Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

UPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CITICOLIN SODIUM AND PIRACETAM IN ITS PHARMACEUTICAL DOSAGE FORM

Alagar Raja. M[1*], B. Rajani[1], David Banji[1], K.N.V. Rao[1], Selva Kumar. D[2]

[1*]Department of Pharmaceutical Analysis and Quality Assurance, Nalanda College of Pharmacy, Cherlapally, Nalgonda-508001, Telangana, India.

[2]School of Pharmacy, Taylors University, Subang Jaya, Malaysia.

ABSTRACT

A simple, accurate, precise, sensitive, rapid UPLC method has been developed and validated for determination of citicolin sodium and piracetam in its pharmaceutical dosage form. Chromatographic separation was achieved on a beh shield rp_{18} (2.1 x 100mm, 1.7µm), by a mobile phase consist of Water: Acetonitrile (pH 2.8, 45:55 v/v). Ratio with a flow rate of 0.3 ml /min. The detection wave length was set at 225 nm. Citicolin and piracetam was subjected to different stress conditions. The degradation products, when any, were well resolved from the pure drug with significantly different retention time values. The method was linear (r^2 = 0.999) at a concentration range of 5-25 µg/ml. The intra and inter day precisions were satisfactory the relative standard deviations did not exceed 2%. The accuracy of the method was proved the mean recovery of citicolin sodium and piracetam was 99.04-101.58%. The proposed method has high throughput as the analysis involved short run-time (3mins). The method met the ICH/FDA regulatory requirements. The proposed method was successfully applied for the determination of citicolin sodium and piracetam with acceptable accuracy and precisions. The results demonstrated that the method can be applied successfully for routine use in quality control industry laboratories.

KEYWORDS

Citicolin Sodium and Piracetam, UPLC, ICH and FDA.

Author for Correspondence:
Alagar Raja. M,
Department of Pharmaceutical Analysis and Quality Assurance, Nalanda College of Pharmacy, Cherlapally, Nalgonda-508001, Telangana, India.

Email: madurairaja@hotmail.com

INTRODUCTION

Citicoline is an intermediate in the generation of phosphatidyl choline from choline. It ischemically 5'-O [hydroxyl ({hydroxyl [2(trimethylammonio) ethoxy] phosphoryl}oxy) phosphoryl] cytidine. Citicoline is a white or off-white amorphous, hygroscopic powder having molecular weight 488.3g/mol1. It helps to improve focus and mental energy and may possibly be

useful in the treatment of attention deficit disorder[1-2]. Piracetam is a cyclic derivative of GABA. It is one of the group of racetams. It is chemically 2-oxo -1-pyrrolidine acetamide, it shares the same 2-oxo-pyrrolidone base structure with 2-oxo-pyrrolidine carboxylic acid(pyroglutamate). It is a fine white crystalline powder having molecular weight 142. 16g/mol. It may enhance, elevate, and improve cognitive functions and abilities linked and associated to the central nervous system, memory development and memory processes. Many people across the world use the nootropic, piracetam, to effectively retain knowledge and improve memory. Piracetam appears to be effective in treating cognitive impairment in alcoholism[3-8]. Piracetam improves the functioning of the (ACh) transmitters and receptors. Both drugs are psychotherapeutic agents, used as psycho stimulant, nontropic and neurotonics. Both drugs are freely soluble in water. These drugs will increase cerebral metabolism and increase level of various neurotransmitters, including acetylcholine and dopamine, exerting its action by activating the biosynthesis of structural phospholipids in neuronal membrane. This drug will increase the blood flow and oxygen consumption in brain. The review of literature regarding quantitative analysis of Citicoline and Piracetam revealed that the attempts were made to develop analytical methods for Citicoline and Piracetam in serum. Some spectrometric methods and LC methods have been reported for the estimation of the individual drugs [9 -13] and one RP- HPLC method in an expensive and time taking way. The focus of the present study was to develop and validate a rapid, stable, specific, and economic RP-HPLC method for the estimation of Citicoline and Piracetam in tablet dosage form.

MATERIALS AND METHODS

Instrumentation

An Ultra High performance liquid chromatography (UPLC) system consisted of Waters Acquity with PDA detector and data-handling system Empower Pro and all pH measurements were performed on a pH meter (Metrohm, model 654 Herisau).

