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Simultaneous Estimation of Paracetamol, Tramadol and Dicyclomine in Bulk and Pharmaceutical Formulations by RP-HPLC Method

G. Sailaja[1], B. Haribabu[*2],CH.Vijaya Saradhi[2]

1. *Department of Chemistry, Singareni Collieries Women's Degree College, Kothagudem, Khammam, Andhra Pradesh, India.*
2. *Department of Chemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.*

ABSTRACT

A novel, simple, precise, accurate and reproducible RP-HPLC method was developed and validated for simultaneous estimation of paracetamol, tramadol and dicyclomine in bulk and pharmaceutical formulations. Separation of paracetamol, tramadol and dicyclomine was successfully achieved on a Inertsil ODS C18 (250mm x 4.6mm x 5 µm). The mobile consisted of ammonium acetate buffer (pH 4.5): methanol (80:20 *v/v*) at a flow rate of 1.0 mL/min. The detection was performed at 271 nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision and robustness. The response was found to be linear in the concentration range of 500 µg/mL to1500 µg/mL for paracetamol; 50µg/mL to150 µg/mL for tramadol; 10 µg/mL to 30 µg/mL for dicyclomine. The LOD and LOQ for paracetamol were found to be 2.712 µg/mL and 9.042 µg/mL, respectively. The LOD and LOQ for tramadol was 0.9009 µg/mL and 3.003 µg/mL, respectively and for dicyclomine it was 0.380 µg/mL and 1.265 µg/mL, respectively. The percentage recovery for paracetamol, tramadol, and dicyclomine were found to be 99.00%, 100.00% and 99.00%, respectively. The excellent percentage recovery values indicate the high accuracy of the proposed method. The method specifically determines the analytes in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, Specificity and Robustness.

Keywords: Paracetamol, Tramadol, Dicyclomine, analysis, HPLC.

*Corresponding Author Email: dr.b.haribabu@gmail.com
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INTRODUCTION

Paracetamol[1] is also known as acetaminophen (Figure 1) it is commonly used for its analgesic and antipyretic effects. Its therapeutic effects are similar to salicylates, but it lacks anti-inflammatory, antiplatelet, and gastric ulcerative effects. Several analytical methods have been reported for the determination of paracetamol including: high-performance liquid chromatography-diode array (HPLC-DAD) with online post-column photochemical derivatization[2], non-suppressed ion chromatography[3], UV-Visible spectrophotometry[4], HPLC[5,6], gas chromatograph-mass spectrometry (GC-MS)[7] as single component or in combinations with other drugs.

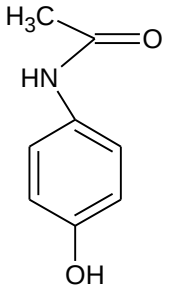

Figure 1: Chemical structure of paracetamol

Tramadol (Figure 2), is a narcotic analgesic proposed for moderate to severe pain[8,9]. It may be habituating. It is also prepared as a variable release capsules, marketed under the brand name Conzip. Tramadol was found to be an effective and well tolerated analgesic agent for the prevention and treatment of moderate to severe pain of various origins. In the literature, for tramadol RP-HPLC[10,11,12], plasma RP-HPLC[13], RP-HPLC method for determination in human plasma, urine and saliva[14], LC-MS-MS[15] and thin layer chromatography[16,17] methods have been reported.

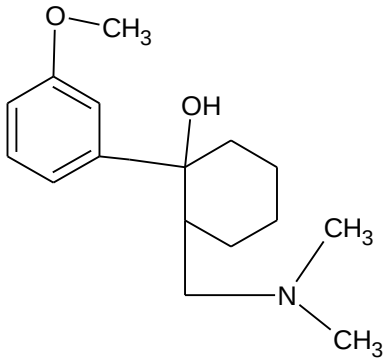

Figure 2: Chemical structure of tramadol

Dicyclomine (Figure 3) is a muscarinic antagonist used as an antispasmodic and in urinary incontinence[18, 19]. It has little effect on glandular secretion or the cardiovascular system. It does have some local anesthetic properties and is used in gastrointestinal, biliary, and urinary tract

spasms. Literature review reveals that few methods are reported for the quantification of dicyclomine either alone or in combination with other drugs. They include gas chromatography[20,21], HPLC[22] and HPTLC[23].

