Review Article

# Comprehensive Review of High-Performance Liquid Chromatography Methods for Simultaneous Determination of Naltrexone Hydrochloride and Bupropion Hydrochloride

**Priyanka Darsi[2], Seetharam Gude[1*], Pravallika Lakshmi Kurra[1], Ravi Shankar. P[2], M Eswarudu[2], Srinivasa Babu P[1]**

1. Department of Pharmaceutics, Vignan Pharmacy College, Guntur, 522213, India.
2. Department of Pharmaceutical Analysis, Vignan Pharmacy College, Guntur, 522213, India.

***Corresponding author's E-mail:** seethasaiofficial@gmail.com

**Received:** 06-06-2024; **Revised:** 28-08-2024; **Accepted:** 10-09-2024; **Published on:** 15-09-2024.

**ABSTRACT**

The combination of Bupropion Hydrochloride and Naltrexone Hydrochloride in a single pharmaceutical formulation has been approved by the U.S. Food and Drug Administration (FDA) for treating obesity. Naltrexone hydrochloride, a semi-synthetic opioid, works by competitively antagonizing mu receptors, while Bupropion hydrochloride, an antidepressant, enhances dopamine activity by inhibiting its reuptake. Naltrexone's effect is mediated through the modulation of pro-opiomelanocortin neurons in the hypothalamus, showcasing the synergistic efficacy of this combination in long-term obesity management. This review focuses on high-performance liquid chromatography (HPLC)-based analytical methods designed for the simultaneous quantification of naltrexone hydrochloride (NTX) and bupropion hydrochloride (BUP). It critically examines a range of analytical techniques, from traditional HPLC methods to innovative, environmentally friendly chromatographic approaches. Each method is analyzed in detail, covering aspects such as mobile phase composition, column selection, and flow rate optimization. Additionally, the review assesses the validation parameters of these analytical methods, ensuring their reliability and accuracy. This comprehensive examination not only highlights the diversity of chromatographic techniques but also underscores the methodological rigor essential for determining these pharmacologically important compounds, thereby advancing the field of pharmaceutical analysis.

***Highlights:*** This review explores a wide range of HPLC-based analytical procedures for the simultaneous estimation of Naltrexone HCl and Bupropion HCl, providing a comprehensive evaluation of validation parameters to enhance the reliability and precision of these methods. The simultaneous estimation of different APIs offers significant advantages in terms of cost, time, energy, and resource savings. Additionally, the review validates HPLC as an accurate, robust, and preferred analytical tool, highlighting its efficacy and robustness in pharmaceutical analysis.

**Keywords:** Naltrexone Hydrochloride, Bupropion Hydrochloride, High Performance Liquid Chromatography (HPLC).

## INTRODUCTION

According to the World Health Organization, obesity has emerged as a significant public health challenge of the twenty-first century, with over 1 billion individuals worldwide classified as obese. This pervasive issue affects not only adults but also an estimated 390 million children, highlighting its extensive reach across age groups. Alarmingly, statistics reveal that one in five women is grappling with obesity, and these numbers continue to escalate. Concurrently, a substantial segment of the population is entangled in the opioid crisis. The American Medical Association reports that up to 19% of individuals are exposed to opioids, either due to medical conditions or addiction. Consequently, the prevalence of obesity and opioid dependence has surged, driven by a combination of lifestyle choices, genetic predispositions, and dietary habits. To address these conditions, various therapeutic strategies are recommended, including lifestyle modifications and structured exercise regimens. Despite these non-pharmacological approaches, medical interventions often become necessary. Medications such as Naltrexone Hydrochloride (HCl) and Bupropion are commonly prescribed by healthcare providers. These pharmaceuticals are recognized for their efficacy in treating obesity and opioid dependence, respectively, and have garnered widespread acceptance on a global scale. However, the potential for abuse and dependency associated with these medications necessitates stringent regulatory guidelines governing their prescription and distribution. Ensuring patient safety and adherence to these regulations requires the precise quantification of these drugs in their pharmaceutical formulations. Accurate measurement and validation of these compounds are imperative to maintain compliance with regulatory standards and to support the safe and effective use of these medications in clinical practice. This underscores the importance of advanced analytical techniques, such as high-performance liquid chromatography (HPLC), which are essential for the reliable and precise determination of Naltrexone HCl and Bupropion in various pharmaceutical products.[1-5]

