بسم الله الرحمن الرحيم
Omdurman Islamic University Journal(OIUJ)
مجلة جامعة أم درمان الاسلامية
https://journal.oiu.edu.sd/index.php/oiuj
https://doi.org/10.52981/oiuj
2024; 20(2):34-43
ISSN: 5361-1858

RP – HPLC Method development and validation of Sodium valproate in pharmaceutical formulations

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To cite this article:

*Ali, M. Masaad[1]; k. Abdelmontalib Osman[1], RP – HPLC Method development and validation of Sodium valproate in pharmaceutical formulations , Omdurman Islamic University Journal, Sudan, **ISSN: 5361-1858**

https://doi.org/10.52981/oiuj.v1i2.1726

Abstract

A rapid, simple, and sensitive HPLC method developed for optimization, validation and determination of sodium valporate tablet in two concentrations. Stability study, chromatographic conditions comprised of a 250 X 4.6 mm column containing 5-µm, packing L_1 (C_{18}), mobile phase consisting of a mixture of 35 volumes of buffer and 65 volumes of acetonitrial with flow rate of 1 mL/min. Detection at 220 nm. The retention time of sodium valporet was 3.35 min. The linear regression analysis data for the calibration plots showed good linear relationship within the concentration range 250-1500 µg/mL. The value of correlation coefficient was found to be 0.999149. The recovery of sodium valproate was 98–102%. The method was validated as per International Council for Harmonization (ICH) guidelines [1]

Keywords: Sodium valproate; Validation; RP -HPLC.

Introduction

Sodium valproate (Fig. 1) is sodium-2-propyl pentanoate, used for its anticonvulsant properties [2]. It is quite dissimilar to other established it has no nitrogen or aromatic moiety, sodium valproate stabilizing electrical activity in the brain, the brain becomes over-stimulated and normal function is disturbed. Sodium valproate prevents epileptic fits by preventing the excessive electrical activity in the brain. This is achieved by increasing the activity of a neurotransmitter called gamma –

aminobutyric acid (GABA) in the brain. Sodium valproate is thought to increase the production and prevent the breakdown of GABA in the brain. This increases the calming activity of GABA in the brain [3] .

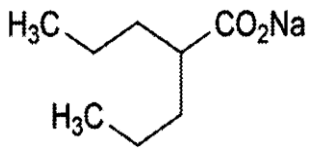


Figure 1: chemical structure of sodium valproate

Sodium valproate in official BP and USP but these pharmacopoeias have adopted gas chromatography (GC) method for quantitative analysis for this drug in formulation. The analytical methods reported in recent scientific literature for the quantification of sodium valproate includes high-performance liquid chromatography (HPLC) with MS detection [3, 4], UV detection [6 -10] and fluorescence detection [11-14], isotope-dilution mass spectrometry [15], and gas chromatography. [16 - 18]

The present work deals with the description of a simple, sensitive, and reliable method for the determination of sodium valproate.

Materials and methods

Chemicals and reagents

The reference standard of sodium valproate from British pharmacopeia chemical references standard of SCIPHARMTECH, INC, sodium valproate tablets were in-house product of formulation research development department, Abdelmonaim pharmaceutical industry Ltd, Sudan, Epilim 200mg and 500mg Sanofi Winthrop Industrie - France. All solvents used were of HPLC grade. Acetonitrile, potassium di-hydrogen phosphate, di-sodium hydrogen phosphate anhydrous, hydrochloric acid and sodium hydroxide were obtained from E. Merck Mumbai, India.

Mobile phase preparation and standard preparation

The buffer weight of monobasic potassium dihydrogen ortho-phosphate KH2PO4 (0.32 W/V%) adjusted pH 3.0 with o-phosphoric acid 35 volumes of buffer and 65 volume of acetonitrile was filtered through 0.45 µm membrane filter (Whatman, India) and sonicated (SOLTECH –Model 2200 MH ,Germany). Weight about 50 mg of sodium valproate BP CRS was weighed accurately in 50 ml volumetric flask with water (HPLC grade), was added, sonicated to dissolve and diluted to the mark to obtain a concentration of 1mg/mL.

