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Validated stability indicating rp-hplc method for the determination of dapoxetine hydrochloride in bulk and pharmaceutical formulations

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ABSTRACT

Objective: The main objective was to develope a new validated RP–HPLC method for the determination of Dapoxetine hydrochloride in dosage form and to apply the developed method for the analysis of Dapoxetine HCl drug in its dosage forms. An isocratic C_{18} (Hypersil BDS, 100 mm x 4.6 mm, 5µ) column was used with mobile phase of composition. **Acetonitrile** : Phosphate buffer (40 : 60 at pH=3.0±0.1) at a flow rate of 1.0 mL/min with UV detection at wavelength of 230 nm for Dapoxetine hydrochloride. The drug was subjected to stress conditions of hydrolysis (acid and base), oxidation, photolysis and thermal degradation. The retention time of the drug was 4.244 minutes. The developed method was validated for specificity, linearity, precision, accuracy, Limit of Detection (LOD), Limit of Quantification (LOQ) and robustness as per International Conference on Harmonization (ICH) guidelines to show the stability indicating power of the method. Linearity was found in the range of 15.0 - 90.0 µg/mL. The percentage recoveries of the drug was ranged from 98 to 102 %.

The proposed method could be used for routine analysis of Dapoxetine HCl drug in its dosage forms. The developed method produces high sensitivity, precision and accuracy

KEYWORDS

Liquid Chromatography;
Dapoxetine hydrochloride;
Dosage form;
Stability indicating validation.

INTRODUCTION

Dapoxetine Hydrochloride (DAP), (S)-N,N-Dimethyl-3-(Naphthalen-1-yloxy)-1-phenyl propan-1-amine, drug is used for the treatment of premature ejaculation[1]. The molecular formula of DAP is $C_{21}H_{23}NO$, molar mass is 305.413 g/mol and its half-life is 1.5-1.6 hrs. DAP is the first compound developed specially for the treatment of premature ejaculation (PE) in men between 18-64 years. It is a white crystalline powder, freely soluble in water, ethanol and acetonitrile. It is a short acting serotonine reuptake inhibitor.

The chemical structure of DAP is given in Figure 1.

A thorough review literature revealed that there are only very few analytical methods reported for the HPLC method analysis of individual DAP drug relatively with high retention time[2,3]. Simultaneous UV, HPLC, HPTLC and spectrophotometric methods for DAP along with other drugs were also reported[4-17]. Hence, it was felt that there is a need of new analytical method

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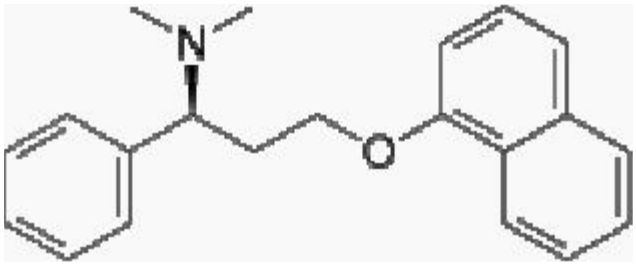

Figure 1 : Structure of DAP

development for the determination of DAP in pharmaceutical dosage form with less retention time at different conditions. Present work is aimed to develop a new, simple, fast, rapid, accurate and reproducible RP-HPLC method for the analysis of DAP with shorter retention time. The developed method was validated according to ICH guidelines.

MATERIALS AND METHODS

Materials

HPLC grade Merck make Ammonium phosphate [$(NH_4)_3PO_4$], Orthophosphoric acid (GR grade), acetonitrile were used in this method. All dilutions were performed in standard class-A, volumetric glassware. For the estimation of commercial formulation, Dapoxetine Hydrochloride equivalent to Dapoxetine 30 mg/60 mg (Sustinex-30/Sustinex-60) tablets were procured from the local market. High pure HPLC grade Milli-Q water was used throughout the analysis.

Instrumentation

Waters HPLC 2 model 2695 series LC chromatographic system consisting of pump with UV-Visible detector with PDA of Waters (2996) make and a fixed injector equipped with 20µL loop was used for the chromatographic separation. The chromatogram was recorded at ambient temperature and peaks quantified by means of Empower 2 software. Chromatographic determination was carried out on an isocratic C_{18} column [Hypersil BDS, 100mm x 4.6mm, 5µ]. Sartorious electronic balance was used for weighing the samples. Ultrasonic bath sonicator was used for degassing and mixing of the mobile phase.

Chromatographic conditions

The mobile phase was composed of acetonitrile and phosphate buffer (pH=3.0±0.1) in the ratio of 40 : 60 (%v/v). [The buffer solution was prepared by dissolving 14.9 gm of ammonium phosphate in 1000 mL water and the pH of the solution was adjusted to 3.0 ± 0.1 using dilute orthophosphoric acid]. It was filtered through a 0.45 µ membrane filter and degassed for 15 minutes. The flow rate of the mobile phase was maintained at 1.0 mL/min. Detection was carried out at 230 nm at ambient temperature.

