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DAPOXETINE: NOVAL ANALYTICAL METHOD DEVELOPMENT, VALIDATION AND IT's FORCED DEGRADATION STUDIES

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ABSTRACT

The aim of the study is to develop novel analytical method for theestimation of dapoxetineusing HPLC and to carry out forced degradation studies. A simple, rapid, sensitive and accurate chromatographic method was developed for dapoxetine using C18Shim-pack (250mm×4.6mm i.e., 5µm particle size) column in low pressure gradient elution mode with the mobile phase comprising of Acetonitrile: Orthophosphoricacid(85:15)pH 4.2, with a flow rate of 1.0 mL/min, injection volume 10µL and the eluent was detected at 291nm usingUV detector.The retention time of dapoxetine was 1.9 minutes. The linearity range is 2-10µg/mL and quantification and detection limit was 7.488 µg/ mL 2.471 µg/ mL.The drug is subjected to forced degradation studies as per the guidelines, it was found that slight change in the peak shape and percentage of drug on subjecting to acid and alkali. The developed method is validated following ICH guidelines. Hence these methods can be used for routine analysis in quality control laboratories.

INTRODUCTION

Dapoxetine is member of naphthalenesand it is chemically dimethyl[(1S)-3-(naphthalen-1-yloxy)-1-phenylpropyl] amine and its molecular formula is $C_{21}H_{23}NO$ with molar mass of 305.18 g/mol. Structure of dapoxetineis given in Fig 1. It is a water-soluble drug with a pKa of 8.6. It is also soluble in DMSO and Ethanol. Dapoxetine is a short acting SSRI drug, used for the treatment of premature ejaculation in men 18-64 years old[1-4]. The action mechanism of the drug is believed to be associated with neuronal serotonin reuptake and further serotonin production potentiation. The central neural ejaculatory network consists of a deeply integrated spinal and brain system. The sympathetic, parasympathetic, and somatic spinal centers act in conjunction to monitor physiological events occurring during ejaculation under the influence of combined sensory genital and cerebral stimuli at the spinal cord level. Experimental data indicates that serotonin (5-HT) plays an inhibitory role in ejaculation in brain downstream pathways[5-7]. As the present methods available are tedious, a rapid and less toxic solvent consumption method was planned to develop and the data obtained was found within the limits provided by ICH guidelines.

1. MATERIALS AND METHODS

Regent and Chemicals: Samples of dapoxetine and working reference standards of Impurities were acquired from RL Fine chemicals, Bangaluru, Karnataka. Acetonitrile (HPLC grade) purchased from Avra

chemicals Pvt. Ltd. Ortho-phosphoric acid (HPLC grade) used in the preparation of buffer of mobile phase purchased from Merck. Milli Pore water was obtained from Thermo Scientific system.

Instrumentation: This method was developed using Shimadzu LC 20AD equipped with UV-Visible detector. The data integration was done using the software Lab solution software. The samples were Sonicated and degassed usingUltrasonicator of model GT sonicand buffer pH was adjusted using pH meter Systronics. The API and formulation were weighed using Shimadzu digital balance.

Method development: The aim of the study was to develop a simple, rapid and robust method for the analysis of dapoxitine. In the development process a systemic procedure was employed like selection of solvent, column, buffer and detector to get an optimized result. From the standard solution linear concentrations are prepared in range of 2- 10 µg/ mL.The drug solution were injected in to the reverse phase chromatographic system with the below conditions.

Chromatographic conditions:

Column : Shim-pack (100 x 4.6 mm, 3.5µ)
Mobile phase : Acetonitrile: Buffer of ortho-phosphoric acid (85:15)
Flow rate : 1.0mL/min
Mode : Low pressure gradient
Injection volume : 10µl
LC stop time : 10 min
Wavelength : 291nm
Detector : UV detector at 291nm
Temperature : 30°C
Retention time : 1.98 min

Preparation of Mobile Phase: It consist of acetonitrile and 0.1% orthophosphoric acid (OPA) which is prepared by diluting 0.5ml of OPA in 500ml of water, the resulting solution has pH 4.2.

Preparation of Stock Solution: A standard stock solution of 100 µg/mLwere prepared by accurately weighing 10mg ofDepoxetineinto a 100mL volumetric flaskand 70mL of methanol was added,sonicated to dissolve and made upto the mark with methanol.

