RESEARCH

Analytical method validation for related substances in sodium valproate oral solution by gas chromatography

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Abstract

Background: Sodium Valproate is the sodium salt of valproic acid (VPA). Valproic acid is mainly used for the treatment of epilepsy. The specific aim of the study is to develop and validate an optimized method for the determination of six related substances such as *N,N*-dimethyl valpronamide, valeric acid, 2-methyl valeric acid, 2-ethyl valeric acid, 2-isopropyl valeric acid and 2-*n*-butyl valeric acid in Sodium Valproate Oral Solution by Gas Chromatography. Chromatographic separations of these six related substances were achieved on DB-FFAP fused silica capillary column (30 m × 0.53 mm) bonded with a 0.5-μm layer of macrogol 20,000 2-nitroterephthalate materials used as stationary phase. The six related impurities were extracted using heptane and monitored by Gas Chromatography coupled with flame ionization detector. The performance of the developed method was assessed by evaluating system suitability, method precision, specificity, linearity and range, ruggedness, accuracy, robustness.

Results: The correlation coefficient was within the acceptance criteria in the range of 0.9998**.** The evaluated concentrations for Sodium Valproate were in the ranges of 5.05–25.27 ppm. The average recovery values were in the range of 92.4–100.4%. Solution Stability experiments were performed to evaluate the degradation behavior of SVS.

Conclusion: A novel, precise and sensitive GC method was developed, validated and optimized for the determination of six related substances in sodium Valproate oral solution. The results obtained from the validation experiments demonstrated that the method is accurate, precise, linear, specific, sensitive and robust. Hence, the proposed method can be an alternative method, for the determination of related substances in sodium valproate oral solution drug substance.

Keywords: Sodium valproate solution, Related substances, Gas chromatography, Flame ionization detector, Validation

Background

Sodium Valproate Oral Solution is chemically known as 2-propylpentanoic acid. The molecular formula of SVS is $C_8H_{16}O_2$ and the molecular weight is 144.211 g/mol. Sodium Valproate is the sodium salt of valproic acid (VPA). Valproic acid is mainly used for the treatment of epilepsy. SVS is an anticonvulsant drug which is used in the treatment of seizure disorders, such as tonic–clonic seizures and simple/complex partial seizures [1] and used in mental disorders such as mood changes, bipolar disorder and also effective against migraine headaches [2]. It is used for aggressive behavior in adults and disability [3], patients with mood changes [4] bipolar disorder [5]. The main action of the drug is gamma-amino butyric acid GABA enhancer and also used for treating alcohol withdrawal [6]. The main action is restoring the balance of certain neurotransmitters in the brain. The related substances present in SVS are *N,N*-dimethyl valpronamide (DMVA), valeric acid (VA), 2-methyl valeric acid (MVA),

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2-ethyl valeric acid (EVA), 2-isopropyl valeric acid (IPVA) and 2-n-butyl valeric acid (BVA) [7]. These impurities present may originate through the manufacturing process of SVS and the acceptance criteria are based on pharmaceutical studies or known safety data [8]. The overdose and high toxicity levels of SV may lead to death in some cases. Appropriate methods can be used to monitor and control the impurity levels in sodium valproate.

The detector used in FID has few advantages which include the ease of operation, reliability, simplicity and versatility. FID detector will not detect signal for common carrier gases such as He, Ar, or N_2 or contaminants in such gases such as O_2 and H_2O. [9] FID connected with temperature programming device is easy to handle and is a best detector used for the routine analysis of organic compounds [10]. The main disadvantage of FID is its destructive nature, so it cannot be connected directly to other GC detectors. FID can be combined and used with other detectors if the carrier gas is split between the FID and the other detector [11].

The literature search reveals that very few analytical methods were reported for the quantification of impurities and the assay of Sodium Valproate. [12]. A specific GC method was developed and validated for the determination of seven related substances in divalproex sodium (DPS) drug substance [13]. A dispersive liquid–liquid micro-extraction coupled with gas chromatography (GC)-flame ionization detector was developed for the determination of valproic acid (VPA) in human plasma. [14] A simple and high throughput method was developed and validated for simultaneous determination of valproic acid and its two toxicant ene-metabolites, 2-enevalproic acid and 4-enevalproic acid in epilepsy patient plasma using liquid chromatography–tandem mass spectrometry [15]. A specific GC–MS method was developed optimized and validated for the determination of five genotoxic impurities, namely Methyl bromide (Me.-Br), Ethyl bromide (Et.-Br), Isopropyl bromide (Ipr.-Br), n-Propyl bromide (n-Pr.-Br) and n-Butyl bromide (n-But.-Br) in Divalproex sodium (DPS) drug substance [16]. Another method was described in a whole blood solid phase extraction of valproic acid, salicylic acid and ibuprofen utilizing butylation for sensitivity and improved chromatography by GC–MS [17]. A rapid, highly efficient, and reliable liquid–liquid micro-extraction (LLME) methods followed by gas chromatography-flame ionization detection for the extraction, preconcentration, and determination of valproate in human plasma and urine samples were developed [18]. A selective ultra-performance liquid chromatographic (UPLC) method for the quantification of valproic acid and its known related impurities using ion pair reagent has been developed [19]. High-performance liquid chromatography with ultra-violet detection (HPLC–UV) and gas chromatography–mass spectrometry (GC–MS) methods were developed and validated for the determination of chlorambucil (CLB) and valproic acid (VPA) in plasma, as a part of experiments on their anticancer activity in chronic lymphocytic leukemia (CLL). [20] A new high-throughput method was developed for analysis of Valproate in human plasma samples by QuEChERS extraction and gas chromatography-tandem mass spectrometry (GC–MS/MS). [21] Though few analytical methods were developed with GC, GC–MS, HPLC, LC–MS, etc., this method indicates the six process-related substances. The main aim of the study was to develop and validate a sensitive and optimized method for the determination of six potential process-related substances in SVS drug substance by GC with FID.

