Pharmaceutical analytical determination of doxepin hydrochloride in tablets using an HPLC–UV method

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Doxepin hydrochloride belongs to a group of substances called tricyclic antidepressants. Accurate quantitative estimation is very important for appropriate dosing. However, methods currently used for selective, sensitive and economical collection, as well as quality-controlled methodologies are not identical. The goal of this article is to develope and validate a straightforward, easy, accurate, and cost-effective HPLC-UV technique for doxepin hydrochloride quantification in tablet dosage form. A C18 column (250 mm × 4.6 mm, 5 µm) with mobile phase acetonitrile: phosphate buffer pH 3.0 (70 : 30, v/v) at a flow rate of 1.0 mL/min was used to create an isocratic technique. Detection was performed at a UV wavelength of 295 nm. It was discovered that the method was compliant with ICH Q2(R1). The authors's technique demonstrated good linearity (r 2 = 0.9998) between 10 and 100 µg/mL. The daily accuracy expressed as a percentage RSD was less than 2.0. The precision of the results for recovery ranged from 98.5 to 101.2. The detection and quantification limits were determined to be 0.8 g per milliliter and 2.5 µg, respectively.
A less expensive and less time consuming HPLC-UV method for the analysis of various pharmaceutical compounds can be used to determine the quality control of doxepin hydrochloride in tablets on routine basis.

Keywords: Doxepin hydrochloride, HPLC-UV, method validation, pharmaceutical analysis, quality control, tricyclic antidepressants, stability-indicating method.

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INTRODUCTION

Doxepin is a tricyclic antidepressant (TCA) belonging to the dibenzoxepin drug class. This medication is used to treat depression, anxiety disorders, and chronic insomnia [1]. The medication works as an antidepressant thanks to its activity as an H1-antihistamine that inhibits the re-uptake of serotonin and noradrenaline. This combination is particularly beneficial for patients experiencing depression accompanied by insomnia [2]. Doxepin Hydrochloride ($C_{19}H_{21}NO{\cdot}HCl$) has a molecular weight of 315.84. It exists as a mixture of geometric isomers (E and Z), with the more pharmacologically active E-isomer, predominating in most pharmaceutical formulations. In addition, its efficacy and safety is dose depended, making them key considerations in this context. Thus, accurate tests must be used for the development of an analytical method.

Literature Review of Analytical Methods

Numerous methods have been developed to assess doxepin hydrochloride in both pharmaceutical dosage forms and living creatures. In recent years, several analyses of different methods has been performed. For example, a stability-indicating RP-HPLC method has been published for the determination of doxepin hydrochloride in both bulk drug substances and pharmaceutical formulations, demonstrating high specificity and sensitivity. Nania et al. (2020) provided a comprehensive review of quantitative and qualitative analytical techniques for doxepin, including spectrophotometric, chromatographic, and electrochemical methods, indicating their advantage and limitations[4]. Moreover, Nagalaxmi and Ajitha (2022) established a stability-indicating RP-HPLC method that enables testing for doxepin in capsules, including forced decomposition studies showing how that plays in certain strains [5]. Earlier, Maślanka and colleagues (2011) evaluated the chemical stability of doxepin, especially in acidic conditions, evaluating degradation pathways [6].

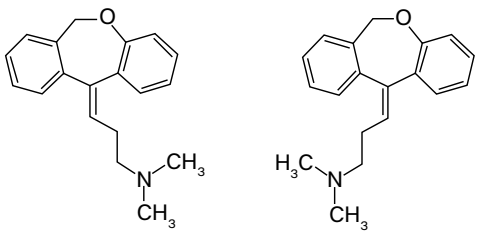

Figure 1. Chemical structure of doxepin, showing (E) and (Z) geometric isomers.

