

DEVELOPMENT AND VALIDATION OF A ROBUST RP-HPLC METHOD FOR SODIUM VALPROATE IN BULK AND TABLET FORMULATIONS

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Abstract

Sodium Valproate is an anticonvulsant drug widely used for the treatment of epilepsy, bipolar disorder, and migraine. It works by stabilizing electrical activity in the brain. The aim of the present study was to develop analytical methods for the estimation of Sodium Valproate in pharmaceutical tablet dosage forms, i.e., Reverse Phase High-Performance Liquid Chromatography (RP-HPLC). This study focuses on the development and validation of a highly sensitive and accurate Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the estimation of Sodium Valproate in bulk and pharmaceutical dosage forms. The method is optimized for separation efficiency and specificity, following International Conference on Harmonisation (ICH) guidelines for method validation. The proposed method shows excellent linearity, precision, accuracy, and robustness, making it suitable for routine analysis in quality control laboratories for the estimation of Sodium Valproate in formulations. The chromatographic separation was performed with HPLC agilent 1100 series with DAD detector. The stationary phase used was C-18 inertsil column of dimension 250mm × 4.6 mm with particle size of 5μ. The mobile phase used was buffer(pH 3.5), acetonitrile in the ratio of (50:50). With retention time 3.178 minutes, linearity in the range of 40-140 (μg/mL) Correlation coefficient 0.9999 at 210 nm. The developed methods were validated according to ICH guidelines and found that the % RSD values for all parameters were found to be within the limit.

Keywords: RP-HPLC, Sodium Valproate, DAD Detector, and Tablet dosage forms.

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INTRODUCTION

Sodium Valproate, chemically known as 2-propylpentanoic acid, is a widely used anticonvulsant and mood-stabilizing drug. Its chemical structure is shown in Figure 1. Sodium Valproate is a salt form of valproic acid and is primarily employed in the treatment of epilepsy, bipolar disorder, and migraine prevention. The drug works by stabilizing electrical activity in the brain and increasing the levels of gamma-aminobutyric

acid (GABA), an inhibitory neurotransmitter. It is widely recognized for its ability to prevent seizure activity and stabilize mood, making it essential in the management of various neurological and psychiatric conditions.

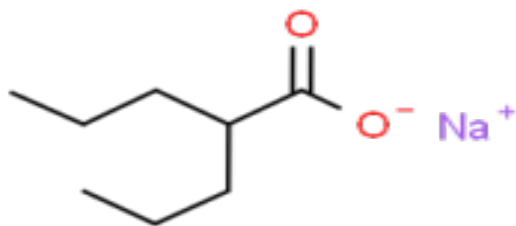


Fig 1: Chemical structure of sodium valproate

Sodium Valproate has a molecular formula of $C_8H_{16}O_2$ and a molecular weight of 166.19 g/mol. Literature surveys reveal that various analytical methods, such as spectrophotometric, chromatographic, and electrophoretic techniques, have been employed for the estimation of Sodium Valproate in biological fluids and pharmaceutical formulations. These include HPLC methods for the quantification of Sodium Valproate in serum, UV spectrophotometric methods for its determination in tablet formulations, and LC-MS/MS techniques for pharmacokinetic studies.

Although several analytical methods have been reported for the estimation of Sodium Valproate, there remains a need for more efficient, accurate, and cost-effective techniques that are suitable for routine pharmaceutical quality control. These methods should minimize the quantity of drug required, reduce time consumption, and ensure high reproducibility. Therefore, the present study aims to develop a highly sensitive, accurate, and reproducible RP-HPLC, HPTLC, and UV-Visible spectrophotometric method for the estimation of Sodium Valproate in bulk and its pharmaceutical tablet formulations.

MATERIALS AND METHODS:

- The chromatographic separation was performed with HPLC agilent 1100 series with DAD detector.
- The stationary phase used was C-18 inertsil column of dimension 250mm × 4.6 mm with particle size of 5 μ .
- The mobile phase used was buffer, acetonitrile in the ratio of (50:50).
- Sodium Valproate with valproic acid was procured from Kausikh Therapeutics, Chennai.

