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UV-Spectrophotometric Methods for Determination of Citicoline sodium and Piracetam in Pharmaceutical Formulation

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ABSTRACT

A simple, specific and reproducible methods have been developed and validated for the simultaneous estimation of Citicoline sodium and Piracetam in pharmaceutical formulation by Second order derivative method for CT and Absorbance Correction method for PM. For Second order derivative method, wavelength selected was 274.60 nm for estimation of CT. For Absorbance Correction method, wavelength selected was 206.8 nm for estimation of CT and PM. The two drugs follow Beer-Lambert's law over the concentration range of 5-50 µg/ml for CT and 4-28 µg/ml for PM. The percent recovery of the drugs was found to be nearly 100 % representing the accuracy of method. Validation of the proposed methods were carried out for its accuracy, precision, linearity range and ruggedness according to ICH guidelines. The proposed methods can be successfully applied in routine work for the determination of CT and PM in combined dosage form.

Keywords: Citicoline sodium (CT), Piracetam (PM), Absorbance Correction method, Second order derivative method

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INTRODUCTION

Citicoline sodium is chemically known as Cytidine 5'-{trihydrogendiphosphate} p'-[2-{trimethylammonio} ethyl] ester inner salt [1]. Piracetam is chemically known as 2-oxo-1-pyrrolidine acetamide [2]. CT is used in treatment of pharmacotherapy of brain insufficiency and other related neurological disorders viz., as stroke, brain trauma and Parkinsonism's disease [3, 4]. PM is used to enhance cognition and memory, slow down brain aging, increases blood flow and oxygen to the brain, aid stroke recovery, and improves Alzheimer's, Down Syndrome, dementia, and dyslexia[2]. A liquid chromatography method for the determination of CT in injection, oral drops and tablet dosage form were reported in the literature [5 to 8]. Spectrophotometric methods like colorimetric by complexation and UV-visible spectrophotometric methods using standard absorptivity value for determination of CT in pharmaceutical dosage form were also reported [9 to 13]. Literature survey revealed HPLC methods were developed for the estimation of PM in biological fluids, Capillary electrophoresis, thin layer densitometric determination, micellar electrokinetic chromatography methods were also developed for the estimation of Piracetam in biological fluids [14]. There is only one spectrophotometric method reported for the analysis of PM in bulk or pharmaceutical formulation [15].

There is no published spectrophotometric method for this combination. The present paper describes a simple, accurate and precise method for simultaneous estimation of CT and PM in combined tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [16]. Since no Spectrophotometric method is reported for simultaneous estimation of CT in presence of PM in combination therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously by simple UV Spectrophotometric methods(Second order derivative method, Absorbance Correction method).

MATERIALS AND METHODS

Instrument

Shimadzu double beam UV-visible spectrophotometer (model 1700) with 1 cm matched quartz cuvettes were used for all absorbance measurements. Sartorius balance was used for weighing the samples**.** All the chemicals used were of AR grade. Double distilled water and Whatmann filter paper (no.41) were used throughout the experimental work.

Materials

Multicomponent tablet Nutam plus (500mg CT and 800mg PM) manufactured by Molecule India Pvt Ltd. All chemicals and reagents used were of AR grade.

Determination of λmax

The standard solutions of 10 µg/ml of Citicoline sodium and Piracetam were individually scanned in the range of 200- 400nm and the λmax was determined. The overlain spectrum of both the drugs is also run. A second order derivative overlay spectrum of CT is shown in figure 1. And Overlay spectra of CT and PM are shown in figure 2.

