WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 1, 521-537. Review Article ISSN 2277–7105

STABILITY INDICATING RP-HPLCMETHOD FOR SIMULTANEOUS ESTIMATION OF PREGABALIN AND EPALRESTAT

G. Indira Priyadarshini*, K. Hadassa, Nagaraju Pappula and V. Mounika

Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi Road, Guntur, Andhra Pradesh, India-522002.

Article Received on
12 November 2024,
Revised on 02 Dec. 2024,
Accepted on 22 Dec. 2024
DOI: 10.20959/wjpr20251-35094

***Corresponding Author**
Dr. G. Indira Priyadarshini
Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi Road, Guntur, Andhra Pradesh, India-522002.

ABSTRACT

A simple, precise, accurate, rapid, robust and economical RP-HPLC method was developed and validated for the assay of Epalrestat and Pregabalin in tablet formulation. This method yielded high recoveries with good linearity and precision. The method was developed using WATERS HPLC 2965 SYSTEM with Auto Injector and PDA 2996 Detector. Software used is Empower 2. Discovery (250 x 4.6 mm, 5µ) column is used as stationary phase with mobile phase containing mixture of buffer: acetonitrile, 50:50 (v/v). The eluted compound was monitored at 241 nm. The developed method was validated for parameters of Specificity, Linearity, Precision, Accuracy, Limit of Detection, Limit of Quantification and Robustness as per approved ICH guidelines. The results obtained after the analysis of drug by the proposed validation parameters were highly reproducible and reliable. The %Assay of Epalrestat and Pregabalin by the proposed method was found to be 98.0%-99.0%. The %RSD of the drug was found to be 0.7 & 0.6 which are within limit. The validation parameters results of the drug were found to be within limit.

KEYWORDS: RP-HPLC method, Validation, Epalrestat and Pregabalin.

1. INTRODUCTION

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Liquid chromatography[3] is an analytical chromatographic technique that is useful for separatingions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second solid or liquid phase to differing degrees due to differences in adsorption, ion

exchange, partitioning or size. These differences will allow the mixture components to be separated from each other by using these differences to determine the time of the solutes through a column.During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography and thin layer chromatography (TLC). However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time pressure liquid chromatography began to be used to decreased flow through time, thus reducing separation time of compounds being isolated by column chromatography. However, flow rates were inconsistent, and the question of whether it was better to have constant flow rate or constant pressure debated. High pressure liquid chromatography quickly improved with the development of column packing materials. Additional convenience of on-line detectors became rapidly a powerful separation technique and is today called as High Performance Liquid Chromatography (HPLC).[1-10]

Classification of hplc[11-20]

Based on modes of chromatography

- Normal phase chromatography
- Reverse phase chromatography

Based on principle of separation

- Adsorption chromatography
- Partition chromatography
- Ion exchange chromatography
- Size exclusion chromatography
- Affinity chromatography
- Chiral phase chromatography

Based on elution technique

- Isocratic separation
- Gradient separation

Based on the scale of operation

- Analytical HPLC
- Preparative HPLC

Reversed Phase-High Performance Liquid Chromatography (RP-HPLC)

As opposed to NP-HPLC, RP-HPLC employs mainly dispersive forces (Hydrophobic or vanderwal's interactions). The polarities of mobile and stationary phases are reversed, such thatthe surface of the stationary phase in RP-HPLC is hydrophobic and mobile phase is polar, where mainly water-based solutions are employed. RP-HPLC is by far the most popular mode of chromatography. Almost 90% of all analysis of low molecular-weight samples are carried out using RP-HPLC. Dispersive forces employed in this separation mode are the weakest intermolecular forces, thereby making the overall background interaction energy in the chromatographic system very low compared to other separation techniques. This low background energy allows for distinguishing very small differences in molecular interactions of closely related analytes. Adsorbents employed in this mode of chromatography are porous rigid materials with hydrophobic surfaces. The majority of packing materials used in RP-HPLC are chemically modified porous silica.[20-26]

2. MATERIALS AND METHODS

2.1 Materials

Epalrestat and Pregabalin pure drugs (API) were obtained from Spectrum Pharma Research solutions, Hyderabad. Combination Epalrestat and Pregabalin tablets were obtained from local pharmacy store. Acetonitrile, phosphate buffer, ammonium acetate buffer, methanol, potassium dihydrogen phosphate buffer, triethyl amine, ortho-phosphoric acid were obtained from Rankemchemicals Ltd., Mumabai, India.

2.2 Instruments

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA 2996Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10 mm and matched quartz was be used for measuring absorbancefor Epalrestat and Pregabalin solutions.

3. METHOD VALIDATION

Table 1: System suitability studies of Epalrestat and Pregabalin.

Property	Epalrestat	Pregabalin
Retention time (tR)	2.172 min	3.013 min
Theoretical plates (N)	5942 ± 63.48	8487 ± 63.48
Tailing factor (T)	1.33 ± 0.117	1.26± 0.117

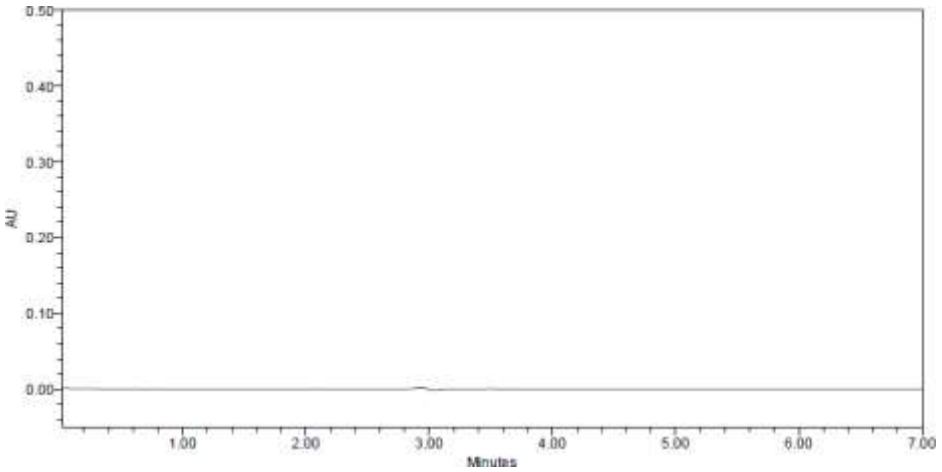

Fig. 1: Blank chromatogram for specificity by using mobile Phase.

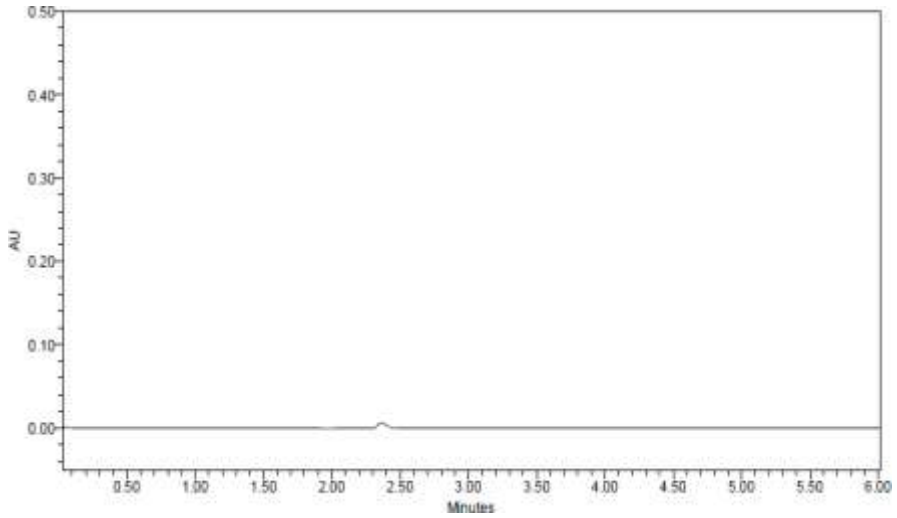

Fig. 2: Placebo chromatogram for specificity by using mobile phase.

