Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(7):1613-1618

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Development and validation of a HPLC analytical assay method for dapoxetine tablets: A medicine for premature ejaculation

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ABSTRACT

A simple, specific, accurate, and precise reverse phase High Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for the estimation of Dapoxetine HCl in bulk and pharmaceutical dosage forms. A Symmetry C18, 3.5 µm column having 250 mm x 4.6 mm in isocratic mode, with mobile phase containing HPLC grade Acetonitrile, Ammonium format (60 : 40 v/v, pH 3.5) was used. The flow rate was 1 ml/min and effluent was monitored at 292 nm. Chromatogram showed a main peak of Dapoxetine HCl at retention time of 5.020 ± 0.0078 min. The Correlation coefficient was 0.999. The method was validated for linearity, sensitivity, precision, accuracy, robustness. The limit of detection and limit of quantitation for estimation of Dapoxetine HCl was found to be 0.142 and 0.471, respectively. Recovery of Dapoxetine was found to be in the range of 98.93-99.91 %. Proposed method was successfully applied for the quantitative determination of Dapoxetine HCl in bulk and pharmaceutical dosage forms. RP-HPLC method for Dapoxetine was Developed and validated according to ICH guidelines.

Key words: Dapoxetine HCl, Premature Ejaculation, HPLC etc.

INTRODUCTION

Recently it has been suggested that Premature Ejaculation (PE) might be associated with the perturbation in seretonergic 5- hydroxytryptamine neurotransmission. It has been proposed that PE may be caused by decreased central serotonergic signaling, hyposensitivity of 5-HT_2C receptor, or hypersensitivity of the 5-HT_{1A} receptor, all of which have been shown to decrease ejaculatory latency time in men. PE is common problem, which may be associated with considerably anxiety frustration and negative impact on affected men and their sexual partners.

Dapoxetine HCl an oral serotonin transporter inhibitor, was effective for the treatment of men with premature ejaculation. Dapoxetine HCl is designated chemically as (S)-N, N dimethyl-3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine, is mainly used as erectile dysfunction as selective short acting potent serotonin reuptake inhibitor (SSRI). Dapoxetine HCl mechanism of action is thought to be inhibition of neuronal reuptake of serotonin and subsequent potentiation of serotonin activity and increase the ejaculation time. Literature survey reveals less information about the determination of dapoxetine HCl by the HPLC method. The developed method was validated as per ICH guidelines Q2 R1. [1, 2, 3]

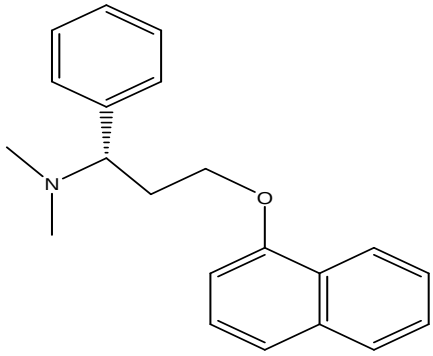

Figure 1. Dapoxetine HCl

EXPERIMENTAL SECTION

Apparatus

A UV- Vis Spectrophotometer (Jasco/ V-630), was used with quartz cells of 10mm path length; HPLC (Jasco 2000 series); Column Symmetry C18, 4.6×250mm, 5µm particle size (Waters); analytical balance (Contech, CB-50 series); ultrasonicator cleaning bath (spectra lab / UCB-400); filter papers 0.45µm.

Materials

Dapoxetine HCl reference standard from and tablet was procured from the market; ammonium formate of laboratory grade (Research-Lab Fine Chem Industries, Mumbai); Acetonitrile of HPLC grade (Merck specialist Pvt. Ltd, Mumbai).

Stock solution of dapoxetine 1000µg/ml was prepared by using 10mg of dapoxetine HCl in 10ml of methanol.