Methods
Instruments and reagents
Sodium Valproate Oral Solution was procured from Apollo pharmacy Tirupati. All the other chemicals of analytical grade were procured from standard suppliers. Gas Chromatography Flame Ionization Detector (GC-FID) GC-HS instrument, with column ID DB-FFAP fused silica capillary column (30 m × 0.53 mm) bonded with a 0.5-μm layer of macrogol 20,000 2-nitroterephthalate stationary phase, helium was used as carrier gas, the volume injected was 1.0 μl, the injector temperature was maintained at 220 °C, the detector temperature was 220 °C, the detector used was Flame Ionization Detector (FID), a split less method was employed, the column flow of Helium was 8.0 ml/min, hydrogen flow was at 35 mL/min, zero air flow was 350 mL/min, makeup flow was at 40 mL/min. Hydrochloric acid (35%, w/v) and HPLC grade heptane were procured from Merck Limited (India). Highly purified water was obtained from Millipore purification system (Merck, India).
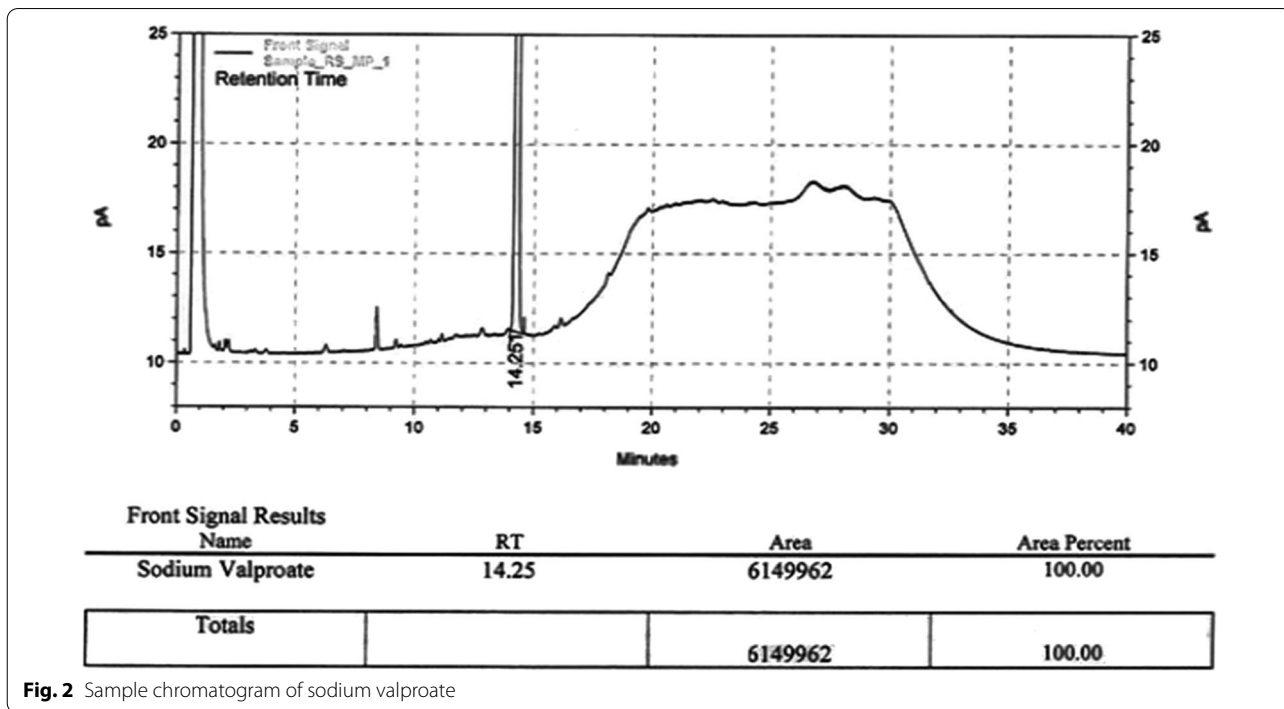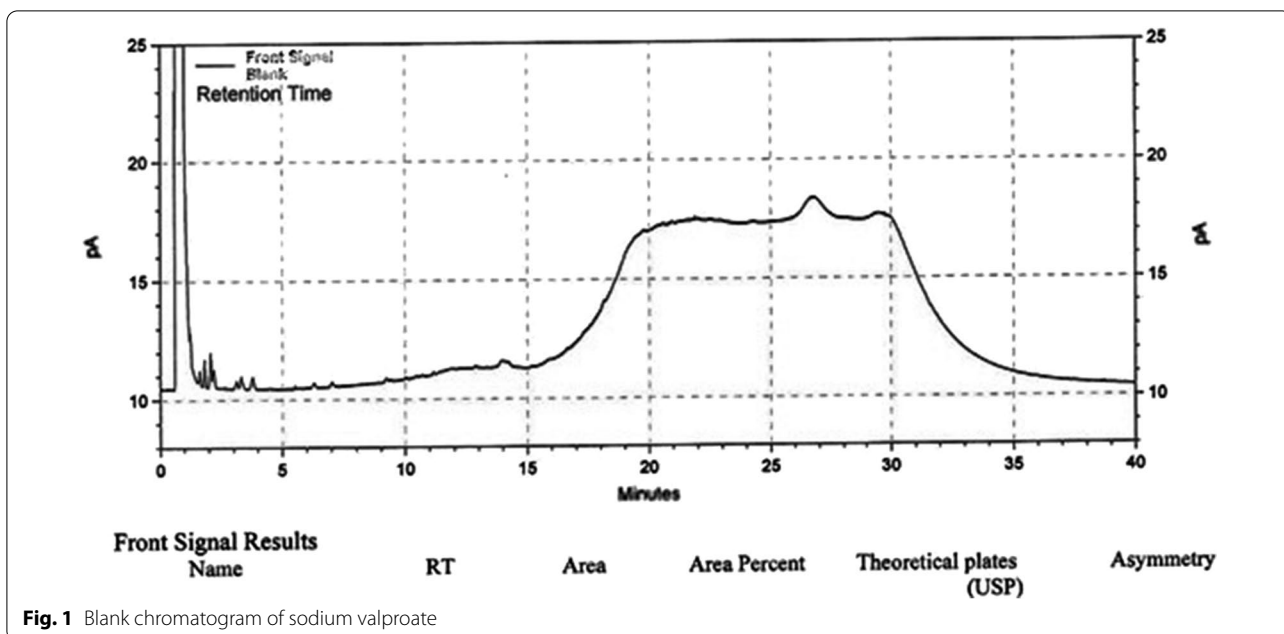
Preparation of solutions
Preparation of blank
The blank solution was prepared by taking purified heptane as a blank solution. The Blank Chromatogram is depicted in Fig. 1.