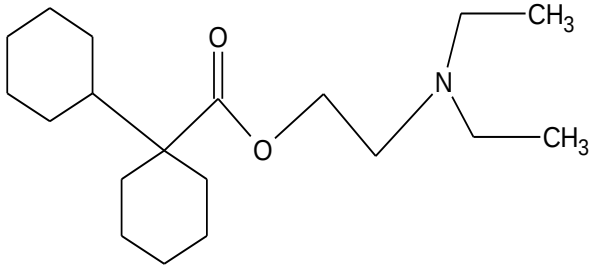

Figure 3: Chemical structure of dicyclomine

From the above literature survey it is very clear that no method has been reported for simultaneous determination of paracetamol, tramadol and dicyclomine by HPLC. So, the present study is designed for the development and validation of simple, precise and accurate HPLC method for the simultaneous determination of paracetamol, tramadol and dicyclomine in pharmaceutical formulations. The proposed method is validated as per ICH guidelines.

MATERIALS AND METHOD

Chemicals and Reagents

Paracetamol, tramadol and dicyclomine were obtained as a gift sample from Lara drugs pvt Ltd., Hyderabad. Ammonium acetate and methanol of HPLC grade was purchased from Merck (India) Ltd., Mumbai. Ortho phosphoric acid of analytical reagent grade was obtained from Sd Fine Chemicals Ltd., Mumbai. Mille Q water was used through out the process.

Chromatographic apparatus and conditions

The development and validation of the assay was performed on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software. The analytical column used to achieve chromatographic separation was Inertsil ODS C18, (250 mm × 4.6; 5µm) column. The mobile phase consisting of ammonium acetate buffer (pH 4.5) and methanol was degassed and pumped from the solvent reservoir in the ratio of 80:20 *v/v*. The flow rate was 1.0 mL/min. The column temperature was maintained at 30°C. The detection was performed at 271 nm and the run time was 14 min. Injection was carried out using a 10 µL loop. Prior to injection of the drug solution the column was equilibrated for at least 15 minutes with the mobile phase.

Standard Solution

500 mg of paracetamol, 50 mg of tramadol and 10 mg of dicyclomine was accurately weighed,

dissolved in mobile phase and diluted to volume in a 100 mL volumetric flask. Pipette out 5.0 mL of the above standard stock standard stock into 25 mL volumetric flask and dilute to volume with mobile phase.

Sample solution

Accurately weigh 1122.20 mg of sample. Transfer the sample powder into 100 mL volumetric flask. Add 10 mL mobile phase and sonicate for 20 minutes. The resulting solution was made up to the volume with mobile phase. Filter through the 0.45 µm filter paper. Transfer 5 mL of the above solution into a 25 mL volumetric flask and made up to the volume with mobile phase.

METHOD VALIDATION

System Suitability

System suitability tests are an integral part of liquid chromatographic method. System suitability was checked on each day of validation to evaluate the analytical system in order to show that the performance of the system meet the standards required by the method. System suitability parameters established are number of theoretical plates, resolution and tailing factor.

Linearity

The linearity of the proposed method was constructed for paracetamol, tramadol and dicyclomine standard solutions by plotting the concentrations of the compound versus peak area response. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Accuracy and Precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried on the selected drugs at three different concentration levels (50%, 100% and 150%). The percentage recovery and standard deviation of the percentage recovery were calculated. The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage relative standard deviation were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage relative standard deviation were calculated.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as composition of mobile phase ratio and temperature of the column, and studying its effects on the performance of the method.

LOD and LOQ

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of paracetamol, tramadol and dicyclomine. The LOQ and LOD values were calculated by using the following formula

(a) LOQ = 10 σ / S

(b) LOD = 3.3 σ / S

Where σ = residual standard deviation of response; S = slope of the calibration curve.

RESULTS AND DISCUSSION

System Suitability Studies

The column efficiency, resolution and tailing factor were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of the selected drug combinations. System suitability parameters may fall within ±2 % Relative standard deviation range during routine performance of the method.

Table 1: System suitability

Parameter	Paracetamol	Tramadol	Dicyclomine
Retention time	2.966	5.547	9.218
Theoretical plates	6580	4178	7534
Tailing factor	1.25	1.07	1.08
% RSD	1.2	1.5	1.0

Linearity and range

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. Paracetamol, tramadol and dicyclomine exhibited linearity n the concentration range of 500-1500 µg/mL, 50-150 µg/mL and 10-30 µg/mL (Figures 4, 5 and 6). The regression equations for the selected drugs are:

Paracetamol: $y = 3857.6x - 6353.8$ ($R^2 = 0.999$)

Tramadol: $y = 27433x + 37474$ ($R^2 = 0.9998$)

Dicyclomine: $y = 80226x - 1265.2$ ($R^2 = 0.9999$)

Where y = peak area and x = concentration of the drug in µg/mL

The results show an excellent correlation exists between areas and concentration of drugs. The results for calibration data are shown in Table 2 and calibration curves are given in Figure 4, 5 & 6.

