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First Derivative Spectroscopic Method for Simultaneous Estimation of Edaravone and Citicoline Sodium in Synthetic Mixture.

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Research Article

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ABSTRACT

A simple, accurate and precise spectroscopic method was developed for simultaneous estimation of Edaravone and Citicoline Sodium in synthetic mixture using first order derivative zero-crossing method. Edaravone showed zero crossing point at 245.60 nm while Citicoline Sodium showed zero crossing point at 271.20 nm. The dA/dλ was measured at 271.20 nm for Edaravone and 271.20nm for Citicoline Sodium and calibration curves were plotted as dA/dλ versus concentration, respectively. The method was found to be linear (r2>0.999) in the range of 1-6µg/ml for Edaravone at 271.20 nm. The linear correlation was obtained (r2>0.999) in the range of 25-150 µg/ml for Citicoline Sodium at 271.20 nm. The limit of determination was 0.032µg/ml and 0.831µg/ml for Edaravone and Citicoline Sodium, respectively. The limit of quantification was 0.098 µg/ml and 2.520 µg/ml for Edaravone and citicoline Sodium, respectively. The accuracy of these method were evaluated by recovery studies and good recovery result were obtained greater than 99% shows first order derivation zero crossing. The method was successfully applied for simultaneous determination of Edaravone and Citicoline Sodium in binary mixture.

INTRODUCTION

Edaravone (EDA) is a neuroprotective agent used for the purpose of aiding neurological recovery following acute brain ischemia and subsequent cerebral infarction [1]. It acts as a potent antioxidant and strongly scavenges free radicals, protecting against oxidative stress and neuronal apoptosis [2,3,4]. It ischemically 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one. Edaravone is a white or off- white amorphous powder having molecular weight 174.20g/mol [5-6] Chemical Structure of Edaravone is in figure.1

Citicoline(CIT), 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. Citicoline's effects may also be explained by the reduction of phospholipase A_2 activity. [7] Citicoline increases phosphatidylcholine synthesis [9,10,11].

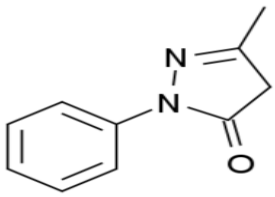

Figure 1: Chemical structure of Edaravone

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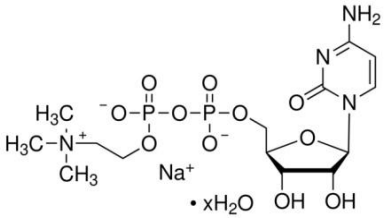

Figure 2: Chemical structure of Citicoline

It is chemically 55'-O[hydroxyl ({hydroxyl [2-(trimethylammonio) ethoxy]phosphoryl} oxy)phosphoryl] cytidine. Citicoline Sodium is a white or off- white amorphous, hygroscopic powder having molecular weight 510.31g/mol [5, 6] Chemical Structure of Citicoline is in figure. 2

Both drugs are Neuroprotctive agents; Both drugs are 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. Finally affect the CNS related disorder.

The review of literature regarding quantitative analysis of Edaravone and Citicoline Sodium revealed that no attempt was made to develop analytical methods for Edaravone and Citicoline Sodium. Some spectrometric methods and chromatographic methods have been reported for the estimation of the individual drugs [8-16]. The focus of the present study was to develop and validate a rapid, stable, specific, and economic RP-HPLC method for the estimation of Edaravone and Citicoline Sodium in Synthetic mixture.

MATERIALS AND METHODOLOGY

- Edaravone and Citicoline Sodium were obtained as gift samples from BDR Pharmaceutical international Pvt. Ltd. Synthetic Mixture contain 1mg of Edaravone and 25mg of Citicoline Sodium.
- A double beam UV/Visible spectrophotometer (Shimadzu model 2450, Japan) with spectral width of 2 nm, 1 cm quartz cells was used to measure absorbance of all the solutions.
- Spectra were automatically obtained by UV-Probe system software.
- An analytical balance (Sartorius CD2250, Gottingen, Germany) was used for weighing the samples.
- Sonicator(D120/2H, TRANS-O-SONIC)
- Class 'A' volumetric glassware were used (Borosillicte)

Standard stock solution for Edaravone

This gives a concentration of 100µg/ml in Distilled Water. Sonicated for 15min and finally volume was made up to the mark with the solvent. From that 10ml was diluted in 100ml of Distilled Water and the volume was made up to 100ml which gives a concentration of 10µg/ml and from that 1ml was diluted with 10ml Distilled Water to obtained the concentration 1µg/ml for selection of analytical wavelength for ZCP method.

