IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 3 Ver. I (May. - June.2017), PP 21-26
www.iosrjournals.org

A Novel Improved RP-HPLC Method for the Estimation of Dothiepin Hydrochloride in Bulk and Pharmaceutical Dosage Form

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***Abstract**: A Simple and precise reverse phase high performance liquid chromatography(RP-HPLC)method was developed and validated for the estimation of Dothiepin hydrochloride in bulk and pharmaceutical dosage form. The optimization is carried out on an Inertsil ODS 3 (250×4.6mm, 5µ particle size) long column with a mobile phase of Formic acid(0.02%): Acetonitrile in the ratio of 72:28v/v at a flow rate of 1ml/min with UV detection at 230 nm using LC solution software. The Linearity of Dothiepin hydrochloride was observed in the concentration range of 2-10µg/ml (R^2=0.999). The proposed method has shown consistent recovery of Dothiepin 99.41-100.85% with the labeled amount in the pharmaceutical formulation. The proposed method was successfully applied for the quantification of active drug in tablet dosage form.*
***Keywords**: Dothiepin hydrochloride, RP-HPLC, ICH guidelines and method validation.*

I. Introduction

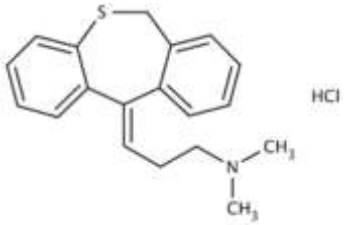

Figure 1: Structure of Dothiepin hydrochloride.

Dothiepin hydrochloride chemical name is 3-dibenzo [b, e] thiepin-11(6H)-ylidene-N,N-dimethyl-1-propanamine hydrochloride[1]. Dothiepin is white to faintly yellow, crystalline powder, odourless or almost odourless [2]. Dothiepin is a tri-cyclic antidepressant with similar actions of amitriptylline. It is freely soluble in water, alcohol and in dichloromethane. Dothiepin (Dosulepin) hydrochloride is readily absorbed from GIT and extensively demethylated by first pass metabolism in the liver to its primary active metabolite desmethyldothiepin. It is excreted in urine or in faeces [3].

Literature survey reveals that Liquid chromatography-Mass spectroscopy(LC-MS)[4], Electrometric[5-6], Conductometric[7], Stability indicating[8-9], UV Spectroscopic[10-11] , RP-HPLC methods[12-16] were reported for the estimation of Dothiepin hydrochloride in bulk, combination dosage forms and in plasma samples. Therefore attempts were made to develop simple RP-HPLC method which is LC-MS compatible for the estimation of Dothiepin hydrochloride in bulk and its dosage form and validated as per ICH guidelines [17-18] .The chemical structure of Dothiepin hydrochloride is shown in Fig 1.

II. Experimental details

Chemicals and reagents

Dothiepin hydrochloride was obtained as a gift sample from Life line formulations Pvt. limited, Vijayawada, India. All the other chemicals needed for the experiment were of HPLC grade and were purchased from MERCK chemicals. The Chemicals required for the experiment are formic acid, ammonium acetate, methanol and acetonitrile.

Instrument and chromatographic conditions

Chromatography was carried out on HPLC Shimadzu prominence technology. The chromatographic system was equipped with LC-10 ATVP series binary pump systems, SIL-20 AHT Auto sampler, SPD-10AVP UV-VIS detector and data acquisition was carried out using LC solution software. The chromatographic separation was performed using Inertsil ODS 3 (250×4.6MM,5µ particle size) long column.

The chromatographic seperation was achieved at an ambient temperature using a mobile phase consisting of 0.02% formic acid and acetonitrile in the ratio of 72:28 v/v which is pumped at a flow rate of 1ml/min .The overall run time was 7min and 10 µl of sample was injected into HPLC System. The eluent was monitored at a wavelength of 230nm using UV-VIS detector.

Preparation of mobile phase

A 0.02% Formic acid solution was prepared by dissolving 200µl of formic acid in 1000ml of HPLC grade water. The solution was filtered through 0.45µm nylon membrane filter to remove all fine particles and sonicated for 30sec to remove any dissolved gases.

