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Original Article

STABILITY INDICATING HPLC METHOD FOR DAPOXETINE HCL IN BULK AND IN FORMULATION

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ABSTRACT

Objective: To develop and validate stability- indicating high performance liquid chromatographic (HPLC) method for estimation of Dapoxetine hydrochloride in bulk and in formulation.

Methods: An isocratic separation was carried out using HiQ-SiL C_{18} (250 x 4.6 mm, 5 µm) column and Methanol: H_2O (80:20 v/v) as mobile phase with quantification carried out at a wavelength of 239 nm. The stability studies under stress condition of hydrolysis (acid, base and neutral), oxidation, photolysis and thermal degradation were also carried out for Dapoxetine hydrochloride.

Results: The retention time for drug was found to be 5.94 ± 0.003 min. The calibration curve was found to be linear between 5-30 µg/ml. The limit of detection and Quantitation were found to be 0.135 µg/ml & 0.410 µg/ml, respectively.

Conclusion: A new simple, accurate, precise and selective stability-indicating high performance liquid chromatographic (HPLC) method has been developed and validated for the determination of Dapoxetine hydrochloride in pharmaceutical dosage form. The proposed method can be applicable for the routine determination of Dapoxetine hydrochloride in bulk and formulation.

Keywords: Dapoxetine Hydrochloride, HPLC, Stability.

INTRODUCTION

Dapoxetine hydrochloride (DAPO), chemically (S)-N,N-dimethyl-3-(naphthalene-1-yloxy)-1-phenylpropan-1-amine (Figure 1) is a selective short acting potent serotonin reuptake inhibitor (SSRI) antidepressant proposed to be used for premature ejaculation. Utility for this indication was envisaged based on delayed ejaculation being a recognized side effect of the SSRI class in treatment of depression [1].

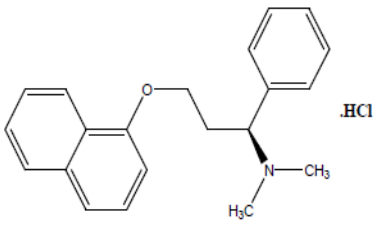

Fig. 1: Structure of Dapoxetine HCl

The literature survey reveals that several UV-VIS Spectro photometric [2-3], HPLC [4-6], Chiral Liquid Chromatographic methods [7] and HPTLC methods [8-10] have been reported for the analysis of DAPO as a single drug or in combination with other drugs in pharmaceutical dosage form. No reports were found for stability-indicating HPLC method for determination of DAPO in bulk and in formulation. This paper describes simple, precise, accurate and sensitive HPLC method development and validation as well as stability studies (hydrolysis, oxidation, photo-degradation and thermal degradation) as per international conference on harmonisation guidelines [11-12].

MATERIALS AND METHODS

Reagents and chemicals

Authentic sample of DAPO was obtained from Inventia Healthcare Pvt. Ltd., East Mumbai. The brand of Sustinex 30 tablets; Emcure Pharmaceuticals Ltd, labeled to contain Dapoxetine HCl equivalent to 10 mg of Dapoxetine were procured form local market. Methanol (HPLC grade) was obtained from S. D. fine chem. Limited (Mumbai, India), HPLC grade water was collected using ELGA water purification system. potassium hydrogen phosphate, sodium hydroxide, o- phosphoric acid (all are AR grade) were purchased from S. D. fine chem. Limited (Mumbai, India).

Instrumentation and Chromatographic condition

Separation was carried out on Jasco HPLC system comprising Model PU 2080 plus pump, equipped with MD 2010 PDA detector and Borwin- PDA software (version 1.5) using HiQ-SiL C_{18} (250 x 4.6 mm, 5 µm) column and Methanol: H_2O (80:20 v/v) as mobile phase with quantification carried out at a wavelength of 239 nm and flow rate of 1 ml/min. All weighing were carried out on Shimadzu balance Model AY-120.

Selection of Detection Wavelength

From the standard stock solution further dilutions were made using mobile phase and scanned over the range of 200 - 400 nm and the spectrum was obtained. It was observed that Dapoxetine HCl showed considerable absorbance at 239 nm (Figure 2)

Preparation of Standard stock solution

Standard stock solution of Dapoxetine HCl was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. From the standard stock solution, 1 ml was further diluted to 10 ml with mobile phase to get 100 µg/ml solution of Dapoxetine HCl.

