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RESEARCH ARTICLE
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ANALYTICAL TECHNIQUES IN NEUROPHARMACOLOGY: SIMULTANEOUS DETERMINATION OF CITICOLINE SODIUM AND PIRACETAM BY UV-SPECTROSCOPY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract
The aim of this research is to develop a UV spectrophotometric method and HPLC method that can estimate both citicoline sodium and piracetam simultaneously in pharmaceutical formulations. A phosphate buffer at pH 6.8 was chosen as the suitable medium for the analysis in the study. Citicoline sodium was found to have a concentration range of 10 to 80µ g/ml, while piracetam's concentration range was 10-80 µg/ml.

Citicoline sodium and piracetam were UV absorbed using 272nm and 207.28 nm wavelengths, with the iso-absorptive point being 219.8nm as reported in the information. The calibration curves were made and showed a linear relationship between concentration and absorbance for both compounds. Within the specified concentration ranges, the method demonstrated excellent precision and accuracy.

The UV estimation method's stability and reproducibility are improved by using a phosphate buffer at pH 6.8.A reverse phase high performance liquid chromatographic method that is simple, fast, sensitive, and validated was developed to estimate Citicoline and Piracetam simultaneously in pharmaceutical dosage forms.

The chromatographic conditions used for the separation was agilent eclipse XDB(4.6 x 250 mm x 5 µm) with mobile phase comprised of Acetonitrile: Ammonium formate pH-3.0 adjusted with OPA (20: 80).

The flow rate was determined at 1.0 ml/min and 269 nm was detected. The analysis revealed that the retention times for Citicoline and Piracetam were determined to be 5.615 and 2.258, respectively. The methodology was formulated with the aim of attaining precision, accuracy, specificity, linearity, system suitability, and stability assessments, incorporating evaluations for limits of detection, quantification, ruggedness, and robustness.

Citicoline sodium and Piracetam exhibited linearity within the ranges of 12.50 to 75.00 µg/ml and 10 to 60g/ml, respectively. All specified parameters fell within the designated limits. The methodologies proposed are deemed suitable for the concurrent analysis of Citicoline and Piracetam in pharmaceutical formulations

KEYWORDS: Citicoline Sodium,Piracetam,UV- spectroscopy method, RP-HPLC,Phosphate buffer,ammonium formate buffer,Acetonitrile

INTRODUCTION

DRUG PROFILE OF CITICOLINE

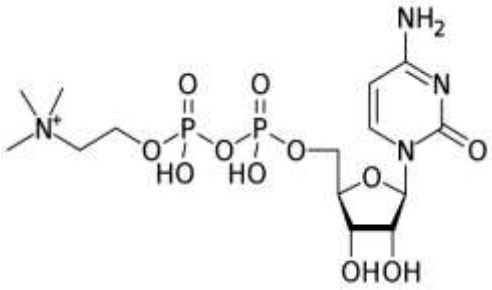


Fig No. 1: Molecular structure of Citicoline

Citicoline plays a role as a donor of choline during the biosynthesis of phosphoglycerides that contain choline. The work has concentrated on diagnosing, treating, and treating a variety of disorders, including bipolar disorder, cocaine abuse, hypomania, mania, and stroke.
Citicoline has demonstrated efficacy in the treatment of stroke, head injury, Alzheimer's disease, and dementia in Parkinson's disease. This medication acts as a protective agent for nerve cells, supplying them with essential nutrients, protecting them from damage, and improving their overall health and function. Citicoline works by boosting the production of phosphatidylcholine, the main phospholipid in the brain, and enhancing the creation of acetylcholine, which positively impacts neurological processes. Stroke and ischemic events can lead to compromised synthesis of brain phospholipids.

DRUG PROFILE OF PIRACETAM

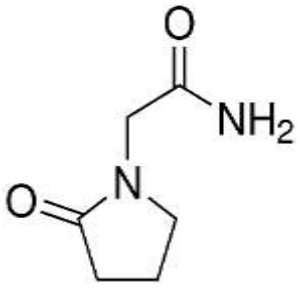


Fig No. 2: Molecular structure of Piracetam

Piracetam is believed to enhance the function of cells in the brain and blood vessels, making it an effective treatment for both aging and specific diseases.

Mechanism of Action:
Piracetam exerts a beneficial influence on the polar components of the phospholipid bilayer, resulting in the formation of mobile lipid complexes that facilitate the rearrangement of lipids, thereby impacting membrane functionality and flexibility. A similar phenomenon has been documented in a research study exploring the effects of beta-amyloid peptides on neuronal growth; while amyloid peptides induce lipid disarray in cellular membranes, leading to neuronal demise, piracetam has shown efficacy in mitigating the destabilizing effects of amyloid peptides.

The researchers propose that piracetam induces a favourable curvature of the membrane by encapsulating the polar moieties present in phospholipids to counteract the adverse curvature induced by amyloid peptides, consequently reducing the susceptibility of membrane destabilization. This mode of action serves to enhance membrane integrity, enabling the maintenance and restoration of the three-dimensional structure or conformation of membrane and transmembrane proteins essential for normal physiological processes such as membrane transport, chemical storage, receptor localization, and activation.

Absorption

Piracetam exhibits direct and time-dependent pharmacokinetic parcels with low intersubject variability over a large range of boluses. The oral administration of piracetam results in rapid-fire and expansive immersion, with the peak tube attention reaching within 1 hour after dosing in subjects who are dieted. After taking3.2 mg piracetam orally, the peak tube attention(Cmax) was 84 g/ mL. Ingestion of food can affect in a 17 reduction in Cmax and a1.5 hour detention in reaching Cmax(Tmax). The cerebrospinal fluid has a Tmax of roughly 5 hours after administration.