Assay of sodium valproate in tablet dosage form 200mg

Ten tablets of sodium valoprate were transferred to a 200 mL volumetric flask, containing 70 mL of (HPLC grade) water**,** sonicated for 20 minutes, shacked for 30 minutes and the volume completed to the mark with (HPLC grade), water and sonicated for 15 minutes. The resulted solution was filtered, and 5 mL of it were diluted to 50mL volumetric flask, then filtered using 0.45μm syringe filter.

The solution was injected at above chromatographic conditions and peak areas were measured to determine the sodium valproate content.

Dissolution study

For the dissolution study of sodium valproate enteric coated tablets, analysis was done using above chromatographic conditions. For this study standard solution of sodium valproate was prepared in dissolution media. For sample preparation, an intact tablet was dissolved in 0.1N HCl media (RPM 100), Apparatus 2 (paddle), at temperature 37 **C**. Sample was collected in dissolution vials after 2 hrs and then decanted the 0.1N HCl media and the pH 6.8 phosphate buffer media. Samples were collected in dissolution vials after different time intervals and filtered through 0.45 μm. The volumes (20μL) of these solutions injected into the chromatograph by auto sampler and peak areas measured.

Results and Discussion

Dissolution study

Figure 2, shows that the peak areas are determined after adjusting of dissolution conditions of standard sodium valproate and its sample form (tablets) by scanning on HPLC – PDA instrument.

Method development

To develop a precise, accurate and suitable HPLC method for the quantitative determination of sodium valproate, different mobile phases and stationary phases employed and the proposed chromatographic conditions found appropriate. Valproic acid (about 1 mg/mL) has a λmax of 220 nm in water (HPLC grade). The wavelength 200 nm was selected instead of 240 nm because of greater peak area at 210 nm when scanned on HPLC -PDA in final mobile phase. The absorption spectrum is presented in Fig. 3.

Method Validation

The proposed method validated for assay of sodium valproate using following parameters.

Specificity

To demonstrate the specificity, potential contaminants were generated by forced degradation. The chromatograms were taken on photo diode array detector and the peak purities were not less than 0.99.

Linearity

Linearity was studied by preparing standard solutions at different concentration levels. When the concentrations of sodium valproate and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r = 0.9999) was observed between the con 250-1500 µg/mL R^2 = 0.999149. The regression equation was found to be y = 39847x + 62325, where y is the peak area and x is the concentration of sodium valproate (Fig 4).

Limit of detection and limit of quantitation

$$\text{Limit of Detection} = \frac{3.3 \times \sigma}{S}$$

$$\text{Limit of Quantification} = \frac{10 \times \sigma}{S}$$

Where: σ = the standard deviation of the response (STEYX), S = the slope of the calibration curve. Based on 'standard deviation the response and slope of the calibration curve specially constructed in a low region of 0.040 to 1.60% of the target analyte concentration. The standard deviation of y-intercepts of the regression lines was used as σ(the standard deviation of the response) and 'S' is the slope of the calibration curve. The LOD and LOQ were found to be 0.4947 and 1.4991µg/mL, respectively.

Method accuracy

To ensure the reliability and accuracy of the method, recovery studies were carried out in triplicate at three concentration levels (50%, 100% and 150%) of test concentration. The recovery of sodium valproate was found to be in the range of 99.54 - 100.33 % [Table 1].

Precision

The intra-day precision of the assay method was evaluated by carrying out six independent assays of sodium valproate (1000µg/mL) test samples against qualified reference standard on same day and these studies were also repeated on six consecutive days to determine inter-day precision. The percentage of RSD of six assay values was calculated = 0.065%. Results are shown in [Table 2].

