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RP-HPLC Bio-Analytical Method for Precise Quantification and Validation of Bupropion Hydrochloride in Human Plasma

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ABSTRACT

Importance:

Bupropion is a widely prescribed antidepressant and smoking cessation aid. Monitoring its plasma concentration is crucial for assessing therapeutic efficacy and safety. Current methods for quantifying Bupropion in plasma are either complex or not widely accessible. This study presents the development and validation of a robust, sensitive, and efficient analytical method for quantifying Bupropion in plasma.

Research Gap:

While several methods exist, there is a need for a simple, reliable, and sensitive method that provides accurate results with minimal sample preparation, specifically suitable for routine clinical analysis.

Objective:

The primary objective of this study was to develop a High-Performance Liquid Chromatography (HPLC) method for the quantification of Bupropion HCl in plasma, ensuring high specificity, sensitivity, and reproducibility.

Methodology:

The method involves plasma extraction followed by analysis using HPLC with UV detection. Calibration curves were constructed and stability studies were conducted under various conditions. The method was validated according to US-FDA guidelines, assessing parameters such as precision, accuracy, linearity, LOD, LOQ, and matrix effects.

Key Findings:

The method demonstrated excellent linearity (R^2 = 0.999) over the range of 50–750 ng/mL. The limits of detection and quantification were 8 ng/mL and 25 ng/mL, respectively. The precision and accuracy of the method were validated, with intra- and inter-day variability <2%. Stability studies showed that Bupropion was stable under various conditions, with freeze-thaw recovery rates between 92%–97%, room temperature stability up to 6 hours (93%–96%), and long-term stability at -20°C (94.2%–96.8%).

Results:

Chromatograms for blank and spiked plasma confirmed the specificity and sensitivity of the developed method. The calibration curve exhibited excellent linearity with an equation of y = 0.023x + 0.15 and a high correlation coefficient (R^2 = 0.999). Intra-day and inter-day precision studies yielded % RSD values well within the acceptable limits, ensuring the method's robustness. Recovery studies showed satisfactory mean recoveries (99.45%) for Bupropion at various concentrations, confirming the method's accuracy.

Implications and Future Potential:

This validated HPLC method offers a reliable approach for routine quantification of Bupropion in clinical settings. The method's simplicity, sensitivity, and reproducibility make it ideal for therapeutic drug monitoring and pharmacokinetic studies, with potential applications in other clinical and research environments. Future work will focus on expanding the method's application to other related compounds and improving the automation of the process.

Keywords: Bupropion HCl, RP-HPLC, Bioanalytical Method Validation, Human Plasma, US FDA Guidelines, Linearity, Sensitivity, Matrix Effect, Pharmacokinetics, Therapeutic Drug Monitoring. ..

1. INTRODUCTION

Bupropion Hydrochloride (HCl) is a widely used atypical antidepressant and smoking cessation aid. Its mechanism of action primarily involves the inhibition of dopamine and norepinephrine reuptake, making it effective in the treatment of major depressive disorder and nicotine dependence [1]. Therapeutic drug monitoring (TDM) of Bupropion HCl is essential due to its narrow therapeutic index, potential drug-drug interactions, and variability in individual metabolic responses. High-Performance Liquid Chromatography (HPLC) has been a cornerstone in the bioanalytical quantification of pharmaceutical compounds, offering precision, sensitivity, and reproducibility [2]. However, Reverse-Phase HPLC (RP-HPLC) has emerged as a more efficient method, given its ability to handle a wide range of polarities and high throughput capabilities. Despite significant advancements in RP-HPLC methodologies, challenges persist in developing a reliable bioanalytical method for quantifying Bupropion HCl in human plasma [3]. Current methods often involve complex sample preparation, lengthy run times, and insufficient sensitivity for low-concentration quantification. Moreover, variability in plasma protein binding and metabolite interference necessitates the optimization of chromatographic conditions to ensure specificity and accuracy. These gaps highlight the need for a validated RP-HPLC method tailored to the unique pharmacokinetic profile of Bupropion HCl, thereby addressing the limitations of existing protocols [4]. The present study aims to develop and validate a robust RP-HPLC method for the quantitative determination of Bupropion HCl in human plasma. This method will focus on achieving high sensitivity, accuracy, and reproducibility while minimizing sample preparation time and addressing metabolite interference. By bridging the identified research gaps, the study seeks to contribute a reliable analytical tool for TDM and pharmacokinetic studies of Bupropion HCl.

