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Stability indicating assay of citicoline monosodium (API) and their degradation products by HPLC

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ABSTRACT

A Simple, selective, rapid, precise and economical RP-HPLC stability - indicating method has been developed and validated for the Quantitative Estimation of Citicoline monosodium (API) and their degradation products. Chromatographic separation was accomplished using C18 column with mobile phase consisting of: Tbahs Buffer: Methanol (95:05): Methanol (95:05, v/v), flow rate was 1.0 ml/min. and the detection wavelength was 270nm.The method was validated for linearity, accuracy, precision, specificity, Robustness. The API was subjected to stress condition of Acid Decomposition (1.0 N HCl refluxed for 12 hrs at 80^{0}C) Alkali Decomposition (1.0 N NaOH refluxed for 12 hrs at 80^{0}C), Neutral hydrolysis (Dist. Water refluxed for 5 days at 80^{0}C) Oxidative decomposition (30% H2O2 for 24 hrs at RT), Thermal decomposition (Drug at 100^{0}C for 24hrs) Photolytic Decomposition (70,000-80,000 lux at 7 days) Percentage assay of degraded products were Acid (20.42, 11.26), Alkali (20.93,8.22), Neutral hydrolysis (22.42), Oxidative decomposition (23.65) respectively and there is no degradation in Thermal and Photolytic Decomposition was found in Degradation studies. Forced degradation study shows that Citicoline monosodium is a labile in acid, alkali, Neutral and oxidative conditions. It is stable to light and dry heat. No interference of degradation product was found at the RT of principle peak. The assay recommended for analysis of the API and degradation products in stability samples. It may be applied to a routine analysis in industries.

Keywords: Citicoline monosodium; RP-HPLC; Stability indicating; Degradation; Method validation.

INTRODUCTION

According to the international conference on harmonization (ICH) guidelines entitled stability testing of new drug substances and products (Q1A) requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. [1] The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension. Photo stability testing should be an integral part of stress testing. The standard conditions for photo stability testing are described in ICH Q1B.[2] Chafetz extensively reviewed the importance of analytical methodology in drug decomposition. A critical examination of assay methods for representative drugs with respect to their usefulness in establishing and monitoring drug stability was provided, and a critical review of some methods for determination of stability was presented.[3] Citicoline monosodium Figure (1) is Cytidine 5' (trihydrogen diphosphate) P' [2-trimethylammonio) ethyl] ester inner salt monosodium salt belongs to medicine known as cerebral vasodilator.[4] Citicoline monosodium (CTM) is primarily used in pharmacotherapy of brain Insufficiency and other related neurological disorders viz., as stroke, brain trauma and Parkinsonism disease.[5] Citicoline monosodium is a white crystalline powder, freely soluble in water but insoluble in ethanol, acetone and chloroform.[6] There was no method based on selected Mobile phase. The results obtained have been statistically validated in accordance with the ICH guidelines. [7] and therefore can be effectively used in quality control of CTM for Stability indicating assay as well as pharmaceutical dosage form. A no of methods for the determination of Citicoline (CT) were reported in the

literature. There was no method based on selected Mobile phase in the present study attempts were made to develop a rapid, economical, precise and accurate method for the Stability Indicating Assay of Citicoline Monosodium (API) and Their Degradation Products by HPLC.

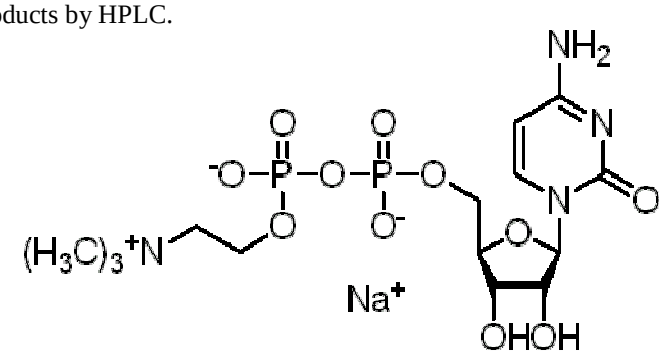

Figure (1): Chemical Structure of Citicoline Monosodium

MATERIALS AND METHODS

Chemicals and Reagents

All the reagents were of analytical-reagent or HPLC grade unless stated otherwise. Citicoline Monosodium was kindly supplied by Torrent Pharmaceutical Limited, (India), Methanol (Merck ltd., Rankem, RFCL Ltd) Tetra Butyl Ammonium hydrogen sulphate (CDH), Acetic acid and Triethylamine (Sd.Fine Chem. ltd). Sodium hydroxide, Hydrochloric acid, Hydrogen peroxide, Triple distilled water (In-House) was used throughout the experiment. . Citicoline Monosodium Tablet (500mg) formulation was purchased from the local market.