Reagents and chemicals

Citicolin sodium and piracetam were obtained as pure standards, samples (tablets containing citicolin sodium and piracetam in the ratio of 50mg; 80 mg respectively) from Discovery intermediates pvt ltd., Hyd. India. HPLC grade solvents, Methanol, Acetonitrile, Orthophosphoric acid, KH_2PO_4 acid was from pharmatrain lab private Ltd., India. Water was deionised and further purified by means of a Milli-Q Plus water purification system, Millipore Ltd (U.S.A).

Chromatographic conditions and measurement procedure

Equipment : ultra-performance liquid chromatography equipped with Auto Sampler and PDA detector
Column : BEH SHIELD RP_{18} (2.1 x 100mm, 1.7µm)
Flow rate : 0.3 mL per min
Wavelength : 225 nm
Injection volume : 3 µl
Column oven : Ambient
Run time : 3 min

Diluents Preparation

Mix a mixture of above Water 450 mL (45%) and 550 ml Acetonitrile HPLC (55%) and degas in ultrasonic water bath for 5 minutes. Filter through 4.5 µ filter under vacuum filtration.

Preparation of standard solution

Accurately weigh and transfer 50 mg of Citicoline and 80 mg of Piracetam working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution

Further pipette 0.1 ml of Citicoline and Piracetam of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3 ml and 3ml of Citicoline and Piracetam of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Preparation of sample solution

Accurately weigh and transfer equivalent to 50 mg of Citicoline and 80mg Piracetam equivalent weight of the sample into a 10ml clean dry volumetric flask add about 70mL of Diluents and sonicate to dissolve it

completely and make volume up to the mark with the same solvent.

Stock solution

Further pipette 0.1 ml of Citicoline and Piracetam of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3 ml and 3ml of Citicoline and Piracetam of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

RESULTS AND DISCUSSION

Selection of wavelength (λ max)

In setting up the conditions for the development of the assay method the choice of detection wavelength was based on the scanned absorption for Citicolin sodium and piracetam. The spectrum was scanned over the range of 225 nm and was obtained by measuring the absorption of 0.1mg/ml solution of Citicolin, piracetam in methanol prepared from stock solution. The spectrum was obtained by using 1cm quartz cell using methanol as reference solution. λ max of Citicolin was was 282nm and λ max of Piracetam was 308. Hence for simultaneous estimation 225nm was selected.

Method development

The wide variety of equipment, columns, eluent and operational parameters involved makes ultra-performance liquid chromatography (UPLC) method development seem complex. The process is influenced by the nature of the analytes and generally follows the following.

Steps

Step 1 - selection of the UPLC method and initial system
Step 2 - selection of initial conditions
Step 3 - selectivity optimization
Step 4 - system optimization
Step 5 - method validation.

Depending on the overall requirements and nature of the sample and analytes, some of these steps will not be necessary during UPLC analysis. For example, a satisfactory separation may be found during step 2, thus steps 3 and 4 may not be required. The extent to which method validation (step 5) is investigated will depend on the use of the end analysis for example, a method required for quality control will require more validation than one developed for a one-off analysis.

Validation of the Method according to ICH Guidelines

Validation of the method was done according to ICH guidelines for Simultaneous Equation method.

Robustness

System suitability and robustness results

Tailing factor for the peaks due to Citicoline and Piracetam in Standard solution should not be more than 2.0. Theoretical plates for the Citicoline and Piracetam peaks in Standard solution should not be less than 2000.

Accuracy

The accuracy study of assay was performed with known amount of drug substance (API) was spiked in placebo at about 50 %, 100 % and 150 % of test concentration in triplicate at each level and was injected each level in duplicate. Amount of drug recovered was quantified and % recovery was calculated from amount found and actual amount added. The accuracy study of this method for estimation of percent assay of Citicolin sodium and Piracetam in tablet dosage was found to be in the range of 100.91 %, 99.40%.

Precision

The method precision study showed that the results of percent assay in six different samples preparations of same sample were within limits (% RSD < 2) as shown in Table No.3. The Intermediate Precision study was performed within laboratory variation by different analysts, on different days, different instruments, and different column by using different standard and sample solution of the same sample as specified in method precision and the results were compared with method precision. The ruggedness study showed that it passes the limits (% RSD < 2).

