Analytical Method Development And Validation Of Divalproex Sodium By Using RP- HPLC

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Keywords:
Reverse Phase High performance liquid chromatography (RP-HPLC), divalproex sodium, Validation, Mobile phase, Method Development.

Abstract
New RP HPLC method was developed for the estimation of Divalproex Sodium pharmaceutical dosage form.. Absorption maxima of the drug in UV–Visible region in different solvents/buffers was determined and different solvents were tried for HPLC method development. Mobile phase was optimized and flow rates for proper resolution and retention times. HPLC method was validated as per ICH guidelines. Among the method's many benefits are its straight forward and mobile phase, inexpensive solvents, quick analysis. HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump. rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and Chrom NAVCFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm x 4.6 mm, 5 µm) column using methanol and 0.1% formic acid in water as mobile phase at flow rate of 1.0 min. Samples were injected using Rheodyne injector with 20 µL. loop. Detection was carried out at 210nm, with a sharp peak at 4.20 minutes for divalproex sodium work .the method exhibits good linearity (r2 = 0.9999). The % RSD values for method precision and intermediate precision studies were found to be less than 2%. The % recovery was found to be within an acceptable limit 98%-102%.Thus, the created method was described as robust, accurate, exact, and linear.

1.Introduction

Valproic acid, supplied as the sodium salt valproate semisodium or divalproex sodium, is a fatty acid with anticonvulsant properties used in the treatment of epilepsy. Typically supplied in the sodium salt form. Divalproex dissociates to the valproate ion in the gastrointestinal tract. This agent binds to and inhibits gamma-aminobutyric acid (GABA) transaminase and its anticonvulsant activity may be exerted by increasing brain concentration of GABA and by inhibiting enzymes that catabolize GABA or block the reuptake of GABA into glia and nerve endings. Divalproex may also work by suppressing repetitive neuronal firing through inhibition of voltagesensitive sodium channels. Valproic Acid is also a histone deacetylase inhibitor and is under investigation for treatment of HIV and various cancers.

High-Performance Liquid Chromatography (HPLC) is an advanced separation technique used to identify, quantify, and purify compounds in a mixture. It works by passing a liquid mobile phase through a column packed with a stationary phase under high pressure. Compounds migrate at different speeds depending on their interactions with the stationary phase, producing a chromatogram for analysis. It has becoming more often used to determine stability studies of polar/ionic, thermally unstable, or non-volatile chemicals because has great sensitivity, specificity, and resolution capabilities. Given the previously mentioned data and the literature review, a unique RP-HPLC technique has been created and

approved for the estimation of divalproex sodium

Chemical structure of divalproex sodium.

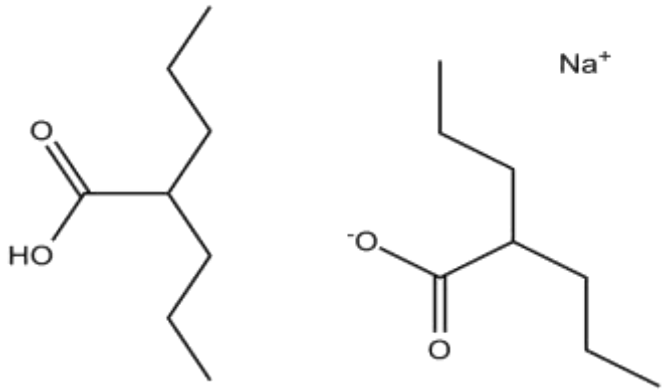

Figure 1: structure of divalproex sodium.

2. MATERIALS AND METHODS

Materials And Reagents:

divalproex sodium was supplied by aarti pharmaceuticals in the Indian state of Maharashtra. It was purchased from a nearby drugstore. Methanol & water were all HPLC-grade reagents employed in the current study. HPLC analysis was performed using the divalproex sodium HPLC-grade water was used in the HPLC study.

Experimental Work:

Instrumentation and Chromatographic Condition

HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump. rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and Chrom NAVCFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm x 4.6 mm, 5 µm) column using methanol and 0.1% formic acid in water as mobile phase at flow rate of 1.0 min. Samples were injected using Rheodyne injector with 20 µL. loop. Detection was carried out at 210nm. And retention time 4.20 minutes for divalproex sodium All weighing was done on Shimadzu balance (Model AY-120)

Mobile phase

Mobile phase was methanol and 0.1% formic acid in water as 90:10 . removal of gases was carried out in ultrasonic water bath for 15 minutes.

