RESEARCH PAPER

RP-HPLC Method Development and Validation for Assay of Paracetamol and Tapentadol in Tablet Dosage Form

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ABSTRACT
This study was aimed to evaluate the mechanisms of action and interaction of the paracetamol-tapentadol combination in tablet dosage form. In this study, a high-performance liquid chromatographic technique for simultaneous identification of paracetamol and tapentadol in a tablet formulation is developed and validated. the invention and validation of a straightforward, precise, and economical RP-HPLC technique determine the analytical parameters of paracetamol and tapentadol by using kromosil C_{18}, 4.6mm*150 column, with mobile phase phosphate buffer: (1.36 gm of potassium dihydrogen orthophosphate add 1 ml of ortho phosphoric acid adjust pH 3.5 with triethylamine) methanol, in the ratio of 60:40v/v the flow rate was adjust 01 ml/minute with UV detection 273nm at the injection volume 10µl and column temperature 40°C.The Retention Time 1.88 min of the paracetamol and 3.90 min of tapentadol were observe respectively, Other replication standard system suitability measurements are consistent and within the range. the other parameters such as accuracy, linearity and range, precision, Robustness, LOD (limit of detection), LOQ (limit of quantification), System suitability, DSC & NMR Recovery was found to be for paracetamol 100.945 % for tapentadol 101% was statistically assessed and validated in accordance with ICH requirements, and it may be used for regular quality control analysis of paracetamol and tapentadol in pharmaceutical dosage forms. The technique showed symmetrical peaks, adequate retention periods, and good resolution between tapentadol and paracetamol. Both medications showed linearity over the chosen concentration ranges, with correlation coefficients that were almost equal to one. While precision experiments revealed low percentage RSD values, accuracy studies demonstrated a reasonable recovery.

Keywords: Analytical method development and method validation, paracetamol, tapentadol, RP-HPLC (High performance liquid chromatography)

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INTRODUCTION

The utilization of the relatively accurately, précising and highly selective High Performance Liquid Chromatographic (HPLC) for The ICH (International Conference on Harmonization of Technical Requirements) of Pharmaceuticals for Human Use criteria were followed in the development and validation of the technique for determining the dose form of paracetamol and tapentadol hydrochloride in tablets**.**Validation is to carried out to ascertain the upper and lower bounds of permitted variability for the keywords circumstances required to execute the technique. This article discusses approaches and a problem that arises during the creation and approval of an HPLC technique[1,2,3,4]**.** A practical and effective method for determining rutin in dosage forms is isocratic RP-HPLC. Among the most precise techniques for both qualitative and quantitative drug item analysis is RP-HPLC (Reverse phase- High Performance Liquid Chromatography), which is also utilized to assess the stability of medicinal products. The procedure created by laboratory testing to determine whether an analytical technique performs distinctive and content the specifications for the purpose for which is referred to as validation [5,6,7,8]. RP-HPLC technique for concurrent determination and examine the developed method's characteristics was verified in the current investigation in accordance with the ICH Q2 (R1) requirements**.** Accuracy**,** precision, specificity**,** linearity, Range, solution stability, Robustness**,** Ruggedness**,** Limits of detection and Quantitation**,** sensitivity, and system appropriateness were all verified for the purpose for which USP (United State Pharmacopeia) rules.10. The validation of forced deterioration was done in accordance with ICH.9,11,12**.** Method development frequently adheres to known procedures, such as choosing a buffer, mobile phase, and column [3,9,10.11,12].

To ensure the the effectiveness, security, and quality of medications, it is essential to demonstrate an analytical method's capacity for quantification [13,14]. The evidence required to confirm that an analytical technique can yield findings that are legitimate, dependable, repeatable, and appropriate for the intended use is

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known as method validation [15,16,17, 18]. The goal of the determination and the stage of the API manufacturing procedure should be reflected in the level of analytical method validation carried out [19,20,21,22].

The following factors led to the development of innovative drug analyzed methods:

- When no approved drugs or drug combinations are listed in the pharmacopoeias.
- When patent regulations prohibit the current drugs from possessing a decorative analysis procedure within the literature [15].

Guidelines for the development of pharmaceutical products have been put into effect by regulatory bodies including the USFDA of the (United States) and the ICH (International Council of Harmonization). By employing the QbD technique, the producer may decrease the total cost of the product and identify quality issues early[17]. The quality, reliability, and dependability of analytical based findings may be evaluated using technique validation results; this is a crucial part of any sound analytical procedure. The method validation [23,24,25]. procedure depends on using equipment that is within specifications, operating as intended, and calibrated correctly. Analytical procedures must be validated or revalidated [26,27].

These days, one of the best tools for analytical method is HPLC high performance liquid chromatography at the contemporary pharmaceutical business, high-performance liquid chromatography (HPLC) is the primary & crucial Analysis based instrument utilized at every stage of drug discovery, manufacture and. Development [26,27,28]. Examples from a project that aims to validate techniques for identifying paracetamol and tapentadol are used to describe and illustrate the processes of the validation process.

A sample (analyte) mixed in a Mobile Phase (solvent) is pushed at highest pressure via a column containing a chromatographic packing material that is immobilized (stationary phase) in High Pressure Liquid Chromatography (HPLC). Trial runs are used to improve and validate these techniques[25,29,61,62].

Paracetamol

The first prescribed medicine, one of the most well-liked and often used medications is paracetamol (acetaminophen, N-acetyl-p-aminophenol, 4-hydroxyacetanilide) as Fig.1. Prescott, L. F. (1980). It was initially provided for sale as a medication in the United Kingdom in 1956. Most widely used substance worldwide possesses exceptional gastric tolerance [33,31,32,63,64].

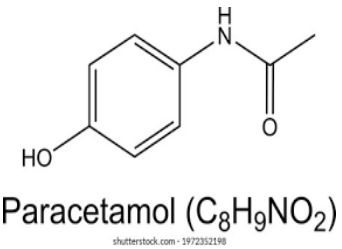


Figure No.1: Structure of Paracetamol

The antipyretic and analgesic properties of paracetamol are thought to be multiple directions, including a minimum of many metabolic pathways. When it comes to purified COX-1 or COX-2 or tissue homogenates, paracetamol is a poor inhibitor of PG production. Acetic anhydride reacts with Para-aminophenol to produce acetaminophen [33,34,35,36,37].

Tapentadol

Tapentadol (Fig.2) act as a synthetic analgesic substance which reduces pain directly. (1R,2R)-3-phenol hydrochloride (3-dimethylamino-1-ethyl-2-methyl-propyl)-phenol hydrochloride] combining two kinds of action, noradrenaline reuptake inhibitor (NRI) and μ-opioid receptor (MOR) agonist which leads to a synergistic impact that is highly analgesic [38,39,40,41].

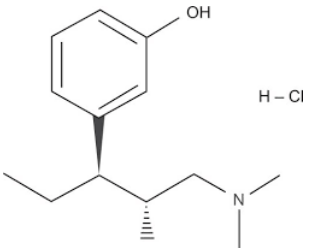


Figure No.2: Structure of tapentadol

Tapentadol has been designated as a class 2 opioid through the FDA (Food and Drug Administration). People Under the Europe, along with severe chronic pain can be treated with Tapentadol PR in 2008 got its initial approval as an immediate-release medication, and in April 2009 it started to be marketed and used. The Extended Release product received approval in 2011 [42,43,65,66].

MATERIALS AND METHODS

Method optimization:

Following technique development, the final findings chosen are displayed in Table 1.

Table No.1: Various Conditions Choose after Method Development and Validation

PARAMETERS	DESCRIPTION
COLUMN	Kromosil, C18,150*4.6mm.5µ
MOBILEPHASE	Buffer- 1.36 gm of KH_2PO_4 Add 1 ml of OPA in 1000 ml Milli -Q-Water (PH-3.5 with TEA) ORGANIC SOLVENT-Methanol
FLOWRATE	0.1mL/min
DETECTION	UV at 273 nm
COLUMNOVENTEMPERATURE	40º
SAMPLE TEMPERATURE	25º
INJECTIONVOLUME	10µL
WAVELENGTH	273nm
RUNTIME	8 min
ELUTION	Isocratic

Preparation of material and method used:

- **Chemical and reagents:**

Working standard and test sample (tablet dosage form) of paracetamol and tapentadol was obtained by Indian pharmacopoeia commission, for the method validation and development respectively.

- **Instrumentation and chromatography:**

Chromatography of paracetamol and tapentadol was carried out using kromosil C_{18}, 4.6mm 150* 5µm by the use of phosphate buffer: methanol as a mobile phase & run time 08 min, flow rate 10µl/min with UV detection range 273nm.

- **Preparation of buffer solution:**

About 1.36gm of KH_2PO_4 (potassium dihydrogen ortho phosphate) and1ml OPA (ortho phosphoric acid) in 1000milli litter of milli Q water, set the pH 3.5±5 with TEA (Triethylamine) respectively.

- **Preparation of Mobile Phase:**

Take buffer solution about 60 ml add into 40 ml of methanol, the ratio of mobile phase is methanol: buffer (40:60v/v) .

- **Preparation of the Diluent:**

Diluent same as mobile phase in which take buffer (60ml) and methanol (40ml), mix well, respectively.

- **Preparation of assay standard solution of paracetamol:**

Accurately weigh about 52.2mg of paracetamol standard powder in 50 milli litter of volumetric flask, to create a concentration of 104ppm add 20 milli litter diluent, shake properly & mix with the help of sonicator and and use diluent to make up the volume. pipette out 5 milli litter of this stock solution into 50 milli litter of volumetric flask add diluent, mix properly with the help of sonicator, fill the diluent up to the point [44,45,46,67,68].

- **Preparation of assay standard solution of tapentadol:**

Accurately weigh about 29.2 milli gram of tapentadol standard powder pore into 50 milli litter of volumetric flask to make concentration about 50 ppm, add diluent, mix well with the help of sonicator fill up the volume up to the mark about 50 milli litter with same diluent. Further pipette out 5 milli litter of this stock solution into 50 milli litter volumetric flask, dilute with diluent, sonicate for 10 min. and adjust the volume to the 50 milli litter with same diluent [47,48,69,70].

- **Preparation of sample solution of paracetamol and tapentadol drug for assay:**

The twenty pills were weighted and ground into a fine powdered. An amount of powdered equal to 3028.2 milli gram of the active ingredient was accurately measured and moved into a one hundred milli Litter volumetric flask. About 70 milli Litter of diluent was mixed and sonicate for twenty minutes. The solution was diluted to make up the volume with diluent and centrifuge the solution with the help of centrifuge, after centrifugation filtered using Whatman filter paper No.0.45µ.

- The analysis of paracetamol, 5 ml of the stock solution should be pipetted into a 50 ml volumetric flask. Diluent (around 20milli litter) was added and sonicate for ten min. the solution was diluted with the diluent up to the mark 50milli litter.

Further dilution of this solution is, pipetting 4 milli litter of Transfer the stock solution into a fifty milli litter of volumetric flask. Diluent (around 20 ml) was added and sonicated about 5 Minute. Solution was diluted with the same diluting agent, till the mark 50milli litter. All solutions were well mixed before being employed in the chromatographic determination. The analysis of tapentadol, pipette out of stock solution of paracetamol and tapentadol to analyze tapentadol about 4ml solution were added into 200 ml of volumetric flask. About 50 milli liter mixed with diluent. And sonicate for ten min. then solution was diluted with the same diluting agent 200 ml up to the

mark. Solution mix well for employed in the chromatographic determination [49,50].

Assay calculation of paracetamol:
Label claim- 325mg/tab
Obtained – 332.02mg/tab
% Assay (potency of test sample) – 102.16%

Assay calculation of tapentadol:
Label claim – 50mg
Obtained – 49.885mg/tab
% Assay (potency of test sample) – 99.77

RESULTS & DISCUSSION

Identification of drug: Purity and Identity of drug was done by using various analytical instruments such as Infrared Spectroscopy, UV-spectroscopy, Differential scanning calorimetry and High Performance Liquid Chromatography and spectras are reported in Fig.3 (a), (b),(c), Fig.4, Fig.5

IR Analysis:

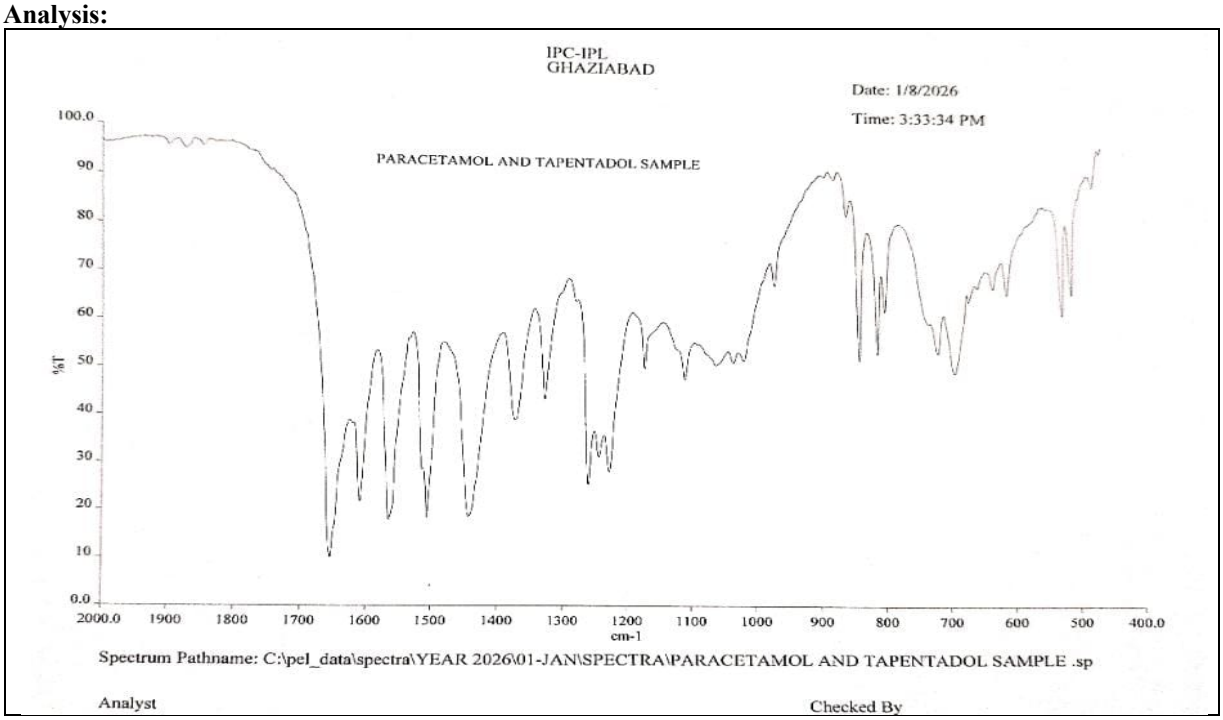


Figure No.3 (a): Infrared Spectroscopy of Paracetamol and Tapentadol with peak

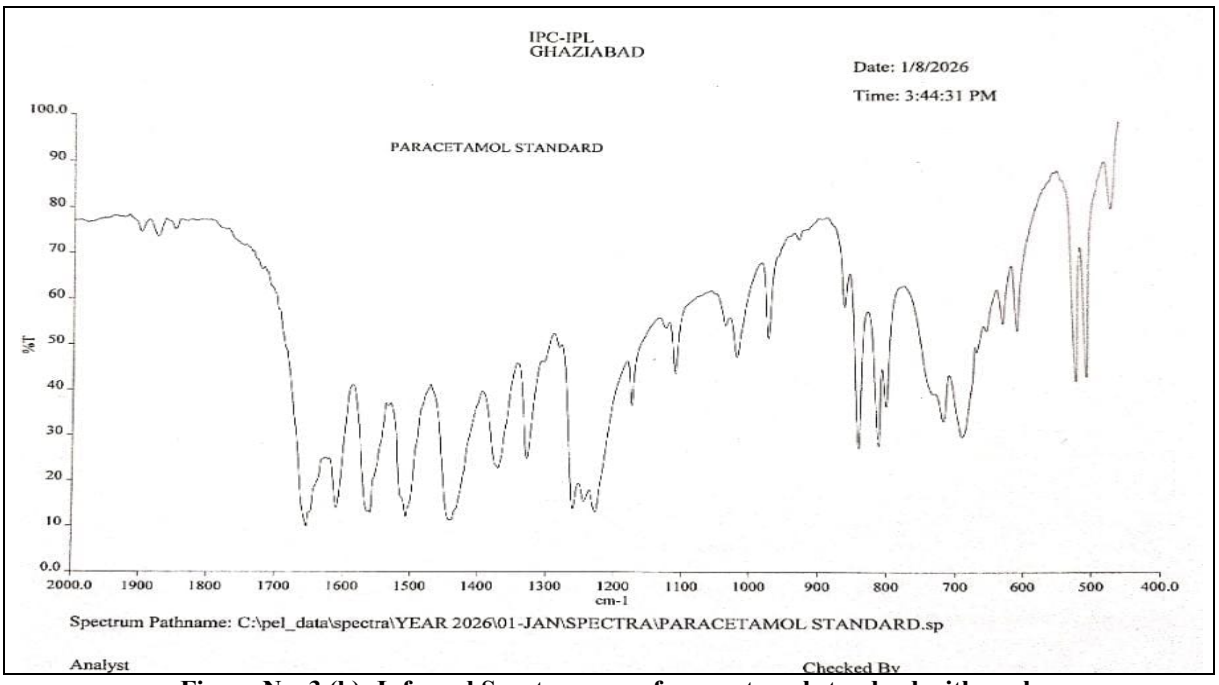


Figure No. 3 (b): Infrared Spectroscopy of paracetamol standard with peak

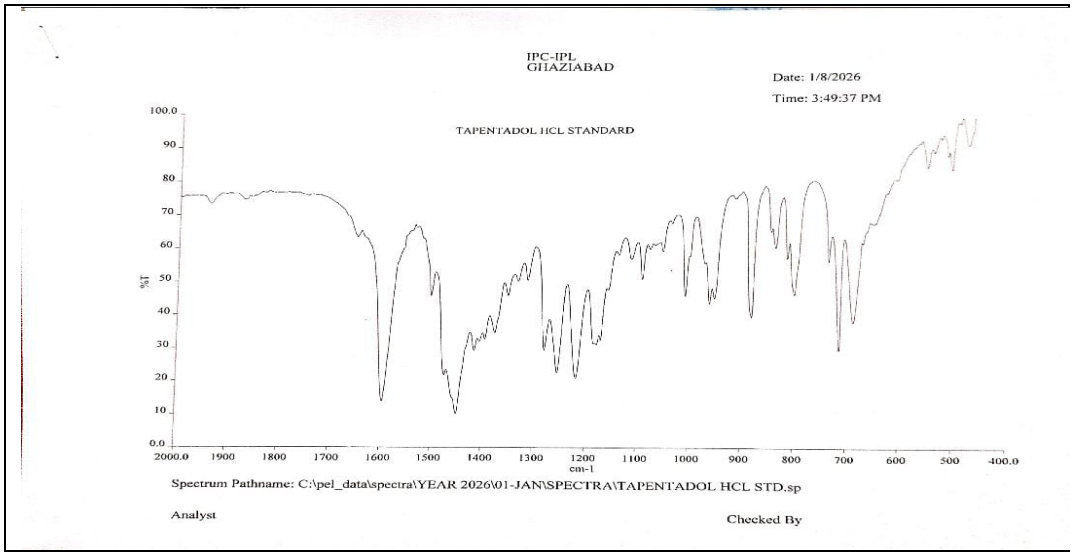


Figure 3 (c): Infrared Spectroscopy of Tapentadol standard with peak

UV Spectroscopy: 1ml of stock solution of paracetamol (52.2mg/ml) and tapentadol (29.1mg/ml) were diluted with diluent into 10ml volumetric flask, with proper shaking respectively. UV spectra of paracetamol and tapentadol are display in Fig.4 (a), (b) and (c) correspondingly.