Preparation of sample solution
The sample solution was prepared by taking a small quantity of the syrup containing 0.50 g of Sodium Valproate and shaken with 10 mL of water, acidified with 2 M sulfuric acid and shaken with three 20 mL quantities of heptane. The combined heptane extracts were washed with 10 mL of water, shaken with anhydrous sodium sulfate, filtered and diluted to 100 mL with heptane. Sample Chromatogram is depicted in Fig. 2.

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Fig. 1 Blank chromatogram of sodium valproate

Fig. 2 Sample chromatogram of sodium valproate

Preparation of placebo solution

The Placebo solution was prepared by shaking 10 mL of water, acidifying with 2 M sulfuric acid and shaking with three 20 mL quantities of heptane. The combined heptane extracts were washed with 10 mL water, shaken with anhydrous sodium sulfate, filtered and diluted to 100 mL with heptane. Placebo Chromatogram is depicted in Fig. 3 (Table 1).

Preparation of standard solution

The standard solution was prepared by diluting 1 volume of solution to 20 volumes with heptane and further

Table 1 Description of analytical method

Instrument conditions	Description
Column ID	DB-FFAP fused silica capillary column (30 m × 0.53 mm) bonded with a0.5-µm layer of macrogol 20,000 2-nitroterephthalate
Carrier Gas	Helium
Injection volume	1.0 µl
Injector Temperature	220 ℃
Detector Temperature	220 ℃
Detector	FID
Split ratio	Split less
Column flow Helium	8.0 ml/min
Hydrogen Flow	35 mL/min
Zero air Flow	350 mL/min
Makeup Flow	40 mL/min

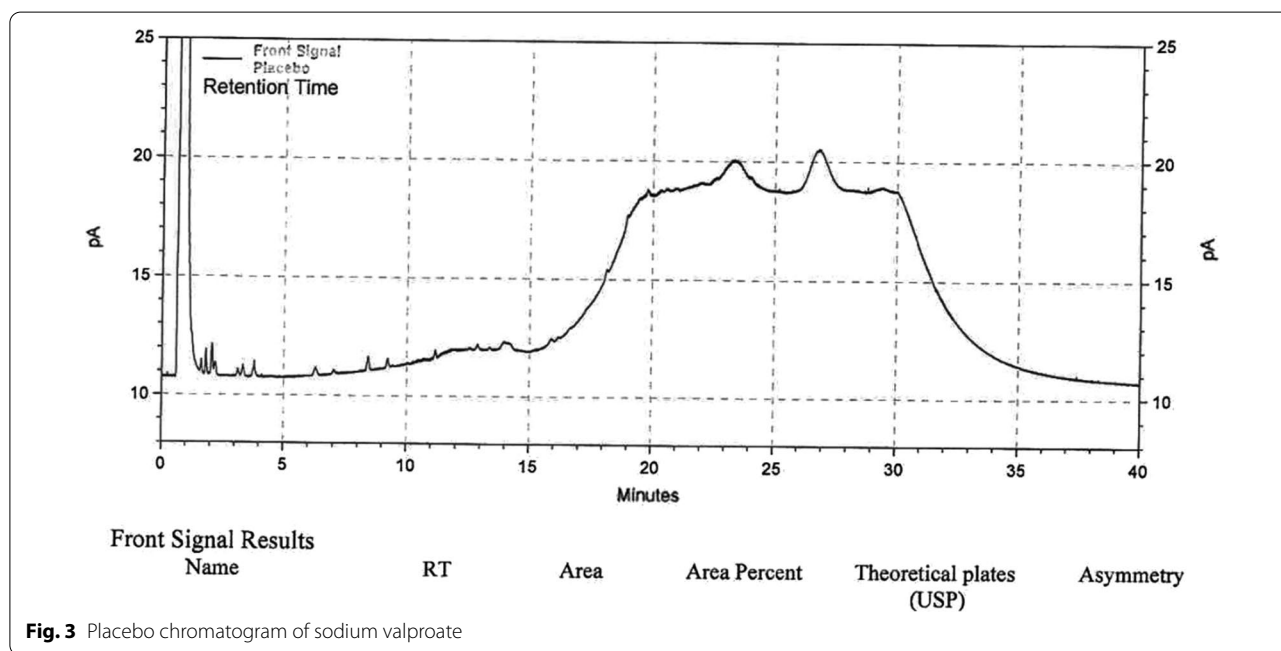| Front Signal Results Name	RT	Area	Area Percent	Theoretical plates (USP)	Asymmetry