CHEMICALS AND REAGENTS:

- Acetonitrile
- Sodium valproate
- Potassium dihydrogen phosphate
- Ortho Phosphoric acid
- Distilled water.

PREPARATION OF BUFFER SOLUTION: 6.8 grams of Potassium dihydrogen phosphate was

taken in 1000ml beaker and dissolved into 1000ml of distilled water. The pH was adjusted to 3.5 with orthophosphoric acid.

PREPARATION OF MOBILE PHASE: The mobile phase was prepared by mixing the buffer and acetonitrile in the ratio of 50:50 and the mixture was degassed in ultrasonicator for 10 min. The mixture was filtered through 0.45 μ nylon filter under vacuum filtration.

CHROMATOGRAPHIC CONDITIONS:

The RP-HPLC method was developed using a mobile phase consisting of a 50:50 mixture of buffer and acetonitrile. The separation was achieved using a C-18 Inertsil column, with a 20 μ L injection volume and a flow rate of 1.0 mL/min. The pH of the mobile phase was adjusted to 3.5 using orthophosphoric acid. The chromatographic run time was set to 20 minutes, and the detection was carried out at a wavelength of 210 nm. These conditions were optimized to ensure efficient separation and accurate quantification of Sodium Valproate in pharmaceutical tablet formulations.

PREPARATION OF STANDARD SOLUTION:

Weigh 50 mg of Sodium valproate and transfer into 50 ml volumetric flask and dissolve with 25 ml of mobile phase and the volume was made up with mobile phase. Take 5ml from this solution and transfer it into 50 ml volumetric flask, dissolve it and makeup with mobile phase. Filter, it using 0.45 μ filter and 10 μ L of the resultant solution was injected for HPLC analysis.

PREPARATION OF SAMPLE SOLUTION:

Accurately weigh, 50mg sodium valproate tablet was transferred it into a 50 ml volumetric flask and dissolve it with 25 ml of mobile phase and the volume was made up with mobile phase. Take 5ml from this solution dissolve in 50ml of mobile phase. The solution was kept under sonicator for complete dissolving of drugs for 15 min. then the solution was made upto mark with mobile phase. Filter the solution using 0.45 μ filter and 10 μ L of the resultant solution was injected for HPLC analysis.

HPTLC METHOD DEVELOPMENT

The developed method for the estimation of Sodium Valproate was validated in accordance with the ICH guidelines, assessing parameters such as system suitability, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness. System suitability testing is a critical component to ensure the chromatographic system performs adequately before sample analysis. The following parameters were evaluated to determine the suitability of the system: Retention Time: The retention time for Sodium Valproate was determined to be 3.178 minutes. Tailing Factor: The tailing factor, which indicates peak symmetry, was calculated to be 0.8, suggesting good peak shape and symmetry.

Number of Theoretical Plates (N): The column efficiency, expressed as the number of theoretical

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plates, was found to be 1136, reflecting the high efficiency of the chromatographic column in resolving the analyte. These system suitability parameters were assessed using a standard chromatogram and confirmed the adequacy of the system for accurate and reproducible analysis of Sodium Valproate.

Specificity testing was performed to assess the ability of the developed RP-HPLC method to distinguish Sodium Valproate from other potential components in the sample, such as excipients and degradation products. This was achieved by comparing the retention times of the standard Sodium Valproate solution with those of the sample solution, blank, and placebo.

The retention time of Sodium Valproate was found to be **unaffected** by the mobile phase, blank solution, or placebo, indicating that no interference from other substances occurred during the chromatographic analysis.

