Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (10):254-261
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

A validated stability indicating high performance liquid chromatographic assay method for simultaneous determination of citicoline and piracetam in tablet formulation

[1]Sanjaykumar B. Bari, [2]Gopal O. Sugandhi, [3]Amol J. Mhaske and [4]Jineetkumar B. Gawad*

[1]H. R. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur
[2]Mylan Laboratories Limited, Nashik
[3]Sandoz India Limited, Kalve
[4]St. John Institute of Pharmacy & Research, Palghar

ABSTRACT

A simple, reliable and stability indicating reversed-phase high-performance liquid chromatographic method has been developed and validated for the simultaneous determination of citicoline and piracetam in their synthetic mixtures and combined tablet formulations. Both drugs were separated on a Chromatopak C_{18} 250 mm × 4.6mm Column packed with 10µm particles. The optimized mobile phase was a 90:10 (v/v/v) mixture of 10 mM potassium phosphate buffer (pH 3.5), pumped at a flow rate of 0.8 ml/min. UV detection was performed at 215 nm. The method was validated in the concentration ranges of 17.5-32.5µg/ml for citicoline and 28-52µg/ml for piracetam, where it demonstrated good linearity with r^2= 0.999 and 0.998 (n = 3), respectively. The method demonstrated to be robust, resisting to small deliberate changes in pH, flow rate and composition (buffer: organic ratio) of the mobile phase. The applicability of the method was demonstrated by determining the drug content of commercial pharmaceutical formulations, where it exhibited good performance.

Keywords: Stability indicating, HPLC, Citicoline, Piracetam

INTRODUCTION

Citicoline sodium (Figure 1) (Cytidine-5'-diphosphocholine monosodium salt, CDP-coline) is an essential precursor in synthesis of phosphatidyl choline. Citicoline, recently became available in the United States as a dietary supplement. [1] Upon oral administration, citicoline gets hydrolyzed and absorbed as choline and cytidine which enter systemic circulation and then crosses blood brain barrier. In the central nervous system cytidine and coline rejoined to form citicoline by CTP-phosphocolinecytidylyl transferase. [1]

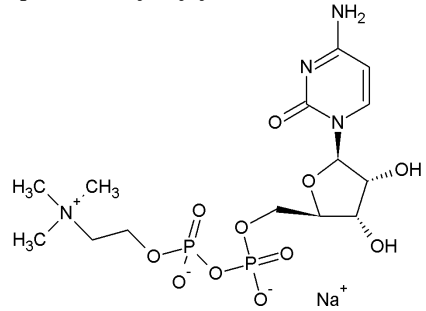

Figure 1: Chemical structure of citicoline sodium

CDP-coline has shown beneficial effects in cerebral ischemia, traumatic brain injury, cognitive impairment, acute ischemic stroke, vascular dementia, [3-7] Citicoline is tolerated well & side effects are rare, not sever and consist mainly of digestive intolerance, gastrointestinal discomfort and restlessness. [4] Piracetam (Figure 2) (2-oxyl-1-pyrrolidinoacetamide) is acyclic derivative of neurotransmitter GABA (γ-aminobutyric acid) used as nootropic psychopharmacological agent to improve cognitive impairments in patients with brain injuries.

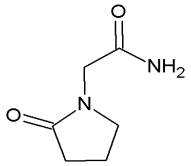

Figure 2: Structure of piracetam

With low toxicity and few side effects, piracetam is effective in treating dementia and cognitive impairment [8], stroke [9] and ischemia [10]. Piracetam enhances neuroplasticity, neuroprotection and brain metabolism [11], has anticonvulsant effects [12], as well as reduces symptoms of clinical depression, anxiety and alcohol withdrawal [13, 14]. Piracetam acts mainly by increasing membrane fluidity in the brain, which may affect receptor binding, and neurotransmitter release [15]. Another proposed mechanism suggests action of piracetam at the benzodiazepine site of the GABAA receptor because flumazenil inhibits its effects [16]. Piracetam can also interact with glutamate receptors, suggesting another potential mechanism for its nootropic action [15]. Several methods were reported in the literature for the individual determination of citicoline and piracetam like UV [17, 20], HPLC [18, 21], HPTLC [19]. Simultaneous HPLC determination of citicoline and piracetam were also published [21] but no reliable cheap, stability indicating method reported ever before which can simultaneously detect both the drugs without getting interfered from degradation products. Therefore, the objective of the reported research was to develop stability indicating assay method and to study degradation of citicoline and piracetam under different International Conference of Harmonization (ICH) recommended stress conditions [22-23].