Thin-layer chromatography methods have also been widely used to analyze doxepin. Several studies have focused on developing TLC-densitometric methods designed to quantify doxepin and clomipramine in pharmaceutical preparations [7]. In 2003, Hassan et al. demonstrated a technique for measuring doxepin HCl using both TLC-densitometric and spectrophotometric methods [8]. Similarly, Walash et al. (2011) developed a TLC-densitometric method for the determiniation of doxepin HCl and its degradation products, indicating its stability in this substance. Maślanka et al. (2008) also described TLC methods for identification and quantitative determination of doxepin in pharmaceutical formulations using a variety of detection systems [10]. Although TLC is simple, inexpensive, and allows for the simultaneous analysis of multiple samples, it is generally less sensitive than HPLC techniques and is therefore more commonly used for qualitative or semi-quantitative analysis.

Current analytical techniques and novelty of the present method

At present, available techniques for doxepin determination include spectrophotometry, capillary electrophoresis [11], and several high-performance liquid chromatography (HPLC) methods, including LC-MS/MS [12]. Although LC-MS/MS techniques are sensitive and selective, they are expensive and require considerable expertise, which limits their use in the QA/QC laboratory, especially in low-resource settings [13]. In contrast, the use of HPLC with UV detection is a practical compromise in view of the analytical performance and implementation. According to the International Council for Harmonization (ICH) Q2(R1) guideline, analytical procedures must be validated to demonstrate their suitability for the intended purpose [15].

The existing study describes a simple, precise, accurate, and economical HPLC-UV method for the estimation of doxepin hydrochloride in tablet dosage forms, which has been fully validated. The developed method differs from previously reported methods in its use of simple isocratic mobile phase, an optimized mobile phase pH that ensures symmetrical peak, less than 8 min analysis time, and its cost-effectiveness and suitability for routine QC applications.

MATERIAL AND METHODS

Reagents and chemicals

A doxepin dosage reference standard (99.8% purity) was obtained from Sigma-Aldrich Company (St. Louis, MO, USA). Methanol and

acetonitrile of HPLC quality were purchased from Fisher Scientific Corp (Fair Lawn, NJ, USA). The plurals are distinguished by the adjectives' beginning form. Because they describe something that already exists, the adjectives in the first phrase adopt their typical forms. Analytical-grade sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2, 30%), and hydrochloric acid (HCl, 37%) were purchased from Merck, Darmstadt, Germany, and used in forced degradation studies. Ultrapure water was prepared using the Milli-Q water purification technology (Millipore, Bedford, MA, USA). Doxepin hydrochloride tablets (25 mg, 50 mg, and 75 mg) from three different manufacturers were purchased from three different local pharmacies.

Chromatographic circumstances and instrumentation

HPLC analysis was performed using an Agilent 1260 Infinity II LC System, equipped with a Thermostatted Column Compartment, an automated sampler, ternary pump, plus variable wavelength detectors (VWD). The authors used OpenLAB CDS ChemStation software to manage the data. For the chromatographic separation, a reversed-phase Agilent Equinox XDB-C18 column (250 mm × 4.6 mm i.d., 5 µm) was used. The mobile phase of the experiment consisted of a 70 : 30 (v/v) solution containing acetonitrile and 25 mM phosphate buffer solutions (pH was adjusted near 3.0 using phosphate acid). The analysis was performed in isocratic mode at a flow rate of 1.0 mL/min. The temperature of the column was kept at 25°C, 20 µL of insertion was created and the UV observation was done at 295 nm.

Preparation of standardized and sample solutions

Standard stock solution

A 1000 µg/mL stock solution containing doxepin hydrochloride was prepared by weighing 100 mg of the reference standard, transferring it to a 100 mL volumetric flask, dissolving it in the solvent phase, and adjusting the volume. The stock solution was further diluted with mobile phase to obtain standard solutions in the concentration range of 10-100 µg/mL, which were used for constructing the calibration curve.

