(RESEARCH ARTICLE)

Simultaneous estimation of naltrexone and bupropion in pharmaceutical dosage form by using UV spectroscopy

Sai datri A *, Lakshmana rao A , Ramnadh B , Valli devi B , Dhana lakshmi CH and Vikas CH

Department of Pharmaceutical Analysis, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, A.P., India.

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Abstract

A sensitive and validated method has been developed for simultaneous estimation of Naltrexone and Bupropion in pharmaceutical dosage form by using UV Spectroscopy, without prior separation, by four different techniques (Simultaneous Equation, Absorbance Ratio method, Dual Wavelength Method and Derivative Spectroscopic Method). The work was carried out on Shimadzu electron UV1800 double beam UV-Visible spectrophotometer. The absorption spectra of reference and test solutions were carried out in 1 cm matched quartz cell over the range of 200 - 400 nm. The linearity ranges for Naltrexone and Bupropion were 2-10 µg/ml. The results of the analysis have been validated statistically and by recovery studies. The proposed procedures are rapid, simple, require no preliminary separation steps and can be used for routine analysis of both drugs in quality control laboratories.

Keywords: Naltrexone; Bupropion; UV spectroscopy; Validation

1. Introduction

Chemically, Naltrexone (Figure 1) is (1S,5R,13R,17S)-4-(cyclopropylmethyl)-10,17-dihydroxy-12-oxa-4-aza pentacycl [9.6.1.0^{1,13}.0^{5,17}.0^{7,18}]octadeca-7(18),8,10-trien-14-one [1]. It is a derivative of noroxymorphone that is the N-cyclopropylmethyl congener of Naloxone. It is a narcotic antagonist that has been proposed for the treatment of heroin addiction. The FDA has approved Naltrexone for the treatment of alcohol dependence.

Chemically, Bupropion (Figure 1) is 2-(tert-butylamino)-1-(3-chlorophenyl) propan-1-one [2]. It is a norepinephrine/dopamine-reuptake inhibitor. It is used most commonly for the management of Major Depressive Disorder, Seasonal Affective Disorder and as an aid for smoking cessation. Thus, the two drugs have effects on two separate areas of the brain involved in the regulation of food intake: the hypothalamus (appetite regulatory center) and the mesolimbic dopamine circuit (reward system) and combinational intake of these two medicines helps in chronic weight management [3].

Literature survey reveals that some Spectrophotometric [5] and HPLC [4-8] methods have been reported for the estimation of Naltrexone and Bupropion in pharmaceutical formulations.

The aim of this paper was to explore the possibility of using techniques of simultaneous equation method, dual wavelength method, isosbestic point method and derivative spectroscopic method for quantifying Naltrexone and Bupropion simultaneously in their mixture forms. The proposed methods are simple, convenient, precise, accurate, and economical than the reported method and validated as per ICH guidelines [7].

* Corresponding author

E-mail address: Sai.dhatri_arige@yahoo.co.in

Figure 1 Chemical structures of analytes (A. Naltrexone and B. Bupropion)

2. Experimental

2.1. Instrumentation

To develop a UV spectroscopic method for simultaneous estimation of Naltrexone and Bupropion, Shimadzu electron UV1800 double beam UV-Visible spectrophotometer was used. The instrument is equipped with Silicon photodiode detector.

2.2. Chemicals and solvents

The working standards of Naltrexone and Bupropion were provided as gift samples from Spectrum Pharma Research Solutions, Hyderabad, India. Combined Naltrexone and Bupropion tablets were purchased from Online International Pharmacy Market.

2.3. Preparation of standard stock and working standard solutions of naltrexone

100 mg of naltrexone was weighed in to 100 ml volumetric flask and dissolved in distilled water and then dilute up to the mark with distilled water to get a concentration of 1000 µg/ml. The solution was diluted accordingly to get a concentration of 100 µg/ml and was kept as the stock solution. The prepared stock solution was diluted with distilled water solution to get working standard solutions of concentrations 2-10 µg/ml.

2.4. Preparation of standard stock and working standard solutions of bupropion

100 mg of Bupropion was weighed and transferred in to 100 ml volumetric flask and dissolved in distilled water and then make up to the mark with distilled water to get a concentration of 1000 µg/ml. The solution was diluted accordingly to get a concentration of 100 µg/ml and was kept as the stock solution. The prepared stock solution was diluted with distilled water solution to get working standard solutions of concentrations 2-10 µg/ml.

