ROBUST ANALYTICAL METHOD DEVELOPED AND VALIDATED FOR THE SIMULTANEOUS ESTIMATION OF VALPROATE AND LAMOTRIGINE BY RP-HPLC

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ABSTRACT

A robust, simple precise and accurate RP-HPLC method was developed and validated for the simultaneous estimation of Lamotrigine and Valproate in a combined dosage form. The stationary phase selected was C18 (150x4.6, 5µm) and the mobile phase comprising of Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v), the flow rate was set at 1.0 ml/min. The analyte was measured in UV detector at 255 nm. The retention time of Lamotrigine and Valproate eluted were to be 2.551 and 4.879 min respectively. The linearity range was found to lie from 10µg/ml to 50 µg/ml of Lamotrigine and 20µg/ml to 100µg/ml of Valproate. The percentage assay of pharmaceutical formulation was obtained for Lamotrigine 100.7% and Valproate 101.4%. The proposed method was validated as per the ICH guideline and the method was linear, precise, accurate and specific.

KEYWORDS

Lamotrigine, Valproate, HPLC, Simultaneous estimation and Robust method.

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INTRODUCTION

Valproic acid (Figure No.1), supplied as the sodium valproate semisodium or divalproex sodium, is a fatty acid with anticonvulsant properties used in the treatment of epilepsy. It may act by increasing gamma-aminobutyric acid levels in the brain or by altering the properties of voltage dependent sodium chennels. It's typically supplied in the sodium salt form. Valproic acid is also a histone deacetylase inhibitor and is under investigation for treatment of HIV and various cancers.

Lamotrigine (Figure No.2) is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorder. For epilepsy it is used to treat partial

seizures, primary and secondary tonic-clonic seizures and seizures associated with lennox-gastaut syndrome. It's also acts as a mood stabilizer. It is the first medication since lithium granted USFDA approval for the maintenance treatment of bipolar type-I. Chemically unrelated to other anticonvulsants, lamotrigine has relatively few side-effects and does not required blood monitoring.

In the literature search, there is little HPLC method which has been cited on Valporic acid in individually and combination with other drug and Lamotrigine is individually and in combination with other drug. There are few simultaneous HPLC methods which has been cited on Valproate and Lamotrigine. There is need to improve method for a simple and robust simultaneous estimation of Valproate and Lamotrigine in HPLC, for reduce the retention time and improve the sensitivity.

EXPERIMENTAL
Chemical and Reagents
Working standards of Valproic acid and Lamotrigine were gifts from KP Labs., Hyderabad, India. Acetonitrile, Methanol and Water (HPLC Grade) were purchased from Merck., Potassium dihydrogen AR Grade were purchased from Merck., Eurepa mf tablets were purchased from local pharmacy.

Chromatographic Condition and Instrumentations used
The method was developed in Waters HPLC (2695) and empower chromatographic software were used whole method development as well as validation. Satorius digital weighing balance (BSA224SC) and Lab India pH meter (AD102U). Double beam UV (UV 3000) and UV Win 5 software used. Inertsil C18 column (250*4.6, 5µm) as a stationary phase, Phosphate buffer (0.05M) pH 4.6: Acetonitrile (30:70 % v/v) used as a mobile phase, detection made at 255nm, Flow rate of mobile phase was fixed at 1.0 ml/min, Injection volume is 20µl and the temperature of the column is ambient.

RESULTS AND DISCUSSION
Wavelength Detection
The detection wavelength was carried out by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individually and combination of both the standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Lamotrigine and Valproate was obtained and the isobestic point of Lamotrigine and Valproate showed absorbance's maxima at 255 nm. The Overlay spectrum of Lamotrigine and Valproate and the spectrums of the individual drugs are shown in Figures No.3, 4 and 5.

Method Development
The chromatographic method development for the simultaneous estimation of Lamotrigine and Valproate were optimized by several trials for various parameters as different column, flow rate and mobile phase (Buffer and Organic phase), finally the optimized chromatographic method was selected for the separation and quantification of Lamotrigine and Valproate in API and pharmaceutical dosage form by RP-HPLC method. There are many trial has been performed by trial and error method among them there are few important chromatographic conditions are listed below.

