Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF DAPOXETINE HYDROCHLORIDE IN TABLET FORMS

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ABSTRACT

A rapid, sensitive, efficient, and reproducible method for the determination of Dapoxetine Hydrochloride has been developed using reverse phase high performance liquid chromatographic method. This method involves separation of Dapoxetine Hydrochloride on reversed phase Hypersil BDS silica, C18, 250×4.6 mm, 5μ column. The elution was done using a mobile phase consisting of Triethylamine buffer and Acetonitrile (pH adjusted 6.0 with Orthophosphoric acid) in the ratio of 20:80 on HPLC Shimadzu Separation Module LC-10AT VP Prominence Liquid Chromatography. An external standard calibration method was employed for quantification. A survey of literature revealed spectrophotometric, capillary electrophoresis and a few chromatographic methods for the determination of Dapoxetine Hydrochloride in bulk drug. Compared to the already reported RP HPLC methods, the current method is rapid, simple and economical for the determination of Purity, accuracy, linearity and Assay of Dapoxetine Hydrochloride in bulk drug and in pharmaceutical dosage forms.

Keywords: RP HPLC, Dapoxetine Hydrochloride, Triethylamine buffer, Analytical validation.
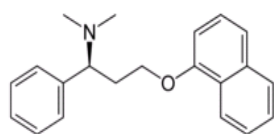
INTRODUCTION

Figure 1: Structure of Dapoxetine

Dapoxetine (S)-N,N-dimethyl-3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine is white powder. Used for the treatment of erectile dysfunction and premature ejaculation in men. Its mechanism of action is to increase the level of serotonin in the central nervous system. It is thought to be related to inhibition of neuronal reuptake of serotonin and subsequent potentiating of serotonin activity. Dapoxetine is rapidly absorbed with maximum plasma concentrations (C_{max}) occurring approximately 1-2 hrs after tablet intake. The absolute bioavailability is 42% (range 15-76%). More than 99% of dapoxetine is bound to human serum proteins. The active metabolite desmethyldapoxetine is 98.5% protein bound with a mean steady state volume of distribution of 162 L. With a short time to maximum serum concentration (about 1 h) and rapid elimination (initial half-life of 1-2 h). By 24 h, plasma concentrations are less than 5% of peak values. Purity determination and assay of Dapoxetine was achieved by HPLC using reverse phase. The presence of impurities, even in small amounts, may affect the efficacy and safety of pharmaceuticals. Methods for detecting and controlling impurities are subject to continuous review and improvement. Characterization of impurities is a crucial aspect of drug development and approval, and is central to quality control.

MATERIALS AND METHODS

Analytical method validation[6]

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures is included in table 1. Other analytical procedures may be considered in future additions to this document.

Analytical Procedure

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

This definition has the following implications:

Identification: To ensure the identity of an analyte.

Purity Tests: To ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Assay (content or potency): To provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Intermediate precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

Detection limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Table 1: Analytical validation parameters

Type of analytical procedures → Characteristics ↓	IDENTIFICATION	TESTING FOR IMPURITIES Quantitation limit		ASSAY - dissolution (measurement only) - content/potency
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Intermediate Precision	-	+	-	+
Specificity	+	+	+	+
Detection Limit	-	-	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristic is not normally evaluated

+ signifies that this characteristic is normally evaluated

Validation of analytical procedures: methodology

This document is complementary to the parent document which presents a discussion of the characteristics that should be considered during the validation of analytical procedures. Its purpose is to provide some guidance and recommendations on how to consider the various validation characteristics for each analytical procedure. In some cases (for example, demonstration of specificity), the overall capabilities of a number of analytical procedures in combination may be investigated in order to ensure the quality of the drug substance or drug product. In addition, the document provides an indication of the data which should be presented in a registration application.

All relevant data collected during validation and formulae used for calculating validation characteristics should be submitted and discussed as appropriate.

Experimental conditions

Experimental conditions employed were tabulated in table 2.