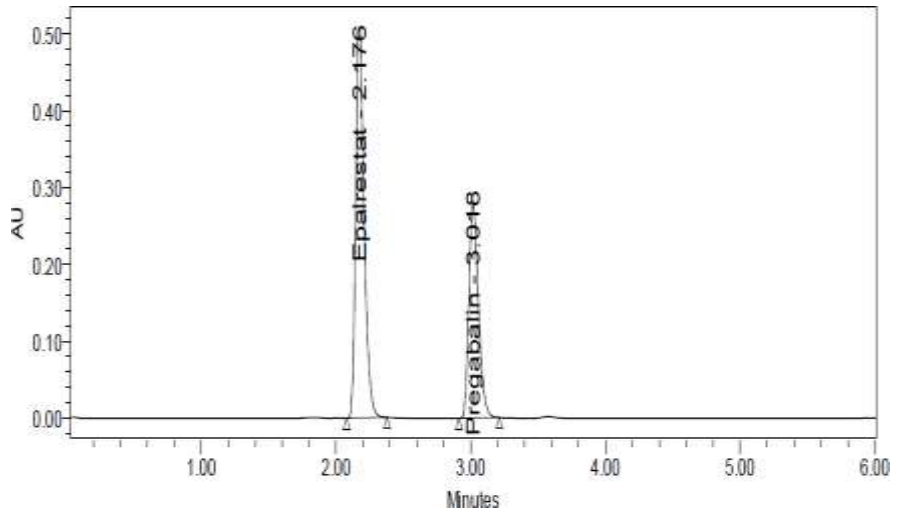

Fig. 3: Standard Chromarogram of Epalrestat and Pregabalin.

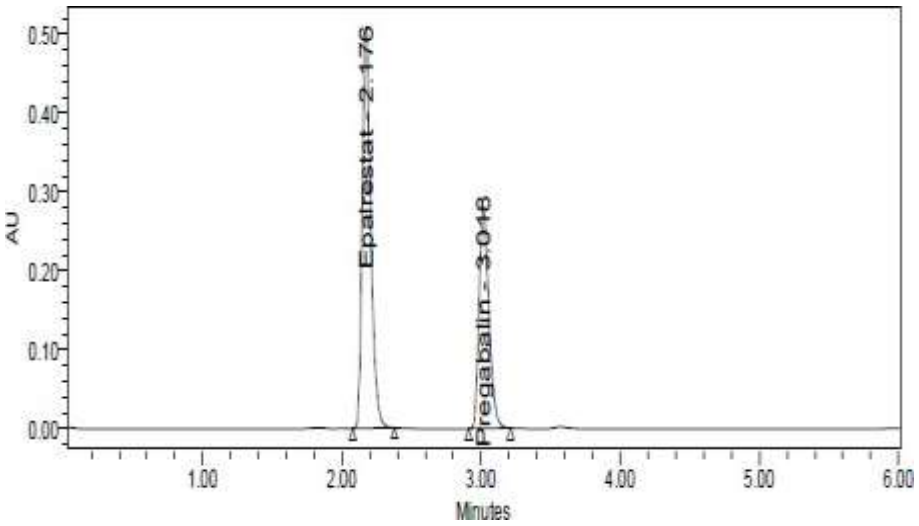

Fig. 4: Sample Chromarogram of Epalrestat and Pregabalin.

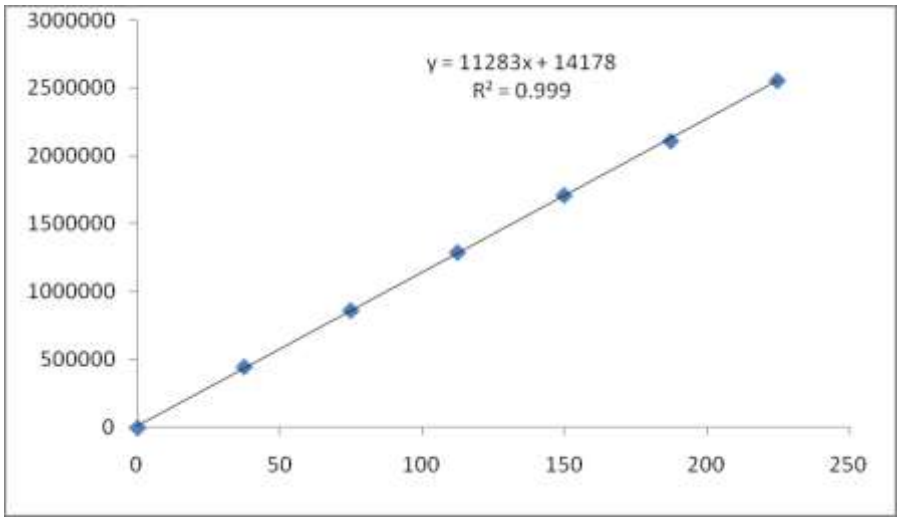

Fig. 5: Calibration curve of epalrestat.

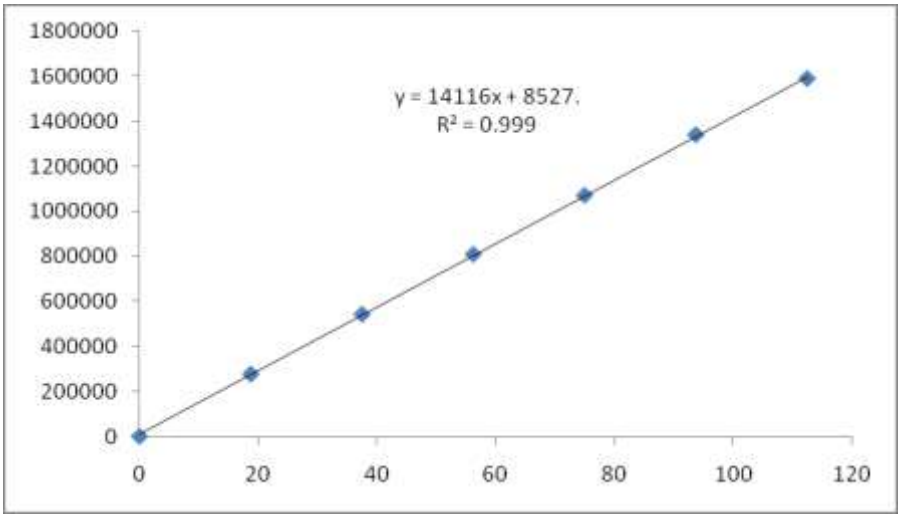

Fig. 6: Calibration curve of Pregabalin.

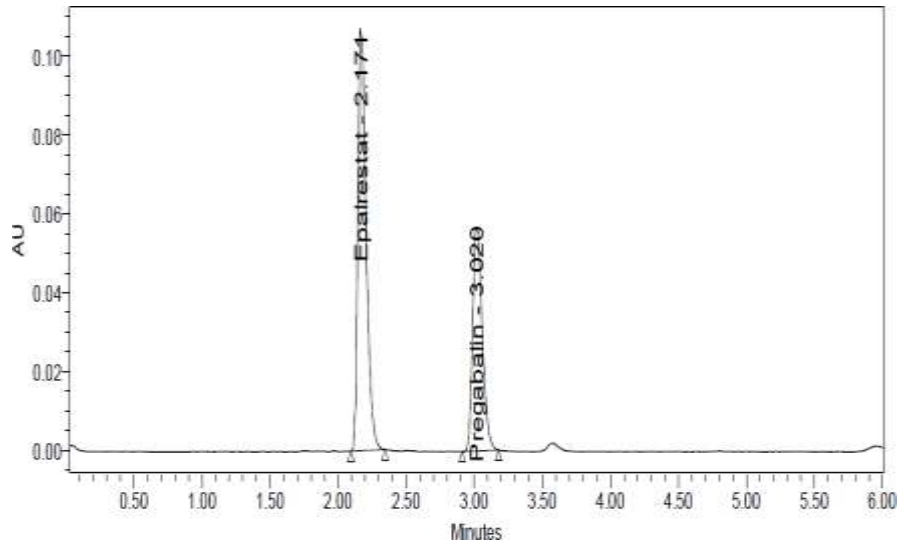

Fig. 7: Linearity 25% Chromatogram of Epalrestat and Pregabalin.

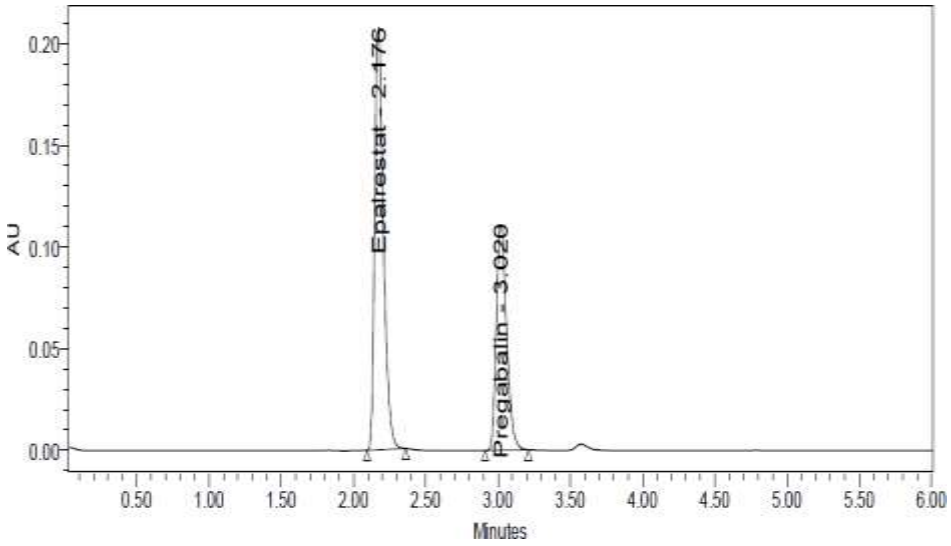

Fig. 8: Linearity 50% Chromatogram of Epalrestat and Pregabalin.

