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Development and validation of analytical method for determination of valproate and valproic acid in bulk and pharmaceutical dosage form by using RP-HPLC

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ABSTRACT

A New RP-HPLC Method for the Simultaneous Estimation of Valproate and Valproic acid in bulk and its Pure and Pharmaceutical Dosage Form as per ICH Guidelines. The Present work was to develop a simple, fast, accurate, precise, reproducible, Reverse Phase High Performance Liquid Chromatographic Method for simultaneous estimation of Valproate and Valproic acid in pure and combined dosage form. Chromatographic separation was done using Symmetry ODS C18 column having dimension of 4.6×250mm having particle size of 5.0µm, with mobile phase consisting of Acetonitrile: Methanol in the ratio 65:45v/v, flow rate was adjusted to 1ml/min and detection wavelength at 256nm. The retention times of Valproate and Valproic acid was found to be 2.256 and 5.427 mins. The proposed method has been validated for accuracy, precision, linearity; robustness and range were within the acceptance limit according to ICH guidelines. Linearity for Valproate and Valproic acid was found in range of 6µg-14µg and 18µg-42µg and correlation coefficient was found to be 0.999 and 0.999% RSD for intermediate precision was found to be 0.5 and 0.3, for repeatability was 0.4 and 0.1, % mean recovery for Valproate and Valproic acid was found to be 101.326% and 100.501% respectively. The method was found to be robust even by change in the mobile phase ±2% and in more and less flow conditions. The developed method can be successfully employed for the routine analysis of Valproate and Valproic acid in bulk and Pharmaceutical dosage forms.

Keywords: Valproate and Valproic acid, RP-HPLC, Validation, Accuracy.

INTRODUCTION

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the *mobile phase*, which carries it through a structure holding another material called the *stationary phase*. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights. Because of these differences, some components of the mixture stay longer in the stationary phase,

and they move slowly in the chromatography system, while others pass rapidly into mobile phase, and leave the system faster .

Based on this approach three components form the basis of the chromatography technique.

- Stationary phase: This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface a solid support".
- Mobile phase: This phase is always composed of "liquid" or a "gaseous component."
- Separated molecules

The type of interaction between stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other. Chromatography methods based on partition are very effective on separation, and identification of small molecules as amino acids, carbohydrates, and fatty acids. However, affinity chromatographies (ie. ion-exchange chromatography) are more effective in the separation of macromolecules as nucleic acids, and proteins. Paper chromatography is used in the separation of proteins, and in studies related to protein synthesis; gas-liquid chromatography is utilized in the separation of alcohol, esther, lipid, and amino groups, and observation of enzymatic interactions, while molecular-sieve chromatography is employed especially for the determination of molecular weights of proteins. Agarose-gel chromatography is used for the purification of RNA, DNA particles, and viruses.

Stationary phase in chromatography, is a solid phase or a liquid phase coated on the surface of a solid phase. Mobile phase flowing over the stationary phase is a gaseous or liquid phase. If mobile phase is liquid it is termed as liquid chromatography (LC), and if it is gas then it is called gas chromatography (GC). Gas chromatography is applied for gases, and mixtures of volatile liquids, and solid material. Liquid chromatography is used especially for thermal unstable, and non-volatile samples .

The purpose of applying chromatography which is used as a method of quantitative analysis apart from its separation, is to achive a satisfactory separation within a suitable timeinterval. Various chromatography methods have been developed to that end. Some of them include column chromatography, thin-layer chromatography (TLC), paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography, high-pressure liquid chromatography, and affinity chromatography.

- Column chromatography
- Ion-exchange chromatography
- Gel-permeation (molecular sieve) chromatography
- Affinity chromatography
- Paper chromatography
- Thin-layer chromatography
- Gas chromatography
- Dye-ligand chromatography
- Hydrophobic interaction chromatography
- Pseudoaffinity chromatography
- High-pressure liquid chromatography (HPLC)

MATERIALS AND METHODS

Valproate from Sura labs, Valproic acid from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck, Triethylamine from Merck

