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NEW VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF VALPROATE AND LAMOTRIGINE IN COMBINED PHARMACEUTICAL DOSAGE FORMS

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Abstract: The present research focuses on the development and validation of a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Valproate and Lamotrigine in combined pharmaceutical dosage forms. The primary objective of this study was to establish a simple, accurate, precise, and cost-effective analytical method suitable for routine quality control and stability analysis. Chromatographic separation was achieved using a Discovery C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 60:40 v/v, pH was adjusted to 3 with 0.1% Ortho phosphoric acid, at a flow rate of 1.0 mL/min. Detection was performed at a wavelength of 230 nm, where both drugs exhibited significant absorbance. Under the optimized chromatographic conditions, sharp and symmetrical peaks were obtained with retention times of 2.365 minutes for Valproate and 3.423 minutes for Lamotrigine, showing excellent resolution and separation. The developed method was validated as per the International Council for Harmonisation (ICH Q2 R1) guidelines. The method demonstrated good linearity over the concentration range of 25–150 µg/mL for Valproate and 12.5–75 µg/mL for Lamotrigine, with correlation coefficients (R^2) greater than 0.999. Accuracy studies showed mean recoveries between 98% and 102%, while precision results confirmed reproducibility with %RSD values below 2. The method also proved to be robust and specific, showing no interference from excipients or degradation products. Hence, the developed RP-HPLC method is rapid, reliable, and suitable for routine simultaneous analysis of Valproate and Lamotrigine in combined dosage forms.

Keywords: *Valproate, Lamotrigine, Method Validation.*

INTRODUCTION

Valproic acid and its derivatives (collectively, valproate) are broad-spectrum antiseizure medications used in the management of several seizure types, including absence seizures and focal (partial) and generalized seizures. Valproate may be used as monotherapy or adjunctive therapy depending on the seizure type and clinical circumstances [1]. It is also used for the treatment of acute manic or mixed episodes associated with bipolar disorder and for the prevention of migraine headaches. Valproate is not used to treat an acute migraine attack.

The precise mechanisms underlying the therapeutic effects of valproate are not completely understood. Its antiseizure activity is thought to involve several mechanisms, including enhancement of inhibitory γ-aminobutyric acid (GABA) neurotransmission and modulation of voltage-gated sodium and calcium channels. These effects reduce neuronal excitability and may decrease the hypersynchronous neuronal activity associated with seizures.

Valproate may also influence intracellular signaling pathways involved in neuronal plasticity and cell survival. Experimental studies have suggested effects on the extracellular signal-regulated kinase (ERK) pathway, including activation of mitogen-activated protein kinase (MEK) and subsequent phosphorylation of ERK1/2. Activation of this pathway can influence downstream targets such as ELK-1 and may increase the expression of proteins including c-Fos, growth-associated protein-43 (GAP-43), B-cell lymphoma 2 (Bcl-2), and brain-derived neurotrophic factor (BDNF), which are involved in neuronal plasticity, growth, and cell survival. However, these molecular effects have primarily been demonstrated in experimental models, and their contribution to the clinical effects of valproate remains incompletely established. Therefore, claims that valproate provides a clinically established

neuroprotective effect or prevents neuronal degeneration in epilepsy, migraine, or bipolar disorder should be interpreted cautiously.

Lamotrigine is a phenyltriazine antiseizure medication used in the treatment of several types of epilepsy and in the maintenance treatment of bipolar I disorder [2]. It is indicated as adjunctive therapy for partial (focal) seizures, primary generalized tonic-clonic seizures, and seizures associated with Lennox-Gastaut syndrome in appropriate patients. Lamotrigine is also used for the maintenance treatment of bipolar I disorder, particularly for the prevention of depressive episodes. It is not considered an established treatment for acute mania.

The precise mechanism of action of lamotrigine is not completely understood. Its antiseizure effects are primarily attributed to inhibition of voltage-gated sodium channels, particularly by stabilizing the inactive state of these channels. This reduces sustained, repetitive neuronal firing and decreases the presynaptic release of excitatory neurotransmitters, particularly glutamate and, to a lesser extent, aspartate. By reducing excessive excitatory neurotransmission and neuronal excitability, lamotrigine helps prevent seizure propagation.

