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Research Article

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Estimation of Sodium Valproate in Tablet Dosage Form by RP-HPLC without Prior Derivatization: Application to Dissolution Studies

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ABSTRACT

A simple, precise and reproducible reverse phase, isocratic high performance liquid chromatographic method was developed and validated for the quantitative determination of sodium valproate in bulk and enteric coated tablet dosage form. The quantification was carried out using a Nova-pack phenyl, 4µm, 150 mm × 3.9 mm i.d. column, with a mobile phase consisting of acetonitrile: buffer (30:70, v/v) (pH 2.5) at a flow rate of 1.2 ml/min and UV detection at 210 nm. The method was validated for specificity, linearity, accuracy, precision, limit of detection, limit of quantification, robustness and solution stability. The linearity of the proposed method was investigated in the range of 50-1500 µg/ml (r = 0.9999). Mean inter- and intra-assay relative standard deviations (RSD) were less than 2.0 %. The proposed method was successfully applied for the analysis of sodium valproate in bulk and pharmaceutical dosage forms. Also, the method was extended for determination of sodium valproate release from *in-vitro* dissolution studies.

Keywords: Sodium valproate; Dissolution; Reversed-phase; HPLC; Dosage forms.

INTRODUCTION

Sodium valproate, chemically sodium-2-propyl pentanoate, is first line drug used for its unique anticonvulsant properties in the treatment of primary generalized seizures, partial seizures and myoclonic seizures. The mode of action is to stabilize electrical activity in the brain by increasing synthesis and decreasing metabolism of gamma amino butyric acid [1].

Sodium valproate is official in BP and USP but these pharmacopoeias have adopted gas chromatography (GC) method for quantitative analysis of this drug in formulation. There are number of analytical methods reported in recent scientific literature for the quantification of sodium valproate in biological matrices either alone or in combination with other drugs. These include high-performance liquid chromatography (HPLC) with MS detection [2-3], UV detection [4-8] and fluorescence detection [9-12], isotope-dilution mass spectrometry [13], and gas chromatography. [14-16]

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No single HPLC method has been reported for the assay and dissolution of this compound in enteric coated tablets. The major advantage of the proposed method is that the assay and dissolution of sodium valproate can be determined on a single chromatographic system with UV detection without the need for prior derivatization.

MATERIALS AND METHOD

Instrumentation

Agilent 1100 series integrated high performance liquid chromatographic system was used for this experiment. Agilent 1100 series system equipped with Agilent1100 series quaternary pump, Agilent 1100 series auto sampler, Agilent 1100 series variable wavelength detector, Agilent 1100 series Column thermostat and controlled by Chem-Station software. The Nova pack phenyl (150 × 3.9 mm), 4µm was used as a stationary phase.

Chemicals and reagents

The reference standard of sodium valproate was obtained from quality control department of Cadila HealthCare Ltd. Ahmedabad, India. Sodium valproate tablets were in-house product of formulation development department, Cadila HealthCare Ltd. Ahmedabad, India. All solvents used were of HPLC grade. Acetonitrile, potassium di-hydrogen phosphate, di-sodium hydrogen phosphate anhydrous and

sodium hydroxide were obtained from E. Merck Mumbai, India. HPLC grade water was obtained by passage through a Milli-Q system (Millipore, Milford, MA, USA).

Chromatographic conditions

The chromatographic column used was a 150 mm × 3.9 mm, Nova pack phenyl, with 4µm particles. The flow rate of the mobile phase was maintained at 1.2 ml/min and the column temperature 45°C. Detection was carried out at 210 nm and the injection volume was 50 µm. Run time was 9 min.

Mobile phase preparation and Standard preparation

The buffer is a mixture of buffer A (0.0019 M citric acid monohydrate and 0.028 M anhydrous Na_2HPO_4) and buffer B (0.05 M KH_2PO_4 and 0.0425 M NaOH) in equal volumes. Buffer and acetonitrile was mixed in the ratio of (70:30), pH was adjusted to 2.5 with o-phosphoric acid and the mobile phase was filtered through 0.45 µm membrane filter (Millipore, USA) and sonicated (Branson sonicator 1510, Germany) prior to use. The mobile phase was used as diluent. About 50 mg of sodium valproate working standard was weighed accurately in 50 ml volumetric flask and mobile phase was added, sonicated to dissolve and diluted to the mark to obtain a concentration of 1 mg/ml.