2.5. Simultaneous equation method (Method-I)

For multi-component system consisting of two components X and Y, each of which absorbs at the $\lambda_{\max}$ of the other, λ_1 is being the wavelength of maximum absorbance of X ($\lambda_{\max}$) and λ_2 being the wavelength of maximum absorbance of Y ($\lambda_{\max}$). In such cases, it can be possible to determine both the components by simultaneous equation method. Standard stock solutions (1 mg/ml) of Naltrexone and Bupropion were prepared by dissolving 25 mg of each in 25 ml distilled water, which was further diluted with distilled water to get the working standard solution (100 µg/ml) of Naltrexone and Bupropion. From this, suitable aliquots are taken and diluted with distilled water to get 10 µg/ml of Naltrexone and Bupropion. The absorption spectra of all the solutions were recorded between 200 and 400 nm. The absorbances were measured for Naltrexone and Bupropion at 204.2 nm (λ_1) (maximum absorbance of Naltrexone) and 210 nm (λ_2) (maximum absorbance of Bupropion), respectively. Wavelengths 204.2 nm and 210 nm were selected for the formation of simultaneous equation (Figure 2). The absorbances were measured at the selected wavelengths. Marketed formulation of Naltrexone and Bupropion were procured. The absorbance of final sample solution was measured against distilled water as blank at 204.2 nm and 210 nm for quantisation of Naltrexone and Bupropion, respectively. The amount of Naltrexone and Bupropion present in the sample solutions were determined by solving the following simultaneous equations.

$$C_x = (A_1 a_{Y2} - A_2 a_{Y1}) / (a_{X1} a_{Y2} - a_{X2} a_{Y1})$$

$$C_y = (a_{X1} A_2 - a_{X2} A_1) / (a_{X1} a_{Y2} - a_{X2} a_{Y1})$$

Where, A_1 , A_2 are abs. of components, a_{X1} , a_{X2} are absorptivity of first drug at λ_1 and λ_2 respectively; a_{Y1} , a_{Y2} are absorptivity of second drug at λ_1 and λ_2 respectively.

Figure 2 Overlay spectra of Naltrexone and Bupropion

2.6. Dual Wavelength Method

The utility of dual wavelength data processing programme is to calculate the unknown concentration of a component of interest present in a mixture containing both the component of interest and an unwanted interfering component by the principle of difference in the absorbance between two points on the mixture spectrum. This is directly proportional to the concentration of the component of interest, independent of the interfering components. The prerequisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows same absorbance whereas the component of interest shows significant difference in absorbance with concentration. Based on this criterion, two wavelengths 233.4 nm and 264 nm were selected as λ_1 and λ_2 for the estimation of Naltrexone as Bupropion shows the same absorbance at these wavelengths. Similarly, wavelengths 257 nm and 283.4 nm were selected as λ_3 and λ_4 for the estimation of Bupropion as Naltrexone shows the same absorbance at these wavelengths. For calibration curve, the standard stock solutions of these drugs were diluted in the concentration range of 2-10 $\mu\text{g/ml}$ (2, 4, 6, 8 and 10 $\mu\text{g/ml}$) for Naltrexone and Bupropion. Absorbances were recorded at selected wavelengths.

2.7. Absorbance ratio method (Q-analysis method)

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length e.g. two dilutions of the same substance give the same absorbance ratio A_1/A_2 . In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in mixture by the absorbance ratio method, absorbance is measured at two wavelengths, one being the λ_{max} of one of the components (λ_2) and the other being a wavelength of equal absorptivity of the two components (λ_1), i.e., an iso-absorptive point. A series of standard solutions of both Naltrexone and Bupropion in the concentration range of 2-10 $\mu\text{g/ml}$ were prepared in distilled water and the absorbances of these solutions were measured at 232 nm (iso-absorptive point), 204 nm (λ_{max} of Naltrexone) and 210 nm (λ_{max} of Bupropion). Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs.

The concentration of two drugs in mixture was calculated by using the following equations:

$$C_x = (Q_m - Q_y / Q_x - Q_y) \times (A_1 / ax_1)$$

$$C_y = (Q_m - Q_x / Q_y - Q_x) \times A_1 / ay_1$$

Where, $ax_1 = A$ (1%, 1 cm) of Naltrexone at 232 nm

$ay_1 = A$ (1%, 1 cm) of Bupropion at 232 nm

$ax_2 = A$ (1%, 1 cm) of Naltrexone at 204 nm

$ay_2 = A$ (1%, 1 cm) of Bupropion at 210 nm

A_1 and A_2 are the absorbances of mixture at 204 nm and 210 nm

C_x and C_y are the concentrations of Naltrexone and Bupropion in gm/100 ml respectively in sample solution.

