CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.10029842

https://www.iajps.com/volumes/volume10-september-2023/46-issue-09-september-23

Available online at: http://www.iajps.com ***Research Article***

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CITICOLINE AND PIRACETAM IN BULK AND TABLET DOSAGE FORM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Kanjarla Bhavani[1]*, Mrs. Bheemagoni Jyothi[1]

[1]DEPARTMENT OF PHARMACEUTICAL ANALYSIS, SREE DATTHA INSTITUTE OF PHARMACY, NAGARJUNA SAGAR ROAD SHERIGUDA, IBRAHIMPATNAM RANGAREDDY - 501510.

Article Received: July 2023 **Accepted: August 2023** **Published: September 2023**

Abstract:

Developed an accurate, precise and reproducible high performance liquid chromatographic method for simultaneous estimation of Citicoline and Piracetam in bulk and tablet dosage forms. Chromatographic separations of the drugs were achieved on a Symmetry ODS C18 (4.6×150mm, 5.0 µm) using a mobile phase consisting of Methanol: TEA Buffer pH-4.8 (35:65) v/v at a flow rate of 1.0 ml/min. The drugs elute were monitored at 276 nm. The retention time obtained for the Citicoline was 2.090 min and for the Piracetam was 5.289 min. The calibration curves were linear over the range of 20-60µg/ml and 25-75µg/ml for Citicoline and Piracetam respectively. The method is validated as per ICH guideline by determining its specificity, accuracy, precision, linearity & range, ruggedness, robustness and system suitability. The results of the study show that the proposed method is simple, rapid, precise and accurate, which is useful for the routine determination of Citicoline and Piracetam in bulk and tablet dosage forms. The method could be applied for determination of in its tablet dosage forms without any interference from excipients or endogenous substances. The proposed method is suitable for routine quality control analysis.

Keywords: *Citicoline and Piracetam, RP-HPLC, Accuracy, ICH Guidelines.*

Corresponding author:

Kanjarla bhavani,

Department of Pharmaceutical Analysis, Sree Datta Institute of Pharmacy, Nagarjuna Sagar Road Sheriguda, Ibrahimpatnam, Rangareddy - 501510.

Email Id- bavani1727@gmail.com.

QR code

Please cite this article in press ***Kanjarla Bhavani*** *et al,* ***Analytical Method Development And Validation For Estimation Of Citicoline And Piracetam In Bulk And Tablet Dosage Form By High Performance Liquid Chromatography,*** *Indo Am. J. P. Sci, 2023; 10 (09).*

INTRODUCTION:

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights. Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into mobile phase, and leave the system faster.

Based on this approach three components form the basis of the chromatography technique.

- Stationary phase: This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface a solid support".
- Mobile phase: This phase is always composed of "liquid" or a "gaseous component."
- Separated molecules

The type of interaction between stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other. Chromatography methods based on partition are very effective on separation, and identification of small molecules as amino acids, carbohydrates, and fatty acids. However, affinity chromatographies (i.e., ion-exchange chromatography) are more effective in the separation of macromolecules as nucleic acids, and proteins. Paper chromatography is used in the separation of proteins, and in studies related to protein synthesis; gas-liquid chromatography is utilized in the separation of alcohol, esther, lipid, and amino groups, and observation of enzymatic interactions, while molecular-sieve chromatography is employed especially for the determination of molecular weights of proteins. Agarose-gel chromatography is used for the purification of RNA, DNA particles, and viruses.

Stationary phase in chromatography, is a solid phase or a liquid phase coated on the surface of a solid phase. Mobile phase flowing over the stationary phase is a gaseous or liquid phase. If mobile phase is liquid, it is termed as liquid chromatography (LC), and if it is gas then it is called gas chromatography (GC). Gas chromatography is applied for gases, and mixtures of volatile liquids, and solid material. Liquid chromatography is used especially for thermal unstable, and non-volatile samples.

The purpose of applying chromatography which is used as a method of quantitative analysis apart from its separation, is to achieve a satisfactory separation within a suitable time interval. Various chromatography methods have been developed to that end. Some of them include column chromatography, thin-layer chromatography (TLC), paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography, high-pressure liquid chromatography, and affinity chromatography.