Experimental and methods

For analytical purposes, yarrow chem goods, based in Mumbai, 400086, India, sent gift samples of piracetam IP and citroline sodium salt. Rankem provided the solvents, which included acetonitrile of HPLC grade. The Milli-Q water that was produced in-house was used to collect the HPLC-grade water. Research-Lab provided other chemicals, all of the best quality and analytical grade, such as ortho phosphoric acid and ammonium formate.

Table No.1: List of Apparatus used in HPLC

S.No	Name	Model	Manufacturer
1.	HPLC	ALLIANCE	Waters e 2695- Empower software2.0versions
2.	pH meter	-	Eutech
3.	Weighing balance	-	Sartouris
4.	Pipettes, beakers and Burettes	-	Borosil
5.	Ultra sonicator	UCA 701	Unichrome
6.	Pump	Isocratic model	--

Methodology

Q-ABORBANCE RATIO METHOD

In UV spectroscopy, the Q Absorbance Ratio Method is a straightforward, sensitive, and cost-effective technique for determining several components of a combination medicinal dosage form at once.

In this process, we utilize the comparison between absorbances at two specific wavelengths. One of these wavelengths signifies an iso-absorptive juncture, where both substances absorb light equally. The second wavelength is associated with the peak absorption of one of the components (λ-max).

The method of absorbance ratio is defined by the following equation.

$$C_x = \left[\frac{Q_m - Q_y}{Q_x - Q_y}\right] x \frac{A_1}{ax^1}$$

Where;

$Q_m = A_2/A_1$

$Q_x=ax^2/ax1$
$Qy=ay^2/ay1$
A1=Absorbance of 'x'drug at Iso –absorptive point
ax^1=Absorptivity of 'x' drug at particular absorbance
Mixture absorbances are Al and A2 at 219.8 nm and 272 nm, respectively; citicoline absorbabilities are axl and ax2 at 219.8 nm and 272 nm, respectively; piracetam absorbabilities are ayl and ay2 at 219 nm and 272 nm, respectively.
The concentrations of citicoline and piracetam are denoted as Cx and Cy, respectively.

Working Wavelength Determination (λmax):
The isobestic wavelength was employed to concurrently determine the concentrations of two drugs. The wavelength where the molar absorptivity of two substances that can be interconverted is equal is referred to as the isobestic point. By utilizing a PDA detector, the spectral region spanning from 200 to 400 nm was examined to identify the wavelength at which the drug solution exhibited maximum absorption when dissolved in a combination of acetonitrile and ammonium formate at pH 3.0, which was modified using OPA (20:80). This analysis was conducted in relation to a reference sample consisting of the identical mixture. At 269 nm, the absorption curve displays the isobestic point. Thus, using the HPLC chromatographic procedure, a detector wavelength of 269 nm was used.

To create a phosphate buffer with a pH of 6.8:
28.80 grams of potassium dihydrogen phosphate and 11.45 grams of potassium dihydrogen phosphate need to be fully dissolved in 900 milliliters of distilled water within a 1000 milliliter volumetric flask. Following 20 minutes of sonication, ensure the compounds are completely dissolved before increasing the volume to 1000 milliliters using purified water.

Setting up the working standard and stock:
10 milligrams of piracetam and citicoline sodium, respectively, were precisely weighed and then placed to a 10 milliliter volumetric flask along with 10 milliliters of methanol. The medication was adequately dissolved to yield a 1000 µg/mL concentration. To achieve a concentration of 100 µg/mL, 10 mL of the stock solution was diluted to 100 mL.

Standard solution preparation: Using a series of 10 mL volumetric flasks, aliquots from the standard stock solution were taken in the range of 1–80 µg/mL. Phosphate buffer pH 6.8 was used to get the volume up to the required level.

Sample solution preparation:
Using a mortar and pestle, two CITISTAR-PM tablets with a 10 mg citicoline content were weighed, their average weight was ascertained, and the tablets were then coarsely ground. The right amount—10 mg of citicoline—was precisely weighed, transferred to a 100 mL volumetric flask, and then the volume was made up by half using methanol.

It was then vigorously shaken for five minutes, sonicated for approximately an hour to improve solubility, and finally completely made up with methanol and filtered through Whatmann filter paper. The resulting filtrate was further diluted to achieve the required concentrations and was used as the sample solution.

Chromatographic conditions:
Conditions for the chromatography: A number of trails were run in order to choose the optimal trail for the optimized procedure.
Preparation of standard solution

The preparation of the stock solution involves the precise weighing of 50 mg of citicoline and 40 mg of piracetam working standard, which are then transferred into a 100 ml clean and dry volumetric flask. Subsequently, diluent is added, followed by sonication to ensure complete dissolution, and the same solvent is used to bring the volume up to the required level. This process constitutes the stock arrangement.

Sample Solution Preparation:
Weigh the sample of citicoline and piracetam precisely, then place it into a 100 mL dry volumetric flask together with 109 mg of it. After adding the diluent and sonicating it for up to 30 minutes to dissolve it fully, centrifuge it for another 30 minutes to bring the volume up to par using the same solvent. After that, a 0.45 micron injection filter (stock solution) is used to filter it. 5 ml of the aforementioned stock solutions should be pipetted into a 50 ml volumetric flask and diluted with diluents to the appropriate level. (40 ppm of piracetam and 50 ppm of citicoline)

Ammonium Format buffer: The procedure involves dissolving 6.30g of ammonium formate in 1 litre of HPLC water, adjusting the pH to 3.0 using OPA, and filtering the mixture through 0.45µ membrane filter paper. Setting Up the Mobile Phase: Acetonitrile: Ammonium fomate pH-3.0 adjusted with OPA taken in a ratio of 20:80 was used to prepare the mobile phase. A 0.45µ membrane filter was used to filter it out of any impurities that would have affected the final chromatogram.

Procedure: Measure the areas for the peaks of citicoline and piracetam, inject 10 µL of the standard, and use the formulas to compute the assay percentage.

SYSTEM SUITABILITY: The theoretical plates for the peaks in Standard solution caused by Citicoline and Piracetam should not be less than 2000. The tailing factor for these peaks should not be greater than 2.0.

