

Novel RP-HPLC Method for the Determination of Paroxetine in Pure Form and in Tablet Formulation

Vijayalakshmi Marella*, Korukonda Lalitha, Maddu Pravallika, Buchi Naidu Nalluri

Department of Pharmaceutical Analysis, KVSr Siddhartha College of Pharmaceutical Sciences, Vijayawada, Andhra Pradesh, INDIA.

ABSTRACT

Aim: To provide simple, rapid, reproducible reverse phase HPLC method for the estimation of paroxetine in pure form and tablet formulation. **Method:** The method is optimised using an Inertsil column C18 (250x4.6 mm, 5µm) with the mobile phase consists of 10mM ammonium formate and acetonitrile in the ratio of 50:50v/v at an isocratic flow rate of 1ml/min. The detection is carried out at 220nm. **Results:** The calibration curve is linear in the concentration range of 5-25 µg/ml. The method is statistically validated for its linearity, precision, accuracy, stability and specificity as per ICH Q2R1 guidelines and the method is found to be robust. The LOD and LOQ values were found to be 0.748 and 2.62 µg/ml respectively. **Conclusion:** Hence the proposed method was found to be precise, accurate, LC-

MS compatible and can easily adaptable for quality control of paroxetine in dosage forms, biological fluids, dissolution studies.

Key words: Paroxetine, RP-HPLC, Isocratic, MS compatible, Statistically validated, Tablet dosage form.

Correspondence

Dr. M. Vijaya Lakshmi,

Assistant Professor, KVSr Siddhartha college of pharmaceutical sciences, Pinnamaneni Polyclinic road, Vijayawada, Andhra Pradesh, INDIA.
Phone no: 8501011122

E-mail: marellavijayalakshmi@yahoo.co.in

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INTRODUCTION

Paroxetine is chemically (-)-Trans-4R-(4'-fluorophenyl)-3S-[(3',4'-methylenedioxyphenoxy)methyl]-piperidine hydrochloride hemihydrate, is a selective serotonin reuptake inhibitor.¹ It is an odourless, off white powder, with a melting point of 120°C to 138°C. It is a phenylpiperidine derivative which is not related to the tricyclic or tetracyclic antidepressants chemically. It is used in the treatment of panic disorder, obsessive-compulsive disorder and panic disorder. It inhibits the reuptake of serotonin at the neuronal membrane, enhances serotonergic receptor sites and potentiates 5-HT in the CNS.²

Literature survey reveals that few LC-MS,¹¹⁻¹³ Chiral HPLC,^{15,16} RP-HPLC^{7-9,14} methods with electrochemical detection, PDA detection were reported for the estimation of biological fluids. Janusz Zukowski *et al* reported a method for the separation of enantiomers in drug. Barry D. Zussman *et al.*,¹⁰ conducted investigation for the determination of paroxetine in human plasma using HPLC with fluorescence detection. Masaroti P *et al*³ reported a selective method for determination of paroxetine in human plasma by LC-MS/MS of ethyl acetate/hexane (50:50% v/v) on C18 column. Vergi -Athanasios *et al.*,⁴ reported a method using acetonitrile/phosphate buffer (pH=3.5) (30:70% v/v) at a flow rate of 1mL/min on Zorbax Eclipse XDB C18 column, using a fluorescence detector for simultaneous determination of paroxetine and its metabolites in plasma. The paroxetine eluted at 10 min and linearity was conducted over a range of 7-200 ng/mL.

N Dominguez *et al.*, conducted bioequivalence studies for the determination of paroxetine in plasma by HPLC on C18 column with fluorescence detector. Later on, Nitasha Agrawal *et al.*⁵ investigated a method for the determination of paroxetine in pharmaceutical preparations using HPLC with electrochemical detection. An optimum mobile phase combination of 40% acetonitrile and 0.01 M phosphate buffer to pH 3 with a flow rate of 1 mL/min and electrochemical detection of 0.9V were used, with a linearity range of 0.5-50 ng/mL. Rapid simple HPLC determination of paroxetine in human plasma using 10 mM phosphate buffer/acetonitrile (40:60%v/v) was reported by Jae-Gook *et al.*⁶ Hence, the present investigation was aimed to develop a simple, economical and rapid RP-HPLC-PDA method for the estimation of Paroxetine in bulk

and its formulations which would be accurate and precise and sensitive. Also the method was developed with LC conditions compatible to MS-detection using a mobile phase combination of 10mM ammonium formate/acetonitrile (50:50%v/v).

