CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com Research Article

A NEW RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LAMOTRIGINE AND VALPROATE IN IT'S PURE AND PHARMACEUTICAL DOSAGE FORM AS PER ICH GUIDELINES

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Abstract:
A novel, accurate, and reliable Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Lamotrigine and Valproate in both pure form and combined pharmaceutical dosage forms. The chromatographic separation was achieved using a Phenomenex Luna C18 column (4.6 × 150 mm, 5 µm) with an isocratic mobile phase of Acetonitrile and Water (45:55 v/v) at a flow rate of 1.0 mL/min. The detection was carried out at 250 nm, and the injection volume was 10 µL. The total run time was 7 minutes, during which both drugs were well resolved with sharp, symmetrical peaks. The method was validated according to ICH Q2(R1) guidelines for parameters including linearity, accuracy, precision, specificity, robustness, LOD, and LOQ. The results demonstrated excellent linearity over the selected concentration ranges for both drugs with correlation coefficients (r^2) close to 1.0. The %RSD for precision studies was well below 2%, indicating good repeatability. Recovery studies confirmed the accuracy of the method, and robustness testing showed the method's reliability under slight variations of analytical conditions.

Keywords: *RP-HPLC, Lamotrigine, Valproate, simultaneous estimation, validation. Phenomenex Luna C18 column.*

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QR CODE

Please cite this article in press ***Y. Shirisha*** *et al.,* ***A New Rp-Hplc Method For The Simultaneous Estimation Of Lamotrigine And Valproate In It's Pure And Pharmaceutical Dosage Form As Per Ich Guidelines*** *, Indo Am. J. P. Sci, 2025; 12(10).*

INTRODUCTION:

High performance liquid chromatography (also known as high pressure liquid chromatography) is a type of column chromatography used to separate, identify, and quantify active ingredients in biochemistry and analysis[1].HPLC mainly utilizes a column that holds packaging material (stationary phase), a pump that moves the mobile phase through the column and a detector that shows the retention time of the molecule. Retention time varies depending on the interaction between the stationary phases the molecule being analysed, and the solvent used.[2]A known amount of the material to be analysed is added to the mobile phase stream and evaluated by a chemical or physical interaction with the stationary phase. The amount of retardation is determined by the type of the analyte as well as the stationary and mobile phase composition. Retention time is the time it takes for a certain analyte to elute (come out of the end of the column). Any miscible combination of water and organic liquids is the most common mobile phase utilised (the most common are methanol & acetonitrile).Gradient elution is used to change the mobile phase composition during the study.[3]

TYPES OF HPLC:

The phase system employed in the process determines the type of HPLC.[3, 4] The following HPLC types are commonly used in analysis:

Normal phase chromatography:

This approach separates analytes based on polarity and is also known as Normal phase HPLC (NP-HPLC). A polar stationary phase and a non-polar mobile phase are used in NP-HPLC. The polar analyte interacts with the polar stationary phase and is retained by it. As the polarity of the analyte rises, so does the adsorption strength, and the interaction between the polar analyte and the polar stationary phase lengthens the elution time.

Reversed phase chromatography:

Reversed phase high performance liquid chromatography (RP-HPLC) consists of a non-polar stationary phase and amoderately aqueous polar mobile phase. RP-HPLCworks on the principle of hydrophobic interactions, the non-polar stationary phase is formed by repulsive forces between a polar eluent, the comparatively non-polar analyte, and the non-polar eluent. When the analyte molecule associates with the ligand in the aqueous eluent, the contact surface area around the non-polar segment of the analyte molecule is proportional to the contact surface area around the non-polar segment of the analyte molecule.

Size exclusion chromatography:

Size Exclusion chromatography, also known as gel permeation chromatography or gel filtration chromatography, is a type of chromatography that separates particles based on their size. It can also be used to figure out the quaternary and tertiary structures of proteins and amino acids. This method is often used to determine the molecular weight of polysaccharides.