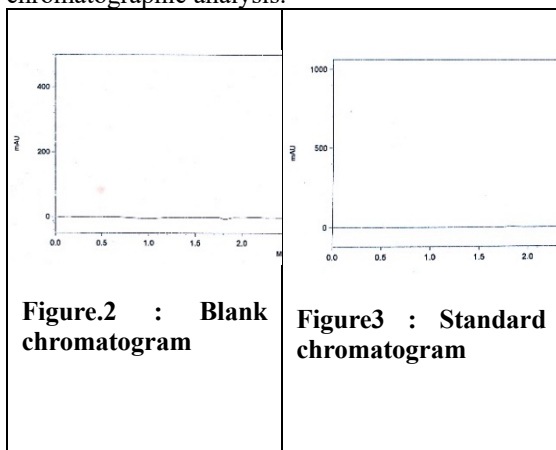
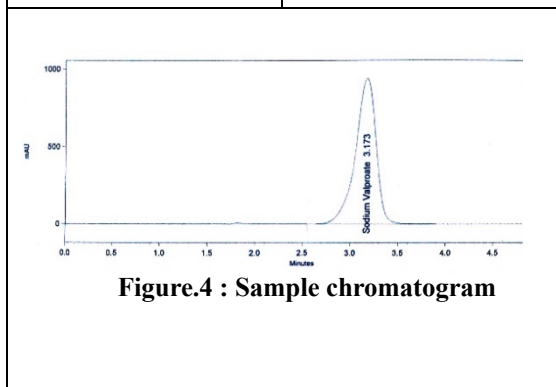


Figure3 : Standard chromatogram



LINEARITY AND RANGE:

For the RP-HPLC method, a calibration curve for Sodium Valproate was constructed by preparing solutions containing concentrations of **50, 75, 100, 125, and 150 µg/ml** from the standard stock solution. An aliquot of 10 µl from each solution was injected into the HPLC system under the optimized chromatographic conditions.

The calibration curve was plotted by correlating the peak area (absorbance) with the concentration of Sodium Valproate. A linear relationship was observed, with a correlation coefficient (r^2) of **0.999**,

indicating excellent linearity of the method within the selected concentration range.

These results demonstrate that the method is suitable for the quantification of Sodium Valproate within the concentration range of **40–140 µg/ml**, making it applicable for both routine and high-precision analysis in pharmaceutical formulations. The calibration curves were shown in figures 5-10. Linearity reports were shown in table -1.

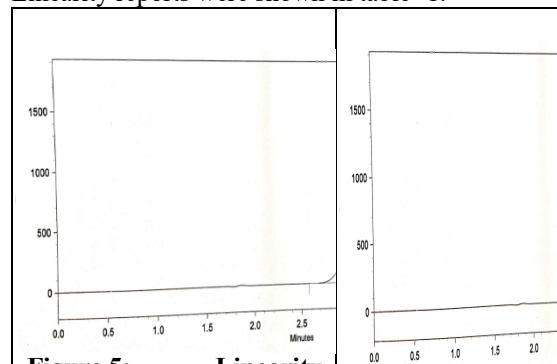


Figure.06: Linearity chromatogram for 60mcg

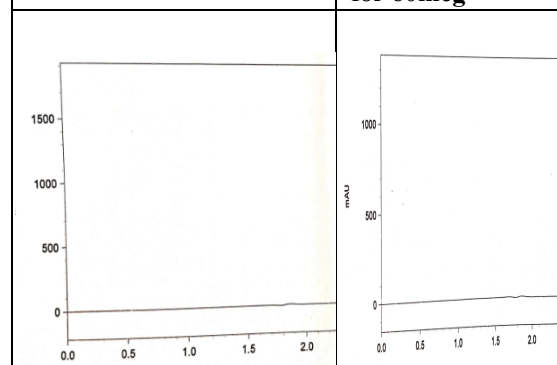


Figure.08: Linearity chromatogram for 100mcg

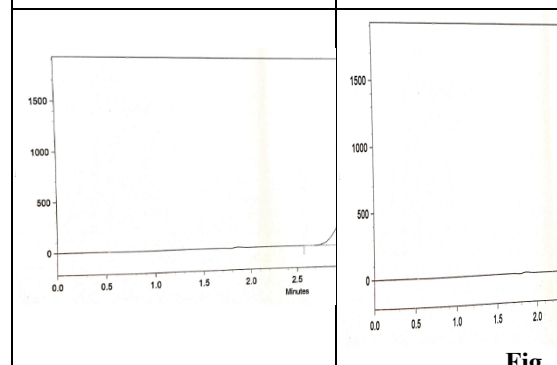


Figure.9: chromatogram 120mcg	Linearity for	chromatogram for 140mcg
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Figure.11: Calibration curve of Sodium valproate

