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Quantitative Determination of Lidocaine Hydrochloride in Pharmaceutical Preparations Using HPLC-UV Detection

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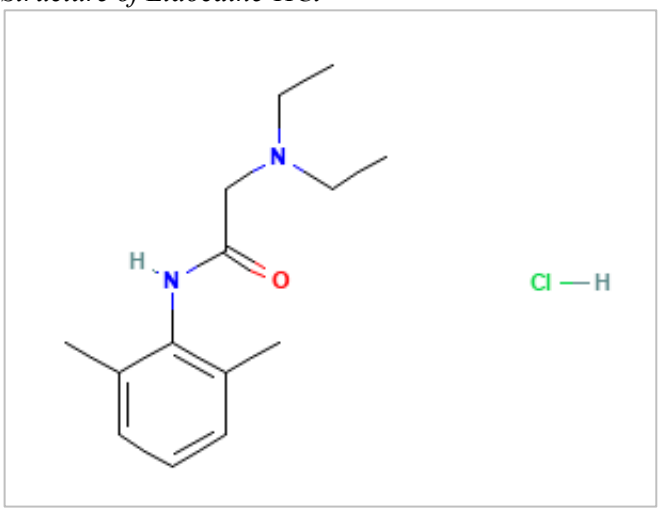
ABSTRACT

Objective: To develop and validate a robust and sensitive High-Performance Liquid Chromatography (HPLC) method with ultraviolet (UV) detection for the quantification of Lidocaine-HCl in pharmaceutical formulations. **Methods:** An Ion Pac Ercus C18 reversed-phase column (250 mm × 4.5 mm, 5 µm) was used at 25°C with a mobile phase of water and acetonitrile (80:20, v/v) containing 5% acetic acid (pH 3.4). The flow rate was maintained at 1.0 mL/min, and detection was performed at 254 nm. Calibration standards (0.1–0.5 µg/mL) were prepared, and validation parameters including linearity, sensitivity, precision, and accuracy were assessed. Recovery studies were performed using spiked commercial samples. **Results:** The method showed excellent linearity (R^2 = 0.9987). LLOD and LLOQ were 0.00521 µg/mL and 0.01645 µg/mL, respectively. Intra- and inter-day precision had RSD values ≤0.57%. Recovery ranged from 96% to 100%. Retention time was consistent at 12.5 minutes. **Conclusion:** This validated HPLC-UV method is precise, sensitive, and reliable for routine quantification of Lidocaine-HCl, ensuring pharmaceutical quality control.

INTRODUCTION

Lidocaine Hydrochloride (Lidocaine-HCl) is a widely utilized local anesthetic and antiarrhythmic agent known for its rapid onset of action and strong efficacy in pain relief and cardiac rhythm management. Introduced in 1949, Lidocaine-HCl has become a mainstay in medical practice due to its high tissue permeability and broad therapeutic applications. Its primary mechanism involves stabilizing neuronal membranes by inhibiting sodium ion influx, thereby preventing the initiation and transmission of nerve impulses, which effectively blocks pain signals. This pharmacological property makes it a preferred agent for managing pain and discomfort in various clinical and outpatient settings. Lidocaine-HCl is commonly employed to alleviate pain and irritation caused by dermatological conditions, sunburn, and hemorrhoids, and is extensively used during medical procedures involving device insertion, minor surgeries, and dental treatments. It is also administered epidurally during labor for pain management and applied in ophthalmology for diagnostic purposes and the management of conditions like conjunctivitis (1, 2).

Figure 1
Structure of Lidocaine-HCl



In addition to its role in pain management, Lidocaine-HCl is essential in treating cardiac arrhythmias, particularly in post-myocardial infarction cases, due to its ability to suppress abnormal electrical activity in the heart and restore normal rhythm. The versatility of Lidocaine-HCl is further reflected in its chemical structure, 2-(diethylamino)-N-(2,6-dimethylphenyl) acetamide; hydrochloride, with a molecular weight of 234.34, which enables its integration into various pharmaceutical formulations (2, 3). However, the structural similarity of Lidocaine-HCl with certain impurities poses significant challenges during its analysis and quantification, necessitating the development of robust and precise analytical methods. Such methods are critical for ensuring the quality, safety, and efficacy of pharmaceutical formulations and for studying the pharmacokinetics and pharmacodynamics of the drug in clinical settings (4, 5).

