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Development and Validation of a RP–HPLC Method For the Determination of Dosulepin In Pharmaceutical Formulation

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ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Dosulepin tablet dosage form. Isocratic elution at a flow rate of 1mL/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted Methanol: Acetonitrile: 0.01M Phosphate buffer in the ratio of 55:20:25 (v/v/v). The UV detection wavelength was 230nm and 20 µL sample was injected. The retention time for Dosulepin was 3.46min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Dosulepin tablet dosage form and bulk drug.

Key Words: Dosulepin, RP-HPLC, Development, Validation, Estimation, 230nm

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INTRODUCTION

Dosulepin is a Tricyclic antidepressant (TCA)[1]. Dosulepin blocks the reuptake of serotonin and norepinephrine in the brain, thereby increasing their levels. It is believed that this action is responsible for its mood-elevating effects. Dosulepin is relatively mild and is used for low-level anxiety, depression and similar disorders, as well as the treatment of chronic and ongoing pain disorders, particularly where insomnia and/or loss of appetite are present. The structure of Dosulepin is given in figure.1

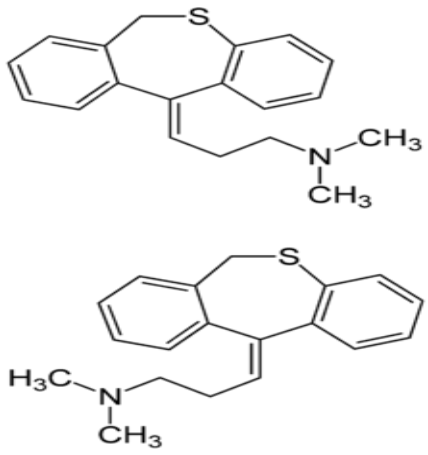

Figure 1: Structure of Dosulepin

Dosulepin works by preventing serotonin and noradrenaline from being reabsorbed back into the nerve cells in the brain. This helps prolong the mood lightening effect of any released noradrenaline and serotonin. In this way, dosulepin helps relieve depression [2].

The dosage of Dosulepin in adults is 75 mg/day in divided doses or as a single dose at night, increasing to 150 mg/day [3]. In certain circumstances, e.g. in hospital use or unresponsive patients, dosages up to 300 mg daily have been used [4]. Dosulepin can cause side effects such as drowsiness. This means it may be useful in treating depression in people who are also anxious and agitated, or who are suffering from disturbances in sleep. The commonly occurred side effects are Dry mouth, Drowsiness, Blurred vision, Constipation, Nausea, Difficulty in passing urine, Drop in blood pressure when going from lying or sitting to sitting or standing, causing dizziness and light headedness (postural hypotension), Sweating, Involuntary muscle movements such as tremors or twitching, Rashes, Confusion or delirium, Headache, Sexual problems, Changes in behaviour etc.

Very few methods have been reported for the estimation of Dosulepin in pharmaceuticals by using different analytical techniques like hplc[2,7], spectrophotometry[3,4,5], LCMS[1], capillary Electrophoresis[8], ion-selective electrode[6], conductometry[9], Potentiometry[10].

MATERIALS AND METHODS:

Materials

Working standard of Dosulepin was obtained from well reputed research laboratories. HPLC grade water, Methanol and Acetonitrile were purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µL loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance.

Determination of wavelength of maximum absorbance

The standard solutions of Dosulepin were scanned in the range of 200 - 400 nm against mobile phase as a blank. Dosulepin showed maximum absorbance at 230nm. So the wavelength selected for the determination of Dosulepin was 230nm.

Chromatographic equipment and conditions

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of DOSULEPIN an isocratic PEAK HPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software. The mobile phase consisted of Methanol: Acetonitrile: 0.01M Phosphate buffer in the ration of 55:20:25 (v/v/v). Injections were carried out using a 20 µL loop at room temperature (20 + 2 °C) and the flow rate was 1 mL/min. Detection was performed at 230nm with 7min runtime.