Instrumentation

HPLC analysis were performed on YoungLin system equipped with quaternary SP930D gradient pump, a vacuum degasser & mixer, an UV730D UV/VIS detector and a rheodyne injector holding 20 µl loop. The signals were acquired and analyzed using Windows XP based YoungLin Autochro-3000 software.

Selection of Separation Variable

Considering the theoretical information and after several trials separation variables were selected which were constant during whole experiment Table (1)

Table (1): Selection of separation variable

Variable	Condition
Column	Nucleosil
Dimension.	250mm x 4.60mm
Particle Size	5µ
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
Buffer : Methanol	95 : 05
Flow rate	1.00 mL/min
Temperature	Ambient
Sample Size	20µl
Diluents	Mobile phase
Detection wavelength	270nm

Mobile Phase Selection and optimization

Initially several exploratory runs given to estimate Citicoline Monosodium, a number of mobile phase in different ratio were tried and the mobile phase found to be most suitable for analysis was: Tbahs Buffer: Methanol (95:05). Tbahs Buffer prepared by dissolve 1.697gm of Tetra Butyl Ammonium Hydrogen Sulfate Dissolve in 1000ml of water, add 2.5 ml Triethylamine and adjust pH 6.0 with diluted acetic acid, taking into consideration the system suitability parameter like Resolution, tailing factor, No. of theoretical plates. The mobile phase was filtered through a 0.45 filter to remove particulate matters and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Preparation of Stock solutions

Accurately weighed 50mg Citicoline Monosodium was transferred into 50 ml volumetric flask and dissolved in Mobile phase, then volume was made up to 50 ml with mobile phase to get a concentration of 1000µg/ml (Stock-A). 10 ml of stock-A was taken in 25 ml volumetric and diluted up to 25ml to get concentration of 400µg/ml (Stock-B).

Finally from stock-B solution different of, 20, 40, 60, 80 and 100µg/ml were prepared for analysis. Linearity was observed by the linear regression equation Figure (2) and correlation coefficient was found to be 0.9999

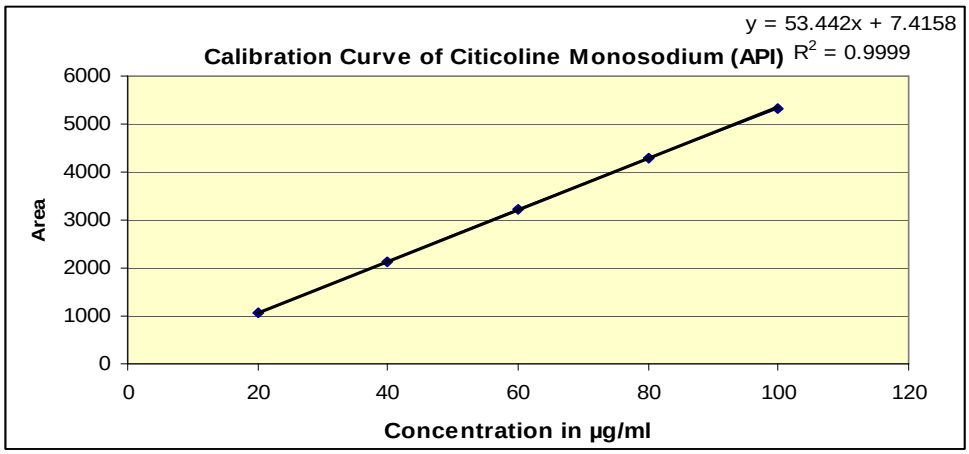

Figure (2): Calibration Curve of Citicoline Monosodium

Chromatographic Conditions

Before the mobile phase was delivered into the system, mobile phase were filtered through 0.45 µm filter and degassed using vacuum. For analysis of samples, the homogeneity was expressed in terms of peak purity and was obtained directly from the special analysis report obtained using the above mentioned software. The chromatographic conditions used for the analysis were given below.The separation of the compound was made on a nucleosil-C 18 column (250 mn x 4.6 mm, 5 µm particle size) using isocratic elution. Wavelength: 270 nm, Injection volume: 20 µl, Flow rate: 1.0 ml/min, Column temperature: 25ºC, Run time: 10 min [Fig.3].