MATERIALS AND METHODS

Ammonium formate of analytical grade, HPLC Milli-Q water and acetonitrile were used. Paroxetine was a gift sample from Sun pharma. The tablets of paroxetine were obtained from local pharmacy.

Shimadzu HPLC system equipped with Inertsil OSD-3 C18, 250 x 4.6 mm, 5 µ column comprising of auto sampler, a PDA detector and LC solutions software was used. 10 mM ammonium formate was prepared by dissolving 157.6 mg of ammonium formate in 250 mL of HPLC grade water, which is used for mobile phase combination with acetonitrile (50:50%v/v) at a flow rate of 1 mL/min. Detection was carried out at 220 nm and peak purity of paroxetine was also determined.

The method which is developed was validated according to ICH guidelines. 10 mg of pure paroxetine was weighed accurately and transferred into a 10 mL volumetric flask. The content was dissolved by using HPLC grade water, after complete dissolution the volume was made up to the mark by using the same which gives 1000 µg/mL of the drug. From this stock solution a serial dilutions were done in order to obtain a concentration range of 5-25 µg/mL.

Estimation of paroxetine

Ten tablets of paroxetine were weighed accurately; average weight was calculated and powdered well. The powder equivalent to 10 mg of the drug was transferred into a 10 mL calibrated standard flask; 5 mL of HPLC grade water was added. The content of the flask was sonicated for 3 min to dissolve paroxetine and made up to the volume with the same and the resulting mixture was filtered through 0.45µm filter. Subsequent dilution of this solution was made with mobile phase to get concentration of 15µg/mL. This solution (15 µL) was injected six times into the HPLC system. The mean value of peak areas of six determinations was calculated and the drug content in the tablet was quantified.

Linearity

The linearity of the calibration curve over a range of 5-25 µg/mL.

Limit of detection and limit of quantification

Limit of detection is the lowest amount of analyte in a sample, which can be detected but not necessarily quantified as an exact value.

$$\text{LOD}=3\sigma/S$$

Where, σ is standard deviation of the intercept and

S is the slope of the calibration curve

Limit of quantification is the lowest amount of analyte in a sample, which can be quantitatively determined.

$$\text{LOQ}=10\sigma/S$$

Precision and accuracy

Repeated injections were used for the determination of intra-day and inter-day variability's. Accuracy was calculated by comparing the standard concentrations with the nominal concentrations.

The HPLC-PDA detector with LC-solution software provides more information regarding the sample composition at more than single wavelength detection. If any impurity or degradation product co-elutes with

the paroxetine peak, that can be detected by comparing with the UV spectra. The peak purity analysis was carried out throughout the study.

The standard paroxetine solution was further diluted in 10mL volumetric flask to get various concentrations ranging from 5-25 µg/mL of drug using mobile phase. From this each calibration standard solutions 10 µL was injected into the HPLC system. The chromatograms were recorded. The concentration of the paroxetine in µg/mL is taken in X-axis and peak area of the individual concentrations of calibration standards was taken in Y-axis. The calibration graph was plotted. This is used for the estimation of paroxetine in tablets.

RESULTS AND DISCUSSION

Paroxetine pure drug is soluble in methanol, ethanol and DMSO. Different mobile phase compositions were tried to elute the drug from the column and adequate resolution is achieved with 10 mM ammonium formate and acetonitrile in the ratio of 50:50 % v/v with Intertsil ODS, C18, 250 x 4.6 mm, 5µm column and this solvent system was found to be most suitable for method development and validation. Paroxetine shows maximum absorbance at 220 nm in the proposed method. A typical chromatogram of paroxetine standard solution is shown in Figure 2a and b respectively. The retention time was 4.559 min. The system suit-