Trial-1: Chromatographic conditions
Column : Agilent C18 (4.6*150mm) 5µm
Mobile phase ratio : Water: Methanol (40:60%v/v) Detection wavelength : 255nm
Flow rate : 1ml/min Injection volume : 20µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Observation: Valproate and Lamotrigine were separated and two individual peaks are separated but they are not clear, then further study has been conducted.

Trial-2: Chromatographic conditions
Column : Thermosil C18 (4.6*150mm) 5µm
Mobile phase rati : Water: Methanol (40:60%v/v)
Detection wavelength : 255nm
Flow rate : 1ml/min Injection

volume : 20µl Column temperature: 40°
Auto sampler temperature : Ambient
Observation: System suitability parameter is being improved when compared to the previous trial. Further trials are conducted for better resolution.

Trial-3: Chromatographic conditions
Column : Agilent C18 5µm (4.6*250mm)
Mobile phase ratio : Phosphate buffer (0.05m) pH 5.0: Methanol (50:50%v/v)
Detection wavelength : 255nm
Flow rate : 1ml/min
Injection volume : 20µl
Observation: There is noticeable improvement in resolution. But Poor peak shape and peak symmetry.

Trial-4: Chromatographic conditions
Column : Inertsil ODS C18 5µm (4.6*250mm)
Mobile phase ratio : Phosphate buffer (0.05M) pH 4.6: MeOH
Detection wavelength : 255nm
Flow rate : 1ml/min
Injection volume : 20µl
Auto sampler temperature : Ambient
Observation: Peak was separated but the qualities of chromatographic parameters are not meet the limit.

Trial-5: Chromatographic conditions: (optimized chromatographic condition)
Column : Inertsil C18 5µm (4.6*250mm)
Mobile phase ratio : Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) Detection wavelength : 255nm
Flow rate : 1.0 ml/min
Injection volume : 20µl Column temperature : Ambient
Observation: The peak was well separated and the qualities of chromatogram like peak symmetry, Resolution are within specified limit. Hence this trial is chosen as optimized Chromatographic condition and this method suitable for both qualitative and quantitative analysis.

Assay Calculations for Lamotrigine and Valproate
The assay study was performed for the Lamotrigine and Valproate. Each three injections of sample and standard were injected into chromatographic system. The chromatograms are shown in Figure No.6. The system suitability parameters for Lamotrigine and Valproate such as theoretical plates, tailing factor and resolution were found to be with Limit. The % purity of Lamotrigine and Valproate in pharmaceutical dosage form was found to be 100.7% and 101.4% respectively.

Method Validation
Accuracy
The accuracy study was performed for 50%, 100% and 150 % for Lamotrigine and Valproate. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery.

Precision
Repeatability
The precision study was performed for five injections of Lamotrigine and Valproate. Each standard injection was injected in to chromatographic system. The area of each Standard injection was used for calculation of % RSD. The results of repeatability are tabulated in Table No.2.

Acceptance Criteria
The % RSD for the area of five standard injections results should not be more than 2.0 %. The Method precision study was performed for the % RSD of Lamotrigine and Valproate was found to be 0.3 and 0.3 respectively (NMT 2.0).

Intermediate precision
The intermediate precision study was performed for five injections of Lamotrigine and Valproate. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD. The Intermediate precision are results in Table No.3.

Acceptance Criteria
The % RSD for the area of five standard injections results should NMT 2.0%. The intermediate

precision was performed for % RSD of Lamotrigine and Valproate was found to be 0.1 and 0.1 respectively (NMT 2.0).

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. The specificity test was performed for Lamotrigine and Valproate. It was found that there was no interference of blank and other degradants in retention time of analytical peak. The chromatograms are shown in Figure No.3. The results of Specificity are tabulated in Table No.4.

Limit of Detection

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula

$$LOD = 3.3 \times \frac{\sigma}{S}$$

Where, σ - Standard deviation (SD), S – Slope

S/N Ratio of **Valproate**

Average Baseline Noise obtained from Blank : 41µV Signal

Obtained from LOD solution : 125 µV

S/N = 125/41= 3.04

Acceptance Criteria

S/N Ratio value shall be 3 for LOD solution.

S/N Ratio of **Lamotrigine**

Average Baseline Noise obtained from Blank : 41 µV Signal Obtained from LOD solution : 124 µV

S/N = 124/41 = 3.02

Acceptance Criteria

S/N Ratio value shall be 3 for LOD solution.

