Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(11):429-435

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Simultaneous analysis of naltrexone hydrochloride and bupropion hydrochloride in bulk and dosage forms by RP-HPLC-PDA method

V. Srikalyani, M. Tejaswi, P. Srividya and Buchi N. Nalluri*

Department of Pharmaceutical Analysis, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, AP, India

---

ABSTRACT

A simple, specific and accurate RP-HPLC method was developed for the simultaneous analysis of Naltrexone hydrochloride (NTX) and Bupropion hydrochloride (BUP) in bulk and dosage forms. A Phenomenex C_{18} column (250 x 4.6mm; 5 µm) with mobile phase containing 0.05% v/v triethylamine (pH 6.5): acetonitrile (45:55% v/v) was used at isocratic mode and eluents were monitored at 215 nm. The retention times of NTX and BUP were 3.4 min and 12.7 min respectively and showed a good linearity in the concentration range of 40-200 µg/mL of NTX and 10-50 µg/mL of BUP with a correlation coefficient of 0.999 and 0.998 respectively. The average recoveries were found to be 98.60% and 98.90% respectively for NTX and BUP. The proposed method was validated as per ICH guidelines and successfully applied to the simultaneous estimation of NTX and BUP in bulk and dosage forms.

Keywords: Naltrexone hydrochloride, Bupropion hydrochloride, Simultaneous estimation, Phenomenax C_{18} column, RP-HPLC, PDA detection, Validation.

INTRODUCTION

NTX is chemically, (5α)-17-(cyclopropylmethyl)-4, 5-epoxy-3, 14-dihydroxymorphinan-6-one hydrochloride used in the treatment of alcoholism and as narcotic antagonist [1,2]. BUP is chemically, (±)-2-(tert-butylamino)-1-(3-chlorophenyl) propan-1-one, an atypical antidepressant and smoking cessation aid. It acts as a norepinephrine and dopamine reuptake inhibitor as well as α3 β4 nicotinic receptor antagonist [3,4]. Presently, combination of these two drugs as a controlled release tablet is under clinical trials for the treatment of obesity.

Literature survey reveals that few methods have been reported on analysis of NTX and BUP individually in pharmaceutical dosage forms and several HPLC methods have been described for the determination of NTX and BUP in biological samples [5-10]. However, there were no validated HPLC-UV/PDA methods reported so far for the simultaneous estimation of NTX and BUP in combination. Hence, the main objective of the present investigation was to develop a validated RP HPLC PDA method for the simultaneous analysis of NTX and BUP in bulk and dosage forms.

EXPERIMENTAL SECTION

Reagents and Chemicals

NTX and BUP were gift samples from Sun Pharma, India. Acetonitrile, water and triethylamine were purchased from E. Merck, Mumbai, India. All the solvents and reagents were of HPLC grade. NODICT® (containing 50 mg of NTX) and BUPRON® (containing 150 mg of BUP) tablets (manufactured by Sun Pharma, Sikkim) were locally purchased.

Equipment

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, and SPD-M20A PDA detector. Data acquisition was carried out using LC solutions software. The chromatographic analysis was performed on Phenomenex C_{18} column (250 × 4.6mm; 5µm).

Chromatographic Conditions

Mobile phase consisting of 0.05% v/v triethylamine (adjusted to pH 6.5 with orthophosphoric acid): acetonitrile (45:55% v/v) was used in isocratic mode and the mobile phase was filtered through nylon disc filter of 0.45µm (Millipore) and sonicated for 3 min in ultrasonic bath before use. The flow rate was maintained at 1.2 mL/min with an injection volume of 20 µL. Eluents were monitored at 215 nm and the separation was achieved at ambient temperature.

Preparation of Stock and Standard Solutions

The stock solutions of NTX and BUP of concentration 1mg/mL were prepared by dissolving 10 mg of each drug separately in a 10mL volumetric flask using methanol as a diluent. The working standard solutions in the concentration ranging from 40-200 µg/mL of NTX and 10-50 µg/mL of BUP were prepared by appropriately diluting the stock solutions with acetonitrile as diluent.