Table 2: Linearity data of paracetamol, tramadol and dicyclomine

Paracetamol		Tramadol		Dicyclomine	
Area	**Amount of drug (µg/mL)**	**Area**	**Amount of drug (µg/mL)**	**Area**	**Amount of drug (µg/mL)**
1920441	500	1399523	50	802884	10
2884314	750	2097220	75	1201968	15
3857215	1000	2787156	100	1601428	20
4819149	1250	3485410	125	2001024	25
5774994	1500	4134551	150	2409018	30

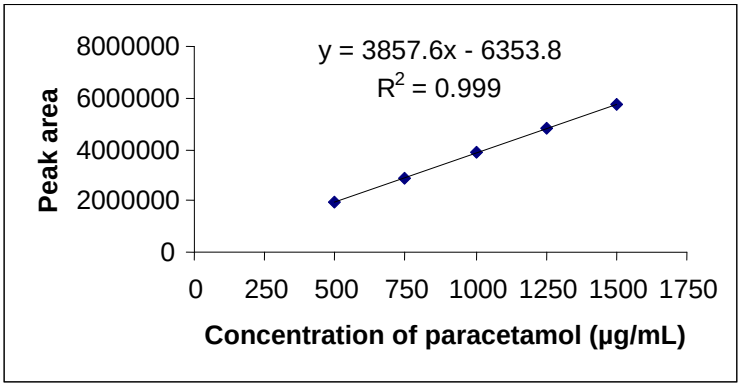

Figure 4: Linearity curve for paracetmol

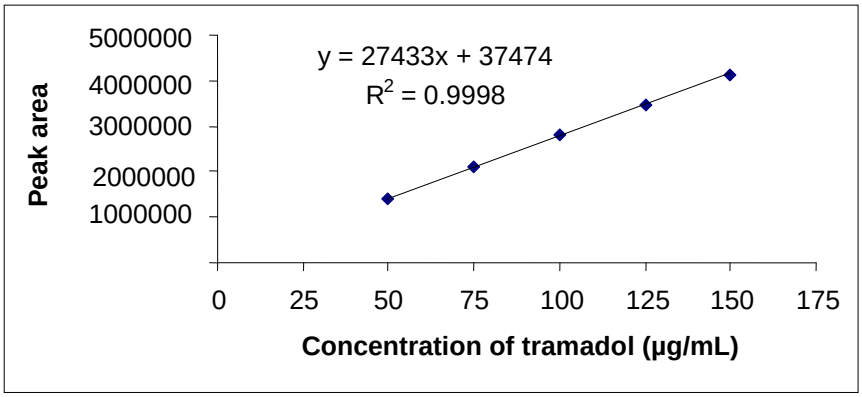

Figure 5: Linearity curve for tramadol

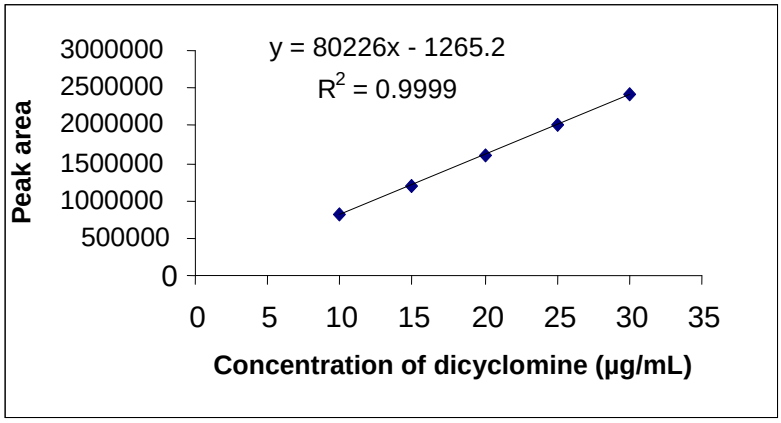

Figure 6: Linearity curve for dicyclomine

Accuracy and Precision

The results of accuracy of proposed methods at three different concentration levels are summarized in Tables 3, 4 and 5. The chromatograms at three different levels are shown in Figures 7, 8 & 9. From the results obtained, added recoveries of standard drugs were found to be accurate.

