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METHOD DEVELOPMENT AND VALIDATION OF DONEPEZIL HYDROCHLORIDE

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ABSTRACT

The aim of the present work was to develop and validate a sensitive, simple, accurate, precise, and cost-effective UV spectrophotometric method for the analysis cholinesterase inhibitor i.e Donepezil hydrochloride. The different analytical performance parameters such as linearity, range, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were determined according to International Conference on Harmonization (ICH) Q2 (R1) guidelines. The study was performed in Acetonitrile, water, and methanol. The peak (λmax) of Donepezil hydrochloride appeared at a wavelength of 231 nm. Beer-Lambert's law was obeyed in the concentration range of 4–20 µg/ml with correlation coefficient (R2) 0.999. The results of the study demonstrated that the developed procedure is accurate, precise, and reproducible (relative standard deviation 2%), while being simple, cheap and less time consuming. Therefore, this method can be suitably applied for the estimation of Donepezil hydrochloride in prepared pharmaceutical formulations.

Keywords *– Acetylcholinesterase inhibitor, Donepezil, Quantitative determination, spectrophotometric method.*

1. INTRODUCTION

To develop ultraviolet spectroscopic method (UV)and validate the method for various parameters such as system suitability, precision, accuracy, linearity, robustness, LOD and LOQ according to the ICH Guidelines. The present work is undertaken with an objective to develop economical, simple, precise, accurate and reproducible method for estimation of Donepezil. Donepezil is a specific noncompetitive reversible inhibitor of acetyl cholinesterase (AChE) and appears to exert its therapeutic effect by enhancing cholinergic function. By inhibiting the hydrolysis of acetylcholine by AChE, donepezil increases acetylcholine concentrations, thus enhancing cholinergic function. As the dementia progresses, fewer cholinergic neurons are thought to remain functionally intact, and the effects of donepezil may be lessened. Donepezil exhibits a relatively high degree of selectivity for neuronal AChE ; at relevant clinical doses, it has only weak inhibitory effects on butyrylcholinesterase (pseudo cholinesterase), an enzyme that is widely distributed in plasma and peripheral tissues. Animal studies have shown that donepezil exhibits tissue selectivity; it significantly inhibits AChE in the brain but causes little inhibition of AChE in smooth, striated, or cardiac muscle [1-13].

2. MATERIALS AND METHODS

2.1 Instruments used

The list of instruments used during the study are summarized below:

Table 1: List of instruments used

S. No	Name of the Instrument	Make and model
1	UV/visible Spectrophotometer	Lab india
2	Electrical Balance	Metler Toledo (ME204)
4	Sonicator	PCI Analytics
5	Distillation Unit	Borosil

2.2 Chemicals and Reagents

Donepezil hydrochloride; Acetonitrile; Water and Methanol AR grade

2.3 Method Development

2.3.1 Selection of Solvent and Detection wavelength

Drug solution of 20µg/ml was scanned over the range of 200-400 nm in UV region using different solvents like hexane, ethanol, cyclohexane, methanol, Water and Acetonitrile. It was observed that the drug showed maximum absorbance in Acetonitrile and Water at 231 nm and hence methanol was used as solvent and 231nm was used as maximum wavelength for detection of Donepezil for further study.

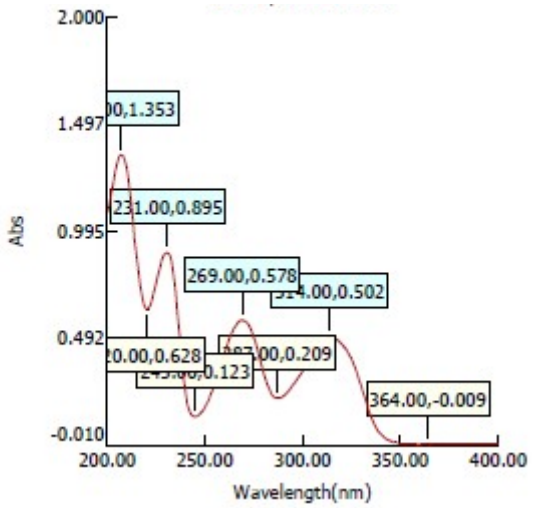

Fig.1: UV Spectrum of Donepezil

2.4 Preparation of Standard Stock Solution

Accurately weighed 100mg of Donepezil was dissolved in 100ml of Acetonitrile and Water, which is considered as a stock solution (1mg/ml). Take 10ml of 1mg/ml solution into 100ml volumetric flask to get 100µg/ml concentration and make up with AR grade methanol and working standard solutions were diluted further to get concentration range 4-20µg/ml.