Although lamotrigine is chemically distinct from phenytoin and carbamazepine, its ability to inhibit voltage-gated sodium channels is broadly similar to that of these antiseizure medications. Its effects on neuronal excitability and neurotransmitter release are also thought to contribute to its therapeutic effects in bipolar disorder. However, the precise molecular mechanisms responsible for its mood-stabilizing effects are not fully established.

A detailed literature survey revealed that several analytical methods based on reverse-phase high-performance liquid chromatography (RP-HPLC) have been reported for the estimation of valproate and lamotrigine, both individually and in combination with other drugs, in various pharmaceutical dosage forms [3–8].

In the present study, chromatographic conditions, including mobile-phase composition, pH, flow rate, and detection wavelength, were systematically optimized to obtain sharp, symmetrical, and well-resolved peaks for both analytes. The proposed method was developed using a C18 reversed-phase column under isocratic conditions, with a mobile phase consisting of a suitable buffer and acetonitrile to achieve efficient separation of valproate and lamotrigine. Detection was performed at an appropriate wavelength at which both drugs exhibited adequate ultraviolet absorbance.

The developed RP-HPLC method was validated in accordance with the applicable International Council for Harmonisation (ICH) Q2(R1) guidelines [9] to establish its reliability, accuracy, and suitability for the intended analytical application. The validation parameters included specificity, linearity, accuracy, precision, robustness, and system suitability. Linearity was evaluated over an appropriate concentration range for both drugs, while accuracy was assessed by recovery studies. System suitability was evaluated using parameters such as theoretical plate count, tailing factor, and resolution to confirm the adequacy and consistency of the chromatographic system.

The proposed RP-HPLC method demonstrated the potential to provide reliable, reproducible, and accurate quantification of valproate and lamotrigine in combined pharmaceutical dosage forms. The method may therefore be suitable for routine quality-control analysis and assay determination of these drugs in pharmaceutical formulations. Its application to stability testing would depend on adequate demonstration of stability-indicating capability through appropriate forced-degradation and related validation studies. Overall, the validated RP-HPLC method provides a practical analytical approach for the simultaneous determination of valproate and lamotrigine and may support consistent quality assessment of their pharmaceutical formulations.

MATERIALS AND METHODS

The present study was carried out to develop and validate a simple, precise, accurate, and economical Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Valproate and Lamotrigine in pharmaceutical dosage forms. The work was performed according to International Council for Harmonisation (ICH Q2 R1) guidelines to ensure reliability and reproducibility for routine quality control analysis. All reagents and chemicals used were of analytical and HPLC grade. Valproate and Lamotrigine pure drug samples were obtained as gift samples from a reputed pharmaceutical manufacturer. Acetonitrile (HPLC grade), phosphate buffer, and orthophosphoric acid were procured from Merck India Ltd., while distilled water was used throughout the analysis. The chromatographic analysis was performed on a Waters HPLC system equipped with an auto-sampler, PDA detector, and Empower 2.0 software for data processing. The separation was achieved using a Discovery C18 column (250 × 4.6 mm, 5 μm particle size) maintained at ambient temperature. During method development, different mobile phase compositions, and flow rates were evaluated to obtain sharp and symmetrical peaks with optimal resolution between both analytes. The final optimized chromatographic conditions consisted of a mobile phase composed of phosphate buffer and acetonitrile in the ratio of 60:40 v/v, and a flow rate of 1.0 mL/min. The detection wavelength was set at 230 nm, and the injection volume was 10 µL. The total run time was about 10 minutes, providing well-resolved peaks for both drugs with retention times of 2.365 minutes for Valproate and 3.423 for Lamotrigine. Optimized Chromatographic Conditions were shown in Table 1.

Table 01: Optimized Chromatographic Conditions

Parameter	Optimized Condition
Column	Discovery C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Phosphate Buffer: Acetonitrile [60:40 v/v]
Flow Rate	1.0 mL/min
Detection Wavelength	230 nm
Injection Volume	10 µL
Run Time	10 minutes
Column Temperature	Ambient
Mode	Isocratic Elution

Preparation of Standard Solutions

Accurately weighed 25mg of Lamotrigine 50mg of Valproate and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (1000µg/ml of Valproate and 500µg/ml Lamotrigine)

Preparation of Sample Solution

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (2000µg/ml of Valproate and 1000µg/ml Lamotrigine)

System Suitability Parameters

Prior to analysis, the system was evaluated for performance consistency. Parameters such as retention time, theoretical plates, tailing factor, resolution, and %RSD were calculated using six replicate injections of the standard solution (Table 02).