Table 3: Accuracy for paracetamol

Accuracy level	Sample weight	µg/mL added	µg/mL found	% Recovery	% Mean
50%	561.10	495.00	495.61	100	100
	561.10	495.00	494.02	100	
	561.10	495.00	494.83	100	
100%	1122.00	990.00	991.65	100	100
	1122.00	990.00	992.24	100	
	1122.00	990.00	990.47	100	
150%	1683.00	1485.00	1484.33	100	100
	1683.00	1485.00	1484.79	100	
	1683.00	1485.00	1485.39	100	

Table 4: Accuracy for tramadol

Accuracy level	Sample weight	µg/mL added	µg/mL found	% Recovery	% Mean
50%	561.10	50.00	49.78	100	100
	561.10	50.00	49.91	100	
	561.10	50.00	49.97	100	
100%	1122.20	100.00	99.55	100	100
	1122.20	100.00	99.41	99	
	1122.20	100.00	99.66	100	
150%	1683.30	150.00	149.73	100	100
	1683.30	150.00	149.72	100	
	1683.30	150.00	149.56	100	

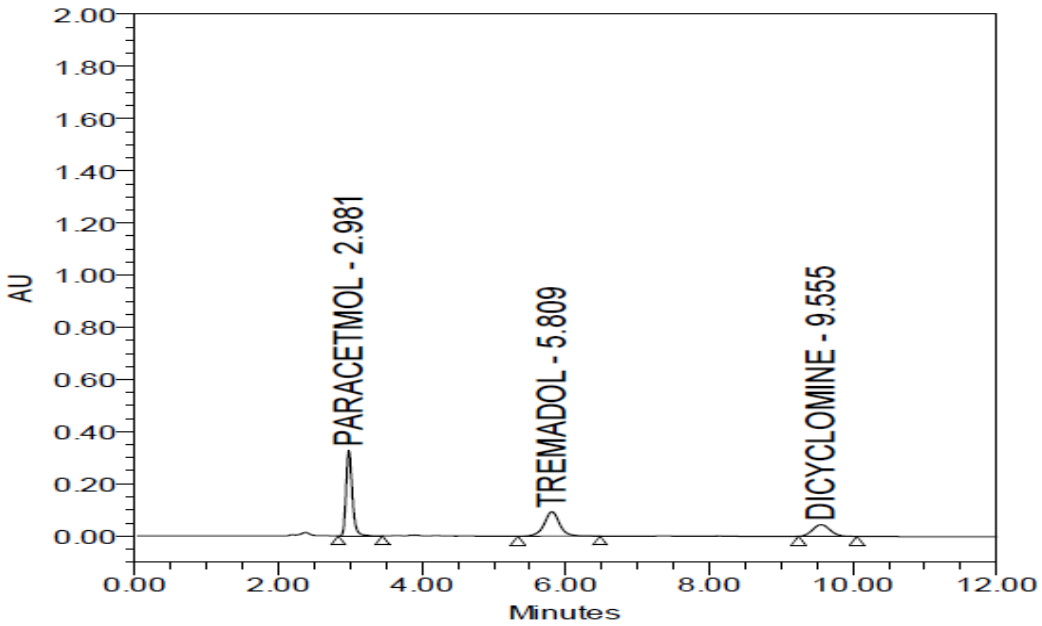

Figure 7: Chromatogram of Paracetamol, tramadol and dicyclomine at 50% level

Table 5: Accuracy for dicyclomine

Accuracy level	Sample weight	µg/mL added	µg/mL found	% Recovery	% Mean
50%	561.10	9.900	9.90	100	100
	561.10	9.900	9.90	100	
	561.10	9.900	9.91	100	
100%	1122.20	19.800	19.85	100	100
	1122.20	19.800	19.86	100	
	1122.20	19.800	19.82	100	
150%	1683.30.	29.700	29.700	100	100
	1683.30	29.700	29.700	100	
	1683.30	29.700	29.700	100	