Table 2: Experimental conditions

Equipment	Dapoxetine
Instrument	HPLC Shimadzu Separation Module LC-10AT VP Prominence Liquid Chromatograph
Detector	UV Visible Detector (SPD 20A Prominence)
Chromatographic data software	LC Solutions
Column	Hypersil BDS silica, C_{18} 250× 4.6 mm. 5μ. column.
P^H meter	Control Dynamics pH meter (Metler Toledo)
Pump	Vacuum filter pump,(NUPORE filtrartion systems, 0.45 μ, 47mm),
Weighing balance	Single pan balance (Metler Toledo)
Sonicator	Ultra sonicator

Table 3: Optimized method

CHROMATOGRAPHIC CONDITIONS		
flow rate	0.5 ml /min	1.0ml/min
Column	RP-C_{18}, 100×4.6mm, 2.7μ	Hypersil BDS silica, C_{18} 250× 4.6mm., 5μ column
Detector wavelength	225 nm	215nm
Column temperature	30°C	25°C
Wave length	225nm	215nm
Injection volume	10μl	10μl
Run time	10 min	12 min
Mobile phase	Water: Acetonitrile (50:50)	Triethylamine buffer P^H6: Acetonitrile (20:80 % v/v)

Analytical method development

Methods are developed for new products when no official methods are available. Alternative methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace most of the drugs in multi component dosage forms can be analyzed by HPLC method.

Instrumental setup and initial studies

The required instrumentation is setup. Installation, operational and performance qualification of instrumentation using laboratory standard operating procedures (SOP's) are verified.

Always new consumables (e.g. solvents, filters and gases) are used, for example, method development is never started, on a HPLC column that has been used earlier.

The analyte standard in a suitable injection / introduction solution and in known concentrations and solvents are prepared. It is important to start with an authentic, known standard rather than with a complex sample matrix. If the sample is extremely close to the standard (e.g., bulk drug), then it is possible to start work with the actual sample.

Optimization

During optimization one parameter is changed at a time and set of conditions are isolated, rather than using a trial and error approach. Work has been done from an organized methodical plan and every step is documented. Final optimized method was tabulated in table 3.

Preparation of solutions

Preparation of Mobile phase: The mobile phase was prepared by mixing Triethylamine buffer and Acetonitrile (pH adjusted 6.0 with Orthophosphoric acid) in the ratio of 20:80. The mobile phase is then sonicated using Ultra-Sonicator to remove the impurities and dissolved gases, as they may lead to unwanted peaks in the chromatogram.

Preparation of Triethylamine buffer: Dissolve 2.0 ml of triethylamine in 1000ml water to produce 0.2% buffer. P^H is adjusted to 6.0 with 10% v/v Orthophosphoric acid.

Preparation of standard stock solution: Weigh and transfer accurately 54 mg of Dapoxetine HCl working standard into a 100 ml volumetric flask, add about 70 ml of diluent, sonicate to dissolve. Dilute to volume with diluent and mix. Transfer 5ml of above solution into 50 ml volumetric flask, dilute to volume with diluent and mix.

Preparation of sample solution: Transfer 5 tablets into a 250 ml volumetric flask add about 140 ml of diluents and sonicate for 15min with intermittent shaking dilute to volume with diluents and mix. Filter the solution thorough 0.45 μ nylon filter. Transfer 2ml of the above solution into a 50 ml volumetric flask, dilute to volume with diluent and mix. Filter the solution through 0.45 μ nylon membrane filter.

Procedure: Separately inject 10 µl of standard and sample solutions into the chromatographic system. Record the chromatograms and measure the peak responses.

Method validation

Linearity

The linearity of the method was demonstrated over the concentration range of 27-81 µg / ml. Aliquots of 27, 40.5, 54, 67.5, and 81 µg / ml were prepared from stock solution and labeled as solution 1, 2, 3, 4 and 5 respectively. The solutions were injected in to HPLC system as per test procedure. A calibration curve was plotted for concentration v/s peak area (Figure 7, 8, Table 6). Acceptance criteria is Correlation Coefficient (r^2) should be not less than 0.9990.

Assay

Assay of marketed formulation was carried by injecting sample corresponding to equivalent weight into HPLC system. And percent purity was found out by following formulae. Recovery studies were carried out. (Figure 12, Table 9)

Calculate the percentage purity of Dapoxetine HCl present in tablet using the formula:

$$\text{Percentage purity} = \frac{Spl_{area} \times Std_{dil} \times Avg\,wt \times P}{Std_{area} \times Spl_{dil} \times L.C \times 100} \times 100$$

Where,

P = (%) potency of Dapoxetine HCl working standard use

L.C = Label claim

Avg wt = Average weight of tablets

Accuracy

The accuracy of the method was evaluated by determination of recovery of Dapoxetine HCl at three levels of concentrations. The sample solutions were spiked with Dapoxetine HCl standard solutions corresponding to 50, 100, and 150% of nominal analytical concentrations. (27μg/ml, 54μg/ml and 81μg/ml). The results showed good recovery within limits (98% – 102%). (Figure 2-4, Table 4). Acceptance criteria is the mean % recovery of the Dapoxetine HCl should be not less than 97.0% and not more than 103.0%.

