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Development and validation of rapid RP- HPLC method for the determination of Paroxetine in bulk and 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 Welchrom C_{18} 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 6.8, acetonitrile in the ratio 50: 50 v/v. An isocratic elution at a flow rate of 1 mL/ min at ambient temperature and using ELICO SL 2203 UV-Visible detector to monitor the eluate at 260 nm. The retention time of Paroxetine is found to be 3.71 min and the calibration curve was linear function of drug in the concentration range of 2-10 µg/ mL (r^2 = 0.9999). The limit of detection and the limit of quantification was found to be 0.059 µg/mL and 0.181 µg/mL respectively.The recovery (Accuracy) studies were performed and the percentage recovery was found to be 99.53 ± 0.6327 %. 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 be feasible 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), obsessive-compulsive 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 of Paroxetine.

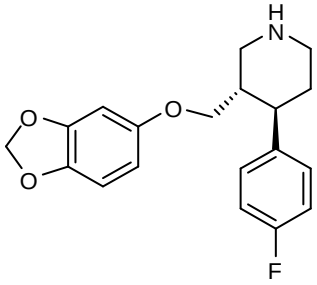

Figure 1: Chemical structure of Paroxetine

MATERIALS AND METHODS

Chemicals and reagents:
An analytically reference standard was kindly gifted by Natco pharma Limited, Hyderabad, India. All the chemicals were analytical grade. HPLC grade acetonitrile and triethylamine were obtained from Merck pharmaceuticals private Ltd., Mumbai, India. Methanol and 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 10 mg containing with labeled amount of 10 mg per tablet is manufactured by Cipla Pvt Ltd.

Instrumentation and Apparatus:
To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Paroxetine Isocratic Shimadzu LC-20AT Prominence Liquid Chromatograph having a Welchrom C_{18} isocratic column, (250 mm × 4.6 mm i.d., particle size 5 µm, maintained at ambient temperature). The wavelength was determined at 260 nm using Shimadzu SPD-20A prominence UV-Vis detector. A manually operating sample Rheodyne injector with 20 µL fixed sample loop was equipped with the HPLC system. The HPLC system was equipped with "Spinchrom" data acquisition software.

Preparation of reagents and standards
Mobile phase
Preparation of phosphate buffer pH 6.8:
Phosphate buffer pH 6.8 was prepared by dissolving 1.488 gm of potassium dihydrogen orthophosphate (KH_2PO_4) and 0.288 gm dipotassium hydrogen phosphate (K_2HPO_4) in 500 mL of HPLC grade water and pH was adjusted to 6.8 with ortho phosphoric acid. Triethylamine is used as column modifier. This solution was filtered through 0.45µ Millipore Nylon filter.

Preparation of mobile phase:
Mobile phase consisting of phosphate buffer of pH 6.8 and acetonitrile (HPLC grade) in the ratio of 50:50 v/v. These solutions were mixed well in the given ratio and sonicated for 15 minutes and filtered under vacuum filtration. The prepared solution was used as mobile phase.

Preparation of diluent:
Phosphate buffer and Acetonitrile (HPLC grade) in the ratio of 50:50 (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 10 mg of drug in to 10 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 2, 4, 6, 8 and 10 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

Parameter	Chromatographic conditions
Instrument	SHIMADZU LC-20AT prominence liquid chromatograph
Column	WELCHROM C_{18} Column (4.6 mm i.d. X 250 mm, 5 µm particle size)
Detector	SHIMADZU SPD-20A prominence UV-Vis detector
Diluents	10 mM Phosphate Buffer (pH-6.8): Acetonitrile (50 : 50 v/v)
Mobile phase	10 mM Phosphate Buffer (pH-6.8): Acetonitrile (50 : 50 v/v)
Flow rate	1 mL/min.
Detection wave length	UV at 260 nm.
Run time	10 minutes
Temperature	Ambient temperature (25 ^{o}C)
Volume of injection loop	20 µL
Retention time (t_R)	3.710 min
Theoretical plates [th.pl] (Efficiency)	11915
Theoretical plates per meter [t.p/m]	238300
Tailing factor (asymmetry)	1.150

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 (2, 4, 6, 8, 10 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 2-10 µg/mL of stock solution. The linearity range was found to be 2-10 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. The column performance and results are shown in Table 3 to Table 7. The Results show that a phenomenal correlation exists between peak area and concentration of drug within the linearity range.

