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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF PAROXETIN HYDROCHLORIDE BY UV SPECTROPHOTOMETRY AND RP- HPLC IN ITS PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, reliable, sensitive, precise, rapid, and reproducible RP - HPLC method was developed and validated for the determination of Paroxetine in pharmaceutical dosage form. Separation was achieved under optimized chromatographic condition on a EZchrom C18 isocratic column, (250 mm × 4.6 mm i.d., particle size 5 µm, maintained at ambient temperature). The mobile phase consisted of phosphate buffer at pH 6.0, acetonitrile in the ratio 60: 40 v/v. An isocratic elution at a flow rate of 1 mL/ min at ambient temperature and using ELICO SL 218 UV-Visible detector to monitor the eluate at 294 nm. The retention time of Paroxetine is found to be 3.20 min and the calibration curve was linear function of drug in the concentration range of 20-100 µg/ mL (r2 = 0.9999). The limit of detection and the limit of quantification was found to be 0.675 µg/mL and 0.795 µg/mL respectively. The recovery (Accuracy) studies were performed and the percentage recovery was found to be 98.60 ± 0.6216%. Analytical validation parameters such as selectivity, specificity, linearity, precision and accuracy were studied and % RSD value for all key parameters was less than 2%. Thus the developed reversed phase HPLC method was found to befeasible for the determination of Paroxetine in bulk and pharmaceutical formulations.

KEYWORDS: RP - HPLC, Paroxetine, Validation, ICH guidelines.

INTRODUCTION

Paroxetine Chemically, (3S, 4R) - 3-[(2H-1, 3-benzodioxol-5-yloxy) methyl]-4-(4-fluoro phenyl) piperidine.[1] Paroxetine drug act by inhibiting reuptake up selective serotonin neurotransmitter. It was the first anti-depressant officially approved in the United States for the treatment of panic attacks. Paroxetine is used to treat depression, panic disorder, generalized anxiety disorder (GAD), premenstrual dysphoric disorder (PMDD), obsessivecompulsive disorder (OCD), social anxiety disorder (also called as social phobia), and post traumatic stress disorder (PTSD). Paroxetine belongs to a group of medicines known as selective serotonin reuptake inhibitors (SSRIs). Paroxetine acts by increasing the activity of the chemical called serotonin in the brain. A thorough review of literature states that few methods such as UV-Spectrophotometric[2-5], HPLC[6-10], HPTLC[11], LC-MS[12-13] and UPLC[14] methods have been reported for the determination of this drug in pharmaceutical dosage forms and biological fluids. However most of the available methods have limitations such as poor resolution, long run time, uneconomical and low sensitivity. So based on the above mentioned reasons infact an attempt has been made to develop a simple, precise, accurate, reproducible and robust RP HPLC method for the determination of Paroxetine in pharmaceutical dosage form. Figure 1 shows the chemical structure.

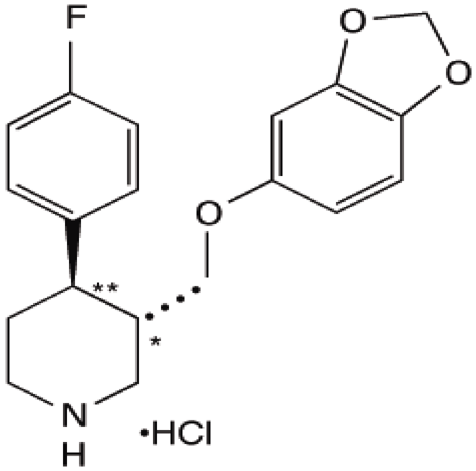

Figure 1: Chemical structure of Paroxetine.

MATERIALS AND METHODS

Chemicals and reagents

An analytically reference standard was kindly gifted by Mylon laboratories pvt.ltd, Hyderabad India. All the chemicals were analytical grade. HPLC grade acetonitrile was obtained from Merck pharmaceuticals private Ltd., Mumbai, India. water utilized were of HPLC grade and purchased from Merck specialties private Ltd., Mumbai, India. Commercial tablets of Paroxetine formulation were procured from local pharmacy. Parotin 20 mg containing with labeled amount of 10 mg per tablet is manufactured by IPCA laboratories Ltd.