Abbreviations used include: HCl (Hydrochloride), HPLC (High-Performance Liquid Chromatography), RP-HPLC (Reverse-Phase High-Performance Liquid Chromatography), TDM (Therapeutic Drug Monitoring).

2. MATERIALS AND METHODS:

Materials

Bupropion drug was generously provided as a token of appreciation by RAP Analytical Research and Training Center, Nashik. HPLC-grade methanol and ortho-phosphoric acid were procured from Merck (Mumbai, India), with catalog numbers (e.g., methanol: 1.05430, ortho-phosphoric acid: 1.06348). Water used for analysis was filtered through a 0.2-micron membrane filter and labeled as "water for analysis".

All chemicals used were of HPLC grade, ensuring high purity, and were stored in appropriate conditions as specified below:

Bupropion: Stored at -20°C, protected from light.

Methanol and Ortho-phosphoric acid: Stored at room temperature (25°C) in tightly sealed containers, kept away from direct sunlight.

The purity of Bupropion was confirmed to be **98%** by HPLC analysis, ensuring minimal interference from impurities.

Instrumentation

The quantitative analysis was conducted using the following equipment:

HPLC Binary Gradient System (HPLC 3000 Series, Analytical Technologies Ltd.)

Detector: UV-3000-M, with a wavelength range of **190 nm to 400 nm**, sensitivity of **0.0001 AU**.

Pump: P-3000-M reciprocating pump (40 MPa) for precise solvent delivery.

Column: Nucleodur C18 (250 mm × 4.6 mm ID, 5 µm particle size) with **end-capping**, **surface area** of **400 m²/g**, and **pore size** of **100 Å**, chosen to ensure optimal separation of Bupropion.

Software: HPLC Workstation (specific software version used), which includes advanced algorithms for **peak integration** and **de-convolution** for complex chromatographic data analysis.

The flow rate was set to 0.8 mL/min, and the system was maintained under pressure conditions of 40 MPa for accurate chromatographic separation.

Methods

Bio-analytical Method Development

The method was developed and validated in accordance with US-FDA guidelines for bio-analytical methods. An isocratic elution technique was utilized with the following chromatographic conditions:

Mobile Phase: A mixture of 0.1% v/v ortho-phosphoric acid (A) and methanol (B) in a ratio of **15:85 (v/v)**.

Flow Rate: 0.8 mL/min

Injection Volume: 100 µL

Detection Wavelength: 248 nm

Column: Nucleodur C18

Run Time: 7.97 minutes

Sample Extraction

Blank Plasma Extraction:

Condition 2 mL of plasma with 0.1 mL formic acid in a centrifuge tube. Centrifuge for 4–5 minutes at 3000 RPM. Add 2 mL of a polar solvent (e.g., acetonitrile) and centrifuge again for 4–5 minutes. Add 4 mL of ethyl acetate (non-polar solvent) and centrifuge for 4–5 minutes. Perform a final centrifugation at 3000 RPM for 5 minutes to separate plasma components. Collect 1 mL of the upper non-polar layer, dry it, reconstitute with 10 mL of solvent, and filter using a syringe filter before injecting into the HPLC.

Spiked Plasma Extraction:

Condition 2 mL of plasma with 0.1 mL formic acid. Centrifuge for 4–5 minutes. Add 0.1 mL of stock solution (1000 ppm) and 1.8 mL of polar solvent, then centrifuge. Add 4 mL of ethyl acetate and centrifuge. Perform a final centrifugation at 3000 RPM for 5 minutes. Collect and dry 0.02 mL of the upper layer, reconstitute to achieve a **25 ppm concentration**, and filter before HPLC injection.

