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Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Bupropion and Zonisamide in Bulk and Tablet Dosage Form

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ABSTRACT

The present research work aims to develop and validate the RP-HPLC method for the simultaneous estimation of bupropion and Zonisamide in bulk and tablet dosage form. Chromatographic separation was achieved by using water inertsil -ODS C18 (250 x 4.6 mm, 5 µ) column with a flow rate of 1.0 ml/min. The injection volume was 20µl. The optimized mobile phase was methanol and phosphate buffer in the ratio of 80:20%v/v. UV detector wavelength monitored at 252 nm and the run time was 8 min. The retention time was found to be 3.226min for Bupropion and 4.522 min for Zonisamide. The linearity was obtained in the range of 20-80 ppm for both drugs. The developed method was validated statistically according to ICH guidelines. The proposed method was accurate, precise, reproducible, and robust and can be employed for routine quality control analysis of pharmaceutical formulations.

INTRODUCTION

Bupropion, (structure present in fig-1) is an antidepressant medication used to treat major depressive disorder and seasonal affective disorder. Bupropion is used to help people stop smoking by reducing cravings and other withdrawal effects. Bupropion selectively inhibits the neuronal reuptake of dopamine, norepinephrine, and serotonin actions on dopaminergic systems that are more significant than imipramine or amitriptyline [1-9].

Zonisamide, (structure present in fig-2) is used with other medications to prevent and control seizures (epilepsy). Zonisamide is a sulfonamide anticonvulsant approved for adjunctive therapy in adults with partial-onset of seizures. Zonisamide may act by blocking repetitive firing of voltage-gated sodium channels leading to a reduction of T-type calcium channel current or by binding allosterically to GABA receptors [10-14].

Literature reveals that few methods have been reported for the estimation of Bupropion and Zonisamide individually and in combination with other drugs by UV (Shubhammahadev et al., 2019) and bioanalytical methods (Nakamura et al., 2001). A few methods have been reported for the simultaneous estimation of Bupropion and Zonisamide by HPLC (Mahalakshmi et al., 2016). The present attempt is made to develop the most reliable method for simultaneous estimation of Bupropion and Zonisamide in bulk and tablet dosage form by RP-HPLC.

MATERIALS AND METHODS

INSTRUMENTS:

The instruments used were HPLC Waters Model NO.2690/5 series Compact System consisting ofInertsil-C18 ODS column along with Electronic balance and sonicator.

CHEMICALS USED:

Gift samples of Bupropion and Zonisamide were procured from PHARMA TRAIN, Hyderabad. HPLC grade water and methanol were purchased from MERCK laboratories, Mumbai. Potassium dihydrogen phosphate ($K_2H_2PO_4$) was obtained from FINAR chemicals Pvt limited, Ahmedabad, and Gujarat.

METHOD DEVELOPMENT:

Mobile Phase: The mobile phase was prepared by taking 200 ml (20%) of Phosphate Buffer and 800 ml (80%) of Methanol, mixed and degassed in an ultrasonic water bath for 10-15 min and then filtered through 0.45µ membrane filter. The pH was adjusted with ortho phosphoric acid.

Preparation of working standard solution:

The solutions were prepared by dissolving 20.0 mg of accurately weighed Bupropion and 25.0 mg Zonisamide in Mobile phase, in two 100.0 ml volumetric flasks separately and sonicated for 20 min. From the above solutions take 10.0 mL from each solution into a 50.0 mL volumetric flask and then makeup with the mobile phase and sonicate for 10min.

The method development was initiated with preliminary chromatographic conditions consisting of column Inertsil-C18, BDS column, flow rate 1ml/min, detection wavelength 252nm. Various compositions of mobile phases were tried for better separation of the analytes and finally optimized with methanol: buffer at 80: 20 ratio. With this mobile phase both the analytes are eluted with good peak symmetry, resolution and the theoretical plate count also meet the acceptance criteria.

Optimized Chromatographic Conditions:

Stationary phase (column)	Inertsil -ODS C18(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol: Buffer (80:20)
Flow rate	1.0 ml/min
Run time	10 min
Column temperature (°C)	Ambient
The volume of the injection loop	20
Detection wavelength	252nm
RT (min)	3.226 min for Bupropion and 4.529 for Zonisamide.

Validation of Analytical method

The developed RP-HPLC method was validated for system suitability, Specificity, Linearity, Accuracy, Precision, and robustness. The validation was done following ICH guidelines Q_2 (R_1).

System Suitability:

A Standard solution was prepared by using Bupropion and Zonisamide. Working standards as per the test method and was injected five times in replicates into the HPLC system. The system suitability parameters like retention times, peak areas, tailing factor, and resolution were evaluated from standard chromatograms.