The resolution of the peaks in standard solution for citicoline and piracetam shouldn't be less than 2.

SUMMARY OF METHOD VALIDATION:
The ability of an analytical procedure to quantify an analyte of interest precisely without influence by known or blank contaminants is known as specificity. Three chromatograms—a blank, a standard, and a sample—were recorded for this purpose. The lack of reaction in the blank chromatogram during the drug retention periods indicates that the drug response was selective.

LINEARITY
The preparation of the stock solution involves the precise weighing of 50 mg of citicoline and 40 mg of piracetam working standard, which are then transferred into a 100 ml clean and dry volumetric flask. Subsequently, diluent is added, followed by sonication to ensure complete dissolution, and the same solvent is used to bring the volume up to the required level. This process constitutes the stock arrangement
.Level-I (12.50 ppm of citicoline and 10.00 ppm of piracetam) Level-II (25.00 ppm and 20.00 ppm of piracetam) Level III (37.50 ppm of citicoline and 30.00 ppm of piracetam) Level IV (50.00 ppm of citicoline and 40.00 ppm of piracetam) Level V (62.50 ppm of citicoline and 50.00 ppm of piracetam) Level VI (75.00 ppm of Citicoline and 60.00 ppm of Piracetam)
The procedure involves quantifying the peak area subsequent to the injection of each standard level into the chromatographic instrument. Following this, a graphical representation is created by plotting the peak area against the concentration, with the concentration depicted on the X-axis and the peak area on the Y-axis. Subsequently, the correlation coefficient is determined to assess the relationship between the concentration and peak area.
Limits of quantification (LOQ) and detection (LOD):

The following formula was used to determine the drug carry's limit of detection (LOD) and limit of quantification (LOQ) in accordance with international conference harmonization (ICH) criteria. LOD is equivalent to 3.3 X σ/S. LOQ is equal to 10 X σ /S.
The linear dose (LOD) and linear quantity (LOQ) of citicoline and piracetam, respectively, were determined to be 0.45µg/mL and 1.50µg/ml and 1.20µg/ml, respectively.

DEGRADATION STUDIES:

Stock preparation:

Weigh the sample of citicoline and piracetam precisely, then place it into a 100 ml dry volumetric flask. Add diluent, sonicate to dissolve it fully, then use the same solvent to get the volume up to the required level.

Acid degradation: Pipette 5 ml of the previously described solution and 1 ml of 1N HCl were added to a 50 ml vacuum flask. After keeping the vacuum flask at 60°C for an hour, the flask was neutralized with 1 N NaOH and diluted with diluent to make 50ml. Using 0.45 micron syringe filters, filter the mixture before transferring it to bottles.

Alkali degradation: In a 50 ml volumetric flask, pipette 5 ml of the aforementioned solution and add 1 ml of 1N NaOH. After an hour at 60 degrees Celsius, the volumetric flask was neutralized with 1N HCl and made up to 50 milliliters with diluent. After using syringe filters with a 0.45 micron filter, pour the solution into vials.

Thermal degradation:A sample of citicoline and piracetam was taken in a petridish and heated to 1050 C in a hot air oven for three hours. Subsequently, the material was extracted, diluted using diluents, and introduced into an HPLC for analysis.

Peroxide degradation:

The pipette A 50 ml vacuum flask was filled with 5 ml of the aforesaid stock solution, 1 ml of 3 percent w/v hydrogen peroxide, and diluent was used to raise the volume to the desired level.

After that, the vacuum flask was kept at 60°C for an hour.The vacuum flask was then allowed to sit at room temperature for fifteen minutes. Using 0.45micron syringe filters, filter the mixture before transferring it to bottles.

Reduction degradation:

The pipette A 50 ml vacuum flask was filled with 5 ml of the above-stock solution, 1 ml of 10% sodium bisulphite was added, and diluent was used to raise the volume to the necessary level. After that, the vacuum flask was kept at 60°C for an hour.

The vacuum flask was then allowed to sit at room temperature for fifteen minutes. Using 0.45micron syringe filters, filter the mixture before transferring it to bottles.

Photolysis Degradation:

A sample of citicoline and piracetam was kept in the photo stability chamber for three hours. Subsequently, the material was extracted, diluted using diluents, and introduced into an HPLC for analysis.

Hydrolysis Degradation:

The pipette A 50 ml vacuum flask was filled with 5 ml of above-stock solution, 1 ml of HPLC grade water, and diluent was used to raise the volume to the necessary level in the flask. After that, the vacuum flask was kept at 60°C for an hour. The vacuum flask was then allowed to sit at room

temperature for fifteen minutes. Using 0.45micron syringe filters, filter the mixture before transferring it to bottles.