Preparation of standard stock solution

Weigh accurately about 10 mg of dothiepin hydrochloride in a clean, dry 10ml volumetric flask and few ml of methanol was added to dissolve completely and then the volume is made up to the mark with methanol to obtain a concentration of 1mg/ml. Further dilutions were carried out with 0.02% formic acid as a diluent to get a series of concentrations 2-10µg/ml.

Method validation

The developed method for the estimation of dothiepin hydrochloride was validated as per ICH guidelines[9-10] for the parameters like linearity, system suitability, specificity, accuracy, precision, robustness, limit of detection(LOD), limit of quantification(LOQ).

Linearity

Aliquots of 40, 80, 120, 160, 200µl of solution was pipetted out from standard stock solution and make up the volume up to 1ml individually with 0.02% Formic acid to get a concentration of 2, 4, 6, 8,10 µg/ml of Dothiepin hydrochloride. The calibration curve is constructed for the dothiepin hydrochloride to check for linearity over the concentration range of 2-10µg/ml. The regression and correlation coefficients were calculated.
Acceptance criteria: Correlation coefficient not less than 0.999

Accuracy

Accuracy was assessed by determining the recovery of the method at three different concentrations (corresponding to 80, 100, 120% of the test solutions) by the addition of the known amount of standard to placebo preparation. At each level three sets were prepared and injected in triplicate.

Precision

The precision is carried out by injecting the standard preparation concentration of 6µg/ml concentration of Dothiepin hydrochloride for six times and the %RSD for six replicate injections was calculated.
Acceptance criteria: The % RSD for the peak areas of standard injections should not more than 2%.

Robustness

To evaluate the robustness of a LC method a few chromatographic parameters are deliberately varied. The parameters include flow rate, percentage of organic phase in mobile phase, wavelength. Only one factor at a time was changed to observe the effect. Thus three injections of standard solution were performed under the change of three chromatographic parameters.

System suitability

The suitability of the proposed method should be tested before each stage of the validation. Five replicate injections of standard solution (6µg/ml) was injected with increased volume of (10-50µl) into HPLC system and the parameters like number of theoretical plates, tailing factor and statistically relative standard deviation was calculated.

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

The LOD is the smallest concentration of the analyte that gives a measurable response and LOQ is the smallest concentration of the analyte which can give the responses that can be quantified accurately. The LOD and LOQ values were determined based on the signal to noise ratios and based on the analytical responses of 10 and 3 times the background noise respectively.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

Whereσ= Standard deviation and s= slope of the equation.

Stability of solution

The stability of the solution was assessed both for test and standard preparation. The solutions were prepared and stored at 5^{O}c and at ambient temperature without protection of light and tested after 24hrs.The responses were determined for the aged sample and was compared with freshly prepared sample.

Specificity

The specificity of the RP-HPLCmethod was evaluated by injecting the blank and standard preparations into the HPLC system. The effect of excipients and other additives in the dosage form of dothiepin hydrochloride under the optimum conditions was evaluated and no interferences were observed.

Analysis of marketed formulation

Twenty tablets were weighed accurately and grounded into fine powder and the powder blend equivalent to 10mg of dothiepin hydrochloride was transferred into a clean and dry 10ml volumetric flask. About 5ml of methanol was added and the solution was sonicated for 3min to extract the Dothiepin and the volume was made upto the mark with methanol. The solution was filtered through 0.45µm nylon membrane filter and the filtrate was appropriately diluted with 0.02% formic acid to make the concentration of 6µg/ml and the results were tabulated.

III. Results and discussion

To develop an effective LC-MS compatible method for the analysis of the dothiepin, various LC-MS compatible mobile phases such as ammonium acetate, ammonium formate was tried. The method is optimized with satisfactory separation and good peak symmetry. The mobile phase composition containing a mixture of Formic acid (0.02%): ACN was used in the ratio of 72:28v/v at a flow rate of 1ml/min with a retention time of 4.38 min.

