RESEARCH ARTICLE

RP-HPLC method to Determination and Validation of Paroxetine Hydrochloride in Bulk and Pharmaceutical Dosage Form

[1]Kumaran S, [2]C Sreedhar, [3]Deepthi D Kini, [4]T Sreenivasa Rao, [5]Harsha K Tripathy

[1]Student, [2]Professor, 3Asso.Professor, [4]Assoc. Professor, [5]Professor
[1]Department of Pharmaceutical Analysis
[1]Karnataka College of Pharmacy, Bangalore - 64, India

Abstract
Purpose: This work makes an attempt to establish a sensitive and accurate method for the development and validation of an analytical method for estimation of Paroxetine hydrochloride in bulk and pharmaceutical dosage form.
Methods: A mixture of 10 mM Ammonium acetate buffer pH 4.5 and methanol in the ratio of 65:35 (v/v %) was used as the mobile phase. An xBridge™ C18 column (250 mm x 4.6 mm, 5µ) was used for the analysis at a flow rate of 1 ml/min, injection volume of 20 µl, run time of 10 mins, and detection wavelength of 291 nm. The repeatability (within-day in triplicates) and intermediate precision (for two days) were carried out by six injections and the obtained results within and between the days of trials were expressed as % RSD. The linearity of the method was determined by the analysis of analyte concentration across a range of 10µg/ml to 60µg/ml.
Results: The %RSD values of precision studies were found to be below the accepted limit of 2%. The method was found to be linear with a correlation coefficient (R^2) of 0.999. The method was also found to be accurate and robust with suitable values. The LOD and LOQ of the method were found to be 0.44 µg/ml and 1.48 µg/ml respectively.
Conclusion: The results of the analysis prove that this method can be used for the routine determination of Paroxetine Hydrochloride in bulk drug and in pharmaceutical dosage forms.
Keywords: Paroxetine, RP-HPLC, method development, validation, LOD, LOQ.

I. Introduction

Paroxetine is a powerful first-line- line inhibitor of serotonin reuptake in a sensory neuron. Paroxetine is a very well antidepressant that is applied in therapeutics around the world. It may have the highest action on serotonin reuptake inhibition when contrasted to other SSRIs. Emotional disturbances, Delayed stress syndrome, premenstrual dysphoric disorder such as depression and anxiety, and migraine are all treated with it. The most effective inhibitor of serotonin reuptake is paroxetine, a phenylpiperidine analog (5- hydroxytryptamine, 5-HT). A selective serotonin reuptake inhibitor is an anti-anxiety that belongs to a group of medications (SSRI).). Paroxetine hydrochloride is an off-white powder with an odorless melting point of 120° to 138°C and solubility of 5.4 mg/mL in methanol.. It treats depression, panic attacks, OCD, anxiety disorders, and post-traumatic stress disorder. It works by assisting in the restoration of a natural substance (serotonin) balance in the brain. The goal of this study is to develop a new UV spectrophotometric method for quantifying Paroxetine in bulk and tablet dosage forms that is simple and quick. However, the need for a rapid, precise, very easy, efficient, time-saving, and highly reliable analytical UV-Spectrophotometric method for routine quality control purposes necessitates the development of a new and improved approach. This method was validated as per ICH guidelines as a result, a simple, perfect, and sensitive UV approach for estimating Paroxetine hydrochloride in pure form and pharmaceutical formulations was proposed. Paroxetine's chemical structure is depicted in Figure 1.
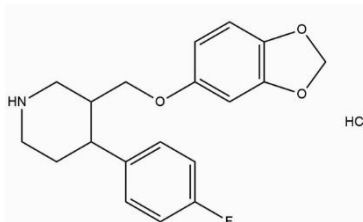
Drug Profile:
IUPAC Name :
$(3S, 4R) - 3 - [(2H - 1,3 - benzodioxol - 5 - yl\ oxy)methyl] - (4\ fluorophenyl)piperidine;\ hydrochloride$
Molecular formula: $C_{19}H_{21}ClFNO_3$
Molecular wt.: 365.8 g/mol
Solubility: Methanol and dimethyl sulfoxide (DMSO)
Pka value: 9.77

Figure 1: Structure of Paroxetine Hydrochloride

II. Materials And Methods

Instruments are used:

- Analytical weighing balance Shimadzu AUX 220
- Agilent Technologies HPLC (LC Compact 1120)
- Direct-Q® MILLIPORE France

Software

Agilent EZ Chrom Elite software package for Windows operating system was used for HPLC data acquisition and processing.