An array of analytical techniques is available for precise drug estimation, with chromatography and spectrometry-based methodologies being particularly prominent. Chromatography is a powerful tool for separating and identifying components within mixtures, applicable in diverse fields such as food, pharmaceuticals, and environmental analysis. It is instrumental in diagnosing diseases, monitoring treatment progress, and identifying new pharmaceuticals. In contrast, spectrometry-based techniques rely on the interactions between electromagnetic radiation and materials, providing detailed insights into atomic and molecular energy levels and transitions. This facilitates the identification and quantification of compounds. Techniques such as UV-Vis, IR, NMR, and mass spectrometry fall under this category and have extensive applications in various scientific disciplines. The choice between chromatography and spectrometry depends on the specific requirements of the analysis: chromatography is primarily used for purification and separation, while spectrometry is essential for identification, structural elucidation, and quantitative analysis. Often, these techniques are used in tandem, complementing each other to achieve comprehensive and accurate results.[6-10]

In the pharmaceutical field, chromatography-based techniques are of paramount importance, playing a crucial role in drug development, product analysis, impurity detection, pharmacokinetics, and bioavailability studies. High-performance liquid chromatography (HPLC) stands out as a cornerstone analytical method, enabling the precise quantification and separation of compounds within pharmaceutical formulations. Known for its high sensitivity, specificity, and selectivity, HPLC has become the preferred method for the simultaneous determination of multiple drugs in pharmaceutical formulations, solidifying its prominence in recent years.

Due to the widespread use of bupropion hydrochloride and naltrexone hydrochloride, extensive research in literature has explored their analytical methodologies. This paper presents a thorough review of HPLC techniques documented in the literature for simultaneous quantification of these compounds in pharmaceutical formulations. The review aims to intricately analyze reported HPLC methods, focusing on stationary and mobile phases, as well as detection methods. Additionally, it comprehensively discusses the validation of these methods, covering aspects such as development, optimization, and validation parameters. The paper navigates through the complexities of method development using HPLC, detailing the influencing factors. It further examines the chemical compositions of both drugs and specifics related to their analysis. The review extends to the various detection methods employed for these compounds, concluding with a comprehensive analysis and summary.[11,12,13,14]

### HPLC Method Development

The development of analytical methods using High-Performance Liquid Chromatography (HPLC) involves a meticulous process of refining and validating techniques to effectively separate, identify, and quantify specific compounds. This study exclusively examines the use of HPLC for the simultaneous determination of naltrexone hydrochloride (NTX) and bupropion hydrochloride (BUP), focusing on assessing the effectiveness and feasibility of HPLC methodologies in accurately quantifying these compounds concurrently.[16]

- **Choosing the Stationary Phase**

The selection of an appropriate stationary phase in HPLC is a critical step in method development, as it directly influences the separation and resolution of compounds. This choice significantly affects analyte selectivity and retention during chromatography. Stationary phases encompass various categories including Reversed-Phase (such as C18, phenyl, C8, C4), Normal-Phase (such as silica, cyano), Size Exclusion, and Ion-Exchange phases. The decision on which phase to use depends on the properties of the analyte and the specific separation criteria required. For instance, in the case of Bupropion Hydrochloride (BUP HCl) and Naltrexone Hydrochloride (NTX HCl), commonly employed stationary phases include $C_{18}$ and $C_8$ columns.[17]

- **Choosing the Mobile phase**

The choice of mobile phase is equally critical in High-Performance Liquid Chromatography (HPLC), as it directly impacts elution time and the separation of analytes. Typically composed of solvents like methanol, acetonitrile, and water, either with or without ion-pairing agents, the mobile phase facilitates the movement of analytes through the chromatographic system. These solvents have demonstrated effectiveness in separating Naltrexone Hydrochloride (NTX HCl) and Bupropion Hydrochloride (BUP HCl). The addition of ion-pairing agents, such as tetrabutylammonium bromide, has been documented to improve analyte retention and separation.[18]

