Research Article

DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR THE DETERMINATION OF DAPOXETINE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATION USING AN EXPERIMENTAL DESIGN

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ABSTRACT

A rapid and sensitive RP-HPLC method with UV detection (230 nm) for routine analysis of Dapoxetine HCl in a pharmaceutical formulation (Priligy®) was developed. Chromatography was performed with mobile phase containing a mixture of buffer [Tri-ethyl amine, pH-4.0 (adjusted with o-phosphoric acid)] and acetonitrile (60:40, v/v) with flow rate was 1.0ml min^{-1}. The procedure was validated for linearity (correlation coefficient=0.9998), accuracy, robustness and intermediated precision. Experimental design was used for validation of robustness and intermediate results in a decrease of the drug found concentration, while the percentage of organic modifier and pH have no important effect on the response. For intermediate precision measure the variables considered were: analyst, equipment and number of days. The R.S.D. value (0.5%, n=6) indicated a good precision of the analytical method. The proposed method was simple, highly sensitive, precise and accurate and retention time less than 5min indicating that the method is useful for routine quality control.

Keywords: Dapoxetine HCl; Validation; Buffer; Calibration.

INTRODUCTION

Dapoxetine HCl is designated chemically as (S)-N, N-dimethyl-3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine with an empirical formula of $C_{21}H_{23}NO$ (Figure 1) and a molecular weight of 305.413 g.[1]

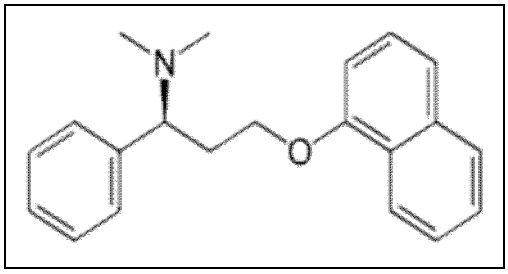


Figure 1: Structure of Dapoxetine

This drug is mainly useful in erectile dysfunction as selective serotonin reuptake inhibitor (SSRI). The drug's mechanism of action is thought to be related to inhibition of neuronal reuptake of serotonin and subsequent potentiation of serotonin activity and increase the ejaculation time.[1]

Literature review of Dapoxetine HCl reveals that a column-switching high-performance liquid chromatographic (HPLC) method is described for the determination of Dapoxetine and its mono- and di-desmethyl metabolites in human plasma[2]. This method is not so specific for estimation of Dapoxetine HCl in its solid dosage form. This paper reports a rapid and sensitive HPLC determination method with UV detection, useful for routine quality control of Dapoxetine HCl in pharmaceutical formulations. The method was validated by parameters such as linearity, accuracy, precision and robustness. Experimental design was used for validation to evaluate the robustness and intermediate precision.[3-5]

MATERIALS AND METHODS

Apparatus

The HPLC system (Shimadzu Corporation, Japan), model Shimadzu VP, consisted of a system controller (CLASS-VP), on-line degasser (LC 2010C, Shimadzu), low pressure gradient valve (LC 2010C, Shimadzu), solvent delivery module (LC 2010C, Shimadzu), auto injector (LC 2010C, Shimadzu), column oven (LC 2010C, Shimadzu), and CLASS-VP software version = SPI, binary pump, auto injector (SIL-10AD VP, Shimadzu), column oven (CTO-10AS VP, Shimadzu) and PDA detector (PDA-SPD-M10A VP, Shimadzu Diode Array Detector) and Chem station (software).

For RP-HPLC method, various columns are available but our main aim is to resolve both the drugs. So the C_{18} column was selected over the other columns. For Dapoxetine HCl Zorbax Eclipse C_{18} (150 x 4.6)mm, 5µ column was chosen to give good peak shape and high resolution as compare to other C_{18} like Kromasil and Inertsil columns. This column has embedded polar groups and which are more stable at lower pH and high Carbon loads, which provide high peak purity and more retention to polar drugs. (Figure 2-3)

Reagents

Dapoxetine HCl Active Pharmaceutical Ingredient (API) and Working Standard were supplied by Sun Pharmaceuticals Industries Limited, India.