Preparation of sample solution (Tablet Formulation Analysis)

Twenty tablets [Sustinex 30; Emcure Pharmaceuticals Ltd, B. No. 16A13003; Mfg. Jun 2013, Exp. may 2015] were weighed and powdered. Tablet powder equivalent to 10 mg of DAPO was weighed and transferred to 10 ml volumetric flask and diluted with methanol. Sonicated for 10 mins and filtered so as to get solution having concentration 1000 µg/ml. 0.1 ml of this solution was further diluted with mobile phase to get the final concentration 10 µg/ml of Dapoxetine HCl. Six determinations were carried out from homogenous sample to determine % assay.

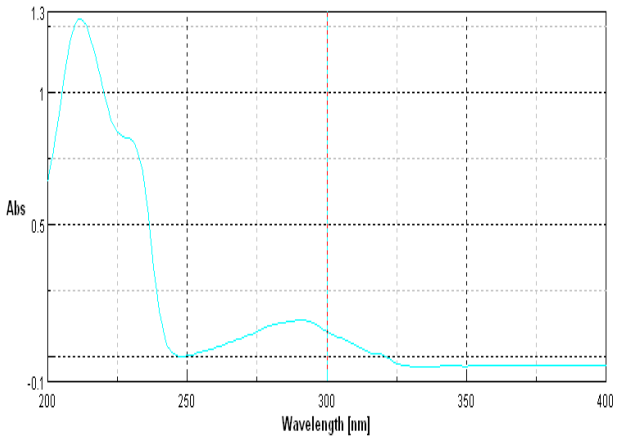

Fig. 2: UV Spectra of DAPO (10 µg/ml)

Stress Degradation Studies Of Bulk Drug

Stress degradation studies were carried under condition of acid, base, neutral hydrolysis, oxidation, dry heat and photolysis. For each study, two samples were prepared (Blank and of DAPO). The blank subjected to stress in the same manner as the drug solution. Dry heat and photolytic degradation were carried out in solid state.

Alkaline hydrolysis

1 ml working standard solution of DAPO (1000 µg/ml) was mixed with 1 ml of 0.1 N methanolic NaOH and 8 ml of Methanol. The solution was kept for 30 min in dark place. The 1 ml of resulting solution was diluted with mobile phase to 10 ml (10 µg/ml) and then was injected into the system.

Acidic hydrolysis

1 ml working standard solution of DAPO (1000 µg/ml) was mixed with 1 ml of 0.1 N methanolic HCl and 8 ml of methanol. The solution was kept for 30 min in dark place. The 1 ml of resulting solution was diluted with mobile phase to 10 ml (10 µg/ml) and then was injected.

Neutral Hydrolysis

1 ml working standard solution of DAPO (1000 µg/ml) was mixed with 9 ml water. The solution was kept for 30 min in dark place. The 1 ml of resulting solution was diluted with mobile phase to 10 ml (10 µg/ml) and then was injected.

Oxidation

1 ml working standard solution of DAPO (1000 µg/ml) was mixed with 1 ml of 30 % solution of H_2O_2 and 8 ml of methanol. The solution was kept for 30 min in dark place. The 1 ml of resulting solution was diluted to 10 ml with mobile phase (10 µg/ml) and then was injected.

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (100^0 C) for a period of 1 hour. Sample was withdrawn after 1 hour and processed as per standard solution preparation procedure mentioned under *Preparation of Standard stock solution* to get 10 µg/ml as final concentration and was injected.

Photo-degradation studies

Photolytic studies were also carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux.Hr. Sample was withdrawn after exposure and processed as per standard solution preparation procedure mentioned under *Preparation of Standard stock solution* to get 10 µg/ml as final concentration and was injected.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The primary target in developing this stability indicating HPLC method was to achieve the resolution between DAPO and its degradation products. To achieve the separation, we used a stationary phase C-18 column and mobile phase methanol and water in ratio 80:20 v/v. The tailing factor obtained was less than two and retention time was 5.94 ± 0.003 (Figure 3). Forced degradation study showed the method is highly specific and no degradation products were eluted at retention time of drug.

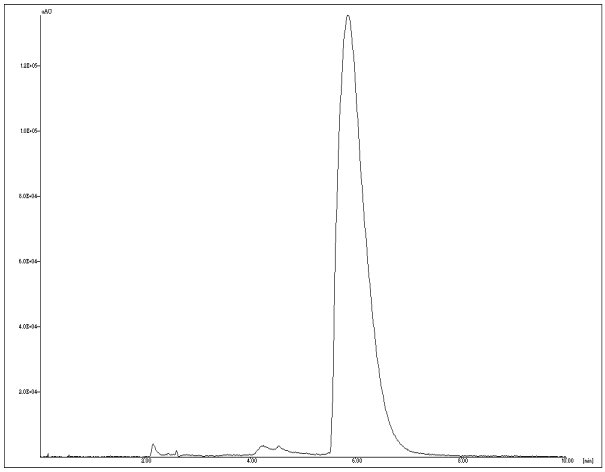

Fig. 3: Chromatogram of Standard Dapoxetine HCl (10 µg/ml)

