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Research Article

RP-HPLC METHOD FOR THE ESTIMATION OF CABERGOLINE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, specific reverse phase liquid chromatographic method was established for the determination of cabergoline in bulk drug and pharmaceutical formulations. Optimum chromatographic separation was achieved within 4.1 minutes by use of Hypersil ODS C18 column (250mm X 4.6 mm, 5 µm) as stationary phase with mobile phase consisted of a mixture of ammonium acetate buffer PH 6, acetonitrile and water (10:50:40) at a flow rate of 1 ml min-1. Detection was performed at 281 nm using UV-Visible detector. The method was validated in accordance with ICH guidelines. Response was a linear function of concentrations over the range of 5-25 µg mL-1 with correlation coefficient 0.999. The method resulted in good elution with acceptable tailing. The developed method can be applied successfully for determination of cabergoline in bulk drugs and pharmaceutical formulations.

Keywords: Reverse phase liquid chromatography, Cabergoline (CBE), Validation, ICH guidelines.

INTRODUCTION

Cabergoline, an ergot derivative, is a potent dopamine receptor agonist on D2 receptors. Rat studies show that cabergoline has a direct inhibitory effect on pituitary lactotroph (prolactin) cells[1]. It is frequently used as a first-line agent in the management of prolactinomas due to higher affinity for D2 receptor sites, less severe side effects, and more convenient dosing schedule than the older bromocriptine. Cabergoline is chemically 1-[(6 allyergoline 8β -yl)]-carbonyl]-1-[3(dimethyl imino)propyl] - 3 ethyl urea. It is White or almost white, crystalline or amorphous powder. Freely soluble in alcohol (96%); slightly soluble in 0.1 M hydrochloric acid; very slightly soluble in hexane; practically insoluble in water[2]. An extensive review of the literature revealed a few UV- Visible spectroscopy[3,4] HPLC[5, 6] and LCMS[7, 8] methods for the estimation of cabergoline. Therefore, attempts were made to develop and validate simple, precise, and sensitive, isocratic reverse phase high performance liquid chromatographic method for the estimation of cabergoline in bulk and its formulations.

MATERIALS AND METHODS

Chemicals and Materials

Cabergoline, Acetonitrile and water (HPLC grade) were purchased from Merck Specialities private limited. Ammonium acetate and glacial acetic acid were purchased from Merck Specialities private limited, cabgolin 0.25tablets were purchased from local pharmacy.

Instruments

Shimadzu HPLC system with LC solutions software equipped with UV detector, Hypersil ODS C18 (250mmX 4.6mm, X5.0µm) column, Digital Ultra Sonic Cleaner (heater), Shimadzu Electronic Balance Type BL-220H, vacuum filtration apparatus.

Chromatographic conditions

The HPLC analysis of CBE was performed using ammonium acetate buffer PH 6, acetonitrile and water (10:50:40) as a mobile phase which was always clarified by filtration

through a nylon filter paper, with pore size equal to 0 .45µm, and degassed through a sonicator, then pumped at flow rate of 1 ml/min, in isocratic mode on Hypersil ODS C18 column (250 mm ×4.6 mm, 5µm). The peak response was monitored at a wave length of 281nm. The sample (20µl) was injected into HPLC system and the data was acquired using LC solutions software.

Preparation of standard solutions

Primary stock solution (100µg/ml) of cabergoline was prepared in a volumetric flask by dissolving accurately weighed amount of CBE in mobile phase. Working standard solution of CBE was prepared by appropriately diluting the respective stock solution with mobile phase.

Preparation of sample solution

1.62g of commercial powdered tablet (cabergoline 5mg) was diluted to 100 ml with mobile phase. It was further diluted to obtain concentration equal to 25µg ml-1 of CBE. The solution was filtered through 0.45 µm nylon filter before analysis.

Validation of the method[9]

The proposed analytical method was validated according to standard ICH guidelines with respect to the following parameters.

Specificity

Specificity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. For specificity, the standard and test samples were injected and the peaks were examined for interference.

Accuracy

The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. Accuracy is determined by replicate analysis of samples containing known amounts of the analyte.

Accuracy was measured using three different concentration levels (50%, 100% and 150%).

Precision

The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots. Repeatability was studied by determination of intra-day and inter-day precision. Intra-day precision was determined by injecting six standard solutions of any of the concentrations on the same day and inter-day precision was determined by injecting the same solutions for three consecutive days. Relative standard deviation (RSD %) of the peak area was then calculated to represent precision.

