Research Article
ISSN: 0974-6943

K.N Patel et al. / Journal of Pharmacy Research 2009, 2(9),1525-1527

Available online through
http://jprsolutions.info

Derivative Spectrophotometric Method for Simultaneous Estimation of nicotine and Bupropion Hydrochloride in Synthetic Mixture by Derivative Spectrophotometric Method

K.N Patel*[1], Dr.J.K.Patel[1] ,I.S.Rathod[2]
[1]*Nootan Pharmacy College, visnagar-384315.*
[2]*Department of Pharmaceutical analysis, L.M.College of Pharmacy, Navrangpura, Ahmedabad-380009.*

Received on: 22-05-2009; Accepted on:26-07-2009

ABSTRACT

The use of first order derivative spectroscophotometry allowed simultaneous determination of nicotine and bupropion hydrochloride in fixed dose combination products. The absorbance values at 251.2 nm and 259.8 nm of first derivative spectrum was used for the estimation of nicotine and bupropion hydrochloride, respectively without mutual interference. This method obeyed Beer's law in the concentration range of 10-30 µg/ml for both nicotine and bupropion hydrochloride. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method.

Keywords: Nicotine, Bupropion hydrochloride, Phosphate buffer, Ultra-violet spectrophotometry, Derivative spectrophotometry.

INTRODUCTION

Nicotine[1-4] (NIC) is CNS stimulant and a cholinomimetic drug. Chemically it is (s) – 3 –(1 – methyl) -2 – pyrrolidinyl) pyridine. Nicotine is official in USP 2003[5].Literature described HPLC method[6], GC method[7], GC-MS method[8] for its determination in plasma, serum and pharmaceutical preparations when present with other drugs. Bupropion hydrochloride [1,2](BUP) is an atypical antidepressive agent and is chemically :(±)-2-(tert-butylamino)-1-(3-chlorophenyl) propan-1-one hydrochloride .It is also official in USP 2003[5].HPLC[9], GC[10] and LC-MS [11]methods have been reported for the estimation of BUP in plasma, and in pharmaceutical preparations No methods were reported for the simultaneous estimation of NIC and BUP in combined dosage forms. The present paper describes a simple, rapid, accurate and responsible method for the simultaneous estimation of NIC and BUP in synthetic Mixture by first order derivative spectrophotometric method.

EXPERIMENTAL

Materials

NIC was gift sample from Nicosulf Industries & Exports Pvt. Ltd and BUP was gift sample from Sun Pharmaceuticals. There was not any market formulation of this both drugs. Methanol AR Grade was procured from Rankem Chemicals.

Equipments:

Derivative spectrophotometric method has been developed for the simultaneous determination of nicotine (NIC) and bupropion hydrochloride (BUP) by using zero crossing first derivative methodology. The derivative UV spectra of standard and test solutions were recorded in 1 cm quartz cells using a Shimadzu UV/Vis-1700 double beam UV/Vis spectrophotometer (Japan) with a fixed slit width of 2 nm. The zero order and first derivative absorption spectra were recorded over the wavelength range 200-400 nm against the solvent blank. Shimadzu Libror AGE 220 balance was used for weighing the samples.Class'A' volumetric glassware were used

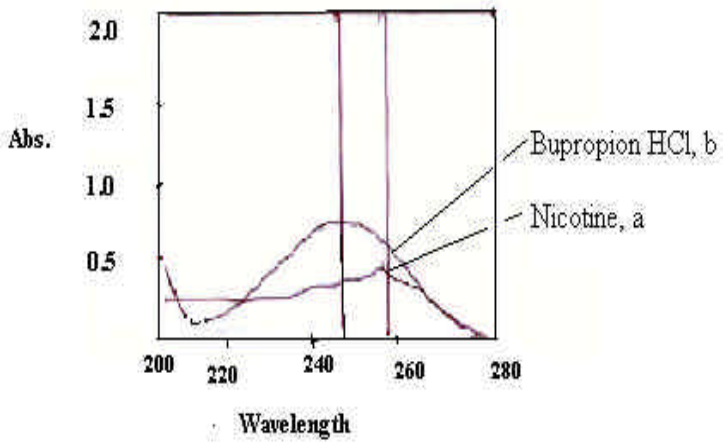
***Corresponding author.**
E-mail: coolkalpesh_77@yahoo.co.in