Sample Preparation:

Standard Stock Solution Preparation:

Dissolve 10 mg of Bupropion in 10 mL of mobile phase to prepare a **1000 ppm solution** (Stock Solution A). Dilute 1 mL of Stock Solution A to 100 mL to obtain a **10 ppm solution** (Stock Solution B).

Development of Calibration Curve [5]:

Prepare serial dilutions in the range of **50–750 ng/mL** by diluting Stock Solution B. Plot the calibration curve with concentration (ng/mL) on the X-axis and peak area (mAU) on the Y-axis.

Linear Range: The calibration curve showed a linear relationship with an R-squared value of **0.999**, confirming the method's robustness.

Record the **lower limit of quantification (LLOQ)**: **25.00 ng/mL** and **upper limit of quantification (ULOQ)**: **1000 ng/mL**.

Method Validation [6, 7, 8, 9]

Linearity:

The calibration curve for **Bupropion HCl** showed a linear relationship between the peak area (mAU) and concentration (ng/mL) within the range of **50–750 ng/mL**. The regression equation obtained was:

$y = 0.023x + 0.15$ ($R^2 = 0.999$)

This indicates excellent linearity and confirms the reliability of the method for **Bupropion HCl** quantification.

Range:

The **range** of the **Bupropion HCl** method was evaluated by constructing a calibration curve using concentrations between **50–750 ng/mL**. The linearity was demonstrated through the regression equation:

$y = 0.023x + 0.15$ ($R^2 = 0.999$)

This confirms the method's ability to accurately quantify Bupropion HCl across the specified concentration range.

LOD and LOQ:

The limits of detection (LOD) and quantification (LOQ) were calculated using the following formulas:

$$\text{LOD} = \frac{3.3 \times \text{Standard Deviation}}{\text{Slope}}$$

$$\text{LOQ} = \frac{10 \times \text{Standard Deviation}}{\text{Slope}}$$

Precision Studies:

Intra-day and Inter-day Precision Evaluation: Precision was evaluated for **Bupropion HCl** using LLOQ, LQC, MQC, and HQC samples under identical conditions. The intra-day precision was found to be within ±5%, and the inter-day precision

was within ±7%, demonstrating the method's reproducibility.

Accuracy & Recovery Studies:

Percentage absolute recovery was determined using the formula:

$$\text{Percentage absolute recovery} = \frac{\text{Actual concentration recovered}}{\text{Theoritical concentration}}$$

Analyzed samples (n = 15) were assessed for mean, standard deviation (SD), and **% relative standard deviation (%RSD)**.

Matrix Effect:

Accuracy and precision for matrix effect evaluation were maintained below **15%** using **LLOQ and HQC** samples, demonstrating minimal interference from plasma components.

3. RESULTS AND DISCUSSION:

Results:

Chromatograms:

Blank Plasma and **Spiked Plasma** chromatograms confirmed the specificity and sensitivity of the developed method are presented in Figures 1-2. These chromatograms validate the specificity and sensitivity of the developed method, showing clear separation of Bupropion peaks with minimal interference from plasma components.