Instrumentation and Apparatus

A HPLC (Agilent technology) consisting of uv detector, symmetry shield C_{18} 250mm×4.6mm,5 µm packing of octadecylsilane chemically bonded to porous silica particles and ezochrome 2 software with computer. The wavelength was determined at 294 nm using UV-Vis detector. A manually operating sample injector with 20 µL fixed sample loop was equipped with the HPLC system.

Preparation of reagents and standards

Mobile phase

Preparation of phosphate buffer pH 6.0

A solution of 13.609 gm of potassium Dihydrogen phosphate was dissolved in 400 ml of HPLC grade water, mix well by using sonicator, and make up the volume to 500 ml with water (Solution-A). A solution of 0.8gm of sodium hydroxide was dissolved in 80 ml of HPLC grade water, mix well by using sonicator, and make up the volume to 100 ml with water (solution-B). The solutions A and B were mixed together and final volume was adjusted to 1000ml with HPLC grade water. The above solution was filtered through a 0.45µ membrane filter and degassed by ultra sonicator.

Preparation of mobile phase

The mobile phase is composed of a mixture of Phosphate buffer (pH-6) and acetonitrile in the ratio of 60:40. For its preparation 600 ml of Phosphate buffer (pH-6) and 400ml of acetonitrile, were mixed well, allowed to equilibrate to room temperature, filtered through 0.45 µm pore size filter and sonicated for 15 min.

Preparation of diluent

Phosphate buffer and Acetonitrile (HPLC grade) in the ratio of 60:40 (v/v) were mixed well and sonicated for 15 minutes. The prepared solution was used as diluent.

Preparation of standard stock and working standard of drug solution

For analysis 1000 µg/ml standard Paroxetine solution was prepared by dissolving 25 mg of drug in to 25 mL of mobile phase and sonicated for 5 minutes then filter with vacuum filtration kit through 0.45 µ millipore filter paper and required concentrations solutions containing 20, 40, 60, 80 and 100 µg/mL of Paroxetine were prepared finally.

Preparation of sample solution for tablets assay

Twenty tablets of Parotin were correctly weighed, crushed and finely powdered. A portion of the powder equivalent to the weight of 10 mg was accurately weighed into 100 ml volumetric flask and 20 mL of mobile phase was added to flask and sonicated for 20 minutes to complete dissolution of drug. It was filtered through whatman filter paper no.42 to remove insoluble materials. The volume of filtrate was diluted to 100 ml with mobile phase (100 µg/mL). The above prepared solution was further diluted to get required concentrations then analyzed following the proposed procedures. The content of the tablet was calculated from plotted calibration graph or using regression equation.

Validation of analytical method

The proposed RP-HPLC method of analysis was validated in pursuance of ICH Q2 (R1) guide lines for the parameters like system suitability, specificity, linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

System suitability

The chromatographic systems used for analysis must pass system suitability limits before sample analysis can commence. Set up the chromatographic system allow the HPLC system to stabilize for 40 minutes. Inject blank preparation (single injection) and standard preparation (six replicates) and record the chromatograms to evaluate the system suitability parameters like resolution (NLT 2.0), tailing factor (NMT 1.5), theoretical plate count (NLT 3000) and % RSD for peak area of six replicate injections of Paroxetine standard NMT 2.0. The parameters such as tailing factor, % RSD and theoretical plates were studied and found satisfactory. The system suitability data and the optimum chromatographic conditions are reported in Table 1.

Table 1: Optimum chromatographic conditions and system suitability data.

parameters	**Chromatographic conditions**
Instrument	Agilent technologies LC compact
Column	EZchrom C18 Column (4.6 mm i.d. X 250 mm, 5 µm particle size)
Detector	UV detector
Diluents	10 mM Phosphate Buffer (pH-6.0): Acetonitrile (60 : 40 v/v)
Mobile phase	10 mM Phosphate Buffer (pH-6.0): Acetonitrile (60 : 40 v/v)
Flow rate	1 mL/min.
Detection wave length	UV at 294 nm
Run time	10 minutes
Temperature	Ambient temperature (25 oC)
Volume of injection loop	20 µL
Retension time (t_R)	3.20 min
Theoretical plates (Efficiency)	13162
Tailing factor (asymmetry)	1.2