Precision (Figure 5, 6, Table 5)

Method precision: Five samples of 54.85 μg/ml solutions were prepared and injected into the HPLC system as per test procedure. Acceptance criteria is the % of Relative standard deviation should not be more than 2.0 %.

System precision: Five samples of 54.85 μg/ml solutions were prepared and injected into the HPLC system as per test procedure. Acceptance criteria is the % of relative standard deviation should not be more than 2.0 %.

Intermediate Precision: Intermediate precision of test method is demonstrated by 5 injections of the same batch (same conc) of samples. Acceptances criteria is %RSD of 6 replicate injections should be not more than 2.0%

System Suitability

A Standard solution of Dapoxetine HCl working standard was prepared as per procedure and was injected six times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections. (Figure 9, Table 7) Acceptance criteria is the % RSD for the retention times of principal peak from 6 replicate injections of each Standard solution should be not more than 2.0 %

The number of theoretical plates (N) for the Dapoxetine HCl peaks should be not less than 2000.

The Tailing factor (T) for the Dapoxetine HCl peaks should be not more than 2.0.

From the system suitability studies it was observed that all the parameters were within limit. Hence it was concluded that the Instrument, Reagents and Column were suitable to perform the assay.

Specificity

Dapoxetine HCl Identification: Solutions of Standard and Sample were prepared as per test procedure and injected into the HPLC system. Acceptance criteria is Chromatogram of standard and sample should be identical with near Retention time.

Blank interference: A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure. Acceptance criteria Chromatogram of blank should not show any peak at the retention time of analyte peak. There is no interference due to blank at the retention time of analyte.

Robustness

The Robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate and mobile phase composition which may differ but the responses were still within the specified limits of the assay. (Figure 10,11, Table 8)

Effect of variation of mobile phase composition

A study was conducted to determine the effect of variation in mobile phase ratio by Changing the ratio of mobile phase i.e. Acetonitrile: Buffer (80:20) by ±2 %. Standard solution was prepared and injected into the HPLC system. The tailing Factor of Dapoxetine HCl standard should not be more than 2.0 for Variation in composition of mobile phase. The % RSD of Dapoxetine HCl standard should be not more than 2.0 for Variation in composition of mobile phase.

Effect of variation in Buffer (P^H)

A study was conducted to determine the effect of variation in Buffer P^H. Standard solution was prepared and injected into the HPLC system by maintaining PH 5.8 and 6.2. The effect of variation in Buffer PH was evaluated. The tailing factor of standard should be not more than 2.0 for Variation in Buffer $P^{H.}$ And the % RSD of Dapoxetine HCl standard should be not more than 2.0 % for variation in Buffer P^H.

Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution was prepared and injected into the HPLC system by keeping flow rates 0.9 ml / min and 1.1 ml / min. The effect of variation of flow rate was evaluated. The tailing factor of standard should be not more than 2.0 for Variation in flow. And the % RSD of Dapoxetine HCl standard should be not more than 2.0 % for variation in flow.

Effect of variation in temperature

A study was conducted to determine the effect of variation in temperature. Standard solution was prepared and injected into the HPLC system by keeping temperature 30°C. The flow rate was evaluated. The tailing factor of standard should be not more than 2.0 for

Int. J. Pharm. Sci. Rev. Res., 14(1), 2012; n° 08, 31-37

ISSN 0976 – 044X

Variation in temperature. And the % RSD of Dapoxetine HCl standard should be not more than 2.0 % for variation in temperature.

Limit of detection (LOD)

The parameter LOD was determined on the basis of response and slope of the regression equation.

Limit of quantification (LOQ)

The parameter LOQ was determined on the basis of response and slope of the regression equation.

RESULTS AND DISCUSSION

Observed results were presented in figure 2 to figure 12 and in table 4 to table 9.
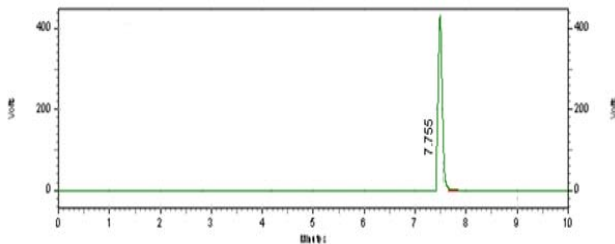
Figure 2: Chromatogram of Accuracy 50%
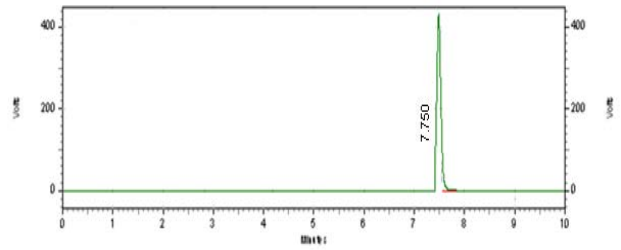
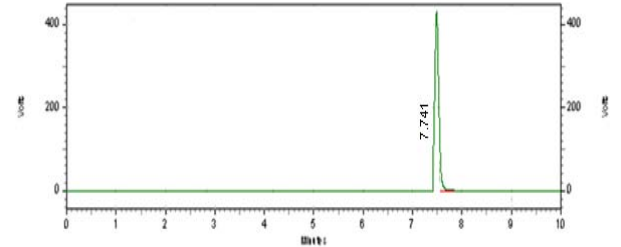