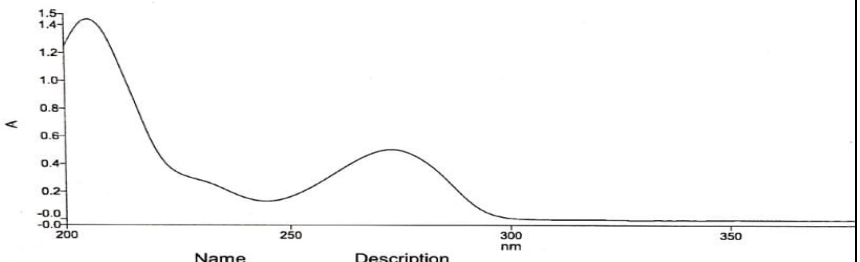
INDIAN PHARMACOPOEIA COMMISION

Instrument ID: IPC/CHEM/INST/060

Data Information: C:\ProgramData\PerkinElmer\UVWinLab\new.mdb

Method Name: Scan Monday, November 08, 2021 10:30 AM
India Standard Time

Analyst: ipc1



Peak Table

Sample ID	Sample Solution 1.Sample
Description	
Threshold	0.010
Abscissa Range	400 - 200 nm
Display Options	Peaks Listed by position

	Position (nm)	Intensity	Type
1	272.95	0.5145	Peak

Print Date & Time : 25-02-26 04:03:35 PM
Analysed By :-
Date :-
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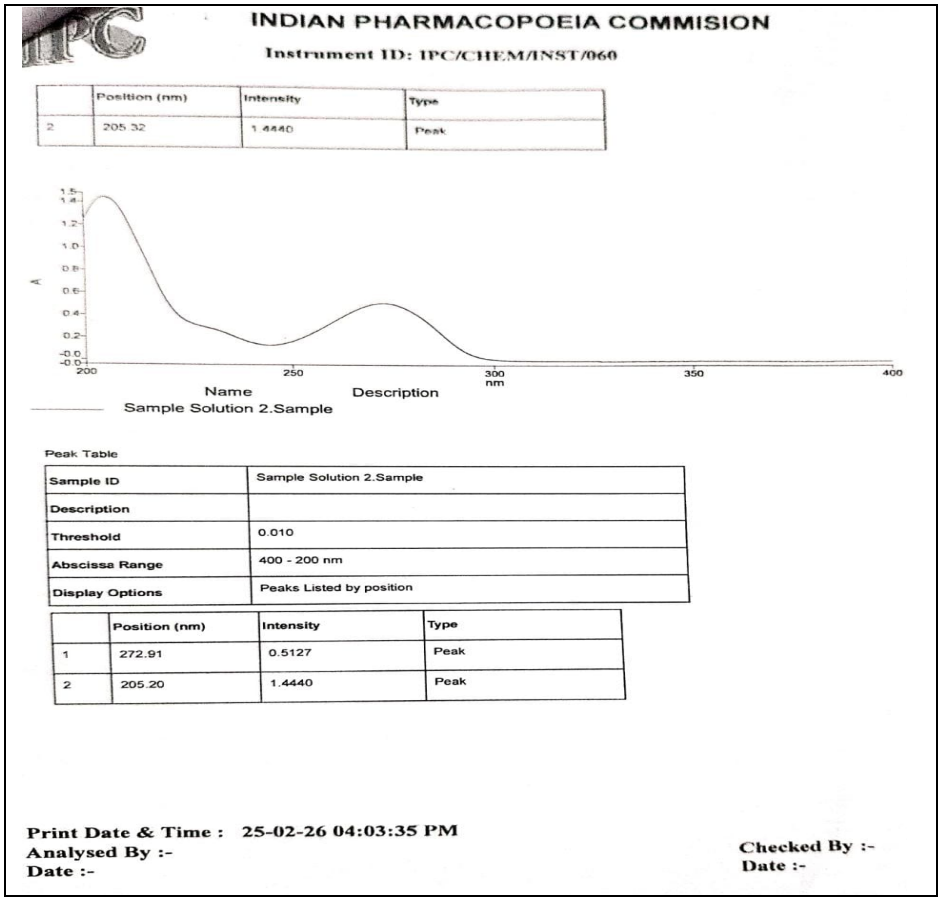
Date :-

Figure No. 4 (a): UV visible Spectroscopy



INDIAN PHARMACOPOEIA COMMISION

Instrument ID: IPC/CHEM/INST/060

	Position (nm)	Intensity	Type
2	205.32	1.4440	Peak

Peak Table

Sample ID	Sample Solution 2.Sample
Description	
Threshold	0.010
Abscissa Range	400 - 200 nm
Display Options	Peaks Listed by position

	Position (nm)	Intensity	Type
1	272.91	0.5127	Peak
2	205.20	1.4440	Peak

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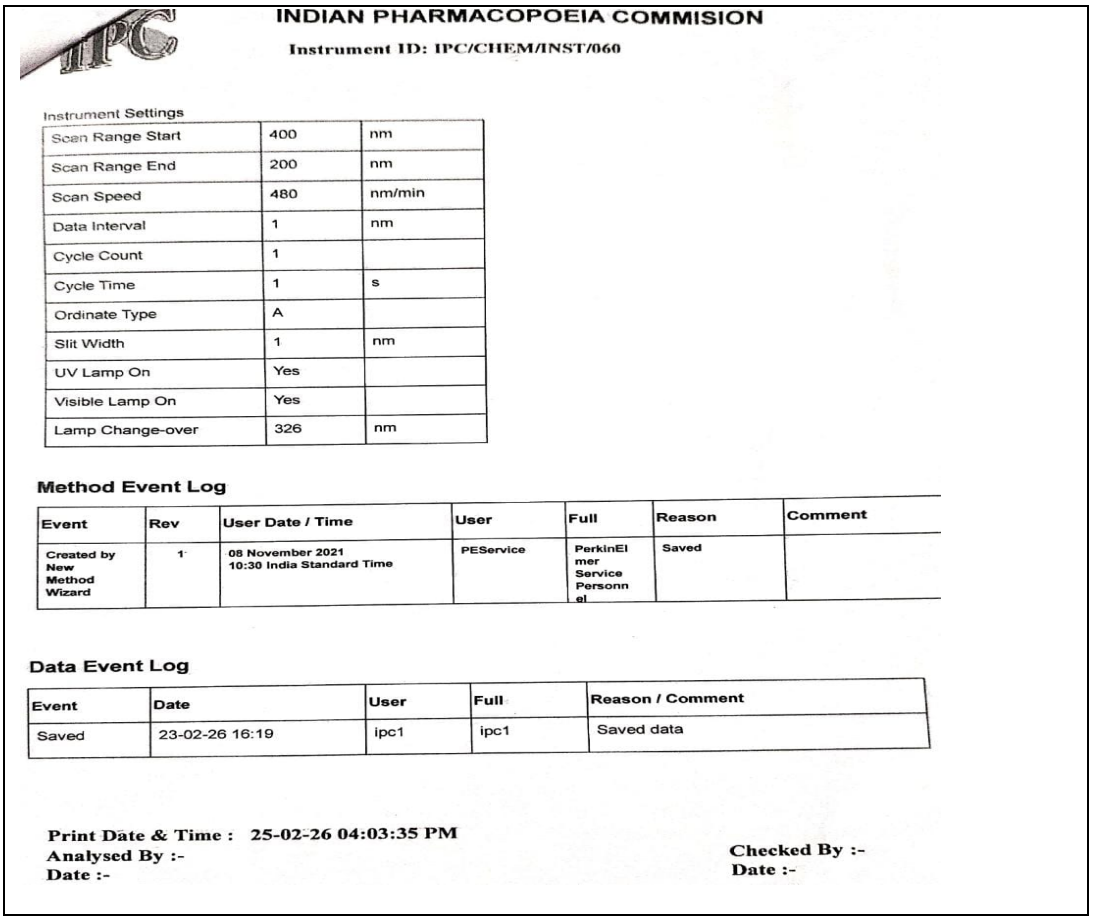
Figure No.4 (b): UV Visible Spectroscopy



INDIAN PHARMACOPOEIA COMMISION

Instrument ID: IPC/CHEM/INST/060

Instrument Settings

Scan Range Start	400	nm
Scan Range End	200	nm
Scan Speed	480	nm/min
Data Interval	1	nm
Cycle Count	1	
Cycle Time	1	s
Ordinate Type	A	
Slit Width	1	nm
UV Lamp On	Yes	
Visible Lamp On	Yes	
Lamp Change-over	326	nm

Method Event Log

Event	Rev	User Date / Time	User	Full	Reason	Comment
Created by New Method Wizard	1	08 November 2021 10:30 India Standard Time	PEService	PerkinElmer Service Personnel	Saved	

Data Event Log

Event	Date	User	Full	Reason / Comment
Saved	23-02-26 16:19	ipc1	ipc1	Saved data

Print Date & Time : 25-02-26 04:03:35 PM
Analysed By :-
Date :-

Checked By :-
Date :-

Figure No.4(c): UV Visible Spectroscopy

Differential scanning calorimetry (DSC)

- **For paracetamol:** 1-2 mg of paracetamol were examined using DSC under nitrogen flow (20 ml/min), heated at a temperature of 10 °C per minute from 30 to 400 °C, and the melting point was ascertained using the thermogram. Result shown in Fig.5.
- **For tapentadol:** 1-2 mg of tapentadol were examined using DSC under nitrogen flow (20 ml/min), heated at a temperature of 10 °C per minute from 30 to 400 °C, and the melting point was ascertained using the thermogram. Results shown in the Fig.6.

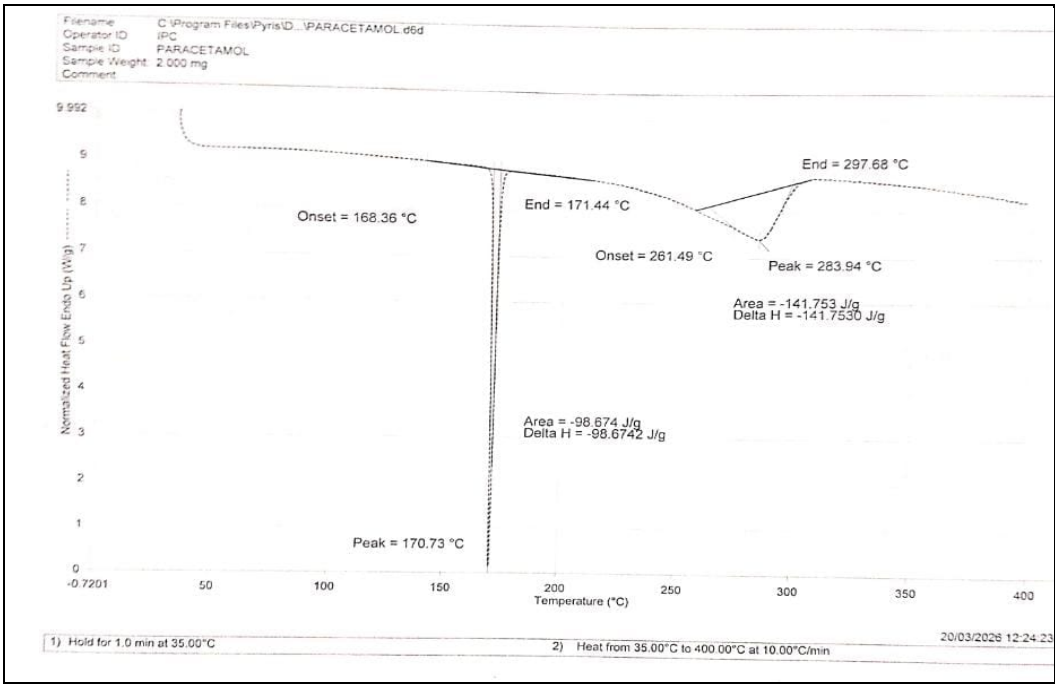


Figure No. 5: Chromatogram of DSC obtained for paracetamol

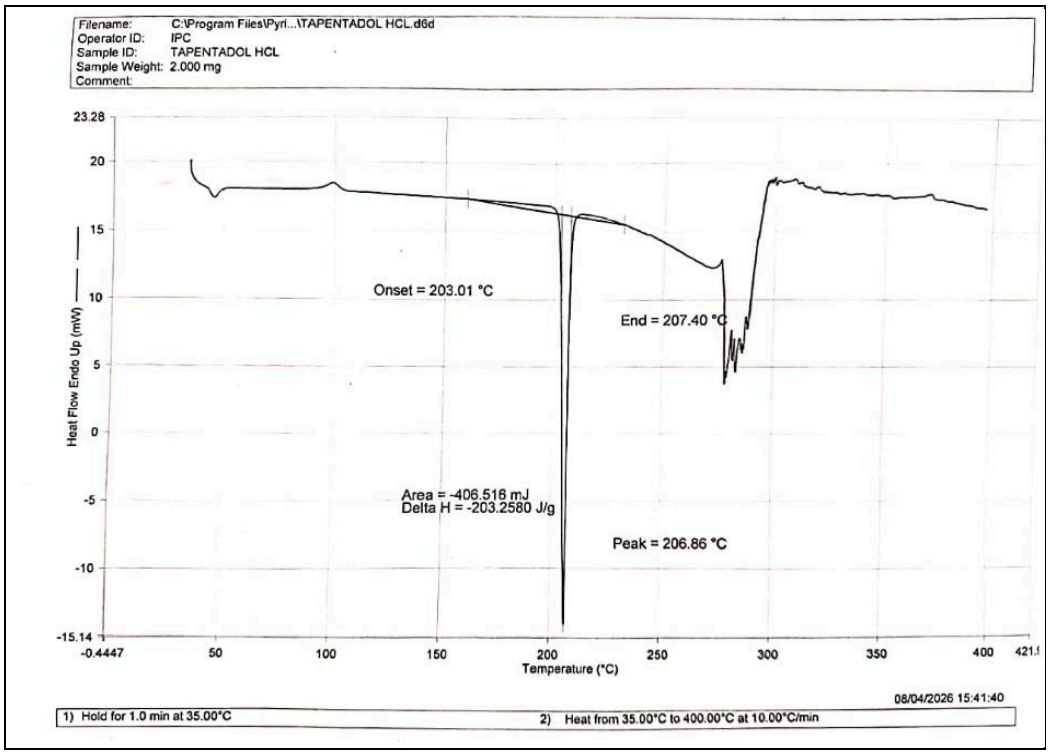


Figure No. 6: Chromatogram of DSC obtained for Tapentadol

Development of Method and Verification of Drug by High Performance Liquid Chromatography:
Technique was developed and validated in accordance to the ICH guidelines,

System suitability:
Standard solution was prepared fresh and injected using a new procedure in order to carry out the system suitability tests [51]. Results are mention in Table 2 (a) and 2 (b).

Table no. 2(a): System Suitability of Paracetamol

S. No.	RT	Area	Assay	TP
1	1.93	2455478	1.39	3738
2	1.93	2451267	1.34	3772
3	1.94	2459719	1.43	3735
4	1.94	2453302	1.42	3843
5	1.93	2454368	1.39	3780
Avg	**1.93**	**2454827**	**1.39**	**3774**
SD	**0.0055**	**3145.05**	**0.0351**	**43.64**
%RSD	**0.283**	**0.128**	**2.516**	**1.156**

Table No.2 (b): System Suitability of Tapentadol

S. No.	RT	Area	Assay	TP
1	4.35	551238	1.22	4274
2	4.35	549448	1.21	4288
3	4.37	551523	1.16	4279
4	4.36	550356	1.14	4182
5	4.36	551253	1.12	4387
Avg.	**4.36**	**550764**	**1.17**	**4282**
SD	**0.0084**	**857.06**	**0.0436**	**72.69**
%RSD	**0.192**	**0.156**	**3.726**	**1.698**

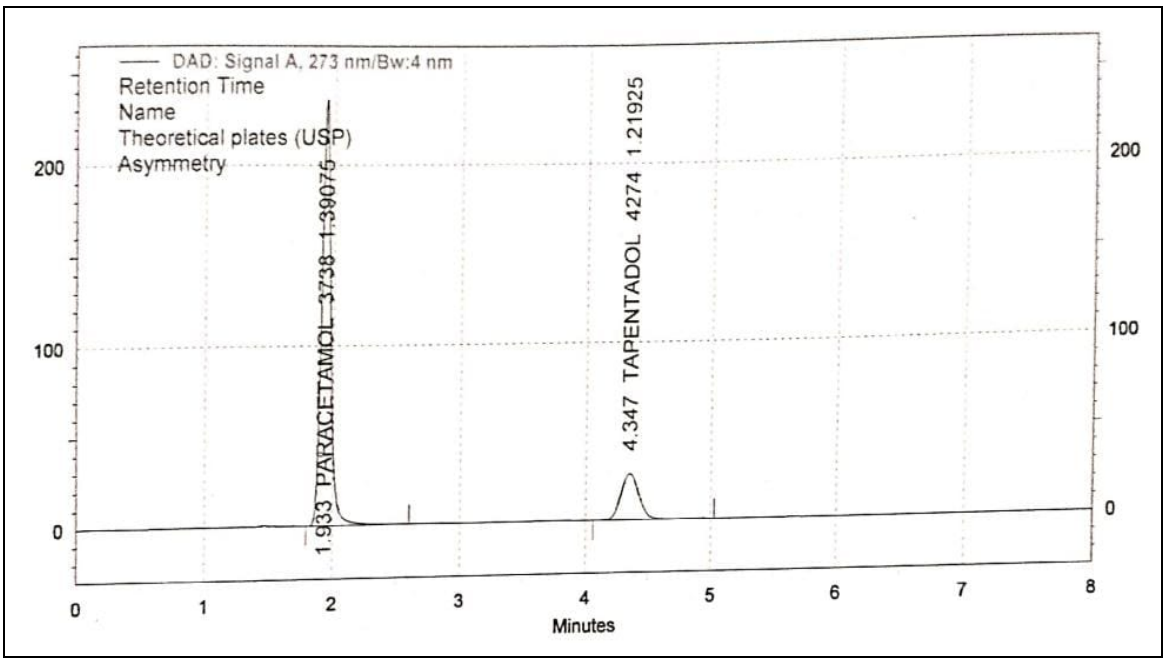


Figure No. 7: Obtained for system suitability of paracetamol and tapentadol

Linearity of paracetamol and tapentadol:
Stock solution of paracetamol [52.2 mg/ml (104 ppm)]. Stock of tapentadol [29.2 mg/ ml [50 ppm)]. This is a stock solution for more dilutions.

- **150 ppm:** pipette out about 3 milli litter of stock solution into a volumetric flask with a capacity of 20 ml. About 5 milli litter of diluting agent was mixed. Sonicated for five Minute. Dilute with same diluent up the mark.
- **120 ppm**: Pipette out 3 ml stock solution into 25 milli litter of volumetric flask. About 10 milli litter diluent was added for shaking then mix with the help of sonicator, solution was diluted with same diluting agent up to the mark.