- Tri Ethyl Amine**:** AR grade, Spectrochem Pvt. Ltd., India
- o-Phosphoric Acid **:** AR grade, Spectrochem Pvt. Ltd., India

- Acetonitrile**:** For HPLC, Spectrochem Pvt. Ltd.,India
- Milli-Q Water**:** In-House Production

Figure 2: Chromatogram of Standard Dapoxetine HCl in Buffer:ACN (60:40) using Inertsil C_{18} Column

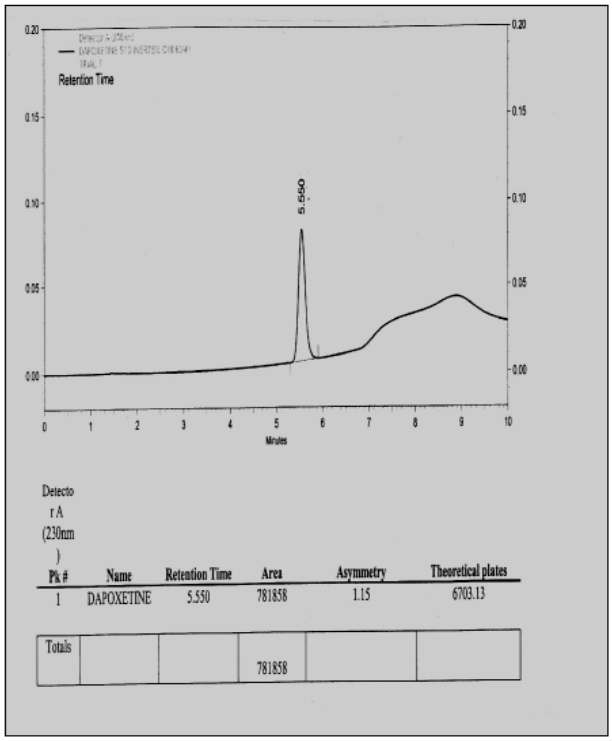
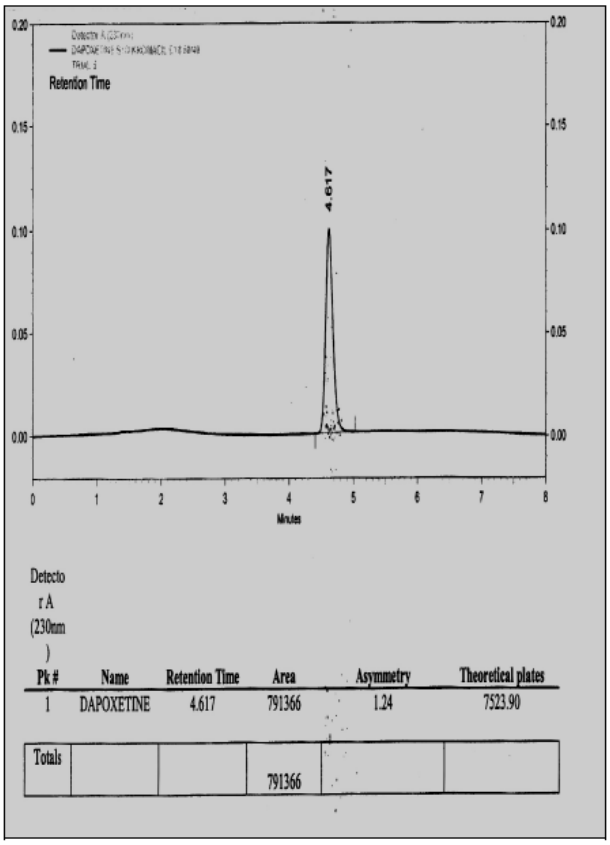


Figure 3: Chromatogram of Standard Dapoxetine HCl in Buffer:ACN (60:40) using KromacilC$_{18}$ Column



Preparation of the Solutions

Standard Stock Solution: Transfer 30mg Dapoxetine HCL in a 100ml volumetric flask. Add about 50ml of mobile phase and sonicate to dissolve. Now make volume up to mark with diluent. Dilute 5ml of this solution to 100ml with mobile phase and mix. Final standard concentration of Dapoxetine HCl is 15ppm.

