STABILITY ASSESSMENT OF PREGABALIN AND ITS RELATED SUBSTANCES BY RP-HPLC

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Abstract:

Pregabalin, approved by the United States Food & Drug Administration for treating neuropathic pain associated to spinal cord injury, post therapeutic neuralgia, fibromyalgia, and diabetic peripheral neuropathy. The primary aim of this article focused upon the stability assessment of pregabalin and its related substances by HPLC through accelerated and long-term stability studies in diverse environments including light, temperature and humidity to identify potential degradation pathways. Evaluation of Accelerated stability studies, Long-term stability studies, Assay method of Enantiomeric purity were done in this study. Inertsil ODS 3V C18, 250 x 4.6mm, 5µmwas used for assay method & for related substances with 30min run time and 11.033 min retention time & 45min run time with 7.317 min retention time for Pregabalin respectively. Hypersil BDS C18, 250x4.6 mm, 5µm was employed for Enantiomeric purity for 40min run time at 10.731 min retention time. All the results were found within the acceptance criteria. The related substances in pregabalin bulk drugs were evaluated for their stability in relation to the active ingredient. The stability data obtainedfrom the studies to formulate recommends for optimal storage conditions and establish expiration dates for pregabalin products.

Key words: Pregabalin, Accelerated and Long-term stability studies, Related substances, & Enantiomeric purity.

1. Introduction:

Pregabalin was sold with the brand Lyrica, an anticonvulsant analgesic & anxiolytic amino acid medication for opioid withdrawal, epilepsy, fibromyalgia, neuropathic pain, generalized anxiety isorder(GAD), & restless legs syndrome [1-12]. It possesses anti-allodynic properties. This drug is a GABA analogue that has the derivatives of γ- aminobutyricacid, an inhibitory neurotransmitter. Pregabalin inhibits certain calcium channels [13-25]. The IUPAC name of Pregabalin is (S)-3-(Aminomethyl)-5-methylhexanoicacid with 159.23g/mol molecular weight & chemical formula $C_{18}H_{17}NO_2$. It reduces pain when administered prior to surgery but results in more sedation & visual disturbances. It is taken orally and stored in airtight containers at NMT25° [26-30].

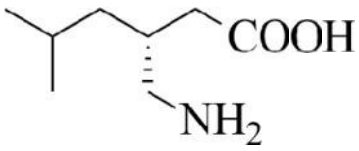

Figure1. Chemical structure of Pregabalin

A review of the literature [31-41] on the pregabalin drug concerning its physical and chemical properties, as well as various analytical methods reported for the estimation of pregabalin, served as the foundation for the assessment of accelerated stability & long-term stability with analytical RP-HPLC for the estimation of Pregabalin in bulk form according to ICH Q1A(R) & Q1E guidelines [42] for its intended application.

2. Experimental work:

2.1 Chemicals and solvents:

Methanol & Acetonitrile of HPLC grade was purchased from JT. Baker, Ernakulam, Kerala, India. Millipore milli-Q water purification system was used for HPLC grade water. Dibasic ammonium phosphate, Orthophosphoric acid, Triethylamine, Acetone, Sodium bicarbonate, Marfey's Reagent from Merck's Peenya Site in Bangalore, India.

2.2 Pregabalin Impurity Standards & Working Standards:

The reference samples of Pregabalin, Mandelic Acid impurity standards, Pregabalin Related Compound A&C impurity standards were provided as a gift sample from Harenzo Life Sciences Private Limited, Hyderabad.

2.3 Instrumentation:

Shimadzu HPLC system having Ai-series LC2050C3D model equipped with an auto sampler with an injection volume of 20 µL was employed. Accuris™ Precision Balance was used for weighing the materials. Digital pH meter (Mettler Toledo, Powai, Mumbai, India) was used for all pH measurements. Ultrasonic bath (Bandelin SONOREX™) was used for sonication of the samples.

Chromatographic conditions: Assay method

Column type	: InertsilODS3VC18,250x4.6mm,5µm
Flow rate	: 1.0ml/min
Column Temp.	: 25ºC
Autosampler	: 10 ºC
Wavelength	: 205 nm
Run time	: 30 minutes
Elution	: Isocratic
Diluent	: Mobilephase

Chromatographic conditions: RelatedSubstances

Column type	: InertsilODS3VC18,250X4.6mm,5µm
Flow rate	: 1.0ml/min
Column Temp.	: 35ºC
Auto sampler	: 10 ºC

Wavelength	: 210nm
Run time	: 45 minutes
Elution	: Gradient
Diluent	: Water

Chromatographic conditions: Enantiomeric Purity method

Column	: Hypersil BDSC18, 250x4.6mm, 5µm
Flow rate	: 2.0mL/min
Column temperature	: 25ºC
Autosampler	: 10 ºC
Wavelength	: 340 nm
Run time	: 40 minutes
Elution	: Isocratic
Diluent	: Water+Acetone (1:1)

2.4 Solution preparations

2.4.1 For Assay method:

Preparation of Mobile phase:

Acetonitrile and water was prepared in 5:95 ratios and filtered through 0.45µm membrane filter or one with finer porosity.

Preparation of Standard solution:

100.0 mg of Pregabalin Standard was weighed accurately and transferred to 50ml volumetric flask. 20 ml of diluent was added to it and sonicated for a while and the final volume was made till the mark with the same diluent.

Sample solution:

100mg Pregabalin sample was weighed accurately into volumetric flask of 50ml. 20ml of diluent was added and sonicated. The final volume was made up with the same diluent.

2.4.2 For Related Substances

Buffer preparation:

Dibasic ammonium phosphate of 5.28 g was weighed accurately in Milli Q water 1000ml & then the solution was adjusted to 6.5±0.05 with dil. Orthophosphoric acid and filtered using 0.45µm membrane filter.

Mobile phase-A for RS method

Mixture of Methanol and Buffer was prepared in 20:80 ratio.

Mobile phase-B for RS method

Methanol and acetonitrile was mixed in10:90 ratio.

Sensitivity solution:

Weighed accurately and transferred about 5.0 mg of the Pregabalin standard to a 100ml volumetric flask and sonicated. 1ml of the solution was taken and diluted to 10ml with the diluent.

Standard solution-1:

2.5 mg of Mandelic Acid impurity was taken and weighed into 10ml volumetric flask and made to the mark with water.

Standard solution-2:

Weighed accurately each 5mg of Pregabalin and Pregabalin Related Compound C impurity standards into a 10ml of volumetric flask and then diluted to the mark with water.

Standard solution:

0.4ml of standard solution-1 & 1ml of standard solution-2 was transferred into 10ml volumetric flask and made till the mark with the diluent.

Sample solution:

100mg sample was accurately weighed into10ml of volumetric flask. 5ml of diluents was added and shaken for 2 to 3 minutes by vortex mixer and made up to the mark with diluent.

2.4.3 Enantiomeric purity method

Buffer preparation:

Transferred 7.2mL of Triethyl amine accurately in milli Q water of 1000ml and the pH was adjusted to 3.0 ± 0.05 with dil. Orthophosphoric acid. The solution was filtered with 0.45µm membrane filter.

Mobile phase:

Acetonitrile & buffer was made in 38:62 ratio.

Derivatizing reagent solution:

30mg of Marfey's reagent was transferred into 10ml volumetric flask and made to the volume with Acetone.

Preparation of 1M Sodium bicarbonate solution:

8.4 gms of $NaHCO_3$ was weighed and dissolved in 100ml water.

Preparation of Blank solution:

Transferred each 0.5 mL of mixture solution (water and Acetone) in the ratio 1:1 into 5 ml volumetric flask and 0.3mLof derivatizing reagent and 50µLof 1M Sodium bicarbonate was added and heated at 55° for about 1 h. Later it was cooled to room temperature and made till the mark with water.

Sensitivity stock solution:

5mg of Pregabalin Standard was weighed into 100ml volumetric flask previously filled with 50ml water and made up to volume with acetone. It was further diluted 1.0 mL into 100ml volumetric flask with 50mL water and finally made till mark with acetone.

Preparation of Sensitivity solution:

1ml of sensitivity stock was transferred to 10ml volumetric flask. 0.6 mL of Derivatizing reagent and 0.1mL of 1M Sodium bicarbonate was added and heated at 55° for about 1 h. Later it was cooled to room temperature and made up finally with water.

