Development of Analytical Method for the Determination of Piracetam in Bulk Drug and Its Pharmaceutical Formulation

Dr. R. Xavier Arulappa[1], M. Jerubin Welsingh[2], Akash. A[3], Anjali. B[4], Aswani B.R[5], Mannya. M[6], Navya. S Kumar[7], Prasobh G. R[8]

[1] *Professor and Head, Department of Pharmaceutical Chemistry and Analysis, Sree Krishna College of Pharmacy and Research Centre, Parassala, Thiruvananthapuram, Kerala, India.*

[2] *Department of Pharmaceutical Chemistry, S. A. Raja Pharmacy College, Tirunelveli, Tamil Nadu, India.*

[3,4,5,6,7] *Department of Pharmaceutical Chemistry and Analysis, Sree Krishna College of Pharmacy and Research Centre, Parassala, Thiruvananthapuram, Kerala, India.*

[8] *Principal, Sree Krishna College of Pharmacy and Research Centre, Parassala, Thiruvananthapuram, Kerala, India.*

Abstract: **This study presents the development and validation of a simple, rapid, and economic UV-visible spectrophotometric method for the estimation of Piracetam in bulk and pharmaceutical formulation. Using Systronics 2202TS spectrophotometer, the method employed a diluent mixture of Methanol and distilled water (40:60) to ensure solubility and stability. The maximum absorbance was observed at 205 nm. The method demonstrated linearity following Beer's Law in the concentration range of 5-25 µg/ml. Validation parameters confirmed high reliability: the assay of Sancetam 800 mg tablets indicated 98.84% purity, while recovery studies yielded a value of 102%. Precision was established with a Relative Standard Deviation of 0.38%, indicating the method is highly reproducible and suitable for routine quality control analysis.**

Keywords: **Analytical Method Validation, Beer's Lambert Law, Linearity, Methanol, Piracetam, Recovery Studies, UV-Visible Spectrophotometry.**

I. INTRODUCTION

Analytical chemistry involves the separation, identification, and quantification of chemical additives in herbal and synthetic materials. It is categorized into qualitative analysis, which identifies components, and quantitative analysis, which estimates their amounts. This field is a universal tool applied in clinical, environmental, and forensic analysis to determine the composition and structure of matter. It plays a crucial role in medicine for diagnosis and in industry for quality control. Furthermore, analytical data is essential in diverse fields ranging from biology and archaeology to space exploration. [1-4]

UV Spectroscopy measures the absorption or reflection of light in the 200–400 nm range. To absorb UV light, a substance must contain a chromophore, making this simple and cost-effective method suitable for analyzing both colored and colorless compounds. [5] The technique is based on the Beer-Lambert law, which states that absorbance is directly proportional to the concentration of the absorbing species and the path length. [6]

II. DRUG PROFILE

Piracetam, chemically known as 2-(2-oxopyrrolidin-1-yl) acetamide, is a prototype nootropic agent used clinically for the treatment of memory impairment, epilepsy, and dementia. Originally developed to treat elderly psychiatric patients, these "smart drugs" focus on improving memory. It is a cyclic derivative of GABA that acts on cognitive function without sedation or stimulation, influencing neuronal and vascular functions. [7-12]

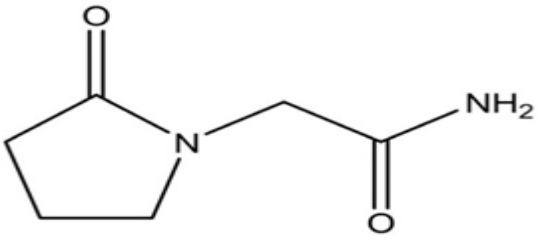

Fig 1: Piracetam

Category	Nootropic
I.U.P.A.C Name	2-(2-oxopyrrolidin-1-yl) acetamide
Molecular Weight	142.2 g/mol
Description	White powder
Chemical Formula	$C_6H_{10}N_2O_2$
Brand Name	SANCETAM
Solubility	Freely soluble in water, Soluble in ethanol

