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Development and Validation of UV Spectrophotometric method for estimation of Dapoxetine HCL in bulk and dosage Form

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Abstract

Dapoxetine HCl, a selective serotonin reuptake inhibitor is a novel drug for premature ejaculation and no spectrophotometric method for its estimation has been reported yet. The aim of present work is to develop and validate simple, accurate, sensitive, reproducible and specific spectrophotometric method for the determination of Dapoxetine HCl, in bulk and its pharmaceutical formulations, using methanol as a solvent. The optimum conditions for the analysis of the drug were established and the developed method was validated with respect to linearity, accuracy (recovery), precision, robustness, ruggedness, LOD, LOQ and specificity. The maximum wavelength (λ max) was found to be 291 nm and a good linearity was observed in the concentration range of 5-60 µg/mL having regression equation, y = 0.0164x – 0.0071 with correlation coefficient of 0.9998. The percentage recovery of Dapoxetine HCl was found to be 99.5489 ±0.1599 and % CV (0.16; n=9) indicated a good precision of the analytical method. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.0239 µg/mL and 0.0724 µg/mL, respectively. Robustness and ruggedness of the method was performed by using different λ max, instruments, apparatus and analysts. The method was found to be simple, accurate, precise, reproducible, economical and robust. Analytical method validation was found to be within an acceptance criteria according to ICH Q2 R1 guidelines. The proposed method can be applied for routine quality control analysis of Dapoxetine HCl.

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INTRODUCTION:-

Dapoxetine hydrochloride is a new chemical entity approved by CDSCO on 13th November, 2010 for the treatment of premature ejaculation (PE) in men of 18 to 64 years[1]. PE is one of the commonly encountered male sexual disorders in clinical practice. In general community, PE has been estimated to occur in 4-39%

of men (McMahon CG, 2007)[2]. Dapoxetine is the only drug with regulatory approval for such treatment [3]. It is already approved for use in several European countries including Finland, Sweden, Portugal, Austria and Germany and is being considered for approval in other European countries. In United States, it is currently in Phase III trials of the Food and Drug Administration (FDA) approval process [3].

Dapoxetine HCl is a selective serotonin (5-HT) reuptake inhibitor and similar in structure to fluoxetine[4]. Chemically, it is the *S* enantiomer of {(+)-(*S*)-*N*,*N*-dimethyl-(α)-[2(1-naphthalenyloxy)ethyl]-benzenemethanamine hydrochloride} [Figure 1]. It is white to off-white crystalline solid having molecular weight 341.9 and molecular formula $C_{21}H_{23}NO{\cdot}HCl$[4].

Analysis is an important component in the formulation development of any drug molecule. A suitable and validated method has to be available for the analysis of drug(s) in the bulk, in drug delivery systems, from release dissolution studies and in biological samples. If a suitable method, for specific need, is not available then it becomes essential to develop a simple, sensitive, accurate, precise, reproducible method for the estimation of drug samples.

The methods for estimation of Dapoxetine HCl given in literature are Dissolution method, RP-HPLC methods and Bioanalytical method. Literature review shows that no spectrophotometric method has been reported so far for its estimation. Literature survey revealed that no method has been reported for the UV Spectrophotometric method development of Dapoxetine HCl and its validation. The UV spectrophotometric method is a very important tool in drug development and quality control. Dapoxetine is available in market at three different dose: 30mg, 60 mg and 90 mg. Various combinations of dapoxetine are also present in the market with tadalafil and sildenafil[8] which shows the immense need of further research in this field. Parameters to set up the UV spectrophotometric method should be researched and defined for drugs that do not possess official monographs [10]. The present paper describes the development and validation of UV spectrophotometric method for quality control of Dapoxetine HCl in bulk and its tablet dosage form.