Procedure:

Development of the method:

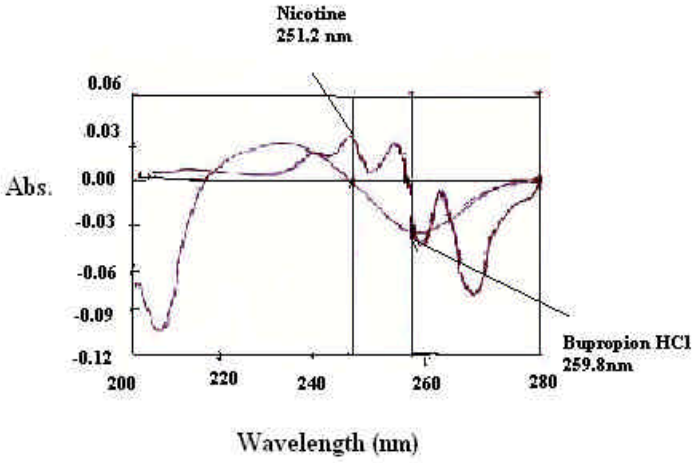
The standard stock solutions (0.05 mg/ml) of NIC and BUP in 7.4.pH phosphate buffer were prepared. Further dilutions were made in 7.4.pH phosphate buffer to obtain concentrations ranging from 10-30 µg/ml for both NIC and BUP.The absorbance of resulting solutions was measured at 259.8 and 251.2 nm for NIC and BUP and the calibration curves were plotted at these wavelengths. The overlain zero order spectra of NIC and BUP (**fig. 1**) showed that the absorption maxima of NIC and BUP lie in close proximity and at absorption maxima of one, another exhibits substantial absorbance. This clearly indicates the existence of spectral interference in estimation of NIC and BUP. To overcome this, spectra of these two drugs were derivatised to first order between 200-400 nm with ? ?= 2 nm.The overlain first derivative spectra of NIC and BUP (**fig. 2**) reveal that BUP concentration is proportional to the first derivative signals at 259.8 nm (zero-crossing point for NIC) and NIC can be estimated at 251.2 nm (zero-crossing point for BUP).

Fig. 1: Overlain zero order spectra of NIC and BUP.



The spectra of the NIC and BUP were taken for their 10 µg/ml solution

Fig. 2: Overlain first derivative spectra of NIC and BUP.



The NIC and BUP were estimated at the marked wavelengths 251.2 and 259.8 nm respectively.

Linearity:

Standard stock solutions were prepared by dissolving 50 mg of each standard drug samples in 100 ml volumetric flask separately and the volume was made up with 7.4 pH phosphate buffer to get a concentration of 0.5 mg/ml.From this, suitable dilutions were made in 7.4 pH phosphate buffer to get the working standard solutions of 10-30 µg/ml for NIC and 10-30 µg/ml for BUP separately. The absorbances of the derivatised spectra were measured at 251.2 nm and 259.8 nm for NIC and BUP, respectively. Six replicate analysis were carried out. Absorbance Vs concentration were plotted to obtain the calibration graph. Both drugs obey the Beer's law with the above concentration range with R2 value of 09983 and 09994 for NIC and BUP, respectively(Figure 2 and 3).

Limit of Detection and Limit of Quantitation:

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the six replicate determinations, y intercept was calculated and the standard deviation of the y intercept was computed. From these values, LOD and LOQ were calculated as follows,

$$\mathbf{LOD = 3.3 \times (SD/slope)}$$

$$\mathbf{LOQ = 10 \times (SD/slope)}$$

Where SD = standard deviation of response

Slope = Average of slope

Analysis of synthetic mixture of NIC and BUP:

Solution in 7.4 pH phosphate buffer containing various proportions of NIC and BUP were prepared and their first derivative spectra were recorded. From the derivative spectra, the absorbance at 251.2 nm and 259.8 nm were noted for the estimation of NIC and BUP, respectively. From these absorbance values, the concentrations of NIC and BUP were determined using calibration graph (**Table 1**).

