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Simultaneous Determination of Piracetam and Citicoline in Combination Drug Products by Rp-Hplc Method

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In this paper a simple, accurate and precise RP-HPLC method for the simultaneous. Estimation of piracetam and citicoline in synthetic mixtures has been developed and validated. Separation of drugs was carried out using buffer and Acetonitryle with proportion of 60:40 %v/v as mobile phase at 5 min. run time and 265nm. The Rt value for piracetam and citicoline was found to be 3.158 and 5.196 min respectively. The developed method has been validated for linearity, accuracy and precision, LOD, LOQ, and system suitability according to ICH guidelines. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate, and precise as it can detected and quantify with very low concentration. The low % RSD values below 2 indicate that the method is precise. The above validation studies revealed the method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of piracetam and citicoline in combined dose products.

Keywords: Piracetam; citicoline; buffer, acetonitryle; isocratic; HPLC.

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1. INTRODUCTION

Piracetam [1-3] (Fig.1.a)1,2, a cyclic subordinate of gamma-aminobutyric corrosive which helps in Alzheimer's and stroke recuperation related conditions [cognition and memory, eases back cerebrum maturing, expands oxygen and blood stream to the brain]. It impacts neuronal and vascular capacities and impacts subjective capacity without going about as a soothing or stimulant. It is synthetically 2-oxo - 1-pyrrolidine acetamide [4-8].

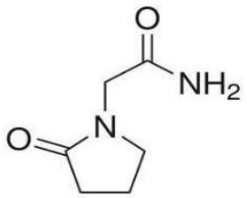

Fig.1.A: Structure of piracetam

Citicoline [9-11] (Fig.1.b) 1-(β-D-ribofuranosyl)cytosine-5' –diphosphate choline,or cytidine 5'- diphosphocholine has a place with the class of "nucleotides" that assume significant jobs to improve memory and other intellectual capacities in patients with constant cerebro vascular sickness and dementia and in elderly individuals. It is artificially 5'- O- [hydroxy ({hydroxy [2-(trimethylammonio) ethoxy] phosphoryl}oxy) phosphoryl] cytidine

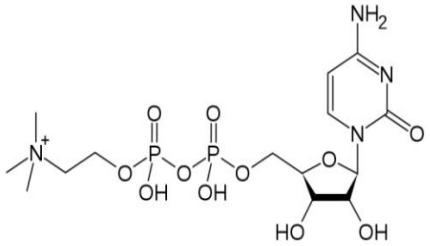

Fig.1. B: Structure of citicoline

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As of late it is demonstrated that the blend of piracetam and Citicoline improved mental execution in Alzheimer's sickness. It is sold in the neighborhood drug store by Intas Pharma with the brand name Prexavon Plus (800mg of piracetam and 500mg of citicoline) [12-16]

A point by point overview of writing uncovered that three RP-HPLC strategies [17-19] for the assurance of these medications was accounted for in pharmaceutical definitions. Thusly in the present examination the creator made an endeavor in this agreement and created and approved a basic, financial, fast, exact, and precise RP-HPLC technique by way of great affectability for measurable investigation of piracetam and citicoline in unadulterated medications as per ICH rules [20].

2. MATERIALS AND METHODS

2.1 Instrumentation

Chromatographic examination was completed by means of utilizing Shimadzu HPLC class VP arrangement, (Shimadzu Corporation, Kyoto, Japan) isocratic high pressure fluid chromatographic framework outfitted with an LC-10AT siphon, a variable wavelength programmable UV/Visible locator SPD-10A, an SCL-10A framework controller. The chromatographic framework uses a Shimadzu class VP arrangement form 5.03 PC program to manage equipment and pile up information. Thermo Scientific C18 column (250x 4.6 mm I.D., 5 µm molecule size) column was utilized. Shimadzu (Tokyo, Japan) electronic gauging balance, model BL 220 H was utilized for gauging the samples [21-25]

2.2 Chemicals and Reagents

Milli-Q water, Acetonitrile (HPLC Grade), Orthophosphoric acid (GR Grade), potassium dihydrogen orthophosphate monohydrate (GR Grade) was acquired from Qualigens Ltd., Mumbai. Every other substance of explanatory grade was obtained from nearby supplies except if indicated.

2.3 Arrangement of Phosphate Buffer

The buffer arrangement was set up by dissolved was precisely gauged 6.8grams of potassium dihydrogen orthophosphate and moved into a dry 1000mL volumetric jar, and dissolved with 1000mL water [HPLC Grade]. The last pH of the buffer was changed in accordance with 2.5 by utilizing orthophosphoric acid.

2.4 Mobile Phase Construction

Set up a separated and degassed blend of buffer and acetonitrile in the proportion of 60:40 %v/v individually [26-29].

2.5 Diluent Construction

Mobile phase is used as diluent in the present assay.

2.6 Arrangement of Stock & Working Standard Solutions

The stock arrangement was set up by weighing precisely 100mg of piracetam and 100mg of citicoline and moved into a dry 100mL volumetric flagon. Around 70 mL of diluent was included and sonicated. The volume was made into the imprint with a similar diluent. From the above-arranged stock

arrangement pipette out reasonable aliquots and moved into a perfect and dry 10mL volumetric jar, the diluent has signified the imprint to get the last convergence of 200-600µg/mL for piracetam and 50-150µg/mL for citicoline individually.

2.7 Construction of Sample Solution

Ten tablets of consolidated portion piracetam (800mg) + citicoline (500mg) tablets (Strocit Plus) produced by Akums Drugs and Pharmaceuticals was secured from the nearby therapeutic drug store was squashed to a fine powder. At that point the example solution was set up by gauging 100mg of the powdered example of piracetam and citicoline and moved into a 100mL spotless and dry volumetric jar and about 70mL of diluent was adjoined and the volume made sufficient with a similar dissolvable.

