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A Comparative Quantitative Study Of Selected Drugs Commercialized In Yemen With HPLC

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Abstract

International free-trade growth and poor regulation of drugs have resulted in the growth of the world's trade in falsified drugs. The best way to avoid this problem is to see technological protection. A new chromatographic method for determining Valproic acid in pharmaceutical preparations was to be developed and validated in this study. The method parameters for validation produced positive results and included range, linearity, accuracy, precision and retrieval. The HPLC method results were calculated by variance analysis (ANOVA). The concluded that the HPLC method proposed is satisfactory for the quantification of Valproic acid in pharmaceutical preparations. This research has provided quantitative evidence of the quality in both formal markets in Yemen of the randomly selected Valproic acid. Samples were tested for quality control, by used high performance liquid chromatography (HPLC). Three samples of Valproic acid were labeled by the same producer but therefore had different packaging, which raised the concern that the packaging could be counterfeited. Two samples of Valproic acid were from the same manufacturer, but the concentration of the active substance differs. However, the other two samples were reported as non-standard because the WHO standards were not complied with. While analyzing the same Valproic acid samples, differences in process, type and proportion of various leading brands were employed. Most of those concerned, with the effect, rather than the technical attributes of medicines including content, appearance and source, have had limited knowledge on good quality medicines and counterfeit medicines. A wide variety of behaviors, reporting such doubts to governments in relation to the medical quality, finding alternative medicines, stopping the use of medicament and taking no more action on such doubts.

Keywords: HPLC, Valproic acid, Anti-counterfeit technology, Yemen, quality counterfeit.

Abbreviations: HPLC: High-Performance Liquid Chromatography; UI: International Unit; LOD: Limit of Detection;

LOQ: Limit of Quantification; CV: Coefficient of Variation

Introduction

The safety, efficacy and quality of medicines are key criteria for the optimal treatment of drugs and are currently received in an era of globalization [1]. The World Health Organization states that medication with questionable quality can be counterfeit or under standard (WHO). WHO defines a falsified medicine as one that is mislabeled in a conscious and fraudulent way as regards identity [2]. Counterfeiting may include branded products and products containing good ingredients or wrong ingredients with insufficient active ingredients or fake packaging, without active ingredients [3]. Counterfeiting may include branded products and products containing good ingredients or wrong ingredients with insufficient active ingredients or fake packaging, without active ingredients [4]. They are legal but do not comply with the specifications due to inadequate production or poor conditions of storage. In recent times, the WHO's term SSFFC has been used to describe both counterfeit and non-substandard medicines at the same time as [5]. This joint definition highlights the importance of identifying both counterfeit and substandard medicines in any proposed medicine quality survey. When using appropriate strategies to combat potential threats of either quality problem, the distinction between counterfeit or non-standard medicines is imperative [6]. Some, however, reject this concept and argue that counterfeit and unusual medicinal products are similar because they claim to be something that they are not really. However, accurate identification, particularly in cases where scarce economic resources are available, could help governments and responsible bodies to assess the necessity of involving local or global enforcement [7, 8]. Reports suggest that the availability of substandard drugs in a number of developing countries is highly frequent mainly due to poor monitoring programs. The World Health Organization estimates that about 10% of all global drug supplies are falsified and unfair, reaching up to 50% of supplies in developing countries and as low as 1% in the developed world [9, 10]. Many new pharmaceuticals are regularly placed on the market and it is becoming increasingly difficult to track the safety of each drug, which has led to the inflow of falsified or non-standard medicines [11]. Fake drugs can be misleading, especially when copied to make it look like the original product, making it difficult for buyers to be suspect [12]. Therefore, analysis methodologies and anti-counterfeit technologies were developed to determine whether good quality products are on the market and to reduce the risk of products that are under-standard to consumers [7-16]. It could be a complex task to determine the exact rates of prevalence of counterfeit or low-standard medicinal products and requires high-quality country-based surveys [17]. Various methods for analyzing adulteration in medicinal products are proposed, such as nuclear magnetic resonance spectroscopy, infrared and high-performance liquid chromatography. Several analytical techniques are suggested to detect adulteration in medicines, such as infrared [18], nuclear magnetic resonance spectroscopy [19] and high-performance liquid chromatography coupled with mass spectrometry (HPLC–MS) [20]. No such method can be considered as a rapid screening method, since most reported HPLC–MS or HPLC–MS-MS methods are preceded by using the standard HPLC as a separation technique. This technique of separation takes time [21]. Due to their importance in quality control in pharmaceutical analysis the development of methods for the determination of drugs in HPLC have been given considerable attention in recent years [22]. In this work, High-quality analysis of three commercial HPLC brands (Legal and Illegal (Smuggled and Falsed) Valproic acid oral solution 200 mg).