Assay of Sodium valproate in tablet dosage form

Twenty tablets were weighed and transferred intact to a 500 ml volumetric flask. About 300 ml of mobile phase was added and sonicated for 30 min after disintegration to effect complete dissolution of sodium valproate, cooled and the solutions were made up to volume with mobile phase. Aliquots of the solution were filtered through a 0.45 µm nylon filter and 5 ml of the filtered solution was transferred to a 100 ml volumetric flask and made up to volume with mobile phase. The solutions were injected at above chromatographic conditions and peak areas were measured to determine the sodium valproate content (Fig. 1).

Dissolution study

For the dissolution study of sodium valproate enteric coated tablets, analysis was done by 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 0.1 N HCL media (RPM 50). Sample was collected in dissolution vials after 2 hrs and then decanted the 0.1 N HCL media and the 6.8 pH phosphate buffer media was loaded and set RPM 50. Samples were collected in dissolution vials after different time intervals and filtered through 0.45 µm HVLP filter. Equal volumes (50 µl) of these solutions were injected into the chromatograph by autosampler and peak areas were measured.

RESULTS AND DISCUSSION

Method development

To develop a precise, accurate and suitable HPLC method for the quantitative determination of sodium valproate, different mobile phases and stationary phases were employed and the proposed chromatographic conditions were found appropriate. Sodium valproate is a sodium salt of valproic acid. Valproic acid (about 1 mg/ml) has a λ_{max} of 213 nm in methanol. The wavelength 210 nm was selected instead of 213 nm because of greater peak area at 210 nm when scanned on PDA in final mobile phase. The absorption spectrum is presented in Fig. 2. System suitability results are as follows. Retention time mean±SEM (n=6) 5.37 ± 0.005, asymmetry 1.66, theoretical plates 6945, capacity factor 3.97.

Method Validation

The proposed method was 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 found to be 0.99 to 1.

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 concentrations of sodium valproate and the respective peak areas in the range 50-1500 µg/ml. The regression equation was found to be $Y = 1321.610\ x + 835.885$, where Y is the peak area and X is the concentration of sodium valproate.

Limit of Detection and Limit of Quantitation

LOD was defined as 3.3σ/S and LOQ was 10σ/S based on 'standard deviation of the response and slope of the calibration curve specially constructed in a low region of 0.05 to 1.0% of the target analyte concentration. [17] 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 0.26 and 0.81 µg/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 99.3-101.1 % [Table 1].

Precision

The intra-day precision of the assay method was evaluated by carrying out six independent assays of sodium valproate (1000 µg/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. Results are shown in Table II.

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 33 h. The same sample solutions were assayed for a 6 h interval up to the study period against freshly prepared solutions. The relative standard deviation was found below 2.0 %. It showed that both standard and sample solutions were stable up to 33 hours at room temperature.

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 and columns. Results are shown in

