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Arun Maruti Kashid

arunkashid2006@gmail.com

Sinhgad Technical Education Society's, Sinhgad Institute of Pharmacy, Affiliated to Savitribai Phule Pune University

Rucha Ravikiran Takalkar

Sinhgad Technical Education Society's, Sinhgad Institute of Pharmacy, Affiliated to Savitribai Phule Pune University

Jayshree Kailas Kokane

Sinhgad Technical Education Society's, Sinhgad Institute of Pharmacy, Affiliated to Savitribai Phule Pune University

Pranav Kailas Deore

Sinhgad Technical Education Society's, Sinhgad Institute of Pharmacy, Affiliated to Savitribai Phule Pune University

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Stability Indicating HPTLC Method Development and Validation for Simultaneous Estimation of Polmacoxib and Paracetamol in Bulk and tablet dosage form

Arun Maruti Kashid^{1*}, Rucha Ravikiran Takalkar², Jayshree Kailas Kokane², Pranav Kailas Deore²

¹Department of Pharmaceutical Chemistry, Sinhgad Technical Education Society's, Sinhgad Institute of Pharmacy, Narhe, Pune, Affiliated to Savitribai Phule Pune University, Maharashtra, India 411041

²Department of Pharmaceutical Quality Assurance, Sinhgad Technical Education Society's, Sinhgad Institute of Pharmacy, Narhe, Pune, Affiliated to Savitribai Phule Pune University, Maharashtra, India 411041

*Corresponding author:

Arun M. Kashid

Department of Pharmaceutical Chemistry

STES's, Sinhgad Institute of Pharmacy, Narhe, Pune, Affiliated to Savitribai Phule Pune University, Maharashtra, India 411041

Contact No: +919011004372

Email ID: arunkashid2006@gmail.com

Abstract

A simple, accurate, reliable, precise and robust HPTLC method was designed for the simultaneous determination of Polmacoxib (POL) and Paracetamol (PCM) in bulk drug and dosage form. Chromatographic separation was performed on silica gel 60 F₂₅₄ plates with a mobile phase of toluene:ethyl acetate:formic acid (5:4:1, v/v/v) and detected densitometrically at 283 nm. POL and PCM were shown to have retention factors (R_f) of 0.66 and 0.50, respectively. With correlation values greater than 0.999, the approach demonstrated excellent linearity over concentration ranges of 80–240 ng/band for POL and 13000–39000 ng/band for PCM. Specificity, accuracy, precision, robustness, sensitivity, LOD, and LOQ were all satisfactory when validation was carried out in accordance with ICH recommendations. Forced degradation studies performed in acidic, alkaline, oxidative, neutral, thermal, and photolytic conditions which confirmed the stability indicating capability of method. It was discovered that the established HPTLC approach was appropriate for regular quality control and stability analysis of polmacoxib and paracetamol in pharmaceutical formulations.

Key words: HPTLC, Polmacoxib, Paracetamol, Simultaneous estimation, ICH

Abbreviations: POL: Polmacoxib, PCM: Paracetamol, HPTLC: High performance thin layer chromatography, ICH: International Council for Harmonisation.

1.Introduction

Millions of individuals worldwide suffer from pain and inflammation, two of the most prevalent clinical disorders that have a major negative influence on quality of life. Pain, inflammation, fever, and musculoskeletal conditions are frequently treated with nonsteroidal anti-inflammatory drugs (NSAIDs). In order to improve drug efficacy, manage pain more effectively and minimize side effects, combination therapy using medications with complimentary modes of action is becoming more and more popular. Because of their analgesic and anti-inflammatory qualities, polmacoxib and paracetamol have become important therapeutic combinations.

A new nonsteroidal anti-inflammatory medication in the class of selective cyclooxygenase-2 (COX-2) inhibitors is polmacoxib [1]. It is mostly used to treat osteoarthritis and inflammatory pain problems. Its chemical name is 2-(4-aminophenyl)-3,4,5-trimethyl-1H-pyrrole derivative. In contrast to traditional NSAIDs, Polmacoxib minimally inhibits cyclooxygenase-1 (COX-1) while selectively inhibiting the cyclooxygenase-2 enzyme that produces inflammatory prostaglandins. This reduces the gastrointestinal side effects that are frequently linked to traditional NSAIDs. Polmacoxib's distinct pharmacological profile includes carbonic anhydrase binding characteristics in addition to selective COX-2 inhibition [2]. Polmacoxib offers efficient pain relief and anti-inflammatory activities with enhanced gastrointestinal tolerability because of its selective action.

Acetaminophen, another name for paracetamol, is one of the most often used analgesics and antipyretics in the world. Chemically called N-(4-hydroxyphenyl) acetamide, it is frequently used to treat fever and mild to moderate discomfort. It is thought that paracetamol works by inhibiting prostaglandin synthesis, mainly in the central nervous system, which results in analgesic and antipyretic effects [3]. When taken at therapeutic dosages, paracetamol has a good safety profile and little peripheral anti-inflammatory activity compared to NSAIDs. Paracetamol is often used in conjunction with anti-inflammatory drugs to improve pain management results because of its effectiveness, safety, and tolerability [4,5]. Figures 1 (a) and (b) show the structures of POL and PCM.

For the treatment of pain and inflammatory illnesses, such as osteoarthritis, rheumatoid arthritis, musculoskeletal pain, and post-operative pain conditions, the combination of polmacoxib with paracetamol provides a synergistic therapeutic strategy. Paracetamol adds quick analgesic and antipyretic effects, enhancing overall therapeutic efficacy, while Polmacoxib offers strong anti-inflammatory action by selective COX-2 inhibition. These fixed-dose combinations have the potential to decrease side effects and lower the necessary dosage of individual medications.

The creation of trustworthy and proven analytical techniques for their simultaneous estimation is crucial to guaranteeing the efficacy, safety, and quality of pharmaceutical dosage forms including these medications. In routine pharmaceutical analysis, stability studies, quality control, and quantitative determination, analytical techniques are essential. High-Performance Thin-Layer Chromatography (HPTLC) has become a popular analytical technique for simultaneous drug quantification because it is quick, affordable, accurate, and sensitive. Benefits of HPTLC include

low solvent consumption, high sample throughput, simple sample preparation, repeatability, and appropriateness for regular quality control analysis.

There are few analytical techniques available for the simultaneous measurement of Polmacoxib and Paracetamol combinations, despite their medicinal significance. Present work is aimed to develop and validate simple, accurate, precise and stability indicating HPTLC method for simultaneous determination of Polmacoxib and Paracetamol in pharmaceutical dosage forms. The developed method should follow International Council for Harmonization (ICH) guidelines for validation parameters of analytical techniques such as specificity, linearity, accuracy, precision, robustness, limit of detection (LOD) and limit of quantitation (LOQ). Therefore, the goal of this study is to create and validate an HPTLC method for the simultaneous quantification of paracetamol and polmacoxib in pharmaceutical formulations.

