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Analytical Technique Optimization and ICH-Directed Verification of a Reverse Phase HPLC Approach for Quantification of Bupropion Hydrochloride

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Abstract

This method was developed for retention time, peak resolution and baseline response using Bupropion Hydrochloride under optimized conditions as validation. The method resulted in two well separated, well defined peaks, a minor peak at 7.5 minutes 12 mV and a main peak at 11.52 minutes 150 mV. At the same time, the baseline has remained stable during the chromatographic run and the fluctuations of noise are very small (0.5 ± 0.4 mV) between 5 and 11 min (0.3 ± 0.2 mV) between 14 and 17 min (0.4 ± 0.2 mV), which indicates that the method was robust and instrumentally consistent. The chromatogram showed sharp, symmetrical peaks and consistent retention times and acceptable signal to noise ratios in the satisfactory analytical method to be relied upon. The developed method is found to be suitable for routine quantitative analysis and quality control applications in pharmaceutical laboratories, and this study provides confirmation of suitability.

Keywords: Bupropion Hydrochloride, RP-HPLC, technique optimization, technique verification, ICH standards, analytical chemistry, pharmaceutical analysis

INTRODUCTION

The development of an analytical method is important to quality, safety, and effectiveness of pharmaceutical compounds [1]. Advanced liquid chromatography (HPLC) has become an essential instrument in the pharmaceutical sector for exact and truthful estimation of major active pharmaceutical ingredients (APIs) [2]. Among the many easy concentrations, RP-HPLC or reverse part HPLC is conventional, as it mixes under the facing, high review, and assemble a good of settle down analytes with large extent calculation proficient nook word [3]. Bupropion Hydrochloride is an atypical antidepressant used commonly for treatment of major depressive disorder and smoking cessation [4]. This development of a quick and validated analytical method for its quantification is essential because of its increasing therapeutic relevance and for strict quality control measures employed during its manufacture [5]. Although a few advanced techniques, like LC-MS and HPTLC, have been described, these are associated with higher operational costs and with instrumentation which may not be available in routine quality control laboratory [6]. Principal aim

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of the present study is described to develop a simple, rapid, cost effective, and reliable RP-HPLC method for the estimation of Bupropion Hydrochloride from its bulk. Finally, the method is also validated in accordance with International Council for Harmonisation (ICH) guidelines to determine important analytical properties, such as linearity, precision and accuracy, sensitivity, and robustness [7]. With this approach, this not only guarantees suitability of the method for routine analysis, but it also plays a part for the guarantee of regulatory requirements for pharmaceutical quality assurance [8]. The chemical structure of Bupropion Hydrochloride is shown in Figure 1.

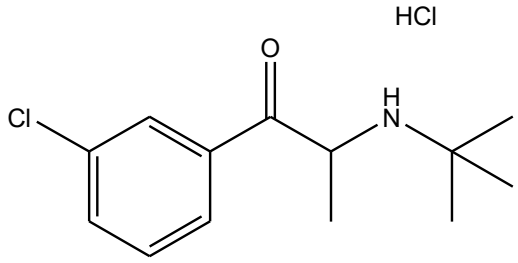

Figure 1. Structure of Bupropion Hydrochloride.

MATERIALS AND METHODS

Chemicals and Reagents

Wockhardt Ltd., Aurangabad, India gave bupropion hydrochloride (analytical grade) as a gift *sample*. Merck Specialities Pvt. Ltd. (Mumbai, India) procured HPLC grade methanol. potassium dihydrogen phosphate and orthophosphoric acid (analytical reagent grade) was purchased *from* Qualigens Fine Chemicals Pvt. Ltd. The Milli-Q water purification system was used throughout the study.

Chromatographic Conditions

The Bupropion Hydrochloride was separated in a Kromasil C18 column of dimensions 250 × 4.6 mm packed with 5 µm particles. Gradient system of phosphate buffer (pH 4.0) and methanol was used for mobile phase. The flow rate set was 1.0 mL/min and the injection volume was 20 µL. The drug was detected at a light absorption of 252 nm, which is the maximum absorbance for the drug. The analysis was done at ambient column temperature with a total run time of 12 minutes for each sample that would provide adequate separation and sharp peak resolution [9].