METHOD VALIDATION

The method was validated according to the ICH guidelines.

Specificity

Specificity studies were carried for pure drugs by comparing the standard and sample solutions with blank (diluent) and placebo. Specificity is a measure of the degree of interference in the analysis of the complex sample mixtures such as analyte mixed with the formulation excipients or the known impurities. Specificity of the method was carried out by comparing chromatogram of the placebo (in house made) with that of the sample for checking any interference peaks.

Linearity

A linear relationship was evaluated across the range of the analytical procedure with a minimum of five concentrations. A series of standard dilutions of NTX and BUP were prepared over a concentration range of 40-200µg/mL (40, 80, 120, 160 & 200µg/mL) and 10-50µg/mL (10, 20, 30, 40 & 50µg/mL) respectively from stock solutions and injected in triplicate. Linearity was evaluated by a plot of peak areas as a function of analyte concentration, and the test results were evaluated by appropriate statistical methods where by slope, intercept, and regression (R^2) & correlation coefficients (R) were calculated.

Precision

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the standard concentration of NTX (80µg/mL) and BUP (20µg/mL). Less than 2% RSD for peak areas indicates the precision of the developed method.

Accuracy

Accuracy was established across the specified range of the analytical procedure. Accuracy (recovery) of the method was tested by spiking 80, 100 and 120% of NTX (80µg/mL) and BUP (20µg/mL) standard concentrations. These solutions were analyzed by developed method in triplicate. The % recovery and the % RSD were calculated at each level of addition.

Robustness
Robustness of the method was determined by altering the experimental conditions such as flow rate and wavelength intentionally. The chromatographic parameters viz., capacity factor, tailing factor, theoretical plate number and % assay were recorded. The flow rate of the mobile phase was maintained at 1.2mL/min. To study the effect of flow rate, the flow rate was changed by ±20% and the effect of wavelength was studied by changing wavelength by ±5nm.

Detection and Quantification Limits
LOD and LOQ were determined by calibration curve method. Standard solutions of NTX and BUP were prepared in the range of 40-200µg/mL and 10-50µg/mL injected (20µL) in triplicate. Average peak area of two drugs was plotted against concentration. LOD and LOQ were calculated by using following equations: LOD = (3.3 ×σ)/m; LOQ= (10.0×σ)/m (Where, σ is the standard deviation of the responses and m is mean of the slopes of the calibration curves).

System suitability
System suitability was carried out by injecting a standard concentration (40µg/mL of NTX and 10µg/mL of BUP) at different injection volumes in the range of 10-50µL. The system suitability test parameters were noted and % RSD was calculated.

Assay
As no combined dosage forms were presently available in the market, individual tablets of NTX (containing 50mg) and BUP (containing 150mg) were used in these studies. Powder blend (from 10 tablets of each brand) equivalent to 10 mg of NTX and BUP were separately weighed and transferred to a 10 mL volumetric flask. 5 mL of methanol was added to solubilize and was sonicated for 5 min and volume was made up to the mark with methanol. The solutions were centrifuged and the supernatant were filtered using nylon disposable syringe filter (13 mm, 0.45 µm). Aliquots of the filtrate of concentration 80µg/mL and 20µg/mL of NTX and BUP were prepared and analyzed in triplicate. The amount present in the each tablet was calculated by comparing the areas of standard NTX and BUP with that of the sample.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions
The present investigation was carried out with a view to develop a RP HPLC PDA method for the simultaneous estimation of NTX and BUP in bulk and dosage forms. Initial trials were carried out with Phenomenex C_{18} column (250 x 4.6 mm; 5µm) using 0.05% v/v formic acid and methanol (60:40% v/v) at a flow rate of 1.2mL/min as mobile phase and acetonitrile as the diluent. The quantification was carried out at 215nm. Under these conditions NTX was eluted at 3.16 min and BUP at 4.88 min. The NTX was almost eluted with the solvent front.