Ion exchange chromatography:

Ion-exchange chromatography (IEC) depend on the attraction between solute ions and charged sites bound to the stationary phase. The ion exchange chromatography is mainly used for the purification of water

Bio-affinity chromatography:

In this method separation based on specific reversible interaction of proteins with ligands. Ligands are covalently attached to solid support on a bio-affinity matrix, retains proteins with interaction to the column-bound ligands. Proteins bound to a bio affinity column can be eluted in two ways:

• Biospecific elution: inclusion of free ligand in elution buffer which competes with column bound ligand.

• Aspecific elution: change in pH, salt, etc. which weakens interaction proteinwith column-bound substrate

History:

Before the invention of HPLC, scientists employed traditional liquid chromatographic methods. Liquid chromatographic methods are inefficient because of the dependence of solvent flow rate on gravity. It can take several hours, or even days, to finish a separation. It was believed that gas stage partition and the study of highly polar high atomic weight biopolymers were not feasible, even though liquid chromatography (LC) was at the time more effective. Because the solutes were thermally unstable, some organic chemists found that GC was unsuccessful. It was therefore expected that other techniques would soon propel HPLC forward. In the 1960s, building on the work of Martin and Synge in 1941, Cal Giddings, Josef Huber, and others predicted that LC could be operated in the high-efficiency mode by lowering the pressing molecule measurement well below the standard LC and GC level of 150 µm and using pressure to increase the versatile stage velocity. These expectations were the subject of much investigation and development in the 1960s and early 1970s. Early efforts were made to enhance LC particles, and the creation of the externally permeable molecule Zipax proved positive for HPLC technology. Throughout the 1970s, a lot of advancements in equipment and machinery were produced. Experts originally constructed a simple HPLC system using injectors and pumps. The reason gas amplifier pumps were ideal was that they didn't require release free seals or

check valves for excellent accuracy and steady flow, and they operated at a constant pressure. The history of HPLC is primarily the story of the development of molecular technology, even though equipment advancements played a big part. Since the introduction of permeable layer particles to boost efficacy, there has been a constant trend towards smaller molecules. However, new issues surfaced as molecule sizes decreased. It is anticipated that the disadvantage of the unnecessary pressure drop will be the challenge of uniformly pressing extremely fine materials and moving diverse liquid through the segment. Generally, each time the molecule size is fully reduced, another cycle of instrument advancement should occur to manage the pressure.[5-10]

EXPERIMENTAL METHODS
INSTRUMENTS USED

1 HPLC WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2 pH meter Lab India
3 Weighing machine Sartorius
4 Volumetric flasks Borosil
5 Pipettes and Burettes Borosil

CHEMICALS USED:

1 Lamotrigine Procured from Sun pharma, provided by Sura Pharma labs
2 Valproate Procured from Sun pharma, provided by Sura Pharma labs
3 Water and Methanol for HPLC LICHROSOLV (MERCK)
4 Acetonitrile for HPLC Merck

HPLC METHOD DEVELOPMENT:
TRAILS
Preparation of standard solution:
Accurately weigh and transfer 10 mg of Lamotrigine and Valproate working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 2.25ml of the above Lamotrigine and 0.45ml of the Valproate stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

RESULTS AND DISCUSSION:
(Optimized Chromatogram) (Standard)

Column : Phenomenex Luna C18 (4.6 x 150mm, 5µm)
Mobile phase : Acetonitrile and water (45:55 %v/v)
Flow rate : 1ml/min
Wavelength : 250 nm
Injection volume : 10 µl
Run time : 7 min

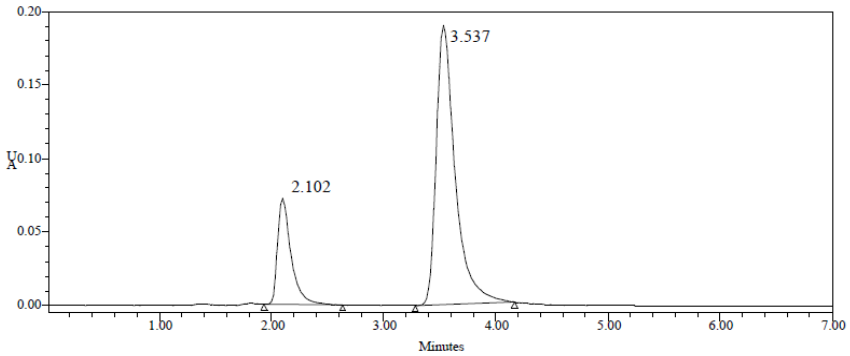

Fig7.5-: Optimized Chromatogram

Table7.5: - Peak results for Optimized Chromatogram

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Lamotrigine	2.102	765789	69584		0.97	5587.0
2	Valproate	3.537	2532158	190049	2.97	1.26	5398.0

