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DEVELOPMENT AND VALIDATION OF A NEW ANALYTICAL UV SPECTROSCOPIC METHOD FOR ANALYSIS OF DONEPEZIL IN BULK POWDER AND TABLET DOSAGE FORM

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Abstract:

A new simple, accurate, rapid, precise, reproducible and cost effective spectrophotometric method for the quantitative estimation of Donepezil in bulk and pharmaceutical dosage form. The developed visible spectrophotometric method for the quantitative estimation of Donepezil is based on measurement of absorption at maximum wavelength 278 nm using methanol as a solvent. The stock solution of Donepezil was prepared, and subsequent suitable dilution was prepared in distilled water to obtained standard curve. The standard solution of Donepezil shows absorption maxima at 278 nm. The drug obeyed beer lambert's law in the concentration range of 0-60 µg/ml with regression 0.999 at 278 nm. The overall % recovery was found to be 100.167% which reflects that the method was free from the interference of the impurities and other excipients used in the bulk and marketed dosage form. The low value of % RSD was indicative of accuracy and reproducibility of the method. The % RSD for precision was found to be 0.864%. The %RSD for inter-day and intra-day precision was found to be 0.807 and 0.881 respectively which is <2% hence proved that method is precise. The results of analysis have been validated as per International Conference on Harmonization (ICH) guidelines. The developed method can be adopted in routine analysis of Donepezil in bulk and tablet dosage form.

Keywords: *Donepezil, UV-Spectrophotometry, Methanol, Accuracy, Precision.*

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INTRODUCTION:

Donepezil is a Cholinesterase Inhibitor. The mechanism of action of donepezil is as a Cholinesterase Inhibitor. In 2016, the global burden of dementia was estimated to be 43.8 million, demonstrating a significant increase from a global prevalence of 20.2 million in 1990 [1]. Donepezil, also known as Aricept, is a piperidine derivative acetylcholinesterase inhibitor used in the management of the dementia of Alzheimer's Disease, and in some cases, is used to manage other types of dementia. Donepezil was first approved by the FDA in 1996, and its extended-release form was approved in combination with [Memantine] in 2014 to manage moderate and severe forms of Alzheimer's dementia. A donepezil transdermal delivery system, Adlarity, was approved by the FDA in March 2022 for the treatment of Alzheimer's dementia [2]. Though it does not alter the progression of Alzheimer's disease, donepezil is effective in managing the symptoms of its associated dementia. Donepezil is the hydrochloride salt of a piperidine derivative with neurocognitive-enhancing activity. Donepezil reversibly inhibits acetyl cholinesterase, thereby blocking the hydrolysis of the neurotransmitter acetylcholine and, consequently, increasing its activity [3]. This agent may improve neurocognitive function in Alzheimer's disease, reduce sedation associated with opioid treatment of cancer pain, and improve neurocognitive function in patients who have received radiation therapy for primary brain tumours or brain metastases. The IUPAC Name of Donepezil is 2-[(1-benzylpiperidin-4-yl) methyl]-5, 6-dimethoxy-2, 3-dihydro-1H-inden-1-one. The Chemical Structure of Donepezil is shown in following figure-1.

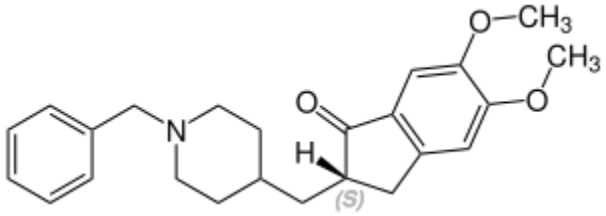

Fig-1: Chemical Structure of Donepezil

EXPERIMENTAL:

Table-1: List of Instruments Used

S.No.	Instruments/Equipments/Apparatus
1	**ELICO SL-159** UV – Vis spectrophotometer
2	Electronic Balance **(SHIMADZU ATY224)**
3	Ultra Sonicator **(Wensar wuc-2L)**
4	P^H Analyzer **(ELICO)**
5	Triple Quartz Distillation Unit **(BOROSIL)**
6	Vaccum filtration Kit **(BOROSIL)**

