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Development and validation of novel UV spectrophotometric and RP-HPLC method for the estimation of paroxetine hydrochloride in bulk and pharmaceutical dosage forms

B. Praveen Kumar*[1], S. Vidyadhara[1], T. E. G. K. Murthy[2], J. Ramesh Babu[1] and R. L. C. Sasidhar[1]

[1]*Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur, A.P.*
[2]*Bapatla College of Pharmacy, Bapatla, A.P.*

ABSTRACT

*The present paper illustrates about development and validation of a new, simple, precise and accurate Spectrophotometric and RP-HPLC method with enhanced sensitivity for the determination of the antidepressant paroxetine (PXT) in bulk and its dosage forms. The drug showed good absorbance in water at 294 nm, hence water is selected as a solvent and validation is performed. Under the optimized conditions, linear relationship with good correlation coefficient (0.9993) was found between the concentration range of 10-50 µg/ml. The limits of detection and quantitation for the method were 0.3 and 0.9 µg/ml, respectively. The precision of the method was satisfactory; the values of relative standard deviations did not exceed 2%. The recovery values were 100.2 ± 1.61%. The chromatographic method was developed on AGILENT HPLC with UV detection. The method was optimized by Kromosil-C_{18}, (250 * 4.6mm, 5µ) column by using Phosphate buffer (pH– 6)**: Acetonitrile (60:40) as a mobile phase with 1 ml/min as flow rate. The detection wavelength is 294 nm. The optimized method was validated with good correlation coefficient (0.9999) was found between the concentration range of 20-100 µg/ml. The limits of detection and quantitation for the method were 0.7 and 2.4 µg/ml, respectively. The precision of the method was satisfactory; the values of relative standard deviations did not exceed 2%. The recovery values were 98.6 - 101.85%. The proposed methods are successfully applied for the determination of PXT in bulk and their dosage forms. The method is having higher sensitivity and wider linear range. The proposed method is practical and valuable for its routine application in quality control laboratories for estimation of PXT.*

Keywords: RP-HPLC, validation, precision, accuracy

INTRODUCTION

Paroxetine (PXT) (3S, 4R)-3-[(2H-1,3-benzodioxol-5-yl oxy) methyl]-(4 fluorophenyl) piperidine is a antidepressant, selective serotonin reuptake inhibitor. Paroxetine likely inhibits the reuptake of serotonin at the neuronal membrane, enhances serotonergic neurotransmission by reducing turnover of the neurotransmitter, therefore it prolongs its activity at synaptic receptor sites and potentiates 5-HT in the CNS. However many method for the estimation of PXT like UV[1-7], Fluorometric [8-10], HPLC [11,12], HPTLC [13] and voltametric methods were reported in the literature. But the present method is more sensitive and precise than the reported methods.

MATERIALS AND METHODS

1.1 Development of UV spectroscopic method

1.1.1 Instruments and chemicals used

Spectral and absorbance measurements were made on an ELICO SL 218 UV Visible double beam spectrophotometer by using 1 cm quartz cells. Axis Ag N 204-PO digital balance was used for weighing the samples. Methanol (analytical grade), supplied by Rankem chemicals Ltd, Hyderabad. Standard drug of Paroxetine HCl (gift samples) was supplied by Mylon laboratories Pvt. Ltd., Hyderabad. Tablets of Paroxetine HCl containing 20 mg of Paroxetine HCl were procured from the market manufactured by IPCA laboratories Ltd.

2.1.2. Selection of wavelength

The solvents like Methanol, Water and Acetonitrile were tried based on the solubility of the drug. Better absorption maximum was found to be 294nm with water. Hence water was selected as optimized solvent for the assay method. In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drug, (100µg/ml) aqueous solution was scanned using spectrophotometer within the wavelength region of 200 – 400nm against water as blank. The resulting spectra were presented (fig 1) and the absorption curve showed characteristic absorption maximal at 294nm.

2.1.3 Preparation of Stock and working standard Solutions:

Standard Paroxetine HCl (100mg) was accurately weighed and transferred to 100 ml volumetric flask. It was dissolved in methanol and diluted up to the mark with Water to obtain a concentration of 1000 µg/ml. From the above solution, 1ml was pipette out into a 10ml volumetric flask and made up to the mark with water to obtain a solution of 100 µg/ml concentration. This solution was used as working standard solution.

