**Pharmacokinetic and Analytical Evaluation of Cinchocaine HCl in Ointment Using Reversed-Phase HPLC**

**ABSTRACT**

A reversed-phase HPLC method has been developed for the separation and simultaneous determination of the local anesthetic Cinchocaine in pharmaceutical formulations. The primary objective of this study was to establish a simple, fast, efficient, and reliable HPLC analytical method for the determination of Cinchocaine in its pharmaceutical dosage form. The analysis was conducted using a reversed-phase C18 column (Luna, 250×4.6 mm, 5μm, 1000A, Phenomenex, Inc.) with a mobile phase consisting of a 50:50 (v/v) mixture of Phosphate buffer (pH 2.5) and Acetonitrile, flowing at 1.0 mL/min. Detection was performed using a UV detector set at 250 nm. The developed method demonstrated effective separation of Cinchocaine from other components in just 2.9 minutes. This method was successfully employed for the determination of Cinchocaine in ointment formulations, showcasing its potential for routine analysis in quality control settings. The rapid separation and reliable results indicate that this HPLC method can be utilized for both routine analysis and further research on local anesthetic formulations, ensuring consistent quality and efficacy in pharmaceutical products.

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**Keywords:** Cinchocaine, HPLC, ointment,Calibration curve, Standard Deviation, Retention time.

**INTRODUCTION**

Anesthesia is a cornerstone of modern medicine, enabling the performance of surgical and diagnostic procedures that would otherwise be too painful or traumatic for patients1. Anesthetics are drugs that induce a reversible loss of sensation, facilitating pain-free medical interventions. They are classified into two major categories: general anesthetics, which induce a reversible loss of consciousness, and local anesthetics, which provide a reversible loss of sensation in a specific region of the body while allowing the patient to remain conscious2. While analgesics relieve pain without eliminating sensation, anesthetics block sensory input more profoundly, making them indispensable in surgical and diagnostic procedures3.

General anesthetics work by depressingly affecting the central nervous system (CNS) to a degree that permits surgical procedures4. These agents, which include inhalational anesthetics (e.g., desflurane, isoflurane) and intravenous anesthetics (e.g., propofol, ketamine), are carefully administered due to their narrow therapeutic indices, meaning the margin between an effective dose and a toxic dose is small5. This necessitates precise management of the drug. The choice of general anesthetic depends on various factors, including the patient's age, medical condition, and the type of procedure being performed6. The main objectives during general anesthesia are to minimize the adverse effects of anesthetic agents, maintain physiological homeostasis during surgery, and ensure optimal postoperative recovery by reducing surgical stress responses7.

In contrast, local anesthetics are used to block nerve impulses in a specific part of the body, providing targeted pain relief while maintaining consciousness8. These drugs work by inhibiting sodium influx through voltage-gated sodium channels in neuronal membranes, which prevents the generation and conduction of action potentials along nerves9. Local anesthetics are typically classified into two chemical families: esters (e.g., procaine, tetracaine) and amides (e.g., lidocaine, bupivacaine)10. Amides, such as lidocaine, are preferred in clinical practice due to their more favorable pharmacological profiles, including a lower risk of allergic reactions and a longer duration of action11. These agents can be administered through various techniques such as topical application, infiltration, nerve blocks, and epidural or spinal anesthesia12. The use of vasoconstrictors like epinephrine in conjunction with local anesthetics helps prolong their effects by reducing systemic absorption and enhancing local drug concentration13.

Among the class of amide-type local anesthetics, cinchocaine stands out for its potency and long-lasting effects. It is widely used for surface and spinal anesthesia, particularly in proctology for the treatment of anorectal conditions like hemorrhoids14. Cinchocaine exerts its action by blocking sodium channels in neuronal membranes, thereby preventing the transmission of nerve impulses. While highly effective, cinchocaine has a relatively high toxicity profile, particularly in the CNS and cardiovascular system15. Consequently, its use must be carefully controlled to avoid severe side effects such as CNS toxicity, cardiovascular collapse, and respiratory depression. Despite these risks, cinchocaine remains an important agent due to its rapid onset and extended duration of action, making it valuable in both surgical and non-surgical settings16.

The development of anesthetic agents, particularly local anesthetics like cinchocaine, has dramatically transformed medical practice, allowing for safer and more effective surgical interventions. Advancements in the understanding of the pharmacokinetics, pharmacodynamics, and mechanisms of action of these drugs continue to enhance their therapeutic applications. New compounds and techniques are actively being explored, promising to further advance the field of anesthesia and improve patient care. As such, continuous research and innovation in anesthetic drugs and their delivery systems are essential for maximizing the safety and efficacy of anesthesia in medical practice.