Specificity:

Bupropion and Zonisamide standard and sample were prepared as per the test method are injected into the chromatographic system. A blank and placebo were also prepared and injected similarly.

Precision:

System precision: Standard solution prepared as per test method and injected in replicates for five times.

Method precision: Prepared six sample preparations individually as per the test method and injected each solution.

Intermediate precision (analyst to analyst variability): A study was conducted by two analysts as per the test method.

Accuracy (Recovery):

Accuracy studies were conducted with triplicate injections as per the test method with an equivalent amount of 50%, 100%, and 150% of Bupropion and Zonisamide. The average % recovery of Bupropion and Zonisamide was calculated and reported.

Linearity:

A Series of solutions were prepared using Bupropion and Zonisamide working standards at concentration levels from 20 ppm to 80 ppm of the target concentration. The solutions were

injected into the HPLC system in replicates and the peak areas were observed. A graph was plotted with peak areas vs concentration and correlation coefficient values were calculated for each analyte.

Robustness:

Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solutions were prepared as per the test procedure and were injected into the HPLC system using flow rates, 1.0ml/min and1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow. The retention times were comparable with those obtained for the mobile phase having flow rates 1.0ml/min.

Limit of Detection and Quantitation (LOD and LOQ):

From the linearity data, the limit of detection and quantitation was calculated, using the following formula.

$$LOD = \frac{3.3\ \sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

$$LOQ = \frac{10\ \sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

RESULTS AND DISCUSSION

System suitability:

The retention time of Bupropion and Zonisamide is found to be 3.617 min and 5.013 min. Three system suitability parameters the tailing factor, theoretical plates, and resolution were studied. The theoretical plate count was more than 2000 and the tailing factor is less than 2

for both the analytes. All the values were present within the limits. The results are shown in table 1&2.

Specificity:

There is no interference observed in the blank. The chromatograms of Standard and Sample were identical with the same retention time.

Precision: Method precision and intermediate precision were performed in six replicate injections and the % RSD of the peak areas was calculated. The % RSD for the area of six standard injections was not more than 2%, which was within the limits. The results are observed in table 3&4.

Accuracy:

The mean percentage recovery was found to be 100.1 0 % for Bupropion and 100.50% for Zonisamide which were within the limits. So the method was accurate and the data was shown in table 5.

Linearity:

A graph was plotted with peak area versus concentration and calculated the correlation coefficient. The correlation coefficient and regression equation of Bupropion was found to be y= 10694.55x+1351.26 (r^2 =0.999) and Zonisamide was found to be y = y= 208538.40 + 57894.84 (r2=0.997). The r^2 values confirmed the method was linear. The results were shown in table 6 and figures 4 & 5.

Robustness:

A study was carried out with variation in flow rate and wavelength to evaluate the robustness of the method. The standard solutions were injected in the selected robust conditions and the system suitability parameters like theoretical plates, tailing factor, and the resolution was observed. The results showed that the theoretical plate count was more than 2000, the tailing factor was less than 2, and the resolution was found more than 2. Hence it indicates that the method is robust. Robustness data was shown in table 7.

Limit of Detection and Quantitation (LOD and LOQ):

The LOD of Bupropion and Zonisamide were found to be 0.34 and 0.25 respectively and the LOD values were found to be 1.05 and 0.77 respectively, were found to meet acceptance criteria.

CONCLUSION

A Rapid, Accurate, linear, specific, sensitive Precise and robust RP-HPLC method has been developed and validated for Bupropion and Zonisamide, in its pure form as well as in tablet dosage form.

Chromatography was carried out on Inertsil-ODS C18 (250 x 4.6mm, 5µm) column using a variety of mobile phase compositions and finally optimized by Methanol: Buffer (80:20)at a flow rate of 1.0ml/min, the detection was carried out at 252nm. The retention times of Bupropion and Zonisamide were found to be 3.226min for and 4.529 respectively. The method was found to be linear in the concentration range of 20 ppm to 80 ppm of the target concentration. The method was precise since the %RSD values of the peak were found to be below **2.** The % recovery values for Bupropion and Zonisamide were found to be 100.1 0 % for 100.50% respectively. The specificity of the method was assessed by injections of standard, sample, and blank solutions separately and the chromatograms were recorded. No interference was observed in the blank chromatogram and the standard and sample chromatogram showed identical Rt. The LOD of Bupropion and Zonisamide were found to be 0.34and 0.25 respectively and the LOD values were found to be 1.05 and 0.77 respectively. The method was also found to be robust under a set of robust conditions.