Table-2: List of Chemicals, Reagents and Standards Used

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	----	----	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	A.R.	Loba Chem; Mumbai.
3.	Ethanol	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Chloroform	99.9%	HPLC	Loba Chem; Mumbai.
5.	Hydrochloric acid	99.9	L.R.	Sd fine-Chem ltd; Mumbai
6.	Sodium Hydroxide	99.9	L.R.	Sd fine-Chem ltd; Mumbai
7.	Sodium nitrite	99.9	L.R.	Sd fine-Chem ltd; Mumbai
8.	MBTH Reagent	99.99	L.R.	Sd fine-Chem ltd; Mumbai
9.	Ferric Chloride	99.99	L.R.	Sd fine-Chem ltd; Mumbai

Analytical Method Development:

Instrumentation:

The Spectroscopic analysis was carried out using Double beam PG Instruments recording UV-Visible Spectrophotometer ELICO SL-159 with 1mm path length matched quartz cells was used for analytical purpose [4].

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Donepezil working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol (1000 ppm).

Further pipette 1ml of the above Donepezil stock solution into a 10ml volumetric flask and dilute up to the mark with Methanol (100ppm).

Further pipette 1ml of the above Donepezil stock solution into a 10ml volumetric flask and dilute up to the mark with Methanol (10ppm) [5].

Preparation of Sample Solution:

Take average weight of the Powder and weight 10 mg equivalent weight of Donepezil sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (1000ppm).

Further pipette 1ml of the above Donepezil stock solution into a 10ml volumetric flask and dilute up to the mark with Methanol (100ppm).

Further pipette 1ml of the above Donepezil stock solution into a 10ml volumetric flask and dilute up to the mark with Methanol (10ppm).

Procedure:

Measure the samples by checking in the UV Spectroscopy and record the absorbance, note the conditions of proper conditions for performing validation parameters as per ICH guidelines [6-9].

RESULTS AND DISCUSSION:

Development of a New Method:

OPTIMIZATION OF METHOD:

Optimization of Selection of Solvent:

It is well known that the solvents do exerts a profound effect on the quality and the shape of the peak. The choices of solvents for UV method development are: Acetonitrile, Ethanol, Chloroform, Acetone, Methanol, Dimethyl sulfoxide (DMSO), Dimethyl formamide etc. First optimize the different solvents [10]. From that solvents Methanol satisfied the all the optimized conditions.

Selection of Wavelength:

The standard solutions are prepares by transferring the standard drug in a selected solvent and finally

diluting with the same solvent. That prepared solution is scanned in the UV wavelength range of 200-400nm.This has been performed to know the maxima of Donepezil, so that the same wave number can be utilized in UV detector for estimating the Donepezil. While scanning the Donepezil solution we observed the maxima at 278 nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450 [11]. The scanned UV spectrum is attached in the following page. The λ_{max} of the Donepezil was found to be 278 nm in methanol as solvent system.