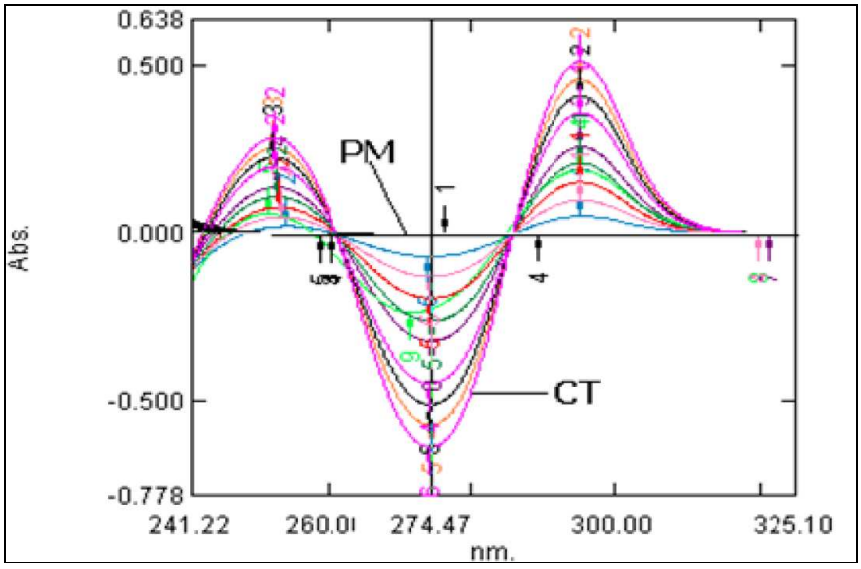

Figure: 1 Second Order Derivative Overlay Spectra of CT

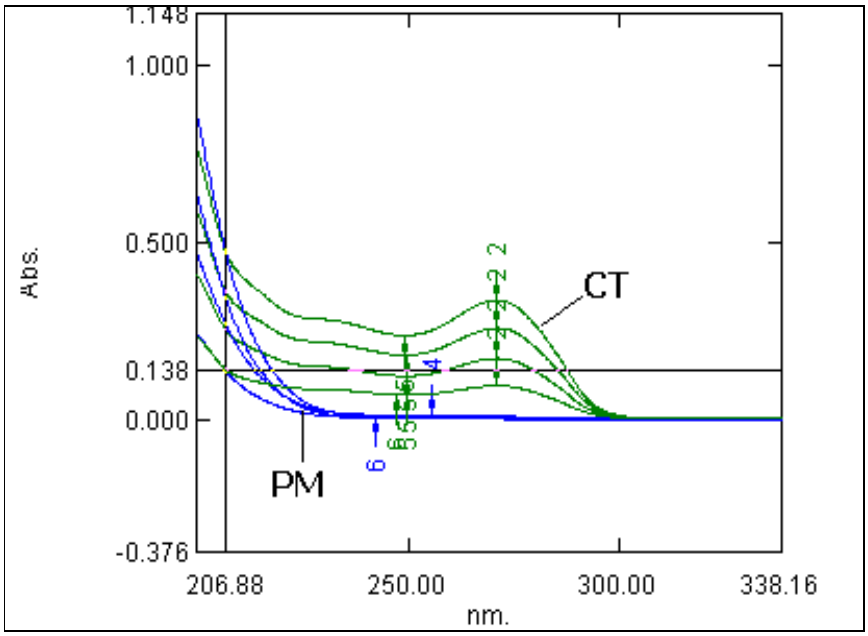

Figure: 2 Overlay Normal Spectra of CT and PM in distilled water

Preparation of standard stock solution

Accurately weighed 100 mg CT and 40 mg PM were transferred separately into 100 ml volumetric flask, dissolved in about 50 ml of double distilled water and volume was adjusted to mark to obtain concentration 1000 µg/ml of CT and 400 µg/ml of PM. From above solution 10 ml solution was transfered into 100 ml volumetric flask, diluted up to the mark with same solvent to obtain final strength of 100 µg/ml of CT and 40 µg/ml of PM.