$$Q_m = A_2 / A_1$$

$$Q_x = ax_2 / ax_1 \text{ and } Q_y = ay_2 / ay_1$$

2.8. Derivative Spectroscopic Method

Derivative spectrophotometry involves the conversion of a normal spectrum (fundamental, zeroth order or D spectrum) to its first, second or higher derivative spectrum by differentiating absorbance of a sample with respect to wavelength λ for higher accuracy.

$$[A] = f(\lambda): \text{zero order}$$

$$[dA/d\lambda] = f(\lambda): \text{first order}$$

$$[d^2A/d\lambda^2] = f(\lambda): \text{second order}$$

The strong positive & negative bands with maximum and minimum at same wavelength of an absorption band as inflection point in absorbance band governs the odd (first & third) derivative spectrum whereas the strong positive & negative band with minimum or maximum at same wavelength as $\lambda_{\max}$ of absorbance band governs the even (second & fourth) derivative spectrum.

Number of bands = Derivative order + 1

The amplitude (D) is directly proportional to the concentration of analyte provided Beer's law is obeyed by D^o spectrum.

In first order derivative spectroscopy, zero crossing point for both drugs is found and the wavelengths are selected in a manner such that at the zero crossing of one drug, the other drug should show substantial absorbance.

The obtained zero order absorption spectra of Naltrexone and Bupropion were converted to first order derivative spectra (Figure 3) by using transformation mode. After observing the first order derivative spectra, zero crossing points of drugs were selected for the analysis of other drugs. The first wavelength selected was 264 nm for Naltrexone (zero crossing) and second wavelength selected was 310 nm for Bupropion (zero crossing).

Figure 3 First derivative overlay spectrum of Naltrexone and Bupropion

Calibration curve for Naltrexone at 310 nm (zero Crossing for Bupropion) and Bupropion at 264 nm (zero Crossing for Naltrexone) was developed to calculate the concentration of sample.

3. Validation of developed methods

3.1. Linearity

A stock solutions were prepared by dissolving 50 mg of the drugs in 50 ml of mobile phase. Then from these stock solutions dilutions of various concentration from 2 to 10 $\mu\text{g/ml}$ were prepared for Naltrexone and Bupropion respectively. Each dilution was analysed in series to construct the calibration curves. Absorbance of each dilution was noted and plotted against the concentration of each dilution.

3.2. Accuracy

Accuracy was determined by calculating %recovery of Naltrexone and Bupropion by standard addition method. The pre-analyzed sample solutions (4 $\mu\text{g/ml}$ of Naltrexone and Bupropion) were spiked with standard drug solutions at three different levels- 50, 100 and 150 %. The resulting mixtures were reanalyzed using the proposed method. The experiment was conducted in triplicates accuracy was reported as % recovery.

3.3. Precision

Precision of the proposed method was calculated by conducting intermediate precision.

3.3.1. Intra-Day Precision

The intra-day precision was determined by estimating the corresponding absorbance of the drug solution (in triplicates) three times on the same day.

3.3.2. Inter-Day Precision

The inter-day precision was established by analysing the drug solution (in triplicates) on three different days.

3.3.3. Analyst-Analyst

The analyst to analyst precision was established by analysing the drug solution by different analyst.

The standard deviation, %relative standard deviation and estimated concentrations based on standard curve were reported for each set of data.

3.4. Robustness

Robustness of the developed method was determined by analysing the drug solution in triplicates by varying the wavelength (± 2). Robustness is reported in %RSD.

3.4.1. LOD and LOQ

Detection limit and Quantitation limit of the drug is calculated by using the calibration curve standards. Detection limit and Quantitation limit were calculated from the equation $3.3\sigma/S$ and $10\sigma/S$ respectively, where σ is the standard deviation of y-intercept and S is the slope of the calibration curve.

3.4.2. Specificity

The specificity of the developed method was seen by analyzing solutions containing excipients and pure drug and demonstrating that the result is unaffected by the presence of the excipients present in it.

3.4.3. Assay

20 tablets were taken and weighed accurately. Then the tablets are crushed to powder. The weight of tablet contents equivalent to 100 mg of Naltrexone and Bupropion was calculated and were taken into 100 ml volumetric flask. 50 ml of distilled water was added to it and soicated for 10 mins and diluted upto the mark to prepare 1 mg/ml solutions. From these solutions, 10 $\mu\text{g/ml}$ dilutions of the drugs were prepared and their absorbances were taken. From the absorbance of the drug solutions, the amounts of each drug in the sample solutions were computed. The results were compared with the label claim of Naltrexone and Bupropion in tablet dosage forms. From the results the average %Assay was calculated.

