SPECTROPHOTOMETRIC DETERMINATION OF CABERGOLINE USING CDNB REAGENT

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ABSTRACT

A new precise, accurate, simple and sensitive spectrophotometric method has been developed for the determination of cabergoline (CAB) in pure and in pharmaceutical formulations. The present developed method is based on the formation of charge-transfer complex between the drug, CAB and 1-chloro-2,4-dinitrobenzene (CDNB) reagent, formed pale yellow color complex. The complex was showed maximum absorbance at 425 nm against blank. The limit of detection and quantitation were 0.1205 mg/ml and 0.4012 mg/ml respectively. The influence of commonly used exipients on the determination of CAB was studied. The linearity was observed between 5-30 µg/ml. The results of analysis were validated by recovery studies, accuracy, precision, LOD, LOQ, robustness and ruggedness, which indicated that the present method can be successfully applied for the determination of CAB in pure and in pharmaceutical formulations.

Keywords: Spectrophotometric method, cabergoline, charge-transfer complex, CDNB.

1. INTRODUCTION

Cabergoline (CAB), *N*-[3-(dimethylamino)propyl]-*N*-(ethylamino)carbonyl-6-(2-propenyl)ergoline-8*β*-carboxamide, (Fig 1) is a dopamine agonist licensed for the treatment of Parkinson's disease as adjunctive treatment with levodopa plus a dopa-decarboxylase inhibitor in patients effected by on-off mobility problems. CAB is a selective, ergoline, dopamine D_2 agonist. It is effective in improving motor function in Parkinson's disease[1]. CAB is effective as mono-therapy in *de novo* patients[2]. In literature, a few methods were reported for the determination of CAB in plasma using electrochemical detection[3], tandem mass spectrometry (TMS)[4-6] and triple-quadrupole mass spectrometry[7,8]. Several extractive spectrophotometric methods were reported for the quality control and assurance of CAB[9,10].

To the best of our knowledge, there is no spectrophotometric method was reported so far with reagent CDNB. Hence, the present method was developed for determination of CAB in bulk and pharmaceuticals formulations using CDNB reagent.

2. EXPERIMENTAL

2.1. Instrumentation

A Shimadzu UV-Visible spectrophotometer (UV-160A) with a matched pair of 10 mm quartz cell was utilized for all measurements. Mettler Toledo analytical balance (accuracy 0.1 mg) was used for weighing all the samples.

2.2. Materials and Reagents

CAB was procured from Sigma-Aldrich. Formulations were purchased from local market. All the used chemicals were analytical grade. Double distilled water is used throughout the experiment. A stock solution of CAB was prepared by dissolving accurately weighed 100 mg of pure drug in 100 ml standar flask by dissolving in methanol and sonicated to get required concentration of 1

mg/ml. From this, further dilutions were made with double distilled water to get required concentrations and used for current investigation.

2.3. Method Development

Standard drug solution was transferred into the cleane and dried volumetric flasks in the concentration range 8-64µg/ml. To each flask, 3 ml of 3% concentration of CDNB solution was added and the contents of all the standard flasks were heated at 98° ± 2°C. All these were cooled to room temperature and the formation of pale yellow colour was observed and the maximum absorbance was measured at 425 nm against the blank. The amount of drug was computed from calibration graph.

3. RESULTS AND DISCUSSION

3.1. Absorption spectrum

Different volumes of drug solution were taken into a clean and dry volumetric flask in range 8-60µg/ml. For this solution, 3% of CDNB was added and entire contents were heated upto 98°±2°C and cooled at laboratory temperature. During this, change in the colour of solution was observed as pale yellow. Maximum absorbance was measured at 425 nm against blank reagent (Fig.2).

3.2. Effect of reagent CDNB concentration

Fresh aliquots of 3% CDNB solution was transferred in the range of 1.0-3.4 ml into the fixed concentration of drug solution containing a series of volumetric flasks. Pale yellow colour was noticed after sometime on the addition of 3.0 ml of reagent and the same volume was considered for further investigation.

3.3. Effect of concentration of the drug

The effect of concentration on the absorbance of pale yellow coloured solution was studied and the linearity was observed in the range of 8-64 µg/ml by addition of fixed volume of reagent with a maximum absorbance at 425 nm against blank, calibration curve was constructed with obtained results and all results were obeyed the Beer's law.

3.4. Analytical method validation

The proposed analytical method was validated according to International conference on harmonization (ICH) guidelines[9,10] for the determination of the drug. The validation parameters such as linearity, accuracy, precision and specificity, Limit of detection (LOD), Limit of quantitation (LOQ), robustness and ruggedness were studied.