Fig. 3 Placebo chromatogram of sodium valproate

diluting 1 volume of the resulting solution to 25 volumes with heptane. Standard Chromatograms RS 1, RS 2, RS 3, RS 4, RS 5 and RS 6, Chromatograms are depicted in Figs. 4, 5, 6, 7, 8 and 9.
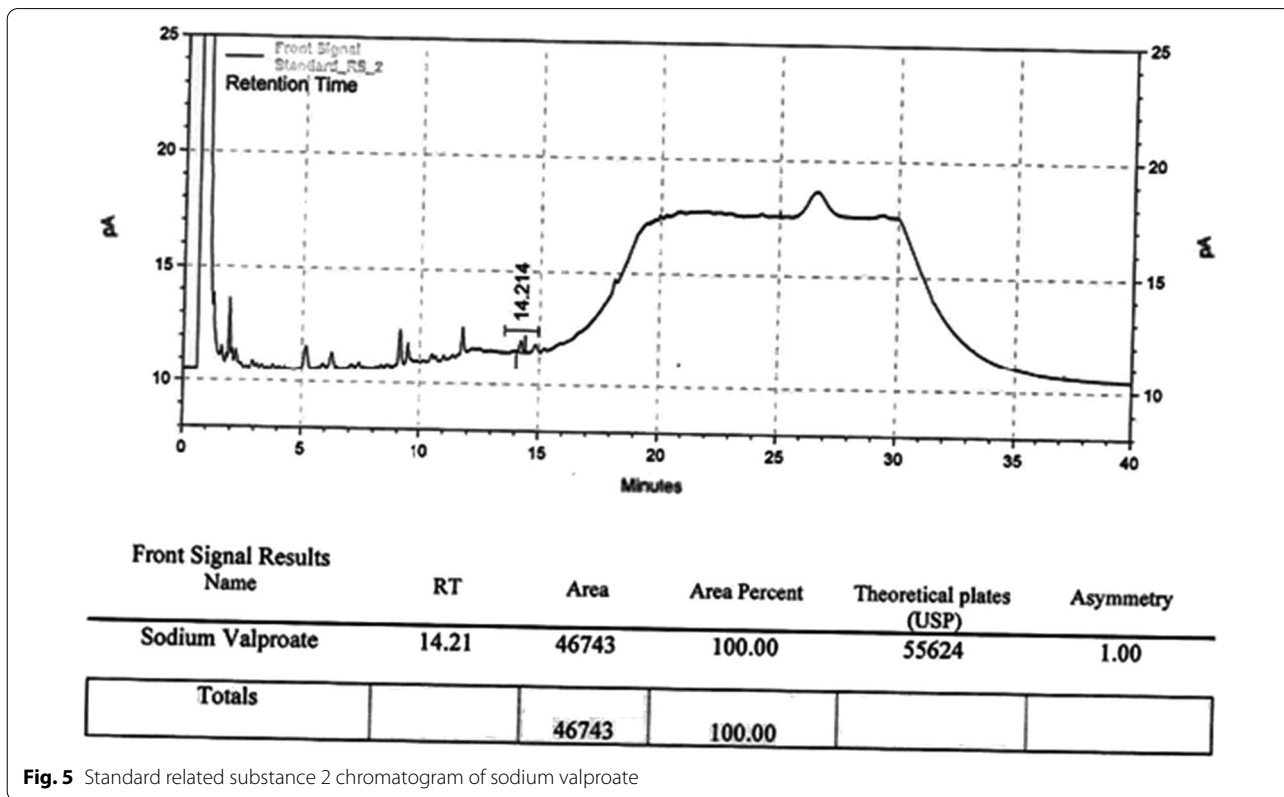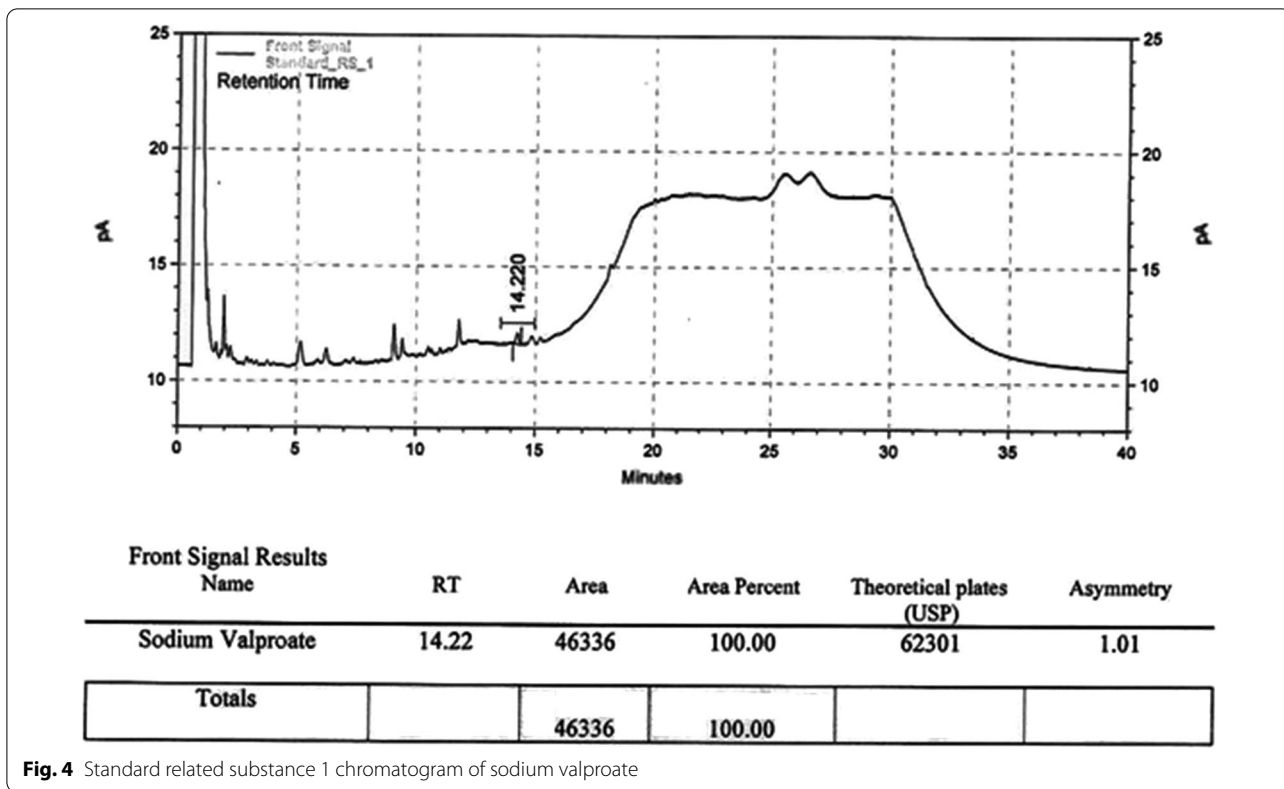
System suitability

