METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DONEPEZIL HYDROCHLORIDE USING DYE DRUG REACTION (EXTRACTIVE SPECTROSCOPY)

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Abstract

The developed method for the estimation of Donepezil Hydrochloride through Dye Drug Reaction (Extractive Spectroscopy) using Bromocresol Green has demonstrated significant promise and reliability. The investigation into the linearity of the drug's reaction with Bromocresol Green revealed a robust correlation between the concentration of Donepezil Hydrochloride and the corresponding absorbance at 672nm. The exceptionally high correlation coefficient ($r^2 = 0.999$) attests to a linear relationship within the concentration range of 10-50 µg/ml.The recovery study, an imperative facet of method validation, showcased compelling outcomes. The mean recovery percentages at different concentration levels (80%, 100%, 120%) were closely aligned with 100%, underscoring the accuracy and dependability of the proposed method.Precision assessment, covering repeatability, intermediate precision (day-to-day and analyst-to-analyst), exhibited minimal standard deviations (% Mean±SD*) for Bromocresol Green across five different concentrations. This underscores the method's consistent and reliable performance under diverse conditions. The method's sensitivity was gauged through the determination of the Limit of Detection (LOD) and Limit of Quantification (LOQ). The obtained low values for LOD (0.75 mg/ml) and LOQ (2.10 mg/ml) underscore the method's adeptness in detecting and quantifying Donepezil Hydrochloride at low concentrations. The application of the developed method to analyze a commercial tablet formulation of Donepezil Hydrochloride yielded satisfactory results. The identified percentage concentration (98.40%) closely matched the labeled concentration (5 mg), affirming the method's suitability for pharmaceutical formulation analysis. In conclusion, the proposed method exhibits significant potential for accurate and reliable estimation of Donepezil Hydrochloride, making it a valuable tool in pharmaceutical analysis.

Keywords: Donepezil Hydrochloride, Bromocresol Green, Extractive spectroscopy, Method development, Validation

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Introduction

Donepezil Hydrochloride, a widely prescribed acetylcholinesterase inhibitor, plays a crucial role in the management of Alzheimer's disease, enhancing cholinergic function and ameliorating cognitive decline. The accurate estimation of Donepezil Hydrochloride in pharmaceutical formulations is imperative for ensuring proper dosage and therapeutic efficacy [1-2]. Several analytical methods have been employed for the quantification of Donepezil Hydrochloride, including HPLC [3], LC/MS/MS [4], HPLC/MS [5-9].

However, the constant quest for improved precision, cost-effectiveness, and environmental sustainability motivates the exploration of alternative methodologies [10].

Spectrophotometry is considered as the most convenient analytical technique in pharmaceutical analysis because of its inherent simplicity and availability in most quality control and clinical laboratories. However, AMD does not possess any chromophore in its molecule, which is the essential requirement for the direct or indirect spectrophotometric analysis [11].

This research introduces a novel approach for the estimation of Donepezil Hydrochloride, utilizing Dye Drug Reaction in conjunction with Extractive Spectroscopy. The method harnesses the unique interaction between Donepezil Hydrochloride and a carefully selected dye reagent, leading to distinctive spectral changes that can be quantitatively analyzed.

Material and Methods

Reagents and chemicals

The working standard of Donepezil Hydrochloride was provided as gift sample from Pharmaceutical Company. The market formulation were procured from local market. Triple distilled water was generated in house. All solvents and reagents were of analytical grade. All the solutions were protected for light and were analyzed on the day of preparations.

Instrument

In UV-spectrophotometric method, Labindia model-3000+ series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells.

Selection of particular dye

Solutions of 100µg/ml of Donepezil hydrochloride was prepared in 0.1 N HCl, in 3 ml of drug solutions add 1 ml dye and extracted with 3ml chloroform and same manner control also prepared shake both the solution and stand aside for 10 min and the colour change compared to control for dye drug reaction.

The reaction with Bromocresol Green suggests that a color change occurred, indicating a change in pH. This dye is sensitive to pH changes in the acidic range. The positive reaction may indicate the presence of an acidic component in the reaction with Donepezil hydrochloride.

Selection of wavelength for linearity

Solutions of 100µg/ml of Donepezil hydrochloride was prepared in 0.1 N HCl , in 3 ml of drug solutions add 1 ml dye and extracted with 3ml chloroform and same manner control also prepared shake both the solution and stand aside for 10 min and compare the colour change compared to control for dye drug reaction. Pipette out the coloured layer of solution and scan between 400 to 800nm as control as blank.

