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Original Article

Development and ICH-guided validation of a robust UHPLC method for the quantitative determination of dothiepin hydrochloride in pharmaceutical dosage form

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ABSTRACT

Objective: To develop and validate a reliable Ultra-High Performance Liquid Chromatography (UHPLC) method for the quantitative estimation of Dothiepin in solid Pharmaceutical dosage form ensuring suitability for routine quality control.

Methods: Chromatographic analysis was carried out using a C18 column (100 mm × 4.6 mm, 3.5 µm) under isocratic elution. The mobile phase consisted of potassium dihydrogen orthophosphate buffer, acetonitrile and tetrahydrofuran in a 40:55:5 (v/v/v) ratio. The buffer was prepared by dissolving 5.0 g of potassium dihydrogen orthophosphate in 1000 mL of water and adjusting the pH with orthophosphoric acid. The flow rate was set at 0.6 mL/min, detection was performed at 232 nm injection volume was 5 µL with a total run time of 5 minutes. Method validation was conducted as per ICH Q2(R1) guidelines.

Results: The method showed excellent linearity over 50% - 150% of the test concentration with a correlation coefficient (R^2) of 0.9999. Precision studies yielded %RSD values of 0.27% (repeatability), 0.70% (short-term precision) and 0.27% – 0.88% (intermediate precision). The assay average was 100.92% reflecting high accuracy. Recovery rates ranged from 100.60% to 101.95% across 80%, 100% and 120% levels. Robustness testing indicated consistent performance despite minor variations in flow rate and wavelength detection. The limits of detection (LOD) and quantification (LOQ) were 1.45 µg/mL and 4.38 µg/mL respectively.

Conclusion: The validated UHPLC method is accurate, precise, robust and linear making it highly suitable for the routine analysis and quality control of Dothiepin in solid dosage formulations.

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Introduction

Dothiepin (also known as Dosulepin) is a tricyclic antidepressant (TCA) primarily used in the treatment of major depressive disorders, anxiety and certain neuropathic pain conditions [1]. Dothiepin is chemically named as 3-(6H-benzo[b][1]benzothiepin-11-ylidene)-N, N- dimethylpropan-1-amine (Figure 1). Its molecular formula is $C_{19}H_{21}NS$. It exerts its pharmacological action by inhibiting the reuptake of norepinephrine and serotonin in the central nervous system, thereby enhancing their synaptic availability [2]. Despite the availability of newer classes of antidepressants, Dothiepin continues to be used in clinical settings due to its efficacy, especially in treatment-resistant depression and certain chronic pain syndromes [3,4]. Given the narrow therapeutic index and potential for toxicity of Dothiepin, accurate and reliable quantification in pharmaceutical formulations are essential to ensure quality, safety and efficacy. Ultra-High Performance Liquid Chromatography (UHPLC) has emerged as a preferred analytical technique over conventional HPLC due to its superior resolution, faster analysis time and reduced solvent consumption [4,5]. A review of the literature reveals the availability of analytical methods such as UV spectrophotometry and HPLC for the determination of Dothiepin, either alone or in combination with other drugs [6-8]. However, no UHPLC-based method has been reported for the selective and accurate estimation of Dothiepin in solid pharmaceutical dosage forms. Moreover, a validated UHPLC method that adheres to the International Council for Harmonisation (ICH) guidelines is lacking. Therefore, the present study aims to develop and validate a simple, accurate, specific and robust UHPLC method for the quantitative determination of Dothiepin in tablet formulations. The method is optimized for routine quality control analysis and validated according to ICH Q2(R1) guidelines for analytical procedure validation [9].

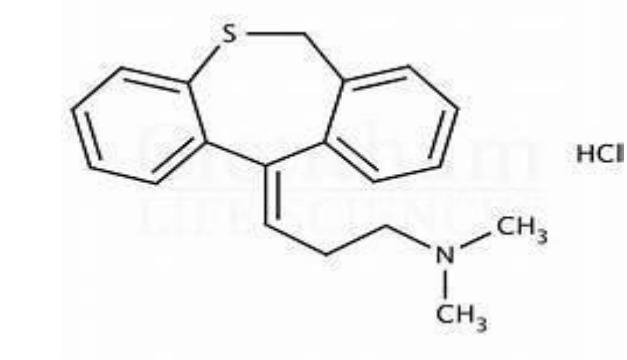

Figure 1: Structure of Dothiepin Hydrochloride.