MATERIALS AND METHODS

Chemicals and Reagents

Citicoline & piracetam combined tablets (Brand Name- Strocit Plus) were manufactured by Akums Drugs & Pharmaceuticals Ltd. Ranipur, Haridwar. Citicoline (Purity 99.8%) and piracetam (Purity 99.5%) standards were obtained from Mepro Pharmaceuticals Pvt. Ltd. Surendranagar, Gujrat. Analytical reagent grade potassium dihydrogen phosphate monobasic (KH_2PO_4), Orthophosphoric acid 85% solution and HPLC grade Methanol were purchased from Merck Mumbai, India Limited. High quality HPLC water was prepared by Milipore purification system.

Chromatographic Equipments and Conditions

Method development and validation work was performed on Shimadzu LC2010HT, system consisting of quaternary gradient pump, auto sampler and PDA detector controlled by computer with ClassVP 5.032 software. Chromatopak C18 (250x4.6mm, 10µ) column was used as a stationary phase. The isocratic mobile phase consists of mixture of Buffer (0.01M KH_2PO_4 pH adjusted to 3.5 with orthophosphoric acid) and methanol in the ratio of 90:10 (v/v) was used in the analysis. Flow rate of the mobile phase is set at 0.8 ml/min with ambient column temperature, 20µl injection volume and UV detection at 210nm.

Standard solutions

Citicoline sodium equivalent to 25mg of citicoline and 40mg of piracetam working standard were weighed then transferred in 100ml volumetric flask. Dissolve the content by sonicating then make up the volume up to mark with mobile phase. Take 1 ml of above solution and makeup volume up to 10ml with mobile phase to obtain solution containing citicoline 25µg/ml and piracetam 40µg/ml.

Sample solution

Twenty tablets were weighed and crushed into fine powder. Take powder equivalent to 25mg of citicoline and 40mg of piracetam. Transfer it into 100ml volumetric flask then add mobile phase. Sonicate for 10 minutes and cool to room temperature make up the volume 100ml with mobile phase. Filter through 0.45µ whatman filter paper. Take 1 ml of this filtrate & makeup the volume 10ml with mobile phase.