Statistical analysis

The result for linearity was calculated using linear regression in Microsoft Excel 2010 software package for Windows operating system. The %RSD was calculated for all values.

Materials used:

Commercially available Paxil tablets (12.5 mg) were procured from Guru Krupa Medicals in Bagalur, Karnataka which was manufactured by IPCA laboratories in Jorethang, Sikkim.

Paroxetine Hydrochloride API got gift sample from Enal Drugs Pvt. Ltd, Jeedimetla, Telangana of these working standard drugs.

Methanol AR grade was got from Karnataka College of Pharmacy in Bangalore, which was manufactured by Merck (India) Ltd, Mumbai.

Preparation of mobile phase

A mixture of 20 mM Ammonium acetate buffer pH 4.5 and methanol in the ratio of 65:35 (v/v%) was used as the mobile phase. Ammonium acetate buffer pH 4.5 was prepared by dissolving 1.5gm of ammonium acetate in 1000 ml of Milli-Q HPLC grade water, adjusting the pH to 4.5 with glacial acetic acid and degassed in ultrasonic water bath for 10 mins and vacuum filtered through 0.22 µm Nylon membrane filter.

Preparation of standard stock solution

A 100mg paroxetine API transferred into 100 ml of volumetric flask and is dissolved in Methanol (HPLC grade) volume were made up to the mark with same solvent. This gave the concentration of 1000µg/ml of Paroxetine Hydrochloride (Stock-1). further dilution 10 ml in 100 ml methanol in volumetric flask gives 100µg/ml. (Stock – 2). The solution is degassed in ultrasonic water for 10 mins and vacuum filtered through 0.22 µm filter.

Preparation of working standard solution

A working standard solution of concentration of 10µg/ml was prepared from the above stock solution using the methanol: Ammonium acetate in a 50:50 ratio as a diluent.

Selection of detected wavelength:

Paroxetine hydrochloride dilutions of 10µg/ml to 60µg/ml were made from the standard stock solution. Using a UV 1700 spectrophotometer and methanol as a blank, the dilutions of Paroxetine were scanned in the UV range of 200-400 nm. The highest absorbance of the drug was discovered at 291 nm, which was chosen as the detection wavelength for Paroxetine determination of solution-2. Figure 2 depicts the Paroxetine hydrochloride spectrum.
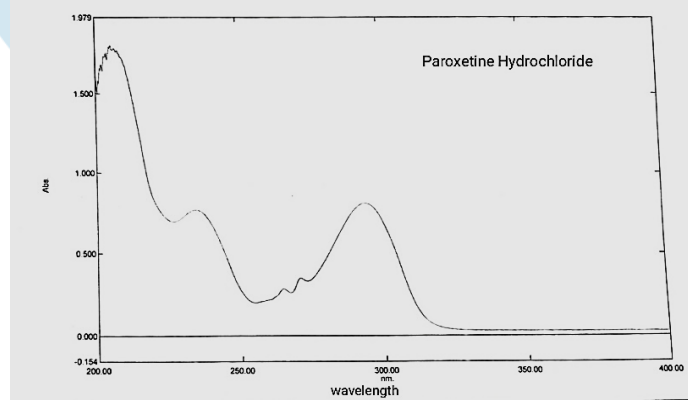

Figure 2: UV Spectrum of Paroxetine Hydrochloride

Chromatographic conditions

xBridge™ C18 column 5µ (250 mm x 4.6 mm) was used for the analysis. The flow rate was set at 1 ml/min with a run time of 10 mins. The injection volume was 20 µl. The detector was set at a wavelength of 291 nm.