2. Experimental

2.1 Materials, reagents, and chemicals

Gift sample of POL (99.7%) and PCM (99.9%) APIs were supplied by Precise Biopharma Pvt. Ltd. (Mumbai, Maharashtra), Ipca Laboratories Ltd. (Mumbai, Maharashtra). Alkem Pharmaceuticals Ltd. manufactured Polmakem-P, a tablet formulation that contained 2 mg of Polmacoxib and 325 mg of Paracetamol. It was bought from a neighborhood pharmacy in Pune, Maharashtra, India. The research used analytical-grade reagents from Loba Chemie Ltd., Mumbai, including methanol, toluene, ethyl acetate and formic acid.

2.2 Instrumentation and Chromatographic conditions

The stationary phase was aluminium backed silica gel 60 F₂₅₄ HPTLC plates (10 × 10 cm) with 20 µm thickness; E. Merck Pvt. Ltd., Mumbai, India). A Camag 10 µL microsyringe (Hamilton, Bonaduz, Switzerland) was used to apply the sample. A Twin Trough glass chamber and Linomat V applicator (CAMAG) were used for chromatographic analysis. Precoated silica gel 60 F₂₅₄ plates were used as the stationary phase for the chromatographic assessment. To remove moisture and ensure compact spot formation, the TLC plates were pre-treated and activated by heating them to 110°C in a hot air oven for 20 minutes prior to sample application. When the sample was applied, distinct bands of 6 mm in length were produced [6].

A TLC Scanner (CAMAG) with visionCATS software was used for detection. A UV Cabinet (CAMAG) was used for visualization. Maximum UV absorption was observed at 323 nm for POL and 250 nm for PCM. The overlay spectra of the tablet and standard formulations showed that the isosbestic point was located at 283 nm. Fig.2 displays the UV absorption spectra of the tablet formulation for POL and PCM as well as the pure medications.

Toluene: ethyl acetate: formic acid (5:4:1 v/v/v) made up the optimal mobile phase. The development chamber was saturated with a mobile phase at room temperature for 10 minutes and a plate was developed up to a migration distance of approximately 80 mm. After development, the plates were allowed to air dry before being subjected to densitometric analysis.

2.3 Preparation of standard stock solution

The concentration range for method optimization was established by using fixed dose combination of POL (2 mg) and PCM (325 mg). By precisely weighing 2 mg of Polmacoxib and 325 mg of Paracetamol, a combination solution was created. After being weighed, the amounts were put into a 10 mL volumetric flask and sonicated for five minutes. The mixed standard solution was then created by adding methanol to the volume. The volume was then adjusted with methanol to establish standard stock solutions with concentrations of 200 µg/mL for POL and 32500 µg/mL for PCM.

2.4 Preparation of sample solution

10 tablets of Polmakem-P containing Polmacoxib (2mg) and Paracetamol (325mg) were accurately weighed and crushed into a fine powder using a mortar and pestle. The average tablet weight was determined and quantity of tablet powder equal to the average tablet weight was accurately weighed and transferred to a 10 ml volumetric flask. The volume was made up with methanol to prepare the sample solution.

2.5 Analytical method validation

To guarantee the method's dependability for analysis, it was validated in accordance with ICH requirements. The following parameters were assessed: specificity, linearity, accuracy, precision, LOD, LOQ, and robustness [7].

2.5.1 Specificity

The ability of an analytical technique to precisely measure the analyte in the presence of additional elements without interference, such as contaminants, degradation products, excipients, or matrix components, is known as specificity [9].

2.5.2 Linearity

Linearity was evaluated by analyzing five concentrations in the ranges (80-240 ng/band for POL and 13000-39000 ng/band for PCM) and the corresponding peak responses were recorded.

2.5.3 Accuracy

2.5.3.1 Assay

The developed HPTLC method was used to assay the commercial tablet formulation containing polmacoxib and paracetamol. The peak regions of the sample and the associated standard solutions were compared in order to perform the analysis. The devised method's suitability for routine quantitative examination of the pharmaceutical formulation was confirmed by the assay results, which showed that the content of both medicines in the tablet was within acceptable limits [12].

2.5.3.2 Percent Recovery

The standard addition method was used to evaluate the accuracy of the novel approach by adding known amounts of POL and PCM to the pre-analyzed tablet sample. The study used three nominal concentration levels: 80%, 100%, and 120%. Each level was tested three times. To perform recovery tests using the over-spotting technique, predetermined doses of popular medications were added to the sample solution.

2.5.4 Precision

An evaluation of precision, encompassing both intraday and interday fluctuations, is part of the validation of an assay and quantitative determination test. The intraday precision was evaluated by measuring three replicates of 0.8 µL concentrations at three different times of the same day: morning, afternoon and evening. To evaluate interday, the

three concentrations were made and examined over the period of three days. % RSD was used to express the precision results.

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

According to ICH Q2(R2) guidelines, the standard deviation of the response and slope technique was used to calculate the limit of detection (LOD) and limit of quantification (LOQ) for POL and PCM. LOD and LOQ were calculated from the calibration curve data using the slope (S) and residual standard deviation (σ) of the regression line. The residual standard deviation was obtained from the linear regression analysis of the calibration data. LOD and LOQ were calculated using $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$, where σ is the response's residual standard deviation and S is the slope of the calibration curve [8].

2.5.6 Robustness

The robustness of the established method was confirmed by the fact that small, intentional changes in the mobile phase composition and saturation duration did not significantly affect the analytical results. This demonstrates that the strategy is stable and dependable under a range of circumstances [9].

2.6 Forced degradation

The stability-indicating character of the developed HPTLC approach for simultaneous measurement of Polmacoxib and Paracetamol was established through forced degradation tests. In accordance with ICH recommendations, specifically International Council for Harmonization Q1(R2), Q1B, and Q2(R2), a range of stress conditions, including acidic, alkaline, oxidative, neutral, thermal, and photolytic settings, were used in the degradation investigations. To assess the degradation behaviour and separation capabilities of the devised approach, the drug mixture solution including Polmacoxib and Paracetamol was exposed to various stress conditions [10].

2.6.1 Acid degradation

The drug solution was treated with 0.1 N hydrochloric acid (HCl) for acid degradation and allowed to sit at room temperature for 30 minutes. The developed HPTLC method was used to analyze the solution after the allotted time. Degradation under acidic conditions was indicated by the densitogram, which displayed degradation products with extra peaks.

2.6.2 Base degradation

The drug solution was subjected to 0.1 N sodium hydroxide (NaOH) at room temperature for 30 minutes in order to cause alkaline degradation. The optimized HPTLC method was then used to evaluate the stressed sample in order to observe degradation behavior and the generation of degradation products.