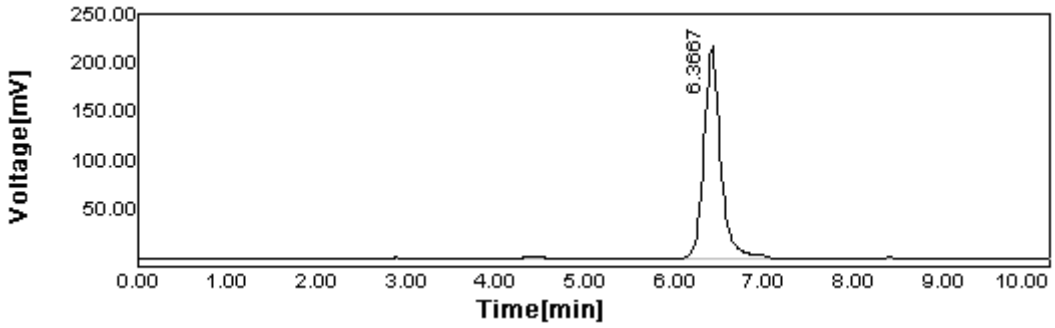

Figure (3): Chromatogram of Citicoline Monosodium (API)

Procedure for Forced Degradation Study

Stress studies were carried out under the conditions mentioned in ICH Q1A (R2) viz dry heat, hydrolysis, oxidation and photolysis. Regulatory guidance in ICH Q2A, Q2B, Q3B and FDA 21 CFR section 211 all requires the development and validation of stability indicating assays. Drug at a concentration of 50µg /ml was used in all degradation studies. Conditions employed for performing stress studies were as follows:

Acid decomposition

Acid decomposition was performed by taking the Different Concentrations (0.01N, 0.1N, 1N, 2N, 5N) of HCl with drug (Citicoline Monosodium) at varied temperature (25^{0}C, 40^{0}C, 70^{0}C, 80^{0}C and 100 ^{0}C) and time period (2, 8, 12, 24 hr). The resulting solution was neutralized by base to avoid any interference of acid and suitably diluted with diluent's to obtain solution of concentration of 50µg/ml. All samples were injected into HPLC and the chromatograms were recorded [Fig.4]. At the end of these studies 1.0 N HCl was used and refluxed for 12 hrs at 80^{0}C in dark in order to exclude the degradative effect of light.

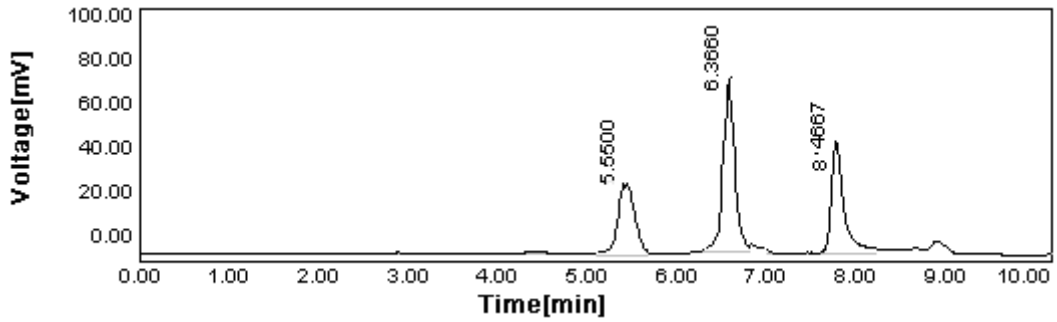

Figure (4): Chromatogram of Citicoline Monosodium and Acid Degradation products

Alkali decomposition

Alkali decomposition was performed by taking the Different concentrations (0.01N, 0.1N, 1N, 2N, 5N) of NaOH with drug (Citicoline Monosodium) at varied temperature (25^{0}C, 40^{0}C, 70^{0}C, 80^{0}C and 100 ^{0}C) and time period (2 hr, 8 hr, 12 hr, 24 hr). The resulting solution was neutralized by acid to avoid any interference of base and suitably diluted with diluent's to obtain solution of concentration of 50µg/ml. All samples were injected into HPLC and the chromatograms were recorded [Fig.5]. At the end of these studies 1N NaOH was used and refluxed for 12 hrs at 80^{0}C in dark in order to exclude the degradative effect of light**.**