Buffer Preparation: Dissolve 30ml of Tri Ethyl Amine in 3000ml of Milli-Q water and adjust pH of this buffer solution to 4.0 with o-H_3PO_4.

Mobile Phase Preparation: Mix the buffer solution and acetonitrile in proportion of 60:40 and sonicate it properly and filter the mobile phase through 0.45µ HVLP Milli-pore filter.

Sample Preparation: 10 intact tablets were weighed accurately to determine average weight of tablets. Then tablets were finely crushed and tablet powder equivalent to about 30mg of Dapoxetine HCl was transferred into 100ml volumetric flask. Then 50ml diluent was added to flask and sonicate for 30minute with intermittent shaking. Make the volume up to mark with mobile phase and mix. Solution was filtered through 0.45µ HVLP Milli-pore filter; collect the filtrate by discarding first few ml of the filtrate. Dilute 5ml of this solution to 100ml with mobile phase and mix to obtain final concentration of 15ppm of the drug.

Placebo Preparation: Weigh and transfer accurately about 96.88mg placebo in 3 different sets of 100ml volumetric flasks. Add 50ml of diluent and sonicate for 30minute with intermittent shaking, then cool to room temperature, make up the volume with diluent and mix. Filter the solution through 0.45µ HVLP Milli-pore filter. Then 5ml of the filtered solution is further diluted to 100ml with diluent.

Calibration Procedure

Calibration graph was found to be linear at range 0.45-22.5µg ml^{-1} six different concentrations of a drug in the range given above were prepared and 10µl of each solution injected in HPLC. The linearity was evaluated by linear regression method. Before injecting solutions, the column was equilibrated for at least 30min. with the mobile phase flowing through system.

Optimized Chromatographic Conditions

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study, standard solution of Dapoxetine HCl was scanned over the range of 200-400nm wavelengths. The drug showed maximum absorbance at three wavelengths of 210nm, 230nm and 292nm. More over API specification of Dapoxetine HCl provided 230nm as detection wavelength. Comparison of chromatograms of these three wavelengths: 210nm, 230nm and 292nm prove that the response peak is better in 230nm than 210nm and

292nm. So, 230nm wavelength was selected for estimation of Dapoxetine HCl in solid dosage form.[6,7]

The standard solution containing 15µg ml^{-1} of Dapoxetine HCl was chromatographed with mobile phase of different ratio of buffer and acetonitrile, 60:40 and 55:45, respectively. (Figure 4-5)

- Column: Zorbax Eclipse C_{18} (150 x 4.6)mm, 5µ
- Detector: 230nm
- Injection Volume: 10µl
- Flow Rate: 1.0ml min^{-1}
- Temperature: 30ºC
- Run Time: 10minute
- Mobile Phase: Buffer:Acetonitrile (60:40)
- Diluent: Mobile Phase

Figure 4: Chromatogram of Standard Dapoxetine HCl Buffer:ACN (55:45) using Zorbax Eclipse C_{18} (150 x 4.6)mm, 5µ Column.