2.6.3 Oxidative degradation

A 3% hydrogen peroxide (H_2O_2) solution was used to decompose oxidative stress. After 30 minutes at room temperature, the drug mixture was examined to evaluate oxidative breakdown and degradant production.

2.6.4 Neutral degradation

The drug solution was subjected to neutral conditions at room temperature for 30 minutes in order to achieve neutral degradation. After that, the sample was examined to look for any hydrolytic stress-related deterioration.

2.6.5 Thermal degradation

The medication sample was subjected to a temperature of 40°C for 15 minutes in a hot air oven in order to perform thermal degradation investigations. The sample was examined to assess the medication's thermal stability following thermal exposure.

2.6.6 Photolytic degradation

The medication sample was exposed to UV light at 254 nm for 24 hours to conduct photolytic degradation tests. The stressed sample was then examined to determine the photostability of polmacoxib and paracetamol.

All degraded samples were applied onto silica gel 60 F₂₅₄ HPTLC plates after stress treatment, and chromatographic separation was performed using the optimized mobile phase, which included toluene: ethyl acetate: formic acid (5:4:1 v/v/v). At 283 nm, densitometric scanning was carried out. The stability-indicating nature of the devised approach was confirmed when the drug peaks were successfully separated from their degradation products.

3. Results and Discussion

3.1 Method optimization

Different mobile phases with different polarity solvents were investigated at varied ratios in order to achieve the best chromatographic separation of POL and PCM. The mobile phase that yielded the most prominent and distinct peaks was toluene:ethyl acetate:formic acid (5:4:1v/v/v). The best results were obtained by detection at 283 nm, with retention factor (R_f) values of 0.66 and 0.50 for POL and PCM, respectively. Figure 3 displays the densitograms of the sample and standard solutions.

3.2 Method validation

3.2.1 Specificity

The specificity was established by comparing the overlay spectra, peak characteristics and R_f values of the standard and sample solutions. The same overlay spectra demonstrated peak purity and absence of excipient interference [11]. Fig. 4 displays the corresponding densitograms.

3.2.2 Linearity

Linearity was performed in the ranges of 80-240 ng/band for POL and 13000-39000 ng/band for PCM. The regression equations for POL and PCM were $y = 9.1998x + 75.726$ and $y = 0.4709x + 7870.2$, respectively. Both compounds showed linearity, with correlation coefficients (r^2) of 0.9993 and 0.9997, respectively. The obtained results confirmed the linearity of the method within the selected concentration range.. Specific data are listed in Table 1, and the corresponding calibration curves are displayed in Fig. 5 (a, b).

3.2.3 Accuracy

3.2.3.1 Assay

POL and PCM had mean percent label claims of 100% and 102%, respectively, in the tablet. Table 2 shows the accuracy findings.

3.2.3.2 Percent Recovery

The accuracy of the recovery research was satisfactory; the mean recovery values for POL and PCM were 99-99.49 and 98-100.99, respectively. These findings confirm that the accuracy requirements are within reasonable bounds. All of the accuracy data is shown in Table 3.

3.2.4 Precision

The accuracy of the proposed method was confirmed with %RSD values for POL and PCM below 2%, and repeatability was evaluated with six replicates. To assess intermediate precision, six more samples were analyzed on different days and Table 4 also displays the consistent outcomes.

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

The LOD and LOQ was performed to see how sensitive the new method is. This was done by checking the deviation of the response and the slope of the calibration curve. The LOD and LOQ for POL and PCM were found to be 0.658 and 1.996 µg/ml and 68.65 and 208 µg/ml. The LOD and LOQ values for POL and PCM are pretty low which means the method is good enough, for analyzing things. The LOD and LOQ results show that the method is sensitive (Table 1).

3.2.6 Robustness

The robustness of the established method was confirmed by the fact that small, intentional changes to the mobile phase composition and saturation duration did not significantly affect the analytical results. This demonstrates that the strategy is stable and trustworthy under a range of circumstances. The results supporting this hypothesis are shown in Tables 5a and 5b.

3.3 Forced degradation

Forced degradation studies were carried out in accordance with ICH Q1A (R2) guidelines under acidic, alkaline, oxidative, thermal, and photolytic settings to evaluate whether the suggested HPTLC method is stability-indicating. Given that no additional peaks were seen in any stressful situations, the medications were stable and the method is unique for the determination of POL and PCM [10].

3.3.1 Acid degradation

The samples were allowed to degrade in 0.1N HCl acid for half an hour at room temperature. 1.80% of the degradation was discovered. Figure 6 (a) and table 6 displays the results and the corresponding degradation data are summarized for investigations on acidic degradation.

3.3.2 Base degradation

Using 0.1N NaOH, the samples were subjected to basic degradation for half an hour at room temperature. There was 4.66% degradation. Figure 6 (b) displays the results for investigations on acidic degradation and in table 6 displays the corresponding degradation data are summarized.

3.3.3 Oxidative degradation

For half an hour at room temperature, the samples were subjected to oxidative degradation using 3% H₂O₂. 10.44% of the deterioration was observed. The results for studies on oxidative degradation are shown in Figure 6 (c) and table 6 displays the corresponding degradation data are summarized.

3.3.4 Neutral degradation

Distilled water was used to carry out neutral degradation while the components were subjected to a temperature for thirty minutes. The percentage of deterioration observed was 4.38%. The results for studies on neutral degradation are shown in Figure 6 (d) and table 6 displays the corresponding degradation data are summarized.

3.3.5 Thermal degradation

Pure POL and PCM samples were heated to 40°C for an hour in a hot air oven as part of the thermal degradation investigation. 7.89% of the deterioration was observed. Figure 6 (e) displays the results for research on thermal degradation and table 6 displays the corresponding degradation data are summarized .

3.3.6 Photolytic degradation

In order to investigate photolytic deterioration, pure POL and PCM samples were exposed to UV radiation for a whole day. The percentage of deterioration found was 6.42%. Figure 6 (f) and table 6 displays the results and corresponding degradation data for research on photolytic degradation.

4. Conclusion

For the simultaneous measurement of Polmacoxib and Paracetamol in bulk and tablet dosage forms, a stability-indicating HPTLC method was effectively designed and validated. In compliance with ICH criteria, the method showed good specificity, linearity, accuracy, precision, robustness, and sensitivity. The technique may successfully separate the pharmaceuticals from their breakdown products under a variety of stress situations, according to forced degradation experiments. As a result, the developed method was found to be reliable, economical, and appropriate for stability studies and regular quality monitoring of pharmaceutical formulations comprising polmacoxib and paracetamol.

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Conflict of Interest

The writers affirm that they have no conflicts of interest.

Authors Contribution

The experiment was carried out by Rucha Takalkar. Jayashree Kokane and Pranav Deore assisted in the study. The research was supervised and guided by Arun Kashid. The final text was reviewed and approved by all authors.

