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Method development and validation for the estimation of Divalproex sodium by using RP-HPLC in bulk and pharmaceutical dosage forms

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ABSTRACT

New RP HPLC method was developed for the estimation of Divalproex Sodium pharmaceutical dosage form. Solubility of Divalproex sodium was determined using various solvents and buffers. 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.

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 voltage-sensitive sodium channels. Valproic Acid is also a histone deacetylase inhibitor and is under investigation for treatment of HIV and various cancers.

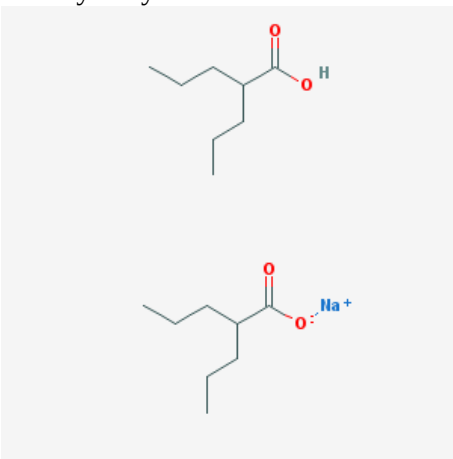

Figure.1.Molecular structure of Divalproex

2. MATERIALS AND METHODS

Divalproex was obtained as a gift sample from Analog Lab, Hyderabad. HPLC grade double distilled water, acetonitrile and all other chemicals were procured from local pharmacy.

Mobile Phase: Mobile phase consisted of 5 mM 1-hexane sulphonic acid sodium salt anhydrous which was filtered through 0.22 µ filter and acetonitrile (HPLC grade) in ratio of 50:50 (V/V). The 5 mM 1-hexane sulphonic acid sodium salt solution was prepared by dissolving 0.94 gm of 1-hexane sulphonic acid sodium salt in 1000 mL of purified water.

Preparation of standard solution: Accurately Weighed and transferred 10mg of Divalproex Sodium

working Standard into a 10 ml clean dry volumetric flask, and 7 ml of HPLC grade Water was added, sonicated for 5 minutes and made up to the final volume with diluent(standard stock).

Preparation of test solution: 5 tablets were weighed and crushed into powder, and transferred into a 250 mL volumetric flask, 130mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtrate, 2.0ml was pippeted out into a 100 ml volumetric flask and made up to 100ml with diluent.

Assay: 20µl of standard and sample solutions were injected into the chromatographic system and the areas of peaks were measured and the % assay was calculated using the formula

$$Assay\% = \frac{AT \times WS \times DT \times P \times Avg.wt}{AS \times DS \times WT \times 100 \times Label\ claim} \times 100$$

Where:
AT = average area count of sample preparation
AS = average area count of standard preparation
WS = weight of working standard taken in mg
WT = weight of working sample taken in mg
DS = dilution of standard
DT = dilution of test sample
P = percentage purity of working standard Label claim in mg/ml. Label claim in mg/ml.

Method validation: Validation parameters like system suitability, linearity, accuracy, precision, specificity, limit of detection, limit of quantitation and robustness were performed as per ICH guidelines.

Linearity: Linearity test solutions were prepared from the stock solution at different concentrations (25 - 150 ppm). 50 µl of each solution was injected into HPLC system and the peak area of chromatogram was noted.

Accuracy: To ensure accuracy of the method, recovery studies were performed by standard addition method at 50%, 100% and 150% level to pre analyzed samples and subsequent solutions were Pre analysed. At each level, three determinations were performed.

System suitability parameters: These tests are an integral part of method development and are used to ensure adequate performance of chromatographic system. Retention time (RT), number of theoretical plates (N), tailing factor (T) and resolution were evaluated.

Precision: Precision of the method was determined in terms of repeatability as method and system precision. It was determined by analysing six samples and assay was performed. % RSD values were also calculated.

Robustness: The drug solution was subjected to small, deliberate changes like flow rate and temperature. The method was followed in accordance to ICH guide lines.

Limit of quantification (LOQ) and limit of detection (LOD): It was calculated based on signal to noise ratio as described in ICH guide lines.

3. RESULTS AND DISCUSSION

Method development and optimization: To optimize the chromatographic conditions, the effect of mobile phase is studied with various solvent system combinations for the determination of Divalproex in bulk and pharmaceutical dosage forms. A mixture of 1-hexane sulphonic acid sodium salt anhydrous and Acetonitrile (50:50%v/v) was selected as it gave best resolution. The effect of flow rate was studied in the range of 0.9 to 1.2 ml/min and 1.0ml/min was preferred to be effective. Under these conditions, the analyte peak obtained was well defined and free from tailing. The retention time (RT) was found to be 3.582min.