Pipette out aliquots of the above stock solution and moved into a perfect and diverse dry 10mL volumetric jar, and diluted with the diluent acquiring centralization of 200-600µg/mL for piracetam and 50-150µg/mL for citicoline separately. 10µL volumes of these standard and test solutions were infused multiple times and the peak areas be documented. The mean and %RSD be determined from the peak areas commonly.

3. RESULTS AND DISCUSSION

3.1 HPLC Technique Improvement

For this the current chosen medications were exposed to chromatographic examination utilizing mobile phases of varying pH, stream rate utilizing under referenced chromatographic conditions. Adjustments in the maintenance time of the medications be recorded by changing of mobile phase, pH, stream rate and temperature. At first, buffer and acetonitrile in the proportion 70: 30 %v/v were attempted however the peak eluted in the dead volume the two peaks were combined. In a while buffer and acetonitrile in the proportion of 65:35 %v/v were attempted and was discovered that the maintenance time and resolution was expanded yet sharp peaks were not acquired. At last buffer and acetonitrile with proportion of 60:40 %v/v at stream pace of 1.0mL/min was attempted. Piracetam and citicoline conferred adequate maintenance time, plates and great resolution at 265nm (Fig.2.c).

3.2 Strategy Validation

The created RP-HPLC technique is approved as per ICH rules [20] for test of piracetam and citicoline utilizing the accompanying parameters.

3.3 Specificity (Blank and placebo interference)

A concentrate to build up the obstruction of blank and placebo were led. Diluent and placebo were infused into the chromatograph in the characterized chromatographic conditions and the blank and placebo chromatograms were recorded. The chromatogram of blank solution indicated no peaks at the maintenance time of piracetam and citicoline peak (Fig.2.a). This demonstrates that the diluent solution utilized in test readiness doesn't meddle in the estimation of piracetam and citicoline in tablets. Additionally, the chromatogram of placebo solution (Fig.2.b) demonstrated no peaks at the maintenance time of piracetam and citicoline peak showing that the placebo utilized in test readiness doesn't meddle in the estimation of piracetam and citicoline in their definitions.

3.4 Linearity and Detector Response

The standard bend was acquired in the concentration scope 200-600µg/mL for piracetam and 50-150µg/mL for citicoline. Assessments of two medications be executed through PDA finder at 265nm and the particular peak areas were recorded for every one of the peaks and are given in Figs.3.a-e. The linearity was assessed by straight relapse examination. Slope, intercept and relationship coefficient [r2] of standard bend be schemed for piracetam and citicoline (Fig.3.f&g) and determined individually. The slope and intercept of an incentive for alignment bend was y = 9114x + 11114 (R2 = 0.999) for piracetam and y = 46409x + 53294 (R2 = 0.999) for citicoline. These outcomes uncovered that an amazing connection subsists sandwiched between peak area and

concentration of medications inside the concentration run showed previously. The LOD esteem for piracetam and citicoline were seen as 5.439µg/mL and 4.380µg/mL, separately (Figs.4.a & b and Table.2)

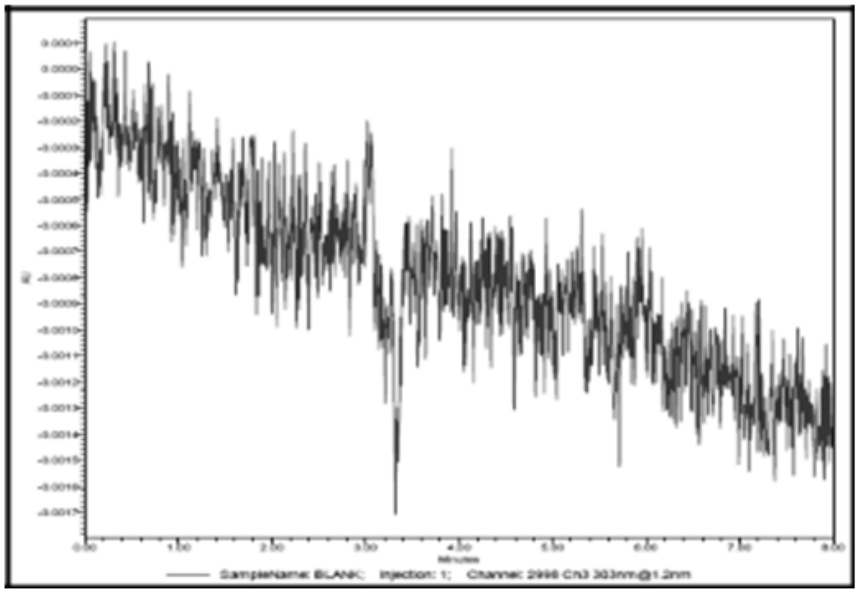

Fig: 2(a). Characteristic HPLC chromatogram demonstrating the no interference of blank for piracetam and citicoline

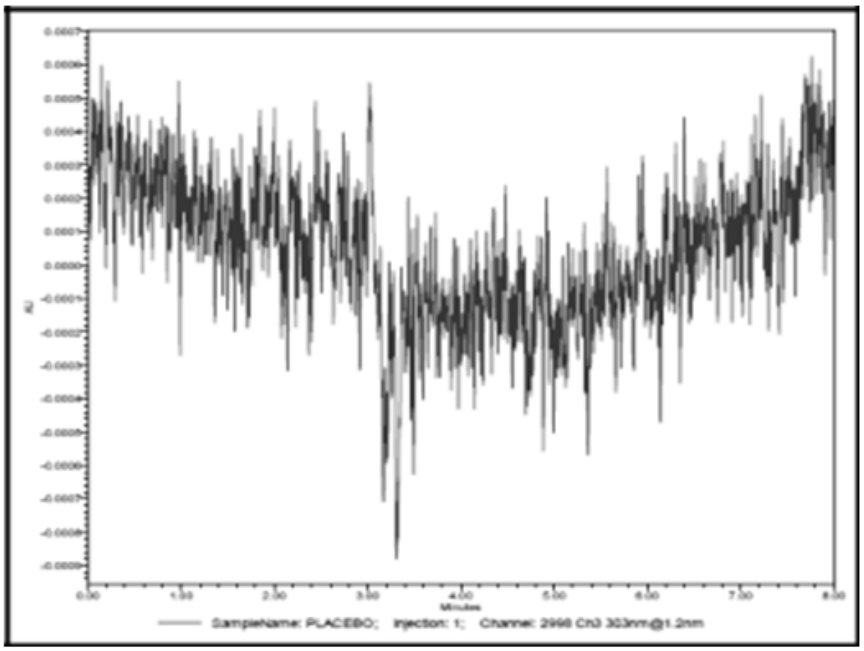

Fig. 2 (b). Characteristic HPLC chromatogram viewing the no interference of placebo for piracetam and citicoline