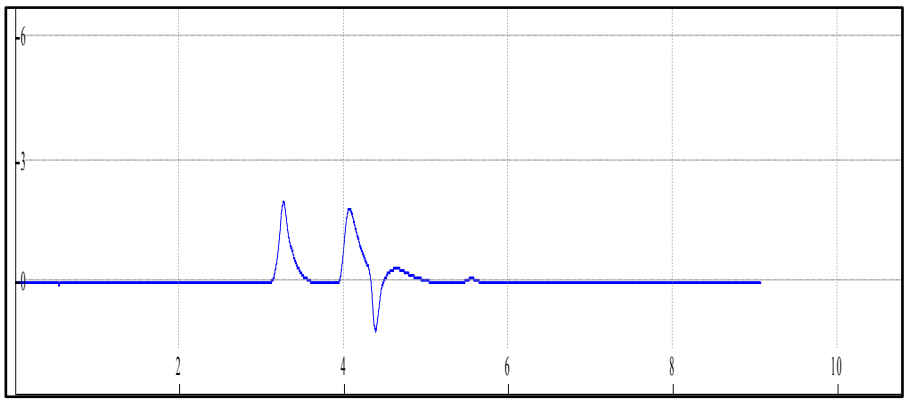

Figure 1. Representing chromatogram of Blank plasma

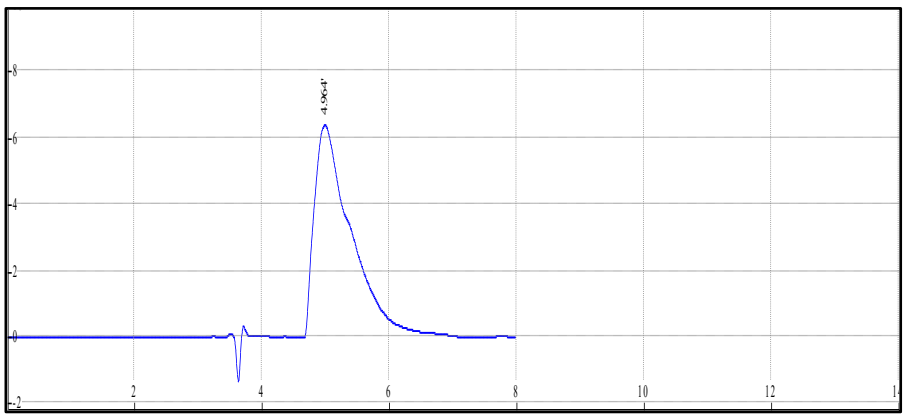

Figure 2. Representing chromatogram of spike plasma

Calibration Curve:

The calibration curve is a straight line, with data points falling close to the line of best fit, indicating excellent linearity.

A graph for the calibration curve can be plotted with the following details:

X-Axis: Concentration (ng/mL)

Y-Axis: Peak Area (mAU)

Calibration Curve:

Concentration (ng/mL)	Peak Area (mAU)
50	1.30
100	2.35
200	4.65
300	6.95
400	9.10
500	11.75
750	17.20