Importance of Analytical Method Validation

To guarantee precise and reliable measurement of pharmaceutical substances, analytical method development and validation are essential. While development entails maximizing the circumstances for efficient separation, validation verifies the method's dependability using metrics including robustness, specificity, accuracy and precision. In Pharmaceutical analysis, these procedures are crucial for regulatory compliance, stability testing, and quality control.

Materials and Methods

Materials

Dothiepin hydrochloride working standard was receive from Pharmafabrikon-Unit II, Madurai. Potassium dihydrogen orthophosphate, orthophosphoric acid, acetonitrile (HPLC grade) and tetrahydrofuran (THF) were obtained from Rankem (India). All reagents and solvents used throughout the study were of analytical or HPLC grade. Commercially available Dothiepin tablet formulations, each labelled to contain 25 mg of the active ingredient were purchased from a local pharmacy.

Instrumentation

The chromatographic analysis was conducted using an Agilent UHPLC system equipped with a quaternary pump, an autosampler and a photodiode array (PDA) detector. The system was operated using Open Lab CHEM STATION software. Other laboratory instruments included a Radwag semi-micro analytical balance, a PCI ultrasonicator, and a Eutech pH meter. All filtration was carried out using 0.45 µm nylon membrane filters.

Preparation of Standard Solution

Accurately weighed 25 mg of Dothiepin hydrochloride was transferred into a clean and dry 100 mL volumetric flask. It was dissolved in a small volume of 0.1 M hydrochloric acid, sonicated to ensure complete dissolution and the volume was made up to the mark with the same diluent to obtain a stock solution of 250 µg/mL. This solution was filtered through a 0.45 µm membrane filter using a vacuum filtration unit. From the stock, 5 mL was pipetted into a 25 mL volumetric flask and diluted to volume with the same diluent to prepare a working standard solution of 50 µg/mL.

Preparation of Tablet Sample Solution

Twenty tablets were accurately weighed, powdered and a portion equivalent to 25 mg of Dothiepin hydrochloride was transferred to a clean, dry 100 mL

volumetric flask. The sample was dissolved in methanol, sonicated for 20 minutes to ensure complete extraction and the volume was made up to the mark with 0.1 M hydrochloric acid. The resulting solution was filtered through a 0.45 µm membrane filter using a vacuum filtration assembly. From this filtrate, 5 mL was transferred into a 25 mL volumetric flask and diluted to volume with the same diluent to yield a final concentration of 50 µg/mL suitable for analysis.

Preparation of Mobile Phase

5.0 g of potassium dihydrogen orthophosphate was accurately weighed and dissolved in 1000 mL of Milli-Q water. The pH was adjusted to pH 3.0 using orthophosphoric acid to reach the required level. The final mobile phase was prepared by mixing 400 mL of this buffer solution with 550 mL of HPLC grade acetonitrile and 50 mL of tetrahydrofuran in the ratio of 400:550:50 (v/v/v). The mobile phase mixture was degassed using an ultrasonication and filtered through a 0.45 µm membrane filter using a vacuum filtration assembly before use.

Assay of Formulation

5 mL each of standard and sample stock solutions were pipetted into separate 25 mL volumetric flasks, diluted to volume with 0.1 M HCl and sonicated for 15 minutes. The solutions were filtered through 0.45 µm membrane filters prior to analysis. Each solution was injected into the UHPLC system in triplicate and chromatograms were recorded. The assay was calculated by comparing the peak area of the sample with that of the standard under the same chromatographic conditions.

Result

Method Development

The RP-UHPLC method for the analysis of Dothiepin was developed through a series of trials by varying chromatographic parameters such as detection wavelength, mobile phase composition, flow rate and stationary phase. Each parameter was adjusted systematically to observe its effect on peak resolution, retention time and overall separation efficiency. The method was optimized to achieve sharp, symmetrical peaks with minimal tailing and acceptable system suitability criteria.

Selection of Wavelength

To identify the optimal detection wavelength, a 50 µg/mL standard solution of Dothiepin was scanned using the UV detector in the UHPLC system. The compound exhibited maximum absorbance at 232 nm, which provided the most suitable signal-to-noise ratio. Therefore 232 nm was selected as the detection wavelength for all subsequent method development and validation procedures.

Selection of Chromatographic Conditions

Initial trials were performed by injecting standard solutions of Dothiepin under various solvent combinations and ratios to establish a mobile phase that offers effective elution and resolution. The choice of mobile phase and its pH were adjusted to ensure peak symmetry and system stability. Several columns were tested, and chromatographic behaviour was compared by altering stationary phase properties and manufacturers. Flow rate and injection volume were also optimized to achieve reproducible retention time and better separation.