- **100 ppm (solution for stock):** pipette 5 milli litter of stock solution into 50 milli litter of suitable volumetric flask. About Diluting agent (20 milli litter) was mixed. Sonicated for five minutes. Dilute with diluent till the mark.
- **80 ppm:** pipette out 8 ml of stock solution (100 ppm) into 10 milli litter volumetric flask. About 5 milli litter diluting agent was mixed. Sonicated for 2 to 3 Minute. Dilute with diluent up to the point.
- **50 ppm:** pipette out 5 ml of stock solution (100 ppm) into a volumetric flask that holds 10 ml. Concerning five milli litter of diluent was added. Sonicated for 2 to 3 Minute. Dilute with diluting agent was added till the point, shake properly.
- **20 ppm:** pipette 2 milli litter solution of stock (100 ppm) in 10 milli litter suitable volumetric flask. About 5 milli litter of same diluting agent was added. Sonicated for 2 to 3 min. dilute with diluting agent up to the point. Shake properly.
- **10 ppm:** pipette out 1 milli litter of solution of stock (100 ppm) into ten milli litter of volumetric flask. Sonicate for 2 to 3 min. dilute with diluent up to the point, Shake properly. the results are mention in Table 3 (a) and 3 (b).

Linearity Calculation

Table No. 3 (a): Paracetamol		
S. No	**Concentration**	**Area**
1	10	240521.3
2	20	389077
3	50	1226381
4	80	1914612
5	100	2332638
6	120	2609317
7	150	3402367

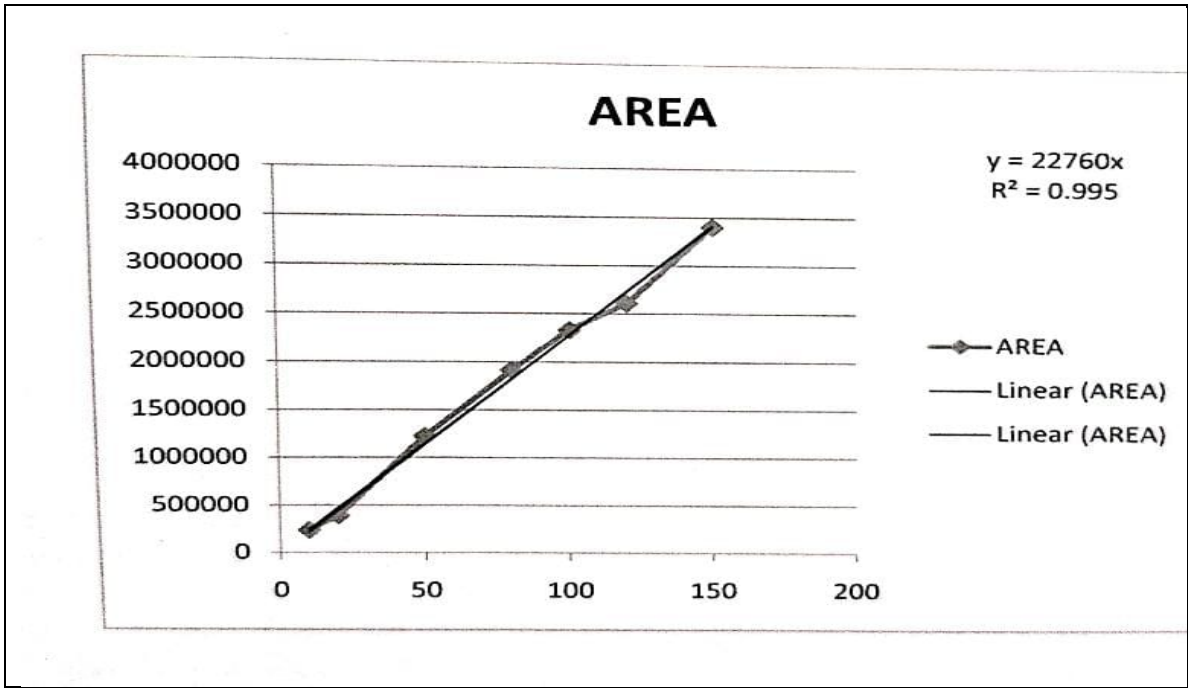


Figure No.8: Linearity graph of Paracetamol.

Table No. 3 (b): Tapentadol		
S. No	**Concentration**	**Area**
1	10	51678
2	20	86443.67
3	50	273377
4	80	417682
5	100	509126.3
6	120	571195.3
7	150	748363

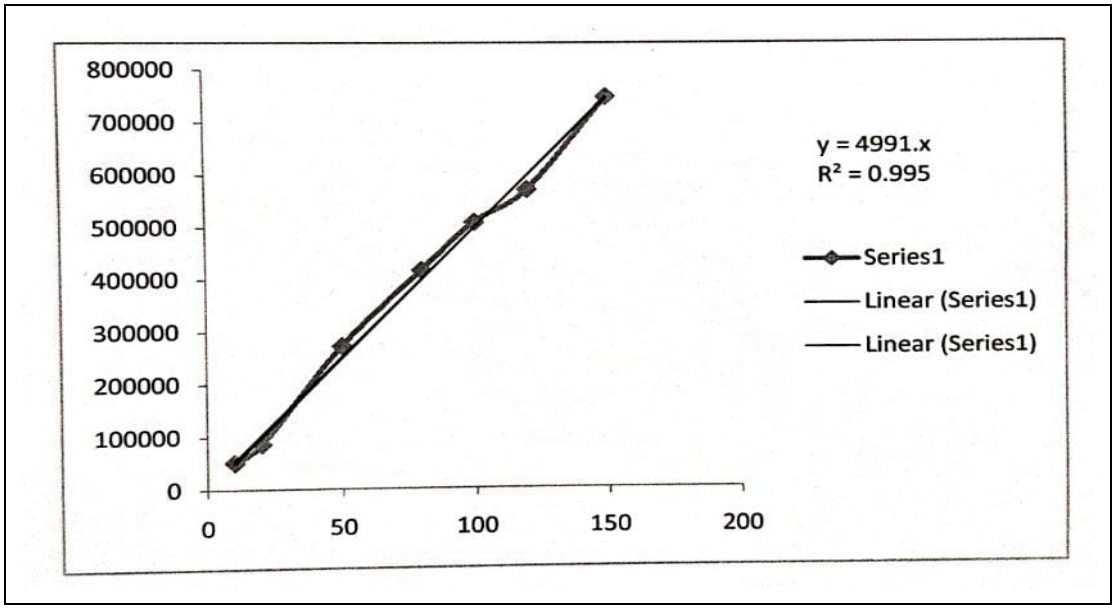


Figure No. 9: Chromatogram of Tapentadol

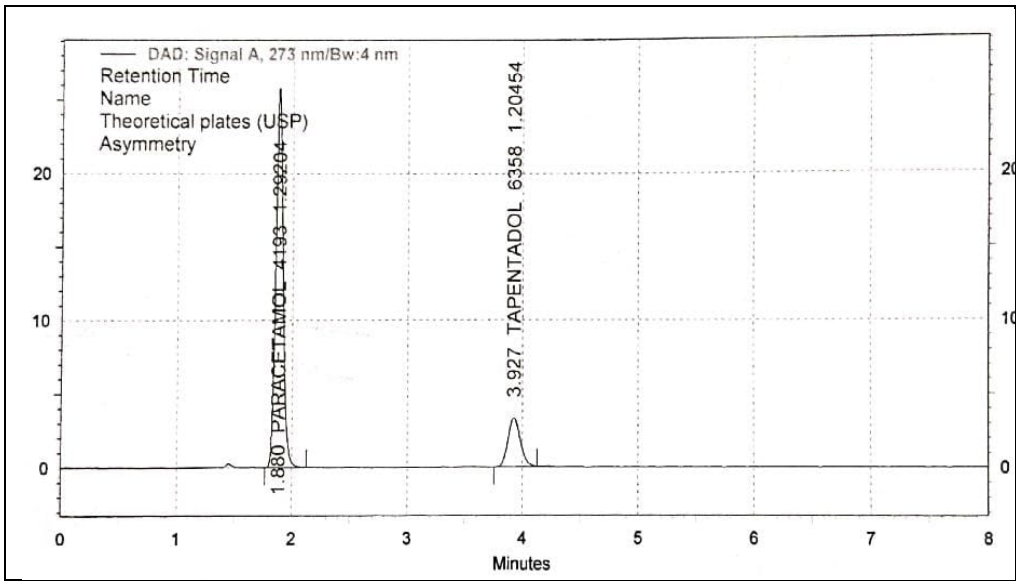


Figure No.10: Chromatogram of paracetamol and tapentadol obtained linearity at 10 % level

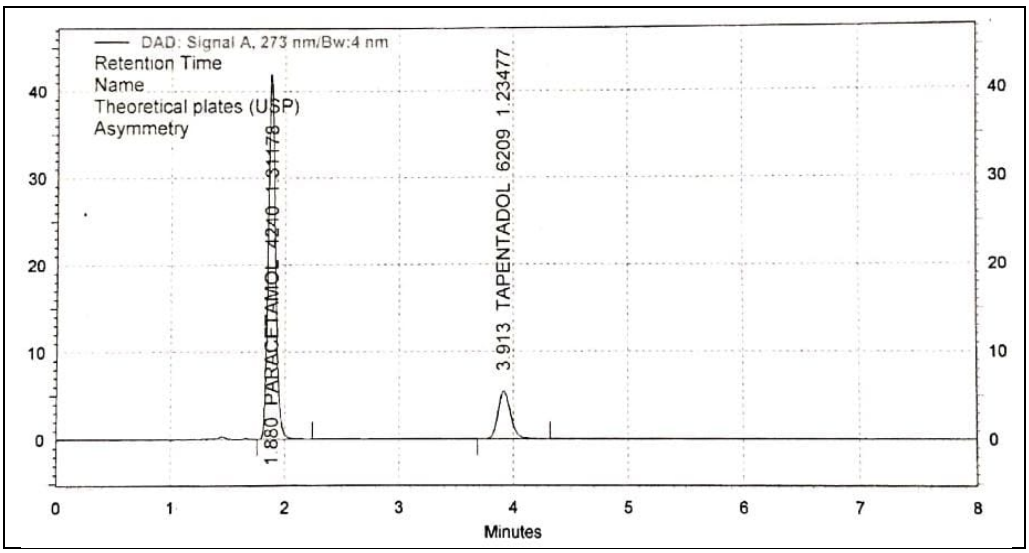


Figure No.11: Chromatogram of paracetamol and tapentadol obtained linearity at 20 % level

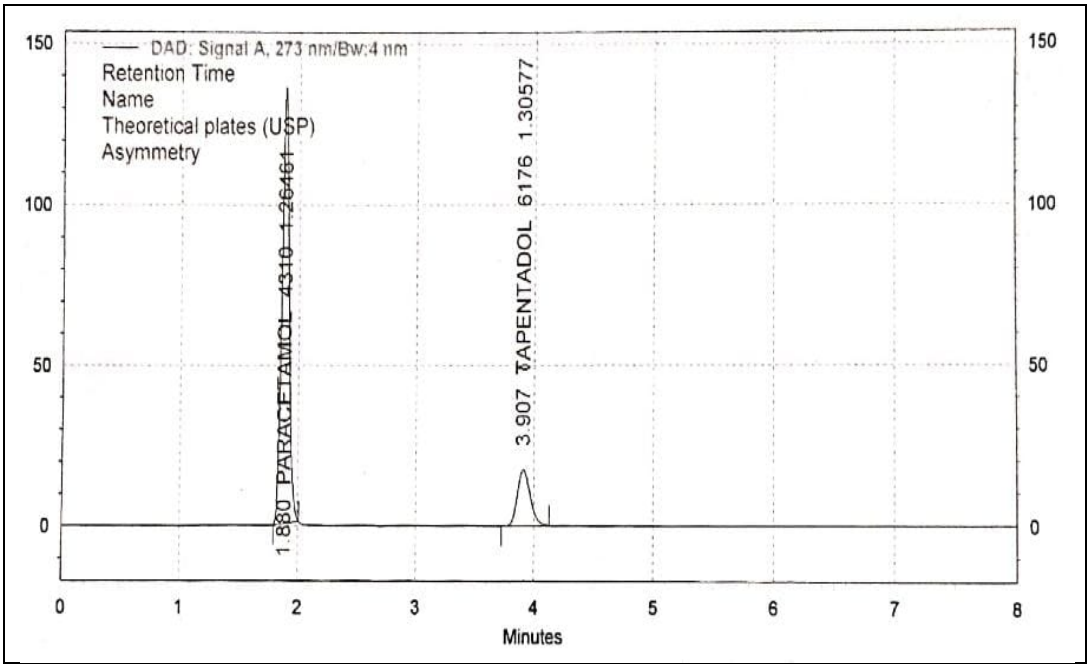


Figure No.12: A Chromatogram of paracetamol and tapentadol obtained linearity at 50 % level

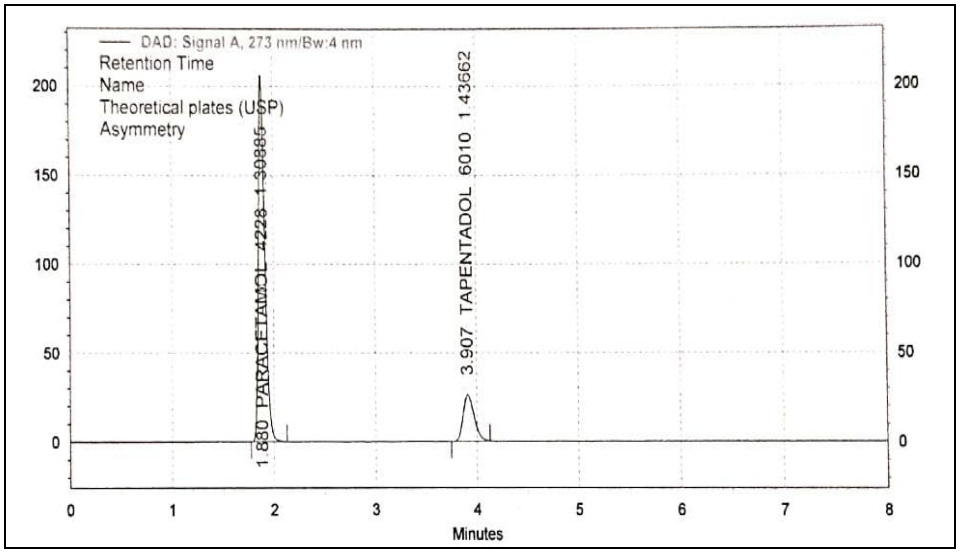


Figure No.13: A Chromatogram of paracetamol and tapentadol obtained linearity at 80 % level

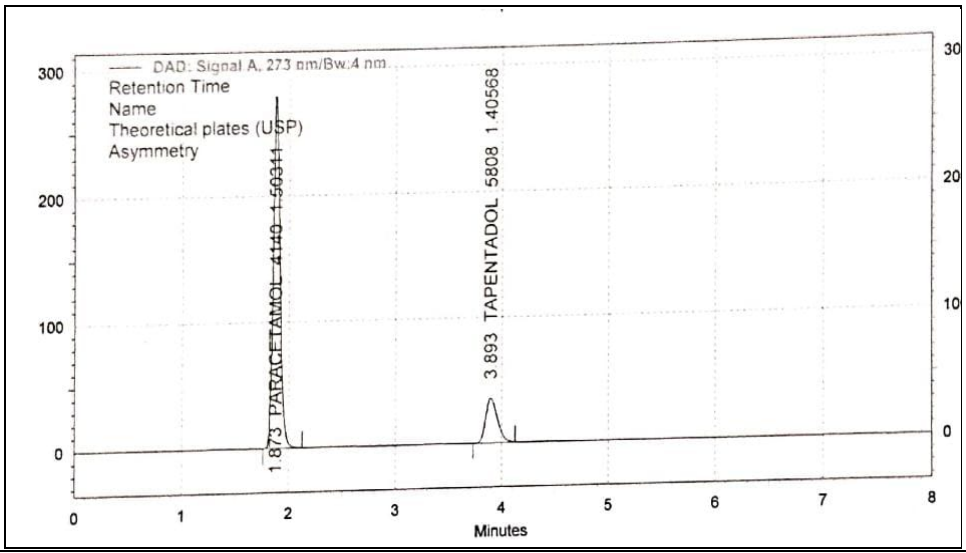


Figure No.14: A Chromatogram of paracetamol and tapentadol obtained linearity at 100 % level

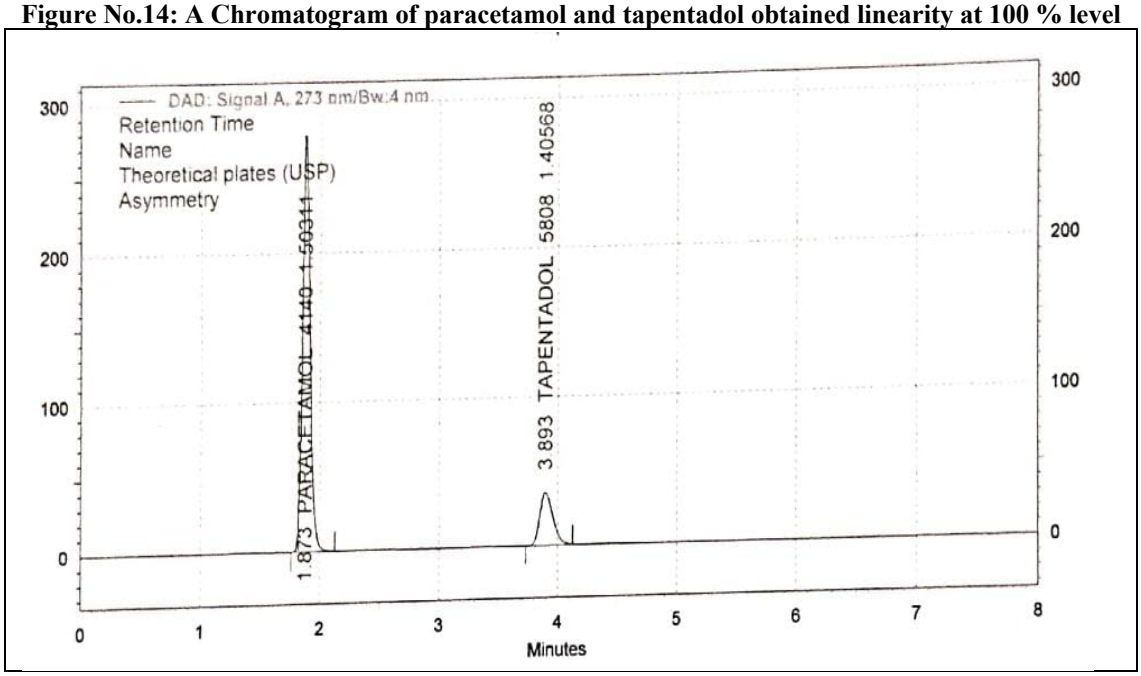


Figure No. 15: A Chromatogram of paracetamol and tapentadol obtained linearity at 120 % level

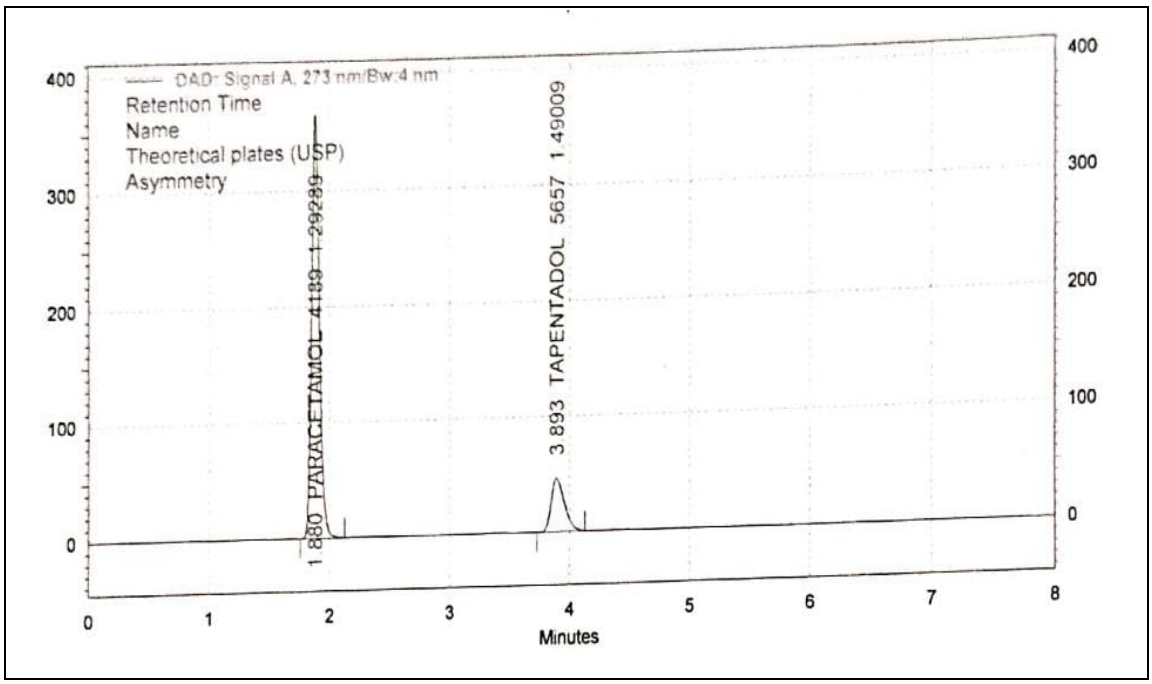


Figure No.16: A Chromatogram of paracetamol and tapentadol obtained linearity at 150 % level

- **Range:**

The analytical Range of method id derived from a research on accuracy and linearity the results are presented in Table 4.