Linearity

Under proposed experimental conditions, the relationship between the area and concentration of Paroxetine was studied. Linearity was checked by preparing standard solutions at 5 different concentration levels of Paroxetine. Standard solutions (20, 40, 60, 80, 100 µg/mL) of Paroxetine were injected into the HPLC system to get the chromatograms. The average peak area and retention time were recorded. The calibration curve was constructed between concentration versus peak area by the prepared concentration of 20-100 µg/mL of stock solution. The linearity range was found to be 20-100 µg/mL and the results are presented in Table 2. The calibration graph of Paroxetine is presented in Figure 2. The standard chromatograms of Paroxetine calibration standards are depicted in Figure 3 to Figure 7.

Table 2: **Calibration data of the proposed method for the estimation of Paroxetine.**

S.No	**Concentration µg/mL**	**Retention time, (t_R) min**	**Peak area,mV.s**
1.	0	0	0
2.	20	3.20	4409845
3.	40	3.22	8102253
4.	60	3.21	12365316
5.	80	3.20	16150514
6.	100	3.20	20648422

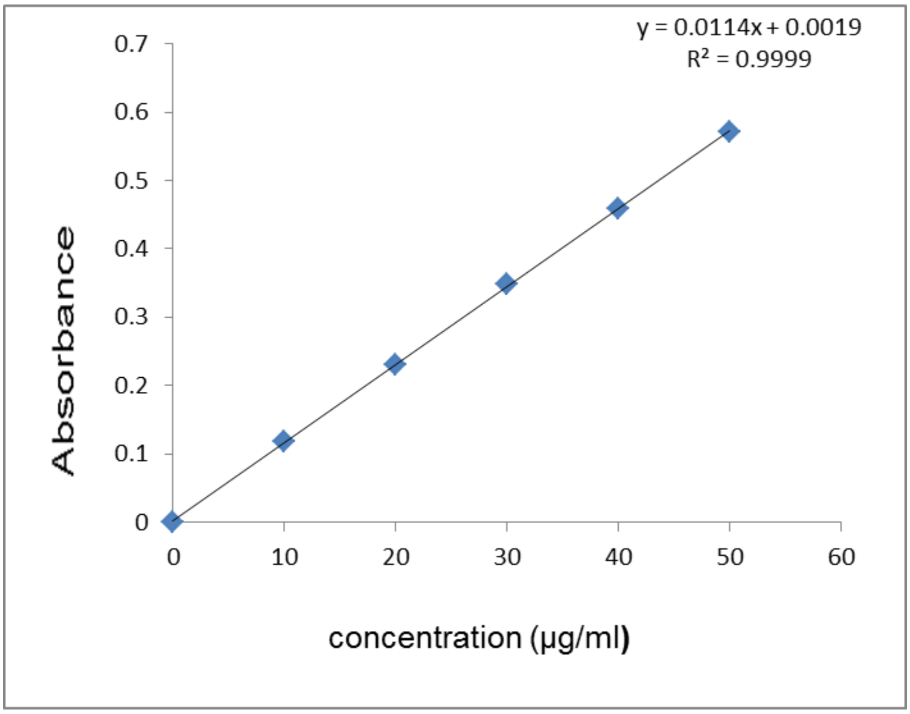

Figure 2: Calibration curve of Paroxetine.