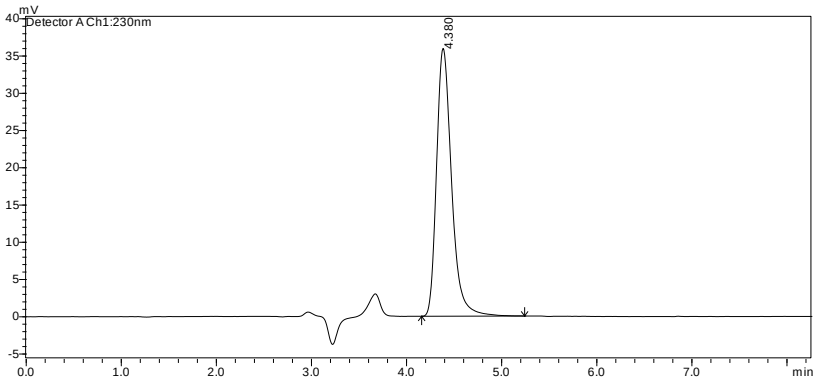

Figure 2 Chromatogram of Dothiepin hydrochloride

Validation of the proposed RP-HPLC method was performed as per ICH guidelinesQ2 (R1). The linearity was established in the concentration range of 2-10µg/ml with a correlation coefficient of 0.999. The % accuracy was found to be 98.3-103.42 which indicates the accuracy of the proposed method. The %RSD value is 0.00051 reveals that the method is precise. The system suitability was carried out by injecting freshly prepared sample solutions and the parameters like %RSD was with in the limit (<2%).

Robustness of the method was studied by changing the chromatographic parameters like flow rate, mobile phase composition etc. Specificity was carried out by injecting blank, placebo and sample but no interferences are observed around the Dothiepin peak. The LOD and LOQ values were found to be 0.0592 and 0.1794 µg/ml respectively. From the above data we can conclude that the proposed method was found to be simple, precise, accurate, specific and robust, hence it is used for the Quality control analysis of Dothiepin in bulk and dosage form.

Table 1 : Optimised chromatographic parametres of HPLC method

Equipment	Shimadzu's prominence HPLC system
Column	Inertsil ODS 3 long (250×4.6mm, 5µm particle size)
Mobile phase	Formic acid (0.02%):ACN(72:28V/V)
Injection volume	10µL
Flow rate	1ml/min
Column temperature	Ambient
Wavelength	230nm
Concentration of standard solution	10µg/ml

Robustness

Robustness was evaluated by making very slight changes in the chromatographic parameters. Different parameters like retention time, theoretical plates and tailing factor were recorded. The results were tabulated in table 2, 3and 4.

Table 2: Robustness data related to flow rate change

S.no.	Flow rate (ml/min)	Retention time (min)	Plate count	Tailing factor
1.	0.8	5.781	4673.447	1.472
2.	1	4.66	3886.573	1.478
3.	1.2	4.076	4387.118	1.410

Table 3: Robustness data related to mobile phase ratio change

S.no	Mobile phase ratio(v/v)	Retention time (min)	Plate count	Tailing factor
1.	74:26	6.425	4387.118	1.443
2.	72:28	4.66	4153.245	1.478
3.	70:30	3.689	8826.306	0.697

Table 4: Robustness data related to wavelength change

S.no.	Wavelength(nm)	Retention time	Plate count	Tailing factor
1.	229	4.32	4150.258	1.458
2.	230	4.66	4153.245	1.478
3.	231	4.40	4152.248	1.452

Linearity

Linearity was obeyed in the concentration range of 2-10µg/ml with a correlation coefficient of 0.999.The calibration curve was shown in figure 3 and the results were tabulated in table 5.

Table 5: Linearity of Dothiepin hydrochloride

S.no	Concentration µg/ml	Peak area
1.	2	79136
2.	4	155469
3.	6	230451
4.	8	308942
5.	10	375680
Regression equation Y=37328x+5967.3		
Regression coefficient R^2=0.999		
Correlation coefficient R^2=0.999		

