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Research Article

Development and Validation of RP-HPLC Method for Determination of Dothiepin Hydrochloride in Bulk and Pharmaceutical Dosage Form

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ABSTRACT
A RP-HPLC method has been developed and validated for the estimation of Dothiepin in bulk and pharmaceutical dosage form. The isocratic separation was achieved on a Shodex C18 column (250×4.6 mm, 5µm).The method was developed using mobile phase comprising of a mixture of 0.05 M phosphate buffer (pH adjusted to 2.6) and methanol (35:65, v/v) at a flow rate of 1.0 mL/min. The analyte was monitored with UV detector at a wavelength of 231 nm. The retention time of Dothiepin hydrochloride was found to be 6.78 min. The method was validated according to ICH guidelines for various parameters like accuracy, precision, specificity, linearity, robustness, LOD and LOQ. Linearity was observed in the concentration range of 0.5-5 µg/mL with a correlation coefficient of 0.999. The limit of detection and limit of quantification for Dothiepin hydrochloride were found to be 0.01µg/mL and 0.1µg/mL respectively. The proposed method is simple, accurate, precise and robust therefore can be used for routine analysis of Dothiepin hydrochloride in bulk drug and pharmaceutical formulation.

Key words: RP-HPLC, Method Development, Dothiepin Hydrochloride, ICH guidelines, Validation

1. INTRODUCTION

Dothiepin hydrochloride (Dosulepin hydrochloride) is a tricyclic antidepressant which s chemically 3-dibenzo [b,e]thiepin-11(6H)-ylidene-N,N-dimethyl-1-propanamine hydrochloride.[1] The chemical structure of the drug is shown in Fig. 1**.** It possesses marked anticholinergic and sedative properties and is also known to prevent reuptake of noradrenaline and serotonin at nerve terminals. It is a safe and effective agent for the treatment of major depressive disorder.[2] Although the onset of action is comparable to that of other tricyclic antidepressants, dothiepin may cause fewer intolerable side effects and have less cardiotoxicity than other compounds.[3] Literature survey reveals that there are several analytical methods reported for the determination of dothiepin alone and in combination however there is no method reported for the determination of this drug in bulk. Hence the present work aims to introduce a novel RP-HPLC method for the determination of dothiepin hydrochloride in its bulk form. This method is very simple in application in comparison with the previously reported methods and at the same time it offers a high degree of accuracy and precision.

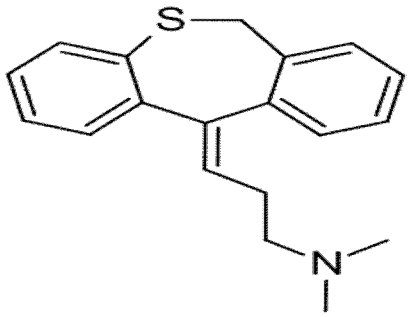

Fig. 1: Chemical structure of Dothiepin hydrochloride

2. MATERIALS AND METHODS

2.1 Materials

Dothiepin hydrochloride was obtained as a gift sample from ACME Formulation Pvt Ltd. Nalagarh, Himachal Pradesh. HPLC grade methanol, monobasic potassium dihydrogen o-phosphate and o-phosphoric acid were purchased from Molychem Manufacturers & Importers of Laboratory Reagents & Fine Chemicals. All other chemicals & reagents used were of analytical grade purchased from Molychem India. Double distilled water used in the study was prepared in house using borosil glass distillation unit.

2.2 Instrumentation

The analysis was performed using Agilent 1200 series quaternary pump HPLC system equipped with variable wavelength programmable UV detector with precision loop injector (Rheodyne 20 µl). 50µL Hamilton injection syringe was used for sample injection. The data was processed using Chemstation (B.02.01) software. UV method analysis was performed on a double beam Jasco V-630 spectrophotometer with Spectramanager software. All chemicals were weighed using Shimadzu electronic balance, Measurement of pH of buffer solutions was made using Equip-tronics digital pH meter with magnetic stirrer All solutions used in HPLC analysis were filtered using a 0.45µm nylon membrane filtration apparatus with vacuum pump. Oscar Ultrasonics bath sonicator was used for degassing the mobile phase.

2.3 Chromatographic Conditions

The chromatographic separation was performed in a Shodex C 18-4E column (5 µm; 250 × 4.6 mm, Showa Denko America Inc., USA). The mobile phase comprised of a mixture of 0.05 M phosphate buffer (pH adjusted to 2.6) and methanol (35:65, *v/v*) at a flow rate of 1.0 mL/min with iscocratic elution. The injection volume was 20 µL and the run time was 10 min. Detection was carried out at 231 nm.

2.4 Preparation of Standard Stock Solution

Accurately about 10 mg of dothiepin hydrochloride was weighed and transferred to a 10 ml volumetric flask. The volume was then made up to the mark with methanol to get a standard solution of dothiepin at a concentration of 1000µg/mL.

2.5 Preparation of Working Standard Solutions

Working standard solutions for HPLC injections were prepared on a daily basis Aliquots of the standard stock solution were taken and diluted with the mobile phase to get solutions in a concentration range of 0.5 -5µg/mL.