Preparation of linearity samples: The solutions were prepared from the above stock solution by diluting with methanol. Pipettedout 0.2, 0.4, 0.6, 0.8, 1.0 ml in 10ml volumetric flasks and make up the volume using methanol to get the concentration ranging from 2-10µg/mL of dapoxetine.

Marketed formulation sample Preparation: Dapoxetine tablets (Duralast®30 from Sun Pharma) was purchased from local pharmacy store. Ten tablets were weighed, powdered and quantity equivalent to 100 mg of Dapoxetine were loaded into 100ml volumetric flask, 70 mLof methanol was added, sonicated to dissolve and made upto the mark with methanol. The resulting solution was filtered through 0.45µ nylon filter and stored.

RESULTS AND DISCUSSION-

METHOD VALIDATION: The proposed method was validated as per ICH guidelines for accuracy, precision, robustness, linearity & range and Limit of detection (LOD), Limit of quantitation (LOQ) & system suitability. **Fig. 2-5** gives depiction of the chromatograms obtained after optimization of method.

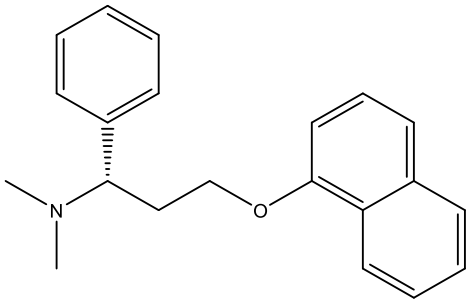

Fig 1: Structure of dapoxetine

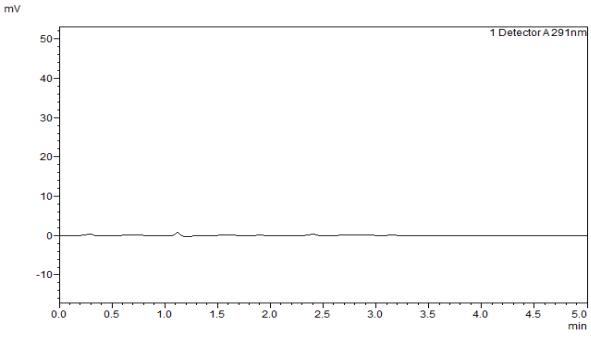

Fig. 2: LC graph of Blank

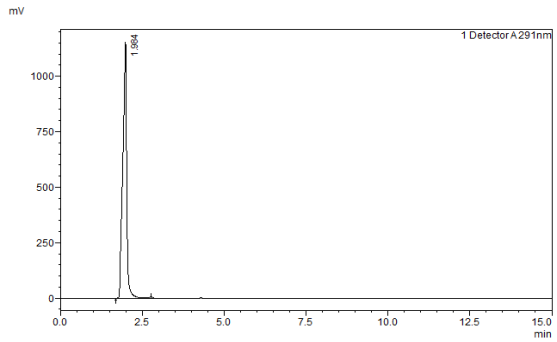

Fig. 3: LC graph of dapoxetine standard

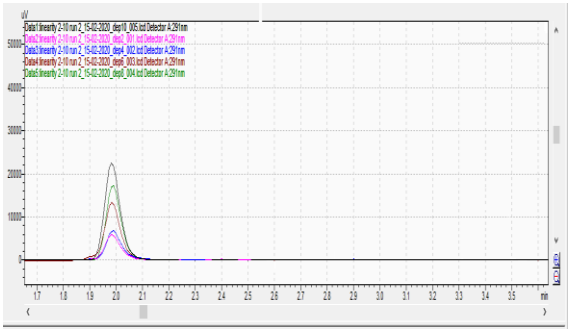

Fig. 4: Overlay chromatogram for dapoxetine standard

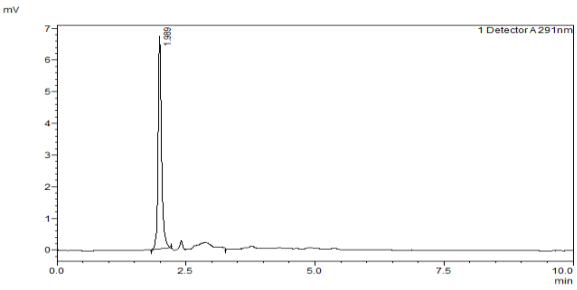

Fig. 5: LC graph of marketed formulation

i. **System suitability:** Reproducibility of chromatographic system was assessed by this test. To assess the effectiveness freshly prepared stock solutions are injected into system. The results of system suitability are tabulated in table 1.