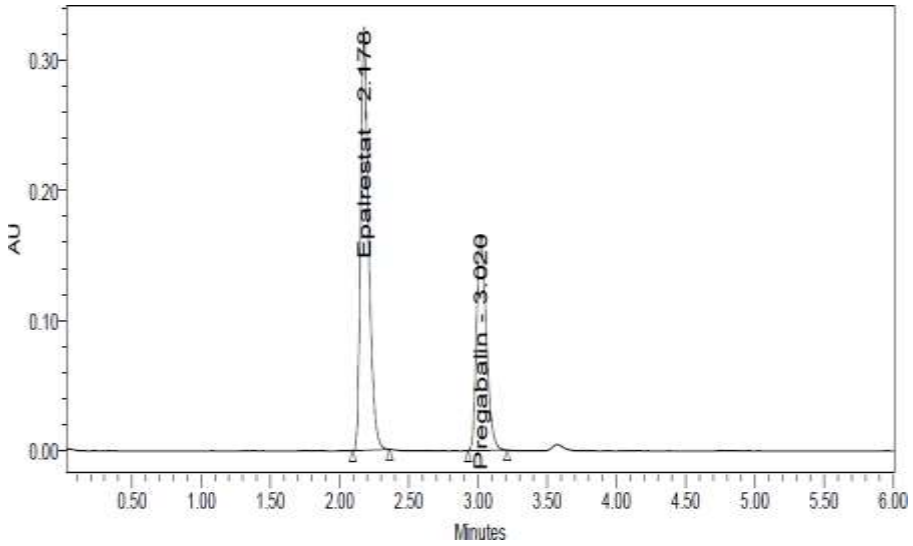

Fig. 9: Linearity 75% Chromatogram of Epalrestat and Pregabalin.

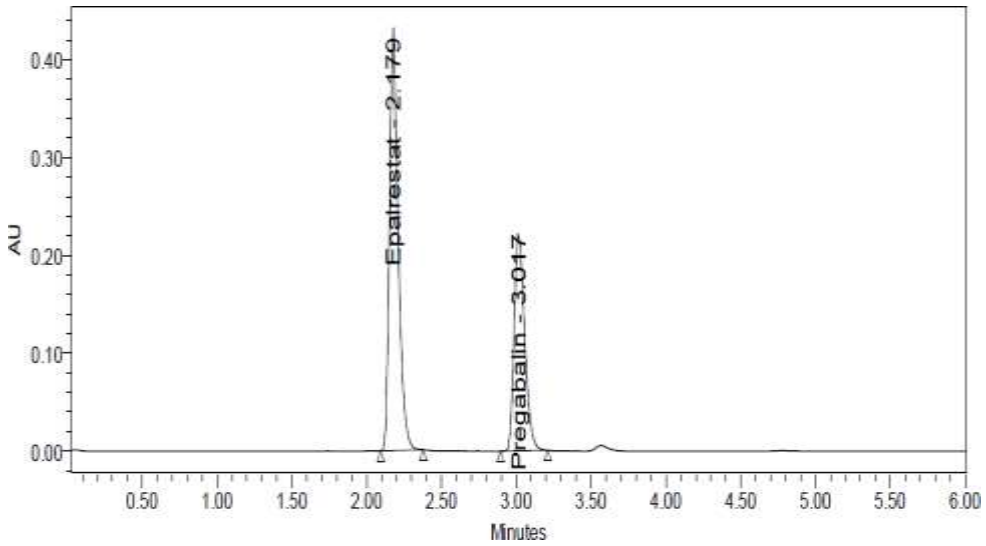

Fig. 10: Linearity 100% Chromatogram of Epalrestat and Pregabalin.

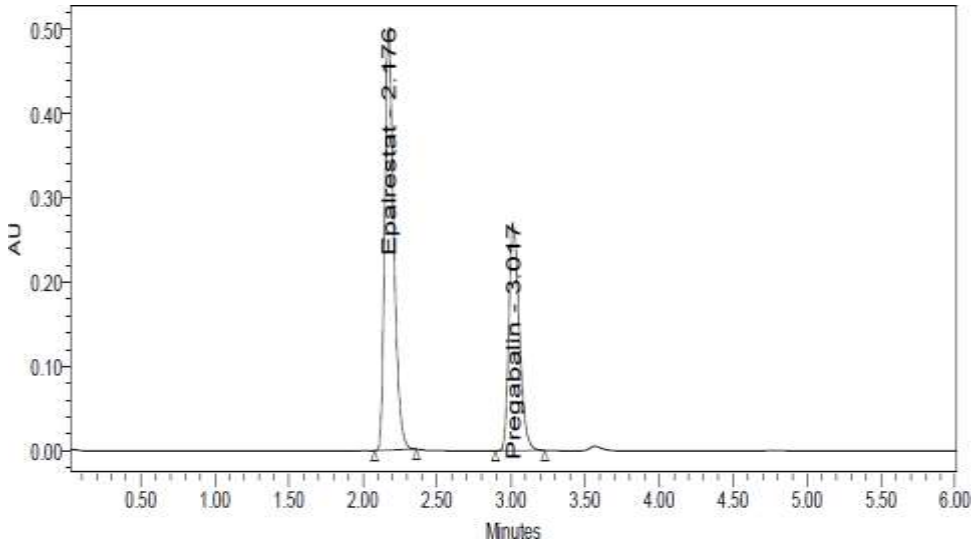

Fig. 11: Linearity 125% Chromatogram of Epalrestat and Pregabalin.

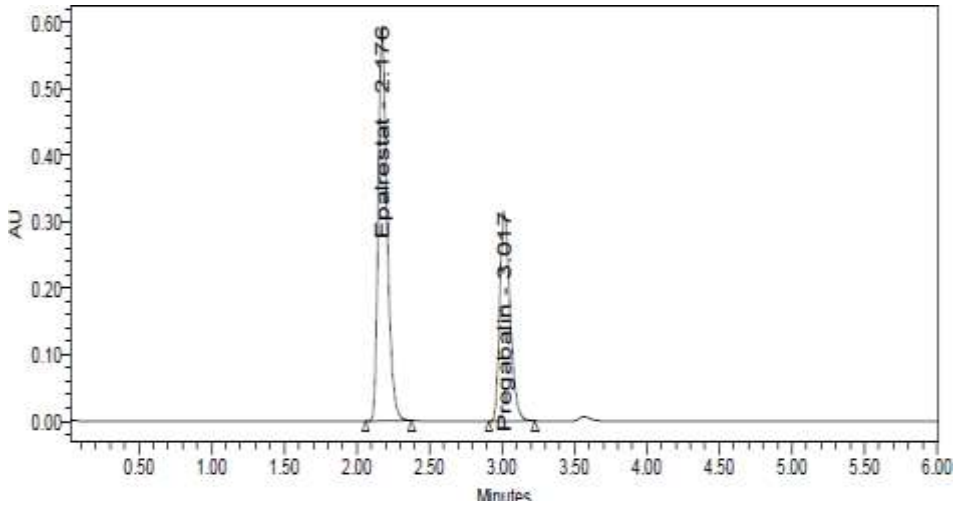

Fig. 12: Linearity 150% Chromatogram of Epalrestat and Pregabalin Precision.

