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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE ESTIMATION OF NICERGOLINE IN MARKETED FORMULATIONS

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ABSTRACT

A simple, selective, accurate, and economical reverse phase high performance liquid chromatography (RP-HPLC) was developed for estimation of nicergoline in pharmaceutical formulations. Chromatographic separation achieved isocratically on a C18column (ODS, C18, 5µ, 250×4.6 mm i.d.) with mobile phase containing methanol, acetonitrile and 1.0 % ortho phosphoric acid in the ratio 80:18:2 v/v/v. The flow rate was 1.0mL/min and effluent was monitored at 265nm. The retention time was 3.128min. The method was validated in terms of linearity, accuracy and precision. The linearity curve was found to be linear over 1.0 - 6.0 µg/mL. The limit of detection and limit of quantification were found to be 0.3 µg /ml and 0.9 µg /ml respectively. The proposed method was successfully used to determine the drug content of marketed formulations.

Keywords: Nicergoline, HPLC, linearity, validation.

INTRODUCTION

Nicergoline (Ergoline-8-methanol,10-methoxy-1,6-dimethyl-,8-(5-bromo-3-pyridinecarboxylate) is an ergot derivative used to treat cognitive, affective, behavioral disorders of older people[1] and use in acute myocardial infarction with diastolic hypertension[2-4]. Literature survey reveals that a few HPLC methods[5-6], Spectrofluorimetry[7], HPTLC method[8] and spectrophotometric methods[9-10] have been reported for the estimation of nicergoline in bulk and pharmaceutical formulations. In the present investigation a new RP-HPLC method has been reported for the estimation of nicergoline from marketed formulations.

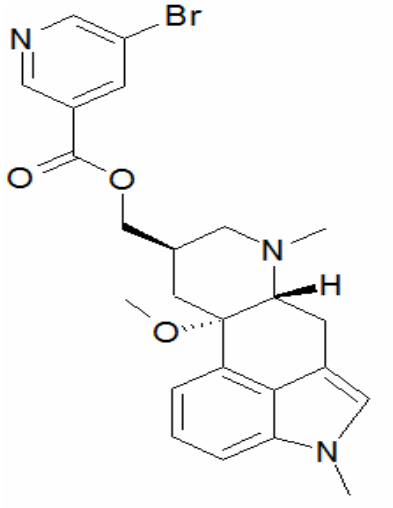


Fig.-1: Structrue of Nicergoline

EXPERIMENTAL

Chemicals and materials

The pharmaceutical grade pure sample of Nicergoline was procured from CELON Laboratories limited, Andrapradesh. Acetonitrile and methanol solvent of analytical grade were obtained from E Merck Ltd, Mumbai, India. Orthophosphoric acid AR grade was procured from Qualigens Fine Chemicals, Mumbai, India. The HPLC grade water was obtained from a Milli-Q RO water purification system.

Equipment and apparatus

Shimadzu (LC 8200AHT) isocratic HPLC system equipped with isocratic liquid pump and UV-Visible spectrophotometric detector was used for the analysis. The data was recorded using window based single channel soft ware. The purity determination performed on a stainless steel column 250 mm long, 4.6 mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5 µm diameter (ODS, C18, 5µ, 250×4.6mm i.d). A Downer electronic balance was used for weighing the materials.

Preparation and assay of standard stock solution

An accurately weighted sample of 10 mg of nicergoline was dissolved in methanol to give standard stock solution of 100µg/ml. A series of working standard solutions (1.0µg/mL – 6.0µg/mL were obtained by diluting the stock solutions with mobile phase (methanol, acetonitrile and 1% ortho phosphoric acid in the ratio 80:18:2 v/v/v). All the volumetric flasks containing nicergoline were wrapped with aluminium foil and stored in the dark.

Preparation and assay of pharmaceutical formulations

Ten tablets of nicergoline were ground to fine powder. Accurately weighed powder sample equivalent to 10mg of nicergoline was dissolved in methanol in a 100 mL volumetric flask. The flask was placed in an ultrasonic bath at room temperature for 10 min. After sonication, the solution was allowed to stand for 5.0 min. 1.0 mL was transferred into a 100 mL volumetric flask and diluted to the mark with mobile phase. A sample of 20 µL of this solution was directly injected. The average content of the tablets was determined either from the calibration graph or using the corresponding regression equation.

