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Validated Hptlc Method for Simulteneous Determination of Citicoline sodium and Piracetam in Combined Dosage Form

[1]Mr. Sanjay S. Malgundkar, [2]Dr. Saira Mulla
[1,2,]*Shri Jjt University, Vidyanagari, Churu, Jhunjhunu Road,Chudela, District-Jhunjhunu, Rajasthan-333001*

Abstract: *A simple, accurate and precise HPTLC method for simultaneous estimation of Citicoline sodium and Piracetam in a bulk and in combined dosages form is described in this paper. After optimization a mobile phase of Methanol: Water (16:4 v/v) was used during validation of an analytical method. Aluminum plate coated with the silica Gel 60 F_{254} was used as stationary phase. Densitometric evaluation of the separated bands was performed at wavelength 212 nm. The Rf values of Citicoline sodium and Piracetam were 0.30 and 0.68 respectively. The validated method was linear over the concentration range of 400 ng to 3200 ng /spot and 1200 ng to 3600 ng/spot of Citicoline sodium and Piracetam respectively. Precision of the method was evaluated by inerday and intraday RSD. The results were Citicoline sodium: Inter day RSD of peak response 0.62 % and Intraday RSD f peak response 1.05 % and for Piracetam : Interday RSD of peak response 1.44 % and Intraday RSD of peak response 1.06 %. Accuracy was determined in terms of percentage recovery at three concentration levels. The results are for Citicoline sodium: 97.66 %, 99.70 % and 97.91 % and for Piracetam: 98.52 %, 97.61 and 96.64 % respectively. Specificity was determined by spectral analysis of Citicoline sodium and Piracetam and overlaying the standard spectra and sample spectra respectively .There was no any interference of mobile phase and diluents at the Rf values of Citicoline sodium and Piracetam. Validation was done in accordance with the ICH Guidelines.*

Keywords: *High performance thin layer chromatography, microgram, nanogram, Citicoline sodium and Piracetam .*

I. Introduction

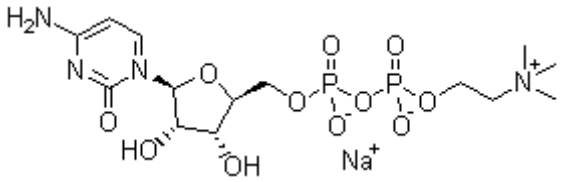
Citicoline sodium is chemically Cytidine 5'-(trihydrogen diphosphate) P'-[2-(trimethylammonio)ethyl] ester inner salt monosodium salt. Molecular formula $C_{14}H_{25}N_4NaO_{11}P_2$. Molecular weight of citicoline sodium is 510.31 and CAS Number 33818-15-4.

Citicoline sodium (INN), also known as cytidine diphosphate-choline (CDP-Choline) & cytidine 5'-diphosphocholine is a psychostimulant/nootropic. It is an intermediate in the generation of phosphatidylcholine from choline. Research studies suggest that CDP-choline supplements increase dopamine receptor densities and suggest that CDP-choline supplementation helps prevent memory impairment resulting from poor environmental conditions. Preliminary research has found that Citicoline sodium supplements help improve focus and mental energy and may possibly be useful in the treatment of attention deficit disorder. Citicoline sodium improves visual function in patients with glaucoma, amblyopia, and non-arteritic ischaemic optic neuropathy.

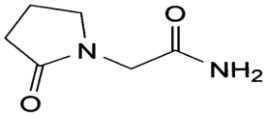
Piracetam is 2-oxo-1-pyrrolidine acetamide . Molecular formula of Piracetam is $C_6H_{10}N_2O_2$ and Molecular weight is 142.2 g/mole. Also CAS number of Piracetam is 7491-74-9. It is a nootropic in the racetams group. It shares the same 2-oxo-pyrrolidone base structure with 2-oxo-pyrrolidine carboxylic acid (pyroglutamate). Piracetam is a cyclic derivative of gamma–aminobutyric acid (GABA). Piracetam is prescribed by doctors for some conditions, mainly myoclonus, but is used off-label for a much wider range of applications. Piracetam improves the function of the neurotransmitter acetylcholine via muscarinic cholinergic (ACh) receptors, which are implicated in memory processes. Piracetam is thought to increase cell membrane permeability. It has been found to increase oxygen consumption in the brain.