- **Selection of Detection Method: Impact on Sensitivity and Specificity:**

The choice of detection method is crucial as it defines the sensitivity and specificity of the analytical approach. Commonly used methods for quantifying Bupropion Hydrochloride (BUP HCl) and Naltrexone Hydrochloride (NTX HCl) include UV-Visible spectroscopy, valued for its simplicity and widespread availability. Alternatively, mass spectrometry provides enhanced sensitivity, specificity, and selectivity, particularly advantageous for analyzing these compounds in complex matrices.[19]

- **Method Optimization**

Optimization is a detailed process aimed at adjusting multiple parameters to achieve the desired separation, resolution, and sensitivity in chromatography. Parameters such as wavelength, mobile phase composition, pH, flow rate, column temperature, and injection volume are meticulously optimized. This critical step enhances method performance by minimizing analysis time, improving peak shape, and maximizing compound separation efficiency. After method development and optimization, validation becomes essential to ensure the accuracy, precision, and robustness of the HPLC method. Various factors are carefully studied and evaluated during the validation process.[20]

- **Method Validation**

Method validation is a crucial step in pharmaceutical product development to assess the appropriateness of the test for its intended use. It confirms the quality, reliability, and consistency of analytical results. Method validation involves conducting various types of tests to ensure accuracy and precision.

***Linearity:***

Linearity in analytical methods assesses the method's ability to accurately reflect changes in substance concentration. It ensures that the method behaves predictably across a range of concentrations, similar to tuning a musical instrument to hit precise notes. Imagine this as a musical scale where each note corresponds accurately to its position, enabling precise measurement of substances across different concentration levels. This is achieved by testing various standard solutions at different concentrations and plotting a calibration curve. This visual guide helps maintain accuracy in measurements, regardless of the concentration being analyzed.

***Range:***

The concentration range critical to the precision and accuracy of an analytical method is a fundamental parameter in its characterization. This range is established through careful assessment, involving systematic analysis of standard solutions spanning a gradient of concentrations. Subsequently determining the limits of detection (LOD) and quantitation (LOQ) is essential in this process. These metrics define the lower boundaries of reliably detectable analyte concentrations (LOD) and the minimum levels accurately quantifiable (LOQ). Therefore, these critical thresholds meticulously define the method's scope, ensuring its capability to effectively and accurately measure analytes within a specified concentration range.[21]

***Accuracy:***

Accuracy is a crucial measure in evaluating the reliability of measured values against actual values within analytical methodologies. In analytical methods, accuracy indicates how closely acquired results match the true quantity being measured. Assessing accuracy typically involves using spiked samples, where a known amount of the analyte is intentionally added to a sample. The percentage recovery, which reflects the successful retrieval of the analyte, is then evaluated. A method is considered accurate if the percentage recovery falls within predefined acceptable limits, indicating that measured values closely align with the true concentrations of the analyte.[22]

***Precision:***

Precision, a vital component of method evaluation, assesses an analytical method's reproducibility. This involves analyzing spiked samples and calculating the percentage relative standard deviation (%RSD). This metric quantifies the variability or dispersion in results obtained from multiple measurements of the same sample. Evaluating precision in this manner provides essential insights into the method's consistency and reliability, allowing researchers to gauge its reproducibility and uniformity.[23]

***Selectivity:***

Selectivity, a crucial parameter in analytical methodology, enables the discrimination between target analytes and interfering substances. It reflects the method's ability to accurately measure the desired analyte in the presence of other compounds or potential interferences. The key lies in the method's proficiency in detecting and quantifying the target analyte while minimizing the impact of extraneous factors. High selectivity ensures that the analytical method can reliably distinguish and quantify the desired analyte with minimal disruption from other constituents, thereby enhancing the dependability and accuracy of the measurements.[24]

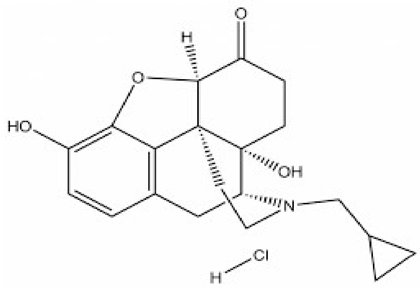
***Robustness:***

Robustness is a parameter used to produce consistent results under varying method conditions. In an analytical context, robustness refers to a method's ability to maintain consistent and reliable results despite variations in experimental conditions. This involves assessing the method's resilience to changes in factors such as temperature, pH, and other environmental variables. Evaluating robustness entails deliberately introducing controlled changes to these conditions and observing their effects on the method's performance. By systematically examining how different conditions impact outcomes, researchers can determine the method's ability to maintain accuracy and precision across diverse settings. This assessment validates the method's reliability and applicability under realistic and fluctuating conditions, thereby enhancing confidence in its use within analytical practice.[25]