Preparation of standard stock solution

About 10mg of divalproex sodium was accurately weighed and transferred into 25 mL volumetric flask. 70 mL of diluent was added and then sonicated in ultrasonic water bath for 30 minutes. The solution was cooled and volume was made up to the mark with diluent. Resulting solution was used as test solution.

Preparation of test solution

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Selection of analytical wavelength

It is the characteristic of a compound which helps to provide the electronic structure of the compound or analyte. The structural analysis of divalproex sodium was carried out under UV ranging from 200-400nm using the standard solution

3. RESULT AND DISCUSSION

Table 1: Final reversed phase High performance liquid Chromatographic Conditions

Sr No	Parameter	Condition Used for Analysis
1	Column	Phenomenex luna
2	Mobile phase	methanol
3	Flow rate	1.0
4	Detection wavelength	210nm
5	Sample injector	20µl
6	Column temperature	30^0 c
7	Retention time	4.2min

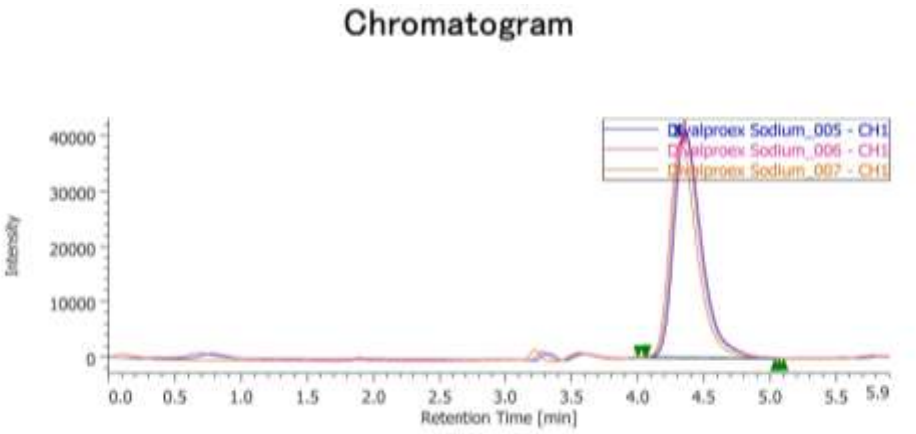


Figure 2 HPLC chromatogram of standard

Divalproex Sodium

Observation: fluticasone propionate eluted at 4.20 minutes with acceptable chromatography

Conclusion: Method can be used for further analysis and will be subjected for validation.

Method development

The proposed chromatographic method was found to be suitable for the effective preparation with good resolution, peak shape given in the figure. The mobile phase composed of methanol at flow rate 1.0ml/min was selected as it gave well resolved peaks of standard fluticasone propionate**.** The optimum wavelength 210nm selected for detection and quantitation.

Validation of analytical method

Validation of proposed analytical method involves linearity and range, precision, accuracy, limit of detection (LOD), limit of quantitation (LOO) and robustness study. It was validated according to ICH Q2(R1) guideline.

System suitability

System suitability: System suitability is the evaluation of the component of an analytical system to show that the performance of a system meets the standard required by a method. System suitability study was performed before each validation run. Area, Retention time (RT), Tailing factor and Theoretical plates were determined. Tailing factor for the divalproex sodium in standard solution should not be more than 2.0. Theoretical plates for the Fluticasone Propionate peaks in standard solution should not be less than 2000.

Parameter	Divalproex Sodium	Acceptance Criteria
Retention time	6 min	±10
Theoretical plate	NLT 2000	>2000
Tailing factor	NMT 2.0	<2.00
% RSD	NMT 2.0	<2.00

Table no 2:System suitability data for divalproex sodium

linearity

The calibration curves were found to be linear for the concentration range of 5-40 ppm. The standard working curve equation for drug was found to be y = 35733x + 93754 with correlation coefficient value R^2 =0.9979 . The results of linearity are given in the table and figure.