High-pressure liquid chromatography (HPLC):

Using this chromatography technique, it is possible to perform structural, and functional analysis, and purification of many molecules within a short time, this technique yields perfect results in the separation, and identification of amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules, In HPLC, mobile phase passes through columns under 10–400 atmospheric pressure, and with a high (0.1–5 cm//sec) flow rate. In this technique, use of small particles, and application of high pressure on the rate of solvent flow increases separation power, of HPLC and the analysis is completed within a short time.

Essential components of a HPLC device are solvent depot, high- pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled

Essential components of a HPLC device are solvent depot, high- pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled with the aid of a computerized system, and material is accrued.

Instrumentation of HPLC

1. Solvent Reservoir

Mobile phase contents are contained in a glass resorvoir. The mobile phase, or solvent, in HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.

2. Pump

A pump aspirates the mobile phase from the solvent resorvoir and forces it through the system's column

and detecter. Depending on a number of factors including column dimensions, particle size of the stationary phase, the flow rate and composition of the mobile phase, operating pressures of up to 42000 kPa (about 6000 psi) can be generated.

3. Sample Injector

The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).

4. Columns

Columns are usually made of polished stainless steel, are between 50 and 300 mm long and have an internal diameter of between 2 and 5 mm. They are commonly filled with a stationary phase with a particle size of 3–10 µm.

Columns with internal diameters of less than 2 mm are often referred to as microbore columns. Ideally the temperature of the mobile phase and the column should be kept constant during an analysis.

5. Detector

The HPLC detector, located at the end of the column detects the analytes as they elute from the chromatographic column. Commonly used detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors.

6. Data Collection Devices

Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.

MATERIALS AND METHODS:

Citicoline & Piracetam Procured from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck, Triethylamine from Merck.

HPLC METHOD DEVELOPMENT:

TRAILS

Selection of chromatographic methods:

The proper selection depends upon the nature of the sample, (ionic or ion stable or neutral molecule) its molecular weight and stability. The drugs selected are polar, ionic and hence reversed phase chromatography was selected.

Optimization of Column:

The method was performed with various columns like HypersilC_{18} column, X- bridge column and X-terra (4.6 ×150mm, 5µm particle size), Symmetry ODS C_{18} (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Mobile Phase Optimization:

Initially the mobile phase tried was Water: Methanol and Water: Acetonitrile and Methanol with TEA Buffer with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer pH-4.8 (35:65) v/v respectively.

Preparation of the Citicoline and Piracetam standard solution:

Preparation of standard solution: (Citicoline)

Accurately weigh and transfer 10 mg of Citicoline, working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.

Preparation of standard solution: (Piracetam)

Accurately weigh and transfer 10 mg of Piracetam working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.

Further pipette 0.4 ml of Citicoline, 0.5ml of Piracetam from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)

Mobile phase : Methanol: TEA Buffer pH-4.8 (35:65)
Column : Symmetry ODS C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 276 nm
Column temp : Ambient
Injection Volume : 10 µl
Run time : 10 minutes

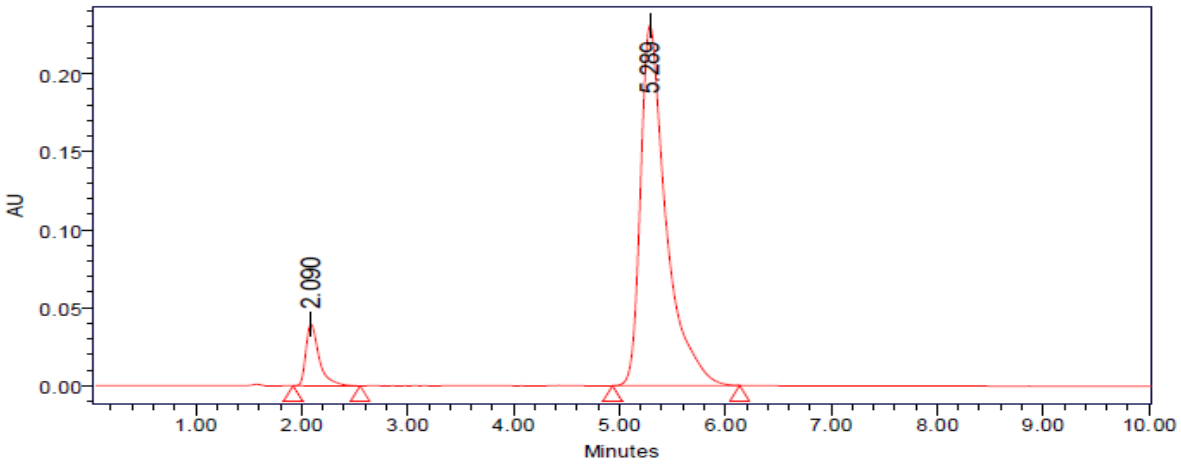

Fig-: Optimized Chromatogram

Table: - Peak Results for Optimized Chromatogram

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Citicoline	2.090	327989	39785		1.72	5657
2	Piracetam	5.289	3576856	232354	9.80	1.77	5869