Standard stock solution-1:

Weighed about 5mg Pregabalin Standard to 5ml volumetric flask containing 2.5ml water and made till the volume with acetone.

Standard solution-2:

5mg of Pregabalin Related Compound A was weighed into 10ml volumetric flask with 5ml water to dissolve and made up with acetone. Further it was diluted1.0mL in 10ml volumetric flask and 5mL of water was added and filled to mark with acetone.

Standard solution:

0.5ml each of standard stock solutions-1&2 were transferred into a 5ml volumetric flask. 0.3mL of Derivatizing reagent and 50µL of 1M Sodium bicarbonate were added and heated at 55° for about 1hour. The mixture was cooled to room temperature and made up with water.

Sample stock solution:

10mg sample was weighed accurately to 10ml volumetric flask containing 5ml water was added and made up with acetone.

Preparation of Sample solution:

0.5 ml of sample stock solution was transferred into 5ml of volumetric flask. 0.3ml of Derivatizing reagent & 50µL of 1M Sodium bicarbonate was added and heated at 55° for about 1 h. The mixture was allowed to cool at room temperature and the final volume was made till the mark with water.

2.5 Accelerated Stability Studies

The samples were charged in the Stability Chamber to conduct Accelerated Stability Studies. This study lasted for 6 months and samples were withdrawn for testing at specified intervals of 0, 1, 2, 3, and 6 months. Testing was conducted with Assay, Related substances and Enantiomeric purity by HPLC and the stability data was generated in accordance with shelf-life specifications and standard test procedures. The data was recorded and evaluated accordingly.

2.6 Long-term Stability Studies

The samples were charged in the Stability Chamber to conduct Long-term Stability Studies. This study lasted for 16 months and the samples were withdrawn for testing at specified time points of 0, 1, 2, 3, 6 9, & 12months. Testing was conducted with Assay, Related substances and Enantiomeric purity by HPLC.

Table 1. Stability testing intervals & tests

S. No	Stability Studies	Stability Condition Temperature & Humidity	Test intervals In Months	Performed test
1.	Accelerated	40°C±2°C& 75%±5%RH	0,1, 2, 3, and 6	Assay, Related substances & Enantiomeric purity
2.	Long-term	25°C±2°C& 60%±5%RH	0, 1, 2, 3, 6,9 & 12	

Table 2. Schedule of analysis over the period of study

Stability Studies	Date of Stability Charging	Due date of Analysis of Month					
		1	2	3	6	9	12
Accelerated	04.09.2022	4.10.2022	04.11.2022	04.12.2022	04.03.2023		
Long term	04.09.2022			04.12.2022	04.03.2023	04.08.2023	04.11.2023

Withdrawal of samples from the stability chambers:

With drawing from the stability samples from the respective stability chambers within ±3 days of sampling due date.

Time period for analysis:

The analysis of the stability sample was performed within a30-dayperiod from the exact date of analysis, and that was the time period for subsequent analyses for the test intervals.