Preparation of solutions for tablet analysis

Twenty tablets from each brand were ground into a fine powder. The average weight was measured for the solution to prepare the test of commercial tablets. A quantity of the powder equal to 25 mg of doxepin monohydrate was weighed and placed in a 50 mL volumetric flask. Approximately 30 mL of mobile phase was added to the flask, and the mixture was sonicated for 30 minutes for optimal solubility of the active ingredient. The solution was then diluted to volume with the mobile phase, mixed thoroughly, and filtered through a 0.45µ syringe filter. The filtrate was diluted from 1.0 mL to 10 mL using the mobile phase, resulting in an aggregate concentration of 50 µg/mL (for 100% assay target). The concentration fell within the method's verified calibration range.

Method validation

The developed approach has been verified in line with the ICH Q2(R1) standards.[15]. The method has been tested for specificity, consistency, range, accuracy, precision, LOD and LOQ.

Specificity and forced corrosion studies

Specificity was evaluated by measuring a blank (mobile phase), a standard, and a sample solution to check for interference at the retention time of doxepin. The conducted forced degradation studies validated the stability, indicating the nature of the method. Doxepin solutions were subjected to various stress conditions including acidity (1 M HCl, 80°C, 2 h), basicity (1 M NaOH, 80°C, 2 h), oxidation (3% H_2O_2, room temp., 24 h), heating (80°C, 48 h), and photolysis (UV light, 254 nm, 48 h) show in Table 1.

Table 1. Forced degradation study of doxepin hydrochloride and stability-indicating specificity

Stress Condition	Time/Condition	% Degradation*	Observations (Degradation Products)
Acidic	1 M HCl, 80°C, 2 h	5.2 ± 0.8	Minor unknown peak observed at Rt 4.2 min
Basic	1 M NaOH, 80°C, 2 h	15.3 ± 1.2	Two unknown peaks observed at Rt 6.1 min and 9.8 min
Oxidative	3% H_2O_2, RT, 24 h	8.7 ± 1.0	One unknown peak observed at Rt 5.8 min
Thermal	Dry Heat, 80°C, 48 h	< 2.0	No significant degradation
Photolytic	UV light (254 nm), 48 h	3.1 ± 0.5	Minor unknown peak observed

*% Degradation was calculated using the formula: [(Peak Area of Control - Peak Area of Stressed Sample) / Peak Area of Control] × 100. Results are mean ± SD (n=3).

Once the exposure time was over, the acidic and basic samples were cooled, and afterwards neutralized to a pH of approximately 6–7 using 1 M NaOH and 1 M HCl respectively, and then injected. The neutralization step is of utmost importance in order to prevent possible damage to the silica-based C18 column from extremes in pH and to ensure consistent chromatographic behavior and retention time. The proposed HPLC method was subsequently used to analyze diluted stressed samples.

Linearity and range

Linearity was evaluated by analyzing six working standard solutions of doxepin hydrochloride at concentrations of 10, 20, 40, 60, 80, and 100 µg. Each concentration was administered three times. Graphing mean peak area and respective concentration allowed for the construction of a calibration curve and to determine the regression equation and correlation coefficient (r^2).

Accuracy (recovery)

The standard addition method was used for recovery studies to establish the accuracy of the method. Known amounts of doxepin standard were added to preanalyzed tablet samples at three different concentration levels (80%, 100% and 120% of asssay concentration, 50 µg mL).

The base sample solution was prepared from powdered tablets as described previously.

80% level (target: 40 µg/mL): To a portion of powdered tablets equivalent to 20 mg of doxepin HCl, 10 mg of pure doxepin HCl reference standard was added.In a 100 mL volumetric flask, the mixture was dissolved in the mobile phase to obtain a stock solution with a concentration of 300 µg/mL. This solution was diluted at a ratio of 1 : 7.5 (i.e., 4 mL to 30 mL) to obtain a concentration of 40 µg/mL as stock.

To powdered tablets equivalent to25 mg of doxepin HCl, 25 mg of pure standard was added to obtain the 100% level (target: 50 µg/mL). A stock solution with a concentration of 500 µg/mL was prepared in a 100 mL volumetric flask. This solution was diluted 1 : 10 to achieve a concentration of 50 µg/mL.

