Original Article

Method Development and Validation for the Estimation of Dothiepin Hydrochloride by using RP-HPLC in PURE and Tablet Dosage Form

Prasanthi Thayi[1*], Lakshman Rao Atmakuri[2], Nandini Mandada[3], Hemanth Mandava[3], Bhuvaneswari Mandru[3], Chaitanya Manne[3]

[1]Department of Pharmaceutical Analysis, Associate Professor, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, Krishna District, Andhra Pradesh, INDIA.
[2]Department of Pharmaceutical Analysis, Professor and Principal, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, Krishna District, Andhra Pradesh, INDIA.
[3]Student, V.V. Institute of Pharmaceutical Sciences, Gudlavalleru, Krishna District, Andhra Pradesh, INDIA.

ABSTRACT

Aim: A simple, sensitive, accurate and precise RP-HPLC method was developed for the determination of Dothiepin HCl (DTH) in pure and tablet dosage form. **Methods:** The method was developed by using Phenomenex C_{18} (250 X 4.6 mm, 5 μm) and the mobile phase composed of buffer (0.1M sodium acetate): acetonitrile in the ratio of 50:50 v/v. The buffer pH was adjusted to 2.8. The retention time for Dothiepin HCl was found to be 3.44 min. Linearity range for Dothiepin HCl was found to be 10-60 μg/mL and the regression equation was found to be y = 14691x-12844. % RSD for intra- and inter-day precision was found to be 0.27% and 0.84%. Average mean recovery was found to be 99.94%. LOD and LOQ values obtained for Dothiepin HCl were found to be 0.825 μg/mL and 2.498 μg/mL respectively. **Conclusion:** The results are analysed statistically and are found to be satisfactory. Hence this method can be successfully employed for analysis of Dothiepin HCl in tablet dosage form.

Key words: Dothiepin HCl, RP-HPLC, Linearity, Dosage form, Precision.

INTRODUCTION

Dothiepin HCl (Figure 1) formerly known as Dosulepin, is a tricyclic antidepressant drug prescribed for the treatment of depression of and associated anxiety/panic disorders. It is chemically (3E)-3-(6*H*-benzo[c] [1] benzothiepin-11-ylidene)-N, N-dimethylpropan-1-amine; hydrochloride.[1-2] It is also useful in chronic pain disorders and insomnia. It acts as a Serotonin–Norepinephrine Reuptake Inhibitor (SNRI) and also has other activities including antihistamine, antiadrenergic, antiserotonergic, anticholinergic and sodium channel -blocking effects. Dothiepin HCl inhibits the reuptake of biogenic amines, increasing available neurotransmitter levels at the synaptic cleft. The use of Dothiepin is only recommended in patients who are intolerant or unresponsive to alternative antidepressant therapies.[3-4]

A survey of literature[5-15] found that few HPLC methods were reported for estimation of Dothiepin HCl in pharmaceutical dosage forms. However the reported methods required long run time, hence there is an attempt has been made to develop a simple, rapid and accurate RP-HPLC method for estimation of Dothiepin HCl in tablet dosage form.

MATERIALS AND METHODS

Instrument

Agilent 1260 infinity binary pump HPLC with open lab software was used for chromatographic studies.

Submission Date: 17-07-2018;
Revision Date: 31-10-2018;
Accepted Date: 18-01-2019

DOI: 10.5530/ijper.53.2.39

Correspondence:
Ms. Prasanthi Thayi,
Department of Pharmaceutical Analysis, Associate Professor, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, Krishna District, Andhra Pradesh, INDIA.
Phone: 8374259526
E-mail: prasanthi8585@gmail.com

www.ijper.org

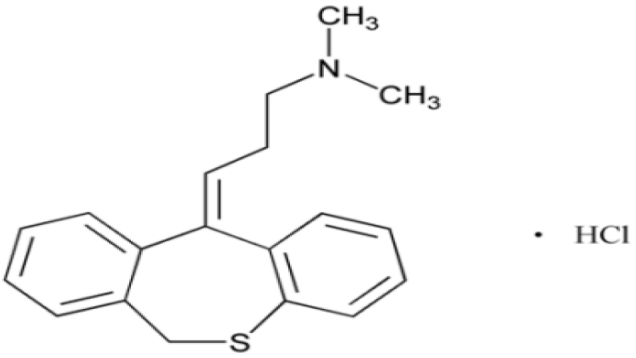


Figure 1: Structure of Dothiepin HCl.