The LOD was performed for Lamotrigine and Valproate was found to be 2.95and 3.04 respectively.

Limit of Quantization

S/N Ratio of Valproate

Average Baseline Noise obtained from Blank : 41 µV   Signal Obtained from LOQ solution : 412µV

S/N = 412/41 = 10

Acceptance Criteria

S/N Ratio value shall be 10 for LOQ Solution.

S/N Ratio of Lamotrigine

Average Baseline Noise obtained from Blank : 41 µV

Signal Obtained from LOQ solution : 405µV

S/N = 405/41 = 9.87

Acceptance criteria

S/N Ratio value shall be 10 for LOQ solution.

The LOQ was performed for Lamotrigine and Valproate was found to be 9.87 and 10 respectively.

Linearity

The linearity study was performed for the concentration of 100ppm to 500ppm and1ppm to 5ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The results are tabulated in Table No.5 and 6 Calibration graph for Lamotrigine and Valproate are shown in Figure No.7, 8.

Acceptance Criteria

Correlation coefficient should be not less than 0.999

Plotting of calibration graphs

The resultant areas of linearity peaks are plotted against Concentration.

Range

The linearity study was performed for concentration range of 10µg - 50µg and 20µg-100µg of Lamotrigine and Valproate and the correlation coefficient was found to be 0.999 and 0.999 respectively.

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

Flow Rate

The robustness was performed for the flow rate variations from 0.8 ml/min to 1.2 ml/min. Standard solution 60µg/ml of Valproate and 30µg/ml of Lamotrigine was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ± 0.2ml/min.

The Organic Mobile phase was varied from 70% to 60%. Standard solution 300 µg/ml of Valproate and 3µg/ml of Lamotrigine was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

The results are summarized. On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

Mobile Phase

Table No.1: Accuracy results of Valproate

S.No	%Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovery	Mean Recovery
1	50%	2332744	5	5.10	101.8%	
2	100%	3132697	10	9.99	99.9%	100.3%
3	150%	3918997	15	14.9	99.1%	

*Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Table No.1.1: Accuracy results of Lamotrigine

S.No	%Concentration (at specification level)	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
1	50%	353867	05	5.0	101.3%	
2	100%	4735088	10	9.94	99.4%	100.0%
3	150%	5911798	15	14.8	99.2%	

*Acceptance Criteria: The % Recovery was within the limit of 95% -105%.

Table No.2: Repeatability results of Lamotrigine and Valproate

S.No	Drug Name	RT	Peak Area	Drug Name	RT	Peak Area
1		2.321	99.6		4.304	101.5
2		2.317	101.5		4.300	102.5
3	Lamotrigine	2.323	100.8	Valproate	4.308	100.8
4		2.322	101.1		4.310	101.1
5		2.324	100.7		4.314	100.7
Mean			100.74			101.32
SD			0.71			0.73
%RSD			0.70			0.72

Table No.3: Intermediate precision results of Valproate and Lamotrigine

S.No	Drug Name	RT	Peak Area	Drug Name	RT	Peak Area
1		2.33	100.9		4.34	100.5
2		2.33	101.8		4.33	101.2
3		2.33	101.4		4.33	100.4
4	Lamotrigine	2.33	101.1	Valproate	4.34	100.8
5		2.33	101.3		4.33	100.9
Mean			101.3			100.76
SD			0.34			0.32
%RSD			0.33			0.32

Table No.4: Specificity results of Lamotrigine and Valproate

S.No	Peak name	R_t	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Lamotrigine	2.237	2513799	394185	2632	1.8	
2	Valproate	4.342	2963381	162758	2614	1.6	5.23

Table No.5: *Linearity Results for* Valproate

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	892464
2	II	40 ppm	1904884
3	III	60 ppm	2906620
4	IV	80 ppm	3800672
5	V	100 ppm	4738193
Correlation Coefficient			0.9993

Table No.6: *Linearity Results for* Lamotrigine

S.No	Linearity Level	Concentration	Area
1	I	10 ppm	907953
2	II	20 ppm	1730043
3	III	30 ppm	2553693
4	IV	40 ppm	3283876
5	V	50 ppm	4144232
Correlation Coefficient			0.9991

Table No.7.1: System suitability results for Valproate (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		Retention Time	USP Tailing
1	0.8	2.099	1.7
2	1.0	2.237	1.1
3	1.2	2.322	1.8

Table No.7.2: System suitability results for Lamotrigine (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		Retention Time	USP Tailing
1	0.8	4.134	1.6
2	1.0	4.342	1.1
3	1.2	4.445	1.5

Table No.7.3: System suitability results for Valproate (Mobile phase)

| S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		Retention Time	USP Tailing
1	10% Less	2.086	1.22
2	Actual	2.228	1.2
3	10% More	2.399	1.2

* Results for actual Mobile phase composition (45:55Buffer: ACN) have been considered from Accuracy standard.

