Advances in Bioresearch
Adv. Biores., Vol 16 (1) January 2025: 16-25

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.1.1625

Advances in Bioresearch

ORIGINAL ARTICLE

Development and Validation of RP-HPLC Method for Determination of Dapoxetine and Its Inherent Impurities in Pharmaceutical Dosage Forms

Subhranshu Panda[1], Tattari Praveenkumar[2*], Alavala Manikanta kumar[3*]
[1, 2]School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India.
[3]Department of Quality Control, M/s Aurobindo Pharma Limited, Visakhapatnam.
*Corresponding author email: praveenpark8@gmail.com

ABSTRACT
A reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the identification and quantification of Dapoxetine and its inherent related substances in finished dosage forms. Chromatographic separation was achieved on a Discovery Column (250 mm x 4.6mm, 2.7mm) with the mobile phase (0.01N Potassium dihydrogen Ortho Phosphate as buffer and Acetonitrile in isocratic mode in the ratio 65:35). The HPLC flow rate was 1.0 ml/ min and peaks were monitored at 230 nm using a UV detector. The column temperature was kept constant at 30°C, and the injection volume was 10 µl. The run time of the method was 10 min. The method was validated as per the International Conference on Harmonization (ICH) guidelines. Linearity was recorded at various concentrations ranging from 0.25 to 1.5 µg/ml for all the Dapoxetine impurities. Linearity, regression value, recovery, %relative standard deviation (RSD) of method precision values were found within the acceptance limits. The method for related substances in Dapoxetine was found to be specific, linear, accurate, precise, rugged, and robust. The validated method was suitable for the quantification of the Related Substances in Dapoxetine drug products. The method can be applied for routine analysis of Dapoxetine drug substance and drug products in labs.
Keywords: Dapoxetine, Discovery, Inherent, method validation

Received 19.10.2024 Revised 23.11.2024 Accepted 13.12.2024

How to cite this article:
Subhranshu P, Tattari P, Alavala MK. Development and Validation of RP-HPLC Method for Determination of Dapoxetine and Its Inherent Impurities in Pharmaceutical Dosage Forms. Adv. Biores. Vol 16 [1] January 2025. 16-25

INTRODUCTION

Antidepressants are medications used to treat major depressive disorder, some anxiety disorders, some chronic pain conditions, and to help manage some addictions.

Popular drugs available in the market

- Citalopram
- Sertraline
- Paroxetine
- Escitalopram
- Dapoxetine

Mechanism of action of selected drug for method validation is that Dapoxetine is a short-acting selective serotonin reuptake inhibitor (SSRI) that increases the serotonin levels in the brain, thereby increasing the time to ejaculate and improving the control over ejaculation.

Dapoxetine is a selective serotonin reuptake inhibitor

Dapoxetine IUPAC Name: (S)-N, N-Dimethyl-3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine
Molecular formula is $C_{21}H_{23}NO$.
Molecular weight: 305.4 g/mol