Linearity

The linearity of this method for assay determination was carried out by analyzing in the range from about 5-25, 8-40 of test concentration. Peak responses of the components on Y axis and the corresponding concentrations on X axis were drawn and the correlation coefficient (r) estimated. The linearity study showed that the calibration curve for citicolin

sodium and piracetam was found to be linear with correlation coefficient (r^2) values 0.999 and 0.999 respectively. Linearity plot of citicolin sodium and piracetam were shown in Figure No.4 and Figure No.5 respectively.

Table No.1: System Suitability and Robustness results for Citicolin Sodium

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.25	13665	0.95
2	0.3	12966	0.89
3	0.35	11569	1.05

Table No.2: System Suitability and Robustness Results for Piracetam

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.25	22001	1.16
2	0.3	23459	1.08
3	0.35	23892	1.05

Table No.3: Robustness (Change in Organic Composition in the Mobile Phase) Results for Citicolin Sodium

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	11646	1.09
2	*Actual	12966	0.89
3	10% more	10254	1.26

Table No.4: Robustness (Change in Organic Composition in the Mobile Phase) Results for Piracetam

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	25016	1.1
2	*Actual	23459	1.08
3	10% more	23536	1.19

Table No.5: Accuracy Results for Citicolin Sodium and Piracetam

S.No	Drug	% Con	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
1	Citicolin sodium	50%	22057	25	25.28	101.29%	100.91%
		100%	43141	50	50.59	101.18%	
		150%	66636	75	74.44	99.25%	
2	Piracetam	50%	106481	40	39.34	98.36%	99.40%
		100%	214518	80	80.66	100.82%	
		150%	326302	120	118.81	99.01%	

Table No.6: Precision of Citicolin Sodium and Piracetam

S.No	Injection	Area of citicolin	Area of piracetam
1	Injection-1	319587	612067
2	Injection-2	319507	615813
3	Injection-3	319430	613182
4	Injection-4	318618	618956
5	Injection-5	319220	612277
6	Average	319272.4	614459
7	Standard Deviation	390.5	2922.7
8	% RSD	0.122297	0.475662

Table No.7: Intraday precision (Ruggedness)

S.No	Injection	Area of citicolin	Area of piracetam
1	Injection-1	310429	616775
2	Injection-2	313105	619141
3	Injection-3	311593	611258
4	Injection-4	312573	610228
5	Injection-5	313726	615564
6	Injection-6	311649	613788
7	Average	312179.2	614459
8	Standard Deviation	1191.0	3379.5
9	% RSD	0.38152	0.549991

Table No.8: Linearity Results of Citicolin Sodium and Piracetam

S.No	Linearity level	Citicolin sodium		Piracetam	
		Conc. (ppm)	Area	Conc. (ppm)	Area
1	I	5 ppm	112755	8ppm	216885
2	II	10 ppm	222086	16 ppm	427620
3	III	15 ppm	327096	24 ppm	618211
4	IV	20 ppm	421061	32 ppm	833028
5	V	25 ppm	532535	40 ppm	1019094
Correlation Coefficient		0.999		0.999	

Table No.9: LOD Results of Citicolin Sodium and Piracetam

S.No	Drug	LOD	
1	Citicolin sodium	Concentration (µg/ml)	0.456
		Retention time(t_R)	0.960
		Height(µv)	174
		Area(µv)	9945
2	Piracetam	Concentration(µg/ml)	0.207
		Retention time(t_R)	1.524
		Height(µv)	175
		Area(µv)	5333

Table No.10: LOQ Results of Citicolin Sodium and Piracetam

S.No	Drug	LOQ	
1	Citicolin sodium	Concentration (µg/ml)	1.54
		Retention time(t_R)	0.963
		Height(µv)	588
		Area(µv)	33607
2	Piracetam	Concentration(µg/ml)	0.697
		Retention time(t_R)	1.529
		Height(µv)	589
		Area(µv)	17949