2.2. Development of HPLC method:

2.2.1 Instruments and chemicals used:

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. Axis Ag N 204-PO Digital Balance was used to weigh samples with accuracy. 1.5 LH Ultra Sonic Bath sonicator was used to degas the mobile phase. Membrane filters of pore size 0.45 µm were used to filter the samples solution. Acetonitrile (HPLC grade) was supplied by Merck specialities Pvt., Ltd. Water (HPLC grade) was supplied by Merck specialities Pvt., Ltd. Potassium Dihydrogen Phosphate Buffer, Standard drug of Paroxetine HCl (gift samples) were supplied by Mylon laboratories Pvt.ltd., Hyderabad. Tablets of Paroxetine HCl containing 20 mg of Paroxetine HCl were procured from market manufactured by IPCA laboratories Ltd.

2.2.2 Method development:

The pure drug of Paroxetine HCl was injected in to the isocratic HPLC system and run at different solvent systems. Different mobile phases like methanol and water, Acetonitrile and water, methanol and Acetonitrile were tried in order to find the best conditions for the elution of Paroxetine HCl. It was found that Potassium Dihydrogen phosphate buffer (pH-6) and Acetonitrile give satisfactory results as compared to other mobile phases. The mobile phase system was tried with different proportions and using different flow rates. Finally, the optimal composition of the mobile phase was determined to be 20 mM potassium di hydrogen phosphate buffer (pH– 6) and Acetonitrile in the ratio of 60:40v/v at a flow rate of 1.0ml/min on kromosil C18 (250 x 4.6 mm id; 5 µ). The detection wavelength was selected as 294 nm based on UV spectrum. The run time was set as 10 minutes.

2.2.3. Preparation of Standard Solution:

25mg of Paroxetine HCl was dissolved into 25ml of mobile phase to get 1000µg/ml solution; sonicate for 10 min and mix and used as standard stock solution.

2.3. Method Validation[14-15]:

2.3.1 System Precision and System Suitability:

The standard solution of 100µg/ml was prepared by diluting 1 ml of the standard solution to 10 ml with the mobile phase. It was injected six times into the HPLC system. The system suitability parameters were evaluated and found to be within the limits. The RSD for the peak areas from six replicate injections of Paroxetine HCL was found.

2.3.2 Linearity:

The linearity of the method was demonstrated over the concentration range of 10-50 µg /ml for UV method. A Series of dilutions were made by using the working standard solution. From the working standard solution 1, 2, 3, 4 and 5ml were pipetted out into a 10ml volumetric flasks and diluted with water and finally make up to the volume with water. The resulting solutions were labelled as 10, 20, 30, 40 and 50 µg /ml. The linearity was calculated by the

least square regression method. At the maximum absorption wavelength, calibration curve was drawn by plotting graph between absorbance and concentrations.

For the evaluation of linearity by HPLC five different concentrations of standard solutions were prepared. The concentration range that prepared were between 20 to 100 µg/ml for Paroxetine HCl. 0.2, 0.4, 0.6, 0.8 and 1 ml of standard stock solution were taken and made upto 10 ml with the mobile phase.. A graph is plotted to "concentration of Paroxetine HCL" versus "area of Paroxetine HCl found".

2.3.3. Precision:

The precision of an analytical method is the degree of agreement among the individual test results obtained when the method is applied repeatability to multiple sampling of the same homogenous sample under prescribed conditio

For UV method intra-day precision a set of six determinations containing 50 µg/ml (100% concentration) were prepared and analyzed at 294nm on the same day at 0hrs, 2hrs, 4hrs, 6hrs at room temperature and refrigerator and %relative standard deviation (%RSD) was calculated and reported. inter-day precision a set of six determinations containing 50 µg/ml were prepared and analyzed at same time on three different days at their selected analytical wavelength of 294nm. The variation of the results on different days was analyzed and %RSD was calculated and reported.

For HPLC method the precision of test procedure was evaluated by performing the six replicate applications of standard solution of 100 µg/ml concentration. The % relative standard deviation of Paroxetine HCl was found to be within the limits.

2.3.4 Accuracy (Recovery):

For UV method the accuracy of the method was evaluated through standard addition method. In this, the volume of the test solution was taken as constant and standard Paroxetine HCl solution was taken in increased amounts equivalent to 50%, 100% and 150% to each test solution. Known amount of Paroxetine HCl test solution of 5µg/ml concentration was added in standard sample for 5, 15 and 25µg/ml. The percent recovery of the solutions was determined and calculated.