Method development

Preparation of standard stock solutions

Standard stock solution of Dapoxetine was prepared by dissolving 60 mg in 50 mL volumetric flask, 60 mL of diluent was added and sonicated to dissolve and diluted to volume with diluent. 10 mL of standard stock solution was transferred into 100 mL volumetric flask and diluted up to the mark with the diluent.

Preparation of sample solutions

Transferred grinded sample quantitatively equivalent to 60 mg of Dapoxetine drug into 100 mL volumetric flask, added 60 mL of diluent, sonicated to dissolve and diluted to mark with diluents. The solution was filtered through 0.45 µ filter paper.

Preparation of assay sample solution

10 mL of standard stock solution was transferred into 100 mL volumetric flask and make up to the mark with diluents. Same procedure was repeated for remaining three preparations.

Method validation

The developed HPLC method for the determination of DAP was validated as per the ICH guidelines.

System suitability and system precision

System suitability for chromatographic separation was checked on each day of validation to evaluate the components of the analytical system in order to show that the performance of the system, meet the standards required by the method. System suitability parameters established for the developed method include number of theoretical plates-NLT 2000 (efficiency), Tailing factor (NMT 2.0). The HPLC system was equilibrated using the initial mobile phase composition, followed by 5 injections of the standard solution of 100% concentration containing 30 µg/mL DAP. These 5

TABLE 1 : System suitability parameters for DAP by proposed method

Name of the compound	Retention Time	Area	Height	USP Tailing	USP Plate count
DAP	4.238	4115436	442325	1.12	4667

TABLE 2 : Chromatographic conditions for DAP

Column	C_{18}, 100mm X 4.6 mm, 5µ,
Flow rate	1.0 mL /min
Wavelength	230nm
Column temperature	30°C
Injection volume	20 µL
Run time	10 minutes
Diluent	Mobile phase
Elution	Isocratic
Needle wash	Water: Acetonitrile 90:10 (v/v)

consecutive injections were used to evaluate the system suitability on each day of method validation. The result was given in the TABLE 1.

Specificity

Blank interference

Specificity studies include application of the proposed method for blank, placebo solution, sample solution (control sample), standard solution. A study to establish the interference of blank was conducted. Diluent was injected into the chromatograph in the above defined chromatographic conditions (TABLE 2) and the blank chromatogram was recorded. Chromatograms of Blank solution (Figure 2) and placebo solution (Figure 3) showed no peaks at the retention time of DAP peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of DAP in Sustinex tablets. Similarly typical representative chromatogram of standard was also shown Figure 4.

Forced degradation studies

The specificity studies also include deliberate degradation of the tablet sample by exposure to stress conditions. Forced Degradation study was carried out by treating the sample under the acidic, alkaline, thermal and photolytic conditions. Weighed twenty tablets of DAP and powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 60 mg was transferred into 100 mL volumetric flask. The contents of the flask were sonicated for about 15 min for complete solubility of the drug and the volume was made up to 100 mL with mobile phase. Then the mixture was filtered through a 0.45µ membrane filter. The results pertaining to these degradation conditions were given in TABLE 3.

Linearity and range

In the concentration range of 15.0 – 90.0 µg/mL for DAP, standard curve was obtained. A statistical method known as linear regression analysis was used