3. Results and Discussion

3.1 Accelerated stability study for HPLC Assay method:

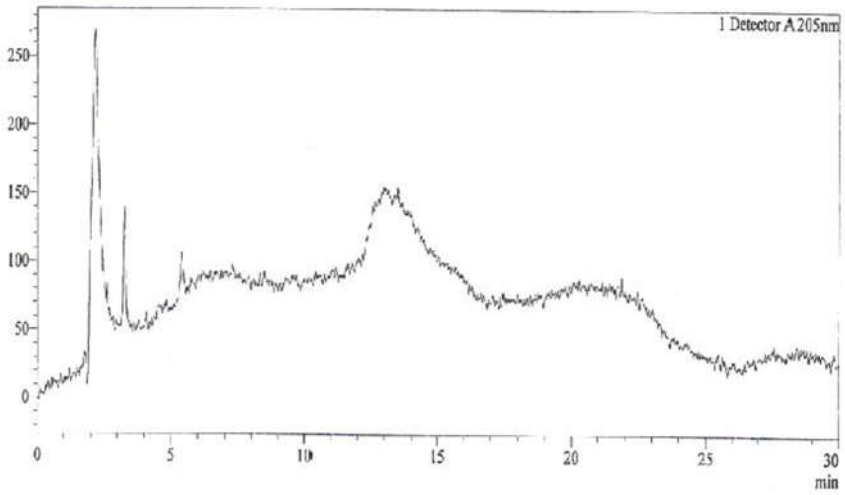

Figure 2. Blank solution chromatogram

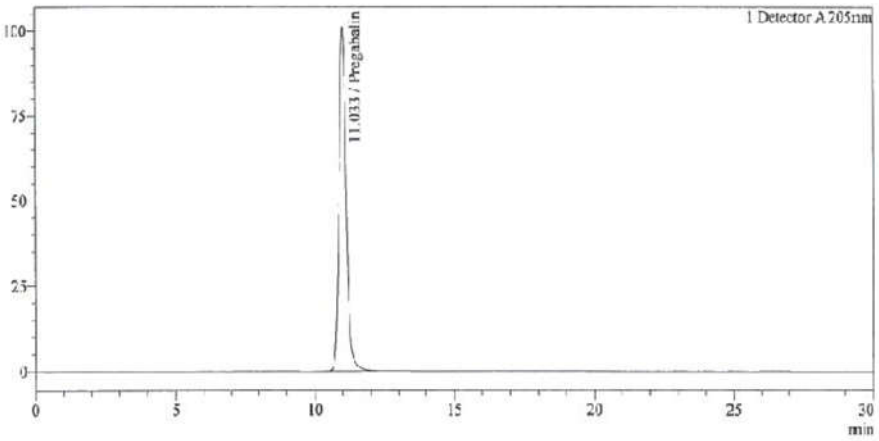

Figure 3. Initial standard chromatogram

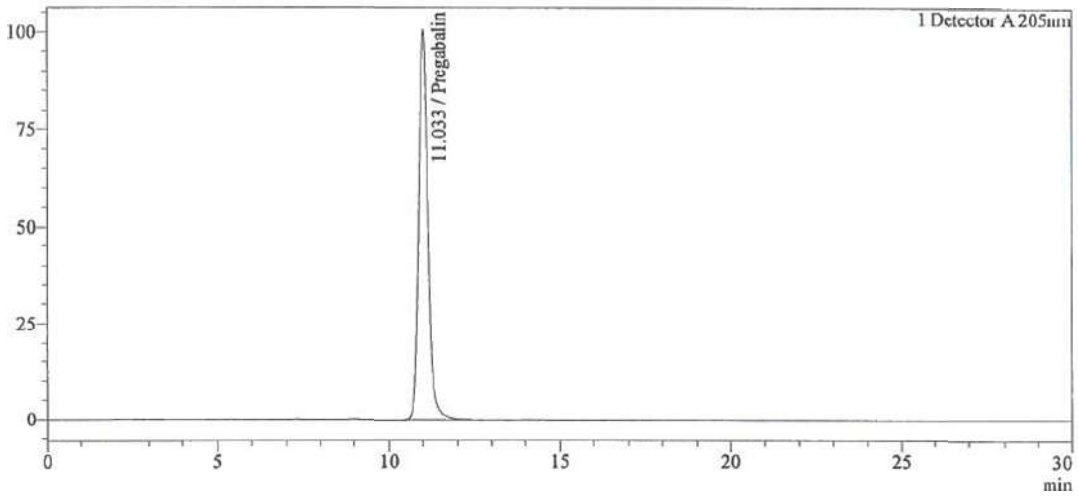

Figure 4. Initial sample chromatogram

3.2 Accelerated stability study for Related substances by HPLC:

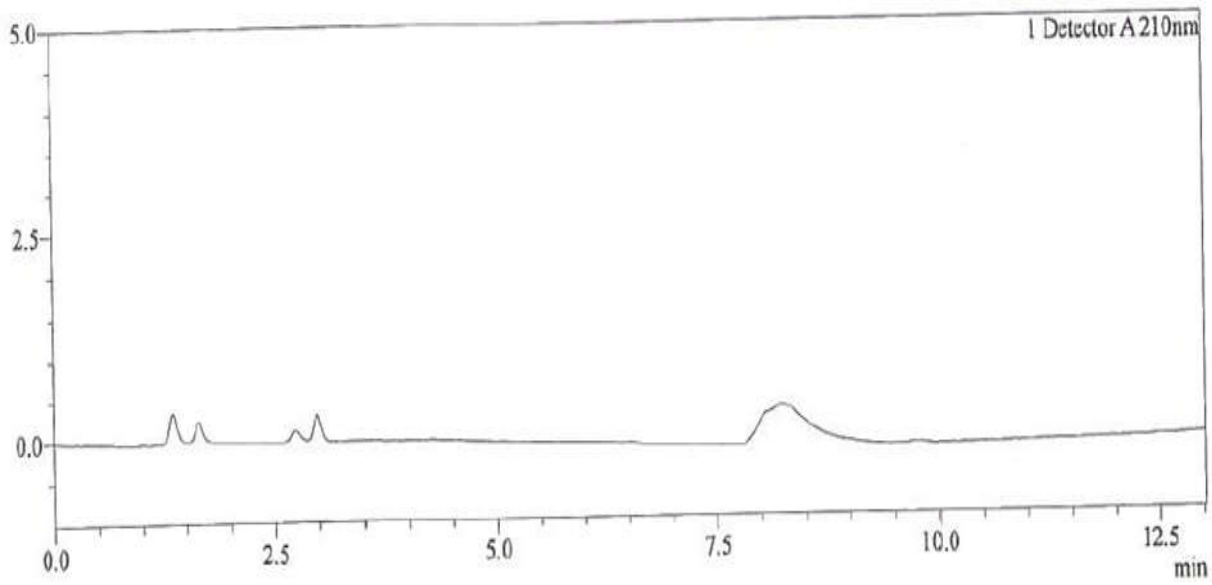

Figure 5. Chromatogram for blank solution

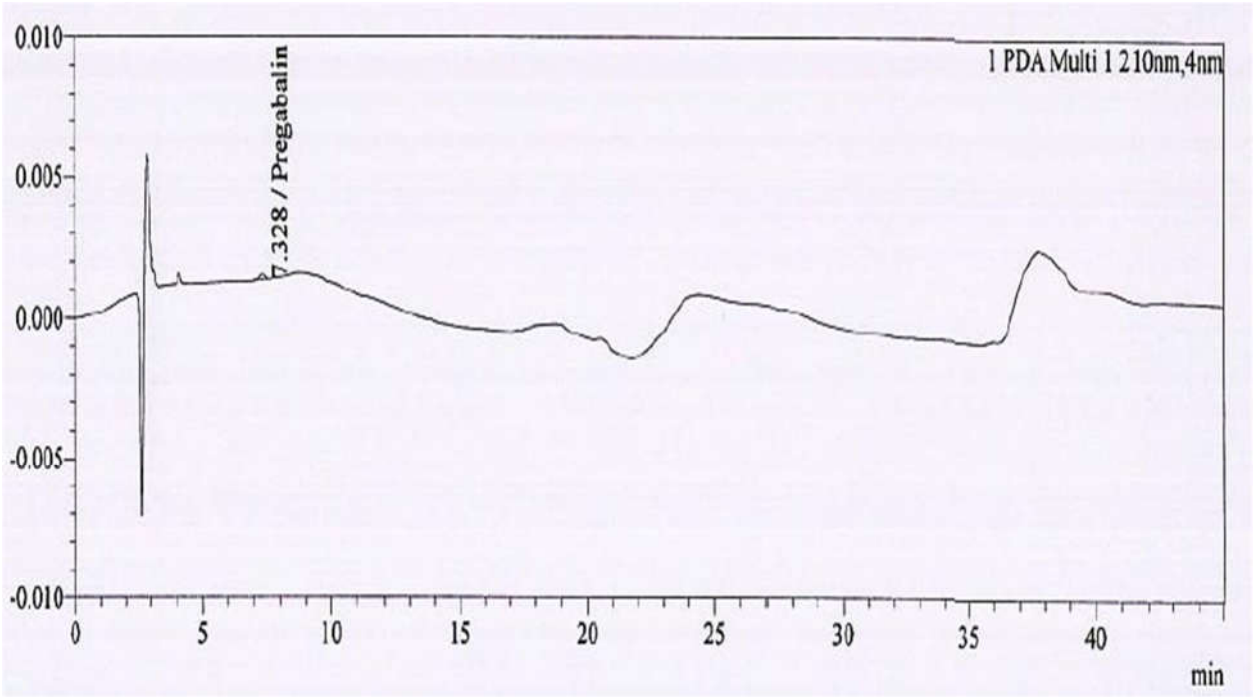

Figure 6. Initial month sensitivity solution chromatogram

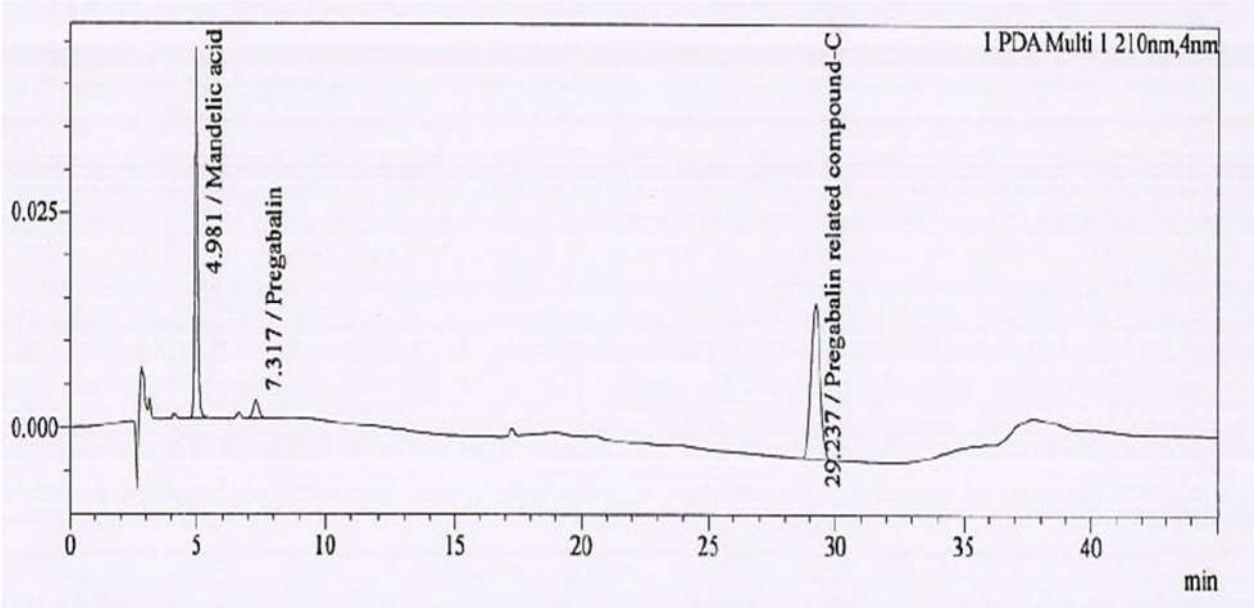

Figure 7. Initial month standard solution chromatogram

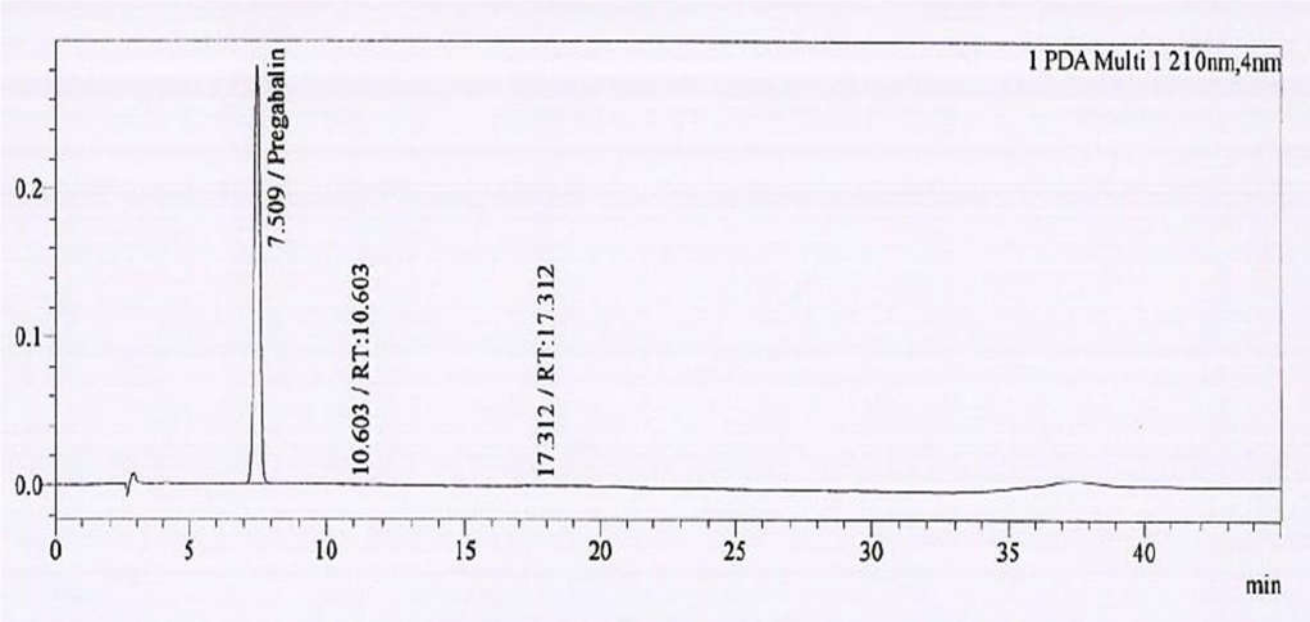

Figure 8. Initial month sample solution chromatogram

3.3 Accelerated stability study for Enantiomeric purity by HPLC:

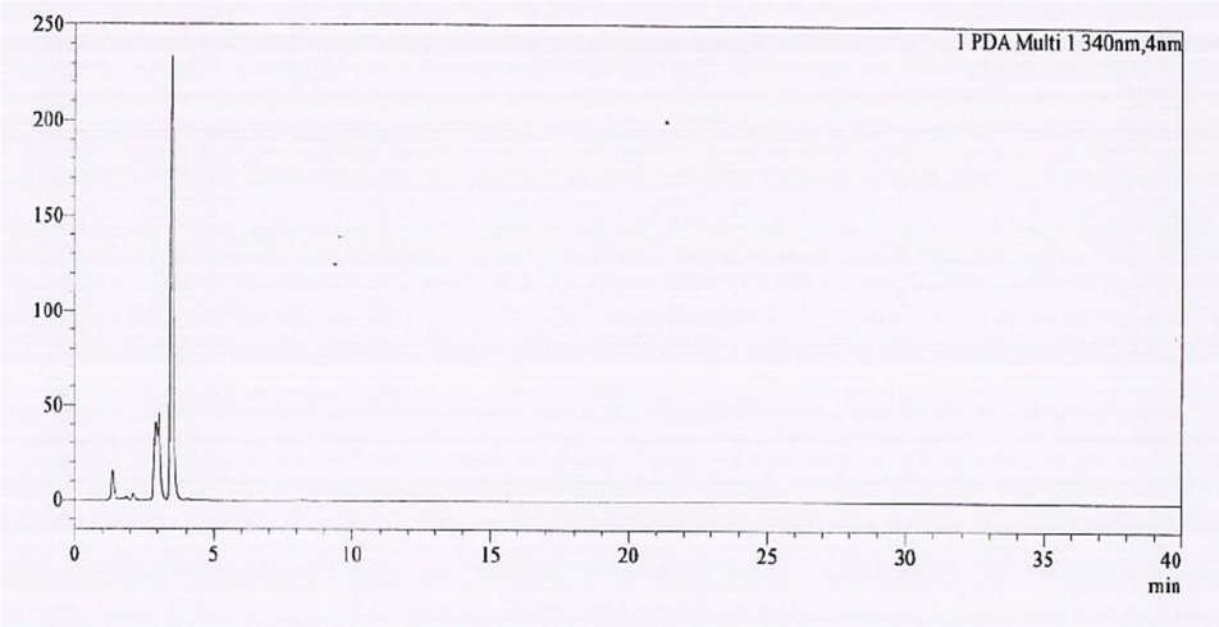