For HPLC method Drug accuracy was performed by spiking with equivalent amount of Paroxetine HCl raw material into each volumetric flask for each spike level to get the concentration of Paroxetine HCl equivalent to 50%, 100%, and 150% of the labelled amount of Paroxetine HCl as per the test method.

2.3.5 Limit of Detection (LOD):

The parameter LOD was determined on the basis of intercept and slope of the regression equation. It was calculated by using the following formula

LOD= 3.3 X S.D of y-intercepts / mean of slopes

2.3.6 Limit of Quantification (LOQ):

The parameter LOQ was determined on the basis of intercept and slope of the regression equation. It was calculated by using the following formula

LOQ= 10 X S.D of y-intercepts / mean of slopes

2.3.7. Robustness:

Robustness examines the effect of variation in operational parameters on the analysis results. For the determination of a method's robustness, parameters like 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 within the method's robustness range. It is the Measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides indication of its reliability during its normal usage.

i. Effect of Flow Rate Variation of HPLC method:

Robustness of the method was checked by changing the flow rate from 0.8 ml/min and 1.2 ml/min instead of 1.0 ml/min by injecting of standard in 0.8 ml/min and 1.2 ml/min flow rate. The system suitability parameter of Paroxetine HCl standard is found within the limits. The method has been found to be robust from the flow rate 0.8 ml/min to 1.2 ml/min.

ii. Effect of wavelength Variation of HPLC method:
Robustness of the method was checked by changing the wavelength from 292nm and 296nm instead of 292nm by injecting of standard in 292nm and 296nm wavelength. The system suitability parameter of Paroxetine HCl standard is found within the limits.

2.3.8. Ruggedness:
The Ruggedness of an analytical method is degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different analysts, instruments. 50 µg/ml standard solution was prepared as mentioned in the precision, the absorbances were measured by different analyst and different instrument. The results were noted down and tabulated.

2.3.9. DETERMINATION OF PAROXETINE HCl IN TABLETS (UV)
The proposed method was applied to analyze commercially available dosage forms. The working test solution of any concentration in the linearity range was prepared and the absorbance was measured. Blank solution was prepared and absorbance was measured at 294 nm. The amount of Paroxetine HCl was calculated from the calibration curve. The readings were taken in triplicate and performing the same experiment for two times.

Acceptance criteria for % Recovery should be between 97.5 to 102% of Paroxetine hydrochloride in its tablets. Since the percent RSD of percent recovery was found to be below 2%, the assay parameter was passed.

2.3.10. ESTIMATION OF PAROXETINE HCL IN TABLETS (HPLC)
Weigh 20 tablets and crush them to powder. Weigh accurately tablet powder equivalent to 25mg of Paroxetine HCl and transfer in to 25ml volumetric flask, add 5ml of mobile phase, keep on shaking for 5 minutes. Sonicate for 10 minutes with occasional shaking in between. Make up the volume with mobile phase. and mix well. A portion of the solution was filtered through a membrane filter of 0.45µm, discarding the first 1-2 ml. Pipette 0.6 ml from 25 ml volumetric flask and diluted to 10 ml with mobile phase.The amount of drug was calculated from the calibration curve.

Table 1: linearity data of UV method

CONCENTRATION (µg/ml)	ABSORBANCE		
	A1	A2	A3
10	0.117	0.108	0.127
20	0.230	0.228	0.216
30	0.348	0.354	0.339
40	0.458	0.461	0.441
50	0.571	0.567	0.564
Slope	0.011	0.011	0.011
Y- intercept	0.001	0.001	0.003
Correlation	0.999	0.999	0.998

Table 2: Intraday precision of Paroxetine HCl for UV method

Concentration	Intraday precision			
	0hrs	2hrs	4hrs	6hrs
50 µg/ml	0.570	0.562	0.552	0.567
	0.572	0.560	0.564	0.562
	0.575	0.564	0.555	0.559
	0.578	0.563	0.560	0.552
	0.572	0.560	0.563	0.557
	0.573	0.565	0.565	0.553
Mean	0.573	0.562	0.559	0.558
S.D	0.0028047	0.0034641	0.005269	0.005645
%RSD	0.449	0.661	0.949	1.012

Table 3: Interday precision of Paroxetine HCl for UV method

Concentration (µg/ml)	Inter-day precision		
	Day 1	Day 2	Day 3
50 µg/ml	0.565	0.553	0.566
	0.570	0.568	0.563
	0.561	0.556	0.560
	0.564	0.561	0.551
	0.568	0.563	0.558
	0.562	0.561	0.554
Mean	0.565	0.560	0.558
S.D	0.0034641	0.0053107	0.00557
%RSD	0.601	0.949	0.981