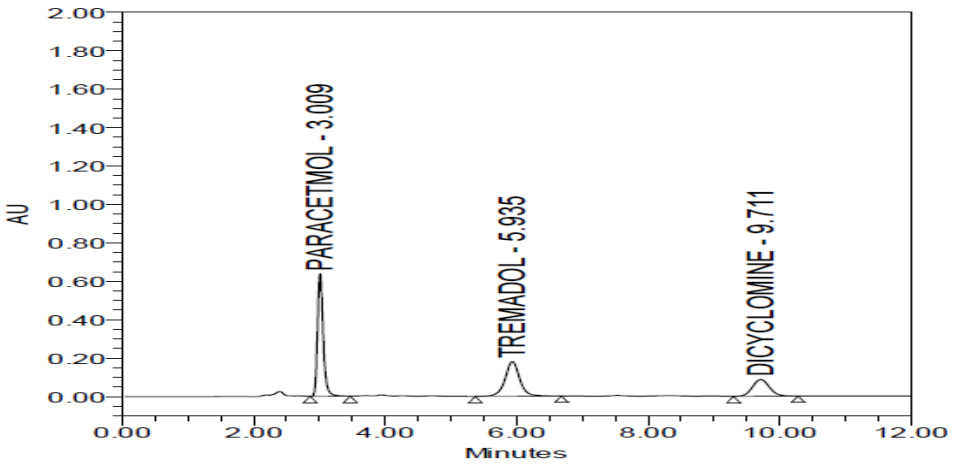

Figure 8: Chromatogram of Paracetamol, tramadol and dicyclomine at 100% level

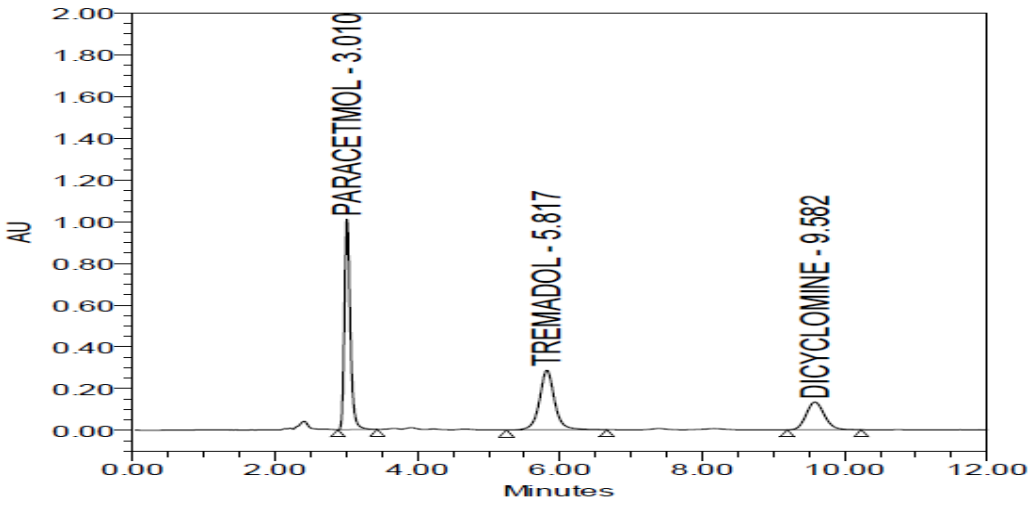

Figure 9: Chromatogram of Paracetamol, tramadol and dicyclomine at 150% level

The precision of the method was demonstrated by inter-day and intra-day variation studies. The results of the precision studies are tabulated in the Table 6. From the results obtained, the developed method was found to be precise for the simultaneous determination of paracetamol, tramadol and dicyclomine.

Table 6: Precision of the method

Sample Wt (mg)	Paracetamol		Tramadol		Dicyclomine	
	Area	%Assay	Area	%Assay	Area	%Assay
1122.20	3851426	99	2784791	100	1600051	99
1122.20	3850125	99	2784837	100	1600419	99
1122.20	3855730	99	2780005	99	1600562	99
1122.20	3859173	99	2786554	100	1600038	99
1122.20	3851961	99	2785015	100	1603002	99
1122.20	3855794	99	2789989	100	1605115	99

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions such as column temperature and mobile phase flow rate. It was observed that there were no marked changes in the analytical performance of the method. The results are shown in Table 7. The results demonstrated that the proposed method is robust.