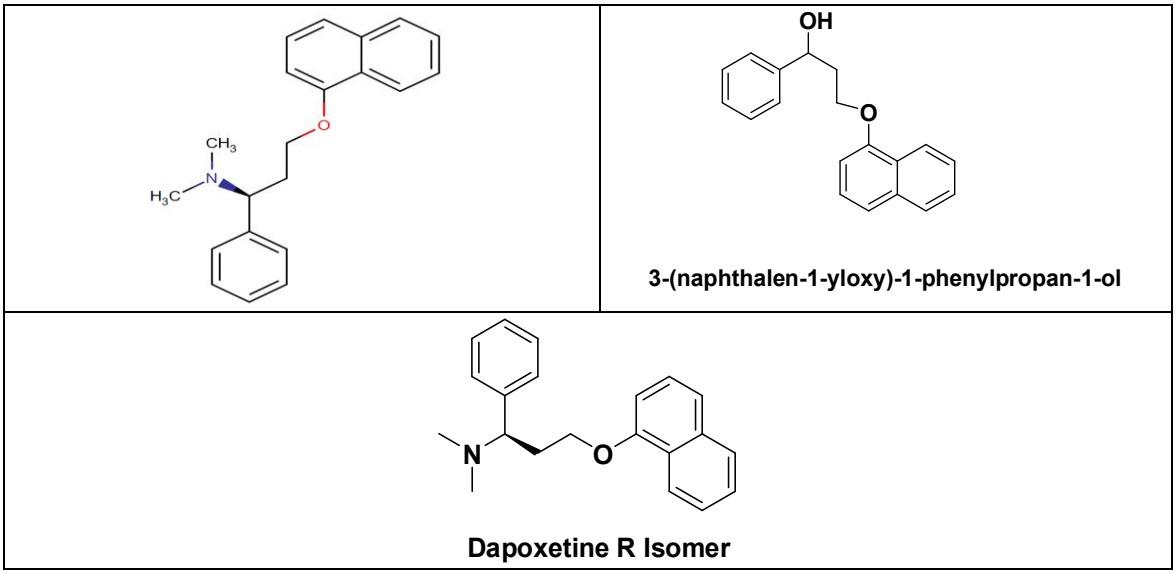

Figure 1: Dapoxetine Inherent Impurities

A detailed literature survey shows that few analytical methods [1,9] for determination of Dapoxetine and its impurities were available by UV (Ultra violet) [2, 11, 15] High performance liquid chromatography [3,5,7] HPLC-MS/MS Method [6], UPLC-MS/MS [4] but there is no method available with these impurities (Figure 1). The present study describes a novel method of determination of inherent impurities in Dapoxetine which is accurate, simple, reproducible and cost saving method which can be adopted for routine analysis at quality control labs in pharma industries, which is in line with ICH-Q2B Guidelines [17,18,19].

MATERIAL AND METHODS

Instruments used: Electronics balance of Denver, Ultrasonicator of Labman, and Vacuum pump of Crompton and High-performance liquid chromatography 2695 system with PDA detector with Empower 2 software was used for Method validation.

Chemicals and Reagents: Acetonitrile, Methanol (HPLC Grade) were purchased from M/s Merck Chemicals division and that of Potassium dihydrogen Ortho phosphate, Triethyl Amine, Ortho-Phosphoric acid and diSodium hydrogen Ortho phosphate was procured from M/s Rankem avantor.

Standards and API of Dapoxetine were gifted by M/s Synergene active ingredients, Vizag and Dapoxetine tablets were arranged by M/s Spectrum labs, Hyderabad.

Preparation of Mobile phase

Preparation of 3.5%w/v of Disodium hydrogen ortho phosphate

Transferred 3.5gms of Disodium hydrogen ortho phosphate to 100ml volumetric flask and diluted to 100ml with milli-Q water.

Preparation of 0.01 Potassium dihydrogen Phosphate buffer

Dissolved 1.369gms of potassium dihydrogen phosphate to 100ml volumetric flask and made up to 100ml with milli-Q water and then adjusted the pH with 3.5%w/v disodium hydrogen ortho phosphate.

Further taken above solution of 100ml and diluted to 1000ml with milli-Q water and degas.

Mobile phase Preparation

Used 0.01M Potassium di hydrogen phosphate as mobile phase solution A.

Used Acetonitrile as Mobile Phase B

Diluent: Water: Acetonitrile (50:50 %v/v)

Tablet average Weight: 190mg

Standard Preparation (Concentration=1.0ppm)

Transfer 10mg of Dapoxetine into 50ml volumetric flask, dissolve and dilute volume with diluents. (Concentration=200ppm)

Further 2.5ml of above stock transfer into a 50ml volumetric flask, dilute to volume with diluent. (Concentration=10ppm)

Again, further dilute above solution 5.0ml into 50ml volumetric flask, dilute to volume with diluent. (Concentration=1ppm)

Sample preparation (Concentration=200ppm)
Crush not less than 10 tablets into fine powder, weigh [20] equivalent to 20mg of Dapoxetine and transfer into a 100ml volumetric flask. Add about 35 mL of diluent, sonicate for 20 minutes with intermittent shaking. Attain to room temperature. Dilute up to the volume with diluent and mix well.
Placebo solution: Weight and transfer placebo powder equivalent to 20 mg of Dapoxetine into a 100 mL of volumetric flask, add about 35ml of diluent, sonicate for 20minutes with intermittent shanking . Attain to room temperature. Dilute up to the volume with diluent and mix well.