Validation parameters

Precision

Method reproducibility was demonstrated by repeatability and intermediate precision measurements of peak area and peak symmetry parameters. The repeatability (within-day in triplicates) and intermediate precision (for 2 days) were carried out at a single concentration level. Six injections were made and the obtained results within and between the days of trials were expressed as % RSD.

Accuracy

The accuracy of the method was determined by calculating recoveries of Paroxetine hydrochloride by the standard addition method. In the pre-quantified sample solution (10 µg/ml), a known amount of standard solutions of Paroxetine hydrochloride (80%, 100%, and 120%) were added. The quantity of Paroxetine hydrochloride was measured using a calibration curve.

Linearity

The linearity of the method was determined by the analysis of analyte concentration across a range of 10µg/ml to 60µg/ml of Paroxetine hydrochloride and area was plotted graphically as a function of analyte concentration.

$$Accuracy = \frac{Amount\ of\ sample\ conc.\ found - Amount\ of\ Test\ Conc.taken}{Amount\ of\ standard\ conc.added} \times 100$$

Robustness

The robustness of the method was studied by deliberate changes in the method like alteration of flow rate and wavelength of detection.

$$Percent\ Assay = \frac{calculated\ qty\ of\ test\ sample\ (mg)}{weight\ of\ test\ sample\ (mg} \times 100$$

Limit of Detection (LOD):

The LOD parameter was calculated using the regression equation's intercept and slope. It was calculated using the formula below.

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

Limit of Quantification (LOQ):

The intercept and slope of the regression equation were used to determine the LOQ parameter. It was calculated using the formula below.

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

Estimation of Paroxetine:

Preparation of stock solution

A tablet is powdered equivalent to10mg of active ingredient is transferred into a 10 ml volumetric flask and is dissolved in Methanol: Ammonium acetate buffer in a 50:50 ratio of diluent (HPLC grade) volume was made up to the mark with the same solvent. This gave the concentration of 1000 µg/ml of Paxil (Stock-1). further dilution 1 ml in 10 ml methanol: Ammonium acetate diluent in volumetric flask gives 100µg/ml. The solution is degassed in ultrasonic water for 10 minutes and vacuum filtered through a 0.22 µm filter.

Preparation of working standard solution

A working standard solution of concentration of 40µg/ml was prepared from the above stock solution using the methanol: Ammonium acetate in a 50:50 ratio as a diluent.
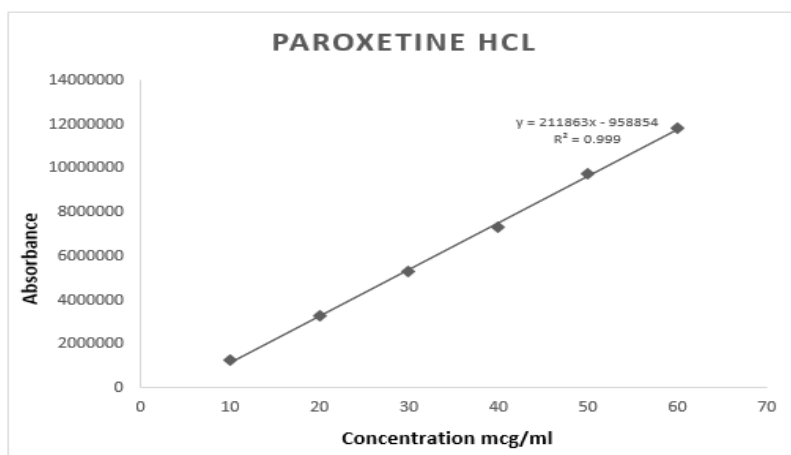

Figure 3 : Linearity chromatogram

Results and Discussion

1. Linearity

For linearity analyte concentration for Paroxetine Hydrochloride was taken across 10µg/ml to 60µg/ml of Rifapentine. They were prepared using HPLC-grade methanol: Ammonium acetate buffer (50:50) ratio as solvent. Then tested at 291 nm. Absorbance is plotted graphically as a function of analyte concentration.

