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Original Article

# DEVELOPMENT OF UV SPECTROPHOTOMETRIC METHOD FOR THE ANALYSIS OF ACETYLCHOLINESTERASE INHIBITOR

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**ABSTRACT**

**Objective**: The aim of this research paper was to develop a simple, sensitive, rapid, accurate and economical Ultra Violet spectrophotometric method for the Analysis of Acetylcholinesterase Inhibitor.

**Methods:** The study was performed in pH 7.4 phosphate buffer and presence of the drug was analysed using UV spectrophotometer. Various analytical parameters such as linearity, range, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were determined according International Conference on Harmonization (ICH) guidelines.

**Results:** Absorbance maximum (ʎmax) of drug in pH 7.4 phosphate buffer were found to be 270.5 nm**.** Beer's law was obeyed over the concentration range of 2 - 20 mg/ml with a correlation coefficient ($R^2$) value of 0.999. % RSD values below 2 at different concentration levels for Intra and inter-day precision indicated that the proposed spectrophotometric method is highly reproducible. LOD and LOQ were 0.401 and 1.22 µg/ml respectively signifying that it can be adopted for routine quality testing.

**Conclusion:** The results of the study demonstrated that the developed method is accurate, precise and reproducible while being simple, cheap and less time consuming and hence can be suitably applied for the analysis of donepezil in pharmaceutical preparations.

**Keywords:** Acetylcholinesterase inhibitor, Donepezil, Quantitative determination, UV spectrophotometric method.

## INTRODUCTION

In this study, the donepezil was selected as Acetylcholinesterase Inhibitor. Donepezil occurs as a white crystalline powder and chemically known as 2-[(1-benzyl-4-piperidyl) methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one with molecular weight of 379.492 g $mol^{-1}$ (Fig. 1) [1]. It is a piperidine-based, reversible and non-competitive inhibitor of the enzyme acetylcholinesterase [2]. It produces obvious and long-lasting inhibition of brain cholinesterase without marked effects on cholinesterase in peripheral tissues and increases the brain content of acetylcholine in vivo [3]. It is the second drug approved by the FDA for the treatment of mild to moderate dementia of the Alzheimer's type. Alzheimer disease is a neurodegenerative disorder characterized by progressive loss of memory followed by complete dementia [4].

A consistent pathological change in Alzheimer's disease is the degeneration of cholinergic neuronal pathways that project from the basal forebrain to the cerebral cortex and hippocampus. The resulting hypofunction of the cholinergic systems is thought to account for some of the clinical manifestations of dementia [5]. Donepezil is postulated to exert its therapeutic effect by enhancing cholinergic function and acetylcholine levels of the brain. This is accomplished by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by acetyl cholinesterase [6]. The recommended initial dose of donepezil is 5 mg taken once daily [7]. A 50% inhibition of acetylcholinesterase activity is obtained at a plasma drug concentration of 15.6 ng/ml, and the inhibition plateaus at the plasma concentration of donepezil higher than 50 ng/ml [8].

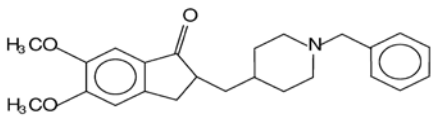


**Fig. 1: Structure of donepezil**

Several analytical techniques have been used for the analysis of donepezil in pharmaceutical preparation. These include high performance liquid chromatography (HPLC) [9-11] fluorescence (FL) [12] or mass spectrometric (MS) detector [13], solvent extraction spectrophotometry [14], spectrofluorimetry [15] and colorimeter [16] etc. In the luminosity and fact of above literature a simple UV spectrophotometric method for the analysis of donepezil has been developed. The proposed method offers several advantages over HPLC methods which are very sensitive but need sophisticated instrumentation & expert hands [9-13] and extraction procedure always not quantitative [14].

The present procedure neither requires any extraction nor any elaborate equipment and the method is less time consuming. So, the aim of this research work was to develop a UV spectrophotometric method for the determination of donepezil in pharmaceutical preparations. UV spectrophotometry is still popular because of the inherent simplicity, low cost, sensitivity, speed and reliability for determination of drugs in pharmaceutical preparations.

