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Research Article

An Isocratic Method for Quantification of Valproic Acid and Its Related Impurities Using Ion Pair Reagent by Ultraperformance Liquid Chromatography

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A selective ultraperformance liquid chromatographic (UPLC) method for the quantification of valproic acid and its known related impurities using ion pair reagent has been developed. The method includes reversed-phase Acquity HSS T3 column with 100 mm × 2.1 mm i.d. and 1.7 μ particle size. The mobile phase consists of acetonitrile, 5 mM 1-hexanesulphonic acid sodium salt, flow rate is 0.6 mL/min, and UV detection is performed at 215 nm. A system suitability test (SST) was developed to govern the quality of the separation. The developed method has been validated further with respect to linearity, accuracy, precision, selectivity, LOD, LOQ, and Robustness.

1. Introduction

Valproic acid is chemically known as 2-propylpentanoic acid. Chemical structures of valproic acid and its pharmacopeial-related impurities are given in Figure 1. Valproic acid (VPA) is a chemical compound that has found clinical use as an anticonvulsant and mood-stabilizing drug, primarily in the treatment of epilepsy, bipolar disorder, and less commonly, it is also used to treat migraine headaches and schizophrenia [1–4]. To our knowledge, there is no paper describing an ultraperformance liquid chromatographic (UPLC) method that allows the separation of valproic acid and its related known impurities in bulk drugs. However, there are the UPLC-MS/MS method for the simultaneous determination of valproic acid and other 21 antiepileptic drugs for the routine therapeutic drug monitoring [5]. Furthermore we have used an ion pair reagent to resolute the valproic acid and its impurities more specifically, that is 2-isopropylpentanoic acid which is a structural isomer of valproic acid. Some articles are available on isocratic HPLC methods for the determination of valproic acid [6–10]. Gas chromatographic and colorimetric methods are also available in the literature for quantification of valproic acid [11–15]. Methods for determination of valproic acid by HPLC, LC-MS, or LC-MS/MS from biological matrix have also been published [16–26]. In addition, the capillary electrophoresis methods for the determination of valproic acid in different biological samples are also available [27–30]. Applying the above methods for quantification of impurities in a bulk valproic acid sample gives poor separation. Hence, the objective of this study is to develop a more selective LC method for the analysis of valproic acid and its related impurities. Besides this, it is accurate, precise, selective, robust, and linear with a reasonable analysis time. Furthermore, the developed method is applicable for the analysis of drug substances as well as commercially available drug products containing valproic acid. This work also deals with the validation of methods for the quantification of valproic acid and its impurities.

2. Materials and Methods

2.1. Reagents and Chemicals. HPLC grade acetonitrile (ACN) was purchased from Fisher Scientific (Leicester, UK). 1-hexane sulphonic acid sodium salt anhydrous was procured from Spectrochem Pvt. Ltd. (India). Dematerialized water was further purified by filtering through Milli-Q Elix-3

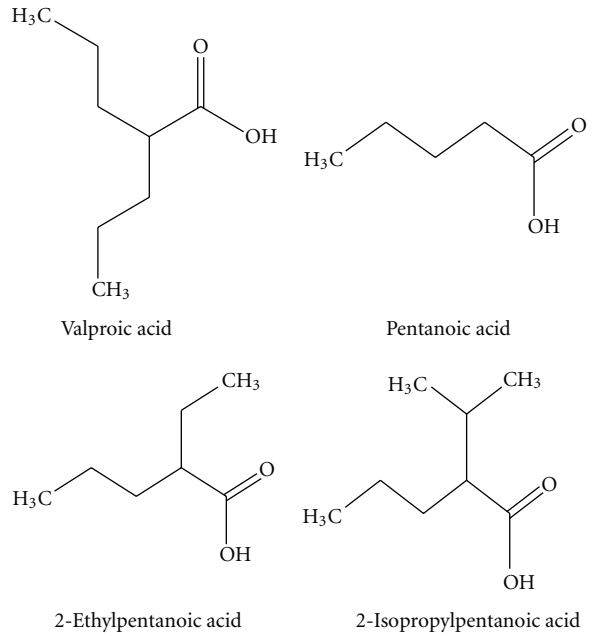

Figure 1: Chemical structure of valproic acid and its related impurities.

purification system (Millipore, Milford, MA, USA). Valproic acid working standards and its related impurities were provided by Parth laboratories Pvt. Ltd.(Rajkot, India).

2.2. Preparation of Standard Solutions. A mixture of acetonitrile and water (1 : 1) was used as sample diluent for all sample preparations. Stock solution of valproic acid and its related impurities were prepared by dissolving accurately 100 mg of each in 100 mL of sample diluent. Further pipette out 10 mL of this solution and dilute it up to 50 mL with sample diluent. This mixture contains 0.2 mg/mL valproic acid and its related impurities.