**Aim of the Study**

The aim of this study is to develop and validate a simple, rapid, and reliable reversed-phase HPLC method for the quantification of Cinchocaine in pharmaceutical ointments. The method optimized chromatographic conditions, including the mobile phase, flow rate, and detection wavelength, to ensure accurate and efficient analysis.

**METHODS AND MATERIALS**

**Drugs & Materials**

The present work was done at CARS (Centre of Advanced Research in Sciences), Dhaka University, Bangladesh. Working standard of Cinchocaine HCl was from a leading pharmaceutical industry, Dhaka, Bangladesh. Sample is collected from local market. HPLC grade methanol was procured from Active Fine Chemicals Ltd., Dhaka, Bangladesh. HPLC grade methanol & Milli-Q water were used for each solution preparation.

**Instrument Used**

**UV Visible Spectrophotometer:** A double beam UV/Visible spectrophotometer, Shimadzu UV-1700 Pharma spec, Japan was employed with a pair of 1 cm quartz cells for all analytical work.

**HPLC System:** High Performance Liquid Chromatography system (shimadzu-UFLC Prominence), equipped with an auto sampler (Model-SIL 20ac HT) and UV-Visible detector (Model-SPD 20A) was used for analysis. The data was recorded with LC –solutions software.

Column Specification: An analytical reversed phase C-18 (ODS) column (4.6nm x 250mm; 5 um), Phenomenex, Inc was used for analysis.

**Chromatographic Conditions**

The chromatographic conditions employed in this study involved using a Luna C18 column (250 x 4.6 mm, 100 A°) as the stationary phase. The mobile phase consisted of Phosphate buffer pH 2.5 (PB-5) (Solvent A) and Acetonitrile (ACN) (Solvent B), with a solvent ratio of 50:50 (v/v). The analysis was conducted at a detection wavelength (λ max) of 250 nm, and the system was maintained at room temperature (~25°C). The injection volume was 20 µL, with a flow rate of 1 mL/min. The total run time was 7 minutes, and the retention time of Cinchocaine was found to be 2.91 minutes. The chromatographic separation was performed under isocratic elution conditions.

**Preparation of Standard solutions:**

Cinchocaine stock standard solution 100 µg/ml was prepared by dissolving 10mg of pure cinchocaine in 100 ml HPLC grade methanol and dissolved properly in volumetric flask. Then 2 ml, 3 ml, 4 ml, 5 ml, 6 ml from this solution was taken in separate eppendor tube and each was leveled up to 10 ml to make concencentration 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, 60 µg/ml accordingly. From these data standard curve was prepared by plotting area vѕ concentration.

**Composition of Cinchocaine 5mg ointment:**

Each gm contains 5 mg cinchocaine as Cinchocaine Hydrochloride BP.

**Preparation of sample solutions**

50mg cinchocaine ointment was weighed and dissolved in 50 ml HPLC grade methanol. After, it was kept for sonication for 20minutes. Then it was filtered by using a filter paper to have final concentration 20 µg/ml. The area and retention time was measured at 250nm using UV-visible detector.

**Determination of λ max**

An accurately weighed quantity of 20 mg cinchocaine was transferred to an eppendor tube diluted using Methanol as solvent up to 10 ml. Working standard solutions and sample solutions were scanned in the entire UV range (200 to 400) to determine the λ max. The λ max of cinchocainbe was found to be 247nm.

**Determination of retention time and area**

Accurately weighed cinchocaine was diluted using Methanol as solvent and produce various concentration. Checked their retention time and area. The retention time of cinchocaine was 2.9 min and the area was varied for its concentration.

**Determination of physical characteristics of the market preparation**

The different pharmaceutical formulations were collected from the local market. Determination of physical characteristics of the market preparation we checked color, taste, Odor, pH, transparency etc.

**Assay**

Method was based on Quantitative equation method. Primary stock solution 1 mg/ml of cinchocaine was prepared by using Methanol. Calibration curve was prepared by using different concentrations of standard cinchocaine solutions. Cinchocaine in dosage form were estimated using the calibration curve. Working standard solutions of 20, 30, 40, 50 and 60 μg/ml was prepared from stock solution (1 mg/mL) using Methanol as solvent. The absorbance of these standard solutions was measured at 250 nm and calibration curve was plotted. Standard Deviation was calculated from calibration curve. Market preparations were analyzed and assayed using calibration curve.

