Kayali A. An overview on analytical method validation, Ankara Univ. Eczacilik Fak. Derg. 2005; 41–57.doi:10.22270/ujpr.v5i1.362
5. Ravisankar P, Naga Navya C, Pravallika D, Navya Sri D. A review on step-by-step analytical method validation. IOSR Journal of Pharmacy. 2015;5(10):7-19.
6. Patil RN, Deore BL, Gurav DG. Analytical method development and validation. Indian Journal of Pharmacy & Drug Studies. 2024;3(4).
7. Ravisankar P, Gowthami S, Rao GD. A review on analytical method development. Indian journal of research in pharmacy and biotechnology. 2014 May 1;2(3):1183.
8. Sawale VS, Umamaheshwari D. A review on novel analytical techniques used in method development and validation of pharmaceuticals. Journal of Pharmaceutical Sciences and Research. 2020 Feb 1;12(2):321-8.
9. Lal B, Kapoor D, Jaimini M. A review on analytical method validation and its regulatory perspectives. Journal of Drug delivery and therapeutics. 2019 Mar 1;9(2):501-6.
10. Shrivastava S, Deshpande P, Daharwal SJ. Key Aspects of Analytical Method Development and Validation. Journal of Ravishankar University (Part-B: Science). 2018;31(1):32-39.
11. Kakad SB, Kolhe MH, Dukre TP. A review on pharmaceutical validation. International Journal of Pharmaceutical Quality Assurance. 2020 Sep 25;11(3):338-42.
12. Shinde SS, Khulbe DP. Analytical Method Validation: A Comprehensive Review of Current Practices. South Eastern European Journal of Public Health. 2023; 21:244-254.
13. Doltade M. and Saudagar R. The Analytical Method Development and Validation: A Review, J. Drug Deliv. Ther. 2019; 9: 563–570.
14. Chandran S, Singh RP. Comparison of various international guidelines for analytical method validation. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2007 Jan 1;62(1):4-14.
15. Chavan SD, Desai DM. Analytical method validation: a brief review. World Journal of
16. Advanced Research and Reviews. 2022;16(2):389–402. doi:10.30574/wjarr.2022.16.2.1165.

17. Murkute PS, Patil PH, Sananp GS, Kathar NP, Pimple AP. A review on high performance liquid chromatography (HPLC). International Journal for Research Trends and Innovation. 2022;7(2):98–103.
18. Dandekar PM, Zore MG, Sheikh AI, Jaware AS, Sarnaik AG, Sawde AA, Achal B and Lode. A review on high performance liquid chromatography (HPLC). International Journal of Advanced Research in Science, Communication and Technology. 2024;4(3):348–354. doi:10.48175/ijarsct-18251.
19. Supriya K, Asma Parveen S, Prapurna Chandra Y. A review on experimental designs in HPLC method development and validation. Journal of Innovations in Applied Pharmaceutical Sciences. 2025;10(1):7–11. doi:10.37022/jiaps.v10i1.655.
20. Gupta S, Verma P, Mishra AP, Omar N, Mathur R. A review on novel analytical method development and validation by RP-HPLC method. Indian Journal of Forensic Medicine & Toxicology. 2021;15(4):3479-3486. doi:10.37506/ijfmt.v15i4.17798.
21. Pawar DL, Thange T. A review on high performance liquid chromatography (HPLC). International Journal of Research Publication and Reviews. 2022;3(7):3014–3028.
22. Nomula AB. A review on instrumentation of high performance liquid chromatography (HPLC). World Journal of Pharmaceutical Research. 2025;14(6):29–54. doi:10.20959/wjpr20256-35522.
23. Khan H. Analytical method development in pharmaceutical research: Steps involved in HPLC method development. Asian Journal of Pharmaceutical Research.2017;7(3):203 .
24. Arote KS, Salade DA, Patil NV, Patil VV, Pawar AR. A review on: analytical method development and validation and it's QbD approach. International Journal of Novel Research and Development. 2022;7(9):365–378.