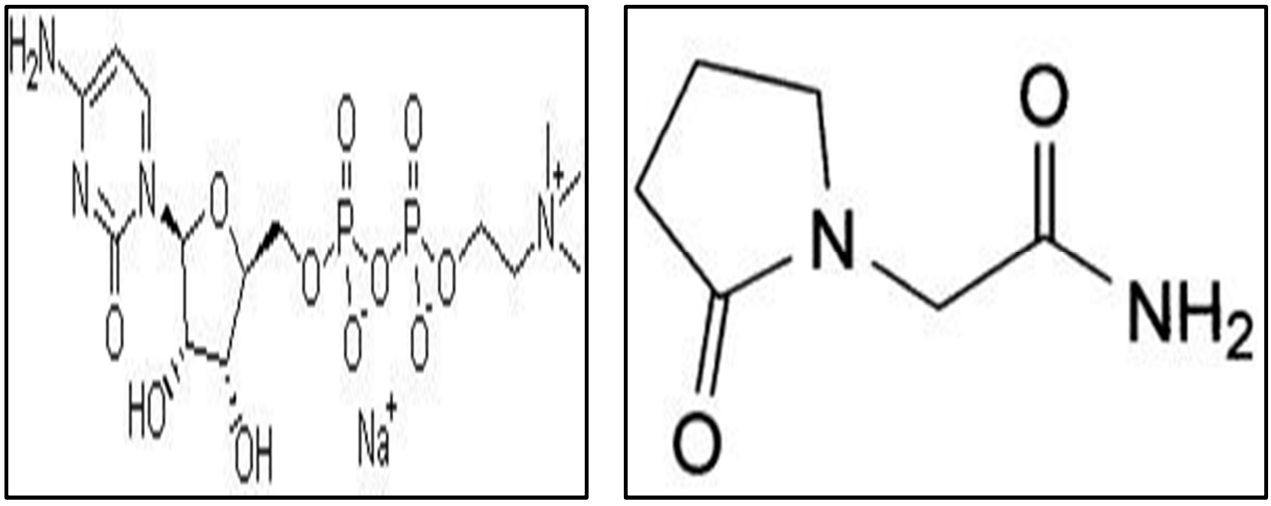


Figure No.1: Molecular structure of Citiocoline sodium and Piracetam