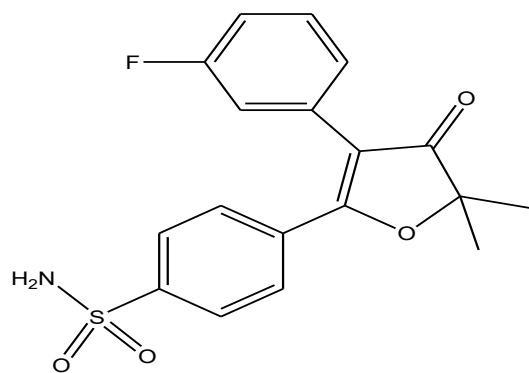
Ethical Approval

As no animal study is involved, ethical approval is not required

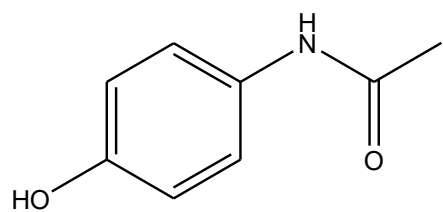
5. Key References

1. Lee MC, Song YW, Seo YI, Kim HA, Lee CK, Yoo MC. (2017) A randomized, multicenter, phase III trial to evaluate the efficacy and safety of polmacoxib compared with celecoxib and placebo for patients with osteoarthritis. *Clin Orthop Surg* 9:439–457. <https://doi.org/10.4055/cios.2017.9.4.439>
2. Ghosh P, Ghosh A, Das S (2024) Polmacoxib: A review of the newer non-steroidal anti-inflammatory drug in osteoarthritis. *Cureus* 16:e58614. <https://doi.org/10.7759/cureus.58614>
3. Graham GG, Scott KF (2005) Mechanism of action of paracetamol. *Am J Ther* 12:46–55. <https://doi.org/10.1097/00045391-200501000-00008>

4. Graham GG, Davies MJ, Day RO, Mohamudally A, Scott KF (2013) The modern pharmacology of paracetamol: Therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. *Inflammopharmacology* 21:201–232. <https://doi.org/10.1007/s10787-013-0172-x>
5. Mallet C, Desmeules J, Pegahi R, Eschalier A (2023) An updated review on the metabolite (AM404)-mediated central mechanism of action of paracetamol. *J Pain Res* 16:1027–1045. <https://doi.org/10.2147/JPR.S393809>
6. Renger B, Végh Z, Ferenczi-Fodor K (2011) Validation of thin-layer and high-performance thin-layer chromatographic methods. *J Chromatogr A* 1218:2712–2721. <https://doi.org/10.1016/j.chroma.2011.01.059>
7. Shabir GA (2003) Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *J Chromatogr A* 987:57–66. [https://doi.org/10.1016/S0021-9673\(02\)01536-4](https://doi.org/10.1016/S0021-9673(02)01536-4)
8. Shrivastava A, Gupta VB (2011) Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chron Young Sci* 2:21–25. <https://doi.org/10.4103/2229-5186.79345>
9. Ferenczi-Fodor K, Végh Z, Nagy-Turák A, Renger B, Zeller M (2001) Validation and quality assurance of planar chromatographic procedures in pharmaceutical analysis. *J AOAC Int* 84:1265–1276. <https://doi.org/10.1093/jaoac/84.4.1265>
10. Blessy M, Patel RD, Prajapati PN, Agrawal YK (2014) Development of forced degradation and stability indicating studies of drugs—A review. *J Pharm Anal* 4:159–165. <https://doi.org/10.1016/j.jpha.2013.09.003>
11. Ferenczi-Fodor K, Renger B, Végh Z (2010) The frustrated reviewer—Recurrent failures in manuscripts describing validation of quantitative TLC/HPTLC procedures for analysis of pharmaceuticals. *J Planar Chromatogr Mod TLC* 23:173–179. <https://doi.org/10.1556/JPC.23.2010.3.1>
12. Kashid AM, Shinde AM, Pere VB, Tathe SV (2024) Stability-indicating high-performance thin-layer chromatography method development and validation for dapagliflozin propanediol monohydrate, vildagliptin, and metformin hydrochloride in active pharmaceutical ingredient and its tablet dosage form. *J Planar Chromatogr Mod TLC* 37:387–398. <https://doi.org/10.1007/s00764-024-00311-1>

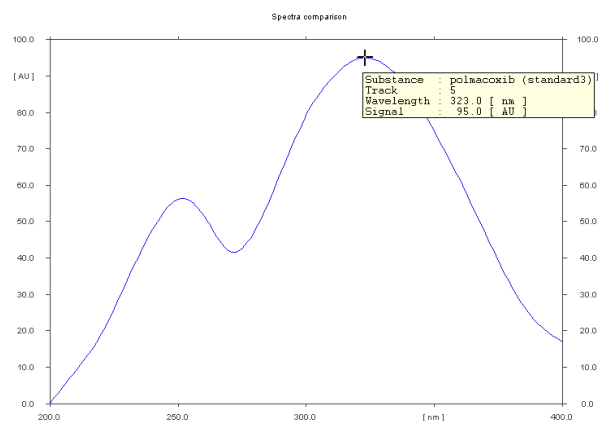


(a)

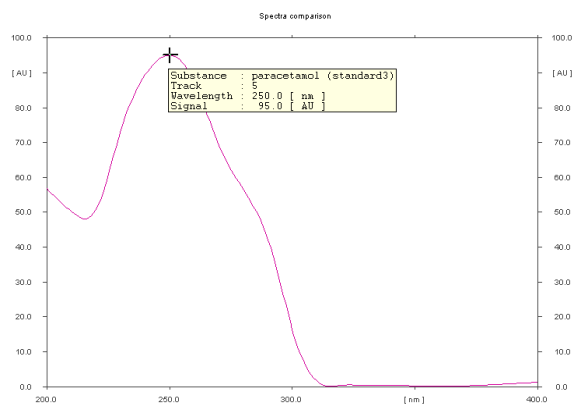


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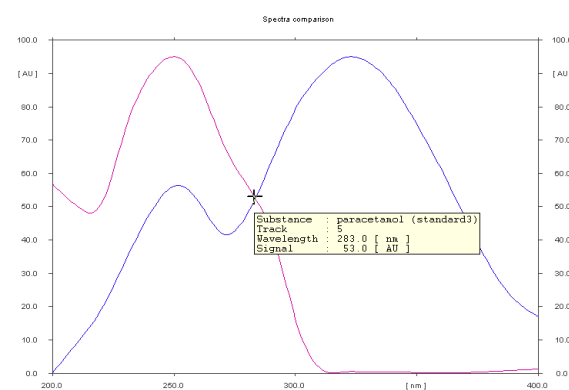
Fig.1 Structures of **(a)** Polmacoxib **(b)** Paracetamol