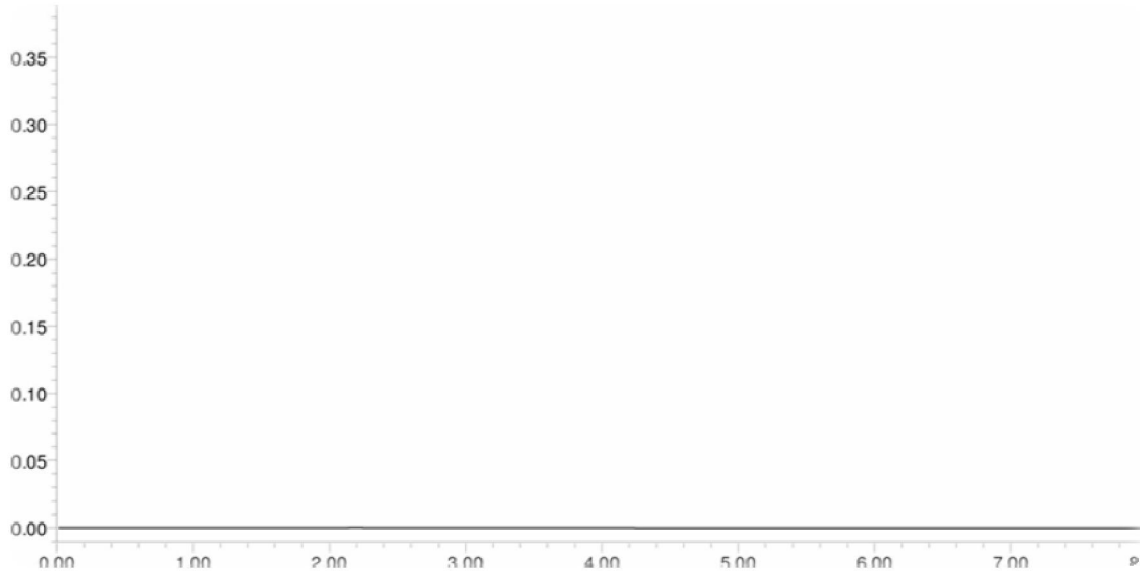

Figure 2 : Chromatogram of DAP Blank

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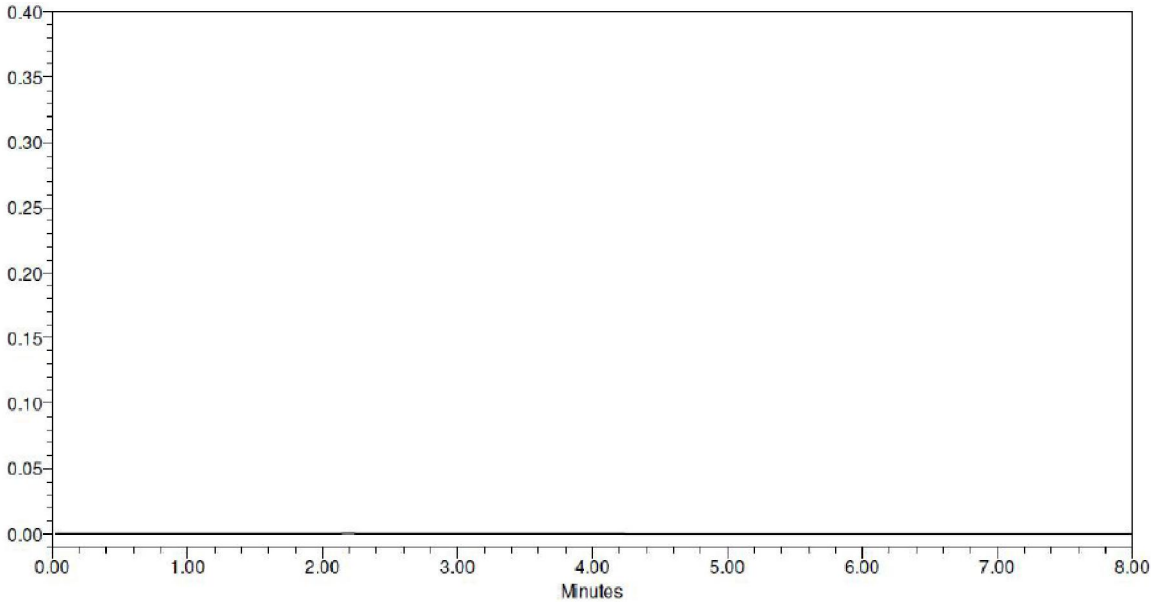