**Drug Details**

***Naltrexone Hydrochloride***

Naltrexone hydrochloride, an FDA-approved medication, belongs to the class of opioid receptor antagonists and is primarily used to treat alcohol and opioid dependence. Formulated as the salt derivative of naltrexone, it offers enhanced solubility and stability. Naltrexone hydrochloride is a key ingredient in various generic products such as RiVia, Vivitrol, and Depade. In addition to its role as an opioid antagonist, it is also utilized for appetite suppression to reduce food cravings.[26]



***Bupropion Hydrochloride***

In the pharmaceutical industry, Bupropion is recognized as a versatile antidepressant with additional benefits in reducing cravings and aiding smoking cessation. Its mechanism of action involves modulating neurotransmitter levels in the brain, particularly norepinephrine and dopamine. The hydrochloride formulation of Bupropion, known as Bupropion hydrochloride, is the salt variant of the compound.[27]

Bupropion hydrochloride functions as a dopamine and norepinephrine reuptake inhibitor, promoting a sense of satiety. This compound is classified as an aminoketone, noted for its high lipophilicity. It exerts its effects by blocking nicotine receptors and inhibiting the reuptake of epinephrine and norepinephrine. Commercially, it is available under various brand names such as Wellbutrin, Zyban, and Forfivo XL.[28]


**Combination of Naltrexone hydrochloride and Bupropion hydrochloride**

To harness potential synergies and leverage complementary mechanisms of action, Naltrexone hydrochloride and Bupropion hydrochloride salts have been combined into a single dosage form. This combination aims to enhance weight loss outcomes by capitalizing on the distinct therapeutic properties of both active pharmaceutical ingredients (APIs). The different mechanisms underlying each compound's action form the basis for their effective collaboration in weight management.[29]

Naltrexone acts as an opioid receptor antagonist, blocking the effects of opioids in the body and reducing cravings for food and potentially addictive substances. In contrast, Bupropion affects the balance of specific neurotransmitters, such as norepinephrine and dopamine, which are crucial in controlling appetite and reducing food intake. The combination of these medications utilizes their individual strengths, creating a synergistic effect to support weight loss efforts.[30]

Commercially available as Contrave, this medication combines Naltrexone hydrochloride and Bupropion hydrochloride salts and is FDA-approved for chronic weight management in overweight or obese adults with weight-related comorbidities such as hypertension or type 2 diabetes. Although the precise mechanism of action for weight loss facilitated by Contrave remains partially understood, it is believed to involve appetite regulation, reduction of food cravings, and modulation of energy balance.[31]

**Analytical methods for Naltrexone hydrochloride and Bupropion hydrochloride**

Naltrexone hydrochloride and Bupropion hydrochloride can be quantified using both HPLC and spectrometry-based methods.

- **HPLC based methods for Naltrexone hydrochloride and Bupropion hydrochloride**

High-Performance Liquid Chromatography (HPLC) methods have long been established for individually analyzing Naltrexone hydrochloride and Bupropion hydrochloride, which are key components in pharmaceutical production. With the incorporation of these compounds into combined pharmaceutical formulations, there is a need to develop simultaneous detection methods. Simultaneously quantifying these compounds is crucial for maintaining the quality and consistency of combination drug formulations that include Naltrexone hydrochloride and Bupropion hydrochloride. This paper emphasizes the critical role of accurate and reliable simultaneous estimation techniques in upholding rigorous standards of product quality.[32]

A variety of analytical techniques exist in both literature and industrial applications for this purpose, encompassing bulk drugs and single pharmaceutical dosage forms. High-Performance Liquid Chromatography (HPLC)-based methods have particularly gained prominence due to their exceptional sensitivity, selectivity, and efficiency in handling multiple samples. This review focuses on delineating the available analytical methods, highlighting the importance and benefits of utilizing HPLC-based techniques for simultaneously estimating these medications.[33,34]