Materials and Methods

Quality control study (Assay)

- Qualitative analysis of assay of three commercial brands of Valproic acid oral solution 200 mg (Legal and Illegal (Smuggled and counterfeit) by HPLC.
- Identification: Valproic Acid USP.

United States Pharmacopeia (USP) Reference Standard
Synonym: 2-Propylpentanoic acid, Valproic acid

- CAS Number 99-66-1

- Linear Formula $(CH_3CH_2CH_2)_2CHCO_2H$
- Molecular Weight 144.21
- Action and use: Antiepileptic
- Definition: 2-Propylpentanoic acid
- Content: 99% to 101%.
- Characters:
- Appearance: colorless or very slightly yellow clear liquid.
- Solubility: very slightly soluble in water miscible with ethanol 96% and with methylene chloride. It dissolve in dilute solutions of alkali hydroxides.

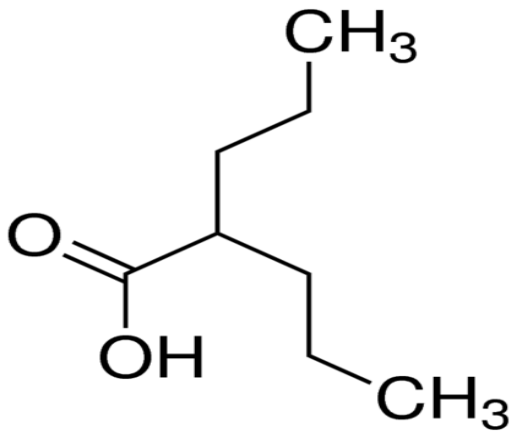

Figure 1. Valproic Acid USP.

Definition

Valproic Acid Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled. Amount of valproic acid ($C_8H_{16}O_2$). It is prepared with the aid of Sodium Hydroxide.

Identification

A- The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay. B-Sample: Volume of Oral Solution equivalent to 250 mg of valproic acid. Analysis: Place the Sample into a separator, add 40 mL of water and 2 mL of hydrochloric acid, mix, and extract with 40 mL of n-heptane. Filter the n-heptane layer through glass wool into a beaker, and evaporate the solvent completely on a steam bath with the aid of current of air. Transfer 2 drops of the residue to a test tube containing 0.5 mL each of potassium iodide solution (1 in 50) and potassium iodate solution (1 in 25). Acceptance criteria: A yellow color is produced.

Qualitative analysis (Assay) Valproic Acid Oral Solution (Procedure)

Buffer: 3.5 g/L of monobasic sodium phosphate in water an adjust with phosphoric acid to a pH of 3.5.
Mobile phase: Acetonitrile and Buffer (45:55)
Diluent: Acetonitrile and water (45:55)
System suitability solution: 0.5 mg/mL of USP Valproic Acid RS and 50 µg/mL of USP Valproic Acid related compound B RS in diluent.
Standard solution: 0.5 mg/mL of USP Valproic Acid RS in diluent.
Sample solution: Nominally 0.5 mg/mL of valproic acid in diluent from a suitable volume of oral solution chromatographic system.

Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15.0-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: System suitability solution and standard solution
[NOTE— the relative retention times for valproic acid related compound B and Valproic acid are 0.90 and 1.0, respectively.]
Suitability requirements:
Resolution: NLT 2.0 between valproic acid related compound B and valproic acid, System suitability solution
Tailing factor: NMT 1.5, Standard solution
Relative standard deviation: NMT 1.0%, standard solution analysis
Samples: standard solution and sample solution
Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) in the portion of oral solution taken:
Result = (r U / r S) × (C S / C U) × 100
r U = peak response from the Sample solution
r S = peak response from the Standard solution
C S = concentration of USP Valproic Acid RS in the Standard solution (mg/mL).
C U = nominal concentration of valproic acid in the Sample solution (mg/mL).
Acceptance criteria: 90.0%–110.0%

Method validation

The method was validated by defining linearity, range, precision, specificity and accuracy of the following operating characteristics [23]. Intraday and interday during three different days, the accuracy and precision of the analysis and the linearity of the calibration curve were determined. The accuracy was the relative standard deviation (RSD, percent) of each curve. The statistical information was calculated by variance analysis (ANOVA).

Linearity

A dilution of 5.0 mL of the reference solutions in 50 mL flasks and a completion of the final volumes with the mobile phasing (10.0 UI/mL) was carried out in an effort to evaluate the validity of the assay. Mobile phase dilution with 0.1, 0.5, 1.0, 2.0, 3.0 and 3.5 UI/mL were suitable for this solution. Each concentration was performed with triplicate injections.

Limit of detection (LOD) and quantification (LOQ) values:

By using the calibration line, LOD and LOQ are directly calculated. The LOD and LOQ factors 3.3 and 10.0 were multiplied by the residual defect and incline ratio respectively (corresponding to the standard error of the slope).

Results and Discussion

Name of the companies which investigated of Valproic acid oral solution 200 mg (Debakine®Sanofi company) (Legal and Illegal (Smuggled and counterfeit) which widely distributed in Yemen Markets in Table 1.

Company	Trade name	Dosage form	Strength
(Sanofi legal France)	Debakine®	Oral solution	200 mg

(Sanofi smuggled) -Turkish)	Debakine®	Oral solution	200 mg
(Sanofi smuggled) - France)	Debakine®	Oral solution	200 mg

Result quality control study (Assay): The Table 2 show name of the companies which investigated of Valproic acid oral solution 200 mg (Debakine® 200 mg Sanofi company) (Legal and Illegal (Smuggled and counterfeit) which widely distributed in Yemen Markets.

Company	Trade name	Dosage form	Strength
(Sanofi legal France)	Debakine®	Oral solution	200 mg
(Sanofi smuggled) -Turkish)	Debakine®	Oral solution	200 mg
(Sanofi smuggled) - France)	Debakine®	Oral solution	200 mg

Three commercial brands of Valproic acid 200 mg (Legal, and Illegal (Smuggled and counterfeit) marketed in Yemen. One drug (Debakine® are registered in supreme board of drugs and medical appliance (Table 1, 2). Identification and assay of Valproic acid oral solution 200 mg (Debakine® 200 mg Sanofi company) (Legal and Illegal (Smuggled and counterfeit) which widely distributed in Yemen Markets.

The normal range of assay of Valproic acid oral solution 200 mg content is (90-110%). The assay content of one type of Debakine® 200 mg oral solution company commercial brands of Valproic acid 250 mg comply with BP requirements namely Debakine® (Sanofi Legal – franc - Yemen) 92.1% whereas Debakine® (Sanofi Smuggled – franc - Turkish) and Debakine® (Sanofi Smuggled – France - Egypt) (88.82% and 32.97%) did not comply with BP requirements because these drugs were Smuggled and counterfeit and the bad storage play a main role on the stability of drug and that cause degradation of the products.