Figure 3 : Chromatogram of DAP of placebo solution

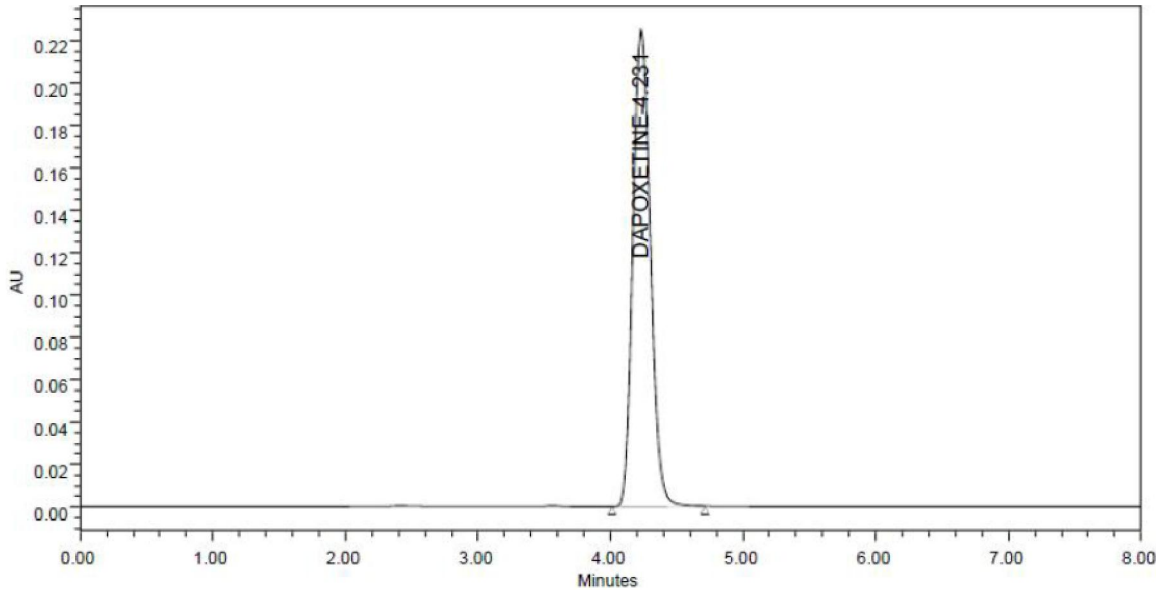

Figure 4 : Standard Chromatogram of DAP

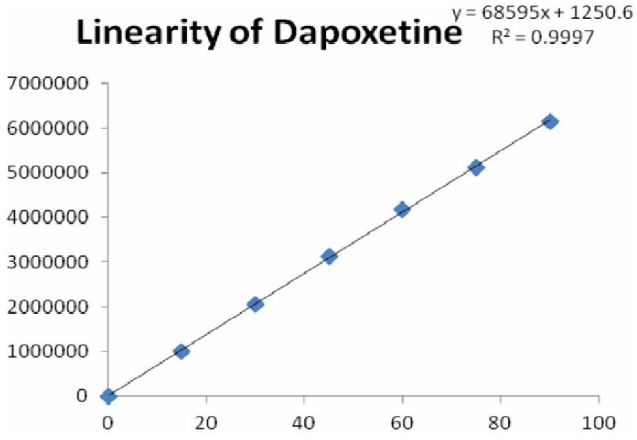

Figure 5 : Calibration curve for DAP

to evaluate the linearity of the curve. To assess the linearity of the proposed method slope, intercept and correlation coefficient [r^2] of standard curve was calculated which is equal to 0.999 and was given in Figure 5. The results were given in the TABLE 4. From the data obtained (For DAP), the method was found to be linear within the proposed range. The linearity chromatograms were given in Figure 6.

Accuracy

Accuracy is defined as the closeness of results obtained by that method to the true value for the sample. Accuracy is expressed in terms of percentage recovery.

TABLE 3 : Forced Degradation data for DAP

Condition	Time (hours)	Retention time (min)	Retention time of additional degradation peak (min)	% Degradation	% of Active drug Present after Degradation
Acid Degradation (5 N HCl)	02 80^0C	4.154	2.360	1.12	88.72
			2.831	0.10	
			3.040	0.35	
			3.717	9.71	
Alkaline Degradation (5 N NaOH)	02 80^0C	4.202	2.848	0.44	97.84
			3.192	0.10	
			3.536	1.61	
Thermal Degradation	24 100^0C	4.208	2.360	0.43	99.34
			3.548	0.23	
Photo Degradation	12	4.201	3.532	0.63	99.37

TABLE 4 : Linearity studies for DAP by proposed method

Concentration(µg/ml)	Area (µv^{2}sec)
15.0	992973
30.0	2051143
45.0	3133432
60.0	4176627
75.0	5115459
90.0	6146430

Recovery % is determined by the standard addition method. In the present study recovery studies were carried out at 50%, 100% and 150% spiked levels. The results of Recovery % were given in TABLE 5 and Chromatograms of accuracy were presented in Figure 7. As the recovery results are found between 98% to 102%, the study proves that the method was accurate for the estimation.

Precision

The closeness of replicate results obtained from analysis of the same homogeneous sample is known as precision of the method. The precision of the method was assessed by six replicate injections of 100% test concentration. The precision was expressed in terms of standard deviation and %RSD. The %RSD of individual % from six sample preparations should not be more than 2.0. The results were given in TABLE 6 and the chromatograms were shown in Figure 8. The system precision was also analysed and the results were given

TABLE 5 : Accuracy data for Dapoxetine

S.No	Dapoxetine		
	Area(µv^{2}sec)		
	50%	100%	150%
Injection 1	3063123	4115436	5115560
Injection 2	3061654	4112023	5115786
Injection 3	3063033	4110034	5115904
Average	3062603	4112498	5115750
*% Recovery	98.66	100.39	99.62