Chemicals

Dothiepin HCl was purchased from Spectrum Pharma Research Solutons, Hyderabad, India. HPLC grade acetonitrile, analytical grade sodium acetate, ortho phosphoric acid were purchased from E. Merck (India) Ltd., Mumbai. Dothiepin HCl tablets were purchased from local market. Triple distilled water was used throughout experiment.

Dothiepin HCl standard stock preparation

Weigh and transfer accurately about 100 mg of Dothiepin HCl working standard into a 100 mL clean dry volumetric flask, add about 20 mL of mobile phase, sonicate for 5 min and dilute to volume with mobile phase.

Diluted standard

Pipette out 1 mL of the Dothiepin HCl standard stock solution and dilute to 10 mL with mobile phase.

RESULTS

Method development

Initially method development was started with the selection of wavelength of detection. The UV spectrum of DTH in mobile phase was noted using UV spectrophotometer. The maximum absorbance was noticed at 230 nm. This wavelength was used for detection of DTH.

The proposed method was developed by several concurrent trails in order to establish the preferred chromatographic conditions which would be helpful to conduct a complete validation study. The mobile phase for consisting of 0.1M sodium acetate (pH 2.8): acetonitrile (50:50 v/v) at 1 mL/min flow rate and detection wave length 230 nm was optimized which gave sharp peak, minimum tailing factor with short run time for DTH. The retention time for DTH was found to be 3.44 min.

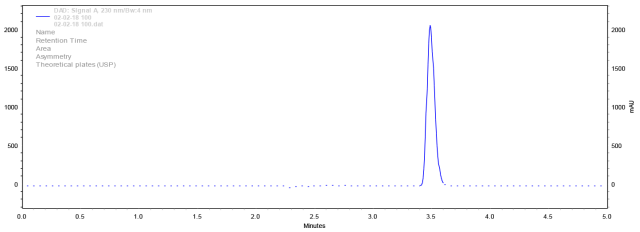


Figure 2: Optimized Chromatogram.

Optimized chromatographic conditions

Buffer preparation

Dissolve 1.3 g of sodium acetate in 100 mL of water. Adjusted the pH to 2.8 using ortho phosphoric acid and the solution is filtered and sonicated for 5 min.

Mobile phase: Acetonitrile: buffer (0.1M sodium acetate)

Ratio: 50:50 v/v

Column: ODS C_{18} (250×4.6 mm×5μm)

Wavelength: 230 nm

Flow rate: 1.0 mL/min

Observation

Peak was observed at 3.44 min and the peak shape was good with low asymmetric factor. Hence it was optmized.

The optimized chromatogram was shown in Figure 2.

VALIDATION

System Suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated and the values are depicted in Table 1.

Linearity

Linearity was performed by preparing standard solutions of Dothiepin HCl at different concentration levels i.e., 5-15 μg/mL. The absorbance was measured at 230 nm. Each measurement was carried out in triplicate. Linearity was proven by regression analysis by the least square method. The straight line in the calibration curve (Figure 3) obeyed linearity in the concentration range of 5-15 μg/mL for Dothiepin HCl. The correlation coefficient, linearity results were presented in Table 2.

Precision

The precision of the method was confirmed by intra-day and inter-day analysis. The concentration used for the precision studies is 10 μg/mL and was assumed as 100%. To study the intra-day precision, the analysis of

Table 1: System suitability results for Dothiepin HCl.

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	3.44	925783	13120	1.07
2	3.44	921463	13399	1.07
3	3.46	926216	13256	1.08
4	3.44	923374	13336	1.12
5	3.44	925145	13194	1.12
6	3.44	919965	13198	1.13
7	3.44	928698	13180	1.08
8	3.45	925364	13202	1.12
9	3.44	925128	13142	1.11
10	3.44	924985	13165	1.12
Mean	3.443	924612.1	-	-
%RSD	0.196	1.174236	-	-

Table 2: Linearity results for Dothiepin HCl.

S. No.	Conc. (µg/mL)	Peak area	Statistical Analysis	
1	0	0	Slope	14691
2	5	693191	Intercept	12844
3	7.5	1101627	Regression equation	y = 14691x -12844
4	10	1466730		
5	12.5	1802094	Correlation coefficient	R^2 = 0.999
6	15	2205094		