25. Panchumarthy R, Anusha S, Supriya K, Ajith Kumar U. Fundamental chromatographic parameters. International Journal of Pharmaceutical Sciences Review and Research. 2019;55(2):46–50.
26. Latey JG. Analytical method development and validation of API by using suitable analytical technique - a review. TANZ Research Journal. 2025;11(4):13-30.
27. Verma R, Gupta R, Waghmare P, Kare SK, Kar AK, Mistry UK, Bhole M, Deshpande S, Patil S, Waghmare Sr P, Kar A. Evaluation of Polmacoxib 2 mg for the Management of Hip and Knee Osteoarthritis: A Prospective, Single-Arm, Multicenter, Open-Label Study. Cureus. 2025 Oct 18;17(10).
28. Khan S, Agrawal P, Mehta A, Gupta N. Clinical perspectives on the effectiveness and
29. safety of polmacoxib in pain management: a survey of clinicians across India. International Journal of Medical Research Professionals. 2025;11(2):11–14.
30. Adake VV, Nitave SA. Polmacoxib: a promising NSAID for pain and inflammation. World Journal of Pharmaceutical Research. 2023;13(1):889–902. doi:10.20959/wjpr20241- 30853.
31. Gunjal VS, Pawar RR, Sharma AD. Review of Safety and Efficacy of Polmacoxib: A
32. Novel Dual Inhibitor of Cyclo-oxygenase 2 and Carbonic Anhydrase in Osteoarthritis and Acute Painful Conditions. The Journal of the Association of Physicians of India. 2025 Oct 1;73(10):88-92.
33. Easwaran R, Mistry UK, Bhole M, Peethambaran K. Polmacoxib: A review of the newer non-steroidal anti-inflammatory drug in osteoarthritis. Cureus. 2024 Apr 17;16(4).

34. Kanojia M, Sodiyal N, Patil S. Polmacoxib and its evaluation parameters. OEIL Research Journal. 2025;23(7).
35. Chandrashekara S. A Narrative Review Examining the Clinical Safety and Efficacy of Polmacoxib: Current Evidence and Upcoming Prospects, Discover Med., 2024; 1(1): 129.
36. Assali M, Abualhasan M, Zohud N, Ghazal N. RP- HPLC method development and validation of synthesized codrug in combination with indomethacin, paracetamol, and famotidine. International Journal of Analytical Chemistry. 2020. doi:10.1155/2020/1894907.
37. Jóźwiak-Bębenista M, Nowak JZ. Paracetamol: mechanism of action, applications and safety concern. Acta Poloniae Pharmaceutica – Drug Research. 2014;71(1):11–23.
38. McCrae JC, Morrison EE, MacIntyre IM, Dear JW, Webb DJ. Long-term adverse effects of paracetamol–a review. British journal of clinical pharmacology. 2018 Oct;84(10):2218-30.
39. Bannwarth B, Péhourcq F. Pharmacological rationale for the clinical use of paracetamol:pharmacokinetic and pharmacodynamic issues. Drugs. 2003;63(Suppl 2):5–13.
40. Ogemdi IK. A Review on the Properties and Uses of Paracetamol. International Journal of Pharmacy and Chemistry. 2019 Sep 21;5(3):31-5.
41. Gupta P. C. Method Validation of Analytical Procedures, PharmaTutor J. Pharm. Educ. Res. 2015; 3(1): 32–39.
42. Chikanbanjar N., Semwal N. and Jyakhwa U. A Review Article on Analytical Method Validation, J. Pharma Innov. 2020; 1(2): 11–21.40.
43. Patil M. S., Patil R. R., Chalikwar S. S., Surana S. J. and Firke S. D. Analytical Method Development and Validation: A Review, Int. J. Pharm. Biol. Sci. Arch. 2019; 7(3): 70–81.