Result of forced degradation studies

Degradation was studied for DAPO under stress conditions like base, acid, oxidation dry heat and UV and Fluorescence. The drug peak is well resolved from peak of degradation products Summary of stress degradation results is given in Table 1. A well resolved peak was observed for product of alkali induced degradation (D1) at RT 4.21 min. (Figure 4), products of acid induced degradation (D2 and D3) at RT 2.56 and 4.23 mins (Figure 5), products of neutral degradation (D4) at 4.68 min (Figure 6) and products of oxidative condition (D5, D6 and D7) at 2.61, 4.22 and 4.65 min, respectively (Figure 7). No peak of degradation was observed under dry heat and photolytic degradation studies.

Peak purity results greater than 990 indicate that peak of DAPO is homogeneous in all stress conditions tested. The unaffected assay of drug in the tablet confirms the stability indicating power of the method.

Table 1: Summary of stress degradation study of DAPO RS

Sr. No.	Stress Degradation Condition	Percent recovered For DAPO (%)
1	Base (0.1 N NaOH, kept for 30 min)	75.27
2	Acid (0.1 N HCl, Kept for 30 min)	58.48
3	Neutral (kept for 30 min)	86.99
4	H_2O_2, 30% (kept for 30 min)	79.15
5	Dry Heat (100^0C for 1 hr.)	98.89
6	Photo stability [UV, 200 watt hrs/square meter Florescence, 1.2 million Lux. Hrs]	95.83

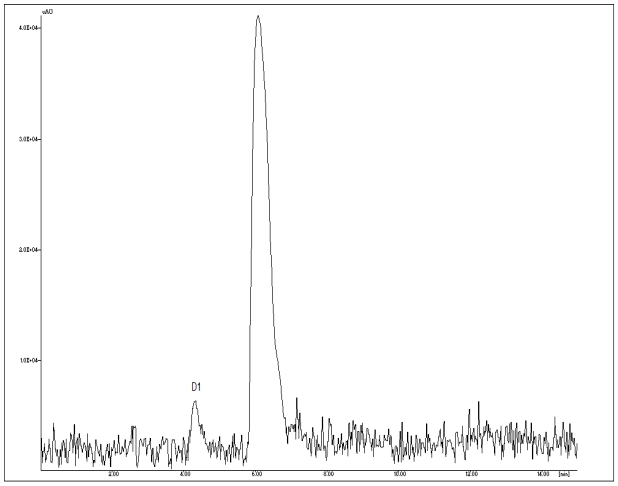

Fig.4: Chromatogram of DAPO (10 µg/ml) after alkaline hydrolysis

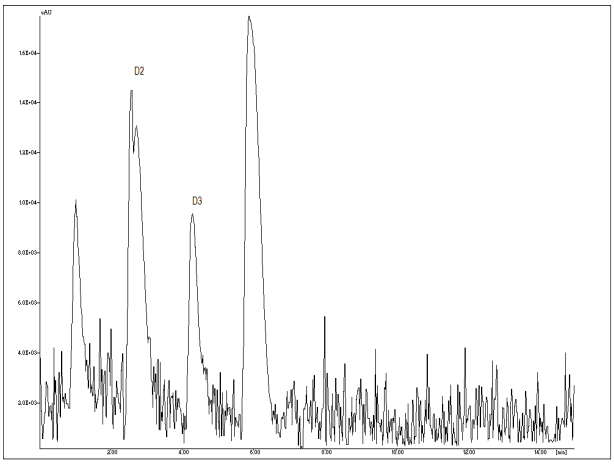

Fig. 5: Chromatogram of DAPO (10 µg/ml) after acid degradation

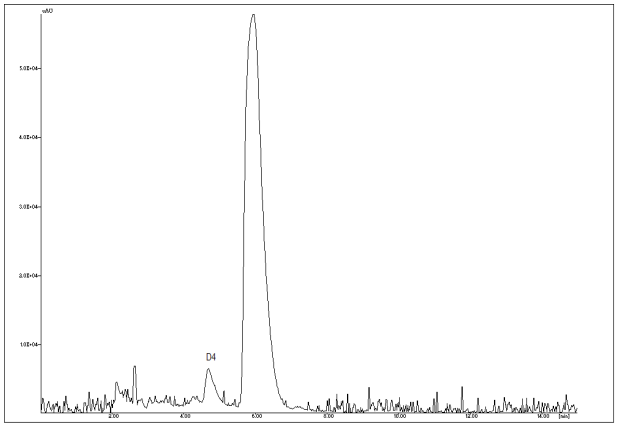

Fig. 6: Chromatogram of DAPO (10 µg/ml) after neutral hydrolysis

METHOD VALIDATION

Linearity

From the standard stock solution (100 µg/ml) of Dapoxetine HCl further dilutions were made with mobile phase to obtain range of solution containing six different concentrations. Six replicates per concentration were injected. The linearity (relationship between peak area and concentration) was determined over the concentration range of 5-30 µg/ml of DAPO. (Figure 8)

