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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF VALPROIC ACID IN DISSOLUTION STUDY OF ITS FORMULATION

MUGDHA KARDE*[1], HARSHAL PAWAR[2], RACHEL GEEVARGHESE[3], JAIKISHAN KHATRI[4]

[1]Pharmaceutics, Dr. L.H. Hiranandani college of Pharmacy, Smt. CHM College Campus, Opp. Railway Station, Ulhasnagar – 421 003, Dist. Thane (Maharashtra), India, [2]Department of Pharmacognosy, [3,4]Department of Pharmaceutics, Dr. L.H. Hiranandani college of Pharmacy, Smt. CHM College Campus, Opp. Railway Station, Ulhasnagar – 421 003, Dist. Thane (Maharashtra), India.
Email: mugdha.karde@yahoo.com

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ABSTRACT

A simple, precise and reproducible reverse phase, isocratic high performance liquid chromatographic (HPLC) method was developed and validated for the quantitative determination of Valproic Acid in the dissolution study of Pharmacosomes. The quantification was carried out using a Zorbax Eclipse XBD- C18 (4.6 × 150mm, 5 µm) column, with a mobile phase consisting of Acetonitrile: Citric acid buffer (50:50, v/v) (pH 3) at a flow rate of 1.5 ml/min and UV detection at 210 nm. The method was validated for specificity, method precision, linearity, recovery, robustness, ruggedness and solution stability. The linearity of the proposed method was investigated in the range of 50-400 µg/ml (r = 0.9997). The proposed method was successfully applied for determination of the Valproic Acid in dissolution study of Pharmacosomes.

Keywords: Valproic Acid, Pharmacosomes, Dissolution, Reversed-phase, HPLC.

INTRODUCTION

Valproic Acid (2-propylpentanoic acid) is an anticonvulsant used to control seizures. Its structure is different from most of the other antiepileptic drugs. Its molecular formula is $(CH_3CH_2CH_2)_2CHCOOH$ and molecular weight is 144.2[1].

Valproic acid is classified as Class I drug, even though it is slightly soluble in water (1.3mg/ml). It is very soluble in acetone, methanol, alcohol, chloroform, benzene, ether, organic solvents, miscible with dichloromethane and dissolves in dilute solutions of alkali hydroxides. It is available as slightly viscous clear liquid with boiling point of 220°C[1].

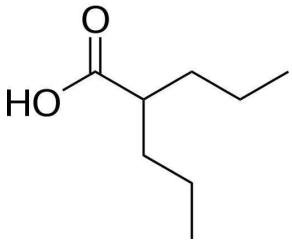

Fig. 1: Structure of Valproic Acid[2]

Valproate is believed to affect the function of the neurotransmitter GABA in the human brain. Valproic acid inhibits GABA transaminase by binding to it and thus enhances the neurotransmission of GABA in brain[3, 4].Valproic acid also blocks the voltage-gated sodium channels and T-type calcium channels. These mechanisms make Valproic Acid a broad-spectrum anticonvulsant drug.

Valproic Acid has been used in combination with Sodium Valproate. These formulations show smaller differences in the pharmacokinetics and accessibility in market [5]. Valproic Acid single or in combination with sodium Valproate is available in different dosage forms; capsule, tablet, enteric-coated tablet, sprinkle, liquid, intravenous, suppository and controlled-release formulations [6].

The methods reported in monographs for the assay and dissolution of Valproic Acid is gas chromatography[7,8]. Many other methods are also used for the estimation of Valproic Acid, this involves, high throughput LC- MS[9], high-performance liquid chromatography (HPLC) with MS detection[9,10], and high-performance liquid chromatography (HPLC) with fluorescence detection[11], isotope-dilution mass spectrometry[12], capillary electrophoresis[13]. Literature survey revealed that no such simple RP-HPLC method is reported for the estimation of Valproic Acid in dissolution study of its formulation like pharmacosomes[14].

The objective of the present study was to develop a simple, less time consuming and economical analytical method for estimation of Valproic Acid in dissolution study of its formulation.

MATERIALS AND METHODS

Reagents and chemicals

Valproic acid USP (Purity = 99.8%) was obtained from Ipca pharmaceuticals, Mumbai as gift sample. The formulation of pharmacosomes was prepared in the Pharmaceutics research laboratory of Dr. L. H. Hiranandani college of Pharmacy. Acetonitrile (HPLC grade, Merck), Citric acid monohydrate (GR, Merck), dibasic sodium phosphate(GR, Merck) were procured from SR Traders. Orthophosphoric acid (88% GR, Merck), sodium hydroxide (analytical grade), potassium dihydrogen phosphate (Molychem) and water (HPLC grade) were used during analysis.