Table No.4: Range Data

S. No.	Name	Range
1	Paracetamol	10-150
2	tapentadol	10-150

- **Accuracy:**

The correctness of the procedure was established using recovery tests for the medication of paracetamol and tapentadol at the concentration of 50%, 100%, 150%, in standard solution using the diluent. the obtain RT of paracetamol 1.93 and tapentadol 4.46 with % RSD 0.004 For Paracetamol and tapentadol 0.010 at the choosen time for accuracy of paracetamol and tapentadol is 8 min, given favorable area of paracetamol and tapentadol peaks. Trials were

conducted in multiple instances, and recovery percentages were determined. The results are mention in Table 5, Table 6, Fig. 17 (a), (b), (c) and Fig.18 (a), (b), (c).

Table No.5: Accuracy (Recovery) study for Paracetamol

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	50%	104	52	154	3495668	156.1187	101.3758	101.427	0.225
		104	52	154	3506043	156.5821	101.6767		
		104	52	154	3490609	155.8921	101.2291		
2	100%	104	52	154	4654555	207.8754	101.8997	101.763	0.138
		104	52	154	4641724	207.3023	101.6188		
		104	52	154	4648648	207.6115	101.7704		
3	150%	104	52	154	5872283	262.2598	100.8692	100.945	0.083
		104	52	154	5875868	262.42	100.9308		
		104	52	154	5881938	262.691	100.035		
							Avg.	**101.378**	
							SD	**0.411**	
							%RSD	**0.004**	

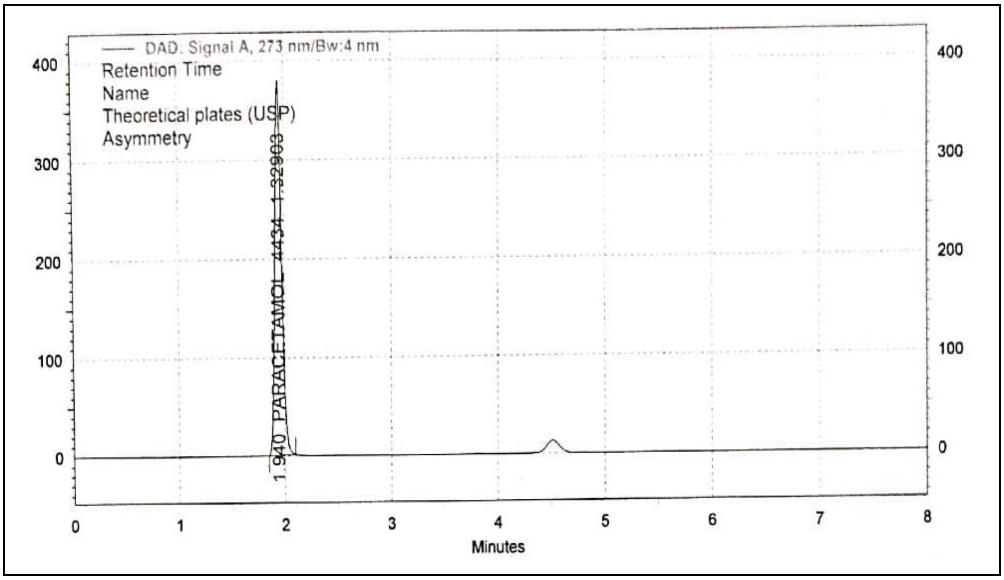


Figure No.17 (a): Chromatogram obtained at 50% level

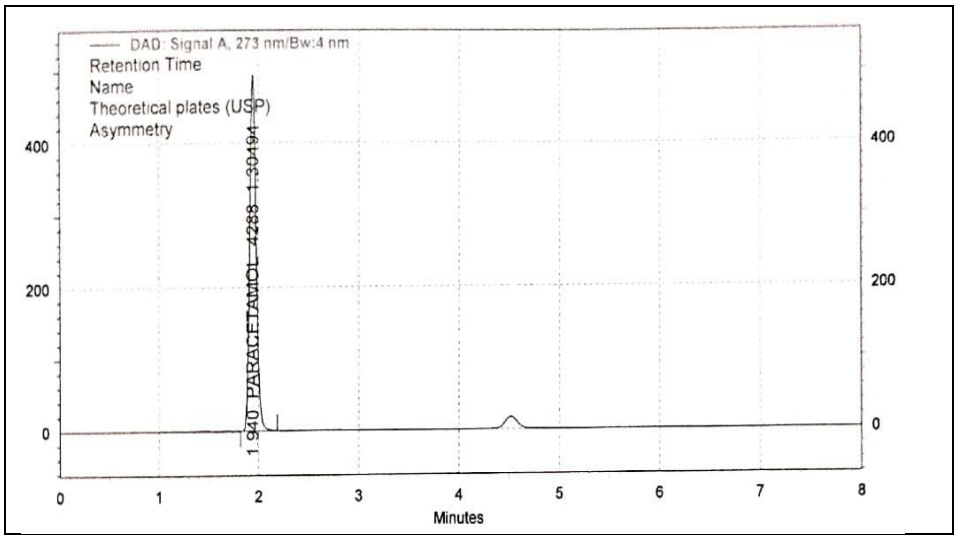


Figure No.17 (b): Chromatogram obtained at 100% level

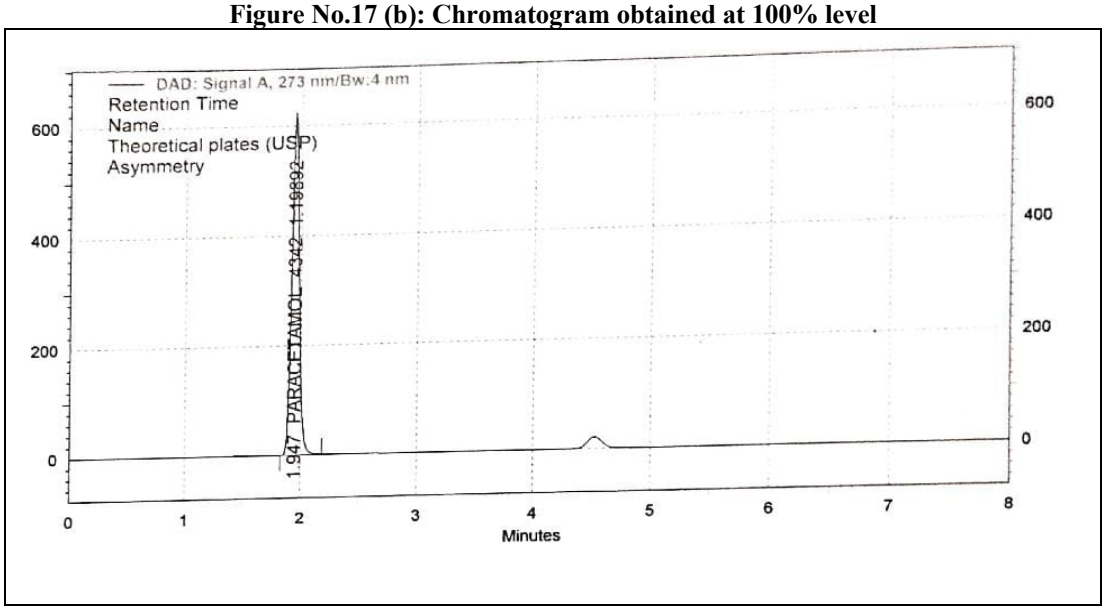


Figure No. 17(c): Chromatogram for the accuracy of paracetamol obtained at 150% level

Table No.6: Accuracy (Recovery) study for Tapentadol

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	50%	50	25	75	775084	74.71318	99.6175	99.820	0.184
		50	25	75	777878	74.9825	99.9766		
		50	25	75	777014	74.89922	99.8656		
2	100%	50	50	100	1026949	98.99137	98.9913	99.069	0.105
		50	50	100	1027333	99.02838	99.0283		
		50	50	100	1028980	99.18714	99.1871		
3	150%	50	75	125	1310333	126.3078	101.0462	101.129	0.250
		50	75	125	1308786	126.1587	100.9269		
		50	75	125	1315080	126.7654	101.4123		
							Avg	**100.006**	
							SD	**1.042**	
							%RSD	**0.010**	

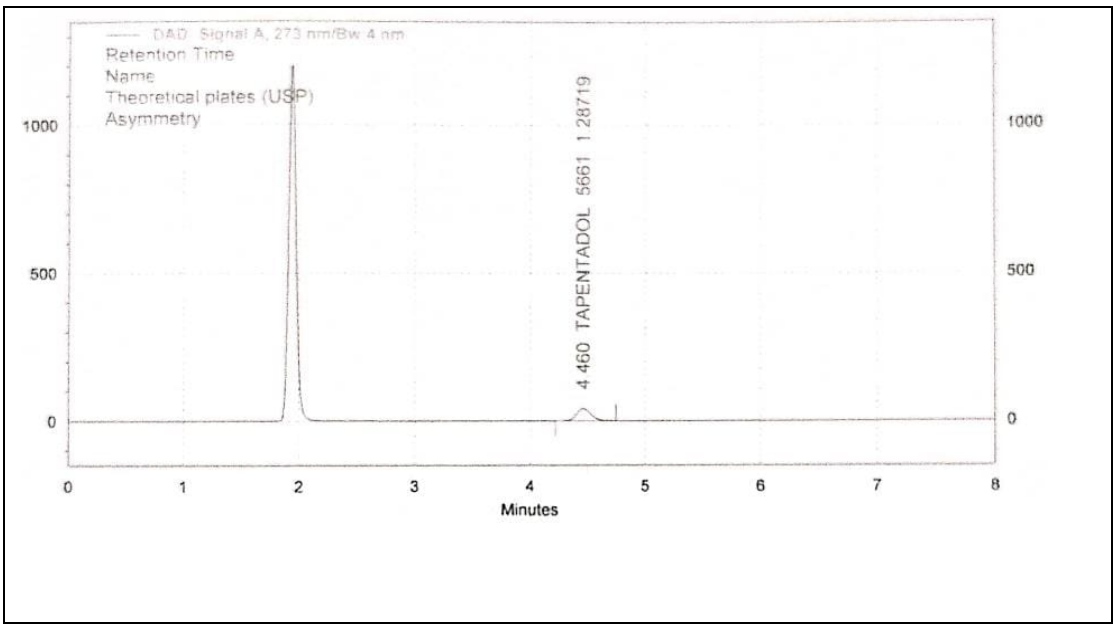


Figure No. 18 (a): A Chromatogram for the accuracy of tapentadol obtained at 50% level

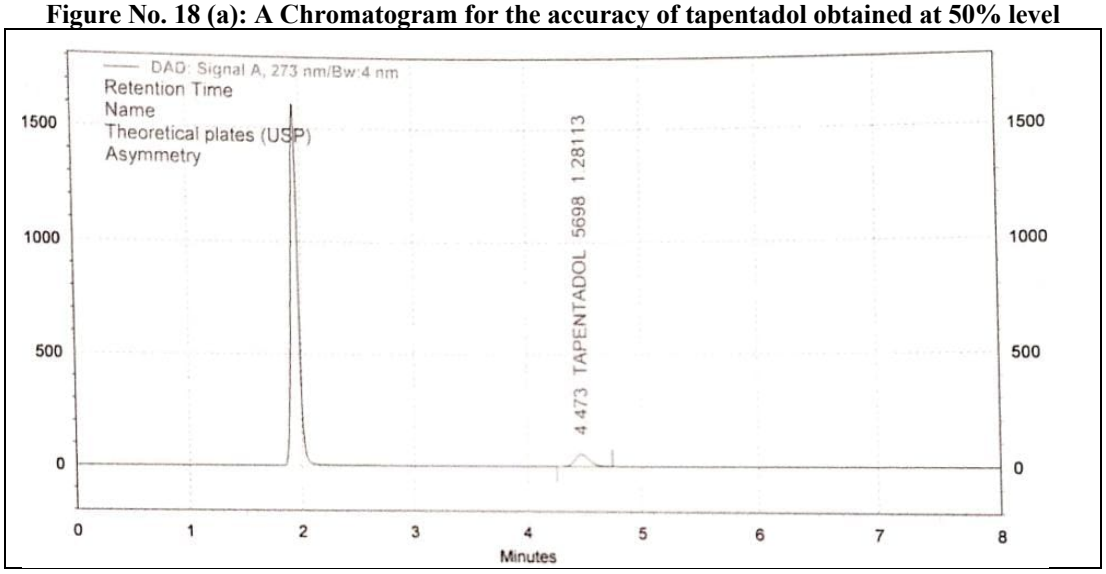


Figure No.18 (b): A Chromatogram for the accuracy of tapentadol obtained at 100% level

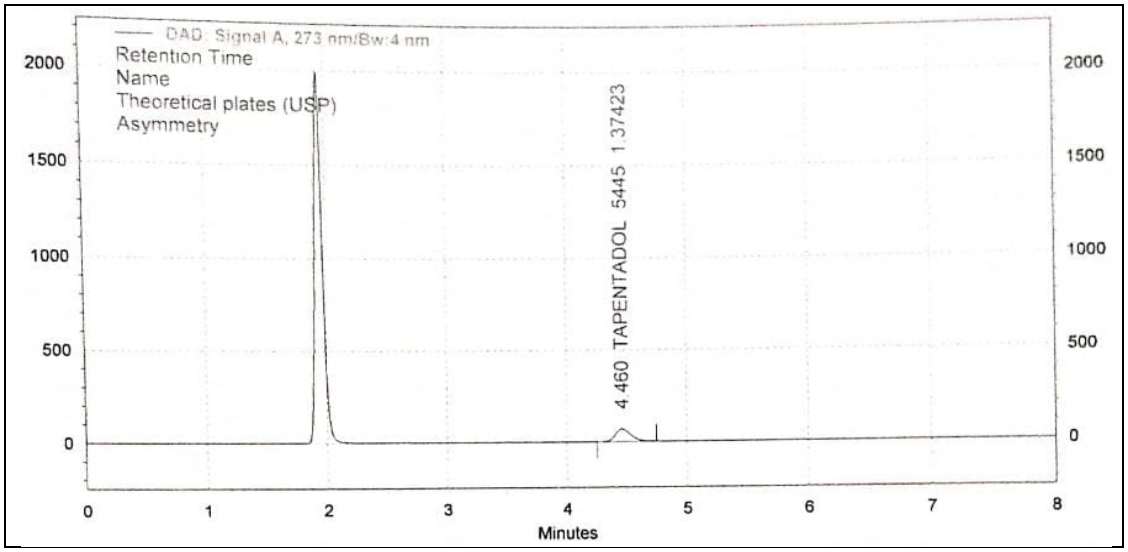


Figure No. 18 (c): A Chromatogram for the accuracy of tapentadol obtained at 150% level

PRECISION:

The precision is a procedure in which we identify the how much system is precise. Many samplings of the same homogenous sample conducted in accordance with the guidelines. The process's intra-day and inter-day precision was assessed. For intra-day precision for paracetamol and tapentadol testing were conducted three times in a single day, for inter-day precision, they were performed on three different days. The findings were given as a percentage of RSD [52,53].

(a) Repeatability:

The level of accuracy of paracetamol and tapentadol achieved under the same operational circumstances in a relatively brief period of time that is expressed by repeatability, additionally known as intra-assay precision[49]**.** There are three replicates of injection to determine the degradability of drug. Solution contains three concentration level, 10%, 100%, 150%. This level prepared in the way as prescribed, calculated Mean, SD & RSD%. Results of paracetamol and tapentadol are shown in Table 7, 8 and Fig. 19(a), (b) and (c).