Figure 3: Chromatogram of Accuracy 100%

Figure 4: Chromatogram of Accuracy 150%

Table 4: Accuracy table

Sample ID	Concentration	Percentage Recovery	Mean % recovery	Standard deviation	Relative standard deviation
1	50%	98.9			
2	50%	98.7	98.8	0.1	0.10121
3	50%	98.8			
4	100%	98.7			
5	100%	99.4	99.0	0.3656	0.3642
6	100%	98.9			
7	150%	99.2			
8	150%	99.1	99.2	0.15275	0.15393
9	150%	99.4			

The mean recovery for Dapoxetine was found to be 99.0%. The acceptance limits should be between 98.0-102.0%
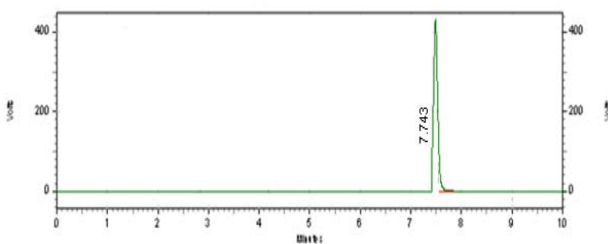

Figure 5: Chromatogram of Method precision System precision

Figure 6: Chromatogram of system precision

Table 5: Method precision

Parameters	Method precision	System precision
Avg. area	3233238	3336726
SD	0.45	6084.28
% RSD	0.46	0.18

The % RSD values for 5 injection samples of Dapoxetine was found to be 0.46 which is well within the acceptance criteria limit of less than 2%
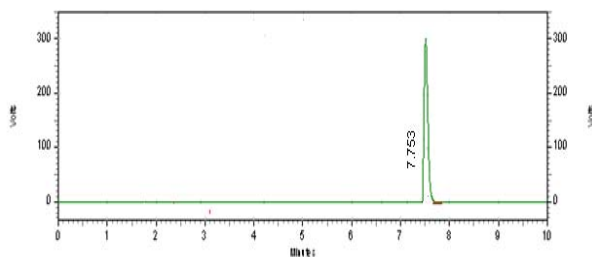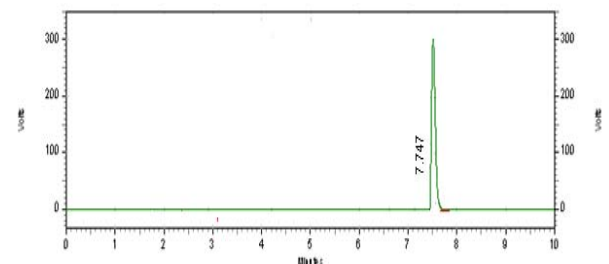

Figure 7: Chromatogram of Linearity

Int. J. Pharm. Sci. Rev. Res., 14(1), 2012; n° 08, 31-37

ISSN 0976 – 044X
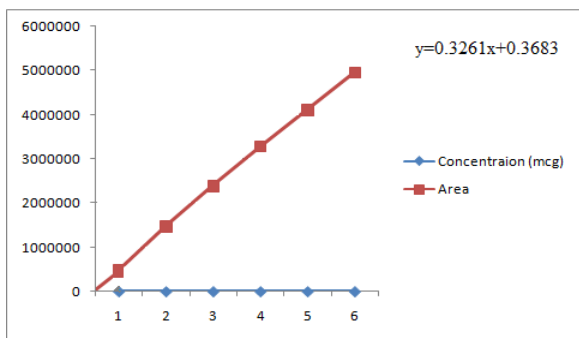
Figure 8: Linearity plot for Dapoxetine

Table 6: Linearity table

Concentration (mcg/ml)	Area
27	1678273
40.5	2491686
54	3285198
67.5	4112682
81	4951514
Correlation coefficient (r^2)	0.99995