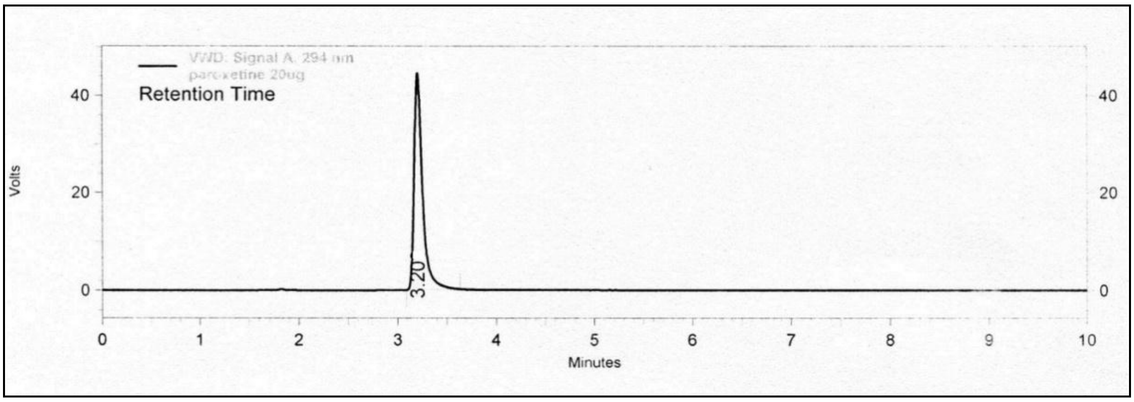

Figure 3: Standard chromatogram of Paroxetine (20 µg/mL).

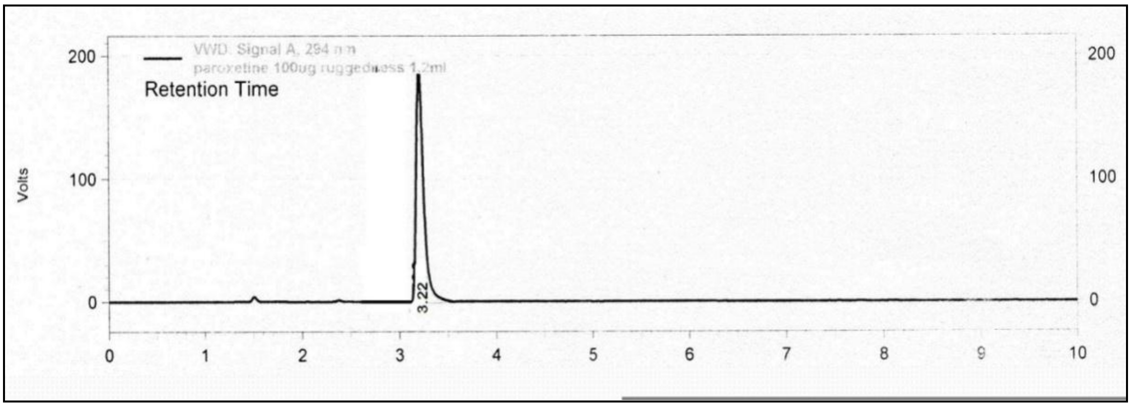

Figure 4: Standard chromatogram of Paroxetine (40 µg/mL).

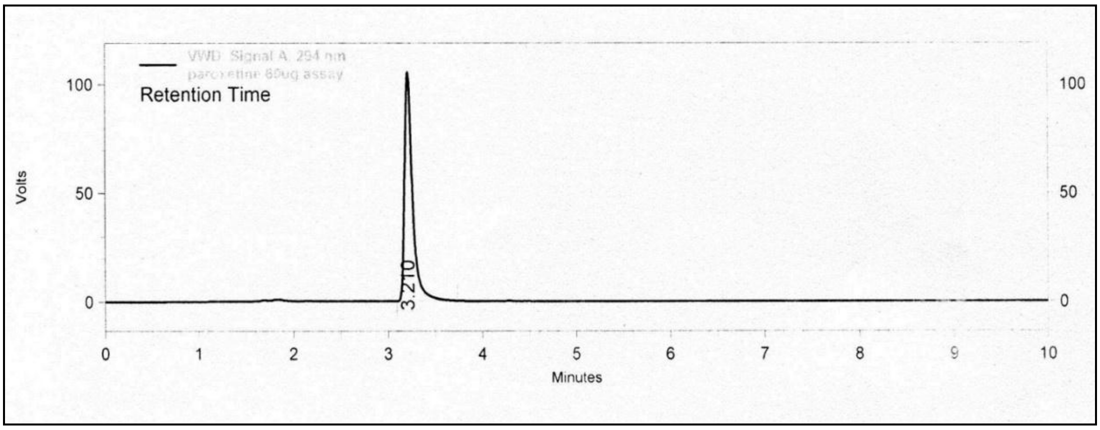

Figure 5: Standard chromatogram of Paroxetine (60 µg/mL).