The primary objective of this literature review is to comprehensively outline High-Performance Liquid Chromatography (HPLC) methodologies that have been established and validated for the simultaneous quantification of Naltrexone hydrochloride and Bupropion hydrochloride across various pharmaceutical dosage forms. The paper aims to provide a thorough analysis of HPLC techniques, covering their development, validation, and practical application in accurately determining drug concentrations within pharmaceutical formulations. Recognized as a widely utilized analytical method, High-Performance Liquid Chromatography (HPLC) has witnessed the development and validation of numerous methodologies for effectively and precisely simultaneously detecting Naltrexone hydrochloride (NTX) and Bupropion hydrochloride (BUP) in diverse pharmaceutical formulations.

**Table 1:** Summary of HPLC methods for simultaneous quantification of naltrexone hydrochloride and bupropion hydrochloride

| S. No | Method | Mobile Phase | Column | UV-Detection (nm) | Flow rate (mL/min) |
|---|---|---|---|---|---|
| 1 | RP-HPLC | ACN:TEA(55:45) | C-18 | 215 | 1.2 |
| 2 | RP-HPLC | ACN:KH2PO4 (70:30) | Nuceosil C-18 | 214 | 1.35 |
| 3 | RP-HPLC | ACN:methanol:water (20:60:20) | Chromosil C-18 | 254 | 1 |
| 4 | RP-HPLC | Triethanolamine:SLS:propanol | RP-C18 | 210 | 1.2 |
| 5 | RP-HPLC | Bufer:CAN (60:40) | C-18 | 224 | 1 |
| 6 | RP-HPLC | N,N-Diisopropylethylamine:water (25:75) | C-18 | 251 | 1.2 |

- **RP-HPLC based methods**

RP-HPLC typically utilizes stationary phases such as octadecylsilane (C18) or octyl silane (C8), combined with a mixture of water (as the aqueous phase) and an organic solvent such as acetonitrile or methanol (as the organic phase). These same stationary and mobile phase combinations were used for the analysis of Naltrexone hydrochloride (NTX) and Bupropion hydrochloride (BPU).[35,36]

## DISCUSSION

In the pursuit of simultaneously determining Bupropion HCl and Naltrexone HCl, various alternative analytical methods have been explored, including spectrophotometric techniques. While these methods offer simplicity and cost-effectiveness, it is important to acknowledge their inherent limitations in terms of sensitivity and selectivity compared to chromatographic approaches.

Spectrophotometric methods, despite their straightforward nature and economic viability, may struggle to achieve the necessary sensitivity for accurately quantifying Bupropion HCl and Naltrexone HCl. Additionally, their ability to distinguish between the target analytes and potential interfering substances may be less robust compared to chromatographic methods, which offer greater discriminatory power.

Nevertheless, spectrophotometric methods serve as valuable alternatives for preliminary analyses and screening purposes, where extremely high sensitivity and selectivity may not be critical requirements. Their simplicity and cost-effectiveness make them particularly suitable for rapid routine analyses or situations where handling large numbers of samples is necessary.

As the field of pharmaceutical analysis advances, it is crucial to carefully consider the specific analytical needs of each study. The choice between spectrophotometric and chromatographic techniques should be based on factors such as sensitivity, selectivity, precision, and accuracy, ensuring that the selected method aligns with the desired level of analytical performance and objectives of the research.

**Table 2:** A comprehensive comparison of various analytical techniques for drug estimation.

| | UV-Vis | HPLC |
|---|---|---|
| Mechanism | Absorbance of 1 component at a time is measured | Separation of more than one component take place at a time |
| Accuracy & Precision | Less precise and accurate | High accuracy and precision |
| Cost of Analysis | Very low | High |
| Reagents/mobile phase/ diluents | Polar solvents mostly | Multiple combinations of Buffers, Organic and polar solvents (HPLC Grade) |
| Instrument operation | Easy operation | complex |
| Parameters | Qualitative | Quantitative |
| Run time | Compared to HPLC, analysis is complete in less time | High run time (depends upon elution of the component) |
| Resolution | Low resolution | Higher resolution |
| Sensitivity and selectivity | Limited sensitivity and selectivity | Highly sensitive and selective |
| Solvent consumption | Depends on cut-of value | Low |
| Injection volume | 2.5–4 mL | 2 µL |
| Advantages | • Quick and accurate<br>• Non-destructive | •Quick and precise quantitative analysis<br>• Extremely precise, fast and automated technique |
| Disadvantages | • Only for solutions<br>• Not for solid/gaseous samples | • Expensive as it requires large electric supply and organic solvents<br>• Difficulty in trouble shooting |