Optimized Chromatographic Conditions

Based on systematic method optimization trials, the final UHPLC conditions for the determination of Dothiepin were finalized as shown in Table 1. A distinct peak was observed at 2.43 minutes with sharp symmetry and a low asymmetry factor confirming good peak shape. Hence the method was finalized under these conditions. The optimized chromatogram is shown in Figure 2.

Table 1. Optimized Chromatographic conditions for Dothiepin Analysis.

Parameter	Optimized Condition
Instrument	UHPLC (Agilent) with OpenLab ChemStation
Flow Rate	0.6 mL/min
Mobile Phase	Buffer: Acetonitrile: Tetrahydrofuran (400:550:50 mL)
Detector/ Wavelength	UV Detector at 232 nm
Run Time	5 min
Injection Volume	5 µL
Column Temperature	Ambient
Diluents	0.1 M HCl for standard and sample preparation
Mode of separation	Isocratic

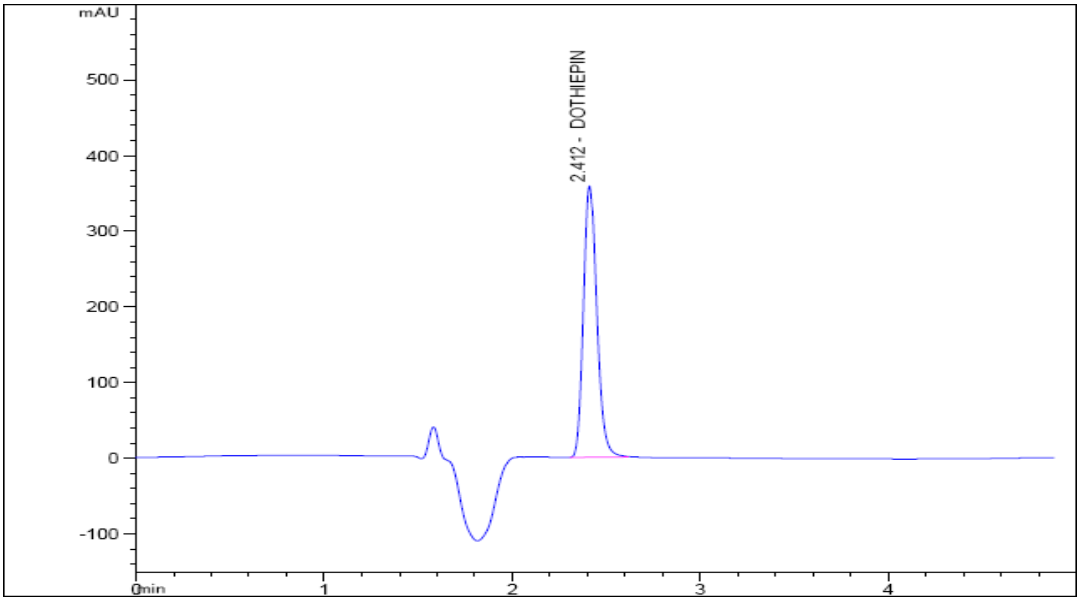

Figure 2: Optimized Chromatogram.

Table 2: System suitability result for Dothiepin HCl.

Details	**Dothiepin**			
Injection No.	Retention Time	Peak Area	Theoretical Plates	Tailing Factor
Injection -01	2.420	1706.451	5725	1.169
Injection -02	2.431	1714.256	5716	1.155
Injection -03	2.433	1702.086	5711	1.126
Injection -04	2.418	1709.410	5769	1.156
Injection -05	2.455	1700.256	5728	1.139
Mean	2.43	1706.492	5730	1.149
SD	0.015	5.637	22.95	0.017
%RSD	0.61	0.33	0.40	1.45