A suitable amount of powdered tablets (equivalent to about 30 mg of doxepin HCl) was supplemented with 30 mg of pure standard. The stock solution was 600 µg/mL prepared by dissolving the mixture in a 100 mL volumetric flask. A dilution of 1 : 10 was achieved to give a final concentration of 60 µg/mL.

Every concentration was made, examined three times, and the recovery% was calculated.

Precision

At two levels, precision was evaluated which are repeatability and intermediate precision. The repeatability was determined by injecting six replicated trials of a 50 µg/mL standard solution on the same day. Intermediate precision has been evaluated on three different days of analysis. The outcomes were expressed as the percent relative standard deviation (%RSD).

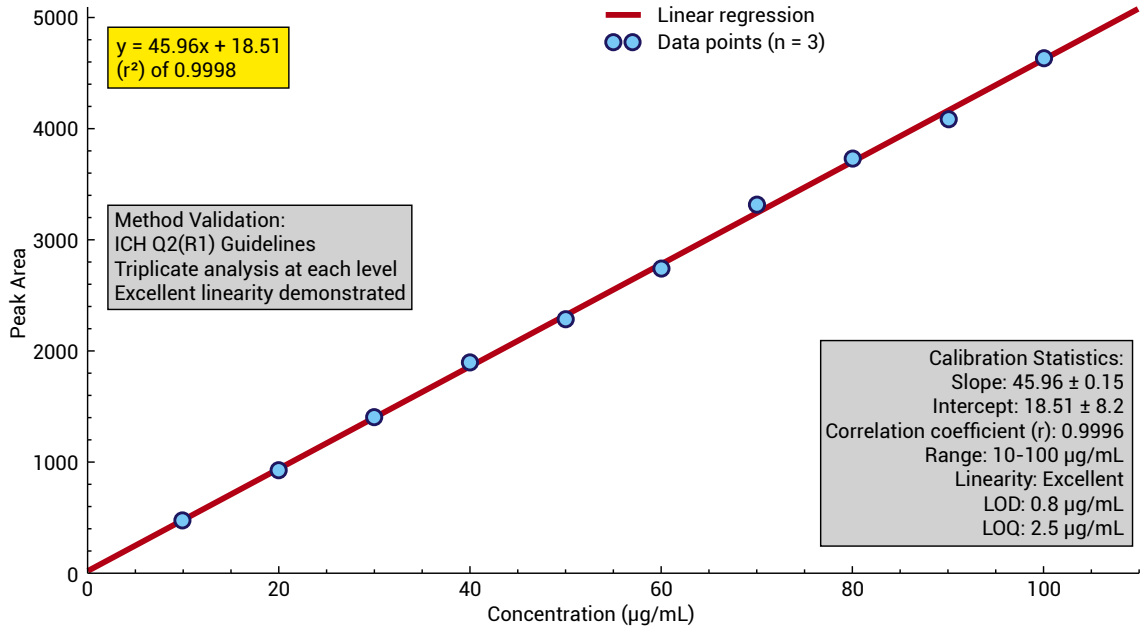

Figure 2. Calibration curve for doxepin hydrochloride (10–100 µg/mL). The regression equation is y = 45.96x + 18.51, with a correlation coefficient (r^2) of 0.9998.

LOD and LOQ

The limits of detection (LOD) and quantitation (LOD) were calculated based on the calibration curve's slope and the standard deviation of the response, using the equations LOD = 3.3 × (σ/S) and LOQ = 10 x (σ/S), where S is the calibration curve's slope and σ is the regression line's intercept standard deviation.

RESULTS AND DISCUSSION

Method improvement and optimization

The main aim was to develop a simple and rapid HPLC method for the analysis of doxepin. The UV spectrum of Doxepin showed maximum absorbance of 295 nm, which was chosen for detection due to high sensitivity. Different types of mobile phase compositions were checked. A mixture of acetonitrile andphosphate buffer (pH 3.0) (70 : 30, v/v) provided the best results; a symmetric peak (asymmetry factor ≈ 1.08) with a suitable retention time (≈ 7.8 min) has a high theoretical plate count, indicating the suitability of the column.