RESULTS AND DISCUSSION

Chromatographic conditions

Chromatographic separation was performed on a Shimadzu (LC 8200AHT) isocratic HPLC system equipped with isocratic liquid pump and UV- Visible spectrophotometric detector was used for the analysis. The data was recorded using window based single channel soft ware. The purity determination performed on a stainless steel column 250 mm long, 4.6 mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5 µm diameter (ODS, C18, 5µ, 250×4.6mm i.d) with the mobile phase containing of methanol, acetonitrile and 1.0% ortho phosphoric acid in the ratio 80:18:2 v/v/v at a flow rate 1.0 mL/min at ambient temperature. The elution was monitored at 265nm and the chromatographic conditions employed for the analysis of nicergoline are shown in Table.1. The typical chromatogram of nicergoline was shown in Fig.2.

Range and linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity of the method was observed within the expected concentration range demonstrating its suitability for analysis (Fig.3). The correlation coefficient (R^2) was found to be 0.9991 and value of intercept was less than 25 of the response of 100% of the test concentration in. The results showed that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above (Table.2).

Table-1: Optimized chromatographic conditions

Chromatographic parameters	Peak HPLC
Elution	Iso cratic
Mobile phase	Acetonitrile: water : 1.0 % ortho phospharic acid (70:27:3 v/v/v)
API Concentration	3 µg/ml
Column	ODS C-18 RP (4.6 mm i.d x 250 mm)
Flow rate	1 min/ ml
Detection	UV at 265 nm
Injection volume	20 µl
Temperature	Ambient
Retention time	3.128 minutes
Run time	7 minutes
Area	132023.2 mAU
pH	5.7
Theoretical plates	3978
Pressure	30-35 Mpa
Tailing factor	1.83

Table-2: Calibration of the RP HPLC for the estimation of Nicergoline

Concentration (µg)	Area (mAU)
1.0	59223.6
2.0	111331.7
3.0	167099.1
4.0	220805.4
5.0	279570.5
6.0	339255.7
Regression equation :	Y = a X + b
Slope (a) :	55959.52
Intercept (b) :	356.0133
Correlation coefficient :	0.9991

Table-3: Precision data of HPLC method

Day	Precession Area Mean	R.S.D.
Day- 1	227318.3	0.288
Day-2	229406.7	0.092

All the values are the averages of five determinations

Table-4: Results of Recovery studies of tablet containing Nicergoline studies

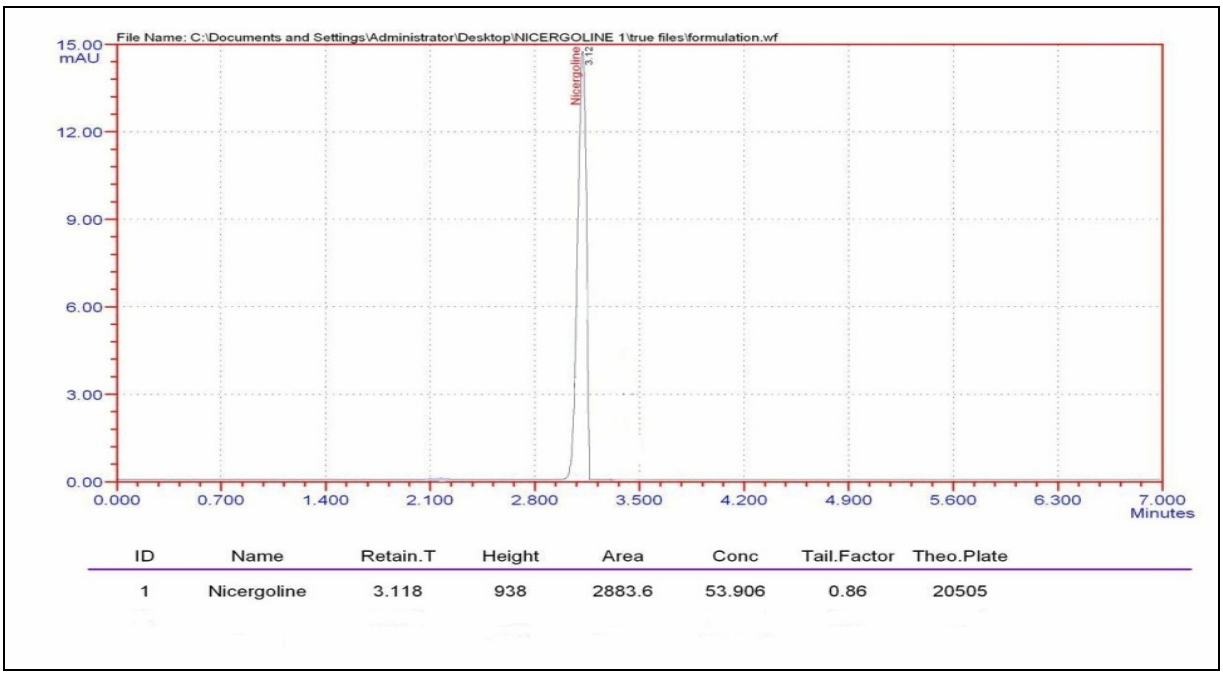
Pharmaceutical formulation	Amount of nicergoline		% of Recovery
	Labelled	Found	
Tablet - 1	30 mg	30.55 mg	101.85 %

