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Research Article

Method Development and Validation of Anti-depressant Bupropion by RP-HPLC

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Abstract:
Bupropion is an atypical antidepressant and smoking cessation aid. The drug is a non-tricyclic antidepressant and differs from most commonly prescribed antidepressants such as SSRIs, as its primary pharmacological action is thought to be norepinephrine-dopamine reuptake inhibition. A new sensitive and rapid HPLC method was developed for the determination of buproprion in bulk and pharmaceutical dosage forms; it was validated according to ICH and FDA guidelines. The HPLC analysis was performed on the Alliance Waters e2695 Separations Module system equipped with a Waters X – Bridge C-18 5µm, 4.6 X 150 mm column 5, with a mixture of Acetonitrile: Ammonium bicarbonate (5mM) pH-9 adjusted with 1% Ammonium hydroxide (50:50, %v/v). Flow rate was 1 ml/min quantification was done by PDA detector at 254nm. The proposed method showed linearity in the concentration range of 50 to 250 ppm for Bupropion. The linear regression equation of Bupropion was found to be y = 6E+06x + 91344 and correlation coefficient value was found to be 0.997 indicating a high degree of linearity for the drug. The proposed method was stable except the basic condition during analysis. The stability of the analyte solution was determined at interval of 1^{st}, 2^{nd}, 3^{nd}, 4^{th} and 5^{th} day.
The low values of %recovery and %C.V. showed that the method is precise within the acceptance limit of 5% (according to ICH guidelines).The limit of detection **(**LOD) of Bupropion was 0.5 ppm and limit of quantification (LOQ) was 2.0 ppm respectively. The proposed method showed excellent linearity, accuracy, precision, specificity, robustness, LOD, LOQ, and system suitability results within the acceptance criteria.

Introduction

HPLC is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phase through the column, and a detector that shows the retention times (RT) of the molecules. RT varies depending on the interactions between the stationary phase, the molecules being analyzed and different type of solvents were used.[1] Analytical method validation

guarantees that different HPLC analytical techniques will produce trustworthy and reproducible findings. It is an important step in the development of new dosage forms since it offers details on accuracy, linearity, precision, detection, and quantitation limits. The ICH guideline states that "the objective of an analytical procedure's validation is to show that it is suitable for its intended purpose." Providing the necessary authorities with the validation data is now required during the medication development process. ICH and USP guidelines are among the rules for validating analysis methods [2–5].

A review of the literature turned up a few methods for determining buproprion in both bulk drugs and pharmaceutical preparations [6-11]. In this study, a new sensitive and quick HPLC technique was created and validated in accordance with ICH and FDA regulations for the measurement of buproprion in pharmaceutical dosage forms.

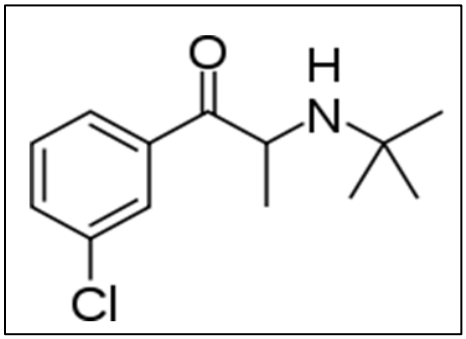

Figure 1: Stucture of Bupropion

2. Materials and Methods

2.1. Instrumentation

Alliance Waters e2695 Separations Module system was used for liquid chromatography method development and validation, equipped with a pump (Waters 2774 pump), and a Waters X – Bridge C-18 5µm, 4.6 X 150 mm column, and the detector consisted of UV/VIS operated at 277 nm. Empower Pro was used for data processing and evaluation.

2.2. Chemicals and Reagents

A pharmaceutical grade sample of buproprion (assigned purity 99.4%) was purchased from Balaji drugs (Ahmedabad). Acetonitrile HPLC grade and ammonium carbonate were purchased from Merck (Merck Serono Amman, Jordan). The double distilled water was obtained from a local pharmaceutical company.

2.3. Chromatographic Conditions

The mobile phase was prepared by dissolving 1.0 gm ammonium carbonate in 1000 ml water. From the previous solution, 450 ml was mixed with 550 ml of acetonitrile. Prior to use the mobile phase was filtered through 0.45 μm membrane filters and degassed by sonication for 10 min.

2.4. Preparation of Standard Solution

A standard solution of buproprion was prepared by dissolving an accurately weighed amount of buproprion (10mg) in 100ml of the mobile phase.

2.6. Method Validation

The method was validated as per ICH and FDA guidelines, and the validation parameters included specificity, linearity, range, accuracy, precision, sensitivity (LOQ and LOD), and robustness [9, 12].

2.6.1. Specificity

Specificity is one of the significant features of HPLC, and it refers to the ability of the analytical method to discriminate between the analyte and the other components in the complex mixture [13]. Specificity of the method was evaluated by injecting 10 μl

solutions of standard, sample, blank, and placebo separately.