2.3. Instrumentation and Liquid Chromatographic Conditions. Waters Acquity UPLC (Switzerland) includes a binary solvent manager, a sample manager, PDA detector, and Empower 2.0 version software for data acquisition. A Waters Acqutiy HSS T3 column with 100 mm × 2.1 mm i.d. and 1.7 μ particle size was used to achieve the best separation; flow rate was set at 0.6 mL/min while the injection volume was 10 μL. Based on the absorption maxima observed for all the four components, the detection wavelength was set at 215 nm. The Spinco. Ltd. ultrasonic bath was used for the mobile phase and sample degassing. The Sartorius microbalance was used for the weighing purpose.

2.4. Mobile Phase Preparation. Mobile phase A consisted of 5 mM 1-hexane sulphonic acid sodium salt anhydrous which was filtered through 0.22 μ filter; mobile phase B consisted of acetonitrile (HPLC grade). The isocratic mode was set at 50 : 50 (V/V) ratio. 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.

Table 1: Data for method precision.

	Area			
Injection	PA	2-EPA	2-IsoPPA	VPA
SET 01	224100	663931.5	672628.5	850128.5
SET 02	212925.5	631144	639404.5	821765
SET 03	213801	633675.5	641733.5	823815.5
SET 04	214931.5	635104	643164.5	824633
SET 05	218389.5	649573	654524	838073
SET 06	218432.5	642225.5	642627.5	830001
Mean	217096.7	642608.9	649013.8	831402.7
Std dev	4134.018	12416.7	12715.01	10893.97
% CV	1.904229	1.932233	1.959127	1.310312

3. Results and Discussion

3.1. Method Development. The column selection is the most important part in the method development to achieve maximum sensitivity, resolution, and speed. After performing several trials on different columns, the maximum separations was achieved on Waters Acquity HSS T3 column with 100 mm × 2.1 mm i.d. and 1.7 μ particle size column. The compatibility of the method to ICH guideline was achieved by the isocratic elution at 50 : 50 (V/V) ratio of mobile phase A and B. The selectivity of the valproic acid and its related impurities can be altered by changing the pH of the buffer. The premethod validation experiments were performed to avoid uncertainty at the time of method validation. The chromatograms of the standard and sample are given in Figure 2.

3.2. Method Validation

3.2.1. Accuracy. The accuracy experiment was performed by recovery study at three levels 150%, 100%, and 50% of concentration. The recovery was found between 98 to 102% which is suitable to the ICH guideline Q2 (A).

3.2.2. Precision. The method precision was assessed using multiple preparations of a single sample. Six different preparations of valproic acid and its related impurities were analyzed in triplicate on the same day. The method precision data is shown in Table 1. New solutions were prepared and analyzed on each of two successive days for intermediate precision study which is given in Table 2. The relative standard deviation values obtained for the peak areas of valproic acid, pentanoic acid, 2-ethylpentanoic acid and 2-isopropylpentanoic acid were not more than 2.0%.

3.2.3. Linearity. The linearity of the method was evaluated by analyzing eight solutions in the concentration range between 50 to 400 μg/mL for each solution of valproic acid and its related impurities. The peak areas obtained from different