Observation: From the above chromatogram it was observed that the Lamotrigine and Valproate peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

Column : Phenomenex Luna C18 (4.6 x 150mm, 5µm)
Mobile phase : Acetonitrile and water (45:55 %v/v)
Flow rate : 1ml/min
Wavelength : 250 nm
Injection volume : 10 µl
Run time : 7 min

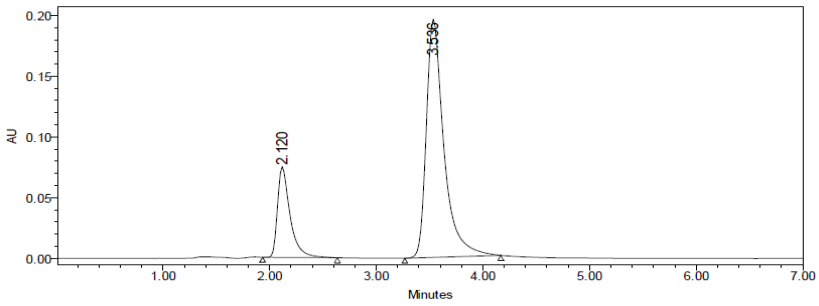

Figure7.6-: Optimized Chromatogram (Sample)

Table7.6: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Lamotrigine	2.120	775684	13124		0.99	6365.0
2	Valproate	3.536	2658478	937405	5.06	1.23	7458.0

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION

Blank:

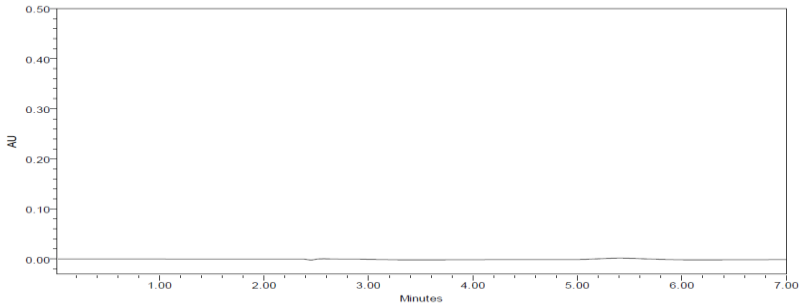

Fig7.7: Chromatogram showing blank (mobile phase preparation)

System suitability:

Table7.7: Results of system suitability for Lamotrigine

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Lamotrigine	2.117	765843	69587	5589	1.9
2	Lamotrigine	2.118	766594	69854	5576	1.6
3	Lamotrigine	2.116	765487	70211	5658	1.6
4	Lamotrigine	2.109	765928	69213	5642	1.7
5	Lamotrigine	2.102	765426	69558	5685	1.6
Mean			765855.6			
Std. Dev			466.6522			

% RSD			0.060932			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table7.8: Results of system suitability for Valproate

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Valproate	3.547	2534658	190058	5365	1.2	2.07
2	Valproate	3.539	2536854	190052	5348	1.4	2.05
3	Valproate	3.547	2535879	190078	5389	1.5	2.0
4	Valproate	3.565	2533564	190035	5347	1.6	2.01
5	Valproate	3.537	2534214	190085	5364	1.6	2.01
Mean			2535034				
Std. Dev			1183.309				
% RSD			0.046678				

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitated Lamotrigine and Valproate in drug product.