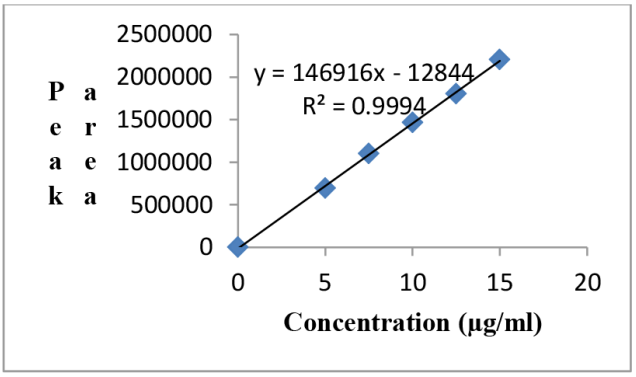


Figure 3: Linearity curve of Dothiepin HCl.

drugs was repeated for six times in the same day and for inter-day precision the analysis of drugs was carried out for six days. Six replicate standard solution of Dothiepin HCl was measured with the same concentration and the %RSD was calculated. Results of intra-day inter-day precision were given in Table 3.

Accuracy

Accuracy was performed in triplicate as per test method with equivalent amount of Dothiepin HCl into each volumetric flask for each spike level to get the concentration equivalent to 50%, 100% and 150% of the labeled amount as per the test method. The average % recovery of was calculated. The accuracy results were tabulated in Table 4.

Table 3: Intra and inter-day results for Dothiepin HCl.

S. No.	Intra-day Time (Hours)	Peak area	Inter-day Days	Peak area
1	0	1466625	1	143498
2	3	1466692	2	144225
3	6	1465653	3	145928
4	9	1465699	4	145925
5	12	1456742	5	143569
6	15	1467718	6	143285
Mean		1464855	Mean	144405
SD		4046.582	SD	1219.6945
%RSD		0.27	%RSD	0.84

Table 4: Accuracy results for Dothiepin HCl.

Recovery/ Spike level at about (%)	Amount of DTH added (ppm)	Conc. found (µg/mL)	% Recovery	% Mean recovery
50	5	5.05	101.0	99.66
50	5	4.97	99.40	
50	5	4.93	98.6	
100	10	9.95	99.5	99.86
100	10	9.99	99.9	
100	10	10.02	100.2	
150	15	15.14	100.9	100.32
150	15	15.12	100.8	
150	15	14.98	99.86	

Ruggedness

Ruggedness of the method was confirmed by the analysis of samples was done by different analysts. Samples of Dothiepin HCl at 10 μg/mL concentration were analyzed by different analysts. It was observed that there were no marked changes in absorbance, which demonstrated that the developed method was rugged in nature.

Robustness

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision. The robustness results were furnished in Table 5.

Table 5: Robustness results of Dothiepin HCl.

S. No.	Parameter	Optimised	Used	Rt (min)	Peak area	%RSD
1	Flow rate	1 mL/min	0.8 mL/min	3.51	924936	0.35
			1.0 mL/min	3.44	924684	0.75
			1.2 mL/min	3.41	925382	0.95
2	Wavelength	230 nm	232 nm	3.42	914685	0.59
			230 nm	3.44	925146	0.75
			228 nm	3.46	923214	0.69

Table 6: LOD and LOQ of Dothiepin HCl.

Parameter	Measured value (µg/mL)
Limit of detection	0.825
Limit of quantification	2.498

Table 7: Assay results of Dothiepin HCl formulation.

Formulation	Label claim	Amount found	%Assay
DOTHEP	50 mg	49.62 mg	99.24

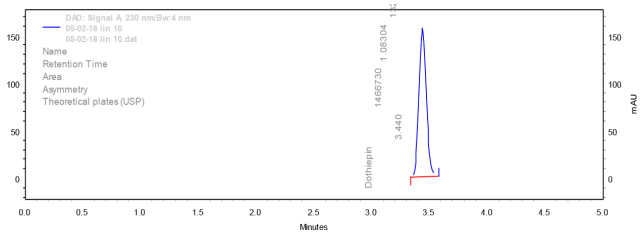


Figure 4: Sample Chromatogram.

Limit of detection and Limit of quantification (LOD and LOQ)

LOD and LOQ values are calculated from calibration curve method and the LOD and LOQ of Dothiepin HCl is given in Table 6.

Estimation of Dothiepin HCl tablet dosage forms

5 tablets of Dothiepin HCl (Dothe) were weighed and crushed them to a fine powder and a quantity of tablet powder equivalent to 50 mg of DTH was transferred to 50 mL volumetric flask and dissolved in mobile phase and the volume was adjusted up to the mark with mobile phase. The mixture was allowed to stand for 30 min with intermittent sonication to ensure complete dissolution. The resulting solution was filtered through a 0.22 μm membrane filter. The filtrate was diluted further with mobile phase to get the working sample solution. The assay results were shown in Table 7 and sample chromatogram was represented in Figure 4.