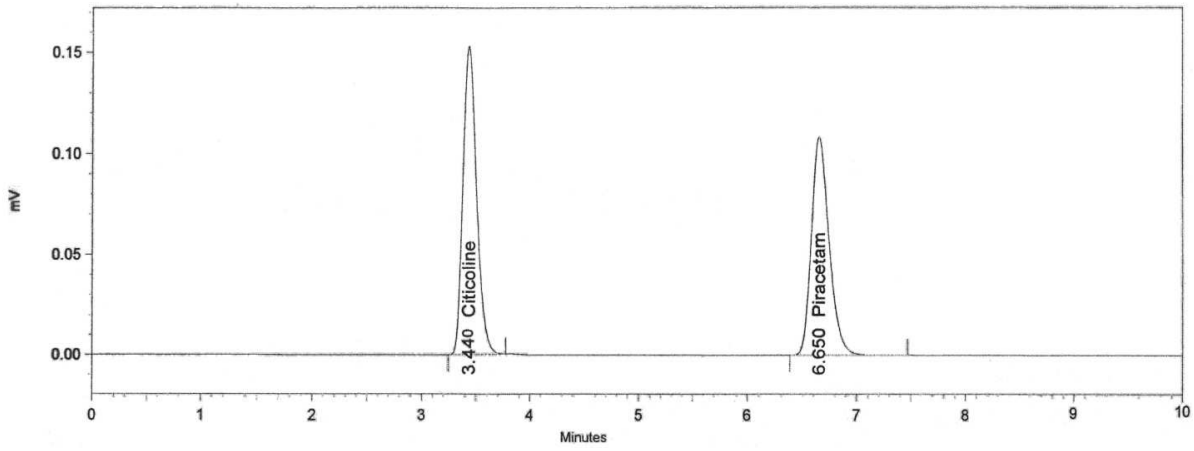

Figure 3: Chromatogram showing well resolved peaks of citicoline and piracetam

VALIDATION

Linearity

Linearity of the proposed method was evaluated according to the ICH guidelines by the analysis of working solutions of citicoline sodium and piracetam at different concentrations. Taking into account the purpose of the assay, the linear ranges were 17.5-32.5µg/ml for citicoline and 28-52µg/ml for piracetam. These covered the range from 70% to 130% of the expected concentrations of the analytes in the tablet samples.

Precision

System precision was determined by performing injection repeatability test and % RSD was calculated. Method precision (intra-day precision) was evaluated by carrying out six independent assays of test samples against a reference standard. The percentage of RSD of six assay values obtained was calculated. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts and different days in the same laboratory.

Accuracy

The accuracy of the method was determined by measuring the drug recoveries by the standard addition method, in order to determine eventual positive or negative interferences produced by the excipients in the formulation. Known amounts of each drug, corresponding to 80%, 100% and 120% of the label claim were added to placebo and their percentage recovery calculated. Each set of additions was repeated five times.

Robustness

Robustness was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 mL/min to 0.8 mL/min and 1.2 mL/min. The organic strength was varied by ±2%, pH was varied by ±0.2 units, Detection wavelength varies by ±5nm. Standard solution was injected six times in replicate for each change. Percentage RSD values estimated were found to be less than 2 indicate robustness of the method.

FORCE DEGRADATION STUDIES

Selectivity of the method was demonstrated after observing that the excipients did not produce absorption peaks in the chromatogram and did not interfere with the exact determination of the analyte in the accuracy assay. Interference from the degradation in the analyte determination was studied by observing sample under various stressed conditions. The purpose of stability indicating assay method is to provide evidence that the analytical method is efficient in determination of drug substances in commercial drug product in the presence of its degradation products. Stress study was carried out under the degradative conditions of acid, base, Peroxide and Photo degradation.

Acidic degradation:

Tablet powder (equivalent to 25 mg of citicoline and 40 mg of piracitam) and placebo was weighed and transferred in two separate 50ml flasks. Then 50 ml of 1M HCl was added to it and solution was kept for 1 hour at 80ºC after which 5 mL of solution was neutralized with 1M HCl. The solution was then diluted with mobile phase to give working solution of 25µg/ml of citicoline and 40µg/ml of piracetam.