Table No.7.4: System suitability results for Lamotrigine (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		Retention Time	USP Tailing
1	10% Less	4.636	1.56
2	Actual	4.342	1.1
3	10% More	4.134	1.6
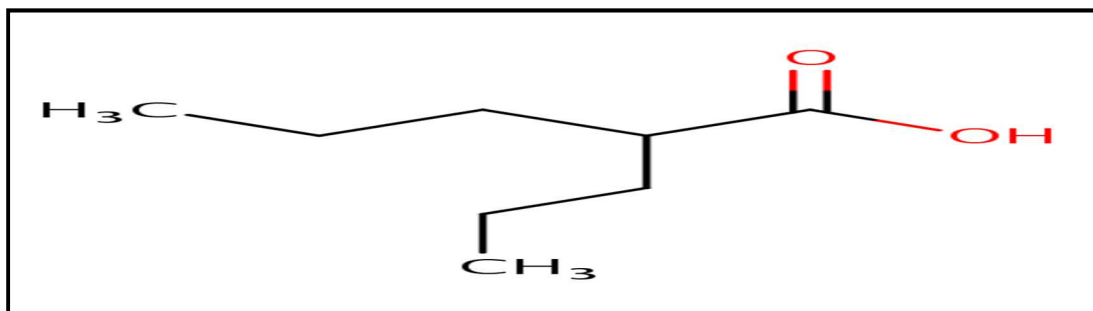
Figure No.1: Valporic acid
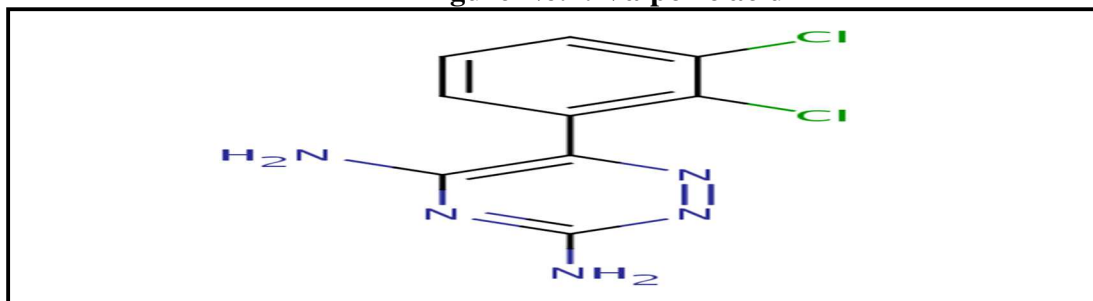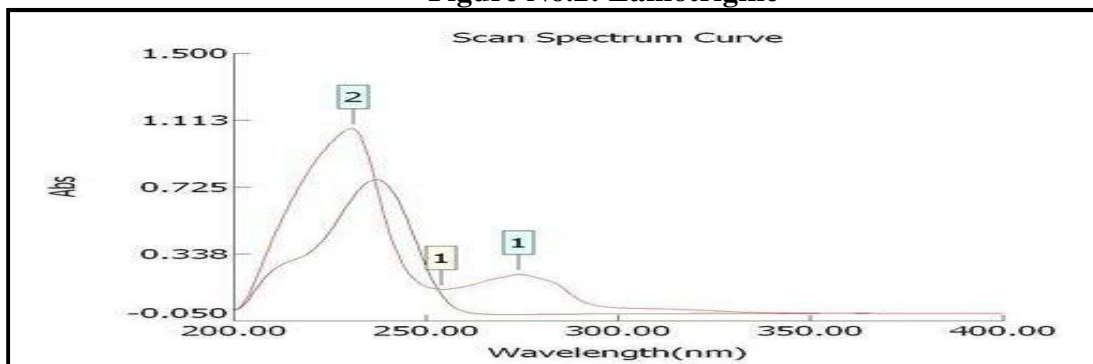

