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RP-HPLC Method Development and Validation for the Determination of Bupropion Hydrochloride in a Solid Dosage Form.

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ABSTRACT

A simple, rapid, precise, sensitive, cost effective and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of bupropion HCl in a solid dosage form. The proposed RP-HPLC method was developed on phenomenex Luna^{R} (C-18 250 × 4.6 mm, 5µm) column with a mobile phase composed of methanol : acetate buffer (pH-6) in the ratio of 80:20 in isocratic mode at a flow rate 1.0 mL/min. Detection was carried out using UV detector at 251 nm. The retention time under optimized chromatographic conditions was found to be 3.19 minutes. A good linear response was observed in range of 15-90 µg/mL. The interday and interaday precision was found to be within limits. The proposed method has adequate sensitivity, reproducibility and specificity for determination of bupropion HCl in solid dosage form. LOD (Limit of detection) and LOQ (Limit of quantitation) for bupropion HCl were found to be 5 µg/mL and 15 µg/mL.

INTRODUCTION

Bupropion, chemically (±)-2-(*tert*-butylamino)-1-(3-chlorophenyl)-propan-1-one, previously known as amfebutanone is an atypical antidepressant and also used as an smoking cessation aid. It acts as a nor-epinephrine and dopamine reuptake inhibitor as well as α_3, β_4 receptor antagonist [1,2,3]. Bupropion hydrochloride belongs to the chemical class aminoketone. Effectiveness of bupropion hydrochloride in long-term use (more than 6 weeks) has not been systematically evaluated in controlled trials. Therefore, the physician who elects to use Bupropion hydrochloride sustained release tablets for extended periods should periodically re-evaluate the long-term usefulness of the drug for the individual patient. The drug is available in tablet form (100mg, 150mg and 450mg) and is official in any pharmacopoeia. A few methods of analysis of Bupropion hydrochloride have been reported using different techniques such as validated liquid chromatographic method for the quantitation of Bupropion hydrochloride in human plasma using liquid-liquid extraction, solid-phase extraction liquid chromatography-mass spectrometry [4,5], spectrophotometric estimation of Bupropion hydrochloride in bulk drug and dosages forms. Most of these methods are considered tedious. The HPLC methods using the most commonly available columns and detector like UV are preferred. The present study describes the determination of Bupropion hydrochloride in pharmaceutical dosage forms by using RP-HPLC, C18 column with UV detectors.

MATERIALS AND METHODS

Instruments

The HPLC system consisted of HPLC 10AT-VP of Shimadzu Corp. Ltd.,Kyoto, Japan. LC-20AT Pump of prominence series, UV detector SPD-20 A of Shimadzu Corp.Ltd.,Kyoto, Japan and manual injection of 20 µL fixed loop, Rheodyme, USA was used. The data was analyzed by using spinchrome CFR chromatography software and separation was carried out on phenomenex C18 column (250 mm × 4.6 mm, 5 µm), Japan. Weighing of reagents was done on microbalance of Mettler Toledo International, Switzerland and Spinix vortex was used for the mixing of samples.

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Working standard and Reagents

A gift sample of pure Bupropion HCl was provided by Intas lab. Ahmedabad. Water and methanol used for preparation of mobile phase were of HPLC grade, procured from Qualigens fine chemicals, Mumbai. All others chemicals and solvents used were of Analytical grade. Bupropion HCl tablets were procured from local pharmacy.

Preparation of mobile phase

In the mobile phase, mixture of methanol and ammonium acetate buffer (pH 6.0) was used in the ratio of 80:20. Firstly ammonium acetate buffer was prepared by dissolving 200 g of ammonium acetate in 600 mL of water and then 8.2 mL of glacial acetic acid was added to it. pH was adjusted, if necessary, using 10 M ammonia or 5 M acetic acid and finally diluted with water to 1000 mL.

Afterwards 200 mL (20%) of ammonium acetate buffer (pH 6.0) was transferred into a 1000 mL reagent bottle and 800 mL (80%) of methanol was added to it, mixed well and sonicated for 10 minutes. The mobile phase so obtained was filtered through 0.45µm filter and stored at room temperature.

Preparation of Calibration plot

Stock solution (50µg/mL) of bupropion was prepared by dissolving in mobile phase. The stock solution of was further diluted with mobile phase to give the series of standard dilutions for preparation of calibration curve. The different concentration of sample solution was injected in the concentration range of (15, 30, 45, 60, 75, 90µg/mL) of drug substance and the calibration curve is given in fig 1.