Table.1.Optimized chromatographic parameters

Flow rate	1.0 ml/min
Column	Agilent SB C18, 50 x 4.6 mm, 5µ
Detector Wave Length	210nm
Column temperature	30°C
Injection Volume	50µL
Run time	8min
Standard Concentration	100ppm
Diluent	1-hexane sulphonic acid sodium salt anhydrous and Acetonitrile (50:50%v/v)

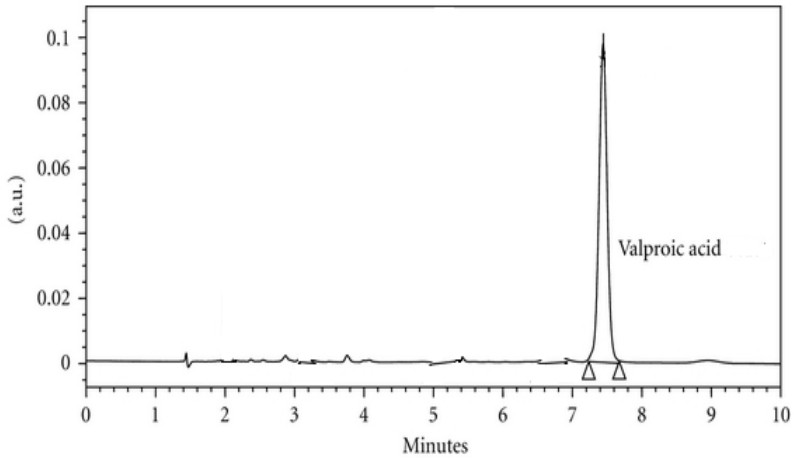

Figure.2.Chromatogram for Divalproex standard

System suitability studies: The system suitability method acceptance criteria set in each validation run were capacity factor >2.0, tailing factor ≤ 2.0 and theoretical plates > 2000. In all cases, the relative standard deviation (RSD) for analyte peak area < 2.0%. The results were within acceptable criteria and indicates efficient performance of column.

Method precision: The results obtained were within acceptable criteria.

Linearity: A calibration graph was obtained by plotting graph between peak area versus concentration. Excellent correlation was obtained between peak area and concentration with $R^2 = 0.999$ for active ingredient.

Accuracy: The closeness of obtained value to true value indicates that the proposed method is accurate.

Robustness: The results obtained were not affected by varying the chromatographic conditions, indicating the method is robust.

Table.2.System suitability parameters

Retention time	Area	Plate Count	Asymmetric factor
3.582	1170889	3260	1.02
3.529	1168745	3187	1.03
3.482	1166845	3167	1.03
3.441	1163697	3162	1.04
3.395	1159830	3265	1.03
3.41	1156249	3264	1.01
Avg: 3.473	1164376		
%RSD 1.24			

Table.3.Method precision results

S.No	% Assay
1	101.91
2	98.98
3	98.64
4	98.12
5	100.14
6	99.27
Avg	99.51
SD	1.23
%RSD	1.24

Table.4.Linearity results for Divalproex

Concentration (ppm)	Peak area
25	253651
50	5812157
75	8523421
100	1136759
125	1418785

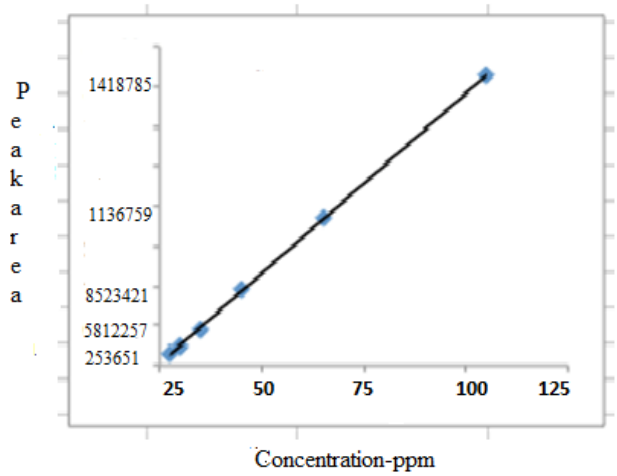

Figure.3. Calibration curve for Divalproex

Table.5.Accuracy studies of Divalproex

%spiked level	% recovery	SD	%RSD
50%	100.28	0.332	0.33
50%	100.9		
50%	100.8		
100%	98.84	1.437	1.43
100%	101.51		
100%	101.1		
150%	100.57	0.605	0.601
150%	100.01		
150%	100.6		

Table.6.Robustness study for Divalproex

Parameters studied	Retention time	Peak area	Asymmetric factor	Theoretical plates	
Flow rate	0.9ml/min	4.218	1444020	1.03	3054
(1.0ml/min)	1.1ml/min	2.702	938356	1.01	3001
Column temp.	25^{O}C	3.194	1152247	1.02	3009
(30^{o}C)	35^{O}C	3.11	1158745	1.06	3039

4. CONCLUSION

The analytical method proposed in this project was found to be specific, precise, accurate, rapid and economical. The developed method was validated in terms of accuracy, linearity, robustness and precision in accordance with ICH guidelines. The method is cost effective due to short retention time which enabled analysis of Divalproex with small amount of mobile phase. The method was found to be precise and accurate from the recovery studies. The method is sensitive due to low detection and quantitation limits. Robustness data indicate that the method is not susceptible to small changes in chromatographic conditions. This method was successfully applied for estimation of drug as well as impurity in bulk and dosage forms. Hence, this method can be readily used in quality control laboratories for the routine pharmaceutical analysis.

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