The different analytical performance parameters such as linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were determined according to ICH Q2 (R1) guidelines [17].

## MATERIALS AND METHODS

### Instruments

A Shimadzu UV visible spectrophotometer (UV -1800, Shimadzu Corporation, Kyoto, Japan) was used for all absorbance measurements with one cm matched quartz cells and Shimadzu electronic balance (AUX 220, Shimadzu Corporation, Kyoto, Japan) was used for weighing the sample.

### Materials

Donepezil was received as a gift sample from Jubilant Life Sciences Ltd. Noida, UP (India), Potassium dihydrogen phosphate was

purchased from Qualigens fine chemicals, Mumbai (India), and Sodium hydroxide was purchased from Fisher scientific, Mumbai (India). All chemicals and reagents used were of analytical grade. Double distilled water was used to prepare solutions wherever required and it was filtered before use through a 0.22 µm membrane filter.

**Methods**

**Preparation of phosphate buffer pH 7.4**

The phosphate buffer pH 7.4 was prepared as per specifications given in Indian Pharmacopoeia [18].

**Preparation of 0.2 M potassium dihydrogen phosphate solution**

27.218 gm of potassium dihydrogen phosphate was dissolved in 1000 ml of distilled water to produce 0.2M solution of potassium dihydrogen phosphate.

**Preparation of 0.2 M NaOH solution**

8 gm of sodium hydroxide was dissolved in 1000 ml of distilled water to produce 0.2M sodium hydroxide solution

**Preparation of buffer**

50 ml of 0.2 M potassium dihydrogen phosphate was placed in 200 ml of volumetric flask, and then pH was adjusted to 7.4 0 ± 0.05 by adding 39.1 ml of 0.2 M NaOH solution. Finally, the volume was made upto 200 ml with distilled water, and then filtered through 0.22 µm membrane filter.

**Determination of wavelength of maximum absorption and preparation of calibration curve**

A standard stock solution of drug sample (100 µg/ml) was prepared by dissolving 10 mg of the drug in small quantity methanol in a 100 ml of volumetric flask and then volume was made upto mark with phosphate buffer (pH 7.4). The dilutions of this stock solution were made by diluting the required aliquot with phosphate buffer to obtain solutions in the range of 2- 20 µg/ml. An UV spectroscopic scanning (200– 400 nm) was carried out with drug solutions to determine the absorbance and maximum absorption ($\lambda$ max) using phosphate buffer (pH 7.4) as blank.

**Validation Procedure**

Method was validated according to International Conference on Harmonization (ICH) guidelines [17] in terms of linearity, range, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ).

**Linearity and range**

Linearity is the ability of the method to obtain test results that are directly proportional to analyte concentration within a given range. The range of an analytical method is the interval between the upper and lower concentration of analyte for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

To study the linearity, serial dilutions of donepezil were suitably prepared in the concentration range of 2 - 20 µg/ ml in phosphate buffer of pH 7.4. The absorbance of each solution was scanned at 270.5 nm using phosphate buffer as blank. Calibration curve was constructed by plotting concentration versus absorbance on x and y axis respectively. Linearity was determined by the regression equation. This experiment was repeated 3 times.

Range is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.

**Precision**

The precision was determined at two levels as per ICH, Q2 (R1) guidelines i. e. Repeatability and intermediate precision [19].

Repeatability of drug sample was determined as intraday variation (3 concentrations/3 replicates each, three times a day / a minimum of 9 determinations covering the specified range for the procedure) whereas intermediate precision was determined by interday variation (for three different days) for the determination of donepezil at three different concentration levels of 6, 12 and 18 µg/ml in triplicate. The % RSD was calculated for absorbance to obtain the intraday variation and interday variation.

**Accuracy as recovery studies**

Accuracy is the closeness of the test results obtained by the analytical method to the true value. The method was further validated to check the sensitivity of the method to determine donepezil in the presence of excipients. The accuracy of the method was evaluated by standard addition method. Pre-analysed samples of donepezil (8 µg /ml) were spiked with the extra 50%, 100% and 150 %, of the standard drug and the mixtures were analysed by the proposed method. The experiment was performed in triplicate. The % recovery of each sample and % RSD was calculated at each concentration level.