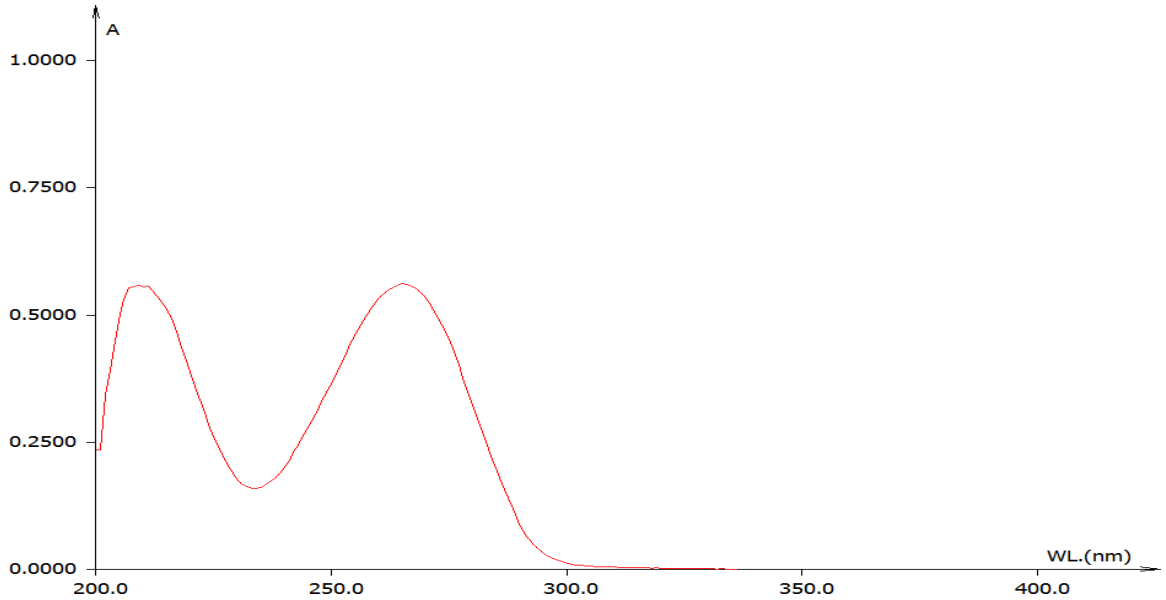

Fig-2: UV Spectrum of Donepezil at 278nm

Preparation of Calibration Curve for Donepezil:

Standard solutions of Donepezil in the concentration range of 10 µg/ml to 60 µg/ml were obtained by transferring (1,2,3,4 and 5,6, ml) of Donepezil stock solution (100 ppm) to the series of clean and dry 10 ml volumetric flasks [12]. The volumes in each volumetric flask were made up with the solvent system and mixed.

The absorbencies of the solutions were measured at 278 nm against the solvent system as blank and calibration curve is plotted. The Lambert-Beer's Law is linear in concentration range of 10 to 60 µg/ml at 278 nm for Donepezil. The results are shown in Table no.2.

Table-2: Results of Calibration Curve

Concentration (µg/ml)	Absorbance (n=6)
0	0
10	0.214
20	0.415
30	0.631
40	0.844
50	1.035
60	1.236

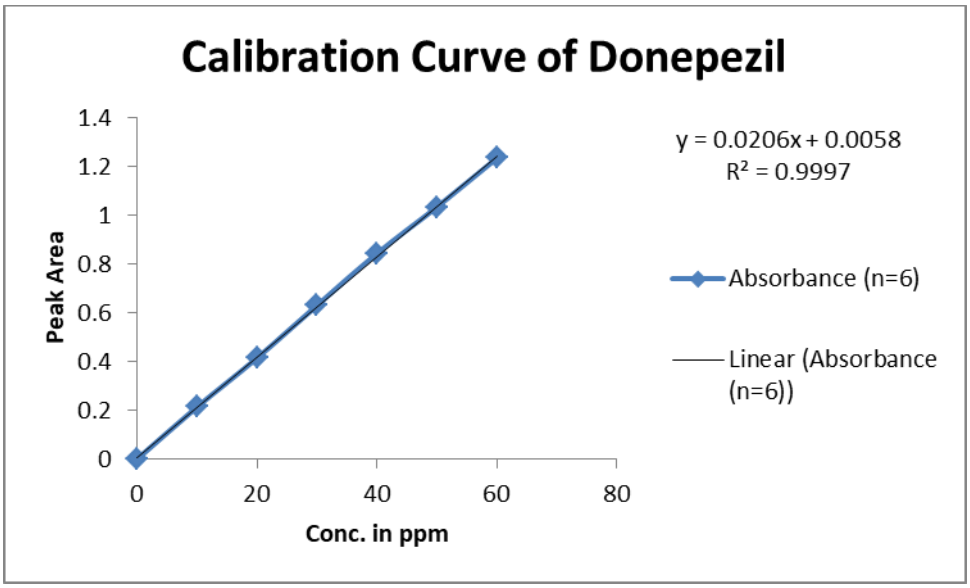

Fig-3: Calibration Curve for Donepezil at 278 nm

Result & Discussion:

For Donepezil the Beer- Lambert's law is obeyed in concentration range of 10 to 60 µg/ml at 278nm. Moreover, in the linearity study at consecutive wavelength, the linear regression equation for Donepezil, calibration curve at 278nm was calculated by y = 0.0206x + 0.0058 (R^2 = 0.9997).