Assay (Standard):

Table7.9-: Peak results for assay standard

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Lamotrigine	2.102	759868	71255		1.7	5689	1
2	Valproate	3.537	2458754	215654	2.04	1.6	5362	1
3	Lamotrigine	2.105	759458	72541		1.7	5748	2
4	Valproate	3.552	2465885	226565	2.00	1.6	5452	2
5	Lamotrigine	2.112	759245	72584		1.7	5584	3
6	Valproate	3.560	2489578	221542	2.04	1.6	5456	3

Assay (Sample):

Table7.10: Peak results for Assay sample

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Lamotrigine	2.120	756985	68958		0.98	7253	1
2	Valproate	3.536	2569856	198564	2.06	1.23	8836	1
3	Lamotrigine	2.120	758745	69857		1.05	6530	2
4	Valproate	3.537	2598654	195682	2.04	0.99	7270	2
5	Lamotrigine	2.102	756848	69588		1.7	7586	3
6	Valproate	3.537	2587454	192541	2.04	1.6	8371	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Weight of standard}} \times \frac{\text{Weight of standard}}{\text{Dilution of sample}} \times \frac{\text{Dilution of sample}}{\text{Purity}} \times \frac{\text{Purity}}{\text{Weight of tablet}} \times 100$$

Standard area Dilution of standard Weight of sample 100 Label claim

The % purity of Lamotrigine and Valproate in pharmaceutical dosage form was found to be 99.8%.

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Lamotrigine:

Concentration µg/ml	Average Peak Area
0	0
15	205035
30	381239
45	561128
60	740162
75	909922

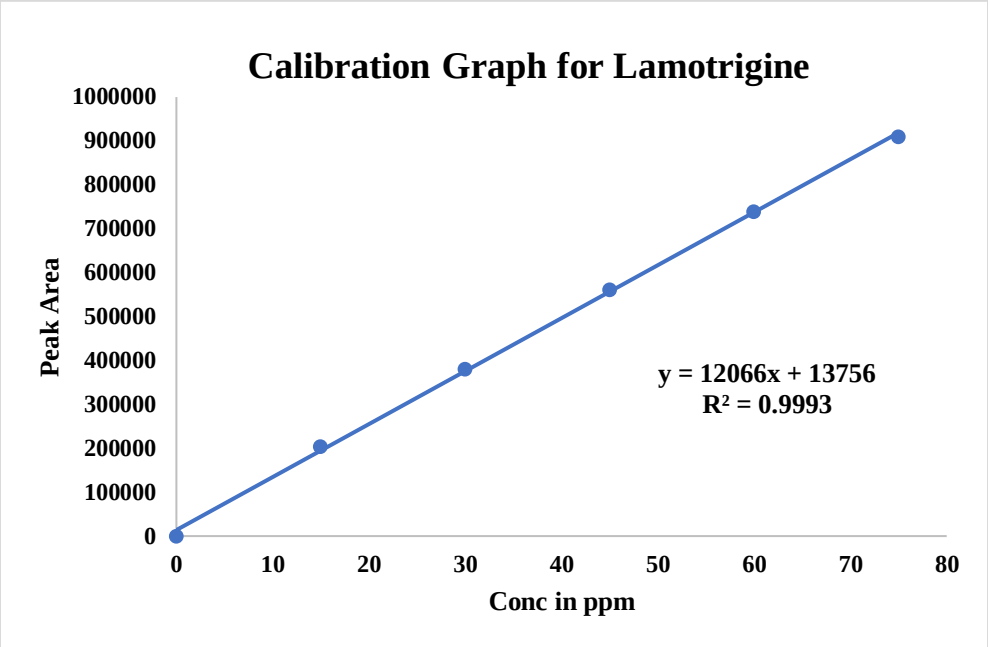

Figure-7.24: Calibration Graph for Lamotrigine

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Lamotrigine is a straight line.

Y = mx + c

Slope (m) = 12066

Intercept (c) = 13756

Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 13756. These values meet the validation criteria.