All the values are the averages of three determinations

Limits of detection and quantitation

The detection limit (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a

concentration that gives a signal-to-noise ratio of 2:1 or 3:1.The lower limit of detection for nicergoline is 0.3 µg /mL in reference material and formulation. Limit of Quantitation (LOQ) is the lowest amount analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of 10:1 can be taken as LOQ of the method (USP 2004). The LOQ values were found to be 0.9 µg /mL for raw material, formulations.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	Nicergoline	3.118	938	2883.6	53.906	0.86	20505

Fig.-2: Chromatogram of Nicergoline

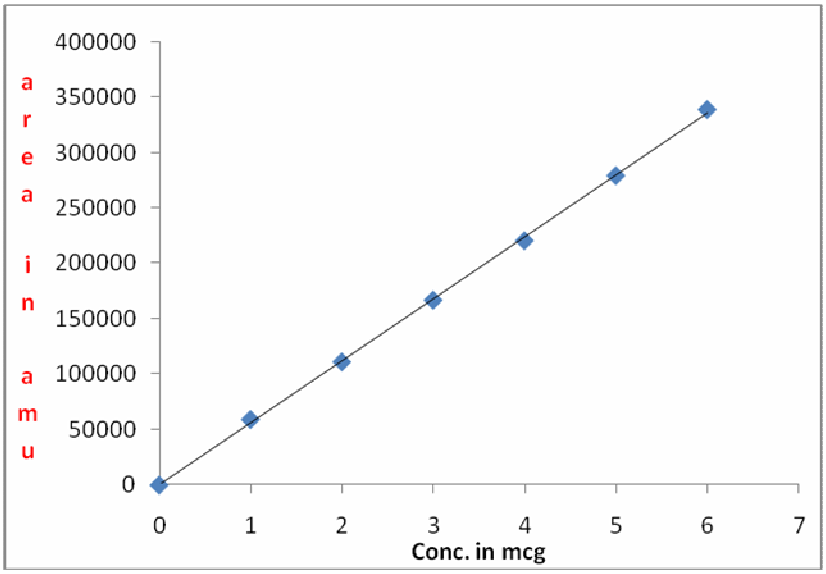


Fig.-3: Linearity of Nicergoline

Precision

Precision is the degree of reproducibility or repeatability of the analytical method under normal operating conditions. The method passed the test for repeatability as determined by %RSD of the area of

the peaks of five replicate injections at 100% test concentration. The results of intra-and inter-day variation are shown in (Table.3).

Accuracy (Recovery studies)

The accuracy of an analytical method is the closeness of test results obtained by that method to true value. In case of the assay of a drug in a formulated product, accuracy may be determined by application of the analytical method to synthetic mixtures of the drug product components to which known amount of analyte has been added within the range of method. If it is not possible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product (*i.e*. "to spike"). In our studies, the later technique was adopted and nicergoline was spiked in drug product. The result of accuracy given in (Table.4) revealed that the method was found accurate.

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010AHT), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil C18, Phenomenex and Gemini C18. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, are rugged and robust.

CONCLUSION

The results of our study indicate that the proposed RPHPLC method is simple, rapid, precise and accurate. The developed HPLC method was found suitable for determination of nicergoline in bulk drug and in marketed formulations without any interference from the excipients. Statistical analysis proves that, the method is repeatable and selective for the analysis of nicergoline. It can therefore be concluded that use of the method can save much time and money and it can be used in small laboratories with very high accuracy and a wide linear range.

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