Table 3. Identification and assay of Valproic acid oral solution 200 mg (Debakine® 200 mg Sanofi company) (Legal and Illegal (Smuggled and counterfeit) which widely distributed in Yemen Markets**.**

	legal Debakine®	**Illegal Turkish Debakine®**	**Illegal Egypt Debakine®**
Area of the peak1	247957	168648	88735
Area of the peak2	255949	286133	89743
Area of the peak3	248848	270530	90816
Av. area peaks	250918	241770.3333	89764.66667
Av. standard	272184	272184	272184
%	92.1%	88.82%	32.97%
Limit	90.0% - 110.0%		
Result	Confirm	not confirm	not confirm

The physicochemical characteristics of Valproic acid oral solution, such as solubility and polarity, have influenced chromatographic conditions in this trial. In order to rapidly assess the quality of Valproic acid in pharmaceuticals, the described mobile phase was developed.

In order to allow for the correct determination of medicines by HPLC the wavelength of 215 nm was selected. A sharp and symmetrical peak with good baseline resolution and minimal tailing was obtained, making it possible to measure the peak area ratio accurately.

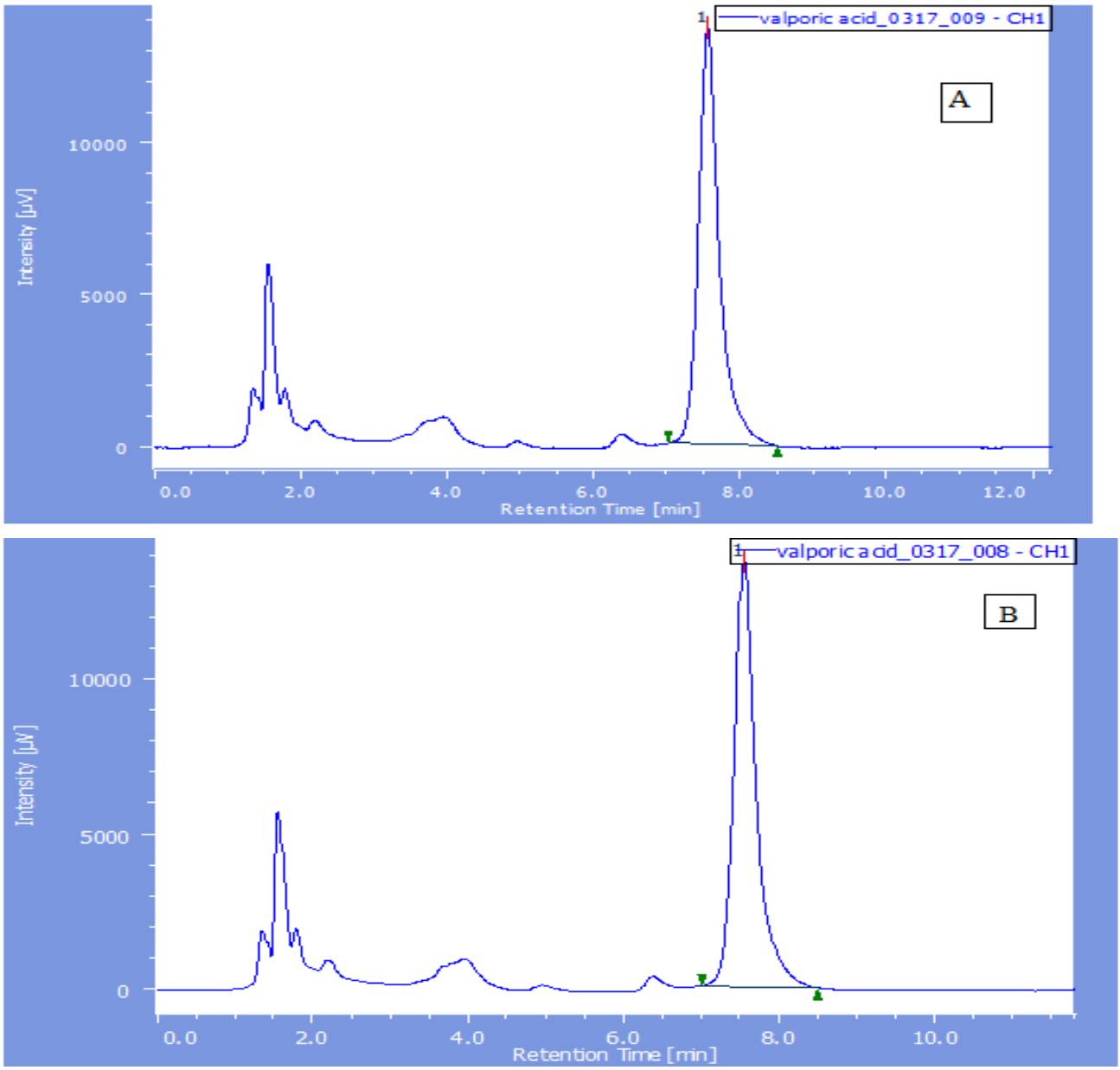

Figure 2. Identification and assay of reference standard of Valproic Acid at UV 215 nm, column: c8 (15 cm × 4.6 mm - 5-µm packing L7) flowrate: 1ml/min (A: peak Area 272299) and (B: peak Area 272069).