Chromatographic conditions

Mobile phase	**:**	0.01N kH2PO4: Acetonitrile (65:35 v\v ratio)
Flow rate	**:**	1 ml/min
Column	**:**	Discovery250 mm x 4.6mm, 2.7µm.
Detector wave length	**:**	230 nm
Column temperature	**:**	30°C
Injection volume	**:**	10µL
Run time	**:**	10min
Diluent	**:**	Water: Acetonitrile 50:50

Method Development study
The aim of the research work study was to develop a simple, robust, accurate and sensitive HPLC method [10,12,13,14] for the determination of Dapoxetine and its inherent impurities [8]. Initially various mobile phases and stationary phases were tested to obtain the separation.
In development trail 01, Impurity B peak not eluted and that of Impurity A and Dapoxetine peak was blunt, so stopped the run after 10mins and made new trail with changing the mobile phase to OPA: Methanol (50:50). In development trail 02, Impurity B peak not eluted and that of Impurity A and Dapoxetine peak was sharp, so stopped the run after 10min and made new trail by changing the mobile phase to KH_2PO_4: Methanol (60:40). In development trail 03, all the impurities peaks along with Dapoxetine peak was eluted with sharp peak shape but system suitability criteria not met, hence stopped the run after 10mins and made new trail by mobile phase ratio to KH_2PO_4: Acetonitrile (60:40). In development trail 04, all Impurities and Dapoexetine peak were resolved properly with good peak shape but all the peaks came close to each other and system suitability criteria was not proper and made new trail by changing the mobile phase ratio to OPA: Acetonitrile (40:60). In development trail 05, Impurity B peak was blunt and Impurity A and dapoxetine peaks were sharp, hence again new trail was made by changing mobile phase ratio to 0.01M Potassium dihydrogen ortho Phosphate: Acetonitrile (65:35). Basing on development trail 05 we had optimised the conditions as follows Mobile phase ratio to 0.01M Potassium dihydrogen ortho Phosphate: Acetonitrile (65:35), Column Discovery250 mm x 4.6mm, 2.7µm flow rate 1.0ml/min detection at 230nm and run time as 10mins and found that all the impurities were resolved , peak shape and base line found satisfactory. Hence this finalised method was used for Method validation of Dapoxetine. (Figure 2)

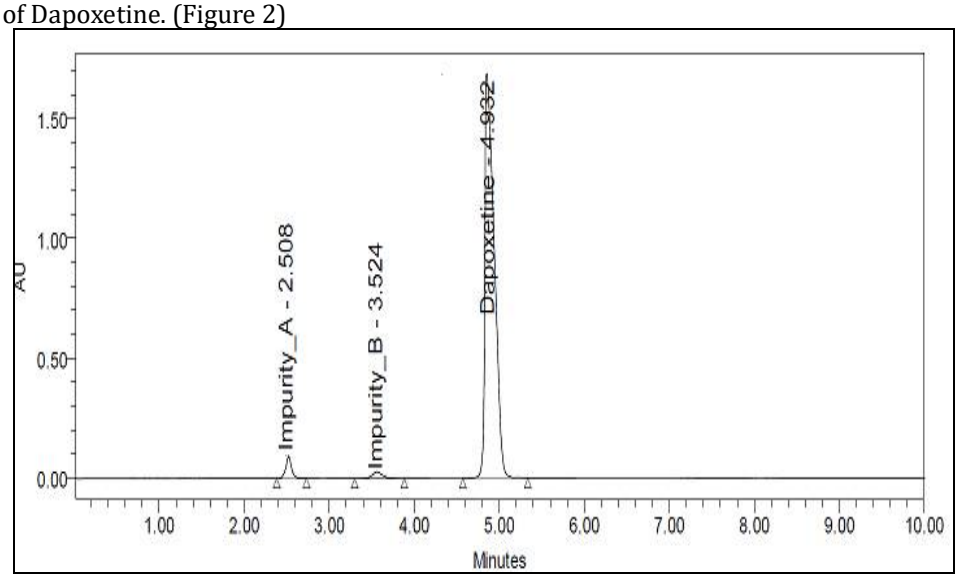

Figure 2: Optimised Chromatogram

RESULTS AND DISCUSSION

Method Validation: The method was validated [16] in accordance with recognized ICH guidelines

System suitability: Prepared the standard solution as per methodology and injected six times into the chromatographic system and obtained % RSD from six replicate injections was 0.8. The observed tailing factor for Dapoxetine peak from the first injection of standard solution was 1.2 and that of theoretical plates is 7258, suitability results are given in Table 1.