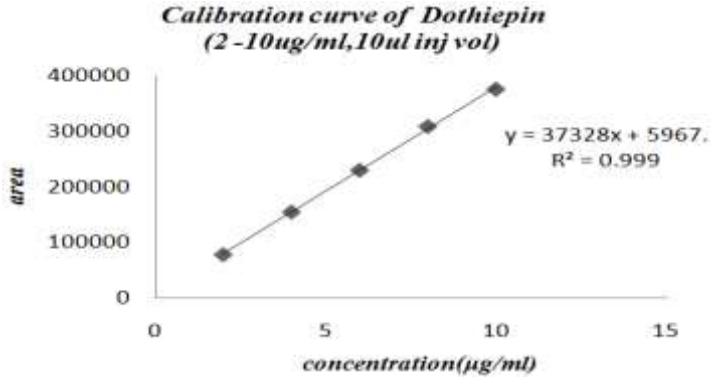

Figure 3 : Calibration curve of Dothiepin hydrochloride

Accuracy

Accuracy of the proposed method was carried out by carrying out the recovery studies by standard addition method. The % recovery was found to be 99.41-100.85 and the results were tabulated in table 6.

Table 6 Accuracy data

S.no	Level of recovery	Amount present(µg/ml)	Amount added(µg/ml)	Amount recovered(µg/ml)	%Recovery+S.D
1.	80	6	4.8	5.95	99.41+0.197
2.	100	6	6	5.98	99.73+0.443
3.	120	6	7.2	6.06	100.85+0.215

Precision

Precision was carried out by injecting repeatedly the standard 6µg/ml solution for 6 times and the %RSD was within the limits <2 for peak areas and less than 1 for retention time. The data was evaluated and theresults were tabulated in table 7.

Table 7 Precision data

S.no	Sample	Retention time(min)	Area
1.	Injection 1	4.66	230451
2.	Injection 2	4.676	230450
3.	Injection 3	4.688	230452
4.	Injection 4	4.687	230453
5.	Injection 5	4.691	230451
6.	Injection 6	4.685	230450
Average		4.681	230451
Standard deviation		0.01155	1.6905
% RSD		0.2467	0.00051

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

The LOD and LOQ values were calculated based on the values of slopes and intercepts of the calibration curve. The Limit of detection and the Limit of quantification values for the estimation of Dothiepin hydrochloride was found to be 0.0592 and 0.1794µg/ml respectively.

Stability in sample solution

There were no interferences in the chromatogram of Dothiepin hydrochloride reveals the stability of drug in the sample solution.

System suitability

System suitability was performed by injecting the sample solution at increasing injection volume into the HPLC system. The parameters like retention time, peak area, theoretical plates, tailing factor was evaluated. The values are summarized in Table 8.

Table 8: System suitability data

S.no.	Injection volume(µl)	Retention time (min)	Tailing factor	Theoritical plates(N)
1.	10	4.66	1.478	230451
2.	20	4.71	1.402	411727
3.	30	4.721	1.365	619099
4.	40	4.75	1.324	817548
5.	50	4.768	1.291	1030441
Mean		4.7218	1.372	3109266
%RSD		0.87889	5.28739	10.20345
Limits			< 2	>2000

Analysis of marketed formulation

The assay of Dothiepin in marketed formulation was evaluated by comparing the area of the standard Dothiepin with the tablet sample. The percent assay was found to be 99.06 which is found to be in limits and the results were tabulated in Table 9.

Table 9: Assay

S.no	Drug	Labelled claim (mg)	Mean±SD	%RSD
1.	Dothiepin hydrochloride	25	99.06 + 0.8016	0.809

Table 10 Summary of validation parameters

S.no	Parameter	Dothiepin
1.	Linearity(R2)	0.999
2.	Accuracy(Mean)	99.996 %
3.	Precision(%RSD)	0.0051
4.	Robustness	Within the acceptance criteria
5.	Assay	99.06%
6.	Theoritical plates System suitability Tailing factor %RSD	3109266 1.372 0.8788
7.	LOD and LOQ	0.059221 and 0.179456µg/ml.
8.	Specificity	No interferences are observed due to blank and placebo.

IV. Conclusion

A novel RP-HPLC method has been developed which is LC-MS compatible and validated as per ICH Q2(R1) guidelines for the parameters like linearity, accuracy, precision, robustness, specificity and assay. No interferences are observed from any other excipients of dosage form and the developed method has been successfully applied for the routine quality control analysis.

Acknowledgement

The authors are thankful to management and principal of K.V.S.R. Siddhartha college of pharmaceutical sciences, Vijayawada, India and sincere thanks to lifeline formulations for providing the drug sample to carry out the research work.

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