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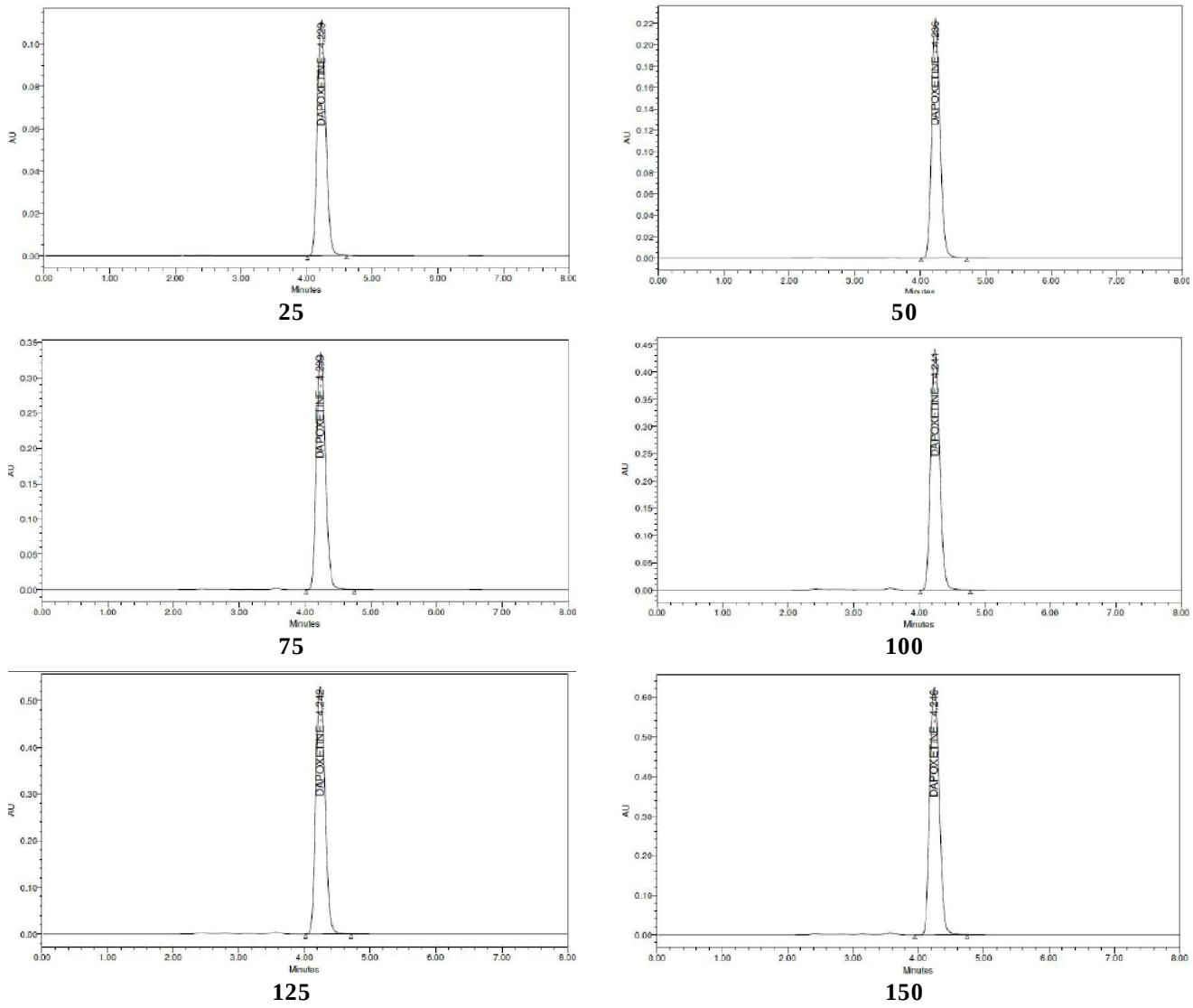

Figure 6 : Linearity Chromatograms of DAP

TABLE 7 and the corresponding chromatograms were represented in Figure 9.

Ruggedness

Degree of reproducibility of test results obtained by analyzing the same sample under variety of normal test conditions such as different analysts, instruments, days, reagents, column etc., The Ruggedness of the method was verified by analyzing the six samples of same batch for method precision as per test method on two different days. The analyst's prepared six sample of the same batch on two different day's. Calculated %RSD on two different days in six samples for ruggedness results with the method precision. The results of ruggedness were given in TABLE 8 and the chromatograms were given in Figure 10. The % of RSD of ruggedness was less than 2.0 and hence the method was rugged.

LOD and LOQ

The formulae 3.3 σ/S and 10 σ/S were used to calculate LOD and LOQ respectively. Where σ is the mean of standard deviation of y intercepts of the three calibration curves and S is the mean of slopes of the calibration curves. The LOD and LOQ values of DAP are 1.746 and 5.2911 µg/mL respectively.

Robustness

The ability of the developed method to remain unaffected by the small changes in the parameters is known as Robustness. Robustness was assessed by varying the parameters such as percent organic content,

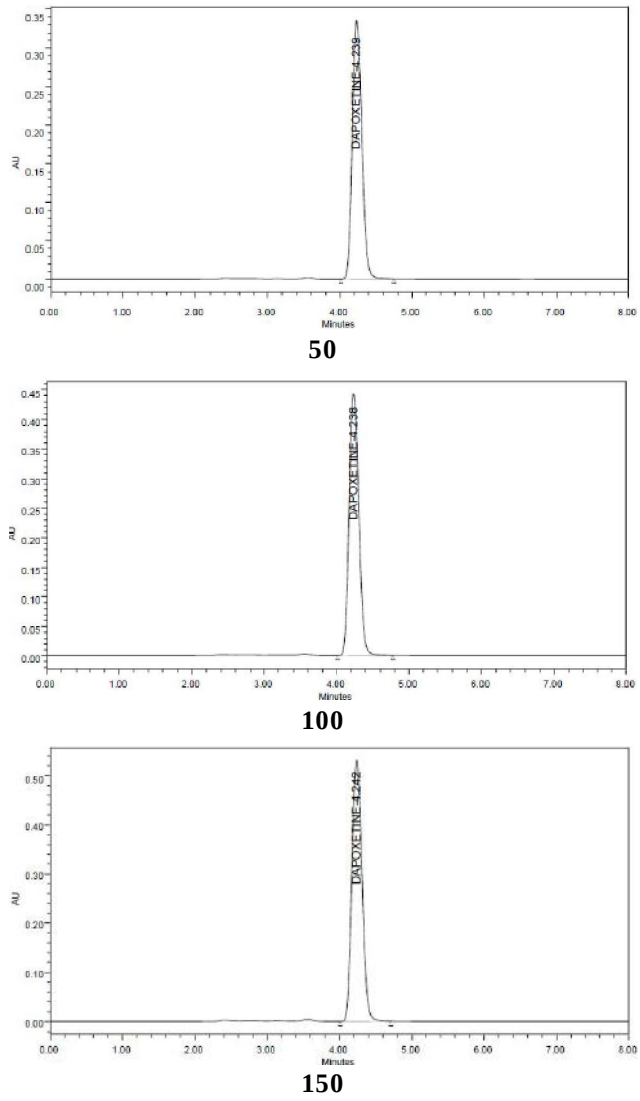

Figure 7 : Accuracy Chromatograms of DAP

pH of the mobile phase, buffer concentration, temperature, injection volume and flow rate. In the present investigation, a variation of ± 0.1 mL/min in the flow rate, change in temperature were adopted to study Robustness. The results were tabulated in TABLE 9 and chromatograms of robustness were given in Figure 11.