METHOD VALIDATION:

Validation of the Developed Method According to I.C.H guidelines

Following parameters were taken into consideration for validation of proposed method:

Linearity

Method: As per Test Assessed under Above Linearity (Plotting a Calibration Curve)

Preparation of Dilutions of Donepezil for Linearity Study

Standard solutions of Donepezil in the concentration range of 10 µg/ml to 60 µg/ml were obtained by transferring (1,2,3 and 4,5,6, ml) of Donepezil stock solution (100ppm) to the series of clean & dry 10 ml volumetric flasks. The volumes in each volumetric flask were made up with the solvent system and mixed [13].

The Absorbances of the solutions were measured at 278 nm against the solvent system as blank and calibration curve is plotted. The Lambert-Beer's Law is linear in concentration range of 10 to 60 µg/ml at 278 nm for Donepezil. The results are shown in Table no.3.

Table-3: Results of Linear Curve

Concentration (µg/ml)	Absorbance (n=6)
0	0
10	0.214
20	0.415
30	0.631
40	0.844
50	1.035
60	1.236

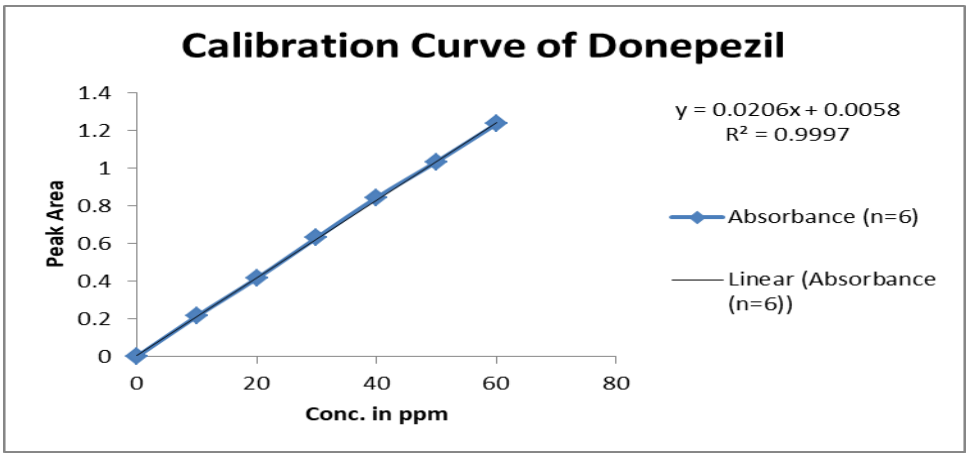

Fig-4: Calibration Curve for Donepezil at 278 nm.

Result & Discussion:

Linearity range was found to be 10-60 µg/ml for Donepezil at 278 nm. The correlation coefficient was found to be 0.9997, which shows good linearity between above range. The slope was found to be 0.0206 and intercept was found to be 0.0058 which was close to zero intercept.

Range

Range of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. It includes working range, linearity range and target range and 100% concentration or test concentration [14]. The range my developed method concentrations are 10-60 µg/ml.

Accuracy

The accuracy is nothing but the comparison of obtained value with the standard value. After completion of analysis of Donepezil containing 3 group 3 replicates with the bulk and Marketed pharmaceutical dosage form.

Method: The accuracy of the developed method can be studied by preparing the solutions of various concentrations i.e. 80%, 100% and 120%. In these concentrations the amount of marketed pharmaceutical dosage form was kept as constant and the quantity of pure drug (API) is varied [15]. The prepared solutions in triplicates and here determined is percentage recovery of pure drug. The results obtained from the accuracy studies are shown in Table-4.

