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Development and Validation of First Order derivative UV Spectrophotometric Method for Simultaneous Estimation of Bupropion and Naltrexone in Combination

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ABSTRACT

UV spectrophotometric method has been developed using Water as a solvent in First Order Derivative method determination was carried out at 262.33 nm λmax ZCP of Naltrexone and 227.19nm λmax ZCP of Bupropion. The calibration curves were linear 10-60 µg/ml and 1-6 µg/ml for Bupropion and Naltrexone at their respective wavelength. Both the drugs were found in good agreement with the label claimed in the marketed formulation. In Combination both the drugs were estimated as 98.6% and 99.4% Bupropion and Naltrexone respectively. In this method drugs obey Beer's law using the concentration range 10-60µg/ml and 1-6 for BUP and NAL respectively. The correlation coefficient of BUP and NAL was found to be 0.999 and 0.999 respectively. The results of Recovery study BUP and NAL were found to be within range of 98-102%. Precision study showed that %RSD was within range of acceptance limits (<2%). The method was validated as per ICH Q2 (R1) guideline.

Keywords: Bupropion(BUP),Naltrexone(NAL), First order derivative method, UV Spectrophotometry.

INTRODUCTION

NTX is chemically, (5a)-17-(cyclopropylmethyl)-4, 5-epoxy-3, 14-dihydroxymorphinan-6-one hydrochloride used in the treatment of alcoholism and as narcotic antagonist major metabolite of naltrexone, 6-ß-naltrexol, is also an opiate antagonist and may contribute to the antagonistic activity of the drug. BUP is chemically, (±)-2-(tert-butylamino)-1-(3chlorophenyl) propan-1-one, an atypical antidepressant and smoking cessation aid. The increase in norepinephrine may attenuate nicotine withdrawal symptoms and the increase in dopamine at neuronal sites may reduce nicotine cravings and the urge to smoke it acts as a norepinephrine and dopamine reuptake inhibitor as well as α3 ß4 nicotinic receptor antagonist [1,3]. Presently, combination of these two drugs as a controlled release tablet is under clinical trials for the treatment of obesity. Both drugs are formulated together in the form of tablet dosage form for treatment of Obesity. The chemical structures of both drugs [4-9] were shown in Fig. 1.

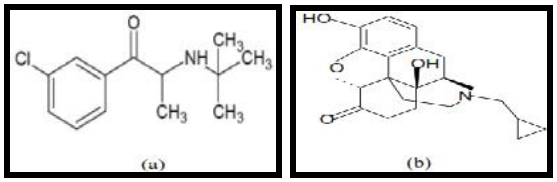

Fig. 1: Chemical structure of (a) Bupropion and (b) Naltrexone

From literature survey it reveals that various analytical methods have been reported for estimation of Bupropion and Naltrexone individually or in combination with other drugs either as API or in pharmaceutical dosage form. So the purpose of this work was to develop a simple, precise, accurate and sensitive Simultaneous Equation Method for determination of Bupropion and Naltrexone in Combination.

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MATERIALS AND METHODS

Instruments:

The instrument used was double beam UV- visible spectrophotometer (Shimadzu, model 1800, software: UV-Probe 2.34) having two matched quartz cell with 1 cm path length. Sonication of sample solutions was done using ultrasonic cleaner.

Materials:

Bupropion (BUP) drug sample was procured from Cadila Pharmaceuticals, Ahmedabad and Naltrexone (NAL) drug sample was gifted by Intas Pharmaceutical, Ahmedabad.

Method:

Preparation of standard stock solution:

The stock solution having 1000µg/ml concentration of Bup and NAL were prepared separately by dissolving accurately weighed 100mg of both drugs in 100 ml Water. Further dilutions of standard stock solutions of both drugs were made with Water to get the working standard stock solutions of 100µg/ml concentration of BUP and NAL.

Method Development (First Order Derivative Method):

Selection of scanning range and sampling wavelength:

The standard stock solution of BUP and NAL were diluted with Water individually to get the concentration of 10µg/ml for both and was scanned in UV range 200-400 nm. The λ of both the drugs were found to be 250nm and 281nm respectively for BUP and NAL respectively in normal UV spectra shown in figure 2.