Chromatographic system

Analysis was conducted on Symmetry C18 4.6×250mm, 5µm particle size column at 292 nm. The samples were introduced through a Rheodyne injector valve with 20-µl sample loop. The mobile phase consisted of acetonitrile and ammonium formate (60:40, v/v), filtered through a filter, degassed in ultrasonic bath, and pumped at a flow rate of 1.0 ml/min.

Analytical method development

Different mobile phases were investigated to develop the suitable HPLC method for the analysis of dapoxetine HCl in formulations. For the selection of media the criteria employed was sensitivity of the method, ease of sample preparation, miscibility of the drug, cost of solvents and applicability of method to various purposes. Retention time and peak area of dapoxetine HCl in the selected medium at respective wavelengths were determined and compared with the reference standards and formulation also.

RESULTS AND DISCUSSION

Preparation of calibration curve

Stock solution was prepared using 10mg of dapoxetine HCl in 10ml of methanol and further dilutions were done with mobile phase. Solutions were sonicated for 5 min in ultrasonic clean bath and manually 20µl was injected through the Rheodyne injector. Five concentrations were taken for the calibration curve at 292nm. Results were shown in figure 2. [1, 4, 5]

Specificity and selectivity

6 tablets of dapoxetine HCl formulation were triturated in mortar pestle and 10mg equivalent weight of dapoxetine HCl was transferred in 10 ml volumetric flask and volume was adjusted with methanol. Peak area and retention time of formulation was compared with the API of the drug.