The system suitability parameters were determined by the following method. Standard solutions were prepared as per the test method and injected into GC system. The system suitability parameters such as tailing factor, Theoretical plate and relative standard deviation for peak response of six replicate injections of standard solution were calculated and found to be within the limits. The results are summarized in Tables 2 and 3.

Precision

The precision of test method was evaluated by analyzing six samples and injected into GC system. The Related Substance in sample was calculated. The relative standard deviations of six preparations in each content were found to be within the acceptance criteria. The results are summarized in Table 3. Figure 4 SRS 1, Fig. 5 SRS 2, Fig. 6 SRS 3, Fig. 7 SRS 4, Fig. 8 SRS 5 and Fig. 9 SRS 6.
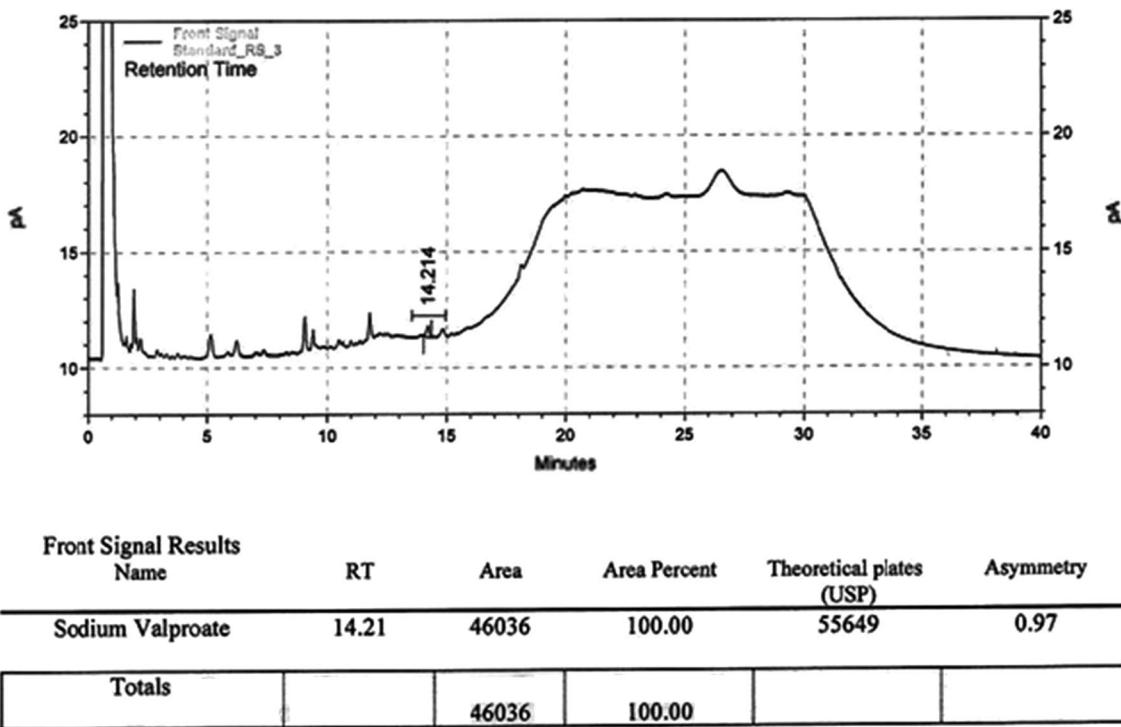
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Front Signal Results

Name	RT	Area	Area Percent	Theoretical plates (USP)	Asymmetry
Sodium Valproate	14.22	46336	100.00	62301	1.01
Totals		46336	100.00		

Fig. 4 Standard related substance 1 chromatogram of sodium valproate

Front Signal Results

Name	RT	Area	Area Percent	Theoretical plates (USP)	Asymmetry
Sodium Valproate	14.21	46743	100.00	55624	1.00
Totals		46743	100.00		

Fig. 5 Standard related substance 2 chromatogram of sodium valproate

Front Signal Results

Name	RT	Area	Area Percent	Theoretical plates (USP)	Asymmetry
Sodium Valproate	14.21	46036	100.00	55649	0.97
Totals		46036	100.00		