Standard and sample solutions

A 10 mg amount of Dosulepin reference substance was accurately weighed and dissolved in 10 mL mobile phase in a 10 mL volumetric flask to obtain 1000 ppm concentration solution. Required concentrations were prepared by serial dilution of this solution.

A composite of 20 (Dothepin-75mg) tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Dosulepin was accurately weighed and quantitatively transferred into a 100 mL volumetric flask. Approximately 25 mL mobile phase was added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 60ppm.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation, the quality of the chromatograph was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2500. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

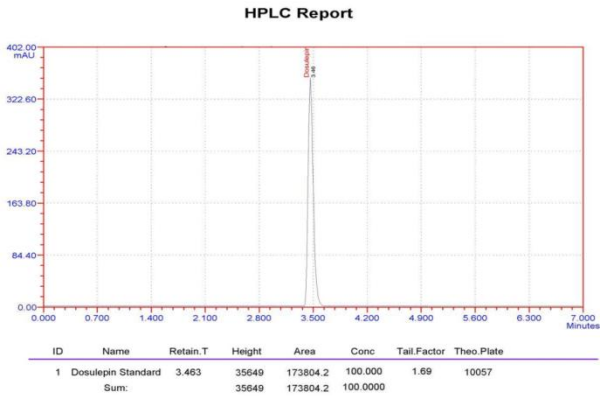

Figure.2: Standard chromatogram of Dosulepin

Table.1 System suitability parameters of DOSULEPIN

Parameter	Condition
Mobile phase	Methanol: Acetonitrile:0.01M Phosphate buffer 55:20:25 (v/v/v)
Pump mode	Isocratic
*p*H	5.9
Diluents	Mobile phase
Column	Zodiac C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	230nm
Injection Volume	20 µL
Flow rate	1.0mL/min
Run time	7 minutes
Retention Time	3.46 minutes
Pump Pressure	11.9MPa

Linearity

Linearity was performed by preparing standard solutions of Dosulepin at different concentration levels including working concentration mentioned in experimental condition i.e 60ppm. 20µL of each concentration was injected into the HPLC system. The peak responses were read at 230nm and the corresponding chromatograms were recorded. Linearity plot of concentration over areas was constructed and given in Figure 3. Linearity results were presented in Table.2

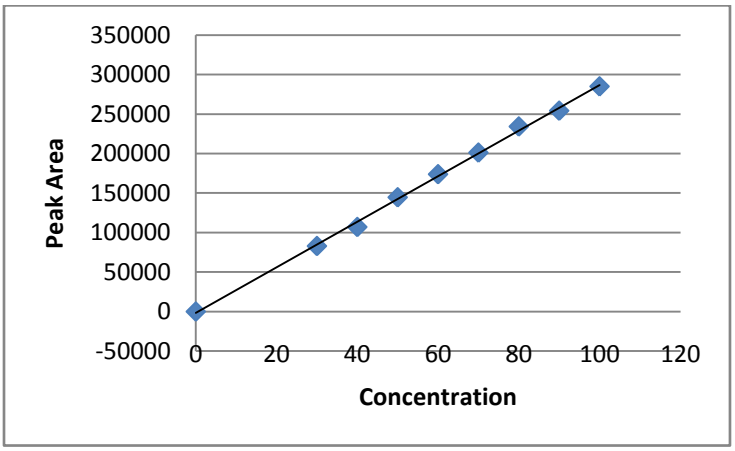

Figure 3: Calibration curve of Dosulepin

Table.2 Linearity results

Level	Conc. of Dosulepin In ppm	Peak Area
Level – 1	30	82941
Level – 2	40	107030
Level – 3	50	144597
Level – 4	60	173804
Level – 5	70	201134
Level – 6	80	234165
Level – 7	90	254162
Level – 8	100	284910
Range: 30ppm to 100ppm		Slope: 2883.027 Intercept: -1825.69 Cc: 0.999208

Precision:

Precision is the degree of repeatability of an analytical method under normal Operational conditions. Precision of the method was performed as intraday precision and Inter day precision.

Intraday precision

To study the intraday precision, six replicate standard solutions (60ppm) of Dosulepin were injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.80, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 3.