Figure No.2: Lamotrigine

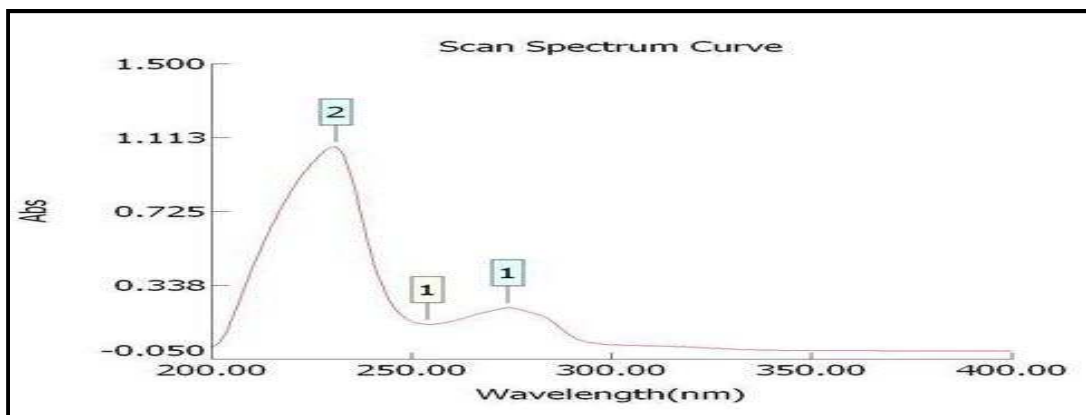**Figure No.3: Overlay spectrum of Lamotrigine and Valproate**