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
2.	2	3.71	56.629
3.	4	3.71	113.258
4.	6	3.71	170.533
5.	8	3.71	226.921
6.	10	3.71	280.779

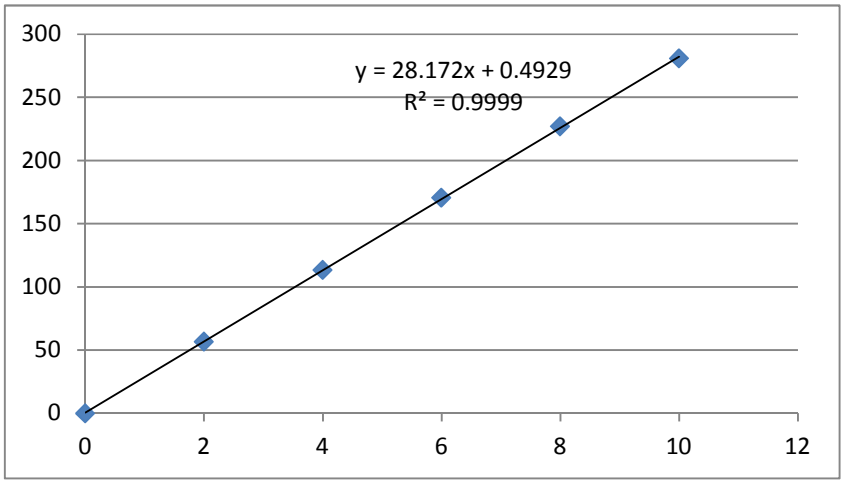

Figure 2: Calibration curve of Paroxetine

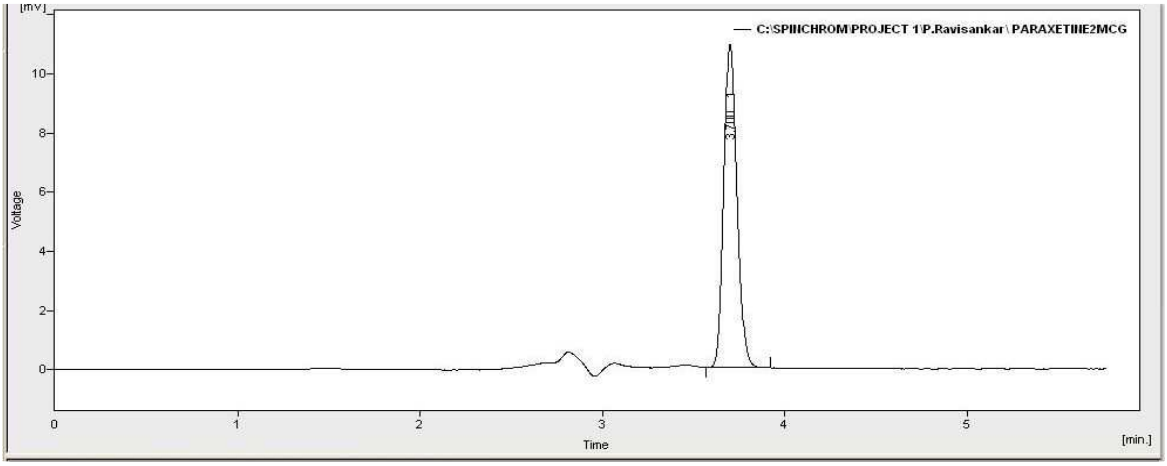

Figure 3: Standard chromatogram of Paroxetine (2 µg/mL)

Table 3: Column performance and result table (2 µg/mL)

Column Performance Table (From 50% - C:\SPINCHROM\PROJECT 1\P.Ravisankar\ PARAXETINE2MCG) Result Table (Uncal - C:\SPINCHROM\PROJECT 1\P.Ravisankar\ PARAXETINE2MCG)

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]		Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.100	0.083	1.150	0.00	10921	218427	-	1	3.100	56.629	10.976	100.0
									Total	56.629	10.976	100.0