Instrumentation

Agilent 1200 series integrated high performance liquid chromatographic system equipped with quaternary pump, manual sampler, single wavelength detector, column thermostat and controlled by Chem-Station software was used for HPLC analysis. The Zorbax Eclipse XBD- C18 (4.6 × 150mm, 5 µm) analytical column was used as a stationary phase.

Formulation Development

The pharmacosomes (Strength: 250mg) of Valproic Acid were prepared by thin film hydration method. The excipients used were soya phosphatidylcholine (Lipoid Gm, Germany) and Cholesterol (analytical grade). The drug lipid complex was dissolved in dichloromethane of analytical grade and evaporated in rotary evaporator to get the thin film which was further hydrated to obtain the vesicles[15, 16]. These vesicles were dried and used for the further analysis. The assay of the prepared pharmacosomes was performed using previously validated analytical method and the contents results were used to calculate the percentage release on each time of dissolution profile.

Dissolution Study

Dissolution test of prepared Valproic Acid formulation was performed in Electrolab dissolution test system (n=6), dissolution

apparatus no. 2 (paddle). The dialysis membrane was used for the dissolution of formulation. The media used was phosphate buffer pH 7.5, according to the USP. The temperature of bath was maintained at 37°C and speed of the paddle was set at 100rpm. The aliquots of sample were withdrawn at end of dissolution. These samples were filtered and injected in HPLC injection port for analysis.

Citrate buffer and Acetonitrile were used to prepare mobile phase. Citrate buffer was prepared using Citric acid monohydrate and dibasic sodium phosphate. The pH of the buffer was adjusted at 3.0± 0.5. The prepared buffer solution was filtered through 0.45 µm membrane filter and degassed by sonication before use. It was found that the ratio of Citrate buffer: Acetonitrile at 70: 30 gave the retention time of 19 minutes. After the ratio was adjusted at 50:50, the retention time was reduced to 3.1 -3.3 minutes with good peak shape. This also reduced the amount of mobile phase required and thus reduces the cost of analysis. The flow rate was adjusted from 0.5ml/min to 1.5ml/min that yield the optimum column back pressure.

HPLC method development

The identity of the drug is confirmed by UV and IR spectroscopy. The maximum absorbance of Valproic Acid was observed at 213nm. The UV spectrum and IR spectrum of the drug is shown in fig. 2 and 3 respectively.

