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Reverse Phase High Performance Liquid Chromatographic method for the estimation of Valproic acid in bulk drug and soft gelatin capsules

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Abstract

A simple, precise and reproducible reverse phase high performance liquid chromatographic method for the estimation of valproic acid in bulk and soft gelatin capsules was developed and validated. The quantification was carried out using a Nova-pack phenyl, 4µm, 150 mm × 3.9 mm i.d. column, with an isocratic elution at a flow rate of 1.2 ml/min and UV detection at 210 nm. The mobile phase composition was acetonitrile: buffer (30:70, v/v) (pH 2.5). 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 10-1500 µg/ml. Mean inter- and intra-assay relative standard deviations (RSD) were less than 2.0%. The proposed method was successfully applied for the analysis of valproic acid in bulk and pharmaceutical dosage forms.

Key Words: Valproic acid, HPLC, Validation, Dosage form

Introduction

Valproic acid, chemically 2-propyl pentanoic acid 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].

Valproic acid is official in USP but this pharmacopoeia has 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 valproic acid 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]. Only one method [17] has been reported an HPLC method for the determination of this compound in pharmaceutical dosage forms, which requires precolumn derivatization and internal standard, which in turn leads to laborious sample preparation and lengthy analytical procedure. Chromatography of valproic acid without prior derivatization would significantly simplify the method and thus shorten the analysis time. In the present work, the derivatization is omitted due to using simple but optimized chromatographic conditions.

Results and discussion

Method development

To develop a precise, accurate and suitable HPLC method for the quantitative determination of valproic acid, different mobile phases and stationary phases were employed and the proposed chromatographic conditions were found appropriate (Table 1). System suitability results are as follows. Retention time 5.38 ± 0.006 (n=6); theoretical plates 8447; Asymmetry 1.55 and; Capacity factor 3.98.

Table 1. Chromatographic Conditions

Column	Nova pack phenyl (150mm × 3.9 mm), 4µm
Detector	210 nm
Injection volume	50 µL
Flow rate	1.2 ml/min
Temperature	45° C
Run time	9 min
Mobile phase	Buffer: Acetonitrile (70:30)

Method Validation:

The proposed method was validated for assay of valproic acid 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 valproic acid and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r = 0.9994) was observed between the concentrations of valproic acid and the respective peak areas in the range 10-1500 µg/ml. The regression equation was found to be Y = 1439.818 X + 1152.147, where Y is the peak area and X is the concentration of valproic acid.

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 [18]. 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 valproic acid was found to be in the range of 98.6-100.5% (Table 2).

Precision

The intra-day precision of the assay method was evaluated by carrying out six independent assays of Valproic acid (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 3.

Table 2. Accuracy

Level (%)	Drug Added (mg)	Drug recovered (mg)	%Recovery Mean (n=3)	%RSD of Assay (n=3)
50	123.30	123.88	100.5	0.7
100	249.67	247.17	99.0	1.0
150	374.07	368.91	98.6	1.8

Table 3. Inter and intra-day precision

Concentration (µg/ml)	Intra-day precision		Inter-day precision	
	%Assay	% RSD of assay	%Assay	% RSD of assay
1000	100.1±0.282	0.7	100.4±0.653	1.6

All values are mean ± SEM (n=6)

Standard and sample 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 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 4. 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.

Table 4. Method robustness (N=5)

Condition	Change	% RSD
Temperature	Normal	0.02
	-5°C	0.65
	$+5^0$C	0.04
p^H	Normal	0.02
	-0.2 unit	0.03
	+0.2 unit	0.05
Flow rate	Normal	0.02
	-10%	0.06.
	+10%	0.07
Organic phase	Normal	0.02
	-2%	0.02
	+2%	0.05

Method Ruggedness

Ruggedness test was determined between two different analysts, instruments and columns. Results are shown in Table 5 and the values of percentage RSD were below 1.0%, showed ruggedness of developed analytical method.

Table 5. Method ruggedness

% Assay Mean ± SEM (n=6)	% RSD of Assay(n=6)
Day 1, Analyst 1, Instrument 1 & Column-1	
100.1±0.282	0.7
Day 2, Analyst 2 , Instrument 2 & Column-2	
99.1±0.244	0.6

Assay of Valproic acid in pharmaceutical dosage forms:

The content of 20 capsules of valproic acid was mixed and weighed. A portion of capsules content equivalent to 100 mg of Valproic acid was accurately weighed into 100 ml volumetric flask and about 50 ml of mobile phase was added. The volumetric flask was sonicated to effect complete dissolution of Valproic acid, cooled and made up to volume with mobile phase. The solution was filtered through a 0.45 µm nylon filter. The solutions were injected at above chromatographic conditions and peak areas were measured (Fig. 1 and Fig. 2). The % assay was found to be 100.1±0.282 (n=6).