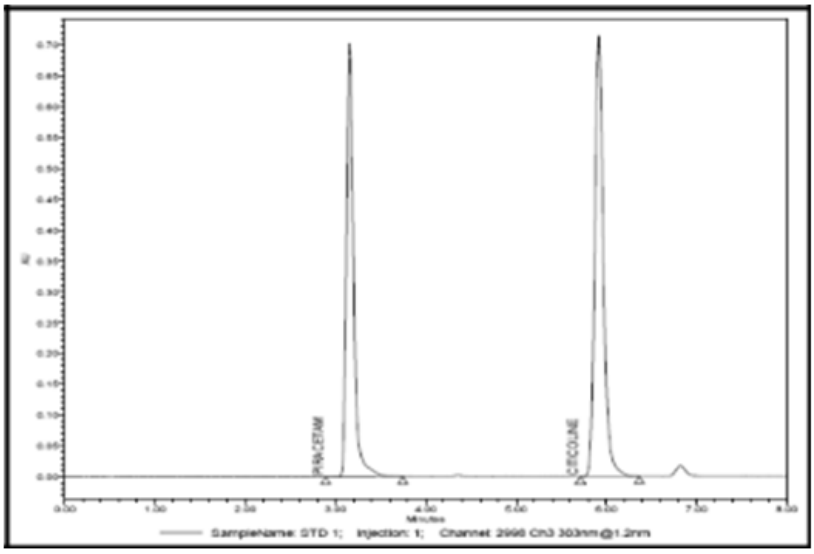

Fig. 2 (C). Characteristic HPLC chromatogram illustrating the peaks of piracetam and citicoline

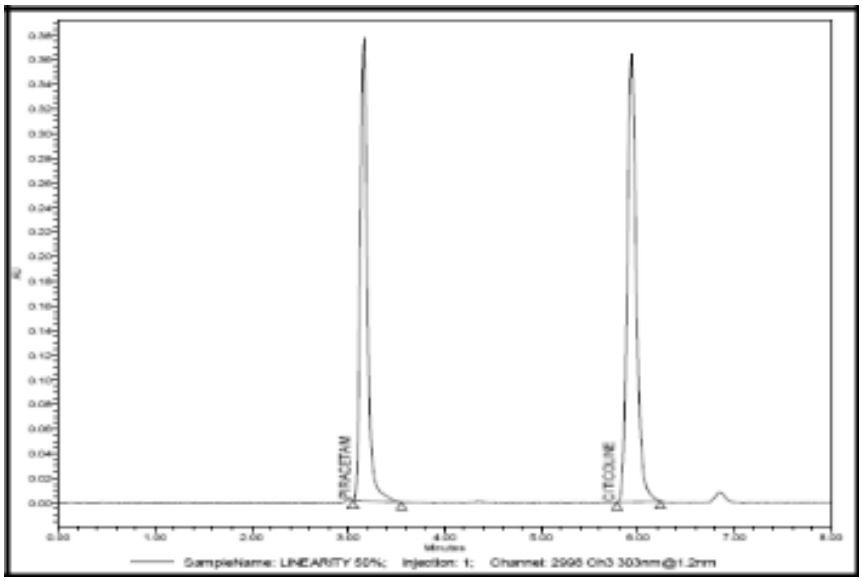

Fig. 3 (a). Chromatogram of piracetam and citicoline at 50% conc.level

Table 1. System suitability parameters of piracetam and citicoline

Name of the Compound	Retention Time	Theoretical Plates	Tailing Factor	USP Resolution
Piracetam	3.158	8719	1.334	-
Citicoline	5.916	18971	1.230	17.235

3.5 Precision

The strategy exactness study for six example arrangements in promoted tests demonstrated a %RSD of 0.570 for piracetam and 00.109 for citicoline separately uncovering high exactness of the proposed RP-HPLC technique (Figs.5.a-f and Table.3) correspondingly.

Table 2. Linearity review of piracetam and citicoline

	Piracetam			Citicoline	
% Level	Conc.	Area	% Level	Conc.	Area
(Approx.)	µg/mL		(Approx.)	µg/mL	
50	200	1937582	50	50	2393874
75	300	2841565	75	75	3554530
100	400	3766491	100	100	4643540
125	500	4646669	125	125	5813531
150	600	5592241	150	150	7065500
Slope		9114	Slope		46409
RSQ(r2)		0.9999	RSQ(r2)		0.9999
LOD (µg/mL)		5.439	LOD (µg/mL)		4.380

Table 3. System precision review of piracetam and citicoline

System correctness by projected technique		
Piracetam		Citicoline
Set-1	3777338	4753434
Set-2	3801876	4754767
Set-3	3805118	4746865
Set-4	3751215	4757894
Set-5	3802141	4762103
Set-6	3772886	4752105
*Over All Avg.	3785096	4754528
*Over All Std Dev.	21587.84	5187.621
*Over All %RSD	0.570	0.109