Table 02: System Suitability Parameters

Parameter	Acceptance Criteria	Observation
Retention Time (min)	Consistent	2.367 (Valproate), 3.416 (Lamotrigine)
Theoretical Plates (N)	≥ 2000	6032 (Valproate), 8253 (Lamotrigine)
Resolution (Rs)	≥ 2.0	7.5
Tailing Factor	≤ 2.0	1.35 (Valproate), 1.36 (Lamotrigine)
%RSD (Peak Area)	≤ 2.0%	0.54%

The system suitability results confirmed the efficiency and reproducibility of the chromatographic system. Linearity studies demonstrated an excellent correlation between concentration and peak area within the tested ranges for both drugs, with correlation coefficients greater than **0.999**. The method exhibited satisfactory accuracy, with recovery results between **98% and 102%**, and high precision, with %RSD values below **2.0%** for both intra-day and inter-day studies. Minor variations in chromatographic parameters such as pH, wavelength, and flow rate did not significantly affect the results, confirming the robustness of the method. In conclusion, the developed RP-HPLC method was proven to be simple, rapid, precise, accurate, and cost-effective for the simultaneous estimation of Valproate and Lamotrigine. It can be effectively applied for routine quality control, stability testing, and assay determination of pharmaceutical formulations containing these drugs.

RESULTS AND DISCUSSION

The developed Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Valproate and Lamotrigine in pharmaceutical formulations was successfully optimized and validated according to the International Council for Harmonisation (ICH Q2 R1) guidelines. The objective was to establish a simple, accurate, and reproducible analytical method capable of determining both drugs simultaneously with high sensitivity and resolution. During method development, several trials were conducted by varying the mobile phase composition, and detection wavelength to obtain sharp, symmetrical, and well-separated peaks for both analytes. Various combinations of phosphate buffer and acetonitrile were studied in different ratios (45:55, 60:40, and 70:30 v/v) under isocratic conditions. The optimized chromatographic conditions were achieved using a Discovery C18 column (250 × 4.6 mm, 5 µm) with a mobile phase of phosphate buffer and acetonitrile (60:40 v/v), and a flow rate of 1.0 mL/min. The detection wavelength was set at 230 nm, which provided optimum response for both drugs. Under these optimized conditions, Valproate and Lamotrigine were well resolved with retention times of 2.386 minutes and 3.435 minutes, respectively. The chromatogram showed sharp, symmetrical peaks with good baseline separation, confirming the suitability of the method for routine analysis (Figure 01).

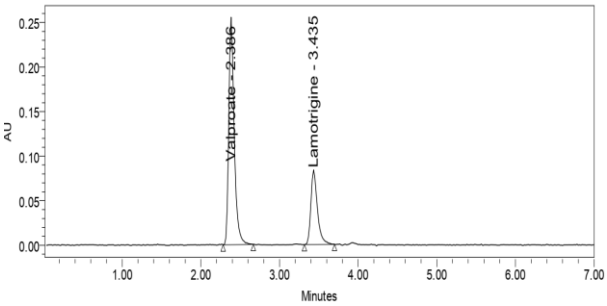

Figure 01: Optimized Chromatogram for Valproate and Lamotrigine

The optimized chromatogram demonstrated that both drugs were adequately resolved, with good peak symmetry and no significant interference from formulation excipients. The resolution (Rs) between the two peaks was 7.5, indicating excellent separation. The theoretical plate counts for valproate and lamotrigine were 6032 and 8253, respectively, demonstrating good column efficiency. The tailing factors were 1.34 for valproate and 1.35 for lamotrigine, both of which were within the generally acceptable limit of ≤2.0, indicating satisfactory peak symmetry. The percentage relative standard deviation (%RSD) of the peak areas for six replicate injections was 0.54%, demonstrating good injection repeatability and compliance with the predefined system suitability criterion.