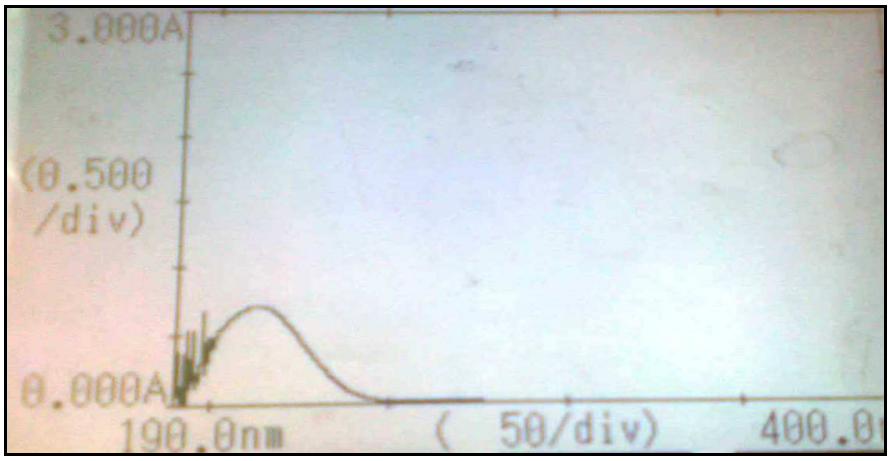

Fig. 2: UV spectrum of Valproic Acid

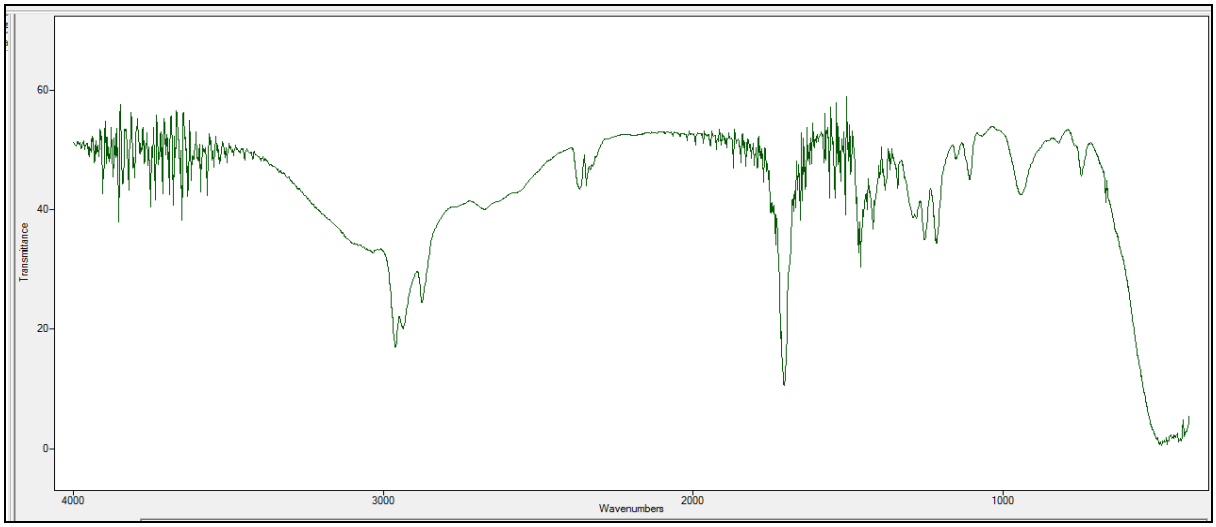

Fig. 3: IR spectrum of Valproic Acid

The final optimized HPLC parameters are represented in Table 1.

Table 1: Optimised HPLC parameters

Column	**Zorbax Eclipse XBD - C18 (4.6X150mm, 0.5µ)**
Injection volume	20µl
Mobile phase ratio- citrate buffer : ACN	50:50
Detector	UV visible
Wavelength	210nm
Flow rate	1.5ml/min
Retention time	About 3.1 minutes
Run time	6 minutes
Temperature	25° C

Preparation of Standard solution

Standard stock solution of Valproic Acid was prepared by dissolving 100 mg of Valproic Acid in 100 ml phosphate buffer pH 6.8 in standard volumetric flask. The solution was sonicated for 20 min. The resulting stock solution was further diluted with pH 6.8 buffer so as to get final concentration of 250 µg/ml.

System suitability testing

System suitability testing is used to verify that the precision / reproducibility of the system is adequate for the analysis to be performed. Parameters such as therotical plates, tailing factor and reproducibility (%RSD for area of five replicates) were determined and compared against the specifications. Five replicate injections of the standard solution were made into HPLC system. The mean, SD and % RSD were calculated.

Method Validation

The HPLC method was validated in terms of system precision, specificity, method precision, linearity, recovery, robustness, ruggedness and solution stability.

System precision

Five replicate injections of the standard solution were made into HPLC system. The mean, SD and % RSD were calculated.

Specificity

The dissolution tests specificity was evaluated by preparing samples of the placebo of the formulation. These samples were transferred to separate vessels with 900 ml of the dissolution medium and stirred for 12 hr at 100 rpm using the Paddle (with dialysis membrane) apparatus. The interference of the excipients of each formulation was evaluated.

Method precision

Dissolution was performed on six units of single batch and samples were analyzed as per the test method.

Linearity

Linearity of response was performed using the drug solution in the range of 50µg/ml to 400 µg/ml (about 20% - 160% of the standard concentration of 250 µg/ml).

Recovery

The recovery studies were carried out by adding a known quantity of drug with preanalysed sample and contents were reanalyzed by the proposed method. The recovery studies were carried out in triplicate at three concentration levels (80%, 100% and 120%) of test concentration.

Robustness

Robustness of the proposed method was estimated by changing the flow rate by ± 0.2 ml/min and changing the wave length by ±2nm.

Solution stability

The solution stability of Valproic Acid was carried out by leaving the test solutions in a tightly capped volumetric flask at room temperature for 24h. The same sample solutions were injected at different time interval up to the study period against freshly prepared solutions

Ruggedness

Ruggedness of the method (intermediate precision) was estimated by preparing six dissolution sample solutions as per the proposed method and each sample was injected in duplicate using different analyst on different days.

RESULTS AND DISCUSSIONS

System Precision and Sysyem Suitability

System precision was evaluated by injecting Standard solution of Valproic Acid. Standard deviation and % relative standard deviation for Valproic Acid for the peak areas from five replicate injections of standard are verified at every stage. The % relative standard deviation (% RSD) of Valproic Acid peak was found less than 1. The area of 5 replicate injection & calculated % RSD is shown in Table 2.