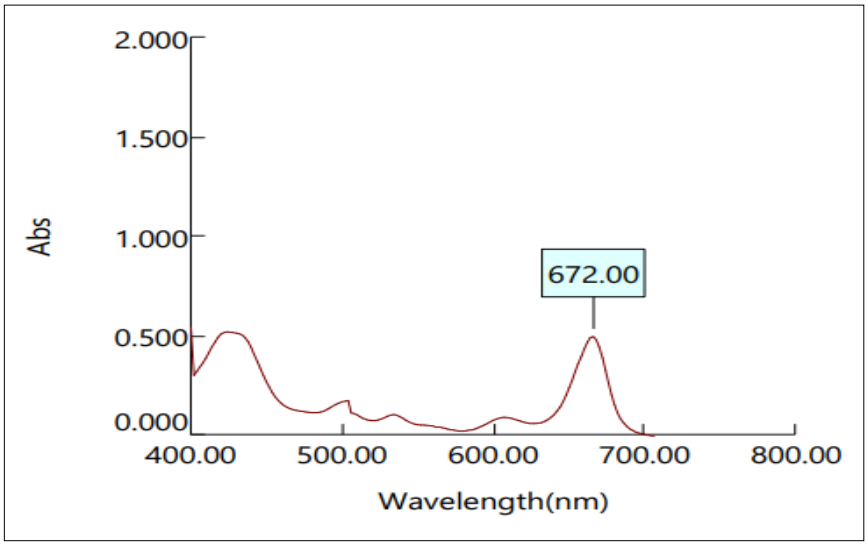

Fig. 1: Determination of λ max of Donepezil hydrochloride

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of drug in 80`mL mixed water the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark with water to get a concentration of 1000µg/ml (Stock-A) for drug.

Preparation of Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of Donepezil hydrochloride and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with 0.1 N HCl that gave concentration of 100 µg/ml (Stock-B).

Preparation of Working Standard Solution (For reaction with bromocresol green)

Aliquots of 1ml, 2ml, 3ml, 4ml and 5ml withdrawn with help of pipette from standard stock solution (Stock-B) in 10 ml volumetric flask and volume was made up to 10 ml with 0.1 N HCl. This gave the solutions of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml respectively for Donepezil hydrochloride. Take 3ml of each standard and react with 1 ml (2%) bromocresol green and add chloroform (3ml) was added to each volumetric flask; the flask was shaken well for thorough mixing of two phases and was allowed to stand for clear separation of the layers. The absorbance of the separated chloroform layers were measured against the reagent blank at 672nm and a calibration curve was drawn for the standard dilutions.

Analysis of tablet sample

Twenty marketed tablets of Donepezil hydrochloride was weighed and ground to a fine powder; amount equal to 10mg of Donepezil hydrochloride was taken in 10 ml volumetric flask, dilute suitably to 10µg/ml. Take 3 ml of this solution in three different volumetric flasks, Then 1 ml of dye solution (2%, Bromocresol green) was added and 3 ml chloroform in flask, the flask was kept aside for about 10 min. Pipette out the colored layer and the absorbance was observed at selected wavelengths and the concentrations were obtained from calibration curve method.

Validation of developed method

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to absorbance of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 10 to 50µg/ml) and abasorbance for each concentration were recorded three times and mean absorbance was calculated.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The stock solution was prepared. The precision are established in three differences:

4.5.3.1 Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 10, 20, 30, 40 and 50µg/ml for Donepezil hydrochloride indicates the precision under the same operating condition over short interval time.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Results and Discussion

The developed method for the estimation of Donepezil Hydrochloride using Dye Drug Reaction (Extractive Spectroscopy) with Bromocresol Green exhibited promising results, as depicted in the obtained data. The linearity study demonstrated a strong correlation between the concentration of Donepezil Hydrochloride and the absorbance at 672nm. The high correlation coefficient (r2 = 0.999) indicates a linear relationship over the concentration range of 10-50 µg/ml table 1.

The recovery study, a critical parameter for method validation, yielded satisfactory results. The mean recovery percentages at different concentration levels (80%, 100%, 120%) were close to 100%, indicating the accuracy and reliability of the proposed method table 2.

Precision, assessed through repeatability, intermediate precision (day-to-day and analyst-to-analyst), demonstrated low standard deviations (% Mean±SD*) for Bromocresol Green at five different concentrations. This signifies the method's ability to generate consistent and reliable results under varying conditions table 3.

The method's sensitivity was evaluated through Limit of Detection (LOD) and Limit of Quantification (LOQ). The low values for LOD (0.75 mg/ml) and LOQ (2.10 mg/ml) indicate the

method's capability to detect and quantify Donepezil Hydrochloride at low concentrations table 4.

The application of the developed method to analyze a commercial tablet formulation of Donepezil Hydrochloride exhibited satisfactory results. The percentage concentration found (98.40%) closely aligned with the labeled concentration (5mg), confirming the method's applicability for pharmaceutical formulation analysis table 5.

Table 1: Results of linearity of drug react with Bromocresol green

Parameter	**Bromocresol green**
λ_{max} (nm)	672nm
Concentration (µg/ml)	10-50
Correlation Coefficient (r^2)*	0.999
Slope (m)*	0.012
Intercept (c)*	0.003

*Value of five replicate

Table 2: Results of recovery study of Donepezil hydrochloride react with Bromocresol green

% Level	**% MEAN±SD***
	Bromocresol green
80%	99.81±0.073
100%	99.58±0.324
120%	99.53±0.353

***** Value of three replicate and three concentrations

Table 3: Results of precision

Parameter	**% Mean±SD***
	Bromocresol green
Repeatability	99.528±0.099
Intermediate precision	
Day to day precision	99.192±0.185
Analyst-to-Analyst	98.982±0.231

***** Value of five concentrations

Table 4: LOD and LOQ of Donepezil hydrochloride

Name	**LOD (µg/ml)**	**LOQ (µg/ml)**
Donepezil hydrochloride	0.75	2.10

Table 5: Analysis of tablet formulation of Donepezil hydrochloride

Conc. Present (mg)	**Donepezil hydrochloride**	
	Conc. Found (mg)	**% Conc. Found**
5	4.92	98.40

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