Validation procedure

Specificity

According to chromatograms of blank, standard, and sample solution, the method is specific. No peak overlap from excipients was seen at the retention time of doxepin (7.8 min). According to the forced degradation studies, stability indicating capacity of the method was confirmed as all degradation product peaks were well resolved from the main doxepin peak.

Linearity, distance, LOD and LOQ

Linearity was repeated within the concentration range of 10–100 µg/mL. The linear regression equation y = 45.96x + 18.51, which has a correlation coefficient (r^2) of 0.9998, can be used to explain the calibration curve (Figure 2). Peak area and concentration are closely related. The method's LOD and LOQ values were determined to be 0.8 and 2.5 µg/mL, respectively, suggesting that it is sensitive enough for doxepin measurement in drug formulation.

Accuracy and Precision

The recovery results ranged from 98.5% to 101.2% (Table 2 and Figure 3) confirming the accuracy of the method. As the obtained values fell within the acceptable range of 98–102%, the method can be considered free of systematic errors. The method's precision (%RSD) for reproducibility and intermediate precision was less than 2.0%, indicating its precision.

Application to commercial formulations

The doxepin hydrochloride content of three distinct commercial tablet brands was successfully ascertained using the approved approach. The assay findings (Table 3) indicated that the amount of doxepin in all tested formulations was within the permissible pharmacopeial limits (usually 90–110% of the labeled amount), indicating the usefulness of the approach for routine quality control.

Cost-effectiveness analysis

The proposed HPLC-UV method is more cost--effective than the method described by Rodriguez-Martinez et al. (2020) [16] for several reasons.

The mobile phase consists of simple isocratic binary system (acetonitrile:buffer). Using ternary/ quaternary gradient systems can be a complex method for TCA separation as might be inferred from Rodriguez-Martinez et al. However, the contrary is true for many methods. Gradient elution requires more complex, expensive gradient pumps and various solvent reservoirs and consumes a larger variety of (often more expensive) useful solvents.

Table 2. Evaluation of analytical methods (relative errors, precision, linearity, and sensitivity).

Parameter	Result
Linearity Range (µg/mL)	10–100
Regression Equation	y = 45.96x + 18.51
Correlation Coefficient (r^2)	0.9998
Accuracy (% Recovery, n = 3)	98.5% – 101.2%
Precision (%RSD)	Intra-day: 0.78%; Inter-day: 1.25%
LOD (µg/mL)	0.8
LOQ (µg/mL)	2.5
Retention Time (min)	7.8 ± 0.1

Table 3. Assay of doxepin hydrochloride in commercial tablet formulations.

Brand	Labeled Amount (mg)	Found Amount (mg)*	Assay (%)*
Brand A	25	24.8 ± 0.3	99.2
Brand B	50	50.8 ± 0.7	101.6
Brand C	75	73.8 ± 0.8	98.4

*Mean ± SD (n = 6).