Table 4: Accuracy of Paroxetine HCl for UV method

Recovery level	Amount added(µg/ml)		Amount found (µg/ml)	%recovery (%w/w)
	Standard	Test		
50%	5	5	9.82	98.20%
100%	15	5	19.13	99.13%
150%	25	5	30.08	100.2%
Mean Recovery	98.20-100.2%			

Table 5: Assay of Paroxetine HCl in Tablets for UV method

Brand Name	Label claim	Absorbance	Amount found	%Purity (% w/w)
PARI	20 mg	0.345	19.82mg	99.13

Table 6: Ruggedness of Paroxetine HCl (50 µg/ml) for UV method

S.No	Analyst 1	Analyst 2	Instrument 1	Instrument 1
1	0.572	0.556	0.567	0.558
2	0.567	0.569	0.561	0.548
3	0.546	0.559	0.558	0.553
4	0.569	0.562	0.569	0.549
5	0.563	0.556	0.561	0.545
6	0.570	0.564	0.556	0.550
Mean	0.5676	0.5610	0.5623	0.5505
S.D	0.003326	0.005059	0.005046	0.00450
% R.S.D	0.581	0.901	0.889	0.817

Table 7: System suitability of Paroxetine HCl for HPLC Method

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	20656629.5	3.2153
S.D	123191.48	0.001542
% RSD	0.5963	

Table8: Validation Data of System Suitability for HPLC Method

S.No	Parameters	Results
1	No. of Theoretical plates	13162
2	Tailing factor	1.2
3	Retention time	3.20± 0.01
4	%RSD	0.5963

Table 9: Linearity Data of Paroxetine HCl for HPLC Method

Concentration (µg/ml)	Area	R_t (min)
20	4409845	3.20
40	8102253	3.22
60	12365316	3.21
80	16150514	3.20
100	20648422	3.20
Slope (m)	20389	
Intercept (b)	84593	
Correlation coefficient (r)	0.999	

Table 10: Precision of Paroxetine HCl (100 µg/ml) for HPLC Method

No of Injection	Peak area
	Intraday
1	20619734
2	20864738
3	20658921
4	20541765
5	20896473
6	20739856
Std	140026.6623
Mean	20720247.83
% RSD	0.675

Table 11: Accuracy of Paroxetine HCl for HPLC Method

Recovery level	Amount Added (µg/ml)		Amount Found (µg/ml)	% Recovery (%w/w)
	Std	Test		
50%	10	10	19.73	98.6
100%	30	10	39.71	99.27
150%	50	10	61.11	101.85
Mean % Recovey	98.6 - 101.85			

Table 12: Robustness of Paroxetine HCl at variable flow rates (100 µg/ml) for HPLC Method

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

Table 13: Robustness of Paroxetine HCl at variable Wavelengths (100 µg/ml) for HPLC Method

wavelength (nm)	Retention time (min)	Peak Area
292	3.210	20451547
296	3.227	20719018