Table 1: Drug profile of Piracetam

Several official analytical procedures for the estimation of Piracetam in raw materials and pharmaceutical dosage forms are reported in standard compendia such as the BP and IP. A review of the available literature indicates that only a limited number of HPTLC, HPLC, and spectroscopic methods have been described for its quantitative determination. Therefore, the objective of the present study is to develop a simple, rapid, reproducible, and cost-effective spectrophotometric method for the estimation of Piracetam in bulk drug and in its pharmaceutical formulation.

III. METHOD AND MATERIALS

INSTRUMENTS:

Spectral measurements were carried out using a Systronics 2202TS UV–Visible double-beam spectrophotometer, equipped with a 2 nm spectral bandwidth and a wavelength accuracy of ±4 nm, along with automatic wavelength correction. A pair of 10 mm quartz cells was used for all measurements. A Kinglab KAB 203E analytical single-pan balance was employed for weighing. All glassware used in the procedures were soaked overnight in a mixture of chromic acid and concentrated sulfuric acid, thoroughly rinsed with distilled water, and dried in a hot air oven.

MATERIALS:

Piracetam was kindly gifted by Sahana Pharmaceuticals, Nagercoil, Tamil Nadu, India. Commercially available tablets were obtained from the local market. Methanol (AR grade) and deionized water were obtained from Shiv Scientific Industries, Tirunelveli, Tamil Nadu, India.

PREPARATION OF DILUENT:

Methanol (AR) and deionized water is mixed in the ratio 40:60 and this is used as diluent for the routine analytical purpose.

PREPARATION OF STANDARD STOCK SOLUTION:

100 mg of pure Piracetam was weighed and transferred into 100 ml standard flask. To this, add diluent to make it dissolve and then make up the volume up to the mark with the diluent [Standard stock solution – I (1 mg/ml)].

PREPARATION SERIAL DILUTIONS:

1 ml of stock solution-I was pipetted in 10 ml volumetric flask and make up to 10 ml with diluent. The concentration of solution was 100 µg/ml (stock solution-II). From stock solution-II, various aliquots of 0.5 ml, 1 ml, 1.5 ml, 2 ml and 2.5 ml were pipetted into a 10 ml volumetric flask followed by the addition of diluent. The volume is made upto 10 ml with diluent to produce concentration in the range of 5-25 µg/ml.

SELECTION OF WAVELENGTH FOR ANALYSIS OF PIRACETAM:

Accurately measured 1 ml of Standard Stock Solution I was transferred into a 10 ml volumetric flask and diluted to volume with the diluent to obtain Solution II (100 µg/ml). From this solution, 1 ml was pipetted into another 10 ml volumetric flask and diluted to volume with the same diluent, yielding a final concentration of 10 µg/ml. This solution was used for the initial spectral scan in the UV range of 190-230 nm against a reagent blank to determine the wavelength of maximum absorbance.

DETERMINATION OF WAVELENGTH OF MAXIMUM ABSORBANCE:

The working standard solution was scanned in the UV range of 190–230 nm using a 1 cm quartz cell against a solvent blank. The obtained UV spectrum of Piracetam showed a distinct absorption peak, with 205 nm selected as the λmax for its estimation. At this wavelength, Piracetam exhibited maximum absorbance, shown in Fig: 2.

PROPOSED METHOD FOR ASSAY OF FORMULATION:

Twenty Piracetam tablets were accurately weighed, and the average tablet weight was calculated. The tablets were finely powdered using a glass mortar and pestle. A quantity of tablet powder equivalent to 100 mg of Piracetam was accurately weighed and transferred into a 100 mL volumetric flask. The powder was dissolved in a suitable diluent, filtered, and the residue was washed thoroughly with the same diluent. The volume was then made up to 100 mL with diluent to obtain Solution I.