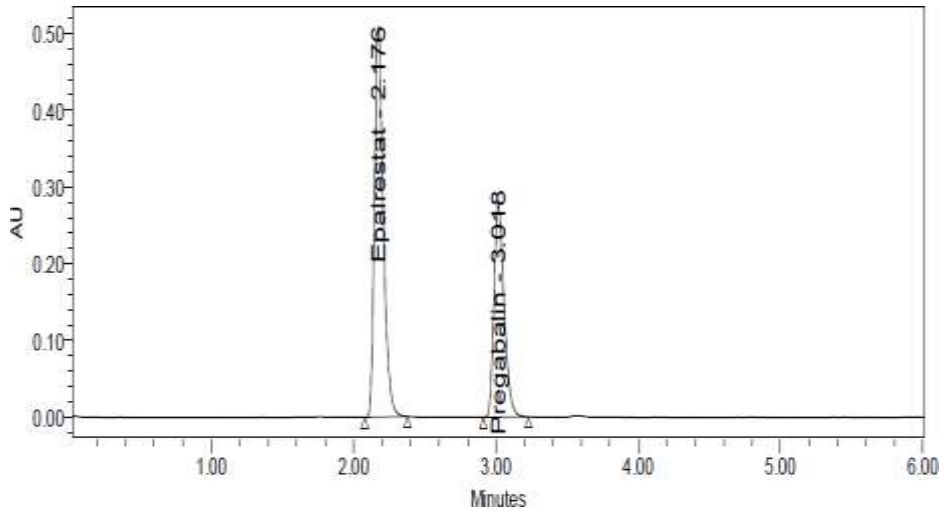

Fig. 13: Chromatogram of method precision.

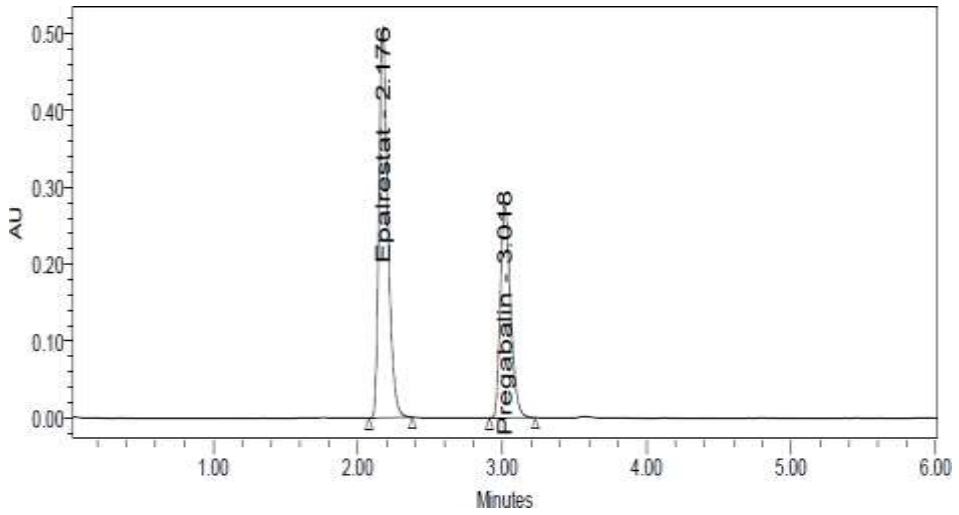

Fig. 14: Repeatability Chromatogram of Epalrestat and Pregabalin.

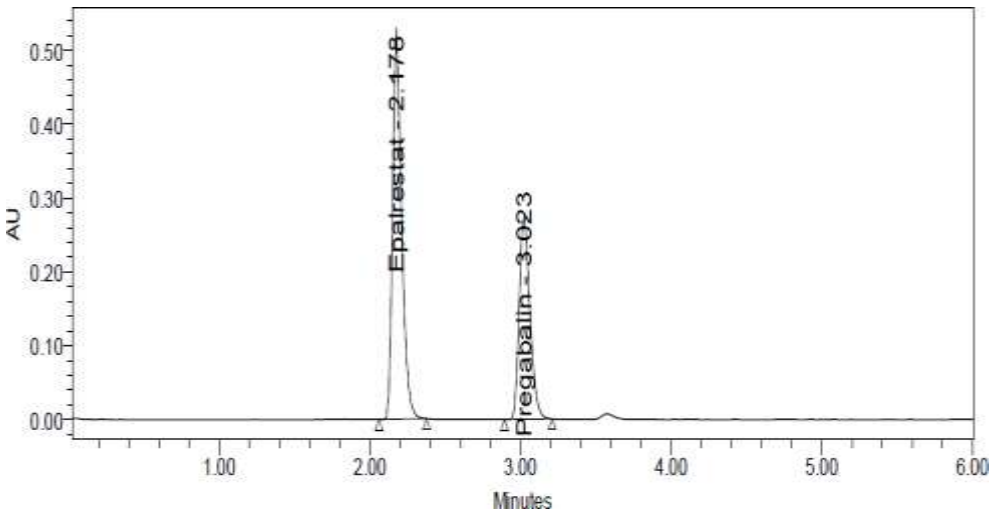

Fig. 15: Inter-day Precision Chromatogram of Epalrestat and Pregabalin.

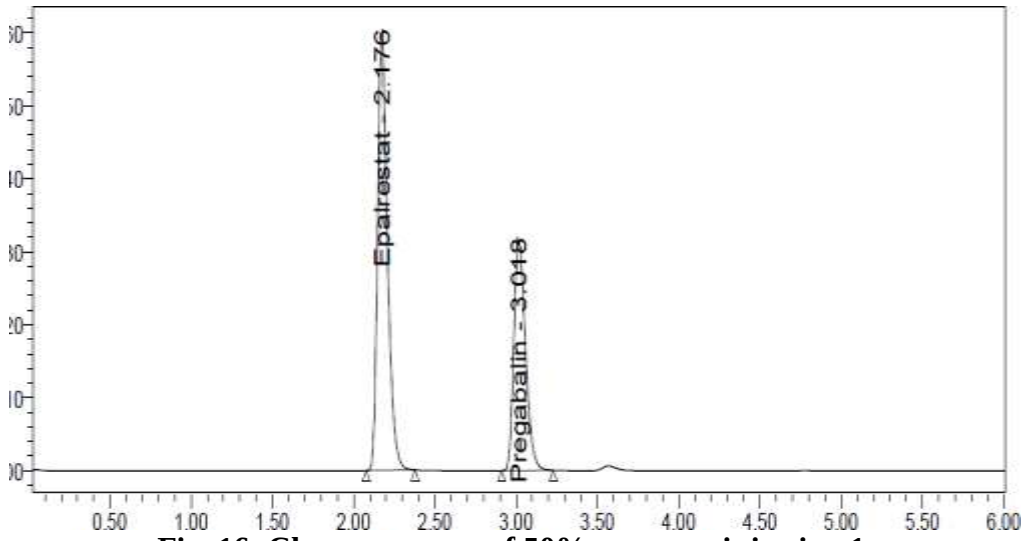

Fig. 16: Chromatogram of 50% recovery injection 1.

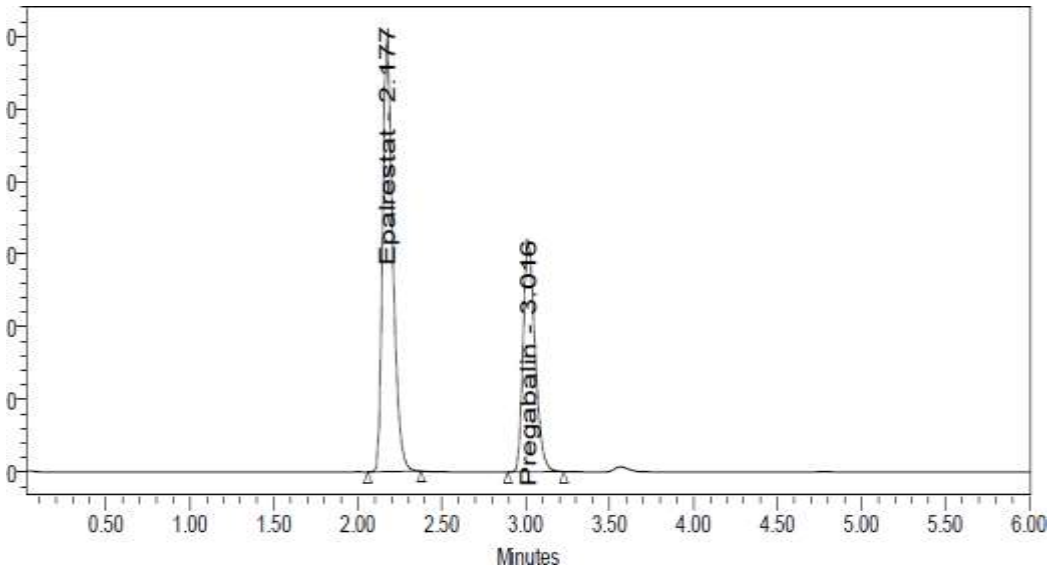

Fig. 17: Chromatogram of 50% recovery injection 2.