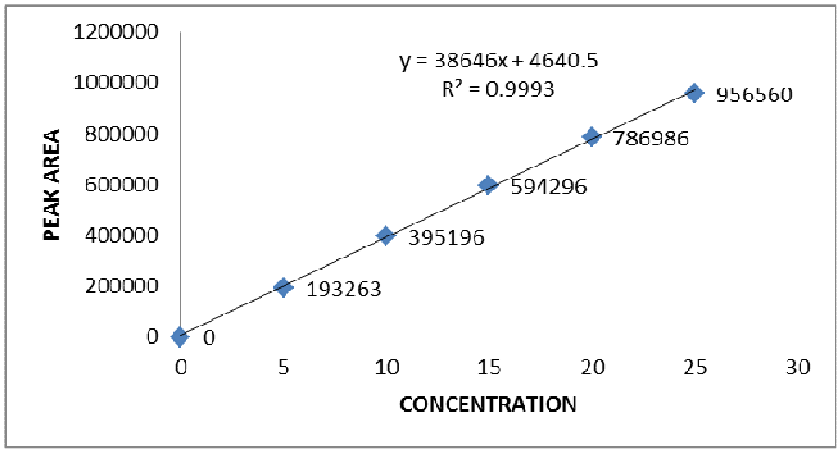

Figure No.2: Linearity graph for dapoxetine HCl

Analytical Validation

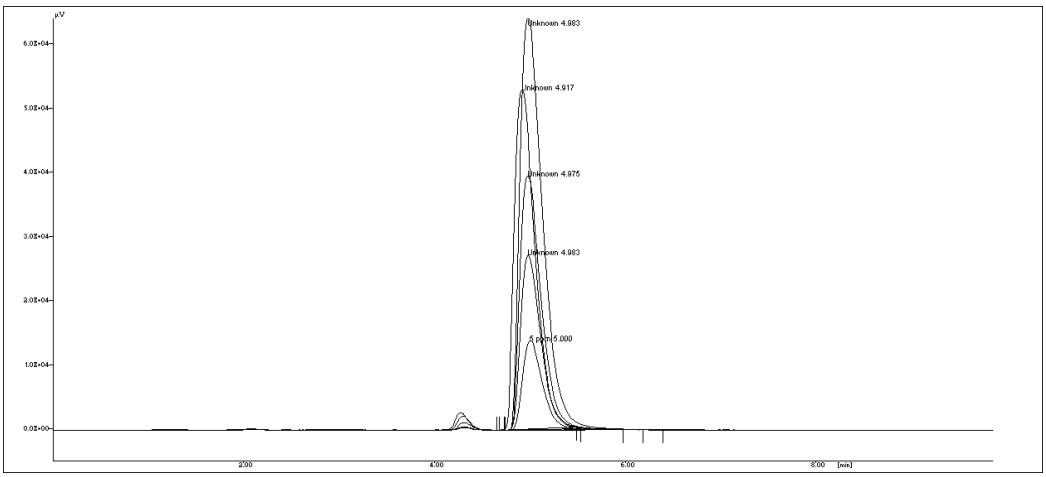

Figure No. 3: Overly spectra for dapoxetine HCl

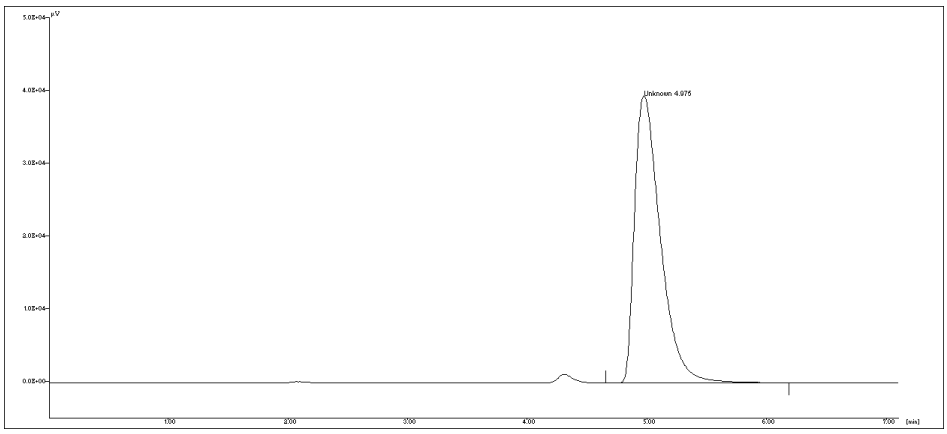

Fig No. 3: Typical chromatogram of dapoxetine HCl (15µg)

Sensitivity

The sensitivity of measurement of dapoxetine HCl by use of the proposed method was estimated in terms of the limit of detection (LOD) and the limit of quantification (LOQ). The LOD and LOQ were calculated by the use of signal to noise ratio. In order to estimate the LOD and LOQ values, the blank sample was injected six times and the peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level, while ten times the noise value gave the LOQ. LOD and LOQ were found to be 0.142 and 0.471, respectively.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between the series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Method of precision was accessed on two levels, intra and intermediate precision. Solutions of known concentration were prepared in replicates and were injected to get the peak area. Results obtained are shown in table 1 and 2.

Intraday precision

Table No.1: System precisions results (Intraday precision) for dapoxetine HCl

Concentration µg/ml	Peak area	SD	%RSD
12	315016	3275	0.69
17	671513	2396	0.35
27	1042231	11402	1.08

Interday precision

Table No. 2: System precision results (Interday precision) result for dapoxetine HCl

Concentration µg/ml	Peak area	SD	%RSD
12	477228.2	4501.233	0.93
17	685053	7213.66	1.04
27	1052097.9	12644.25	1.19

Repeatability

Demonstration of precision was done under two categories. The injection repeatability (System Precision) was assessed by using six injections of the standard solution of Dapoxetine and the % RSD of the replicate injections was calculated. [7, 8, 9]

Table No. 3: Repeatability results for dapoxetine

Sr. No.	Concentration (µg/ml)	Peak Area
1	7	271874
2	7	276145
3	7	278414
4	7	278452
5	7	277665
6	7	276145
Mean		276449.2
Std. Dev		2468.49
%RSD		0.8929

Accuracy

Accuracy of 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. Accuracy should be established across the specified range of 98-102 %. For accuracy three concentrations of five replicates were prepared and injected to get the peak area. [7, 8, 10, 12]

