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HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ASSAY OF DAPOXETIN HYDROCHLORIDE TABLETS

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ABSTRACT

Dapoxetin hydrochloride is a new beginning in the treatment of Premature Ejaculation as dapoxetine is a unique medicine in tablet form designed to be taken only when needed.. In the present study an HPLC method was developed and validated for the assay of Dapoxetin hydrochloride tablet. The chromatographic system was equipped with Xterra C18 (4.6 x 150mm, 5 µm, Make:Waters) column and detector set at 230 nm, in conjunction with a mobile phase of Disodiam hydrogen phosphate buffer and Acetonitrile in the ratio of 65:35 (pH 4.0, adjusted with orthophosphoric acid) at a flow rate of 1.0ml/min and the tablet volume set at 20 µl with 9 minutes of runtime. The described method was linear over a concentration range of 10.0-100.0 µg/ml for the assay of Dapoxetin hydrochloride with a with good linearity response of 0.999. The retention time of Dapoxetin hydrochloride was 2.6 min ± 0.02. The results of analysis were validated. The results of the study showed that the proposed HPLC method was simple, rapid, precise and accurate, which is useful for the routine determination of Dapoxetin hydrochloride tablets.

INTRODUCTION

Dapoxetin is a unique medicine in tablet form designed to be taken only when needed. It heralds a new beginning in the treatment of PE. Chemically it is - *S*)-*N*,*N*-Dimethyl-3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine

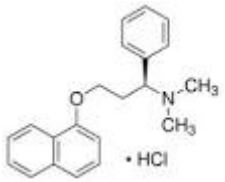


Figure 1: Chemical structure of Dapoxetin Hydrochloride

There are several methods to estimate dapoxetin, including high performance liquid chromatography (HPLC), high performance thin layer chromatography, capillary electrophoresis2, and spectrophotometry [6]. The present work describes a high performance liquid chromatographic method for the assay of Dapoxetin hydrochloride tablet, which can be used for the quality control of this formulation. The benefit of this method is that by doing one column analysis one can save time and resources. This study achieved satisfactory results in terms of selectivity, linearity, precision, and accuracy under simple chromatographic conditions.

Objective: Objective of this work comprise a whole hearted logically approached attempt to an elaborate study of method development, validatable, transferable, robust, reliable, accurate and precise methodology for the assay of Dapoxetin hydrochloride tablet within our laboratory.

MATERIALS AND METHODS

Chemicals and reagents:

Dapoxetin hydrochloride was obtained as a gift sample from Lee Pharma Ltd, Hyderabad, Disodiam hydrogen phosphate, was purchased from Genis enterprises, Abids, Hyderabad, Acetonitrile HPLC Grade was purchased from Rankem, India. All other chemicals were of analytical reagent grade.

Chromatographic conditions:

Chromatographic separation was performed on a Xterra C18 (4.6 x 150mm, 5 µms Make:Waters, software:Empower) with UV- detection of 230 nm at ambient temperature. The tablet volume was 20µl with a flow rate of 1.0 ml/min per minute and a run time of 5 minutes.

Mobile phase and solutions:

The mobile phase consisting of a binary mixture of acetonitrile and buffer adjusted to pH 4.0 with orthophosphoric acid in a ratio of 65:35. Degassed by ultrasonic water bath and filtered through 0.45µ membrane filter.

Standard and Sample Solution Preparation

Accurately weighed and transferred 30.0 mg of Dapoxetinpowder working standard into a 100 mL volumetric flask, added about 70 mL of diluents(mobile phase) and sonicated to dissolve it completely and made volume up to the mark with the same solvent (Stock solution). Further pipetted 2 ml of the above stock solution out into a 10ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45µm filter. Similar procedure was also carried out for sample preparation.

Calibration:

Five different concentrations (15,30,60,90,120 µg/ml) of Dapoxetinsolutions were prepared for linearity studies. The responses were measured as peak areas and plotted against concentration.

Estimation of Dapoxetinfrom tablet formulation [7]:

Inject 20 µL of the standard, sample into the chromatographic system and measure the area for the Dapoxetinpeak and calculate the %Assay by using the formulae.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = Peak Area of Dapoxetin obtained with test preparation
AS = Peak Area of Dapoxetin obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

System Suitability:

Tailing factor for the peak due to Dapoxetin in Standard solution should not be more than 1.5. Theoretical plates for the Dapoxetin peak in Standard solution should not less than 2500.

Validation Parameters [7,8]

Precision:

Preparation of stock solution:

Accurately weighed and transferred 30.0mg of Dapoxetin working standard into a 100 mL volumetric flask, added about 70 ml of mobile phase and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution).