Table No. 7: Repeatability of Paracetamol

S. No.	level	conc. µg/l	Area	Avg. Area	SD	% RSD
1	10%	5	240690	240521.3	233.37	**0.097**
	10%	5	240255			
	10%	5	240619			
2	100%	50	2319681	2328685	7803.3	**0.335**
	100%	50	2332891			

	100%	50	2333483			
3	150%	75	3404722	3413587	10646.2	**0.312**
	150%	75	3410643			
	150%	75	3425395			

Table No. 8: Repeatability of Tapentadol

S. No	level	conc. µg/l	Area	Avg. Area	SD	% RSD
1	10%	5	51738	51678	149.33	**0.289**
	10%	5	51508			
	10%	5	51788			
2	100%	50	514654	510802	3528.6	**0.691**
	100%	50	510026			
	100%	50	507726			
3	150%	75	748306	745423	4338.8	**0.582**
	150%	75	747530			
	150%	75	740433			

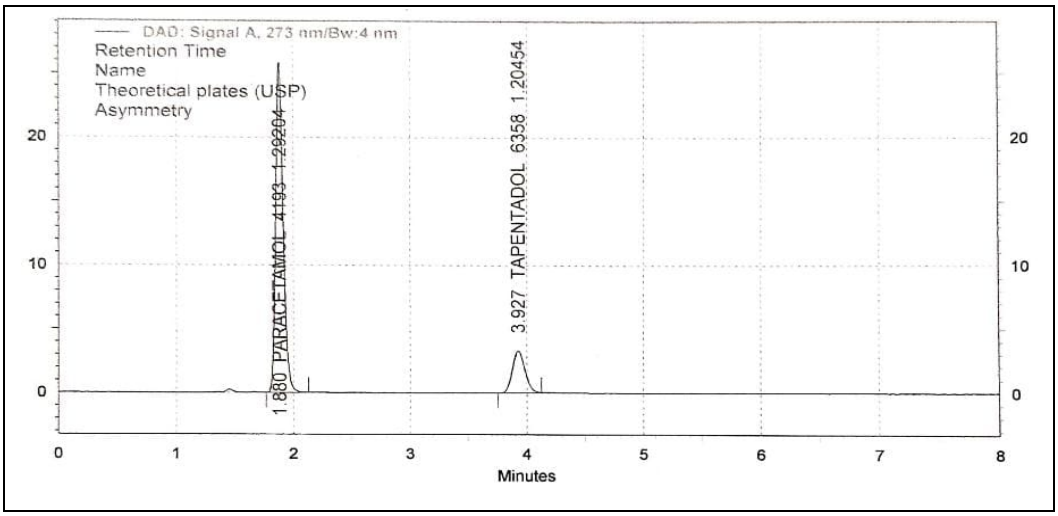


Figure No.19 (a): Chromatogram of Repeatability obtained for Paracetamol and Tapentadol at the level of 10 %

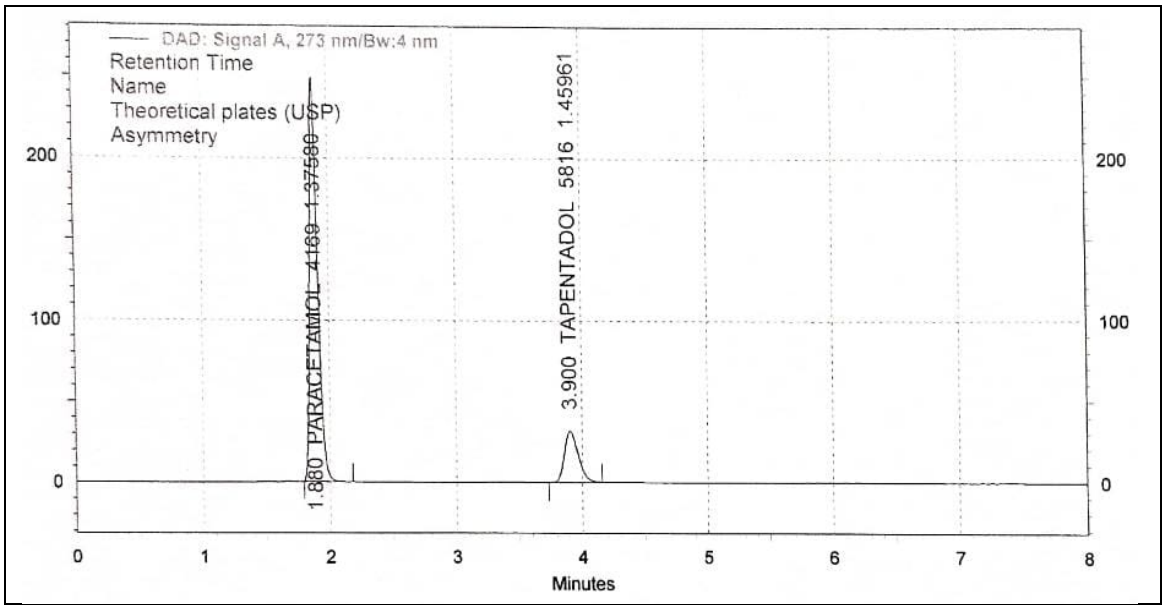


Figure No.19 (b): Chromatogram of Repeatability obtained for paracetamol and tapentadol at the level of 100 %

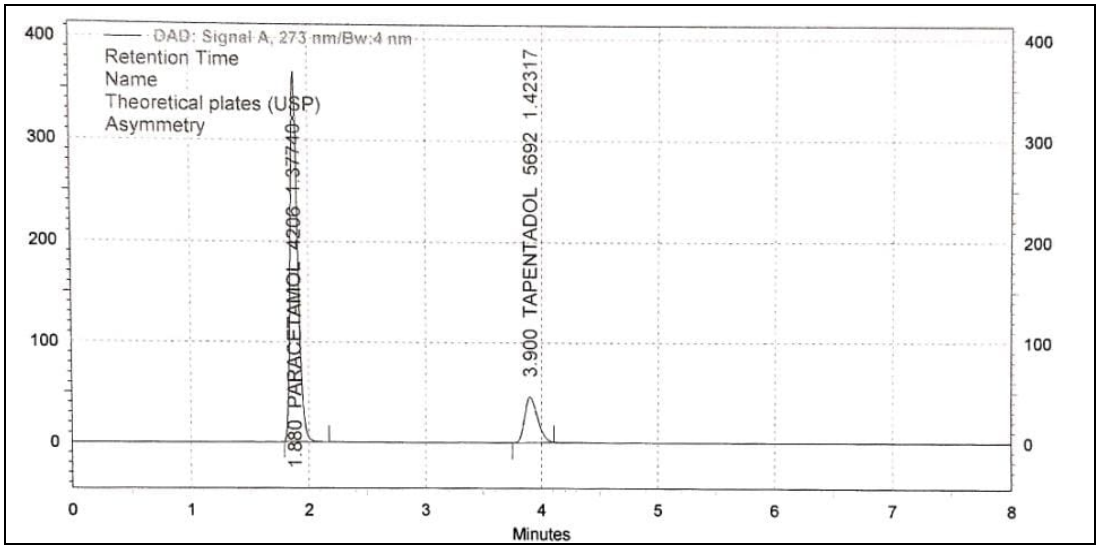


Figure No.19 (c): Chromatogram of Repeatability obtained for Paracetamol and Tapentadol at the level of 150 %

Method Precision:

The stability of the established technique for measuring analyte in a sample is confirmed by method precision. A blank solution, five injections of 100 ppm of standard solution and six replicates of the test solutions (about 100 ppm) were used to assess this. To evaluate consistency, the RSD for paracetamol 0.389 and tapentadol 0.524 was computed in the Table 9, Fig.20 (a), (b) and (c).

Table No.9: Method Precision Data for Paracetamol

Paracetamol			**Tapentadol**		
S. No.	**Area**	**RT**	**S. No**	**Area**	**RT**
1	2225350	1.93	1	502735	4.45
2	2239442	1.93	2	503104.5	4.445
3	2229627	1.935	3	499255.5	4.45
4	2241087	1.93	4	506010.5	4.43
5	2248999.5	1.935	5	504712	4.45
6	2242145	1.93	6	499932.5	4.435
Avg.	**2237775**	**1.932**	**Avg.**	**502625**	**4.44**
SD	**8713.3**	**0.003**	**SD**	**2633.0**	**0.009**
% RSD	**0.389**	**0.134**	**% RSD**	**0.524**	**0.197**

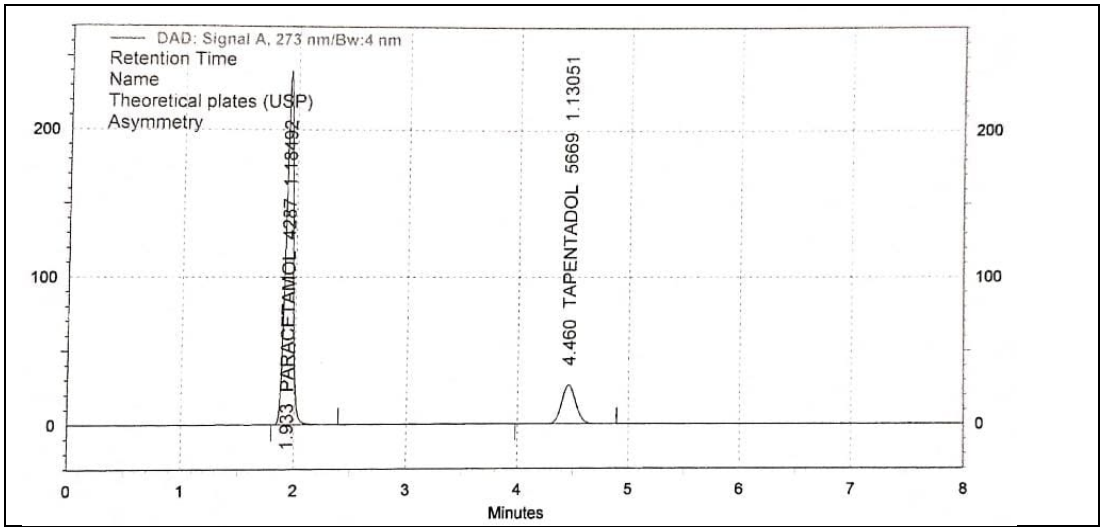


Figure No.20 (a): Chromatogram of method precision obtained for standard of Paracetamol and Tapentadol

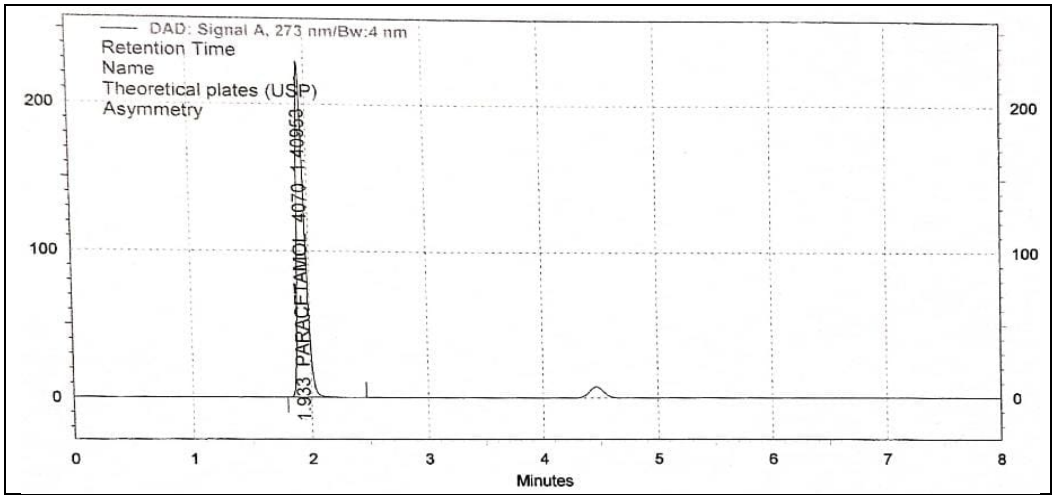


Figure No.20 (b): Chromatogram of method precision obtained for Paracetamol

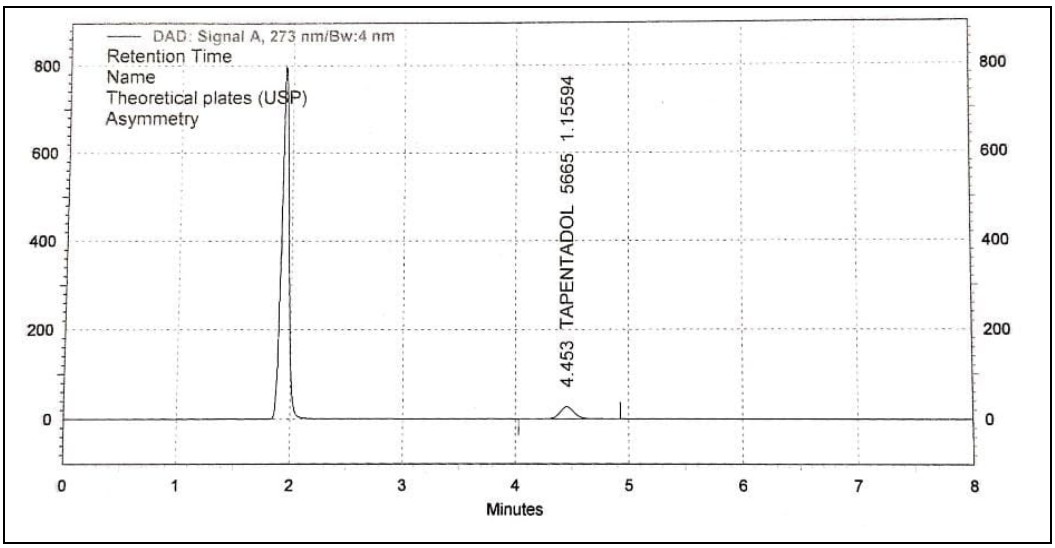


Figure No.20 (c): Chromatogram of method precision obtained for Tapentadol

Intermediate precision:

Analysing the appropriate responses three times over the course of 48 hours on the same day and three separate days allowed for the determination of the suggested approach for paracetamol and tapentadol [54, 55]. The results are given in a Table 10 and Fig. 21 (a), (b), (c).

Table No. 10: Intermediate Precision Study for Paracetamol and Tapentadol

I Intermediate Precision For Paracetamol			**I Intermediate Precision For Tapentadol**		
S. No	**Conc. (µg/ml)**	**ASSAY%**	**S. No**	**Conc. (µg/ml)**	**Area**
1	104	99.06	1	50	99.34
2	104	99.80	2	50	100.10
3	104	99.05	3	50	100.68
4	104	99.71	4	50	99.98
5	104	99.65	5	50	99.81
6	104	99.84	6	50	100.47
Avg.		**99.518**	**Avg.**		**100.063**
SD		**0.365**	**SD**		**0.478**
% RSD		**0.367**	**% RSD**		**0.477**

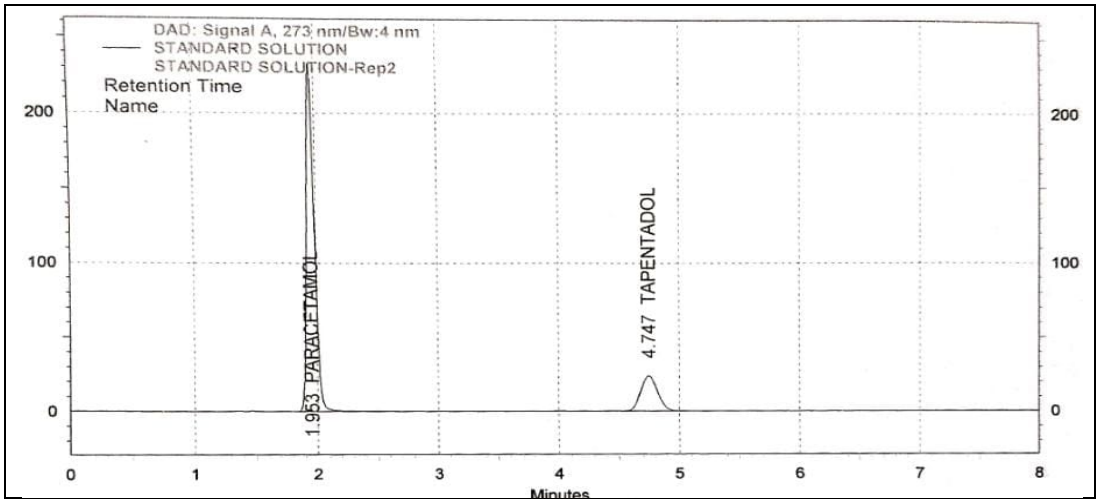


Figure No. 21(a): Chromatogram of intermediate precision obtained for the standard of Paracetamol and Tapentadol

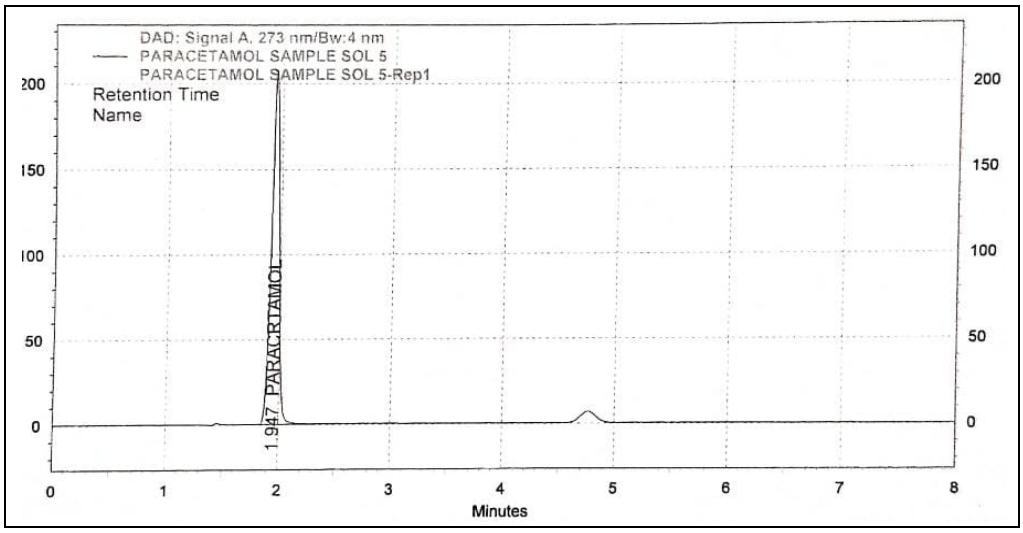


Figure No. 21(b): Chromatogram intermediate precision obtained for test sample of Paracetamol

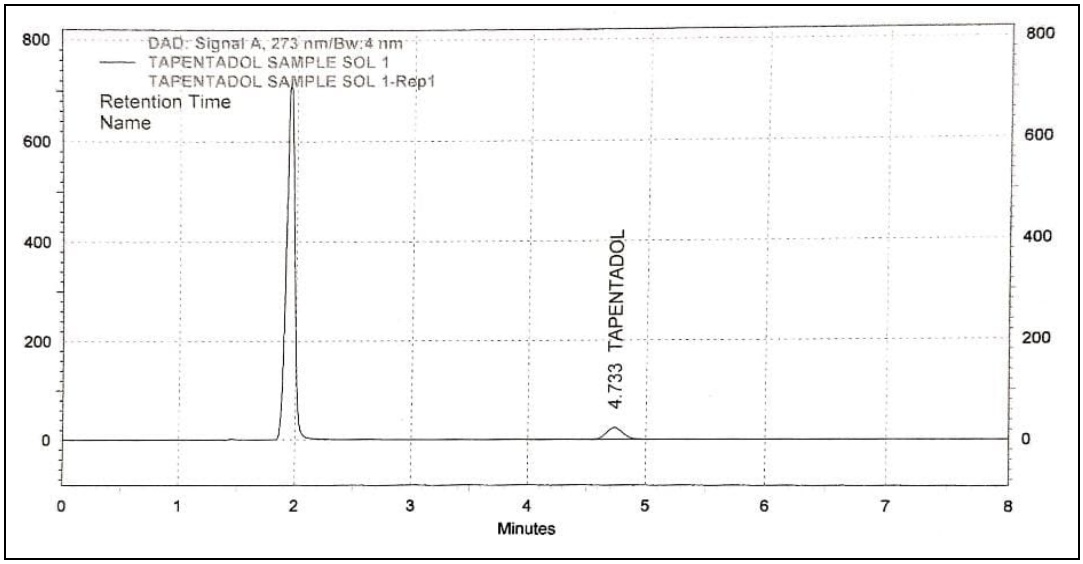


Figure No. 21(c): Chromatogram of intermediate precision obtained for test sample of Tapentadol

System Precision:
Six replicate injections were made into the HPLC instrument in compliance with the test procedure, which required the preparation of a reference solution using paracetamol and tapentadol. The proportion of the relative standard deviation (%RSD) for paracetamol 0.07918 and tapentadol 0.152 was calculated [52]. Data shown in Table 11 and Fig. 22 (a), (b).