In the other trial, methanol was replaced with acetonitrile and mobile phase combination of 80:20% v/v at a flow rate of 1.2mL/min and under these conditions, NTX was eluted at 3.61 min and BUP at 6.70 min. However, the resolution between the solvent front and the NTX peak was not satisfactory. In further trials, 0.05% v/v of formic acid was replaced with 0.05% v/v triethylamine (adjusted to pH 6.5 with orthophosphoric acid) keeping acetonitrile as organic modifier at a ratio of 50:50% v/v and the flow rate is 1.2mL/min. Under these conditions the NTX was eluted at 5.14 min and BUP at a longer retention of 20.23 min.

Finally, the mobile phase was maintained at a ratio of 45:55% v/v of 0.05% v/v triethylamine and acetonitrile with a flow rate of 1.2mL/min in order to achieve proper resolution of the both NTX and BUP peaks respectively. Under these conditions the NTX and BUP peaks were eluted at 3.47 min and 12.34 min respectively. Both the peaks were symmetrical and tailing factor was within the limits. For quantitative analytical purpose wavelength was set at 215 nm, which provided better reproducibility without interference. The peak purity indices were also found to be greater than 0.9999 and this indicating peak purity of the both the drugs SAL and AMB used in the analysis. A sample chromatogram of NTX and BUP were given in Figure 1 along with UV spectra.

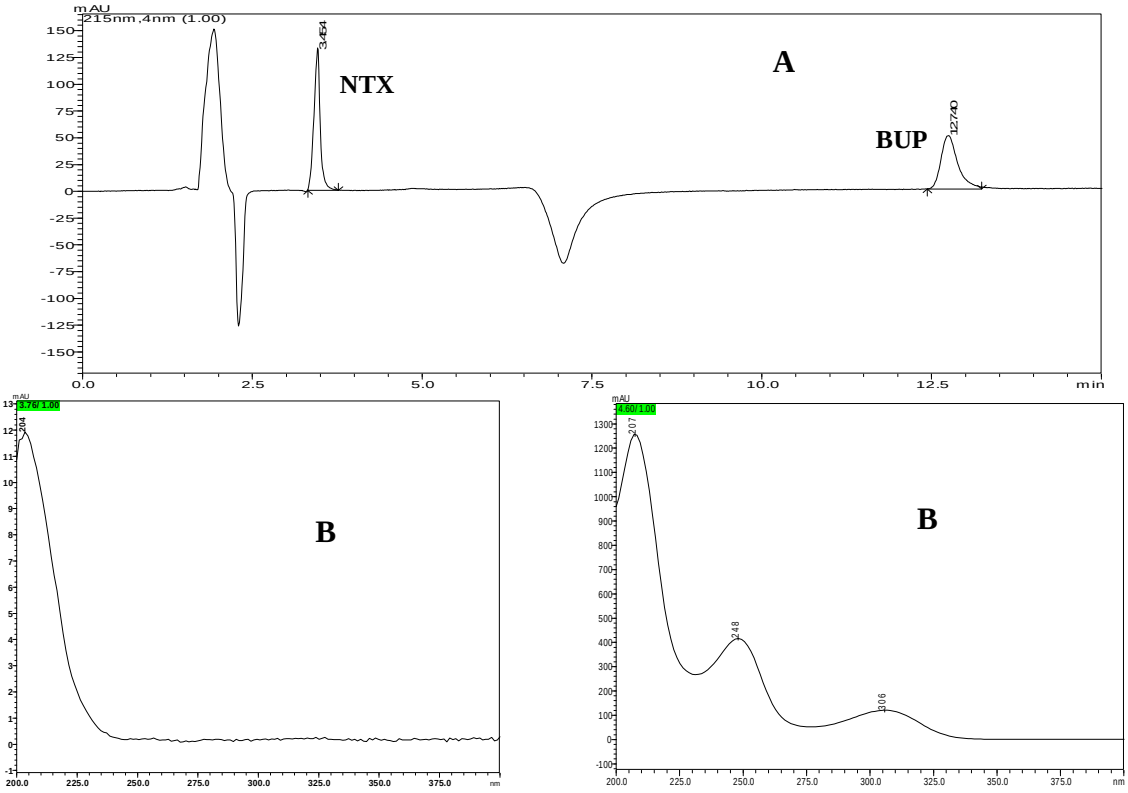

Fig. 1 Standard Chromatogram of NTX (40µg/mL) and BUP (20µg/mL) mixture (A); and (B) UV spectra of NTX and BUP

