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Validated UV spectrophotometric method for quantitative analysis of paroxetine in bulk and pharmaceutical dosage form

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ABSTRACT

A simple, precise, accurate, economical and reliable UV spectrophotometric method has been developed for the estimation of Paroxetine in tablet dosage form. The Paroxetine shows maximum absorbance at 293 nm in water and obeys Beer's law in the concentration range of 2-10 µg /mL with good correlation coefficient (r^2 = 0.9992). The results of analysis were validated by recovery studies. The percentage recovery method was found to be 99.53-100.41 %. The relative standard deviation was found to be < 2.0 % in all cases. The Proposed spectrophotometric method was validated as per the ICH Q2 (R1) guidelines. The method was successfully applied to pharmaceutical formulation because no chromatographic interferences from the tablet excipients were found. The proposed method was found to be accurate and reliable for routine quantification of Paroxetine in bulk form and pharmaceutical formulations.

Key words: Paroxetine, UV spectrophotometry, Validation, Tablet dosage forms.

INTRODUCTION

Paroxetine is chemically known as (3S, 4R) - 3-[(2H-1, 3-benzodioxol-5-yloxy) methyl]-4-(4-fluoro phenyl) piperidine[1,2]. Paroxetine is developed specifically for the treatment of depression, generalized anxiety disorder (GAD), panic disorders, and post traumatic stress disorder (PTSD) [3] and premenstrual dysphoric disorder (PMDD). Paroxetine acts by inhibiting reuptake up of selective serotonin neurotransmitter [4]. Paroxetine was the first anti-depressant [5] for the treatment of panic disorders.

Literature survey revealed that not many analytical methods published to describe the quantification of Paroxetine in biological fluids includes UV- Spectrophotometric [6], HPLC[7-11], TLC, HPTLC [12] and Ultra Performance Liquid Chromatography [13]. The target of this study is to develop a new, simple and fast analytical method by UV spectrophotometric method to quantify Paroxetine in bulk and its tablet dosage forms. However the requirement of fast, precise, very simple, efficient, time saving and highly reliable analytical UV- Spectrophotometric method for routine quality control purpose always necessities to see a new and better method. Hence, it was proposed to develop a simple, trouble-free, fast, perfect, and sensitive UV method for the concurrent estimation of Paroxetine in pure form and pharmaceutical formulations. This work describes the validation parameters stated by the International Conference on Harmonization [ICH] guidelines Q2 (R1). Figure 1 shows chemical structure of Paroxetine.

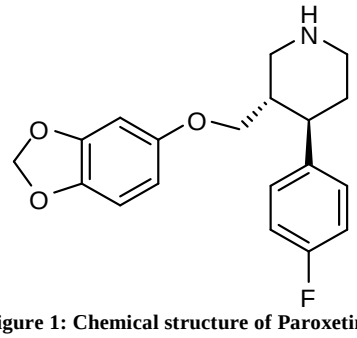

Figure 1: Chemical structure of Paroxetine

MATERIALS AND METHODS

Selection of solvent

A number of trails were done to find out the ideal solvent system for dissolving the drug. The solvents such as double distilled water, acetonitrile and methanol were tried based on the solubility of the drug. Maximum absorption of the drug was found to be 293 nm in double distilled water. So distilled water was selected as optimized solvent in this spectrophotometric method.

Instruments used

ELICO Double beam SL 210 UV-VIS spectrophotometer was used to record the absorption spectra. Spectrophotometer with 1 cm matched quartz cells were used for the estimation of Paroxetine.

Reagents and Materials

Paroxetine standards obtained as a gift sample from Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India. Parotin tablets containing 10 mg of Paroxetine tablets are obtained from local pharmacy. Analytical grade double distilled water used throughout the experiment was given by Vignan Pharmacy College, Vadlamudi, Guntur Dist.