Instruments and Equipment

Chromatographic analysis was carried out on the PerkinElmer HPLC system fitted with UV-visible detector, gradient pump, Rheodyne manual injector (20 µl injection loop). Isolation was carried out using a Kromasil C18 column (250 mm × 4.6 mm, 5 µm particle size). Data handling and collection were executed using Total Chrome Navigator software version 6.3.1. The additional equipment consisted of a PerkinElmer UV–visible spectrophotometer, Labindia digital pH meter and Shimadzu AUX220 analytical balance as well as micropipettes from Erba Biohit.

Preparation of Mobile Phase

The moving solution comprised of phosphate buffer (10 mM, pH 4.0 adjusted with orthophosphoric acid) and methanol. The ingredients were deaerated and passed through a 0.45 µm membrane filter being used [10].

Preparation of Standard Solution

Following the preparation of a stock Bupropion Hydrochloride solution by dissolution of 10 mg quantity in 10 mL of methanol, producing a strength of 1000 µg/mL, a series of serial dilution of Bupropion Hydrochloride were performed to prepare the working standard of 10, 50, and 100 µg/mL Serial dilutions of stock solution in the strength span of 20–120 µg/mL were made in the mobile phase and used as working standard solutions [11].

Preparation of Buffer

The phosphate buffer solution in the mobile phase was prepared by dissolving 1.36 grams of potassium dihydrogen phosphate (KH_2PO_4) in 1 liter of ultrapure Milli-Q water and diluted to 1 liter to make the buffer solution as 10 MM. The acidity level of the buffer was precisely set to 4.0 using a standardized digital pH meter and diluted orthophosphoric acid. After formulation, the buffer solution was further passed through a 0.45 µm membrane filter and subjected to ultrasonic waves to degas it to use in chromatographic analysis [12].

Detection of Wavelength

By using a UV visible spectrophotometer, the UV absorption spectrum for Bupropion Hydrochloride was recorded between 200 and 400 nm. As the detection wavelength for HPLC analysis, the λmax was found at 252.2 nm [13].

HPLC METHOD DEVELOPMENT AND VALIDATION

HPLC method development was carried out to develop an efficient, reliable, and reproducible analytical technique for the estimation of Bupropion Hydrochloride. Optimization of Different chromatographic factors, including moving phase composition, buffer acidity, liquid velocity, and sensing wavelength, were systematically optimized to achieve a sharp, well-separated, and symmetrical peak with minimal asymmetry [14]. Ultimately, a gradient elution system comprising phosphate buffer (pH 4.0) and methanol was established after multiple experimental attempts, with detection carried out at 252.2 nm. A thorough verification of the technique was conducted following the guidelines set by the International Council for Harmonisation (ICH Q2(R1)) [15]. Consequently, validation aspects, including proportionality, reproducibility, correctness, detection threshold (LOD), quantification threshold (LOQ), and resilience, were assessed. Critical evaluation was made for each parameter of the method so that it complies with regulatory standards and is suitable for routine analysis in pharmaceutical quality control setting (Figure 2) [16].