Limit of detection and limit of quantitation

Limit of quantitation is the concentration of the analyte that give a signal-to-noise (S/N) ratio of 10:1 at which analyte can be readily quantified with accuracy and precision. Limit of detection is the concentration of the analyte that give signal-to-noise (S/N) ratio of 3:1 at which analyte can be readily detected.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Robustness

Premeditate variations were performed in the experimental conditions of the proposed method to assess the method robustness. For this intention, minor changes were made in mobile phase composition, flow rate and ph of buffer solution. The effect of these changes on chromatographic parameters such as retention time, tailing factor and number of theoretical plates was then measured.

RESULTS AND DISCUSSION

In this work we have proposed a simple, fast, and accurate RP-HPLC method for the determination of cabergoline. For optimization of the chromatographic conditions and to obtain symmetrical peak, various chromatographic conditions such as composition of mobile phase, mobile phases with different pH, were applied to cabergoline.

Optimization of mobile phase and pH

Method development process was initiated with different ratios (20:80, 30:70, 40:60 and 50:50) of water and acetonitrile at different PH. With acetonitrile and water, broad peaks of cabergoline were observed. The peak tailing of cabergoline may be due to the unwanted interactions between drug and silanol residues of stationary phases. The peak broadening of CBE may be due to low polarity of the mobile phase. Then polarity of the mobile phase was increased by using ammonium acetate buffer, PH 6 in the mobile phase composition. Further chromatographic experiments were performed on Hypersil ODS C18 using ammonium acetate buffer PH 6, acetonitrile and water in the ratio of 10:50:40. Highly symmetrical and sharp peak of cabergoline was obtained with ammonium acetate buffer PH 6, acetonitrile and water in the ratio of 10:50:40 on Hypersil ODS C18 column (with better retention time,

peak shapes and theoretical plates). The variations in the composition of the mobile phase had substantial influences on peak shape, tailing factor, retention factor and theoretical plates.

Finally, ammonium acetate buffer PH 6, acetonitrile and water (10:50:40) was selected which provided symmetrical peak with acceptable tailing of cabergoline using Hypersil ODS C18 column. Under the mentioned chromatographic conditions highly symmetrical and sharp peak of cabergoline was obtained at retention time 4.1min.

System suitability parameters

To ensure the validity of the system and analytical method, system suitability test was performed. RSD (Relative Standard Deviation) of cabergoline from the six consecutive injections of standard solutions was 2.2. The tailing factor for cabergoline peak was 1.44 and the theoretical plate count was found to be 6711 with retention time 4.1 min, thus reflecting good peak symmetry.

The developed chromatographic method was validated using ICH guidelines[9]. Validation parameters included linearity, accuracy, precision, robustness, specificity, limit of detection and quantitation.

Linear calibration plot for the proposed method was obtained in concentration ranges of 5-25 µg mL-1 (5, 10, 15, 20, and 25µg mL-1). The linear regression equation (r2 =0.999) was found to be Y = 21193 X + 1336 in which Y is the dependent variable, X is independent variable, 21193 is slope (denoted by a) which shows change in dependent (Y) variable per unit change in independent (X) variable and 1336 (denoted by b) is the Y-intercept i.e., the value of Y variable when X = 0. The Y-intercept provides with an estimate of the variability of the method. For example, the ratio percent of the Y-intercept with the variable data at nominal concentration is used to estimate the method variability.

The limit of detection (LOD) and quantization (LOQ) were determined and the values were found to be 23.2µg/ml and 70.3µg/ml respectively.

Accuracy of the developed method was performed by the standard technique. Three levels of solutions (50, 100 and 150%) of the nominal analytical concentrations were prepared and analysed by the developed method. Percentage recoveries along with standard deviation and relative standard deviations for each analyte are given in [Table 1]. Recovery studies showed the method to be highly accurate and suitable for intended use.

Intra-day precision was determined by injecting six standard solutions of any one of the concentrations on the same day and inter-day precision was determined by injecting the same solutions for three consecutive days. Relative standard deviation (RSD %) of the peak area calculated to represent precision which was found to be 2.4 and 2.2 for intra and inter day precision respectively.

Robustness of the method was performed by slightly varying the chromatographic conditions. The results showed that slight variations in chromatographic conditions had negligible effect on the chromatographic parameters.

The method was found to be specific as the commercial tablet hasn't shown any interference with the drug peak at retention time 4.1min.

CONCLUSION

A simple, fast and accurate RP-HPLC method is described for the determination of cabergoline in pharmaceutical formulations. The developed method was validated by testing its linearity, accuracy, precision, limits of detection and quantitation and specificity. So, it is concluded that the method can be successfully used for routine analysis of cabergoline in bulk and in formulations.