**Average of six determinations*

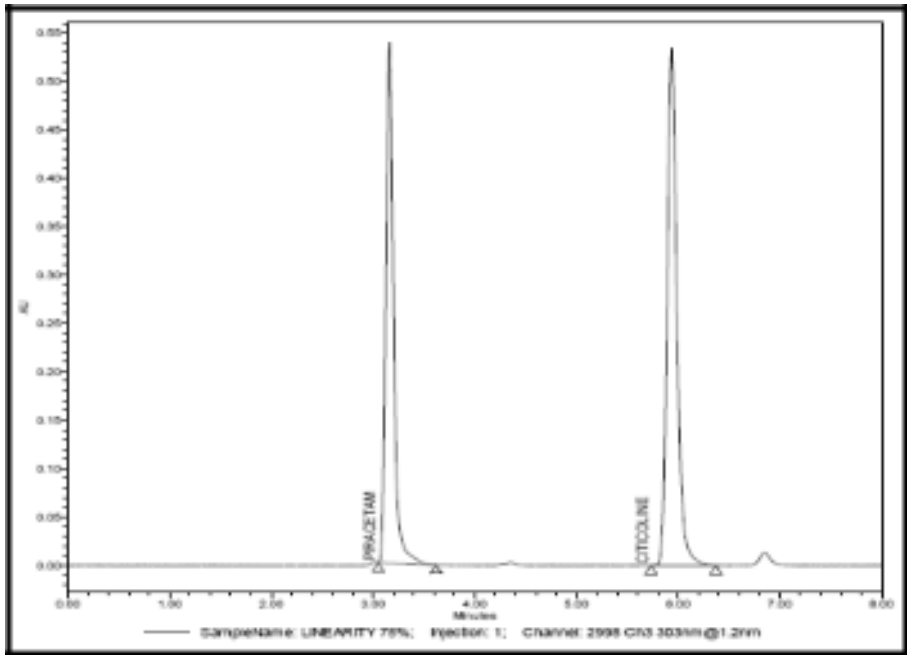

Fig. 3 (b). Chromatogram of piracetam and citicoline at 75% conc.level

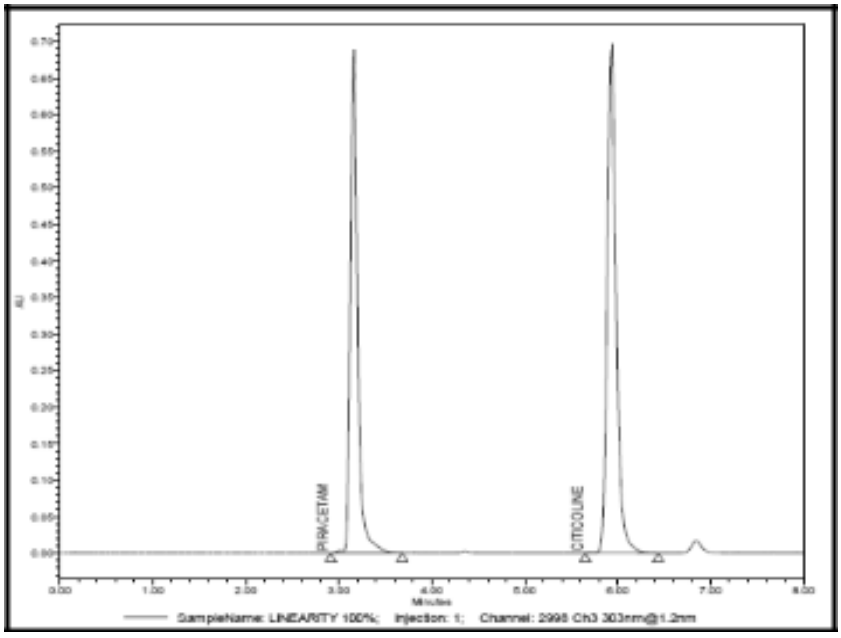

Fig. 3 (c). Chromatogram of piracetam and citicoline at 100% conc.level

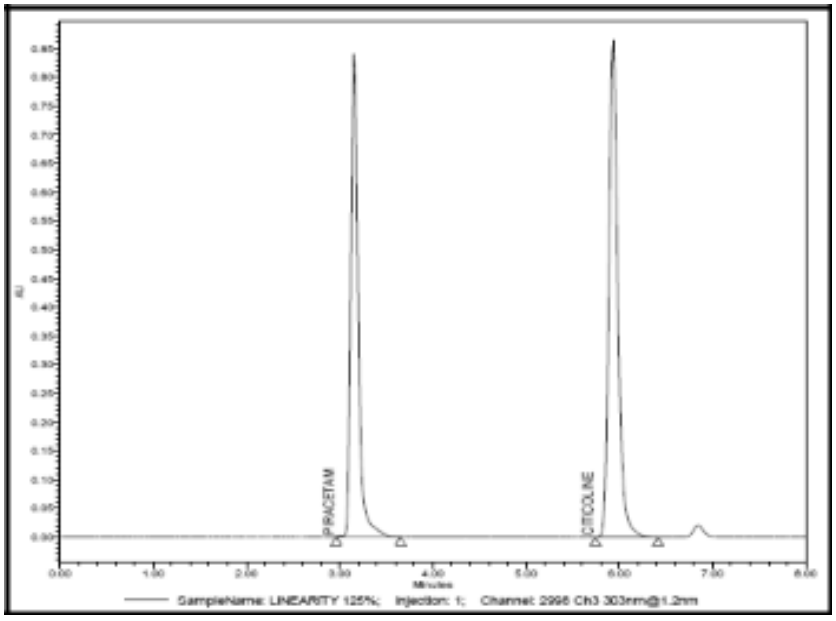

Fig. 3 (d). Chromatogram of piracetam and citicoline at 125% conc.level

3.6 Exactness

Exactitude of the strategy was resolved on three concentration levels via recuperation tests. The recuperation was done in triplicate arrangement with on composite mix gathered from 10 tablets of piracetam and citicoline, broke down according to the proposed strategy. The %RSD was extended from 0.053-0.253 for piracetam and 0.008-0.053 for cticoline with rate recuperations ran of 99.9% for piracetam and for citicoline separately. From the information announced in Figs.6.a-c and Table.4, uncovered that the created RP-HPLC technique was seen as exact for piracetam and citicoline test.