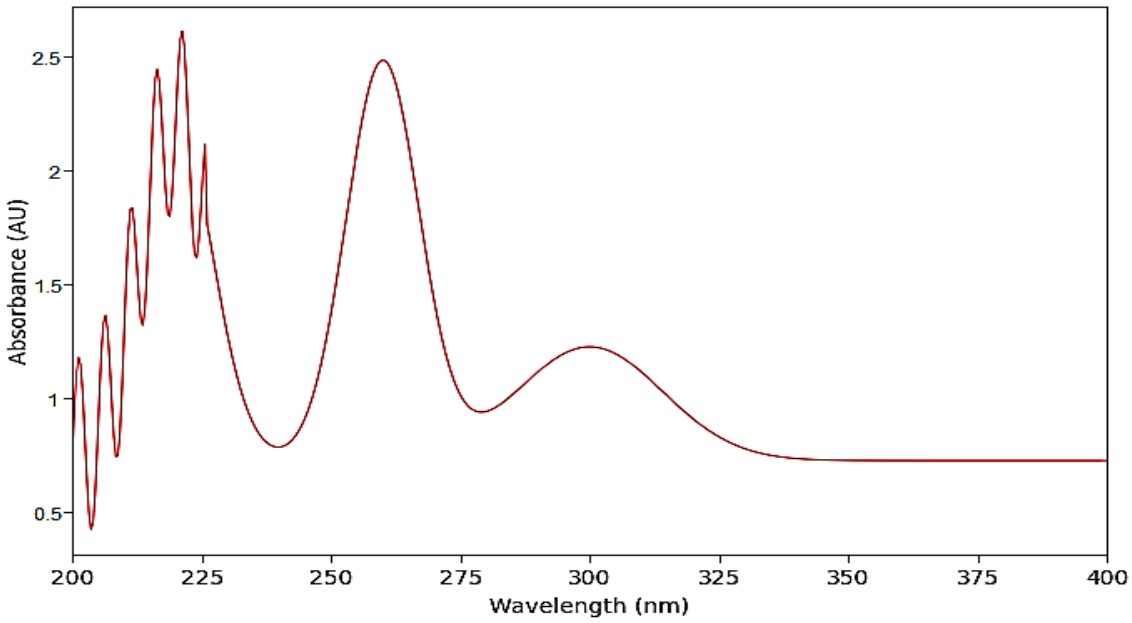

Figure 2. UV-Vis absorption spectrum.

RESULT AND DISCUSSION

Chromatographic Performance

The developed RP-HPLC method provided effective and sharp separation of Bupropion Hydrochloride with a well-resolved, symmetrical peak. The retention time was found to be approximately 11.52 minutes, confirming the suitability of the mobile phase composition and gradient program (Figure 3) [17].

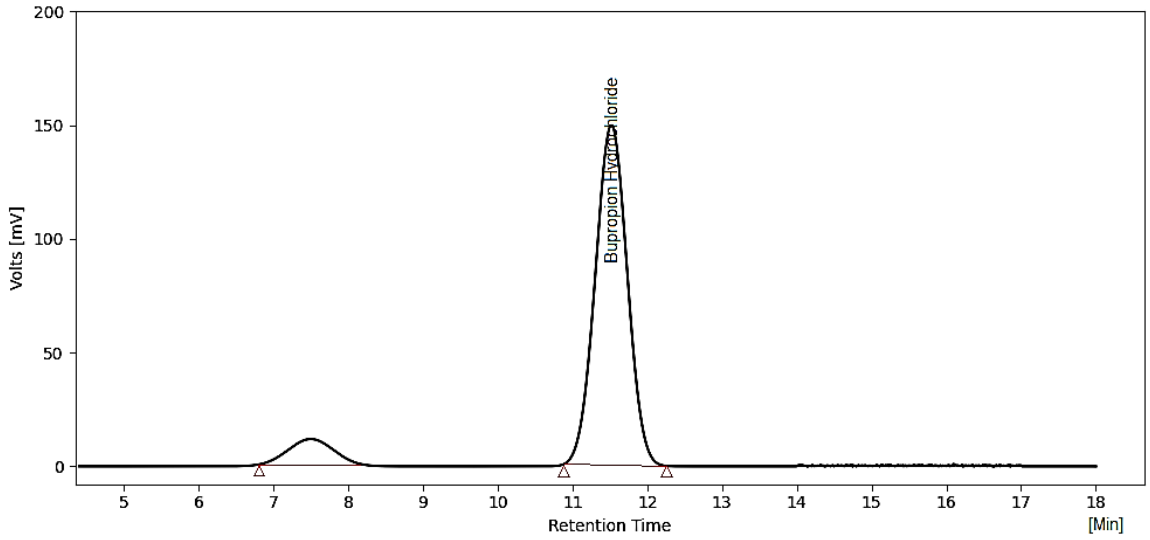

Figure 3. Chromatogram showing retention peak.