Table No. 11: System Precision study for Paracetamol and Tapentadol

System Precision for Paracetamol			**System Precision for Tapentadol**		
S. No.	**Concentration (µg/ml)**	**Area**	**S. No**	**Concentration (µg/ml)**	**Area**
1	104	2314443	1	50	505269
2	104	2316918	2	50	507212
3	104	2318450	3	50	505571
4	104	2316918	4	50	505571
5	104	2314132	5	50	506060
Avg		**2316172**	**Avg**		**505937**
SD		**1833.94**	**SD**		**767.282**
% RSD		**0.0791**	**% RSD**		**0.152**

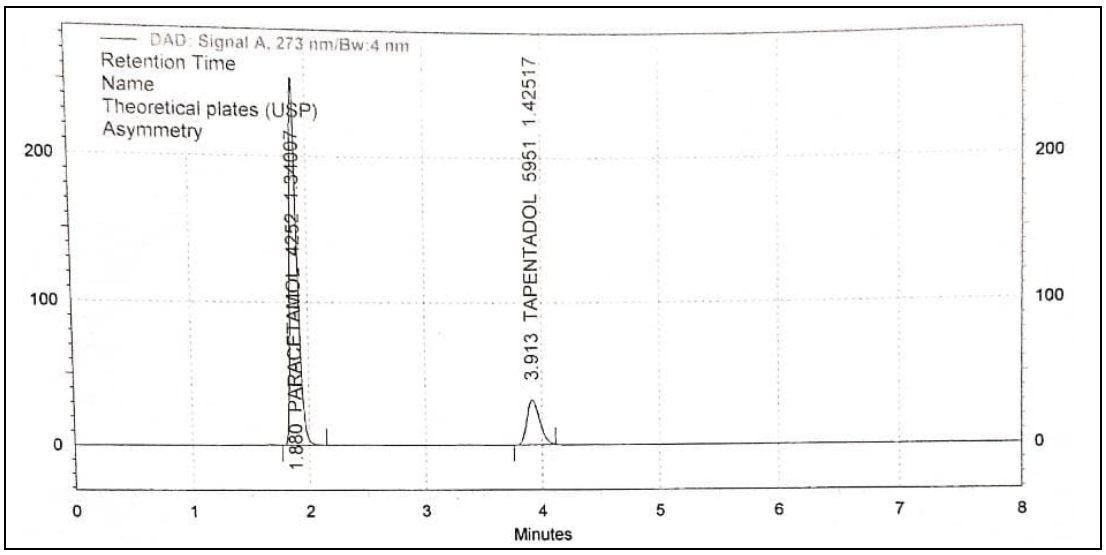


Figure No.22 (a): Standard for system precision of Paracetamol and Tapentadol

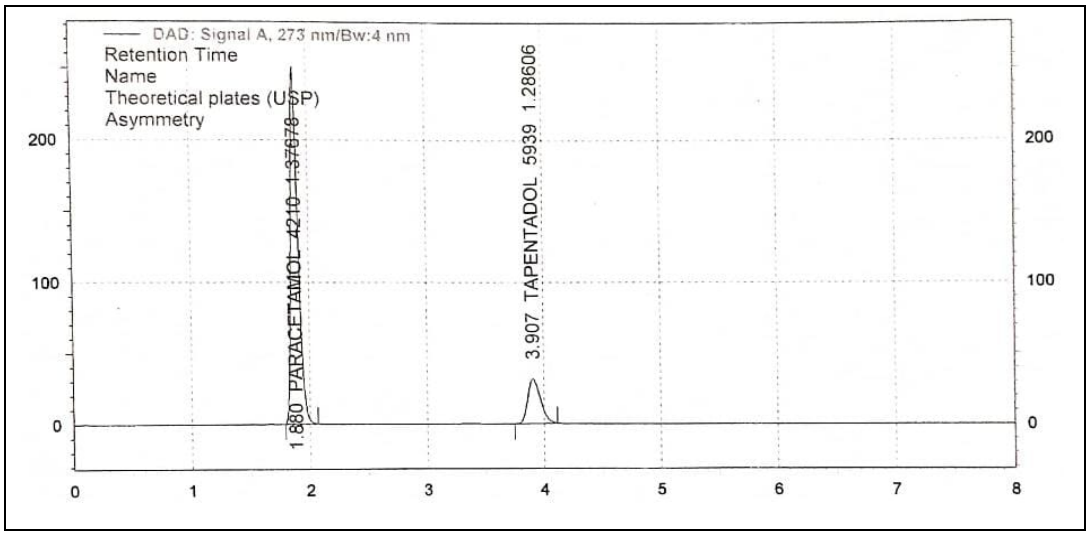


Figure No.22 (b): Chromatogram obtained for system precision of paracetamol and Tapentadol

Specificity:

Capacity of the procedure to precisely quantify the analyte response when all possible sample components are present is known as specificity[53]**.** Single parameter can be analyzed of paracetamol and tapentadol. Results are presented in Fig. 23 (a), (b), (c), (d) and (e) for paracetamol respectively.

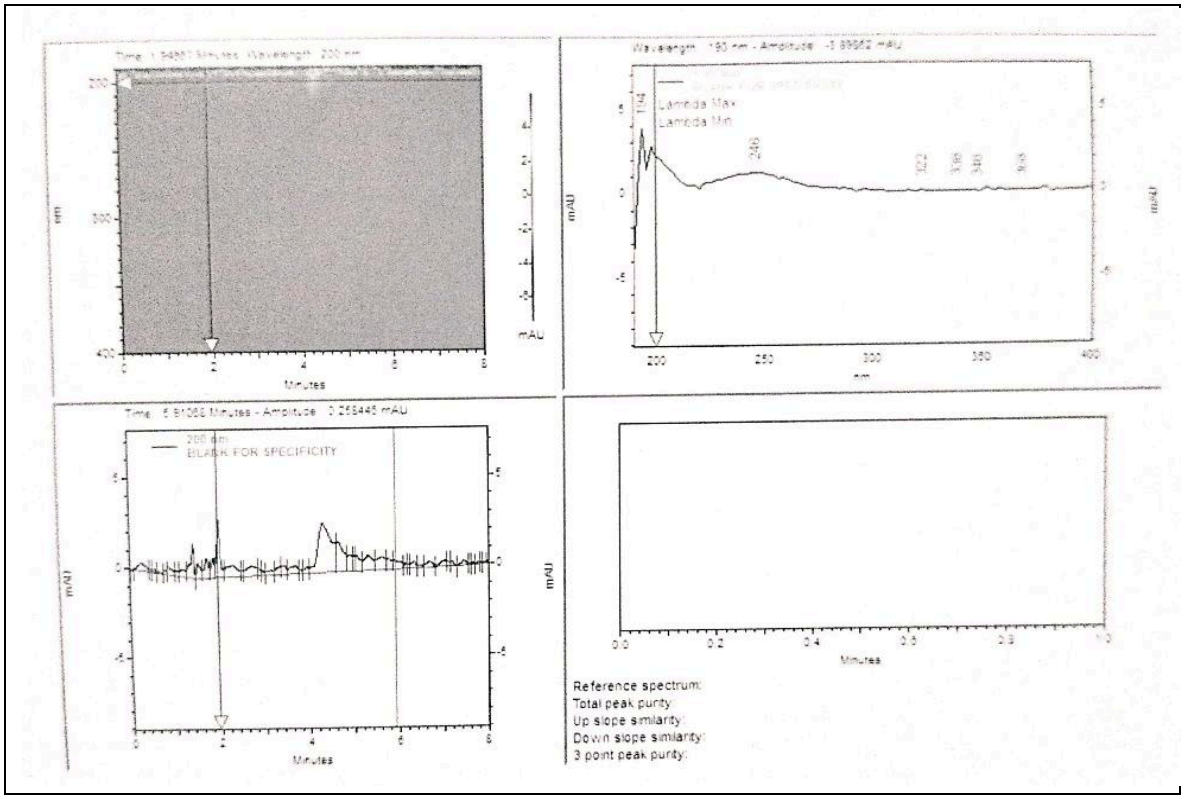


Figure No. 23 (a): Blank for specificity

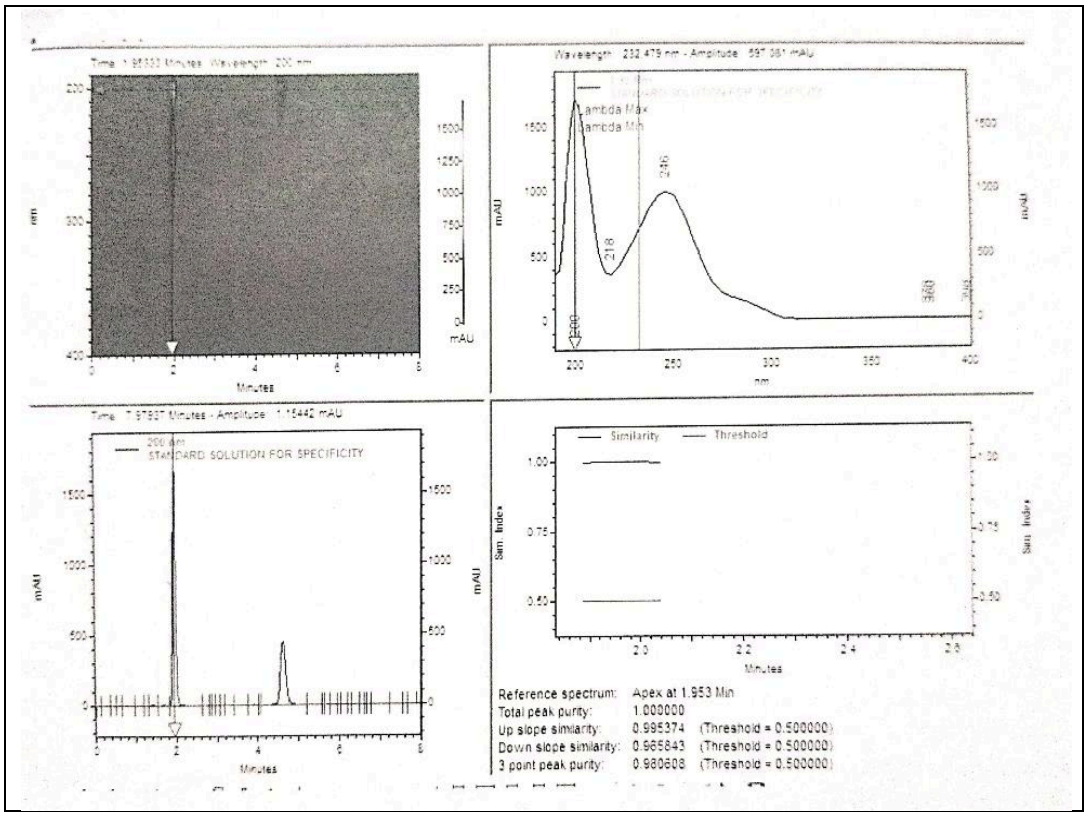


Figure No. 23 (b): Standard solution for specificity of paracetamol

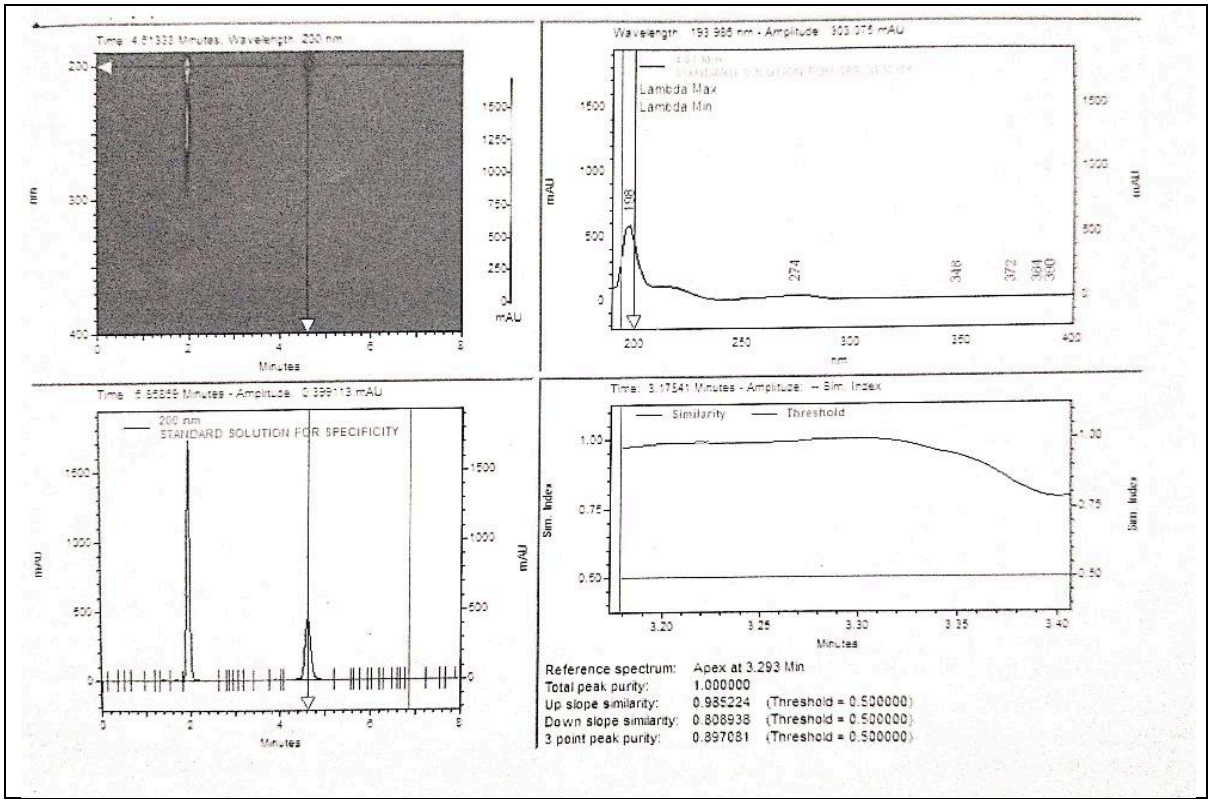


Figure No. 23 (c): Standard solution for specificity tapentadol

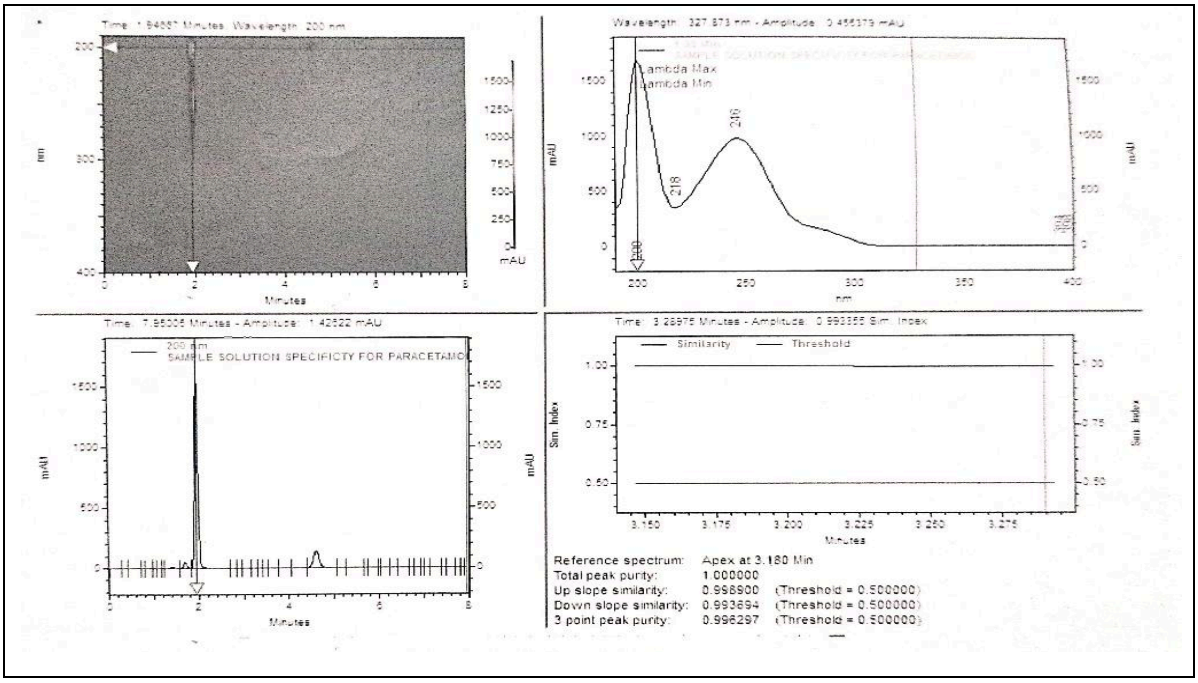


Figure No. 23 (d): Sample solution specificity for paracetamol

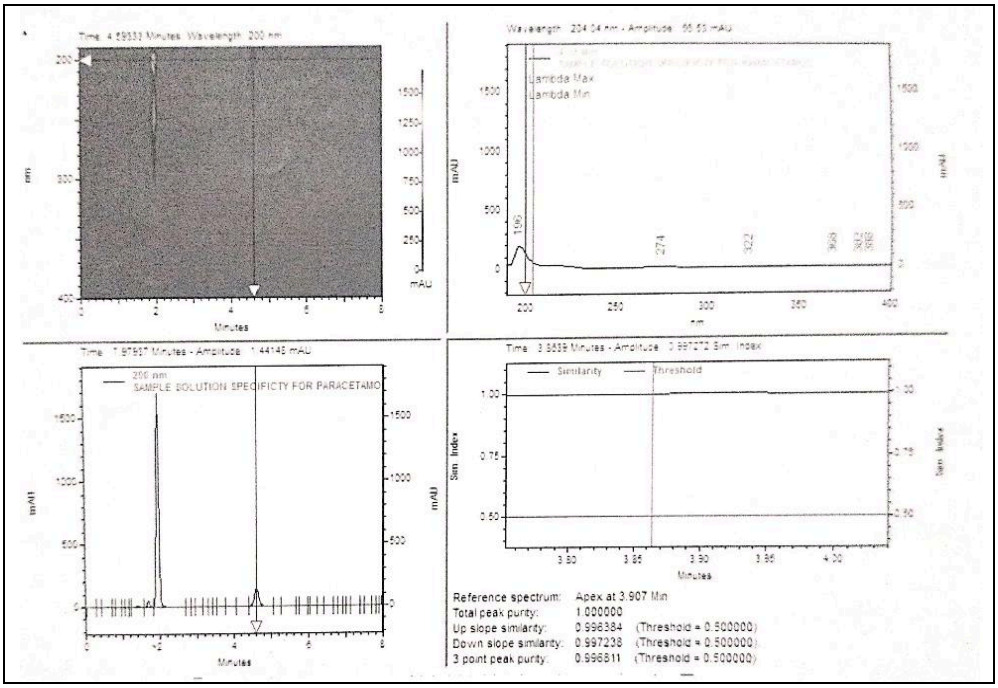


Figure No. 23 (e): Sample solution specificity for tapentadol

Robustness:

It showed durability by carefully adjusting the method's chromatographic settings, assessing the system analysis parameters from the standard solution, and calculating the absolute variation between the mean % assay of various conditions and the method condition from the sample solution. The ability of the analytical approach to withstand minor but intentional changes in process is measured by its robustness[55,56,57]. The % RSD of paracetamol and tapentadol are mention in the Table 12 (a) and (b), Fig. 24 (a),(b),(c),(d),(e) and (f).