DISCUSSION

The present RP-HPLC method was developed for estimation of Dothiepin HCl by using Phenomenex C_{18} (250 x 4.6 mm, 5 μm) as stationary phase with mobile phase containing mixture of 0.1M sodium acetate (pH 2.8) and acetonitrile (50:50 v/v). The eluted compound was monitored at 230 nm. Dothiepin HCl peak was eluted at 3.44 min. The developed method was validated for parameters of specificity, linearity, precision, accuracy, limit of detection, limit of quantification and robustness as per approved ICH guidelines and validation results are summarized in Table 8.

Table 8: Method Validation Summary.

S. No.	Parameter	Observation
1	System suitability	The %RSD for retention time is 0.19% and for peak area is 1.17%
2	Precision	%RSD for Intra and Inter-day Precision 0.27 and 0.84
3	Accuracy	%Mean recovery is between 99.66-100.32%
4	Linearity	Regression equation: y=14691x-12844; R^2= 0.999
5	LOD and LOQ	LOD: 0.825 µg/mL and LOQ: 2.498 µg/mL
6	Ruggedness	%RSD value is below 2%
7	Robustness	The % variation change in wavelength and flow rate is within limits

CONCLUSION

A new, reversed-phase HPLC method has been developed for analysis of Dothiepin HCl in commercial formulation. The developed RP-HPLC method was found to be simple, accurate, sensitive and precise proving reliability of the method. The method is very simple and involving no complicated sample preparations. The run time was relatively short, i.e. 3.44 min, which enables rapid quantitation of many samples in routine and quality control analysis of formulations. The optimized solvent system was used throughout the experimental work and no interference from any excipient was observed. The developed RP-HPLC method was validated as per the ICH guidelines. These results have shown that method could find practical application as a quality-control tool for analysis of Dothiepin HCl in pharmaceutical dosage forms in quality-control laboratories.

ACKNOWLEDGEMENT

Authors are very much thankful to management and Principal of V.V. Institute of Pharmaceutical Sciences for providing resources and facilities to carry out the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

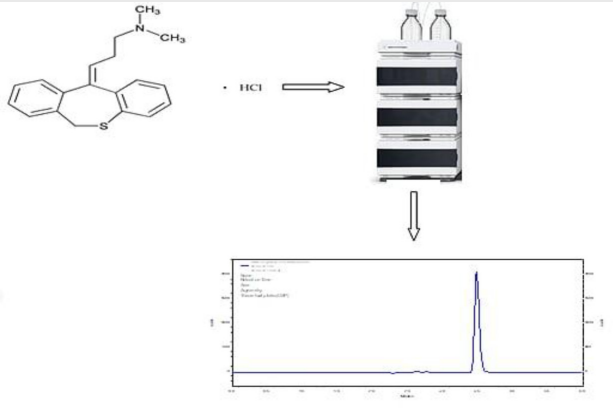
ABBREVIATIONS

RP-HPLC: Reverse Phase High Performance Liquid Chromatography; **HCl:** Hydrochloride; **UV:** Ultra Violet; **RSD:** Relative Standard Deviation; **ICH:** International Conference on Harmonization.