Standard stock solution for Citicoline Sodium

This gives a concentration of 250µg/ml in Distilled Water. From that 1ml was diluted in 10ml of Distilled Water and the volume was made up to 10ml which gives a concentration of 25µg/ml for selection of analytical wavelength for ZCP method.

Preparation of Standard Mixture Solution (EDA + CIT)

1ml of standard stock solution of EDA (10µg/ml) and 1ml of standard Stock solution of CIT (250µg/ml) were pipetted out into two 10ml volumetric flasks and volume was adjusted to the mark with Distilled Water to get 1µg/ml of EDA and 25µg/ml of CIT.

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Preparation of test solution

The preparation of synthetic mixture was as per patent [17]:

- Water for Injection : 7.5 ml (finally upto 10ml)
- Cystein HC1 : 0.75mg
- HydroxyPropyl Beta Cyclodextrene :100mg
- Edaravone : 10mg
- Sodium Bisulphite : 10mg
- Citicoline Sodium:250mg
- Disodium EDTA : 0.2mg

All the excipients were mixed in 10ml volumetric flask and sonicated for 15min. make up the volume with Distilled Water. The solution was filtered through Whatman filter paper No. 42.
Finally the solution had concentration 100µg/ml for EDA and 2500µg/ml for CIT from that pipette out 2ml in 100ml volumetric flask and make up to the mark with Distilled Water.

Table 1: Calibration data for EDA and CIT at 271.20nm and 245.60nm, respectively. *(n=6)

Sr. No	Concentration (µg/ml)		Absorbance* (271.20nm)±SD EDA	Absorbance* (245.60nm)±SD CIT
	EDA	CIT		
1	1	25	-0.01332±0.00058	-0.03655±0.00315
2	2	50	-0.02735±0.00063	-0.06858±0.00339
3	3	75	-0.04012±0.00095	-0.10410±0.00316
4	4	100	-0.05477±0.00065	-0.13583±0.00456
5	5	125	-0.05477±0.00086	-0.16953±0.00490
6	6	150	-0.08070±0.00092	-0.20623±0.00413

Table 2: Intraday precision data for estimation of EDA and CIT *(n=3)

Conc. (µg/ml)		Absorbance* (EDA) Avg. ± SD(271.20nm)	% RSD	Absorbance* (CIT) Avg.±SD(245.60nm)	% RSD
EDA	CIT				
2	50	-0.0274 ± 0.00025	0.912	-0.0695 ± 0.00060	0.863
3	75	-0.0405 ± 0.00040	0.987	-0.1051 ± 0.00077	0.738
4	100	-0.0548 ± 0.00036	0.657	-0.1356 ± 0.0085	0.630

Table 3: Interday precision data for estimation of EDA and CIT *(n=3)

Conc. (µg/ml)		Absorbance* (EDA) Avg.± SD(271.20nm)	% RSD	Absorbance* (CIT) Avg.±SD(245.60nm)	% RSD
EDA	CIT				
2	50	-0.0272 ± 0.00020	0.764	-0.0685 ± 0.00060	0.875
3	75	-0.0402 ± 0.00036	0.896	-0.1048 ± 0.00096	0.910
4	100	-0.0548 ± 0.00036	0.653	-0.1371 ± 0.00111	0.812

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Table 4: Recovery data of EDA *(n=3)

Conc. of EDA from formulation (µg/ml)	Amount of Std.EDA added (µg/ml)	Total amount of EDA (µg/ml)	Total amount of EDA found (µg/ml) Mean* ± SD	% Recovery (n=3)	% RSD EDA
2	1.6	3.6	3.62 ± 0.022	100.05	0.727
2	2.0	4.0	4.00 ± 0.018	100.12	0.468
2	2.4	4.4	4.39 ± 0.022	99.95	0.595