RESULTS AND DISCUSSION

In present study, a new analytical method known as reversed phase high performance liquid chromatography (RP-HPLC) method was adopted for the determination of Sustinex – 30/60 tablets in combined dosage form. The column used in this method was Hypersil BDS C_{18}, (100 mm X 4.6 mm, 5µ) with a flow rate of 1.0 mL/min at a wavelength 230 nm and Column temperature was maintainted at 30°C. The mobile phase preparation was done by using buffer ammonium phosphate (pH=3.0±0.1). The mobile phase combination was Buffer: ACN in the ratio of 60:40 (%v/v). The run time was set for 10 minutes. The retention time of DAP was 4.244 minutes. The developed method

TABLE 6 : Data for Method precision of Dapoxetine

S.NO	Dapoxetine	
	Retention time	Area
Injection 1	4.224	4143653
Injection 2	4.229	4145094
Injection 3	4.223	4140924
Injection 4	4.226	4148705
Injection 5	4.226	4145655
Injection 6	4.228	4154897
Average	4.226	4146488
Standard deviation	0.002	4842.7
% RSD	0.054	0.12

TABLE 7 : Data for System precision of Dapoxetine

S.NO	Dapoxetine	
	Retention time	Area
Injection 1	4.228	4181645
Injection 2	4.232	4144483
Injection 3	4.235	4175729
Injection 4	4.238	4183926
Injection 5	4.243	4180750
Injection 6	4.243	4091977
Average	4.237	4159752
Standard deviation	0.006	36294.5
% RSD	0.142	0.87

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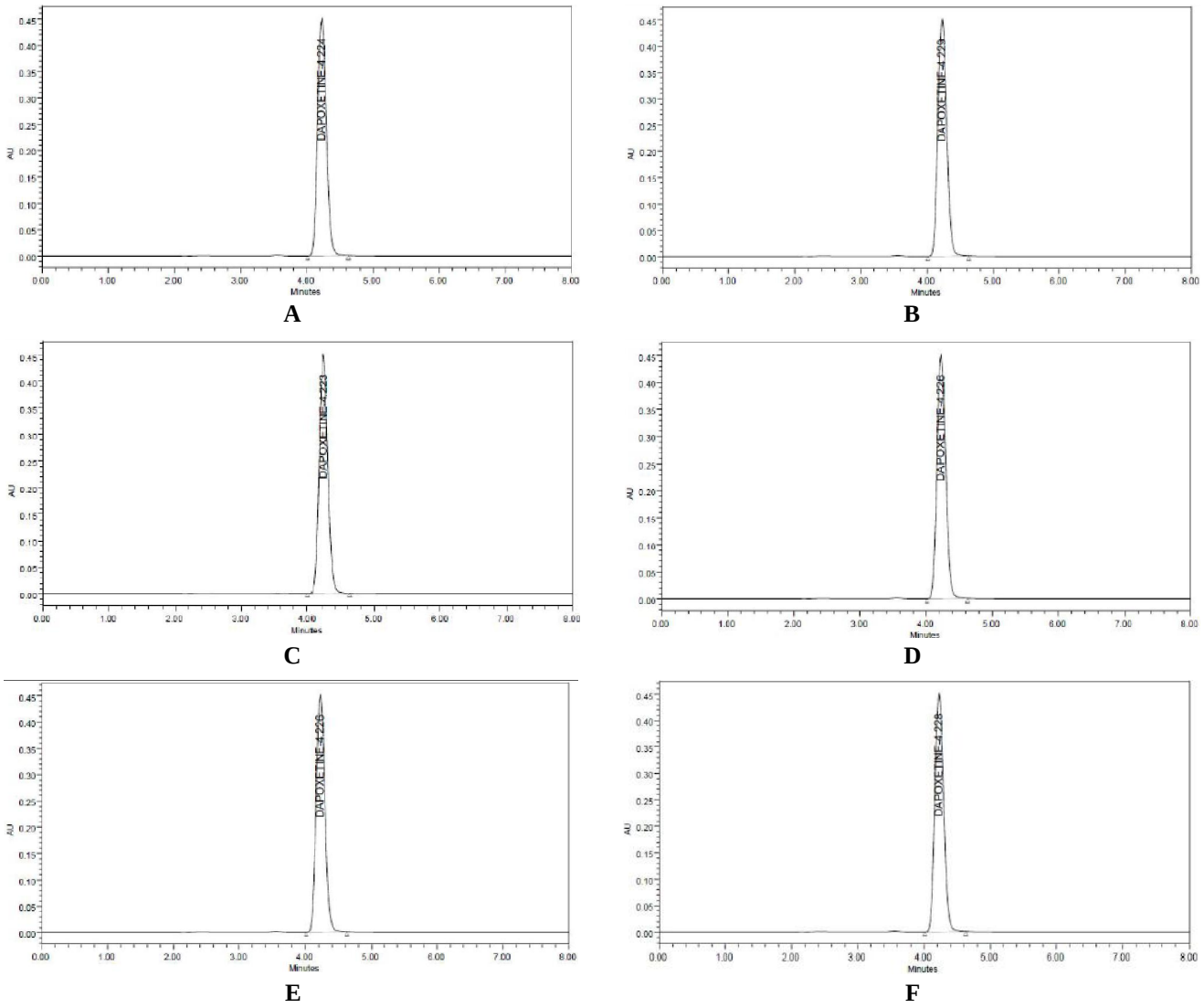