The correlation coefficient (r^2) should be not less than 0.999.
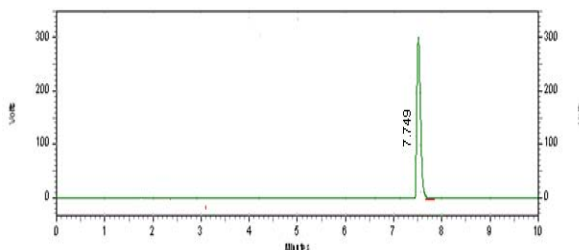
Figure 9: Chromatogram of system suitability

Table 7: System suitability

Parameters	Dapoxetine
Tailing factor (T)	1.51
Theoretical plates (N)	5996
Retention time (R_t)	7.749
% RSD	0.26

The tailing factor was found to be 1.5 which is found to be in the acceptance criteria of not more than 2. The numbers of theoretical plates were found to be 5996 which was found to be acceptance criteria of not less than 2000. The % RSD value was found to be 0.26 which is found to be in acceptance criteria limit of less than 2%
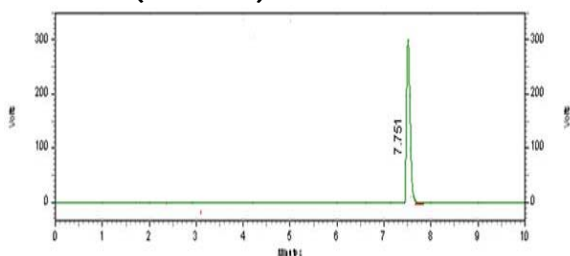
Robustness (More flow)

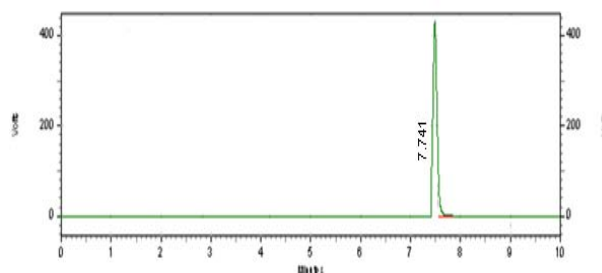**Figure 10:** Chromatogram of robustness (more flow)
Less flow

Figure 11: Chromatogram of robustness (less flow)

Table 8: Method robustness

Proposed variations	Test Parameter	Tailing factor	Theoretical plates
Variation in Mobile phase	+2 %	1.53	6111
	-2 %	1.68	5286
Variation in P^H	+ 0.2	1.55	5931
	- 0. 2	1.56	5656
Variation in Flow Rate	+ 0.2	1.34	2766
	- 0.2	1.27	2717

Assay of Dapoxetine
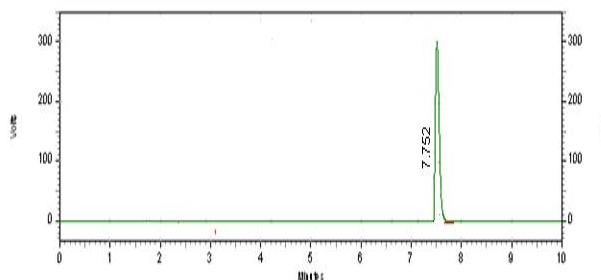
Figure 12: Chromatogram of Dapoxetine sample (assay**)**

Table 9: Assay parameters

Peak Name	Retention time	Area
Sample	7.752	3219470
Standard	7.743	3223092

$$\% \text{ Assay} = \frac{Spl_{area} \times Std_{dil} \times Avg\ wt \times P}{Std_{area} \times Spl_{dil} \times L.C \times 100} \times 100 = \mathbf{98.3\%}$$

Percentage Label Claim = 98.3 %

CONCLUSION

Dapoxetine HCl is a short-acting selective serotonin reuptake inhibitor developed for the on-demand treatment of premature ejaculation. The proposed analytical method is simple, economical, rapid, sensitive, reproducible and accurate for the estimation of Dapoxetine HCl. A newer RP-HPLC method was developed for formulations. The proposed method gives reliable assay results with short analysis time using mobile phase of Acetonitrile: Triethylamine (P^H 6) (80:20 % v/v)

The system suitability parameter also reveals that the values within the specified limit for the proposed method. Theoretical plate for Dapoxetine HCl was found to be not

less than 3000. The precision of the System and Method were checked and found to be within limits. This indicates that the method is precise. From the linearity studies, the specified range for Dapoxetine HCl was found to be within limits. It was evaluated by the visual inspection of the plot of Peak area vs. Concentration.

Thus the above studies and findings will enable the quantification of the drug for future investigation in the field of analytical chemistry.

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