Table 4. Data of recovery studies of piracetam and citicoline

Piracetam						
Spiked Level	**Sample Weight**	**Sample Area**	**µg/mL added**	**µg/mL found**	**% Recovery**	**% Mean**
50%	445.65	1930511	297.400	294.57	99	
50%	445.65	1933448	297.400	295.00	99	
50%	445.65	1933360	297.400	294.98	99	99
50%	445.65	1937578	297.400	295.60	99	
50%	445.65	1937268	297.400	295.55	99	
50%	445.65	1930834	297.400	294.62	99	
100%	891.30	3767261	594.800	590.05	99	
100%	891.30	3761646	594.800	589.23	99	99
100%	891.30	3767760	594.800	590.12	99	
150%	1337.00	5590388	892.234	883.55	99	
150%	1337.00	5597102	892.234	884.52	99	
150%	1337.00	5595283	892.234	884.26	99	99
150%	1337.00	5590260	892.234	883.53	99	
150%	1337.00	5593445	892.234	883.99	99	
150%	1337.00	5596057	892.234	884.37	99	
Citicoline						
Spiked Level	**Sample Weight**	**Sample Area**	**µg/mL added**	**µg/mL found**	**% Recovery**	**% Mean**
50%	445.65	2365530	29.920	29.91	100	
50%	445.65	2364559	29.920	29.89	100	
50%	445.65	2362622	29.920	29.85	100	100
50%	445.65	2363962	29.920	29.88	100	
50%	445.65	2368356	29.920	29.96	100	
50%	445.65	2366308	29.920	29.92	100	
100%	891.30	4628661	59.840	59.77	100	
100%	891.30	4626493	59.840	59.73	100	100
100%	891.30	4627916	59.840	59.76	100	
150%	1337.00	7081986	89.764	89.45	100	
150%	1337.00	7082015	89.764	89.45	100	
150%	1337.00	7088800	89.764	89.58	100	100
150%	1337.00	7086699	89.764	89.54	100	
150%	1337.00	7085157	89.764	89.51	100	
150%	1337.00	7084297	89.764	89.49	100	