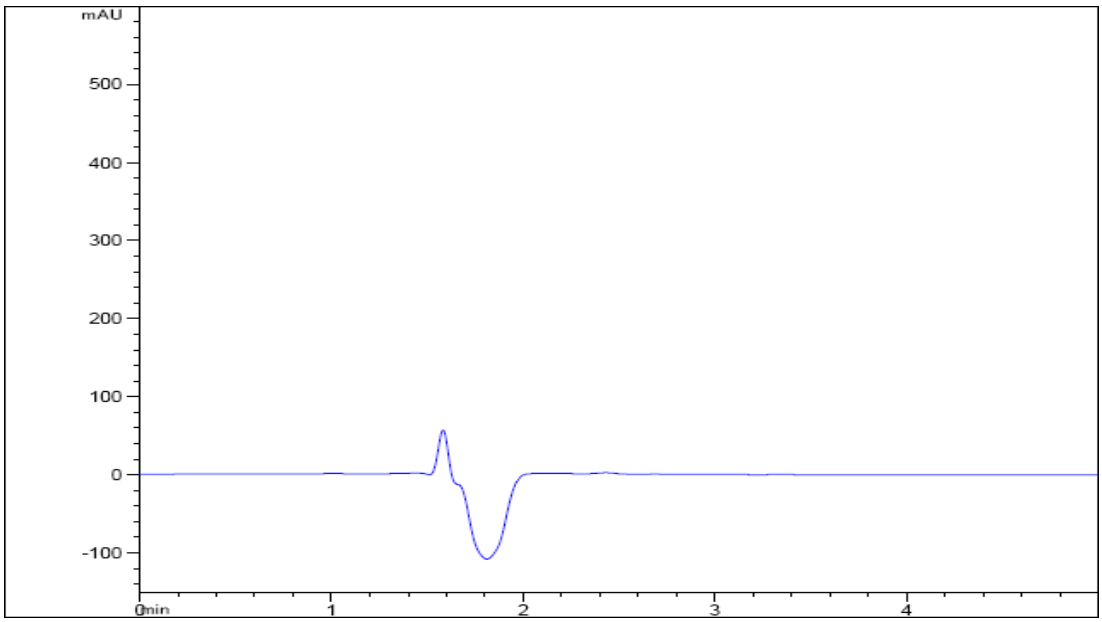

Figure 3: Chromatogram of blank.

Method Validation System Suitability

System suitability was evaluated by injecting five replicates of the standard solution before sample analysis. Parameters such as retention time, theoretical plate count, tailing factor and percentage relative standard deviation (%RSD) of peak area were assessed and the results are summarized in Table 2.

Table 3: Specificity results for Dothiepin HCl.

Sample ID	Dothiepin	
	Retention Time	Area
Blank	2.412	No peak observed
Placebo	2.412	No peak observed
Standard	2.412	1751.651
Sample	2.407	1808.003

Specificity

Specificity was demonstrated by injecting blank, placebo, standard and sample solutions to ensure there was no interference at the retention time of Dothiepin (approximately 2.43 minutes). No peaks were observed 3.

in the blank (Figure 3) or placebo (Figure 4) chromatograms, confirming the method's ability to accurately measure Dothiepin without interference from excipients or solvents. The retention time and peak area data are summarized in Table

Table 4: Linearity result for Dothiepin HCl.

S. No.	% Level	Conc (µg/mL)	Peak area	Statistical Analysis	
1	50%	24.82	890.558	Slope	35.8730
2	70%	34.74	1234.657	Intercept	2.1835
3	100%	49.62	1788.892	Regression Equation	Y = 35.8730x – 2.1835
4	125%	62.03	2220.448	Correlation Coefficient	$R^2 = 0.9999$
5	150%	74.44	2666.448		