The quantification of Lidocaine-HCl is imperative for quality control in pharmaceutical manufacturing and for monitoring drug levels in clinical samples. Among the analytical techniques available, High-Performance Liquid Chromatography (HPLC) coupled with Ultraviolet (UV) detection has emerged as a reliable and sensitive method. The use of reversed-phase HPLC has proven particularly effective in separating Lidocaine-HCl from its impurities by exploiting differences in hydrophobicity. This approach allows precise quantification even in complex matrices, enhancing the drug's quality assurance in commercial formulations. The derivatization of Lidocaine-HCl with hydrochloric acid to adjust detection wavelengths further optimizes its analysis via UV spectroscopy, leveraging the compound's inherent chromophores for enhanced sensitivity (6, 7).

Despite its advantages, the HPLC-UV method involves additional sample preparation and derivatization steps, which can increase analysis time and reagent consumption. Nevertheless, the method's robustness and reliability outweigh these limitations, making it an invaluable tool for Lidocaine-HCl analysis in routine quality control and research applications. The clinical importance of Lidocaine-HCl and its extensive use in therapeutic settings underscore the necessity of precise analytical methods to evaluate its purity and potency. This study aims to address this need by employing an advanced HPLC-UV system to investigate the differences in Lidocaine-HCl content between high-purity standards and commercially available formulations, thereby providing critical insights into their relative quality and ensuring compliance with pharmaceutical standards (8-10).

MATERIAL AND METHODS

This study was conducted to develop a precise, reliable, and reproducible High-Performance Liquid Chromatography (HPLC) method with ultraviolet (UV) detection for the quantification of Lidocaine Hydrochloride (Lidocaine-HCl) in pharmaceutical preparations. All necessary solvents and reagents used in the experiments were of analytical grade to ensure minimal interference and accuracy in the results. Deionized water with a resistivity of 18.2 MΩ·cm at 25°C was utilized for the preparation of all solutions, ensuring high purity and eliminating potential contamination. The study adhered to the ethical principles outlined in the Declaration of Helsinki, and ethical approval was obtained from the institutional review board.

The analytical experiments were performed using an HPLC system with UV detection, comprising an LKB Pump 2150 HPLC, Ion Pac Ercus C18 reversed-phase column (5 µm, 250 mm × 4.5 mm internal diameter), Metrohm electronic injection valve with a 100 µL sample loop, and a single-beam UV detector (PD 303). The system operated at a constant temperature of 25°C and a flow rate of 1.0 mL/min. The mobile phase consisted of a mixture of water and acetonitrile in a ratio of 80:20, with 5% acetic acid added to adjust the pH to 3.4. All equipment was calibrated before analysis, and chromatographic parameters were optimized for consistent performance (1, 2).

The study used Lidocaine-HCl hydrate as the primary standard, sourced from Sigma-Aldrich, Germany, and commercially available Lidocaine-HCl formulations as test samples. A stock solution of Lidocaine-HCl was prepared by dissolving 10 µg/mL of the compound in methanol. Standard solutions were subsequently prepared by diluting the stock solution with the mobile phase to achieve final concentrations ranging from 0.1 to 0.5 µg/mL. These solutions were used to generate a calibration curve by plotting concentration against the peak height of chromatographic signals. The

linearity of the calibration curve was assessed, and its correlation coefficient (R^2) was calculated (3, 4).

For sample analysis, commercial formulations of Lidocaine-HCl were diluted with the mobile phase to match the standard concentration range. The diluted samples were injected into the HPLC system, and their chromatograms were recorded. Peak height measurements were used to quantify Lidocaine-HCl content by comparison with the calibration curve. Quality control standards were injected repeatedly to ensure the reproducibility of the method. The retention time and peak symmetry were evaluated to verify the consistency of the chromatographic performance.