Preparation of60 µg/ml solution: Further pipetted 2 ml of the above stock solution out into a 10ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45µm filter.

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of soame dimensins.

Accuracy:

Preparation of Standard stock solution:

Accurately weighed and transferred 30.0 g of Dapoxetin Working standard into a 100 mL volumetric flask, added about 70 mL of diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution).

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weighed and transferred 30.0mg of Dapoxetin API sample into a 100 mL volumetric flask, added about 70 ml of diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of the above stock solution out into a 10ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45µm filter.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer30.0 mg of Dapoxetin API sample into a 100 mL volumetric flask add about 70 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.(Stock solution). Further pipette 2 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer30.0 mg of Dapoxetin API sample into a 100 ml volumetric flask add about 70 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Dapoxetin and calculate the individual recovery and mean recovery values.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%.

Linearity:

From stock solution the following preparations were made-

Preparation of Level – I (15 µg/ml):

0.5ml of stock solution has taken in 25 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (30 µg/ml):

1ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (60 µg/ml):

2ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (90 µg/ml):

3ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (120 µg/ml):

4ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Limit of detection:

Preparation of 10µg/ml solution:

Accurately weigh and transfer 10 mg of Dapoxetin API sample into a 100 ml volumetric flask add about 70 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Pipette 1 ml of stock solution into a 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of 0.2% solution At Specification level (0.02µg/ml solution):

Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Further pipette 0.2mL of above diluted solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Limit of quantification:

Preparation of solution was same as in case of **Limit of detection**

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method.

a) The flow rate was varied at 0.7 ml/min to 0.9 ml/min. Standard solution 30 µg/ml was prepared and analysed using the varied flow rates along with method flow rate.

b) The Organic composition in the Mobile phase was varied from 30% to 40%.

Standard solution 60µg/ml was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

RESULTS AND DISCUSSIONS

System Suitability Results:

1) Tailing factor Obtained from the standard tablet is 1.24

2) Theoretical Plates Obtained from the standard tablet is 5116

Assay Results: % Recovery of Dapoxetin tablet = 98.5% **(see Table 1 and Figure 2).**

Table 1: Results for estimation of Dapoxetinhydrochloride

SL NO	SampleName	Peak Name	RT	AREA	HEIGHT (µV)	USP Plate Count	USP Tailing
1	Dapoxetin standard	Dapoxetin	2.852	857516	140935	5116.78	1.24
2	Dapoxetin sample	Dapoxetin	2.887	876285	141502	5018.53	1.25

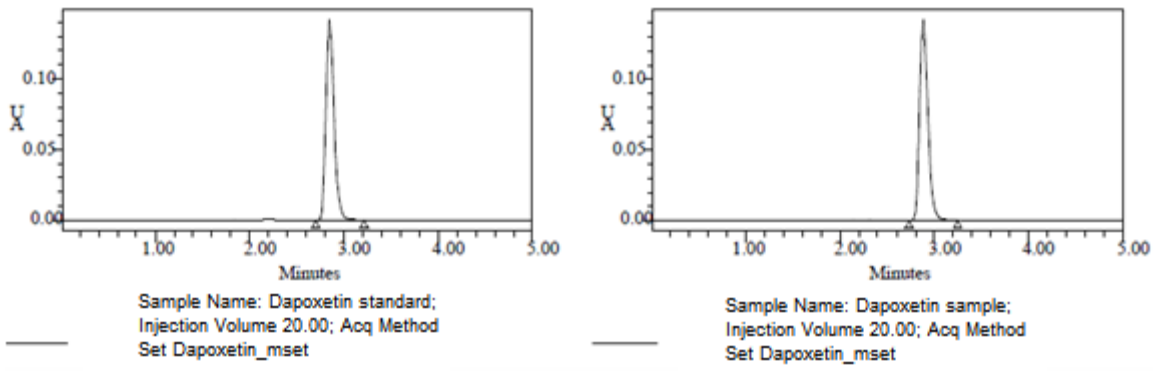
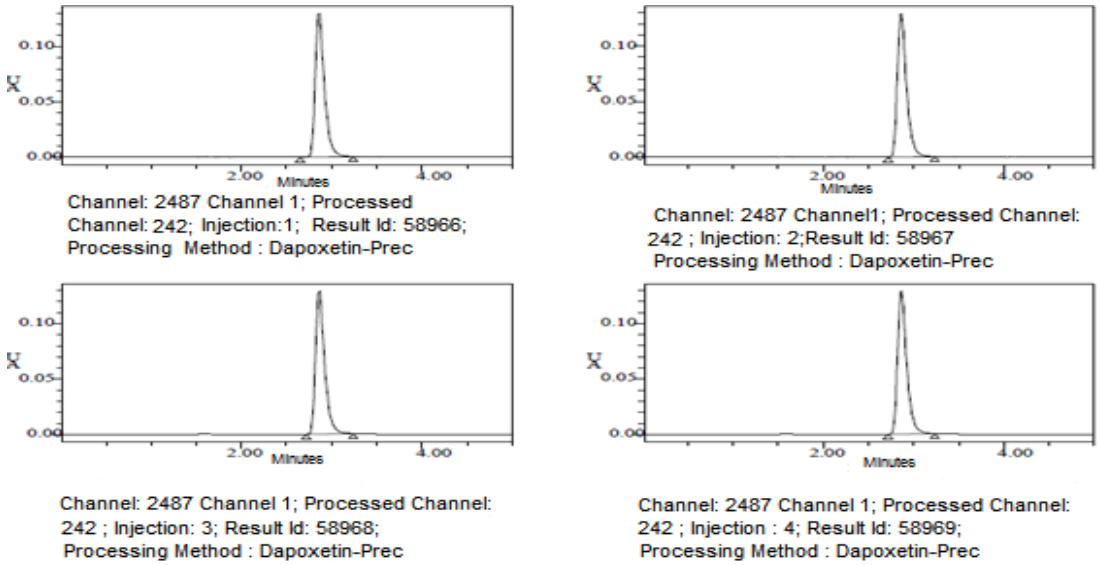


