



## Extractive Spectrophotometric Determination of Nicergoline Through Ion-pair Complexation Reaction

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(Received: December 05, 2012; Accepted: January 25, 2013)

### ABSTRACT

Two simple, sensitive, selective, accurate and economical spectrophotometric methods (A and B) have been described in the present work for the determination of Nicergoline in bulk drug and pharmaceutical formulations (tablets). Methods – A/B are based on the formation of orange red / yellow colored ion-association complexes between Nicergoline and Tropaeolin ooo (TPooo) / Alizarine Red S (ARS) in acid medium followed by their extraction with chloroform, exhibiting absorption maxima at 490nm / 430nm, and obeying Beer's law in the concentration range of 2.5–12.5µg/ml. Statistical analysis of the results of the proposed methods reveals high accuracy and good precision. The proposed methods could be successfully extended to the commercial pharmaceutical formulations containing Nicergoline.

**Key words:** Nicergoline, Tropaeolin ooo, Alizarine Red S, Ion-pair complex, Visible spectrophotometry.

### INTRODUCTION

Nicergoline (Ergoline-8-methanol, 10-methoxy-1,6-dimethyl-, 8-(5-bromo-3-pyridinecarboxylate) is an ergot derivative used to treat cognitive, affective behavioural disorders of older people<sup>1</sup> (Fig. 1). It has been used to treat symptoms of mental deterioration associated with cerebrovascular insufficiency and in peripheral vascular disease. The side effects of nicergoline are usually limited to nausea, hot flushes, mild gastric upset, hypotension and dizziness<sup>2</sup>. Literature survey revealed that spectrophotometric<sup>3-6</sup>, electrochemical<sup>7</sup>, capillary electrophoretic<sup>8</sup>,

spectrofluorimetric<sup>9</sup>, HPLC<sup>10-12</sup>, LC-MS<sup>13</sup> and radioimmunoassay<sup>14-15</sup> methods have been developed for its determination. The chromatographic, voltammetric and electrophoretic methods utilized delicate and /or expensive instruments that are not available in most quality control laboratories. Visible spectrophotometry may serve as a useful alternative to many of the aforesaid sophisticated techniques, because of its cost effectiveness, ease of operation, sensitivity, fair accuracy and precision, and wide applicability. The extraction spectrophotometric procedures are popular for their sensitivity and selectivity in the assay of drugs<sup>16</sup> and spectrophotometric

techniques are the most widely used in pharmaceutical analysis<sup>17</sup>. In addition, the analytically important functional groups in Nicergoline have not been exploited properly so far, hence, in the present study, an attempt has been made to develop sensitive and flexible extractive visible spectrophotometric method involving ion pair complex formation for the determination of Nicergoline in pure and dosage formulations using the acidic dyes (ARS & TPoo).

## MATERIALS AND METHODS

### Instrument

A Systronics digital spectrophotometer (model-106) provided with 1 cm matched quartz cells was used for all absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements. All spectroscopic measurements were carried out at room temperature ( $25 \pm 4^\circ\text{C}$ ).

### Materials and Reagents

Chemicals used were of analytical reagent grade and distilled water was used throughout the investigation.

### TPoo solution (0.2%)

Prepared by dissolving 200mg of Tropaeolin ooo (Benzenesulfonic acid, 4-[2-(2-hydroxy-1-naphthalenyl)diazenyl]-, sodium salt, Fluka make) in 100ml of distilled water.

### ARS solution (0.2%)

Prepared by dissolving 200mg of Alizarine Red S (9,10-dihydro-3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonic acid sodium salt, BDH make) in 100ml of distilled water.

### HCl solution (0.1M)

Prepared by diluting 8.6ml of conc HCl to 100ml with distilled water in a volumetric flask.

### Preparation of standard solutions

The standard stock solution of Nicergoline was prepared by dissolving 25mg of the drug in 25mL of the methanol to get 1.0mg/mL solution. Final working standard solution of 100 $\mu\text{g/mL}$  of Nicergoline was prepared by diluting 1.0mL solution of the above solution to 10mL with distilled water. Calibration curves were constructed by plotting

optical densities of the coloured solution (developed by following the proposed methods) versus concentrations of Nicergoline and the regression equations were calculated.

### Preparation of Marketed formulations

In case of marketed formulations, two accurately weighed tablets were crushed to a fine powder and an amount equivalent to 10mg of Nicergoline was added into different 100mL volumetric flasks and volume was made up with methanol. The samples were filtered through a 0.45 $\mu\text{m}$ -membrane filter. The drug content of this solution was obtained by applying the proposed procedure to aliquot containing different volumes of solution drugs.