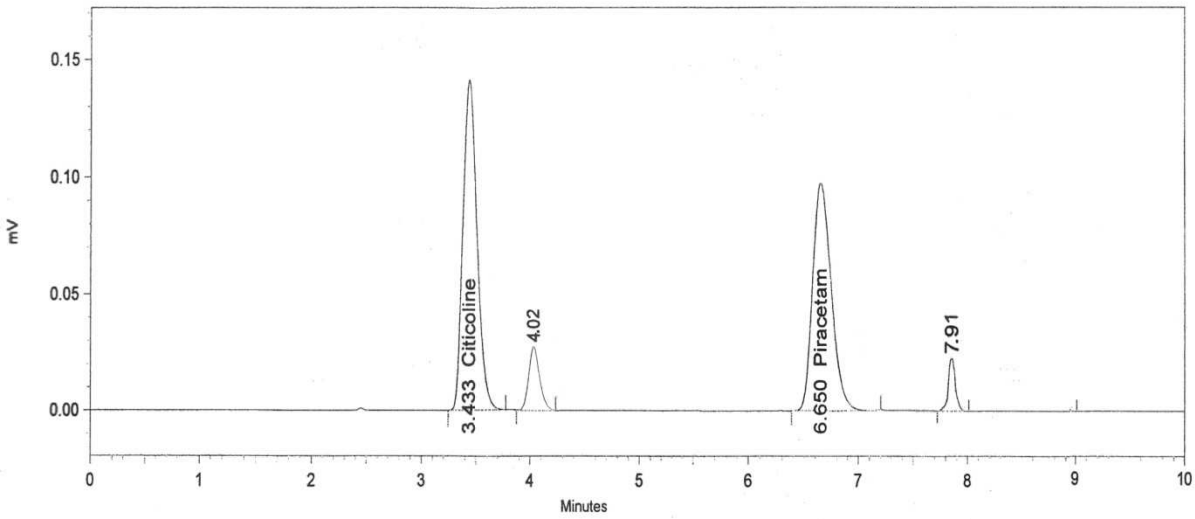

Figure 4: Chromatogram of acid degraded sample

Alkaline Degradation:

Tablet powder (equivalent to 25 mg of citicolin and 40 mg of piracetam) and placebo was weighed and transferred in two separate 50ml flasks. Then 50 ml of 0.5N NaOH was added to it and solution was kept for 1 hour at 80ºC after which 5 mL of solution was neutralized with 0.5M HCl. The solution was then diluted with mobile phase to give working solution of 25µg/ml of citicoline and 40µg/ml of piracetam.

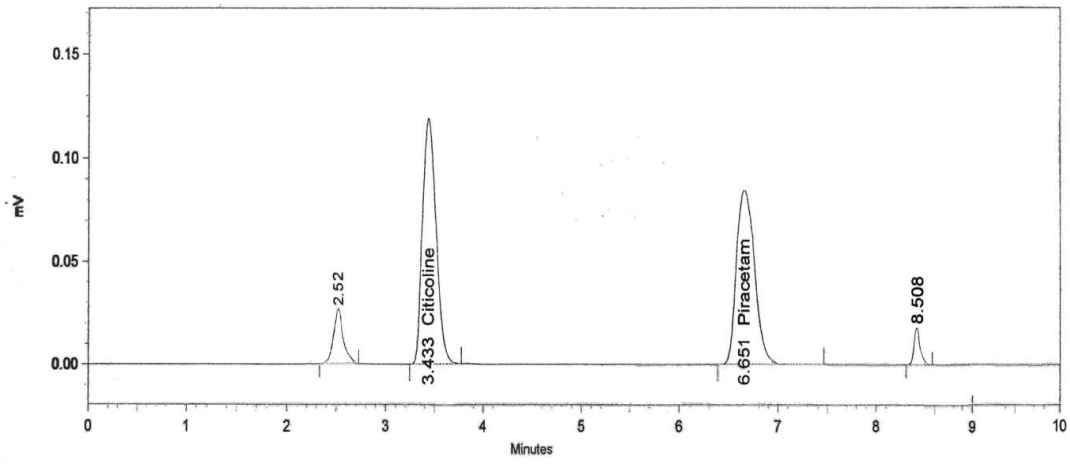

Figure 5: Chromatogram of base degraded sample

Oxidative degradation:

Tablet powder (equivalent to 25 mg of citicolin and 40 mg of piracetam) and placebo was weighed and transferred in two separate 50ml flasks. Then 50 ml of 3% H_2O_2 was added to it and solution was kept for 8 hour at Room temperature. The solution was then diluted with mobile phase to give working solution of 25µg/ml of citicoline and 40µg/ml of piracetam.