RESULTS AND DISCUSSION

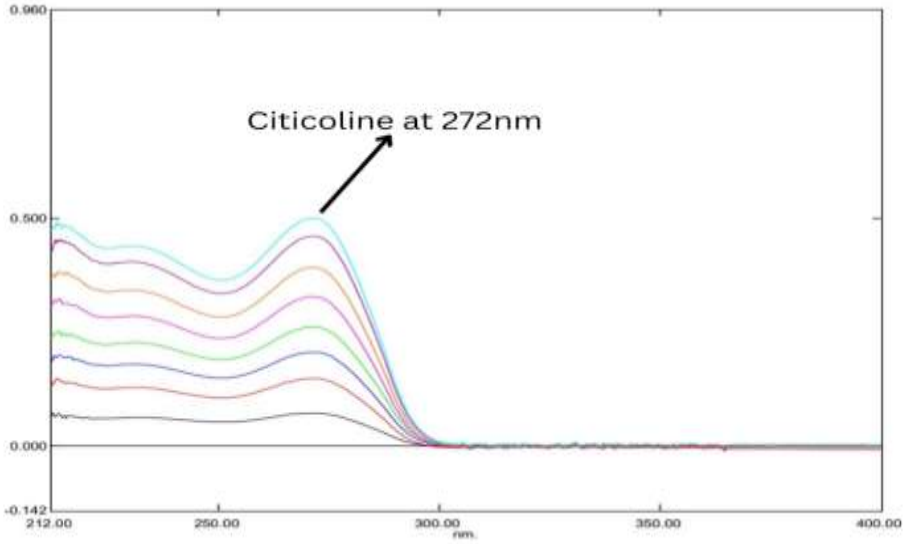


Figure:1 Citicoline sodium λmax 272nm

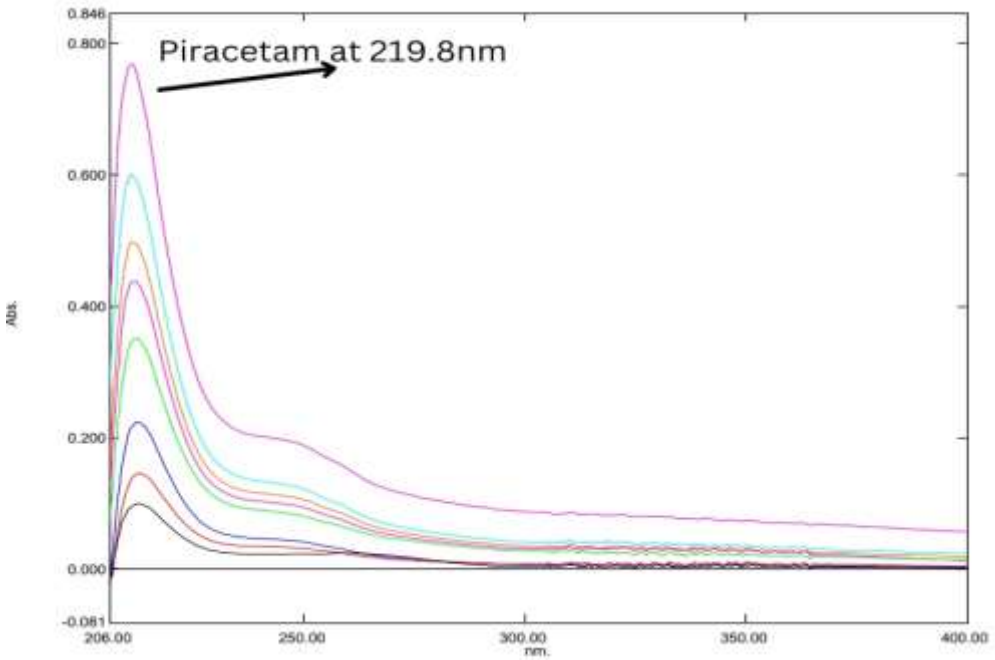


Figure:2 Piracetam λmax 219.8 nm

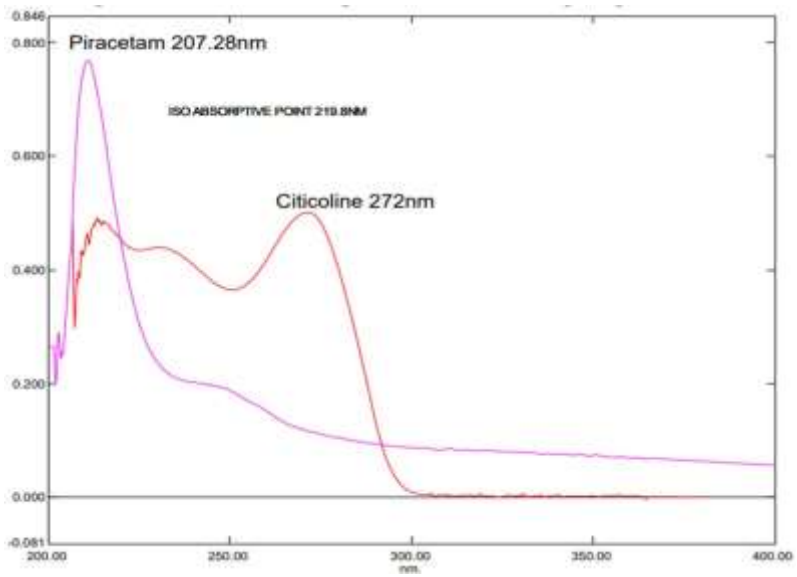


Figure:3 combined spectrum of citicoline sodium and piracetam

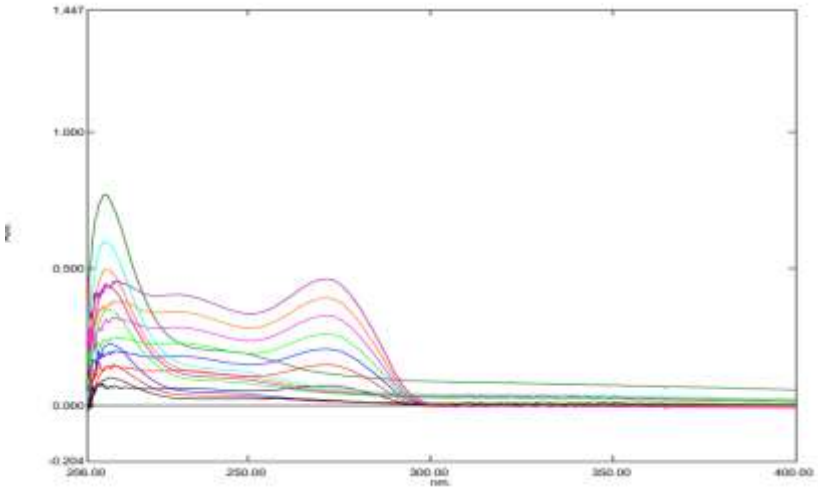


Figure 4: Combined spectrum of citicoline and spectrum

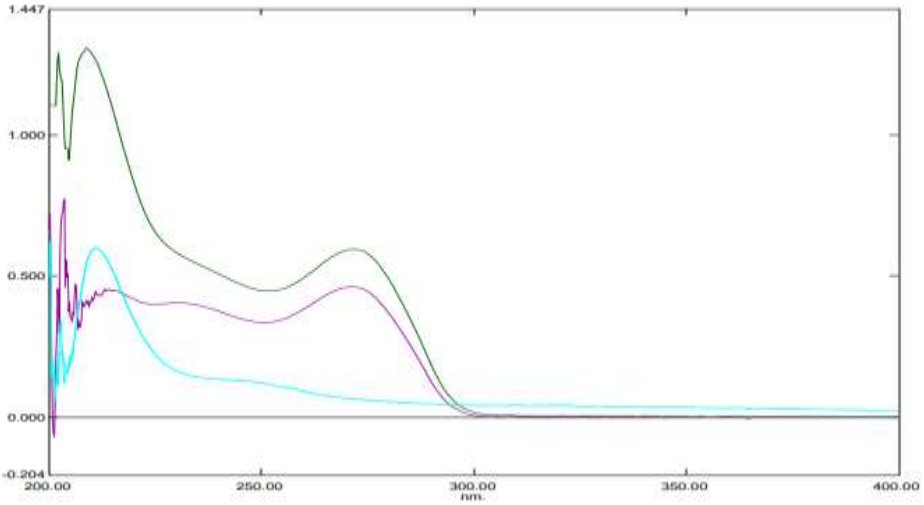
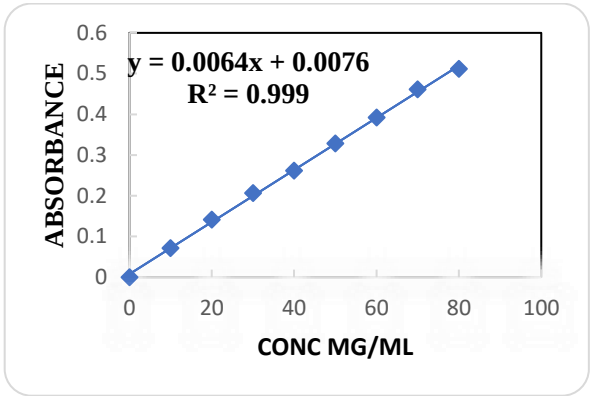