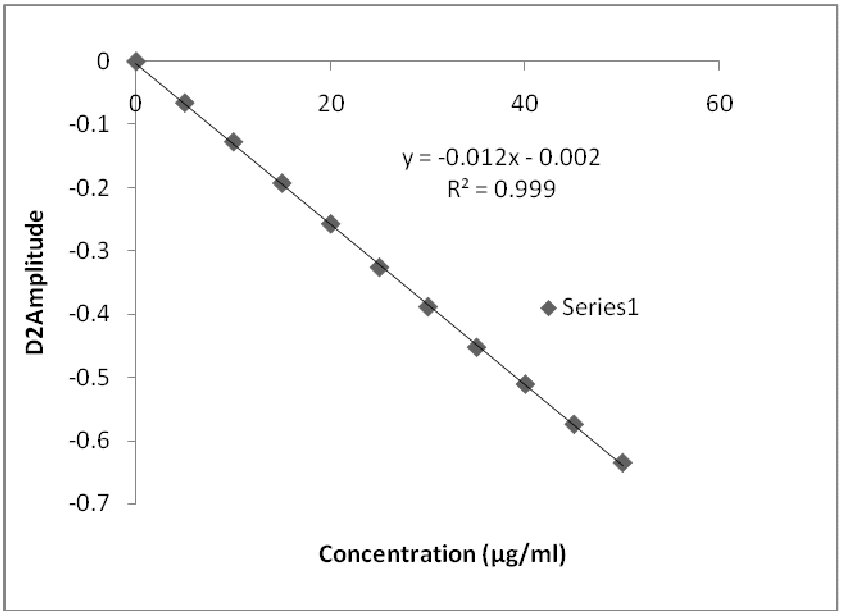

Figure: 3 Calibration Curve of CT (5-50µg/ml) at 274.60 nm

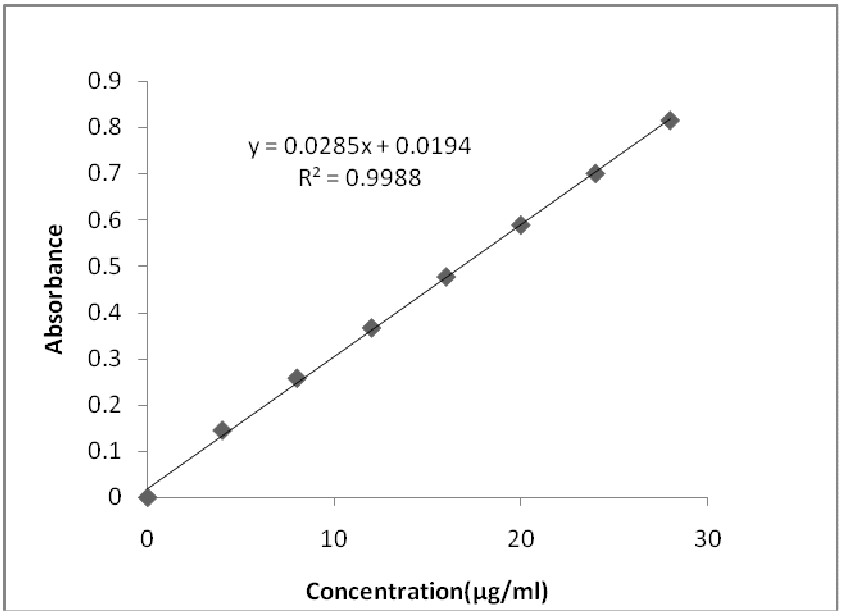

Figure: 4 Calibration Curve of PM (4-28µg/ml) at 206.8 nm

Study of calibration curve of CT and PM

Different aliquots were pipetted out from standard stock solution into a series of 10 ml volumetric flasks and the volume was made up to the mark with double distilled water to get concentrations of 5,10,15,20,25,30,35,40,45 and 50 µg/ml of CT. The solutions were scanned on Spectrophotometer (Shimadazu -1700) in the UV region of 200 - 400 nm. The obtained spectra were transformed into second order derivative, using UV probe software of instrument, at $\Delta\lambda$ = 16 and scaling factor 200. The amplitudes of the corresponding troughs were measured at 274.60 nm**.** The same solutions were measured at 206.8 nm and absorbances were noted and calibration curve was plotted. Calibration curves are shown in figure 3. In similar manner PM (40 µg/ml) solution was diluted to get concentrations of 4,8,12,16,20,24 and 28 µg/ml of PM. The absorbances of solutions were measured at 206.8 nm

and calibration curve was plotted as absorbance v/s concentration of PM. Calibration curve is shown in figure 4. Results of CT and PM are shown in Table 1.

Table: 1 Results of CT and PM estimation

Parameter	CT at 274.60nm	CT at 206.8 nm	PM at 206.8nm
Linear Range (µg/ml)	5-50	5-40	4-28
Slope	0.0128	0.0238	0.0285
Intercept	0.0021	0.0101	0.0194
Correlation coefficient (r^2)	0.9998	0.9991	0.9988
Molar absorptivity (l/mol.cm)	-	1.2145×10^4	1.4543×10^4
Sandell's sensitivity (µg/cm2/0.001AU)	-	0.042	0.035
Limit of Detection (µg/ml)	0.74	0.40	0.42
Limit of Quantitation (µg/ml)	2.26	1.23	1.27
Intraday Precision (%RSD)	1.29,0.12,0.52	-	2.27,1.28,1.33
Interday Precision (%RSD)	1.97,1.94,2.69	-	2.16,3.24,1.55

Application of proposed method for tablet formulation

For analysis of commercial formulation; twenty tablets (Nutam plus, 500mg CT and 800mg PM) were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 100 mg of CT was transferred into 100 ml volumetric flask, dissolved in about 50 ml of double distilled water and volume was adjusted to mark with same solvent then filtered through Whatmann filter paper no. 41. 1ml of resulted solution was further diluted with double distilled water to get final concentration 10µg/ml of CT and 16 µg/ml of PM. This solution was scanned on Spectrophotometer (Shimadazu -1700) in the range 200 - 400 nm and spectrum was derivatised into second order derivative and amplitude of the troughs was recorded at 274.60 nm and absorbance was also measured at 206.8 nm. The concentration of CT was calculated from linear regression equation at 274.60 nm while the concentration of PM was calculated from linear regression equation at 206.8 nm after correcting the absorbance. The results are shown in Table 2.