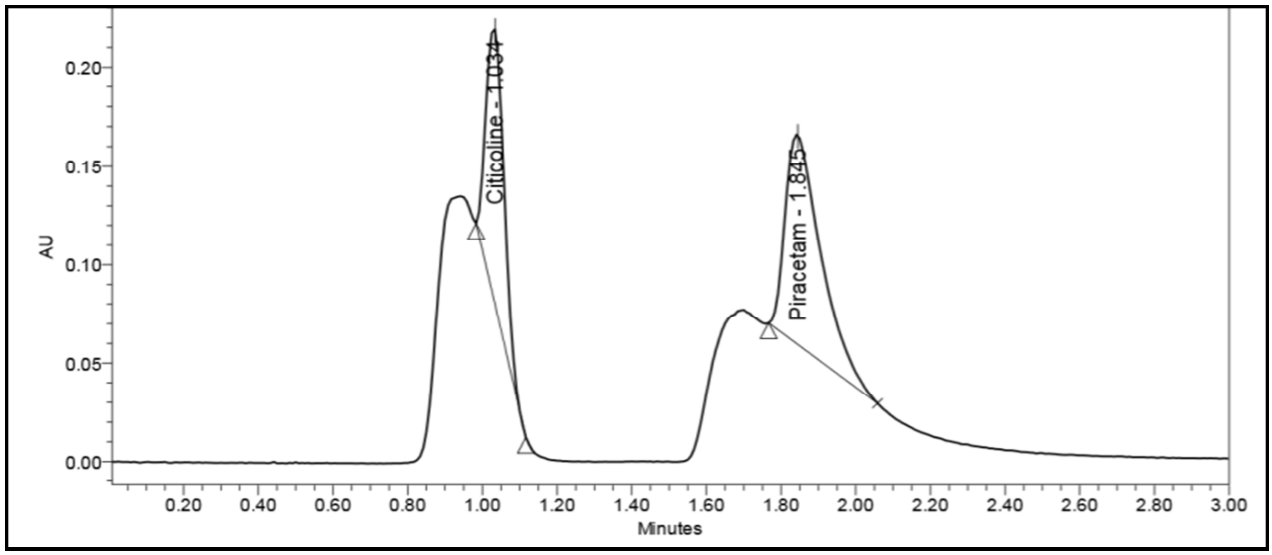


Figure No.2: Chromatogram of Citicolin Sodium