WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 4, 1399-1409. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF ZONISAMIDE AND BUPROPION IN SYNTHETIC MIXTURE

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Article Received on
07 Feb. 2017,

Revised on 27 Feb. 2017,
Accepted on 19 March 2017

DOI: 10.20959/wjpr20174-8228

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of Zonisamide and Bupropion in synthetic mixture. The method is based on the simultaneous equations for analysis of both the drugs using methanol as solvent. Zonisamide has absorbance maxima at 238.5 nm and Bupropion has absorbance maxima at 246 nm in methanol. The linearity was obtained in the concentration range of 4-28 µg/ml and 4-28 µg/ml for Zonisamide and Bupropion, respectively. The concentrations of the drugs were determined by solving simultaneous equations at both the wavelengths. The mean recovery was 99.68 ± 1.06 and 98.96 ± 0.59 for Zonisamide and Bupropion, respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of Zonisamide and Bupropion in synthetic mixture. The results of analysis have been validated statistically and by recovery studies.

KEYWORDS: Zonisamide, Bupropion, Recovery, Simultaneous equations method, Validation.

INTRODUCTION

Zonisamide (ZON) (Figure 1) is chemically 1,2-benzoxazol-3-methane sulphonamide; $C_8H_8N_2O_3S$[1] is a anti-epileptic agent used in the treatment of anticonvulsant.[2] It is official

in Indian Pharmacopoeia(IP)[3] and United Sate Pharmacopoeia(USP).[4] IP[3] and USP[4] describe liquid chromatography method for its determination. Literature survey reveals HPLC[5-12], HPTLC[13] spectrophotometric[14-16] methods for the determination of ZON in alone and HPLC[17] and spectrophotometry[18] method for determination of ZON in combined dosage forms with other drug. Bupropion (BUP) (Figure 2) is chemically 2-(tert-butylamino)-1-(3-chlorophenyl) propan-1-one[19], is a antidepressant type. It is used for Depressant.[20] This drug is official in USP.[21] USP describe LC method for its estimation. Literature survey reveals HPLC[22-26], spectrophotometry[27-29], LC[30] and titrimetric[31] methods for determination of BUP in alone and first order derivative spectrophotometric[32-33], UPLC-MS/MS[34], HPLC[35-37] methods for estimation of BUP in combination with other drugs. The combination of ZON and BUP has been shown to be effective in the weight loss. The combination was generally more effective than ZON, BUP and placebo alone. This combination of ZON and BUP is not official in any pharmacopoeia, so no official method is available for the determination of these two drugs in combined dosage forms. Literature survey reveals no spectrophotometric method for the simultaneous determination of ZON and BUP in combined dosage form. The present manuscript explain simple, sensitive, accurate, precise, rapid and economic zero order derivative spectrophotometric method for simultaneous determination of ZON and BUP in synthetic mixture.

MATERIALS AND METHODS

Apparatus

A shimadzu double beam UV-Visible spectrophotometer model 1700 (Japan) with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was appointed to measure absorbance of all the solutions. Spectra were automatically acquired by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 5, Mumbai, India) was appointed in the study.

Reagents and Materials

Pure sample of ZON was provided as a gift sample from BDR life sciences pvt. Ltd and BUP was obtained from Intas pharmaceuticals limited. Synthetic mixture (5000 mg) of ZON (400 mg) and BUP (300 mg) was made in laboratory by incorporating commonly used excipients (4300) like lactose, talc, magnesium stearate. Methanol AR Grade was received form S.D fine Chemicals Ltd, Mumbai, India. Whatman filter paper no 41. All the chemicals used were of analytical grade.

Preparation of standard stock solutions

An accurately weighed quantity pure powder of ZON (10 mg) and BUP (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of ZON (100 µg/ml) and BUP (100 µg/ml).

METHODOLOGY

Standard drug solutions having concentration 10 µg/ml was scanned separately in the range of 200 nm to 400 nm. Maximum absorbance was noticed at 238.5 nm and 246 nm by ZON and BUP respectively. So these two wavelengths was preferred as an analytical wavelength. In this method, the absorbances of the solutions were measured at the λmax of both the drugs. The criteria are that the ratios [(A2/A1) / (ax2/ax1)] and [(ay2/ay1) / (A2/A1)] should lie outside the range 0.1 - 2.0. For this measurement, the standard solutions of ZON and BUP (10 µg/ml) were scanned separately in the range of 200 - 400 nm against Methanol as a blank. Data were recorded at an interval of 1 nm. (Figure 3) indicates the overlain spectra of the two drugs. Absorbance was measured at selected wavelengths i.e. 238.5 nm and 246 nm absorption maxima for ZON and BUP respectively. The absorbance and Absorptivity values at the particular wavelengths were calculated and substituted in the following equation to get the concentration