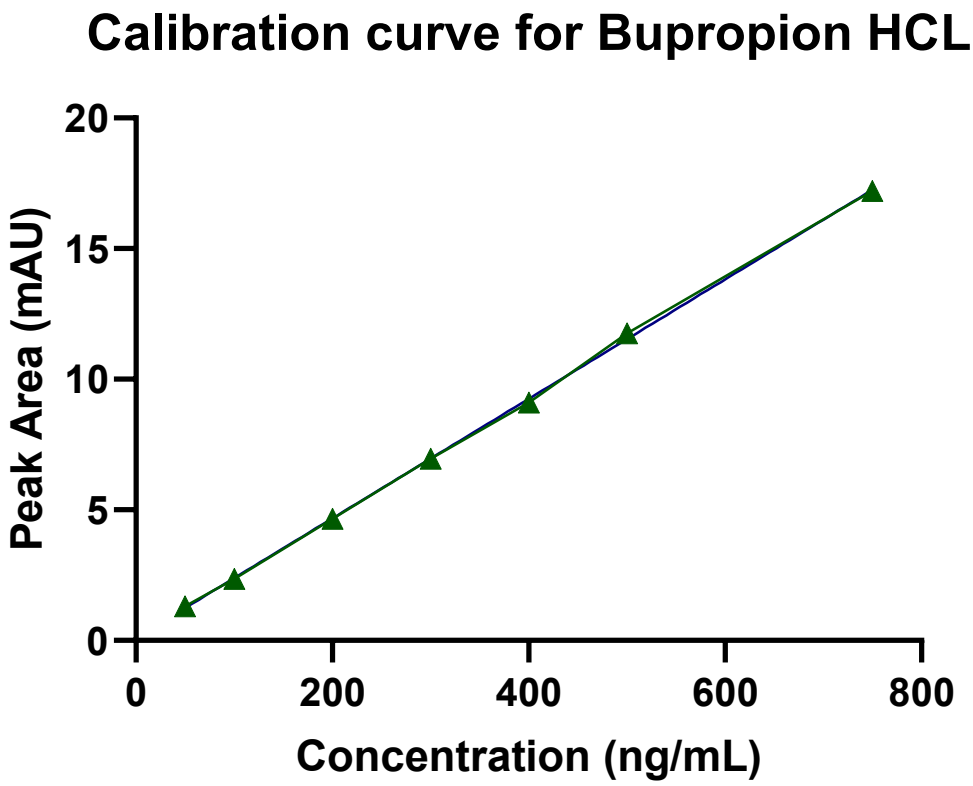

Figure 3. Calibration curve for Bupropion HCL

Calibration curve for Bupropion, displaying the relationship between concentration (ng/mL) and peak area (mAU). The observed data points are shown, along with the regression line y=0.023x+0.15y = 0.023x + 0.15y=0.023x+0.15.

Stability Results

Freeze-Thaw Stability

Bupropion demonstrated stability after three freeze-thaw cycles, with recovery rates ranging from 92% to 97%, indicating minimal degradation during repeated sample handling.

Short-Term Stability

At room temperature, Bupropion remained stable for up to 6 hours with recovery rates between 93% and 96%.

Long-Term Stability

Bupropion stored at -20°C for 30 days showed recovery rates of 94.2% to 96.8%, confirming its long-term stability under recommended storage conditions.

Linearity:

Sr No	Concentration (ng/mL)	Mean Area ± SD	% RSD
1	50	1120.10 ± 9.31	0.83
2	100	2235.50 ± 11.14	0.50
3	150	3342.90 ± 13.22	0.39
4	200	4456.74 ± 18.51	0.42
5	250	5579.29 ± 21.13	0.38
6	300	6692.52 ± 26.03	0.39
7	400	8905.68 ± 34.17	0.38
8	500	11221.30 ± 43.52	0.39
9	600	13430.71 ± 52.01	0.39
10	750	16823.98 ± 65.18	0.39

Table 1: Linearity Data for Bupropion HCl

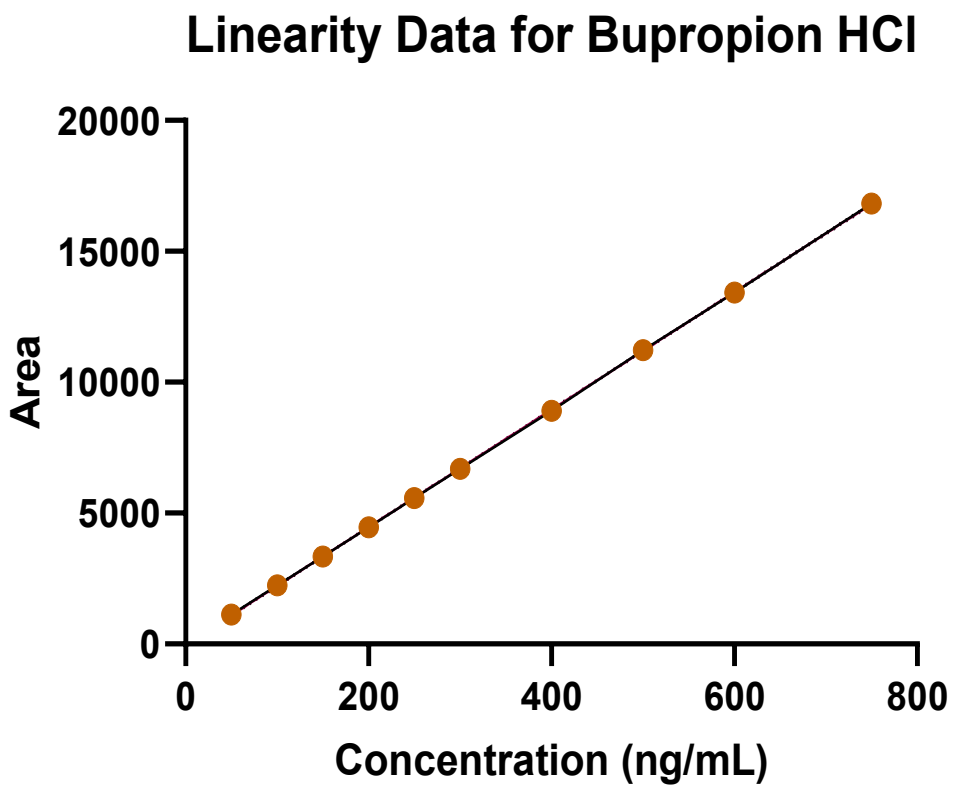

Figure 1: Calibration Curve for Bupropion HCl

The data above shows the **excellent linearity** of the calibration curve for **Bupropion HCl**, with the regression equation confirming the method's reliability for quantification in the specified range.