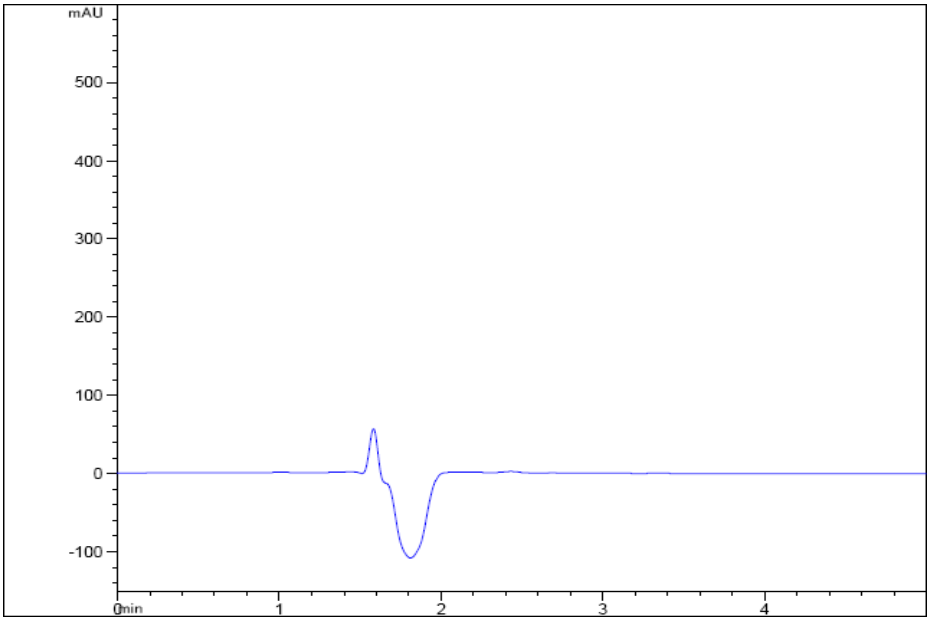

Figure 4: Chromatogram of placebo.

Linearity

Linearity was established across five concentration levels: 50%, 70%, 100%, 125%, and 150% of the target concentration (50 µg/mL). Each level was injected in duplicate. Linearity was evaluated using linear regression analysis (least squares method). The calibration curve (Figure 5) demonstrated a strong linear response over the tested concentration range. The correlation coefficient (R^2) and linearity data are presented in Table 4.

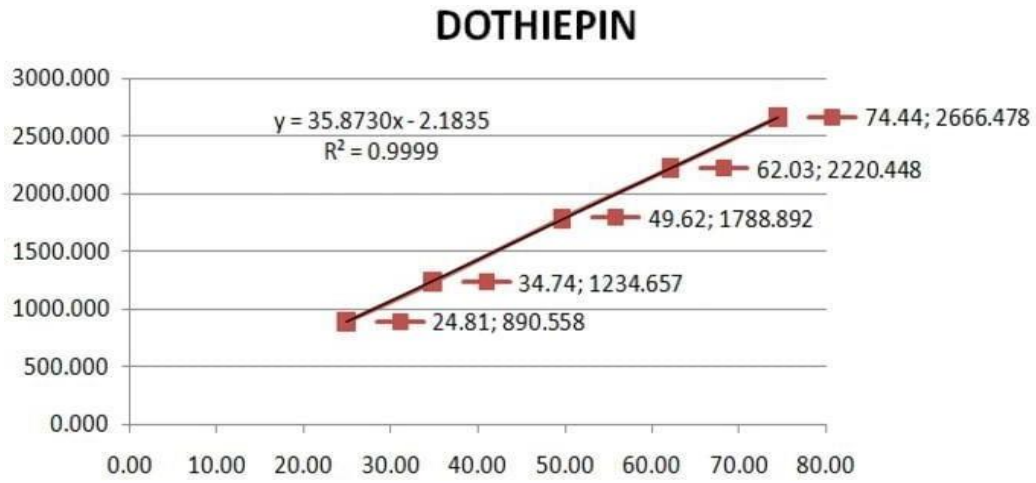

Figure 5: Calibration curve for Dothiepin.

Table 5. Precision result for Dothiepin HCl.

Parameter	Mean	Standard deviation	%RSD
Repeatability	104.17	0.2381	0.27%
Intermediate Precision-Day 1	103.64	0.8697	0.84%
Intermediate Precision-Day 2	104.26	0.9148	0.88%

Precision

Repeatability was evaluated by analysing six independently prepared Dothiepin samples at the same concentration on the same day. Intermediate precision was assessed by performing the analysis on two different days (Day 1 and Day 2), using different analysts and instruments. The %RSD values obtained for both repeatability and intermediate precision were within the acceptable limits as per ICH guidelines, indicating the method's precision and reproducibility. The detailed results are presented in Table 5..

Table 6: Accuracy result for Dothiepin HCl.

Sample ID	Sample wt (mg)	Sample Area	Calculated Content (in mg)	Calculated Content (in %)
80% Sample-01	83.41	1433.925	25.151	100.60
80% Sample-02	83.36	1436.640	25.214	100.86
80% Sample-03	82.57	1435.611	25.439	101.76
100% Sample-01	104.65	1809.709	25.300	101.20
100% Sample-02	104.87	1808.612	25.487	101.93
100% Sample-03	103.91	1810.165	25.482	101.95
120% Sample-01	123.60	2152.797	25.482	101.93
120% Sample-02	124.31	2150.315	25.308	101.23
120% Sample-03	124.68	2151.789	25.250	101.00
			Mean	101.27
			SD	0.494
			% RSD	0.49 %

Accuracy

Accuracy was assessed through recovery studies conducted at three concentration levels 80%, 100% and 120% of the target concentration. Each level was prepared and analysed in triplicate and the percentage recovery of Dothiepin was calculated. The results are summarized in Table 6. The mean recovery ranged from 100.60% to 101.95%, which falls within the acceptable range.