MATERIALS & METHOD:

Instruments and Materials

Only AR grade reagents and solvents were used. The pure drug Dapoxetine HCl was obtained as a gift sample from Sunrise remedies Pvt. Ltd. Gandhinagar, with 100.03% w/w assay value and was used without further purification. The spectrophotometer used was Shimadzu UV Visible spectrophometer 1800 model with spectral bandwidth of 3 nm and wavelength accuracy (with automatic wavelength correction) of 0.5 nm, connected to computer loaded with UV Probe software. Digital weight balance of Sartorius (Model no.CD2250) and Sonicator of Trans-o-sonic (Model no. D120/1H) were also used. All the apparatus and instruments were calibrated and validated as per calibration and validation protocol specified before starting the experimental work.

Selection of media

Main criteria for media selection are solubility and stability i.e. drug should be soluble as well as stable for sufficient time in selected media. Though the literature reported media for this drug are water, methanol, chloroform, acetonitrile and 0.1 M HCl, but it gives the most accurate linearity in methanol compared to other solvents. Thus methanol has been selected as an analytical media for present research work.

Preparation of standard stock solution

Stock solution was prepared by dissloving 100 mg of Dapoxetine HCl in 100 mL of methanol to get 100 µg/mL solution. Stock solution was further diluted to

produce 100 µg/mL. This solution was further diluted for the preparation of calibration curve.

Preparation of calibration curve

The absorbance of the solutions containing Dapoxetine HCl at 10µg/ml was determined and absorbance maxima of Dapoxetine HCl was detected at 210 nm, 230 nm and 291 nm among which 291 nm [Figure 2] was chosen for its good linearity(correlation coefficient = 0.9998). Calibration curve was prepared in methanol at λmax 291 nm using Shimadzu UV Visible spectrophotometer 1800 model. For this, from the stock solution (100 µg /mL), serial dilutions of 10, 20, 30, 40, 50 and 60 µg /mL were prepared and absorbance was taken at λmax 291 nm. Averages of such 3 sets of values were taken for standard calibration curve, and solutions were scanned in the range of 200-400 nm against blank. The calibration curve was plotted. The optical characteristics are summarized in Figure 3.

Preparation of sample solution

The proposed method was successfully applied for the determination of Dapoxetine HCl in tablet dosage form. Ten tablets were weighed and powdered. The amount of tablet powder equivalent to 10 mg of Dapoxetine HCl was weighed accurately and transferred to 60 mL methanol and kept for 5 min in sonicator and volume was made up to 100 mL with the same solvent. The solution was then filtered through whatmann filter paper # 41. This filtrate was diluted suitably with methanol to get the solution of 10 µg/mL concentration .The absorbance was measured against blank. The drug content of the preparation was calculated using standard calibration curve. Amount of drug estimated by this method is given in Table 1.

Method validation

The UV spectrophotometric method used to analyze the Dapoxetine HCl samples in methanol as an analytical medium was validated for specificity, linearity, precision and accuracy, according to ICH Q2 R1 guidelines. All absorbance were determined at 291 nm.

RESULTS AND DISCUSSION

Linearity

The linearity of the response of the drug was verified at 5 to 100 µg/mL concentrations, but linearity was found to be between 5-60 µg/mL concentration. The calibration graphs were obtained by plotting the absorbance versus the concentration data and were treated by linear regression analysis (Table 5). The equation of the calibration curve for Dapoxetine HCl obtained y = 0.0164x - 0.0071, the calibration curve was found to be linear in the aforementioned concentrations. The correlation coefficient (r^2) of determination was 0.9998.

Precision

Assay of method precision (intra-day precision) was evaluated by carrying out three independent assays of test samples at three concentrations of Dapoxetine HCl. The intermediate precision (inter-day precision) of the method was also evaluated by carrying out the assay on three consecutive days in the same laboratory. The relative standard deviation (RSD) and assay values obtained for three concentrations (10 µg/mL, 15 µg/mL and 20 µg/mL) were 0.36, 99.16 ; 0.21, 99.41 and 0.29, 99.33 respectively (Table 2). All the data are within the acceptance criteria of 2%.

Accuracy (Recovery study)

The accuracy of the method is the closeness of the measured value to the true value for the sample. Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of pure drug to its tablet formulation. The recovery was performed by preparing concentration of 10 µg/mL of Dapoxetine HCl sample solution. Three samples were

prepared for each recovery level of 80%, 100% and 120% spiking of standard solution. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The accuracy was calculated as the percentage of the drug recovered from the formulation matrix. The percentage recoveries obtained [Table 3] were considered acceptable [8].

