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METHOD DEVELOPMENT AND VALIDATION OF DONEPEZIL HYDROCHLORIDE BY USING UV SPECTROPHOTOMETRIC METHOD

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Abstract: Simple, specific, accurate and cost economic UV spectrophotometric methods were developed and validated for determination of Donepezil Hydrochloride. Instead of using organic solvents, mixture of Acetonitrile and water was used during method development and validation. Donepezil hydrochloride standard solution was scanned in the UV range (400-200nm) in a 1cm quartz cell in a double beam UV spectrophotometer. The standard solution of Donepezil Hydrochloride showed maximum absorption at wavelength 231 nm. The method obeys Beer's law in the concentration range from 4-20µg/ml. The correlation coefficient was found to be 0.9983and regression of the curve was found Y=0.0376x+0.0185 with excellent recovery 99.66-100.83%. Limit of detection and limit of quantification were found to be 0.197µg/ml and 0.6µg/ml respectively. The ruggedness and robustness were performed. The method was validated for several parameters like accuracy, precision as per ICH guidelines. Statistical analysis proved that the methods are repeatable and specific for determination of the drug. These methods can be adopted in the routine assay analysis of Donepezil Hydrochloride in API and pharmaceutical dosage form.

Keywords: Uv- Spectrophotometer, Donepezil Hydrochloride, ICH guidelines, Validation, Concentrations, Method development

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INTRODUCTION

Donepezil Hydrochloride(RS)-2-[(1-Benzyl-4-piperidyl)methyl]-5,6-dimethoxy-2,3dihydroinden-1-one, is a centrally acting reversible acetyl cholinesterase inhibitor. Its main therapeutic use is in the treatment of Alzheimer's disease where it is used to increase cortical acetylcholine. Donepezil is postulated to exert its therapeutic effect by enhancing cholinergic function. This is accomplished by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by acetyl cholinesterase. Donepezil hydrochloride is not yet official in any pharmacopeia, where, only few analytical methods have been reported for its determination in pharmaceutical formulations. Such methods include HPLC, colorimetric methods. Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity, and low cost. This study presents a new spectrophotometric method for the determination of Donepezil hydrochloride pharmaceutical formulations. Accordingly, the objective of this study was to develop and validate the UV-spectrophotometric method for the estimation of Donepezil hydrochloride in pharmaceutical formulations as per ICH guidelines.

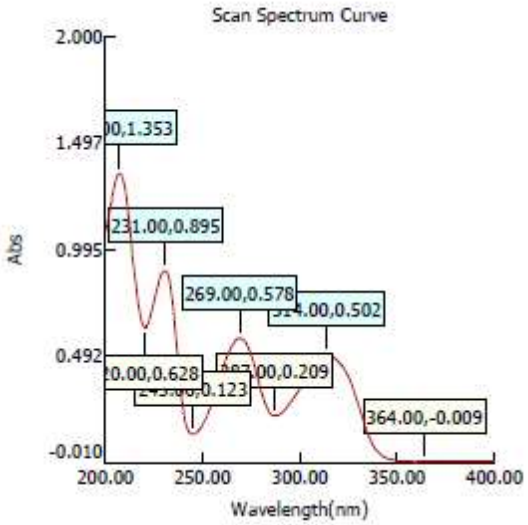
MATERIALS AND METHODS:

Sr.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

METHOD DEVELOPMENT

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.



UV Spectrum of Donepezil

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.

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 Acetontrile and Water. This gives dilutions of 4, 8, 12, 16 and 20µg/ml solutions respectively.

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 as reported in the table no 1

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 as shown in the table no .2

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 as shown in the table no.3

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 as shown in the table no .4,5

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 as shown in the table no 6

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.

System suitability:

Table.1 system suitability in Uv

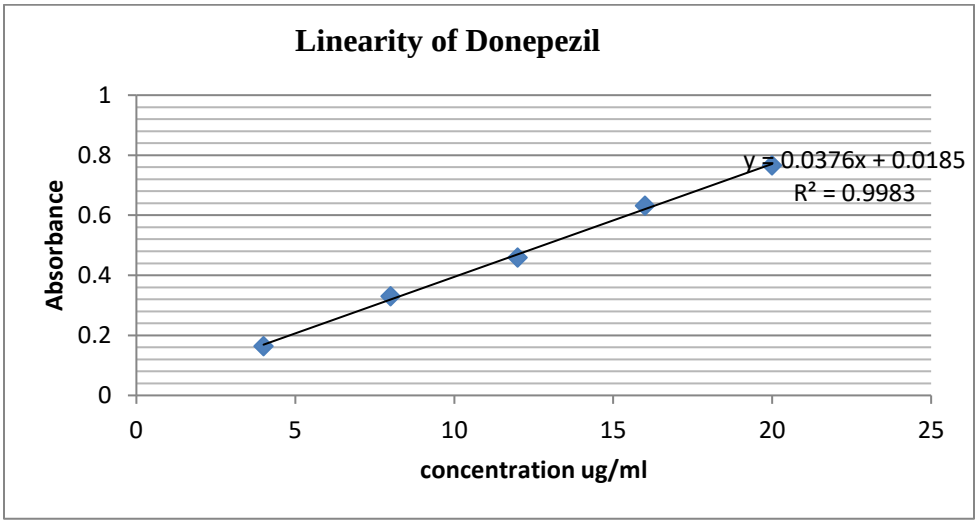
Concentration(µg/ml)	Absorbance
4	0.164
8	0.330
12	0.459
16	0.631
20	0.766
Correlation coefficient	0.999
Slope(m)	0.037
Intercept(c)	0.01

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

Linearity:

Table.2 Results for linearity in UV

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	



Calibration curve of Donepezil in UV

Inference:

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

Accuracy:

Table.3 Results for accuracy in UV

S.No	Concentration Level (%)	Amount added(µg/ml)		Amount found(µg/ml)	%Recovery	Statistical parameters
		Std drug	sample			
1	50	8	12	19.98	99.66	Mean=100.21
2		8	12	20.01	100.16	SD=0.587
3		8	12	20.05	100.83	%RSD=0.59
4		12	12	24.01	100.12	Mean=100.24

5	100	12	12	23.99	99.87	SD=0.453
6		12	12	24.06	100.75	%RSD=0.45
7	150	16	12	28.01	100.08	Mean=99.97
8		16	12	28.01	100.08	SD=0.19
9		16	12	27.97	99.75	%RSD=0.19

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

PRECISION:

Intraday precision:

Table 4 Results for Intraday precision in UV

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

Interday precision:

Table. 5 Results for interday precision in UV

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

LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD & LOQ):

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

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

Table. 6 Results for robustness in UV

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

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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