and Piracetam

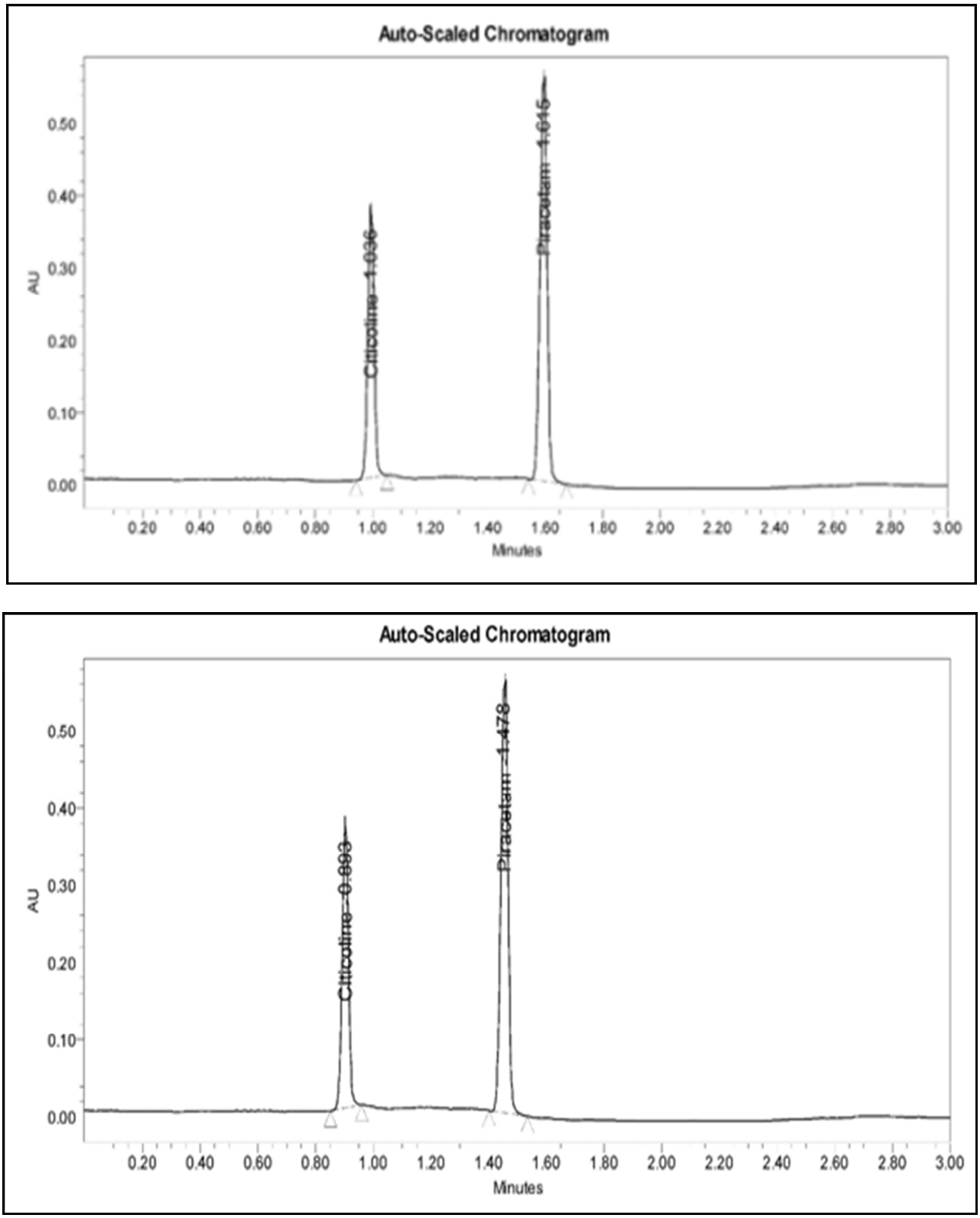
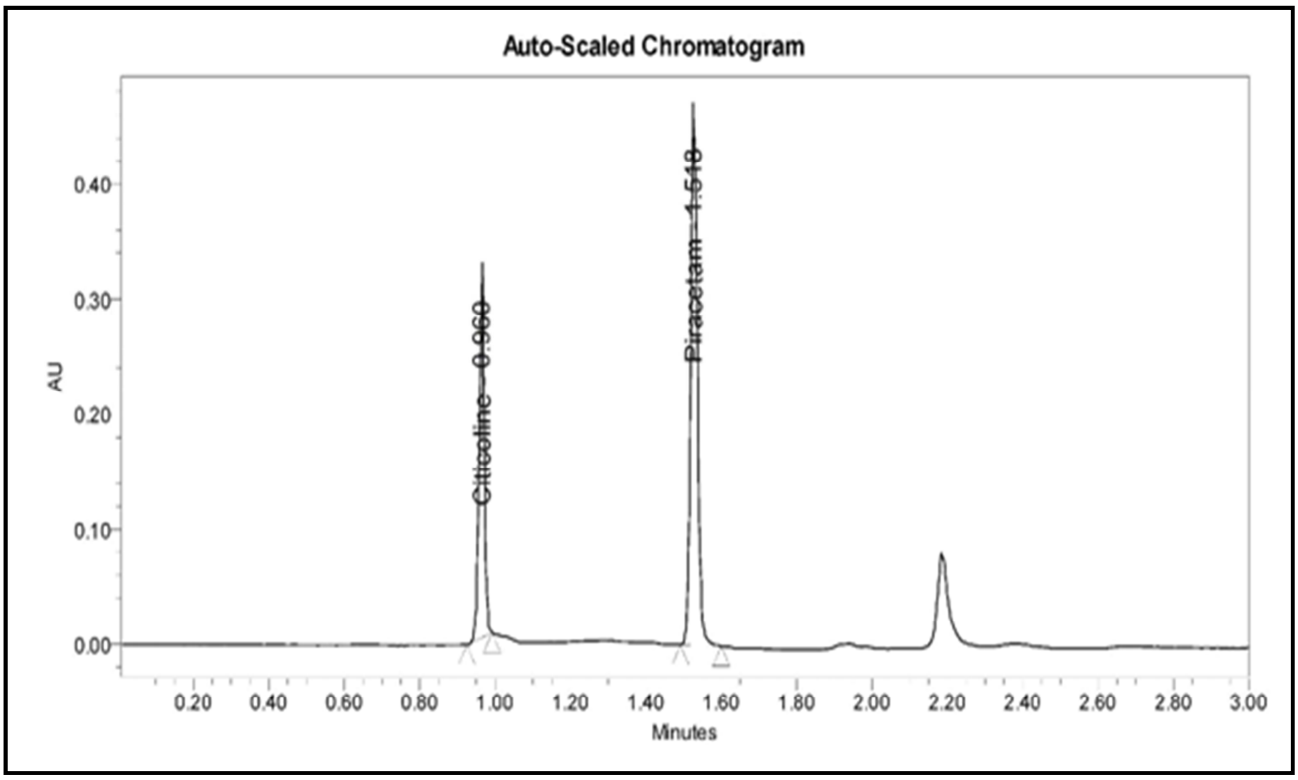