Concentration	Area
0	0
20	804561
40	1584637
60	2324151
80	2984631
100	3678945
120	4287263

Table no 03: linearity of Divalproex Sodium

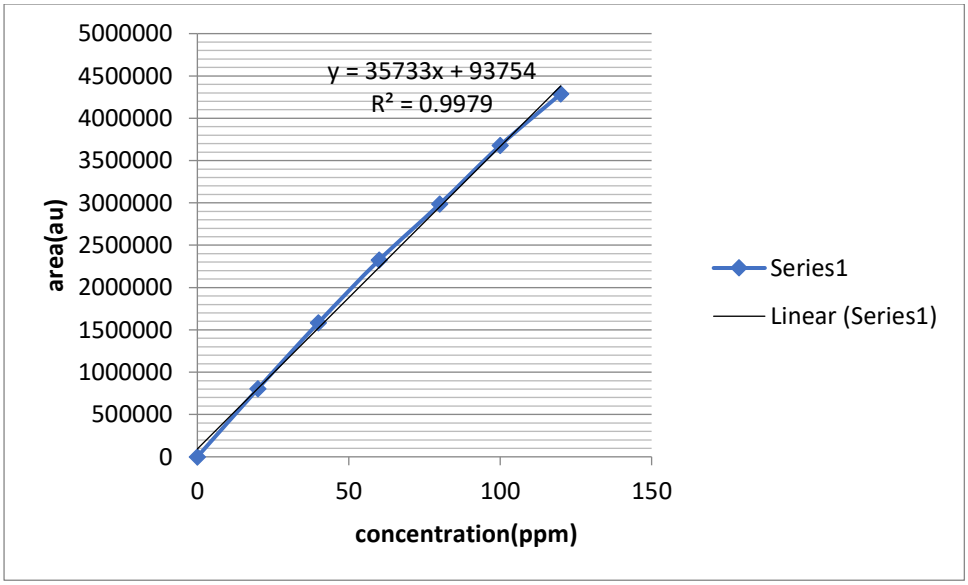


Figure 3: Linearity curve of standard Fluticasone Propionate

Recovery studies

The accuracy was determined from recovery studies. A known but varying amount of sample was spiked into pre-analyzed sample test solution at 50%, 100% and 150% recovery levels of working concentration in triplicate. The spiked test solution was analyzed according to the proposed procedure. The percentage recoveries were calculated against respective levels and mentioned in Table 4.

Analyte	Recovery level	% Recovery	Average % Recovery
Sample	50%	100.4	100.60
	50%	100.62	
	50%	100.8	
	100%	100.5	100.22
	100%	98.97	
	100%	101.2	
	150%	100.5	101.04
	150%	101.44	

	150%	101.2	

Table no 4: Recovery of Divalproex Sodium.

Method precision
The six test solutions were prepared separately. Each was analyzed as per proposed procedure. The % assay, average and %RSD was calculated and tabulated in the Table 05

Sample no	% Assay of Sample
1	101.91
2	98.98
3	100.42
4	100.22
5	100.14
Mean	100.334
% RSD	1.04