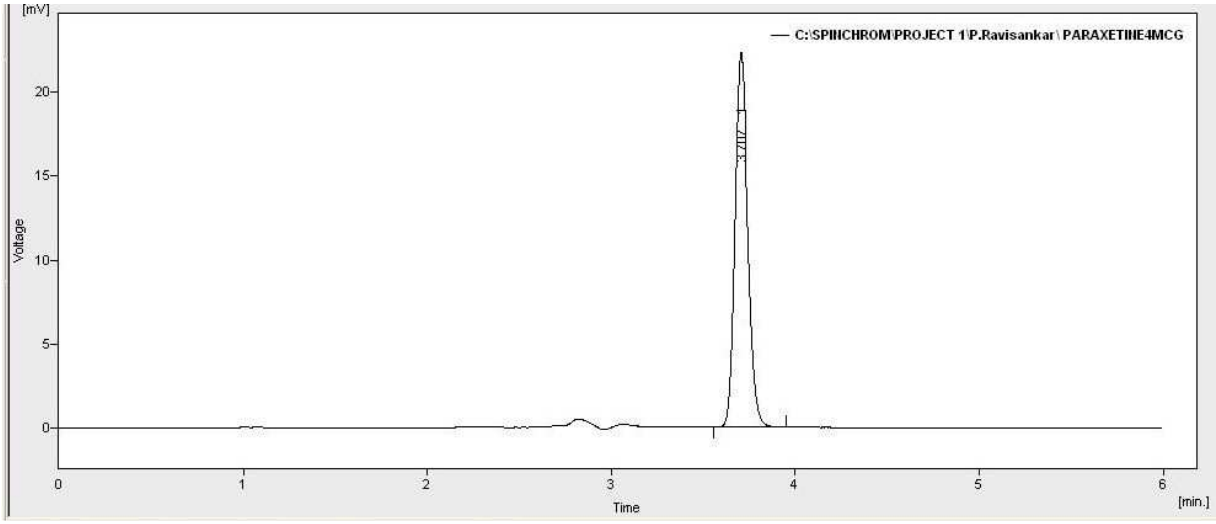

Figure 4: Standard chromatogram of Paroxetine (4 µg/mL)

Table 4: Column performance and result table (4 µg/mL)

Column Performance Table (From 50% - C:\SPINCHROM\PROJECT 1\P.Ravisankar\ PARAXETINE4MCG) Result Table (Uncal - C:\SPINCHROM\PROJECT 1\P.Ravisankar\ PARAXETINE4MCG)

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]		Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.707	0.083	1.150	0.00	10961	219215	-	1	3.707	113.258	22.319	100.0
									Total	113.258	22.319	100.0

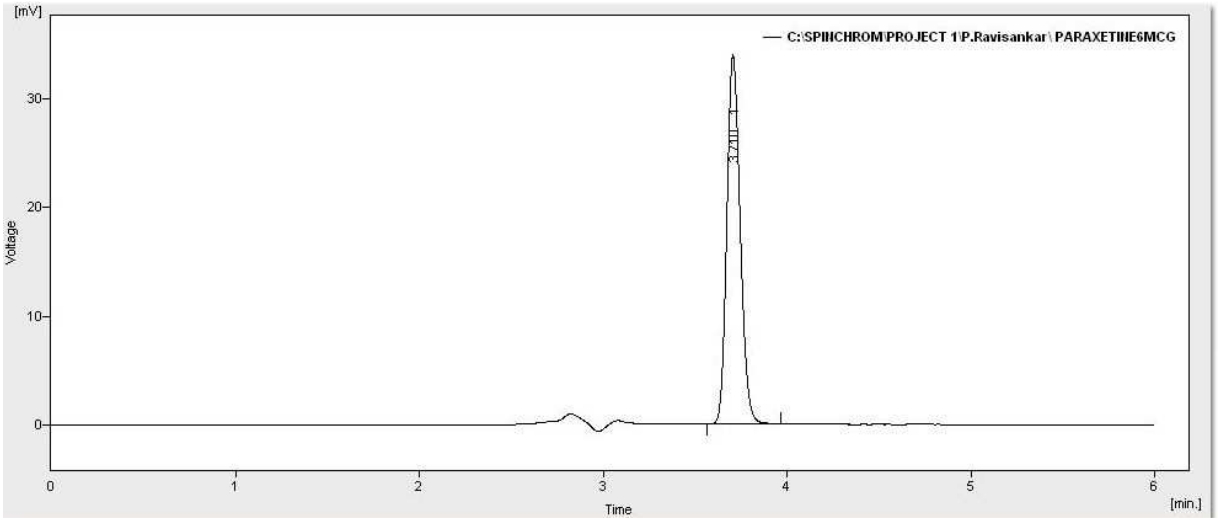

Figure 5: Standard chromatogram of Paroxetine (6 µg/mL)