In nine different 10 ml volumetric flasks, 1 ml of the pre-analysed tablet solution (10 µg/ml) was taken and added 1, 2, 3 ml of standard solution of bulk (API) mixture (100µg/ml) and the volume was made up to 10 ml with methanol.

The results are shown in Table no.4.

Table-4: Data of Recovery studies

Level of Recovery	Sample Conc. (µg/ml)	Standard Conc. Added (µg/ml)	Total Conc. (µg/ml)	Amount Recovered (µg/ml)	% Recovery	Mean % Recovery ± SD	% RSD
80%	8	10	18	18.025	100.138	100.1273 ± 0.200213	0.199959
80%	8	10	18	17.986	99.922		
80%	8	10	18	18.058	100.322		
100%	10	10	20	20.064	100.320	100.165 ± 0.195768	0.195445
100%	10	10	20	19.989	99.945		
100%	10	10	20	20.046	100.230		
120%	12	10	22	22.075	100.340	100.210 ± 0.253385	0.252854
120%	12	10	22	21.982	99.918		
120%	12	10	22	22.082	100.372		

Result & Discussion:
The results obtained for the accuracy study (recovery method) from three sample studies (n = 3) for each level indicated that the mean of the % recovery was 100.1273% and 100.165% and 100.210% and %R.S.D was found to be 0.199959%, 0.195445% and 0.252854% for Donepezil in mixture (Donepezil- 10 µg/ml). Here the mean % recovery is in between 98-102 % thus showing that the analytical technique has a good recovery study [16].

Precision:
The precision of developed analytical method said to be the closeness of agreement between a series of measurement obtained from the multiple sampling of the homogenous sample solution under the prescribed experimental conditions. The precision of the developed method can be analysed by the 5 or 6 different homogenous solutions and the respective are noted down [17]. The results are shown in the precision is % RSD. The results obtained from the precision studies are shown in the Table-5.

The precision can be divided into following types. 1. Repeatability and 2. Intermediate precision. In this first one is Repeatability or Intra-day precision was determined on six replicates of same sample solutions on the same day. Inter-day precision was estimated by analyzing newly prepared sample solutions in triplicate over the 3 consecutive days. Both inter day and intraday precision was expressed as % RSD. The % RSD values for intraday precision for Method A was found to be within the limits. The % RSD for inter day precision for Method A found to be within the limits. The results were summarized in Table-5. The low value of % RSD for the method indicates the high precision of the method.

Repeatability
Repeatability was assessed using:
Six time repetition of target concentration 100 % that is (10µg/ml).
Intermediate precision can be assessed by intra-day and inter day analysis [18].

Method: In the study of the repeatability precision which was conducted on the solution which has the concentration value 100 % of the target concentration (n = 6).
The results are shown in Table-5.

Table-5: Data of Repeatability of Absorbances

S.No.	Conc. (µg/ml)	Wavelength (nm)	Absorbance
1	10	278	0.214
2	10	278	0.217
3	10	278	0.216
4	10	278	0.219
5	10	278	0.215
6	10	278	0.218
Mean ± S.D.	**0**	**0**	**0.2165**
Standard Deviation	**0**	**0**	**0.001871**
% RSD	**0**	**0**	**0.864124**

Result & Discussion:
Repeatability study showed a R.S.D of 0.864124% for Donepezil. Thus it is concluded that the analytical technique has a good repeatability precision as R.S.D for the drug were less than 2 %.

Intermediate Precision:

Intra-Day & Inter-Day:
The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Donepezil revealed that the proposed method is precise [19].

Table-6: Intra-Day and Inter-Day Precision for Method

Con. taken (µg/mL)	Observed Conc. of Donepezil (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Con. found (µg/mL)	% RSD	Con. found (µg/mL)	% RSD
8	7.983	0.986	8.068	0.598
10	10.014	0.752	10.054	0.964
12	12.136	0.685	12.365	1.083

Robustness:

Robustness of the method was determined by carrying out the analysis under different temperature condition i.e. at 23°C, 25°C and at 28°C. The respective Absorbances of 10µg/ml were noted and the result was indicated as % RSD (following tables) [20].