Literature survey reveals that the several analytical methods viz. High performance liquid chromatography and UV-VIS spectrophotometric method have been reported for estimation of Citicoline sodium and Piracetam as an individual drug substance and in the combination drug products.

A simple, accurate and precise HPTLC method for simultaneous estimation of Citicoline sodium and Piracetam in the combined dosages form has been developed.



(a)



(b)

Figure 1: Molecular structure of Citiocoline sodium (a) and Piracetam (b)

II. Materials and Methods

2.1 Chemicals and Reagents

Citicoline sodium and Piracetam working standards were provided by M.J. Chempharma PVT Ltd. and Trichem Healthcare Ltd through Mr. Vikrant Tamse-Senior purchase Manager as a noble gift samples for validation study. Tablet samples were procured from the market. All other chemical reagents used during validation study were manufactured by Merck Chemicals, India and were of analytical grade.

2.2 Instrumentation

HPTLC instrument, Make: CAMAG was used for optimization and validation of analytical method. CAMAG Make HPTLC instrument possesses automatic TLC sampler 4 (ATS4) connected with the win CATS 4 software , CAMAG visualizer , CAMAG TLC scanner and Integrator controlled by win CATS4 software. Precoated silica Gel 60 F_{254} on aluminum plates were used as a stationary phase. CAMAG twin trough glass chamber with stainless steel lid was used for development of TLC.

During optimization of Method, various solvents viz. n-butanol, methanol and water in the variable compositions were used. However separation was not achieved. Hence the method was optimized with the methanol and water. Initially methanol and water in 1:1 proportion was used. To achieve proper resolution and peak shape, Methanol and water in a composition of 16:4 v/v was used as a mobile phase. The peak shape as well as resolution between Citicoline sodium and Piracetam was proper. Hence the validation was done by using methanol and water as a mobile phase in a composition of (16:4 v/v). The TLC chamber was saturated for 30 minutes. A deuterium lamp was used in the UV range of 190 to 400 nm as a source of radiation. A slit dimension was 6.00 x 0.45 mm, micro, scanning speed was 20 mms^{-1} and data resolution at 100µm/step. CAMAG automatic TLC sampler-4 (ATS) was used for the application of sample on the silica gel 60 F_{254} TLC plate. The plates were developed in the CAMAG TLC chamber upto 80 mm. Run time of the analysis was 25 minutes. After development, TLC plate was dried in a current of hot air with the help of hair dryer to evaporate the mobile phase. The TLC plate was then dried on a CAMAG hot plate at 120°C for 5 minutes. The contents of Citicoline sodium and Piracetam were evaluated by comparing the peak areas with linear regression.

III. Standard solution preparation

10 mg of Citicoline sodium and 10 mg of Piracetam standards were accurately weighed and transferred to separate 10 mL volumetric flasks. 5 mL of Methanol and 1 mL of water was added to each flask and sonicated for 5 minutes to dissolve the standards. Then diluted to 10 mL with methanol (Stock solution 1 and stock solution 2 for Citicoline sodium and Piracetam respectively) 2 mL each from stock solution 1and stock solution 2 were pipetted out in two separate 10 mL capacity volumetric flask and diluted up to the mark to obtain the concentration of 0.2 mg /mL of standard Citicoline sodium and 0.2 mg / mL of standard Piracetam respectively.

IV. Sample solution preparation

Marketed samples of Citicoline sodium and Piracetam are available in individual dosage as well as in the combined dosage forms. Citicoline sodium in tablet dosage form with different brand names contains 500 mg of active Citicoline . Piracetam in a tablet dosage form with different brand names contains 800 mg of Piracetam in each tablet. Citicoline sodium and piracetam in a combined dosage form contains 500 mg and 800 mg of Citicoline and piracetam respectively. To determine the content, 10 tablets were taken and average weight was recorded. The tablets were triturated to make a powder and pooled sample was used for analysis. A sample

weight equivalent to 500 mg of Citicoline sodium and 800 mg of Piracetam was weighed, dissolved in 10mL of methanol and 2.5 ml of water, sonicated for 5 minutes and diluted to 25 ml with methanol. Proportionate dilutions were done to get the final concentration of Citicoline sodium and Piracetam as 0.2 mg/ mL and 0.32 mg/ mL respectively. The solution was filtered through whatman filter paper No. 42 to get the clear solution.