Table.1: Results for Linearity

S.NO	Concentration	Peak area
1	40	949244
2	60	1354244
3	80	1759244
4	100	2164244
5	120	2569244
6	140	2982454
Correlation coefficient		0.9999

LOD and LOQ

Detection limit and Quantification limit was calculated based on the SD of the response and the slope of the calibration curve. LOD and LOQ was estimated as per ICH guidelines in terms of limit of detection $LOD=3.3 \times \sigma/S$ and $LOQ=10 \times \sigma/S$, where, σ is the standard deviation and S is the slope

ACCURACY

The accuracy of the developed RP-HPLC method was assessed through recovery studies, where known amounts of Sodium Valproate were spiked into the sample solution. The recovery was evaluated at three different concentration levels: 50%, 100%, and 150% of the target concentration. The amount of Sodium Valproate recovered was determined by comparing the measured concentration with the amount added, and the results were used to calculate the percentage recovery. The % RSD values were found to be less than 2%. The results were shown in table 2.

Table.2 : Results for % Recovery

Concentration	Amount added	Amount recovered	% Recovery	Mean recovery	SD	%RSD
50	24.8	24.6	100.81	100.73	0.20	0.2
	25.03	24.9	100.52			
	24.6	24.36	100.98			
100	49.5	49.43	100.14	100.06	0.75	0.7
	50.09	49.67	100.34			
	49.9	50.23	99.34			
150	74.33	74.33	100.26	100.26	0.11	0.1
	75.03	74.7	100.44			
	74.26	74.05	100.28			

PRECISION

The precision was carried out for both the inter day and intraday. By examining the six replicates of the prepared samples on the same day and three days apart, respectively, the intraday and interday precision was assessed. The percentage RSD were calculated and shown in the table no 3

Table.3; Results for Intraday and inter day precision

S.no	Intraday precision	Inter day precision
1	2327363	2327928

2	2332166	2333147
3	2333195	2333055
4	2336425	2333371
5	2336468	2334808
6	2338733	2335062
Mean	2334058	2332895
SD	4059.898	2582.333
% RSD	0.17	0.11

ROBUSTNESS: Robustness is assessed by adjusting the parameters, such as flow rate, mobile phase composition, wavelength, etc., and seeing any impact on the method's output.

Table.4 : Robustness of the developed method change in flow rate, mobile phase composition and wavelength

Parameters	Flow rate			Wavelength			Mobile phase composition		
	0.8	1.0	1.2	208nm	210nm	212nm	1	2	3
Retention time	3.984	3.73	2.64	3.178	3.178	3.173	3.919	3.173	2.64
Tailing factor	0.88	0.88	0.9	0.88	0.88	0.88	0.88	0.88	0.9
Plate count	11167	11117	1111	1122	1128	1119	1119	1110	1109

CONCLUSION:

The majority of sodium valproate estimation techniques employed in the past were laborious and time-consuming. Thus, an HPLC technique in reverse phase was created and verified. According to the system appropriateness and applicability results, the suggested method can be used for routine laboratory analysis with a shorter retention time. Based on the validation parameters that verify the acquired findings fulfill the predefined acceptance requirements, the suggested technique is linear, exact, accurate, precise, robust, specific, and selective.

In conclusion, this study employed the RP-HPLC methodology to create an analytical method and validate sodium valproate with valproic acid. We have effectively established an effective chromatographic separation method for the estimation of sodium valproate and valproic acid in pharmaceutical formulations.

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