Overall, the system suitability results confirmed that the developed RP-HPLC method exhibited satisfactory precision, efficiency, peak symmetry, and chromatographic resolution, making it suitable for the simultaneous determination of valproate and lamotrigine.

Linearity and Calibration Curve

The linearity of the developed method was evaluated by preparing a series of standard solutions over the concentration range of 25–150 µg/mL for valproate and 12.5–75 µg/mL for lamotrigine. Calibration curves were constructed by plotting the concentration of each drug against its corresponding peak area. Both drugs exhibited excellent linearity over the investigated concentration ranges, with correlation coefficients (R^2) greater than 0.999, indicating a strong linear relationship between analyte concentration and chromatographic response.

The linear regression equations obtained were **y = 12032x + 13101** for valproate and **y = 8993.4x + 1948.7** for lamotrigine. The corresponding calibration data and curves are presented in **Table 03 and Figures 02–09**, respectively.

Table 3: Linearity table for Valproate and Lamotrigine

Valproate		Lamotrigine	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
25	320715	12.5	114461
50	604208	25	235418
75	935882	37.5	334186
100	1234676	50	446830
125	1505358	62.5	565355
150	1807453	75	678167

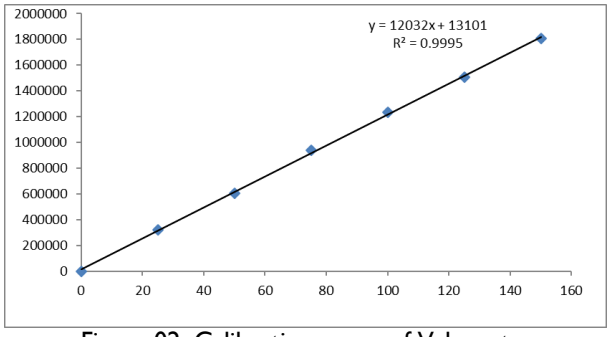

Figure 02: Calibration curve of Valproate

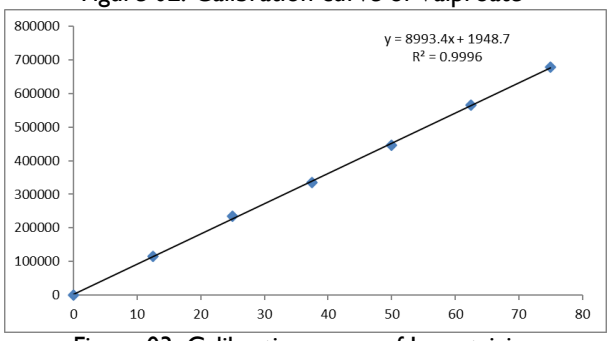

Figure 03: Calibration curve of Lamotrigine

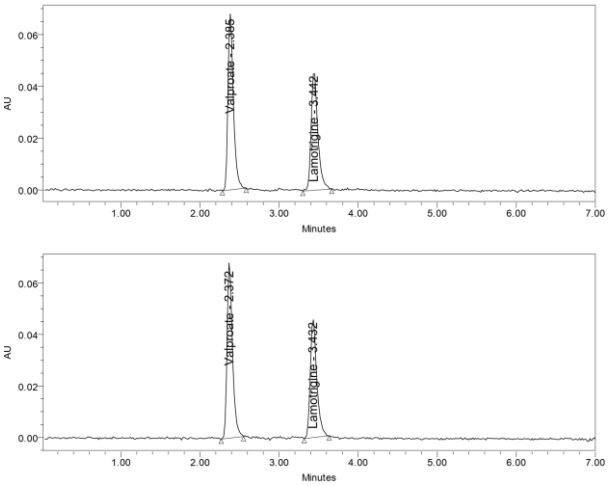

Figure 04: Linearity 25% Chromatogram of Valproate and Lamotrigine

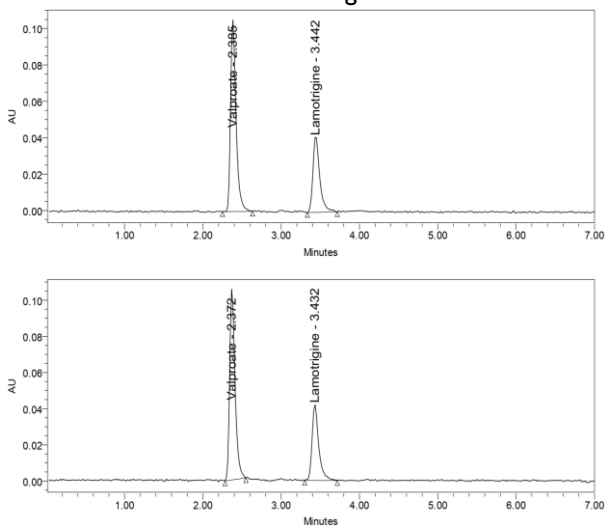

Figure 05: Linearity 50% Chromatogram of Valproate and Lamotrigine

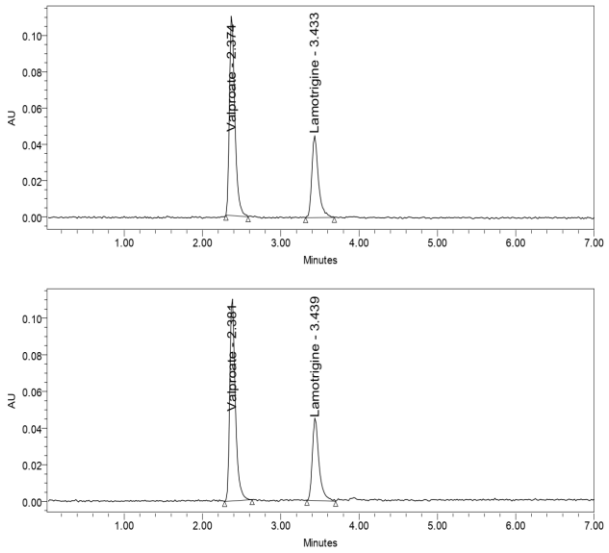