2.5 Preparation of Dilutions for Calibration Curve Construction

Dilute the working standard solution (100µg/ml) by pipetting 10ml of working standard solution into 100ml volumetric flask and filling up the volume with methanol to make 10µg/ml concentration solution. Now pipette 0.4, 0.8, 1.2,1.6 and 2ml of 10ml solutions into 10ml volumetric flasks and make up the volume to get 10ml with Acetonitrile and Water. This gives dilutions of 4, 8, 12, 16 and 20 µg/ml solutions, respectively.

2.6 Validation Parameters

Validation parameters are calculated according to International Conference on Harmonization (ICH) guidelines - validation of analytical procedures: text and methodology Q2 (R1).

System Suitability

System suitability is checked by taking absorbance of six replicates of 12µg/ml concentration and the percentage relative standard deviation is found to be less than 2.

Linearity and Range

The standard calibration curve was constructed between concentrations $v_{s.}$ absorbance and the linearity was found in the range from 4µg/ml to 20µg/ml region i.e. the absorbance values in the range from 4µg/ml to 20µg/ml. The regression equation and correlation coefficient were calculated and found to be within the required limits.

Accuracy

To the pre-analyzed sample three different amounts of 50%, 100% and 150% of working standard was added, at each level 3 replicate samples were prepared, and samples were analyzed to determine percentage recovery from the sample. Percentage recovery is calculated for all the nine readings from the ratio of amount of drug added by amount of drug found. Further statistical parameters such as mean, standard deviation and percentage relative standard deviation are calculated for percentage recovery data. The results were found within the limits.

Precision

The precision was determined for Donepezil in terms of intraday and interday precision. Sample solution of 100µg/ml was prepared from stock solution and injected into the system six times at two different times in a day (intraday) and between two days (interday). Statistical parameters such as mean, standard deviation and percentage relative standard deviation are calculated. The percentage assay of each individual sample is between 97% - 102% and percentage RSD is NMT 2%. Hence, the results were found within the limits.

Robustness

Robustness of the method was determined by changing the wavelength ±1nm from actual wavelength 231nm.The %RSD of the absorbance was found to be less than 2.

Ruggedness

The ruggedness of an analytical method is degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times, different assay temperatures, different days, etc. Ruggedness is normally expressed as a lack of influence on test results of operational and environmental variables of the analytical method. The standard stock solution and sample stock solution were prepared by different analysts on different days and the absorbances of the resulting solutions were measured. The value should be between 92-102%.

Specificity

The specificity of an analytical method is its ability to measure accurately and specifically the analyte in the presence of compounds that may be expected to be present in the sample matrix. The specificity of the analytical method was determined by analyzing the placebo solution under the same experimental conditions as the assay.

Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value under the stated, experimental conditions. Several approaches for determining the detection limit are possible, depending on whether the

procedure is non-instrumental or instrumental. A specific calibration curve was studied using samples containing an analyte in the range of detection limit. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

Limit of Quantitation

It is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Several approaches for determining the quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental. A specific calibration curve was studied using samples containing the analyte in the range of quantitation limit. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

3. RESULTS AND DISCUSSION

UV Spectroscopic method was developed for Donepezil. The drug wavelength at maximum absorbance was found to be 231 nm for 12µg/ml solution.

3.1 System suitability:

The results of system suitability in UV Spectrophotometer are summarized in Table-2.

Table-2: Results of system suitability

Concentration(µg/ml)	Absorbance
4	0.164
8	0.330
12	0.459
16	0.631
20	0.766
Correlation coefficient	0.999
Slope (m)	0.037
Intercept(c)	0.01

Inference: The obtained experimental values in system suitability trials (n=6) were found to be within the limits proposed by ICH guideline.

3.2 Linearity

The results of Linearity are summarized in Table-3. The calibration curve constructed is represented in Fig. 2.

Table-3: Results for linearity

S. No	Concentration(µg/ml)	Absorbance	Statistical parameters
1	12	0.438	Mean=0.447 SD=0.006 %RSD=1.52
2		0.449	
3		0.459	
4		0.446	
5		0.447	
6		0.445	

Inference:

The results indicate that an excellent correlation exists between the absorbance and concentration of drug.