Table1: System suitability results

Injections	Peak area of Dapoxetine
1	395083
2	392344
3	394050
4	398403
5	390348
6	390246
Mean	393412
SD	3119.7
%RSD	0.8

Specificity: Specificity is demonstrated by checking the blank, placebo, known and degradant impurities interference with the analyte peak.

Prepared blank, placebo, standard solution, sample solution, Impurities spiked sample solution and individual impurities solutions as per method and injected into HPLC system to evaluate the peak purity and interference of any peak with Dapoxetine and known impurities. All blank and placebo peaks were well separated from known impurities and Dapoxetine peak. All known peaks were separated with each other and Dapoxetine peak. Specificity results are addressed in table 2 and the specificity chromatograms and peak purity plots are shown from figure.3 to figure.6. Blank, placebo and impurities have not shown any interference with Dapoxetine and Known degradant impurities. Hence the above method is specific.

Table 2: Specificity results

Name of the Active/Impurity	Retention time (min)	Peak purity	
		Purity angle	Purity threshold
Impurity A	2.527	0.557	0.583
Impurity B	3.623	2.731	3.147
Control sample (Dapoxetine)	4.884	3.560	4.171

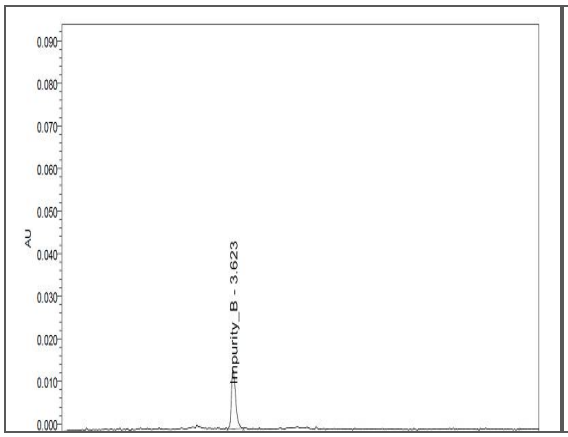

Figure 3: Peak purity of Impurity B

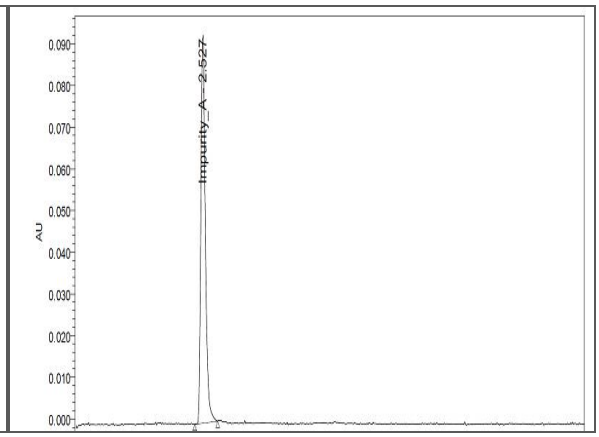

Figure 4: Chromatogram of Impurity A

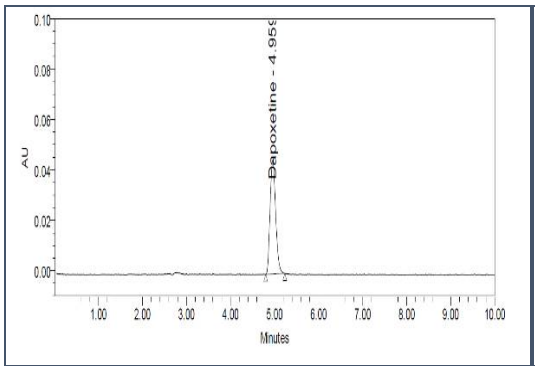

Figure 5: Peak purity of Dapoxetine standard

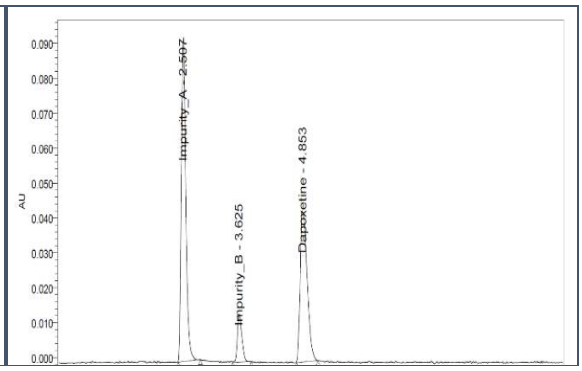

Figure 6: Chromatogram of standard and Impurity mixture

Forced degradation

Forced degradation of Dapoxetine in Dapoxetine tablets was carried out, to confirm that, during the stability study or throughout the shelf life, any degradation product if found will not interfere with Dapoxetine and known impurities peaks and also the forced degradation study will help to identify the type of degradation pathway (whether oxidative, alkali hydrolysis, acid hydrolysis, photolytic, dry heat and humidity) for each of the degradants. Dapoxetine tablets was forcefully stressed by exposure to acid hydrolysis, base hydrolysis, peroxide, Hydrolysis, UV and thermal. Control and stressed samples were injected into the HPLC system and evaluate the Peak purity, interference of degradants and mass balance.