Linearity for Estimation of Paroxetine Hydrochloride:

By using the working standard, aliquots of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, and 60 µg/ml, were prepared with methanol: Ammonium acetate buffer (50:50) ratio as solvent and Six dilutions of each of the above-mentioned concentrations were prepared separately and from these six dilutions, 20µl of each concentration were injected into the HPLC system. Then their chromatogram was recorded.
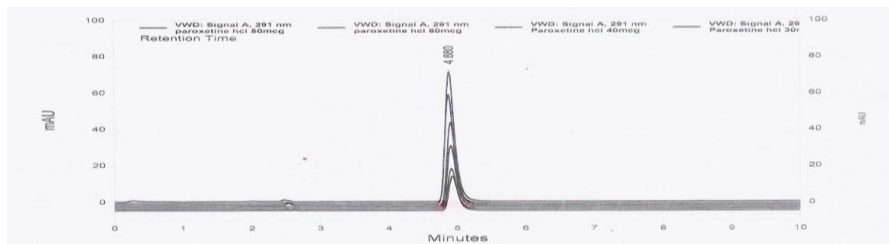

Figure 4: Linearity graph of Paroxetine Hydrochloride

Concentration (μg/ml^{-1})	Area
10	1278565
20	3265169
30	5287550
40	7344048
50	9764914
60	11797813

Table 1: Linearity data for Paroxetine Hydrochloride

2. Precision

The precision of the analytical method was studied by analysis of multiple sampling of a homogenous sample. The inter-day (between 2 days) and intraday (at the same days: morning and evening) precision were carried out. The variation of results was calculated and % RSD was determined.

CALCULATION

Morning				
Injection (40 μg/ml)	Areas	Average	Sd	RSD (%)
1	7377996			
2	7396903			
3	7359668	7379904.83	12165.51	0.16
4	7384919			
5	7382495			
6	7377448			

Table 2: Intraday precision of Paroxetine Hydrochloride (morning)

Day 2				
Injection (40 μg/ml)	Areas	Average	Sd	RSD (%)
1	7300496			
2	7301447			
3	7351878	7348400.33	38832.18	0.53
4	7380584			
5	7388959			
6	7367038			

Table 3: Intraday precision of Paroxetine Hydrochloride (day 2)

3. Accuracy

The accuracy for estimation of Paroxetine Hydrochloride using methanol was determined by adding a known amount of the analyte. The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay

Level of Percentage Recovery	80%	100%	120%
Amount present (mg per tablet)	12.5	12.5	12.5
Amount of standard drug added (mg)	32	40	48
Area Response	5832714 5881403 5862345	7332714 7381403 7462345	8867016 8858072 8858050
Mean	5858820	7392154	8861046
Standard Deviation	24535.08	65480.81	5170.18
RSD	0.42	0.89	0.06
Total amount recovery (mg)	32.3	43.2	48.7
% Recovery	100	100.4	100.1

Table 4: Accuracy for Paroxetine Hydrochloride

4.Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. A signal-to-noise ratio of 3:1 and 10:1 was considered to calculate LOD and LOQ.

Name of drug	LOD µg/ml	LOQ µg/ml
Paroxetine	0.44	1.48

Table 5: LOD and LOQ for estimation of Paroxetine Hydrochloride

5.Robustness

The robustness of analytical procedures describes its capability to remain unaffected by small and deliberate variation in the chromatographic conditions and found to be unaffected by small variation ±0.1ml/min inflow rate of mobile phase, and wavelength ±1nm results are shown.