HPLC method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Valproate and Valproic acid working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the Valproate and 0.3ml of the Valproic acid stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Optimized chromatographic conditions:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 35ºC
Column : Symmetry ODS C18 (4.6×250mm, 5µm) particle size
Mobile phase : Acetonitrile: Methanol (65:45v/v)
Flow rate : 1ml/min
Wavelength : 213 nm
Injection volume : 20 µl
Run time : 10 min

Validation

Preparation of buffer and mobile phase

Preparation of mobile phase

Preparation of mobile phase

Accurately measured 650 ml (65%) of Acetonitrile, 450 ml of Methanol (450%) were mixed and degassed in digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Acetonitrile: Methanol (65:45v/v)
Column : Symmetry ODS C18 (4.6×250mm, 5µm) particle size
Flow rate : 1 ml/min
Wavelength : 213 nm
Column temp : 35ºC
Injection Volume : 20 µl
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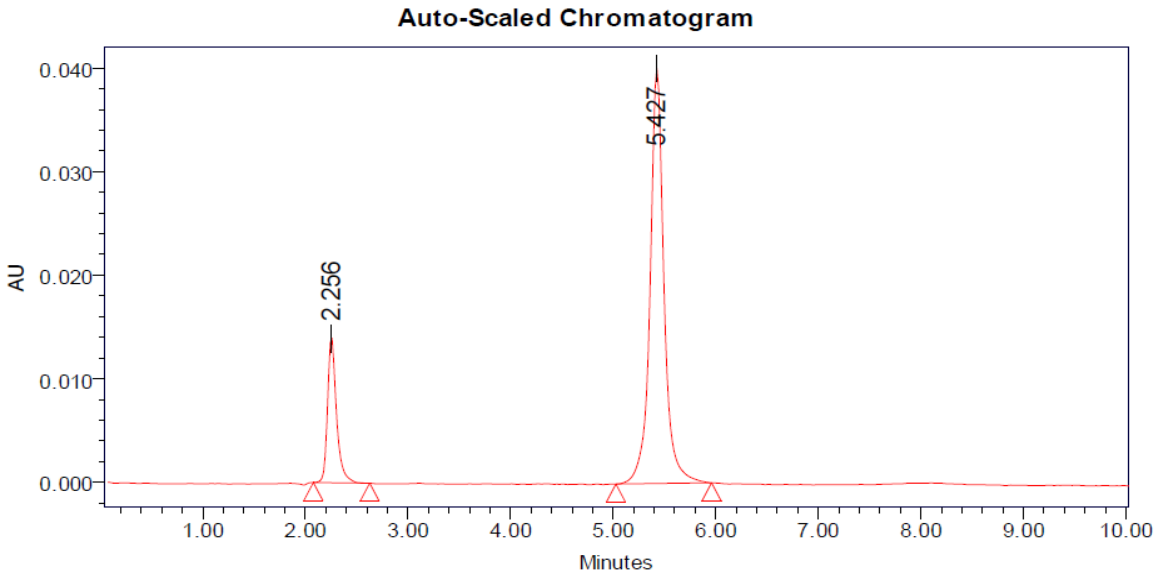


Fig 1: Optimized Chromatogram

Table1: Peak Results for Optimized Chromatogram

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Valproate	2.256	86895	14256		1.32	5635
2	Valproic acid	5.427	385689	41254	16.27	1.03	9452

From the above chromatogram it was observed that the Valproate and Valproic acid peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

