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Research Article

Analytical Method Development and Validation for the Simultaneous Estimation of Valproate and Lomitrigine by RP-HPLC Method

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ABSTRACT

A simple precise and accurate reverse phase high performance liquid chromatographic technique was developed and validated for the simultaneous estimation of Lamotrigine and Valproate in a combined dosage form Symmetry Agilent C18 (4.6*150mm) 5µm column in isocratic mode was used with the mobile phase comprising of Water and Methanol in the ratio of 40:60v/v, the flow rate was set at 1ml/min. The analyte was monitored with dual wavelength UV detector at 255nm. The retention time of Lamotrigine and Valproate was found to be 2.551 and 4.879 min respectively. The linearity range was found to lie from 10µg/ml to 50µg/ml of Lamotrigine, 20µg/ml to 100µg/ml of Valproate. Percentage recoveries were obtained in the range of for Lamotrigine 98.8% and for Valproate 98.5%. The proposed method is precise, accurate, selective, reproducible and rapid for the simultaneous estimation of Lamotrigine and Valproate in combined form.

Keywords: Lamotrigine, Valproate, UV, HPLC

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CONTENTS

1. Introduction

Lamotrigine is an antiepileptic drug belonging in the phenyltriazine class. It is used in the treatment of both epilepsy and as a mood stabilizer in bipolar disorder. Lamotrigine is the first medication since lithium granted Food and Drug Administration (FDA) approval for the maintenance treatment of bipolar type I. Lamotrigine has relatively few side-effects and does not require laboratory monitoring. While it is indicated for epilepsy and bipolar disorders, there is evidence that lamotrigine could have some clinical efficacy in certain neuropathic pain states. Valproate (VPA) and its valproic acid, sodium valproate, and valproate semisodium forms are medications primarily

used to treat epilepsy and bipolar disorder and prevent migraine headaches.[2] They are useful for the prevention of seizures in those with absence seizures, partial seizures, and generalized seizures.

2. Methodology

Instrumentation: The instrument used was HPLC Alliance Waters model No. 2695 separation module.2487 UV detector, Software- EM power. The stationary phase used was Agilent C18 column (4.6×150mm)5µ. Semi micro balance–Model number Sartorius ME235P, Sonicator (Enertech)-SE60US, PH meter Lab India, UV/VIS spectrophotometer UV3000 Lab India Software-UVWin5

Materials and reagents

Lamotrigine and Valproate were gift samples provided by Dr. Reddy's Laboratories Hyderabad, Potassium dihydrogen orthophosphate, Methanol, Acetontrile, Water were supplied by Merck.

Method development

Five trials were made by changing the mobile phase ratios and solvents Water: Methanol(40:60%v/v) Agilent C18(4.6*150mm) 5µm Water: Methanol (40:60%v/v) Thermosil C18(4.6*150mm) 5µm Phosphate buffer (0.05m) pH5.0:Methanol (50:50%v/v)Phosphate buffer (0.05M) pH4.6 :MeOH Phosphate buffer (0.05M) pH 4.6:CAN (30:70%v/v). Finally, the mobile phase optimized mobile phase ratio was Water and Methanol in the ratio of 40:60v/v Symmetry Agilent C18(4.6*150mm)5µm column.

Chromatographic conditions

The chromatographic conditions were successfully developed for the estimation of Lamotrigine and Valproate in a combined dosage form Symmetry Agilent C18 (4.6*150mm) 5µm column in isocratic mode was used with the mobile phase comprising of Water and Methanol in the ratio of 40:60v/v, the flow rate was set at 1ml/min. The analyte was monitored with dual wavelength UV detector at 255nm.

3. Results and Discussion

Table 1 Accuracy results of Valproate

% Concentration (at specification Level)	Area	Amount added(mg)	Amount found (mg)	% Reco	Mean Recovery
50%	2332744	5	5.10	101.8%	100.5%
100%	3132697	10	9.99	99.9%	
150%	3918997	15	14.9	99.1%	

Table 2 Accuracy results of Lamotrigine

% Concentration (at specification level)	Area	Amount Added(mg)	Amount Found(mg)	%Recovery	Mean Recovery
50%	353867	5	5.0	101.3%	
100%	4735088	10	9.94	99.4%	100.0%
150%	5911798	15	14.8	99.2%	

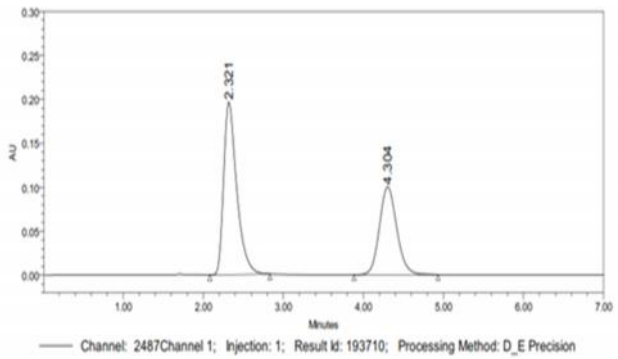

Figure 1 Chromatogram of Standard Inj

Table 3 Repeatability results of Lamotrigine

Name: lamotrigine

	Name	RT	Area	Height (µV)
1	lamotrigine	4.304	1501417	100275
2	lamotrigine	4.300	1486940	100079
3	lamotrigine	4.308	1490656	98257
4	lamotrigine	4.310	1487329	98165
5	lamotrigine	4.314	1490384	98153
Mean			1491345	
Std. Dev.			5881.4	
% RSD			0.39	