Sl.no.	Parameter	Optimized	Used	Retention time(mins)
1	Flow rate	1 ml/min	0.9 ml/min	4.84
			1.1ml/min	4.37
2	Detection wavelength	291 nm	289nm	4.85
			293 nm	4.84

Table 6: Robustness of Paroxetine Hydrochloride

Parameter	Method	Acceptance Criteria
λ max (nm)	291	-
Linear Range (µg/ml)	10-60	-
Correlation Coefficient (r^2)	0.999	-
Limit of Detection (µg/ml)	0.44	-
Limit of Quantification (µg/ml)	1.48	-
Number of Theoretical Plates per meter	6127	NLT 2000
Tailing Factor	1.566	NMT 2

Table no.7: Optimum conditions in RP-HPLC method for the estimation of Paroxetine Hydrochloride.

Assay

Preparation of Sample Solutions:

Tablets containing (12.5mg) twenty tablets of Paroxetine hydrochloride were weighed and finely powdered. A quantity of powder equivalent to 50 mg of Paroxetine hydrochloride was weighed and transferred to a 50 ml volumetric flask containing 30 ml Ammonium acetate. The mixture was sonicated for 20 min. The volume was made up to 50 ml with Ammonium acetate. The contents were filtered through a 0.45 µ membrane filter. Further dilutions were made to get a concentration of 40µg/ml. Twenty microliters of the test and standard solutions were injected separately and chromatograms were recorded for up to 10 min. The proposed method was found to be specific and no interference from tablet excipients was observed.

Injection 40 µg/ml	Peak Area
1	7396903
2	7349719
3	7386214
4	7310977
5	7327082

6	7311960
Average	7347142.50
Standard Deviation	37302.82
RSD (%)	0.51

Table 8: Assay of Paroxetine Hydrochloride

Conflict Of Interest
This is a non-funding research work. There were no conflicts of interest.

References

[1]. Surekha ML, Gandla K, Ashwini GL. RP-HPLC Method Development and Validation for estimation of Paroxetine Hydrochloride in Tablet Dosage form. Journal of Pharmacy Research 2012; 5(3):1768-1770.
[2].B. Praveen Kumar, S. Vidyadhara, T. E. G. K. Murthy, J. Ramesh Babu and R.L. C. Sasidhar. Development and validation of novel UV spectrophotometric and RP-HPLC method for the estimation of paroxetine hydrochloride in pharmaceutical dosage forms. Der Pharmacia Lettre 2016; 8 (11):1-9.
[3].Satchitkrishna R, Dhara P, Dhananjay M. Development and validation of RP- HPLC method for simultaneous estimation of paroxetine hydrochloride and etizolam in pharmaceutical dosage form, Robert Skibiñski and Genowefa Misztal "Determination of Moclobemide, Paroxetine, and Fluvoxamine Tablets by HPLC" Acta poloniae pharmacuetica-Drug Research 2011;58(2):97-100.
[4].Scholze P, Zwach J, Kattinger A, Pifl C, Singer EA, Sitte HH: Transporter mediated release: a superfusion study on human embryonic kidney cells stably expressing the human serotonin transporter. J Pharmacol Exp Ther 2000; 293(3):870-8.
[5].Davis BA, Nagarajan A, Forrest LR, Singh SK: Mechanism of Paroxetine (Paxil) Inhibition of the Serotonin Transporter. Sci Rep 2016; 6(1).
[6].Foster RH, Goa KL: Paroxetine: a review of its pharmacology and therapeutic potential in the management of panic disorder. CNS Drugs 1997; 8(2):163-88.
[7].Zhou SF, Zhou ZW, Yang LP, Cai JP: Substrates, inducers, inhibitors and structure-activity relationships of human Cytochrome P450 2C9 and implications in drug development. Curr Med Chem 2009; 16(27):3480-675.
[8].Uttamsingh V, Gallegos R, Liu JF, Harbeson SL, Bridson GW, Cheng C, Wells DS. Graham PB, Zelle R, Tung R: Altering metabolic profiles of drugs by precision deuteration: reducing mechanism-based inhibition of CYP2D6 by paroxetine. J Pharmacol Exp Ther 2015; 354(1):43-54.