Selection of detection wavelength:

Appropriate dilutions of Paroxetine were prepared from the standard stock solution. Utilizing ELICO Double beam SL 210 UV VIS spectrophotometer, the dilutions of Paroxetine were scanned in UV range of 200 - 400 nm using double distilled water as a blank. It was observed that the drug showed maximum absorbance at 293 nm which was selected as the detection wavelength for the estimation of Paroxetine. The spectrum of Paroxetine is shown in Figure 2.

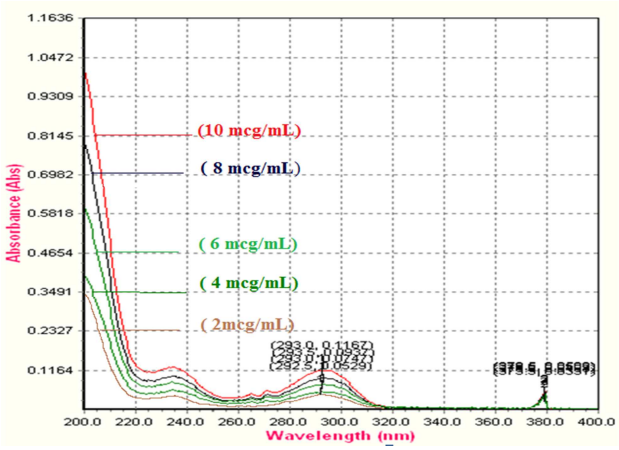

Figure 2: UV Spectrum of Paroxetine

Preparation of standard drug solutions:

An accurately weighed 10 mg of Paroxetine pure drug was dissolved and transferred in 10 mL volumetric flask containing 7.5 mL double distilled water and sonicated well. Then the volume was adjusted up to the mark with

double distilled water to obtain the stock (primary) solution of 1000 µg/mL. From the above stock solution, secondary standard solution with a concentration of 100 µg/mL was prepared. Aliquots of 0.2 to 1.0 mL portions of standard solutions were transferred to a series of 10 mL volumetric flasks and volume in each flask was adjusted to 10 mL with double distilled water to get the working standard solutions.

Preparation of Calibration curve:

Aliquots of standard drug (0.2 mL to 1.0 mL) solution in double distilled water were transferred into a series of 10 mL volumetric flasks and the solution was made up to 10 mL with water. After setting the instrument for its spectral properties the solutions were scanned in the wavelength ranging from 200 nm - 400 nm. The wavelength of maximum absorption for Paroxetine was found at 293 nm. Calibration data is presented in Table 1. Calibration curve was prepared by plotting concentration of Paroxetine on X-axis and their respective absorbance's on Y-axis. The calibration curve is shown in Figure 3. The optical characteristics are presented in Table 2.

Table 1: Linearity data for Paroxetine

Concentration(µg/mL)	Absorbance
0	0
2	0.0369
4	0.0529
6	0.0747
8	0.094
10	0.118

Table 2: Optical characteristics, regression data of the proposed method

Parameter	Result
λmax (nm)	293
Beer's law limits (µg / mL)	2-10
Molar absorptivity (L.mole^{-1} cm $^{-1}$)	3989.996
Sandell's sensitivity (µg/cm2/0.001 absorbance unit)	0.082531
Regression equation (Y= a+bc) ; Slope is	0.01171
Standard deviation of slope (S_b)	0.000164621
Intercept (a)	0.0014
Standard deviation of intercept (S_a)	0.000996828
Standard error of estimation (S_e)	0.001377316
Correlation coefficient (r^2)	0.9992