Linearity

The method demonstrated outstanding proportionality within the strength span of 20–120 µg/mL, with a correlation factor (R^2) of 0.9996, indicating a strong linear relationship between drug concentration and peak area. This confirms that the method is highly capable of accurate quantitative estimation over a wide range of concentrations. The high R^2 value demonstrates the excellent linear behavior of the method, which is a critical parameter in quantitative analytical procedures and indicating a strong linear relationship between concentration and peak area as shown in Figure 4 (Table 1).

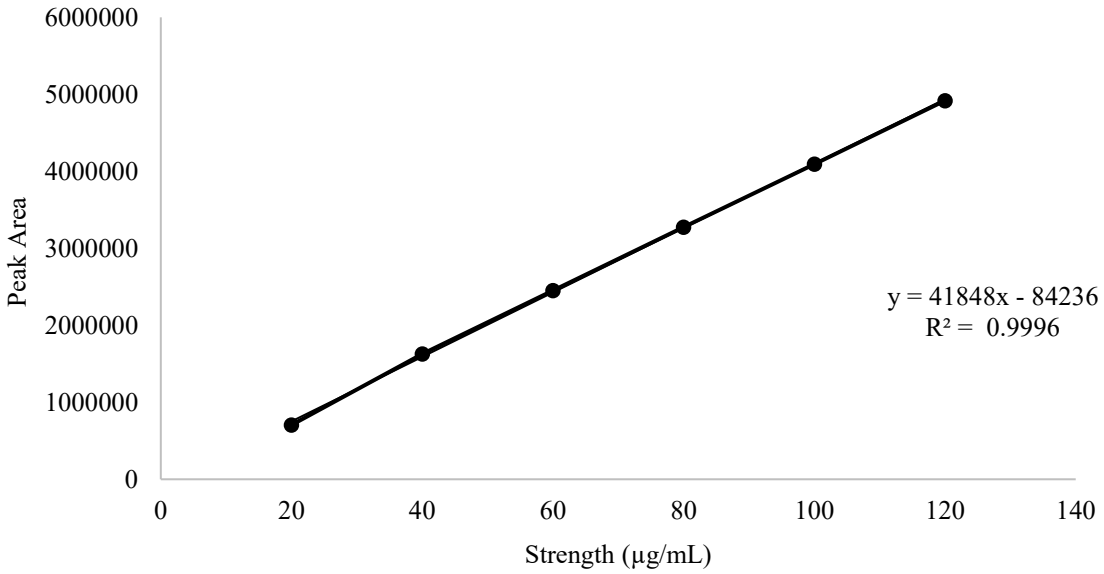

Figure 4. Calibration curve of Bupropion Hydrochloride.

CALIBRATION CURVE OF BUPROPION HYDROCHLORIDE

Precision

The method demonstrated excellent precision, with %RSD values for both same-day and different-day evaluations below 1.5%, well within the permissible range limits. This confirms that the method produces consistent and repeatable results under normal operating conditions. The low %RSD values indicate minimal variability and confirm that the method is precise and reproducible (Figure 5) (Table 2).

Table 1. Linearity parameters of Bupropion Hydrochloride.

Parameter	Updated Result
Concentration Range	20–120 µg/mL
Slope	41848.27
Intercept	–84235.67
Correlation Coefficient (R^2)	0.9996

Table 2. Modified precision parameters of Bupropion Hydrochloride.