Temperature-23°C

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.215	Mean = 0.216 SD = 0.001789 % RSD = 0.828173
10	0.219	
10	0.214	
10	0.217	
10	0.216	
10	0.215	

Temperature-25°C

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.228	Mean = 0.225833 SD = 0.002787 % RSD = 1.23404
10	0.229	
10	0.227	
10	0.226	
10	0.222	
10	0.223	

Temperature-28°C

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.236	Mean = 0.236833 SD = 0.001835 % RSD = 0.774742
10	0.238	
10	0.234	
10	0.239	
10	0.238	
10	0.236	

Ruggedness:

In the ruggedness study, the influence of small, deliberate variations of the analytical parameters on the absorbance of the drug was examined. The factor selected was a change in the analyst. The Ruggedness of the method was determined by carrying out the analysis by different analyst and the respective absorbance of 10µg/ml was noted [21]. The result was indicated as %RSD (Table No-7).

Table-7: Results Showing Ruggedness for Donepezil

Analyst-1

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.219	Mean = 0.215833 SD = 0.002787 % RSD = 1.291216
10	0.212	
10	0.213	
10	0.218	
10	0.216	
10	0.217	

Analyst-2

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.256	Mean = 0.255333 SD = 0.002338 % RSD = 0.915701
10	0.252	
10	0.254	
10	0.259	
10	0.256	
10	0.255	

Analyst-3

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.262	Mean = 0.265333 SD = 0.002805 % RSD = 1.05707
10	0.268	
10	0.266	
10	0.264	
10	0.269	
10	0.263	

Specificity:
The presence of excipients in formulation does not interfere with the drug Absorbances. Therefore, the proposed method was found specific and selective for the drug[22].

Limit of Detection and Limit of Quantification
The limit of detection (LOD) and the limit of quantification limit (LOQ) are measured by using the following equations:

L.O.D. = 3.3 (SD/S).

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The slope S and the SD may be estimated from the calibration curve of the analyte/sample.

Result & Discussion:
The LOD was found to be 0.369 µg/ml and LOQ was found to be 1.107 µg/ml for Donepezil respectively which represents that sensitivity of the method is high[23].

Analysis of Marketed Formulation (Assay):
Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 10 mg of Donepezil was accurately weighed and transferred to a 10 ml volumetric flask. A few ml of diluent was added and sonicated for 15 min. Volume was made

upto the mark with methanol. An aliquot of 1ml was transferred to a 10ml volumetric flask and the volume was made up to the mark to obtain 10µg/ml of Donepezil. The solution was filtered using 0.45µ Millipore PVDF filter. This solution was prepared six times and the absorbance of each solution was determined at 278 nm and the concentration of drug in sample solution was determined from calibration curve [24-26].

Table-8: Assay Results of Tablet Dosage Form

Marketed Formulation	Actual Amount (mg)	Amount Found ± SD (mg)	% of Drug Found ± SD
Donep 10 Tablet (Alkem Laboratories Ltd)	10mg	9.587 ± 0.635	99.745 ± 0.364

SUMMARY AND CONCLUSION:

The standard solutions of Donepezil in Methanol (10µg/ml) subjected to a scan individually at the series of wavelengths of 200 nm to 400 nm. Absorption maximum of Donepezil was found to be at 278 nm. Therefore, 278nm was selected as λ_{max} of Donepezil for the present study. The calibration curve of Donepezil was found to be linear in the range of 10-60 µg/ml at 278 nm. Therefore, it was clear that Donepezil can be determined without interference of any irrelevant substance in single component pharmaceutical products. The used technique was initially attempted on bulk drugs in their synthetic sample and concentrations were estimated.

The % recovery was carried out at 3 levels, 80%, 100% and 120% of Donepezil standard concentration. Three samples were prepared for each recovery level. The solutions were then analysed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for marketed tablet dosage form. The newly developed method was validated according to the ICH guidelines and the method validation parameters.

The developed method was subjected to do the various method validation parameters such as specificity, accuracy, precision, linearity and range, limit of detection and limit of quantification, robustness and ruggedness etc.

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