3.4.1. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well defined mathematical proportional to the concentration of analyte in sample within a given range (Fig. 3). For studying the linearity of the method, different concentrations of the drug solution were prepared and calibration plots were constructed. From the plots drawn between the concentration of the drug and absorbance within the concentration range, the regression equations were computed. The optical characters like Beer's law limit, Sandell's sensitivity, molar absorptivity, are given in the Table 1.

3.4.2. Robustness and ruggedness

To study the robustness of the proposed method, a few analytical parameters like concentration of the drug, concentration of the reagent and shaking time were interchanged. Even after that, it was noticed that the results were unaffected. The method of ruggedness is studied as the percentage of relative standard deviation for the present method developed by two different analysts from two different instruments in two different days and it was observed that there is no significant difference between the two analysts and instruments. Hence the developed analytical method is robust and rugged.

3.4.3. Accuracy

The accuracy of an analytical method is the close agreement between the accepted and obtained value. The obtained accuracy results proved that the recovery values in drug and in pharmaceutical formulations were within the acceptance criteria and details were presented in the Tables 2 and 3.

3.4.4. Precision

Precision of a method is a measure of the ability to create reproducible results. It is evaluated using six separate determinations for repeatability, precision and reproducibility. The intra and inter day precision were evaluated and found % RSD is less than 1.0 that proves that there is no considerable difference for the assay which is tested in inter-day and intra-day from pharmaceutical ingredients and results were presented in Tables 2 and 3.

3.4.5. Recovery

For recovery studies, the selected drug samples taken in various concentrations were analyzed by the proposed method and the recovery percentages were found to be more precise which shows the accuracy and selectivity of the proposed method. The average recovery results were summarised in the Table 4.

3.4.6. Specificity and selectivity

To assess the developed of the method, the effect of excipients in its dosage forms like starch, lactose, glucose, sugar, talc etc. were studied. The results indicated that there was no interference from the excipients in its dosage forms. The results are shown in Table 5.

4. APPLICATIONS

Blood and urine samples were collected from healthy donors, and centrifuged at 3000 rpm per min. for nearly 10 mins. The resulted solutions were filtered and preserved in the absence of light at a temperature of 4^{o}C. From these solutions, various concentrations of the drug CAB were analyzed with the help of proposed analytical method and these results were recorded in Table 4. Hence, the proposed method can be successfully applied to recover CAB in biological samples, viz. urine and blood due to its high accuracy and good recoveries.

5. CONCLUSIONS

A simple, accurate and reliable spectrophotometric method for determination of CAB in pure and in pharmaceutical formulations was developed. By using this method it is possible to determine CAB with good precision and accuracy. The linearity of the calibration standards of the drug by the reported method was good from the result of correlation coefficient. The overall recovery of the drug by the proposed methods was satisfactory. LOD, LOQ, molar absorbity and Sandell's sensitivity values were calculated which indicated that the proposed analytical method was accurate, simple and reproducible for the estimation of CAB in pure and in pharmaceutical formulations.