When compared to the previous methods reported the present method is rapid due to less Rt.

The accountability of the method was assessed and documented by validation as per ICH guidelines. The results of validation indicate that the method is suitable and can be adopted for routine analysis of Bupropion and Zonisamide as a part of regular quality control analysis.

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Table No. 1(a): Data of System Suitability for Bupropion

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.227	429817	15231.845	1.15124
2	3.220	429036	15292.721	1.18749
3	3.221	429254	15225.754	1.17877
4	3.222	429816	15742.816	1.12460
5	3.226	429844	15236.789	1.18744
Mean	3.2232	429553.4	15345.99	1.165908
SD	0.00314	380.8659	-------	-------
% RSD	0.096627	0.88666	-------	------

Table No. 1(b): Data of System Suitability for Zonisamide

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	4.522	842634	6452.5421	1.28754
2	4.516	842071	6421.3458	1.24512
3	4.519	842682	6474.2186	1.22485
4	4.518	842371	6488.1744	1.27841
5	4.529	842627	6471.4152	1.25921

Table No. 2(a): Repeatability Data of Bupropion

	Injection	Peak Areas of Bupropion	% Assay
Concentration 40ppm	1	429012	99.97
	2	429874	100.17
	3	429087	99.99
	4	429781	100.15
	5	429567	100.1
	6	429856	100.2
Statistical Analysis	Mean	429464	100.76
	SD	395.484	0.091
	% RSD	0.092	0.091

Table No. 2(b): Repeatability Data of Zonisamide

	Injection	Peak Areas of Zonisamide	%Assay
Concentration 40ppm	1	842245	100.28
	2	842684	100.33
	3	842724	100.33
	4	842845	100.35
	5	842712	100.33
	6	842365	100.32
Statistical Analysis	Mean	842642	100.32
	SD	230.32	0.026
	% RSD	0.027	0.025

Table No. 3(a): Data of Repeatability (Method precision) for Bupropion

	Injection	Peak Areas of Bupropion	%Assay
Concentration 40ppm	1	429547	100.09
	2	429781	100.11
	3	429634	100.11
	4	429781	100.15
	5	429089	99.99
	6	429574	100.09
Statistical Analysis	Mean	429567.7	100.09
	SD	254.955	0.053
	% RSD	0.059	0.036

Table No. 3(b): Data of Repeatability (Method precision) for Zonisamide

	Injection	Peak Areas of Zonisamide	%Assay
Concentration 40ppm	1	842785	100.34
	2	842892	100.35
	3	842732	100.33
	4	842987	100.36
	5	842759	100.34
	6	842905	100.35
Statistical Analysis	Mean	842843.3	100.345
	SD	99.72	0.010
	% RSD	0.011	0.010

Table No. 4(a): Data of Intermediate precision for Bupropion

	Injection	Peak Areas of Bupropion	%Assay
Concentration 40ppm	1	429481	100.08
	2	429951	100.19
	3	429830	100.16
	4	429479	100.08
	5	429087	99.99
	6	429875	100.17
	Mean	429617.2	100.1117
Statistical analysis	**SD**	329.2199	0.075741
	% RSD	0.076631	0.075656

Table No. 4(b): Data of Intermediate precision for Zonisamide

	Injection	Peak Areas of Zonisamide	% Assay
Concentration 40ppm	1	842358	100.29
	2	842084	100.26
	3	842384	100.29
	4	842587	100.32
	5	842380	100.29
	6	842927	100.36
Statistical Analysis	**Mean**	842453.3	100.3017
	SD	282.1019	0.034303
	% RSD	0.033486	0.034199

Table No. 5 (a): Data of Accuracy of Bupropion

Concentration % of the spiked level	Area	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	214823	20	19.96	99.80	**MEAN**	99.77
50%Injection 2	214776	20	19.96	99.78		
50% Injection 3	214694	20	19.95	99.74	**%RSD**	0.0306
100 % Injection 1	429472	40	40.03	100.08	**MEAN**	100.10
100 % Injection 2	429652	40	40.05	100.12		
100% Injection 3	429674	40	40.05	100.12	**%RSD**	0.023
150% Injection 1	338265	60	60.10	100.17	**MEAN**	100.20
150% Injection 2	338566	60	60.15	100.26		
150% Injection 3	338309	60	60.11	100.18	**%RSD**	0.048