Fig. 6 Standard related substance 3 chromatogram of sodium valproate

Front Signal Results

Name	RT	Area	Area Percent	Theoretical plates (USP)	Asymmetry
Sodium Valproate	14.21	46580	100.00	55451	1.03
Totals		46580	100.00		
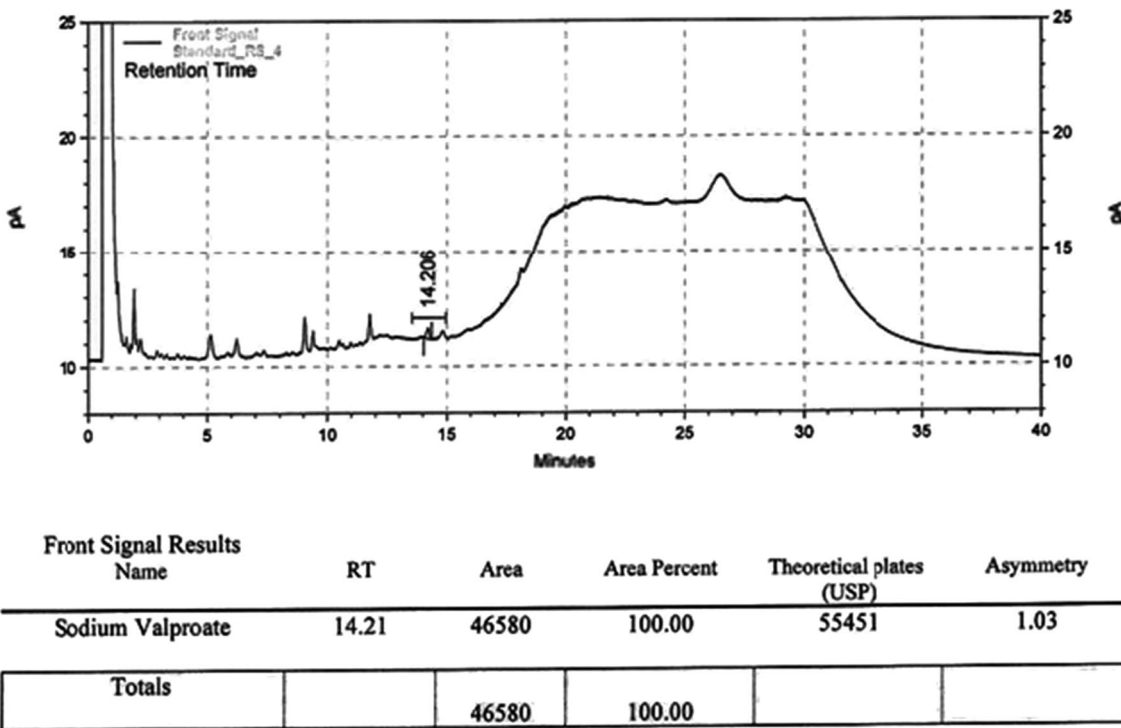
Fig. 7 Standard related substance 4 chromatogram of sodium valproate

Front Signal Results

Name	RT	Area	Area Percent	Theoretical plates (USP)	Asymmetry
Sodium Valproate	14.20	46100	100.00	56103	0.98
Totals		46100	100.00		
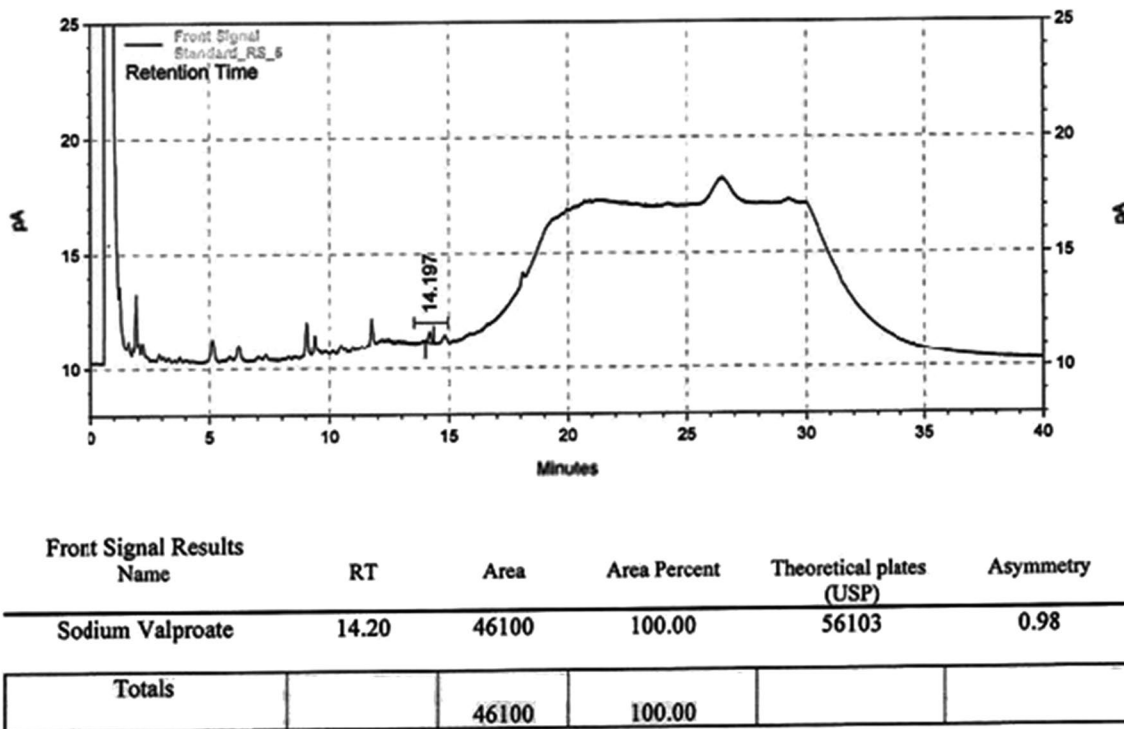
Fig. 8 Standard related substance 5 chromatogram of sodium valproate

