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Research Article

A new RP-HPLC method development and validation for simultaneous estimation of lamotrigine and zonisamide in pharmaceutical tablet dosage formulations

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ABSTRACT

A simple, specific, rapid, accurate and sensitive RP-HPLC method was developed and validated for the simultaneous estimation of Lamotrigine and Zonisamide in bulk drug and pharmaceutical tablet dosage form. The separation was achieved by using C_{18} column (250mm×4.6mm, 5µm particle size), Inertsil ODS using a mobile phase of mixed phosphate buffer (pH 3) adjusted with ortho phosphoric acid : acetonitrile (50:50 v/v), with a flow rate of 1.0 ml/min at an ambient temperature of 30˚C and at 232 nm as detection wave length. Rt values (Rt = retention time) of Lamotrigine and Zonisamide were found to be 2.666 min and 3.958 min respectively. Lamotrigine and Zonisamide follow Linearity in the concentration range of 5-30µg/ml with a correlation coefficient of 0.9998 and 0.9997 for Lamotrigine and Zonisamide respectively. Percentage purity of Lamotrigine and Zonisamide were found to be 99.45% and 99.66 %w/v respectively. The proposed method has been validated for specificity, linearity, range, accuracy, precision and robustness were within the acceptance limit according to ICH Q2(B) guidelines and the developed method can be employed for determination Lamotrigine and Zonisamide in the bulk and combined pharmaceutical dosage form(tablets).

Keywords: Lamotrigine; Zonisamide; RP-HPLC; Validation.

INTRODUCTION

Lamotrigine is chemically 6-(2,3-dicholro phenyl)-1,2,4-triazine-3,5-diamine its chemical formula is $C_9H_7Cl_2N_5$, Molecular weight is 256.09, pKa value is 5.7. It is white to pale cream coloured powder, soluble in water and slightly soluble in 0.1MHCl at 25˚C. Its melting point is 216-218˚C. It belongs to category anticonvulsants, calcium channel blockers, and anti-depressants. Literature survey revealed that few spectrophotometric methods (Navdeep Saini *et al*.,2011, Fadhil M *et al*., 2013) HPLC methods (Ankit Patel *et al*., 2012, Geetharam Yanamadala *et al*.,2014, Sanjay Bais *et al*., 2013, Selvadurai Muralidharan *et al*.,2012, Mathrusri Annapurna M *et al.,* 2010) has been reported for the estimation of Lamotrigine. The structure of Lamotrigine is shown in Figure 1.

Zonisamide IUPAC is 1,2-benzoxazol-3-yl-methyanesulfonamide, its chemical formula is $C_8H_8N_2O_3S$, Molecular weight is, 212.226, pKa value is 10.2. It is available in white to pale yellow coloured crystalline powder. It is freely soluble in acetone, sparingly soluble in methanol, slightly soluble in ethanol, very slightly soluble in water, ether and chloroform. Melting point is 164-168˚C, belongs to category antioxidants and anti-convulsants. Literature survey revealed that few spectrophotometric methods (Tanvi A *et al*., 2013) HPLC methods (Ananda Reddy *et al*., 2011) has been reported for the estimation of Zonisamide. Only one method has been reported earlier for simultaneous estimation of Lamotrigine and Zonisamide (Krishna Chaitanya Prasad M *et al.,*2013). The structure of Zonisamide is shown in Fig.2.

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MATERIALS AND METHODS

Instrumental and analytical conditions

Reagents and Chemicals

Pure samples of Lamotrigine and Zonisamide were provided as gift samples by Glaxo Smith Kline's pharmaceutical Pvt. Ltd and Elisa pharmaceutical India Ltd. respectively. The chemicals used are of HPLC grade. Phosphate buffer and acetonitrile were procured from Thermo Fischer Scientific India Pvt. Ltd. Milli Q Water was used in the buffer preparation.

Equipment

Waters HPLC system (e2695 gradient system) with Empower-2 software, 2489 UV/Vis detector was used for the present analysis.