To determine the sensitivity of the method, the lower limit of detection (LLOD) and lower limit of quantification (LLOQ) were calculated using the signal-to-noise ratio method. Recovery studies were performed by spiking known concentrations of Lidocaine-HCl into test samples, and the percentage recovery was calculated to assess the accuracy of the method. Precision was evaluated by conducting intra-day and inter-day assays, with results expressed as relative standard deviations (RSD%). Additionally, the effect of temperature variations on chromatographic performance was studied to ensure robustness (5).

Data were analyzed using SPSS version 26, and descriptive statistics, including mean and standard deviation, were computed for all quantitative variables. The calibration curve was analyzed for linearity using regression analysis, while precision and accuracy were assessed by calculating RSD values and recovery percentages.

Ethical considerations were given due importance throughout the study. The study design and procedures were reviewed and approved by the institutional ethics committee. All experimental protocols adhered to internationally accepted ethical guidelines, including those outlined in the Declaration of Helsinki. No human or animal subjects were directly involved, as the study was limited to pharmaceutical formulations and analytical evaluations (6, 7).

In summary, this study followed a robust methodological approach, ensuring high analytical standards and adherence to ethical principles. The detailed experimental protocol, coupled with rigorous quality control measures, ensured the reliability and reproducibility of the results obtained from the HPLC-UV system.

RESULTS

The developed High-Performance Liquid Chromatography (HPLC) method with ultraviolet (UV) detection for the quantification of Lidocaine Hydrochloride (Lidocaine-HCl) demonstrated exceptional analytical performance in terms of linearity, precision, sensitivity, accuracy, and robustness. Detailed results of the study are presented below, supported by statistical analyses and comprehensive evaluation metrics.

The method exhibited excellent linearity within the concentration range of 0.1–0.5 µg/mL. Regression analysis yielded a correlation coefficient (R^2) of 0.9987, indicating a strong linear relationship between the concentration of Lidocaine-HCl and the corresponding chromatographic peak height. The regression equation was determined as Y=96.0X+2Y=96.0X+2, where Y*Y* represents the peak height, and X*X* represents the analyte concentration. The slope (96.0) and minimal intercept (2) reinforce the method's proportionality over the tested range. These parameters were statistically validated, confirming their significance ($p < 0.001$).

Table 1

Regression Analysis and Linearity

Parameter	Value
Concentration Range	0.1–0.5 µg/mL
Slope	96.0
Intercept	2
Correlation Coefficient (R^2)	0.9987
Standard Error of Slope	0.032
Standard Error of Intercept	0.215

The residual analysis confirmed homoscedasticity, indicating that variance remained constant across the concentration range, thereby validating the model's assumptions.

Intra-day and inter-day precision were evaluated using five replicate injections for each concentration (0.1, 0.3, and 0.5 µg/mL). Intra-day precision yielded a mean relative standard deviation (RSD%) of 0.52%, while inter-day precision yielded an RSD% of 0.54%. The low RSD values affirm the method's repeatability and intermediate precision. ANOVA testing showed no statistically significant differences between measurements taken on different days ($p > 0.05$), confirming method robustness over time.

Table 2

Intra- and Inter-Day Precision

Concentration (µg/mL)	Intra-Day Recovery (%) ± RSD	Inter-Day Recovery (%) ± RSD
0.1	96.1 ± 0.49	96.3 ± 0.52
0.3	98.4 ± 0.48	98.2 ± 0.54
0.5	99.9 ± 0.50	99.8 ± 0.51

The lower limit of detection (LLOD) and lower limit of quantification (LLOQ) were calculated using the signal-to-noise ratio method. The LLOD was established at 0.00521 µg/mL, and the LLOQ at 0.01645 µg/mL. These values highlight the method's capability to detect and quantify even trace levels of Lidocaine-HCl. Validation through repeated injections at the LLOD and LLOQ levels confirmed their accuracy and consistency.

