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Stability Indicating HPTLC Method for Dapoxetine HCL in Bulk and in Formulation

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ABSTRACT

A new simple, accurate, precise and selective stability-indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for the determination of Dapoxetine hydrochloride in bulk and in formulation. Chromatographic separation was performed on aluminum plate precoated with Silica Gel 60 F_{254} using Ethyl Acetate: Methanol (9:1 v/v) as the mobile phase with saturation time 20 min, followed by densitometric scanning at 239 nm. This system was found to give compact spot for Dapoxetine hydrochloride (Rf value 0.78 ± 0.005) and specificity in accordance with international conference on harmonization (ICH) prescribed stress conditions. The calibration curve was found to be linear between 100-700 ng/band. The proposed method was found to be accurate, precise, reproducible, specific and sensitive and applicable for the determination of Dapoxetine in bulk and in formulation. The drug was subjected to stress condition of hydrolysis (acid, base and neutral), oxidation, photolysis and thermal degradation.

Keywords: Dapoxetine hydrochloride, HPTLC, Stability.

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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].

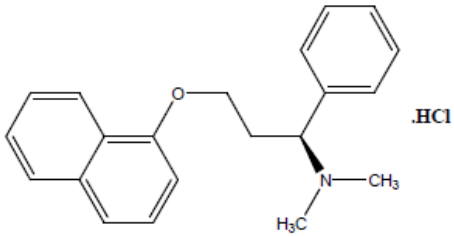

Figure 1: Structure of Dapoxetine HCl

The literature survey reveals that several UV-VIS Spectrophotometric[2,3], HPLC[4-6], Chiral Liquid Chromatographic method[7] and HPTLC[8-10] methods 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 HPTLC method for determination of DAPO in bulk and in formulation. This paper describes simple, precise, accurate and sensitive HPTLC method development and validation as well as stability study (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 Healthe care Pvt. Ltd. East, Mumbai.The brand of Sustinex 30; Emcure Pharmaceuticals Ltd, labelled to contain Dapoxetine HCl equivalent to 10 mg of Dapoxatine was procured form local market. Methanol, Ethyl acetate, sodium hydroxide, hydrochloric acid (all AR grade) were purchased from S. D. Fine Chem. Limited (Mumbai, India).

Instruments and Chromatographic Conditions

Chromatographic separation of drug was performed on Aluminium plates precoated with silica gel 60 F_{254}, (10 cm × 10 cm with 250 µm thickness) purchased from E-Merck, Darmstadt, Germany. Samples were applied on the plate as a band with 6 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm) and a densitometric

scanning was performed using Camag TLC scanner 3 at 239 nm, operated by winCATS software (Version 1.4.3, Camag). Chamber saturation time was 20 min. Migration distance was 90 mm, slit dimensions were 5.00 x 0.45 mm and Deuterium lamp was used as a radiation source.

Selection of Detection Wavelength

From the standard stock solution further dilutions were done 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).

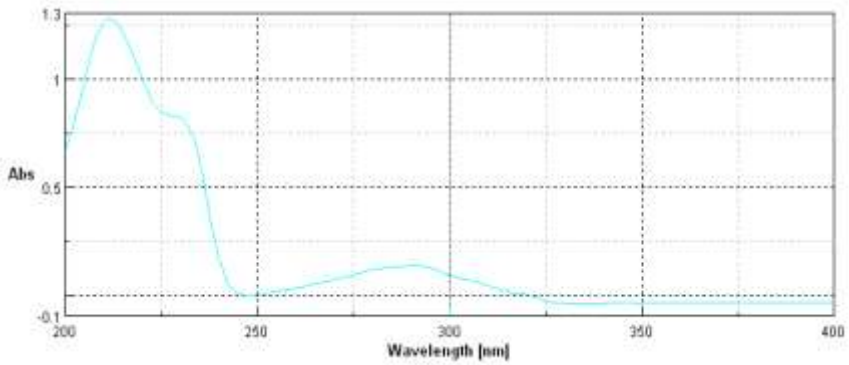

Figure 2: UV Spectra of DAPO (10 µg/ml)

Preparation of Standard Stock Solution

Standard stock solution of DAPO 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 methanol to get solution 100 µg/ml.

Mobile Phase Optimization

Method development for DAPO was started with the development of densitogram with neat solvents and combinations of methanol AR Grade, Ethyl Acetate AR Grade, Chloroform in various proportions. Finally, Ethyl acetate: Methanol (9:1 v/v) was selected as a mobile phase since acceptable peak was obtained at Rf 0.78 ± 0.005 (Figure 3).