Chromatographic conditions

Inertsil ODS C18 column (250mm×4.6mm, 5µm particle size) was used for separation. The mobile phase consists of an aqueous solution of mixed phosphate buffer (pH 3 adjusted with 50:50%v/v ratio of ortho-phosphoric acid and acetonitrile. The flow was adjusted to 1ml/min. Ambient temperature was maintained throughout the analysis. The UV detection was achieved at 232 nm which is the isobestic point shown in Fig.3. The injection volume was 20µL.

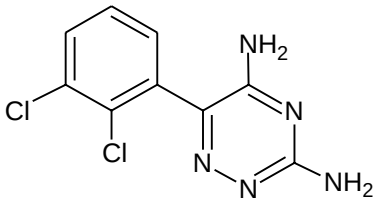


Figure 1: Chemical structure of Lamotrigine

Preparation of analytical solutions

Preparation of missed phosphate buffer solution

3.25gms of 0.02M potassium dihydrogen phosphate and 0.69gms of 0.03M dipotassium hydrogen phosphate dissolved in 1000ml of water adjusted to pH 3 using ortho phosphoric acid, sonicated and filtered.

Preparation of mobile phase

Mixture of phosphate buffer {pH 3 adjusted with ortho phosphoric acid (50%)} and acetonitrile (50%) was degassed in ultrasonic water bath for 5min. Filtered through 0.22µ filter under vacuum filtration.

Diluent preparation: Mobile phase was used as the diluent.

Preparation of the individual Lamotrigine standard preparation

10mg of drug is taken dissolved in 50ml of diluent. 1ml was pippeted out and made up to 10ml to get a 20ppm concentration solution.

Preparation of individual Zonisamide standard preparation

10mg of drug was taken and dissolved in few ml of diluents, sonicated and the volume was made up to 50ml. 1ml was pippeted out and made up to 10ml to get a 20ppm concentration solution.

Preparation of sample solution

26mg of both drugs were taken and a few ml of diluent was added, sonicated and made up to the volume 50ml. consider 1ml of that stock solution and make up to 10ml and make up the volume with diluent. The concentration is 20ppm.

Method Development and Validation of HPLC

The method developed was validated as per ICH guidelines with respect to following parameters such as specificity, linearity, accuracy, precision, robustness and system suitability.

Specificity

The specificity of the method developed was carried out to determine whether there is any interference of impurities in retention time of analytical peak. Forced degradation studies were carried out by using acidic (0.1M HCl), basic (0.1M NaOH) conditions, and in presence of heat and UV light.

Linearity

The linearity of the method was established by spiking a series of sample mixtures of Lamotrigine and Zonisamide solutions of six different concentration levels 5-30µg/ml are injected in to the HPLC system. The calibration curve was plotted for the standard solutions by plotting their response ratios (ratios of the peak area of the analytes) against their respective concentrations linear regression was applied and slope-a, intercept-b, correlation coefficient-R^2 and standard error were determined shown in (Figure 4 and 5).

Accuracy

The accuracy was assessed in terms of percentage recovery, the accuracy study was performed for 80%, 100% and 120% for Lamotrigine and Zonisamide. Standard and sample solutions were injected in to HPLC system in triplicate and percentage recoveries of Lamotrigine and Zonisamide were calculated. The area of each level was used for calculation of %recovery.

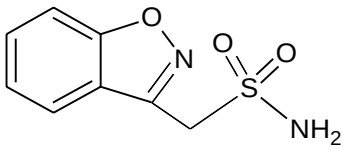


Figure 2: Chemical structure of Zonisamide

Precision

Precision express the closeness of agreement between the series of measurements obtained from multiple sampling of same homogeneous samples under the prescribed conditions. Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision/ruggedness (show the degree of reproducibility of test results obtained by analyzing the sample under variety of normal test conditions such as analyst to analyst variation and instrument to instrument variation).