Table 4 Repeatability results of Valproate

Name: valproate

	Name	RT	Area	Height (µV)
1	valproate	2.321	2235319	196999
2	valproate	2.317	2240678	198254
3	valproate	2.323	2249490	195128
4	valproate	2.322	2245822	196164
5	valproate	2.324	2251694	195887
Mean			2244601	
Std. Dev.			6656.8	
% RSD			0.30	

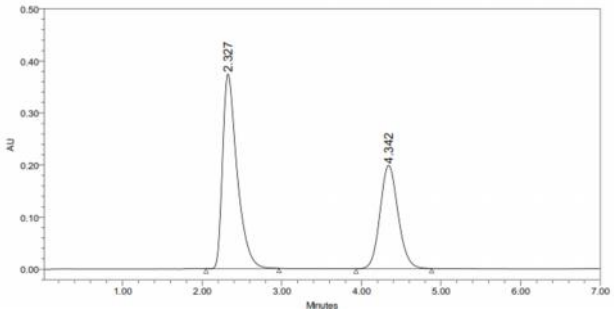

Figure 2 Chromatogram of Standard Injection

Table 5 Ruggedness results of Lamotrigine

Name: lamotrigine

	Name	RT	Area	Height (µV)
1	lamotrigine	2.328	2194758	189693
2	lamotrigine	2.326	2195700	190025
3	lamotrigine	2.327	2196191	189862
4	lamotrigine	2.326	2195326	190700
5	lamotrigine	2.331	2200951	189426
Mean			2196585	
Std. Dev.			2496.0	
% RSD			0.11	

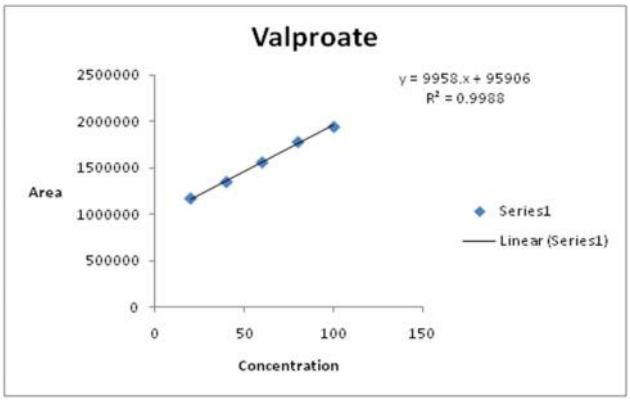

Figure 3 Calibration curve of Valproate

Table 6 Ruggedness results of Valproate

Name: valproate

	Name	RT	Area	Height (µV)
1	valproate	4.335	1456296	95623
2	valproate	4.336	1457422	95150
3	valproate	4.334	1456513	95165
4	valproate	4.337	1454579	95298
5	valproate	4.340	1451483	95251
Mean			1455259	
Std. Dev.			2347.6	
% RSD			0.16	

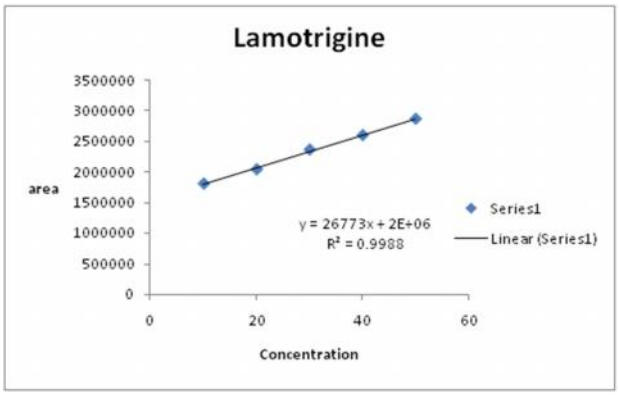

Figure 4 Linearity Results (for Valproate): (for Lamotrigine)

Table 7 Linearity results of Valproate

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	892464
2	II	40 ppm	1904884
3	III	60 ppm	2906620
4	IV	80 ppm	3800672
5	V	100 ppm	4738193
	Correlation Coefficient		0.99932

Table 8 Linearity results of Lamotrigine

S.No	Linearity Level	Concentration	Area
1	I	10 ppm	907953
2	II	20 ppm	1730043
3	III	30 ppm	2553693

4	IV	40 ppm	3283876
5	V	50 ppm	4144232
	Correlation Coefficient		0.99916

Table 9 System suitability results for Valproate (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	1748.5	1.22
2	1.0	1548.2	1.2
3	1.2	1948.0	1.2

Table 10 System suitability results for Lamotrigine (Flow rate)

S.No	Flow Rate (ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	883.3	1.56
2	1.0	1234.0	1.1
3	1.2	969.2	1.6

4. Conclusion

A new method was established for simultaneous estimation of Lamotrigine and Valproate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Lamotrigine and Valproate by using Xterra C185µm (4.6*250mm) column, flow rate was1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: CAN (55:45%v/v) (pH was adjusted with orthophosphoricacid), detection wavelength was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The retention times were found to be 2.399mins and 3.907mins. The %purity of Lamotrigine and Valproate was found to be 100.7% and 101.4% respectively. The system suitability parameters for Lamotrigine and Valproate such as theoretical plates and tailing factor were found to be 1.3, 5117.5 and 1.4, 3877.3 the resolution was found to be 8.0. The analytical method was validated according to ICH guidelines (ICH, Q2(R1)). The linearity study for Lamotrigine and Valproate was found in concentration range of 1µg-5µg and 100µg-500µg, correlation coefficient (r2) was found to be 0.999and 0.999,%mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, %RSD for intermediate precision was0.5and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Lamotrigine and Valproatein API and Pharmaceutical dosage form.

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