Recovery studies (Accuracy):

It is defined as the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. It is measure of exactness of analytical method. Accuracy should be expressed as % recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals. Accuracy should be established across the specified range of the analytical procedure. It was determined by calculating the recovery of nicotine and bupropion HCl by standard addition method. To the fixed amount of solution (10 µg/ml of nicotine and bupropion hydrochloride) an increasing aliquots from working standard solution of nicotine and bupropion hydrochloride were added. The solutions were measured at 251.2 nm for nicotine and 259.8 nm for bupropion HCl and % recovery of the sample were calculated (**Table 2 and 3**).

RESULT AND DISCUSSION:

Zero-order absorption spectra of nicotine and bupropion hydrochloride showed overlapping peaks that interfere with the simultaneous determination of this formulation (Fig. 1). Derivative spectroscopy, based on a mathematical transformation of the spectra zero-order curve into the derivative spectra, allows a fast, sensitive and precise resolution of a multicomponent mixture and overcomes the problem of over-

Table: 1.Analysis of Synthetic Mixtures of NIC and BUP:

Sample No.	Concentration of NIC (µg/ml)		% Recovery	Concentration of BUP(µg/ml)		%Recovery
	Theoretical	Experimental		Theoretical	Experimental	
1	10	9.82	98.2	10	9.92	99.2
2	15	14.88	99.2	15	15.08	100.5
3	20	19.57	97.8	20	19.87	99.3
4	25	25.22	100.8	25	25.12	100.4
5	30	29.36	97.8	30	29.36	97.8

Table: 2.Accuracy data of determination of Bupropion HCl in the presence of Nicotine (10µg/ml) using first derivative spectroscopy.

Amount of Nicotine (µg/ml)	Amount of added Bupropion HCl (µg/ml)	Total amount found Mean ± S.D.	Accuracy (%)
10	10	09.82±0.23	98.2
10	15	14.88±0.003	99.2
10	20	19.57±0.004	97.8
10	25	25.22±0.002	100.8
10	30	29.36±0.007	97.8

Table: 3.Accuracy data of determination of Nicotine in the presence of Bupropion HCl (10µg/ml) using first derivative spectroscopy.

Amount of Bupropion HCl (µg/ml)	Amount of added Nicotine (µg/ml)	Total amount found Mean ± S.D.	Accuracy (%)
10	10	9.92±0.003	99.2
10	15	15.08±0.003	100.5
10	20	19.87±0.0012	99.3
10	25	25.12±0.042	100.4
10	30	29.36±0.055	97.8

Table: 4.Validation of the proposed method

Sample No.	Parameters	Experimental Values NIC	BUP
1	Precision (%C.V.)		
	1.Repeatability	2.45%	2.34 %
	2.Intraday precision	0.64-2.51%	1.35-2.15%
	3. Interday precision	1.57-4.16%	2.00-2.24 %
2	Linearity Range	10-30µg/ml	10-30µg/ml
3	Accuracy(%Recovery)	97.8-100.8%	97.8-100.5%
4	Limit of Detection(µg/ml)	0.39µg/ml	0.23µg/ml
5	Limit of Quantification(µg/ml)	1.17µg/ml	0.68µg/ml
6	Correlation coefficient	0.9967	0.9990

lapping of a multi-component system. Derivative spectroscopy on the basis of zero-crossing measurements involves measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelength of the derivative spectra of individual components, which should be only a function of the concentration of other component. The spectroscopic parameters including derivative order, wavelength and ?? values should be optimized to obtain maximum resolution, sensitivity and reproducibility. In this study first-derivative technique (D_1) traced with ??= 2 nm was used to resolve the spectral overlapping. Zero-crossing points of 220-280 nm is presented in fig. 2. The optimums D1values without interference for nicotine and bupropion hydrochloride were 251.2 and 259.8 nm, respectively (Fig. 2).