Table No. 4: Accuracy results for dapoxetine H C l

Concentration µg/ml	Peak area	Concentration Found	Accuracy
7	276510	7.03	100.49
17	671362	17.25	101.48
22	854793	21.99	99.99

Robustness

Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of mobile phase composition (60:40 v/v), flow rate by 1 ml/min (0.9 and 1.1 ml/min), Variation of wavelength by 292 nm (290nm and 294), Variations in pH by 3.5 (3.3 and 3.7) had no significant effect on the retention time and chromatographic response of 15 µg/ml solution, indicating that the method was robust, The result was shown in table. no: 5, 6, 7 and 8. [7, 8, 12, 13]

Mobile Phase

Table No. 5: Results of robustness (mobile phase) for dapoxetine HCl

Buffer	Acetonitrile	Peak area	Retention time
40	60	594296	5.008
38	62	599351	4.658
42	58	598926	5.142

Wavelength

Table No. 6: Result for robustness (wavelength) for dapoxetine HCl

Wavelength	Peak area	Retention time
290	594965	4.89
292	594296	5.00
294	587166	4.94

Flow rate

Table No. 7: Result for robustness (flow rate) for dapoxetine HCl

Flow rate	Peak area	Retention time
0.9 ml/min	65444.2	5.45
1 ml/min	594296	5.00
1.2 ml/min	527334	4.21

pH of mobile phase

Table No. 8: Result for robustness (pH of mobile phase) for dapoxetine HCl

pH	Peak area	Retention time
3.3	592143	4.99
3.5	594296	5.00
3.7	600917	5.01

Recovery

The recovery of the method was determined by use of standard additions at three different levels, i.e. multiple-level recovery studies. Preanalysed samples of Dapoxetine H C l were spiked with extra 80, 100 and 120 % of the standard dapoxetine HCl the mixtures were reanalyzed by the proposed method and the % recovery was determined. Values were found to be within the limits and are presented in table. no: 9. [7, 8, 14]

Table No. 9: Recovery result of dapoxetine HCl by RP-HPLC

Amount of sample (µg/ml)	Amount of drug added (µg/ml)	Amount Recovered (µg/ml)	% Recovery ± SD
15	12	11.96	98.88 ± 0.31
15	15	14.88	99.01 ± 0.58
15	18	17.72	98.46 ± 0.41

Specificity

Chromatogram of Dapoxetine HCl showed peak at a retention time of 5.0min. The mobile phase designed for the method resolved the drug very efficiently; The Retention time of Dapoxetine HCl was 5.0 ± 0.0078min. The wavelength 292 nm was selected for detection because; it resulted in better detection sensitivity for the drug. The peak for Dapoxetine from the tablet formulations was identified by comparing its retention time and peak area with those of standard dapoxetine HCl. Recovery was found to be 99.28 ± 0.004. [7, 8, 11, 14]

Table No. 9: Specificity result for dapoxetine HCl by RP-HPLC

Sample	Label Claim (mg)	Amount found (mg)	Recovery ± SD (%)	Retention time
Tab	30	29.95	99.28 ± 0.004	5.020 ± 0.0078 min

CONCLUSION

In the present investigation RP-HPLC method for the quantitative estimation of Dapoxetine HCl in bulk drug and pharmaceutical formulations has been developed and validated as per ICH guidelines. The developed RP-HPLC method is more sensitive, accurate and precise as compared to the previously reported methods. There was no any interference of excipients in the recovery study.

The low value of %RSD, high theoretical plates, good retention time and sensitivity suggested that the developed method is sensitive. Thus the reported method is of considerable importance and has great industrial applicability for quality control and analysis of dapoxetine HCl from bulk drug and formulations.

Acknowledgement

The authors are thankful to Inventia HealthCare Pvt. Ltd. Mumbai, India for providing the gift sample. The authors are highly thankful to Dr. C. R. Kokare, Principal, Sinhgad Institute of Pharmacy, Narhe, Pune, India for providing all facilities to carry out the work.

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