REFERENCES

1. The British Pharmacopoeia. London: British Pharmacopoeia Commission. 2009;4:721.
2. The Indian Pharmacopoeia. Indian Pharmacopeia Commission, Ghaziabad, India. 2014;1619-20.
3. David H, Keith M, John B, Roger B. Comparative pharmacology of Dothiepin, its metabolites and other antidepressant drugs. Drug Dev Res. 1992;27(2):121-35.
4. Lancaster SG, Gonzalez JP. Dothiepin: A review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in depressive illness. Drugs. 1989;38(1):123-7.
5. Vijayalakshmi M, Srinavyatha M, Prasanna ML, Buchi NN. A novel improved RP-HPLC method for the estimation of Dothiepin HCL in bulk and pharmaceutical dosage form. IOSR J Pharm Bio Sci. 2017;12(3):21-6.
6. Anita A, Komal G, Mayuri N, Priya M, Paraag G. Development and validation of RP-HPLC method for determination of Dothiepin HCl in bulk and pharmaceutical dosage form. Int J Chem Pharm Sci. 2013;1(1):9-13.
7. Rambabu C, Rao V, Umamaheswa K. Development and validation of a RP-HPLC method for the determination of Dosulepin in pharmaceutical formulation. Am J Pharm Tech Res. 2012;35(3):721-5.
8. Umamaheswar K, Naganjaneyulu T, Rambabu C. Extractive spectrophotometric methods for the determination of Dothiepin in pure and pharmaceutical formulations. Global J Pharm Pharm Sci. 2017;2(2):1-5.
9. Wel HS. Extractive colorimetric method for the determination of Dothiepin HCl and Risperidone in pure and in dosage forms. Chem Pharm Bull. 2008;56(8):1092-6.
10. Abdellatef HE, Henawee MM, Ayad MM. Spectrophotometric and spectrofluorimetric methods for analysis of Tramadol, Acebutolol and Dothiepin in pharmaceutical preparations. Spectrochimica Acta Part A Molecular and Biomolecular Spectroscopy. 2016;65(5):1087-92.
11. Rahman SAM, Basaviah K, Vinay KB. Validation of UV spectrophotometric methods for the determination of Dothiepin HCl in pharmaceutical dosage form and stress degradation studies. J App Spect. 2012;79(5):780-7.
12. Reddy LSK, Reddy PSLN, Reddy SK. Validated stability indicating Liquid Chromatographic method for simultaneous estimation of Dosulepin and Methylcobalamin in combined pharmaceutical dosage form. Orient J Chem. 2014;30(3):1-6.
13. Radhika S, Ragin S. Stability indicating RP-HPLC method for simultaneous estimation of Dosulepin Hydrochloride and Methylcobalamin in tablet dosage form. Int J Appl Pharm Sci. 2017;9(4):69-75.
14. Usharani G, Chandrashekar B, Devanna N. Simultaneous estimation of Dosulepin and Methylcobalamin in bulk and pharmaceutical formulation by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). IOSR J Pharm Biol Sci. 2014;9(3):55-9.
15. Aruna P, Ajitha A, Maheshwar UV. Analytical method development and validation of simultaneous estimation of Dosulepin and Methylcobalamin in tablet dosage form by RP-HPLC. Int J Pharm. 2014;4(3):267-74.

PICTORIAL ABSTRACT



SUMMARY

- RP-HPLC method was developed and validated for the assay of Dothiepin HCl in tablet formulation. Analysis of Dotiepin HCl was carried out by using Phenomenex C18 (250 x 4.6 mm, 5 µm) as stationary phase with mobile phase containing mixture of 0.1M sodium acetate (pH 2.8) and acetonitrile (50:50 v/v) and the compound was detected in very short time i.e 3.44 min. The method was validated as per ICH guidelines and the results were satisfactory. So the developed method was simple, precise, accurate and appropriate for the determination of Dothiepin HCL in bulk and tablet dosage forms.

ABOUT AUTHORS

Ms. Prasanthi Thayi1: Associate Professor, Department of Pharmaceutical Analysis, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru Krishna District.

M Nandini, has completed B. Pharmacy in V. V. Institute of Pharmaceutical Sciences, Gudlavalleru Krishna District.

M Bhuvaneswari, has completed B. Pharmacy in V. V. Institute of Pharmaceutical Sciences, Gudlavalleru Krishna District.

M Chaitanya, has completed B. Pharmacy in V. V. Institute of Pharmaceutical Sciences, Gudlavalleru Krishna District.

Dr. A. Lakshmana Rao: he is currently the Principal of V. V. Institute of Pharmaceutical Sciences, Gudlavalleru. He has 18 years of Teaching, Research and administrative experience for B.Pharmacy, M.Pharmacy, Pharm.D. and Ph.D. Programs. He has 238 publications in various reputed national and international journals. His academic achievements include 8 guest lectures, authored 2 books, guided 8 candidates for Ph.D. and five are currently under his guidance for Ph.D. He has filed a patent on Preparation of Rosuvastatin Formulation for Treating Human Oral Squamous Cell Carcinoma. He is Ratified as Principal by Jawaharlal Nehru Technological University-Kakinada, Kakinada, as an Associate Professor by Andhra University, Visakhapatnam. He is Approved Research Director for Ph.D. Programmes, adjudicator of Ph.D. and M.Pharmacy Thesis in Pharmaceutical Sciences of various Universities in India and Abroad.

M Hemanth, completed B. Pharmacy in V. V. Institute of Pharmaceutical Sciences, Gudlavalleru Krishna District.

Cite this article: Thayi P, Atmakuri LR, Mandada N, Mandava H, Manne C. Method Development and Validation for the Estimation of Dothiepin Hydrochloride by using RP-HPLC in PURE and Tablet Dosage Form. Indian J of Pharmaceutical Education and Research. 2019;53(2):310-5.