**Limit of detection and limit of quantitation**

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be detected, but not necessarily quantified as an exact value. The limit of quantitation (LOQ) is the lowest concentration of an analyte that can be quantitatively determined with acceptable precision and accuracy under the stated operational conditions of the method.

LOD and LOQ of the drug were calculated using the following equations as per ICH guidelines.

$LOD = 3.3 \times \sigma/S$..... (1)

$LOQ = 10 \times \sigma/S$..... (2)

Where $\sigma$ = the standard deviation of the response; S= the slope of the regression line.

**RESULTS AND DISCUSSION**

**Wavelength of maximum absorption and preparation of calibration curve**

The wavelength of maximum absorption ($\lambda$max) was found to be 270.5 nm in phosphate buffer (pH 7.4). The calibration curve was prepared in the concentration range of 2- 20 µg/ml. It observed that there was no change in the $\lambda$max of the drug in the concentration range 2- 20 µg/ml (Fig. 2).

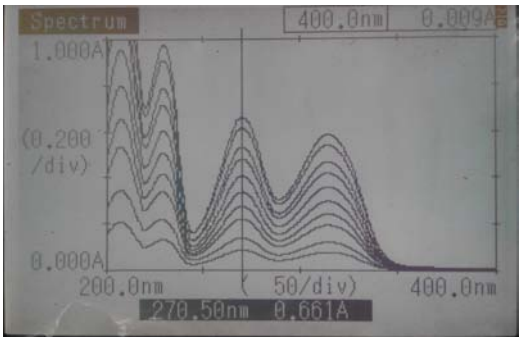


**Fig. 2: Overlay spectra of donepezil in Phosphate buffer (pH 7.4)**

**Method validation**

**Linearity and range**

The absorbance of the drug solutions (2-20 µg/ml) was determined at 270.5 nm by UV spectrophotometer (UV -1800, Shimadzu Corporation, Kyoto, Japan). The mean absorbance range (n=3) was found to be 0.088-0.67 with RSD values below 2 % (Table 1). The calibration curve obtained was evaluated by its correlation coefficient (Fig. 3). The absorbance of the samples in the

concentration range of 2.0 -20 µg/ml was linear with a correlation coefficient ($R^2$) 0.999.

**Precision**

The precision of the proposed method was assessed by analyzing donepezil in three different concentration levels as 6, 12 and 18 µg/ml in triplicate. Results of repeatability **(**intraday precision) and intermediate (interday) precision were expressed in the terms of % RSD.

The intraday and interday precision study of the developed method confirmed adequate sample stability and method reliability where all RSDs were below 2% (Table 2).

**Accuracy as recovery studies**

The standard addition technique was carried out by adding excipients (in the likely range to be used) to be used in the formulation development with the addition of drug at 4 (50 %), 8 (100 %) and 12 (150 %) µg/ml concentrations in sample solution of 8 µg/ml.