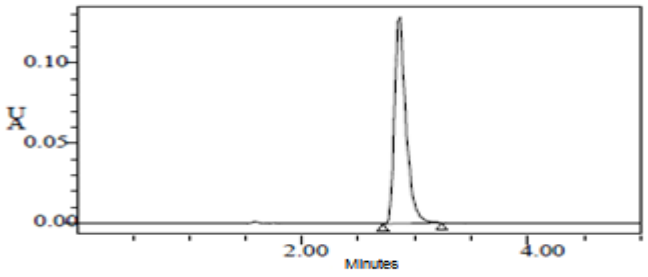
Figure 2: Spectras for Dapoxetin tablet of standard and sample

Precision: The values of the relative standard deviation of five replicate tablets of the standard solutions lie well within the limits (% RSD ≤ 0.26), indicating the tablet repeatability of the method. **(see Table 2 and Figure 3).**

Table 2: Precision -Percentage RSD value

SI.NO	INJ	PEAK	RT	AREA	HEIGHT
1	1	Dapoxetin	2.862	920467	129849
2	2	Dapoxetin	2.863	916697	129018
3	3	Dapoxetin	2.865	916165	129289
4	4	Dapoxetin	2.865	915896	129079
5	5	Dapoxetin	2.864	913893	128288
Mean				916625	
SD				2396.2	
%RSD				0.26	





Channel : 2487 ; Channel 1; Processed Channel : 242; Injection: 5 ; Result Id : 58970 ; Processing Method: Dapoxetin-Prec

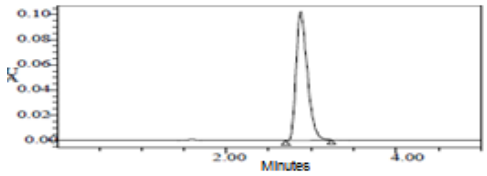
Figure 3: Precision spectras of Dapoxetin

Intermediate Precision/Ruggedness:

The values of the relative standard deviation of five replicate tablets of the standard solutions lie well within the limits (% RSD ≤ 0.07), indicating the tablet repeatability of the method. **(see Table 3 and Figure 4).**

Table 3: Intermediate Precision -Percentage RSD value

TABLET	AREA
1	943341
2	944392
3	945114
4	944696
5	944882
Avg	944484
SD	692.1
%RSD	0.07



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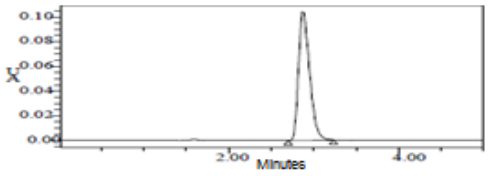

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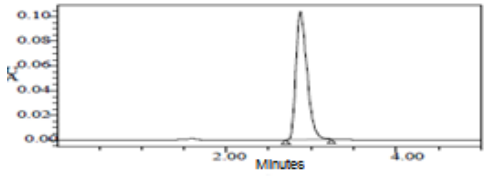

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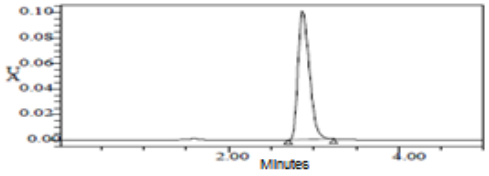