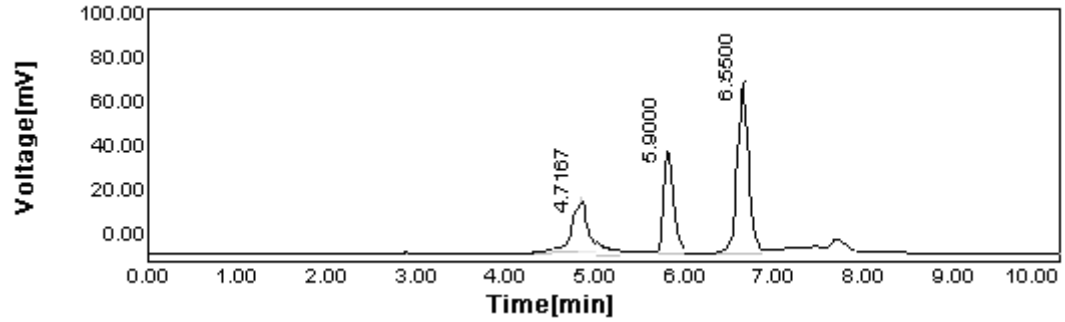

Figure (5): Chromatogram of Citicoline Monosodium and Alkali Degradation products in 1 N NaOH

Neutral decomposition:

Drug (Citicoline Monosodium) was subjected to neutral hydrolysis by subjecting to H_2O at varied temperature (25^{0}C, 40^{0}C, 70^{0}C, 80^{0}C and 100 ^{0}C) and time period (2 hr, 8 hr, 12, hr, 1day, 2 days, and 5 days) the resulting solution with diluent's to obtain solution of concentration of 50µg/ml. All samples were injected into HPLC and the chromatograms were recorded [Fig.6].

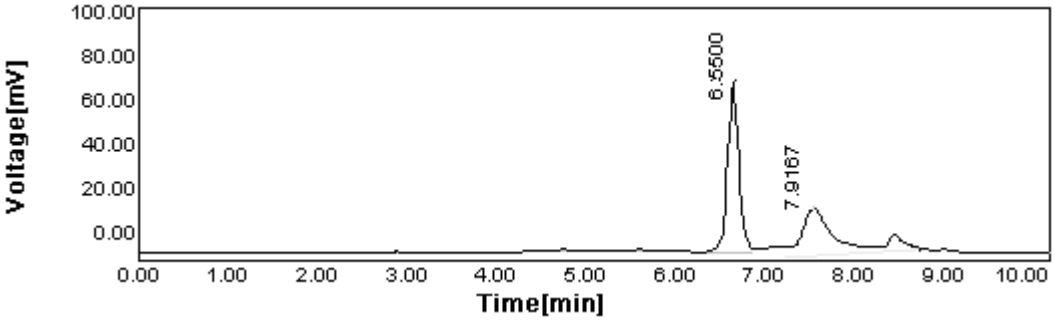

Figure (6): Chromatogram of Citicoline Monosodium and Neutral Degradation products in H2O

Oxidative decomposition:

Oxidative decomposition was performed by taking the drug (Citicoline Monosodium) in 1%, 3%, 10%, and 30 % of H_2O_2 v/v at different temperature and time period (30 min 3 hr, 6 hr, and 24 hr). All samples were injected into HPLC and the chromatograms were recorded [Fig.7]. At the end of these studies 30 % of H_2O_2 was used for 24 hrs at RT.

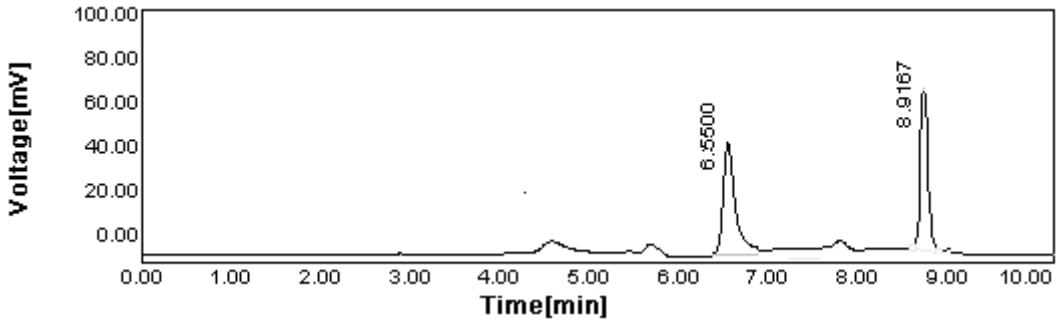

Figure (7): Chromatogram of Citicoline Monosodium and H2O2 Degradation products in 30%H2O2

Thermal decomposition:

Thermal studies were also conducted on solid drug (Citicoline Monosodium), which were heated at varied temperature (40^0C, $60\ ^0$C, $80\ ^0$C, and $100\ ^0$C) and time period (2hrs, 4hrs, 8hrs, 12hrs & 24 hrs) in hot air oven All samples were injected into HPLC and the chromatograms were recorded [Fig.8].

