# STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DONEPEZIL HYDROCHLORIDE USING RP-HPLC

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**Abstract**

The present study outlines a straightforward and cost-effective stability indicating method for the quantitative determination of Donepezil Hydrochloride (DPH) using Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC). The method utilizes a Thermo C18 column with dimensions of 250mm x 4.60mm and a particle size of 5µ, bonded with Octadecylsilane (C18). The mobile phase, comprising 50% Methanol and 50% Acetonitrile, ensures efficient separation. The analysis is performed at an ambient temperature with a flow rate of 1.0 ml/min. A sample size of 20 µl is injected into the system, and detection is carried out at a wavelength of 268nm. The retention time for Donepezil Hydrochloride is found to be 5.680±0.01 minutes. This method provides a reliable and sensitive approach for the determination of Donepezil Hydrochloride, making it suitable for stability-indicating assays in pharmaceutical formulations.

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

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## Introduction

Donepezil Hydrochloride (DPH) is a widely prescribed medication for the treatment of Alzheimer's disease, characterized by cognitive impairment and memory loss. Accurate and reliable estimation of DPH is crucial to ensure therapeutic efficacy and patient safety. In this context, the development of a robust analytical method becomes imperative [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]. High-Performance Liquid Chromatography (HPLC) is a powerful and versatile analytical technique widely used in the pharmaceutical, chemical, and biological sciences for the separation, identification, and quantification of compounds in complex mixtures. HPLC operates on the principles of chromatography, utilizing a liquid mobile phase to move a sample through a packed stationary phase. The efficiency and speed of separation in HPLC are significantly enhanced by high pressure, allowing for the analysis of a wide range of compounds with varying polarities. The technique provides superior resolution, sensitivity, and precision, making it an indispensable tool for quality control, drug development, and research. HPLC has evolved over the years, with various modes such as reverse-phase, normal phase, and ion-exchange chromatography, each offering unique advantages for specific applications [10-11]. The current research aims to develop and validate an RP-HPLC method for the estimation of Donepezil Hydrochloride in pharmaceutical formulations. The method will be validated according to regulatory guidelines, ensuring its accuracy, precision, linearity, specificity, and robustness.

## Material and Methods

### Reagents and chemicals

The working standard of Donepezil Hydrochloride was provided as gift sample from Pharmaceutical Company. The market formulation was procured from local market. Methanol, acetonitrile were procured from Rankem, RFCL Limited, New Delhi, India. Ammonium acetate AR, sodium dihydrogen phosphate AR and ortho-phosphoric acid AR grade were procured from
Central Drug House (P) Limited, New Delhi, India. The 0.45µm pump nylon filter was obtained from Advanced Micro devices (Ambala Cantt, India). All other chemical used were of analytical grade. Triple distilled water was used for whole experiment was generated in house. All the solutions were protected for light and were analyzed on the day of preparations.

### Instrument

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data. Weighing was done on a Digital Micro Balance (CX-265) manufactured by Citizen Scale (I) Pvt. Ltd.

### Selection of mobile phase

Initially to estimate Donepezil hydrochloride fix dosage form number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

### Selection of Diluent

The diluents employed for sample preparation were found to be compatible with the mobile phase, exhibiting no substantial impact on the retention time and resolution of the analyte. Following several trial runs, methanol was identified as the optimal diluent for the task.

### Preparation of Stock Solution:

Accurately weighed 10mg API of DPH was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

### Preparation of Sub Stock Solution:

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (methanol) to give concentration of 100µg/ml of DPH respectively (Stock-B).

### Preparation of Different Solution

0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml, for DPH.

**Linearity and Calibration Graph**

To establish the linearity of analytical method, a series of dilution ranging from 1-5 µg/ml for DPH were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 268.0 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

**Validation of developed Method**

**Linearity**

The linearity of an analytical procedure refers to its capacity, within a specified range, to yield test results that are directly proportional to the area of the analyte in the sample. To assess linearity, a calibration plot was constructed through the analysis of five different concentrations (ranging from 1 to 5µg/ml for Donepezil Hydrochloride). The areas for each concentration were recorded three times, and the mean area was calculated. The regression equation and correlation coefficient of the curve are provided, and the standard calibration curve of the drug is depicted in Figure 4.32. The response ratio, or response factor, was determined by dividing the Area Under Curve (AUC) by the respective concentration.