2.6 Assay of Tablet Formulation

Twenty marketed tablets of Dothiepin hydrochloride (PROTHIADEN, 50mg) were accurately weighed and triturated. The average weight per tablet was calculated and tablet powder equivalent to 10mg of dothiepin was weighed and transferred into 50ml volumetric flask. 70ml of methanol was added to dissolve the contents of the flask with the aid of ultrasonication for 10mins and volume was made up to 100ml with methanol to get a stock solution with concentration of 100µg/mL. 1ml of the stock solution was further diluted up to 10ml with the mobile phase to get a final concentration of 5µg.ml. The resulting solution was subjected to chromatographic analysis. From the results obtained the percentage assay of the drug was calculated.

2.7 Method Validation

The method was validated as per ICH guidelines to demonstrate that it is suitable for the intended purpose. The method was validated for system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness[12, 13].

2.8 System Suitability

System suitability parameters were studied to ensure that the instrument is suitable for the intended purpose. Retention time, tailing factor and theoretical plates were evaluated. The drug solution was injected five times into chromatographic system under the optimized conditions and the parameters were evaluated.

2.9 Linearity

Series of dilutions were prepared from the standard stock solution of Dothiepin in the concentration range of 0.5 - 5µg/mL. 20µL of each of these solutions was then injected into the column and the chromatographic characteristics were studied under the optimized conditions.

2.10 Specificity

Marketed tablets of Dothiepin Hydrochloride were analyzed to determine the specificity of the optimized method in the presence of excipients. The chromatograms were observed for the interfering peaks at the retention time of Dothiepin hydrochloride.

2.11 Accuracy

The recovery studies for the method were carried out by standard addition method. It was evaluated at three concentration levels (80, 100 and 120%) and the percentage recoveries were calculated.

2.12 Precision

The precision of the method was determined by intra and inter day precision studies. This was evaluated by injecting three different sample preparations of Dothiepin from a single formulation at three different concentration levels on the same day (Intra day) and on three different days (Inter day). From the resulting data the % Relative standard deviation was calculated.

2.13 Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined based on the standard deviation of the response and the slope of the calibration curve. The sensitivity of the method was established by the LOD and the LOQ values.

2.14 Robustness

Robustness was established by introducing small deliberate changes in the HPLC optimized conditions which include the change in wavelength, flow rate and percentage of methanol in mobile phase. This was studied using three replicates at a concentration level of 5µg/mL.

3. RESULTS AND DISCUSSION

A simple RP-HPLC method has been developed for determination of dothiepin hydrochloride. The method was optimized to provide a good separation of the component (acceptable theoretical plates) with a sufficient sensitivity and suitable peak symmetry in a short run. For this purpose, the analytical column, solvent selection, mobile phase composition, flow rate, and detector wavelength were studied. The chromatographic separation was achieved using an RP C18 column. Our experiments using methanol along with low pH phosphate buffer as mobile phase gave a good peak shape (peak symmetry) and resolution for dothiepin hydrochloride. The optimized chromatographic conditions are given in Table - 1. The representative chromatogram of standard Dothiepin Hydrochloride is shown in Fig. 2.

Table - 1: Optimized chromatographic conditions

Parameters	Optimized conditions
HPLC system	Agilent 1200 series quaternary pump system with Chemstation software
Column	Shodex C-18-4E (5 µm; 250 × 4.6 mm)
Mobile phase	Methanol: Phosphate buffer (pH 2.6; 0.05M) (65:35 V/V)
Flow rate	1mL/min
Detection wavelength	231nm
Injection volume	20 µL
Concentration of Standard Dothiepin hydrochloride	5 µg/mL

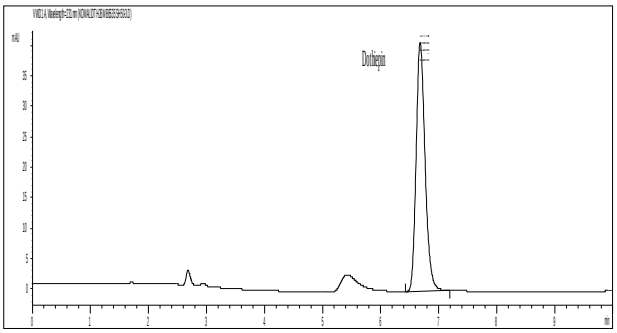

Fig. 2: Representative chromatogram of standard Dothiepin hydrochloride

The chromatogram for the assay of marketed tablets showed a single peak for Dothiepin hydrochloride at a retention time of 6.78 min. The representative chromatogram is depicted in Fig. 3.