Table 2: Data of System Precision

Injection	Area of STD
1	148.104
2	147.45
3	147.046
4	146.387
5	148.64
Mean	**147.525**
SD	**0.8**
% RSD	**0.5**

The results of system precision and system suitability are presented in Table 3.

Table 3: Results of System Precision and System Suitability Testing

Parameters	Value
System precisions	% RSD for 5 replicate injections of standard < 1
Retention time of Valproic Acid	About 3minutes
Theoretical plates	> 5000
Tailing factor	< 2

Specificity

The representative chromatograms of Placebo (Fig.4) and Sample (Fig.5) indicated that there was no interference from blank (dissolution media) and placebo at the retention time of Valproic Acid.

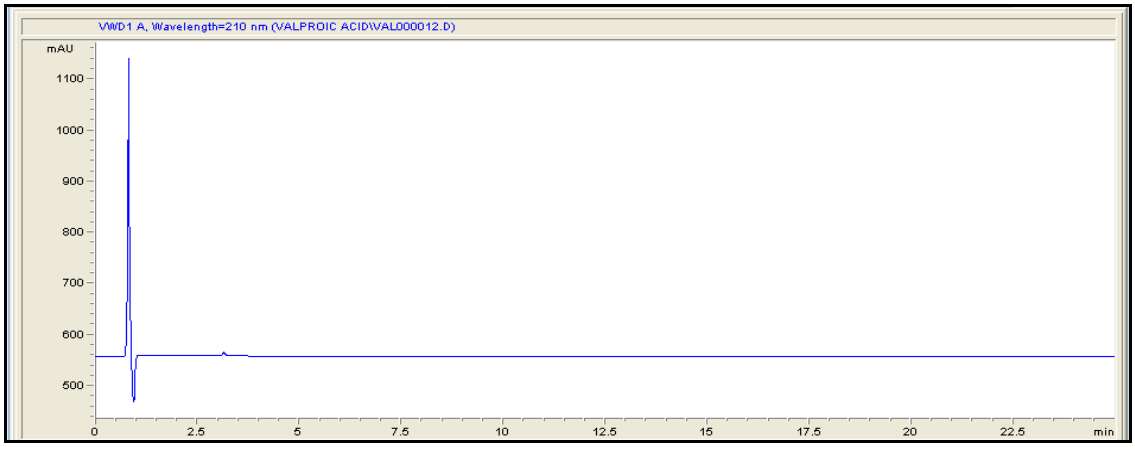

Fig. 4: Representative Chromatogram of placebo

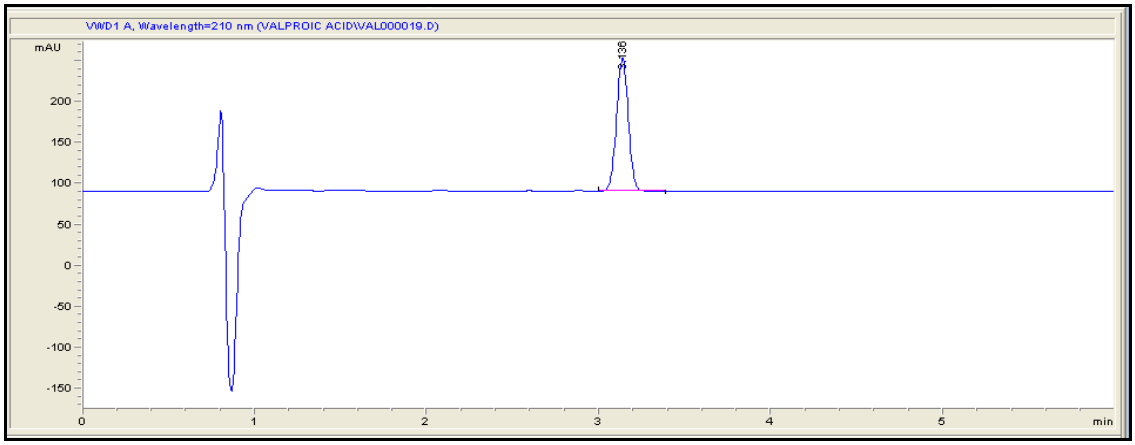

Fig. 5: Representative Chromatogram of Valproic Acid in sample solution

Method precision

The % RSD value of less than 1 indicated good precision of the method. The results of method precision are summarized in Table 4.

Linearity

The results of the linearity are tabulated in Table-5. Calibration curve (Fig.6) was plotted using different concentration values versus their respective mean peak areas. The slope, intercept, r^2 and regression equation was obtained. The representative linear equation was *y=0.6098x-1.2006*, where *x* is concentration and *y* is the peak absolute area. The correlation coefficient was 0.9997, indicating good linearity.