Table No.12 (a): Robustness study for paracetamol by Change in wavelength, flow Rate and column temperature

Drug	Parameters	Variance	RT	Area	%SD	%RSD
PARACETAMOL	Wavelength	-2	1.98	2590579	6517.01	0.251
			1.97	2596343		
			1.98	2595360		
			1.97	2596777		
			1.97	2581280		
	Wavelength	+2	1.97	2049629	2144.5	0.105
			1.97	2048635		
			1.97	2053832		
			1.97	2050442		
			1.98	2052611		
	Flow Rate	-2	2.47	2835892	2584.62	0.091
			2.48	2832544		
			2.48	2837284		
			2.48	2836442		
			2.47	2840596		
	Flow Rate	+2	1.65	1890031	1980.221	0.105
			1.65	1893318		
			1.65	1890296		
			1.65	1888694		
			1.64	1888280		
	Column Temperature	-2	1.99	2255810	1129.8	0.050
			1.99	2256179		
			1.99	2258049		
			1.99	2257494		
			1.99	2258352		

	Column Temperature	+2	1.95	2284209	3447.04	0.151
			1.95	2288837		
			1.95	2284484		
			1.96	2281849		
			1.95	2279591		

Table No.12 (b): Robustness study for Tapentadol by Change in wavelength, flow rate and column temperature

Drug	Parameters	Variance	RT	Area	%SD	%RSD
TAPENTADOL	Wavelength	-2	4.85	464052	**3689.72**	**0.791**
			4.85	465403		
			4.85	465241		
			4.85	465479		
			4.85	459360		
	Wavelength	+2	4.84	501191	**626.17**	**0.125**
			4.83	501561		
			4.83	502685		
			4.84	502323		
			4.84	502390		
	Flow Rate	-2	6.09	608840	**824.254**	**0.135**
			6.11	610452		
			6.10	609834		
			6.11	610528		
			6.10	610969		
	Flow Rate	+2	3.99	412185	**2381.376**	**0.580**
			3.99	411576		
			4.01	409476		
			4.01	407468		
			3.98	413505		
	Column Temperature	-2	4.97	472900	**2156.1**	**0.454**
			4.97	475060		
			4.97	476150		
			4.97	478102		
			4.97	473192		
	Column Temperature	+2	4.69	500448	**675.20**	**0.135**
			4.68	501216		
			4.69	501529		
			4.69	500212		
			4.69	499943		

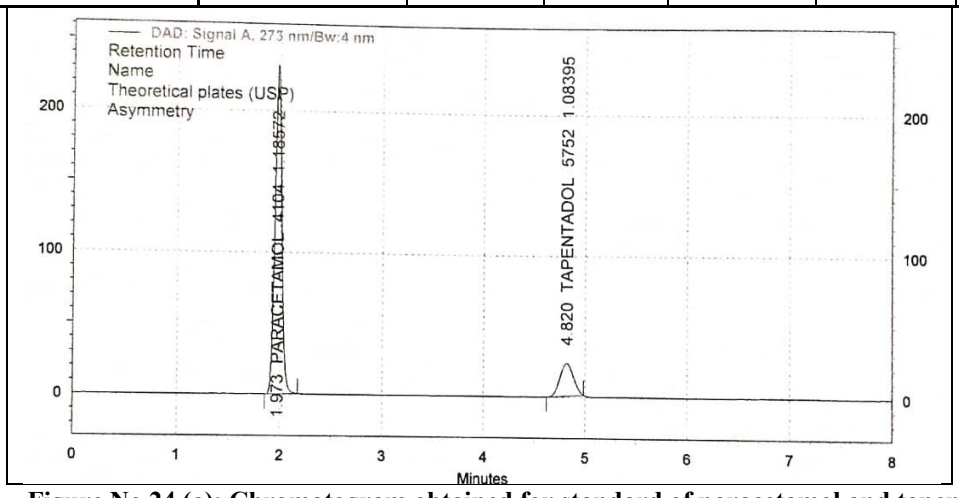


Figure No.24 (a): Chromatogram obtained for standard of paracetamol and tapentadol

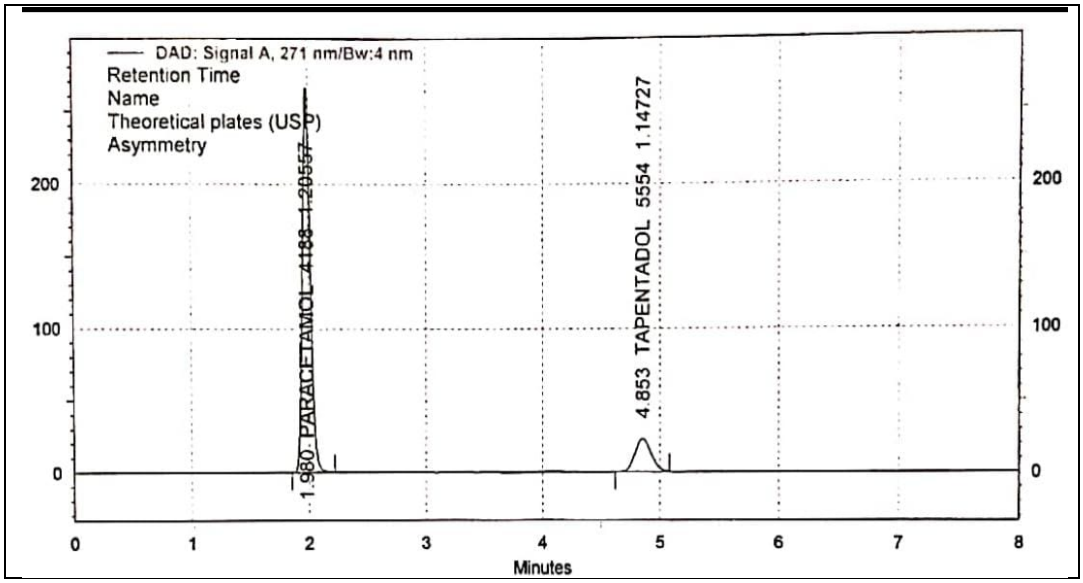


Figure No.24 (b): Chromatogram obtained for low flow rate

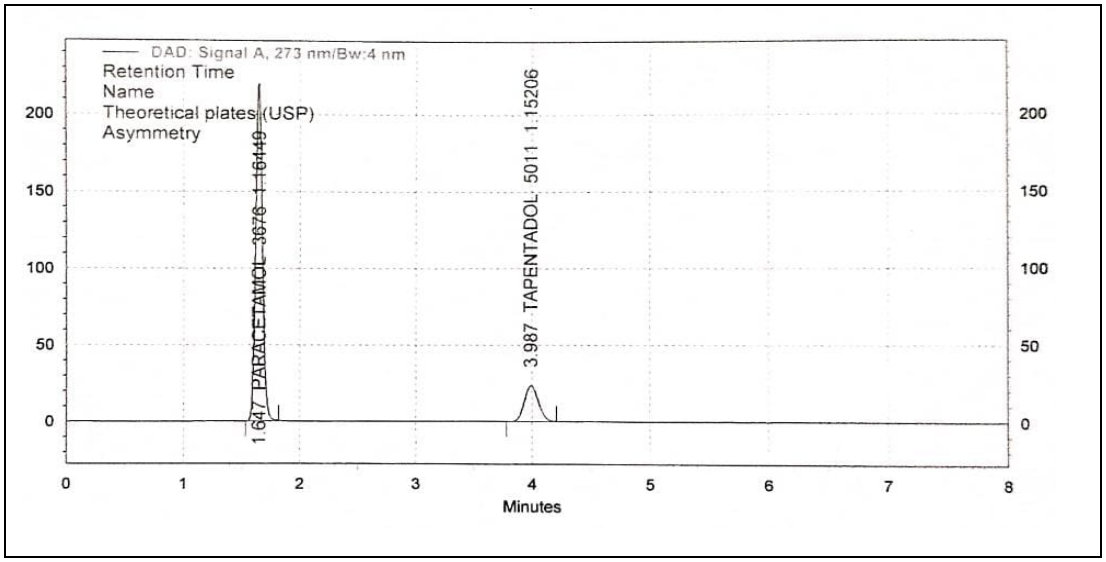


Figure No.24 (c): Chromatogram obtained for high flow rate

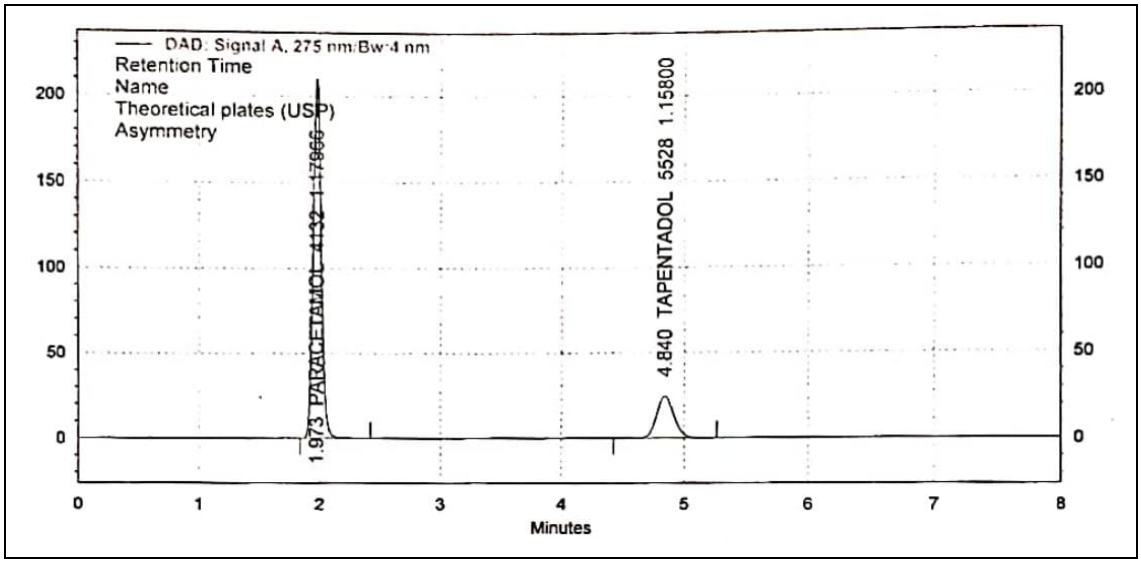


Figure No.24 (d): Chromatogram obtained for High Wave Length

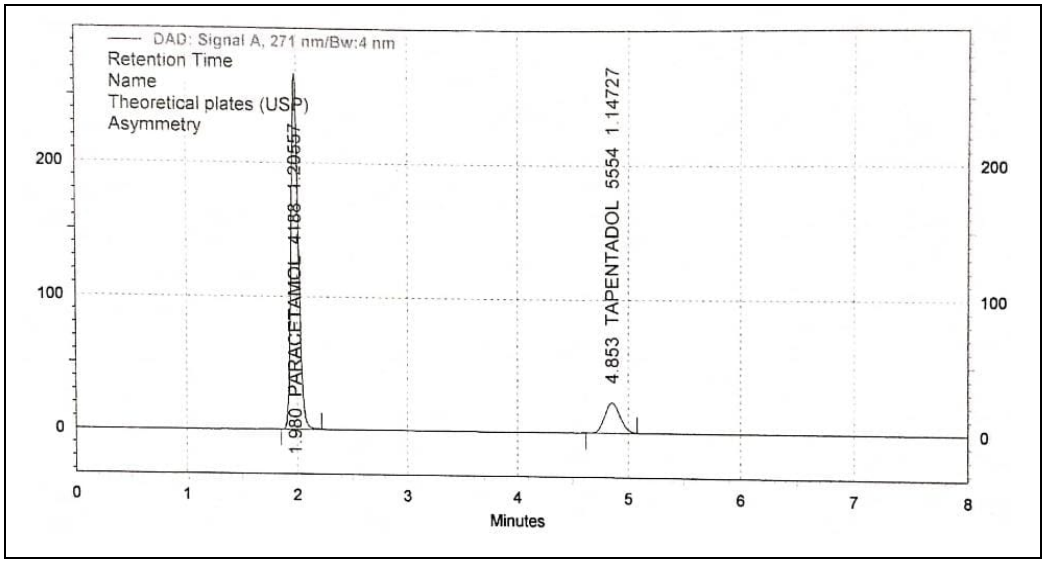


Figure No.24 (e): Chromatogram obtained for low wave length

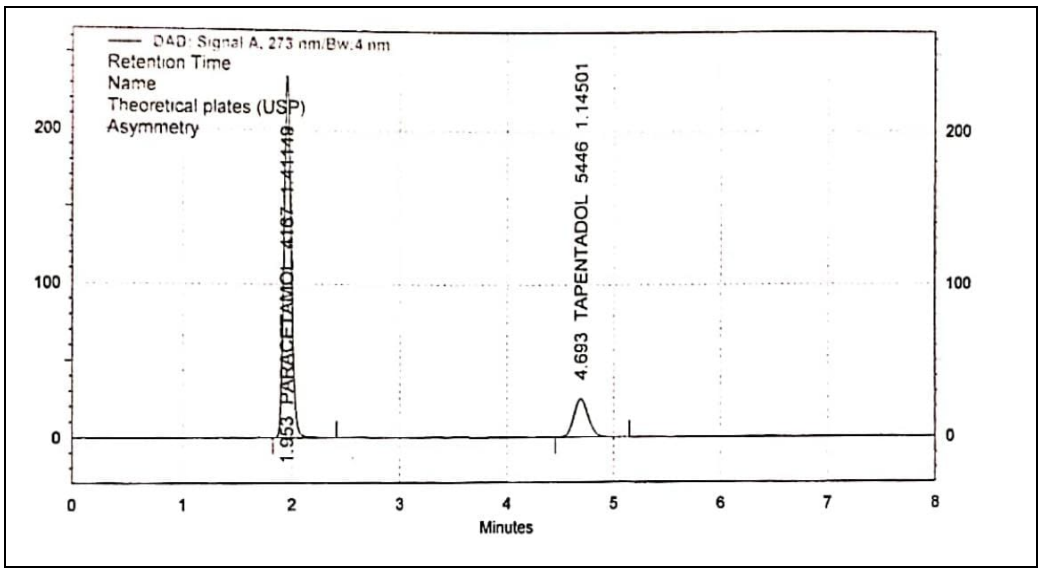


Figure No.24 (f): Chromatogram obtained for high temperature

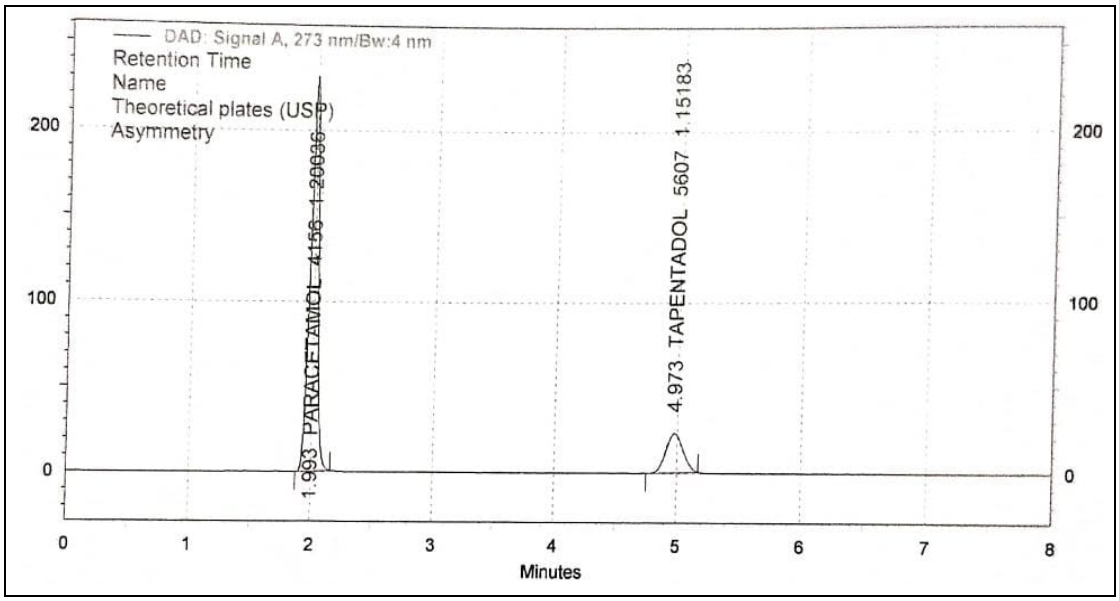


Figure No.24 (g): Chromatogram obtained for low temperature LOD: (Limit of Detection)

The lower concentration that produces a discernible peak area is known as LOD[54]. Determined the presence of water content in paracetamol and tapentadol. Results are mention in Table 13 and Fig.25.

TableNo.13: LOD of Paracetamol and Tapentadol				
Paracetamol			**Tapentadol**	
S. NO.	**RT**	**AREA**	**RT**	**AREA**
1	1.92	72754	4.21	15610
2	1.91	73191	4.21	15463
3	1.92	73153	4.21	15531
4	1.93	73234	4.23	15573
5	1.92	73121	4.21	15858
6	1.91	73031	4.20	15234
Avg.	**1.92**	**73081**	**4.21**	**15545**
SD	**0.0075**	**174.20**	**0.0098**	**203.28**
RSD%	**0.392**	**0.2384**	**0.2334**	**1.3077**

Limit of Quantification (LOQ):

For lowest concentrations of chemicals in sample matrices, the quantitation limit is a parameter of quantitative assays that is specifically utilized to recognized contamination and/or degradation substance present in the paracetamol and tapentadol**.** Results are mention in Table 14 and Fig.26.

TableNo.14: LOD of Paracetamol and Tapentadol				
Paracetamol			**Tapentadol**	
S. No.	**RT**	**Area**	**RT**	**Area**
1	1.92	224287	4.21	48735
2	1.91	224170	4.19	48644
3	1.91	224552	4.18	48433
4	1.92	224675	4.20	49524
5	1.92	224490	4.20	48999
6	1.91	224774	4.20	49151
Avg.	**1.92**	**224491**	**4.20**	**48914**
SD	**0.0055**	**229.05**	**0.0103**	**392.89**
RSD%	**0.286**	**0.1020**	**0.2461**	**0.8032**

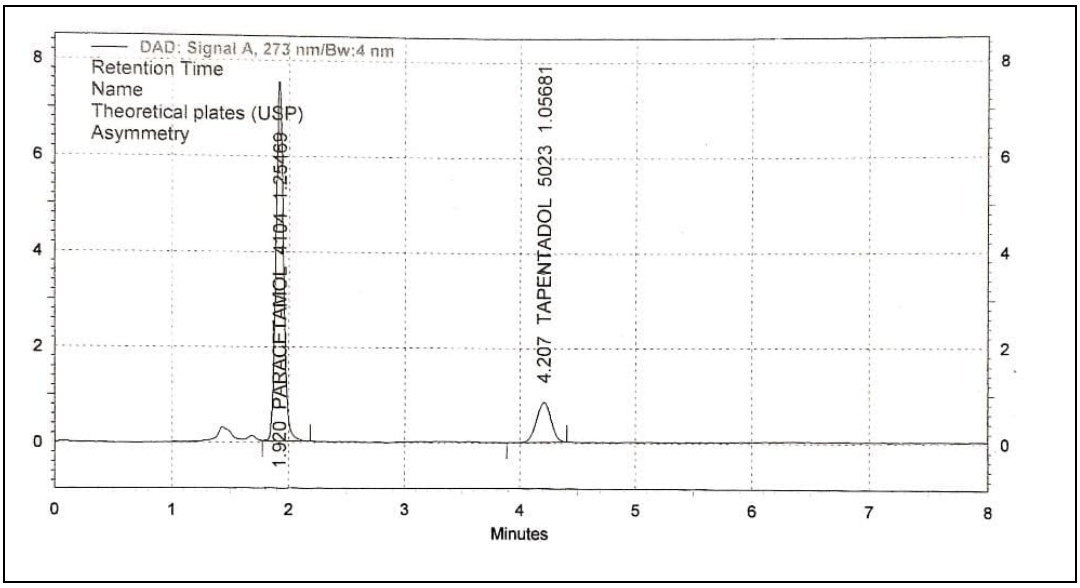


Figure No.25: Chromatogram of LOD obtained for paracetamol and tapentadol

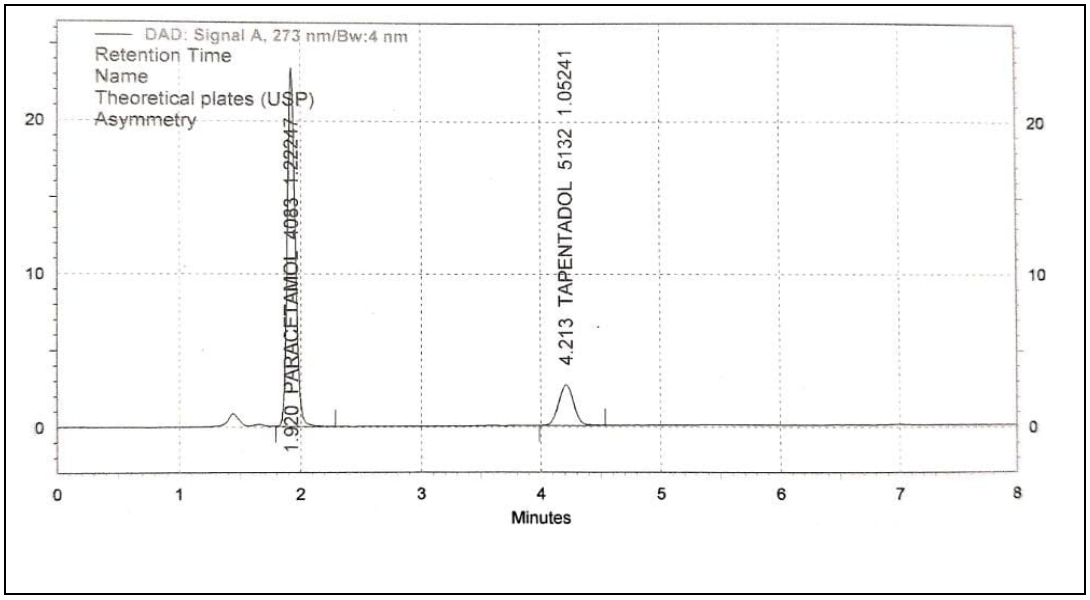


Figure No.26: Chromatogram of LOQ obtained for paracetamol and tapentadol.