Valproate

Concentration µg/ml	Average Peak Area
0	0
10	757881
20	1457881
30	2132457
40	2901811

50	3501811

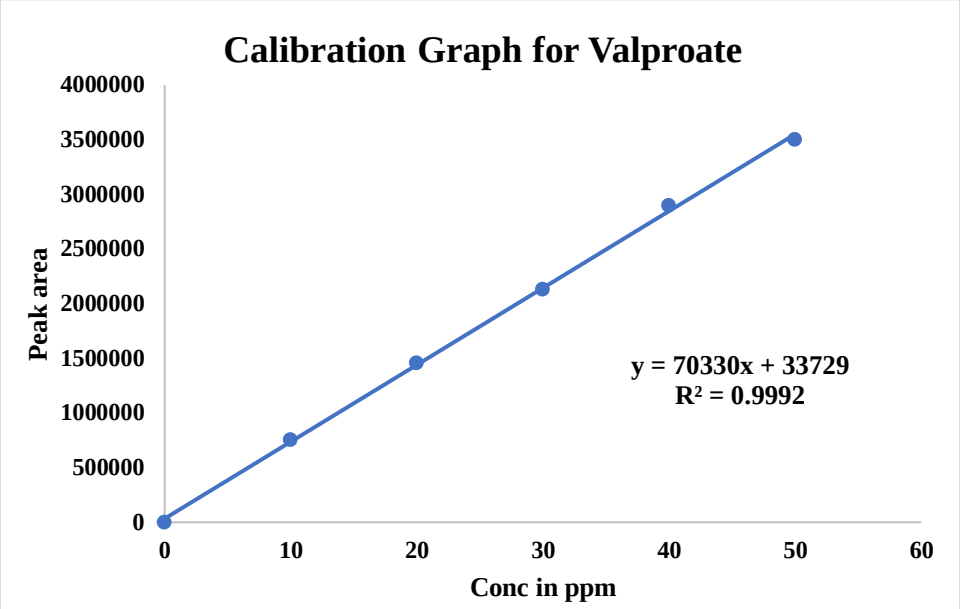

Figure-7.25: Calibration Graph for Valproate

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Valproate is a straight line.

Y = mx + c

Slope (m) = 70330

Intercept (c) = 33729

Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 33729. These values meet the validation criteria.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table7.11: Results of Repeatability for Lamotrigine:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Lamotrigine	2.108	766854	702564	5685	1.6
2	Lamotrigine	2.105	765884	698789	5584	1.4
3	Lamotrigine	2.113	765842	701235	5521	1.6
4	Lamotrigine	2.109	768985	700124	5525	1.9
5	Lamotrigine	2.109	765845	698986	5578	1.7
Mean			766682			
Std. Dev			1357.973			

% RSD			0.177123			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table7.12-: Results of method precision for Valproate**:**

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Valproate	3.552	2569865	2231111	5365	1.6
2	Valproate	3.550	2578474	2674210	5425	1.6
3	Valproate	3.564	2568985	2231261	5368	1.5
4	Valproate	3.564	2586845	2421301	5359	1.5
5	Valproate	3.565	2545898	2324710	5498	1.6
Mean			2570013			
Std. Dev			15309.45			
% RSD			0.595695			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

Day 1:

Table7.13-: Results of Intermediate precision for Lamotrigine

S.no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Lamotrigine	2.108	758955	68986	5785	1.6
2	Lamotrigine	2.105	759869	68957	5698	1.4
3	Lamotrigine	2.113	758985	68545	5689	1.6
4	Lamotrigine	2.109	756894	68952	5781	1.9
5	Lamotrigine	2.109	759854	68595	5785	1.7
6	Lamotrigine	2.102	756985	68952	5693	1.6
Mean			758590.3			
Std. Dev			1339.793			
% RSD			0.176616			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.