Recovery

The results of recovery study are summarised in table-6.The percent recovery of Valproic Acid was found to be between 96.63- 101.88%.

Robustness

Robustness of the proposed method was estimated by changing the flow rate from 1ml to 1.5 ml to 1.7ml/min and changing the wave length (±2nm). The System suitability parameters were found to be within acceptable limits. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions. The results of robustness are represented in Table-7.

Solution stability

The cumulative relative standard deviation was found below 2.0 %. It showed that both standard and sample solutions were stable up to 24 hours at room temperature.

Ruggedness

The relative standard deviation was found below 2.0%. The results are shown in Table 8.

Table 4: Results of Method Precision

Sample	% Dissolution
1.	95.97
2.	96.76
3.	95.93
4.	96.72
5.	95.99
6.	97.50
Mean	**96.48**
SD	**0.629**
%RSD	**0.65**

Table 5: Data of Linearity

Concentration (ppm)	Injection 1 (peak area)	Injection 2 (peak area)	Mean (peak area)
50	29.348	29.098	29.223
100	61.026	59.216	60.121
150	92.667	90.413	91.540
200	120.342	119.858	120.100
250	149.152	149.112	149.132
300	182.461	180.225	181.343
350	215.432	212.506	213.969
400	241.624	243.500	242.562

Table 6: Results of Recovery Study

Levels %	Drug added (mg)	Drug recovered (mg) mean (n=2)	%Recovery mean (n=2)	% RSD mean (n=2)
80	200	193.25	96.63	0.60
100	250	254.69	101.88	0.90
120	300	302.58	100.86	0.69

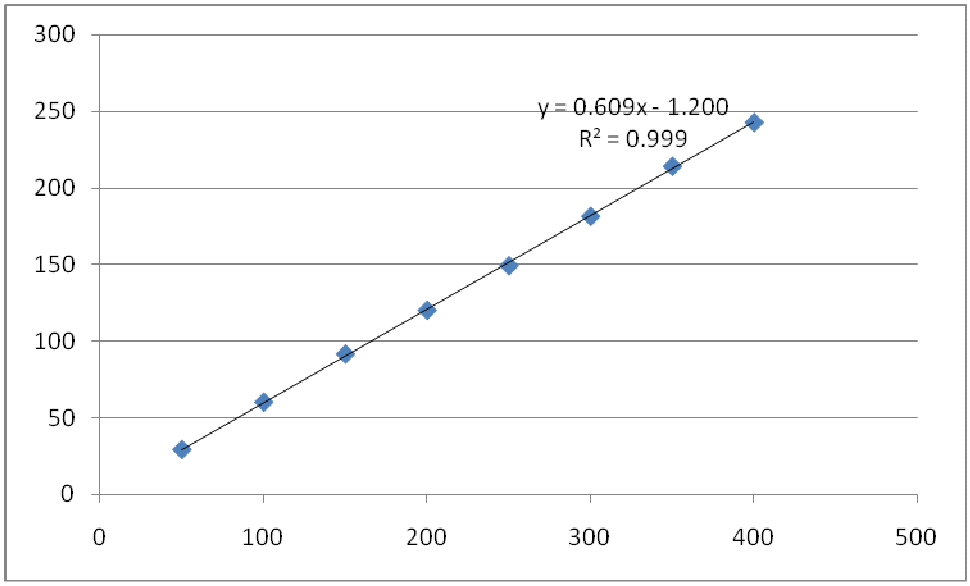

Fig. 6: Calibration curve of Valproic Acid concentration versus mean peak area

Table 7: Results of Robustness study

Parameters	Variables	Area (n=3)	%RSD
Standard	-	135.408	0.3
Wavelength	208nm	127.530	0.9
	210nm	129.348	0.2
	212nm	128.120	0.7
Flow rate	1.3ml/min	128.682	0.7
	1.5ml/min	129.348	0.2
	1.7ml/min	127.819	0.8

Table 8: Results of Ruggedness (Intermediate Precision)

S. No.	Analyst	Label claim (mg)	Amount estimated (mg)	Mean ±SD (n=6)	% RSD
1.	A	250	241.20	241.20 ± 1.57	0.65
2.	B	250	241.87	241.87 ± 1.86	0.70

CONCLUSION

The validated HPLC method was found to be specific, linear, precise and accurate. The major advantage of the proposed method is that the dissolution of Valproic Acid can be determined on a single chromatographic system that is HPLC with UV detection without the need for prior derivatization. The stated analytical method can be successfully used for in vitro dissolution and routine analysis of formulations of Valproic Acid in quality control laboratory.

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