Figure 06: Linearity 75% Chromatogram of Valproate and Lamotrigine

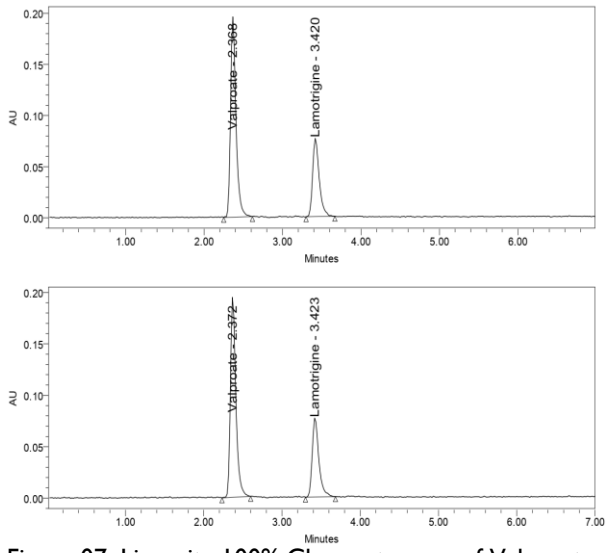

Figure 07: Linearity 100% Chromatogram of Valproate and Lamotrigine

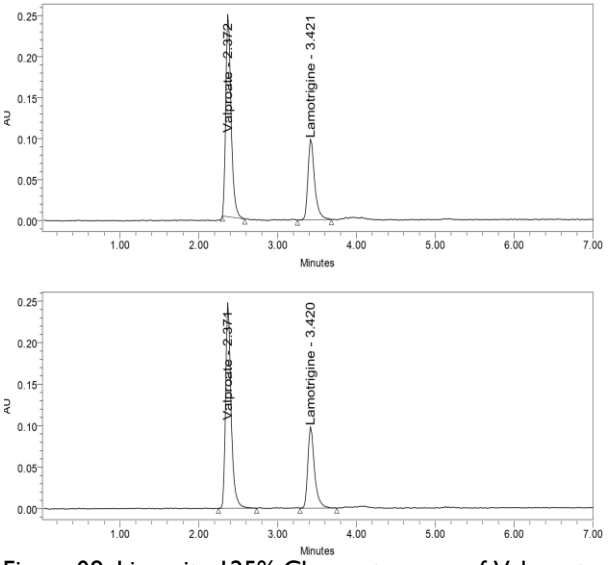

Figure 08: Linearity 125% Chromatogram of Valproate and Lamotrigine

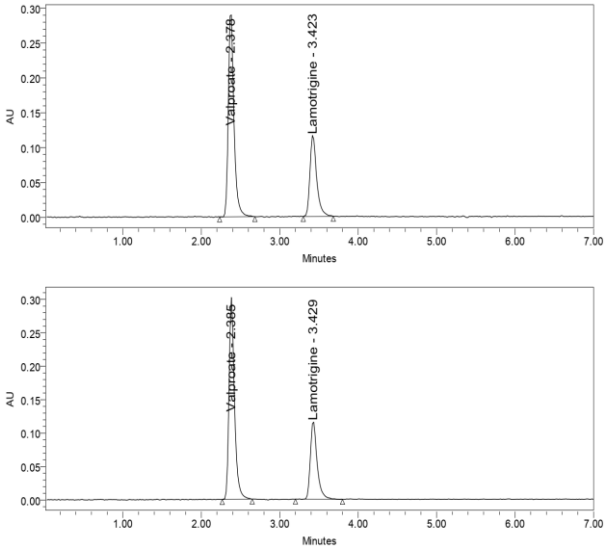

Figure 09: Linearity 150% Chromatogram of Valproate and Lamotrigine

Accuracy: Accuracy of the method was evaluated through recovery studies conducted at three levels: 50%, 100%, and 150% of the target concentrations. The percentage recoveries for Valproate and Lamotrigine ranged between **98.01% and 100.35%**, indicating that the method is highly accurate and free from interference by excipients in the formulation.

Precision: Precision studies, including intra-day and inter-day variations, were performed to assess the repeatability and intermediate precision of the method. The %RSD values for peak areas were found to be less than **2.0%** for both drugs, demonstrating the excellent precision and reproducibility of the method.

LOD and LOQ: The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined based on the standard deviation of the response and the slope of the calibration curve. The LOD values were 0.45 µg/mL for Valproate and 0.38 µg/mL for Lamotrigine, while the LOQ values were 1.36 µg/mL and 1.15 µg/mL, respectively. These low values indicate the high sensitivity of the method for detecting and quantifying both analytes.

Robustness: The robustness of the method was tested by introducing small, deliberate variations in method parameters such as flow rate, mobile phase composition, and temperature. These changes did not significantly affect the chromatographic performance, as the retention times and peak areas remained consistent with %RSD values below 2.0%, confirming that the method is robust.