### Proposed procedure for methods A and B

Different portions of standard Nicergoline solution (for Method A & B) were delivered in to a series of 80ml separating funnels and then 6.0 ml of 0.1M HCl and 2.0ml of 0.2% dye solution (TPoo and ARS in Method A and Method B respectively) were added successively and the total volume of aqueous phase in each separating funnel was made to 15.0ml with distilled water. To each separating funnel 10ml of chloroform was added and the contents were shaken for 2min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was determined at  $\lambda_{\text{max}}$  490nm and 430nm respectively in Method A (TPoo) and Method A (ARS) against chloroform blank.

## RESULTS AND DISCUSSION

Ion pair extraction spectrophotometry has received considerable attention for quantitative estimation of many pharmaceutical compounds. This technique depends on the reaction of a drug that has basic cationic nitrogen and an anionic dye, where a highly colored ion-pair complex is formed<sup>18-19</sup>. An important advantage of the extractive spectrophotometric method is that it can be applied to the determination of an individual component in the presence of routine excipients and filling materials. This aspect of spectrophotometric analysis is of great interest since it offers distinct possibilities for the assay of a particular component in a complex dosage formulation. This lead the authors to develop

two accurate, selective, precise, and inexpensive procedures for the determination of Nicergoline in bulk drug and pharmaceuticals based on ion-pair complex formation using Tropaeolin ooo (TPoo) / Alizarine Red S (ARS) as chromogens.

#### Absorption Spectra of extracted coloured ion-pair complexes

In the present investigation, the nitrogen on pyridine ring of Nicergoline enabled it to develop yellow and orange-red colours by the formation of ion association complexes with chromogens –

TPoo (Method A) and ARS (Method B) respectively. These complexes have absorption maxima ( $\lambda_{\max}$ ) at 490nm and 430nm for method A(TPoo) and method B(ARS) respectively against chloroform blank (Fig. 3 and 4). Cañamares *et al.*<sup>20</sup>, (2006) have shown that alizarin red S can exist in three different forms (neutral absorbing at 433 nm, monoionized absorbing at 526 nm, and di-ionized absorbing at 567 nm) based on different pHs 3.4, 10.0, and 12.8, respectively. Nemodruk and Karalova<sup>21</sup> have stated that alizarin red S has a yellow color in acid solutions (pH < 5).

**Table 1: Statistical analysis of calibration graphs and analytical data in the determination of the nicergoline using the proposed methods**

Parameter	Method A	Method B
$\lambda_{\max}$ (nm)	490	430
Beer's law limits ( $\mu\text{g/ml}$ )	2.5 – 12.5	2.5 – 12.5
Molar absorptivity ( $1 \text{ mol}^{-1} \cdot \text{cm}^{-1}$ )	$3.658 \times 10^3$	$3.489 \times 10^3$
Sandell's sensitivity ( $\mu\text{g} \cdot \text{cm}^{-2} / 0.001 \text{ absorbance unit}$ )	0.0389	0.0328
Optimum photometric range (mg/ml)	3.0 – 9.0	3.5 – 10.0
Slope (b)(From regression equation, $Y=a+bc$ )	0.0310	0.0360
Standard deviation on slope ( $S_b$ )	0.0002463	0.002571
Intercept (a)	0.001	0.003
Standard deviation on intercept ( $S_a$ )	0.0001423	0.0002674
Standard error on estimation ( $S_e$ )	0.002366	0.002033
Correlation coefficient (r)	0.9995	0.9998
Relative standard deviation (%)*	1.006	1.674
% Range of error (confidence limits)		
0.05 level	0.8415	1.4999
0.01 level	1.244	2.218
LOD	0.01848	0.02853
LOQ	0.1848	0.2853

\* Average of six determinations considered

**Table 2: Estimation of Nicergoline in pharmaceutical formulations**

Sample	Labelled amount (mg)	Amount obtained (mg)		% Recovery of Proposed methods	
		Proposed methods*		Proposed methods	
		Method-A	Method-B	Method-A	Method-B
Sermion	10	9.992	9.978	99.92	99.78

\*Average of six determinations

### Reaction Mechanism

Nicergoline being a base, forms an ion association complexes with acidic dyes – Tropaeolin ooo (TPooo) and Alizarine Red S (ARS) which are extractable into chloroform from the aqueous phase. In acidic medium, the nitrogen on pyridine of Nicergoline gets protonated and is expected to attract the oppositely charged part – anionic dye and behave as a single unit being held together by electrostatic attraction. The probable reaction mechanism of ion association complex is represented in scheme (Fig. 2)