Figure No.3: Chromatogram for less flow and Chromatogram for more flow



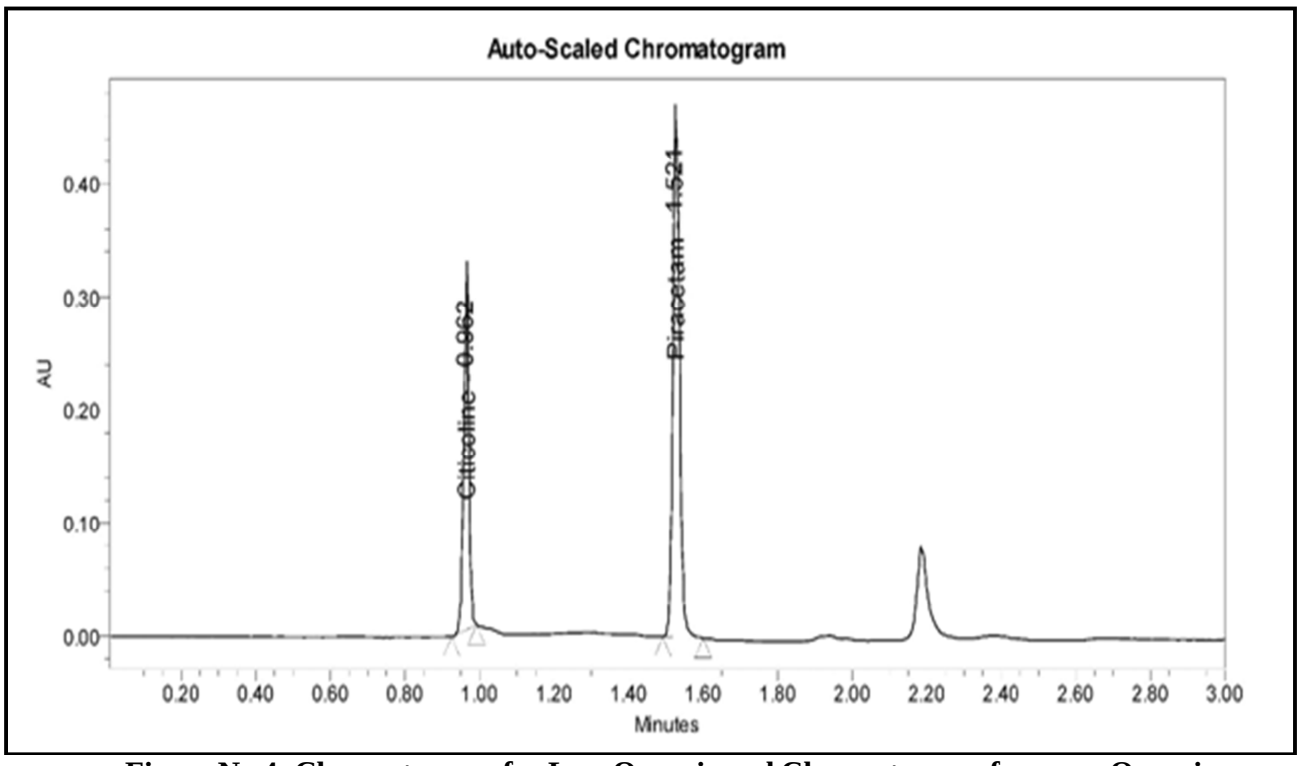


Figure No.4: Chromatogram for Less Organic and Chromatogram for more Organic

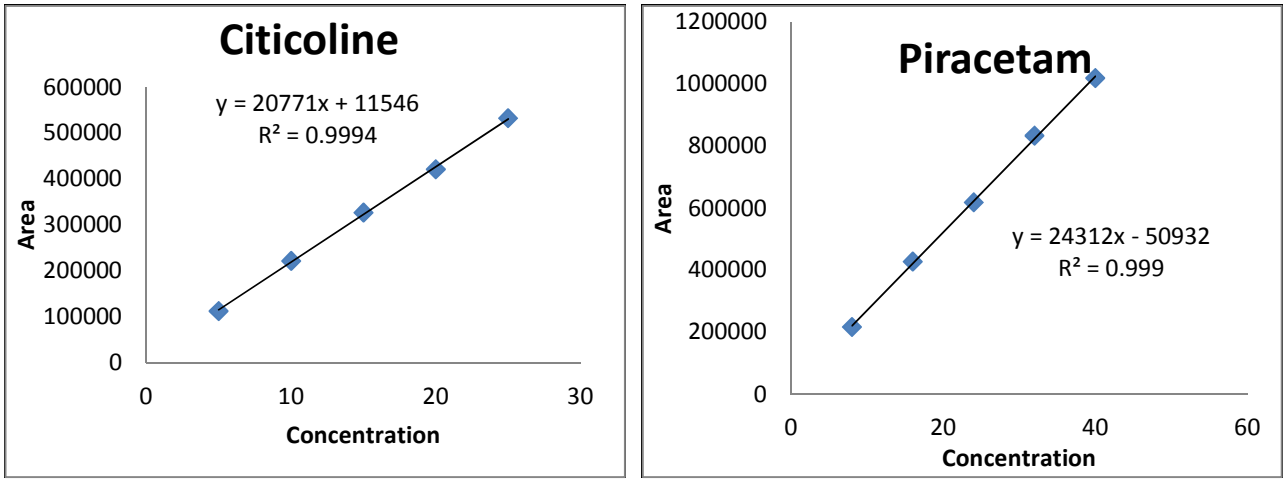


Figure No.5: Linearity Calibration Graph of Citicolin Sodium and Piracetam

CONCLUSION

This intended study can be concluded as the proposed method is economical, simple, ultra-fast, sensitive and reliable and is found to be more accurate, precise, specific, stability indicating, rugged and robust hence it can be employed for routine estimation of tablets containing citicolin sodium and piracetam. Conventional reported HPLC methods may be replaced by the proposed UPLC method because of its superiority in cost effectiveness, Savings of analysis time per sample and better detection. For faster samples testing routinely in QC lab the validated method may be used.

