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DEVELOPMENT AND VALIDATION OF DAPOXETINE IN PURE AND SOLID DOSAGE FORM BY HPTLC METHOD

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ABSTRACT

An HPTLC method has been developed for the estimation of Dapoxetine in bulk and solid dosage form. It employs pre coated plates by using silica gel 60 F 254(2×10 cm, thick ness of layer 0.2 mm) as stationary phase and the mobile phase comprises of Acetonitrile: Ethyl acetate (9:1v/v). The developing solvent was run up to 50 mm in Camag chamber. Densitometic scanning was then performed with Camag TLC scanner-3 at λ max 292 nm. The R_f value was found to be 0.54. The linearity and range for Dapoxetine was found to be 100-600ng/µl and the method was found to be accurate with 99.16% -100.33% with %RSD 0.5936. The %RSD for interday and intraday precision was 0.304389 and 0.5471 respectively. Correlation coefficient was found to be 0.9995 and the method was validated as per ICH guide lines.

Keywords: HPTLC, Dapoxetine, Validation, Camag TLC scanner, Silica gel 60 F 254.

INTRODUCTION

Dapoxetine is used for the treatment of premature ejaculation. It's a selective serotonin reuptake inhibitor. Ejaculation is controlled by both serotonin, dopamine, primarily the 5-HT_1a and 5-HT_2 c receptors. It works by inhibiting the reuptake of the serotonin transporter. It inhibits the reuptake transporters of dopamine and nor epinephrine. Dapoxetine is chemically N, N-dimethy l-3-(naphthalene-1-yloxy)-1-phenylpropan-1-amine. The empherical formula was $C_{21}H_{23}NO$ and its molecular weight is 341.88 g/mol, Brands; PRILYXET (30mg) and structural formula shown in Fig. 1. Dapoxetine is white to half white powder and it's freely soluble in water. Literature survey shows several HPLC determinations. Literature survey revealed that no HPTLC method has been reported for the estimation of Dapoxetine. The present investigation has been made to develop a HPTLC method for the estimation Dapoxetine in pure and solid dosage forms.

MATERAILS AND METHODS:

Dapoxetine was procured from Alkem Laboratories, Hyderabad, India. PRILYXET (30mg) was purchased from local pharmacy. Reagents in this assay were of analytical grade.

Apparatus

The instrument used for the estimation was Camag TLC scanner 3, Camag Automatic TLC sampler 4, Camag UV cabinet with dual wavelength UV lamp, Hamilton 100 µl HPTLC syringe and Camag twin trough glass chamber (20×10).

Preparation of mobile phase

A mixture of Acetonitrile: Ethyl acetate (9: 1 v/v) previously filtered through 0.45 µm filter paper was used as a mobile phase.

Prepartion of standard stock solution (100 NG/µL)

10 mg of Dapoxetine raw material was weighed and transferred in to a 10 ml volumetric flask and dissolved in methanol. Pipetted out 1 ml of above solution in to a 10 ml volumetric flask and the volume were made up to the mark with methanol to give a solution containing 100 ng/µl Dapoxetine.

Calibration curve for dapoxetine (100-600 NG/µL)

Camag Automatic TLC sampler spotter was used containing a syringe having capacity of 100 ng/µl. Mixed stock solution having concentration of 100 ng/µl of Dapoxetine was filled in the syringe under nitrogen stream and it was applied in form of band of desired concentration range for each of drug on a single plate having concentration of 100 to 600 ng/µl each of Dapoxetine. Plate was developed using Acetonitrile: Ethyl acetate (9: 1 v/v) and the spots were dried by using hair drier. Developed plates were subjected to densitometic measurements in absorbance mode at wavelength 292 nm using Camag TLC Scanner 3. A plot of peak area vs. concentration for drug

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was obtained. A spectrum of drug was recorded in the range of 100-600 ng/µl and purity of chromatographic peak was checked by scanning each individual peak at 3 different positions (peak start, peak apex and peak end). Calibration curve was shown in Fig. 2.

Determination of dapoxetine from solid dosage form
Sample preparation

Twenty tablets of formulation (Prilyxet 30) containing 30 mg of Dapoxetine were accurately weighed and finely powdered. The powdered tablet equivalent to 10 mg of Dapoxetine was transferred into a 100 mL volumetric flask, added 25 mL of methanol and sonicated for 15 min, then shaken vigorously for few min and finally made up to the mark with methanol. The above solution was collected by filtering it through 0.45 µm filter paper. This solution was used for the estimation of Dapoxetine.

Estimation of dapoxetine in solid dosage form

3ng/µl of above solution was spotted on a TLC aluminum sheets silica gel 60 F 254 and the plates were allowed to develop in twin trough chamber 20x10 cm using Acetonitrile: Ethyl acetate (9: 1 v/v), the solvent front position is noted and the plates are then removed and allowed it to dry in hair drier for 5 min. The spots are then detected using Camag TLC scanner 3 and the peak area obtained at the detecting wavelength 292 nm and the amount of Dapoxetine was calculated by using the regression equation.