Table 5: Column performance and result table (6 µg/mL)

Column Performance Table (From 50% - C:\SPINCHROM\PROJECT 1\P.Ravisankar\ PARAXETINE6MCG)

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	3.710	0.080	1.150	0.00	11915	238291	-

Result Table (Uncal - C:\SPINCHROM\PROJECT 1\P.Ravisankar\ PARAXETINE6MCG)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.710	170.533	34.056	100.0
	Total	170.533	34.056	100.0

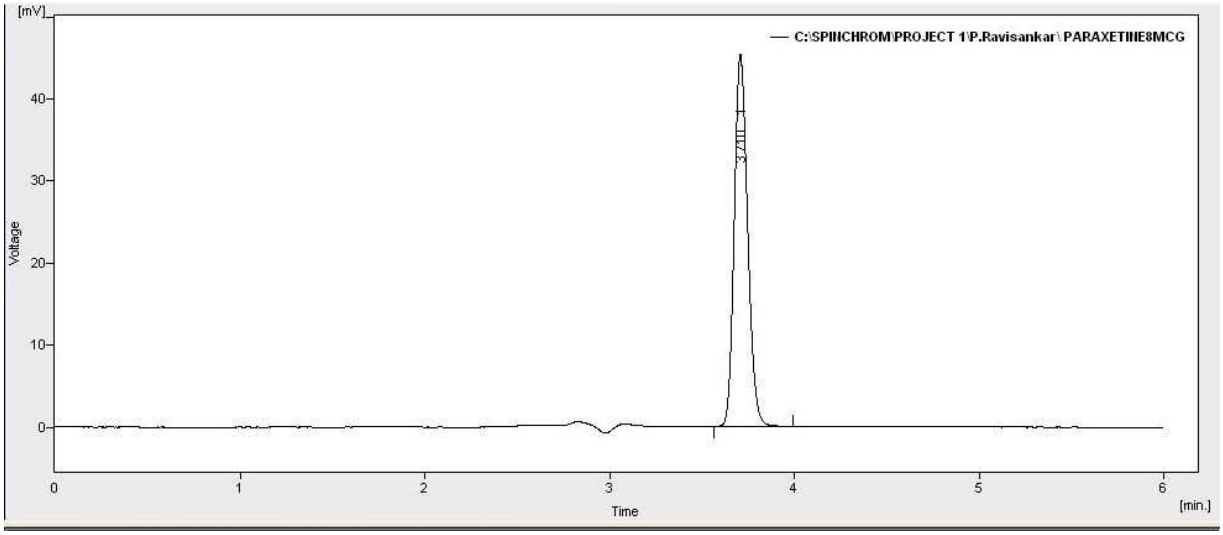

Figure 6: Standard chromatogram of Paroxetine (8 µg/mL)

Table 6: Column performance and result table (8 µg/mL)

Column Performance Table (From 50% - C:\SPINCHROM\PROJECT 1\P.Ravisankar\ PARAXETINE8MCG)

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	3.710	0.080	1.116	0.00	11915	238291	-

Result Table (Uncal - C:\SPINCHROM\PROJECT 1\P.Ravisankar\ PARAXETINE8MCG)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.710	226.921	45.355	100.0
	Total	226.921	45.355	100.0