Table 7: Robustness of the method

Sample no.	Sample Name	Retention time	Peak area	Theoretical plates	USP Tailing
Paracetamol					
1	Temp-1	3.277	3970125	6768	1.27
2	Temp-2	2.689	3895730	5968	1.27
3	Flow-1	3.212	3891961	6228	1.28
4	Flow-2	2.671	3961426	6554	1.27
Tramadol					
1	Temp-1	5.850	2864837	4018	1.06
2	Temp-2	5.221	2800005	3981	1.06
3	Flow-1	5.921	2835015	3990	1.05
4	Flow-2	5.261	2864791	3826	1.09
Dicyclomine					
1	Temp-1	9.957	1620419	6397	1.12
2	Temp-2	9.164	1560562	7373	1.07
3	Flow-1	9.904	1573002	6426	1.07
4	Flow-2	9.076	1640051	7155	1.12

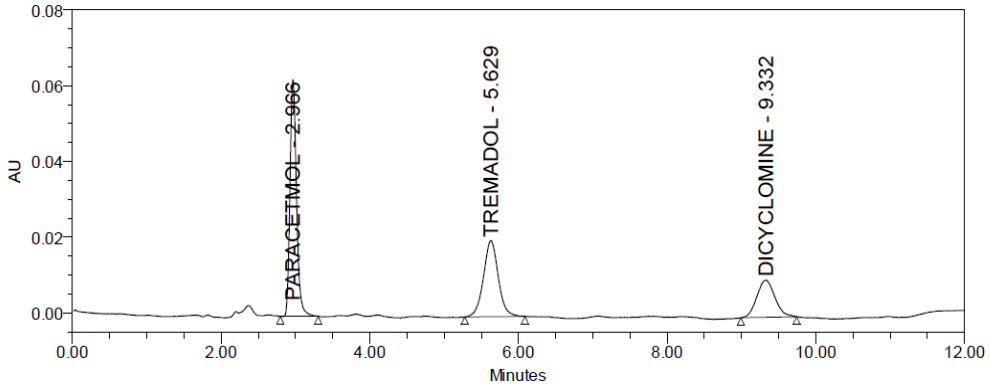

Figure 10: Chromatogram of LOD

Limit of quantification and limit of detection

Limit of quantification (LOQ) and limit of detection (LOD) gives information about the sensitivity of the method. The LOD and LOQ values for the paracetamol, tramadol and dicyclomine are presented in Table 8. The chromatograms of LOD and LOQ are shown in Figures 10 and 11, respectively. The results indicated that the proposed method possess sufficient sensitivity.

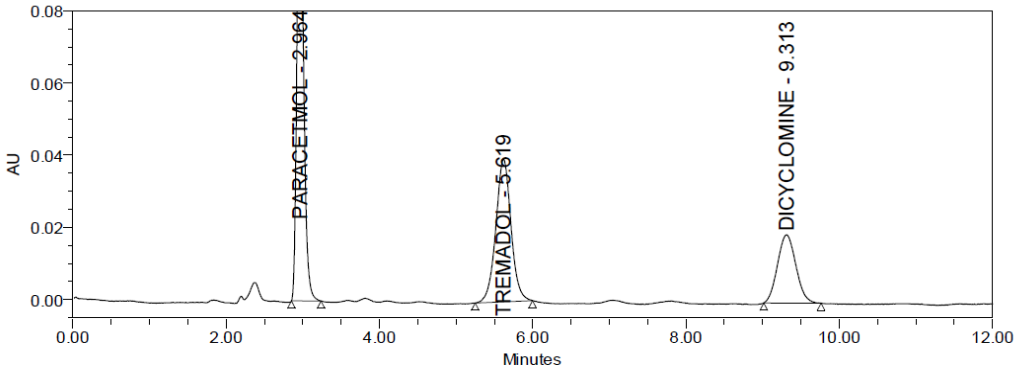

Figure 11: Chromatograms of LOQ

Table 8: LOD and LOQ for paracetamol, tramadol and dicyclomine

Sample Type	Sample name	RT	Area	Value
LOD	Paracetamol	3.47	379727	2.712
LOQ	Paracetamol	9.744	777836	9.042
LOD	Tramadol	3.94	277359	0.9009
LOQ	Tramadol	9.503	544959	3.0030
LOD	Dicyclomine	3.07	165093	0.380
LOQ	Dicyclomine	9.78	314666	1.265

CONCLUSION

A HPLC with UV detection method was developed and validated for the simultaneous determination of paracetamol, tramadol and dicyclomine in combined tablet dosage forms. The developed method was found to be simple, precise, accurate and sensitive for the simultaneous estimation. The method can easily and conveniently adopt for routine quality control analysis of paracetamol, tramadol and dicyclomine in pure and in its pharmaceutical dosage forms.

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