Standard and sample solution stability

The solution stability of sodium valproate was carried out by leaving the test solutions in a tightly capped volumetric flask at room temperature for 12 h. The same sample solutions were assayed for five days at 2-5 **C** in refrigerator interval up to the study period against freshly prepared solutions. The relative standard deviation found below 2.0 %.

Method robustness

This was done by small deliberate changing in flow rate, pH of mobile phase, mobile phase ratio and column oven temperature. Results are shown in Table III. Results show that the contents of the drug were not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust.

Method Ruggedness

Ruggedness test was determined between two different analysts, instruments, day, Results are shown in table IV and the values of percentage RSD were below 1.0%, showed ruggedness of developed analytical method.

Results of dissolution study

The mean (n = 6) percentage dissolution of sodium valproate in HCl 120 min and ripples by 6.8 pH phosphate buffer at 45 min from tablet dosage form was found within the limit. Analytical RP-HPLC method was developed and validated for the determination of sodium valproate enteric coated tablet dosage form. The developed method was found to be simple, precise and accurate and can be applicable for the routine quality control analysis of sodium valproate in tablet dosage form. The advantages of the method are short run time, simplicity of sample preparation, no need of derivative formation, which require longer time for analysis. The other advantage of the method is the common chromatographic conditions adopted for both the assay and dissolution studies. As a result, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing; that are typically associated when different chromatographic conditions are used.