Channel: 2487 Channel 1 : Processed Channel : 242; Injection : 4; Result Id: 59025; Processing Method : Dapoxetin-I.Prec

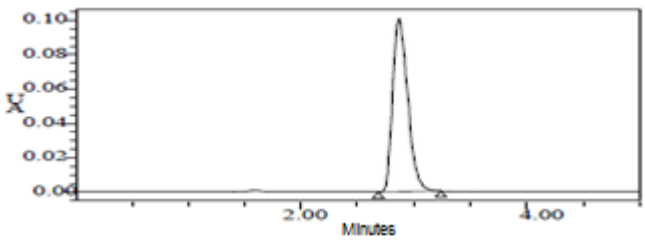


Figure 4: Intermediate precision spectras

Accuracy: The values of the relative standard deviation of three replicate tablets of the standard solutions lie well within the limits (% RSD ≤ 0.04, 0.13, 0.06), indicating the tablet repeatability of the method. **(see Tables 4-7 and Figures 5-7).**

Table 4: Accuracy for 50% solution -Percentage RSD value

SL	TABLET	PEAK NAME	RT	AREA	HEIGHT
1	1	Dapoxetin	2.868	536616	71986
2	2	Dapoxetin	2.867	536681	71804
3	3	Dapoxetin	2.868	537013	70917
Mean				536769	
SD				213.0	
%RSD				0.04	

Table 5: Accuracy for 100% solution -Percentage RSD value

SL NO	TABLET	PEAK	RT	AREA	HEIGHT
1	1	Dapoxetin	2.866	912212	121926
2	2	Dapoxetin	2.866	913332	122199
3	3	Dapoxetin	2.867	914668	122067
MEAN				913404	
SD				1231.3	
%RSD				0.13	

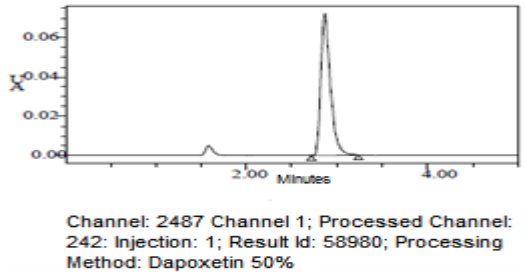
Table 6: Accuracy for 150% solution -Percentage RSD value

SL NO	TABLET	PEAK	RT	AREA	HEIGHT
1	1	Dapoxetin	2.864	1561028	208194
2	2	Dapoxetin	2.862	1559476	208265
3	3	Dapoxetin	2.865	1561159	209917
Mean				1560554	
SD				935.4	
%RSD				0.06	

Acceptance Criteria for accuracy: The % Recovery for each level should be between 98.0 to 102.0%. From the experimental value the recovery was found to be within the limit. **(see table 6).**

Table 7: Percentage Recovery

%CONTENT RATION	MEAN AREA	AMOUNT ADDED(MG)	AMOUNT FOUND(MG)	%RECOVERY	MEAN RECOVERY
50%	536769	15.2	15.36	101.05	
100%	913404	26.1	26.15	100.2	100.4
150%	1560554	44.5	44.68	100.4	



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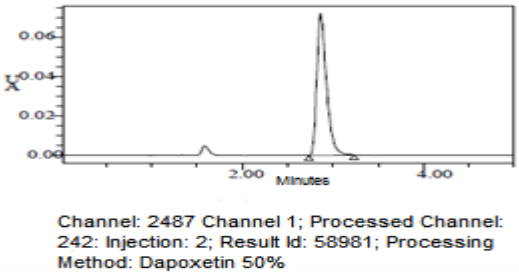

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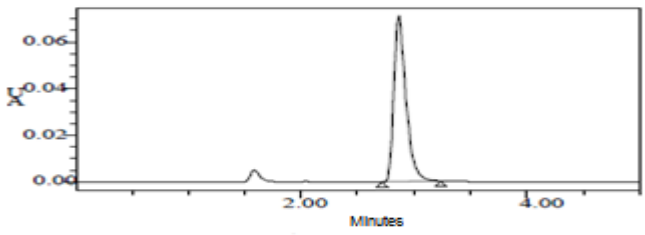
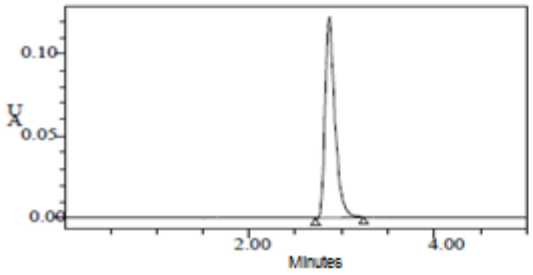