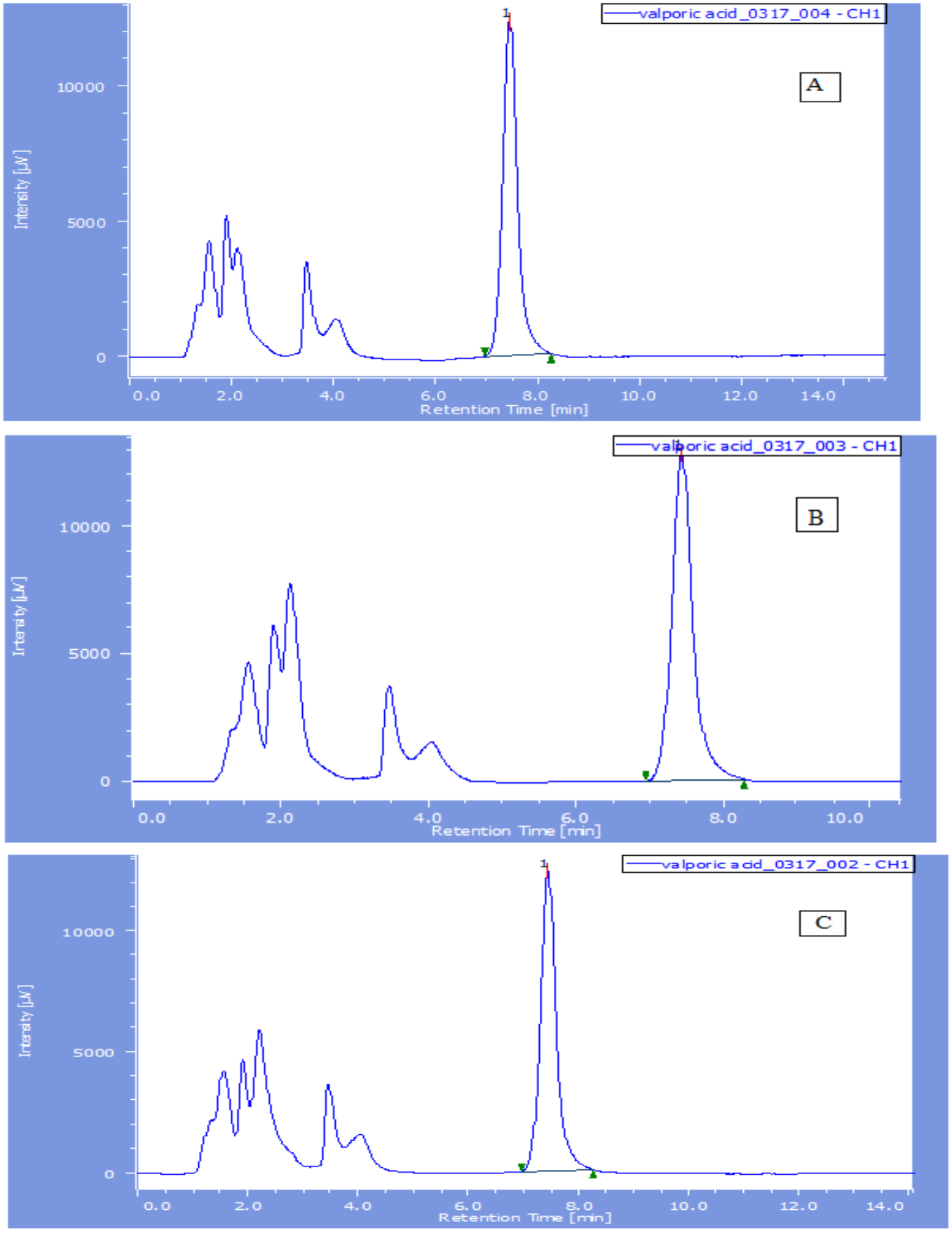

Figure 3. Identification and assay of (legal Debakine®) Yemen Valproic Acid at UV 215 nm, column: c8 (15 cm × 4.6 mm - 5-µm packing L7) flowrate: 1ml/min (A: peak Area 247957), (B: peak Area 255949) and (C: peak Area 248848).