Front Signal Results

Name	RT	Area	Area Percent	Theoretical plates (USP)	Asymmetry
Sodium Valproate	14.20	46744	100.00	59663	1.06
Totals		46744	100.00		
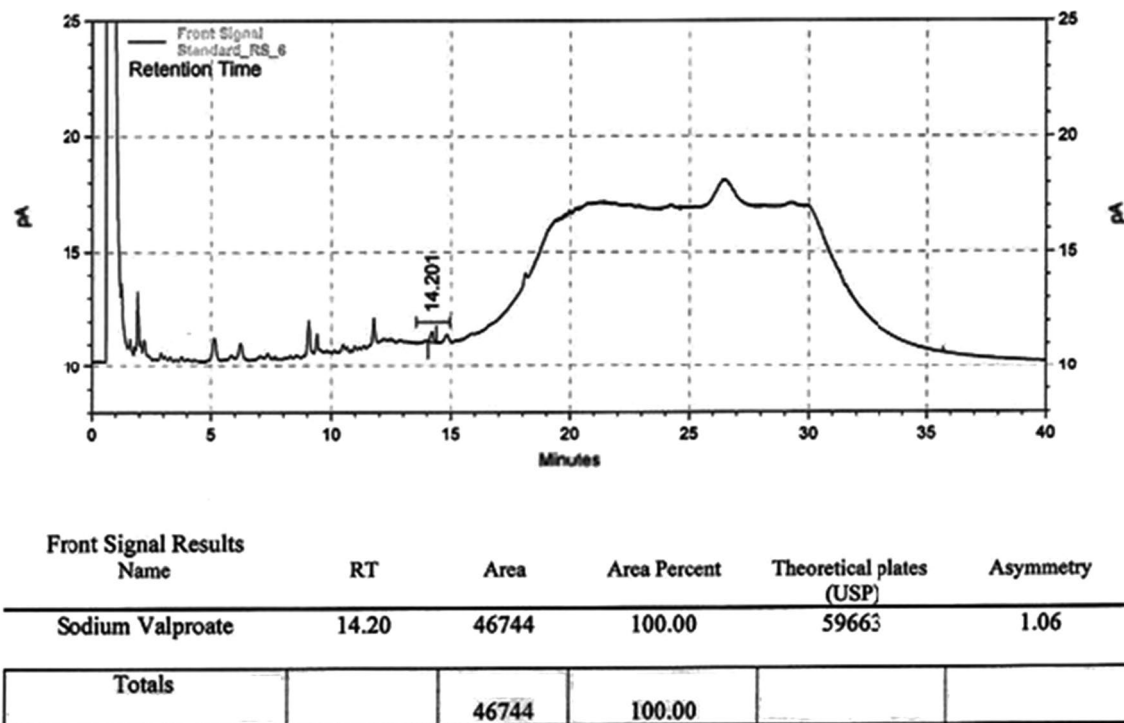
Fig. 9 Standard related substance 6 chromatogram of sodium valproate

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Table 2 System suitability parameters

System suitability parameters	Observed value	Acceptance criteria
The % RSD of six replicate standard injections	1.3	NMT 2.0%
The tailing factor for the Sodium Valproate peak from the first standard injection	1.3	NMT 2.0
The Theoretical plate for the Sodium Valproate peak from the first standard injection	64,907	NLT 2000

NMT Not more than, *NLT* not less than

Table 3 Precision results for sodium valproate

Injection no.	Peak area
01	46,474
02	47,395
03	46,519
04	45,581
05	46,362
06	46,182
Average	46,419
% RSD	1.3

Table 5 Linearity for sodium valproate

% Level	Concentration in ppm	Area response
LOQ %	5.05	23,250
80%	7.08	37,471
100%	10.11	46,714
150%	15.16	70,556
200%	20.22	95,163
250%	25.27	116,419
Correlation coefficient	0.999	
y-intercept	2141	

Table 4 Specificity for sodium valproate

Name of the peak	Retention time in minutes
Sodium valproate	14.25

Results

Specificity

Specificity was established by taking the blank, placebo, standard solution, test solution and individual standard solutions as per method of analysis and injected into GC system. Chromatograms were evaluated for the interference of blank peaks at the retention time of known peaks in all the solutions. The results are summarized in Table 4

Linearity

The linearity of test method was determined by preparing the standard solutions from LOQ % to 250% of the targeted concentration and analyzed as per the method. The correlation coefficient and Y-intercept were calculated and found to be within the acceptance criteria. The results are summarized in Table 5.

Based on the linearity, precision and accuracy data, the test method was taken from LOQ % to 250% of the target concentration. The evaluated concentration for Sodium Valproate is (i.e., 5.05–25.27 ppm). Linearity of Sodium Valproate is depicted in Fig. 10.

Accuracy/recovery

The accuracy of test method of Sodium Valproate was evaluated from the spiked Placebo. Samples were prepared by spiking Sodium Valproate with Placebo at different levels ranging from LOQ %, 100%, 150% and 250% of the target concentration of known standards. The sample solutions were prepared in triplicate at LOQ %, 100%, 150% and 200% spike levels and calculated the recovered Sodium Valproate content from the placebo sample. The results are summarized in Table 6.
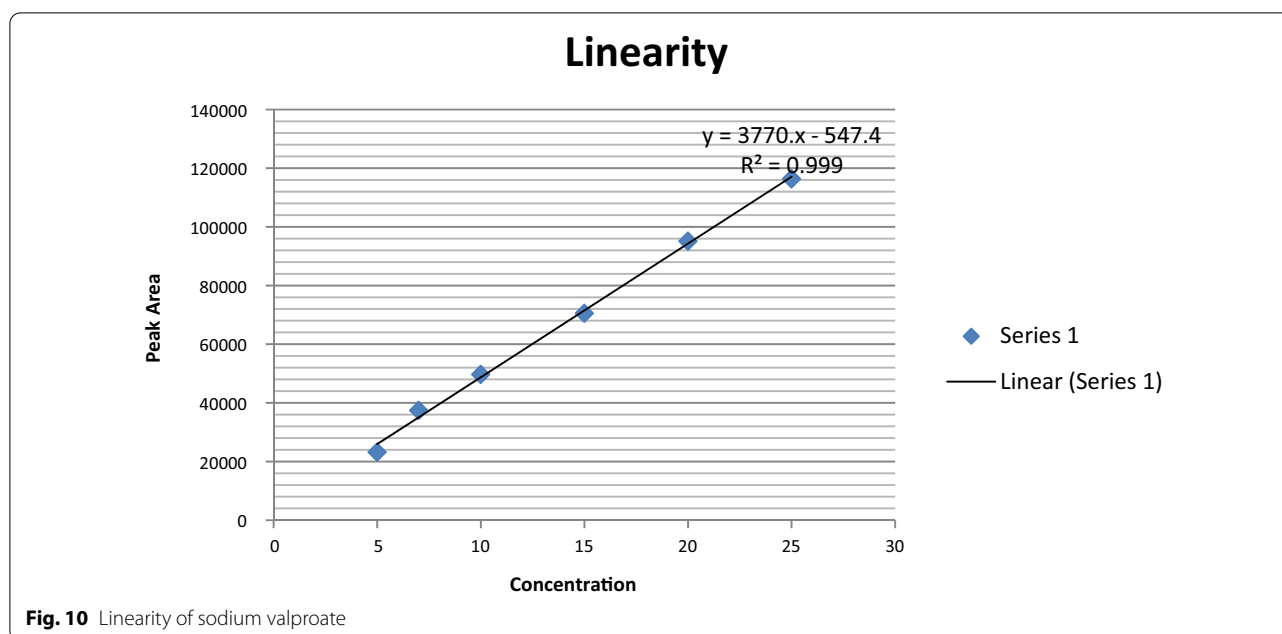
Robustness