METHOD VALIDATION

Specificity

The specificity of the method was established by injecting the solutions of diluent, placebo, standard and test sample (formulation) individually to examine any interference. From the 3D plots of placebo and test samples shown in Figure 2, it can be inferred that there were no co-eluting peaks at the retention time of NTX and BUP. These results show that peak of analyte was pure and the excipients in the formulation did not interfere with the analysis. The peak purity indices for sample and standard were found to be greater than 0.999 and this confirms specificity of the method.

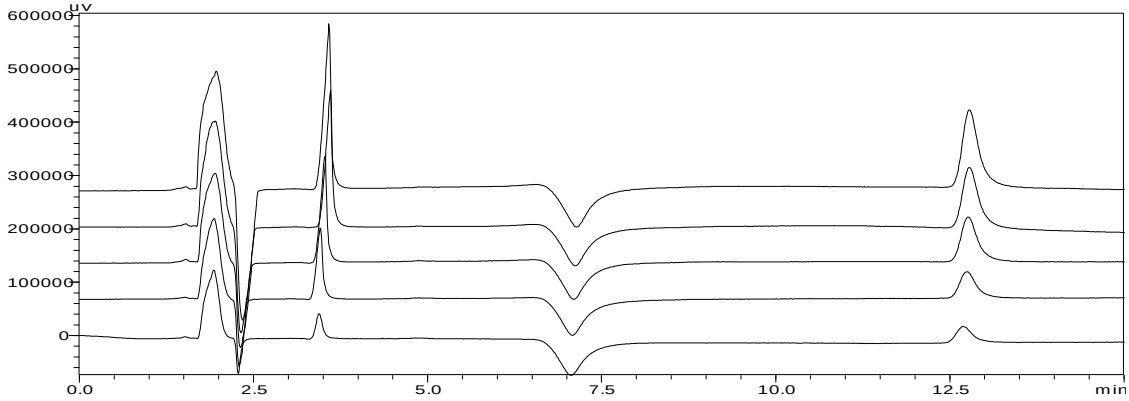

Fig. 2 Overlaid NTX (40-200µg/mL) and BUP (10-50µg/mL) standard chromatograms

Linearity

A linear relationship evaluated across a concentration range 40-200µg/mL of NTX and 10-50µg/mL of BUP in triplicate (n=3). The concentration range was selected based on 80, 100 and 120% of the test concentration for assay. Peak area and concentration data was subjected to least square regression analysis. The correlation coefficients (R) were found to be 0.999 and 0.998 respectively for NTX and BUP and indicate a good linearity within the concentration range selected. The data of the calibration curve was given in Table 1 and chromatograms were shown in Fig 2.

Precision

Precision studies were carried out in terms of repeatability. Repeatability of standard application was assessed by using six replicates of concentration at 80µg/mL of NTX and 20µg/mL of BUP and the data was given in Table-2. The % RSD was found to be less than 2 for peak areas; this shows the closeness of the data values to each other, indicating the method was precisied.

Accuracy

Accuracy of the proposed method was ascertained by performing recovery studies using the standard addition method by spiking the known quantities of standards at 80, 100, and 120% to the test solution of 80µg/mL of NTX and 20µg/mL of BUP. The analyte peak is evaluated by 3D plots of the chromatogram in order to confirm the existence of components at 3.4 min and 12.7 min elution time of NTX and BUP respectively and shown in Figure 3. The recoveries were found to be 99.15-101.73%, 99.04-100.04%, and 98.24-99.50% at 80, 100 and 120% respectively for NTX and BUP. These results indicate a good accuracy of the method to that of the labeled claim. The obtained recovery results were given in Table 1.