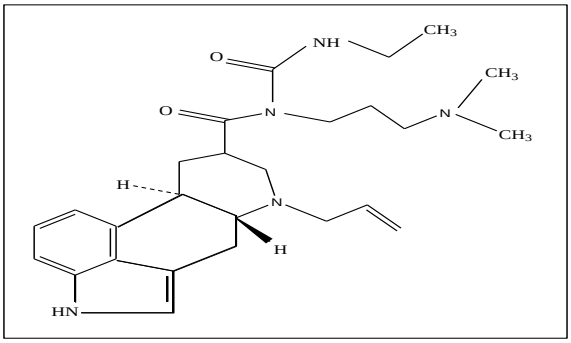

Figure 1: Structure of CAB

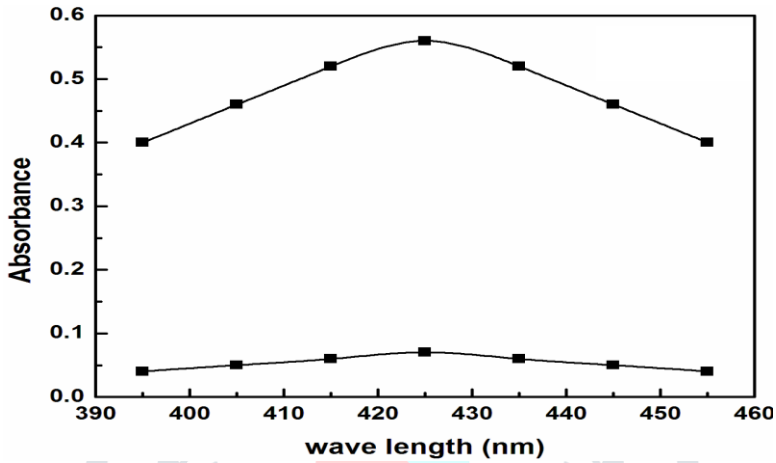

Figure 2: Absorption spectrum of CAB with CDNB

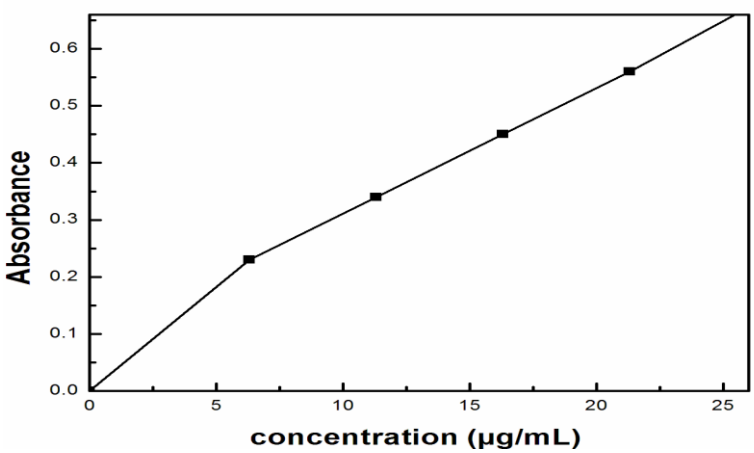

Figure 3: Calibration plot of CAB (Method C)

Table 1: Spectral characteristics of the drug with reagent

λmax (nm)	Beer's law limit (µg/ml)	Molar absorbance ($L.mol^{-1}$ cm^{-1})	Sandell's sensitivity	Correlation coefficient (r^2)	Slope (m)	Intercept (c)	%RSD	Colour	LOD	LOQ
425	5-30	$2.629X10^4$	0.0018	0.9943	0.0249	0.0384	0.1786	Pale yellow	0.1205	0.4012

Table 2: Evaluation of accuracy and precision results of the proposed method in bulk form

Taken mg/ml	Intra day				Inter day			
	*Found mg/ml	Reco-very %	± SD	% RSD	*Found mg/ml	Recov-ery%	± SD	% RSD
2	1.97	98.67	0.012	0.59	1.99	99.33	0.006	0.29
4	3.96	98.92	0.015	0.39	3.96	98.92	0.015	0.39
6	5.96	99.39	0.006	0.10	5.96	99.28	0.021	0.35

***Average of six determinations**

Table 3: Evaluation of accuracy and precision results of the proposed method in pharmaceutical dosage form

Pharmaceutical formulation	Taken mg/ml	Intra day				Inter day			
		*Found mg/ml	Reco-very %	± SD	% RSD	*Found mg/ml	Recov-ery%	± SD	% RSD
dostinex	4	3.96	99.00	0.010	0.25	3.95	98.67	0.021	0.53
cabaser	6	5.96	99.33	0.020	0.34	5.95	99.22	0.012	0.19
cabgolin	8	7.95	99.42	0.006	0.07	7.96	99.54	0.021	0.26

***Average of six determinations**

Table 4: Method accuracy from recovery assay

Sample	Added mg/ml	*Found mg/ml	Recovery%	±SD	%RSD
Blood samples	0.2	0.20	98.17	0.001	0.59
	0.4	0.40	99.17	0.001	0.29
	0.6	0.59	99.06	0.003	0.42
	0.8	0.79	99.00	0.002	0.25
Urine samples	0.4	0.40	99.08	0.002	0.39
	0.6	0.60	99.28	0.002	0.26
	0.8	0.80	99.54	0.002	0.19
	1	0.99	98.67	0.006	0.59

***Average of six determinations**

Table 5: Determination of CAB in presence of excipients

Excipients	Amount taken mg/ml	*Found mg/ml	Recovery %	±SD	RSD%
Glucose	5	4.97	99.33	0.015	0.31
Sucrose	10	9.95	99.53	0.012	0.12
Lactose	15	14.85	99.02	0.035	0.24
Dextrose	10	9.95	99.50	0.010	0.10
Talc	15	14.87	99.13	0.026	0.18
Starch	20	19.63	98.17	0.229	1.17

***Average of six determinations**

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