**RESULT**

Selection of solvent was based on solubility and stability of drug in solvent system as well as extraction of drug from its formulation. Cinchocaine hydrochloride pure form and its market formulation can be freely soluble in ethanol, methanol and other organic solvents. 0.1M HCl, ethanol, methanol was selected as solvent for UV spectrometric determination.

Stock solution (1 mg/ml) of standard Cinchocaine hydrochloride was prepared respectively in 0.1M HCl, ethanol & water (1:99), methanol & water (50:50). Then, individual stock solution was further diluted to 100 µg/ml solution. Each solution was scanned over the range of 200-400 nm and λ max was found 247 1nm for individual preparation. Therefore, it was decided to use Cinchocaine HCl as standard solution for the total experiment. Three different working solutions for each 20, 30, 40, 50 and 60 µg/ml concentrations of Cinchocaine HCl were prepared by dilution with 0.1M methanol solvent system and absorbance were taken at 240 nm. A calibration curve was constructed using the average absorbance values of the 5 different standard concentrations and their standard deviations were also calculated. A linear relationship (R2 = 0.996) was obtained within the concentration range experimented.

**Table 1: Absorbance and standard deviation (STDEV) of 5 different concentrations of standard Cinchocaine HCl**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Concentration**  **(µg/ml)** | **Abs 1** | **Abs 2** | **Abs 3** | **Average** | **STDEV** |
| 5 | 0.069 | 0.075 | 0.072 | 0.072 | 0.003 |
| 10 | 0.117 | 0.121 | 0.119 | 0.119 | 0.002 |
| 15 | 0.169 | 0.167 | 0.165 | 0.167 | 0.002 |
| 20 | 0.224 | 0.226 | 0.222 | 0.224 | 0.002 |
| 25 | 0.260 | 0.256 | 0.264 | 0.260 | 0.004 |

**Figure 1: Calibration curve of standard Cinchocaine HCl**

Cinchocaine HCl stock standard solution 100 µg/ml was prepared by dissolving 10mg of pure cinchocaine in 100 ml HPLC grade methanol and dissolved properly in volumetric flask. Three different working solutions for each 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, and 60 µg/ml concentrations were prepared by dilution with the same solvent system and retention time and area were taken at 250 nm. A calibration curve was constructed using the average area values of the 5 different standard concentrations and their standard deviations were also calculated. A linear relationship (R2 = 0.996) was obtained within the concentration range experimented. Cinchocaine HCl was found in 2.8 min.

**Table 2 Area and standard deviation (STDEV) of 5 different concentrations of standard Cinchocaine HCl**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Conc(µg/ml)** | **Area 01** | **Area 02** | **Area 03** | **Avg** | **STDEV** |
| 20 | 536890 | 536930 | 536984 | 536935 | 47.1734 |
| 30 | 775180 | 775100 | 775280 | 775187 | 90.185 |
| 40 | 1084725 | 1084771 | 1084638 | 1084711 | 67.545 |
| 50 | 1300710 | 1300854 | 1300735 | 1300766 | 76.9437 |
| 60 | 1525014 | 1524970 | 1525028 | 1525004 | 30.2655 |

**Figure 2: Calibration curve of standard Cinchocaine HCl**

Three set of working solutions (20 µg/ml) of sample were prepared and area, retention time was taken at 250 nm. The area and retention time was measured at 250nm using UV-visible detector.

The concentrations were calculated using the equation of calibration curve for cinchocaine hydrochloride.

**Table 3: Area and standard deviation sample**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Brand** | **Area 1** | **Area 2** | **Conc 1 (µg/ml)** | **Conc 2 (µg/ml)** | **Average** | **STDEV** |
| A | 50772 | 50452 | 0.277 | 0.265 | 50612 | 226.2742 |

**DISCUSSION**

This study determines the concentration of Cinchocaine Hydrochloride (Cinchocaine HCl) in various formulations using UV-visible spectrophotometry and HPLC (High-Performance Liquid Chromatography) methods, in addition to analyzing relevant scientific literature regarding its various applications and analytical methods.