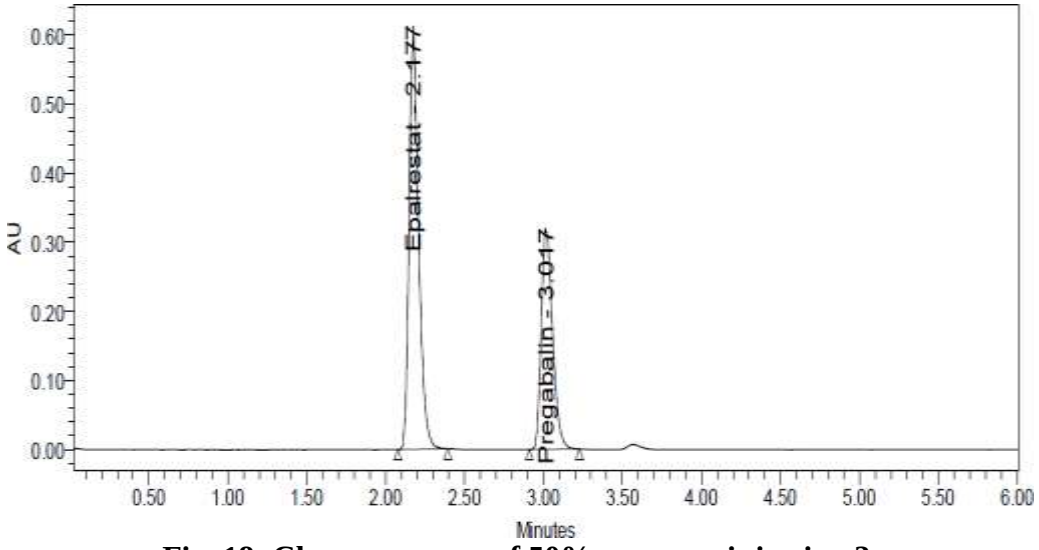

Fig. 18: Chromatogram of 50% recovery injection 3.

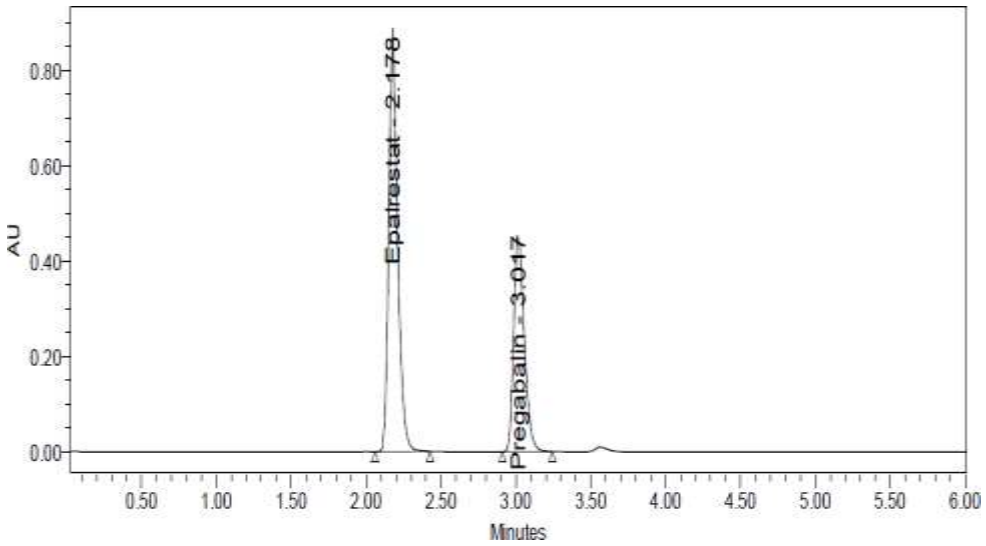

Fig. 19: Chromatogram of 100% recovery injection 1.

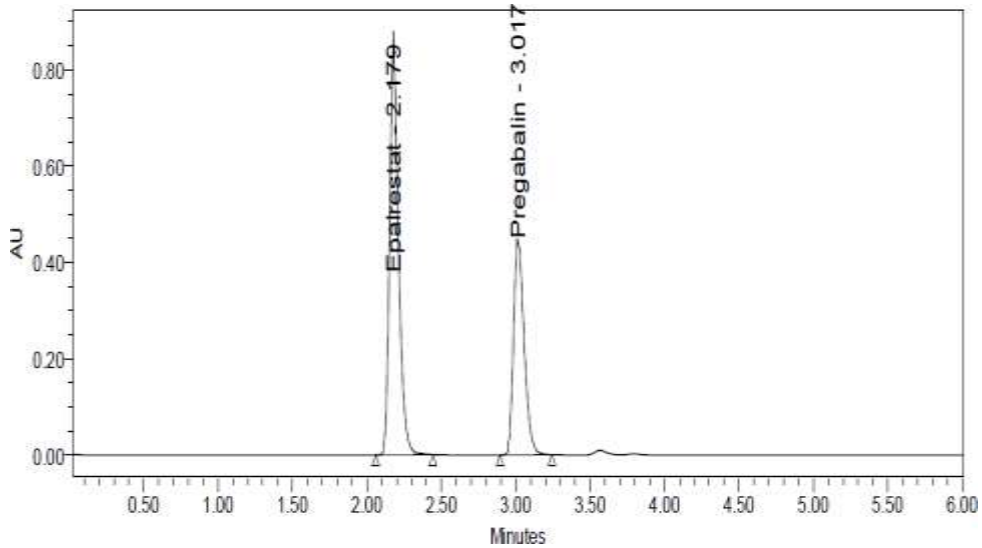

Fig. 20: Chromatogram of 100% recovery injection 2.

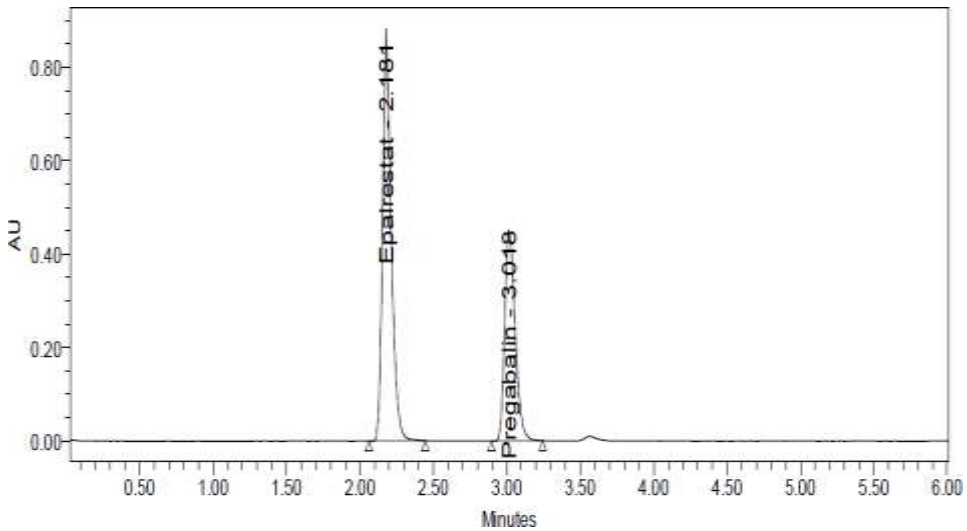

Fig. 21: Chromatogram of 100% recovery injection 3.

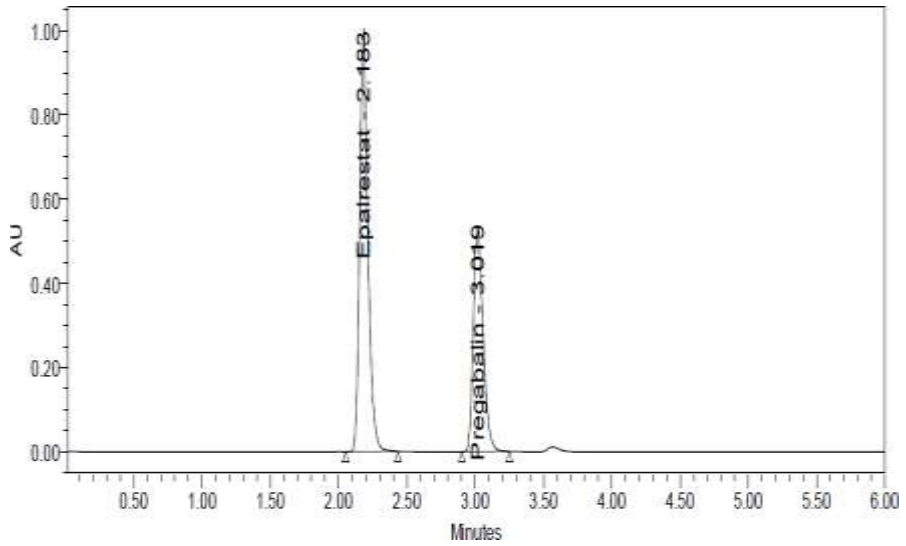

Fig. 22: Chromatogram of 150% recovery injection 1.