LOD & LOQ

The absorbance of ten solutions containing 10 µg/mL were measured at 291 nm and calculated according to equation of LOD [3.3 x MSD/ average response] and LOQ [10 x MSD/ average response]. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.0239 µg/mL and 0.0724 µg/mL, respectively.

Robustness and Ruggedness

It were performed by using different instrument, apparatus, λmax, and analysts .Result were expressed in %CV [Table 4].

Determination of Active Ingredients in Tablets

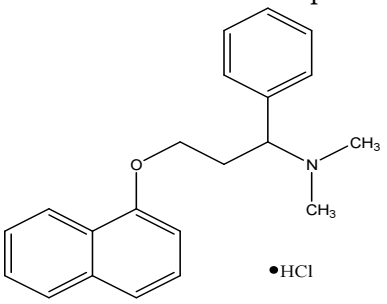
The validated method was applied to the determination of Dapoxetine HCl in Tablets. Six tablets were assayed and the results are shown in (Table no. 1) indicating that the amount of drug in tablet samples met with requirements (99–102% of the label claim).

The development of a simple, rapid, sensitive, and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary tedious sample preparations, the cost of materials and labour. Dapoxetine HCl is a UV absorbing molecule with specific chromophores in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectrophotometric method. The stock and sample solutions of Dapoxetine HCl were prepared in methanol. The λmax of the drug for analysis was determined by taking scans of the drug sample solutions in the entire UV region. The absorption spectrum of Dapoxetine HCl in methanol shows three λmax at 210 nm, 230 nm and 291 nm [Figure 2], among which 291 nm was selected for its good linearit [r^2= 0.9998]. Calibration curve data was constructed in the range of the concentrations of 5-100µg/ml, but Beer's law obeyed in concentration range of 5-60 µg/ml. The regression equation was found to be y= 0.0164x - 0.0071 with correlation coefficient (r^2) of 0.9998. Performing replicate analyses of the standard solutions was used to assess the accuracy, precision, and reproducibility of the proposed method. The selected concentration within the calibration range was prepared in methanol and analyzed with the relevant calibration curve to determine the intra and inter day variability. The proposed method can be successfully applied for assay in tablet dosage forms without any interference. The assay showed that the drug content of this product to be in accordance with the label claim (Table 1). The repeatability as well as interday precision was found to be within the specified limits[8] [Table 2].The recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy of the method (Table 3). The obtained results demonstrate the validity and accuracy of the proposed method for the determination of drug in tablet. In order to check the accuracy and precision of the developed method and to prove the absence of interference by excipients, recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulations of all drugs. It was found that the sample solution was stable up to 24 hr in which no decomposition was observed. These results reveal that the developed method have an adequate precision and accuracy, and consequently, can be applied to the determination of Dapoxetine HCl tablet in pharmaceuticals without any interference from the excipients.

CONCLUSION

A spectrophotometric method for quantifying Dapoxetine HCl in formulation samples has been developed and validated. The proposed methods can be successfully employed for Dapoxetine HCl estimation both in Bulk and in tablet dosage forms without any interference in quality control. Analysis by this method was found to be simple, accurate, reproducible, reliable, precise, and in good agreement with labelled claim of the drug. The sample solution was stable for 24 hr. In summary, the proposed method can be used for the drug analysis in routine quality control.