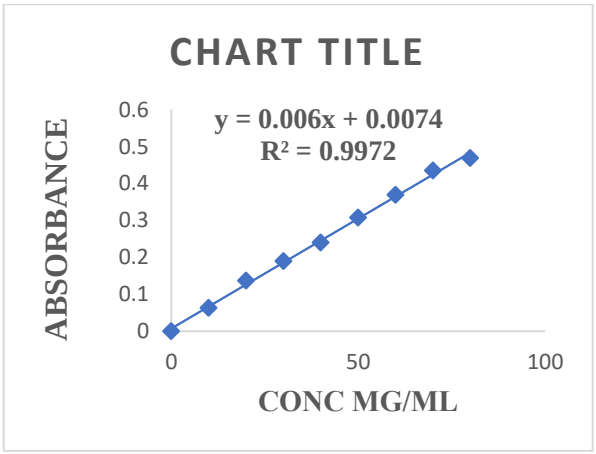


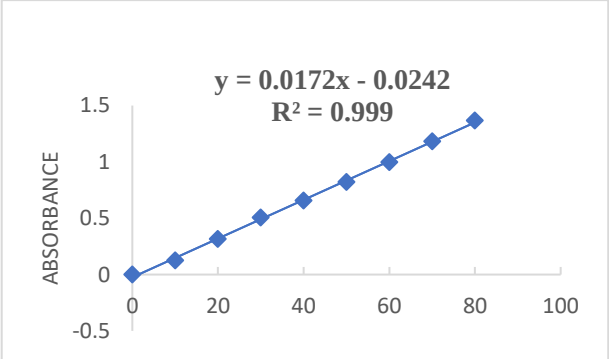
Figure5: Assay Of Citicoline Sodium And Piracetam Tablets



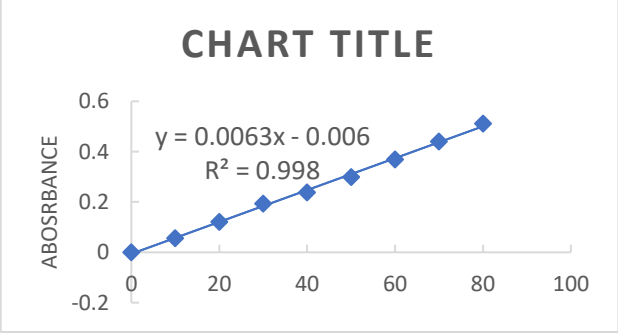
Graph1: Calibration curve of citicoline sodium at 272nm



Graph2:calibration curve of citicoline sodium at iso-absorptive point 219.8nm



Graph3: Calibration curve of piracetam at 272nm



Graph4: calibration curve of piracetam iso absorptive point at 219.8nm

Determination of Working Wavelength (λ_{max}):