Table 1: System suitability parameters

Parameters	**Results**
Retention time	1.98 min
Peak area	14988
Tailing factor	0.816
Theoretical plates	2451

ii. **Linearity:** The linearity of an analytical approach is the ability to deliver findings that are strictly proportionate to the analyte concentration of particular samples.It was performed as per ICH Q2 between the range of 2- 10 µg/ ml and as shown in the **table 2** and **fig. 6**. It is concluded that it is well between the acceptance criteria i.e., R^2 is 0.9966.

Table 2: **Dapoxetinelinearity data**

Concentration µg/ mL	Peak area
2	15004
4	30337
6	52197
8	73918
10	94389

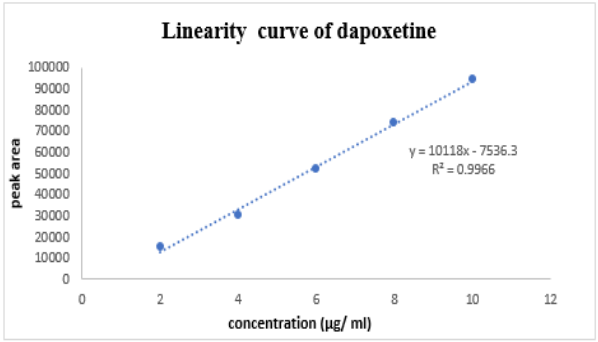

Fig. 6: Linearity graph for dapoxetine standard

iii. **Precision:** Precision of an analytical method was performed by using three different concentrations ie. 2, 6 and 10µg/ mL and performed six injections. The results for precision are given in the **table 3 & 4**.

iv. **Recovery:** It was achieved in three separate concentration levels, i.e. 50%, 100% and 150%, of standard solution for the drug.The results of recovery studies are tabulated in **table 5**.

Table 3: Intraday precision data of dapoxetine (n=6)

Concentration (µg/ ml)	Peak area	Concentration (µg/ ml)	Peak area	Concentration (µg/ ml)	Peak area
2	15004	6	52197	10	94389
2	15098	6	53284	10	93896
2	15158	6	52891	10	93289
2	15412	6	53208	10	95189
2	15306	6	53499	10	93689
2	14997	6	53698	10	95889
Average	15162.5		53129.5		94390.16667
Std deviation	152.4989071		485.8315037		897.5003095
% RSD	1.005763608		0.914428902		0.950840899

Table 4: Interday Precision Data of dapoxetine (n=3)

Concentration (µg/ ml)	Peak area	Concentration (µg/ ml)	Peak area	Concentration (µg/ ml)	Peak area
2	15101	6	52175	10	92484
2	15246	6	53462	10	93578
2	15218	6	52598	10	93005
2	15394	6	53468	10	94849
2	15325	6	53254	10	93787
2	14848	6	53272	10	94982
Average	15188.66667		53038.16667		93780.83333
Std deviation	177.3110888		483.8141574		904.4938579
% RSD	1.167390744		0.912200002		0.964476243

Table 5: Dapoxetine-Recovery data

Level of recovery (%)	Amount of formulation (µg/ ml)	Amount of Pure drug (µg/ ml)	Total amount of drug (µg/ ml)	Peak area	Difference	%Recovery	Mean
50	4	2	6	45451	30447	100.3625935	99.72311
	4	2	6	45257	30253	99.72311039	
	4	2	6	45931	30927	101.9448199	
100	4	4	8	61112	30775	101.4437815	100.8175
	4	4	8	60922	30585	100.8174836	
	4	4	8	61046	30709	101.2262254	
150	4	6	10	82856	30659	101.0614102	99.97693
	4	6	10	82527	30330	99.97692587	
	4	6	10	82855	30658	101.0581139	

Table 6: LOD and LOQ of dapoxetine

Parameter	Result
LOD	2.471 µg/ mL
LOQ	7.488 µg/ mL

Table 7: Robustness data of dapoxetine at 289nm

Wave length(nm)	Concentration (µg/ mL)	Peak area
289	6	51495
	6	50976
	6	52115
	Average	51528.66667
	Std deviation	465.6037896
	%RSD	0.903582064