In force degradation studies fortunately all generated impurities have not interfered with the Dapoxetine peak, known impurities peaks and also with each other. The purity angle of Dapoxetine and its known impurities is less than the purity threshold. Forced degradation chromatograms shows peak purity, peak threshold, assay and degradations and the results for forced degradation studies were addressed in the table 3.

Table 3: Forced degradation Studies

Sample Name	Impurity A (%w/w)	Impurity B (%w/w)	Single max. Unknown (% area)	Purity Angle	Purity Threshold	% assay
Control sample	ND	ND	ND	0.152	0.366	100.0
0.01N HCl / 24hrs at Bench top.	ND	ND	0.28	0.352	1.352	99.72
0.01N NaOH / 24hrs at Bench top	ND	ND	0.07	0.358	1.301	99.93
3.0%H_2O_2 for 24hrs on Bench top	ND	ND	3.08	0.307	0.791	100.0
Water /40°C For 24Hrs	ND	ND	ND	0.316	0.870	100.0
UV for 24hrs	ND	ND	ND	0.443	0.839	100.0
Thermal 105°C for 6 Hours	ND	ND	ND	0.270	0.822	100.0

Linearity and RRF establishment

A series of known impurity and Dapoxetine from LOQ to 150% of specification level and injected into HPLC system as per method. Linearity was conducted by preparing the five levels of linearity solutions and Plot a graph of concentration versus response for impurity solutions and standard solutions.

Relative response factors for all individual impurities established based on slope method and calculate the RRF values from the linearity data.

Calculate the relative response factor for all the known impurities using following formula.

$$\text{Factor (RRF) of impurity} = \frac{\text{Slope of impurity solution}}{\text{Slope of standard solution}}$$

The obtained all known impurities and Dapoxetine correlation coefficient were not less than 0.999. All the linearity data and RRF values are addressed from table 4 and table 5 and the linearity graphs are shown from figure.7 to figure.9.

Table 4: Linearity results of Impurity A and Impurity B

Linearity levels	Impurity A		Impurity B	
	Concentration (ppm)	Area response	Concentration (ppm)	Area response
25%	0.25	92965	0.25	23600
50 %	0.50	183431	0.50	47690
75%	0.75	286384	0.75	71418
100 %	1.00	375604	1.00	95314
125%	1.25	465865	1.25	118018
150 %	1.50	552513	1.50	140611
Correlation Coefficient (r)	**0.99**		**0.99**	
Square Correlation Coefficient (r^2)	0.999		0.999	
Slope	369630.32		93706.70	
Y-Intercept	2700.35		781.68	
Relative response factor	0.96		0.24	