Effect of flow variation

To determine the robustness the flow variation of test method, the blank, placebo, standard and test solutions, were prepared as per the test method and injected with the variation in gas flow (i.e., low and high flow variation), the system suitability parameters were evaluated and calculated for the Related Substance in sample as per the above variant test method.

Solution stability

The solution stability of test method was evaluated, by taking the standard and test as mentioned in the test method and kept on bench top. Standard and test solutions were injected at initial stages 17th hours and 34th hours. The % difference of the content of Sodium

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Fig. 10 Linearity of sodium valproate

Valproate solvents in standard and samples were calculated against the initial injection. The results are summarized in Tables 7 and 8.

Discussion

This work aimed to develop an efficient optimized method for the determination of six process related impurities in Sodium Valproate oral solution. Because of volatility, polar and ionic nature of SVS and its impurities, a GC method with FID by following liquid injection method was chosen. Trial experiments were conducted to select the suitable solvent for extraction using heptane and hence heptane was selected as extraction solvent. In addition, heptane is especially suitable for acidic and basic extraction conditions. 2 M H_2SO_4 was used to acidify the SVS sample solution. Finally, for extraction heptane was finalized with 2 M H_2SO_4. By using DB-FFAP fused silica capillary column (30 m × 0.53 mm) bonded with a 0.5-μm layer of macrogol 20,000 2-nitroterephthalate materials used as stationary phase to provide very inert column that can accommodate the demanding analysis of acids dissolved in water. Elution of analytes was investigated using helium as carrier gas, with the constant column pressure of 75 kPa and keeping the column oven temperature initially 80 °C is maintained for 5 min and then increased to 190 °C at a rate of 10 °C/min, followed by holding at 190 °C for 15 min. From this experimental trial, it was observed that all analytes were not

Table 6 Accuracy/recovery studies for sodium valproate

Recovery level	Sample no.	'ppm' added	'ppm' recovered	% Recovery
LOQ %	1	0.5044	0.5057	100.25
	2	0.5044	0.5119	101.48
	3	0.5044	0.5049	100.09
100%	1	1.0089	1.0091	100.02
	2	1.0089	1.0011	99.23
	3	1.0089	1.0020	99.32
150%	1	1.5133	1.4821	97.94
	2	1.5133	1.4877	98.31
	3	1.5133	1.4803	97.82
250%	1	2.5222	2.3883	94.69
	2	2.5222	2.3515	93.23
	3	2.5222	2.3285	92.32
			Average	97.89
			% RSD	2.00

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Table 7 Solution stability for Standard

Solution stability: standard	
Intervals	Area % difference of Sodium Valproate
Initial	–
17 Hours	0.4
34 Hours	0.5

Table 8 Solution stability for Sample

Solution stability: sample			
Intervals	Area % difference of sodium valproate	Any secondary impurities	Total impurities
Initial	–	ND	ND
17 Hours	− 2.8	ND	ND
34 Hours	− 2.6	ND	ND

ND Not detected

clearly separated. Satisfactory separation and better peak shapes were achieved within a reasonable time by flushing helium as the carrier gas with a constant pressure of 90 kPa and initial column oven temperature of 190 ℃ is maintained for 28 min and then increased to 220 ℃ at a rate of 10 ℃/min, followed by holding at 220 ℃ for 1.7 min.

The validation protocols such as accuracy, precision, linearity, specificity, sensitivity and robustness were observed to be within the acceptance limit. The correlation coefficient was within the acceptance criteria in the range of 0.999. The evaluated concentrations for Sodium Valproate were in the ranges of 5.05–25.27 ppm. The average recovery values were in the range of 92.32–101.48%. Solution Stability experiments were performed to evaluate the degradation behavior of SVS. The validation parameters such as % RSD of six replicate standard injections found to be 1.3% was observed to be within the limit. The tailing factor for the Sodium Valproate peak from the first standard injection was observed as 1.05 and was within the acceptance limit. The Theoretical plate for the Sodium Valproate peak from the first standard injection was found to be 64,907 and was observed to be within the limit. Finally, a new novel optimized method was developed and validated, with better peak shape and satisfactory separation was achieved on chromatographic conditions.

Conclusions

In this study, a novel, simple, highly sensitive and optimized cost-effective method was developed and validated consisting of six related substances in sodium valproate oral solution with GC with FID. Although numerous methods have been developed for determination of SV such as GC–MS, HPLC, LC–MS, etc., in this study six related substances were detected by using Gas Chromatography with Flame Ionization Detector. Compared with current methods, the most advantageous aspect of our method is its simple, rapid and highly sensitivity. So the method can be an efficient alternative method for the determination of related impurities. Furthermore, the proposed method is simple and user friendly and its

potential application makes it attractive for the routine analysis for the determination of related substances in sodium Valproate oral solution.

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