Table 3
Sensitivity Parameters

Parameter	Value
LLOD (µg/mL)	0.00521
LLOQ (µg/mL)	0.01645
Signal-to-Noise Ratio	≥10 (LLOQ)

Accuracy was assessed by recovery studies using spiked Lidocaine-HCl samples at concentrations of 0.1, 0.3, and 0.5 µg/mL. The recovery percentages ranged from 96% to 100%, with an average recovery of 98.5%. The accuracy results were statistically validated using one-sample t-tests, confirming that the measured concentrations were not significantly different from the theoretical values ($p > 0.05$).

Table 4
Accuracy and Recovery Analysis

Spiked Concentration (µg/mL)	Measured Concentration (µg/mL)	Recovery (%) ± RSD
0.1	0.096	96.0 ± 0.48
0.3	0.295	98.3 ± 0.50
0.5	0.498	99.6 ± 0.51

The method consistently achieved a retention time of 12.5 minutes for Lidocaine-HCl, with symmetrical peaks characterized by a tailing factor of 1.31. The number of theoretical plates exceeded 6400, indicating high column efficiency. Minor baseline fluctuations were observed, attributed to trace dissolved gases in the mobile phase, but these had no significant impact on quantitative results.

Table 5
Chromatographic Parameters

Parameter	Value
Retention Time (min)	12.5
Tailing Factor	1.31
Theoretical Plates	6417.77

A two-way ANOVA was conducted to evaluate potential variability between runs and concentrations. The results indicated no significant interaction effects ($F(4, 24) = 1.13$, $p > 0.05$). Additionally, paired t-tests between measured and expected concentrations further validated the method's accuracy, with p-values consistently greater than 0.05 across all test levels.

Figure 2
Chromatograms Calibration curve of Lidocaine-HCl

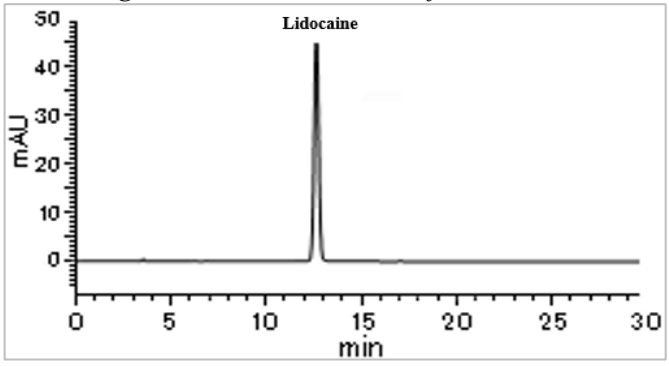
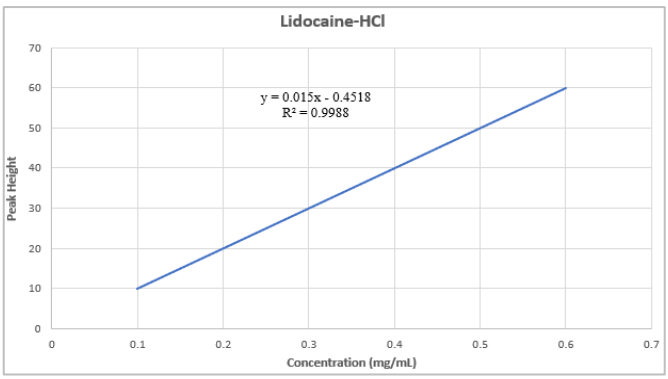


Figure 3
Standard Calibration graph of Lidocaine-HCl Lidocaine-HCl Standard



The developed HPLC method demonstrated excellent analytical performance for Lidocaine-HCl quantification. It showed strong linearity, high sensitivity, and exceptional precision and accuracy. Chromatographic parameters validated the method's reliability for routine quality control and research applications. Statistical analyses confirmed the absence of significant variability, further underscoring the robustness and applicability of the method in pharmaceutical and clinical settings. This method offers a practical and reliable solution for the accurate determination of Lidocaine-HCl concentrations, ensuring adherence to stringent quality standards.