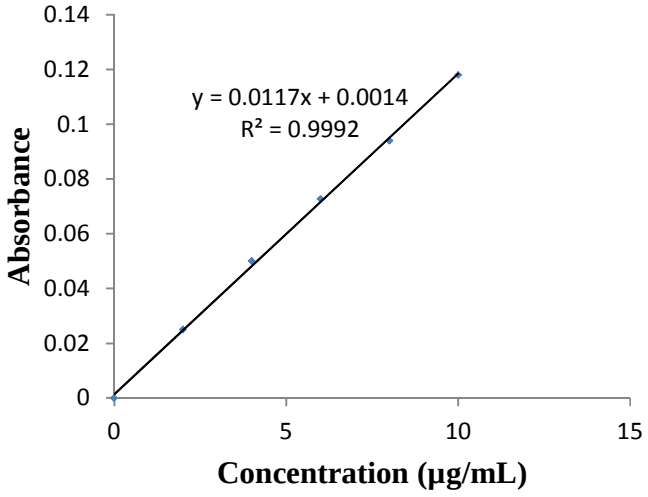

Figure 3: Calibration curve of Paroxetine by UV method

Noise and smoothing:
Noise is an unwanted (or) unknown signal i.e., received by the detector in UV spectrophotometry. Paroxetine standard solutions were scanned using ELICO SL-210 spectrophotometer and the spectrograms were analyzed using Spectra treats software version 3.3. Paroxetine standard solutions having a concentration range of 2- 10 mcg/ mL were scanned and from the spectrum it is observed that the frequency of noise increased with decrease in concentration. In order to reduce the noise and the increase the smoothing box car method was applied. The spectrum before and after smoothing are given in figures 4 and 5. From the figure it is observed that noise is reduced and the spectrum was observed to be a smooth line. Reduction of noise and smoothing of spectrogram will provide good correlation and regression analysis during development of calibration curves.

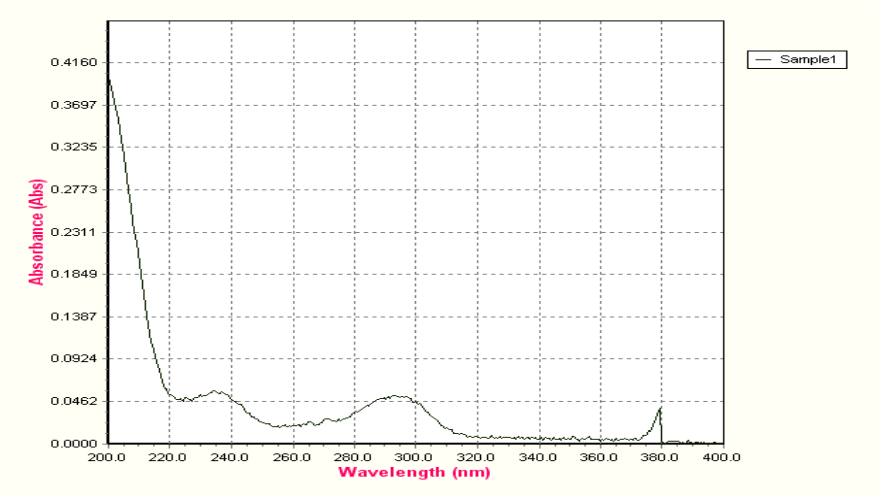

Figure 4: Paroxetine at 4 mcg/mL before box car smoothing using spectra treats® software