2.6.2. Linearity

To evaluate the linearity and range of the method, different standard solutions were prepared by diluting the standard stock solution with the mobile phase in deferent concentrations of buproprion: 50, 100, 150, 200 and 250 μg/ml. Three injections from each concentration were analysed under the same conditions. Linear regression analysis was used to evaluate the linearity of the calibration curve by using the least square linear regression method.

2.6.3. Sensitivity

Limit of detection (LOD)/limit of quantitation (LOQ) of buproprion were determined by analysing different solutions of buproprion and measuring the signal-to-noise ratio. The limit of detection (LOD) is the concentration that gives a signal-to-noise ratio of approximately 3 : 1, while the limit of quantification (LOQ) is the concentration that gives a signal-to-noise ratio of approximately 10 : 1 with %RSD () of less than 10%.

2.6.4. Accuracy

The accuracy of the assay method was determined by recovery studies at three concentration levels (50%, 100%, and 150%), i.e., 50, 100, and 150 μg/ml, and three samples from each concentration were injected. The percentage recovery of added buproprionand RSD were calculated for each of the replicate samples.

2.6.5. Precision

The system precision and method precision (repeatability) of the proposed methods were determined by several measurements of standard solution and sample solution, respectively [19–22]. System precision was established by ten measurements of the standard solution at the 100% concentration levels on the same day. Method precision was established by six assay determinations of the sample solution at the 100% concentration levels on the same day [13]. The RSD of obtained results was calculated to evaluate repeatability results.

2.6.6. Robustness

Robustness of the method was verified by applying minor and deliberate changes in the experimental parameters, for example:(i)Column temperature: ±5°C(ii)Flow rate: ±0.2 mL/min(iii)Wavelength: ±3 nm(iv)Mobile phase composition, organic composition ±5%

Change was made to evaluate its effect on the method. Obtained data for each case was evaluated by calculating %RSD and percent of recovery.

2.6.7. Stability of Analytical Solutions

The stability of analytical solutions was determined by analysing the standard and sample preparations at 0 h and after one day in refrigerator and at ambient room temperature 30°C. Three injections from each solution were analysed, and the average of the peak and the RSD were calculated.

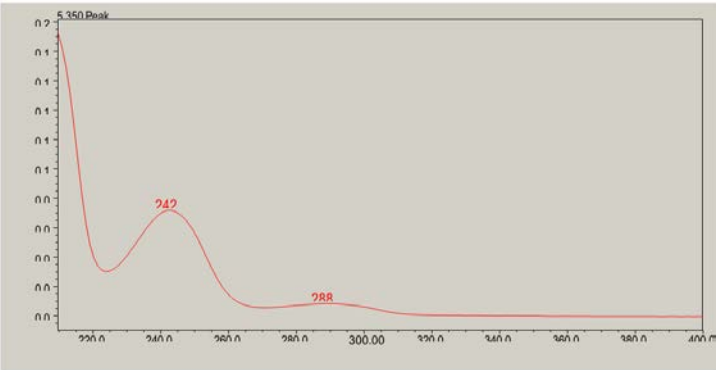

Figure 2: UV Spectra of Bupropion

Result and Discussion

Method development and validation

An acceptable separation was achieved by using buffer of pH 9.0 along with the organic phase in a gradient mode shown in Table 1. The developed method was validated with respect to linearity, precision, accuracy, LOD, LOQ and robustness.

Table 1: Gradient Program of Mobile Phase

TIME (min)	FLOW ml/min	BICARBONATE	ACETONITRILE
00.00	1.00	50%	50%
1.00	1.00	50%	50%
6.00	1.00	10%	90%
8.5	1.00	10%	90%
9.00	1.00	50%	50%
10.00	1.00	50%	50%

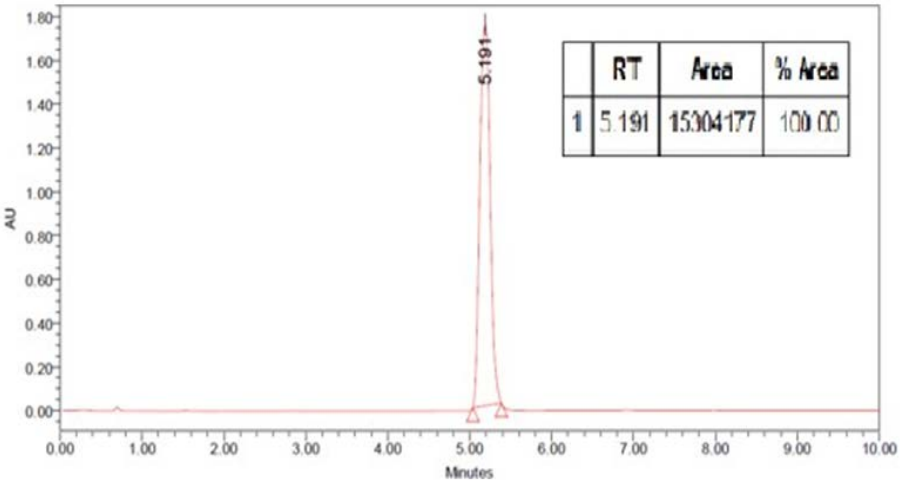

Figure 3: Chromatogram Shows the Response of Bupropion after AnalyticalMethod Development.