### Optimisation of reaction variables

The effect of the dye concentration was studied by measuring the absorbance of solutions containing a fixed concentration of Nicergoline (12.5  $\mu\text{g mL}^{-1}$ ) and different amounts of the dye solution and the study showed that 2.0 mL of 0.2% dye solution was necessary to yield a maximum absorbance. It was noticed that the maximum color intensity was observed using hydrochloric acid. Also, the formed ion-pair complex was found to be pH independent since no remarkable changes were observed while using different concentrations of HCl such as 0.05, 0.1, 0.3, and 0.5 M. Further, 6.0 mL of 0.1 M HCl gave reproducible results, and it was fixed throughout the study. The optimization of the reaction conditions was investigated. Among various organic solvents studied (benzene, toluene, cyclohexane, carbon tetrachloride, and chloroform), chloroform was preferred because of its selective and quantitative extraction of the drug-dye ion-pair from the aqueous phase, greater stability of the extracted ion-pair complex and short time required for the separation of the two phases hence, it was used in this experiment. The ratio of aqueous to organic phase on extraction was taken as 3:2. Shaking times ranging from 1 to 4 min produced no change in absorbance, and so two minutes shaking time was selected. Only one extraction was sufficient to achieve quantitative recovery of the complex as a second extraction with identical quantity of chloroform yielded negligible absorbance. The absorbance of the chloroform phase was stable for 2 - 3 h. The absorbance was measured periodically at an interval of 15 min for 3 h. The composition of the ion-pair complex formed between Nicergoline and TPooo/ARS was established by applying Job's method<sup>21</sup> of continuous variations. The plot reached

a maximum value at a mole fraction of 0.5 which indicated that a 1 : 1 (drug : acid dye) ion-pair complex was formed.

### Validation of the proposed methods:

Validation of the proposed spectrophotometric methods was done according to ICH guidelines<sup>22</sup>.

### Linearity and Range

The linearity of the method was determined at five concentration levels ranging from 2.5  $\mu\text{g mL}^{-1}$  to 12.5  $\mu\text{g mL}^{-1}$  for Nicergoline. The calibration curve was constructed by plotting optical densities of coloured ion complexes against various concentrations of Nicergoline. The regression equations derived using the least-squares method<sup>24</sup> for the calibration curves of Nicergoline were  $Y=0.031x+0.001$  ( $R^2=0.9995$ ) for Method A (TPooo) and  $Y=0.036x+0.003$  ( $R^2=0.9998$ ) for Method B (ARS) respectively. The results showed an excellent correlation between optical density and concentration of drugs within the concentration range indicated above (Table.1) for both the methods proposed. The calibration curves are shown in figures 5 and 6.

### Sensitivity (LOD and LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve. The values of LOD and LOQ for Method A (TPooo) and Method B (ARS) are given in Table 1.

### Precision and Accuracy

The precision of the method was demonstrated by intraday variation studies. In the intraday studies, six repeated estimations of standard and sample solutions were made and the optical densities of the drug and Relative standard deviation (%) were calculated and found to be within permissible limits. The accuracy of the method was determined by recovery experiments. The

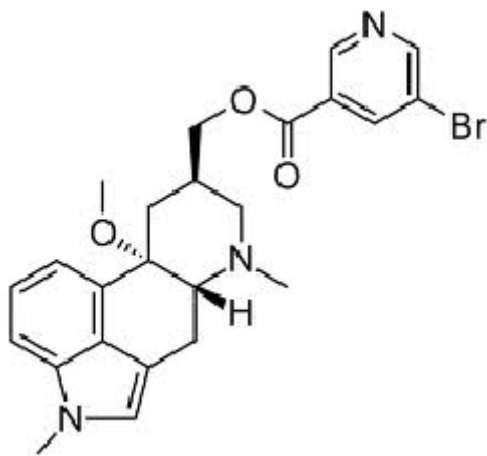


Fig. 1: The chemical structure of Nicergoline

recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table 1.

#### Analysis of pharmaceutical preparations

Commercially available Nicergoline tablets were subjected to analysis by the proposed methods. Results obtained by the proposed methods [Method A(TPoo) and Method B(ARS)] (Table 2) for Nicergoline confirm the suitability of these methods for commercial pharmaceutical formulations. The methods were successful to the determination of the active drug in pharmaceutical formulations without any detectable interference from the excipients present in the tablets.