Strength (µg/mL)	Same Day %RSD	Different Day %RSD
40	1.42	1.33
60	1.25	1.18
80	1.36	1.21

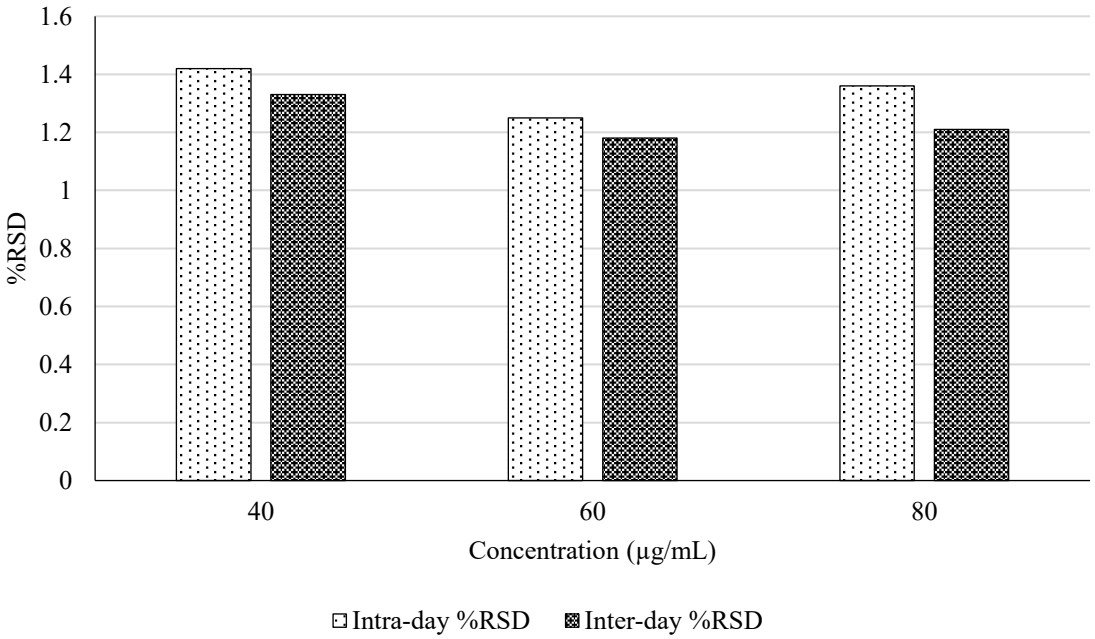

Figure 5. Comparative bar graph illustrating intra-day and inter-day precision (%RSD).

Accuracy

Three concentration levels were studied (i.e., 80%, 100%, and 120%), and for each strength level, three series of electrochemical evaluations were conducted. The findings revealed that the percentage recovery ranged from 99.27 to 100.96%, with %RSD below 1.5%, demonstrating the high precision of the technique. The elevated recovery rates and minimal %RSD confirm that the method effectively quantifies the drug content when introduced at different levels. This establishes the method's reliability and suitability for regular quantitative assessment in quality control facilities. Figure 6 presents the percent recovery results of Bupropion Hydrochloride at different spiked levels (80, 100, or 120%) and high accuracy response of the method (Table 3).

Table 3. Percent recovery of Bupropion Hydrochloride.

Level (%)	Amount Added (µg)	Amount Found (µg)	% Recovery	%RSD
80	80	79.84	99.80	1.28
100	100	100.96	100.96	1.12
120	120	119.12	99.27	1.35