Precision was determined by taking six independent sample solution preparation from a single lot of formulations of Lamotrigine (20µg/ml) and Zonisamide (20µg/ml) and injected into the HPLC system. The retention time and peak area was determined and expressed as mean and %RSD calculated from the data obtained, which are found to be within the specified limits.

Table 1: Linearity results for Lamotrigine and Zonisamide

Lamotrigine		Zonisamide	
Conc (µg/ml)	Area	Conc(µg/ml)	Area
5	585755	5.00	412721
10	1169290	10.00	817979
15	1769290	15.00	1241822
20	2407298	20.00	1685524
25	3032751	25.00	2122302
30	3662826	30.00	2561306

Table 2: System Precision values for Lamotrigine and Zonisamide % RSD results for Lamotrigine

S.No.	Injection	Peak name	R_t	Area	Height
1	Injection -1	Lamotrigine	2.524	1763951	244937
2	Injection-2	Lamotrigine	2.524	1794350	250754
3	Injection-3	Lamotrigine	2.527	1792044	255248
4	Injection-4	Lamotrigine	2.526	1792044	255248
5	Injection-5	Lamotrigine	2.524	1783951	244937
6	Injection-6	Lamotrigine	2.524	1794350	250754s
Average			1786782		
Standard deviation			11825.36		
%RSD			0.662		

Table 3: Showing %RSD results for Zonisamide

S.No.	Injection	Peak name	R_t	Area	Height
1	Injection-1	Zonisamide	4.629	2575632	268443
2	Injection-2	Zonisamide	4.629	2576930	275252
3	Injection-3	Zonisamide	4.630	2613729	282668
4	Injection-4	Zonisamide	4.631	2613729	282668
5	Injection-5	Zonisamide	4.629	2575632	268443
6	Injection-6	Zonisamide	4.629	2570930	275252
Average			2585120		
Standard deviation			18722.21		
%RSD			0.724		

Table 4: Robustness showing results for Lamotrigine and Zonisamide

S no	Parameter	RT	Area	RT	Area
1	Standard	2.526	1728698	4.629	2574325
2	Robustness-Flow-1	2.828	2137383	5.254	3043043
3	Robustness-Flow-2	2.261	1688920	4.137	2413944
4	Robustness-Oven Temp-1	2.555	1932082	4.738	2755918
5	Robustness-Oven Temp-1	2.523	1945900	4.516	2970931

Robustness

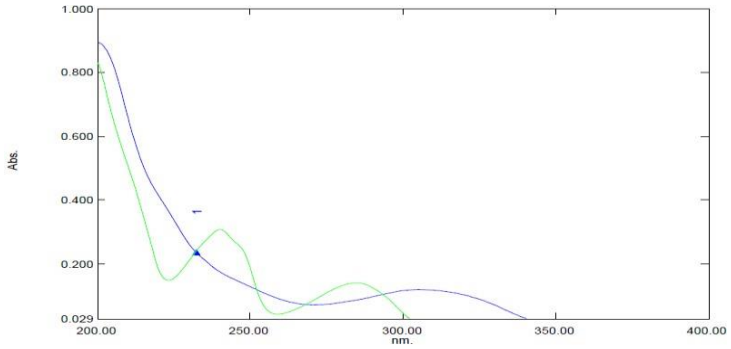
The robustness of newly developed method was assessed by evaluating the influence of small deliberate variations in procedure variables such as flow rate (±5%) and change in wave length (±5nm). The robustness was evaluated by changing the flow rate from 0.9ml/min to 1.1ml/min and the method is found robust only in low flow rate conditions and even by changing the mobile phase ±5%.

System Suitability

System suitability tests were carried out on freshly prepared standard stock solutions of Lamotrigine and Zonisamide, it was calculated by injecting standards in six replicates at 6min interval and the values were recorded.