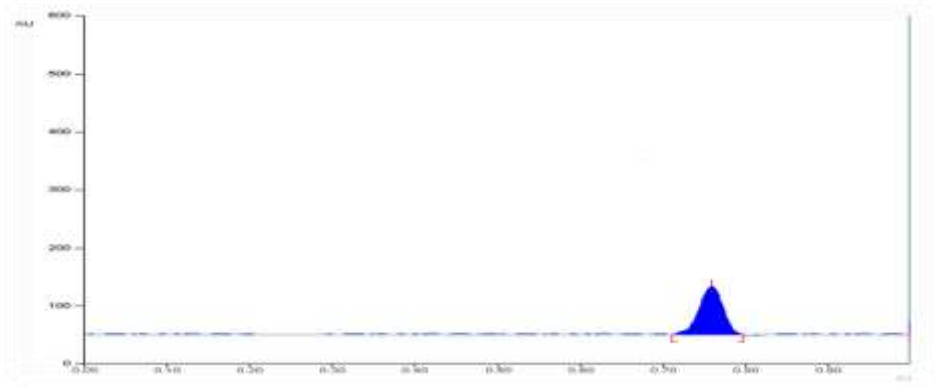

Figure 3: Chromatogram of Standard Dapoxetine HCl (200 ng/band)

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 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 4 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition

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 4 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition

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 4 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

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 4 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

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 100 µg/ml as final concentrations. The 4 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

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 hrs. Drug was withdrawn after exposure and processed as per standard solution preparation procedure to get 100 µg/ml as final concentrations. The 4 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

RESULTS AND DISCUSSIONS

Stress Degradation Study

The degradation study indicated that DAPO is susceptible to acid, base, oxidative degradation and to some extent to neutral, thermal and photo degradation. Results of the stress degradation studies are presented in table 1. A well resolved peak was observed for product of alkali induced degradation (D1) at Rf 0.70 (Figure 4), products of acid induced degradation (D2 and D3) at Rf 0.41 and Rf 0.56 (Figure 5), products of neutral degradation (D4) at Rf 0.68 (Figure 6) and products of oxidative condition (D5, D6 and D7) at Rf 0.21, Rf 0.42 and Rf 0.72, respectively (Figure 7). The overlain spectra of the drug peak and peak for product of degradation, is shown resolved.

Table 1: Summary of stress degradation study of DAPO

Sr. No.	Stress Degradation Condition	Percent recovered For DAPO (%)
1	Base (0.1 N NaOH, kept for 30 min)	78.86
2	Acid (0.1 N HCl, Kept for 30 min)	54.26
3	Neutral (kept for 30 min)	91.74
4	H_2O_2, 30% (kept for 30 min)	68.14
5	Dry Heat (100^0C for 1 hr.)	97.96
6	Photo stability [UV, 200 watt hrs/square meter Florescence , 1.2 million Lux. Hrs]	98.68

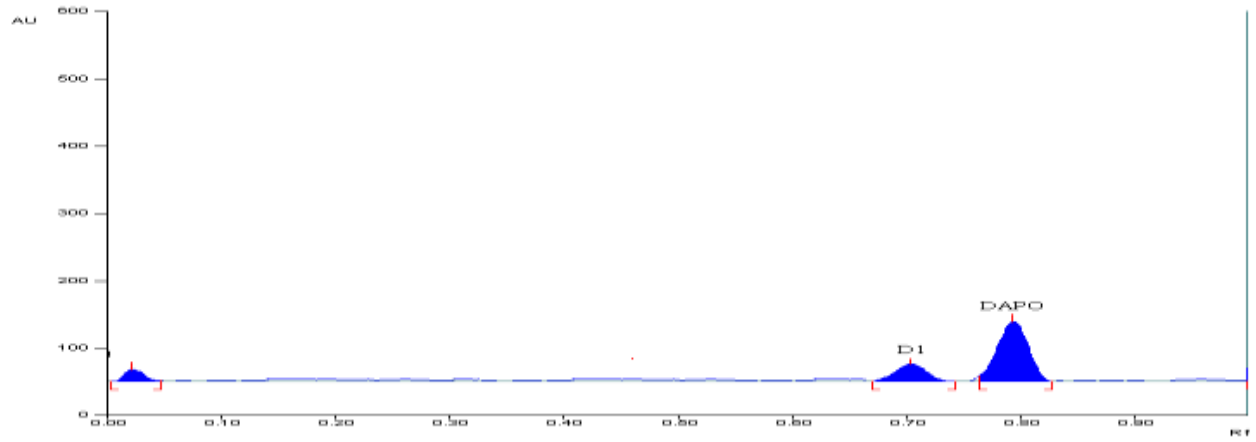

Figure 4: Chromatogram of DAPO (400 ng/band) after alkaline hydrolysis

Method Validation

Linearity

From the standard stock solution (1000 µg/ml) of DAPO, 1 ml was further diluted to 10 ml with methanol to get solution 100 µg/ml. Different volumes were applied on TLC plate to obtain linear range. Six replicates per concentration were applied. The linearity (relationship between peak area

and concentration) was determined over the concentration range 100-700 ng/band (Figure 5).