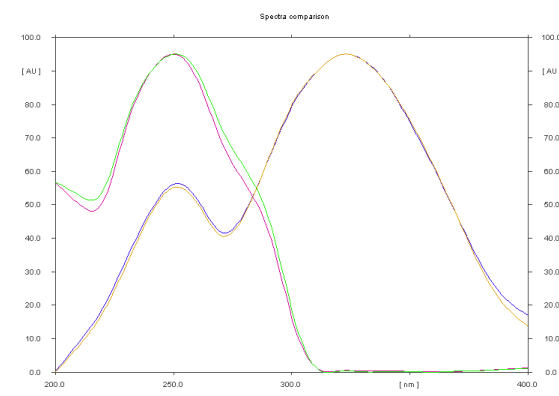
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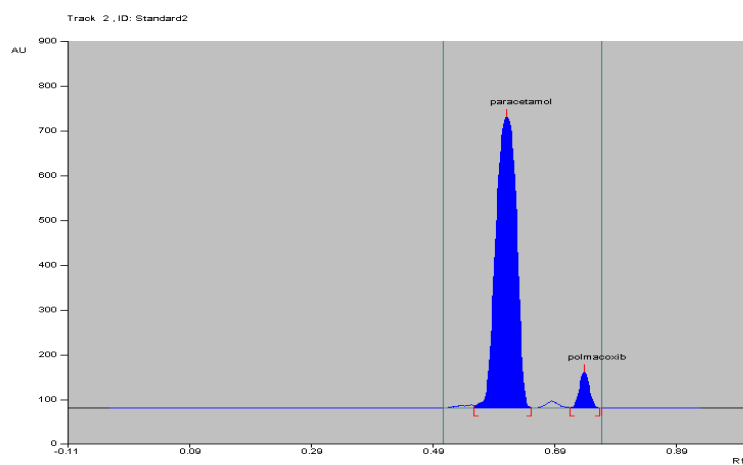


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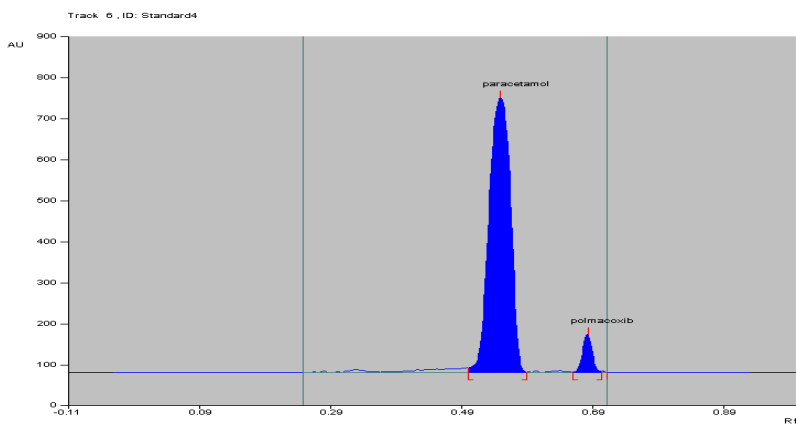


(d)

Fig. 2 (a) UV spectra of Polmacoxib, (b) UV spectra of Paracetamol, (c) Overlay of UV spectra of API of Polmacoxib and Paracetamol, (d) Overlay UV spectra of API and Tablet of Polmacoxib and Paracetamol.



(a)



(b)

Fig. 3 (a) Standard mixture of Paracetamol and Polmacoxib **(b)** Sample mixture of Paracetamol and Polmacoxib containing optimized mobile phase Toluene: Ethyl acetate: Formic acid (5:4:1 v/v/v)

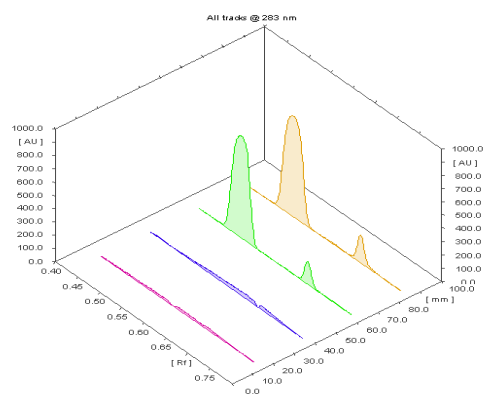
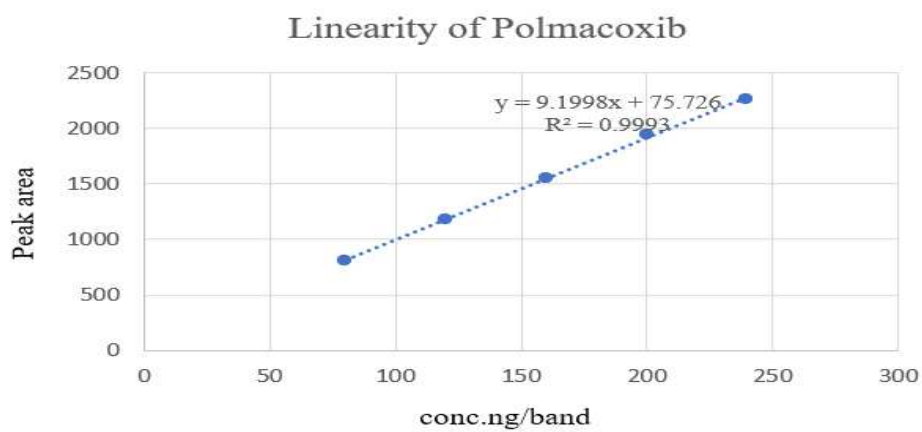
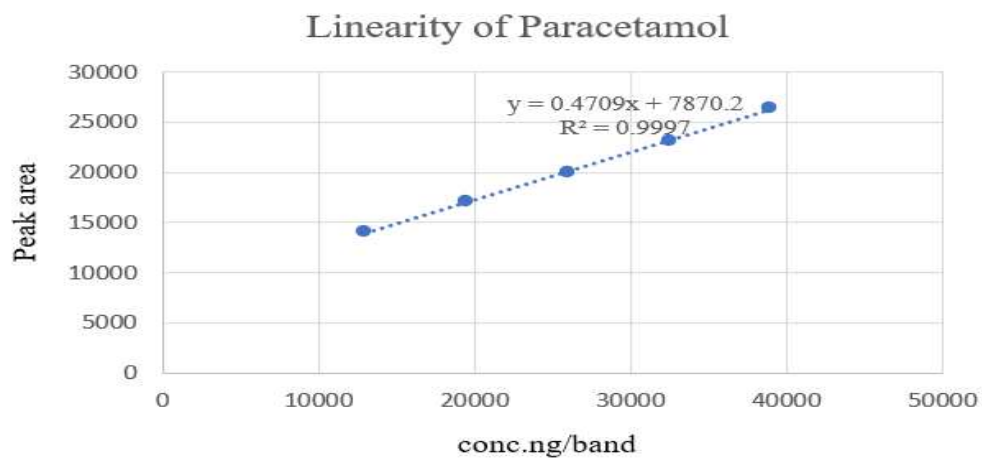