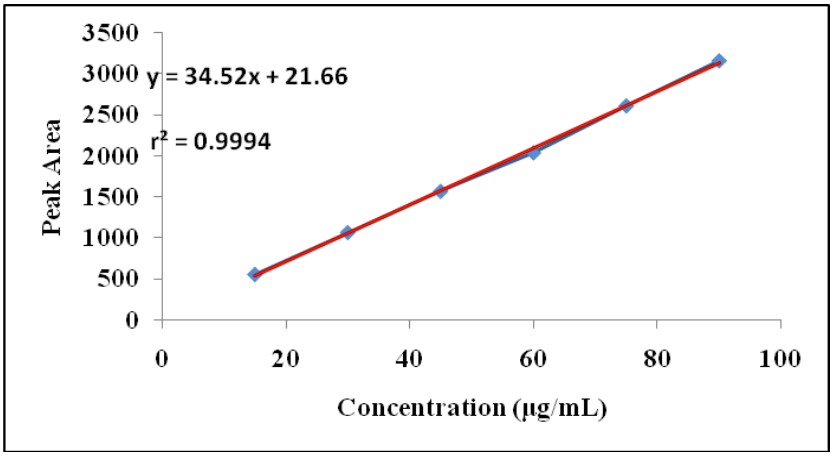

Figure 1: Linear calibration curve of Bupropion HCl

Sample solution preparation

For analysis of commercial formulation, 20 tablets containing Bupropion HCl (100 mg) were taken and crushed to a fine powder. The powder equivalent to 50 mg of Bupropion HCl (204 mg) was accurately weighed and transferred into a 100 mL volumetric flask. Sufficient amount of mobile phase was added, sonicated and remaining volume was made up to the mark with mobile phase. Aliquot of 5mL from this solution was transferred to a 50 mL volumetric flask and volume was made up with mobile phase to get final concentration of 50 ppm. Prepared solution was finally filtered through 0.45µm filter.

Method Validation Studies

Linearity

Linearity of method was determined by preparing standard solutions at different concentration levels. The calibration curve of drug over the concentration range 15-90 µg/mL was plotted and its linearity was evaluated by linear regression analysis.

Specificity/ Selectivity

Specificity of the method was evaluated by analyzing the blank, standard and sample solution. One injection of 20 µL blank solution was given onto HPLC system to check for the interference from the mobile phase as well as gradient. Then two injections of standard solution containing 50 µg/mL drug were injected and the peak purity of analyte peaks was monitored [6]. (Fig 2).

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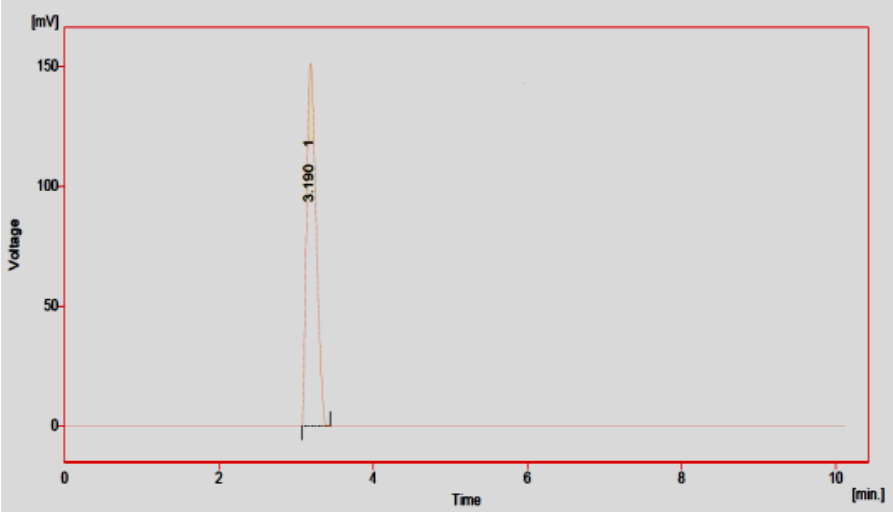

Figure 2: Typical chromatogram of standard (Bupropion HCl)

Precision

The precision of the method based on intra-day repeatability was determined by giving six replicate injections of same concentrations. Precision was calculated for both system as well as method.

System precision

The system precision was determined by giving six replicates injections of the same concentration (50µg/mL). The peak responses were measured and percent relative standard deviation (%RSD) of the series of measurements was calculated [7].

Method precision

The method precision was determined by giving six injections of different sample prepared from same homogenous blend of marketed tablet of bupropion HCl (50µg/mL). The peak responses were measured and percent relative standard deviation (%RSD) of the series of measurements was calculated [7].