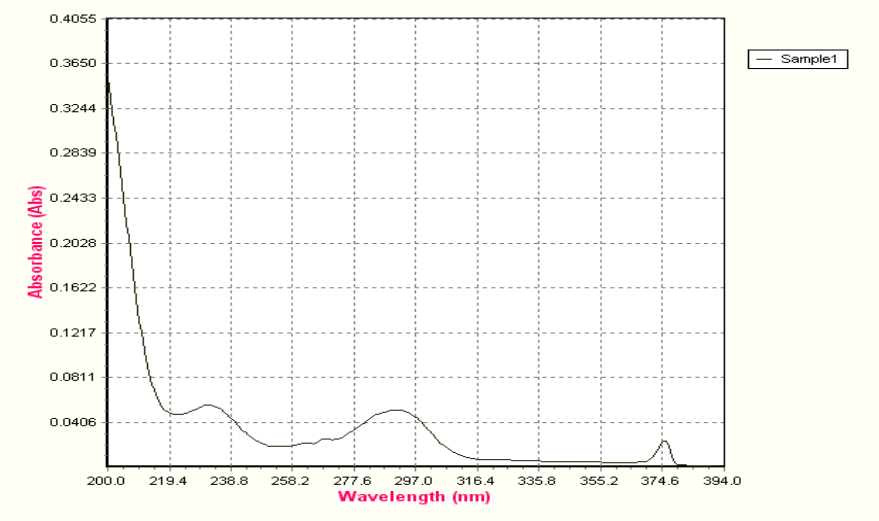

Figure 5: Paroxetine at 4 mcg/mL after box car smoothing using spectra treats® software

Validation of the developed method: [14,15]
The proposed UV method of analysis was validated in pursuance of ICH Q2 (R1) for the parameters like system suitability, specificity, linearity, precision, accuracy, and robustness, limit of detection (LOD) and limit of quantitation (LOQ).

Precision:
The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. Precision was

determined by intra-day and inter-day study. The repeatability of the method was evaluated by carrying out the assay 3 times on the same day and intermediate precision was evaluated by carrying out the assay on 3 consecutive days for the sample solution. The percent relative standard deviation (% RSD) was calculated. The results obtained are given in Table 3.

Table 3: Results of precision study

Parameter	Intra-day	Inter-day		
		Day -1	Day -2	Day -3
Mean % recovery	0.73	0.725	0.727	0.733
SD	0.00144	0.001419	0.00153	0.00155
% RSD*	0.198	0.1944	0.2104	0.2114

**average of 6 determinations*

Accuracy (Recovery studies):

The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted true value. Accuracy studies were performed at three different levels (50%, 100% and 150%) by standard addition method and the samples were analyzed in triplicate by the proposed method. The recovery studies were carried out by adding known amount of pure drug Paroxetine at 50%, 100% and 150% of preanalyzed formulation and the proposed method was followed. From the amount of Paroxetine found, % recovery was estimated. The results obtained are given in Table 4.

Table 4: Results of accuracy study

Recovery levels %	Amount of standard drug solution added (µg/mL)	Amount of the drug formulation added (µg/mL)	Amount recovered (µg/mL)	Mean Percent recovery ±SD $	% RSD*
50 %	3	5	7.98	99.75±0.213	0.198
100 %	6	5	10.98	99.81± 0.205	
150 %	9	5	13.98	99.85±0.173	

$ = Standard deviation; ******* *= Average of six determinations*

Ruggedness

Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents and so on. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method. The results obtained are shown in Table 5.

Table 5: Ruggedness results

Parameter	Instrument-1 (Systronics model 2203)	Instrument-2 (Elico SL 159)	Analyst -1	Analyst -2
Mean	0.73	0.735	0.73	0.735
SD*	0.00144	0.00162	0.00144	0.00162
% RSD#	0.198	0.220	0.198	0.220

** = Standard deviation; % RSD# = % Relative standard deviation*

Robustness

According to ICH the robustness is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. The most important aspect of robustness is to develop methods that allow for expected variations in the separation parameters. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be 0.within the method's robustness range. The absorbance was measured and assay was calculated for six times. The results of robustness are presented in Table 6.

Table 6: Results for Robustness study

Parameter	λ_{max} 1	λ_{max} 2
Mean	0.73	0.735
SD*	0.00144	0.00162
% R.S.D#	0.198	0.220

** = Standard deviation; % RSD# = % Relative standard deviation*

LOD and LOQ:
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Limit of Detection and Limit of Quantitation were calculated using following formula LOD = 3.3(SD) / S and LOQ = 10 (SD) / S, where SD = standard deviation of response (absorbance) and S = slope of the calibration. The results of LOD and LOQ are shown in Table 7.