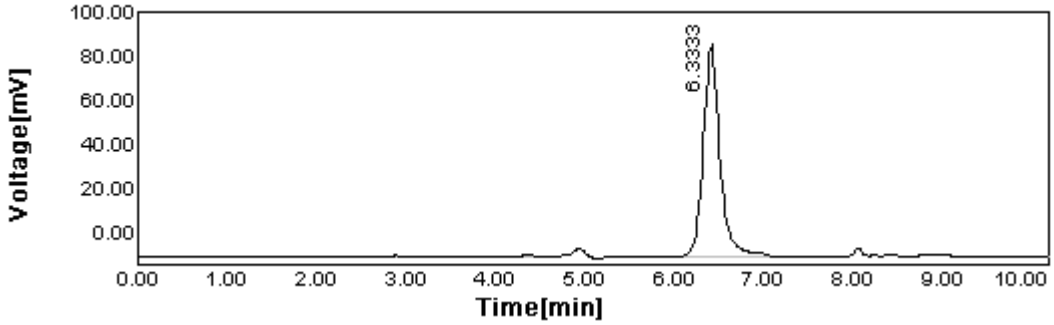

Figure (8): Chromatogram of Citicoline Monosodium Thermal Degradation products in 100^0C

Photolytic decomposition:

Photo degradation studies were performed by exposing the drug (Citicoline Monosodium) to sunlight for 1day, 2 days, 3days and 7days. Samples were withdrawn at appropriate time period and all samples were injected into HPLC and the chromatograms were recorded. [Fig.9].

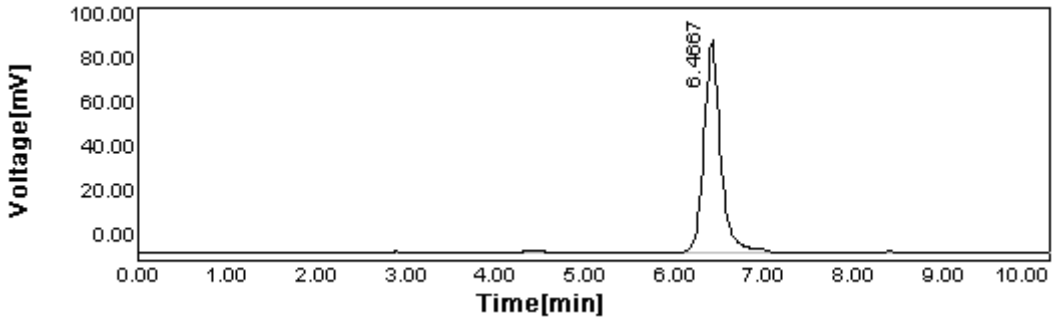

Figure (9): Chromatogram of Citicoline Monosodium and Photolytic Degradation products in sun light

Preliminary Separation Studies

The initial analysis of different stressed samples was performed on HPLC system using a C-18 column and mobile phase composed of Tbahs Buffer (pH 6): MeOH (95:05).It was filtered through 0.45 µm nylon filter and sonicated before use. The injection volume was 20µl and the flow rate was set at 1ml/min. The detection was carried out at 270nm.

Optimization Studies

The separation of drug from their degradation products were optimized by varying the ratio &/or nature of organic modifier. Finally method was developed using mobile phase composed of Tbahs Buffer (pH 6): MeOH (95:05) in that drug and degradation products showing good elution.

RESULTS

Results of Forced Degradation Study

Degradation of Citicoline Monosodium (CTM) was observed in Acid hydrolysis, Alkali hydrolysis, Neutral hydrolysis, Oxidative conditions there was no degradation seen in Thermal and Photolytic conditions. The degradation behaviour of Citicoline Monosodium (CTM) in various stress conditions was shown in Figure.(4-9) and the results were shown in Table (2).

Table (2): Degradation characteristics of Citicoline Monosodium.