Channel: 2487 Channel 1; Processed Channel: 242; Injection: 3; Result Id: 58982; Processing Method: Dapoxetin 50%

Figure 5: Accuracy spectras for 50% solution



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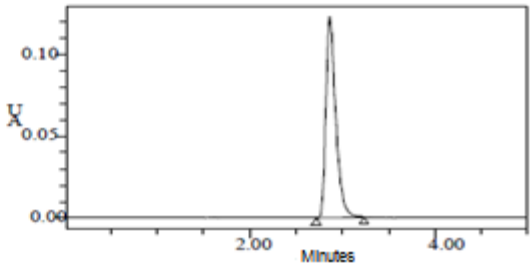

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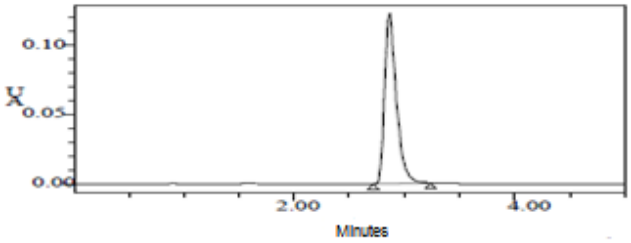
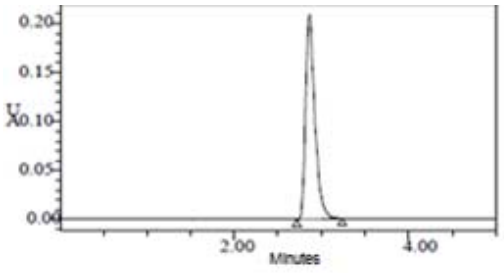
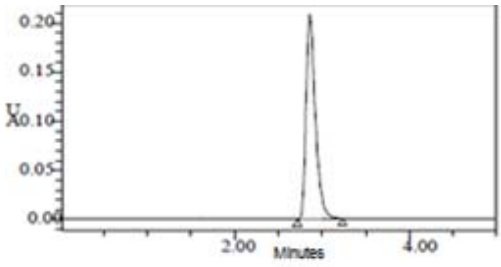


Channel: 2487; Channel 1; Processed Channel: 242; Injection: 3; Result Id: 58986; Processing Method: Dapoxetin 100%

Figure 6: Accuracy spectras for 100% solution



Channel: 2487 Channel 1: Processed Channel: 242; Injection : 1; Result Id: 58988; Processing Method: Dapoxetin 150%



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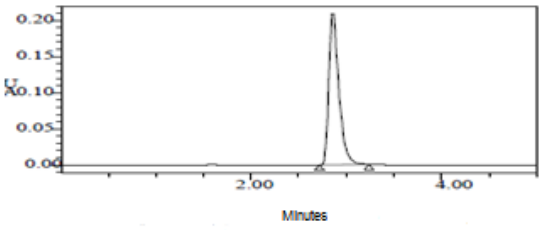

Channel : 2487; Channel 1; Processed Channel 242; Injection: 3; Result Id: 58990: Processing Method: Dapoxetin acc 150%

Figure 7: Accuracy spectras for 150% solution

Linearity: Correlation coefficient should be not less than 0.999. From the experimental value the correlation coefficient was found to be the same. **(see Table 8 and Figure 8).**

Table 8: Linearity-Correlation coefficient value

SL NO	LINEARITY LEVEL	CONC	Area
1	I	10µg/ml	224319
2	II	25µg/ml	547841
3	III	50µg/ml	963014
4	IV	75µg/ml	1472471
5	V	100µg/ml	1822719
6	VI	125 µg/ml	2161225
Correlation Coefficient			0.999