**Specificity**

The specificity of the method was conducted to definitively determine the presence of the analyte and to evaluate the potential existence of components such as impurities, degradation products, and matrix components.

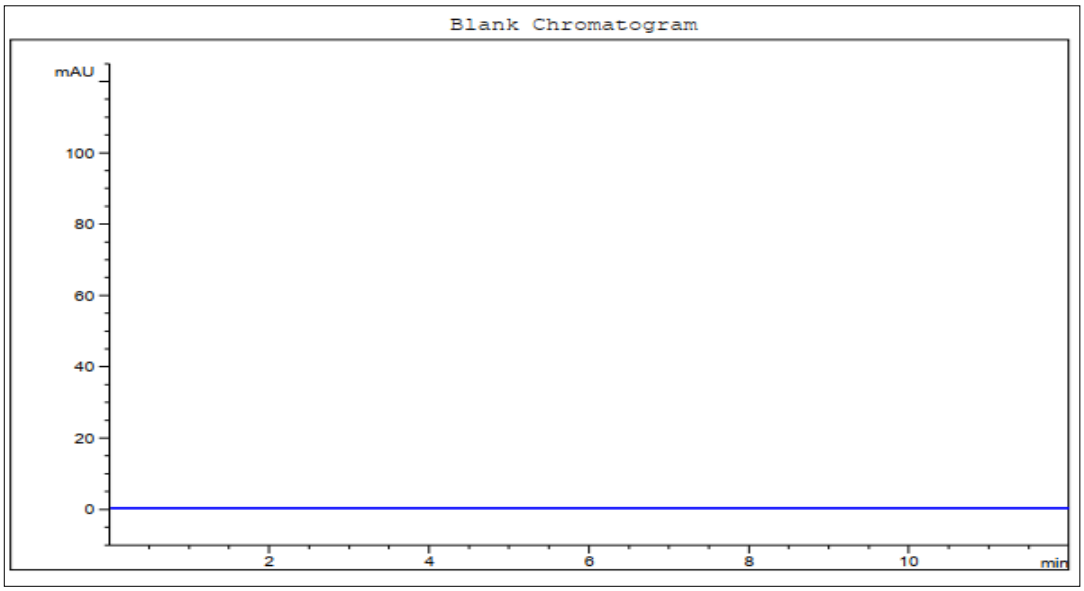


**Figure 1: Chromatogram of Blank**

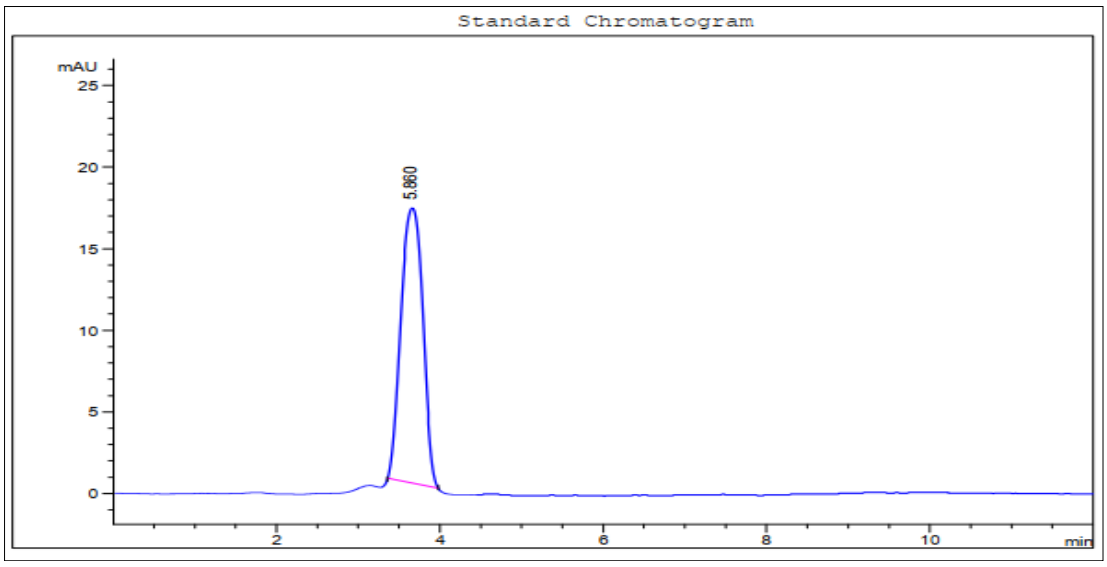


**Figure 1: Chromatogram of Standard Drug**

**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:

**Repeatability**

The repeatability was performed for five replicate at five concentrations in linearity range 1, 2, 3, 4 and 5µg/ml for DPH indicates the precision under the same operating condition over short interval time.

**Intermediate Precision**

**Day To Day Precision**

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations.

**Robustness**

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Acetonitrile: Methanol (50:50 % v/v) to (45:55 % v/v).

**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.

**Analysis of drug in tablet Sample**

Twenty tablets (DONECEPT-5) were precisely weighed, and their collective mean weight was determined. Subsequently, the tablets were ground into a fine powder, and a precisely measured quantity of the powder, equivalent to 10mg of DPH, was transferred to a 10 ml volumetric flask containing methanol. The solution underwent sonication for 25 minutes, and the final volume was adjusted using the mobile phase. Following sonication, the mixture was filtered through a 0.45 µm filter. The resulting stock solution was further diluted with methanol to obtain a sample solution with a drug concentration of 10µg/mL DPH. The amounts of DPH in the tablet formulation were then calculated by extrapolating the area value from the calibration curve.

**Forced degradation studies**

To assess the method's stability-indicating capability, forced degradation studies were performed on the drug powder, and subsequent analysis was conducted using HPLC with a UV detector. Each forced degradation sample, comprising 20µl, was injected for analysis.