RESULTS AND DISCUSSIONS

The present investigation "A new RP-HPLC method development and validation of simultaneous estimation of Lamotrigine and Zonisamide" was proceeded with wavelength selection (232nm). From several trials an optimized method was selected for simultaneous estimation of Lamotrigine and Zonisamide and mentioned under chromatographic conditions earlier. The linearity was determined as linearity regression of the claimed analyte concentration of the range 5-30µg/ml for Lamotrigine and 5-30µg/ml for Zonisamide. Standard curve was plotted by taking peak area on Y-axis concentration on X-axis the results were shown in **Table 1,** the graph was linear and the correlation coefficient was found to be 0.9998 and 0.9997 for Lamotrigine and Zonisamide respectively.



No.	Wavelength	Absorbance	Description
1	232.20	0.237	Isobestic Poi

Figure 3: UV spectra showing Isobestic point of Lamotrigine and Zonisamide.

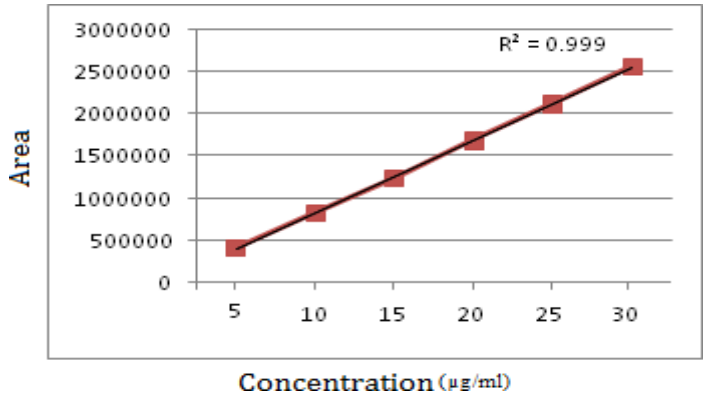


Figure 4: Showing linearity for Zonisamide

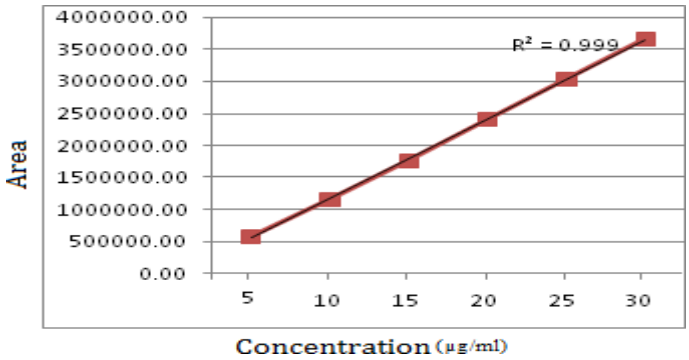
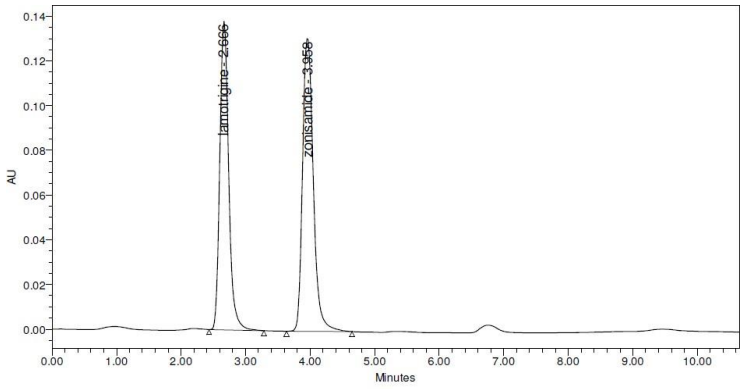


Figure 5: Showing linearity for Lamotrigine



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	lamotrigine	2.666	1314834	137870	46.394		1.21	1903.01
2	zonisamide	3.958	1519215	131554	53.606	4.75	1.27	2923.71