## CONCLUSION

This study explores the benefits of simultaneously assessing active drug ingredients, offering efficiency in method usage and cost-effectiveness. It provides detailed insights into Naltrexone hydrochloride and Bupropion hydrochloride, focusing on High-Performance Liquid Chromatography (HPLC) techniques for their simultaneous estimation. The paper comprehensively examines various methodologies, including different stationary phases, mobile phase compositions (notably Acetonitrile and water), optimal detection wavelengths, and essential validation parameters. The octadecylsilane-based C18 column is highlighted as particularly effective for detecting both drugs accurately. Additionally, alternative methods incorporating acids and other agents are discussed, along with associated challenges and considerations. Overall, the findings underscore HPLC-based methodologies as robust and precise analytical tools for simultaneously determining Active Pharmaceutical Ingredients (APIs), specifically Naltrexone hydrochloride and Bupropion hydrochloride, in pharmaceutical formulations.

**Source of Support:** The author(s) received no financial support for the research, authorship, and/or publication of this article

**Conflict of Interest:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## REFERENCES

1. Abdel-Gawad SA, El-Gamal RM. Simultaneous determination of naltrexone and bupropion in their co-formulated tablet utilizing green chromatographic approach with application to human urine. Saudi Pharmaceut J. 2018;26(2):169–76.
2. Apostolidi A, Protopapa C, Siamidi A, Vlachou M, Dotsikas Y. Dissolution assay of Bupropion/Naltrexone hydrochloride salts of bilayer composition tablets following the development and validation of a novel HPLC method. Materials. 2022;15(23):8451.
3. Awosemo OT. Development and Validation of LC−MS/MS Assay for 5-Amino-1-Methyl Quinolinium: Application to Pre-clinical Pharmacokinetic Studies. (Doctoral dissertation), Texas Southern University, 2020.
4. Belanger JM, Paré JJ, Sigouin M. High performance liquid chromatography (HPLC): principles and applications. In: Techniques and instrumentation in analytical chemistry: Elsevier; 1997;18:37–59.
5. Betz JM, Brown PN, Roman MC. Accuracy, precision, and reliability of chemical measurements in natural products research. Fitoterapia. 2011;82(1):44–52.
6. Branch SK. Guidelines from the international conference on harmonisation (ICH). J Pharmaceut Biomed Anal. 2005;38(5):798–805.
7. Ch PR, Chaitanya D, Prasanthi B. RP-HPLC and spectrophotometric methods for the simultaneous estimation of Bupropion HCL and Naltrexone HCL. Int J Pharmaceut Sci Res. 2015;6(7):2982–90.
8. Costa R, Oliveira NG, Dinis-Oliveira RJ. Pharmacokinetic and pharmacodynamic of bupropion: integrative overview of relevant clinical and forensic aspects. Drug Metab Rev. 2019;51(3):293–313.
9. Dey S, Kalyan K, Samyuktha B, Sahoo SK, Mohapatra S, Murthy P, Kumar D. Development and validation of a UV–VIS spectrophotometric method for the estimation and degradation monitoring of cefadroxil in bulk and pharmaceutical dosage forms. Int J Chem Res. 2010;3:29–34.
10. Dharuman J, Vasudevan M, Somasekaran K, Dhandapania B, Ghodea PD, Thiagarajana M. RP-HPLC method development and validation for the simultaneous estimation of ofoxacin and tinidazole in tablets. Int J PharmTech Res. 2009;1(2):121–4.
11. Dighe SA, Siddheshwar SS, Shinde GS. A review on analytical method for determination of venlafaxine hcl in bulk and pharmaceutical dosage form. Res J Sci Technol. 2021;13(3):208–12.
12. Dubey SS, Duggirala M. High performance liquid chromatography with PDA detector for combined determination of emtricitabine, tenofovir and efavirenz. Pharm Lett. 2015;7(10):85–91.
13. FDA. Bioanalytical method validation guidance for industry. J Appl Bioanal. 2018;5:33-39.
14. Fekete S, Guillarme D. Ultra-high-performance liquid chromatography for the characterization of therapeutic proteins. TrAC, Trends Anal Chem. 2014;63:76–84.
15. Gandhimathi M, Anandakumar K, Cheriyan A, Ravi T. Simultaneous estimation of metformin and gliclazide in tablets using reverse phase high performance liquid chromatography. Indian J Pharm Sci. 2003;65(5):530–1.
16. Ghoneim MM, El-Desoky HS, Abdel-Galeil MM. Voltammetry of naltrexone in commercial formulation and human body fuids: QUANTIFICATION and pharmacokinetic studies. Bioelectrochemistry. 2011;81(2):65–73.
17. Greenway FL, Dunayevich E, Tollefson G, Erickson J, Guttadauria M, Fujioka K, Group N-S. Comparison of combined bupropion and naltrexone therapy for obesity with monotherapy and placebo. J Clin Endocrinol Metab. 2009;94(12):4898–906.
18. Greig SL, Keating GM. Naltrexone ER/bupropion ER: a review in obesity management. Drugs. 2015;75:1269–80.
19. Guideline I. Stability testing of new drug substances and products. Q1A, Current step 2003;4():24-29.
20. Guideline IHT. Validation of analytical procedures: text and methodology. ICHHT Guideline J Q2 (R1). 2005;1(20):05-12.
21. Haritha A, Kumar PBR, Priya RV, Sekhar K. Analytical method development and validation for simultaneus estimation of naltrexone hydrochloride and bupropion hydrochloride in oral dosage form (tablets) by RP-HPLC techniques. J Global Trends Pharmaceut Sci. 2015;6(2):2600–6.
22. Khan S, Berendt R, Ellison C, Ciavarella A, Asafu-Adjaye E, Khan M, Faustino P. Bupropion hydrochloride. In: Profles of drug substances, excipients related methodology, 2016;41:1–30.
23. Koll K, Reich E, Blatter A, Veit M. Validation of standardized high-performance thin-layer chromatographic methods for