Figure 9. Blank solution chromatogram

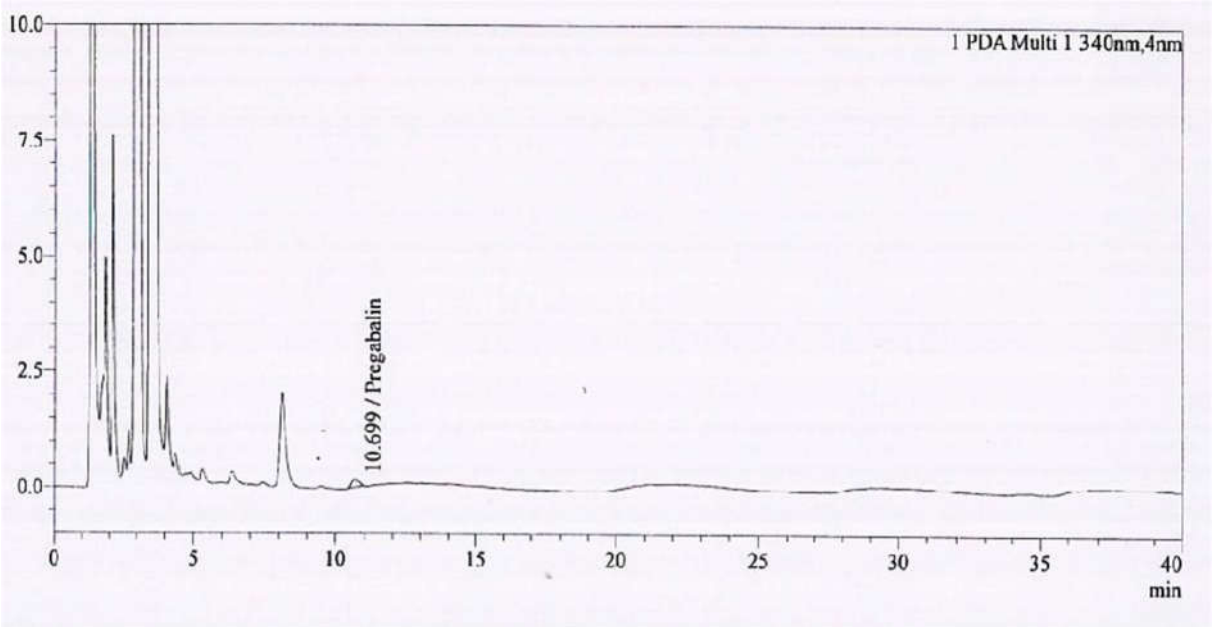

Figure 10. Sensitivity solution chromatogram

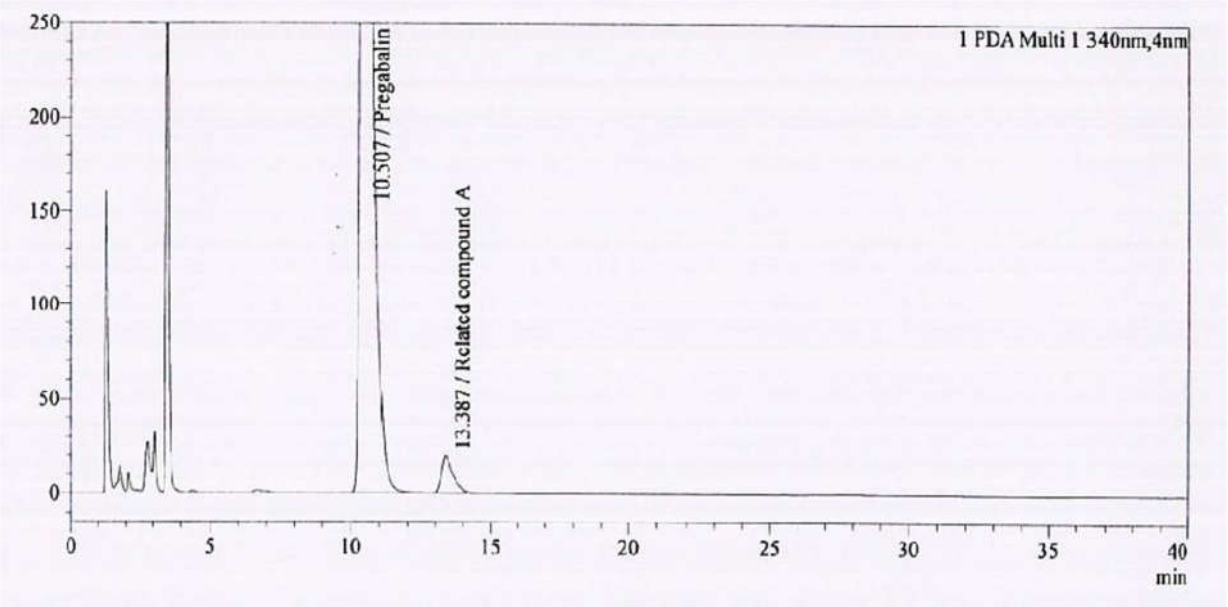

Figure 11. Chromatogram for Standard

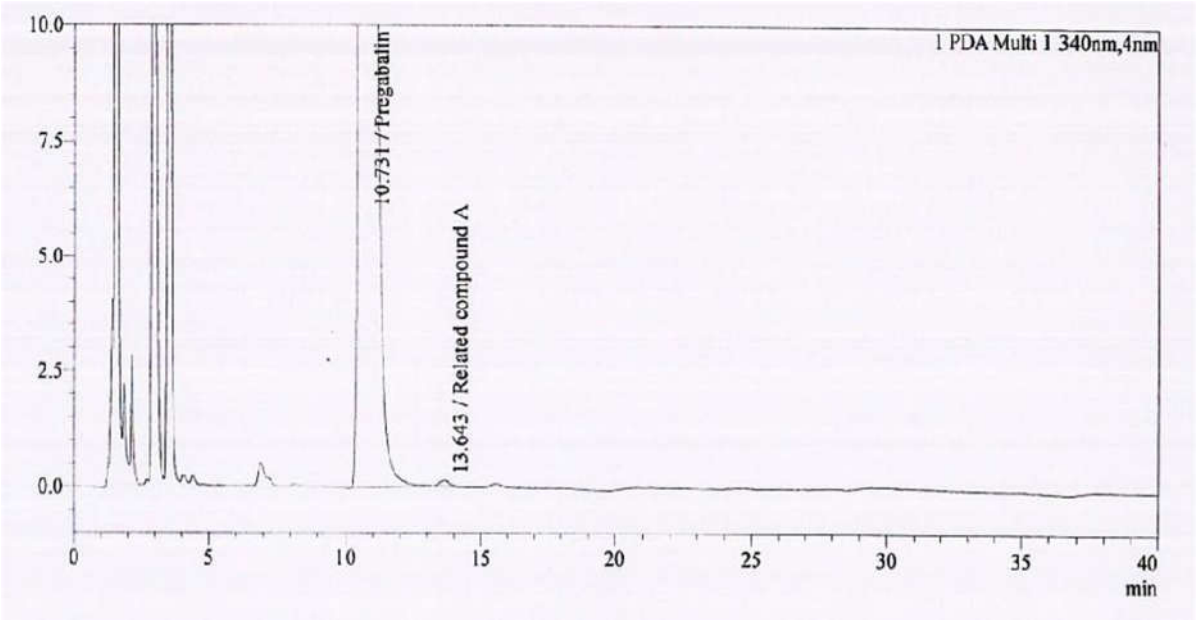

Figure 12. Chromatogram for Initial month Sample

3.4 Long-term stability assay by HPLC:

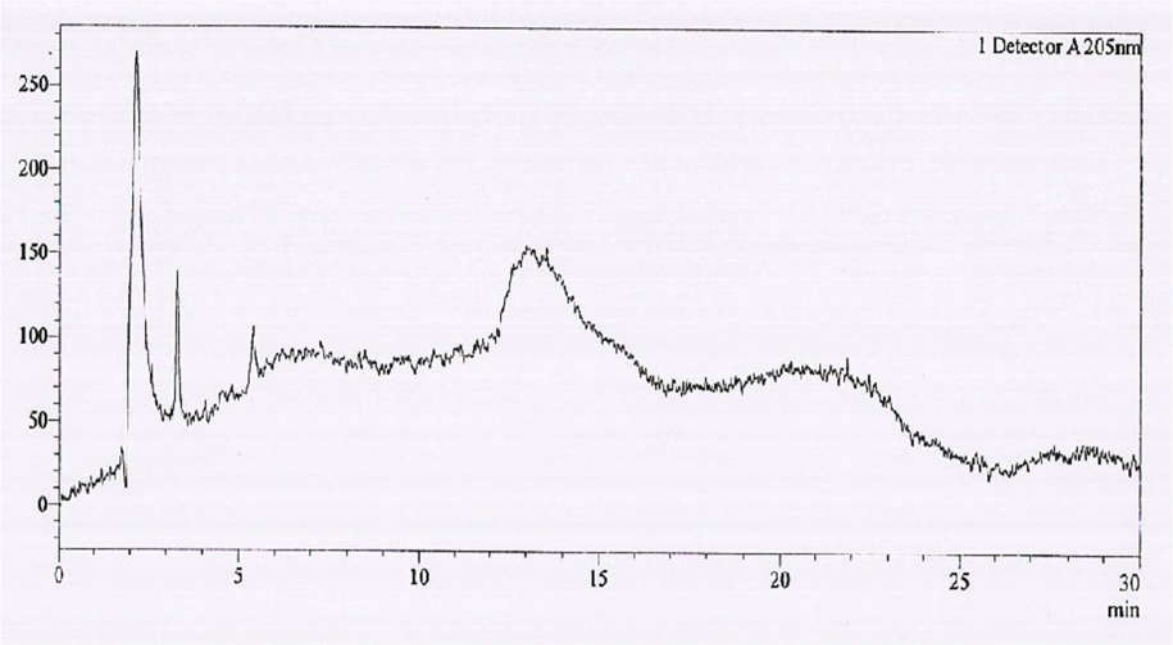

Figure 13. Chromatogram for Blank

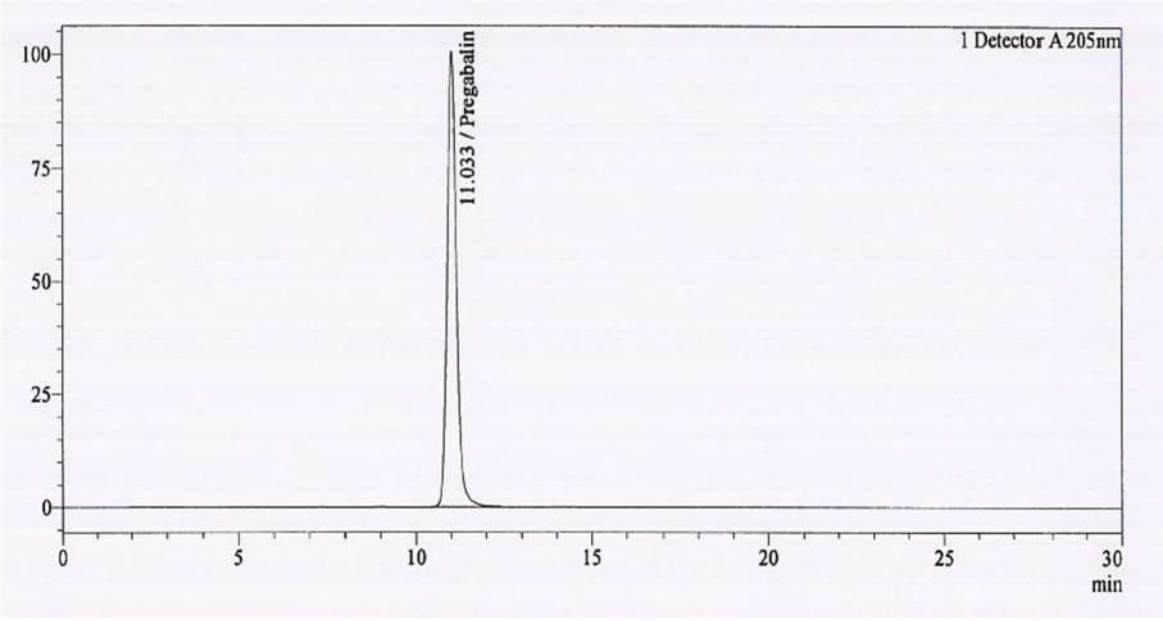

Figure 14. Chromatogram for Standard solution

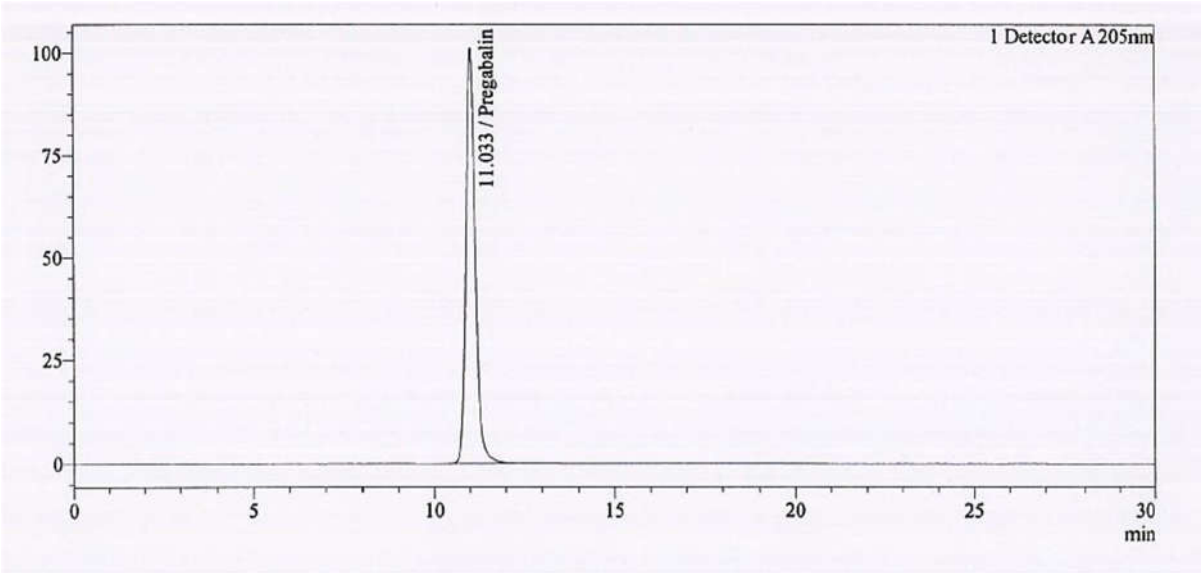

Figure 15. Chromatogram for Initial month sample

3.5 Long-term stability for Related substances by HPLC

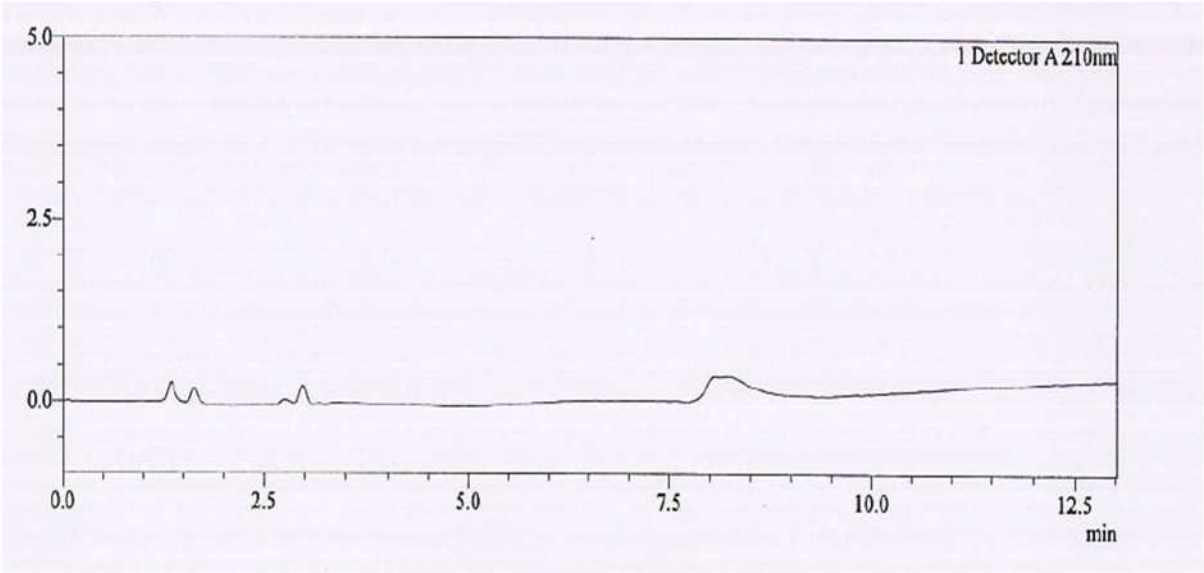

Figure 16. Blank solution chromatogram

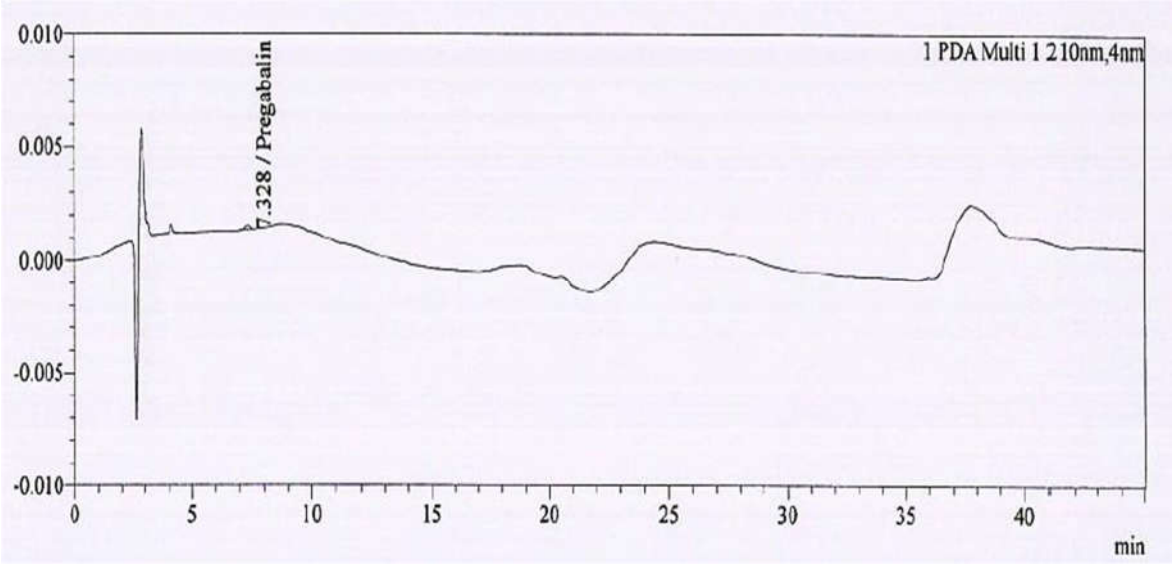

Figure 17. Sensitivity Solution Chromatogram

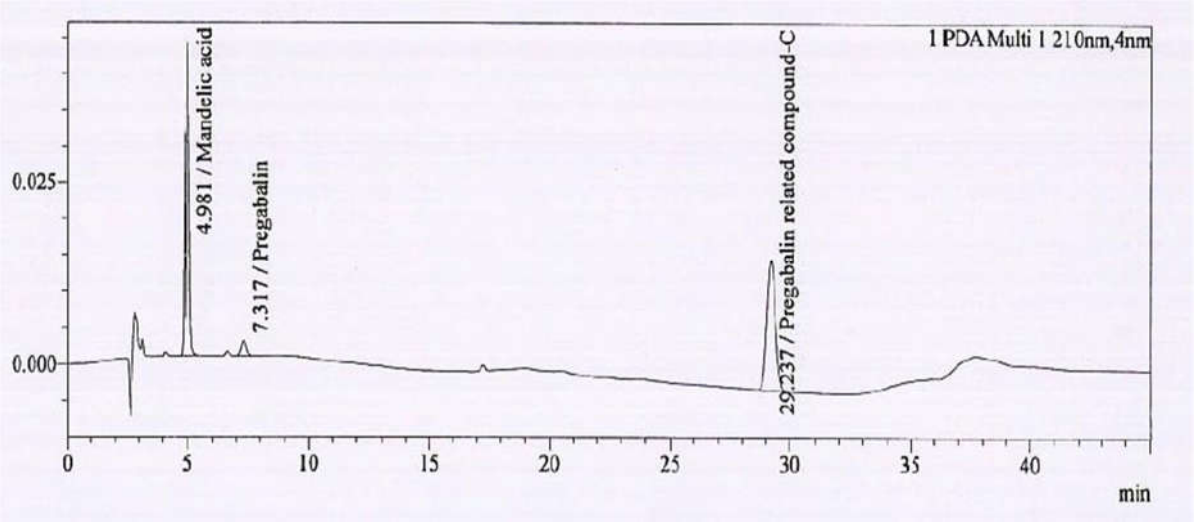

Figure 18. Standard Chromatogram