Table 5: Recovery data of CIT *(n=3)

Conc. of CIT from formulation (µg/ml)	Amount of Std.CIT added (µg/ml)	Total amount of CIT (µg/ml)	Total amount of CIT found (µg/ml) Mean* ± SD	% Recovery (n=3)	% RSD CIT
50	40	90	90.21 ± 0.257	100.23	0.285
50	50	100	99.57 ± 0.571	99.57	0.573
50	60	110	110.28 ± 0.428	100.25	0.3387

Table 6: LOD and LOQ data of EDA and CIT *(n=10)

Conc. (µg/ml)		Absorbance* (EDA) Avg. ± SD(271.20nm)	% RSD	Absorbance* (CIT) Avg.±SD(245.60nm)	% RSD
EDA	CIT				
1	25	-0.01332 ± 0.00013	0.988	-0.03773 ± 0.00035	0.935
LOD (µg/ml)		0.032		0.831	
LOQ (µg/ml)		0.098		2.520	

Table 7: Robustness and Ruggedness data of EDA and CIT *(n=3)

Conc. (PPM)	Edaravone (Mean* ±% RSD)			
	Instrument 1	Instrument 2	Stock – 1*	Stock – 2*
2	-0.0273 ± 0.863	-0.0271 ± 0.927	-0.0264 ± 0.860	-0.0275 ± 0.912
3	-0.0405 ± 0.290	-0.0404 ± 0.755	-0.0410 ± 0.700	-0.0406 ± 0.982
5	-0.0549 ± 0.471	0.0551 ± 0.553	-0.5507 ± 0.342	-0.0549 ± 0.820
	Citicoline Sodium (Mean* ±% RSD)			
50	-0.0696 ± 0.338	-0.0701 ± 0.931	-0.0687 ± 0.514	-0.0690 ± 0.806
75	-0.1056 ± 0.713	-0.1062 ± 0.438	-0.1051 ± 0.470	-0.1055 ± 0.621
100	-0.1357 ± 0.138	-0.1361 ± 0.669	-0.1374 ± 0.308	-0.1368 ± 0.692

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Table 8: Analysis data of commercial formulation *(n=3)

Sr. No.	Formulation		Absorbance (267.00nm) EDA	% Assay EDA±SD*	Absorbance (258.40nm) CIT	% Assay CIT±SD*
	EDA	CIT				
1			-0.0268		-0.0710	
2	2	50	-0.0269	99.87 ± 0.776	-0.0715	99.52 ± 0.861
3			-0.0272		-0.0722	

Table 9: Summary of validation parameters

PARAMETERS	Edaravone	Citicoline Sodium
Concentration range(µg/ml)	1-6	25-150
Regression equation	y = -0.0134x - 0.0002	y = -0.0014x - 0.0019
Correlation Coefficient(r^2)	0.9996	0.9996
Accuracy(%Recovery) (n=3)	100.04	100.01
Intra-day Precision (%RSD) (n=3)	0.657-0.987	0.630-0.863
Inter-day precision (%RSD) (n=3)	0.653-0.896	0.812-0.910
LOD(µg/ml)	0.032	0.831
LOQ(µg/ml)	0.098	2.520
Ruggedness and Robustness	0.290-0.982	0.138-0.931
% Assay	99.87	99.52

75, 100µg/ml of CIT) and the %RSD of assay (inter-day and intra-day) was calculated. The results of study are shown in Table no 2 and 3.

Accuracy

The accuracy of the method was determined by spiking of EDA and CIT to prequantified sample solutions of EDA (2µg/ml) and CIT (50µg/ml) in triplicate at three concentration level of 80, 100, 120% of the specified limit. The percentage recoveries of EDA and CIT were calculated and the result is nearer to 100% shown in Table no 4 and 5.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were evaluated by standard deviation of response and slope method. LOQ and LOD were calculated by the equation LOD = 3.3 × *N/B* and LOQ = 10 × *N/B*, where "*N*" is standard deviation of the absorbance, and "*B*" is the slope of the corresponding calibration curve. The limit of detection (LOD) were found to be 0.032µg/ml for EDA and 0.831µg/ml for CIT and respectively and limit of quantitation (LOQ) were found to be 0.098µg/ml for EDA and 2.520µg/ml for CIT presented in Table no 6.