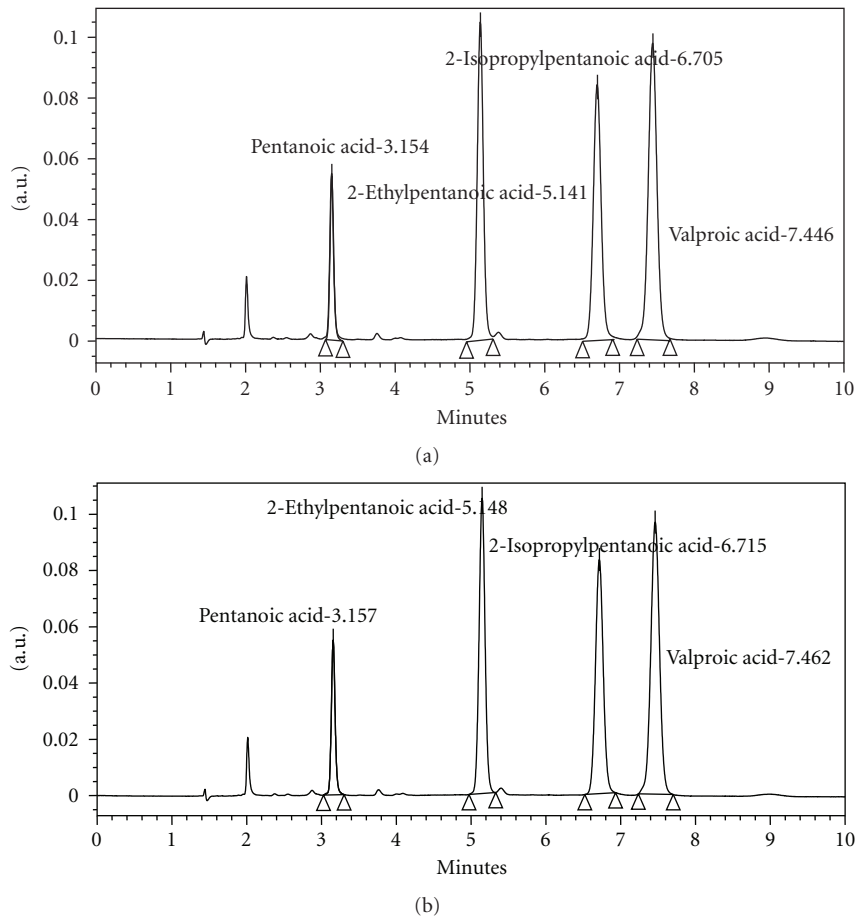

Figure 2: Chromatograms of working standards and sample of valproic acid with its related impurities, where (a) chromatograms of reference standard of valproic acid (0.2 mg/mL) and impurities (0.2 mg/mL). (b) Chromatograms of sample of valproic acid (0.2 mg/mL) and impurities (0.2 mg/mL).

Table 2: Data for intermediate precision.

	Area			
Injection	PA	2-EPA	2-IsoPPA	VPA
SET 01	210954.0	623938.5	629944.0	813293.0
SET 02	208416.5	616521.5	623969.5	799387.0
SET 03	208213.0	617099.5	621781.0	800629.0
SET 04	208638.5	615928.5	622960.5	799801.5
SET 05	210549.5	626428.0	631619.5	813999.0
SET 06	211420.5	636014.5	636014.5	818790.0
Mean	209698.7	622655.1	627714.8	807649.9
Std dev	1431.1	7849.7	5673.5	8665.1
% CV	0.7	1.3	0.9	1.1

Table 3: Data for robustness.

	% CV			
Condition	PA	2-EPA	2-IsoPPA	VPA
Column change	0.1	0.1	0.2	0.1
MP ratio change (56 : 44)	0.9	0.05	0.1	0.4
MP ratio change (54 : 66)	0.5	0.8	0.3	0.1
Flow rate change (1.10 mL/min)	1.6	1.4	1.1	0.6
Flow rate change (1.3 mL/min)	1.4	0.3	0.2	0.1

concentrations of the drugs were used to calculate linear regression equations. These equations are $y = 211.6x - 1217$, $y = 238.6x + 12028$, $y = 250.4x - 8400$, and $y = 321.8x - 7153$ with correlation coefficients of $R^2 = 0.998$, $R^2 = 0.997$, $R^2 = 0.999$ and $R^2 = 0.99$ for pentanoic acid, 2-ethylpentanoic acid, 2-isopropylpentanoic acid and valproic acid, respectively. The high values of the correlation coefficients are indicative of linear relationships between the analyte concentration and peak area.

3.2.4. Limit of Detection and Limit of Quantification. The limit of detection (LOD) and limit of quantification (LOQ) were established by evaluating the minimum level at which the analyte could be readily detected and quantified accurately. The LOD and LOQ for each component are 0.5 μg/mL and 2.0 μg/mL, respectively. Signal-to-noise ratio is more than 3.3 for LOD and more than 10 for LOQ.

Table 4: Summary of validation results observed by the proposed method.

Parameter	PA	2-EPA	2-IsoPPA	VPA
LOD (μg/mL)	0.5	0.5	0.5	0.5
LOQ (μg/mL)	2.0	2.0	2.0	2.0
Accuracy (%)	99.12–99.80	98.27–101.1	100.01–101.9	98.56–101.35
Precision (RSD, %)				
Method precision	0.61	1.21	0.90	1.07
Intermediate precision	0.91	0.33	0.56	1.34
Linearity				
Range (μg/mL)	50–400	50–400	50–400	50–400
Correlation coefficient	0.998	0.997	0.999	0.995
Slope	211.6	238.6	250.4	321.8
Intercept	−1217	12028	−8400	−7153
Theoretical plate	35426	21329	17200	31128
Asymmetry	1.06	1.23	1.02	1.31

3.2.5. Selectivity. The selectivity of the method was evaluated by injecting blank matrix, each individual impurity, the valproic acid standard solution, and the spiked solution to check the interference of the diluent as well as the standard solution on each other. The method is proved to be highly selective that there is no interference on any component.

3.2.6. Robustness. The influence of five (k) chromatographic parameters on the separation was investigated. The parameters examined were the amount of ACN in the mobile phases, the amount of buffer solution in the mobile phases, different column lots, different flow rates, and difference in pH of buffer. There is no such impact of the small changes on the above parameters observed which suggests that the method is highly robust. The results obtained are shown in Table 3.

4. Conclusions

The proposed isocratic ion-pair UPLC method shows a good separation of valproic acid from its impurities present in bulk drug. The robustness study indicates that mainly the amount of ion pair reagent in the mobile phase solution should be monitored carefully to ensure the best chromatography since this has a significant effect on the separation. The method was shown to be selective, precise, sensitive, and linear which is proven by the result of the validation summary shown in Table 4. The method can be used for the quantification of valproic acid and their pharmacopeial-related impurities. It can be applicable for the analysis of drug substances and drug products in pharmaceutical dosage form also.

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