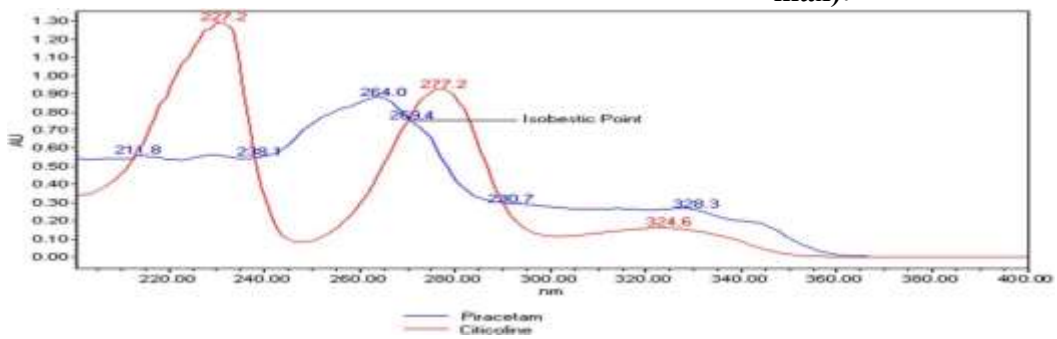


Figure6: PDA - Spectrum of Piracetam & Citicoline

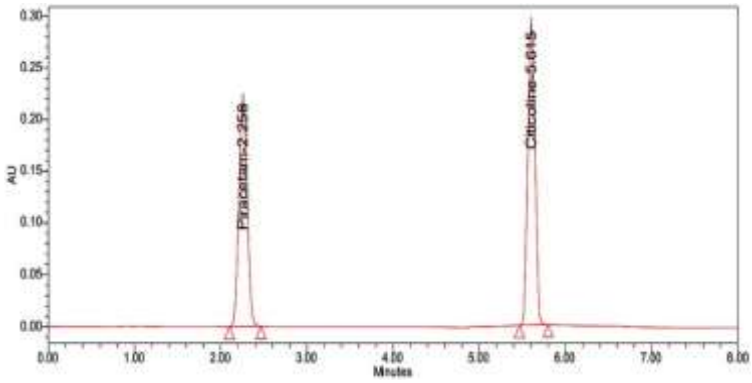


Figure7:Optimized chromatogram

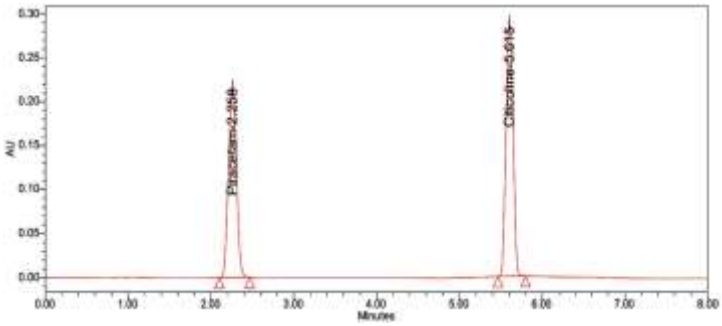
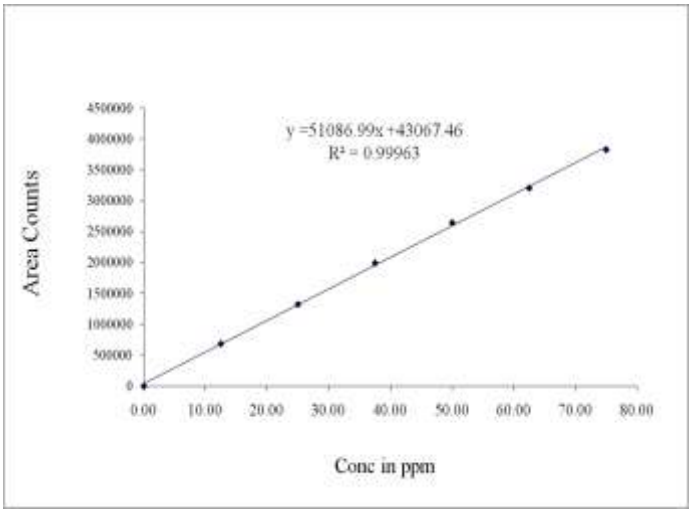
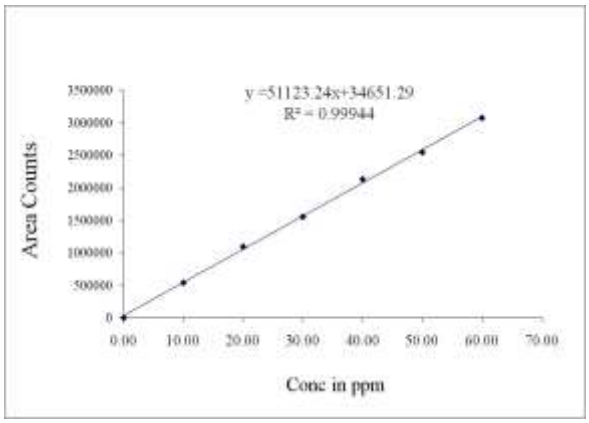


Figure8: Chromatogram of standard



Graph5: Calibration curve for Citicoline



Graph6: Calibration curve for Piracetam

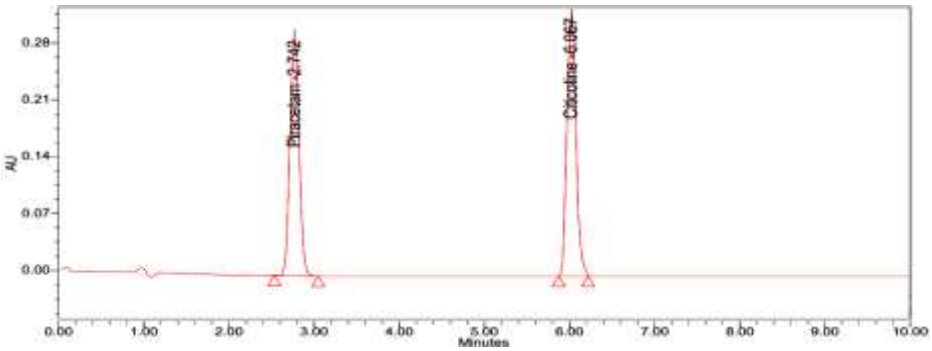


Figure9: Chromatogram for less flow rate (0.9 ml)