Robustness and Ruggedness

Robustness was done by different instrument and difference in preparation of stock solution. The result was decided by %RSD which is in the limit which is mentioned in table no 7.

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Assay

As per the test solution preparation, the solutions were prepared and further proceed for UV Spectrum.

A zero order derivative spectrum of the resulting solution was recorded and processed to first derivative spectra. A first order derivative spectrum of the sample solution was recorded and the absorbance at 271.20nm and 245.60nm were noted for estimation of EDA and CIT, respectively. The concentrations of EDA and CIT in formulation were determined using the corresponding calibration graph. The result was as per the Table 8.

Summary Table

Summary Table was as per the table no 9.

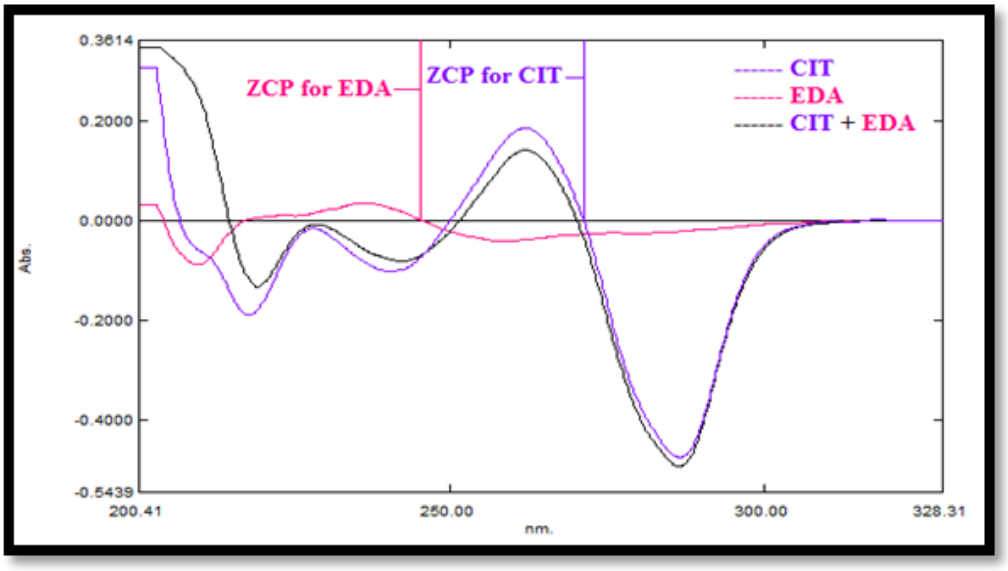

Figure 3: Overlain first order spectra of EDA and CIT in 1:25 ratios, respectively with the combination solution (1:25)

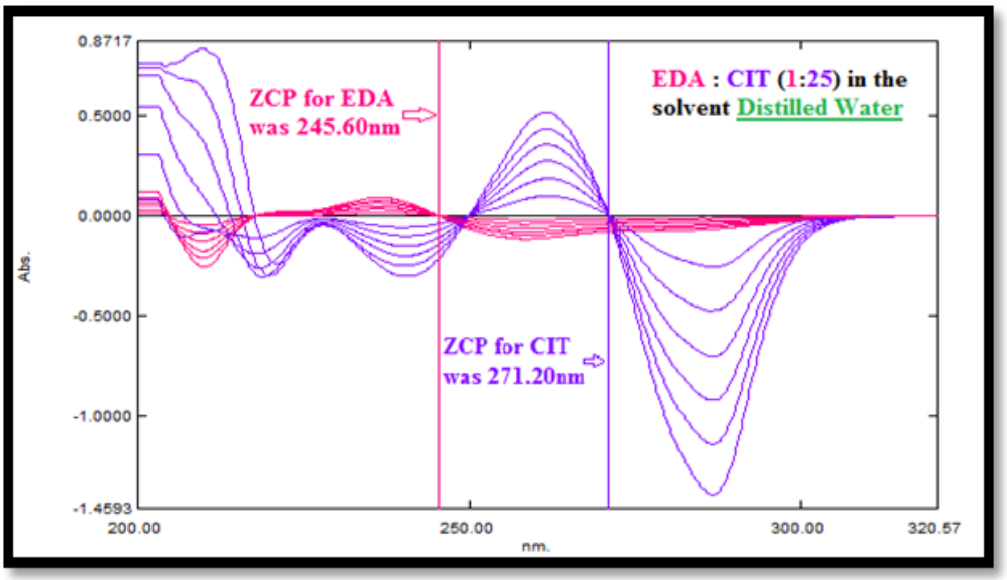

Figure 4: Overlain linear first order spectra of EDA (Pink) and CIT (Blue) in 1:25 ratios