Table7.14-: Results of Intermediate precision for Valproate

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Valproate	3.552	2659852	190025	5485	1.5	2.04
2	Valproate	3.550	2648574	190048	5421	1.6	2.03
3	Valproate	3.564	2659865	190054	5468	1.6	2.01
4	Valproate	3.564	2658547	190078	5487	1.6	2.05
5	Valproate	3.565	2648981	190016	5492	1.6	2.02
6	Valproate	3.537	2654652	190057	5463	1.6	2.03
Mean			2655079				
Std. Dev			5242.086				
% RSD			0.197436				

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

Day 2:

Table7.15: Results of Intermediate precision Day 2 for Lamotrigine

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Lamotrigine	2.102	766895	69858	5586	1.5
2	Lamotrigine	2.105	765988	69854	5636	1.6
3	Lamotrigine	2.112	766532	69824	5432	1.6
4	Lamotrigine	2.113	766214	69875	5468	1.6
5	Lamotrigine	2.109	765897	69854	5546	1.9
6	Lamotrigine	2.109	765245	69848	5507	1.7
Mean			766128.5			
Std. Dev			567.7234			
% RSD			0.074103			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.

Table-7.16: Results of Intermediate precision for Valproate

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Valproate	3.537	2653254	190110	5428	1.6	7.98
2	Valproate	3.552	2648985	190058	5452	1.6	6.4
3	Valproate	3.560	2658213	190142	5498	1.6	8.9
4	Valproate	3.564	2653652	190031	5442	1.5	8.3
5	Valproate	3.564	2648978	190058	5489	1.5	7.5
6	Valproate	3.565	2658985	190047	5463	1.6	5.3
Mean			2653678				
Std. Dev			4313.355				
% RSD			0.162543				

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table-7.20: The accuracy results for Lamotrigine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	392891.7	5	5.027	100.540%	100.351%
100%	781996	10	10.026	100.260%	
150%	1171988	15	15.038	100.253%	

Table-7.21: The accuracy results for Valproate

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	204962	15	15.156	101.040%	100.93%
100%	365018	30	30.378	101.260%	
150%	521064.3	45	45.218	100.484%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Result:

Lamotrigine:
0.6µg/ml
Valproate**:**
0.8µg/ml

LIMIT OF QUANTITATION

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Result:

Lamotrigine:
1.8µg/ml
Valproate**:**
2.4µg/ml

Robustness

Table-7.22: Results for Robustness

Lamotrigine:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	765789	2.102	5587	1.7
Less Flow rate of 0.9 mL/min	758698	2.330	5458	1.7
More Flow rate of 1.1 mL/min	7689584	1.950	5696	1.7
Less organic phase	758412	2.290	5586	1.4
More organic phase	769852	1.998	5355	1.5

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Valproate**:**

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2532158	3.537	5398	1.6
Less Flow rate of 0.9 mL/min	2458692	3.885	5329	1.7
More Flow rate of 1.1 mL/min	2658642	3.263	5256	1.7
Less organic phase	2452148	4.435	5214	1.2
More organic phase	2653894	3.009	5524	1.0

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

8. SUMMARY AND CONCLUSION:

A new, simple, accurate, and precise Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Lamotrigine and Valproate in their pure and combined pharmaceutical dosage forms, in accordance with ICH Q2(R1) guidelines.

The chromatographic separation was achieved using a Phenomenex Luna C18 column (4.6 × 150 mm, 5 µm), with a mobile phase of Acetonitrile and Water in the ratio of 45:55 v/v, at a flow rate of 1.0 mL/min. The detection was carried out at a wavelength of 250 nm, and the injection volume was set at 10 µL. The total run time was 7 minutes, providing clear and sharp peaks with adequate resolution between the two drugs. The method was validated for various analytical parameters as per ICH guidelines including linearity, accuracy, precision, specificity, LOD, LOQ, robustness, and system suitability. Both drugs showed good linearity within their respective concentration ranges, and the %RSD values were

within acceptable limits, indicating the method's reliability.

CONCLUSION:

The newly developed RP-HPLC method is found to be simple, rapid, specific, and reproducible for the simultaneous estimation of Lamotrigine and Valproate in bulk and combined dosage forms. The method demonstrates excellent accuracy, precision, and robustness, meeting all validation criteria as per ICH Q2(R1) guidelines.

Due to its short runtime, cost-effectiveness, and high sensitivity, this method is highly suitable for routine quality control and stability studies in pharmaceutical industries involving Lamotrigine and Valproate formulations.

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Holy Mary Institute of Technology and Science (College of Pharmacy), Keesara - Bogaram - Ghatkesar, Telangana, Telangana, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Pharma Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

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