Table 8: Robustness data of dapoxetine at 291nm

Wave length(nm)	Concentration (µg/ ml)	Peak area
293	6	51487
	6	52646
	6	52481
	Average	52204.66667
	Std deviation	511.9181792
	%RSD	0.980598502

Table 9: Ruggedness data of dapoxetine by changing analyst
By changing the analyst

C	T1	T2	Mean	SD	%RSD
1	51633	52698	77982	753.068722	0.965695573
2	52958	52116	79016	595.3839098	0.753497912
3	52156	52849	78580.5	490.0249994	0.623596184
4	52318	51658	78147	466.6904756	0.597195638
5	52415	51865	78347.5	388.9087297	0.496389457

Table 10: Ruggedness data of dapoxetine by changing the instrument
By changing the instrument

C	T1	T2	Mean	SD	%RSD
1	51495	52086	51790.5	417.9001077	0.806904949
2	50976	51478	51227	354.9676042	0.692930689
3	51498	52065	51781.5	400.9295449	0.774271786
4	52115	52769	52442	462.4478349	0.881827228
5	52345	51747	52046	422.8498551	0.812454089

Table 11: forced degradation study data of dapoxetine

Stress condition	% Assay
As such sample	94.75
Refluxed with 0.5N HCl solution for about 14 hrs	93.38
Refluxed with 0.5N NaOH solution for about 10 min	93.75
Refluxed with 15% Hydrogen peroxide for about 15 min.	94.02
Thermal Degradation	94.02