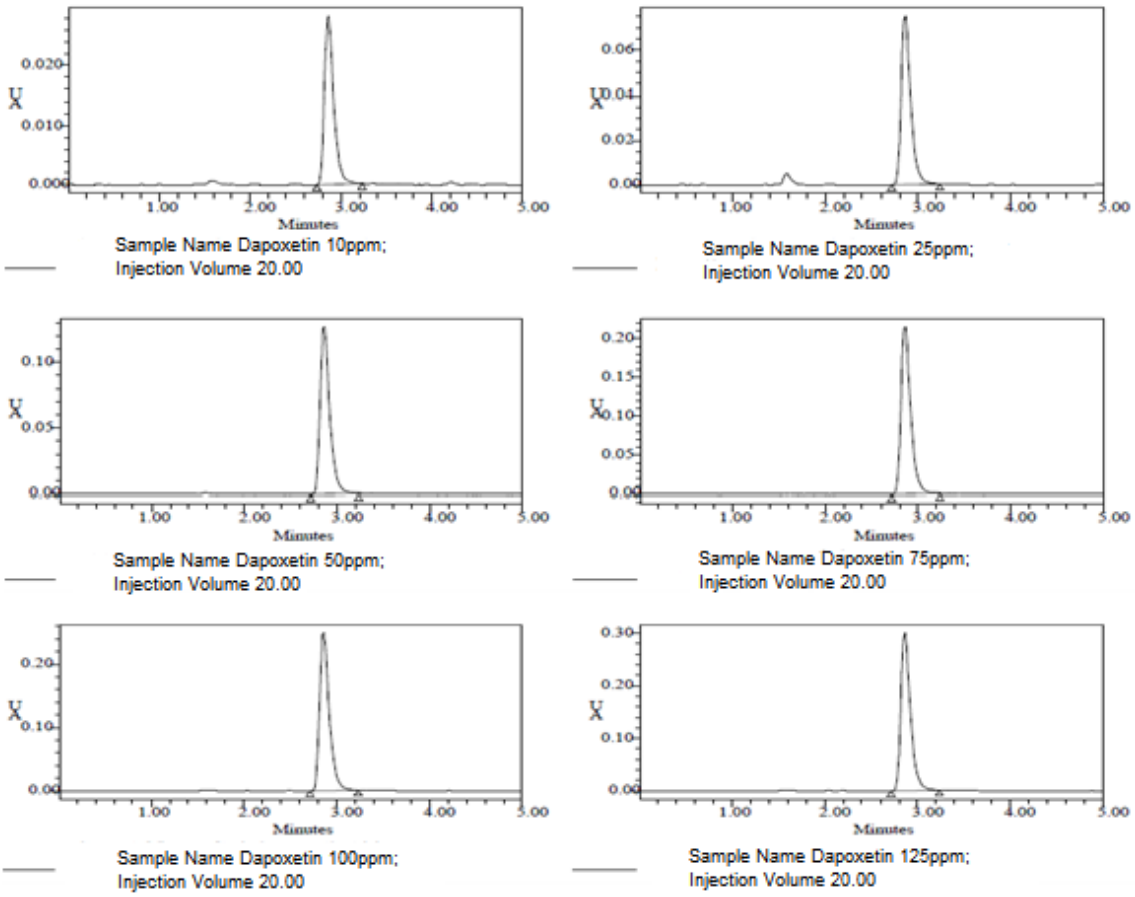

Figure 8: Linearity spectras of Dapoxetin at various concentrations

Limit of detection: S/N Ratio value should be 3 for LOD.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 24 µV

Signal Obtained from LOD solution (0.2% of target assay concentration): 69 µV

S/N = 69/24 = 2.87.

From experimental value the S/N ratio was found to be within limit i.e. 2.87. **(Figure 9).**

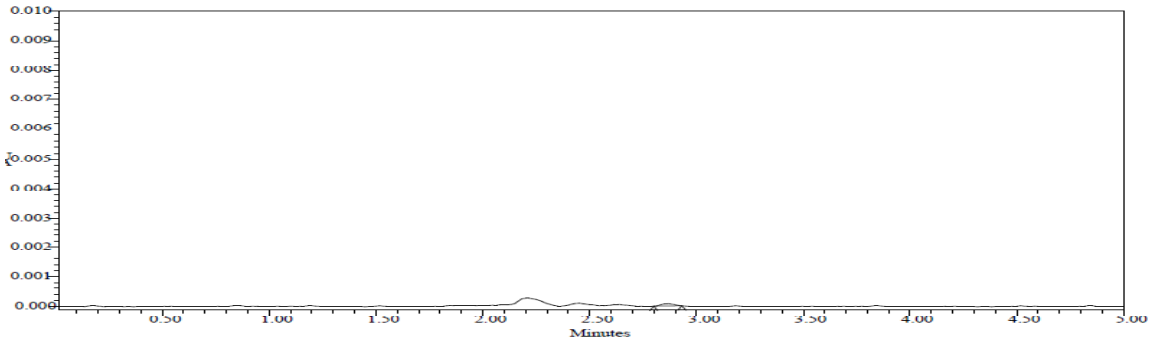

Figure 9: Spectra for limit of detection

Limit of quantification: S/N Ratio value should be 10 for LOQ solution

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 24 µV

Signal Obtained from LOD solution (0.8% of target assay concentration) : 253µV

S/N = 253/24 = 10.54

From experimental value S/N ratio was found to be within limit i.e.10.54.**(Figure 10).**