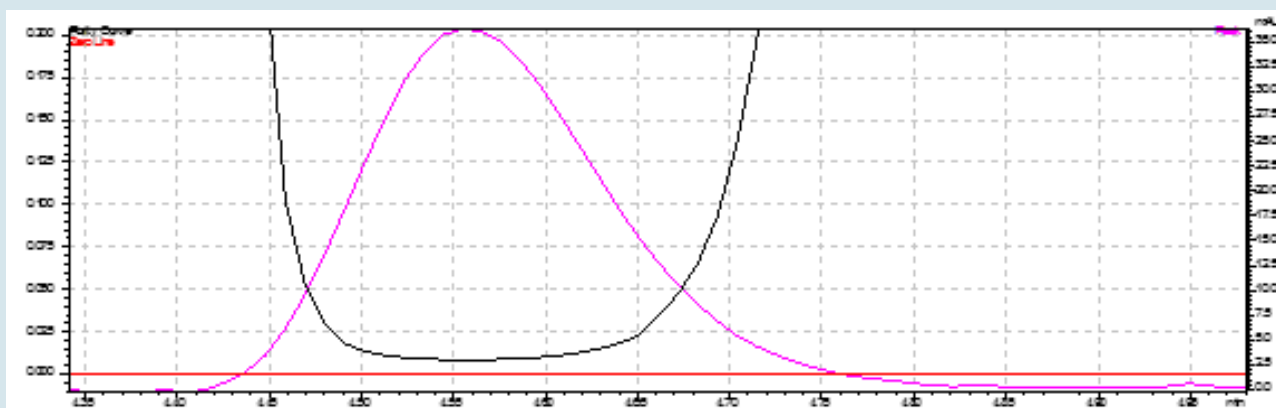


Figure 1: Peak purity profile of paroxetine.

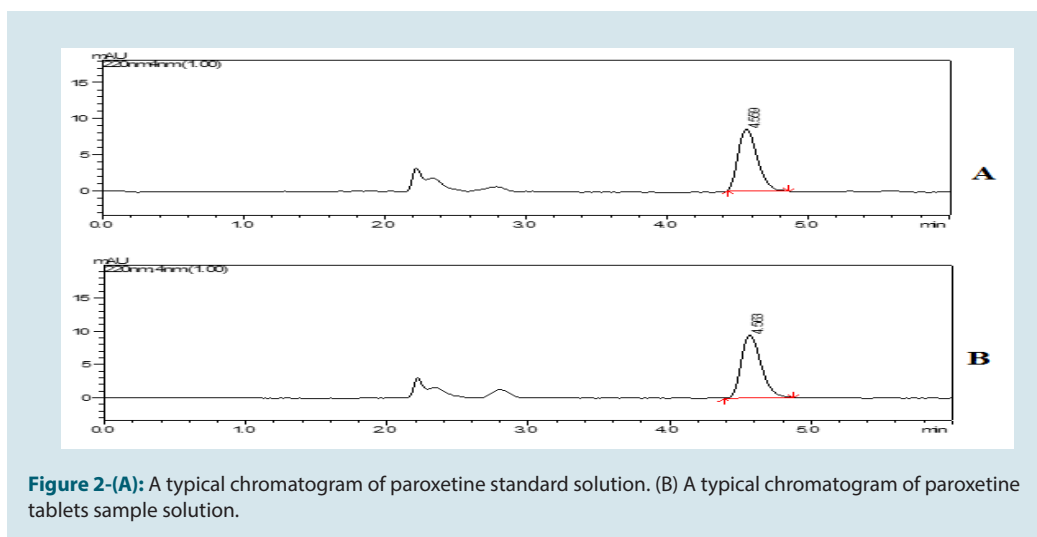


Figure 2-(A): A typical chromatogram of paroxetine standard solution. (B) A typical chromatogram of paroxetine tablets sample solution.

ability tests were carried out on freshly prepared standard stock solution. These parameters indicate good sensitivity and selectivity of the developed method.

Linearity

In the present developed HPLC method, the standard and sample preparation involves simple and rapid extraction procedure and requires less time. A good relationship was obtained in the concentration range of 5-25 µg/mL. Linear regression analysis report is given in Table 1. The proposed method was used to estimate the amount of paroxetine in tablets.

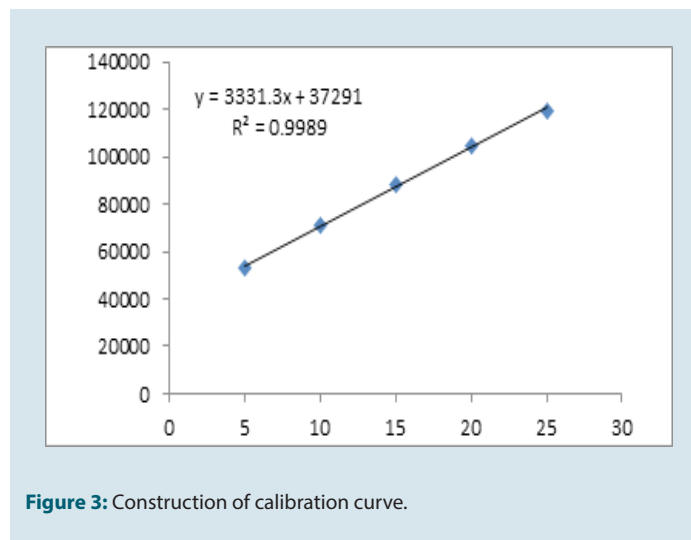


Figure 3: Construction of calibration curve.