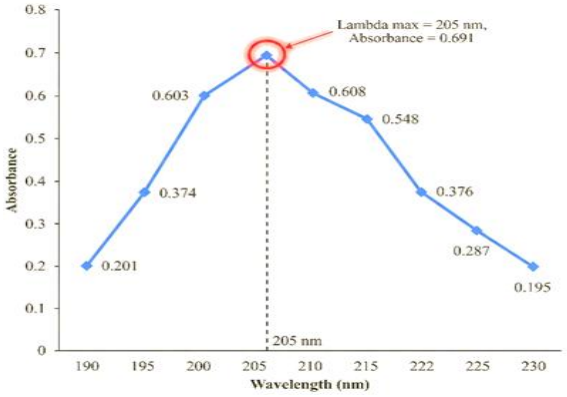

Fig 2: Absorption spectral data for Piracetam (10 µg/ml)

An aliquot of 1 ml from Solution I was pipetted into a 10 mL volumetric flask, and the volume was adjusted to the mark with diluent to obtain Solution II. Further, 1 ml of Solution II was transferred into another 10 mL volumetric flask and diluted to volume with diluent to yield a final drug concentration of 10 µg/ml. The absorbance of the resulting solution was measured at 205 nm against a reagent blank using a UV–Visible spectrophotometer. The assay was performed in five replicates, and the absorbance values were recorded.

The standard solution was prepared using pure Piracetam reference standard following the same dilution procedure to obtain a final concentration of 10 µg/ml. The absorbance of the standard solution was measured under identical experimental conditions. The results obtained were tabulated and presented in Table 2.

Sl. No	Brand Name	Wt. of tablet powder (mg)	Wt. of std drug (mg)	Std Abs	Avg. Wt. of tablet (mg)	Test Abs	Content drug in tablet (mg)	Avg. content (mg)
1	SANCETAM 800	130	100	0.691	1035	0.683	786.93	790.76
					1036	0.684	788.85	
					1037	0.684	790.76	
					1038	0.685	792.68	
					1039	0.686	794.60	

Table 2: Assay results for Piracetam

IV. METHOD VALIDATION [13-16]

The proposed method was validated for various parameters such as linearity and range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, sensitivity, and specificity according to ICH Q2 (R1) Guideline and USP guidelines.

LINEARITY AND RANGE:

Linearity refers to the ability of an analytical procedure to produce results that are directly proportional to the concentration of the analyte within a specified range. The analytical range is defined as the interval between the upper and lower concentrations of the analyte for which the method has been shown to provide acceptable precision, accuracy, and linearity.

In this study, linearity was evaluated across the concentration range of 5–25 µg/ml by performing triplicate analysis at each level. The absorbance values obtained for each concentration were recorded, and a calibration curve was constructed by plotting concentration (µg/ml) versus absorbance. The linear regression equation and correlation coefficient were generated using UV Probe software and the corresponding plot is shown in Fig: 3.

ACCURACY:

Accuracy refers to the closeness of agreement between the value obtained from the analytical procedure and the accepted reference or true value, and is often

described in terms of trueness. The accuracy of the proposed method was evaluated through recovery studies. This was performed by spiking the standard working solution into the sample solution (formulation). At each concentration level, the final amount of Piracetam was determined, and three replicate measurements were carried out. The percentage recovery was then calculated and expressed as mean ± standard deviation.

PRECISION:

Precision refers to the degree of reproducibility among individual test results when an analytical procedure is repeatedly applied to multiple samples of a homogeneous material. It is commonly expressed in terms of the standard deviation of the measurements.