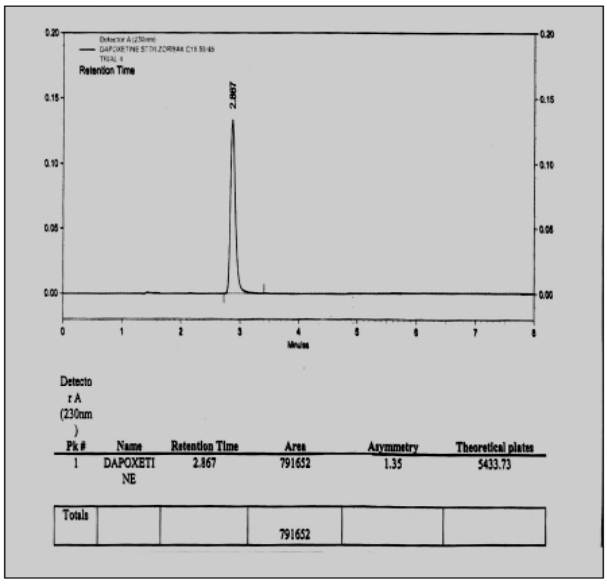
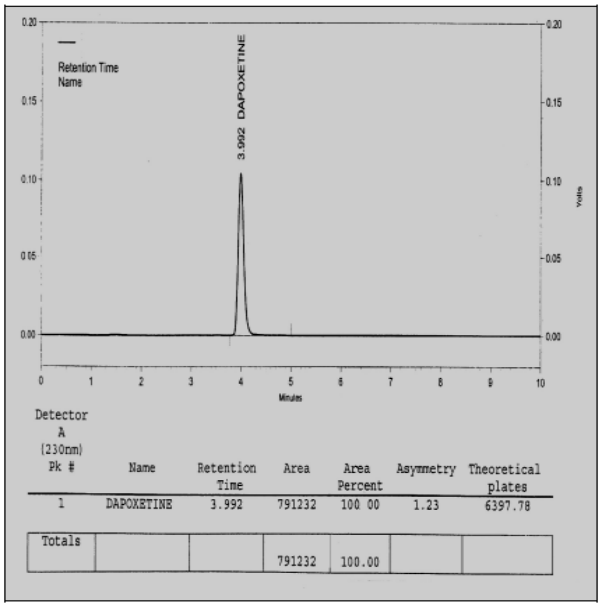


Figure 5: Chromatogram of Standard Dapoxetine in Buffer ACN (60:40) using Zorbax Eclipse C_{18} (150 x 4.6)mm, 5µ Column.



RESULTS AND DISCUSSION

System Suitability

Table 1: System Suitability and System Precision

Parameters (n=5)	Dapoxetine HCl
Retention Time (min)	4.18 ± 0.010
Theoretical Plates	7227
Asymmetry	1.17
Capacity Factor	41.00
%RSD	0.1

Stability of the Solution

Table 2: Results of Standard Solution Stability

Time (Hour)	Area	% Difference
	Dapoxetine HCl	Dapoxetine HCl
0 (Initial)	778506	---
4	774732	0.7
8	784949	0.8
12	786559	1.0
16	786738	1.1
20	787161	1.1
24	787737	1.2
Mean %RSD		**0.9833**
± SD		**0.1771**

Table 3: Results of Sample Solution Stability

Time (Hour)	Area	%Difference
	Dapoxetine HCl	Dapoxetine HCl
0 (Initial)	781989	---
4	778744	-0.4
8	784271	0.3
12	786418	0.6
16	787534	0.7
20	786106	0.5
24	787029	0.6
Mean %RSD		**0.3833**
± SD		**0.3715**

Linearity

Table 4: Linearity Data of Dapoxetine HCl

Linearity Range	Stock Solution to be Taken (ml)	Final Volume (ml) with diluent	Final Concentration of Dapoxetine HCl (µg ml^{-1})	Area
20%	1.0	100	3.0	155234
50%	2.5	100	7.5	383265
80%	4.0	100	12.0	603815
100%	5.0	100	15.0	747897
120%	6.0	100	18.0	895685
150%	7.5	100	22.5	1142989