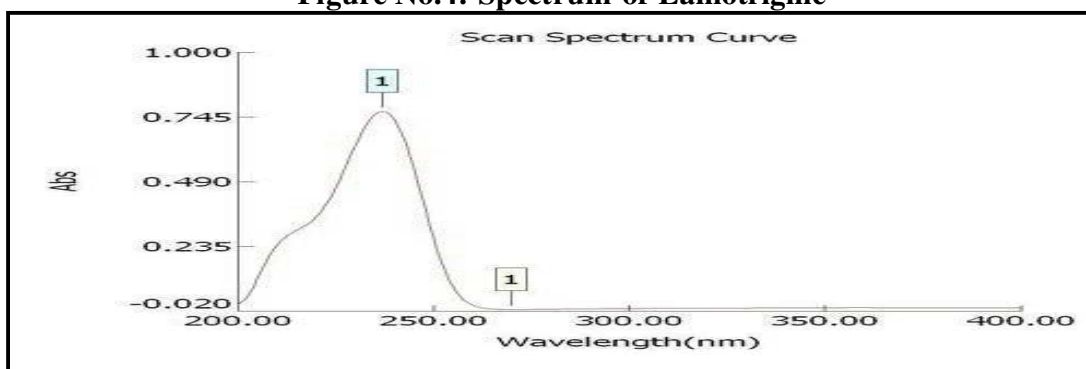**Figure No.4: Spectrum of Lamotrigine**

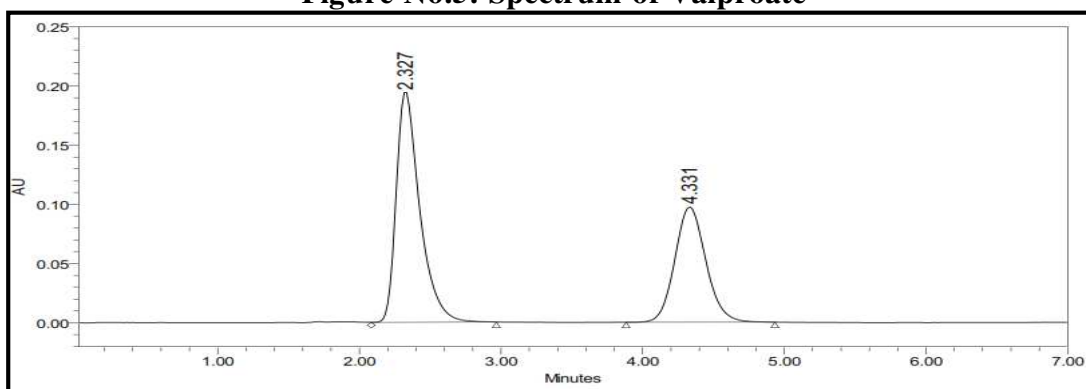
Figure No.5: Spectrum of Valproate

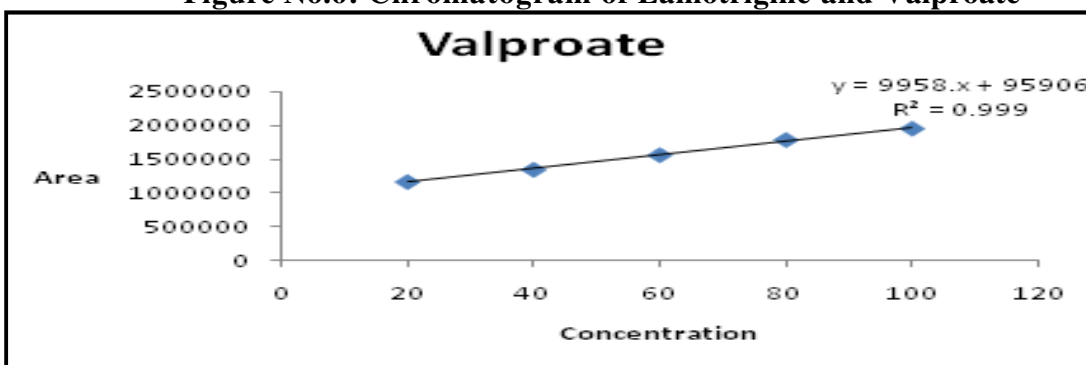**Figure No.6: Chromatogram of Lamotrigine and Valproate**

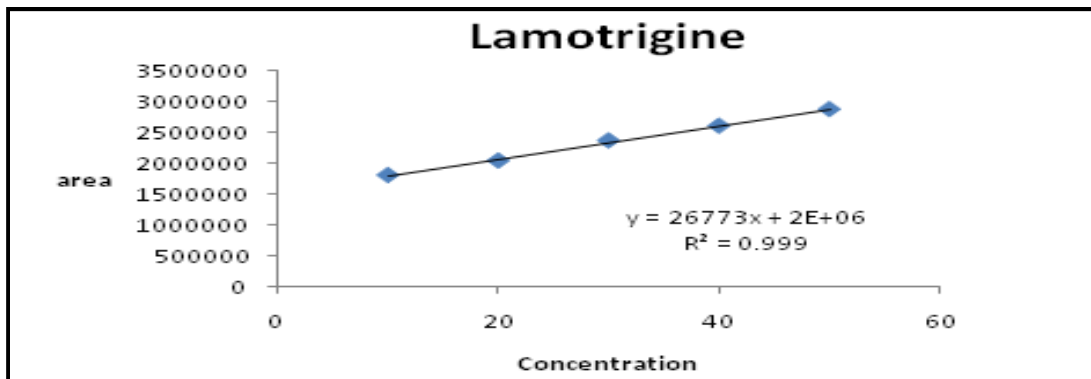**Figure No.7: Calibration curve of Valproate**

Figure No.8: Calibration curve of Lamotrigine

SUMMARY AND CONCLUSION

A new analytical method was established for simultaneous estimation of Lamotrigine and Valproate by RP-HPLC method. The chromatographic conditions were successfully developed and optimized for the separation of Lamotrigine and Valproate by using Xterra C18 5µm (4.6*250mm) column, flow rate was 1.0 ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection was carried out at 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.

The retention times were found to be 2.399 Min and 3.907 Min and the % purity of Lamotrigine and Valproate was found to be 100.7% and 101.4% respectively. The system suitability parameters for Lamotrigine and Valproate such as theoretical plates and tailing factor were found to be within the specified limit and satisfied.

The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Lamotrigine and Valproate was found in concentration range of 10 µg to 50 µg and 20 µg to 100 µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively. The precision study was precise, robust and repeatable. LOD value was 2.95 and 3.04ng/ml,

LOQ value was 9.87 and 10ng/ml respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Lamotrigine and Valproate in API and Pharmaceutical dosage form.

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CONFLICT OF INTEREST
Authors have declared that no competing interests exist.

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