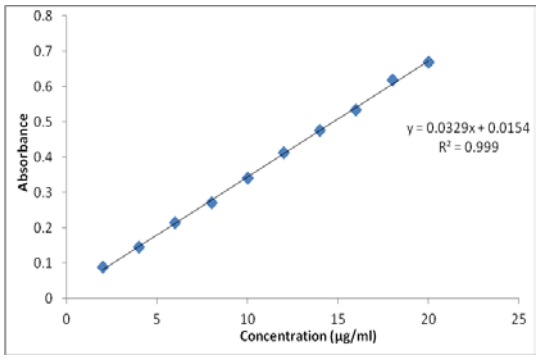


**Fig. 3: Calibration curve of donepezil in phosphate buffer (pH 7.4)**

**Table 1: Calibration curve data for donepezil in phosphate buffer (pH 7.4)**

| Concentration (µg/ml) | Mean absorbance at 270.5 nm± SD (n = 3) | Regressed absorbance | Equation of Line |
|---|---|---|---|
| 2 | 0.088±0.004 | 0.081 | **1. Equation of Line** |
| 4 | 0.144±0.004 | 0.147 | y=0.0329x + 0.0154 |
| 6 | 0.214±0.004 | 0.213 | **2. Correlation coefficient** |
| 8 | 0.270±0.005 | 0.279 | $R^2$ = 0.999 |
| 10 | 0.342±0.005 | 0.344 | **3. Slope** |
| 12 | 0.412±0.005 | 0.41 | m = 0.0329 |
| 14 | 0.477±0.006 | 0.476 | **4. Intercept** |
| 16 | 0.533±0.005 | 0.542 | c = 0.0154 |
| 18 | 0.620±0.030 | 0.608 | |
| 20 | 0.670±0.009 | 0.673 | |

**Table 2: Precision of proposed method**

| Concentration (µg /ml) | Intraday precision (repeatability) | | Interday (intermediate) precision | | |
|---|---|---|---|---|---|
| | Mean absorbance at 270.5 nm ± SD (n=3) | RSD (%) | Day | Mean absorbance at 270.5 nm ± SD (n=3) | RSD (%) |
| 6 | 0.214±0.004 | 1.866 | 1 | 0.215±0.004 | 1.86 |
| | | | 2 | 0.211±0.002 | 0.95 |
| | | | 3 | 0.219±0.005 | 1.37 |
| 12 | 0.412± 0.005 | 1.215 | 1 | 0.428±0.004 | 0.93 |
| | | | 2 | 0.414±0.006 | 1.44 |
| | | | 3 | 0. 423±0.007 | 1.65 |
| 18 | 0.62±0.003 | 0.484 | 1 | 0.623±0.005 | 0.80 |
| | | | 2 | 0.634±0.003 | 0.47 |
| | | | 3 | 0.630±0.001 | 0.16 |

**Table 3: Accuracy as recovery of the proposed method**

| Percent of standard spiked to the sample | Concentration | | | % of drug recovered | % RSD |
|---|---|---|---|---|---|
| | Sample (µg /ml) | Total including spiked sample (µg /ml) | Spiked sample determined (µg/ml) ± SD (n = 3) | | |
| 50 | 8 | 12 | 11.83±0.21 | 98.50 | 1.77 |
| 100 | 8 | 16 | I5.94±0.29 | 99.63 | 1.82 |
| 150 | 8 | 20 | 19.56±0.21 | 97.80 | 1.07 |

**Table 4: UV spectrophotometric parameters of donepezil**

| S. No. | Parameters | Results |
|---|---|---|
| 1. | Absorption maxima (nm) | 270.5 |
| 2. | Linearity range (µg/ml) | 2-20 |
| 3. | Regression equation | y = 0.0329x + 0.0154 |
| 4. | Slope | 0.0329 |
| 5. | Intercept | 0.0154 |
| 6. | Correlation coefficient ($R^2$) | 0.999 |
| 7. | Recovery (%) | 97.80-99.63 |
| 8. | LOD (µg/ml) | 0.401 |
| 9. | LOQ (µg/ml) | 1.22 |

The proposed method afforded recovery of 97.80-99.63 % after spiking the additional standard drug solution to the previously analysed test solution.

The value of % recoveries and % RSDs are shown in table 3. The high % recoveries indicated no interference of excipients that are used to prepare formulations of donepezil i. e. solid lipid nanoparticles.

**Limit of detection (LOD) and limit of quantitation (LOQ)**

LOD and LOQ of this method were determined by the standard deviation method. The value of LOD and LOQ was found to be 0.401and 1.22 µg/ml respectively. A summary of all results is given in table 4.

**CONCLUSION**

The results and the statistical parameters demonstrated that the proposed UV spectrophotometric method is simple, rapid, specific, accurate and precise. Therefore, this method can be used to determine donepezil quantitatively for routine analysis in pharmaceutical preparations without interference of commonly used excipients and related substances. It has been proved that the method is selective and linear between concentration range 2-20 µg/ml. The value of LOD and LOQ was found to be 0.401and 1.22 µg/ml respectively.

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**CONFLICT OF INTERESTS**

The author declares that there is no conflict of interests regarding the publication of this paper."

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