Table 5: Linearity results of Dapoxetine

Linearity levels	Dapoxetine	
	Concentration (ppm)	Area response
25%	0.25	98306
50 %	0.50	196897
75%	0.75	292450
100 %	1.00	394837
125%	1.25	494639
150 %	1.50	583155
Correlation Coefficient (r)	**0.99**	
Square Correlation Coefficient (r^2)	0.999	
Slope	390840.88	
Y-Intercept	1394.84	
Relative response factor	NA	

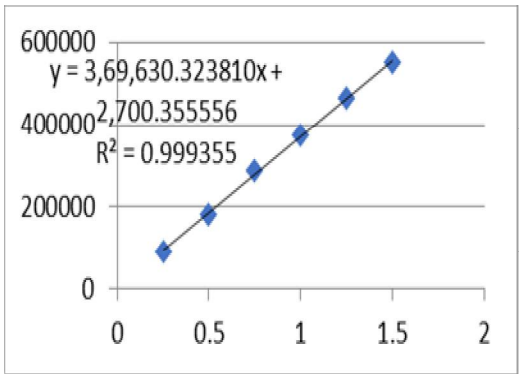

Figure 7: Linearity graph of Impurity A

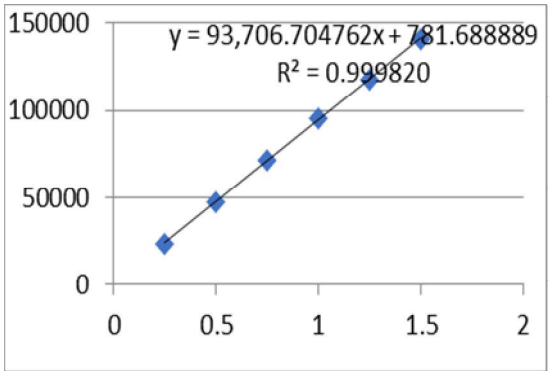

Figure 8: Linearity graph of Impurity B

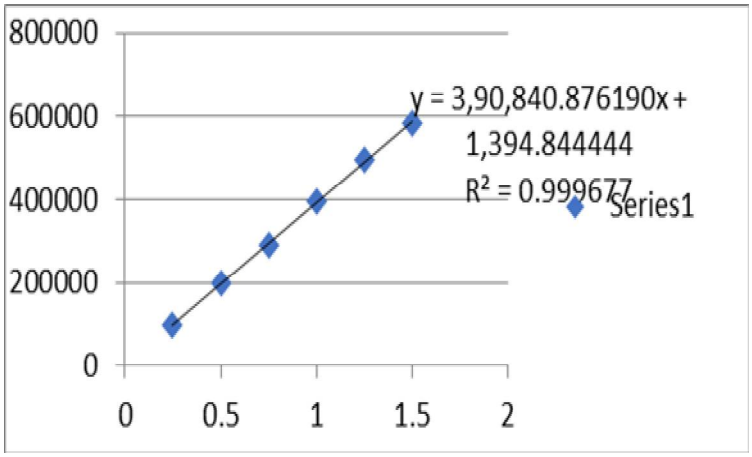

Figure 9: Linearity graph of Dapoxetine

Establishment of LOD and LOQ

The LOD and LOQ values of all known impurities and Dapoxetine were determined by using Standard deviation of the response and Slope.

From the above linearity curve, standard deviation on response and slope of known impurities and main peak were calculated at different concentrations.

LOD and LOQ values are expressed as a known concentration of Dapoxetine and its known impurities at a low concentration for LOQ 10:1, for LOD 3:1 were quantitated or detected by HPLC method but LOD and LOQ is determined here by signal to noise ratio method.

The LOD and LOQ values and concentrations are addressed in table 6.

Table 6: Signal to noise ratio of LOD and LOQ

Name of the Active/Impurity	LOD (S/N)	LOQ (S/N)	LOD Conc. in ug/ml	LOQ Conc. in ug/ml
Impurity A	3.7	36.4	0.017	0.052
Impurity B	3.9	24.8	0.014	0.042
Dapoxetine	3.4	36.5	0.08	0.024

Precision

System precision: It is demonstrated by calculating %RSD for retention time and peak areas of Dapoxetine peak from six replicate injections of standard solution preparation. The system precision results are addressed in table 7.