Robustness

Robustness was evaluated by introducing small, deliberate changes to critical method parameters, including flow rate (±0.1 mL/min) and detection wavelength (±1 nm). These variations did not significantly affect the system performance. The %RSD values obtained under each altered condition were below 2.0%, indicating that the method is robust and reliable under varied analytical conditions. The results are presented in Table 7.

Table 7: Robustness result of Dothiepin HCl.

S. No.	Condition	%RSD of Dothiepin Assay
1	Flow rate (-) 0.5	0.52 %
2	Flow rate (+) 0.7	0.47 %
3	Wavelength (-) 231	0.68 %
4	Wavelength (+) 233	0.77 %

Table 8: Assay result of Dothiepin HCl.

Sample	Label Claim (mg/tablet)	Amount Found (mg/tablet)	% Assay
1	25	25.23	100.92

Table 9: Summary of validation parameter.

Parameter		Limit	DOTHIEPIN
Linearity: Regression equation (Y = mx+c)		R^2 not less than 0.999	R^2 = 0.9999
Assay (% mean assay)		90.0% -110.0%	100.92%
Specificity		No interference of any peak	Complies
Repeatability %RSD		RSD NMT 2.0%	0.27%
Repeatability (Short Interval) %RSD		RSD NMT 2.0%	0.70%
Intermediate Precision Day-01 %RSD		RSD NMT 2.0%	0.27%
Intermediate Precision Day-02 %RSD		RSD NMT 2.0%	0.88%
Accuracy %		98% - 102%	100.60%-101.95%
Robustness	**Flow Minus**	RSD NMT 2.0%	0.52%
	Flow Plus	RSD NMT 2.0%	0.47%
	Wavelength Minus	RSD NMT 2.0%	0.68%
	Wavelength Plus	RSD NMT 2.0%	0.77%
LOD		-	1.45 µg/mL
LOQ		-	4.38 µg/mL

Sensitivity (LOD and LOQ)

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated based on the signal-to-noise ratio. The LOD for Dothiepin HCl was found to be 1.45 µg/mL and the LOQ was 4.38 µg/mL demonstrating the method's sensitivity for low-concentration detection.

Assay of Marketed Formulation

The assay of the marketed Dothiepin HCl tablet formulation was performed using the developed UHPLC method. A single test sample solution was injected in five replicates, and the average assay result was found to be 100.92% indicating compliance with pharmacopeial specifications. The results including the label claim, amount of drug found, and percentage assay are summarized in Table 8.

Discussion

The UHPLC method developed in this study offers a significant improvement over previously reported analytical techniques for the quantification of Dothiepin. Traditional HPLC methods typically involve longer run times and higher solvent consumption, whereas the present UHPLC method provides rapid elution (retention time 2.43 minutes) with sharp, symmetrical peaks, thereby increasing throughput and reducing analysis cost.

The method also demonstrated excellent linearity, precision, accuracy and robustness as validated according to ICH Q2(R1) guidelines. Its low limits of detection (LOD = 1.45 µg/mL) and quantification (LOQ = 4.38 µg/mL) confirm the method's sensitivity, making it suitable for detecting even low levels of Dothiepin in Pharmaceutical dosage forms. A summary of all validation parameters and their compliance with ICH Q2(R1) guidelines is provided in Table 9.

Compared to conventional UV or HPLC methods reported in earlier studies, this UHPLC approach offers superior performance with shorter analysis time and better resolution. Notably, there are limited or no prior studies reporting a validated UHPLC method specifically for Dothiepin, underscoring the novelty and relevance of this work.

In the context of routine quality control, this method is highly applicable due to its operational simplicity, speed and reproducibility. Laboratories can benefit from reduced solvent use and increased sample throughput, which are critical for cost-effective pharmaceutical analysis.

Conclusion

A simple and rapid UHPLC method was successfully developed and validated for the quantification of Dothiepin in solid dosage forms. The method offers substantial advantages over traditional HPLC including shorter run time, improved sensitivity and lower solvent usage. With full compliance to ICH Q2(R1) guidelines and excellent performance across all validation parameters, the method is highly suitable for routine quality control and batch release applications in pharmaceutical analysis.

Author Contributions

All authors contributed equally to this research. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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Ethical Approvals

This study does not involve experiments on animals or human subjects.

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