Table No. 5(b): Data of Accuracy for Zonisamide

Concentration % of the spiked level	Area	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	421682	20	19.94	99.70	**MEAN**	99.5733
50% Injection 2	421972	20	19.96	100.56		
50% Injection 3	421883	20	19.52	100.43	**%RSD**	0.050
100 % Injection 1	842792	40	40.14	100.24	**MEAN**	100.29
100 % Injection 2	842375	40	40.12	100.21		
100% Injection 3	842064	40	40.10	100.19	**%RSD**	0.044
150% Injection 1	1264894	60	60.38	100.28	**MEAN**	100.47
150% Injection 2	1265091	60	60.39	100.29		
150% Injection 3	1267420	60	60.50	100.29	**%RSD**	0.455

Table No. 6(a): Linearity of Bupropion

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	10695
20	214909	y-Intercept	1351
30	322364	Correlation Coefficient	0.999
40	429817		
50	537272		
60	644726		
70	752181		
80	852634		

Table No. 6(b): Linearity of Zonisamide

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	20854
20	421437	y-Intercept	5789
30	632156	Correlation Coefficient	0.999
40	842875		
50	1053594		
60	1264313		
70	1475032		
80	1655750		

Table No. 7 (a): Effect of variation in flow rate Bupropion:

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	427284	1.099	**Flow 1.0 ml**	429128	1.128	**Flow 1.2 ml**	430215	1.121
	427852	1.103		429827	1.112		430127	1.122
	427085	1.111		429028	1.121		430875	1.124
	427148	1.117		429127	1.124		430257	1.123
	427982	1.119		429745	1.123		430127	1.099
	427985	1.118		429656	1.321		430654	1.098
Avg	427470	1.110	**Avg**	429371	1.1221	**Avg**	430320	1.118
SD	416.705	0.008	**SD**	382.11	0.006	**SD**	315.244	0.010
%RSD	0.097	0.097	**%RSD**	0.0889	0.542	**%RSD**	0.073	0.961

Table No. 7 (b): Effect of variation in flow rate Zonisamide

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	8415687	1.238	**Flow 1.0 ml**	8413761	1.128	**Flow 1.2 ml**	430215	1.121
	8410875	1.230		8415687	1.112		430127	1.122
	8413761	1.240		8429846	1.121		430875	1.124
	8419612	1.238		8410874	1.124		430257	1.123
	8410874	1.241		8413761	1.123		8413761	1.099
	8414111	1.276		8419612	1.321		8415687	1.098
Avg	8414162	1.238	**Avg**	8424624	1.124	**Avg**	8455126	1.245
SD	3667.56	0.004	**SD**	3533.91	0.010	**SD**	3669	0.010
%RSD	0.043	0.346	**%RSD**	0.0419	0.828	**%RSD**	0.0433	0.860

Table No. 8: Limit of Detection and Quantitation (LOD and LOQ):