LIMIT OF DETECTION (LOD):

The limit of detection is the lowest amount of analyte in a sample that can be detected, though not necessarily quantified with accuracy. The LOD for the proposed method was determined by preparing solutions of different concentrations in the range of 5–10 µg/ml. It was calculated using the formula:

$$LOD = 3.3 \times (SD / S)$$

where:
SD = standard deviation of the response
S = slope of the calibration curve

LIMIT OF QUANTIFICATION (LOQ):

The limit of quantification is the lowest concentration of analyte that can be quantitatively determined with acceptable precision and accuracy. The LOQ was calculated using the standard deviation of the response and the slope of the calibration curve:

$$LOQ = 10 \times (SD / S)$$

where:
SD = standard deviation
S = slope

ROBUSTNESS:

Robustness is a measure of the method's ability to remain unaffected by small, deliberate variations in analytical conditions, indicating its reliability during routine analysis.

To assess robustness, a 10 µg/ml standard solution of Piracetam was analysed by varying the wavelength by ±1 nm from the selected λmax, i.e., at 204 nm and 206 nm.

RUGGEDNESS:

Ruggedness measures the reproducibility of results under normal but variable conditions such as different analysts, instruments, or days.

For evaluating ruggedness, a 10 µg/ml standard solution of Piracetam was prepared and analysed by different analysts, and the results were compared.

V. RESULT AND DISCUSSION

LINEARITY AND RANGE:

The calibration curve demonstrated excellent linearity over the concentration range of 5-25 µg/ml. Linear regression analysis of absorbance against concentration produced the regression equation $Y = 0.0689X + 0.0013$, with a high coefficient of determination ($R^2 = 0.9999$), confirming the linear response of the method. The measurements were performed at the maximum absorption wavelength of 205 nm, indicating the suitability of the method for quantitative analysis within the studied range.

ACCURACY:

The percentage recovery and %RSD were calculated to evaluate the accuracy and precision of the method. The mean percentage recovery and %RSD values were found to be 102% and 0.38% respectively, within acceptable limits (≤2%) indicating that the developed method is accurate and precise for the estimation of piracetam. The mean, standard deviation, and percentage relative standard deviation (%RSD) were calculated, and the results are presented in the table.

PRECISION:

The repeatability of the method was evaluated by a precision study. The percentage relative standard deviation for piracetam was found to be 0.38%, indicating good repeatability of the developed analytical method.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION:

The limit of detection (LOD) and limit of quantification (LOQ) were calculated in accordance with ICH Q2(R1) guidelines using the standard deviation of assay results and the slope of the

calibration curve. The LOD and LOQ values were found to be 1.82 µg/ml and 5.53 µg/ml, respectively, indicating adequate sensitivity of the developed UV spectrophotometric method for the estimation of Piracetam.

ROBUSTNESS:

The percentage relative standard deviation obtained under these varied conditions was found to be within acceptable limits (≤2%), indicating that minor changes in wavelength did not significantly affect the analytical response. These findings confirm the robustness and reliability of the proposed UV spectrophotometric method for routine analysis of Piracetam.

RUGGEDNESS:

The absorbance measurements obtained under these conditions were compared, and the percentage relative standard deviation was calculated. The % RSD values were found to be within acceptable limits (≤2%), indicating that the results were consistent and reproducible. This demonstrates that the proposed UV spectrophotometric method is rugged and reliable for the routine estimation of piracetam in tablet dosage forms.

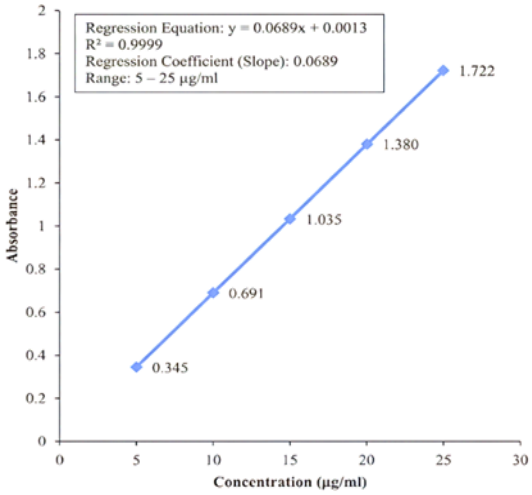

Fig 3: Calibration curve for Piracetam

VI. APPLICATION OF THE PROPOSED METHOD

The proposed method was successfully developed and validated for the estimation of Piracetam in pharmaceutical formulation. The results obtained using this method were compared with those from a reference method, demonstrating its reliability and suitability for routine analysis.