Table 14: Assay of Paroxetine HCl in Tablets by HPLC Method

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

Figure 1: UV spectrum of paroxetine

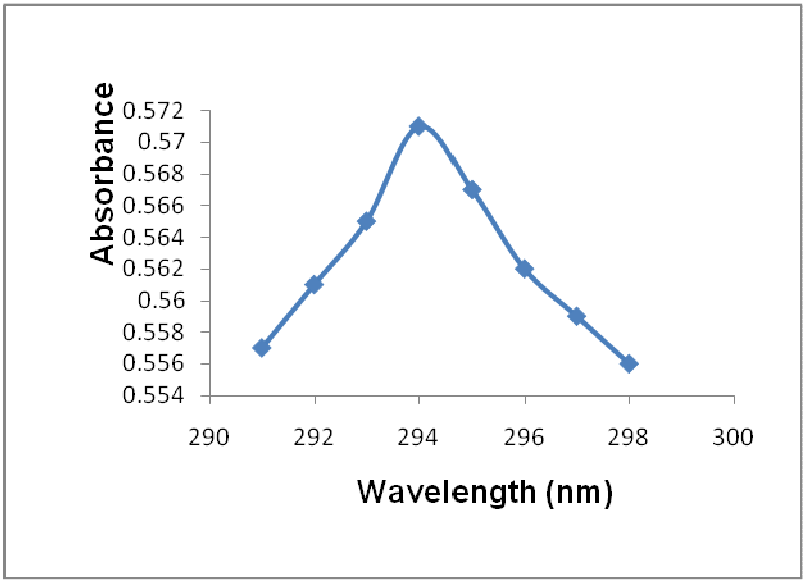


Figure 2: Linearity of paroxetine by UV method

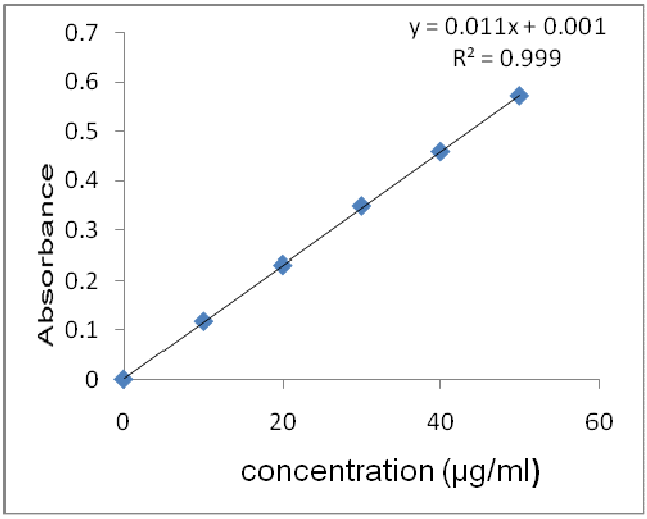


Figure 3: Representative chromatogram of standard Paroxetine HCl

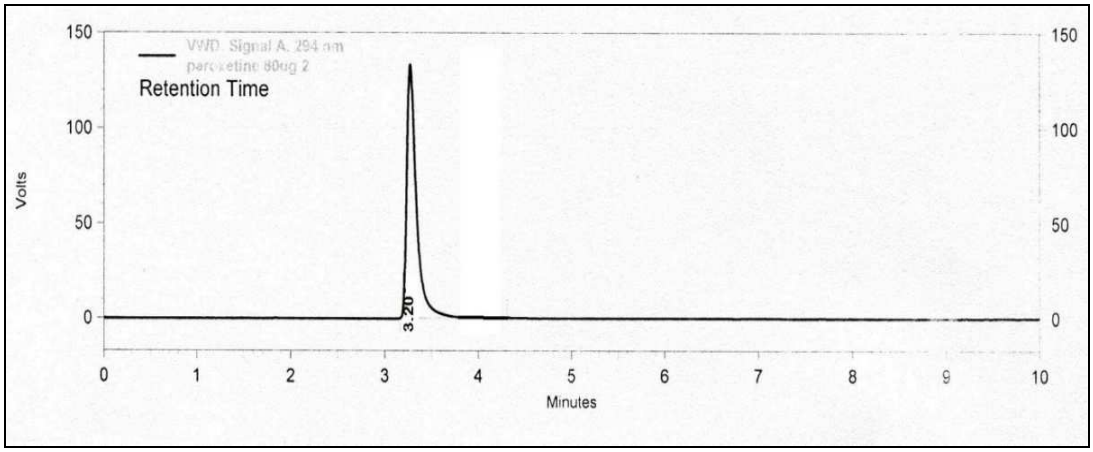
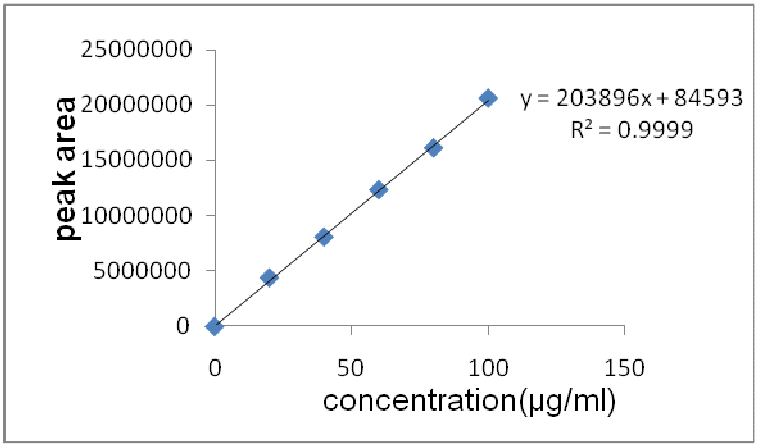


Figure 4: Linearity graph of Paroxetine HCl by HPLC



RESULTS AND DISCUSSION

3.1 Results of UV spectroscopic method:

3.1.1 Linearity:

The linearity was plotted between 10-50 µg/ml and the R^2 value was found to be 0.999. The table with results was shown in table 1. The linearity graph was shown as fig 2.