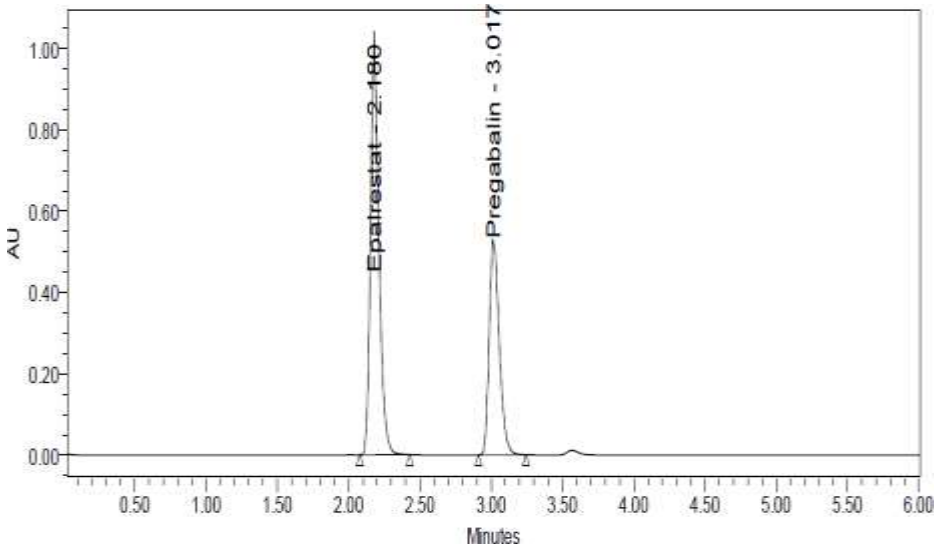

Fig. 23: Chromatogram of 150% recovery injection 2.

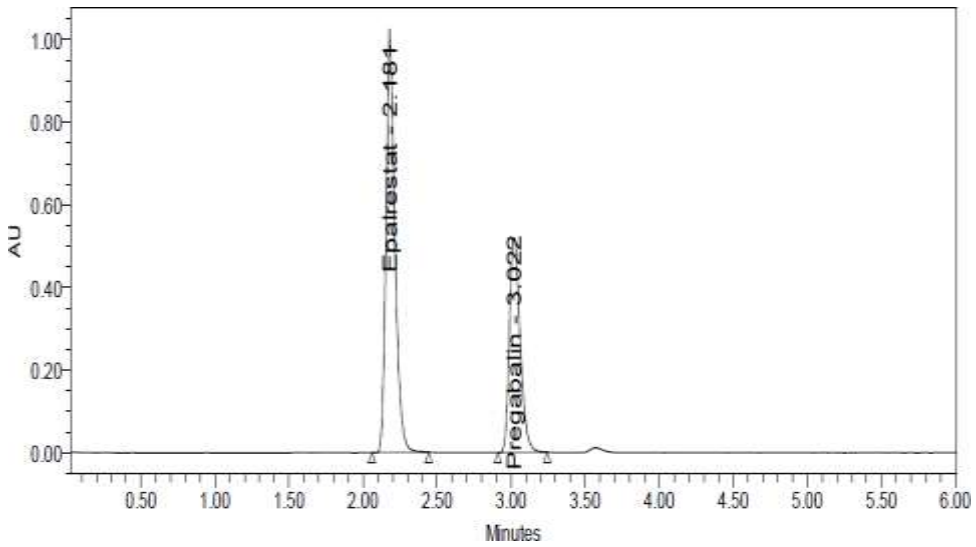

Fig. 24: Chromatogram of 150% recovery injection 3.

Table 2: Table of accuracy.

Sample	Concentration (µg/ml)	Recovery (%)	% RSD
Epalrestat	75	100.04	0.98
	150	100.11	0.62
	225	100.82	0.40
Pregabalin	37.5	100.72	0.55
	75	100.55	0.67
	112.5	99.62	0.71

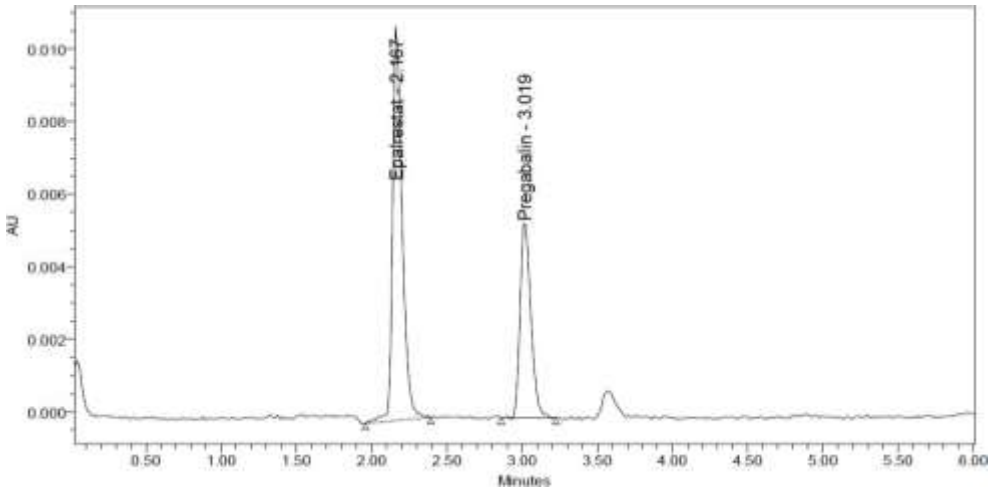

Fig. 25: LOD Chromatogram of Epalrestat and Pregabalin.

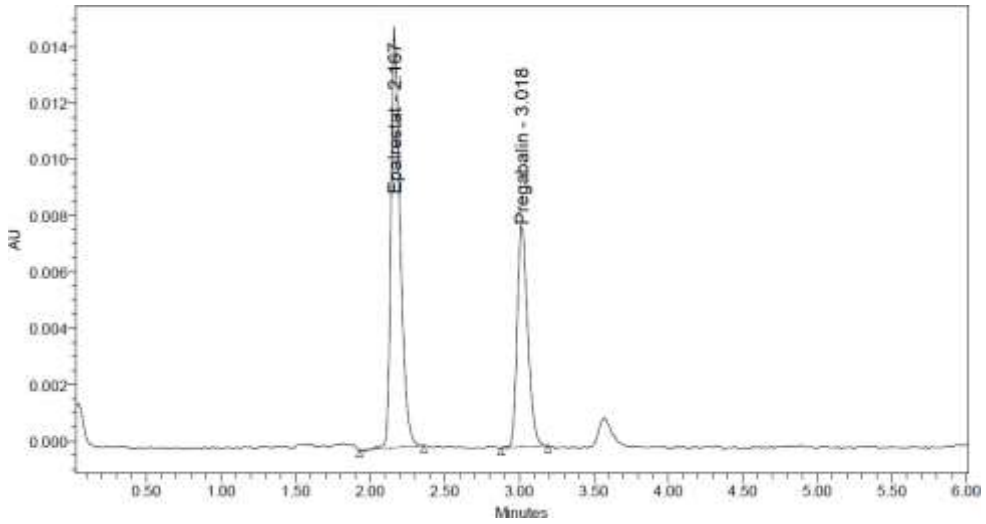

Fig. 26: LOQ Chromatogram of of Epalrestat and Pregabalin.

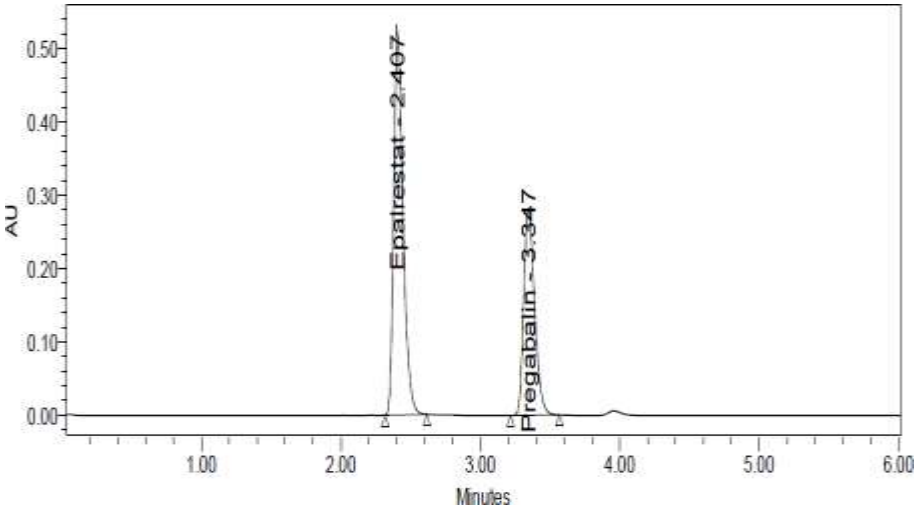

Fig. 27: Flow minus Chromatogram of Epalrestat and Pregabalin.