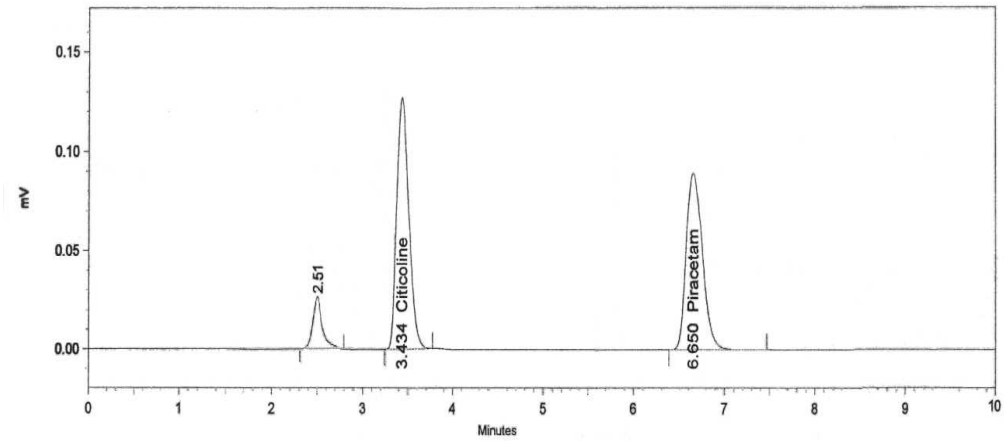

Figure 6: Chromatogram of peroxide degraded sample

Photo degradation:
Tablet powder (equivalent to 25 mg of citicolin and 40 mg of piracetam) and placebo was exposed to the short wavelength (254 nm) and long wavelength (366 nm) UV light for 48 h. The working solution was then prepared using mobile phase to give concentration 25µg/ml of citicoline and 40µg/ml of piracetam.

RESULTS AND DISCUSSION

Method Development
Various solvent compositions were tested to obtain well resolved sharp peaks of citicoline and piracetam. Buffer: methanol (90:10 v/v) was found to give well resolved and sharp peaks for citicoline and piracetam with a retention time of 3.44 & 6.65 min respectively. A wavelength of 210 nm selected for quantification of both drugs, good resolution of peaks with good selectivity. The optimised chromatogram is shown in figure 3.

Optimized Chromatographic Conditions:
Column- Chromatopak C18 (250x4.6mm, 10µ)
Mobile phase- Buffer pH 3.5 (0.01M Potassium dihydrogen phosphate) : methanol (90:10 v/v).
Flow rate- 0.8ml/min
Run Time- 10min
Detection wavelength- 210 nm

Linearity study
The calibration curve for citicoline sodium and piracetam was found to be linear in the range of 17.5-32.5µg/ml and 28-52µg/ml with a correlation coefficient of 0.999 and 0.998 respectively. The equation obtained for the calibration curve of citicoline and piracetam was y = 27497x – 35846 and y = 30719x – 9720 respectively. The statistical data of linearity is shown in Table 1.

Table 1: Statistical analysis for the calibration curve of Citicoline and Piracetam

Sr. no.	Concentration in [µg/ml]		Mean Peak area		% RSD	
	Citicoline	Piracetam	Citicoline	Piraceatm	Citicoline	Piracetam
1	17.5	28	445317	856414	0.67	0.22
2	20	32	513313	971910	0.70	0.24
3	22.5	36	577426	1080681	0.70	0.75
4	25	40	656502	1220775	0.38	0.77
5	27.5	44	725622	1354851	0.20	0.43
6	30	48	789445	1466174	0.51	0.54
7	32.5	52	853427	1582340	0.63	0.38

Precision
The relative standard deviations in repeatability analysis (% R.S.D.) were 0.19 for citicoline sodium and 0.20 for Piracetam, which are well within the acceptable limit of less than 2.0%. The % R.S.D's. values for citicoline and piracetam were found to be 0.24 and 0.39 for intra-day precision and 0.22 and 0.38 for inter-day precision. The Intra-day and Inter-day precision data shown in Table 2.

Table 2: Results of precision studies (Intra-day and Inter-day)

Sample No.	Assay as % of Label claim			
	Analyst 1 (Intra-day precision)		Analyst 2 (Inter- day precision)	
	Citicoline	Piracetam	Citicoline	Piracetam
1	99.7	100.0.	101.5	100.2
2	100.2	99.8	100.0	99.8
3	101.0	99.9	99.9	100.7
4	99.9	100.1	100.2	100.5
5	99.8	100.8	99.7	101.3
6	100.3	99.7	100.1	99.8
Mean	99.95	100.05	100.0333	100.15
% RSD	0.24	0.39	0.22	0.38

Accuracy

The accuracy of the method was determined by calculating recoveries of citicoline and piracetam by method of standard addition. The mean recoveries found to be 100.33 and 100.10 for citicoline and piracetam respectively (Table 3). The high recovery values indicate that the method is accurate.