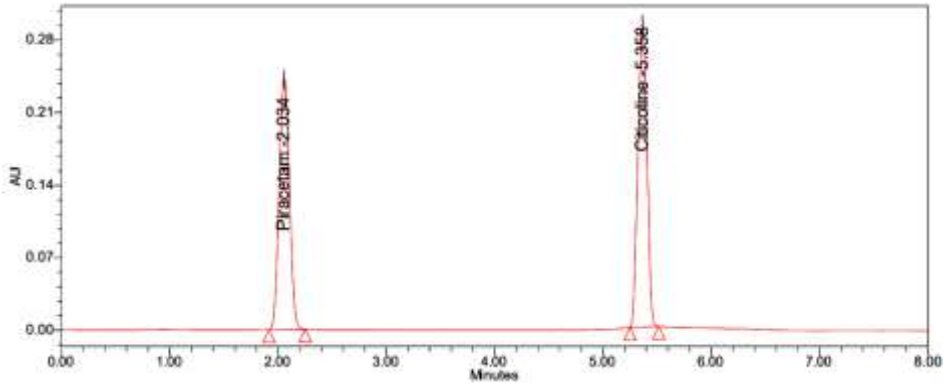


Figure10: Chromatogram for more flow rate (1.1 ml)

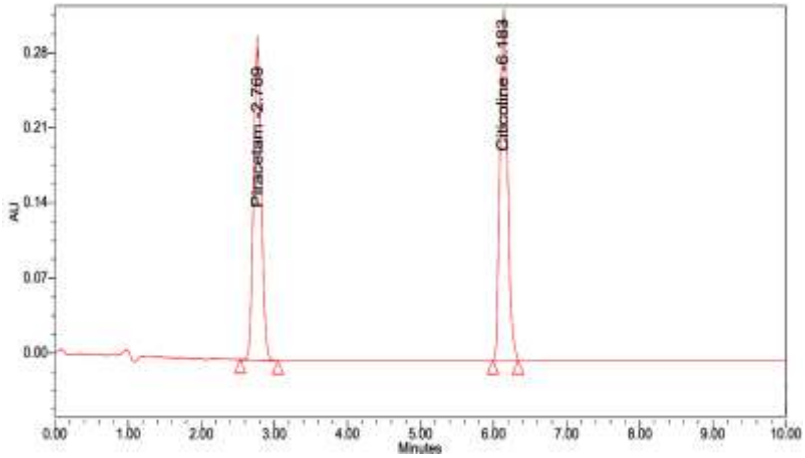


Figure11: Chromatogram for less Organic Phase (18:82)

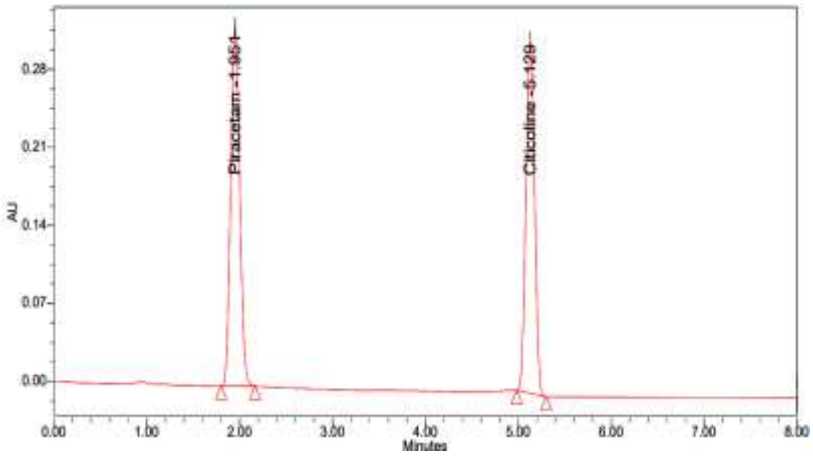


Figure12: Chromatogram for more Organic Phase (22:78)

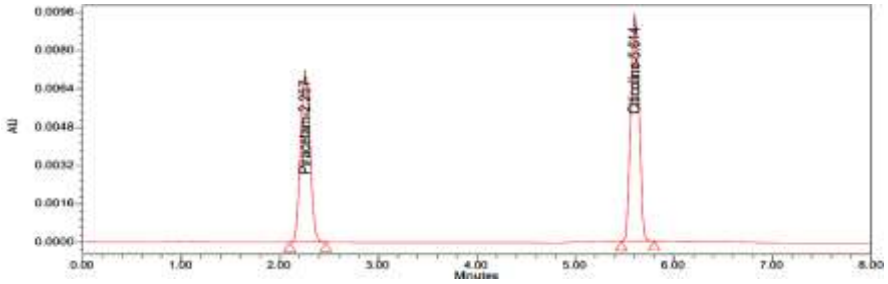


Figure13: Chromatogram for LOQ

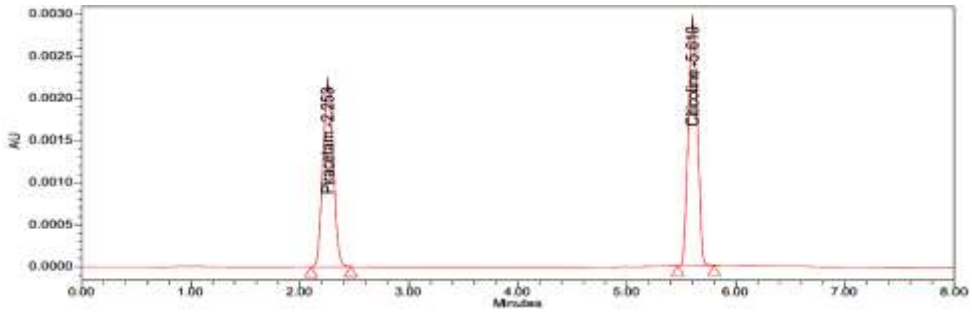


Figure14: Chromatogram for LOD

Trial	Mobile Phase	Mobile phase ratio	Detector wavelength	Flow rate (mL/min.)	Run time	Remarks
1.	Acetonitrile: 0.1% Formic acid	70:30	200-400nm	1ml/min	6min	Retention time is not within the limit
2.	Acetonitrile: 0.1% Formic acid	60:40	269nm	1ml/min	5min	Resolution not within the limit
3.	Acetonitrile: 0.1% Formic acid	50:50	269nm	1ml/min	10min	Tailing is not within the limit
4.	Acetonitrile: Ammonium formate pH-3.0 adjusted with OPA	30:70	269nm	1ml/min	6min	Plate count is not within the limit
5.	Acetonitrile: Ammonium formate pH-3.0 adjusted with OPA	25:75	269nm	1ml/min	6min	Response of the peaks are very high