Observation: From the above chromatogram it was observed that the Citicoline and Piracetam peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

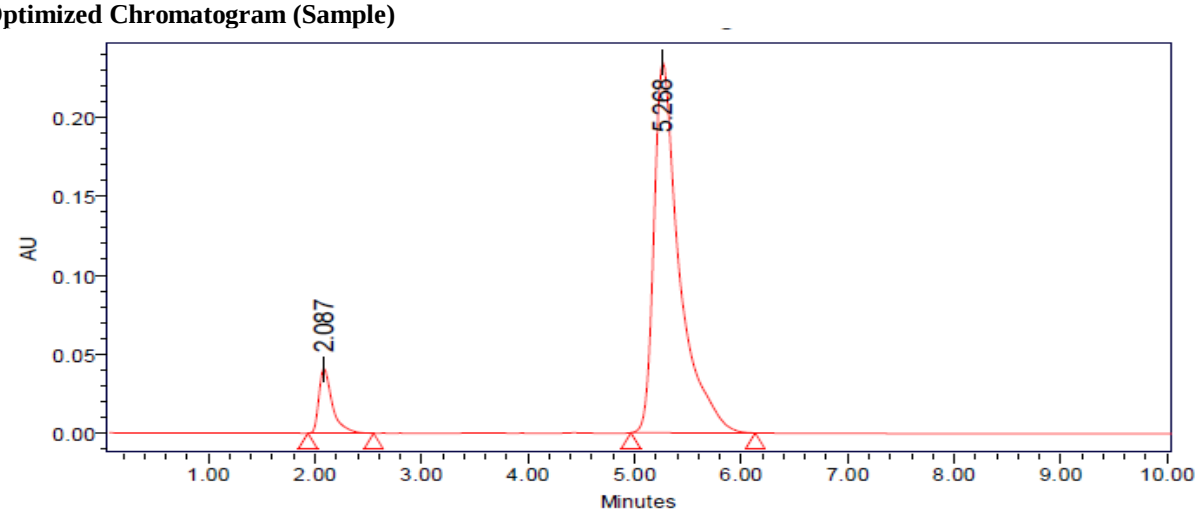

Figure-: Optimized Chromatogram (Sample)

Table: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Citicoline	2.087	312548	41236		1.75	5568
2	Piracetam	5.268	3498965	236584	9.83	1.94	5847

Table-: Results of system suitability for Citicoline

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Citicoline	2.090	325896	39689	5653	1.42
2	Citicoline	2.090	326989	39689	5695	1.42
3	Citicoline	2.089	327985	39698	5598	1.44
4	Citicoline	2.089	329477	40198	5569	1.43
5	Citicoline	2.085	325858	40259	5612	1.47
Mean			327241			
Std. Dev			1527.944			
% RSD			0.466917			

Table-: Results of system suitability for Piracetam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Piracetam	5.289	3576859	232352	5785	1.46	9.80
2	Piracetam	5.289	3585695	232365	5915	1.47	9.81
3	Piracetam	5.338	3596885	232451	5895	1.48	9.81
4	Piracetam	5.327	3565874	231653	5987	1.40	9.83
5	Piracetam	5.262	3598654	233658	5861	1.43	9.82
Mean			3588946				
Std. Dev			3585486				
% RSD			11360.78				

Assay (Standard):

Table-: Peak results for assay standard

S no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Citicoline	2.090	328966	39586		1.70	5563	1
2	Piracetam	5.289	3574898	232356	9.80	1.77	5665	1
3	Citicoline	2.089	327898	39568		1.66	5584	2
4	Piracetam	5.338	3569854	232548	9.93	1.83	5646	2
5	Citicoline	2.089	328657	40526		1.68	5584	3
6	Piracetam	5.327	3565874	232547	9.91	1.86	5783	3

Assay (Sample):

Table-: Peak Results for Assay Sample

S no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Citicoline	2.088		40365		1.69	5569	1
2	Piracetam	5.276		232565	9.75	1.89	5658	1
3	Citicoline	2.087		41245		1.72	5548	2
4	Piracetam	5.268		235685	9.82	1.91	5864	2
5	Citicoline	2.085		40898		1.75	5496	3
6	Piracetam	5.262		234588	9.78	1.95	5754	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Citicoline and Piracetam in pharmaceutical dosage form was found to be100.2%.