Validation of the method
Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 20 tablets were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels taking into consideration percentage purity of added bulk drug samples. Accuracy was determined by calculating the recovery. The method was found to be accurate with 99.16% -100.33% recovery of Dapoxetine. The results are shown in Table 3.

Precision

Variation of results within the same day (intraday), variation of results between days (interday) was analyzed. Intraday precision was determined by analyzing Dapoxetine for three times in the same day at 292 nm. Inter day precision was determined by analyzing Dapoxetine daily for three days at 292 nm. Precision was calculated as intra and inter day variation for the drug. The method was found to be precise with % RSD 0.54711 for intraday (n=3) and %RSD 0.304389 for interday (n=3) for Dapoxetine. The results are shown in Table 4.

RESULTS AND DISCUSSION

Activated the TLC plates were prior to use. The chromatographic conditions were maintained, Precoated Silica gel 60 F254 (20×10 cm) aluminum sheets used as stationary phase. Acetonitrile: Ethyl acetate (9:1v/v) as mobile phase for Dapoxetine. Samples were applied using Camag Automatic TLC sampler 4. Developed plates were subjected to densitometeric measurements in absorbance mode at wavelength 292 nm using Camag TLC Scanner 3. A plot of peak area vs. concentration for drug was obtained. A spectrum of drug was recorded in the range of 100-600 ng/µl and purity of chromatographic peak was checked by scanning individual peak at 3 different positions (peak start, peak apex and peak end). Calibration graph was plotted with peak area vs. concentration. R_f value of Dapoxetine was found to be 0.53.Detection was carried out at 292nm. The proposed method has been validated for assay of Dapoxetine in bulk and tablet dosage forms using following parameters. Linear calibration plots were obtained over the calibration ranges tested i.e.100 to 600 ng/µl. The corresponding linear regression equation was Y= 1288.04881 x + 131.6392.

Table 1. Optical Characteristics of Dapoxettine By HPTLC Method

Optical parameter	Value
λmax (nm)	292 nm
Beer's law limit (µg/mL)	5-30
Correlation coefficient (r)	0.9995
Regression equation (Y=mx+c)	Y= 1288.04881 x + 131.6392
Slope (m)Intercept (c)	1288.04881
Sandell's sensitivity	131.6392
(µg/cm^2/0.001 A.U.)	7.76461
Standard error of mean	4.18044

Table 2. Assay Results of Marketed Formulation (PRILYXET 30)

Sample	Lable claim (mg)	Amount found (mg)	Percentage obtained	S.D	%RSD	S.E
6	30	29.96	99.877	0.869768	0.871089	0.02416

Table 3. Summary of Validation Parameters of Dapoxetine By HPTLC

Parameter	Values	S.D	%RSD
Recovery	99.66-100.6%	0.4703	0.4697
Precision			
Interday(n=3)	100.6%	0.450925	0.050103
Intraday(n=3)	100.1%	0.69282	0.692128
Rugged ness			
Analyst 1	99.03%	1.039447	0.692128
Analyst 2	100.5%		
Instrument 1	99.3%	0.070711	0.071173
Instrument 2	99.3%		

Fig. 1 Structure of Dapoxetine

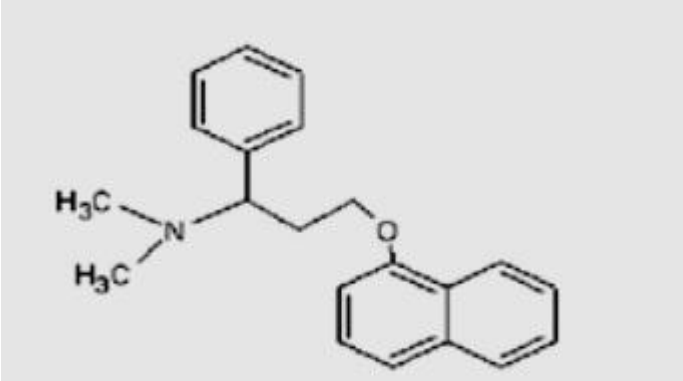


Fig. 2. CALIBRATION CURVE OF DAPOXETINE (100-600 ng/µl)

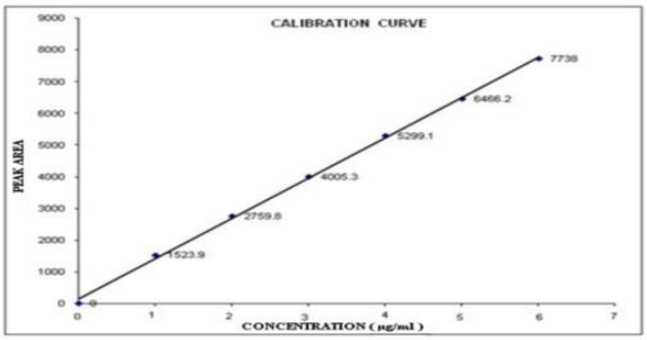


Fig. 3. Chromatogram For Estimation Of Dapoxetine In Solid Dosage Form (300 ng/µl)