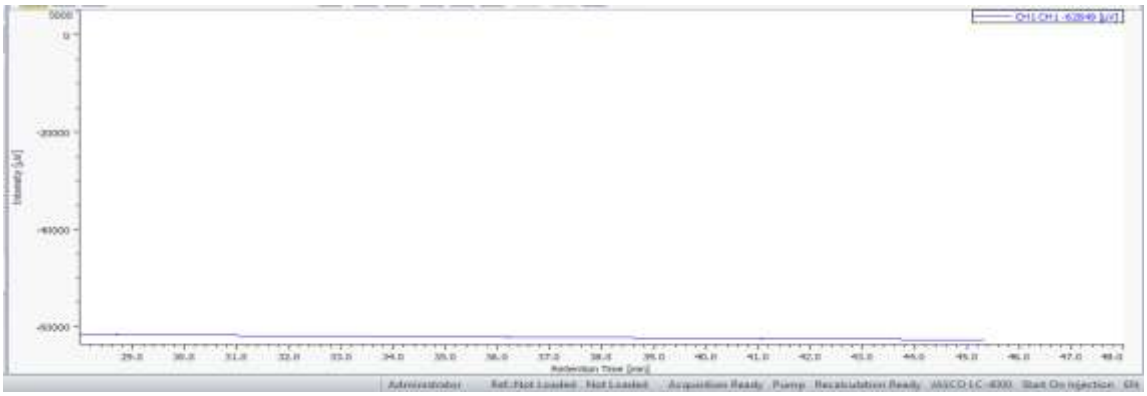
Table no 05 Method precision

Intermediate precision
The intermediate precision was determined by comparing two independent analysis on 2 different days. The data of the 1" analysis was taken from the analysis of 'Method precision.

Name of analyte	Sr.no	Assay (% w/w analysis-1)	Assay (% w/w analysis-2)
Sample	1	101.91	100.21
	2	98.98	101.63
	3	100.42	100.43
	4	100.22	98.99
	5	100.14	100.12
	6	99.51	100
	Average	100.24	100.23
	% RSD	0.996	0.847
	Overall % RSD	0.9215	

Table no 6: Intermediate precision

Secificity
Specificity The specificity of the method for Assay is demonstrated by injecting following solutions into the HPLC system.



Diluent as a blank

Figure no 04: HPLC Chromatogram of blank solution

Test solution

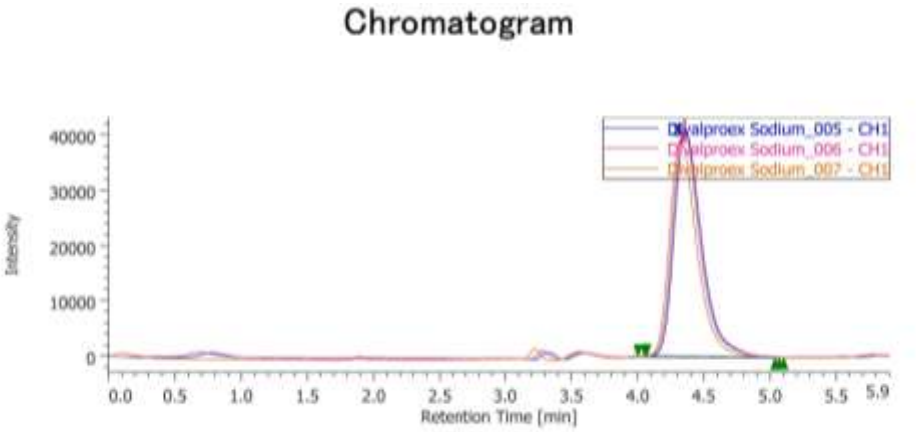


Figure no 05: HPLC Chromatogram of test solution

Robustness

The influences of slightly changed parameters of the chromatographic conditions were tested according to ICH guidelines to demonstrate robustness of the method. The tests are carried out by injecting Blank and standard solution by varying same of the parameters of chromatography mentioned below.

Table no 7: Robustness parameters

Sr no.	**Parameters**	**Working parameter**	**- changes**	**+ changes**
1	Flow	0.8 ml/min	1.0 ml/min	1.1 ml/min
2	Wavelength	210		220

Table no 8: Robustness study with change in wavelength

Changes in wavelength (nm)		
	210nm	**220nm**
1	594313	598449
2	608052	594025
3	602356	602511
4	608425	596231
5	594025	592419
Mean	601434	596727
SD	7055.5653	3953.65
% RSD	1.1	0.7

Table no 9: Robustness study with change in flow rate

Change in Flow (min)			
	0.8 ml/min	1.0 ml/min	1.1 ml/min
1	697358	497044	704189
2	710427	501599	702854
3	714890	500514	714629
4	707564	513568	692510
5	703124	502419	702734
Mean	706672	503028.8	703383.2

SD	6741.10	6237.64	7842.91
% RSD	0.9	1.2	1.1

CONCLUSION

The Specificity of the HPLC test for Assay of was proved by chromatographic comparison and method was found to be specific. The linearity of the proposed method was determined from the correlation coefficient and the method was found to be linear and within the range of 50 to 150% of working concentration The accuracy of the method was calculated by recovery study & the proposed method was found to be accurate as all the parameter of the method complies as per the acceptance criteria.

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