Figure 6: Standard Chromatogram of Lamotrigine and Zonisamide

Table 5: Accuracy results for Lamotrigine

%Concentration (at specific level)	Average area	Amount added (standard + tablet powder)(mg)	Amount found(mg)	%Recovery	Mean recovery
80%	1374384	10 +6	15.994	99.96%	99.96%
100%	1718370	10+10	19.997	99.98%	
120%	2061602	10+14	23.991	99.96%	

Table 6: Accuracy results for Zonisamide

%Concentration (at specification level)	Average area	Amount add (standard + tablet powder)(mg)	Amount found (mg)	%Recovery	Mean recovery
80%	2041329	10+6	15.997	99.98%	99.97%
100%	2551005	10+10	19.991	99.96%	
120%	3062186	10+14	23.997	99.99%	

Table 7: System suitability Parameters for Lamotrigine and Zonisamide

	Lamotrigine	Zonisamide
R_t	2.666	3.958
Tailing factor	1.21	1.27
Resolution	4.75	4.75
Plate count	1903.01	2923.71
Assay value	99.45	99.66

Table 8: Showing ruggedness charts 1 and 2

Ruggedness Day 1(Intraday precision)					Ruggedness Day 2(Inter day precision)				
S.No	Lamotrigine		Zonisamide		S.No	Lamotrigine		Zonisamide	
	Rt	Area	Rt	Area		Rt	Area	Rt	Area
1	2.528	1728985	4.629	2460258	1	2.529	1738621	4.627	2477328
2	2.527	1728984	4.628	2460257	2	2.528	1738620	4.626	2477327
3	2.526	1728983	4.627	2460256	3	2.527	1738629	4.625	2477326
4	2.525	1728982	4.626	2460255	4	2.526	1738628	4.624	2477325
5	2.524	1728982	4.625	2460254	5	2.525	1738627	4.623	2477324
6	2.523	1728980	4.624	2460253	6	2.524	1738626	4.622	2477323
Avg	2.526	1728983	4.627	2460256	Avg	2.527	1738629	4.625	2477326
Std dev	0.0019	1.9	0.0019	1.87	Std dev	0.0019	1.8708	0.0019	1.8708
RSD	0.074	0.000	0.040	0.000	RSD	0.074	0.000	0.040	0.000

The precision of the method was ascertained from determinations of peak areas 6 replicates of sample solution.

The % relative standard deviation for system precision of Lamotrigine and Zonisamide was found to be 0.662 and 0.724 respectively and presented in table 2. The % relative standard deviation for the method precision of Lamotrigine and Zonisamide was found to be 0.583 and 0.675 and presented in Table 3. The accuracy study was performed in 80%, 100% and 120%. The percentage recovery was determined for Lamotrigine and Zonisamide was found to be 99.96 and 99.99% the results were presented in Table 4 & 5.

The robustness were carried out with minor but deliberate changes in parameters i.e., detection wavelength, column temperature and flow rate were presented in Table 6.

Theoretical plate's values and tailing factor for Lamotrigine and Zonisamide were within the acceptance limits i.e theoretical plates more than 2000 and tailing factor not more than 2. The results were presented in Table 7.

Ruggedness

Trial-1

10mg of both the drug is taken in a 50ml volumetric flask. Pippete out 1ml of solution and make up to 10ml the concentration of solution was 20ppm.

Trial-2

10mg of both the drug is taken in a 50ml volumetric flask. Pippete out 1ml of solution and make up to 10ml the concentration of solution was 20ppm.

CONCLUSION

The newly developed method was found to be simple, precise, accurate reproducible and rapid for determination of Lamotrigine and Zonisamide in pharmaceutical tablet dosage form. The newly developed method was validated according to ICH guidelines for parameters such as specificity, linearity, accuracy, precision, robustness and system suitability values were found to be within limits. The validation study indicates that method can be considered suitable for carrying out

quality control and routine analysis of Lamotrigine and Zonisamide pharmaceutical tablet dosage form.

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