Method validation:

Linearity:

As shown in Figure 4, The response for the drug was found to be linear in the investigated concentration range. The values of slope and correlation coefficient (R 2) were 913448 and 0.997, respectively. The linearity data are shown in Table 2.

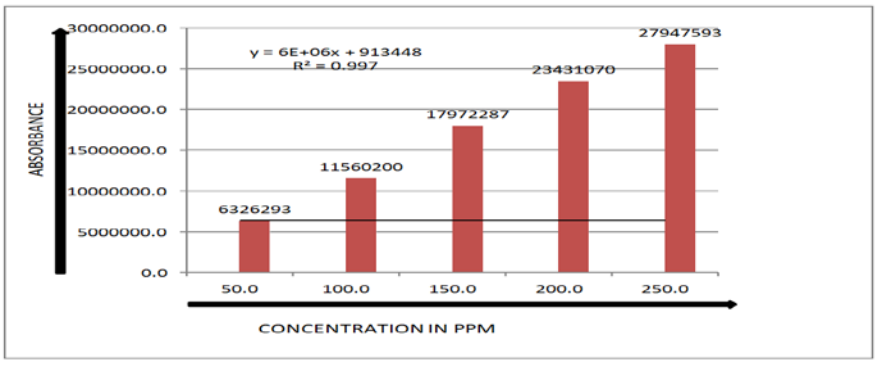

Figure 4 Linearity graph b/w absorbance & concentration

Table 2: Linearity data

CONC IN PPM	Area	R^2
50.0	6326293	0.997
100.0	11560200	
150.0	17972287	
200.0	23431070	
250.0	27947593	

Accuracy:

In order to determine accuracy we observe % recovery for that three different sample of Bupropion is prepared one is control sample having 150 ppm concentration other is spiked sample in which 2 mg of drug is added in the standard solution and third one is the standard stock solution of Bupropion five runs of each were made and % recovery of control sample and spiked sample were calculated As shown in Table 4, the percent recovery ranged between 97% to 93%.

Table 3: Area of Standard, Control & Spiked Solution for % Recovery Study

Area			% Recovery	
Standard Sample	**Control sample**	**Spiked Sample**	**Control sample**	**Spiked sample**
410804	438380	432345	93%	95%
431406	435478	445451	99%	96%
425147	429656	437496	98%	97%
400601	415298	427483	96%	93%
425913	432065	445451	98%	95%
Total =2093871	2150877	2188246		
Mean = 418774.2	430175.4	437649.2	96.8%	95.2%
S.D.= 12697.7	8952.799	7953.522		
% RSD= 3.03	2.08	1.81		

Precision:

Intra-day precision data are enlisted in Table 4. The RSD values ranging from 1.04% to 1.82% for intra-day precision studies respectively, confirmed that the method was sufficiently precise.

Table 4: Precision Data

Run	Area	
	150 ppm	**200 ppm**
First	12004445	20617355
Second	12737660	19541293
Third	12261417	24275707
Fourth	12261417	24285864
Fifth	12728013	20340924
Sixth	12663329	19541293
Total	87477055	128602436
Average	12496722	21433739
SD	315538.6	224654.5
% RSD	1.822816	1.04813

Limit of detection (LOD) and Limit of quantitation (LOQ):

The LOD and LOQ was calculated with standard deviation of response and slope of calibration curve. The LOD and LOQ for bupropion HCl was found to be 1.70 ng mL -1 and 2 ng mL -1, respectively.

Robustness:

In robustness, method was checked by changing the chromatographic condition like flow rate (+0.1 and - 0.1ml/min) and temperature (+5 and -5nm). After changes it was observed that the peak symmetry and peak response was found to be adequate. The data obtained in robustness study are shown in Table 5, indicating that the test method was robust in variable condition.

Table: 5 Robustness Parameter of Bupropion

Robustness Data Table				
S. No.	**Temp**	**Flow**	**R.T.(min)**	**Area**
1	25^0C	0.9ml/min	5.526	6985492
2		1.0 ml/min	5.346	3938026
3		1.1 ml/min	4.817	11994681
4	30^0C	0.9ml/min	5.545	9668377
5		1.0 ml/min	5.199	12316977
6		1.1ml/min	4.803	12508314
7	35^0C	0.9ml/min	5.611	5457399
8		1.0 ml/min	5.187	10490406
9		1.1ml/min	4.803	12373100

Conclusion:

The developed method was found to be rapid, accurate, precise and reproducible. The method was linear over a wide concentration range, economical and utilizes a mobile phase which can be easily prepared. All these factors make this method suitable for the estimation of bupropion. The developed method can be used for the routine analysis and assay of bupropion in quality control laboratories.

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