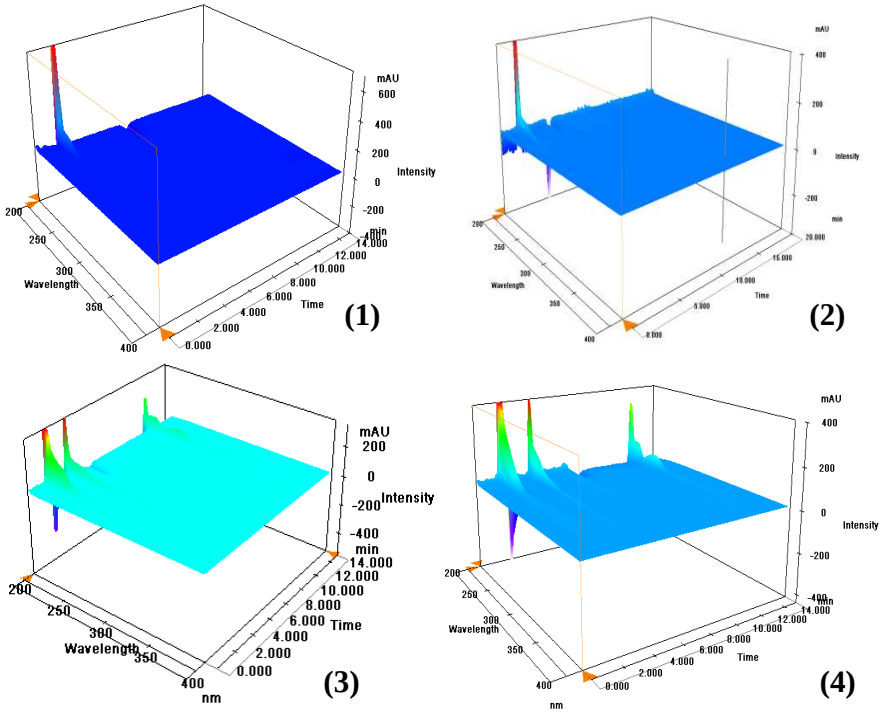

Fig 3. 3D plots of Placebo (1), Diluent (2), Standard (3) and Sample (4)

Robustness

As part of the robustness, a deliberate change in the flow rate and wavelength was made to evaluate the impact on the method. Retention times were significantly changed with flow rate and no change in the retention time was observed in wavelength change. However % assay values were within limits and these results indicated minor changes in the flow rate and wavelength didn't affected the assay results. The results were given in Table 2.

Table 1. Linearity, Precision and Accuracy data

Validation data of NTX and BUP			
	Parameters	**NTX**	**BUP**
Linearity(n=3)	**Range**	40-200µg/mL	10-50µg/mL
	Regression equation	Y=11632x-1152	Y=53152x-7026
	Regression coefficient(R^2)	0.999	0.998
	Correlation coefficient(R)	0.998	0.997
Accuracy(n=3)	**% Level of Addition**	**Mean % Recovery (RSD)**	**Mean % Recovery (RSD)**
	80	99.59 (1.95)	100.03 (1.08)
	100	99.12 (0.89)	101.07 (1.02)
	120	99.82 (0.64)	100.09 (0.86)
Precision(n=6)		**NTX**	**BUP**
System Precision	**Average Peak area of the standard sample (RSD)**	839193 (1.94)	9404 (1.13)
Method Precision	**Average peak area of the Assay sample (RSD)**	855010 (0.81)	8891 (0.16)