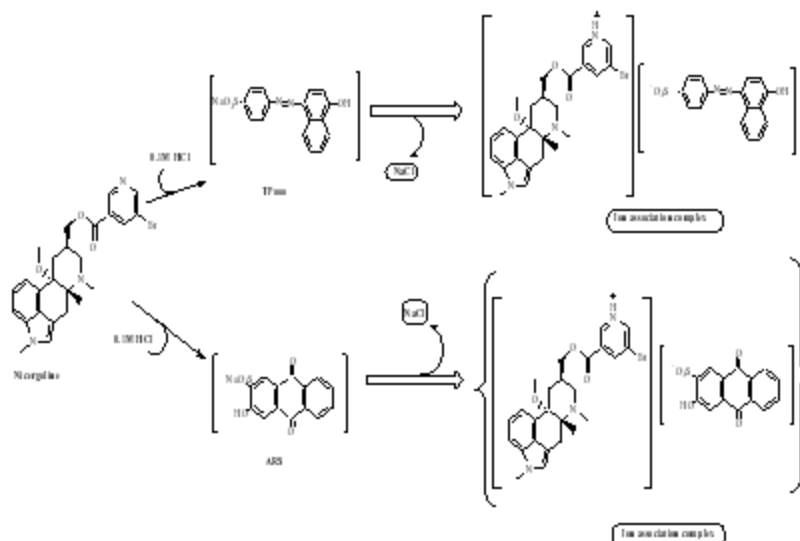


Fig. 2: Reaction mechanism of ion association complex of Nicergoline with TPoo and ARS

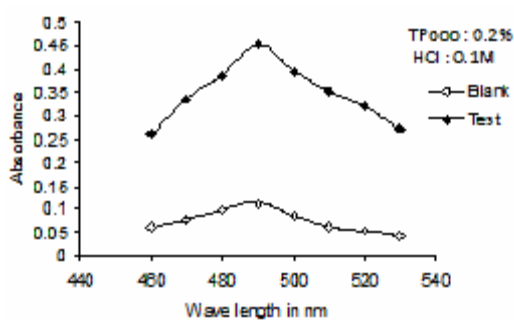


Fig. 3: Absorption spectrum of Nicergoline – TPoo complex

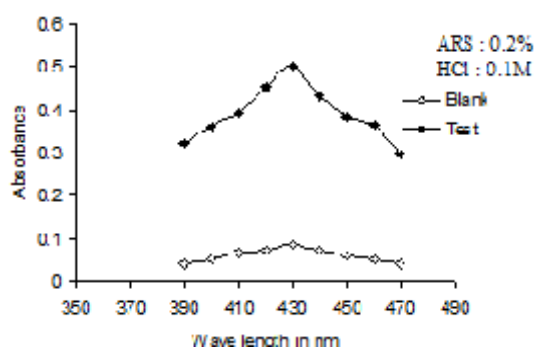


Fig. 4: Absorption spectrum of Nicergoline - ARS complex

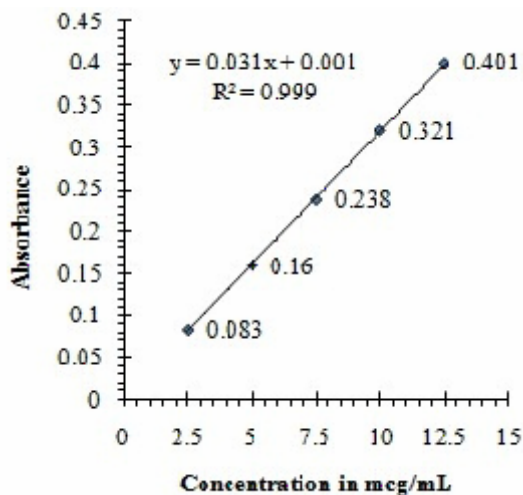


Fig. 5: Calibration curve for Nicergoline estimation by method A (Tpoou)

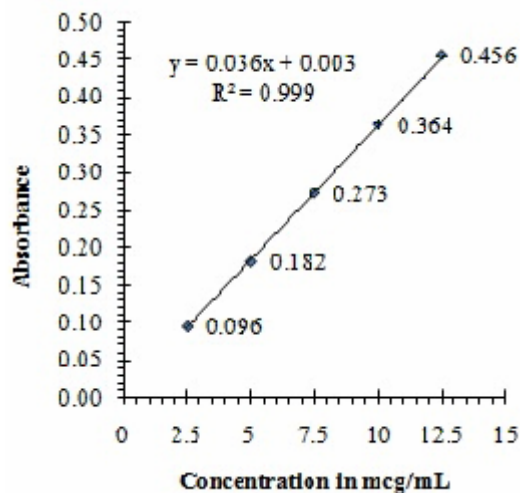


Fig. 6: Calibration curve for Nicergoline estimation by method B (ARS)

### CONCLUSION

Nicergoline was estimated successfully by the developed extractive spectrophotometric methods, both as a pure compound and as a pharmaceutical formulation. The proposed methods are suitable and valid for application in laboratories lacking of liquid chromatographic or other

sophisticated instruments. These methods are simple, rapid, accurate, and does not involve any critical reaction conditions, or tedious sample preparation. It is unaffected by slight variations in experimental conditions such as pH, dye concentration, shaking time and temperature. Hence, the proposed methods can be used for the routine analysis of the cited drug in their available dosage forms.

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