Drug	Lod	Loq
Bupropion	0.34	1.05
Zonisamide	0.25	0.77

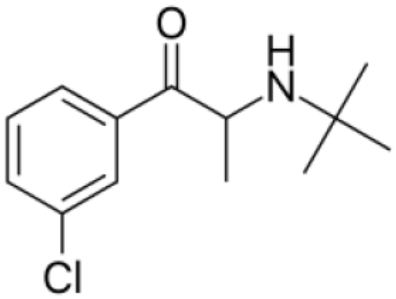

Figure No. 1: Chemical structure of Bupropion

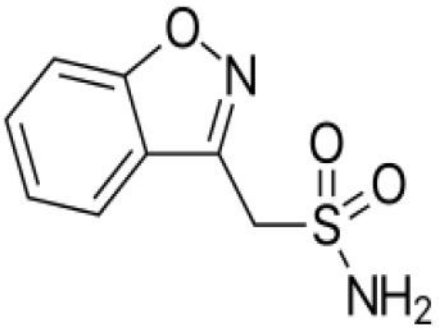

Figure No. 2: Chemical structure of Zonisamide

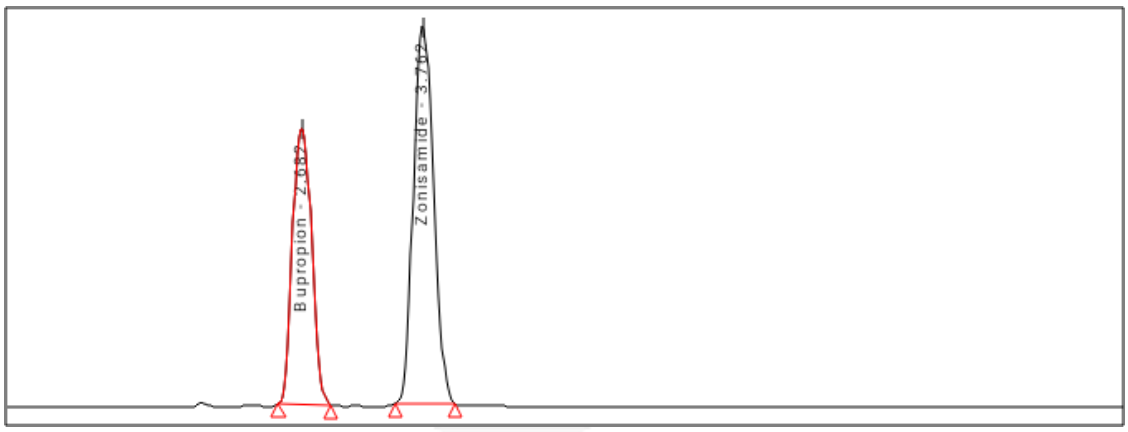

Figure No. 3: Optimized Chromatogram for Bupropion and Zonisamide

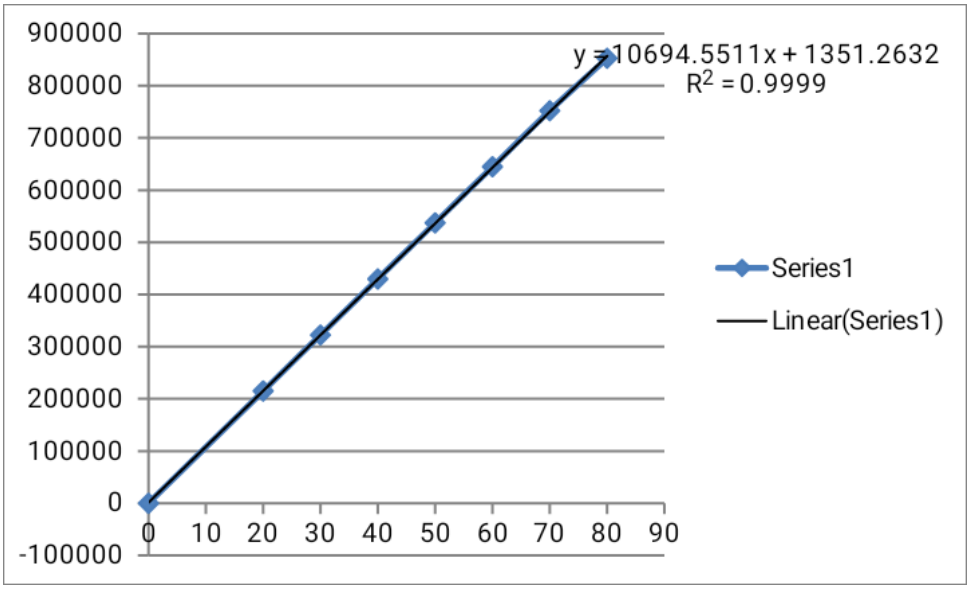

Figure No. 4: Calibration curve of Bupropion

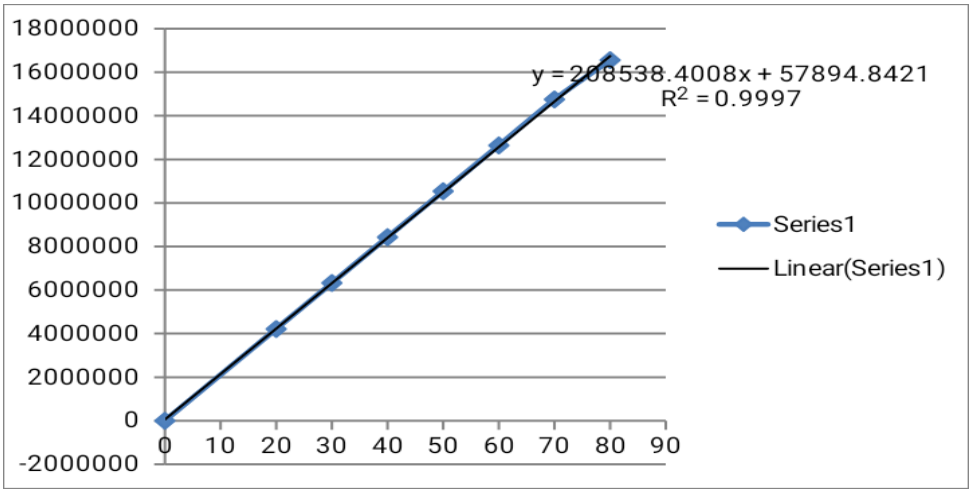

Figure No. 5: Calibration curve of Zonisamide

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