Table 2: Robustness data

Drug	Parameter range	Retention time (min)	Theoretical plates (N)	Tailing factor	Capacity factor (k)	% Assay
			Wavelength (nm)			
NTX	210	3.5	5274.5	1.0	1.1	98.5
	215	3.54	6079.5	1.0	0.8	100.2
	220	3.5	5317.1	1.3	0.8	100.3
BUP	210	12.7	14152.6	1.4	5.6	101.2
	215	12.7	14886.4	1.3	5.6	100.3
	220	12.7	14086.0	1.3	5.6	99.15
			Flow rate (mL/min)			
NTX	1.0	4.5	5282.3	1.42	0.99	98.6
	1.2	3.7	4895.4	1.37	0.94	101.2
	1.4	3.4	4906.2	1.39	1.1	99.8
BUP	1.0	13.8	13827.6	1.47	5.54	101.8
	1.2	12.7	13040.7	1.39	5.62	100.2
	1.4	11	13633.8	1.38	5.79	98.2

Detection and Quantification Limits

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve. LOD for NTX and BUP was found to be 0.326 and 0.436 µg/mL respectively. LOQ for NTX and BUP was found to be 0.990 and 1.321 µg/mL respectively.

System suitability:

System suitability studies were carried out by injecting a 40µg/mL standard of NTX and 10µg/mL of BUP respectively at injection volumes ranging from 10-50µL. With increment of injection volumes, the % RSD for tailing factor and theoretical plate number were calculated and were found to be within limits.

Assay

Assay of NTX and BUP in tablets was performed by the proposed method and the % assay was calculated as an average of 3 determinations. These results indicate that the present HPLC method can be successfully used for the simultaneous assay of NTX and BUP respectively in bulk and dosage forms. The assay values were found to be within the limits and the data was given in Table 3.

Table 3: Assay Results (n=3)

Formulation	Labeled amount(mg)	Amount found(mg) (Mean ± SD)	%Assay	% RSD
NODICT®	50 mg	47.69±0.11	98.2	1.19
BUPRON®	150 mg	146.51±0.34	98.9	1.2

Stability of the Stock Solution

The stability of the stock solution was determined by analyzing the samples under refrigeration (8±1°C) at different time intervals up to 48hrs. The % variation in assay values at different time intervals were found 0.825 for NTX and

0.546 for BUP from the initial zero time interval solution, thus indicating that the solutions were stable for a period of 48hrs when stored at 8±1°C.

CONCLUSION

The proposed RP-HPLC-PDA method was validated as per International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of NTX and BUP using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The method provides selective and simultaneous quantification of NTX and BUP without interferences from diluent and placebo. Overall, the proposed method is highly sensitive, reproducible, reliable, rapid and specific and can be employed in quality control for simultaneous estimation of NTX and BUP in bulk and in dosage forms that may available in near future.

Acknowledgements
The authors are thankful to the Sun Pharma India for providing gift samples and the Siddhartha Academy of General and Technical Education, Vijayawada, for providing facilities to carry out this research work**.**

REFERENCES

[1] The United State Pharmacopoeia, 26th Revision, Rockville MD: US Pharmacopoeial Convention Inc, **2004**; 1277.
[2] The Merck Index: An encyclopedia of chemicals, drugs, and biological. 13th Edition, Merck research laboratories, **2001**; 1141.
[3] The United State Pharmacopoeia, 26th Revision, Rockville MD: US Pharmacopoeial Convention Inc, **2004**; 279.
[4] Indian Pharmacopoeia, Vol - I, **2010**; 1748.
[5] N Billa; KK Peh; KH Yeun. *J Chromatogr B*., **1997**, 701, 140-145.
[6] M Sabine; SW Toennes; F Gerold; W Jakel. *J Pharm Biomed Anal*., **2004**, 35, 169-176.
[7] HY Yan. *Talanta*., **2007**, 71(4), 1553-1559.
[8] S Prakash; A Faheem; A Sonawane; D Gowrisankar. *Der Pharmacia Lettre*., **2010**, 2 (2), 294-299.
[9] I Bhattacharyya; SP Bhattacharyya; S Sen. *Int J Pharm Technol*., **2010**, 2 (2), 224-232.
[10] RT Sane; M Francies; SMA Khedkar; A Moghe; P Patil. *Indian Drugs.*, **2003**, 40(4), 231.