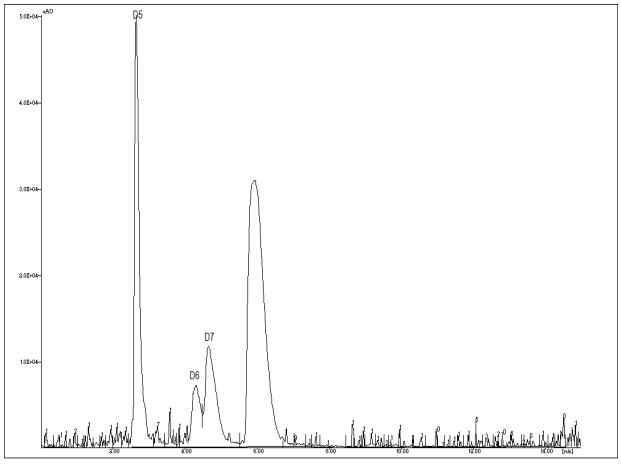

Fig. 7: Chromatogram of DAPO (10 µg/ml) after oxidation

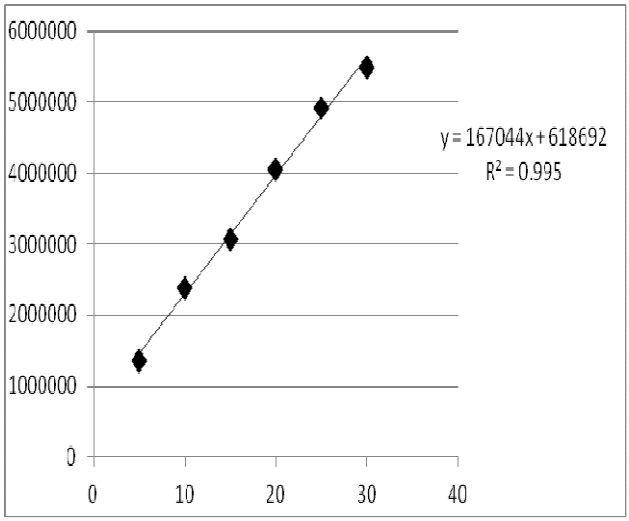

Fig. 8: Calibration curve for DAPO

Precision

The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intra- day studies, 3 replicates of 3 different concentrations (10, 20, 30 µg/ml) of DAPO were analyzed in a day and percentage RSD was calculated.

For the inter day variation studies, 3 replicates of different concentrations were analyzed on 3 consecutive days and percentage RSD were calculated. For intraday precision and inter-day precision % RSD found to be 0.18 and 0.17 respectively.

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels of 50, 100 and 150 %. Basic concentration of sample chosen was 10 µg/ml of DAPO from tablet solution. These solutions were injected in stabilized chromatographic conditions in triplicate to obtain the chromatograms. The drug concentrations of DAPO were calculated by using linearity equation of DAPO. Results of recovery studies are shown in Table 2.

Table 2: Recovery study of DAPO

Level	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery*± SD	Mean ± SD
50 %	5	4.94	99.57 ± 1.09	101.28 ± 1.55
100 %	10	10.52	102.60 ± 1.83	
150 %	15	15.42	101.67 ± 0.67	

Specificity: The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 997, indicating the no interference of any other peak of degradation product, impurity or matrix.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 0.135 µg/ml and 0.410 µg/ml respectively.

Robustness

Robustness of the method was checked by carrying out the analysis under conditions during which flow rate, wavelengths were altered and the effect on the area was noted. The method is robust as % RSD found to be less than 2. (Table 3)

Table 3: Robustness study

Drug	% RSD Found For Robustness Study (peak area)					
	Flow Rate(1 $mLmin^{-1}$)			Wavelength (nm)		
	0.9	1.0	1.1	239	240	241
DAPO	0.0826	0.2341	0.0999	0.2341	0.1312	0.2598

CONCLUSION

The developed method is stability indicating and can be used for assessing the stability of drug in bulk drug and pharmaceutical dosage form. The developed method is specific, selective, robust, rugged and precise.

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