DISCUSSION

The developed RP-HPLC method proved to be simple, rapid, and reliable for the simultaneous determination of Valproate and Lamotrigine. The results demonstrated that the method is accurate, precise, and sensitive, with excellent resolution and reproducibility. All validation parameters complied with ICH guidelines, confirming that the method is suitable for the routine quality

control, assay, and stability testing of pharmaceutical formulations containing Valproate and Lamotrigine. The sharp and symmetrical peaks with distinct retention times reflect the efficiency of the optimized chromatographic conditions. Overall, this method offers a robust analytical tool for simultaneous estimation in combined dosage forms, ensuring consistent pharmaceutical quality and regulatory compliance.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Valproate and Lamotrigine in Tablet dosage form. Retention time of Valproate and Lamotrigine were eluted at 2.365 min and 3.423 min respectively. %RSD of the Valproate and Lamotrigine were and found to be 0.6 and 0.7 respectively. %Recovery was obtained as 99.61% and 99.52% for Valproate and Lamotrigine respectively. LOD, LOQ values obtained from regression equations of Valproate and Lamotrigine were 0.45, 1.36 and 0.15, 1.15 respectively. Regression equation of Valproate is y = 12032x + 13101 and y = 8993.4x + 1948.7 of Lamotrigine. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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INFORM CONSENT AND ETHICAL STATEMENT

Not applicable

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Not Declared

AUTHOR CONTRIBUTION

All authors are contributed equally.

CONFLICT OF INTREST

Not Declared

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