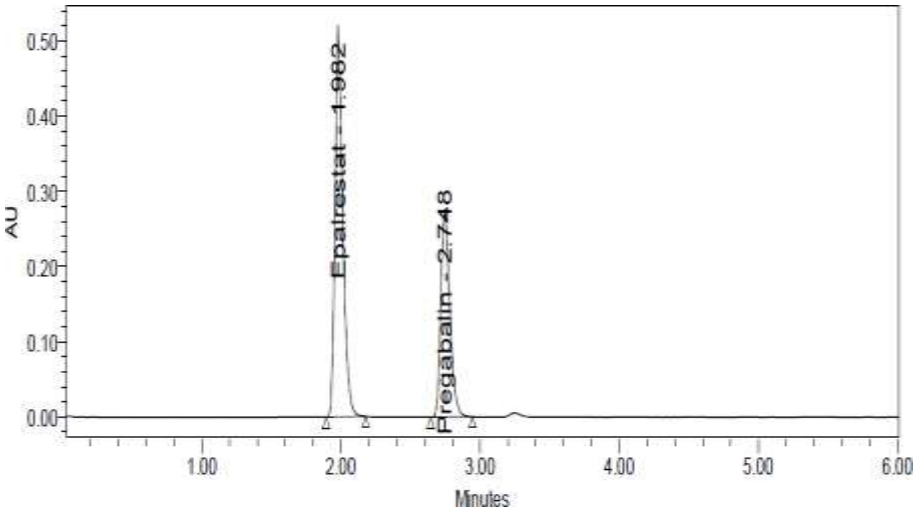

Fig. 28: Flow plus Chromatogram of Epalrestat and Pregabalin.

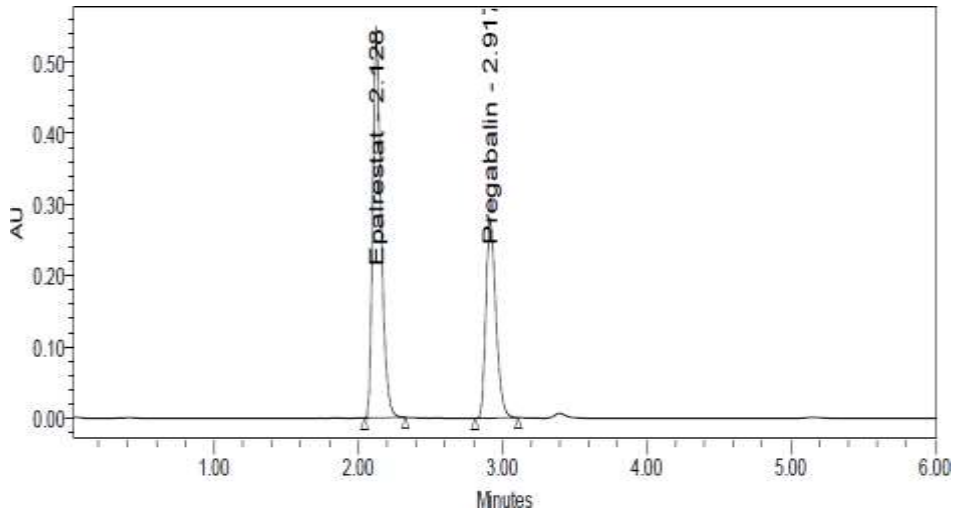

Fig. 29: Mobile phase minus Chromatogram of Epalrestat and Pregabalin.

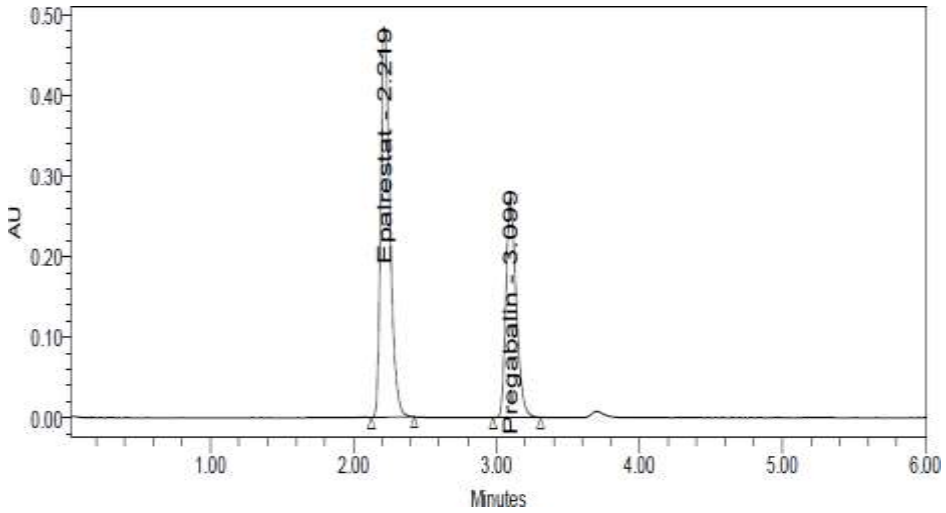

Fig. 30: Mobile phase plus Chromatogram of Epalrestat and Pregabalin.

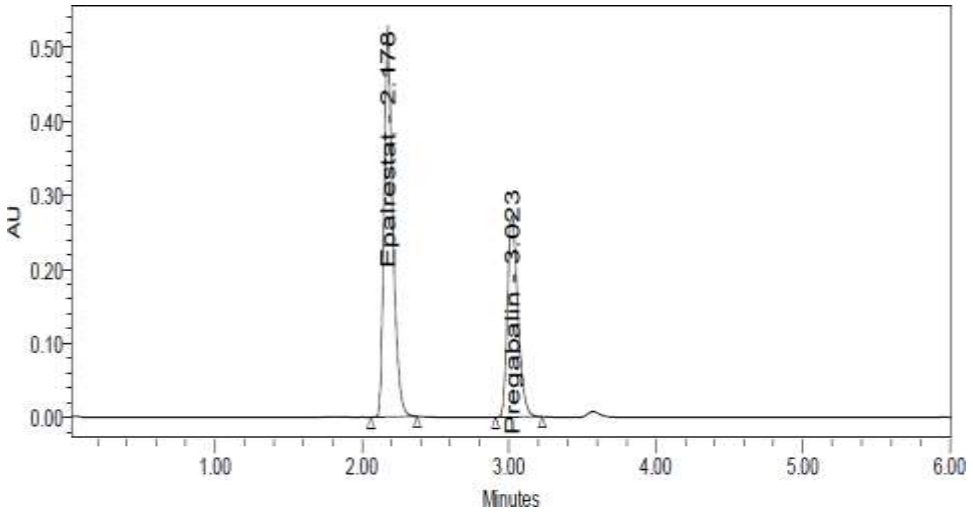

Fig: 31: Temperature minus Chromatogram of Epalrestat and Pregabalin.

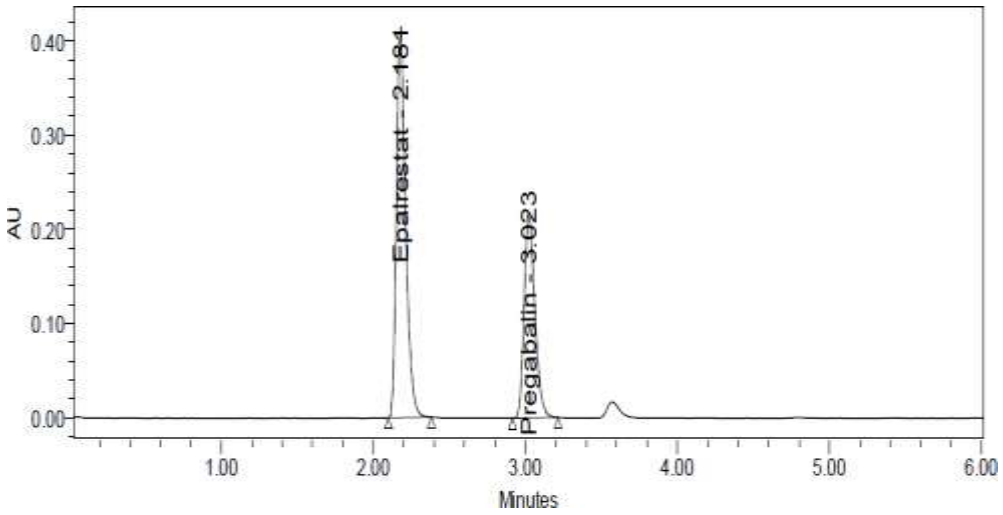

Fig. 32: Temperature plus Chromatogram of Epalrestat and Pregabalin.