Table 5: Results of Linearity, LOD and LOQ Study

Parameters	Dapoxetine HCl
Linearity Range	0.45-22.5µg ml^{-1}
Linearity Equation	y = 50381.01 x + 1388.65
Correlation Coefficient	0.9998
L.O.D.	0.15µg/ml
L.O.Q.	0.45µg/ml

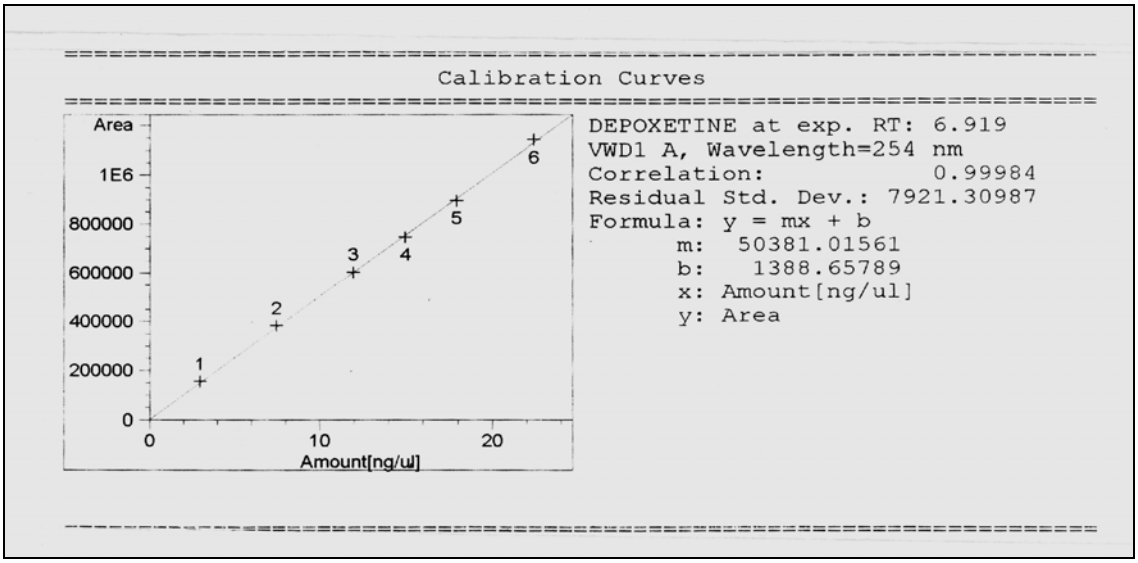


Figure 6: Linearity Calibration Curve of Dapoxetine HCl

Accuracy and Repeatability

Demonstrate the accuracy of the test method by preparing recovery samples at the level of 50%, 100%, and 150% of target concentration. Prepare the recovery samples in triplicate in each level.

Table 6: Preparation of Recovery Sample

Recovery Level	Wt of Placebo to be taken (mg)	Wt of API to be Spiked (mg)	Mobile Phase to be Added (ml)	Sonication Time (min)	Make up with mobile phase (ml)	Final concentration of Dapoxetine HCl (µg/ml) Recovery Level
50%	111.88	15	50	30	100	40
100%	96.88	30	50	30	100	80
150%	81.88	45	50	30	100	120

Dilute 5ml of this solution to 100ml with mobile phase and mix.

Table 7: Results of Accuracy Data of Dapoxetine HCl

For Dapoxetine HCl					
Level	**Amount of Drug Added (mg)**	**Amount of Drug Recovered (mg)**	**Recovery (%)**	**Mean ± SD (%)**	**% RSD**
50 %	14.91	14.90	99.90	99.93 ± 0.124	**0.2**
	14.97	14.97	100.1		
	15.01	14.98	99.8		
100 %	29.92	30.01	100.3	100.03 ± 0.44	**0.6**
	29.82	29.64	99.4		
	29.87	30.00	100.4		
150 %	44.74	44.49	99.4	99.13 ± 0.20	**0.3**
	44.93	44.44	98.9		
	44.73	44.32	99.10		