Table 7: System precision results

Injection	Dapoxetine Retention time	Peak area of Dapoxetine
1	4.860	395083
2	4.903	392344
3	4.956	394050
4	4.959	398403
5	4.973	390348
6	4.987	390246
Mean	4.94	393412
SD	0.05	3119.7
%RSD	1.0	0.8

Method precision

Method precision was evaluated by injecting spiked known impurities on drug product at specification level. % RSD values for Retention time and peak area responses of individual impurities should not be more than 10%.

%RSD of Impurity A found 0.8 and 0.8 and Impurity B found 0.8 and 0.1. The data demonstrated that the values are met the acceptance criteria. Hence the method was found Precise and the results are addressed from table 8.

Table 8: Method precision for Impurity A and Impurity B

Injection	Impurity A Retention time	Peak area of Impurity A	Impurity B Retention time	Peak area of Impurity B
1	2.495	377373	3.618	71110
2	2.503	371489	3.629	71195
3	2.522	374465	3.647	71294
4	2.528	376147	3.653	71294
5	2.535	379604	3.672	71294
6	2.547	371936	3.702	71256
Mean	2.52	375169	3.65	71241
SD	0.02	3161.4	0.03	74.74
%RSD	0.8	0.8	0.8	0.1

Accuracy

The accuracy was evaluated by measurement (n=3) applying the method to the sample spiked with known amounts of known impurities corresponding to 50 %, 100 % and 150 % of specification. The recovery data of all known impurities obtained from a study of formulation from 50% level to 150 %. The test sample were prepared at each % level and tested against standard according to the description of the methodology. The total average recovery for Impurity A is 101.74% with 0.8 % RSD and Impurity B is 100.29 % with 0.6 % RSD.The accuracy results are addressed in table 9.

Based on the impurities recovery results, it is concluded that there was no interference from excipients present in the formulation and the method is accurate.

Table 9: Accuracy results (% Recovery)

Level	Impurity A	Impurity B
50 % Mean % Recovery	101.19	99.60
50 % % RSD	0.1	0.3
100 % Mean % Recovery	102.73	100.53
100 % % RSD	0.4	0.4
150 % Mean % Recovery	101.30	100.75
150 % % RSD	0.4	1.3
Overall Mean % Recovery	101.74	100.29
RSD of overall % Recovery	0.8	0.6

Solution stability

Spiked sample solution was found stable up to 24 hours at room temperature with the difference in 10% individual known impurity from initial to time intervals. Solution stability[17] results are addressed in table 10.

Table 10: Spiked Sample solution stability at Room temperature

	At Room temperature (25°C) %Difference		
Hours	**Impurity A**	**Impurity B**	**Dapoxetine**
Initial	NA	NA	NA
24 Hours	0.45	0.15	4.65

Mobile phase stability

The mobile phase was found stable for 6 days at bench top condition, no haziness of mobile phase was observed.

Robustness

Robustness of the method was assessed by varying the instrumental conditions such as column temperature (± 5°C) , flow rate ((±0.1mL) and Organic variation of mobile phase(±5%).

The deliberate changes in the method have no significant changes in retention time, relative retention time and no distorted chromatography was observed for Dapoxetine and its known impurities. This indicates that the method is robust. Results for robustness studies are addressed in the table 11.

Table 11: Robustness studies for spiked sample

RRT of Impurities in spiked sample			
parameter	**Variation**	**Impurity A**	**Impurity B**
Original conditions	None	0.50	0.76
Mobile phase variation	Organic 5% minus	0.50	0.78
	Organic 5% plus	0.54	0.73
Flow Rate mL/min	0.9mL/min	0.54	0.75
	1.1mL/min	0.49	0.73
Column oven temperature	25°C	0.51	0.77
	35°C	0.54	0.73

CONCLUSION

A validated reverse phase HPLC method concluded that the method is suitable, specific, linear, accurate, precise and robust. The range of the analytical method is from 50% to 150% of its specification limit and it can be used for intended purpose. This method is suitable for routine analysis for determination of inherent impurities in Dapoxetine drug substances and Pharmaceutical dosage forms.

ACKNOWLEDGMENTS

This research article is made possible through the help and support from M/s Synergene Active Ingredients, Hyderabad & M/s spectrum labs, Hyderabad.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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