Table 7: Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Parameter	Results
Limit of Detection (LOD)	0.38814 µg/mL
Limit of Quantitation (LOQ)	1.17618 µg/mL

Procedure for assay of pharmaceutical formulations:
Twenty tablets of Paroxetine (Parotin) marketed formulations were weighed and powdered in glass mortar. A quantity of tablet powder equivalent to 100 mg of Paroxetine was transferred to 100 mL volumetric flask and ultrasonicated for 20 minutes and volume was made up to the mark with distilled water. The solution was then filtered through a Whatman filter paper No 41. The filtrate was appropriately diluted further to obtain concentration in between linearity range. The absorbance of the resulting solution was measured at 293 nm and the amount of Paroxetine was determined by referring to the calibration plot. Assay results are presented in Table 8.

Table 8: Assay results of Paroxetine

S.NO.	Formulation	Labeled amount	Amount found *(mg) (mean ± SD)	% Assay	% RSD*
1	Parotin	10 mg	9.987 ± 10	99.87	0.226

** Average of six determinations.*

RESULTS AND DISCUSSION

For the selection analytical wavelength, Paroxetine solution were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 - 400 nm by ELICO Double beam SL 210 UV-VIS spectrophotometer. The λ_{max} of 293 nm was selected for the determination of Paroxetine and the absorption maxima curve was shown in Figure 2. The calibration curve for Paroxetine were prepared in the concentration range of 2-10 µg/mL. The proposed method obeyed Beer's law in the concentration range of 2-10 µg/mL with good correlation coefficient of r^2 = 0.9992. Calibration data is presented in Table 1. Beer's law range was confirmed by the linearity of the calibration curve of Paroxetine is shown in Figure 3**.** The optical characteristics and the data concerning to the proposed method is represented in Table 2. Accuracy studies were carried out by recovery study using standard addition method at three different concentration levels (50, 100 and 150 %). The known amount of standard drug solution of Paroxetine to pre-analyzed tablet sample solution at three different concentration levels. The resulting solutions were analyzed by the proposed methods. The recovery study results was found to be in the range of 99.53 to 100.41 percentages with percentage RSD less than 2 (Table 4). The same solutions of recovery study was further determined on same day at three different times and on three different days for intra-day and inter-day precision study. The precision of the method was found to be good with % RSD less than 2 which indicates that the method was precise and the results are presented in Table 3**.** Ruggedness was performed by changing two different analysts and two instruments and the results are tabulated in Table 5. It reveals that the proposed method was found to be rugged. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influences of the variables were determined. The absorbance was measured and assay was calculated for six times. The results of robustness are presented in Table 6. The results are within the specified limits which states that this method is robust. The LOD and LOQ were found to be 0.38814 µg/mL and 1.17618 µg/mL respectively which shows that this method was very sensitive as they were within the permitted levels. The LOD and LOQ results are shown in Table 7. The developed method was eventually utilized in analysis of tablet formulation and were found to be within the proposed limits and also the mean % assay value was found to be 99.93 %. The assay results are given in Table 8. The developed method has good linearity, accuracy and precision results indicates that the high quality of the method.

CONCLUSION

The developed and validated UV spectrophotometric method was found to be economical due to the use of double distilled water as a solvent throughout the experiment. None of the usual excipients employed in the formulation of Paroxetine dosage forms interfered in the analysis of Paroxetine by the proposed method. The system suitability parameters and system precision are determined and found within the limits. The plot is drawn between the concentration and absorbance which is found to be linear in the concentration range of 2 - 10 µg/mL with good correlation coefficient greater than r^2 = 0.9992. Low % relative standard deviation and high percent of recovery indicates that the method is highly precise and accurate. Thus, the developed method for Paroxetine was found to be simple, precise, accurate and cost effective and it can be effectively feasible for routine sample analysis of Paroxetine in pharmaceutical dosage forms.

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