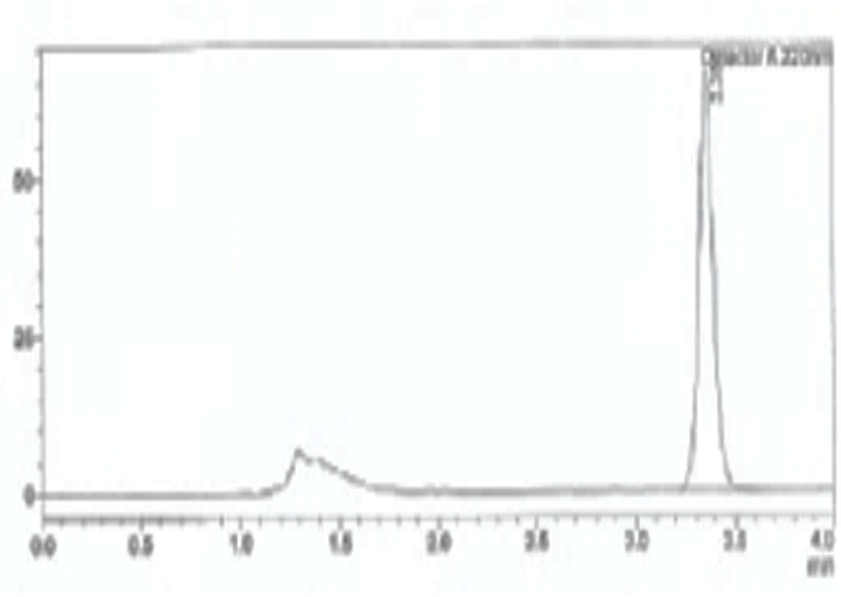


Fig. 2 Chromatogram of sodium valproate in sample solution

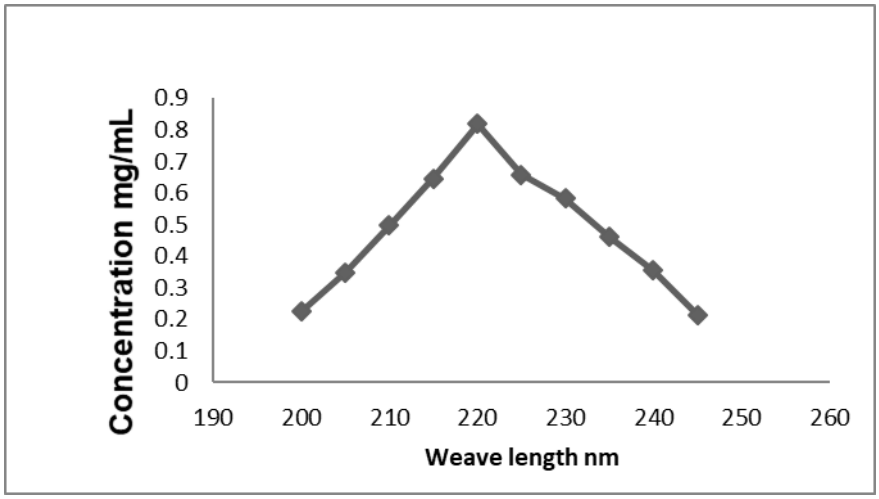


Figure: 3 Wavelength Vs concentration

Table 1: shows accuracy

Level %	drug added(mg)	Drug recovered (mg)	%Recovery mean (n=6)	RSD % assay n=6
50	**50.0141**	**49.850**	**49.9515**	0.234%
100	**100.0072**	**100.074**	**100.0815**	0.143%
150	**150.000**	**148.1712**	**148.17116**	0.310%

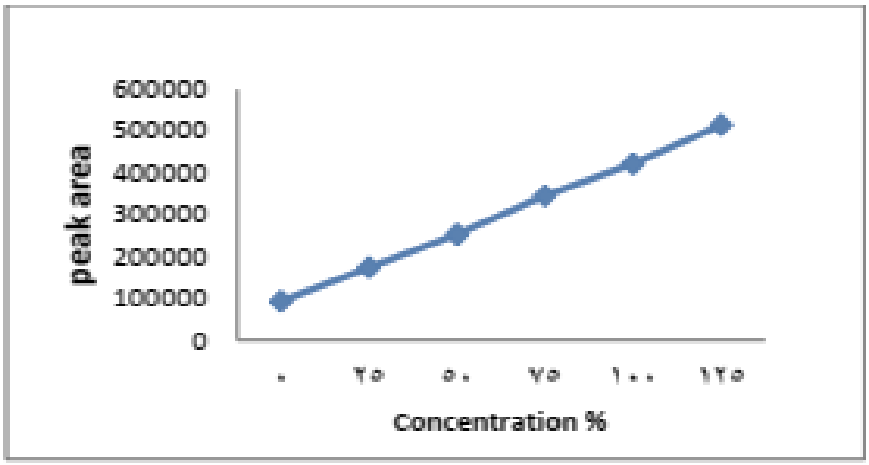


Fig 4 Linearity curve for sodium valproate

Table 2: Stability of solution

No	Peak Area (Freshly)	Peak Area(after day)	Concentration mg/mL freshly	Concentration mg/mL after day	Different < 5.0%	Average
	STD 340957	STD 340621				
1	93446	93136	0.5	0.442	0.483%	0.556%
2	341631	339834	1.0	0.9947	0.526%	
3	513529	513024	1.5	1.495	0.66%	

Table 3: Method ruggedness

No	**Instrument**		**Day**	**Analyst**
	No 32	**No 33**		
Assay	101.0433	102.0033	98.71%	99.635
RSD%	0.976%	0.225%	0.26%	0.25%

Table 4 Linearity study of sodium valproate standard

Linearity %	**Mean Area**	**Standard dev**	**%RSD**
1 (25%)	93321	8.485281	0.0091
2 (55%)	173701.5	71.41778	0.041
3 (75%)	253198.5	14.84924	0.0058
4 (100%)	345602	168.2914	0.0486
5 (125%)	422127.5	64.34672	0.0152
6 (150%)	518819	968.7363	0.186

Correlation Coefficient: NLT 0.999

R^2 = 0.999149

Acknowledgements

The authors are thankful to Abdel Moneim Pharmaceutical Industries Company for all facilities to complete this research work