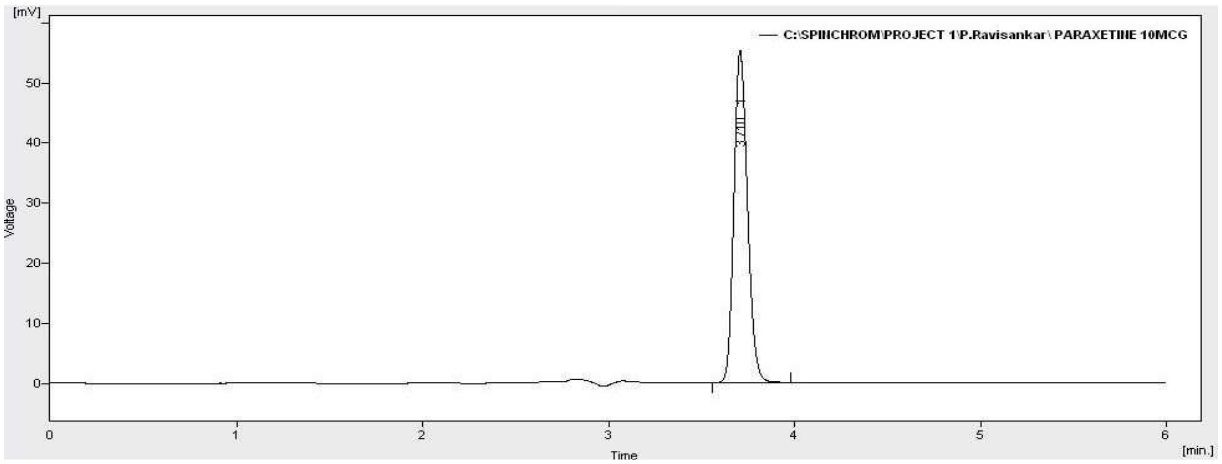

Figure 7: Standard chromatogram of Paroxetine (10 µg/mL)

Table 7: Column performance and result table (10 µg/mL)

Column Performance Table (From 50% - C:\SPINCHROM\PROJECT 1\P.Ravisankar\ PARAXETINE 10MCG)

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff.f [t.p./m]	Resolution [-]
1	3.710	0.080	1.116	0.00	11915	238291	-

Result Table (Uncal - C:\SPINCHROM\PROJECT 1\P.Ravisankar\ PARAXETINE 10MCG)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.710	280.779	56.745	100.0
	Total	280.779	56.745	100.0

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 8.

Table 8: Specificity study for Paroxetine

Name of the solution	Retention time (t_R) min.
Mobile phase	No peaks
Placebo	No peaks
Paroxetine 10 µg/mL	3.71 min.

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 9 and 10 respectively.

Table 9: Results of precision study (intra-day) for Paroxetine

Sample	Concentration (µg/mL)	Injection no.	Peak area (mV.s)	% RSD[#]
Paroxetine	3	1	170.536	0.0017
		2	170.53	
		3	170.537	
		4	170.533	
		5	170.537	
		6	170.532	

[#] *Acceptance criteria < 2.0.*

Table 10: Results of precision study (inter-day) for Paroxetine

Sample	Concentration (µg/mL)	Injection no.	Peak area (mV.s)	% RSD[#]
Paroxetine	3	1	170.533	0.0015
		2	170.532	
		3	170.53	
		4	170.534	
		5	170.536	
		6	170.537	

[#] *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 11.

Table 11: Recovery data for Paroxetine

S.NO	Level of spiking of standard	Amount added to Sample (previously analyzed) conc. (µg/mL)	Amount Found conc. (µg/mL)	% recovery*	% RSD
1	25%	10	9.981	99.81	0.26
2	50%	20	19.989	99.945	0.17
3	100%	40	39.976	99.94	0.060

**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 12 indicated that the small change in the conditions did not significantly affect the determination of Paroxetine.

Table 12: Robustness results of Paroxetine

S. No	Parameter	Optimized	Used	Retention time (t_R), min	Plate count$^{\$}$	Peak asymmetry$^{\#}$	Remark
1.	Flow rate (±0.2 mL/min)	1.0 mL/min	0.8 mL/min	3.75	11,639	1.290	*Robust
			1.0 mL/min	3.71	11,915	1.25	*Robust
			1.2 mL/min	3.65	11,660	1.210	*Robust
2.	Detection wavelength (±5 nm)	260 nm	265 nm	3.71	11,915	1.25	Robust
			260 nm	3.71	11,915	1.25	Robust
			255 nm	3.71	11,915	1.25	Robust
3.	Mobile phase composition (Acetonitrile : Water)	50:50 v/v	55:45v/v	3.79	11,649	1.203	*Robust
			50:50 v/v	3.71	11,915	1.25	*Robust
			45:55v/v	3.75	11,640	1.203	*Robust

*Acceptance criteria (Limits): $^{\#}$Peak Asymmetry < 1.5, $^{\$}$ Plate count > 3000, * Significant change in Retention time.*

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 13. The results of LOD and LOQ supported the sensitivity of the developed method. Summary of validation parameters are shown in Table 14.