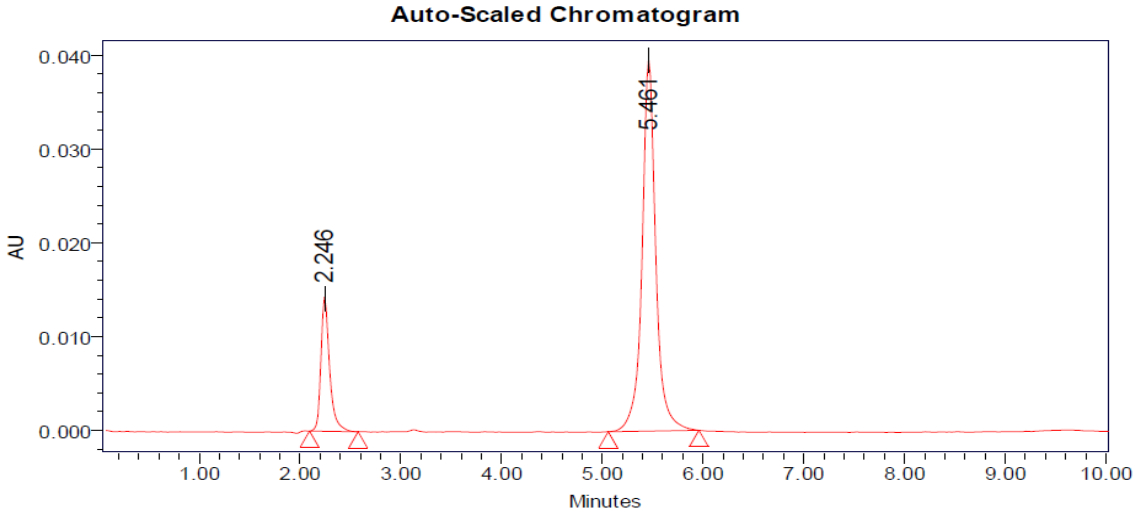


Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Valproate	2.246	87584	14254		1.32	5648
2	Valproic acid	5.461	398565	41365	16.42	1.02	9416

- *Resolution between two drugs must be not less than 2.*
- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

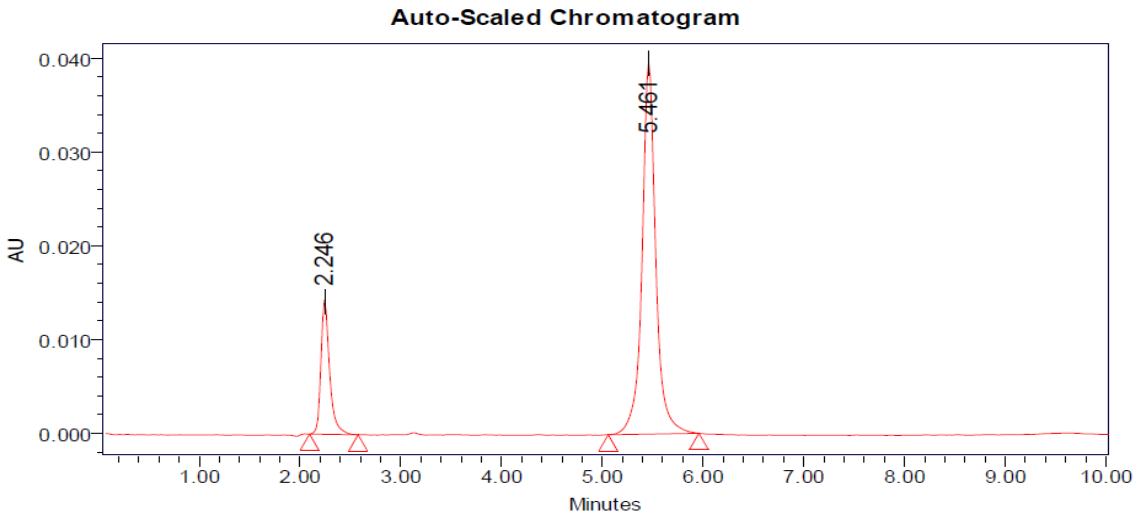


Fig 3: Optimized Chromatogram (Sample)

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- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

Assay (Standard)

Table 4: Peak results for assay standard

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Valproate	2.256	86598	14352		1.32	5682
2	Valproic acid	5.427	385698	41254	16.27	1.03	9458
3	Valproate	2.249	86574	14289		1.38	5642
4	Valproic acid	5.430	385452	41321	16.13	1.05	9487
5	Valproate	2.248	86548	14326		1.40	5654
6	Valproic acid	5.443	385471	41259	16.19	1.05	9487

Assay (Sample)

Table 5: Peak results for Assay sample

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Valproate	2.247	86985	14352		1.36	5688	1
2	Valproic acid	5.452	386587	41365	16.42	1.38	9547	1
3	Valproate	2.246	86852	14269		1.32	5628	2
4	Valproic acid	5.461	385784	41298	16.42	1.04	9587	2
5	Valproate	2.243	86542	14325		1.03	5642	3
6	Valproic acid	5.466	385983	41354	16.48	1.02	9658	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Valproate and Valproic acid in pharmaceutical dosage form was found to be 99.9 %.