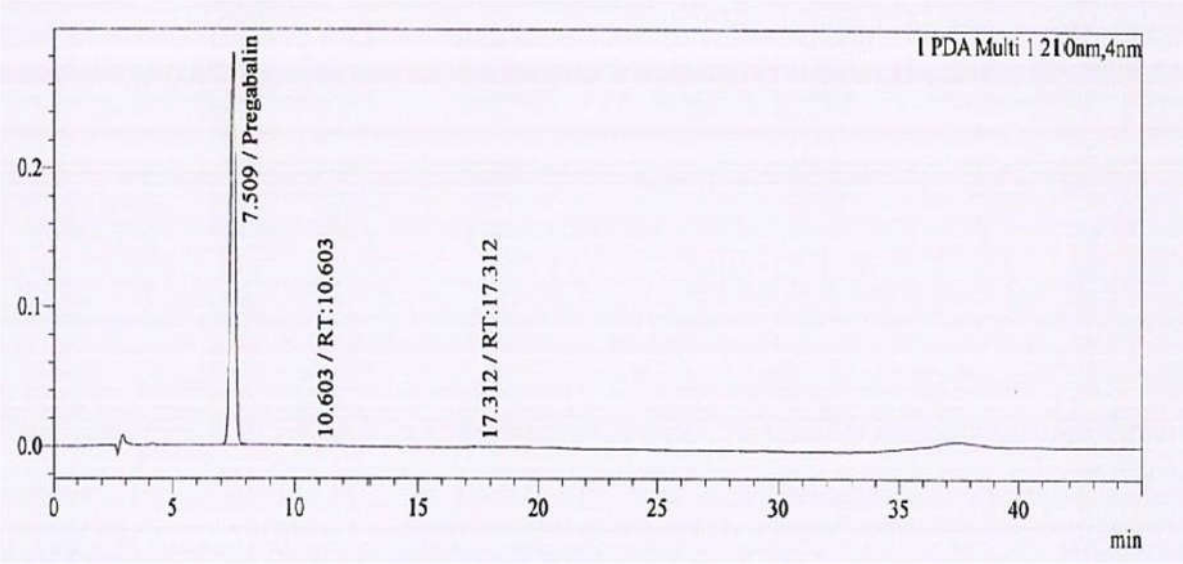

Figure 19. Sample Chromatogram

3.6 Long-term stability for Enantiomeric purity by HPLC

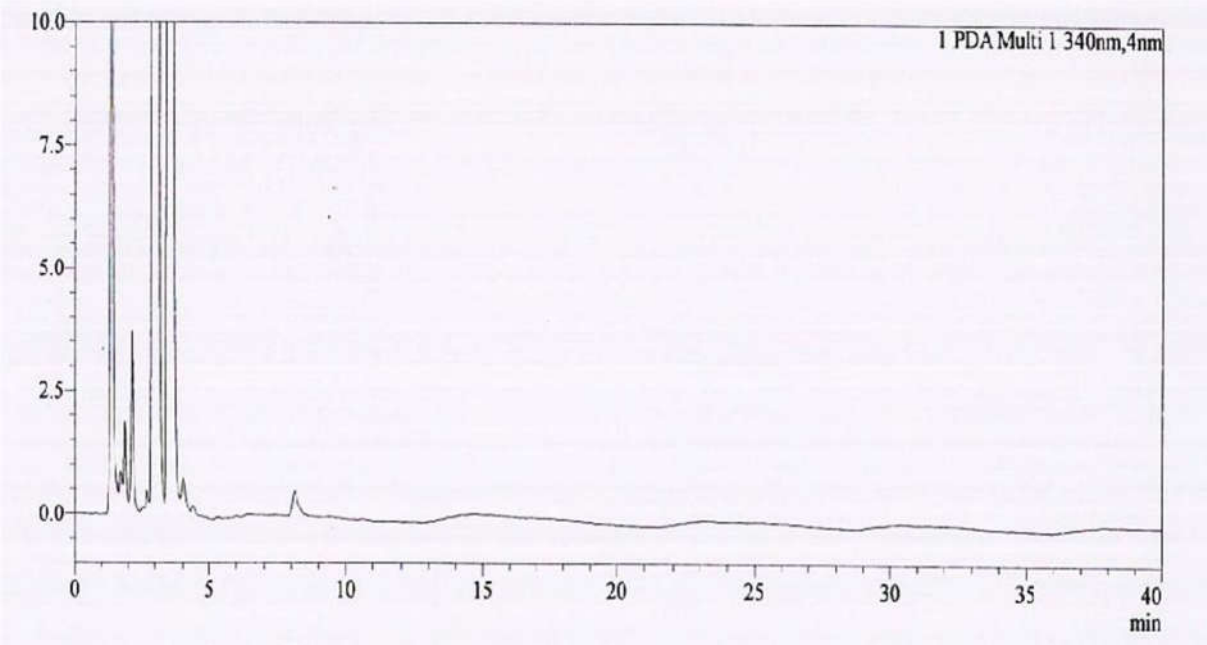

Figure 20. Blank Solution Chromatogram

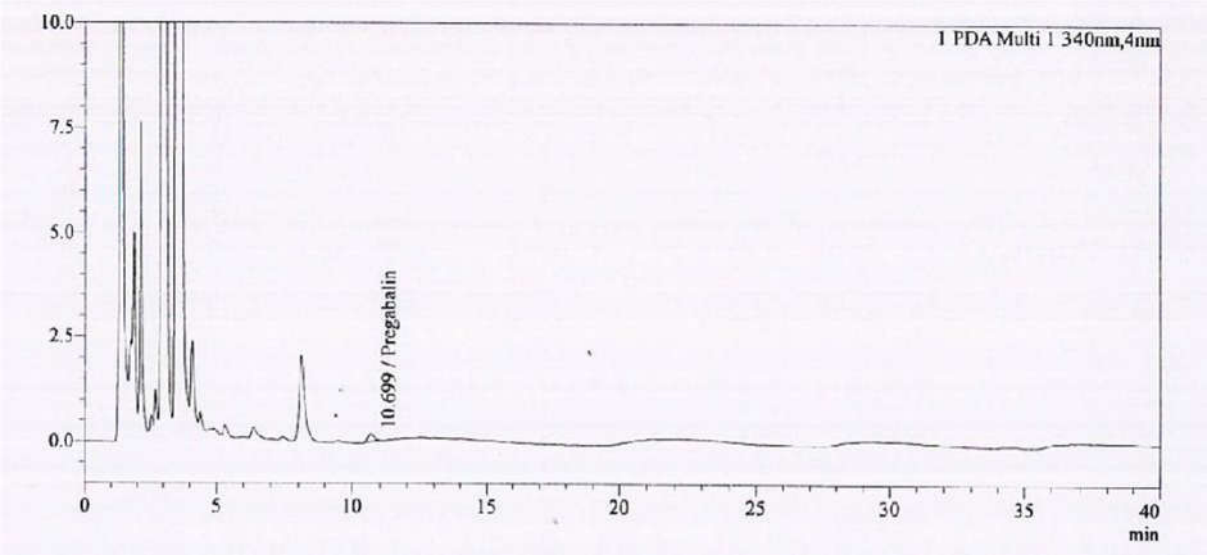

Figure 21. Sensitivity Solution Chromatogram

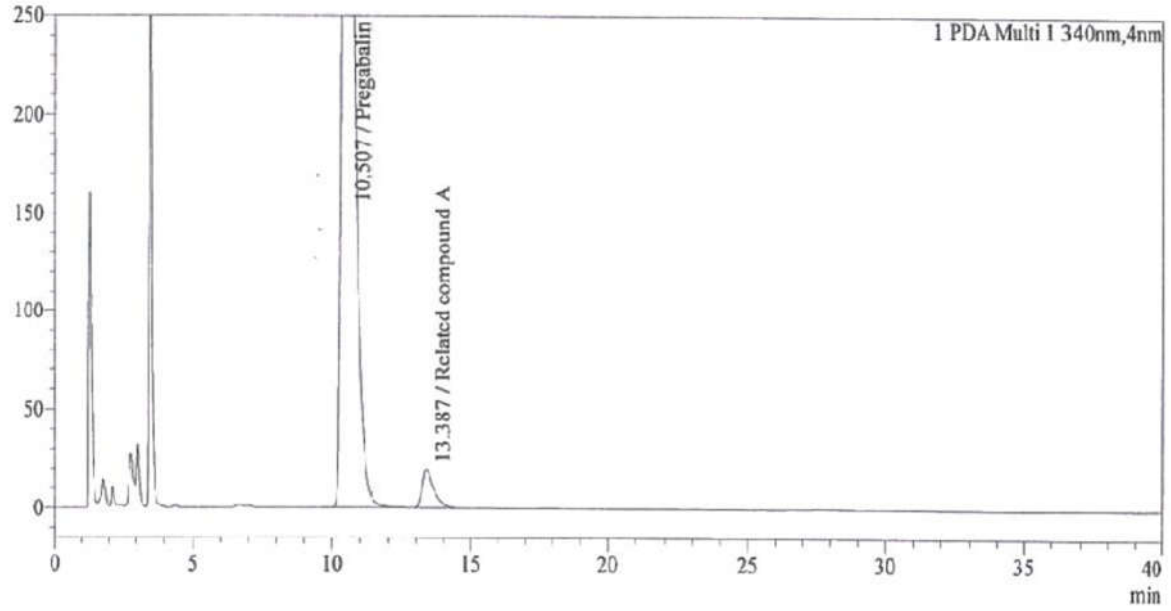

Figure 22. Standard Chromatogram

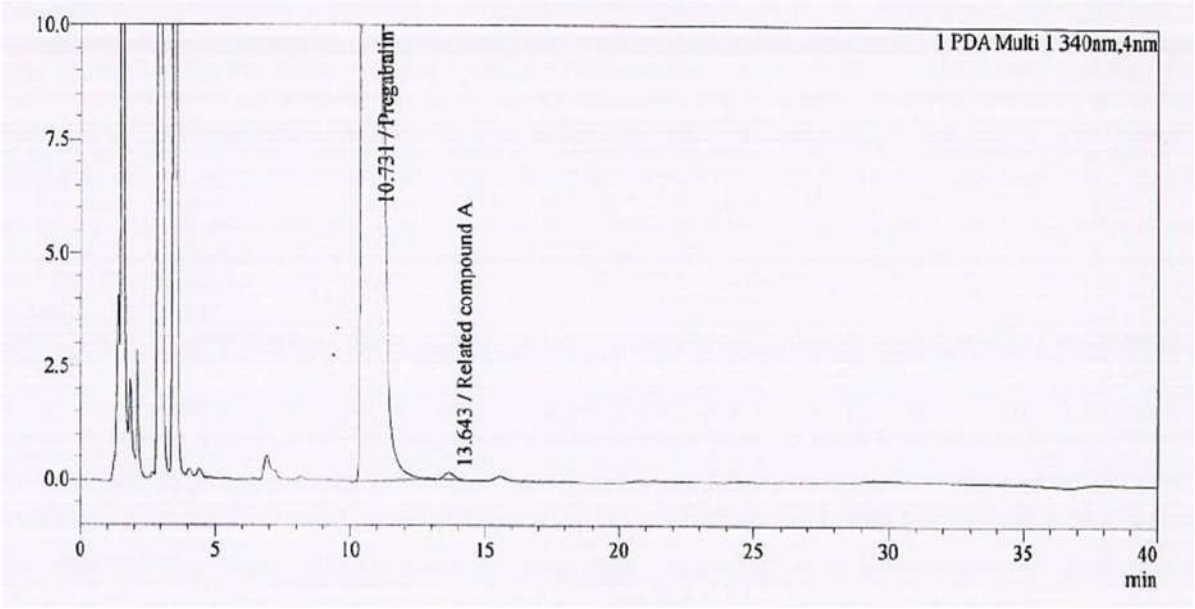

Figure 23. Initial month sample Chromatogram

Table 3. Evaluation of Accelerated Stability Studies Summary Report

Test	Months				
	Initial	**1st**	**2nd**	**3rd**	**6th**
Assay by HPLC on dried basis(%w/w)	99.8	100.1	99.9	99.7	100.0
Peak area	1835554	1835584	1835596	1835352	1835574
Related Substances by HPLC (%w/w)					
Mandelic acid	ND*	BDL	ND	BLOD*	BDL
Peak area	0.00	311	0.00	897	0.00
Isobutylglutaricacid	ND	ND	ND	ND	ND
Peakarea	0.00	0.00	0.00	0.00	0.00
Isobutylglutarmonoamide	ND	ND	ND	ND	ND