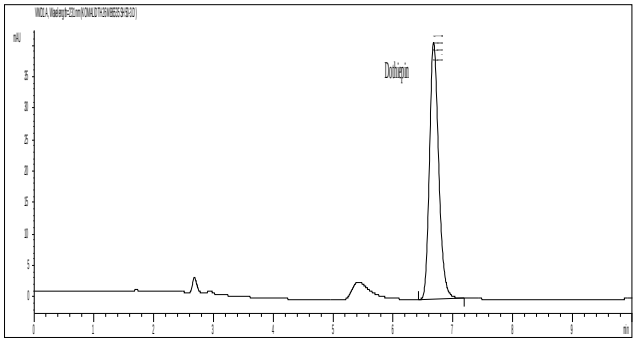

Fig.3: Representative chromatogram of Dothiepin hydrochloride in tablet formulation

The report for the assay of Dothiepin Hydrochloride tablets is presented in Table -2.

Table -2: Report for assay of Dothiepin hydrochloride

Drug	Amount present (mg/Tablet)	Amount found (mg/Tablet)	% Label claim
Dothiepin hydrochloride	50	49.47	98.94

The proposed method was found to be simple. The linearity data is tabulated in Table - 3. Calibration curve of peak area against concentration was found to be linear in the concentration range of 0.5 - 5µg/mL as shown in Fig. 4 with the regression equation y= 109.0x +2.507 and the correlation coefficient of 0.999.

Table - 3: Linearity data

Concentration (µg/mL)	Peak Area (mAU*s)
0.5	55.78
1	110.23
2	218.16
3	338.32
4	439.41
5	543.24

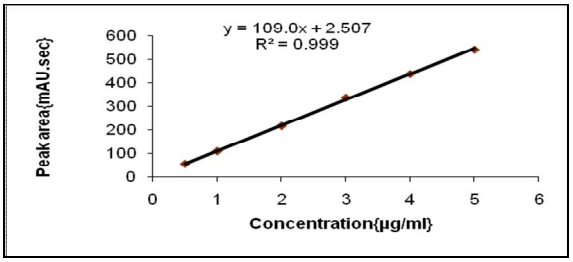

Fig. 4: Calibration curve of Dothiepin hydrochloride

System suitability parameters indicate high column efficiency with large number of theoretical plates (.>2000). The tailing factor was found to be 0.92 which does not exceed the critical value (2). The average retention time was found to be 6.78 min. No interference was seen from any of the excipients of the marketed tablet of dothiepin hydrochloride indicating the specificity of the method. The results of recovery studies are tabulated in Table - 4. Good recovery of the spiked drug was obtained at each added concentration, and the %RSD was found to be in the range of 0.24-0.80.

Table - 4: Recovery data of Dothiepin hydrochloride

%Spike level	Amount added (mg)	Amount found (mg)	% Recovery	Mean % Recovery ± SD (n=3)	%RSD
80%	40.00	40.36	100.9	99.99±0.791	0.791
	40.00	39.82	99.56		
	40.00	39.81	99.52		
100%	50.00	50.29	100.59	100.78±0.245	0.243
	50.00	50.03	100.06		
	50.00	50.35	100.70		
120%	60.00	60.9	101.50	101.54±0.330	0.384
	60.00	61.17	101.95		
	60.00	60.71	101.18		

SD: Standard deviation, %RSD: % Relative standard deviation

The data for precision is represented in Table - 5. The %RSD was found to be 0.28-0.97 for intraday and 0.49 – 1.24 for inter day precision studies. Thus the developed method was found to be accurate and precise as the % RSD value was less than 2.

Table - 5: Precision data for Dothiepin hydrochloride

Sr. No.	Conc. (µg/mL)	Intraday precision		Inter day precision	
		Mean*± SD	%RSD	Mean*± SD	%RSD
1	4	497.26±23.74	0.28	492.53±21.89	0.49
2	5	550.48±49.18	0.44	551.35±48.92	0.97
3	6	679.84±59.61	0.97	678.28±60.02	1.24

SD: Standard deviation, %RSD: % Relative standard deviation

The limit of detection and limit of quantification for Dothiepin hydrochloride were found to be 0.01µg/mL and 0.1µg/mL respectively. The results of robustness study are given in Table - 6. It was found that there was no drastic change in the resolution of Dothiepin hydrochloride when deliberate changes were introduced in the optimized chromatographic conditions thus confirming robustness of the developed method.

Table - 6: Results of Robustness Study

Parameter	Optimized	Variation	Mean peak area (mAU*s)	Mean Retention time (min)	Mean No. of theoretical plates	Mean Tailing factor
Detection wavelength	231nm	229nm	539.17	6.77	9453	0.89
		--	542.18	6.78	9742	0.92
		232nm	536.82	6.74	9271	0.90
Flow rate	1mL/min	0.8mL/min	540.72	7.02	9819	0.91
		--	542.18	6.78	9742	0.92
		1.2mL/min	541.36	6.56	9736	0.92
% of methanol in mobile phase	65%	63%	548.74	6.85	9581	0.90
		--	542.18	6.78	9742	0.92
		67%	546.25	6.72	9628	0.91

4. CONCLUSION

A validated RP-HPLC analytical method has been developed for the determination of Dothiepin hydrochloride in bulk and pharmaceutical dosage form. The proposed method is accurate, precise, specific and suitable to use for the routine analysis of Dothiepin hydrochloride in either bulk API powder or pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS and GC–MS.

5. ACKNOWLEDGEMENTS

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