Table:1 Trails in optimization of chromatographic condition

	Piracetam	Citicoline

Results: % Degradation results	Area	% Assay	% Deg	Purity Angle	Purity Threshold	Area	% Assay	% Deg	Purity Angle	Purity Threshold
Control	2135654	100	0	1.399	3.024	2641673	100	0	0.847	4.745
Acid	1902178	89.1	10.9	1.384	3.028	2319234	87.8	12.2	0.821	4.711
Alkali	1871236	87.6	12.4	1.383	3.032	2297134	86.9	13.1	0.839	4.726
Peroxide	1829782	85.7	14.3	1.371	3.039	2255165	85.3	14.7	0.894	4.783
Reduction	2103205	98.5	1.5	1.343	3.061	2371783	89.8	10.2	0.877	4.762
Thermal	1889782	88.5	11.5	1.306	3.055	2593481	98.1	1.9	0.808	4.798
Photolytic	2119452	99.2	0.8	1.368	3.014	2571821	97.3	2.7	0.842	4.732
Hydrolysis	2122838	99.4	0.6	1.377	3.021	2618748	99.1	0.9	0.865	4.757

Table2: Forced degradation studies

Parameter	Citicoline						
	Condition	Retention time(min)	Peak area	Resolution	Tailing	Plate count	%RSD
Flow rate Change (mL/min)	Less flow (0.9ml)	6.067	2550564	18.86	1.03	7419	0.40
	Actual (1.0ml)	5.615	2648751	19.08	0.97	7325	0.27
	More flow (1.1ml)	5.358	2846481	18.84	0.93	7260	0.56
Organic Phase change	Less Org (18:82)	6.183	2326501	19.51	0.99	7465	0.42
	Actual (20:80)	5.618	2631498	19.06	0.92	7388	0.27
	More Org (22:78)	5.129	2965248	17.67	0.86	7211	0.50

Table3: Robustness of citicoline

Parameter	Piracetam					
	Condition	Retention time(min)	Peak area	Tailing	Plate count	% RSD
Flow rate Change (mL/min)	Less flow (0.9ml)	2.742	2043678	1.10	12527	0.35
	Actual (1.0ml)	2.258	2141485	1.05	12470	0.25
	More flow (1.1ml)	2.034	2339482	1.00	12372	0.27

Organic Phase change	**Less Org (18:82)**	**2.769**	**1842748**	**1.13**	**12589**	**0.65**
	Actual (20:80)	**2.252**	**2139174**	**1.07**	**12461**	**0.25**
	More Org (22:78)	**1.951**	**2245475**	**1.02**	**12310**	**0.81**

Table4: Robustness of piracetam

Parameter		**Citicoline Sodium**	**Piracetam**
λ(nm), ZCP		**272nm**	**272nm**
Linearity (µg /mL)		**10-80(µg /mL)**	**10-80(µg /mL)**
Correlation coefficient		**0.999**	**0.999**
Regression Equation		**y = 0.0064x + 0.0076**	**y = 0.0172x - 0.0242**
LOD (µg /mL)		**0.0051(µg /mL)**	**0.0136(µg /mL)**
LOQ (µg /mL)		**0.0156(µg /mL)**	**0.0412(µg /mL)**
Precision (% RSD)	**Intra Day**	**100.47±0.3855,0.3838**	**100.16±0.0699,0.0698**
	Inter Day	**100.47±0.3855,0.3838**	**100.2±0.07,0.07**
Accuracy (% Recovery)		**100.07±0.1147,0.1146**	**100.01±0.0106,0.0106**
Assay (% w/w)		**98.12**	**100.01**

Table5: Summary Of Validation Parameters For Uv Method

Parameter		**Citicoline Sodium**	**Piracetam**
Detection wavelength (nm)		**269nm**	
Linearity (µg/mL)		**12.50-75.00(µg /mL)**	**10-60(µg /mL)**
Retention Time		**5.615**	**2.258**
Tailing Factor		**0.97**	**1.05**
Correlation coefficient		**0.99963**	**0.99944**
Regression Equation		**Y=51086.99x+43067.46**	**Y=51123.24x+34651.29**
LOD (µg/mL)		**0.45(µg /mL)**	**0.36(µg /mL)**
LOQ (µg/mL)		**1.50(µg /mL)**	**1.20(µg /mL)**
Precision (%RSD)	**Intra Day**	**0.63**	**0.62**
	Inter Day	**0.54**	**0.61**
Accuracy (% recovery)		**100.2**	**100.0**
Assay (% w / w)		**100.2**	**99.7**

Table6: Summary Of Validation Parameters For Rp – Hplc Method Summary Conclusion

The UV and HPLC methods developed for the estimation of selected drugs are simple, fast, accurate, robust and economical. Mobile phase and solvent preparation are simple, economical, reliable, sensitive and less time consuming.

The recovered samples were in good agreement with their respective label claims and showed no formulation recipient intervention in the estimation and can be used in the laboratory for routine analysis. selected drugs. Since the systematic validation parameters of the HPLC method were used

to estimate the selected drugs in their pure form and also showed positive, accurate and reproducible results (without any interference). card from the recipient), it can be inferred that the simple and concise methods proposed will be the most effective. useful for analysis. target. The present study concludes that the testing method indicating stability by RP-HPLC is simple, accurate, precise and specific and does not affect placebo and degradation products. Therefore, they can be used for routine analysis of Piracetam and Citicoline.

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