Peak area	0.00	0.00	0.00	0.00	0.00
Pregabalin related compound C	ND	ND	BDL	ND	BDL
Peak area	0.00	0.00	0.00	0.00	0.00
Any unspecified Impurity	0.03	0.03	0.03	0.03	0.03
Peak area	2546	2553	2658	2749	3015
Total Impurities	0.05	0.05	0.04	0.04	0.04
Peak area	8946	8686	8766	8936	8857
Enantiomeric purity by HPLC(%)					
Pregabalin related compound A	0.04	0.04	0.04	0.03	0.03
Peak area	2152	2421	2632	2445	2442

***ND:-** Not Detected

***BDL:-** Below Discarded Limit

***BLOQ:-**Below Limit of Quantification

***BLOD:-** Below Limit of Detection

Table 5. Evaluation of Long-Term Stability Studies Summary Report

Test	**Months**				
	Initial	**1st**	**6th**	**9th**	**12th**
Assay by HPLC on dried basis(%w/w)	99.8	100.0	99.3	99.4	99.4
Peak area	1911167	1925471	1911025	1935412	1935434
Related Substances by HPLC (%w/w)					
Mandelic acid	ND*	BDL	ND	BLOQ*	BDL
Peak area	0.00	811	0.00	1894	0.00
Isobutylglutaricacid	ND	ND	ND	ND	ND
Peak area	0.00	0.00	0.00	0.00	0.00
Isobutylglutarmonoamide	ND	ND	ND	ND	ND
Peak area	0.00	0.00	0.00	0.00	0.00
Pregabalin related compound C	ND	ND	BDL	ND	BDL
Peak area	0.00	0.00	865	0.00	0.00
Any unspecified Impurity	0.03	0.03	0.03	0.02	0.02
Peak area	2546	1553	1658	2749	3015
Total Impurities	0.04	0.04	0.04	0.1	0.1
Peak area	8946	8686	8766	8936	8857
Enantiomeric purity by HPLC(%)					
Pregabalin related compound A	0.03	0.03	0.03	0.03	0.03
Peak area	2152	2421	2632	2445	2442

Conclusion

In this study, the long-term and accelerated stability of Pregabalin was evaluated through a series of comprehensive assays, including HPLC for assay, related substances, and enantiomeric purity methods. The results from these analyses demonstrated that Pregabalin maintains high purity and quality over the designated stability periods.

The long-term stability tests indicated that Pregabalin remains within acceptable limits for assay and related substances, reflecting its robustness under standard stability conditions. Additionally, the accelerated stability studies corroborated these findings, showing that even under stress conditions; the drug substance retains its integrity and enantiomeric purity.

Overall, the consistent results across both stability testing methodologies confirms that Pregabalin exhibits excellent stability characteristics, supporting its suitability for long-term storage and use in pharmaceutical applications. These findings affirm the quality and reliability of Pregabalin as a therapeutic agent, providing confidence in its performance and safety for patients.

Conflict of Interest

The authors declared that there was no conflict of interest.

Acknowledgement

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