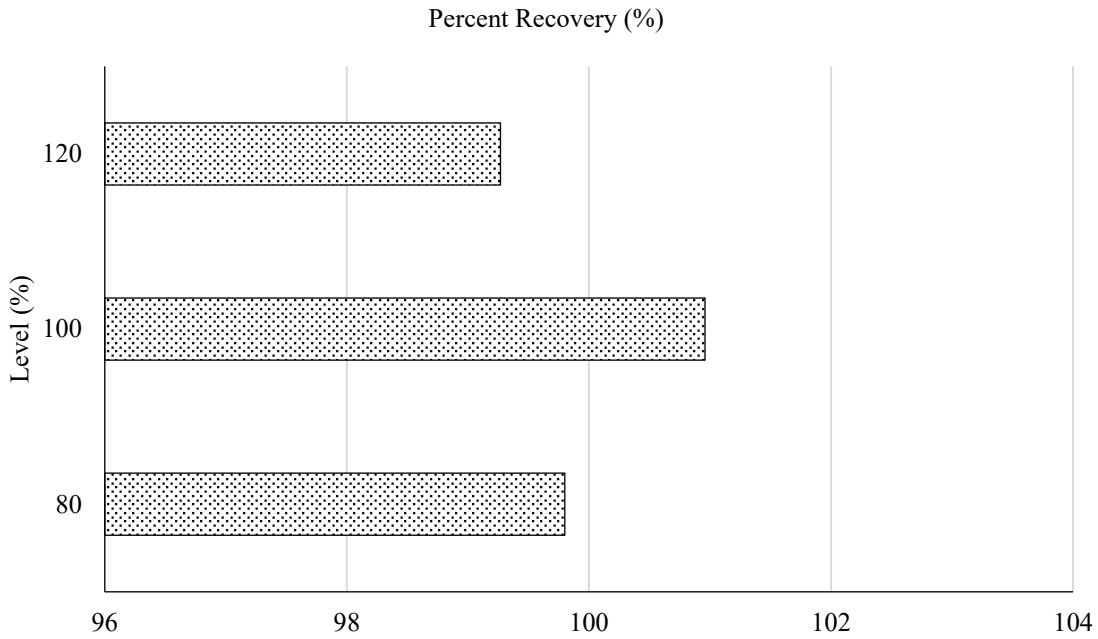

Figure 6. Percent recovery of Bupropion Hydrochloride.

Sensitivity

Variation measure and gradient of the calibration plot were utilized to determine the detection threshold (LOD) and quantification threshold (LOQ) and the sensitivity of the method was evaluated. It was found that the LOD is 3.91ng/mL and LOQ is 7.8 ng/mL. These values indicate that the method is very sensitive for detection of even very low concentrations of Bupropion Hydrochloride. Thankfully, such sensitivity is particularly useful when carrying out trace analysis or impurity detection (Table 4).

Table 4. Result of sensitivity parameters (LOD and LOQ).

Parameter	Value
Limit of Detection (LOD)	3.91 ng/mL
Limit of Quantitation (LOQ)	7.8 ng/mL

Robustness

Robustness was tested by deliberately varying chromatographic conditions, such as flow rate and detection wavelength. The %RSD values were consistently below 1.5%, confirming the method's stability and reliability under minor variations in operational parameters. The minor changes in flow rate and detection wavelength had no significant impact on peak area or retention time, indicating that the method is robust and reliable under slightly altered analytical conditions (Table 5).

Table 5. Result of robustness.

Factor Adjustment	Mean Area	%RSD
Flow Rate: 0.9 mL/min	7952482.21	1.21
Flow Rate: 1.1 mL/min	7937256.78	1.17
Wavelength: +2 nm	7969841.14	1.10
Wavelength: –2 nm	7941983.67	1.24

CONCLUSIONS

RP HPLC method employed in present study was successfully designed and verified for quantifying Bupropion Hydrochloride in raw drug form, offering a straightforward, reliable, precise, and resilient approach. A gradient moving phase of phosphate buffer (pH 4.0) and methanol was optimized to

achieve distinct peak separation and suitable dwell time. The technique was confirmed per ICH Q2(R1) standards for dependability and was found good with excellent linearity (R^2 = 0.9992), very high precision, excellent accuracy, and excellent sensitivity with the very low (LOD) and low (LOQ) values of the method, it showed good robustness as it performed the same well under deliberately varied chromatographic conditions. The attributes of the proposed method render it extremely well suited for use in the routine quality control analysis and batch release testing in pharmaceutical laboratories. Also, it proves to be a cost effective and easy to execute method for industrial scale use in the estimation of Bupropion Hydrochloride.

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Conflict of Interest

The authors declare that there is no conflict of interest associated with this research work.

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