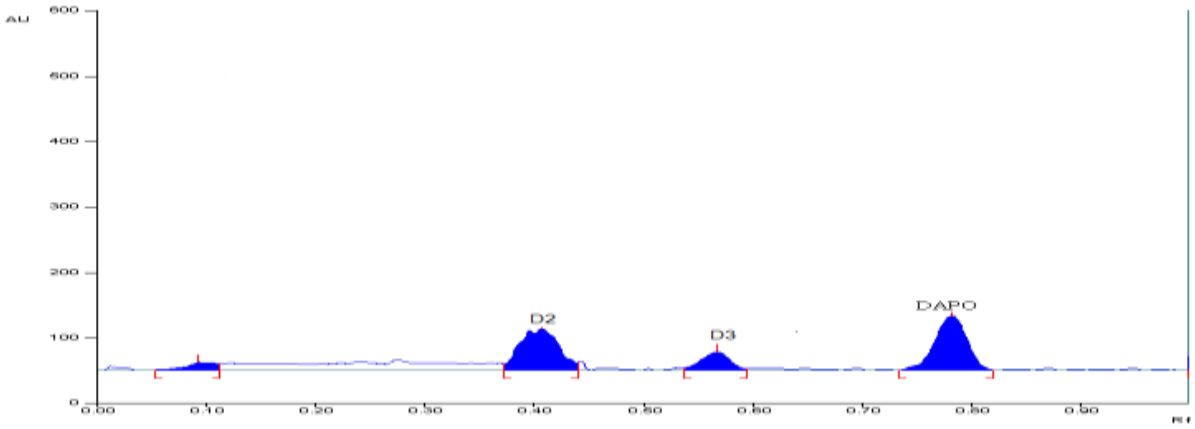

Figure 5: Chromatogram of DAPO (400 ng/band) after acid hydrolysis

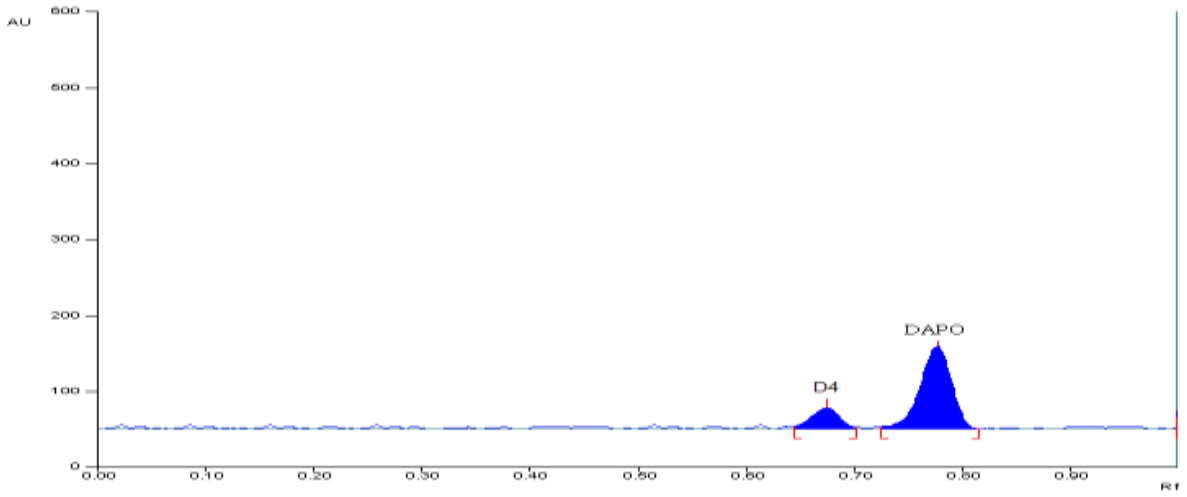

Figure 6: Chromatogram of DAPO (400 ng/band) after neutral hydrolysis

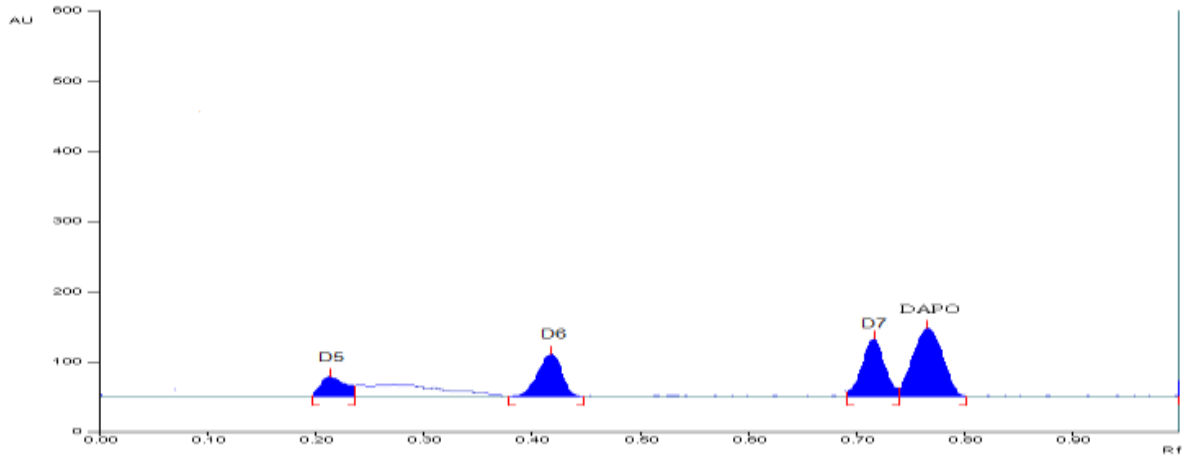

Figure 7: Chromatogram of DAPO (400 ng/band) after oxidation

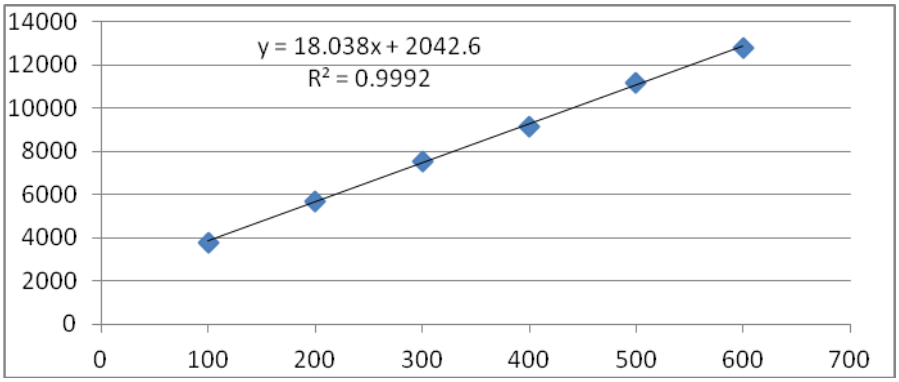

Figure 8: Calibration curve for DAPO

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 (200, 400 and 700 ng/band) 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. The results obtained for Intra-day and Inter day variations are shown in Table 2 and 3.

Table 2: Intra-day precision study DAPO

Concentration (ng/band)	Area* (µV. Sec)	% Recovery ± SD	Mean % Recovery* ± SD	% RSD*
200	5720.67	101.95 ± 0.37	100.37±0.31	0.3088
400	9230.34	99.79 ± 0.31		
700	12805	99.44 ± 0.27		

*Average of three determinations

Table 3: Inter-day precision of DAPO

Concentration ((ng/band)	Area* (µV. Sec)	% Recovery ± SD	Mean % Recovery* ± SD	% RSD*
200	5718	101.87 ± 0.25	100.29±0.47	0.4287
400	9217.67	99.43 ± 1.02		
700	12802.34	99.58 ± 0.13		

*Average of three determination

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 50, 100 and 150 %. Basic concentration of sample chosen was 200 ng/band of DAPO from tablet solution. These solutions were applied under optimized chromatographic conditions in triplicate to obtain the chromatograms. The drug concentrations of DAPO were calculated by using linearity equation of DAPO. The results obtained are shown in Table 4

Table 4: Recovery study of DAPO

Level	Amount added (ng/band)	Amount recovered (ng/band)	% Recovery* ± SD	Mean ± SD
50%	100	98.17	99.39 ± 0.56	99.96 ± 0.68
100%	200	202.97	100.72 ± 0.25	
150%	300	298.80	99.77 ± 0.76	

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

LOD and LOQ were calculated as 3.3σ/S and 10σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD of DAPO found to 15.079 ng/band and LOQ is 45.696 ng/band, respectively.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.995, indicating the no interference of any other peak of degradation product, impurity or matrix.

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which Mobile phase composition and Chamber saturation period were altered and the effects on the area were noted. The results obtained are shown in Table 5.

Table 5: Robustness study of DAPO HPTLC method

Sr. No.	Parameters	Variation	% RSD
1.	Mobile phase composition	± 0.2 ml Methanol	1.074
2.	Chamber saturation period	± 2 mins	0.84

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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