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Research Article

METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC FOR SIMULANEOUS ESTIMATION OF BUPROPION AND ZONISAMIDE

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ABSTRACT

For the simultaneous estimation of the Bupropion and Zonisamide in Pharmaceutical Dosage Form dosage form, a straightforward, accurate, and precise method was developed. Chromatogram was run through Inertsil -ODS C18(250mm x 4.6 mm, 5 µm). Mobile phase containing Methanol: Water taken in the ratio 45:55 was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 275nm. Retention time of Bupropion and Zonisamide were found to be 3.226 min and 4.529. %RSD of the Bupropion and Zonisamide were and found to be 0.09 and 0.02 respectively. %Recovery was obtained as 99.97% and 99.57% for Bupropion and Zonisamide respectively. LOD, LOQ values obtained from regression equations of Bupropion and Zonisamide were 0.34, 1.05 and 0.25, 0.77 respectively. Regression equation of Bupropion is y = 10695x + 1351.3, y = 208538x + 57895 of Zonisamide. Because retention times and run times were reduced, the method developed was simple and cost-effective, and it can be used in regular quality control tests in industries.

Keywords: Bupropion and Zonisamide, RP-HPLC

INTRODUCTION

A norepinephrine and dopamine reuptake inhibitor known as bupropion, it is prescribed to treat major depressive disorder (MDD), seasonal affective disorder (SAD), and to help people quit smoking [1]. Adults with partial-onset seizures can take the sulfonamide anticonvulsant zonisamide as a supplement to their current treatment [2]. The neurotransmitters norepinephrine and dopamine are taken up from the synaptic cleft by enzymes that are weakly inhibited by bupropion. This prolongs the duration of their action within the neuronal synapse and the

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downstream effects of these neurotransmitters. Bupropion particularly binds to the dopamine transporter (DMT) and the norepinephrine transporter (NET) [3]. Zonisamide are indicated as adjunctive therapy in the treatment of partial seizures in adults with epilepsy[4]. These drugs are used for many evaluations of problems like weight loss and anxiety problems. Bupropion is an aminoketone, unicyclic antidepressant. Although the exact mechanism underlying its therapeutic effects is unclear, it does seem to prevent dopamine uptake. The hydrochloride is a therapy option for smoking cessation.[5] Because it does not have the same effects as traditional antidepressants like monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), or selective serotonin reuptake inhibitors (SSRIs), bupropion was initially categorised as a "atypical" antidepressant. Although it is equally as effective as standard first-line depression treatments like SSRIs. zonisamide was effective against tonic extension seizures but ineffective against clonic seizures. It also increased the threshold for generalized seizures and reduced the duration of cortical focal seizures[6]. Use of zonisamide may result in adverse effects that are deadly. Patients receiving sulfonamides like zonisamide have experienced severe responses such Stevens-Johnson syndrome, toxic epidermal necrolysis, fulminant liver necrosis, agranulocytosis, and aplastic anaemia. Acute myopia, secondary angle closure glaucoma, drug response with eosinophilia and systemic symptoms (DRESS), multi-organ hypersensitivity, suicidal behaviour, and ideation are other potential side effects of zonisamide. Before being abandoned, the combination had advanced to phase II clinical trials while being developed by Orexigen Therapeutics[7]. When there is a treatment failure or just partial response, bupropion is occasionally used as an adjunctive therapy to first-line treatments for depression, such as SSRI medicines. When there is a treatment failure or just partial response, bupropion is occasionally used as an adjunctive therapy to first-line treatments for depression, such as SSRI medicines.[8] There are some other rp-hplc methods published[9,10,11].

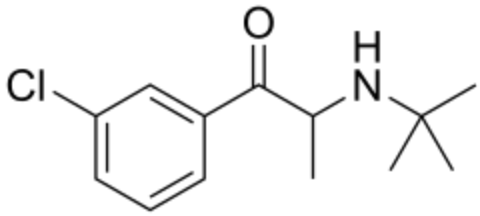

Figure 1. Structure of Bupropion

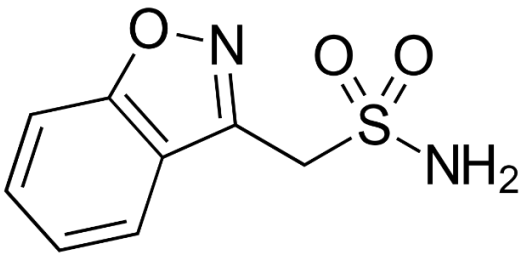

Figure 2. Structure of Zonisamide

A review of the literature revealed that some methods for simultaneous estimation of Bupropion and Zonisamide have been reported, as well as methods for estimation of individual drugs or in combination with other drugs such as UV-Spectrophotometric methods, UPLC, and RP-HPLC. The primary goal of this research is to create a simple, precise, accurate, relatively sensitive, and fast RP-HPLC technique for estimating Bupropion and Zonisamide in bulk and tablet formulations.