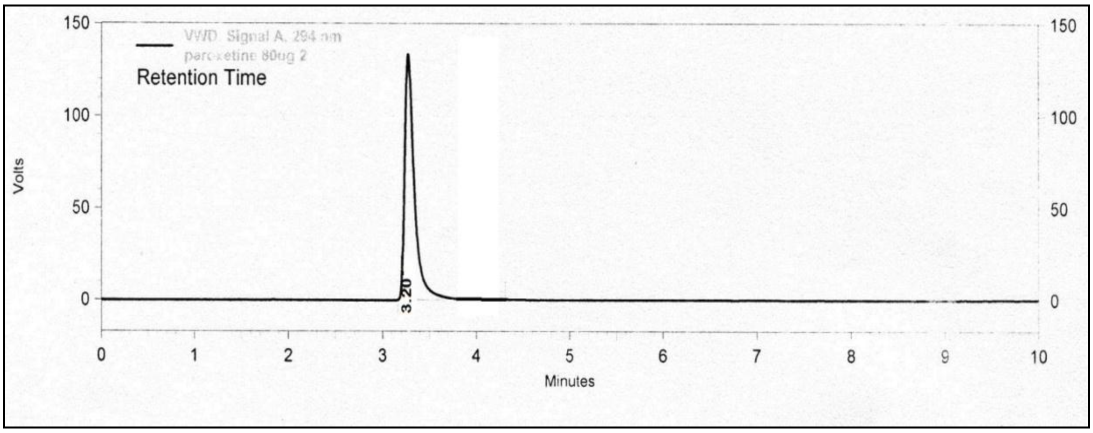

Figure 6: Standard chromatogram of Paroxetine (80 µg/mL).

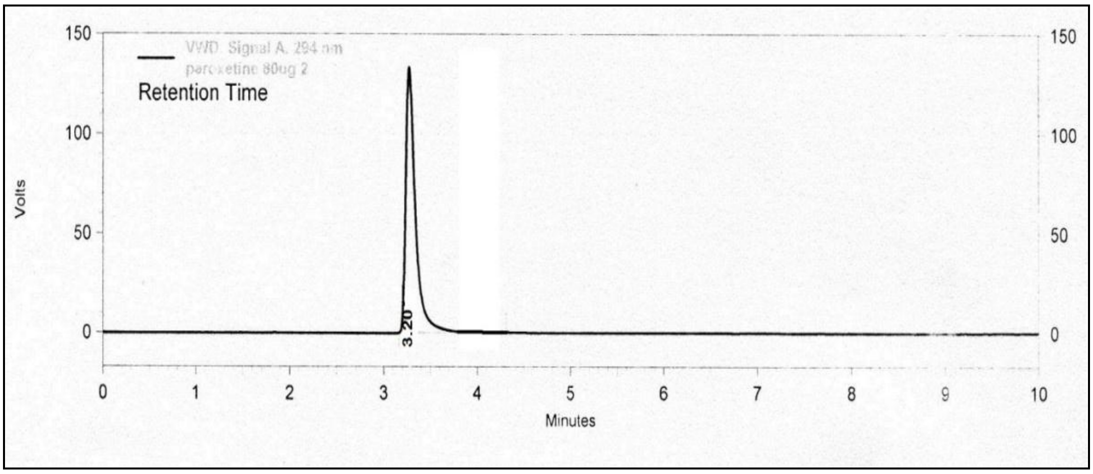

Figure 7: Standard chromatogram of Paroxetine (100 µg/mL).

Specificity

The specificity of the method was determined by the prepared standard, sample solutions and the blank solution were injected and checked for the interference of any other excipients. It was shown that the excipients present in pharmaceutical tablets of Paroxetine did not show any interference with Paroxetine peak because no excipients peaks appear in the chromatogram of the prepared tablet. Furthermore the well-shaped peaks also indicate the specificity of the method. The specificity results are tabulated in Table 3.

Table 3: Specificity study for Paroxetine.

Name of solution	Retension time (t_R)
Mobile phase	No peaks
Placebo	No peaks
Paroxetine 100 µg/mL	3.20

Precision

Precision of the method was evaluated by determining intra-day precision and inter-day precision and express in terms of % RSD (% relative standard deviation). The repeatability was studied by repeating the assay three times in the same day and intermediate precision was studied by repeating the assay on three different days, three times on each day. The results of intra-day and inter-day precision are shown in Table 4 and 5 respectively.