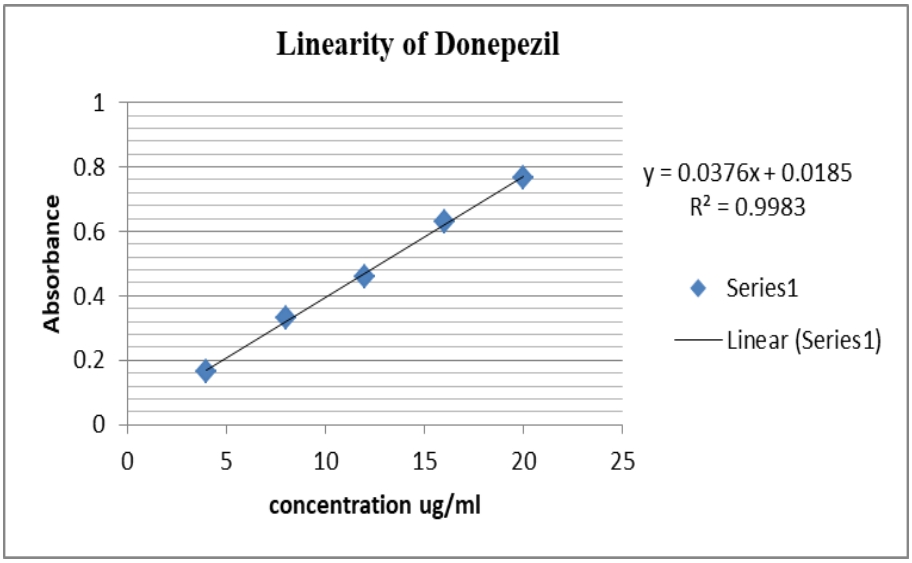

Fig. 2: Calibration curve of Donepezil

3.3 Accuracy

Table-4: Results for accuracy

S. No	Concentration Level (%)	Amount added(µg/ml) Std drug	sample	Amount found(µg/ml)	%Recovery	Statistical parameters
1	50	8	12	19.98	99.66	Mean=100.21 SD=0.587 %RSD=0.59
2		8	12	20.01	100.16	
3		8	12	20.05	100.83	
4	100	12	12	24.01	100.12	Mean=100.24 SD=0.453 %RSD=0.45
5		12	12	23.99	99.87	
6		12	12	24.06	100.75	
7	150	16	12	28.01	100.08	Mean=99.97 SD=0.19 %RSD=0.19
8		16	12	28.01	100.08	
9		16	12	27.97	99.75	

Inference: The results represent the high percent recovery values indicating that the proposed method is accurate.

3.4 Precision

Intraday precision: The results of Intraday precision are represented in Table-5.

Table-5: Results of intraday precision

S. No	Absorbance	%Assay	Statistical parameter
1	0.438	99.81	Mean=99.78 SD=0.069 %RSD=0.07
2	0.449	99.85	
3	0.459	99.79	
4	0.446	99.80	
5	0.447	99.65	
6	0.445	99.81	

Inter-day precision: The results of Inter-day precision are represented in Table-6.

Table-6: Results of inter-day precision

S. No	Absorbance	% Assay	Statistical parameter
1	0.450	100.02	Mean=99.92 SD=0.077 %RSD=0.08
2	0.457	99.93	
3	0.459	99.89	
4	0.452	99.85	
5	0.455	100.01	
6	0.454	99.84	

Inference: The % RSD for Intraday precision and interday precision for Donepezil were found to be 0.07 and 0.08 which indicates the method is precise.

3.5 Limit of Detection and Limit of Quantitation (LOD & LOQ):

The LOD and LOQ of Donepezil Was found as 0.197 µg/mL and 0.6 µg/mL, respectively.

3.6 Robustness

The results of robustness are represented in Table-7.

Table 7: Results of Robustness

Concentration (µg/mL)	S. No	234m	231 nm	227 nm
12	1	0.457	0.459	0.456
	2	0.521	0.458	0.454
	3	0.454	0.459	0.455
	4	0.456	0.457	0.457
	5	0.458	0.458	0.457
	6	0.458	0.459	0.456
	Mean SD	0.454 0.02	0.457 0.0008	0.454 0.001
	%RSD	0.64	0.17	0.25

Inference: All the experimental values for robustness obtained fall into the acceptance criteria

4. CONCLUSION

A simple, precise, economic, accurate and efficient UV Spectroscopic method was developed for Donepezil. The solubility of drug was obtained from the literature survey. The drug wavelength at maximum absorbance was found to be 231 nm. Quantitative linearity was found in the concentration range of 4-20µg/ml. The regression equation for linear range of concentrations was found to be y = 0.037x+ 0.018. The limit of detection and limit of quantification were found at 0.198µg/ml and 0.68µg/ml respectively indicating the sensitivity of the method. The proposed method has been validated according to the ICH guidelines and can be successfully applied to estimate the levels of Donepezil in bulk form.

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