$Cx = (A2\ Ay1 – A1\ Ay2) / (Ay1\ Ax2 – Ay2\ Ax1)$ ----- (1)

$Cy = (A1\ Ax2 – A2\ Ax1) / (Ay1\ Ax2 – Ay2\ Ax1)$ ------ (2)

Where, A1, A2= Absorbances of mixture at λ1 & λ2 respectively,

ax1 = Absorptivity of ZON at 391 nm,

ax2 = Absorptivity of ZON at 303 nm.

ay1 = Absorptivity of BUP at 289 nm.

ay2 = Absorptivity of BUP at 413 nm

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.[38]

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 4-28 µg/ml for ZON and 4-28 µg/ml for BUP. Accurately measured standard solutions of ZON (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8 ml) and BUP (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8 ml) were transferred to a series

of 10 ml of volumetric flasks and diluted to the mark with Methanol. The absorbances of the solutions were measured at 400 and 200 nm against as blank. The calibration curves were assembled by plotting absorbances versus concentrations and the regression equations were calculated.

Method precision (Repeatability)

The precision of the instrument was examined by repeated scanning and measurement of absorbance of solutions (n = 6) for ZON and BUP (18 µg/ml for ZON and 18 µg/ml for BUP) without changing the parameter of the suggested spectrophotometric method.

Intermediate precision (reproducibility)

The intraday and interday precision of the suggested method was determined by analysing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of ZON and BUP (5, 10, 15 µg/ml for ZON and 5, 10, 15 µg/ml for BUP). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of ZON and BUP by the standard addition method. Known amounts of standard solutions of ZON and BUP were added at 50, 100 and 150% level to prequantified sample solutions of ZON and BUP (8 µg/ml for ZON and 6 µg/ml for BUP). The amounts of ZON and BUP were determined by applying acquired values to the respective regression line equations. The experiment was repeated for three times.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.[38]

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of ZON and BUP in synthetic mixture

A quantity of synthetic mixture equivalent to 40 mg of ZON and 30 mg of BUP was transferred to a 100 ml volumetric flask. Methanol (50 ml) was added to the flask and sonicated for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. This solution is wonted to contain 400 µg/ml of ZON and 300 µg/ml of BUP. From this solution (1.0 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of ZON (40 µg/ml) and BUP (30 µg/ml). Then after from this solution (4.0 ml) was taken in to a 10 ml volumetric flask and volume was adjusted up to mark with methanol to get a final concentration of ZON (16 µg/ml) and BUP (12 µg/ml). The responses of the sample solution were measured at 238.5 nm and 246 nm for determination of ZON and BUP, respectively. The amounts of the ZON and BUP present in the sample solution were calculated by fitting the responses into the regression equation for ZON and BUP in the suggested method.

RESULTS AND DISCUSSION

Simultaneous equations method, the primary requirement for developing a method for analysis is that the entire spectra should follow the beer's law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for determination of the drugs were 238.5 nm (λ-max of ZON) and 246 nm (λ-max of BUP) at which the calibration curves were constructed for both the drugs. The overlain UV absorption spectra of ZON (238.5nm) and BUP (246 nm) showing in methanol is shown in Figure 3.

The validation parameters were studied at all the wavelengths for the suggested method. Accuracy was determined by calculating the recovery, and the mean was determined (Table 1). The method was successfully used to determine the amounts of ZON and BUP present in the synthetic mixture. The results obtained were in good agreement with the corresponding labelled amount (Table 2). Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs. Optical characteristics and summary of validation parameters for method is given in (Table 3). By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these two drugs in synthetic mixture.

Table 1: Result of recovery study of ZON and PBUP by developed method

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
ZON	I	8	50	99.91 ±1.01
	II	8	100	99.64 ±1.67
	III	8	150	99.49 ±0.52
BUP	I	6	50	100.22 ±0.38
	II	6	100	98.38 ±0.83
	III	6	150	98.29 ±0.57

S.D. is standard deviation and n is number of replicate.

Table 2: Analysis of ZON and BUP by proposed method

Synthetic Mixture	Label claim (mg)		Amount found (mg)		% Label claim ± S.D. (n = 5)	
1	ZON	BUP	ZON	BUP	ZON	BUP
	40	30	40.38	30.79	100.95 ± 0.32	102.62 ± 0.86

S.D is standard deviation and n is number of replicate.

Table 3: Regression analysis data and summary of validation parameters for the proposed method.