MATERIALS AND REAGENTS

Chemicals and reagents: Bupropion and Zonisamide pure drugs (API), combination Bupropion and Zonisamide NEXLIZET (Zonisamide 10mg, Bupropion 180mg), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen, ortho phosphate buffer, ortho-phosphoric acid. All the above chemicals and solvents provided by Rankem.

INSTRUMENTATION

WATERS HPLC, model: 2695 SYSTEM with Photo diode array detector was used for the development and method validation, with an automated sample injector with software Empower

CHROMATOGRAPHIC CONDITIONS:

Table 1. Chromatographic Conditions:

Flow rate	**:**	**1**ml/min
Column	**:**	Inertsil -ODS C18(250 x 4.6 mm, 5 µ)
Mobile phase:		Methanol: Water taken in the ratio 55:45
Detector	**:**	275.0 nm
Temperature	**:**	Ambient
Inj volume	**:**	20.0µL
Run time	**:**	10.0 mins

PREPARATION OF SOLUTIONS

Preparation of Standard solution: Accurately weighed 10 mg of Bupropion, 40 mg of Zonisamide and transferred to 50ml volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. 100µg/ml of Bupropion and 800µg/ml Zonisamide)

Standard Working solution:

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (10µg/ml of Bupropion and 80µg/ml of Zonisamide)

Sample Working solution: 00.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (90µg/ml of Bupropion and 5µg/ml of Zonisamide)

Method Validation:

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Bupropion (10ppm) and Zonisamide (80ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Table 2. System suitability parameters for Zonisamide and Bupropion

S no	Zonisamide			Bupropion		
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing
1	4.522	6452.5421	1.28754	3.227	15231.845	1.15124
2	4.516	6421.3458	1.24512	3.220	15292.721	1.18749
3	4.519	6474.2186	1.22485	3.221	15225.754	1.17877
4	4.518	6488.1744	1.27841	3.222	15742.816	1.12460
5	4.529	6471.4152	1.25921	3.226	15236.789	1.18744

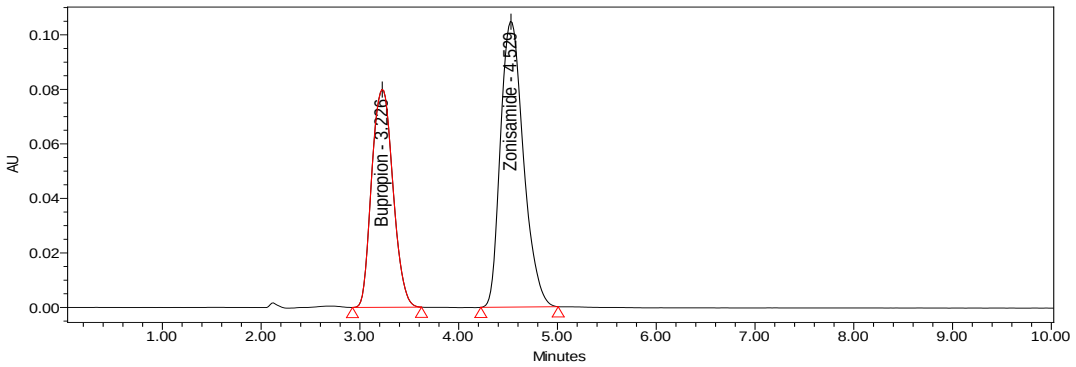

Figure 3. Optimized chromatogram

Typical Chromatogram

Discussion: Bupropion and Zonisamide and were eluted at 3.226 min and 4.529 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated and We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity:

Table 3. Linearity table for Zonisamide and Bupropion

Zonisamide		Bupropion	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
5	163090	2.5	92029
10	316562	5	175574
15	478718	7.5	263323
20	633932	10	351412
25	784529	12.5	433302
30	944352	15	512006