LINEARITY
Citicoline:

Concentration µg/ml	**Average Peak Area**
20	164436
30	255571
40	348687
50	439024
60	534830

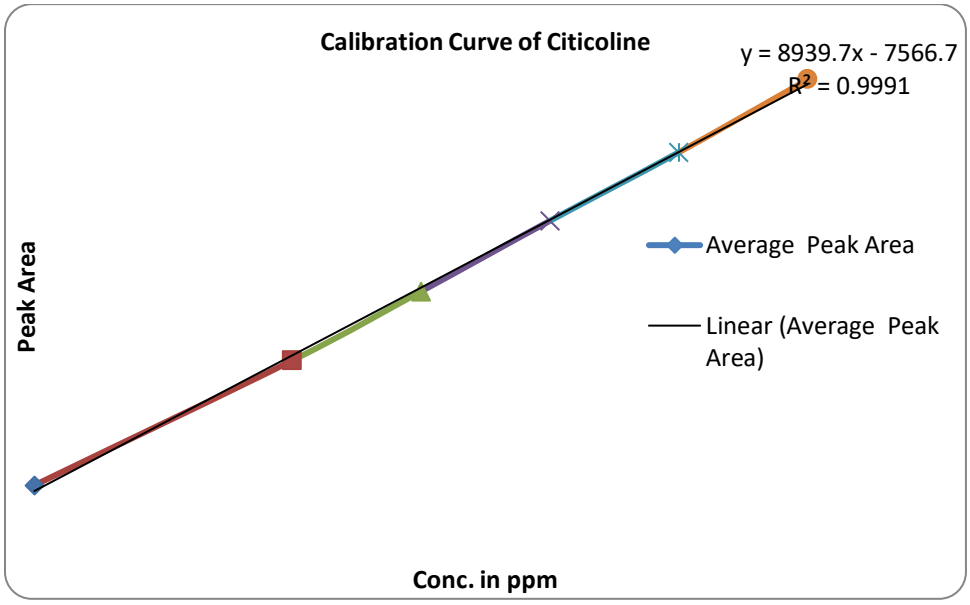

Figure: Calibration graph for Citicoline

Piracetam

Concentration µg/ml	Average Peak Area
25	1782454
37.5	2728974
50	3688678
62.5	4658022
75	5592695