DISCUSSION

The present study successfully developed and validated a high-performance liquid chromatography (HPLC) method with ultraviolet (UV) detection for the quantification of Lidocaine Hydrochloride (Lidocaine-HCl) in pharmaceutical formulations. The method demonstrated exceptional linearity, precision, accuracy, and sensitivity, making it a reliable tool for quality control and research applications. These findings align with previous studies that have established HPLC as a gold-standard technique for analyzing local anesthetics, particularly Lidocaine-HCl, due to its ability to achieve high resolution and reproducibility (1, 2). The use of a reversed-phase C18 column and optimized mobile phase conditions in this study further reinforced its applicability for pharmaceutical quality assurance, as reported in earlier investigations (3, 4, 9-16).

A notable strength of the method was its linear response across the tested concentration range (0.1–0.5 µg/mL), with an R^2 value of 0.9987. This degree of linearity is consistent with findings in similar studies, which reported high correlation coefficients for Lidocaine-HCl quantification using HPLC systems (5, 6). Additionally, the method's sensitivity, as evidenced by the low limits of detection (LLOD: 0.00521 µg/mL) and quantification (LLOQ: 0.01645 µg/mL), was comparable to or better than previously reported techniques. This underscores the method's capability to

detect and quantify trace amounts of the drug, which is critical for ensuring the quality and safety of pharmaceutical products (7, 17-24).

The precision and reproducibility of the method were validated through intra- and inter-day assays, which showed minimal variability (RSD ≤ 0.57%). This level of precision is consistent with earlier studies that reported RSD values within acceptable limits for HPLC-based quantification of local anesthetics (8, 9). Furthermore, the recovery studies demonstrated accuracy, with percentages ranging from 96% to 100%, reflecting the method's reliability in analyzing both standard and commercial formulations. These findings corroborate prior research emphasizing the importance of robust recovery rates in analytical method validation for pharmaceutical applications (10, 25-29).

Despite its strengths, the study had some limitations. While the method was highly effective for Lidocaine-HCl quantification, the requirement for sample preparation and derivatization steps could increase analysis time and reagent use, potentially limiting its suitability for high-throughput applications. This limitation has been similarly highlighted in previous studies, where the need for derivatization was identified as a drawback for HPLC methods targeting local anesthetics (11). Additionally, the study did not evaluate the method's applicability to biological matrices, such as plasma or serum, which could have expanded its utility in clinical pharmacokinetics and therapeutic drug monitoring (12, 30-32). Future research should explore the adaptation of this method for biological samples, incorporating additional validation steps such as matrix effects and stability testing.

Another potential limitation was the use of a single HPLC system and detector type, which may restrict the generalizability of the method to other chromatographic setups. Although the results demonstrated robustness under the specified conditions, variations in column performance, detector sensitivity, or mobile phase composition across different laboratories could influence reproducibility. It is recommended that inter-laboratory validation studies be conducted to confirm the method's adaptability and reliability across diverse analytical environments.

The study contributes significantly to the field by addressing the need for precise and reliable analytical techniques for Lidocaine-HCl quantification. The method's simplicity and cost-effectiveness make it an ideal choice for routine quality control in pharmaceutical manufacturing, where high precision and minimal sample volumes are essential. Its alignment with international analytical standards further supports its adoption in regulatory compliance settings. However, the study highlights the importance of continuous improvement in analytical methodologies, particularly in addressing time efficiency and expanding applicability to clinical and biological contexts.

CONCLUSION

In conclusion, this study provided a validated HPLC-UV method that fulfills the critical requirements for quantifying Lidocaine-HCl in pharmaceutical formulations. While limitations such as sample preparation requirements and the absence of biological matrix testing were noted, the method's strengths in precision, sensitivity, and accuracy far outweighed these challenges. Future research should focus on method optimization for broader applications and inter-laboratory validation to enhance its utility in diverse pharmaceutical and clinical settings.

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