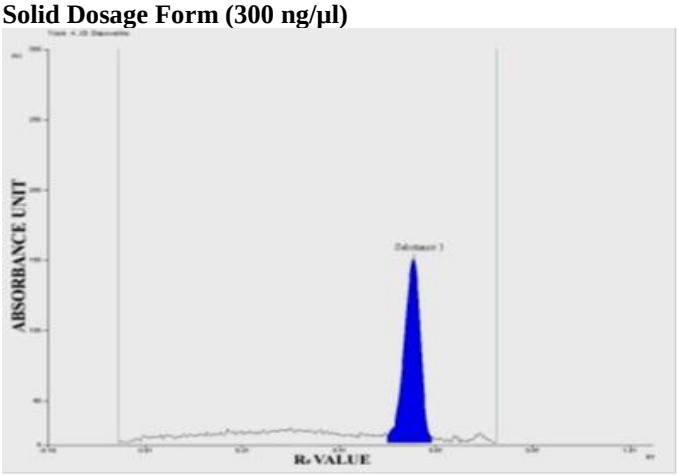
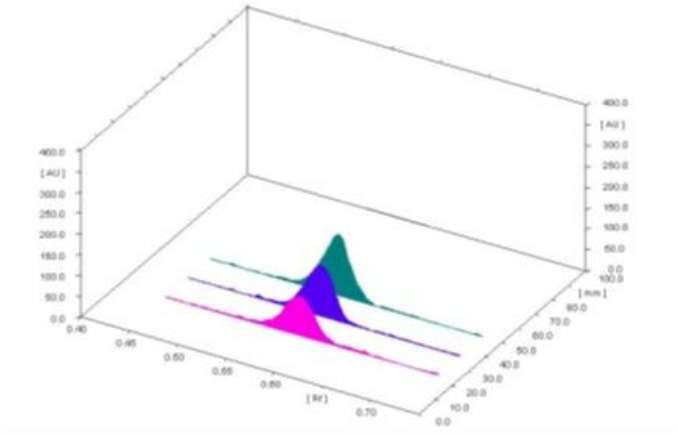


Fig. 4. Chromatogram For Recovery of Formulation (PRILYXET 30) – 2.4 ng/µl, 3.0 ng/µl and 3.6 ng/µl.



CONCLUSION

A new validated HPTLC method has been developed for the Quantitative and Qualitative determination of Dapoxetine in tablet dosage form. The method was completely validated shows satisfactory results for all the method validation parameters tested and method was free from interferences of the other active ingredients and additives used in the formulation. Infact results of the study indicate that the developed method was found to be simple, reliable, accurate, linear, sensitive, economical and reproducible and have short run time which makes the method rapid. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis.

The developed HPTLC technique is simple, precise, specific accurate and from the excipients in the formulation. The statistical analysis proves that method is reproducible and selective for the analysis of Dapoxetine in bulk and tablet formulation.

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REFERENCES

1. Anonymous. The United States Pharmacopoeia. 22nd Revision United States of Pharmacopoeia Convention, Inc, Rock villa, MC, USA, 1995, 1776 – 1777.
2. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry, 4th edition, CBS Publishers and Distributors, New Delhi, (II), 2007, 276-290.
3. Code Q2A, Text on validation of analytical procedures, ICH Harmonized Tripartite guidelines, Geneva, Switzerland, 27October, 1994, 1 – 5.
4. Code Q2B, Validation of Analytical Procedure, Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, and 6th November, 1996, 1 - 8.
5. Dougles A. Skoog, Donald M. West, James F. Hollex and Stanely R. Crouch. Fundamentals of Analytical chemistry, 8th edition, Thomson Asia Pvt. Limited, Singapore, 2005, 2, 5, 11.
6. Gary D. Christian. Analytical chemistry 6th edition, John Willey and Sons Ltd, New Delhi, 2007, 1-11, 20 – 22
7. Gurdeep R. Chatwal and Sham K Anand. Instrumental Methods of Chemical Analysis, 1st edition, Himalaya Publications.
8. Mehtal Pratik, Sahoo Ujjwal, Seth A.K. Development and Validation of a RP-HPLC Method for the Determination of Dapoxetine Hydrochloride in Pharmaceutical Dosage form. *International Journal of Pharmaceutical Sciences Review and Research*, 6, 2011, 76 - 78.
9. Sasmita Kumari Acharjya, Alok Sahu, Srikanta Das, Padhy Sagar and Mathrusri Annapurna. Spectrophotometric Methods for the Determination of Mesalamine in Bulk and Pharmaceutical Dosage Form. *Indian Journal of Pharmaceutical Education and Research*, 1, 2010, 63 – 67.
10. Thyseen AN, Sharma OM, Tianmei SI and Mudambi Ramagopal. Pharmcokinetics of Dapoxetine Hydrochloride in Healthy Chinese, Japanese, and Caucasian Men, *Journal of Clinical Pharmacology*, 50, 2010, 1450 - 1460.
11. Willard HH, Metrit LL, Dean JA and Settle FA. Instrumental methods of Analysis. 7th edition, CBS Publishers and Distributors, New Delhi, 1986, 8, 11. 30. William Kemp. Organic Spectroscopy, 3rd edition, Pal grave Publishers, New York, 1996, 258-260.