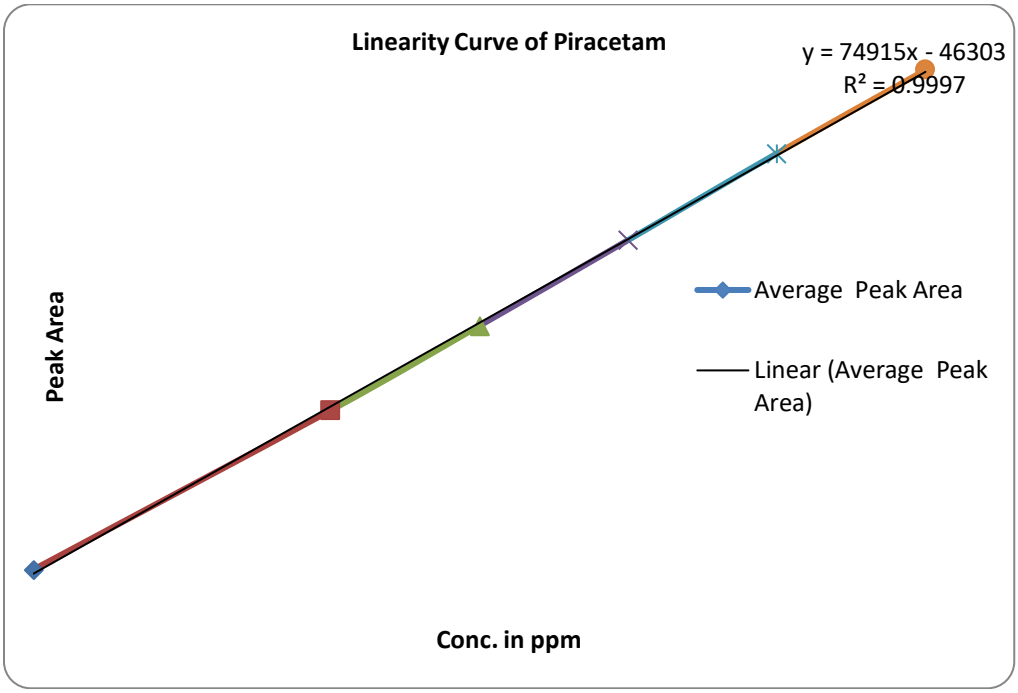

Figure: Calibration graph for Piracetam

REPEATABILITY:

Table-: Results of Repeatability for Citicoline:

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Citicoline	2.086	327689	41697	5081.3	1.8
2	Citicoline	2.083	327978	41402	5144.1	1.8
3	Citicoline	2.083	327879	41540	5118.1	1.8
4	Citicoline	2.081	327868	42256	5147.3	1.8
5	Citicoline	2.081	327859	42143	5101.8	1.8
Mean			327854.6			
Std. Dev			104.2176			
% RSD			0.031788			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table-: Results of method precision for Piracetam:

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Piracetam	5.178	3576985	241253	5969.5	2.0	9.8
2	Piracetam	5.199	3578989	2365824	5865.1	2.0	9.7
3	Piracetam	5.235	3576859	239568	5936.4	2.0	9.9
4	Piracetam	5.202	3578458	2386547	5964.4	2.0	9.8
5	Piracetam	5.206	3579864	241425	5045.6	2.0	9.5
Mean			3578231				
Std. Dev			1296.889				
% RSD			0.036244				

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

Table-: Results of Intermediate precision for Citicoline

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Citicoline	2.083	328986	42365	5556.2	1.6
2	Citicoline	2.083	328898	42685	5524.6	1.6
3	Citicoline	2.089	327789	42544	5465.2	1.6
4	Citicoline	2.083	328758	42685	5464.5	1.6
5	Citicoline	2.082	328869	42256	5589.4	1.8
6	Citicoline	2.080	329687	42365	5565.5	1.8
Mean			328831.2			
Std. Dev			608.8985			
% RSD			0.185171			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2.

Table-: Results of Intermediate precision for Piracetam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Piracetam	5.229	3578659	243659	5252.1	2.2	10.2
2	Piracetam	5.203	3578469	2436521	5256.4	2.1	10.0
3	Piracetam	5.133	3574865	245664	5356.8	2.1	10.0
4	Piracetam	5.229	3574824	243652	5265.6	2.2	10.2
5	Piracetam	5.151	3579861	244254	5235.7	1.5	9.9
6	Piracetam	5.112	3574898	236558	5986.2	1.6	9.9
Mean			3576929				
Std. Dev			2112.55				
% RSD			0.05906				

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Table-: Results of Intermediate precision Day 2 for Citicoline

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Citicoline	2.078	370979	42978	7083.0	1.9
2	Citicoline	2.082	371041	42568	8583.2	1.8
3	Citicoline	2.080	371386	42211	7533.2	1.8
4	Citicoline	2.089	369246	42277	6537.8	1.6
5	Citicoline	2.083	370840	42065	5489.3	1.6
6	Citicoline	2.089	369246	42277	6537.8	1.6
Mean			370456.3			
Std. Dev			954.6004			
% RSD			0.25			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

Table-: Results of Intermediate precision for Piracetam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Piracetam	5.077	3578985	246818	5208.0	1.5	10.1
2	Piracetam	5.151	3578415	242854	5127.6	1.3	10.0
3	Piracetam	5.112	3579864	242955	5269.7	1.5	10.2
4	Piracetam	5.133	3579862	242955	5269.7	1.6	10.2
5	Piracetam	5.203	3578948	242854	5127.6	1.5	10.0
6	Piracetam	5.133	3586775	242955	5269.7	1.6	10.2
Mean			3580475				
Std. Dev			3137.978				
% RSD			0.087641				

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY:

Table-: the accuracy results for Citicoline

%Concentration (at specification Level)	**Area**	**Amount Added (ppm)**	**Amount Found (ppm)**	**% Recovery**	**Mean Recovery**
50%	186584.7	20	20.026	100.13	100.435%
100%	367968.7	40	40.32	100.80	
150%	545922	60	60.225	100.375	