Refernces

References

1. ICH guideline,(2005) Q2 (R1) step 4, Validation of Analytical Procedures: Text and Methodology
2. Ibrahim AA, Omar M, Belal F. Valproic Acid and sodium valproate comprehensive profile, Profiles of Drug Substances. Excipients and Related Methodology, 2005; 32: 209- 232.
3. Gupta RK, Singh UK, Kumar S, Moothan B. Estimation of Sodium Valproate in Tablet Dosage Form by RP-HPLC without Prior derivatization: Application to Dissolution Studies. Int J Pharm Sci Drug Res 2009; 1(2): 103 -106.
4. Deepak S J, Gunta S, Mallika S, Pranav S. A high throughput and selective method for the estimation of Valproic acid an antiepileptic drug in human plasma by tandem LC-MS/MS. Talanta 2007; 72(1): 80-88.
5. Tsukasa M, Masaharu N, Hiroyuki W, Susumu Y, Kenji S.Determination of Valproic acid in serum by Liquid Chromatography Atmospheric Pressure Chemical Ionization Mass Spectrometry. Anal sci 2007; 17: 999.
6. Amini H, Javan M, Ahmadiani A. Development and validation of a sensitive assay of Valproic acid in human plasma by high-performance liquid chromatography without prior derivatization. J. Chromatogr. B Analyt Technol Biomed Life Sci 2006; 830(2): 368-71.
7. Kishore P, Rajani K V, Satyanarayana V, Krishna D R. HPLC determination of valproic acid in human serum. Phammazie 2003; 58(6): 378-80.
8. Lucarelli C, Villa P, Lombaradi E, Prandini P, Brega A. HPLC method for Simultaneous analysis of valproic acid and other common anticonvulsant drugs in human plasma or serum. Chromatographia, 1992; 33: 37- 40.
9. Gentile de Illiano B and Quintana de Gainzarain A. HPLC determination of valproic acid in human plasma by derivatization with *O*-*p*-nitrobenzyl-*N*, *N*-diisopropylisourea. Journal of high resolution chromatography 1989; 12(8): 540-543.
10. Kazuko K, Mikio N, Ryota N, Satoshi K. Direct method for determination of Valproic Acid in Serum by High Performance Liquid Chromatography. Anal sci 1985; 1: 385.
11. Yan Z, Zheng J Yunqiu Y. Simultaneous determination of mycophenolic acid and Valproic acid based on derivatization by high performance liquid chromatography with fluorescence detection.Biomed Chromatogr 2006; 20: 319–326.

12. Shuuji H, Motoki K, Kazuo I, Misa F, Nobufumi O, Takeshi K. Determination of Valproic acid in human serum by High Performance Liquid Chromatography with Fluorescence detection. Biol Pharm Bull 1999; 22(9): 975.
13. Liu H, Forman L J, Montoya J, Eggers C, Barham C, Delgado M.Determination of valproic acid by high performance liquid chromatography with photodiode-array and fluorescence detection. Journal of chromatography: Biomedical applications 1992; 576(1):163-169.
14. Masaharu N, Akira S, Kenji S. Determination of serum valproate by high performance liquid chromatography using fluorescence labeling with 9-ammophenanthrene. Anal sci 1988; 4: 385 - 388.
15. Susanto F, Reinauer H. Determination of Valproic Acid in Serum by isotop-dilution mass spectrometry. Chromatographia, 1995; 41: 407-413.
16. Riva R, Albani F, Baruzzi A. Rapid and simple GLC determination of Valproic acid and Ethosuximide in plasma of epileptic patients. J pharm sci 1982; 71(1): 110.
17. Krogh M, Johansen K, Tonnesen F, Rasmussen KE. Solid-Phase microextraction for the determination of the free concentration of Valproic acid in human plasma by capillary gas chromatography. Journal of chromatography B: Biomedical science and applications 1995; 673(2): 299- 305.
18. Pravin S, Abdorreza M, Naader A. Determination of the Valproic acid in human serum and pharmaceutical preparation by headspace liquid phase microextraction gas chromatography flame ionization detection without prior derivatization. J chromatogr B 2006; 850: 128-133.
19. Crowther JB. Validation of pharmaceutical test methods. In: Handbook of modern pharmaceutical analysis, Academic press, New York, 2001, pp. 415–443.