Table 1: Linear regression analysis	
Parameters	Values
Concentration range, µg/mL	5-25
Slope	3331.3
Intercept	37291
Correlation coefficient	0.9989

Limit of detection and Limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) for paroxetine was found to be 0.748 and 2.62 µg/mL, respectively.

Precision and accuracy

Precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) was found to be 0.44 and 0.921% respectively. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time that was evaluated by analyzing six drug solutions, at the final concentration corresponding to 15 µg/mL of paroxetine during the same day. Intermediate precision was assessed by comparing the estimation on different days by different analysts. The paroxetine concentrations were determined and relative standard deviations (%RSD) were calculated. The accuracy of the developed method was carried out by adding the known amount of paroxetine pure drug to placebo solution and subjected to the proposed method. The study was done at 80, 100 and 120% of test concentrations (15 µg/mL) levels.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of paroxetine from pure form and tablet dosage form. The mobile phase used in this method is simple to prepare and the run time was 7 min, so less time consuming method. The recovery study shows that there is no interference of additives used for the preparation of tablets. Hence, the method can be easily and conveniently applied for routine quality control of paroxetine in its dosage form and can also be used for dissolution studies.

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CONFLICT OF INTEREST

Nil

ABBREVIATION USED

RPHPLC: reverse phase high performance liquid chromatography; LOD: limit of detection; LOQ: limit of quantitation; LCMS: liquid chromatography; mass spectroscopy; RSD: relative standard deviation.

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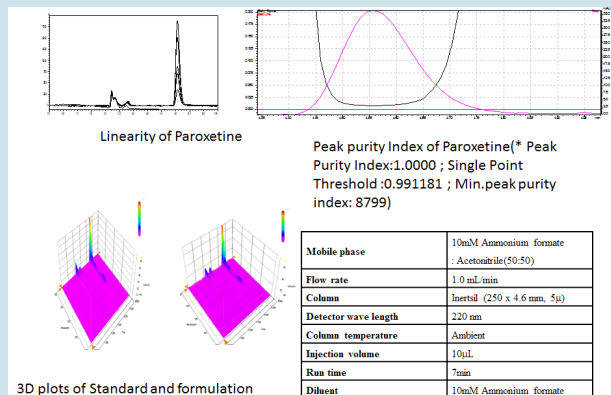
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PICTORIAL ABSTRACT



SUMMARY

- A novel RP-HPLC method is developed and validated for the estimation of paroxetine in pure and dosage forms.
- The method is optimized with LC-MS compatible mobile phase, ammonium formate and acetonitrile(1:1) at 1ml/min using intersil ODS column.
- The drug is detected at 220nm.
- The recovery study shows that there is no interference of additives used for the preparation of tablets.
- Hence, the method can be easily and conveniently applied for routine quality control of paroxetine in its dosage form and can also be used for dissolution studies.

ABOUT AUTHORS

M. Vijayalakshmi: Is working as Assistant Professor at KVSRR Siddhartha College of Pharmaceutical Sciences, Vijayawada, Andhra Pradesh-520010, India.

Maddu Pravalika: Is studying M.Pharm at KVSRR Siddhartha College of Pharmaceutical Sciences, Pinnamaneni Polyclinic Road, Vijayawada, Andhra Pradesh.

Korukonda Lalitha: Is studying M.Pharm at KVSRR Siddhartha College of Pharmaceutical Sciences, Pinnamaneni Polyclinic Road, Vijayawada, Andhra Pradesh.

Buchi Naidu Nalluri: Is PG director and Researcher at KVSRR Siddhartha College of Pharmaceutical Sciences 17 years of Research and Teaching experience, Ph.D and M.Pharm degrees with specialization in Pharmaceutics Supervisory/Administrative Experience.