quality control and stability testing of herbals. J AOAC Int. 2003;86(5):909–15.

24. Lee MW, Fujioka K. Naltrexone for the treatment of obesity: review and update. Expert Opin Pharmacother. 2009;10(11):1841–5.

25. Makowski CT, Gwinn KM, Hurren KM. Naltrexone/bupropion: an investigational combination for weight loss and maintenance. Obes Facts. 2011;4(6):489–94.

26. Milione LJ. Overview of the drug enforcement administration diversion control program. US Att'ys Bul. 2016;64:11-19.

27. Morais JAG, Lobato MdR. The new European Medicines Agency guideline on the investigation of bioequivalence. Basic Clin Pharmacol Toxicol. 2010;106(3):221–5.

28. Neri S. Method development, degradation studies and validation of Bupropion Hydrochloride using reversed phase high performance liquid chromatography: Northeastern Illinois University. 2016.

29. Nikolin B, Imamović B, Medanhodžić-Vuk S, Sober M. High perfomance liquid chromatography in pharmaceutical analyses. Bosn J Basic Med Sci. 2004;4(2):5-11.

30. Patel RB, Patel PU. Development and validation spectrophotometric method for simultaneous determination of zonisamide and bupropion in synthetic mixture. World J Pharmaceut Res. 2017;6(4):1399-06. https://doi.org/10.20959/wjpr20174-8228.

31. Persson B-A, Vessman J. The use of selectivity in analytical chemistry—some considerations. TrAC Trends Anal Chem. 2001;20(10):526–32.

32. Ramnadh B, Vikas C. Simultaneous estimation of naltrexone and bupropion in pharmaceutical dosage form by using UV spectroscopy. World J Biol Pharm Health Sci. 2020;1(1):033–41.

33. Rao M, Prahhaka T, Sankar G, Jyothi N. Development and validation of new stability indicating HPLC method for the determination of valganciclovir in tablet dosage forms. Int J Pharm Sci. 2012;2(4):101–4.