Fig. 4 3D densitogram for specificity

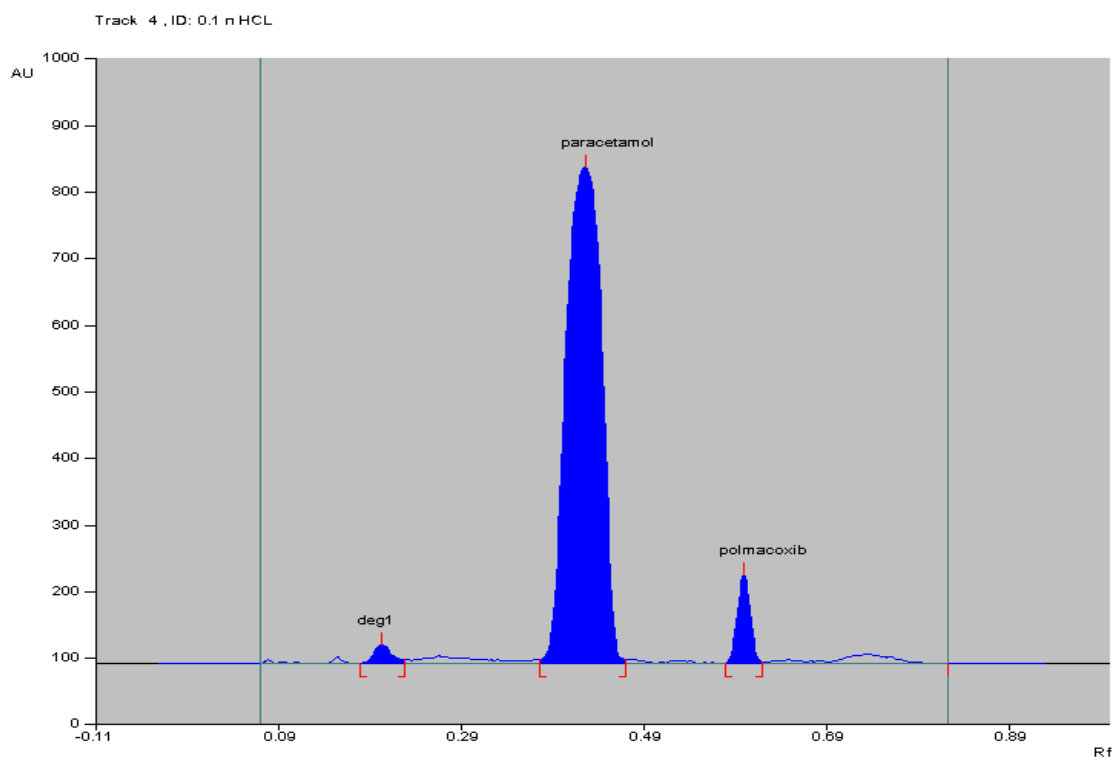


(a)