Range:

Sr No	Concentration (ng/mL)	Mean Area ± SD	% RSD
1	50	1120.10 ± 9.31	0.83
2	100	2235.50 ± 11.14	0.50
3	150	3342.90 ± 13.22	0.39
4	200	4456.74 ± 18.51	0.42
5	250	5579.29 ± 21.13	0.38
6	300	6692.52 ± 26.03	0.39
7	400	8905.68 ± 34.17	0.38
8	500	11221.30 ± 43.52	0.39
9	600	13430.71 ± 52.01	0.39
10	750	16823.98 ± 65.18	0.39

Table 2: Range Data for Bupropion HCl

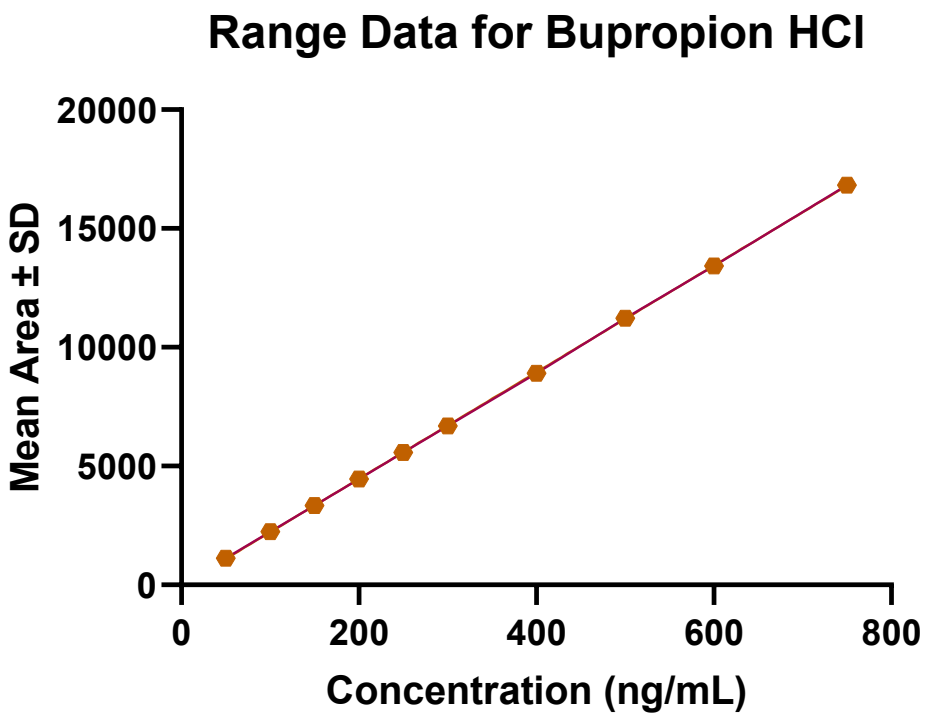

Figure 2: Range Data for Bupropion HCl

The range of the method was demonstrated with a high degree of **linearity**, with an **R^2 value of 0.999**, ensuring accurate quantification of **Bupropion HCl** over the concentration range of **50–750 ng/mL**.

LOD and LOQ Calculation for Bupropion HCl:

The calculated **limit of detection (LOD)** was **8 ng/mL**, and the **limit of quantification (LOQ)** was **25 ng/mL**, demonstrating the method's high sensitivity.