Table: 2 Assay Results of Tablet Formulation (Nutam Plus Tablet)

Label claim: 500mg CT			Label claim: 800mg PM	
Amount present in (mg/tab)		%Amount obtained in (mg/tab)	Amount present in (mg/tab)	%Amount obtained in (mg/tab)
529.8		105.96	740	92.5
523.3		104.66	774	96.7
533.7		106.74	789.5	98.6
531.0		106.20	781	97.6
531.1		106.22	752	94.0
528.5		105.70	781	97.6
Mean ± SD	529.56 ± 3.52	105.91 ± 0.70	769.58 ± 19.29	96.17 ± 2.39
%RSD	0.66	0.66	2.50	2.48

Validation of Spectrophotometric method

Accuracy

To study the accuracy, 20 tablets were weighed and powdered and analysis of the same was carried out in three sets. Recovery studies were carried out by addition of the known amount of PM and CT separately to the sample at three different concentration levels i.e. 50%, 100%, and 150% of respective label claim (standard addition method). The results of % recovery studies are given in Table 3-4.

Table: 3 Results of % Recovery of CT by Standard Addition Method

%Level recovery	Pre-analyzed Sample solution (µg/ml)	Amount of standard drug added (µg/ml)	Amount of standard drug recovered (µg/ml)	%Recovery ±SD(*n=3)	%RSD
50%	30	15	14.71	98.46±0.02	0.02
100%		30	29.58	98.62±0.66	0.66
150%		45	44.50	98.91±0.09	0.09

Precision

The precision is usually expressed as the standard deviation or relative standard deviation (coefficient of variation). Intraday precision was determined by analyzing 4, 16 and 28 µg/ml concentration of PM and 10, 30 and 50 µg/ml concentration of CT for three times in the same day at their selected analytical wavelengths. Inter-day precision was determined by analyzing 4, 16 and 28 µg/ml concentration of PM and 10, 30 and 50 µg/ml

concentration of CT daily once for three consecutive days at their selected analytical wavelengths. The Results of Intraday& Interday Precision are given in Table 1.

Table: 4 Results of % Recovery of PM by Standard Addition Method

%Level recovery	Pre-analyzed Sample solution (µg/ml)	Amount of standard drug added (µg/ml)	Amount of standard drug recovered (µg/ml)	%Recovery ±SD(*n=3)	%RSD
50%	16	8	8.02	100.7±1.09	1.08
100%		16	15.69	98.06±1.42	1.45
150%		24	23.62	98.36±1.51	1.54

Repeatability

Standard solution of PM (16 µg/ml) and CT (30 µg/ml) were prepared six times, absorbance and amplitude of troughs of these solutions were measured at 206.8 nm and 274.60 nm respectively using double distilled water as a blank , relative standard deviation was calculated.

Reproducibility

Ruggedness of the proposed method was determined by analyzing aliquots from homogenous slot by two analyst using same operational and environmental conditions.

Linearity and Range

The linearity of analytical method was determined by studying standard calibration curves which were prepared considering the instrumental relative error. The range of analytical method was decided from the interval between the upper and lower levels of analyte of calibration curves.

RESULTS AND DISCUSSION

Linearity was observed in the concentration range of 5-50 µg/ml for Citicoline sodium and 4-28 µg/ml for Piracetam. Marketed brand of tablet (Nutam plus) was analyzed and amount of drug determined by proposed method ranges from 104 to 107% for CT and 90 to 100% as shown in Table 2. The proposed method was validated as per ICH guideline. The accuracy of method was determined at 50, 100 and 150 % level. The % recovery ranges from 98.44 to 100 for CT and 96.85 to 101.7 for PM. Precision was calculated as interday and intraday variations (% RSD found to be 1.97) for both drugs. From the interday and intraday studies it is supposed that the drug in solution state is stable for a period of 24hr. The proposed method was found to be simple, accurate and rapid for the routine determination of Citicoline sodium and Piracetam in tablet formulation. This method can be successfully used for simultaneous estimation of Citicoline sodium and Piracetam in combined dosage.

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