Development of First order derivative spectra:

The spectral data was then processed to obtain first order derivative spectrum at wavelength interval of 2nm for the range of 200-400nm. It was observed that BUP shows ZCP at 227.19nm and NAL showed ZCP at 262.33nm. At ZCP of BUP (227.19nm), NAL showed a measurable dA/d λ where at ZCP of NAL (262.33nm).BUP showed showed a measurable dA/d λ. Hence thw wavelengths 227.19nm and 262.33nm were selected as wavelengths for determination of BUP and NAL first order derivative method respectively shown in Fig. 3.

Method Validation:

The above proposed method was validated as per the ICH Q2 (R1) guidelines for validation of analytical procedures [10] in order to determine the linearity, Accuracy, Precision, LOD and LOQ.

Linearity and Range: Calibration curve constructed was linear over a selected range of 10-60µg/ml for BUP and 1-6µg/ml for NAL. The calibration curve of absorbance against concentration plotted was shown in figure 4 and 5. Each concentration was repeated six times. Correlation coefficient and regression line equations for BUP and NAL were calculated and were shown in table no.1.

Accuracy: The accuracy of the developed method was determined by finding out the amount of recovery of Bupropion and Naltrexone. For the accuracy standard addition method was used where, as known amount of BUP and NAL were added to the known concentration (22.5µg/ml BUP and 2µg/ml NAL). The amount recovered was found by measuring the absorbance of the solution and was expressed as mean recovery of samples with upper and lower limits of percent relatives of standard deviation. Recovery was done at three different levels i.e. 80%, 100% and 120%, within the linearity range of both the drugs.

Precision:

Repeatability (n=6): For the repeatability study, from the working stock solution, appropriate volume of solution was transferred to a 10 ml volumetric flask and diluted up to mark with methanol such that it gives the concentration of 22.5µg/ml and 2µg/ml of BUP and NAL respectively. The absorbance of the solutions was measured at 262.33nm and 227.19nm respectively. The procedure was repeated six times and % RSD was calculated and shown in Table no. 3.

Intraday Precision (n=3): From the working stock solution, appropriate volume of solution was transferred to a 10 ml volumetric flask and diluted up to mark with Distil.Water such that it gives the concentration of 10, 20 and 30µg/ml of BUP and 1, 2, and 3µg/ml of NAL. The solutions were analysed three times on the same day and % RSD was calculated and shown in Table no. 3.

Interday Precision (n=3): From the working stock solution, appropriate volume of solution was transferred to a 10 ml volumetric flask and diluted up to mark with Distil.Water such that it gives the concentration of 10, 20 and 30µg/ml of BUP and 1, 2, and 3µg/ml of NAL. The solutions were analysed three times on three different days and % RSD was calculated and were shown in table no. 4.

Limit of Detection (LOD) and Limit of Quantification (LOQ): Limit of detection (LOD) is the minimum concentration of the analyte in the sample which can be analysed by the instrument. Limit of quantification (LOQ) is the minimum concentration of the analyte that can be reliably quantified. The Limit of detection (LOD) and Limit of quantification (LOQ) were measured using following formula. The values of LOD and LOQ for BUP and NAL were shown in Table no. 5.

$$LOD = 3.3 \times (SD/Slope)$$
$$LOQ = 10 \times (SD/Slope)$$

Where,

SD = Standard deviation of the Y- intercepts of the 6 calibration curves.

Slope = Mean slope of the 6 calibration curves.

Assay of Combination: Combination Containg both Bupropion and Naltrexone were used For the Study. Combine solution equivalent to 90mg of Bupropion and 8 mg of Naltrexone and transferred in to a 100ml volumetric flask to bring both drug in 9:0.8 ratio and stock solution of this was prepared in Water, Sonicated for 15min, the volume was adjusted up to the mark with same solvent. This stock solution contain Bupropion 900µg/ml and Naltrexone 80µg/ml. From the above solution, pipette out 1 ml into 10 ml volumetric flask and volume adjusted to the mark with Water Then the appropriate dilution of 90µg/ml(BUP) and 80µg/ml(NAL) was made using Water as solvent. From the above solution, pipette out 2.5 ml into 10 ml volumetric flask and volume adjusted to the mark with Water to get 22.5 µg/ml standard stock solution of Bupropion which is equivalent of 2µg/ml of Naltrexone. All the determination were carried out in triplicate. The absorbance of the prepared solution was measured at ZCP of BUP and ZCP of NAL and then the concentration of both the drug was calculated using calibration curve equation. The amount of the drug found in Combination was shown in Table no. 6.