Table 13: Limit of detection and limit of quantitation

Limit of Detection (LOD)	0.059 µg/mL
Limit of Quantitation (LOQ)	0.181µg/mL

Table 14: Summary of validation parameters

Parameter	Result
Linearity range (µg/mL)	2 - 10 µg/mL
Linear Regression equation (Y = a + bX)	Y = 28.17X+0.492
Intraday precision (% RSD)	0.0017
Interday precision (% RSD)	0.0015
% Recovery	99.81 - 99.945 %
LOD (µg/mL)	0.059
LOQ (µg/mL)	0.181
Robustness	Robust

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 15. The results were very close to the labeled value of commercial tablets. The representative sample chromatogram of Paroxetine is shown in Figure 7.

Table 15: Assay results of Paroxetine formulation

S.No	Brand name	Labelled claim	Amount found	Assay ± % RSD*
1	Parotin tablets (Cipla Laborates Pvt Ltd India).	10 mg/tablet	09.821 mg/tablet	99.55 ± 0.40

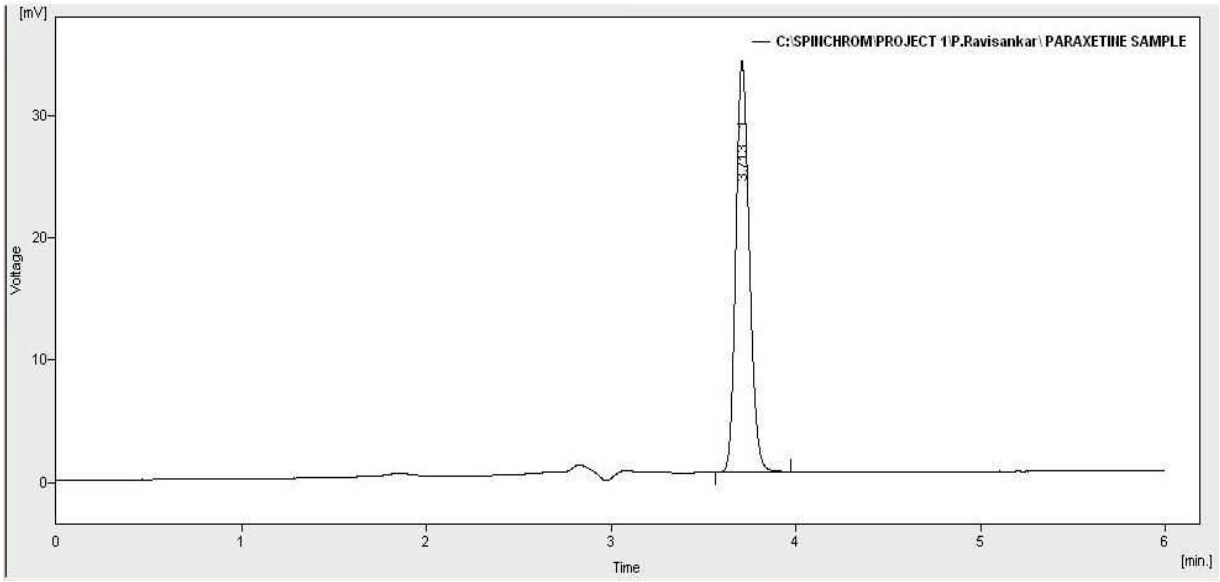

Fig 7: Sample chromatogram of Paroxetine

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, C_{18} 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 - 6.8): Acetonitrile (50 : 50 v/v) at a flow rate of 1mL/min was selected. The retention time for Paroxetine was found to be 3.71 minutes. The correlation coefficient (0.9999) of regression was found almost equal to one in the range of 2-10 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 0.0017 for intra-day precision and 0.0015 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 25 %, 50 % and 100 % 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 0.059 µg/mL and LOQ of 0.181 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 3.71 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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