Figure 8 : Method Precision Chromatograms of DAP

is specific for the determination of DAP and the same was known from the blank, placebo and forced degradation studies, as no other peak was found at the retention time of DAP during these studies. The new HPLC method developed and validated for determination of DAP in pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of drug in its solid combined dosage form by RP-HPLC method. The linearity range for DAP is 2.5 – 15.0 µg/mL, the correlation co-efficient was found to be 0.999. The percentage RSD obtained for system precision of DAP was 0.87. The percentage RSD obtained for method precision of DAP was 0.12. The Limit of detection (LOD) and Limit of Quantification (LOQ) values for DAP are 1.746 and 5.2911 µg/mL respectively.

The Ruggedness of the method has been verified by analyzing the six samples of same batch for method precision as per test method by different analysts using different instruments, different days. The analyst's prepared six samples of the same batch on two different days and calculated %RSD for two different days in six samples for ruggedness results with the method precision. The system suitability was evaluated in each condition and compared the results with method precision which causes the method is robust for change in flow rate and temperature. No peak was observed at the retention time of DAP and the developed method was found to be specific.

The sample solution was injected and the amount of DAP present in the formulation was calculated from the calibration curve. The amount of DAP found in the

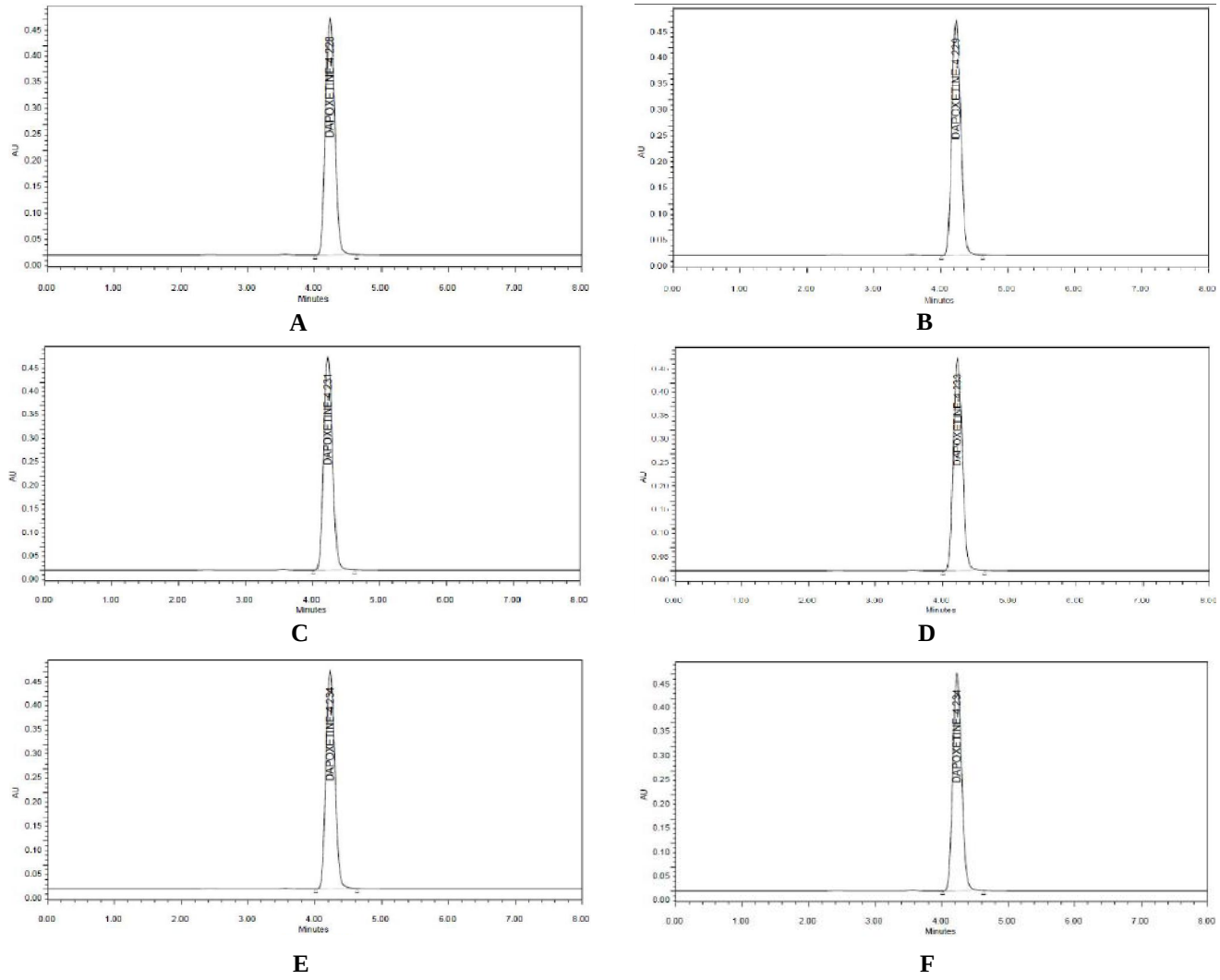