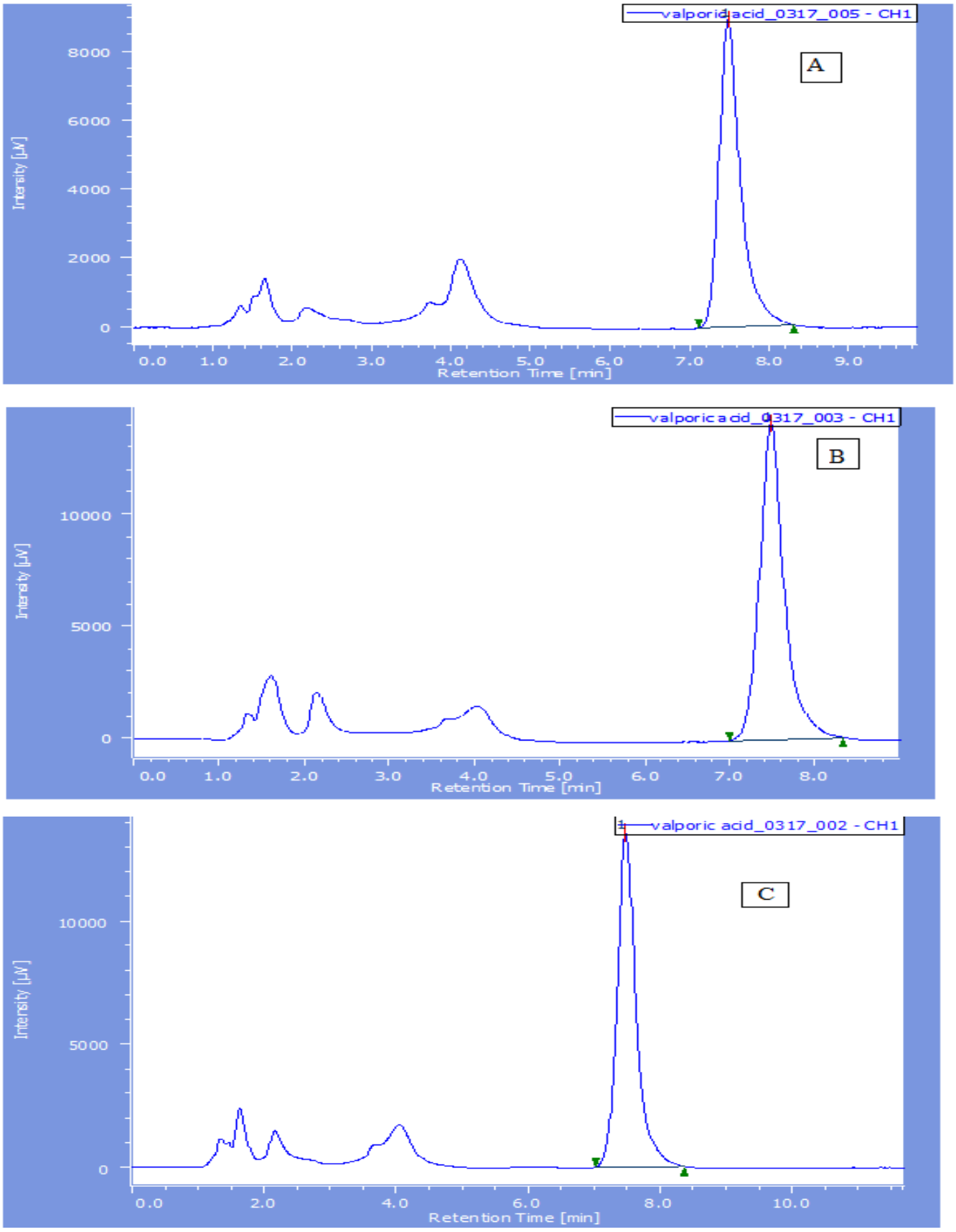

Figure 4. Identification and assay of (illegal Debakine®) Turkish Valproic Acid at UV 215 nm, column: c8 (15 cm × 4.6 mm - 5-µm packing L7) flowrate: 1ml/min (A: peak Area 168648), (B: peak Area 286133) and (C: peak Area 270530).