Table 3: Recovery analysis of Citicoline and Piracetam

Level of Addition %	Amount added [mg]		Percentage Recovered		Average recovery	
	Citicoline	Piracetam	Citicoline	Piracetam	Citicoline	Piracetam
80	20	32	99.90	99.50		
100	25	40	100.14	100.67	100.33	100.10
120	30	48	100.94.	100.14		

Robustness

The robustness of the proposed method was examined against small, deliberate variations of critical parameters suchas the pH, composition of the mobile phase and the flowrate.The pH was varied in the range 3.3-3.7, the composition of mobile phase (90:10 buffer:Methanol) was changed from 88:12 to 92:8% (v/v) and the flow rate effect was evaluated between 0.9 and 1.1ml/min. The detail data of robustness study is shown in table 4.

Table 4: Robustness of the method

Parameters	%RSD for Peak Area of Citicoline*	%RSD for Peak Area of Piracetam*
Mobile phase composition (± 2% organic phase)	0.54	0.72
Flow rate (± 1ml)	0.94	0.81
Detection Wavelength (± 5nm)	0.79	0.67
pH of Mobile Phase (± 2 pH)	0.81	0.92

** mean of three estimations*

4.6 System suitability

System suitability tests were performed in accordance with USP 30 [26] to confirm that the equipment was adequate for the analysis to be performed. The test was carried out by injecting five replicates of a standard solution containing 25 µg/ml and 40µg/ml of citicoline and piracetam respectively. The corresponding observed RSD values were 0.26% and 0.70% which were considered satisfactory, meeting the requirements of USP 30 (R.S.D. < 2%).

Force Degradation Studies

Selectivity of the method was demonstrated after observing that the excipients did not produce absorption peaks in the chromatogram and did not interfere with the exact determination of the analytes in the accuracy assay in addition, chromatograms were completely super imposable with those recorded by simultaneous detection at 210 nm, all of which served as indication that the determination was not interfered by any of the ingredients. No degradation was observed during UV exposure. Degradation was observed in acidic, basic and oxidative conditions. In each condition peak purity is higher than 992 indicate that citicoline and piracetam peak is homogenous in all conditions. The table 5 indicates the degradation condition and peak purity data.

Table 5: Forced Degradation Study

Stress Type	Duration	Peak Purity of Citicoline	Peak Purity of Piracetam
Acidic (1M HCl, 80°C)	1 hour	999	998
Alkali (0.5M NaOH, 80°C)	1 hour	999	999
Peroxide (3% Peroxide)	8 hours	998	999
Photo degradation	48 hours	999	999

Thus method is capable in determination of exact concentration of citicoline sodium and piracetam in presence of its degraded products. The chromatograms of degradation samples are shown on figure 4-6.

Assay of marketed formulation

The drug content of Marketed Dosage form (Strocit Plus- Citicoline 500 mg & Piracetam 800 mg) were calculated six times using proposed method. The percentage content of Citicoline and Piracetamin the tablet formulation was determined as 99.67-100.75% and 99.46-101.73% respectively. Assay results shown in table 6 indicates that method is suitable for analysis of marketed formulation.

Table 6: Marketed Formulation Assay

Brand Name: StrocitPlus **Mfg. By:** Akums Drugs & Pharmaceuticals Ltd. Haridwar.

Drugs	Label claim [mg]	Amount found [mg]	Amount found [%]
Citicoline	500	498	99.6
		503	100.6
		500	100.0
		501	100.2
		504	100.8
		502	100.4
	Mean ± SD	501.33 ± 0.31	100.26±0.23
	% RSD	0.28	0.21
Piracetam	800	800	100.0
		802	101.25
		799	99.87
		803	100.37
		798	99.75
		801	100.12
	Mean ± SD	800.5±0.23	100.22± 0.31
	% RSD	0.31	0.30

CONCLUSION

A simple, specific, precise and accurate HPLC method has been developed for quantitative determination of Citicoline & Piracetam in Tablet formulation. The developed method was validated based on ICH guidelines. Statistical analysis proves that the method is reproducible and selective for the analysis of Citicoline and Piracetam as bulk drug and in pharmaceutical formulations. The degradation study was performed to show the specificity of the method. The advantages of the proposed methods involve a simple procedure for sample preparation and relatively short time of analysis. The proposed HPLC method is suitable for the simultaneous analysis of Citicoline and Piracetam in presence of its degradation products in commercial tablets.