Stressed Parameter				Results of stress degradation			
S. No.	**Stress Condition**	**Stress Temp.**	**Stress Time**	**AUC of API**	**% Assay of API**	**AUC of Degraded Products**	**% Assay of Degraded Products**
1	Acid Decomposition (1.0N HCL)	80^0C	12hrs	1804.58	67.53	(i) 545.62 (ii) 300.86	20.42 11.26
2	Alkali Decomposition (1.0N NaOH)	80^0C	12hrs	1871.54	70.03	(i) 559.24 (ii) 219.63	20.93 8.22
3	Neutral Hydrolysis (Dist. Water)	80^0C	5 days	2051.96	76.79	599.06	22.42
4	Oxidative Decomposition (30% H2O2)	RT	24hrs	2000.52	74.87	631.92	23.65
5	Thermal decomposition	100^0C	24hrs	2651.96	99.25	------	------
6	Photolytic Decomposition	70,000 to 80,000 lux	7 days	2641.07	98.84	------	------

Each reading is the mean of three replicates.

Method Validation

The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, robustness and system suitability in accordance with ICH guidelines Q2A (R1).

Linearity

The curve proved to be linear over a concentration range of 20-100 µg mL-1 (Fig.2). Standard solution were prepared at five concentrations (20, 40, 60, 80,100 µg mL-1) were injected in triplicate. Linear regression of concentration Vs peak area resulted in an average coefficient of determination (R2) 0.9999. The Regression equation is Y= 53.442X+7.4158, Figure. (2).

Precision

The precision of the method was evaluated by carrying out six independent assays of test samples of Citicoline Monosodium. The precision of the method was also evaluated in same day for repeatability of precision and in different days. The results shown in Table.(3), indicates that the method is reproducible.

Table (3): Results of Precision

Precision	% Found	SD	% CV
Repeatability	99.62	0.185	0.187
Intermediate Precision	99.89	0.130	0.13

Accuracy

Accuracy was calculated as the percentage recovery of the known added amount of Citicoline Monosodium reference substance in the sample solutions using three concentration levels (50%, 100%, and 150%). covering the specified range (20, 40, and 60 µg mL-1). The accuracy of the method ranged from 97.85-99.75% indicating that this assay is reliable Table (4).

Table (4): Results of Recovery Study

Percentage Level	% Recovery	SD	%CV
50	97.85	0.2030	1.045
100	99.45	0.0750	0.113
150	99.75	0.1708	0.286

Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered. The ratio of mobile phase was change 95:05 by ± 2 to 97:03 and 93:07 (TBAHS Buffer: Mooch **v**/v) and Changed flow rate by ± 0.1 ml/minute [use flow rate 0.9 ml and 1. 1 ml]. While the other parameters were held constant in chromatographic condition. The % CV was not more than 2% in both conditions, Table (5).

Table (5): Results of Robustness study

Mobile Phase Composition Change				Robustness study when Flow rate Change		
Parameters	**95: 05**	**97: 03**	**93: 07**	**1.0 ml/min**	**0 .9 ml/min**	**1.1 ml/min**
S.D.	0.8215	0.8729	0.8617	0.9530	0.9880	0.9465
%CV	0.038	0.041	0.040	0.045	0.046	0.044

Results of Laboratory samples & Tablet Formulation

In order to confirm the validity of the method, laboratory and Tablet samples containing Citicoline Monosodium were prepared in the range of 20µg/ml – 100µg/ml. The amount of drug present in the standard and Test solution was calculated by using the selected linearity equation and the results are tabulated in the Table (6).

Table (6): Results of Laboratory samples & Tablet Formulation

Parameters	Laboratory samples	Tablet Formulation
% Mean Found	99.74	99.54
S.D.	0.196	0.410
%CV	0.197	0.413

DISCUSSION

Stability-indicating assay method for Quantitative estimation of Citicoline Monosodium (API) and their degradation products and its validation in Tablet Dosage form. Showed good specificity, sensitivity, linearity, precision and accuracy over the entire range of significant, thereby enabling its use in LC-MS/MS

CONCLUSION

Forced degradation study on Citicoline Monosodium was carried out under the conditions of Acid, alkali, Neutral hydrolysis, oxidation, Thermal and Photolytic conditions and the study shows that Citicoline monosodium is a labile in acid, Neutral, alkali, and oxidative conditions. It is stable to light and dry heat. Based on the information generated by forced degradation, a stability-indicating assay method was developed and validated, which separates all the degradation products formed under variety of conditions. The method was found sufficiently linear, precise, accurate, sensitive, robust and specific to the drug. No interference of degradation product at the retention time of principle peak was found in degradation study. Hence, it is recommended for analysis of the drug and degradation products in stability samples by the industry.

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