V. Results and discussions

5.1 Validation of analytical method

ICH guideline was referred to validate an analytical method. Optimized analytical method was validated for the validation parameters: specificity, linearity, accuracy, and precision, LOD, LOQ and Robustness.

5.1.1 Specificity

In specificity, standard Citicoline sodium, Standard Piracetam, sample solution, diluent and mobile phase were separately spotted on the TLC plate. TLC plate was developed in a twin trough CAMAG chamber saturated with the mobile phase (Methanol: Water in a composition of 16:4 v/v). The developed plate was dried using hair dryer to remove the mobile phase and dried on hot plate at 120ºC for 5 minutes. There was no any interference of mobile phase and diluent at the *Rf* value of Citicoline sodium and Piracetam. The spectrum of separated bands of Citicoline sodium and Piracetam was taken. Peak purity of Citicoline sodium and Piracetam was determined by comparing spectrum at three different regions of the spots. i.e Peak start (S) Peak apex(M) and peak end(E) of both the drugs. The bands for Citicoline sodium and Piracetam were confirmed by comparing *Rf* values. The *Rf* values of Citicoline sodium and Piracetam were 0.30 and 0.68 respectively.

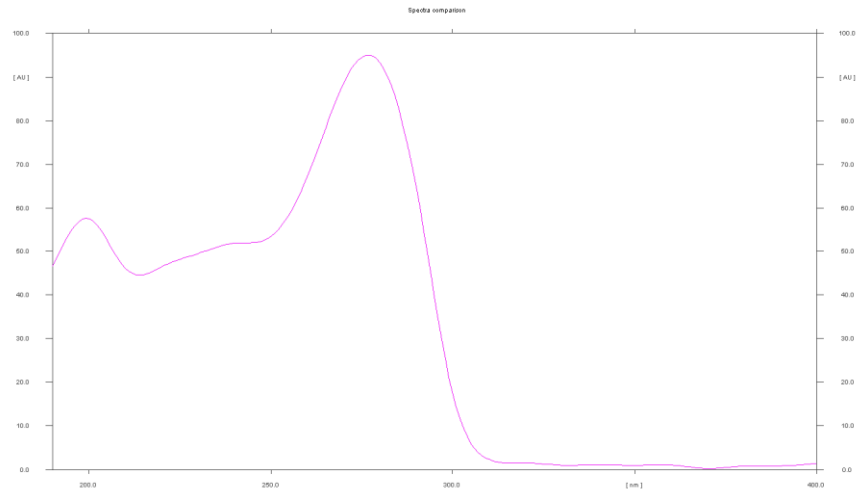


Figure 2: Spectrum of standard Citicoline sodium

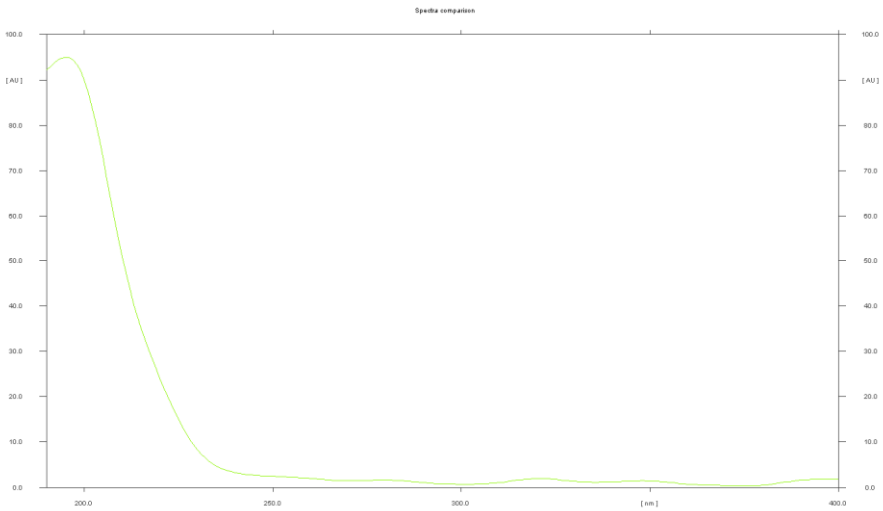


Figure 3 : Spectrum of standard Piracetam

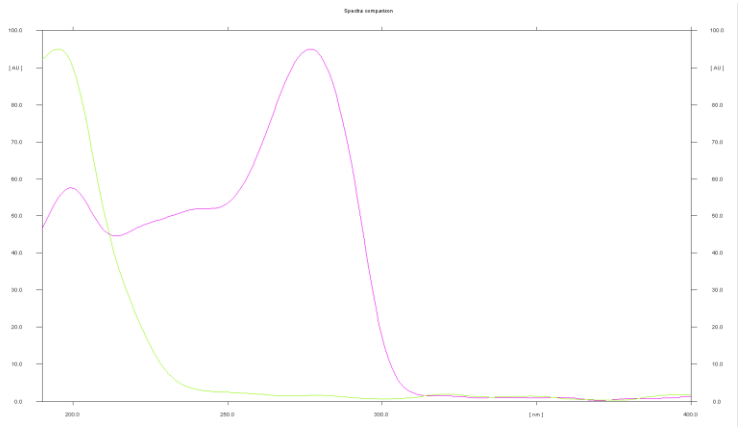


Figure 4. : Overlain spectra of standard Citicoline sodium and standard Piracetam

5.1.2 Accuracy

The accuracy of the Citicoline sodium and Piracetam was determined by performing recovery at three different concentration levels. The known concentrations of the samples were spiked with the standard Citicoline sodium and piracetam with the concentrations 1600 ng, 2000 ng and 2400 ng of Citicoline sodium and 1920 ng, 2400 ng and 2880 ng of Piracetam. The spiked samples were analysed by referring proposed analytical method. The percentage recovery was calculated and was in the range of 97.66 % to 99.70 % for Citicoline sodium and 97.61 % to 98.52 % for Piracetam respectively. The results are tabulated as under:

Table 1. : Percentage Recovery of Citicoline sodium
(n=3)

Sr. No.	Amount of std. Citicoline sodium added in ng	Amount of std. Citicoline sodium recovered in ng	% Recovery	% Relative Standard Deviation
1	1600	1562.56	97.66	1.0
2	2000	1994.0	99.70	1.32
3	2400	2349.84	97.91	0.44

Table 2: Percentage Recovery of Piracetam
(n=3)

Sr. No.	Amount of std. Piracetam added in ng	Amount of std. Piracetam recovered in ng	% Recovery	% Relative Standard Deviation
1	1920	1891.58	98.52	0.44
2	2400	2342.64	97.61	0.31
3	2880	2783.23	96.64	0.88

5.1.3 Precision

Precision of the method was determined by interday and intraday analysis on six determinations of Citicoline sodium and Piracetam standard solutions. The analysis was carried out by referring the developed method. Analytical results obtained are tabulated as under:

Table 3: Precision for the Citicoline sodium
(n= 6)

Conc. of the Citicoline sodium (ng/ band)	Inter-day precision		Intra-day precision	
	Mean area (AU)	% RSD	Mean area (AU)	% RSD
2000	3850	0.62	3384	1.05

Table 4: Precision for Piracetam
(n=6)

Conc. of the Piracetam (ng/ band)	Inter-day precision		Intra-day precision	
	Mean area (AU)	% RSD	Mean area (AU)	% RSD
2400	4570	1.44	3930	1.06

5.1.4 Robustness of the method

During Robustness testing, small deliberate changes in the mobile phase composition were done and effect on the results was examined. Each component of the Mobile phase with ± 0.1 mL change in volume was

made and chromatograms were run. Also ± 5 % variation in the mobile volume was done and chromatograph was run. The robustness of the method was determined at three different concentration levels. The results are tabulated as under:

Table 5: Robustness testing
(n=3)