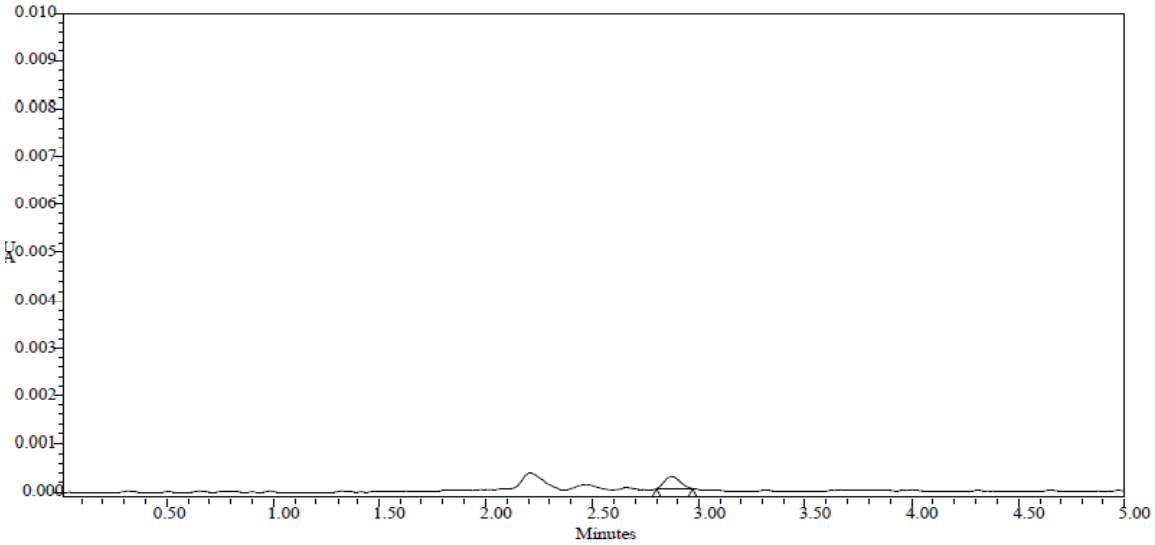

Figure 10: Spectra for limit of quantification

Robustness: When flow rate was varied at 0.7 ml/min to 0.9 ml/min it was found that the variation in flow rate do not affect the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10% **(see Table 9 and Figure 11).**