3.1.2 Precision:

Both intra-day and inter-day precision was performed by analysing 50 µg/ml concentration. The %RSD values in both the cases were not more than 2.0%. The results were shown in table 2 and table 3.

3.1.3 Accuracy:

It is performed over the concentration range of 50%, 100% and 150% and mean recovery was found to be 98.20-100.2%. The results were shown in table 4.

3.1.4 LOD and LOQ:

The LOD and LOQ of PXT were found to be 0.3 and 0.909 µg/ml respectively.

3.1.5 Ruggedness:

Ruggedness was performed by analysing the samples on two different instruments with different analysts. The results were shown in table 6.

3.2. Results for HPLC method:

3.2.1. System suitability:

It is performed by injecting six replicate injections. The %RSD w found to be 0.5963. The results were shown in table 7.

3.2.3. Linearity:

The linearity was plotted between 20-100 µg/ml and the R^2 value was found to be 0.9999. The table with results was shown in table 9. The linearity graph was shown as fig 4.

3.1.2 Precision:

Both intra-day and inter-day precision was performed by analysing 100 µg/ml concentration. The %RSD values in both the cases were not more than 2.0%. The results were shown in table 10.

3.1.3 Accuracy:

It is performed over the concentration range of 50%, 100% and 150% and mean recovery was found to be 98.60-101.85%. The results were shown in table 11.

3.1.4 LOD and LOQ:

The LOD and LOQ of PXT were found to be 0.07 and 0.24 µg/ml respectively.

3.1.5 Robustness:
Robustness was performed by analysing the samples at different wavelengths and varying flow rates. The results were shown in table 12 and 13.

REFERENCES

[1] Janvika M Boda et al., UV - Spectroscopic Method for simultaneous estimation of clonazepam and paroxetine hydrochloride hemihydrate in combined pharmaceutical formulation, *Inventi Rapid pharma analysis & Quality Assurance*, **2012**.
[2] Sharma MC, Smita Sharma, Validated simultaneous spectrophotometric estimation of Paroxetine hydrochloride bulk& tablet dosage form using Ferric Chloride, *Journal of optoelectronics & biomedical materials*, volume 2, p. 185-189, **2010**.
[3] Moinuddin R Syed, Seema H, Jintendra B Naik, *Int j of pharm and pharm Sci*, 2(2); 43-45, **2010**.
[4] Onal A, Kepekci SE, Oztunc A. *J AOAC*, 88(2); 420-5, **2005**.
[5] Darwish I A. *J AOAC*, 88(1); 38-45, **2005**.
[6] Elquda by H.M., et al., *IJRAP*, 8(2); 45-49, **2012**.
[7] Ibrahim A D, Abdine H H, Sawson M A, Al-Rayes L I. *IJAR*, 2009; 1-8, **2009**.
[8] Nawal Alarfaj et al., Spectroflurometric determination of paroxetine hydrochloride in its formulations and human plasma, Pubmed.org, **2006**.
[9] Ibrahim A.Darwish, et.al. *Analytical chemistry Insights*. 2(2); 145-155, **2000**.
[10] Ruchita S. and. Agrawal Y.K. *IJPER*, 27; 59-71, **2012**.
[11] Geetharam Y, Praveen S P. *Int. J of pharm*, 4(1); 448-457, **2014**.
[12] M.Lakshmi Surekha, et.al. A validated RP-HPLC method for the estimation of Paroxetine hydrochloride in bulk and tablet dosage form, *JPR solutions*, **2012**.
[13] Carda-broch, et.al. Determination of Paroxetine HCL in pharmaceutical preparation using HPLC electrochemical detection, *The open analytical chemistry journal*, 1-5, **2013**.
[14] ICH, Q2A, Hamonised Tripartite Guideline, Test On Validation of Analytical Procedures, IFPMA. In: Proceedings of the International Conference on Harmonization, Geneva, March, **1994**. 23.
[15] ICH, Q2B, Hamonised Tripartite Guideline, Validation of Analytical Procedure: Methodology, IFPMA, In: Proceedings of the International Conference on Harmonization, Geneva, March, **1996**.