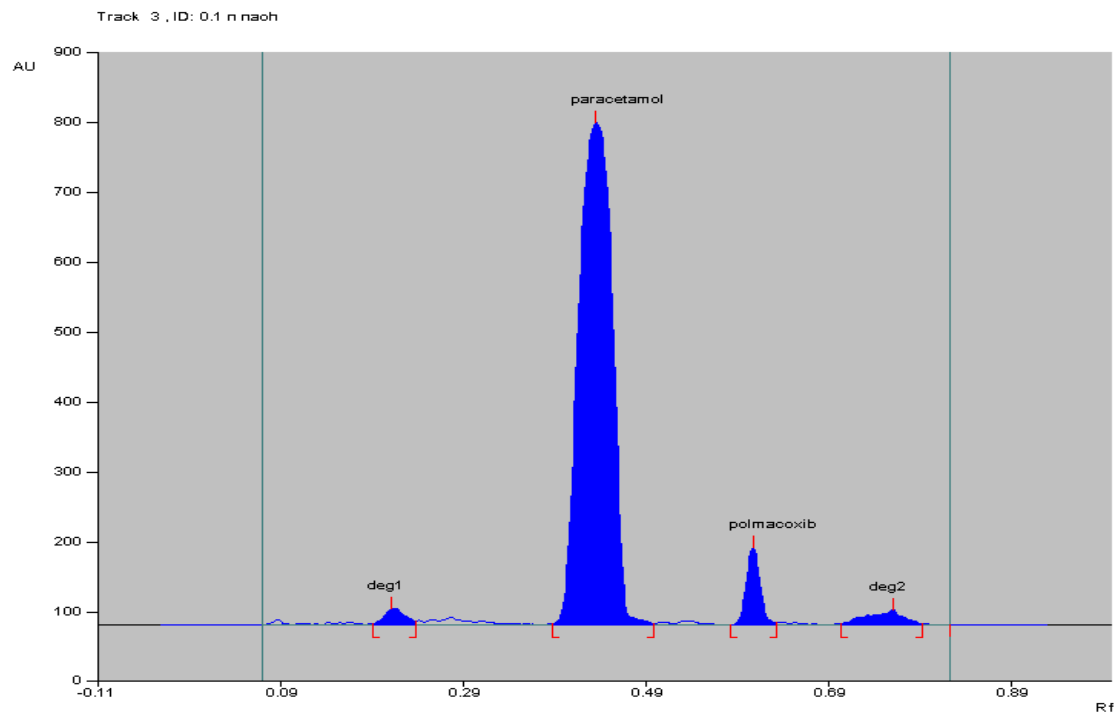


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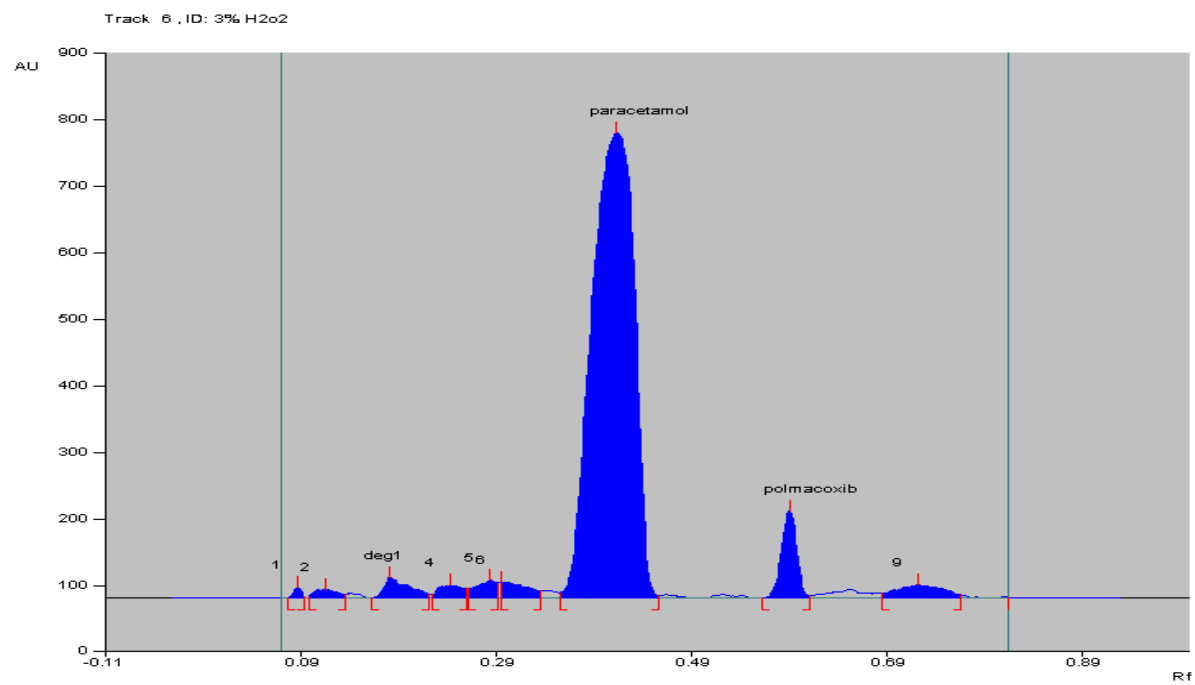
Fig. 5 (a) Calibration curve of Polmacoxib (b) Calibration curve of Paracetamol



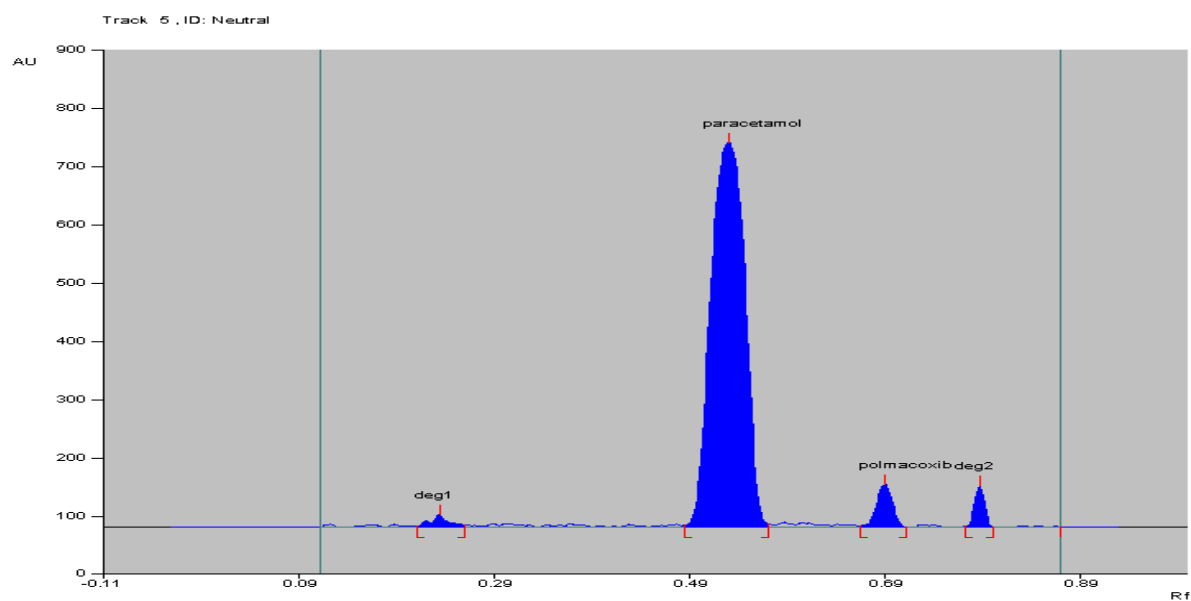
(a)



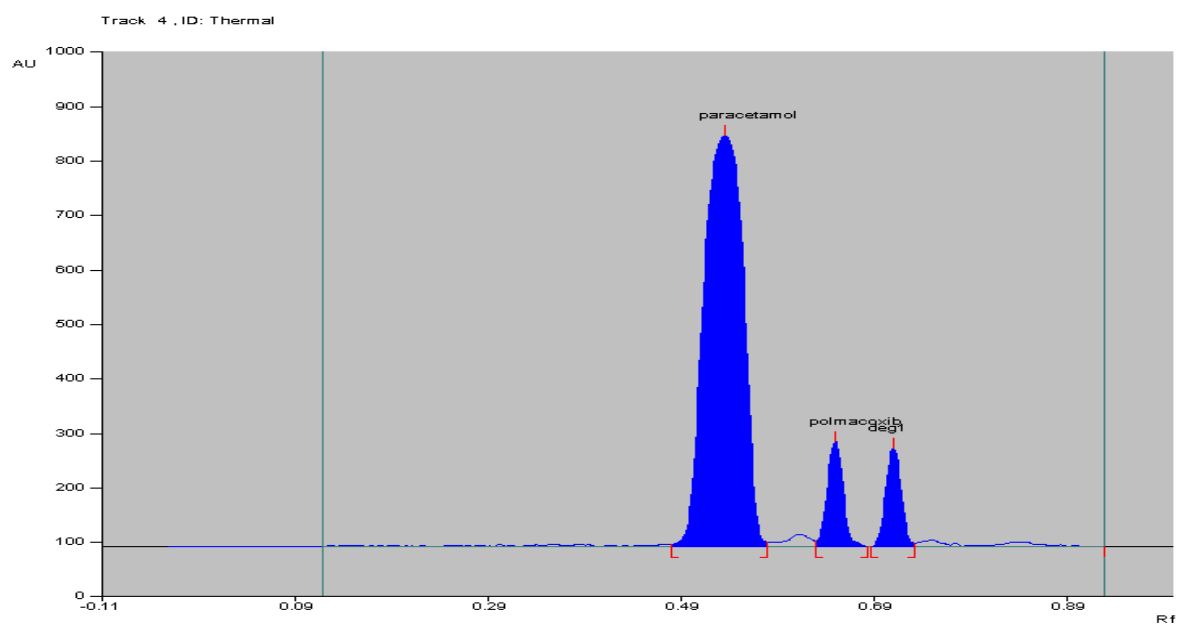
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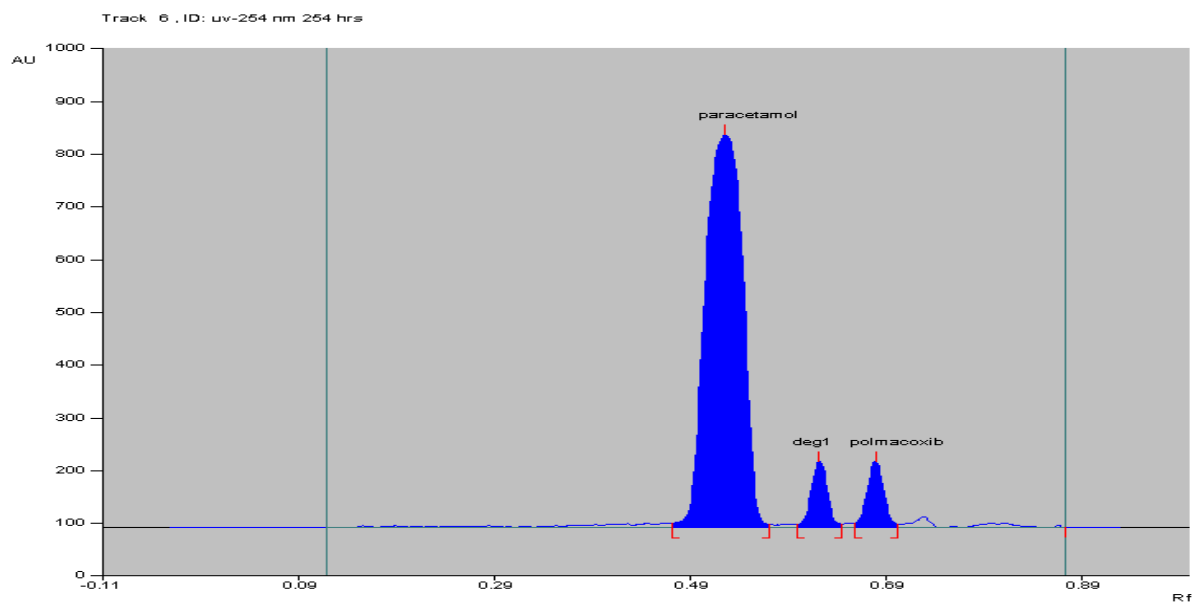
(c)