Table 4: Results of precision study (intra-day) for Paroxetine.

No of Injection	Peak Area	R_t (min)
1	20619734	3.20
2	20864738	3.22
3	20658921	3.21
4	20541765	3.20
5	20896473	3.20
6	20739856	3.21
Mean	20656629.5	3.2153
S.D	123191.48	0.001542
% RSD	0.5963	

Table 5: Results of precision study (intra-day) for Paroxetine.

No of Injection	Peak Area	R_t (min)
1	20648422	3.20
2	20521765	3.210
3	20748960	3.20
4	20543895	3.224
5	20845961	3.218
6	20635615	3.240
Mean	20720247.83	3.2153
S.D	140026.6623	0.001542
% RSD	0.6750	

 # Acceptance criteria < 2.0.

Accuracy/Recovery

The accuracy of the method was found out by standard addition method. A known amount of standard drug was added at 25%, 50% and 100% level. The concentrations were re-analyzed with the above described procedure. The percent recovery of the triplicate solutions was determined and average of the percent recovery was calculated. The recovery results are presented in Table 6.

Table 6: Recovery data for Paroxetine.

Recovery level	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery (%w/w)	Recovery level
	Std	Test		
50%	10	10	19.73	50%
100%	30	10	39.71	100%
150%	50	10	61.11	150%
Mean % Recovey	98.6 - 101.85			

**Average of triplicate injections*

Robustness

Robustness of the method is its ability to remain unaffected by small changes in variety of parameters such as the slight variation in acetonitrile percentage composition of the mobile phase, flow rate, detection wavelength. The results of robustness study is shown in Table 7 indicated that the small change in the conditions did not significantly affect the determination of Paroxetine.

Table 7: Robustness results of Paroxetine.

Flow rate (ml/min)	Retention time (min)	Peak Area
0.8	4.153	26269845
1.2	2.783	17532543

LOD and LOQ

Limit of detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. Limit of detection and limit of quantitation were calculated using following formula LOD = 3.3 σ/S and LOQ = 10 σ/S where, σ is the standard deviation of response and S is the slope of the calibration curve. The LOD and LOQ values are presented in Table 8. The results of LOD and LOQ supported the sensitivity of the developed method. Summary of validation parameters are shown in Table 9.

Table 8: Limit of detection and limit of quantitation.

Parameters	**Results**
Limit Of Detection (LOD)	0.795 µg/ml
Limit Of Quantitation (LOQ)	2.410 mg/ml

Table 9: Summary of validation parameters.

Parameters	**Results**
Detection wave length (nm)	294
R_t (min)	3.2± 0.01
Beer's law range (µg/ml)	20-100
Regression equation	Y=147991x+211606
Correlation coefficient	0.999
Accuracy (%w/w)	98.6 -101.85
Precision(%RSD)	
Intra-day	0.675
Limit of detection (µg/ml)	0.795
Limit of quantitation (µg/ml)	2.410
Robustness(Rt)	
Flowrate (ml/min)	0.8
	1.2
Assay (%w/w)	98.2

Application to commercial tablet

Using the developed RP-HPLC chromatographic method, assay of Paroxetine in tablet was carried out as mentioned in the experimental section. Six replicate determinations were made. Satisfactory results were obtained and were good agreement with the label claim and assay results were shown in Table 10.

Table 10: Assay results of Paroxetine formulation.

Sample	**Absorbance**	**Label claim**	**Amount found**	**%purity (w/w)**
PARI	12150478	20 mg	19.65mg	98.2