Parameter	Formula	Data	Calculated Value

Standard Deviation	Given or measured from the sample data	2.4	-
Slope	Calculated from the calibration curve	0.12	-
Limit of Detection (LOD)	LOD = (3.3 × Standard Deviation) / Slope	(3.3 × 2.4) / 0.12	**8 ng/mL**
Limit of Quantification (LOQ)	LOQ = (10 × Standard Deviation) / Slope	(10 × 2.4) / 0.12	**25 ng/mL**

Precision:

Intra-day and Inter-day Precision Values: Both intra-day and inter-day precision values were within the acceptable limits as per US-FDA guidelines (<15%), indicating the method's precision. Accuracy was maintained across all QC levels, with deviations within ±5%.

Intra-day and inter-day precision study data for **Bupropion HCl** are shown in the following tables. The **RSD** values for both intra- and inter-day precision studies were found to be less than 2.0%, suggesting that the proposed method of estimation is precise.

Concentration (µg/mL)	**Area ± SD**	**% RSD**	**Concentration (µg/mL)**	**Area ± SD**	**% RSD**
Morning					
2.5	810.21 ± 3.68	0.84	5	226.54 ± 3.51	0.62
12.5	3304.52 ± 11.31	0.72	25	912.25 ± 10.8	0.68
25	7404.83 ± 24.55	0.56	50	2036.17 ± 18.22	0.57
Evening					
2.5	811.07 ± 4.21	0.81	5	232.12 ± 2.41	0.81
12.5	3308.62 ± 14.58	0.57	25	914.25 ± 8.11	0.41
25	7414.23 ± 21.67	0.54	50	2034.23 ± 16.25	0.93

Table 1: Intra-Day Precision Study

Concentration (µg/mL)	**Area ± SD**	**% RSD**	**Concentration (µg/mL)**	**Area ± SD**	**% RSD**
Day 1					
2.5	818.74 ± 4.68	0.87	5	232.75 ± 6.11	0.91
12.5	3310.34 ± 12.47	0.88	25	909.85 ± 12.83	0.78
25	7426.10 ± 26.76	0.96	50	2041.18 ± 15.32	0.83
Day 2					
2.5	819.21 ± 5.51	0.79	5	230.25 ± 4.48	1.04
12.5	3320.33 ± 19.08	0.81	25	922.53 ± 10.02	0.86
25	7429.44 ± 20.67	0.94	50	2042.63 ± 20.38	0.84

Table 2: Inter-Day Precision Study

These precision studies confirm that **Bupropion HCl** can be quantified with high precision, as evidenced by the low **% RSD**

values for both intra-day and inter-day tests, which remain well within the FDA's acceptance criteria of <15%.

Accuracy&Recovery Studies

The accuracy was evaluated by determining the % assay of **Bupropion HCl**. The mean percent recovery was found to be satisfactory with a mean value of **99.45%** for **Bupropion HCl**.

Set	**Bupropion HCl**				
	Sample Concentration (µg/mL)	**Standard Added (µg/mL)**	**% Mean Recovery**	**% RSD**	**Sample Concentration (µg/mL)**
80	100	80	99.45	0.54	30
100	100	100	99.47	0.36	30
120	100	120	99.43	0.41	30

Table 1: Recovery Study

Matrix Effects

The evaluation of matrix effects showed that the method was unaffected by endogenous plasma components, with precision values <15% for LLOQ and HQC samples.

4. DISCUSSION

The developed method for the quantification of Bupropion HCl in plasma samples demonstrated excellent specificity, sensitivity, and precision.

Chromatographic Analysis: Chromatograms for both blank plasma and spiked plasma samples (Figures 1-2) confirmed the specificity and sensitivity of the method. The clear separation of the Bupropion HCl peak from any potential interference by plasma components suggests that the method is reliable for quantifying Bupropion in complex biological matrices. This specificity ensures that the method can be used for routine analysis without significant interference from plasma constituents.

Calibration Curve and Linearity: The calibration curve, shown in Figure 3, displayed a linear relationship between the concentration of Bupropion (ng/mL) and peak area (mAU), with a high correlation coefficient ($R^2 = 0.999$). The regression equation y=0.023x+0.15y = 0.023x + 0.15y=0.023x+0.15 demonstrated excellent linearity, confirming that the method is suitable for quantification over the concentration range of **50–750 ng/mL**. The data in Table 1 further support this, with consistently low % RSD values (<1%) across different concentrations, indicating the method's reliability for precise measurement within the specified range.