(d)



(e)



(f)

Fig. 6 Densitogram of (a) acidic degradation (0.1 N HCl), **(b)**basic degradation (0.1 N NaOH), **(c)** oxidative degradation(3% H₂O₂), **(d)** neutral degradation, **(e)** thermal degradation, **(f)** photolytic degradation

Table 1. Results of Linearity, LOD and LOQ of the developed method

Concentration (ng/band)		Peak area	
POL	PCM	POL	PCM
80	13000	808.39	14039.25
120	19500	1174.86	17073.86
160	26000	1548.94	20029.32
200	32500	1940.94	23082.68
240	39000	2265.3	26337.86
Calibration range		80-240	13000-39000
r^2		0.9993	0.9997
LOD/LOQ ($\mu\text{g/ml}$)		0.658 /68.65	1.996/208

Table 2. Assay of the tablet results of the developed method

Drug	Label claim (mg)	Content of drug in sample (mg)	% label claim	Mean % label claim
POL	2	2	100	100%
		2	100	
		2	100	
PCM	325	333.8	102.7	102 %
		333.3	102.5	
		331.3	101,9	

Table 3. Results of Percent recovery of the developed method

Drug	% Composition	Amount added (mg)	Peak area	% Recovery	% RSD
POL	80%	1.6	2713.0	99	0.01
			2713.5		
			2714		
	100%	2	3008.13	99.23	0.009
			3008.43		
			3008.73		
	120%	2.4	3304.92	99.49	0.009
			3305.22		
			3305.52		
PCM	80%	260	30005.10	100.99	0.003
			30006.26		
			30007.42		
	100%	325	32100	98	0.03
			32112		
			32124		
	120%	390	34908	100.69	0.001
			34908.5		
			34909		

Table 4. Results of intraday and interday precision of the developed method

Precision		Peak Area	Mean	SD	% RSD
Intraday precision of PCM	Morning	21398.2	21363.17	39.09	0.1
		21370.3			
		21321			
	Afternoon	19964.2	19886.8	87.47	0.4
		19904.3			
		19791.9			
	Evening	18951.8	19030.27	68.65	0.3
		19079.3			
		19059.7			
Intraday precision of POL	Morning	1459.1	1434.467	21.38	1.4
		1423.6			
		1420.7			
	Afternoon	1025.5	1018.8	7.89	0.7
		1020.8			
		1010.1			
	Evening	972.6	978.5	5.16	0.5
		982.2			
		980.7			
Interday Precision of PCM	Day 1	18566.8	18544.13	20.11	0.1
		18528.4			
		18537.2			
	Day 2	18300.7	18224.9	115.30	0.6
		18092.2			
		18281.8			
	Day 3	18233.8	18282.53	42.21	0.2
		18307.6			
		18306.2			
Interday precision of POL	Day 1	1206.3	1198	8.39	0.7
		1186.5			
		1201.2			
	Day 2	721.7	720.0333	8.03	1.1
		711.3			
		727.1			
	Day 3	622.6	631.2	7.59	1.2
		637			
		634			

Table 5. a Robustness study of Polmacoxib

Conditions		Polmacoxib		
		Mean (n=3)	SD	%RSD
Mobile Phase Composition (± 1 ml)	Toluene: EA: FA (5:3:2 v/v/v)	2159	15.395	0.7 %
	Toluene: EA: FA (4:4:2 v/v/v)	2076.9	21.114	1 %
Saturation Time (± 5 min)	15 min	1366.9	21.002	1.5 %
	5 min	2190.4	17.065	0.7%

Table 5. b Robustness study of Paracetamol

Conditions		Paracetamol		
		Mean (n=3)	SD	%RSD
Mobile Phase Composition (± 1 ml)	Toluene: EA: FA (5:3:2 v/v/v)	26455	65.729	0.2 %
	Toluene: EA: FA (4:4:2 v/v/v)	27556	34.858	0.1 %
Saturation Time (± 5 min)	15 min	19069	84.483	0.4 %
	5 min	25543	281.3	1.1 %

Table 6. Forced degradation results of Polmacoxib and paracetamol

Stress condition	Temperature, time	% Degradation
Acidic (0.1N HCl)	at room temp. for 30 min	1.80 %
Basic (0.1N NaOH)		4.66 %
Oxidative (3%H ₂ O ₂)		10.44 %
Neutral		4.38 %
Thermal	40 °C for 15min	7.89 %
Photolytic (UV Chamber)	254 nm for 24 h	6.42 %