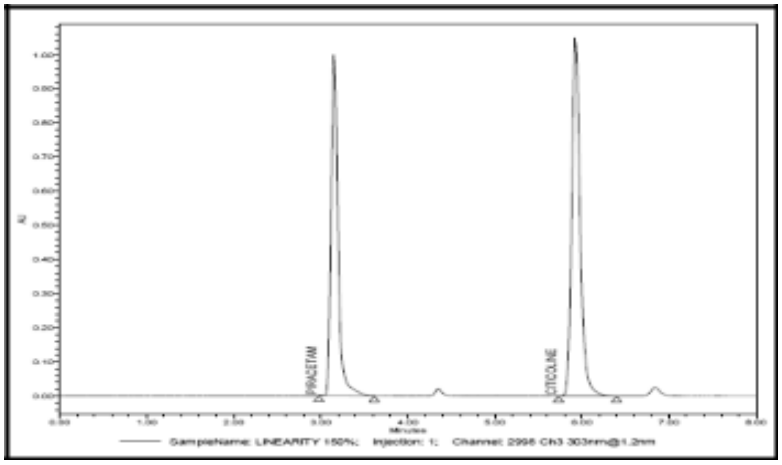

Fig. 3 (e). Chromatogram of piracetam and citicoline at 150% conc.level

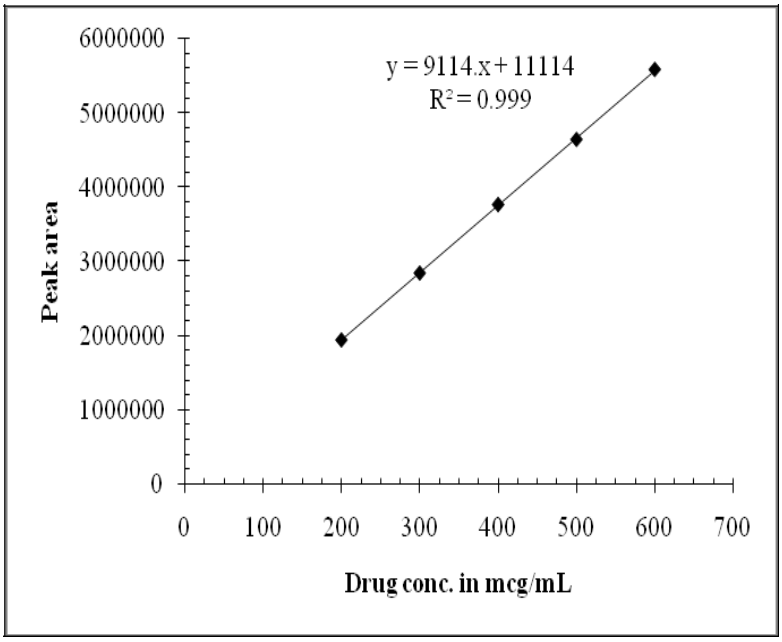

Fig. 3 (f). Linearity curve for piracetam

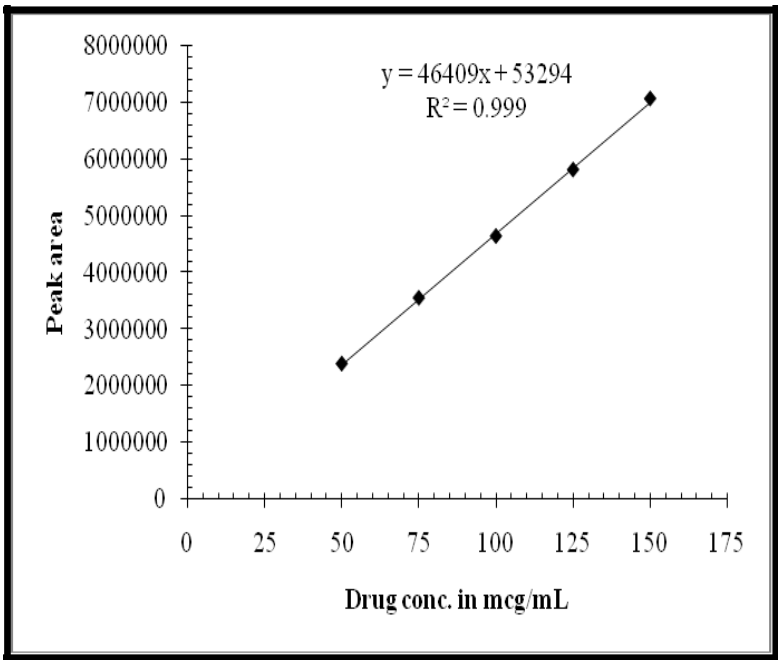

Fig. 3 (g). Linearity curve for citicoline

3.7 Robustness Studies

The vigor investigation of the created examine strategy for piracetam and citicoline was set up in all different conditions. Test estimations of the test planning solution were not influenced and were as per that of real (Table.6). Framework reasonableness parameters were likewise seen as palatable finishing up the heartiness of the created technique.

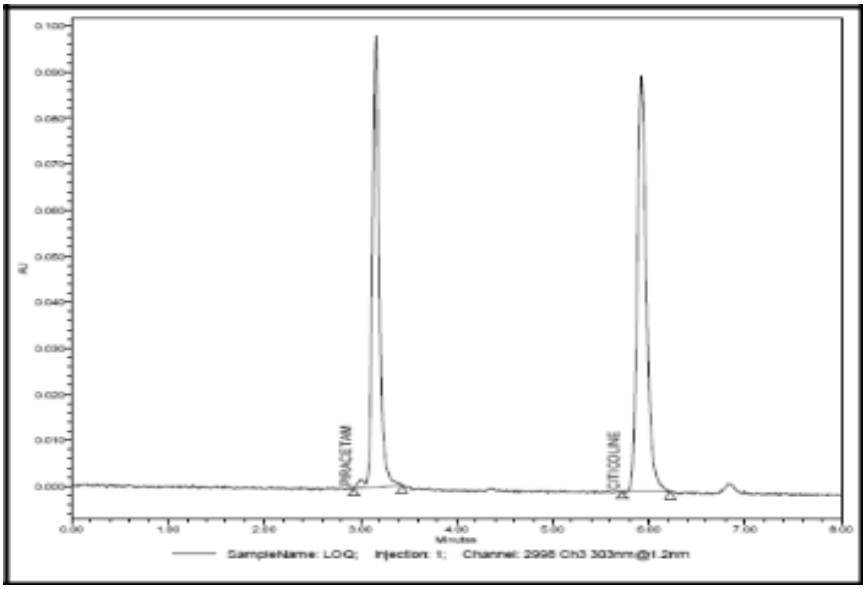

Fig. 4 (a). LOD chromatogram of piracetam and citicoline

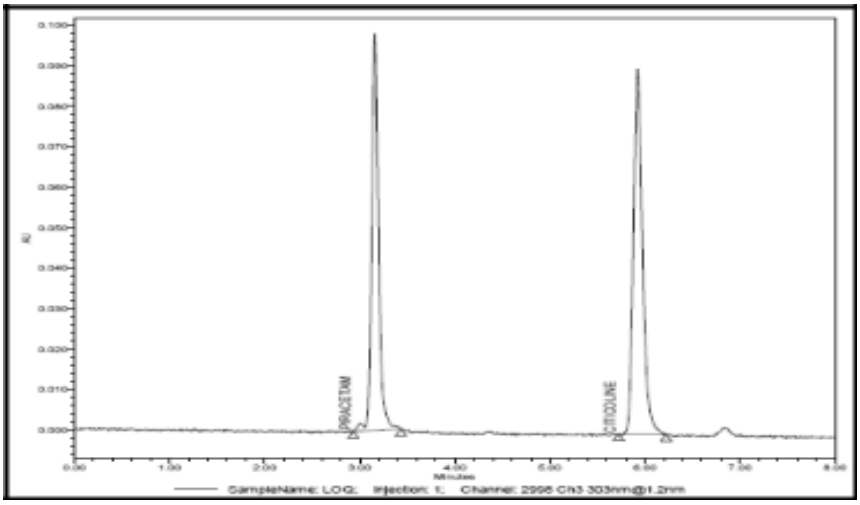

Fig. 4 (b). LOQ chromatogram of piracetam and citicoline

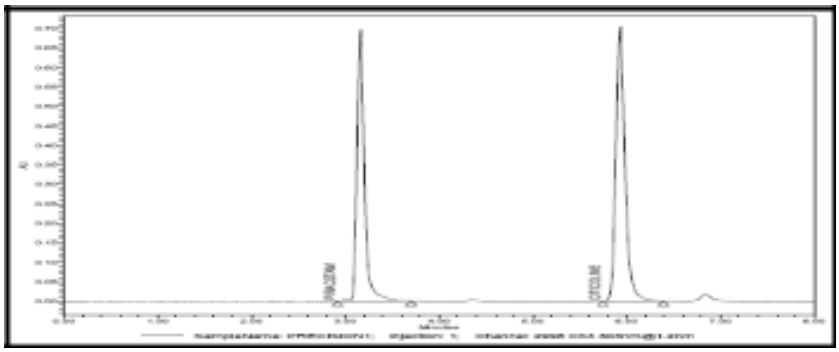

Fig. 5a. Chromatogram of piracetam and citicoline(INJ-1)