Robustness

Table 8: Results of Robustness Study

Parameter	**% RSD (n=5)**		
	Normal Condition	**Changed Condition**	
Temperature	**Normal**	**(-5°C)**	**(+5°C)**
Dapoxetine HCl	0.1	0.0	0.4
pH	**Normal**	**(-0.2 unit)**	**(+0.2 unit)**
Dapoxetine HCl	0.1	0.3	0.1
Flow Rate	**Normal**	**(-10%)**	**(+10%)**
Dapoxetine HCl	0.1	0.1	0.2
Mobile Phase Ratio	**Normal**	**(-2%)**	**(+2%)**
Dapoxetine HCl	0.1	0.1	0.0

Intermediate Precision

Table 9: Method Precision Data of Dapoxetine HCl

Set No.	**% Assay**	**% Assay Mean ± SD**	**% RSD**
	Dapoxetine HCl	**Dapoxetine HCl**	**Dapoxetine HCl**
1.	98.8	99.56 ± 0.44	0.5
2.	100.1		
3.	99.9		
4.	99.3		
5.	99.4		
6.	99.9		

Table 10: Intermediate Precision Data of Dapoxetine HCl

Set No.	**% Assay**	**% Assay Mean ± SD**	**% RSD**
	Dapoxetine HCl	**Dapoxetine HCl**	**Dapoxetine HCl**
1	99.2	99.56 ± 0.58	0.6
2	100		
3	99.8		
4	100		
5	100.1		
6	98.7		

Table 11: Comparison of Results of Method Precision and Intermediate Precision

Analyst	**Analyst-1**	**Analyst-2**
Analysis Date	**26/10/10**	**08/11/10**
System	**HPLC-1**	**HPLC-2**
	Dapoxetine HCl	
	Repeatability	**Intermediate Precision**
% Assay Mean ± SD	99.56 ± 0.44	99.56 ± 0.58
% RSD	**0.5**	**0.6**
% Difference of two Means	0.00	

Table 12: Method Validation Parameters and Their Acceptance Criteria

Validation Parameters	**Acceptance Criteria**
Accuracy/Recovery	Recovery 98-102% (individual)
Precision	RSD < 2%
Repeatability	RSD < 2%
Intermediate Precision	RSD < 2%
Specificity/ Selectivity	No interference, the peak purity index > 0.999
Linearity	Correlation coefficient r^2 > 0.999 or 0.995
Solution Stability	> 12hour
Lower Detection Limit	S/N > 2 or 3
Lower Quantitation Limit	S/N > 10

CONCLUSION

Table 13: Summary of Validation Parameters of Dapoxetine HCl by RP-HPLC

Parameter	**Acceptance Criteria**	**Dapoxetine HCl**
Linearity Range Correlation Coefficient	Correlation coefficient r^2 > 0.999 or 0.995	0.45-22.5µg ml^{-1} r^2= 0.9998
L.O.D.	S/N > 2 or 3	0.15µg ml^{-1}
L.O.Q.	S/N > 10	0.45µg ml^{-1}
Precision	RSD < 2%	%RSD= 0.5
Intermediate Precision	RSD < 2%	%RSD= 0.6
Accuracy	Recovery 98-102% (individual)	% recovery= 99.1-100.1
Specificity	1) No interference from blank, placebo and other degradation products with the main peak. 2) The peak purity index > 0.999	No interference. **Peak purity** 1)Test Sample = 0.9992 2)Spiked Sample = 0.9998 3)Standard Solution = 0.9989
Solution Stability	> 12 hour	Stable up to 24 hour Mean %RSD= 0.98
Robustness	RSD NMT 2% in modified condition	Complies

From the validation results, we can conclude that developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, and robust and hence it can be used for the routine analysis of Dapoxetine HCl in quality control department.

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