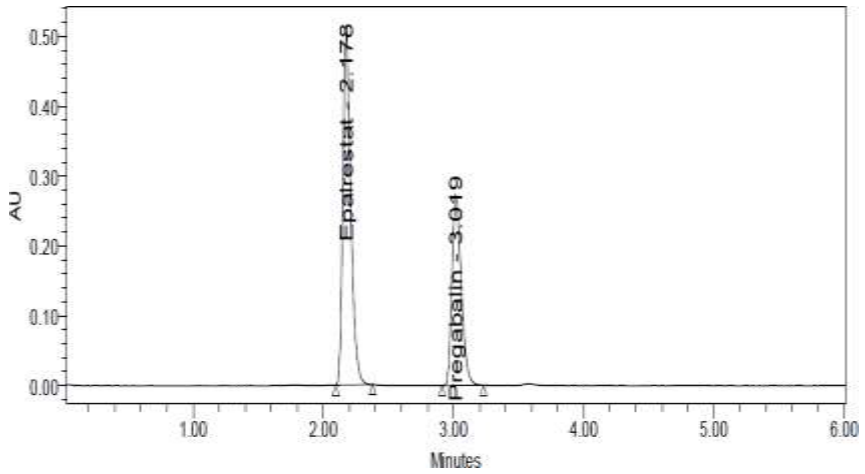

Fig. 33: Assay of tablet formulation.

4. SUMMARY AND CONCLUSION

A simple, precise, accurate, rapid, robust and economical RP-HPLC method was developed and validated for the assay of Epalrestat and Pregabalin in tablet formulation. This method yielded high recoveries with good linearity and precision. The method was developed using WATERS HPLC 2965 SYSTEM with Auto Injector and PDA 2996 Detector. Software used is Empower 2. Discovery (250 x 4.6 mm, 5µ) column is used as stationary phase with mobile phase containing mixture of buffer: acetonitrile, 50:50 (v/v). The eluted compound was monitored at 241 nm. The developed method was validated for parameters of Specificity, Linearity, Precision, Accuracy, Limit of Detection, Limit of Quantification and Robustness as per approved ICH guidelines. The results obtained after the analysis of drug by the proposed validation parameters were highly reproducible and reliable. The %Assay of Epalrestat and Pregabalin by the proposed method was found to be 98.0%-99.0%. The %RSD of the drug was found to be 0.7 & 0.6 which are within limit. The validation parameters results of the drug were found to be within limit. Hence, the overall conclusion of the work is a good approach for obtaining reliable results and found to be suitable for the routine analysis of Epalrestat and Pregabalin in tablet formulation.

Parameters	Epalrestat	Pregabalin
Calibration range (µg/ml)	37.5-225	18.75-112.5
Optimized wavelength (nm)	241	241
Retention time (min)	2.172	3.013
Regression equation (y)	y = 11283x+14178	y = 14116x+8527
Correlation coefficient (r^2)	0.999	0.999
Precision (% RSD)	0.7	0.6
% Assay	98.92%	99.12%
Limit of Detection (µg/ml)	0.10	0.03
Limit of Quantitation (µg/ml)	0.29	0.11

5. REFERENCES

1. A Skoog, DM West, and FJ Holler. Fundamentals of Analytical Chemistry, Saunders College Publishing, 1992; 7: 1-3.
2. KA Corners. Textbook of Pharmaceutical Analysis, A Wiley Inter science Publication, 1967; 1: 475-478.
3. AH Beckett and JB Stenlake. Practical Pharmaceutical Chemistry, CBSPublishers and Distributors, 2002; 2, 4: 157-174.
4. Remington. The Science & Practice of a Pharmacy, Lippincott Williams & Wilkins, 2006; I, 20: 587-613.
5. LR Snyder, JJ Kirkland and LJ Glajch. Practical HPLC Method Development. John Wiley and Sons, INC, 1997; 2: 98-102.
6. LR Snyder and JJ Kirkland. Introduction to Modern Liquid Chromatography. JohnWiley & Sons, New York, 1979; 453-482.
7. ICH. Validation of analytical procedures: Text and methodology. International conferenceon harmonization, IFPMA, Geneva, 1996.
8. HH Willard, LL Merritt, JA Dean and FA Settle. Instrumental Methods of Analysis. CBS Publishers and Distributors. New Delhi, 2006; 7: 580-608.
9. RS Satoskar, SD Bhandarkar and SS Ainapure. Pharmacology and Pharmacotherapeutics. Popular Prakashan, Mumbai, 2001; 17: 578 - 598.
10. N Kannapan, SP Nayak, T Venkatachalam and V Prabhakaran. Analytical RP-HPLC Method for Development and Validation of Pregabalin and Methylcobalamine in Combined Capsule Formulation. *Journal of Applied Chemical Research*, 2010; 13: 85-89.
11. Ki-Ho Jang, Ji-Hyung Seo, Sung-Vin Yim and Kyung-Tae Lee. Rapid and simple method for the determination of Pregabalin in human plasma using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) application to a Bioequivalence study of Pregabalin capsule. *Journal of Pharmaceutical Investigation*, 2011; 41(4): 255-262.
12. A Korolkovas. Essentials of Medicinal Chemistry. Wiley Interscience, New Jersey, 1988; 2.
13. A Gilman and LS Goodman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. McGraw-Hill health professions division, New york, 1996; 9.
14. WO Foye. Foye's Principles of Medicinal Chemistry. Lippincott Williams & Wilkins, New york, 2008; 6.
15. Drugs & Cosmetics Act, 1940 & Rules, 1945, 2nd edition, Susmit publishers, Mumbai,India, 2000.

16. Indian Pharmacopoeia, Ministry of Health & Family Welfare, Government of India, NewDelhi, 1996.
17. The United States Pharmacopoeia- the National Formulary, United States Pharmacopoeialconvention, Rockville, 2007.
18. Jayendra Chundur, Pavan Kumar Chadalawada, Govada Kishore Babu, Srinivasa Babu Puttagunta, Development and Validation of Analytical Procedures for the Simultaneous Estimation of Acyclovir and Zidovudine through UV and RP-HPLC Methods, World Journal of Pharmacy & Pharmaceutical Sciences, 13, 10: 674-683.
19. KL Chaudhari and DG Maheshwari. RP-HPLC method for the estimation of Epalrestat and Methylcobalamin in their combined dosage form. *Indo American Journal of Pharmaceutical Research*, 2014; 4(6): 2697-2705.
20. M Patel, BJ Ladva, V Mahida, BS Nayak, HK Patel and BR Patel. Development and validation of RP-HPLC method for simultaneous estimation of Epalrestat and Methylcobalamin in tablet dosage form. *World Journal of Pharmacy and Pharmaceutical sciences*, 2010; 4(5): 574-584.
21. MA Hinge, NK Patel and RJ Mahida. Development and validation of high performance liquid chromatographic method for simultaneous estimation of Epalrestat and Methylcobalamin in combined dosage form. *Indo American Journal of Pharmaceutical Research*, 2013; 2(3): 2357-2359.
22. PJ Pathi and NA Raju. The estimation of Epalrestat in tablet dosage form by RP-HPLC. *Asian Journal of Pharmaceutical Analysis*, 2012; 2(2): 49-51.
23. Vaishali, V Singh, RK Singh, RK Gupta, SR Swain and J Sahoo. Development and validation of RP-HPLC method for the assay of Pregabalin capsule. *World Journal of Pharmacy and Pharmaceutical Sciences*, 2012; 3(1): 703-711.
24. GB Kasawar and MN Farooqui. Development and validation of HPLC Method for the determination of Pregabalin in Capsules. *Indian Journal of Pharmaceutical Sciences*, 2010; 72(4): 517-519.
25. J Balaji, B Ramachandra and NVS Naidu. Analytical RP-HPLC method for development and validation of Pregabalin in bulk and the determination of Pregabalin in capsule dosage form. *International Journal of Innovative Research in Science, Engineering and Technology*, 2014; 3(4): 512-516.
26. BV Rao, VLS Lanka, DM Rao. Method development and validation studies of Pregabalin drug by RP-HPLC method. Pharma Research Library. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2014; 4(1): 563-567.