Linearity

Chromatographic data for linearity study:

Valproate

Concentration µg/ml	Average Peak Area
6	51476
8	67598
10	84897
12	101114
14	119554

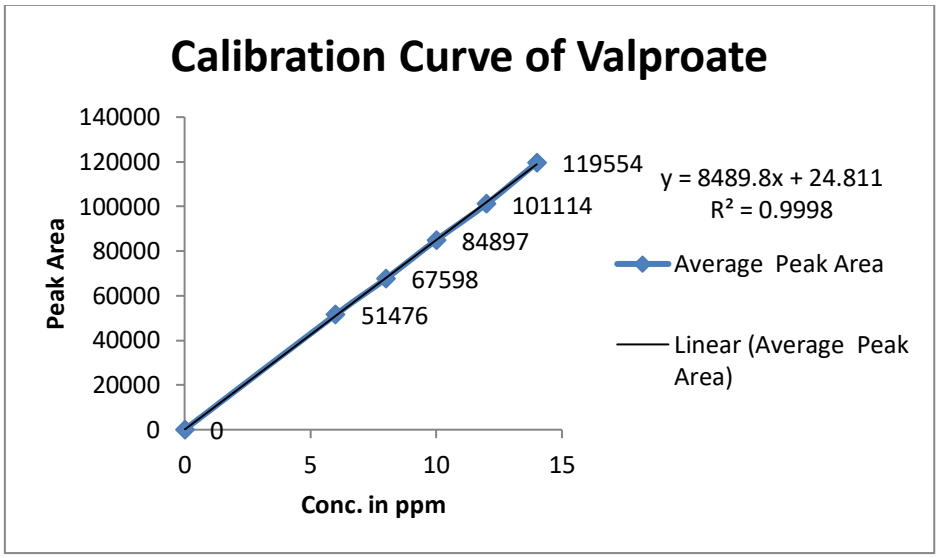


Fig 4: Calibration graph for Valproate

Valproic acid

Concentration µg/ml	Average Peak Area
18	2286598
24	3086587
30	3867579
36	4758517
42	5604874

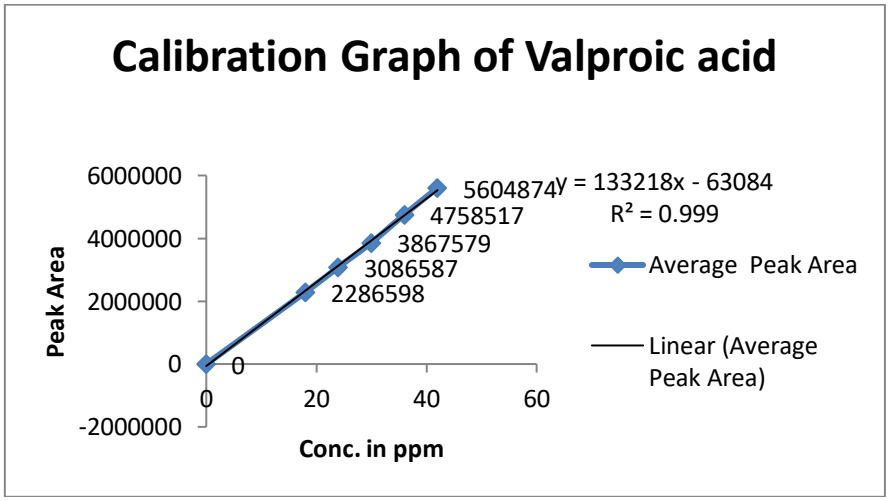


Fig 5: Calibration Graph for Valproic acid

Repeatability

Table 6: Results of Repeatability for Valproate

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Valproate	2.269	86985	14565	5648	1.4
2	Valproate	2.255	86879	14658	5654	1.4
3	Valproate	2.252	86578	14652	5623	1.4
4	Valproate	2.267	86598	14525	5713	1.4
5	Valproate	2.260	86578	14632	5698	1.3
Mean			86723.6			1.4
Std. Dev			194.0703			
% RSD			0.22378			
			86723.6			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 7: Results of method precision for Valproic acid