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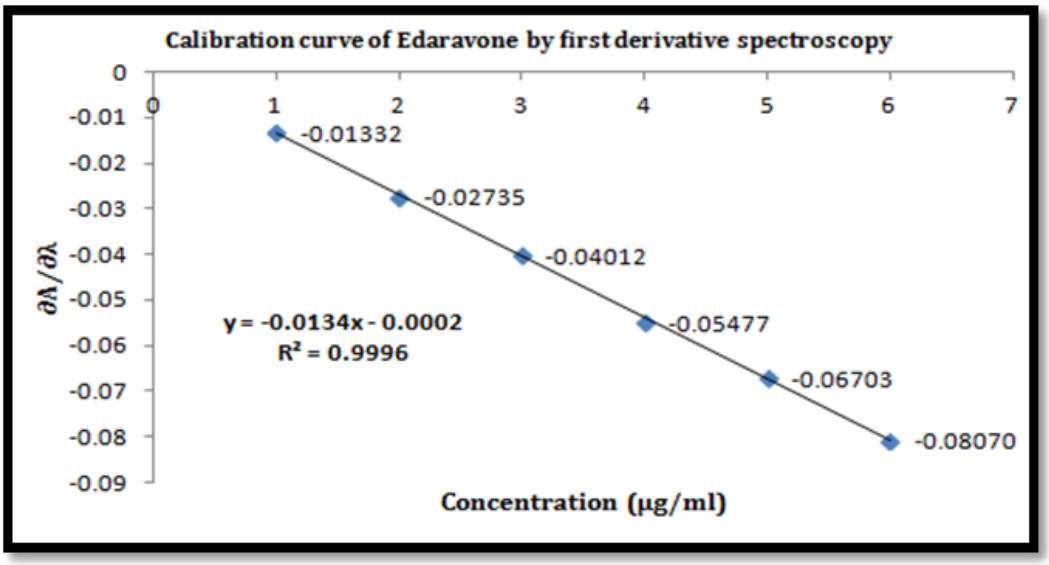