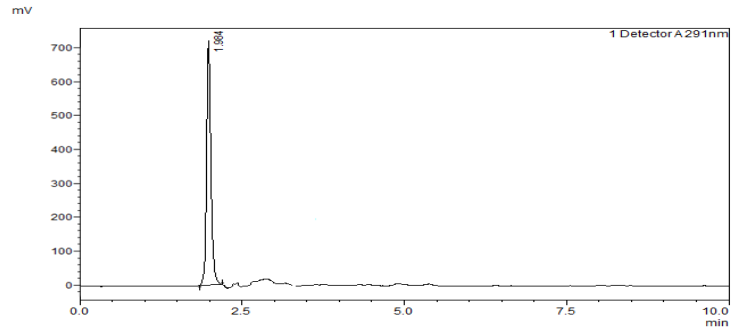

Fig. 7: LC graph of dapoxetine as such

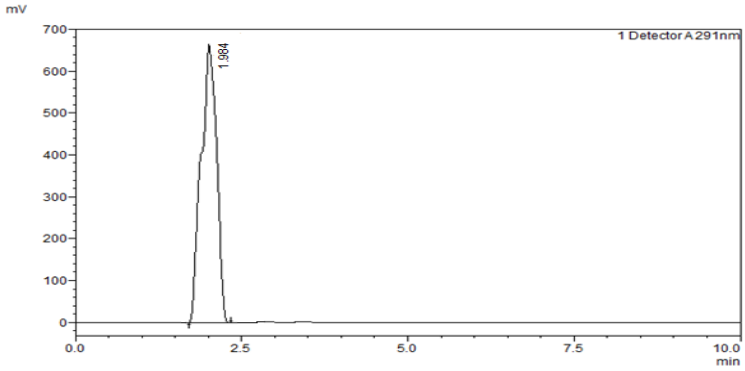

Fig. 8: LC graph of dapoxetine after acid degradation

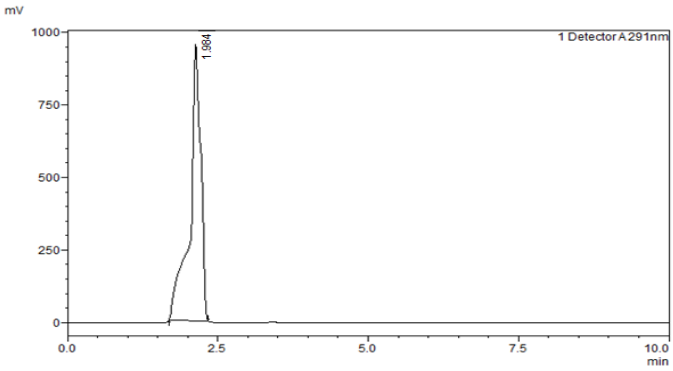

Fig. 9: LC graph of dapoxetine after base degradation

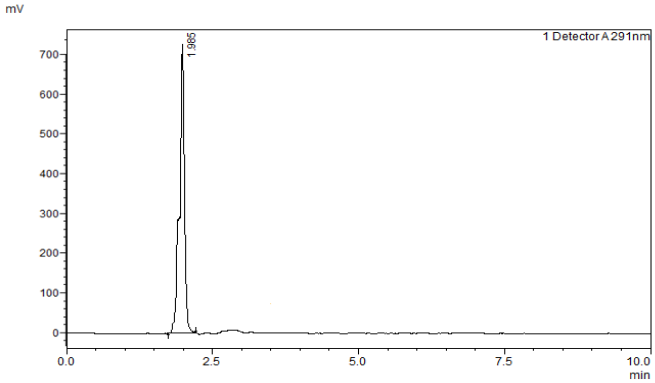

Fig. 10: LC graph of dapoxetine after peroxide degradation

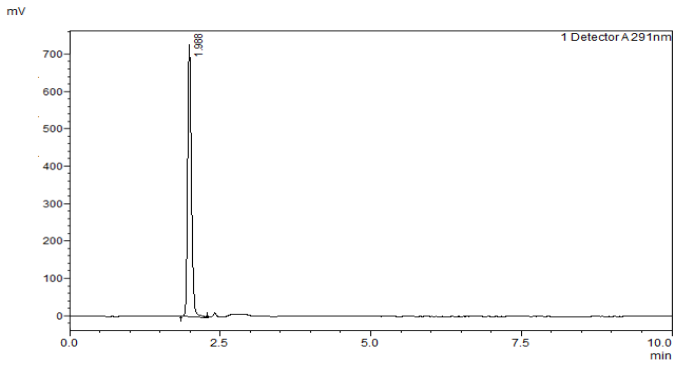

Fig. 11: LC graph of dapoxetine after thermal degradation

v. **Limit of detection (LOD) and Limit of quantification (LOQ):** Limit of detection is the lowest amount of the analyte that can be detected but not necessarily quantitative. Limit of quantification is the lowest amount of analyte that can quantitated. The LOD and LOQ arecalculated by using formula as follow. The results are shown in the **table 6.**

LOD = 3.3 x S.D. / slope, LOQ= 10 x S.D. / slope, Where, S.D. = standard deviation

Robustness: The ability of the analytical method to remain unaffected by small change in parameters. In this method robustness was performed by changing wavelength of drug 291nm, analyst and instrument. The results are given in **table 7& 8**.

Ruggedness: The analytical method's functionality remains uninfluenced while external variables such as researchers, equipment, instruments, reagents and days are intentionally modified. Through this way the researcher and instrument are switched and ruggedness is carried out. The results of ruggedness are tabulated in table **9 & 10**.

Forced Degradation Studies: The stress degradation experiments were conducted in compliance with ICH recommendations Q1A (R2). Stability tests of the dapoxetine drug were performed using a validated analytical method. The results of forced degradation studies are tabulated in **table 11**and dapoxetine was found to be slightly unstable in acid and alkali condition. Unstressed sample's chromatogram is depicted in **fig. 7**.

a. **Acid degradation studies:** The API and formulation was subjected to Reflux with 0.5N HCl solution for about 14 hrs. Following the 10µL of sample injected, the chromatogram obtained is in **fig. 8**.
b. **Basic Degradation Studies / Alkali Degradation Studies:** The API and formulation was subjected to Reflux with 0.5N NaOH solution for about 10 min. Following the 10µL of sample injected, the chromatogram obtained is in **fig. 9.**
a. Oxidation Degradation Studies: The API and formulation was subjected to Reflux with 15% Hydrogen peroxide for about 15 min. Following the 10µL of sample injected, the chromatogram obtained is in **fig. 10.**
b. **Thermal Degradation Studies:** The API and formulation was subjected to dry heated at 105°C for about 2 hrs. Following the 10µL of sample injected, the chromatogram obtained is in **fig. 11.**

CONCLUSION:

For simultaneous estimation in bulk of dapoxetine and formulation, a simple, fast and cost-effective HPLC method has been successfully developed. For the different experimental parameters the suggested procedure was designed and tested. An study of dapoxetine measured the effect of pH of the mobile phase, mobile phase ratio and the

flow rate. In less than 5 minutes, the analyte eluted. In order to assess the content of dapoxetine in routine and stability tests, quality control may easily use the method developed.

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