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Valproic acid	5.274	386598	41236	9475.5	1.1	15.4
2	Valproic acid	5.266	385474	41365	9420.4	1.1	15.6
3	Valproic acid	5.265	386895	41256	9489.4	1.1	15.3
4	Valproic acid	5.278	384574	41329	9383.0	1.1	15.3
5	Valproic acid	5.305	386548	41652	9441.5	1.1	15.3
Mean		5.319	385874	41236	9474.1	1.1	15.3
Std. Dev			385993.8				
% RSD			870.0268				
			0.225399				

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision

Table 8: Results of Intermediate precision for Valproate

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Valproate	2.248	87521	14356	5632.5	1.4
2	Valproate	2.245	86598	14269	5589.4	1.4
3	Valproate	2.242	86987	41352	5658.2	1.4
4	Valproate	2.239	87213	41269	5652.1	1.3
5	Valproate	2.243	86548	41254	5703.3	1.4
6	Valproate	2.246	87548	41365	5648.4	1.3
Mean			87069.17			
Std. Dev			436.918			
% RSD			0.501806			

- *%RSD of Six different sample solutions should not more than 2.*

Table 9: Results of Intermediate precision for Valproic acid

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Valproic acid	5.284	386213	41565	9458	1.1	15.8
2	Valproic acid	5.293	385698	41659	9521	1.1	15.5
3	Valproic acid	5.306	385789	41378	9487	1.0	15.5
4	Valproic acid	5.319	385897	41659	9456	1.1	15.8
5	Valproic acid	5.346	385489	41665	9562	1.1	15.6
6	Valproic acid	5.352	382764	41584	9547	1.1	15.9

Mean			385308.3				
Std. Dev			1269.181				
% RSD			0.329394				

- *%RSD of Six different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Table 10: Results of Intermediate precision Day 2 for Valproate

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Valproate	2.255	87584	14365	5698	1.4
2	Valproate	2.260	86598	14452	5682	1.4
3	Valproate	2.242	86584	14369	5647	1.4
4	Valproate	2.245	86758	14524	5682	1.3
5	Valproate	2.260	86462	14365	5624	1.4
6	Valproate	2.255	86523	14396	5687	1.3
Mean			86751.5			
Std. Dev			419.6998			
% RSD			0.483795			

- *%RSD of Six different sample solutions should not more than 2*

Table 11: Results of Intermediate precision for Valproic acid

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Valproic acid	5.266	396585	41365	9568	1.0	15.5
2	Valproic acid	5.265	398658	41452	9487	1.1	15.8
3	Valproic acid	5.306	399897	41268	9587	1.1	15.6
4	Valproic acid	5.293	395785	41365	9528	1.1	15.9
5	Valproic acid	5.265	396879	41658	9487	1.2	15.1
6	Valproic acid	5.266	396887	41874	9562	1.0	15.3
Mean			397448.5				
Std. Dev			1523.845				
% RSD			0.383407				

- *%RSD of Six different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Accuracy

Table 12: The accuracy results for Valproate

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	42603.33	5	5.015	100.30	101.326%
100%	86533	10	10.190	101.90	
150%	129631.667	15	15.267	101.78	

Table 13: The accuracy results for Valproic acid

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	264159	15	15.094	100.626	100.501%
100%	465304.3	30	30.194	100.646	
150%	663923	45	45.104	100.231	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Valproate

Table 13: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	86895	2.256	5635	1.32
Less Flow rate of 0.9 mL/min	89897	2.505	5852	1.27
More Flow rate of 1.1 mL/min	83526	2.046	5265	1.20
Less organic phase	89865	2.505	5125	1.20
More organic phase	80898	2.046	5253	1.27

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Valproic acid

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	385689	5.427	9452	1.01
Less Flow rate of 0.9 mL/min	398985	5.599	9456	1.03
More Flow rate of 1.1 mL/min	326538	4.576	9658	0.98
Less organic phase	396869	5.599	9454	1.02
More organic phase	341254	4.576	9584	0.99

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Valproate and Valproic acid in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Valproate and Valproic acid was freely soluble in ethanol, methanol and sparingly soluble in water.

Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Valproate and Valproic acid in bulk drug and in Pharmaceutical dosage forms.

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