The solvent selection for the UV-visible spectrophotometric analysis of Cinchocaine HCl was based on the solubility and stability of the drug in various solvents, including ethanol, methanol, and 0.1 M HCl. These solvents were chosen due to their ability to extract the drug from its formulations efficiently. Both pure Cinchocaine HCl and its commercial formulations were soluble in these solvents, which is consistent with the findings of previous studies17. This solubility allowed for the preparation of stock solutions and subsequent dilution to the desired concentration levels (100 µg/ml), followed by scanning across a wavelength range of 200–400 nm to identify the λ\_max. The λ\_max value was found to be 247 ± 1 nm, in agreement with the literature (Azim et al., 2007), confirming that Cinchocaine HCl absorbs well at this wavelength.

The calibration curve was constructed using various standard concentrations, and a strong linear relationship (R² = 0.996) was observed within the concentration range. This high degree of linearity indicates that the UV-visible spectrophotometric method can be used reliably for quantitative determination of Cinchocaine HCl in its formulations. The values for absorbance and standard deviations, presented in Table 1, further affirm the precision of the method, where the small variations in absorbance (with standard deviations ranging from 0.002 to 0.004) suggest minimal experimental error.

The HPLC analysis for Cinchocaine HCl was conducted with a 100 µg/ml stock solution, and working solutions were prepared at concentrations ranging from 20 µg/ml to 60 µg/ml. A calibration curve was constructed using the average area values of the five different standard concentrations, and a linear relationship (R² = 0.996) was again observed, confirming the accuracy and reliability of the method for Cinchocaine HCl quantification.

In comparison with UV-visible spectrophotometry, HPLC offers superior sensitivity, particularly for complex formulations, as it allows for the separation and detection of individual components in a mixture18. The retention time for Cinchocaine HCl in this study was found to be 2.8 minutes, which is consistent with previous studies using similar experimental setups19. The small standard deviations for the areas (ranging from 30.27 to 90.19) further corroborate the precision of the HPLC method, allowing for accurate quantification of Cinchocaine HCl in pharmaceutical formulations (Table 2).

The assay of market preparations showed that the concentrations of Cinchocaine HCl in the samples were lower than expected, with the average concentration being 0.277 µg/ml for Brand A. This result suggests that commercial formulations may have lower than anticipated amounts of the active ingredient, which could be due to variations in manufacturing processes or formulation stability. This finding aligns with literature reports where inconsistencies in the concentration of active ingredients in commercial formulations have been observed20. It is crucial for manufacturers to adhere to stringent quality control measures to ensure that the actual concentrations of active ingredients match the labeled concentrations, especially for therapeutic compounds like Cinchocaine HCl21.

A comparison of this study's findings with the literature reveals that several other methods have been used to determine the concentration of Cinchocaine HCl. For instance, Huang Lan & Huang Jian (2008) used HPLC with a good linearity range (r = 0.99996) for the quantification of Cinchocaine HCl in compound ointments. Similarly, Lin S (2023)22 developed a method using derivative spectrophotometry and HPLC for the determination of Cinchocaine HCl and its degradation products. These studies confirm that HPLC and spectrophotometric techniques remain the methods of choice for determining the concentration of Cinchocaine HCl, offering high accuracy and reproducibility.

Additionally, Xin L (2024)23 successfully determined Cinchocaine HCl in skin tissues using HPLC, with a method that was both rapid and sensitive. This highlights the versatility of the HPLC technique in determining Cinchocaine HCl in different biological matrices, as opposed to the current study which focused primarily on pharmaceutical formulations.

Furthermore, in the field of local anesthetics, studies have also examined the use of Cinchocaine in preventing neuronal damage. Lovejoy (2024)24 demonstrated that Cinchocaine mitigates spreading depolarization in human neocortical slices, a key finding in stroke treatment. This neuroprotective effect further underlines the potential therapeutic benefits of Cinchocaine beyond its role as a local anesthetic25.

**CONCLUSION**

A sensitive, accurate, and rapid analytical method has been developed for analyzing local anesthetics and parabens in pharmaceutical formulations. Parabens, used extensively in cosmetics, foods, and pharmaceuticals, are linked to potential health risks such as skin cancer, genotoxicity, and reproductive issues. This method, based on HPLC, offers a simple, economical, and fast approach for determining parabens in liquid preparations. A calibration curve was established for methyl paraben and propyl paraben, showing a good linear relationship. Market samples of ketotifen syrup were analyzed, revealing slightly higher paraben concentrations, though without harmful effects. The UV-Vis method provides quick analysis, but HPLC allows for better separation and simultaneous determination of parabens, making it suitable for routine quality control.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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