Parameter	Conc. Level in ng spot^{-1} of Citicoline sodium	SD of Peak response of Citicoline sodium	%RSD	Conc. Level in ng spot^{-1} of Piracetam	SD of Peak response of Piracetam	%RSD
Mobile phase composition Methanol : Water (16.1: 4.1)	800	36.4966	1.64	1200	42.0991	1.89
	1200	39.5095	1.64	1600	47.9618	1.52
	1600	22.9420	0.63	2000	24.7049	0.70
Analysis by using 19 ml mobile phase ± 5 % variation in mobile phase volume	800	31.7857	1.40	1200	43.3128	1.77
	1200	24.2693	0.81	1600	50.6195	1.59
	1600	56.7656	1.53	2000	58.1292	1.50

5.1.5 Linearity

A series of standard solutions were prepared from the standard stock solutions of Citicoline sodium and Piracetam. 2 µl to 18µl of Citicoline sodium and 4µl to 20 µl of Piracetam solutions were spotted on the TLC plate. The corresponding concentrations were in the range of 400 ng / spot to 3600 ng /spot and 800 ng/spot to 4000 ng /spot respectively. The method is linear over the range of 400 ng to 3200 ng for Citicoline sodium and 1200 ng to 3600 ng for Piracetam. The linear Correlation coefficient for Citicoline sodium was 0.9987 and 0.9979 for Piracetam respectively.

5.1.6 LOD and LOQ

The Limits of detection (LOD) and Limit of Quantitation (LOQ) were calculated from standard deviation of peak response and slopes of the calibration curve. The Limit of Detection and Limit of Quantitation obtained by this method for Citicoline sodium and Piracetam were LOD= 3.8460 mcg, LOQ= 11.6545 mcg, LOD= 4.2162 mcg and LOQ = 12.7762 mcg respectively. The details are as under:

Table 6: Regression analysis of the calibration curves for Citicoline sodium and Piracetam

Sr. No.	Parameters	Citicoline Sodium	Piracetam
1.	Linearity Range	400 ng to 3200 ng	1200 ng to 3600 ng
2.	Standard Deviation	2.24	1.85
3.	Slope	1.922	1.448
4.	Intercept	439.6	498.8
5.	Regression Coefficient	0.9987	0.9979
6.	Re-gression Equation	1.922*X+439.6	1.448*X + 498.8
7.	LOD	3.8460 mcg	4.2162 mcg
8.	LOQ	11.6545 mcg	12.7762 mcg

5.1.7 Analysis of drug product

Experimental HPTLC results of the amount of Citicoline sodium and Piracetam in the tablet dosage form expressed as a mg of label claim were in good agreement with the label claim.
The drug content was found to be 99.22 % and 97.47 % for Citicoline sodium and Piracetam respectively.

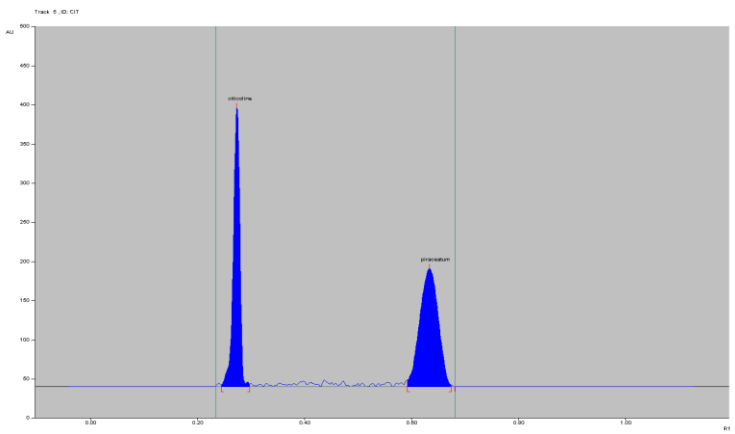


Figure 5. Densitogram of Citicoline sodium (R_f 0.30) and Piracetam (R_f 0.68)

5.1.8 Conclusion

HPTLC analysis is rapidly becoming popular in routine analysis. The advantages of these analytical techniques are low operating cost and high sample throughput. This method may be used for simultaneous determination of Citicoline sodium and Piracetam in routine analysis in drug substances as well as drug products. This method may be used for degradation study of the Citicoline sodium and Piracetam. The proposed HPTLC method is simple, accurate, economically chief and reproducible.

5.1.9 Acknowledgement

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