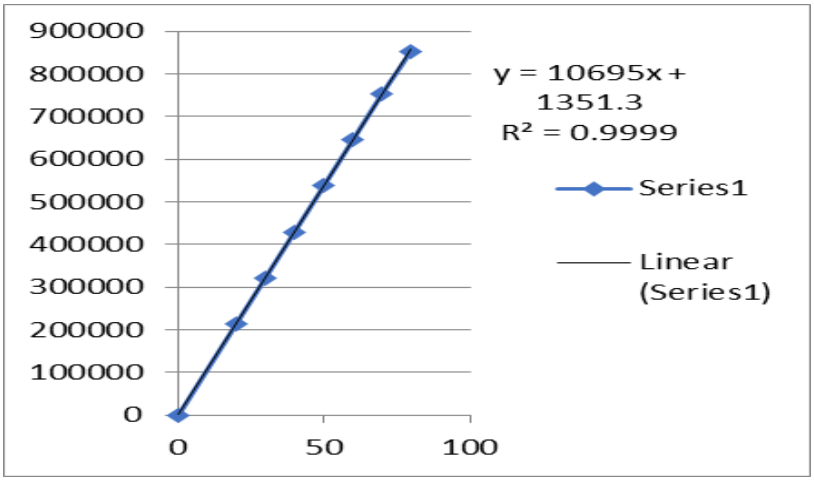

Figure 4. linearity of Bupropion

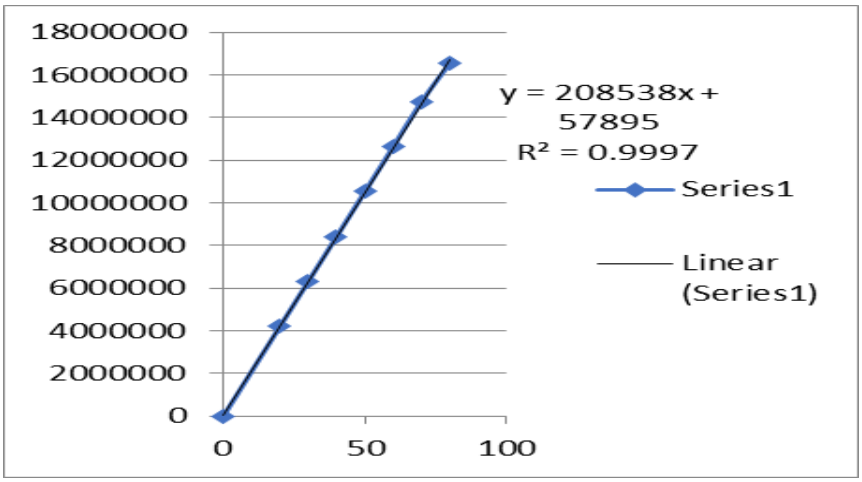

Figure 5. linearity of Zonisamide

Table 4. Accuracy (%Recovery data)

%Level	%Recovery					
	Bupropion			Zonisamide		
	Amount added	Amount found	%Rec	Amount added	Amount found	%Rec
50% Level	20	19.96	99.80	20	19.52	19.52
	20	19.96	99.78	20	40.14	40.14
	20	19.95	99.74	20	40.12	40.12
100%Level	40	40.03	100.08	40	40.10	40.10
	40	40.05	100.12	40	60.38	60.38
	40	40.05	100.12	40	60.39	60.39
150%Level	60	60.17	100.28	60	60.50	60.50
	60	60.17	100.29	60	19.52	1952
	60	60.17	100.29	60	4014	40.14
Mean%			99.77			99.57

System Precision: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.88% and 0.03% respectively for Bupropion and Zonisamide. As the limit of Precision was less than "2" the system precision was passed in this method. Results of peak area are summarized in Table 5.

Table 5. System precision data

Injection	Zonisamide	Bupropion
1	842634	429817
2	842071	429036
3	842682	429254
4	842371	429816
5	842627	429844
Avg	**842477**	**429553.4**
Std dev	**257.5102**	**380.8659**
%RSD	**0.030566**	**0.88666**

The % RSD for the peak areas of Zonisamide and Bupropion obtained from six replicate injections of standard solution was within the limit.

Method Precision: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.05% and 0.01% respectively for Bupropion and Zonisamide. As the limit of Precision was less than "2" the system precision was passed in this method. As the limit of Precision was less than "2" the system precision was passed in this method. Data obtained is summarized in Table 6.

Table 6. Method precision data

Injection	Zonisamide	Bupropion
1	842785	429547
2	842892	429781
3	842732	429634
4	842987	429781
5	842759	429089
6	842905	429574
Avg	**842843.3**	**429567.7**
Std dev	**99.72094**	**254.9554**
%RSD	**0.011831**	**0.059352**

From the above results, the % RSD of method precision study was within the limit for Zonisamide and Bupropion.

Sensitivity

Table 7. sensitivity

Molecule	LOD	LOQ
Bupropion	0.34	1.05
Zonisamide	0.25	0.77

CONCLUSION

The RP-HPLC methodology was used to create and evaluate a new stability indicating analytical approach. The sample preparation is straightforward, uses less mobile phase, and takes very little time to analyse. The results of the study will be highly beneficial for quality monitoring of Zonisamide and Bupropion in pharmaceutical dosage forms. The assay examination of two medications from a combination dosage form using this devised method yielded results that were nearly 100 % accurate. The results of the recovery studies were good, indicating that there was no interference from excipients.

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