Figure 1: Structural formula of Dapoxetine HCL



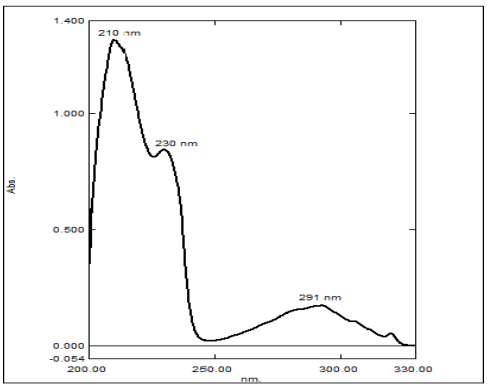


Figure 2: Absorption Spectrum of Dapoxetine HCL

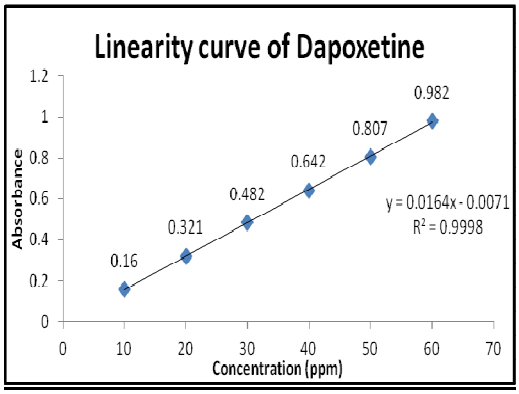


Figure 3: Linearity curve of Dapoxetine HCl in methanol

Table 1: Determination of active ingredients in Dapoxetine HCl tablets

Sample	Label Claim (mg)	Amount found (mg) per tablet	% Label Claim*
Dapoxetine HCl	60 mg	59.6866 ± 0.4007	99.4777 ± 0.6679

(*Average of six determinations)

Table 2: Precision data of Dapoxetine HCL

Sr No	Sample solution concentration (µg/mL)	% Assay Value		% CV		Mean % CV	
		Intraday	Interday	Intraday	Interday	Intraday	Interday
1	20	99.4807± 0.1798	98.6884±0.9345	0.1807	0.9463	0.2093	0.9566
2	30	99.5850±0.2074	98.8243± 1.0442	0.2074	1.0566		
3	40	99.5846±0.2379	98.9616± 0.8578	0.2379	0.8668		

Table 3: Accuracy data for Dapoxetine HCL

Sr. No.	Amount of Drug in formulation (µg/mL)	Amount of drug spiked (µg/mL)	Total Amount (µg/mL)	Amount Recovered (µg/mL)	Mean % Recovery (n = 9)
1	10	8 (80%)	18	17.9166	99.5489 ± 0.1599
2	10	10(100%)	20	19.9166	
3	10	12(120%)	22	21.8958	

Table 4: Robustness & Ruggedness data for Dapoxetine HCL

1. Change in λmax				
λmax (nm)	**Concentration (µg/mL)**	**% Assay**	**SD**	**% CV**
289	10	98.125	0.625	0.6329
291	10	99.375		
293	10	98.750		
2. Change in Instrument				
UV Shimadzu Model No.	**Concentration (µg/mL)**	**% Assay**	**SD**	**% CV**
1800	10	99.375	0.3125	0.3154
2450	10	98.750		
3.Change in Apparatus (Volumetric Flask)				
Volumetric Flask	**Concentration (µg/mL)**	**% Assay**	**SD**	**% CV**
50 mL	10	96.875	1.2500	1.2738
100 mL	10	99.375		
4. Change in Analysts				
Analyst	**Concentration (µg/mL)**	**% Assay**	**SD**	**% CV**
Analyst- I	10	99.375	0.9375	0.9523
Analyst- II	10	97.500		

Table 5: Regression Analysis Data And Summary Of Validation Parameters

Sr no.	Validation Parameter	Result
1	Absorption maxima (nm)	291
2	Linearity range (µg/ml)	5- 60
3	Standard Regression equation	y = 0.0164 x - 0.0071
4	Correlation coefficient (R^2)	0.9998
5	LOD (µg/mL)	0.0239
6	LOQ (µg/mL)	0.0724
7	% Recovery (Accuracy, n = 9)	99.5489±0.1599
8	Precision (%RSD) Intraday (*n*= 9) Interday (n= 9)	 0.2093 0.9566
9	Robustness & Ruggedness (%RSD) Change in λmax Change in Instrument Change in Apparatus (Vol. Flask) Change in Analysts	 0.6329 0.3154 1.2738 0.9523
10	Specificity	A 10 µg /mL solution of candidate drug in methanol at UV detection λ of 291 nm will show an absorbance value of 0.160 ± 0.022

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