ACKNOWLEDGMENT

I thankful to the Nalanda College of pharmacy charlapally, nalgonda. Providing necessary facilities to carry out the above research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Lloyd Snyder, Joseph G, Kirckland. Practical HPLC method development, second edition, *Wiley India pvt.ltd*, 6(2), 2007, 685-706.
2. Manohar A, Potdar. Pharmaceutical quality assurance, *Published by Nirali Prakashan*, 4(1), 2011, 5.1-5.16.
3. ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures, Text and Methodology Q2 (R2), *International Conference on Harmonization, Geneva, Switzerland*, Nov. 2005.
4. Indian pharmacopoeia 2010, addendum, 4(2), 2012, 2841-2843.
5. Handbook of pharmaceutical analysis by HPLC, Edited by Steiner Ahuja and Michael W, dong, separation science and technology, 6(4), 2012 ,19-34.
6. HPLC for pharmaceutical scientists edited by Yuri kazakevich, Rosario lobrutto, *Published by John Wiley and Sons, Inc., Hoboken, New Jersey*, 5(1), 2007, 22-34.
7. Conant R and Schauss A G. Therapeutic Applications of Citicoline for Stroke and Cognitive Dysfunction in the Elderly, A Review of the Literature, *Alternative Medicine Review*, (9), 2004, 17-31.
8. Qureshi I and Endres J R, Citicoline. A Novel Therapeutic Agent with Neuroprotective, Neuromodulatory and Neurodegenerative Properties, *Natural Medicine Journal*, 6(3), 2010, 11-25.
9. Maryadele J O Neil. The Merck Index, An Encyclopedia of Chemicals, Drugs and Biological, *Merck and Co., Inc., Whitehouse Station, NJ*, 4(3), 2006, 2319, 7487.
10. Sweet man S C, Martindale. The Complete Drug Reference, London, *UK, Pharmaceutical Press*, 35(8), 2007, 2072.

11. Sweet man S C, Martindale. The Complete Drug Reference, London, UK, *Pharmaceutical Press*, 35(5), 2007, 330.
12. Tripathi K D. Essentials of Medical Pharmacology, *Jaypee Brothers, New Delhi*, 9(3), 2008, 438, 439.
13. British National Formulary, *British Medical Association and Royal Pharmaceutical Society of Great Britain, London,* 57(12), 2009, 215-322.
14. Babu G, Prasad K, Thejonath Kolla, Vijayabaskaran M. RP-HPLC for simultaneous estimation of Citicoline and Piracetam in Tablet Dosage form, *Int J Pharm Tech Res*, 4(3), 2009, 1311-1313.
15. Sunitha N, Subash I, Marihal C. Development and Validation of an RP-HPLC Method for Simultaneous Estimation of Citicoline and Methylcobalamin, *Journal of Global Trends in Pharmaceutical Sciences,* 5(2), 2014, 1522-1527.
16. Keguang Chen, Xiaoyan Liu, Chunmin Wei *et al.* Determination of Uridine in Human Plasma by HPLC and its Application in Citicoline Sodium Pharmacokinetics and Bioequivalence Studies, *Journal of Bioequivalence and Bioavailability*, 2(3), 2011,72-76.
17. Raveendra Babu G, Sri Madhu Sri M, Prasada Rao M. A Validated, Specific Stability-Indicating RP- LC Method for Citicoline And Its Related Substances in Oral Drops, 2(3), 2010, 652-667.
18. Uttarwar S O, Jadhav R T, Bonde C G. Stability Indicating LC Method for Citicoline Sustained Release Tablet, *International Journal of Pharm tech Research,* 5(2), 2010, 2482-2486.
19. Augustin Curticapea, Silvia Imre. New validated method for piracetam HPLC determination in human plasma, *Journal of Biochemical and Biophysical Methods*, 69(3), 2007, 273-281.
20. Ardhani Dwi Lestari, Andi Tri Prasetyo, Tini Palupi, Evi Umayah, Mochammad Yuwono and Gunawan Indrayanto. HPLC Determination of Piracetam in Tablets, Validation of the Method, *Journal of Liquid Chromatography and Related Technologies* 28(9), 2005, 1407-1416.

Please cite this article in press as: Alager raja M *et al*. UPLC method development and validation for simultaneous estimation of Citicolin sodium and Piracetam in its pharmaceutical dosage form, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 3(2), 2015, 66-75.