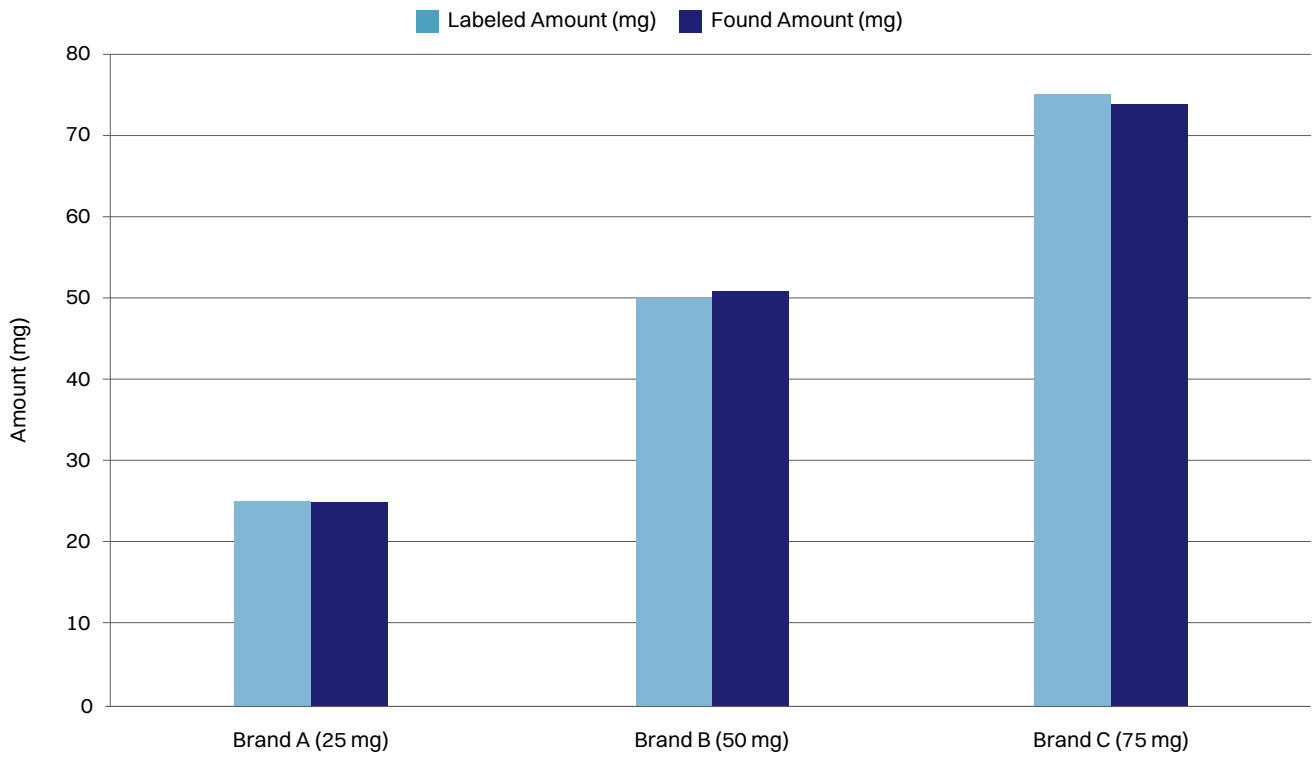

Figure 3. Assay of doxepin hydrochloride.

Faster analyses: Author's method has a run time of less than 8 minutes. The procedure used by Rodriguez-Martinez et al. to separate multiple TCAs takes much longer (approximately 15–20 min). A shorter analysis time means less solvent consumption per sample and higher sample numbers during production, which in turn lowers costs.

Instrumentation: The method is intended for a simple HPLC with a basic UV detector which is commonly available in most QC labs; no specialized or expensive (e.g., diode array, fluorescence) detectors or a mass spectrometer that has higher acquisition and maintenance costs are needed.

Developing and validating a simple isocratic method requires less time and cost than developing and validating a complex gradient method. Additionally, the simpler system requires less maintenance and technical expertise to operate, resulting in long-term cost saving [13]. Considering these factors, the authors' strategy is more cost-effective for routine inspections of a single medicine in the pharmaceutical business.

CONCLUSION

To determine the amount of doxepin hydrochloride in tablets, a straightforward, precise, accurate, and economical stability-indicating HPLC-UV technique has been developed and validated. A thorough validation investigation revealed outstanding analytical performance in accordance with ICH Q2(R1). The method exhibited an linear range of 10–100 grams per milliliter with r^2 = 0.9998, showed good precision (%RSD < 2%), high accuracy (recovery 98.5% to 101.2%), as well as adequate sensitivity (level of detection = 2.5 µg/mL). The method's applicability for routine quality control and stability investigations is demonstrated by its effective application in commercial formulations and its capacity to isolate the test from its degradation products. Because of the procedure's simplicity and low cost, it is a valuable tool for pharmaceutical laboratories with restricted resources.

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