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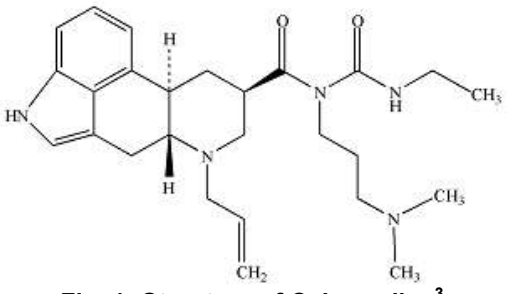

Fig. 1: Structure of Cabergoline[3]

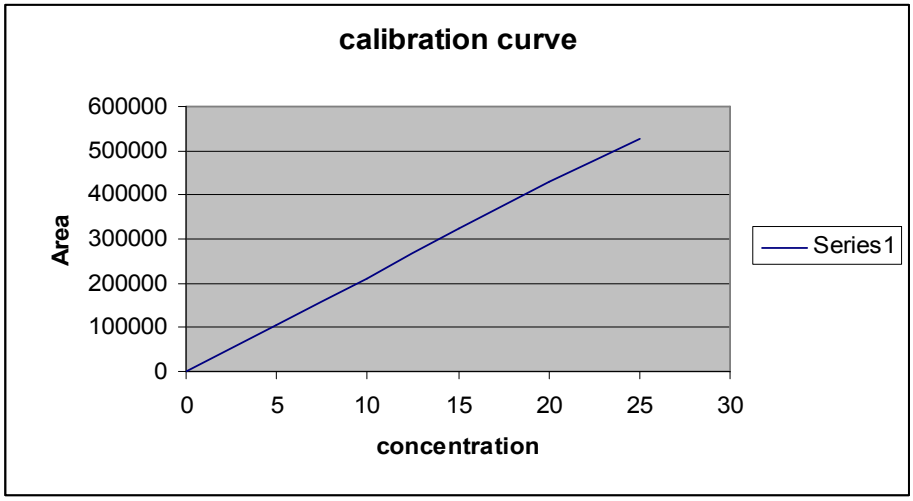

Fig. 2: calibration curve

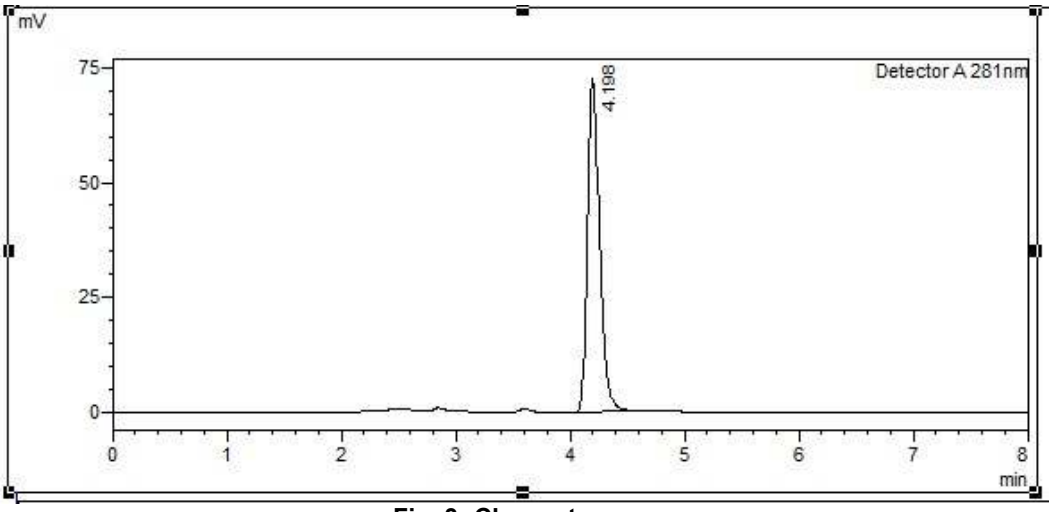

Fig. 3: Chromatogram

Table 1: Accuracy of the proposed HPLC method

Drug	Theoretical content	Amt found	% recovery	Mean recovery
CBE	50% (15µg/ml)	14.8	98.6	96.4
		14.6	97.3	
		14.0	93.3	
CBE	100% (20g/ml)	20.3	101.5	102.6
		20.6	103	
		20.7	103.5	
CBE	150% (25g/ml)	26.3	105.2	104.9
		26.9	107.6	
		25.5	102	Mean = 101.3

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