Table 9: Robustness-Flow rate Variation values

SL NO	FLOW RATE(ML/MIN)	USP PLATE COUNT	TAILING
1	0.7	5132	1.24
2	0.8	5116	1.24
3	0.9	4610	1.21

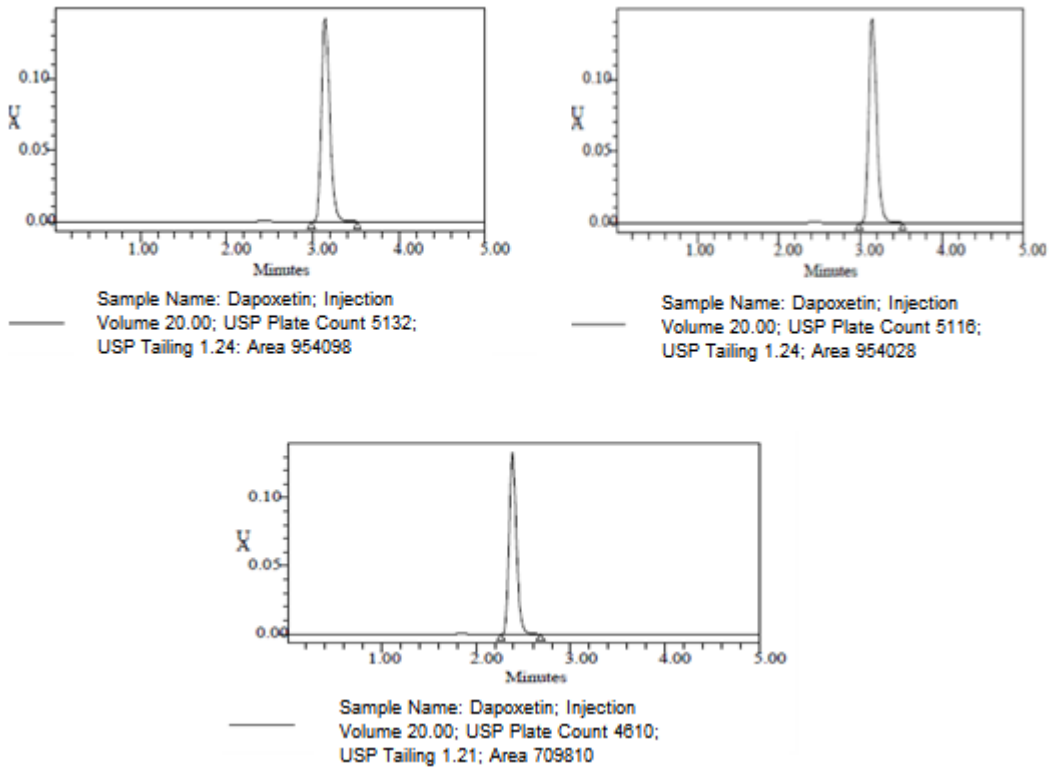

Figure 11: Spectras for robustness with variable flow rates

When organic composition in the Mobile phase was varied from 30% to 40% it was found that in the mobile phase the variation in less organic composition or variation in more organic composition do not affect the method significantly. Hence it indicates that the method is robust even by change in the 10% more organic content in Mobile phase. **(Table 10 and Figure 12).**

Table 10: Robustness-Flow rate Variation values

SL NO	CHANGE IN ORGANIC PART	USP PLATE COUNT	TAILING
1	30 part	3454	1.29
2	35 part	5106	1.25
3	40part	5296	1.19

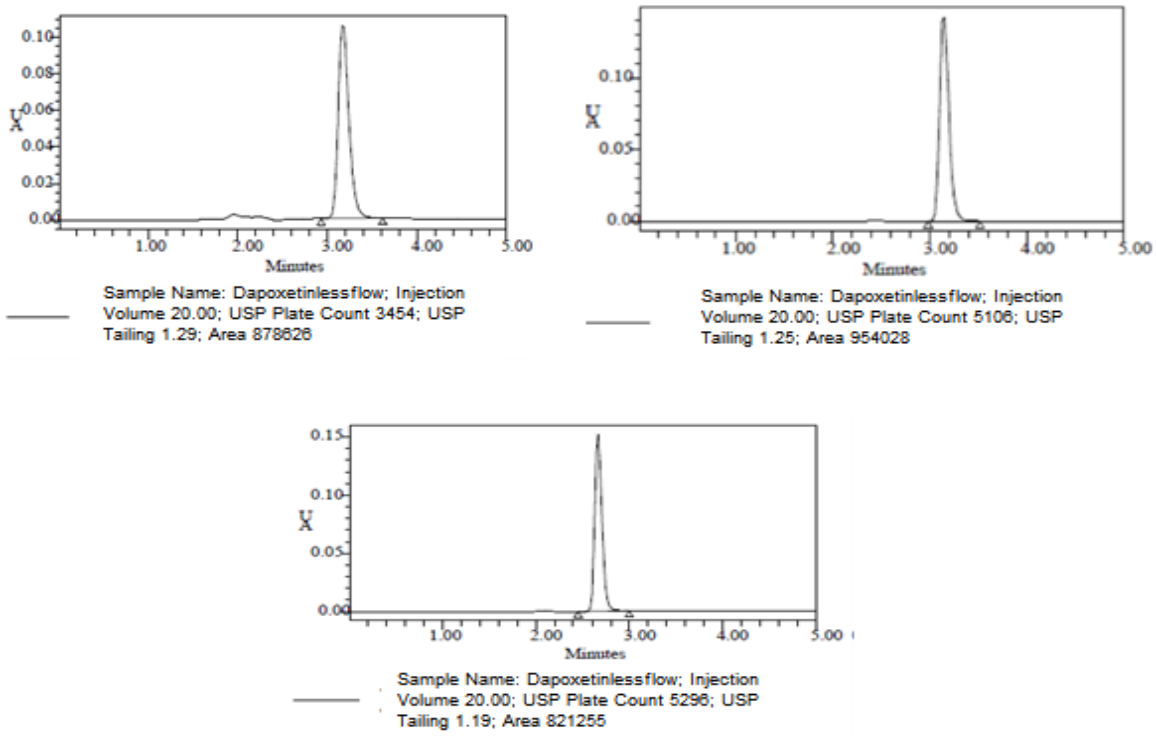

Figure 12: Spectras for robustness with less or more organic solvents

CONCLUSION

More rapid, precise, specific, sensitive, economic, reproducible, isocratic reverse phase HPLC method was developed and validated for quantitative determination of Dapoxetintablet. The calibration curve was constructed at six concentration levels and the method was found to be linear. The correlation coefficient was found to be ≥0.9999. Thus, the proposed analytical method was simple and represents specific procedure for assay of Dapoxetintablet and this HPLC method successfully applicable for regular analysis of Dapoxetinin quality control laboratories.

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