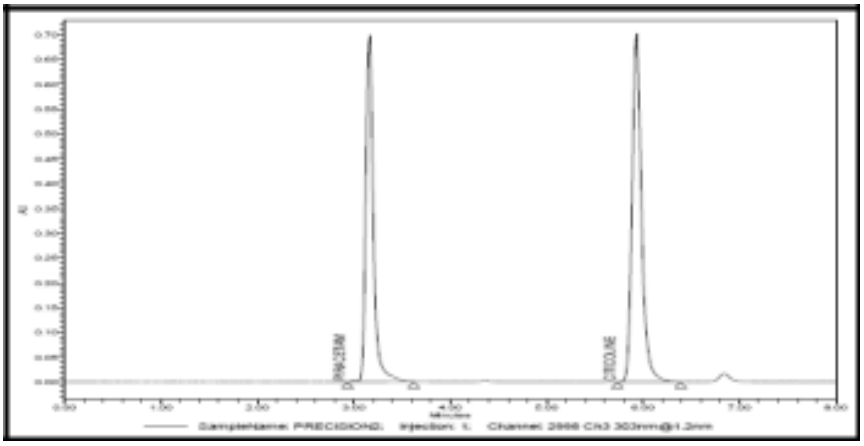

Fig. 5b. Chromatogram of piracetam and citicoline(INJ-2)

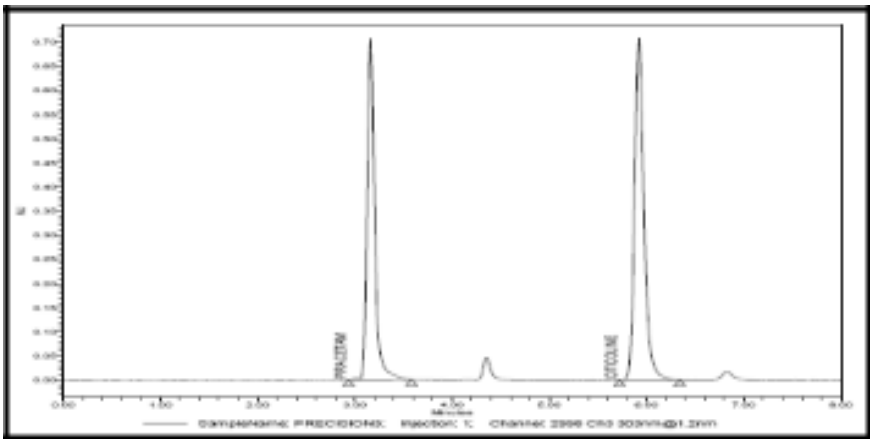

Fig. 5c. Chromatogram of piracetam and citicoline(INJ-3)

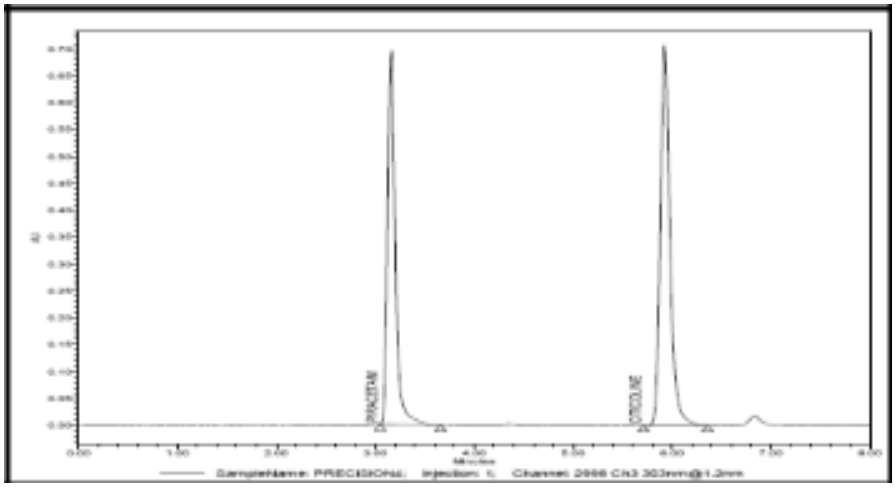

Fig. 5d. Chromatogram of piracetam and citicoline(INJ-4)

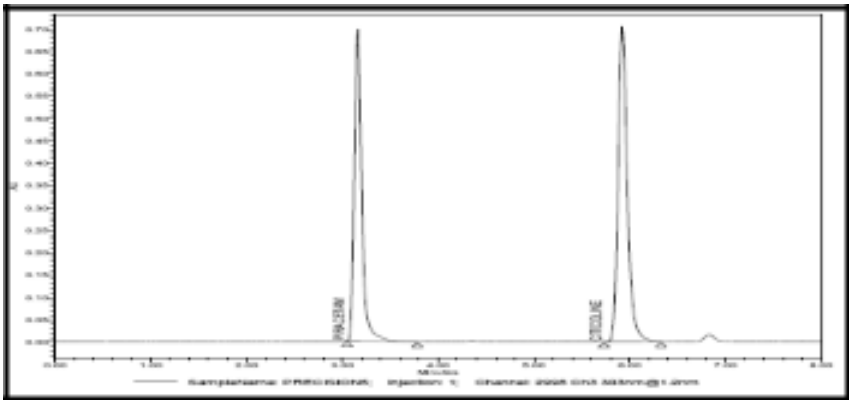

Fig. 5e. Chromatogram of piracetam and citicoline (INJ-5)

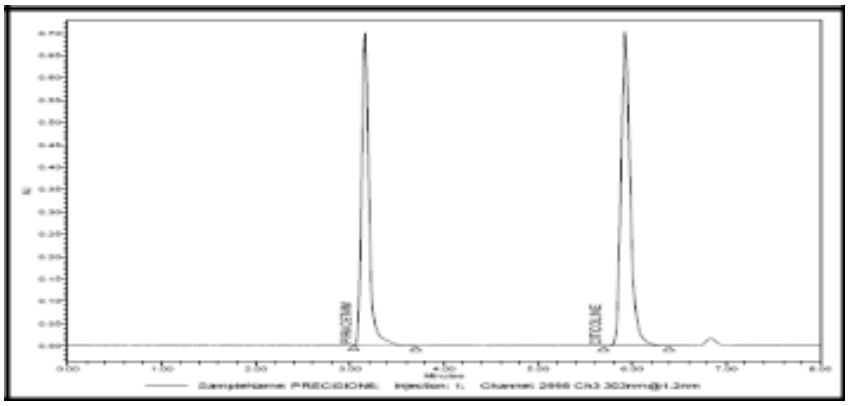

Fig. 5f. Chromatogram of piracetam and citicoline (INJ-6)