The linearity of the method was established form first-derivative spectra by measurement of the absorbance of standard solutions containing varying concentrations of each compound in the presence of constant concentration of the other one. The calibration curves were constructed by plotting the D_1value against nicotine or bupropion hydrochloride concentration at the zero-crossing wavelength of bupropion hydrochloride (251.2 nm) or nicotine (259.8 nm), respectively. The analytical resultsof synthetic mixtures obtained are summarized in Table 1. The linearity of the calibration curves and the adherence of the method to Beer's law are validated by the high value of the correlation coefficient and the value of intercept on ordinate which is close to zero.

The limit of detection that was found to be 0.39 µg/ml and 0.23 µg/ml for nicotine and bupropion hydrochloride.The accuracy and precision were determined by using synthetic mixture of nicotine and bupropion hydrochloride in the laboratory. The mean recoveries and SD are illustrated in Tables 2 and 3. Data of these tables showed a good accuracy and precision over the entire concentration range The data indicate that the proposed derivative spectroscopic method is highly precise during one analysis and between different runs.

The percentage of recovery in each case was calculated. The results obtained from the recoveries of both drugs (Tables 2 and 3) showed excellent accuracy. The influence of excipients was studied by mixing two formulation containing 10 µg/ml of nicotine and10 µg/ml of bupropion hydrochloride. No interference was observed from the presence of excipient in the amounts, which are commonly present in tablet dosage forms. Study of stability of nicotine and bupropion hydrochloride in the solutions during analysis showed that analytes were stable at least for 72 h in solutions. The validation of results are summarized in **Table 4.**

The proposed method was successfully applied to analyze preparation containing nicotine and bupropion hydrochloride.

ACKNOWLEDGEMENTS:

The authors are thankful to Nicosulf Industries & Exports Pvt. Ltd.Dakor and sun pharemaceutical Pvt.Ltd.Baroda, for providing standards sample drug and also the L.M. College of Pharmacy,Ahmedabad for providing the facilities to carry out work.

REFERENCES:

1. The CIMS.Mumbai: CMP Medica India Private Limited; April-July 2005;19
2. Budavari S., O'Neil. M.J., Smith A., Heckelman P.E. eds *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals*, 11th edition, New Jersey, Merck & Co., Inc., 1989,Pg:1523
3. http://www.Rxlist.com [Cited 2008 March 09]
4. http://www.medicinescomplete.com [Cited 2008 March 09].
5. United States Pharmacopoeia 29 (NF24), Rockville MD: United States Pharmacopoeial Convention Inc., 2006.
6. Aqel W. Abu-Qare, Mohamed B. Abou-Donia; "Quantification of nicotine, chlorpyrifos and their metabolites in rat plasma and urine using high-performance liquid chromatography *J. Chromatography. B*; 757 ; 295 –300 (2001
7. Lena Nystrom, Maud Pettersson, Christina R~ngemark; "Simple and sensitive method for determination of nicotine in plasma by gas chromatography"; J. Chromatography B; 701; 124-128(1997
8. Allan S. Xu, Lana L. Peng, James A. Have], Mary E. Petersen, John A. Fiene, James D. Hulse ; " Determination of nicotine and cotinine in human plasma by liquid chromatography-tandem mass spectrometry with atmospheric pressure chemical ionization interface "; J. Chromatography B ; 682 ; 249 -257 (1996)
9. Katarzyna K. Loboz , Annette S. Gross , John Ray, Andrew J. McLachlan; "HPLC assay for bupropion and its major metabolites in human plasma "; J. Chromatography B; 823 ; 115–121 (2005)
10. Sane R.T., Francis M., Khedkar S., Menezrs A.,Moghe A. and Patil P." Gas chromatographic determination of bupropion hydrochloride from its pharmaceutical formulation"Indian Druds, 40(4) April 2003; Page: 231.
11. C. Arellano , C. Philibert , C. Vachoux , J. Woodley , G. Houin ; "Validation of a liquid chromatography–mass spectrometry method to assess the metabolism of bupropion in rat everted gut sacs "; J. Chromatography B; 829 ; 50–55 (2005).

Source of support: Nil, Conflict of interest: None Declared