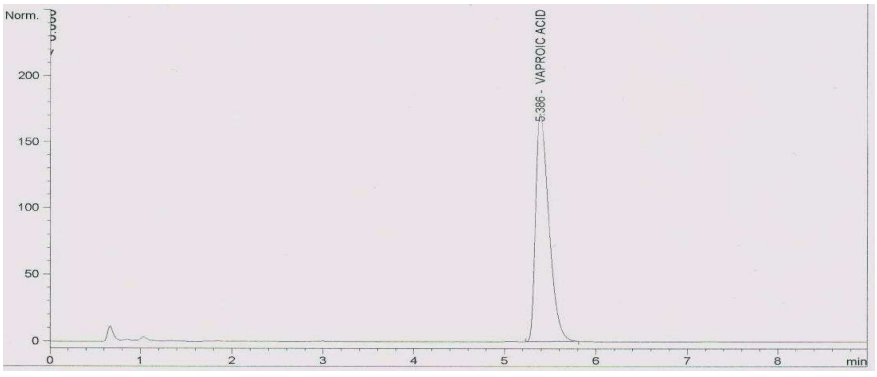

Fig.1 Chromatogram of Valproic acid Standard solution

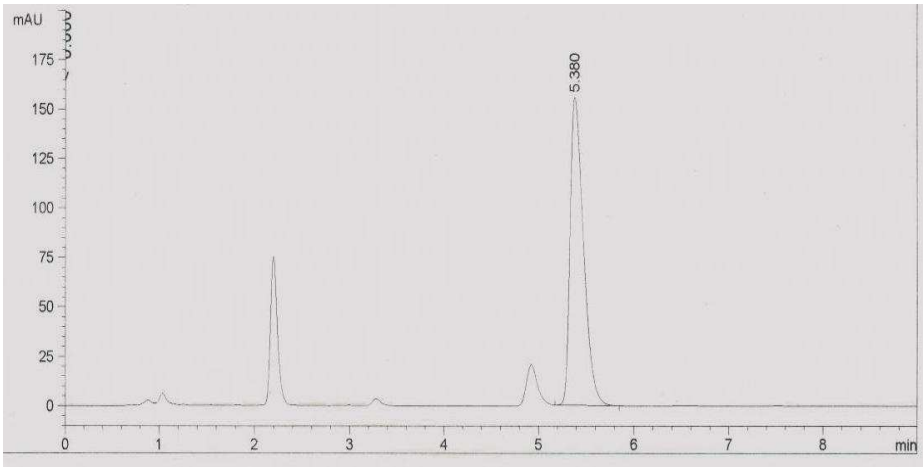

Fig.2 Chromatogram of Valproic acid in Sample solution

Materials and Methods

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 valproic acid was obtained from quality control department of Cadila HealthCare Ltd. Ahmedabad, India. Valproic acid soft gelatin capsules 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 1.2ml/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 valproic acid 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.

Conclusion

Analytical RP-HPLC method was developed and validated for the determination of valproic acid in bulk and its dosage form. The developed method was found to be simple, precise and accurate and can be applicable for the routine quality control analysis of valproic acid in soft gelatin capsules. The advantages of the method are short run time, simplicity of sample preparation, no need of internal standard and derivative formation which require longer time for analysis.

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References

[1] LJ Willmore, *Psychopharmacology*, **2003,** 37, 43.
[2] SJ Deepak, S Gunta, S Mallika, S Pranav, *Talanta*, **2007**, 72(1), 80.
[3] M Tsukasa, N Masaharu, W Hiroyuki, Y Susumu, S Kenji, *Anal sci.*, **2007,** 17, 999.
[4] H Amini, M Javan, A Ahmadiani, *J. Chromatogr. B Analyt Technol Biomed Life Sci*., **2006,** 830(2), 368.
[5] P Kishore, KV Rajani, V Satyanarayana, DR Krishna, *Phammazie*, **2003**, 58(6), 378.
[6] C Lucarelli, P Villa, E Lombaradi, P Prandini, A Brega, *Chromatographia*, **1992**, 33, 37.
[7] BG de Illiano, AQ de Gainzarain, *Journal of high resolution chromatography*, **1989**, 12(8), 540.
[8] K Kazuko, N Mikio, N Ryota, K Satoshi, *Anal sci*., **1985,** 1, 385.
[9] Z Yan, J Zheng, Y Yunqiu, *Biomed Chromatogr*., **2006**, 20, 319.
[10] H Shuuji, K Motoki, I Kazuo, F Misa, O Nobufumi, K Takeshi, *Biol. Pharm. Bull.*, **1999**, 22(9), 975.
[11] H Liu, LJ Forman, J Montoya, C Eggers, C Barham, M Delgado, *Journal of chromatography: Biomedical applications*, **1992,** 576(1), 163.
[12] N Masaharu, S Akira, S Kenji, *Anal sci.*, **1988**, 4, 385.
[13] F Susanto, H Reinauer, *Chromatographia*, **1995**, 41, 407.
[14] R Riva, F Albani, A Baruzzi, *J. pharm sci*., **1982**, 71(1), 110.
[15] M Krogh, K Johansen, F Tonnesen, KE Rasmussen, *J. of chromatography B: Biomedical science and applications*, **1995**, 673(2), 299.
[16] S Pravin, M Abdorreza, A Naader, *J. chromatogr. B*., **2006**, 850, 128.
[17] CA Lau-Cam, RW Roost, *Journal of liquid chromatography and related technologies*, **1997,** 40, 2075.
[18] JB Crowther, Validation of pharmaceutical test methods. In: Handbook of modern pharmaceutical analysis, (Academic press, New York, **2001**) 415.
[19] ICH (1994) Harmonised tripartite guideline, Text on validation of analytical procedures, Q2A. In: International conference on harmonization of technical requirements for registration of pharmaceuticals for human use.
[20] ICH (1996) Harmonised tripartite guideline, Text on validation of analytical procedures, Q2B. In: International conference on harmonization of technical requirements for registration of pharmaceuticals for human use.
[21] JSK Nagarajan, S Muralidharan. *Der Pharmacia Lettre*, **2009,** 1(1), 162-168.
[22] L Sivasubramanian, KS Lakshmi. *Der Pharma Chemica*, **2009,** 1(1), 37-46.