Table-: The accuracy results for Piracetam

%Concentration (at specification Level)	**Area**	**Amount Added (ppm)**	**Amount Found (ppm)**	**% Recovery**	**Mean Recovery**
50%	1925532	25	25.084	100.336	100.284%
100%	3790965	50	49.985	99.970	
150%	5695646	75	75.410	100.546	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table-: Results for Robustness

Citicoline:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	327989	2.090	5698	1.70
Less Flow rate of 0.9 mL/min	302986	2.736	5569	1.82
More Flow rate of 1.1 mL/min	316989	1.673	5598	1.91
Less organic phase	315989	2.736	5651	1.82
More organic phase	308986	1.673	5452	1.91

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Piracetam:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3576856	5.289	5689	1.77
Less Flow rate of 0.9 mL/min	3458978	6.746	5658	1.88
More Flow rate of 1.1 mL/min	3589871	4.032	5245	1.91
Less organic phase	3579124	6.746	5154	1.88
More organic phase	3578698	4.032	5652	1.91

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Citicoline and Piracetam in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Citicoline and Piracetam was freely soluble in ethanol, methanol and sparingly soluble in water.

Methanol: TEA Buffer pH-4.8 (35:65) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Citicoline and Piracetam in bulk drug and in

Pharmaceutical dosage forms.

Acknowledgement:
The Authors are thankful to the Management and Principal, Department of Pharmacy, Sree Dattha Institute of Pharmacy, Ibrahimpatnam, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

BIBLIOGRAPHY:

1. McMurry, John (2011). Organic chemistry: with biological applications (2nd ed.). Belmont, CA: Brooks/Cole. p. 395.
2. Hostettmann, K; Marston, A; Hostettmann, M (1998). Preparative Chromatography Techniques Applications in Natural Product Isolation (Second ed.). Berlin, Heidelberg: Springer Berlin Heidelberg. p. 50.
3. Cuatrecasas P, Wilchek M, Anfinsen CB. Selective enzyme purification by affinity chromatography. Proc Natl Acad Sci U S A. 1968; 61: 636–43.
4. Porath J. From gel filtration to adsorptive size exclusion. J Protein Chem. 1997; 16: 463–8.
5. Harris DC. Exploring chemical analysis. 3rd ed. WH. Freeman & Co; 2004.
6. Regnier FE. High-performance liquid chromatography of biopolimers. Science. 1983:245–52.
7. Sharma BK. Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23th ed .Goel publishing house meerut, 2004,P12-23.
8. H.H. Willard, L.L. Merritt, J.A. Dean, F.A. Settle. Instrumental methods of analysis, 7th edition, CBS publishers and distributors, New Delhi. 1986, P.518-521, 580-610.
9. John Adamovies, Chromatographic analysis of pharmaceutical, Marcel Dekker Inc. New York, 2nd ed, P.74, 5-15.
10. Gurdeep Chatwal, Sahm K. Anand. Instrumental methods of chemical analysis, 5th edition, Himalaya publishing house, New Delhi, 2002, P.1.1-1.8, 2.566-2.570.
11. D. A. Skoog. J. Holler, T.A. Nieman. Principle of instrumental analysis, 5th edition, Saunders college publishing, 1998, P.778-787.
12. Skoog, Holler, Nieman. Principals of instrument al analysis 5th ed, Harcourt publishers international company, 2001, P.543-554.
13. William Kemp. Organic spectroscopy, Palgrave, New York, 2005, P.7-10, 328-330.
14. P.D. Sethi. HPLC: Quantitative analysis pharmaceutical formulations, CBS publishers and distributors, New Delhi (India), 2001, P.3-137.
15. Michael E, Schartz IS, Krull. Analytical method development and validation. 2004, P. 25-46.
16. R. Snyder, J. Kirkland, L. Glajch. Practical HPLC method development, 2nd ed, A Wiley international publication, 1997, P.235,266-268,351-353.653-600.686-695.
17. Basic education in analytical chemistry. Analytical science, 2001:17(1).
18. Method validation guidelines international onference on harmonization; GENEVA; 1996
19. Berry RI, Nash AR. Pharmaceutical process validation, Analytical method validation, Marcel Dekker Inc. New work, 1993; 57:411-28.
20. Anthony C Moffat, M David Osselton, Brian Widdop. Clarke's analysis of drugs and poisons, Pharmaceutical press, London, 2004, P.1109-1110, 1601-1602.