STANDARD METHOD [17]

About 20 tablets of Piracetam were accurately weighed and finely powdered. An amount of tablet powder equivalent to 500 mg of Piracetam was accurately weighed and transferred into a 100 ml volumetric flask. The powder was suspended in about 50 ml of 0.1 N hydrochloric acid, and the mixture was sonicated for 15 minutes to ensure complete extraction of Piracetam. The volume was then made up to the mark with the same solvent (0.1 N HCl). The resulting solution was filtered through Whatman No. 41 filter paper. An appropriate aliquot of the filtrate was further diluted with 0.1 N HCl to obtain a final concentration of 15 µg/ml of Piracetam. The absorbance of the final diluted solution was measured
at 264 nm. This method obeyed Beer's law in the concentration range 10-22 µg/ml with correlation coefficient value of 0.9988. The accuracy of method was accessed by assay and found to be 99.70 % and given in table 3.

Label Claim	% Purity	SD	% RSD
800	99.70	0.838	0.840

Table 3: Assay of Standard method

VII. VALIDATION OF SPECTROPHOTOMETRIC ACCURACY OF PROPOSED UV METHOD:

Accuracy of the proposed method was evaluated through recovery studies using the standard addition method.

STANDARD SOLUTION:

The standard solution was prepared according to the procedure described under standard dilution.

SAMPLE SOLUTION:

The recovery study was carried out on Piracetam tablets. A quantity of powdered tablet equivalent to 100 mg of Piracetam was accurately weighed and dissolved in the diluent. The solution was filtered, and the residue was washed with the same diluent. To this filtrate, 5 ml of the standard solution (1 mg/ml) of pure Piracetam was added. The mixture was shaken thoroughly, and the volume was made up to 10 ml with

the diluent. The resulting solution was then analyzed using the assay procedure for the tablet. The experiment was performed five times, and the results are presented in Table 4. The percentage recovery was calculated using the appropriate formula.

$$\text{Percentage of recovery} = \frac{(A- B)}{C} \times 100$$

Where,

A- Average content from recovery

B- Average content from assay

C- Amount of standard drug added

Table 4: Recovery study results for Piracetam

Sl. No	Brand Name	Wt. of std drug (mg)	Std Abs	Wt. of tablet powder (mg)	Avg. wt. of tablet powder (mg)	Amt of pure drug added (mg)	Abs of recovery sample	Percentage of recovery
1	SANCETAM 800	100	0.691	130	1035	5	0.687	102
2					1036	5	0.688	
3					1037	5	0.689	
4					1038	5	0.690	
5					1039	5	0.691	

λ Max	205 nm
Beer's law limits (µg/ml)	5-25 µg/ml
Slope	0.0689
Correlation coefficient	0.9999
% Relative Standard Deviation	0.38 %

Table 5: Optical characteristics, Precision and Accuracy of the proposed method for Piracetam

VIII. CONCLUSION

The proposed UV–Visible spectrophotometric method is simple, cost-effective, and can be readily applied for the analysis of Piracetam in bulk drug and pharmaceutical dosage form. The method exhibits a wide and reliable dynamic range with excellent accuracy and precision. It does not require any laborious sample pretreatment or cleanup procedures prior to analysis, thereby simplifying the overall methodology. Owing to its operational simplicity and reproducibility, the proposed method is well suited for routine quality control analysis and can be conveniently adopted in research and pharmaceutical industry laboratories for the quantitative estimation of Piracetam in pure and its pharmaceutical formulation.

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CONFLICT OF INTEREST:

The authors declared no conflict of interest.

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