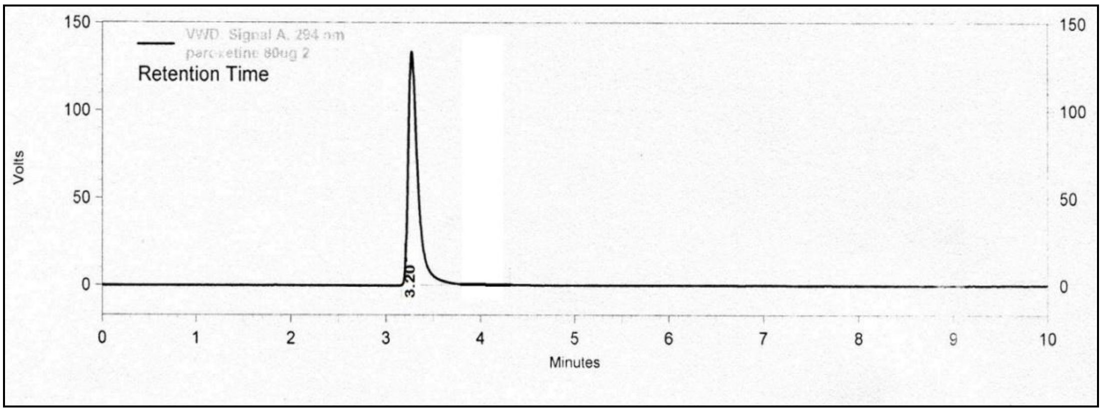

Fig 8: Sample chromatogram of Paroxetine.

RESULTS AND DISCUSSION

The present study was aimed at developing a precise, sensitive, rapid and accurate reversed phase HPLC method for the analysis of Paroxetine in bulk drug and in pharmaceutical dosage forms. In order to achieve extraordinary retention time and peak asymmetry, C18 stationary phase column (250 mm X 4.6 mm i.d, 5 µm particle size) and mobile phase composed of methanol a mixture of 10 mM Phosphate Buffer (pH - 6.0): Acetonitrile (60 : 40 v/v) at a flow rate of 1mL/min was selected. The retention time for Paroxetine was found to be 3.21 minutes. The correlation coefficient (0.9999) of regression was found almost equal to one in the range of 20-100 µg/mL which states that the method was good linear to the concentration versus peak area responses. The comparison of chromatograms of placebo, standard and sample there was no interference observed from the peaks of placebo, standard and sample. It shows that the method is specific. The precision studies were performed and the % RSD of the determinations was found to be 0.6750 for intra-day precision and 0.5963 for inter-day precision which are within the limits which indicates that the proposed method was found to be precise. The accuracy of the method was found to be good with the overall % RSD for recovery at 50%, 100% and 150% levels were all within the limits which indicate that the proposed method was found to be accurate. Method validation following ICH guidelines indicated that the developed method had high sensitivity with LOD of 0.795 µg/mL and LOQ of 2.410 µg/mL. The method was found to be robust even though on slight deliberate variation in the method conditions did have a tiny effect on the peak asymmetry, plate count and retention time and all are within the limits which indicated that the method is robust. Range is the minimum and maximum concentration of the sample at which the analytical procedure gives reproducible results. Range can be determined by linearity, accuracy and precision studies. The retention time of the sample solution of Paroxetine tablet was found to be 3.21 minutes, which is similar to that of the standard solution of Paroxetine. This indicates that there is no drug - excipient interference and the drug is decorously resolved by the developed method. Robustness determines the reproducibility of the test result with small and deliberate variations in the method parameters. The experiment was carried out by slightly changing the ratio of methanol in mobile phase, detection wavelength and flow rate. The effectiveness of the deliberate little variations was observed on the flow rate and mobile phase composition. The statistical data shows no significant variations in the above said parameters which indicate that the method is robust.

The developed method was successfully applied for the determination of Paroxetine in bulk drug and tablet dosage form. The assay result was complied in Table 10 and also shows that there is no interference of the tablet matrix with the drug. The assay results satisfactory results were obtained and were in a good agreement with the label claim. Very low % relative standard deviation shows that this method can be easily utilized for the estimation of Paroxetine in bulk drug and tablet dosage form.

CONCLUSION

The present study envisages Paroxetine as per the ICH guidelines. The good % recovery in tablet forms suggests that the excipients present in the dosage forms have no interference in the determination. The % RSD was also less than 2 % showing high degree of precision of the proposed method. In addition, simple isocratic elution procedure offered rapid and cost-effective analysis of Paroxetine. It can be concluded that the proposed method is a good approach for obtaining reliable results and found to be suitable for the routine analysis of Paroxetine in pharmaceutical dosage forms.

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