**Acid degradation:**

10mg sample of the drug was introduced into a 50 ml round bottom flask. To this, 50 ml of a 0.1 M HCl solution was added, and the contents were thoroughly mixed before being continuously stirred for 8 hours at 80ºC. Samples were extracted, and subsequent dilution resulted in a concentration of 10µg/ml. These diluted samples were then subjected to HPLC analysis, and the percentage degradation was calculated using the drug's calibration curve.

**Alkaline hydrolysis:**

10mg portion of the drug sample was placed in a 50 ml round bottom flask. Subsequently, 50 ml of a 0.1 M NaOH solution was added, and the contents were thoroughly mixed. The mixture was then continuously stirred for 8 hours at 80ºC. Samples were withdrawn, diluted to a concentration of 10µg/ml, and subjected to HPLC analysis. The percentage degradation was subsequently calculated using the calibration curve for the drug.

**Oxidative degradation:**

10mg aliquot of the drug sample was placed in a 50 ml separate round bottom flask. Following this, 50 ml of a 3% hydrogen peroxide solution was added, and the contents were thoroughly mixed. The mixture was left under constant stirring for 24 hours at room temperature. Subsequent to this degradation period, samples were withdrawn, diluted to a concentration of 10µg/ml, and subjected to HPLC analysis. The percentage degradation was then determined using the calibration curve for the drug.

**Thermal degradation:**

10mg aliquot of the drug sample was placed in a petri dish and exposed to an oven at 50ºC for duration of 4 weeks. After the specified time, samples were withdrawn, diluted to a concentration of 10µg/ml, and subsequently subjected to HPLC analysis. The percentage degradation was then determined using the calibration curve established for the drug.