RESULT

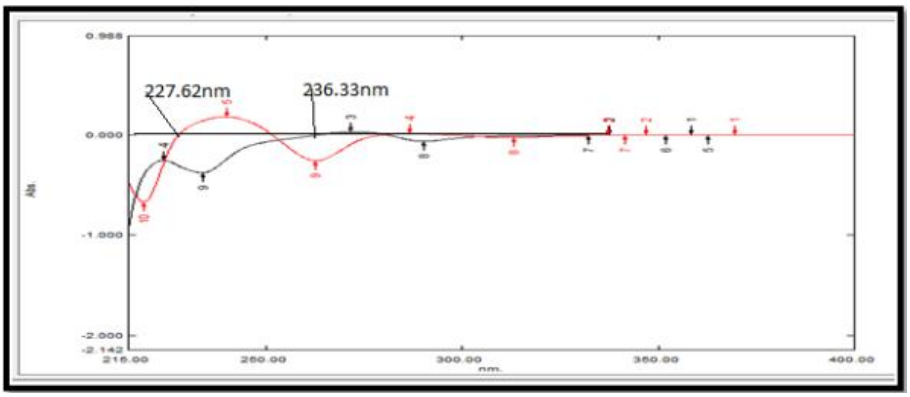

Fig. 2: Overlay spectra of BUP(10µg/ml) and NAL(10µg/ml) in Water.

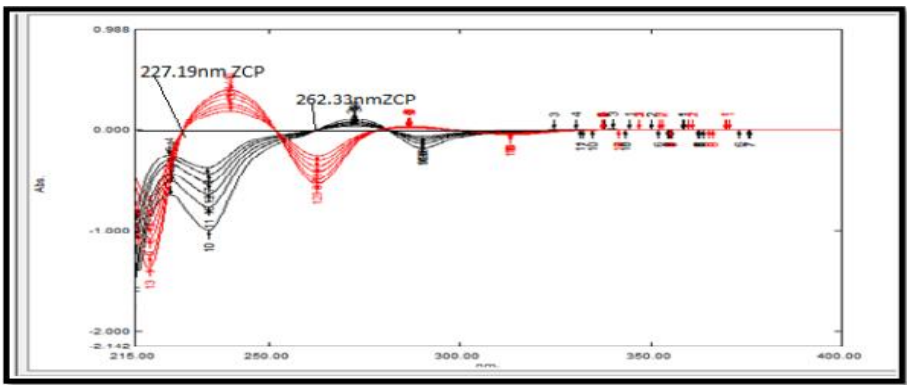

Fig. 3: Overlain linearity spectra of BUP and NAL in Water.