Drug Assay in Formulations using an Established Technique:

Utilized the improved RP-HPLC approach to measure both medicines simultaneously in order to determine if the new method could be reliably applied for genuine tablet compositions. And created a test sample at the same quantities as the standard solution, which contained 52.2µg/ml of paracetamol and 29.1µg/ml of tapentadol. Then, under the previously established chromatographic conditions, we injected 10µl of the standard solution five times and the sample solution two times with an auto-injector. The average peak areas of the standard and test samples were then compared using the following calculation to determine the strength of the tablet formulation [58,59,60]. Results are mention in Table 15.

Assay= Test are × standard weight /Standard area × test weight × 100

Table No.15: Assay Study of Standard and Test Sample

S.No	Sample	Paracetamol	Tapentadol	Avg. Area 1	Avg. Area 2
1	**STD**	2267006	489951		
2	**STD**	2267762	489765		
3	**STD**	2267519	493946	**2266686.8**	**490622.8**
4	**STD**	2265580	489099		
5	**STD**	2265567	490353		
6	**SAMPLE**	2366644	500009	**2370977**	**500115.5**
7	**SAMPLE**	2375310	500222		

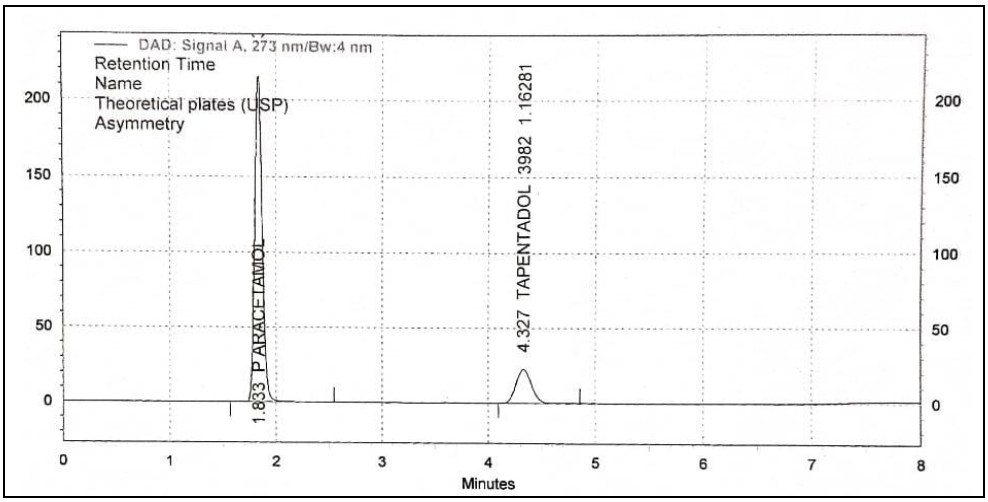


Figure No.27: Standard Chromatogram obtained for assay of paracetamol and Tapentadol

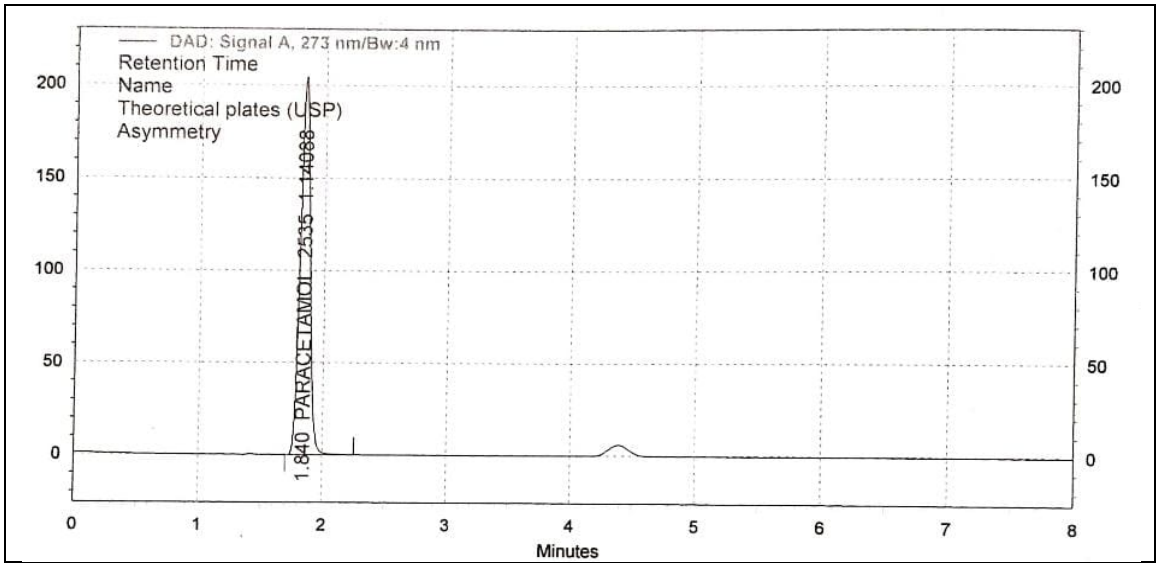


Figure No.28: Standard Chromatogram obtained for assay of paracetamol

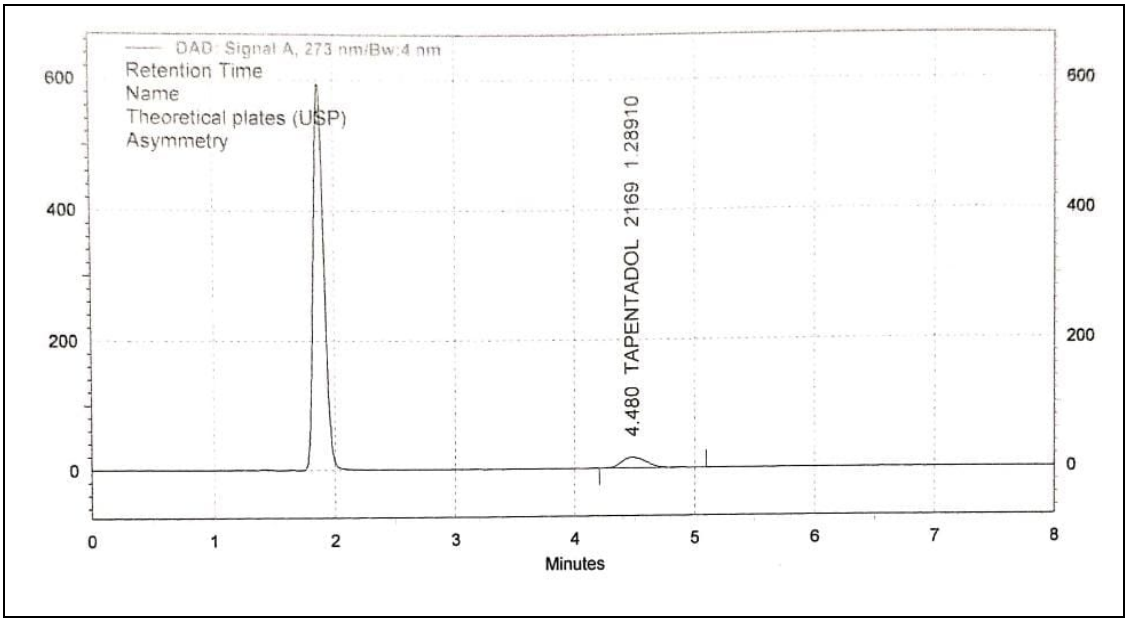


Figure No.29: Standard chromatogram obtained for assay of tapentadol

Stability Study of Paracetamol and Tapentadol:
Since their peak areas varied very little over a 48-hour period, it was determined that both paracetamol and tapentadol were stable under test conditions. (%RSD of paracetamol 0.723 and tapentadol 0.785. The stability data of paracetamol and tapentadol are shown in Table 16 and Fig 30,31,32 and 33.

Table No.16: Stability Study for Paracetamol and Tapentadol

Paracetamol		**Tapentadol**	
Time (hrs)	**Area**	**Time (hrs)**	**Area**
1	2276226	1	498896
3	2287725	3	500784
6	2275018	6	497606
9	2278865	9	498900
12	2272682	12	497532
15	2278445	15	499085
18	2278430	18	499323
21	2274318	21	498034
24	2271974	24	497441
27	2306433	27	505423

30	2274876	30	498998
33	2303712	33	504891
36	2325146	36	510222
39	2312489	39	506801
42	2299660	42	505135
45	2299441	45	503495
48	2276762	48	498305
MEAN	**2287777**	**MEAN**	**501228**
SD	**16550.97**	**SD**	**3932.92**
% RSD	**0.723**	**% RSD**	**0.785**

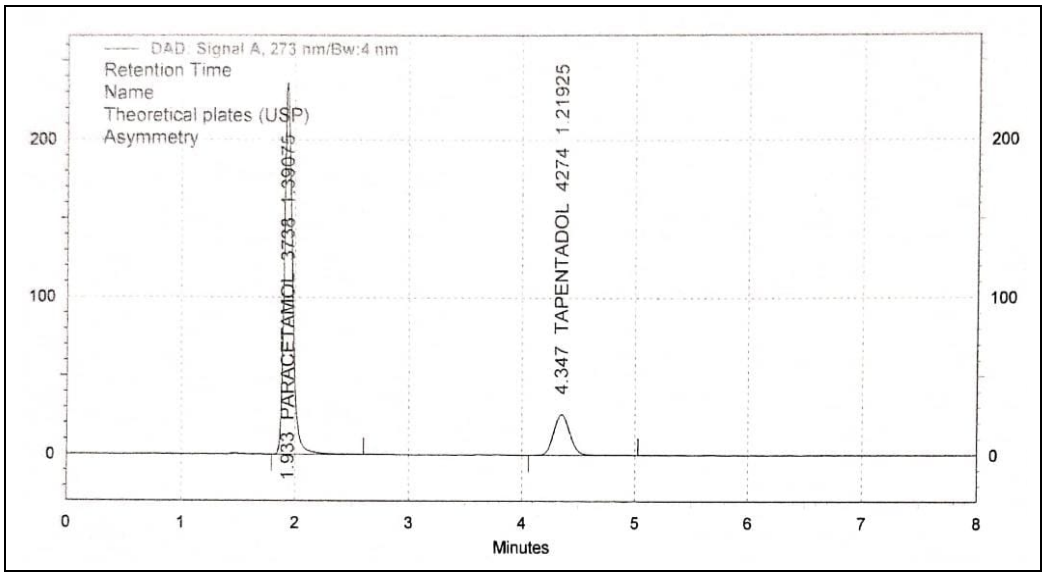


Figure No.30: Chromatogram obtained for stability standard of Paracetamol and Tapentadol

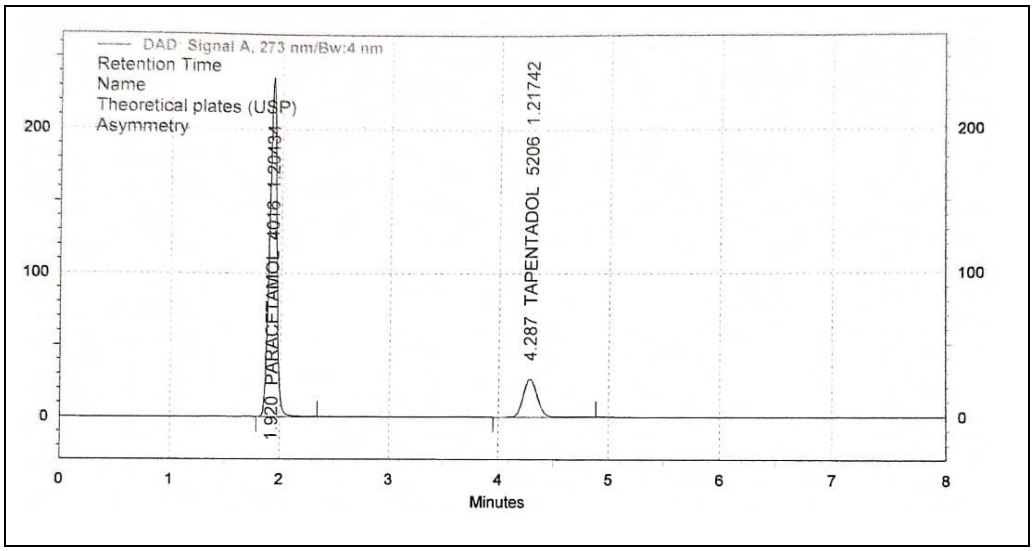


Figure No.31: Chromatogram obtained for (1hour) stability of Paracetamol and Tapentadol test samples

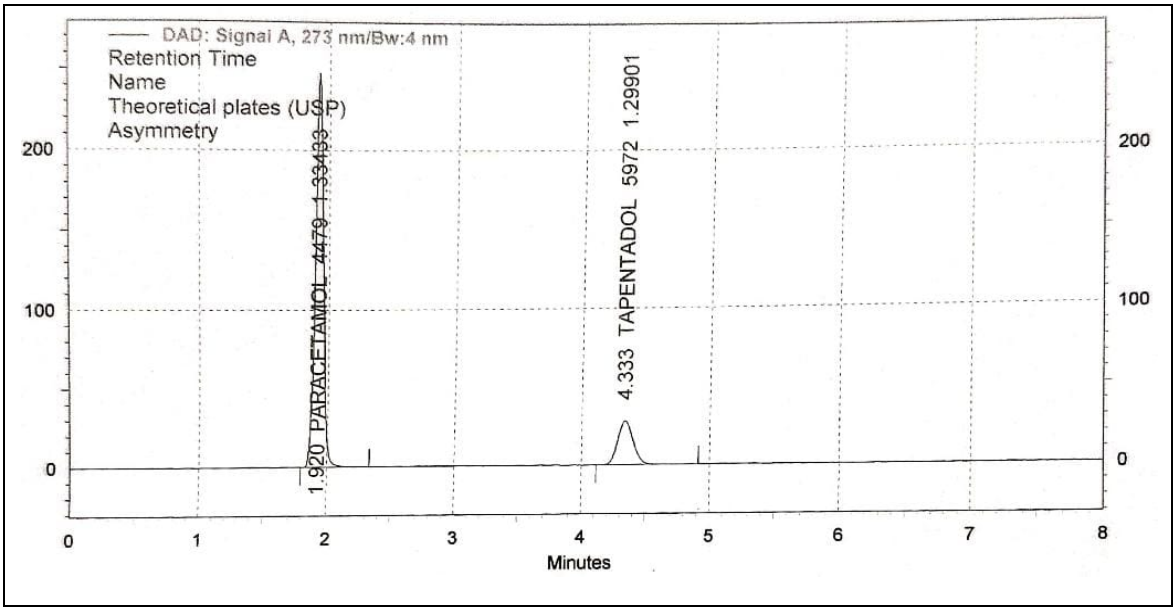


Figure No.32: Chromatogram obtained for (24 hour) stability of Paracetamol and Tapentadol test sample

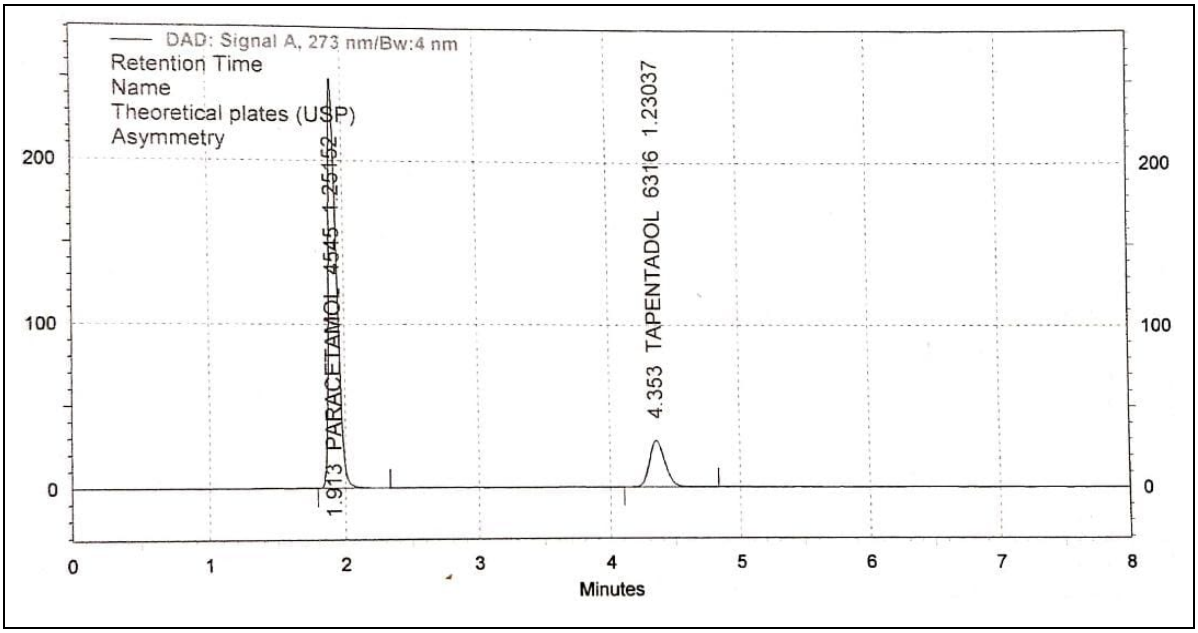


Figure No.33: Chromatogram obtained for (48 hours) of Paracetamol and Tapentadol test sample

CONCLUSION:

This study created and verified a simple, highly accurate, and precise Revers-Phase High Performance Liquid Chromatography/Ultra Violate analytical technique for the concurrent identification of tapentadol and paracetamol. Within the chosen concentration range, validation factors including accuracy, precision, and specificity were assessed. Good repeatability was shown by the % RSD readings being within bounds. It was discovered that the created approach was robust, sensitive, and dependable. As a result, it may be effectively utilized for regular control of quality and examination of pharmaceutical formulations.

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