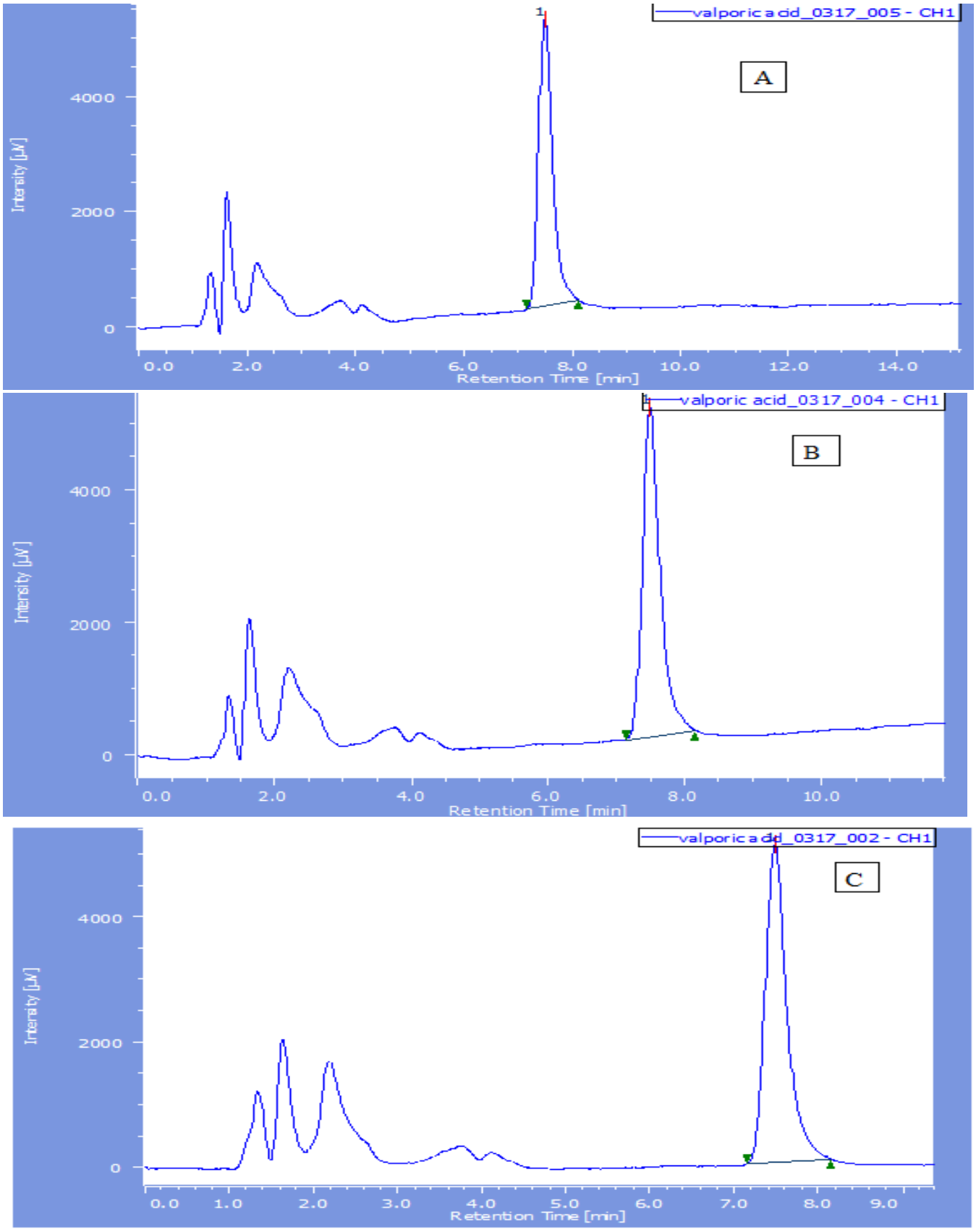

Figure 5. Identification and assay of (illegal Debakine®) Egypt Valproic Acid at UV 215 nm, column: c8 (15 cm × 4.6 mm

- 5-µm packing L7) flowrate: 1ml/min (A: peak Area 88735, (B: peak Area 89743) and (C: peak Area 90816).

The chromatograms of the sample peaks matched the corresponding chromatograph of the standard peaks of drugs, which showed that the peaks of Valproic acid oral solution were pure (Figure 2-5). The R_t was found to be excellent for all solutions during precision studies. The Valproic acid R_t values were respectively 4.8 minutes and 5.0 minutes for reference solutions and pharmaceutical preparations. At 215 nm, there was no interference from the excipient sample. The method has been validated.

The linearity of the concentration was studied by compared with peak area, and the calibration curve showed good linearity within the 0.2 to 3.6 UI/ml concentration range. The equations of regression were calculated using the least squares method.

The calculated LOD and LOQ values were 0.01 UI/mL and 0.05 UI/ mL for Valproic acid oral, 0.01UI/mL and 0.05UI/mL for Turkish Valproic Acid, 0.01UI/mL and 0.05UI/mL for Yemen Valproic Acid and 0.01UI/mL and 0.05UI/mL for Egypt Valproic Acid. The correlation coefficients for Valproic acid orally were larger than 0.992, and the CV was < 1%.