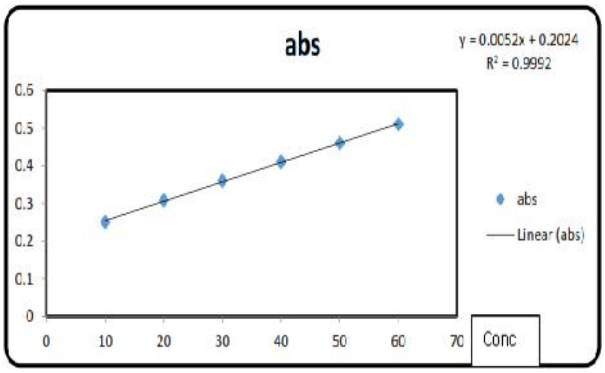

Fig. 4: Linearity graph of Bupropion at ZCP of Naltrexone

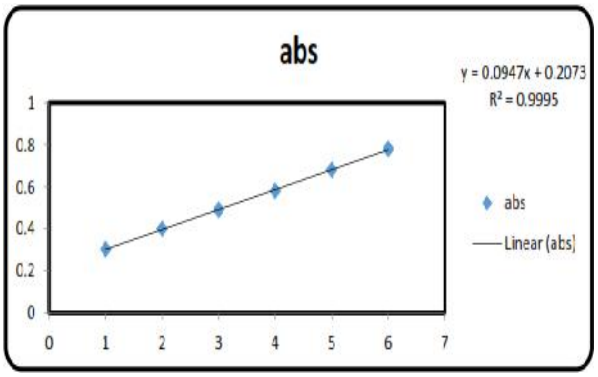

Fig. 5: Linearity graph of Naltrexone at ZCP of Bupropion

DISCUSSION

The present paper describes the estimation of BUP and NAL in combination by First order derivative method. The Beer-Lambert's concentration range was found to be 10-60µg/ml and 1-6µg/ml for both drug BUP and NAL at 227.19nm and 262.33nm respectively. The correlation coefficient was found to be 0.999 for BUP and 0.999 for NAL for proposed method. Precision was determined by studying repeatability, intraday and interday precision. The standard deviation and Relative standard deviation (%RSD) were calculated for both the drugs. The % RSD for proposed method were found to be not more than 2.0% which indicates good intermediate precision. The values of LOD and LOQ were 1.611µg/ml and 4.833µg/ml for BUP and 1.345 µg/ml and 4.078µg/ml for NAL respectively.

Table No. 1: Optical Characteristics.

Parameters	Bupropion	Naltrexone
Beer's law limit (µg/ml)	10-60µg/ml	1-6µg/ml
Regression equation	0.0052x + 0.2024	0.0947x + 0.2073
Slope (m)	0.0052	0.0947
Standard deviation of Intercept (c)	0.016	0.021
Correlation coefficient (R^2)	0.999	0.999

Table No. 2: Results of Recovery studies

Drug	Concentration of STD drug	Recovery level (%)	Amount of drug added(µg/ml)	Amount of drug recovered(µg/ml)	% Mean recovery ±SD
BUP	22.5	80	18	22.5	100.36±0.573
		100	22.5	22.54	100.46±0.670
		120	27	22.58	100.50±0.727
NAL	2	80	1	2.01	100.15±0.825
		100	2	2.04	100.62±0.830
		120	3	2.07	100.66±0.908

Table No. 3: Repeatability, Inter-day and Intra-day precision of NIF and CAN.

Drug	Concentration(µg/ml)	Average ABS±SD	%RSD
REPEATABILITY(n=6)			
BUP	22.5	0.3033 ± 0.00175	0.5731
NAL	2	0.2526 ± 0.00163	0.6463
INTRADAY PRECISION(n=3)			
BUP	10	0.2505± 0.001	0.3992
	20	0.312± 0.002080	0.6672
	30	0.3620±.002646	0.7308
NAL	1	0.303± 0.001	0.3300
	2	0.400333± 0.0020	0.5199
	3	0.493± 0.003	0.6511
INTER DAY PRECISION(n=3)			

BUP	10	0.2573±0.001528	0.5935
	20	0.315±0.002082	0.6622
	30	0.364±0.005292	1.453
NAL	1	0.307±0.002	0.6514
	2	0.404±0.003606	0.8924
	3	0.5016±0.003512	0.70004

*SD = standard deviation, ABS = Absorbance

Table No. 4: Analysis of Synthetic Mixture

Drugs	Label claim (mg/ml)	Conc. Taken for assay(µg/ml)	Concentration found (µg/ml)	% Assay
BUP	90	22.5	22.56	98.95%
NAL	8	2	2.12	99.33%

Table No. 5: Limit of detection (LOD) and Limit of Quantification (LOQ)

Parameters	Bupropion	Naltrexone
LOD (µg/ml)	1.611	1.345
LOQ (µg/ml)	4.833	4.078

CONCLUSION

A simple, accurate and precise UV First order derivative Spectrophotometric method has been developed for the estimation of BUP and NAL in Combination. It has advantage that it eliminates the spectral interference from one of the two drugs while estimating the other drug by at selecting zero crossing point in derivative spectra of each drug at selected wavelength.

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