Figure 5: Calibration curve for EDA at 271.20nm

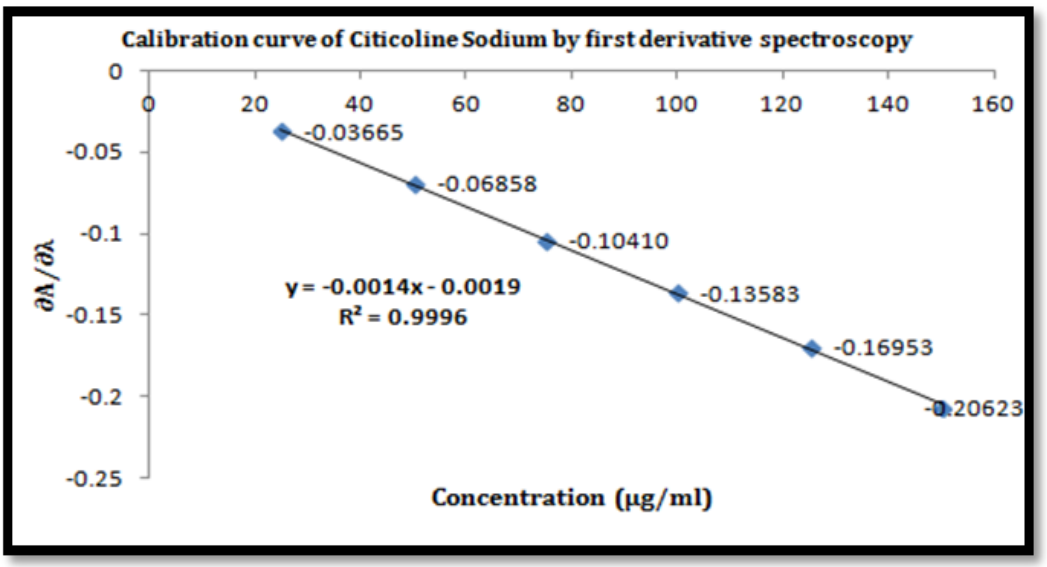

Figure 6: Calibration curve for CIT at 245.60nm

CONCLUSION

A new, First Derivative (ZCP) method has been developed for estimation of Edaravone and Citicoline Sodium in Formulation. The method was validated by employment of ICH[18] guidelines. The validation data is indicative of good precision and accuracy, and prove the reliability of the method.

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REFERENCES

1. Doherty AM. Annual Reports in Medicinal Chemistry. Boston: Academic Press, 2002, pp 37.

2. Watanabe T. et al. Research and development of the free radical scavenger edaravone as a neuroprotectant. Yakugaku Zasshi. 2004; 124(3): 99–111.
3. Higashi Y, Jitsuiki D, Chayama K and Yoshizumi M. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a novel free radical scavenger, for treatment of cardiovascular diseases. Recent Patents on Cardio. Drug Dis. 2006; 1(1): 85–93.
4. Yoshida H. et al. Neuroprotective effects of edaravone: a novel free radical scavenger in cerebrovascular injury. CNS Drug Rev. 2006; 12(1): 9–20.
5. www.chemicalbook.com/ProductMSDSDetailCB1287462_EN.htm and www.chemicalbook.com/ ChemicalProductProperty_US_CB1343597.aspx
6. http://www.lookchem.com/Edaravone/ and http://www.lookchem.com/Citicoline-Sodium/
7. Adibhatla RM, Hatcher JF and Dempsey RJ. Citicoline: Neuroprotective Mechanisms in Cerebral Ischemia. J Neurochem. 2002; 80(1): 12–23.
8. Ping Liao, Zheng-Yu Yan, Zhi-Ji Xu and Xiao Sun. A novel fluorescent assay for edaravone with aqueous functional CdSe quantum dots. Spectrochimica Acta Part A: Mol Biomol Spectro. 2009; 72(5): 75.
9. Gandhimathi M, Kumar MS, Baghla R, Ravi TK. RP-HPTLC Method for the In Vitro Estimation of Edaravone in Human Plasma. Indian J Pharma Sci. 2010; 72(2): 276-282.
10. Li jin-lin et al. Determination of Phenylhydrazine Residues in Edaravone by HPLC. Institute of Medical Information, CAMS, 2009.
11. Patel JA et al. Development and validation of a UV spectrophotometric method for estimation of Citicoline sodium in Bulk and Dosage Form. J Pharm Res. 2010; 3(12): 2876.
12. Babu RG et al. A Validated Specific Stability-indicating RP- LC Method for Citicoline and its Related Substances in Oral Drops. Int J Pharm Tech. 2010; 2(3): 652-667.
13. Panda SS, Mohanta G, Ravi Kumar BV. Estimation of Citicoline Sodium in Tablets by Difference Spectrophotometric Method. Chron Young Sci. 2013, 4, 18-20.
14. Bindaiya S, Argal A. Stability Indicating Assay of Citicoline Monosodium (API) and their Degradation Products by HPLC. Pelagia Res Lib. 2013; 4(3): 109-115.
15. Patel JA, Panigrahi B, Patel CN, Ramalingan B. Stress degradation studies on citicoline sodium and development of a validated stability-indicating HPLC assay. Chron Young Sci. 2011; 2: 150-154.
16. Sharma O and Chand T. Analytical Method Development and its Validation for Estimation of Citicoline Sodium by Reversed Phase High Performance Liquid Chromatography (RP-HPLC). Int J Res Pharma Biomed Sci. 2013; 4(2): 550-558.
17. Shah DM. Novel Injectable Combination. WIPO, WO2011111070A2, 2011.
18. International Conference on Harmonization, Harmonized Tripartite Guideline, Validation of Analytical Procedures Text and Methodology, ICH Q2(R_1), 2005.