**Results and Discussion**

The results obtained from the analytical evaluation of Donepezil hydrochloride (DPH) demonstrate the robustness and reliability of the developed method. In Table 1, the linearity study reveals a strong correlation between DPH concentration and absorbance, with a high correlation coefficient (r2) of 0.999. The slope (m) and intercept (c) values further support the linear relationship, ensuring accurate quantification across the concentration range of 1-5 µg/ml.

Table 2 presents the recovery study, a crucial aspect of method validation. The obtained results at different levels (80%, 100%, 120%) indicate satisfactory accuracy, with % MEAN values for DPH close to 100%. The low standard deviations (% SD) underscore the precision and reliability of the method (97.43±1.243, 98.84±1.213, 95.82±0.081).

Precision, as depicted in Table 3, is evaluated through repeatability, day-to-day precision, and analyst-to-analyst variability. The % MEAN values with minimal standard deviations (Repeatability: 95.82±0.062, Day-to-day precision: 96.81±0.049, Analyst-to-Analyst: 95.29±0.045) confirm the method's consistent performance under varying conditions.

The LOD and LOQ values (Table 4) highlight the method's sensitivity, with low detection limits (LOD: 0.10 µg/ml) and quantification limits (LOQ: 0.35 µg/ml), indicating its capability to detect and quantify DPH at low concentrations.

Table 6 presents the assay of tablet formulations, with the % Assay close to 98.40%, confirming the accuracy of the method in determining the DPH content in the tablets. The low % RSD (0.125) across three determinations emphasizes the precision of the assay.

Finally, Table 7 outlines the forced degradation studies, providing insights into the stability-indicating nature of the method. The recovery percentages under different stress conditions demonstrate the method's ability to distinguish DPH from its degradation products, ensuring the reliability of the analysis.

**Table 1: Results of linearity of Donepezil hydrochloride (DPH)**

| **Parameter** | **DPH** |
|---|---|
| **Concentration (µg/ml)** | 1-5 |
| **Correlation Coefficient ($r^2$)*** | 0.999 |
| **Slope (m)*** | 1232 |
| **Intercept (c)*** | 44.26 |

*Value of six replicate

**Table 2: Results of recovery study**

| **% Level** | **% MEAN±SD*** |
|---|---|
| | **DPH** |
| **80%** | 97.43±1.243 |
| **100%** | 98.84±1.213 |
| **120%** | 95.82±0.081 |

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

**Table 3: Results of precision**

| **Parameter** | **% MEAN±SD*** |
|---|---|
| | **DPH** |
| **Repeatability** | 95.82±0.062 |
| **Intermediate precision** | |
| **Day to day precision** | 96.81±0.049 |
| **Analyst-to-Analyst** | 95.29±0.045 |
| **Robustness** | 95.28 ±0.045 |

***** Value of five replicate and five concentrations

**Table 4: LOD and LOQ of DPH**

| Name | LOD (µg/ml) | LOQ (µg/ml) |
|---|---|---|
| **DPH** | 0.10 | 0.35 |

**Table 6: Assay of tablets formulation**

| | **DPH*** |
|---|---|
| Label Claim (mg) | **5mg** |
| % Found (mg) | 4.92 |
| % Assay | 98.40 |
| % RSD | 0.125 |

*Average of three determination

**Table 7: Results of forced degradation studies of DPH**

| Stress conditions | Drug recovered (%) | Drug decomposed (%) |
|---|---|---|
| Standard drug | 99.85 | 0 |
| Acidic hydrolysis | 86.85 | 13.00 |
| Alkaline hydrolysis | 81.74 | 18.11 |
| Oxidative degradation | 93.45 | 6.40 |
| Photolytic degradation | 92.14 | 7.71 |

## Conclusion

In conclusion, the RP-HPLC method presented in this study is a robust and sensitive technique for the routine analysis of Donepezil Hydrochloride in pharmaceutical formulations. Its stability-indicating nature ensures accurate and reliable results, making it a valuable tool for quality control and research in the pharmaceutical industry.

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