4. Results and discussion

4.1. Linearity

The calibration curves (Figure 4) drawn by using the proposed method were found to be linear in the range (2-10 $\mu\text{g/ml}$ Naltrexone and Bupropion). Table 1 shows the calibration data with regression coefficient and %RSD was found to be less than 2.

Figure 4 Calibration curves of analytes

Calibration curves of A. Naltrexone and B. Bupropion

4.2. Accuracy (recovery)

Accuracy of the methods was determined by using standard addition method and %recovery was found in the range 96-101% and %RSD was within the range for both drugs. Table 1 shows the results of accuracy studies.

Table 1 Summarized results of linearity, accuracy, LOD, LOQ, %assay and Precision

Parameters	Simultaneous Equation		Dual Wavelength		Iso-bestic Point		First Order Derivative	
	Naltrexone	Bupropion	Naltrexone	Bupropion	Naltrexone	Bupropion	Naltrexone	Bupropion
Detection Wavelength	204.2 nm	210 nm	233.4 nm 264 nm	257 nm 283.4 nm	204.2 nm 232 nm	210 nm 232 nm	310 nm	264 nm
Linearity and range	2-10 µg/ml	2-10 µg/ml	2-10 µg/ml	2-10 µg/ml	2-10 µg/ml	2-10 µg/ml	2-10 µg/ml	2-10 µg/ml
Correlation coefficient	0.999	0.999	0.998	0.999	0.982	0.999	0.999	0.999
Slope	0.076	0.076	0.012	0.028	0.135	0.0279	0.076	0.076
Intercept	0.003	0.003	0.004	0.001	3.17	0.002	0.003	0.003
Accuracy	100.21	100.77	99.19	100.04	99.98	100.12	98.67	99.67
Limit of detection	0.13	0.11	0.13	0.11	0.13	0.11	0.13	0.11
Limit of quantitation	0.39	0.35	0.39	0.35	0.39	0.35	0.39	0.35
% Assay	100.9±0.49	97.3±0.20	98.0±0.45	100.4±0.48	99.1±0.42	84.7±0.71	101.40±1.78	95.36±5.15
Precision								
Intra-day (n=3)	97.63±0.80	97.10±1.02	96.00±0.88	97.13±0.83	97.13±0.83	96.43±5.36	97.13±0.83	96.00±0.88
Inter-day (n=3)	87.66±10.41	92.63±7.42	95.36±5.15	101.40±1.78	81.53±17.38	87.36±14.31	101.40±1.78	95.36±5.15
Analyst-Analyst	101.40±0.12	99.33±0.66	100.16±0.76	100.86±2.70	101.0 ±0.81	99.23 ±0.88	100.86±2.70	100.16±0.76

4.3. Limit of Detection and Limit of Quantification

LOD and LOQ were calculated from the calibration curves of the drugs itself and the results are shown in Table 1.

4.3.1. Assay

%Assay of the formulation was calculated by three different techniques and %recovery was found in the range 96-101% and %RSD was within the range for both drugs. Table 1 shows the results of %assay studies.

4.4. Precision

Intra-day precision was assessed by analyzing the drug at 3 different times in the same day. The method passed the test as the %RSD was found to be less than 2. Inter-day precision was assessed by analyzing the drug for three different days. The method passed the test as the %RSD was found to be less than 2. Analyst to analyst Precision was conducted by two different analysts at the same experimental conditions. Results are shown in Table 1.

4.5. Robustness

Robustness studies were performed by varying the detection wavelength (± 2). The method was found to be robust. Results are shown in Table 2.

Table 2 Results of robustness studies

λ_{max} (± 2 nm)	Concentration ($\mu\text{g/ml}$)	Observed absorbance			Mean	SD	%RSD
Naltrexone							
204.2+2	10	0.91	0.9	0.88	0.896	0.0152	1.7
204.2-2		0.9	0.91	0.9	0.903	0.0070	0.78
Bupropion							
210+2	10	0.324	0.333	0.331	0.329	0.0047	0.47
210-2		0.330	0.329	0.337	0.332	0.0043	0.43

4.6. Result of specificity

Specificity studies were performed by spiking the formulation and standard drug using two tailed unpaired t-test.

5. Conclusion

The proposed methods based on simultaneous equation, dual wavelength, absorption ratio and first derivative methods can be used for the simultaneous estimation of Naltrexone and Bupropion in their bulk and pharmaceutical dosage form. The proposed methods are accurate, reproducible, repeatable, linear, precise, selective, reliable and simple to perform. Also, no separation step is required. These results indicate that the proposed method may find practical applications as a quality-control tool in the simultaneous analysis of the two drugs in combined dosage forms in quality-control laboratories.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest is exist.

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