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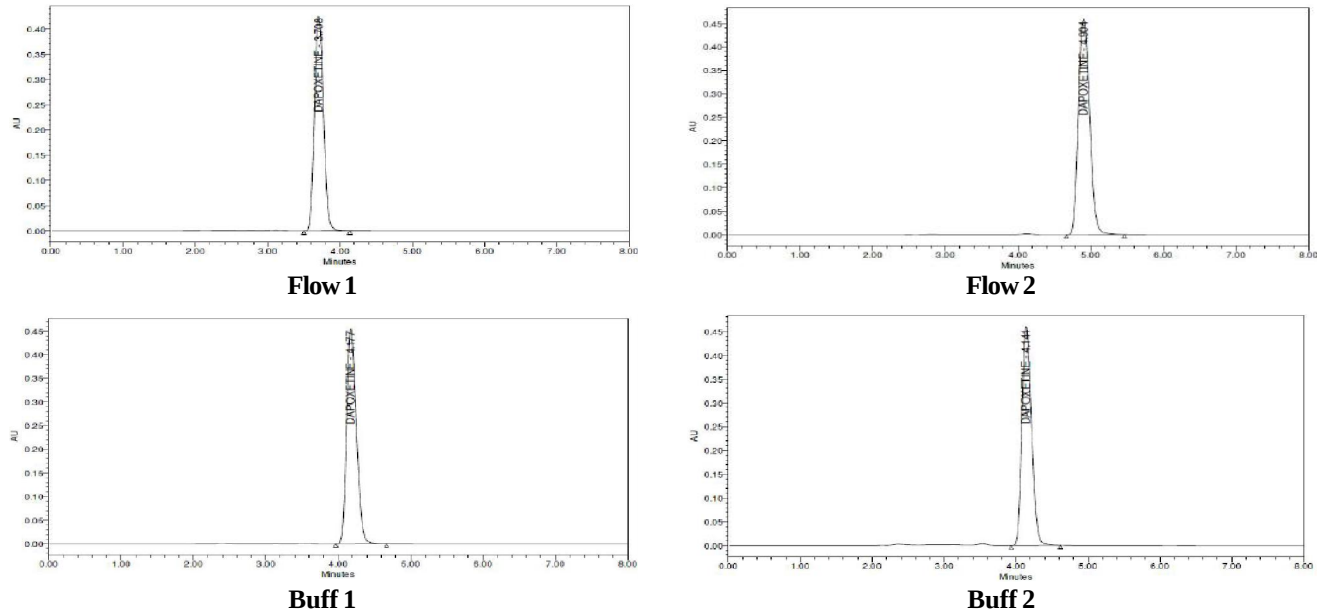
Figure 9 : System Precision Chromatograms of DAP



Flow 1 Flow 2 Buff 1 Buff 2

Figure 10 : Ruggedness Chromatograms (Day 2) of DAP

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TABLE 8 : Data for Ruggedness of Dapoxetine

S.No		Day 1		Day 2	
		Retention Time	Area	Retention Time	Area
1	Injection 1	4.228	4181645	4.228	4172065
2	Injection 2	4.232	4144483	4.229	4179893
3	Injection 3	4.235	4175729	4.231	4174345
4	Injection 4	4.238	4183926	4.233	4168945
5	Injection 5	4.243	4180750	4.234	4168760
6	Injection 6	4.243	4091977	4.234	4165903
Average		4.237	4159752	4.232	4171652
Std Dev		0.006	36294.5	0.003	4981.6
% RSD		0.142	0.87	0.061	0.119

commercial sample as per the developed method was 59.87 mg against to the 60 mg present in the tablet and the assay of DAP was found to be 99.78%.

CONCLUSION

The proposed method for Dapoxetine hydrochloride drug is simple, selective, reproducible and specific with good precision and accuracy. The developed method was proved to be superior to most of the reported methods. The proposed method for estimation of selected drug was successfully applied either in pure form or tablet dosage form. More over the low solvent consumption along with short retention time of 4.244 min. for DAP seems to be cost effective when compared to other developed methods shown in literature review. The proposed method can be used as alternative methods to the reported ones for the routine determination of selected drug under the study in tablet dosage form.

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