The method has been validated by intraday and interday accuracy assessments. In the range of 0.1-3.5UI/mL, the CV on the basis of the peak area ratios for 3 replicates injections were found to be between 0.08 to 1.98% for Valproic acid oral, 0.08 to 1.98% for Turkish Valproic Acid, 0.08 to 1.98% for Yemen Valproic Acid, and 0.07 to 1.03% for Egypt Valproic Acid. Recovery tests have shown that the proposed method is accurate.

Conclusion

The aim of the research was to develop an HPLC assay in pharmaceutical preparations for analysis of Valproic acid. This research has provided quantitative evidence of the quality in both formal markets in Yemen of the randomly selected Valproic acid. The simplest and fastest processes are advantageous for drug analysis in quality control. A quick and precise method for determining Valproic Acid in pharmaceutical preparedness (injection) with HPLC was developed in the current study. The method demonstrated acceptable sensitivity, linearity, accuracy, and precision. The method uses simple reagents with minimum sample preparation and short analytical periods, which encourage their use for routine analysis. The results show that the proposed method may be recommended in pharmaceutical preparations for quality control of valproic acid. All three have been obtained from retail pharmacies. Two samples of Valproic acid were from the same producer but their appearance differs in the active material concentration. The remaining two samples have been reported as standard since they did not meet WHO standards (2015).

Conflict of Interest

The authors declare that there are no conflicts of interest.

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