PARAMETERS	ZON		BUP	
	at 238.5 nm	at 246 nm	at 238.5 nm	at 246 nm
Beer's Law limit (µg/ml)	4 - 28		4 - 28	
Regression equation (y = mx + c)	0.0391x+ 0.0244	0.0303x+ 0.0207	0.0289x+ 0.0247	0.0413x+ 0.002
Slope (m)	0.0391	0.0303	0.0289	0.0413
Intercept (c)	0.0244	0.0207	0.0247	0.002
Correlation Coefficient (r^2)	0.9991	0.9990	0.9990	0.9993
Method precision (Repeatability) (% RSD, n = 6),	0.80	0.87	0.83	0.83
Interday (n = 3) (% RSD)	0.71-0.86	0.83-0.91	0.81-0.99	0.98-0.99
Intraday(n = 3) (% RSD)	0.33-0.66	0.63-0.92	0.20-0.93	0.19-0.75
LOD (µg/ml)	0.09	1.05	1.25	0.37
LOQ (µg/ml)	3.06	3.18	3.81	1.134
Accuracy (Mean % Recovery ± S.D) (n = 3)	99.68 ± 1.06		98.96 ± 0.59	
% Assay ± S.D. (n = 3)	100.95 ± 0.32		102.62 ± 0.86	

RSD = Relative standard deviation. LOD = Limit of detection. LOQ = Limit of quantification. SD=Standard deviation.

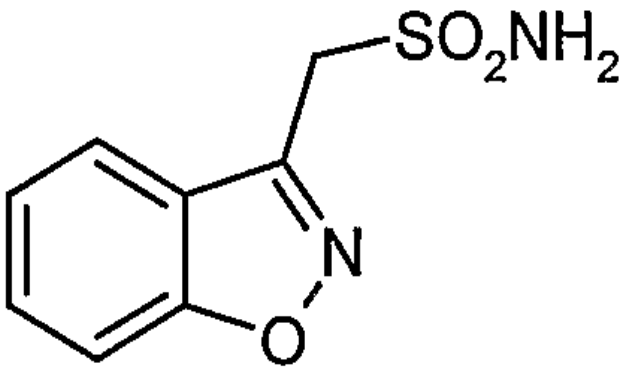

Figure 1: Chemical structure of Zonisamide (ZON)

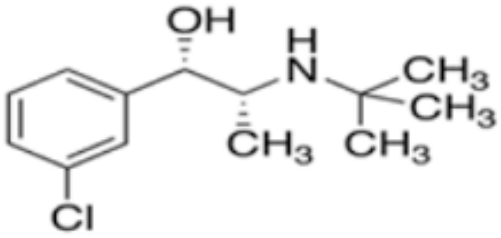

Figure 2: Chemical structure of Bupropion (BUD)

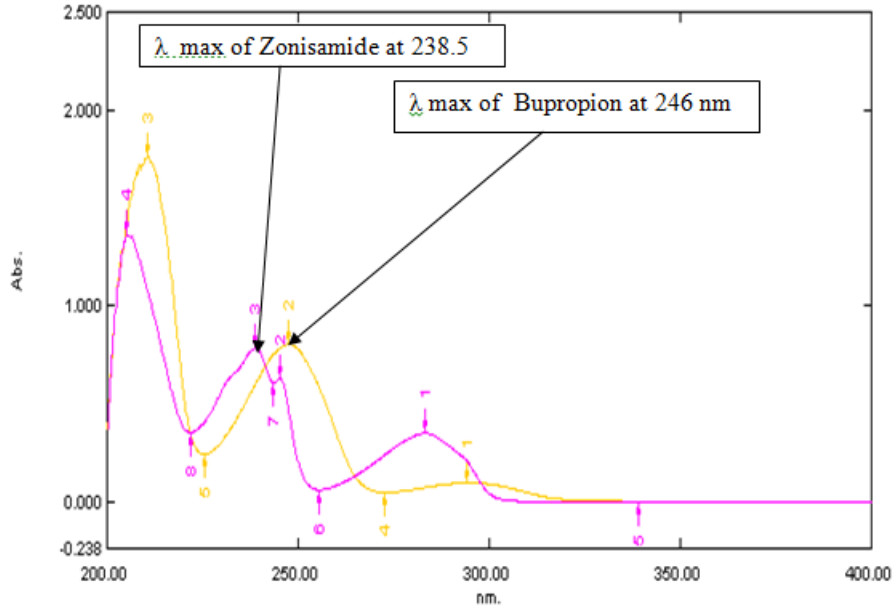

Figure 3: Overlain spectrum ZON (10µg/ml) and BUP (10µg/ml) in methanol

CONCLUSION

Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 4-28 µg/ml and 4-28 µg/ml for ZON and BUP, respectively. The result of the analysis of synthetic mixture by the suggested method is highly reproducible and reliable. The results obtained were in good agreement with the corresponding labelled amount. The additives usually present in the synthetic mixture of the assayed sample did not interfere with determination of ZON and BUP. The method can be used for the routine analysis of the ZON and BUP in synthetic mixture without any interference of excipients.

ACKNOWLEDGEMENT

The authors wish to thank BDR life sciences pvt. Ltd and Intas pharmaceuticals limited for providing ZON and BUP pure drug powder as gift sample for research work. The authors are highly thankful to Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Ganpat vidhyanagar, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

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