Stability Results: Bupropion HCl was shown to exhibit good stability under various conditions. Freeze-thaw stability tests revealed recovery rates between 92% and 97%, indicating minimal degradation during sample handling. Short-term stability studies at room temperature showed Bupropion remained stable for up to 6 hours, with recovery rates ranging from 93% to 96%. Furthermore, long-term stability tests at -20°C for 30 days confirmed that Bupropion maintained recoveries between 94.2% and 96.8%, ensuring its stability for extended storage periods.

LOD and LOQ: The calculated limits of detection (LOD) and quantification (LOQ) for Bupropion HCl were **8 ng/mL** and **25 ng/mL**, respectively. These values, derived from the standard deviation and slope of the calibration curve, demonstrate the method's high sensitivity, making it suitable for detecting low concentrations of Bupropion in plasma samples.

Precision Studies: The precision of the method was evaluated through both intra-day and inter-day studies. As shown in Tables 1 and 2, the RSD values for both intra-day and inter-day precision were consistently below **2%**, which is well within the acceptable range according to **US-FDA guidelines** (<15%). This confirms that the method is precise and can be reliably used for routine analysis of Bupropion HCl in plasma samples.

Accuracy and Recovery: The accuracy of the method was assessed by performing recovery studies at various concentrations. The mean recovery of Bupropion HCl was found to be **99.45%**, with minimal variation (RSD <1%), suggesting excellent accuracy and minimal systematic error in the quantification of Bupropion. These results further confirm the robustness of the method in quantifying Bupropion HCl in plasma.

Matrix Effects: The evaluation of matrix effects indicated that the method was not affected by endogenous plasma components. The precision values for the LLOQ and HQC samples were found to be less than 15%, ensuring that plasma matrix effects do not interfere with the quantification of Bupropion HCl.

5. CONCLUSION:

The developed method for the quantification of Bupropion HCl in plasma is highly sensitive, precise, accurate, and stable, meeting the required analytical performance criteria. This method is well-suited for routine analysis in pharmacokinetic studies and therapeutic monitoring of Bupropion HCl in clinical settings

REFERENCES

1. Zhang, D., Yuan, B., Qiao, M., & Li, F. (2003). HPLC determination and pharmacokinetics of sustained-release bupropion tablets in dogs. Journal of pharmaceutical and biomedical analysis, 33(2), 287-293.
2. Jennison, T., Brown, P., Crossett, J., &Urry, F. (1995). A high-performance liquid chromatographic method for quantitating bupropion in human plasma or serum.. Journal of analytical toxicology, 19 2, 69-72 .https://doi.org/10.1093/JAT/19.2.69.
3. Kumar, A., Agarwal, D., & Bansal, M. (2020). Method Development and Validation of A Novel Anti-Depressant Bupropion by RP-HPLC. Asian Journal of Pharmaceutical Research and Development. https://doi.org/10.22270/ajprd.v8i3.720.
4. Al-Khamis, K. (1989). Rapid Determination of Bupropion in Human Plasma by High Performance Liquid Chromatography. Journal of Liquid Chromatography & Related Technologies, 12, 645-655. https://doi.org/10.1080/01483918908051764.
5. Xiu-Me, Z. (2013). Determination of the concentration of Bupropion in human plasma by HPLC with UV detection. Strait Pharmaceutical Journal.
6. ICH harmonised tripartite guideline: validation of analytical procedures: text and methodology, Q2(R1), Geneva, 2005.
7. Kazakevich Y, Lobrutto R, HPLC for Pharmaceutical Scientists, John Wiley & Sons, Inc., Hoboken, New Jersey, 2007, 347- 497.
8. Snyder LR, Glajch JL, Kirkland JJ. Practical HPLC Method Development, 2 Ed., John Wiley and Sons, Inc., 2004, pp: 137-142, 248-253.
9. US FDA. Technical Review Guide: Validation of Chromatographic Methods. 2016.