Accuracy/Recovery

To ensure the reliability and accuracy of the method, and to study the interference of formulation additives recovery studies were carried out using standard addition method at different concentration levels (80%, 100% and 120%). The sample solution was analyzed by adding a specified amount of the analyte (standard) to the sample, thus increasing its concentration. The resulting increase in peak area due to addition of the standard amount was noted [8,9]. Hence, the concentration or amount of the analyte (Bupropion HCl) in the original sample (X mg/mL) may be calculated using following formula:

$$X = x(A_1) / (A_2 - A_1)$$

Where, X is the amount of drug found after recovery studies (mg)
x is the amount of standard (Bupropion HCl) added to the sample solution (mg)
A_1 is the peak area of sample solution before standard addition
A_2 is the peak area of sample solution after standard addition

Ruggedness

For ruggedness studies, the sample solution was analyzed under a variety of conditions including different laboratories analysts, instruments, reagents, days etc. The results were expressed in terms of % RSD.

RESULTS AND DISCUSSION

Linearity

The data of mean peak area vs. drug concentration at various concentration levels were evaluated by linear regression analysis. Results are shown in table 1 and the calibration curve obtained after plotting drug concentration vs. area is shown by Fig.1.

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The linear regression analysis demonstrated that the chromatograph response obtained from drug was highly linear (r^2-0.9994) in concentration range 15-90 µg/mL of Bupropion HCl.

Precision

The results of system precision and method precision portrays that developed method has sufficient precision. Small percentages of relative standard deviations (%RSD) indicate that method has an acceptable level of precision. The values of %RSD for both precision should be less than 2%.

The values of % Relative standard deviation (% RSD) for system and method precision were found to be 0.58 % and 0.34% respectively. The low percent (%) of RSD indicated that proposed method has good precision.

Table 1: Linear calibration data for Bupropion HCl

S.No.	Concentration (µg/mL)	Mean Peak Area	% RSD
1.	15	555	0.36
2.	30	1067	0.19
3.	45	1567	0.13
4.	60	2040	0.05
5.	75	2612	0.08
6.	90	3163	0.06

$y=34.54X+30.33$, $r^2= 0.9994$

Accuracy/Recovery

The results for accuracy studies vividly reveal that the given method is quite accurate for determination of given drug candidate. The data for accuracy were expressed in terms of percent recoveries of Bupropion HCl. Percentage recovery of drug was found to be 99.98%, 99.95%, 99.71% at 80%, 100% and 120% concentration levels respectively. The high percent of recovery indicates that no interference was produced due to the excipients used in the formulation. Hence the developed method was found to be accurate. The result of recovery is shown in table 2.

Ruggedness

In ruggedness studies, mean area was found to be 1250 with % RSD of 0.83 % as indicated in table 3. The short value of % RSD indicates that the proposed method was sufficiently rugged and no significant changes were observed in results with different analyst, different column and on different day.

Table 2: Recovery data for Bupropion HCl

Level of recovery (%)	Amount added (mg)	Area counts (A_2) (mV*sec)	Amount found (mg)	% Recovery	% Mean recovery ± % RSD
80	40	2478	39.87	99.68	99.98 ± 0.74
		2464	40.33	100.81	
		2481	39.77	99.44	
100	50	2467	50.28	100.57	99.95 ± 0.61
		2482	49.68	99.36	
		2475	49.96	99.92	
120	60	2481	59.66	99.44	99.71 ± 0.34
		2479	59.76	99.60	
		2473	60.05	100.08	

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Table 3: Ruggedness data for Bupropion HCl

Sample code	Area counts (mV*sec)		
	Inj-1	Inj-2	Mean
S1	1248	1256	1252
S2	1252	1260	1256
S3	1262	1258	1260
S4	1244	1248	1246
S5	1248	1250	1249
S6	1240	1238	1239
Mean area count			1250
SD			10.49
% RSD			0.83

CONCLUSION

A simple, selective, rapid and reliable HPLC method was developed for the determination of Bupropion HCl in pure form and in tablets. The optimized analytical conditions and solvent system provided a good separation for Bupropion HCl with a short run time. The method was validated and it demonstrated a wide linear range, a good precision and accuracy. The % RSD for all the validated parameters was found within the acceptance criteria. Developed method was applied to determine the assay of the formulation with excellent percent of recoveries and the assay result obtained was in fair agreement.

Thus, in view of the results obtained, it can be inferred that the developed RP-HPLC method is useful in routine laboratory analysis with a high degree of accuracy and precision and can be successfully applied for the routine quantitative estimation of Bupropion HCl in tablet dosage form.

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