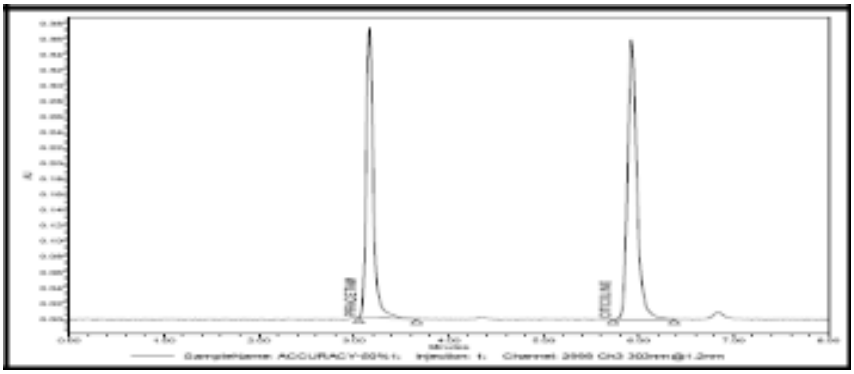

Fig. 6a. Chromatogram of piracetam and citicoline (50%)

3.8 Investigation of Marketed Formulation

Analysis of advertised tablets (Strocit Plus) containing piracetam (800mg) + citicoline (500mg) was completed utilizing the enhanced mobile phase and HPLC stipulations. % sedate substance of tablets by the projected technique intended for piracetam and citicoline was 99.99 and 99.98%, individually. This indicated the estimation of measurement structures was exactly inside the acknowledgment level of 95% to 100%. The outcomes be specified in Table.6.

Table 5. Robustness review of the projected RP-HPLC process

Vigorous provisions		Piracetam		Citicoline	
		Rt	Peak Area	Rt	Peak Area
Flow Rate	0.8 mL/min	4.252	4891243	7.952	6447330
	1.2 mL/min	2.509	3013908	4.738	3769886
Temp	30°C	3.151	3804125	5.965	4715555
	40°C	3.137	3772368	6.062	4703767

Table 6. Examination of marketed tablets by the proposed method

Drug	Labal Claim	Quantity Found*	%Assay
Piracetam	800mg	799.98mg	99.99
Citicoline	500mg	499.99mg	99.99

**Average of six determinations*

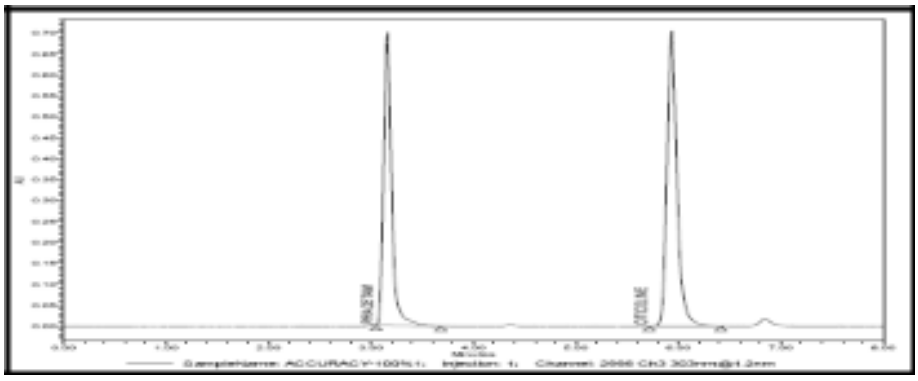

Fig. 6b. Chromatogram of piracetam and citicoline(100%)

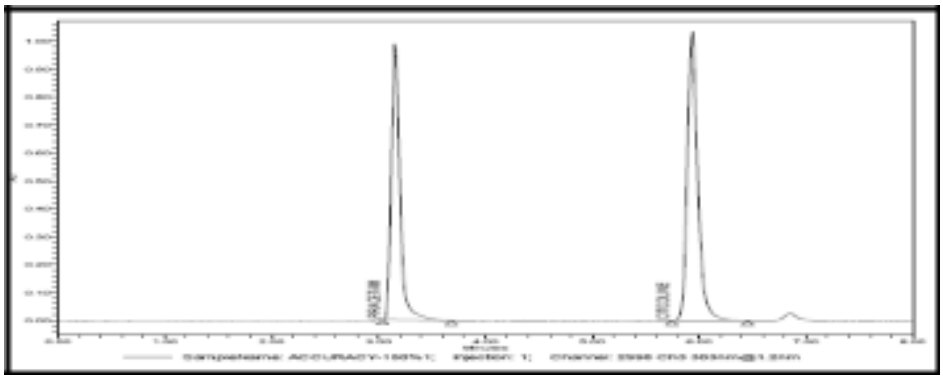

Fig. 6c. Chromatogram of piracetam and citicoline (150%)

4. CONCLUSION

A straightforward, speedy, practical RP-HPLC strategy has been produced for the estimation of piracetam and citicoline and unadulterated and additionally for consolidated measurement structures. The believability of the proposed technique has been set up by approval according to the ICH rules [20]. The point of confinement of location for piracetam and citicoline was seen as 5.439µg/mL and 4.380µg/mL individually demonstrating the great affectability of proposed RP-HPLC strategy for both the medications. The normal % recuperation in plans for piracetum and citicoline was seen as 99.99% individually uncovering that the proposed technique is free

from impedance from excipients present in the detailing. Consequently it very well may be finished up, "that the proposed strategy was a decent approach for acquiring dependable outcomes and seen as appropriate for the standard quality control examination of piracetam and citicoline in unadulterated and likewise in joined measurements structures".

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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