Table.3 Intraday precision Results

Sample	Conc. (in ppm)	Injection No.	Peak Areas	RSD(Acceptance criteria ≤ 2.0%)
Dosulepin	60	1	173804	0.80
		2	171486	
		3	171479	
		4	170112	
		5	171979	
		6	170336	

Inter Day precision

To study the inter day precision, six replicate standard solutions (60ppm) of Dosulepin were injected on third day of sample preparation. The percent relative standard deviation (% RSD) was calculated and it was found to be 1.06, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 4.

Table.4 Interday precision results

Sample	Conc. (in ppm)	Injection No.	Peak Areas	RSD(Acceptance criteria ≤ 2.0%)
Dosulepin	60	1	165321	1.06
		2	164455	
		3	168656	
		4	167682	
		5	164332	
		6	165592	

Limit of Detection and Limit of Quantification

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.75ppm dilution Peak was not clearly observed based on which 0.75ppm is considered as Limit of Detection and Limit of Quantification is 2.5ppm and are given in Table 5.

Table.5 LOD and LOQ

Parameter	Measured Value
Limit of Quantification	2.5ppm
Limit of Detection	0.75ppm

Robustness:

The robustness study was performed by slight modification in flow rate of the mobile phase, pH of the buffer and composition of the mobile phase. Dosulepin at 60 ppm concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The results of robustness study are shown in Table 6.

Table.6 Robustness results

Condition	Mean area	% difference
Unaltered	173804	
Flow rate at 0.8 mL/min	172681	0.65
Flow rate at 1.2mL/min	176071	1.31
Mobile phase: MEOH: Acetonitrile : 0.01M Phosphate buffer		
50:25:25	174683	0.5
60:15:25	175215	0.82
pH of mobile phase at 6.0	175753	1.12
pH of mobile phase at 5.8	175525	0.99

Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analysts on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and presented in Table 7.

Table.7 Ruggedness results

Sample	Conc. (in ppm)	Injection No.	Peak Areas	RSD(Acceptance criteria ≤ 2.0%)
Dosulepin	60	1	171659	1.07
		2	171490	
		3	169767	
		4	166814	
		5	169308	
		6	170453	

Table.8 Recovery Results

Level	Target	Amount of Dosulepin spiked (ppm)	Total in ppm	Amount of Dosulepin recovered(ppm)	% Recovery
	40	10	50	50.4	100.9
50 %	40	10	50	50.5	101.1
	40	10	50	49.7	99.5
	40	20	60	59.7	99.63
100%	40	20	60	59.8	99.81
	40	20	60	59.1	98.6
	40	40	80	79.1	98.89
150%	40	40	80	80.2	100.3
	40	40	80	80.9	101.2

Accuracy:

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed Standard solution. The standard addition method was performed at 50%, 100% and 150% level of 40ppm. The solutions were

analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated and presented in Table 8. Satisfactory recoveries ranging from 98% to 102% were obtained by the proposed method. This indicates that the proposed method was accurate.

Formulation:

For assay 20 tablets of Dosulepin (Dothepin-75mg) were weighed and calculated the average weight. Accurately weighed and transferred the sample equivalent to 10mg of Dosulepin into a 10mL volumetric flask. Added diluent and sonicated to dissolve it completely and made the volume up to the mark with diluents, made homogeneous and filtered through 0.45µm filter. Further pippetted 1mL of the above stock solution into a 10mL volumetric flask and diluted up to mark with diluents and finally 60ppm was prepared and filtered through 0.45µm filter. An aliquot of this solution was injected into HPLC system. Peak area of Dosulepin was measured for the determination and the findings were presented in Table 9.

Table.9: Formulation Analysis

Formulation	Dosage	Concentration	Amount found	% Assay
Dothepin	75mg	60ppm	59.62*	99.36

***Average of five determinations

CONCLUSION

The new HPLC method described in this paper provides a simple, convenient and reproducible method for the determination of Dosulepin in pharmaceutical formulations. The proposed method emphasized is isocratic and the mobile phase is with limited usage of buffer in low concentration. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

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