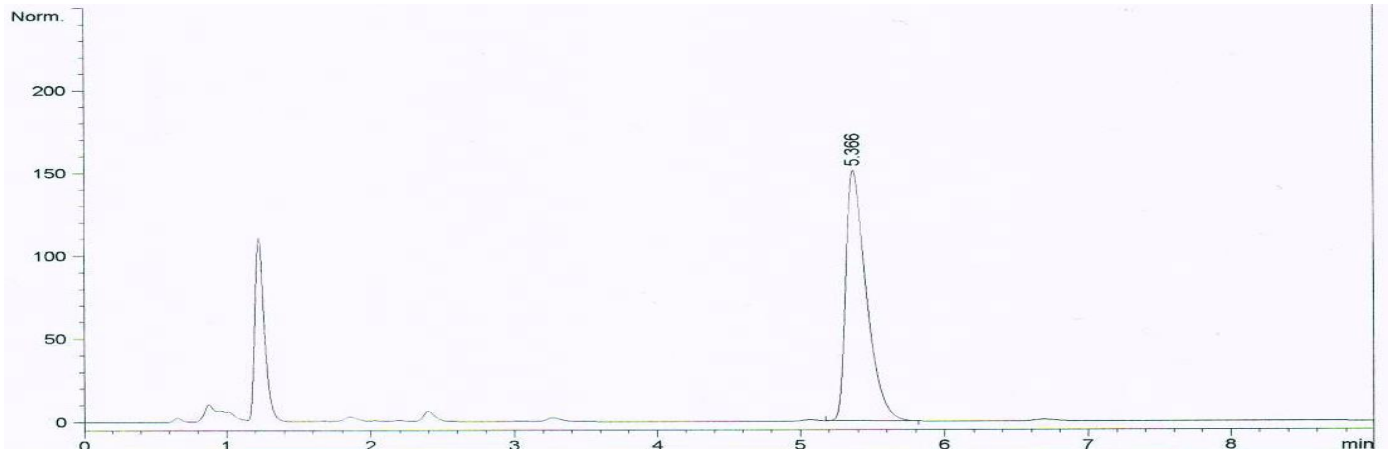

Fig. 1 Chromatogram of Sodium valproate in sample solution

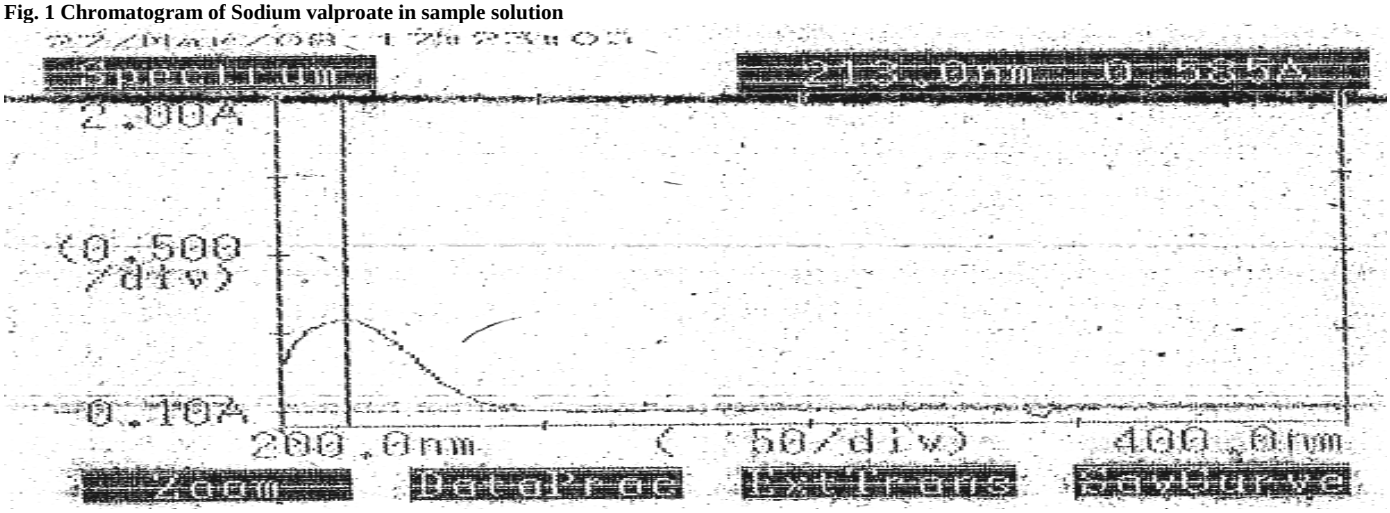

Fig. 2 U.V. Spectrum of Valproic acid

TABLE I: ACCURACY

Level (%)	Drug Added (mg)	Drug recovered (mg)	%Recovery Mean (n=3)	%RSD of Assay (n=3)
50	573.44	570.30	99.3	0.3
100	1146.18	1163.61	101.5	0.5
150	1720.68	1738.88	101.1	0.5

TABLE II: INTER AND INTRA-DAY PRECISION

Concentration (µg/ml)	Intra-day precision		Inter-day precision	
	%Assay	% RSD of assay	% Assay	% RSD of assay
1000	99.2±0.239	0.6	100.1±0.367	0.9

All values are mean ± SEM (n=6)

TABLE III: METHOD ROBUSTNESS (N=5)

Condition	Change	% RSD
Temperature	Normal	0.04
	-5°C	0.12
	+5^{0}C	0.09
p^H	Normal	0.04
	-0.2 unit	0.04
	+0.2 unit	0.12
Flow rate	Normal	0.02
	-10%	0.04
	+10%	0.07
Organic phase	Normal	0.02
	-2%	0.19
	+2%	0.09

TABLE IV: METHOD RUGGEDNESS

% Assay Mean ± SEM (n=6)	% RSD of Assay (n=6)
Day 1, Analyst 1, Instrument 1 & Column-1	
99.2±0.239	0.6
Day 2, Analyst 2, Instrument 2 & Column-2	
99.8±0.204	0.5

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 6.8 pH phosphate buffer in 120 min from tablet dosage form was found within the limit.

Analytical RP-HPLC method was developed and validated for the determination of sodium valproate in bulk and its 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.

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