Acknowledgements

Authors are grateful to Mepro Pharmaceuticals Pvt. Ltd. Surendra Nagar, Gujarat for providing drug sample and also to the Principal, Governing Body of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, (M.S) for providing the laboratory and essential facilities to carry out research work.

REFERENCES

[1] Conant R and Schauss AG, *Altern. Med. Rev*, **2004**, 9(2), 17-31.
[2] Qureshi I and Endres JR, (2010), *Nat. Med. J*, **2010,** 2(1), 11-25.
[3] Da´valos A and Secades J, *American Stroke Association*, **2011**, 42, S36-S39.
[4] Fioravanti M and Buckley AE, *Clin Interv Aging*, **2006**, 1, 247–251.
[5] Clark WM, Williams BJ, Selzer KA, Zweifler RM, Sabounjian LA and Gammans RE, *Stroke: a journal of cerebral circulation*, **1999**, 30, 2592-2597.
[6] Álvarez-Sabín J and Román GC, *American J. of Stroke*, **2011**, 42, S40-S43.
[7] Waegemans T, Wilsher CR, Danniau A, Ferris SH, Kurz A and Winblad B, *Dement Geriatr Cogn Disord*, **2002**, 13, 217-24.
[8] Hankey GJ, Ricci S, Celani MG, Cantisani TA and Righetti E, *American Stroke Association*, **2006**, 37, 2191-2192.
[9] Platt D, Horn J, Summa JD, Schmitt-Ruth R, Kauntz J and Kronert E, *Arch. Gerontol. Geriatr*, **1993**, 16, 149–164.
[10] Winnicka K, Tomasiak M and Bielawska A, *Acta Pol. Pharm*, **2006**, 62, 405–409.
[11] Fedi M, Reutens D, Dubeau F, Andermann E, D'Agostino D and Andermann F, *Arch. Neurol*, **2001**, 58, 781–786.

[12] Dencker SJ, Wilhelmson G, Carlsson E and Bereen FJ, *J. Int. Med. Res*,**1978**, 6, 395–400.
[13] Malykh AG and Sadaie MR, *Drugs*, **2010**, 70, 287–312.
[14] Crews FT, *Psychopharmacol. Bull*, **1982**, 18, 135–143.
[15] Moldavkin GM, Voronina TA, Neznamov GG, Maletova OK and Eliava NV, *Eksp. Klin. Farmakol*, **2006**, 69, 7–9.
[16] Sachan N, Chandra P, Yadav M, Pal D and Ghosh AK, *J. Appl. Pharm. Sci*, **2011**, 1, 191-193.
[17] Chen K, Liu X, Wei C, Yuan G, Zhang R, Li R, Wang B and Guo R, *J. Bioequiv. Availab*, **2011**, 3, 072-076.
[18] Arayne MS, Sultana N, Siddiqui FA, Mirza AZ, Qureshi F and Zuberi MH, *J. Chroma. Sci*, **2011**, 48, 589- 594.
[19] Ovalles JF, Tettey, Miller JH and Skellern GG, *J. Pharm. Biomed. Anal*, **2000**, 23, 757-61.
[20] Prajapati VD, Shah JS and Patel PB, *Inventi Rapid: Pharm Ana & Qual Assur,* **2012**, 12(2), 314-322.
[21] Babu G, Prasad K, Kolla T, Vijayabaskaran M and Latha ST, *Inter. J. PharmTech Res*, **2011**, 3, 1311-1313.
[22] ICH Harmonized Tripartite Guideline (Nov. **2005**) Validation of Analytical Procedures: Text and Methodology Q2 (R1).
[23] The United States Pharmacopeia, 25th ed., United States Pharmacopeial Convention, Rockville, (**2007**) 249-253.