DEVELOPMENT AND VALIDATION OF STABILITY INDICATING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ESTIMATION OF DONEPEZIL HCL FROM BULK DRUG

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ABSTRACT
Stability of Donepezil Hydrochloride (DONE) was investigated using stability indicating Reverse phase high performance liquid chromatography (RP-HPLC) utilizing C-18 column and mobile phase containing Acetonitrile:Water (pH 3.5) in ratio of 40:60 at flow rate of 1 ml min-1. Peaks of donepezil and degradation products were well resolved at retention times < 7 min. Stability was performed in 0.1N hydrochloric acid, 0.1N sodium hydroxide, 3 % hydrogen peroxide, neutral, photolytic and dry heat conditions. Fast hydrolysis was seen in alkaline condition as compared to oxidative and neutral conditions. Methods was validated with respect to linearity, precision, accuracy, specificity and robustness. It was also found to be stability indicating, and therefore suitable for the routine analysis of Donepezil hydrochloride in the pharmaceutical formulation.

Keywords: Donepezil hydrochloride, RP-HPLC, Method Development, Stability studies.

INTRODUCTION
Donepezil is a reversible inhibitor of the enzyme acetylcholinesterase (AChE) approved for use in Alzheimer’s disease.1] The pathogenesis of Alzheimer’s disease attributed some of them to a deficiency of cholinergic neurotransmission. Therefore, AChE inhibitors, which prevent the hydrolysis of acetylcholine, may exert their therapeutic effect by enhancing cholinergic function. The first AChE inhibitor ( tacrine) has been used, however, associated with a high incidence of gastrointestinal (GI) side effects and hepatotoxicity.2] Donepezil is a potent and more selective AChE inhibitor in the central nervous system with little effect on peripheral tissue, therefore, has a lower incidence of GI and cardiovascular adverse effects. The drug produces modest improvements in cognitive scores and has a long half-life allowing once daily dosing. Donepezil is slowly absorbed from the GI tract.3] The present work describes the utility of RP-HPLC in forced degradation-stability study under different chemical conditions.

EXPERIMENTAL
Chemical
Donepezil HCl was gifted by Sun Pharmaceutical Pvt. Ltd. Broda, Gujarat
Methanol HPLC Grade(FINAR)
HPLC Water
HCl AR Grade (MERCK)
NaOH AR Grade (MERCK)
H2O2 AR Grade (RANKEM)
Potassium dihydrogen Phosphate LR Grade(RENKEM)

Acetonitrile HPLC Grade(FINAR)

Instrumentation
Company : Shimadzu
Model No : SPD 10 A-LC
Software : WINCHROME SOFTWARE
Operation : Semi Automatic
Semi micro analytical balance (Sartorius CD2250, Germany) was used for weighing purpose.
HPLC water was obtained using Arium®611VF(Sartorius).
Magnetic stirrer (1 MLH, Remi) was used for mixing purpose.

pH tutor (313927, Eutech Instruments) was used for pH measurement.
Sonication of solutions were done using Ultrasonic cleaner (D 120/1H, Trans-O-Sonic).
Nylon membrane filters (0.22 µm, 47 mm D)

All volumetric glasswares used were calibrated.

DEGRADATION STUDIES
All degradation studies were done at a drug concentration of 50µg ml-1. For acid decomposition studies, drug was dissolved in 0.1N HCl and solution was boiled under reflux for 1 hr. The studies in alkaline conditions were done in 0.1N NaOH boiling under reflux for 1 hr. For study in neutral conditions, drug in water was boiled under reflux for 1 hr. For oxidative conditions, initial studies were done in 3% H2O2.
solution. The solution was kept at room temperature for 1 hr. Photolytic studies were done by exposing solid drug directly to UV light for 2 hr. Thermal decomposition studies were performed by exposing solid sample of drug to dry heat at 70°C for 2 hr in hot air oven.

**HPLC ANALYSIS**

**CHROMATOGRAPHIC SEPERATION**

Standard and sample solutions were injected in column. The chromatogram was run for appropriate time duration with degassed mobile phase, mixture of Acetonitrile: Water pH 3.5 (40:60 v/v), using UV detector (SPD-20AV) at wavelength 230 nm. The chromatogram was stopped after separation was achieved completely. Data related to peak like area, height, retention time, resolution etc was recorded using CLASS-VP software (version 2.31).

### Table 1: Chromatographic conditions of developed method

| Sr no | HPLC Conditions | Results |
|-------|-----------------|---------|
| 1     | Elution         | Isocratic |
| 2     | Mobile Phase    | Acetonitrile: Water pH 3.5 (40:60 v/v) |
| 3     | Diluent         | Acetonitrile |
| 4     | Flow Rate       | 1 ml/min |
| 5     | Detector        | UV Visible |
| 6     | Injection volume| 20 µL |
| 7     | Wavelength      | 230 nm |
| 8     | Column Temperature | Room temperature |
| 9     | Run time        | 10 min |
| 10    | Column          | InertSustainSwift™C18 (250mm×4.6mm i.d.) 5µm |

### STANDARD CHROMATOGRAM

**Blank**

![Blank Chromatogram](image1)

**Donepezil HCl**

![Donepezil HCl Chromatogram](image2)

### CONDITION

**Table 2: Mobile Phase: Acetonitrile: Water pH 3.5(40:60 v/v)**

| Name            | Retention time (min) | Theoretical Plates | Tailing Factor |
|-----------------|----------------------|--------------------|----------------|
| Donepezil HCl   | 5.08                 | 8528.92            | 0.51           |

### Table 3: Observed value for system suitability test

| Sr No | System Suitability Parameter | Donepezil HCl | IP’2007 specification |
|-------|------------------------------|---------------|------------------------|
| 1     | Number of Theoretical Plates (N) | 8528.92 | >2000                  |
| 2     | Resolution                  | -             | >2                     |
| 3     | Tailing Factor (T )        | 0.51          | <2                     |

### VALIDATION OF DEVELOPED RP-HPLC METHOD

#### Specificity

Specificity was measured by injecting a blank solution and another blank solution which was previously spiked with common excipients. No any interference by excipients was observed. The entire base line was found stable.

### Linearity Range

The linearity range for Donepezil HCl was found to be 10-100 µg/ml. Correlation coefficient for calibration curve of Donepezil HCl (DONE) was found to be 0.998.

The regression line equation for Donepezil HCl is as follows:

\[ y = 25514x + 41169 \]

Where, \( y \) = corresponding peak area from CLASS VP software(v2.31)  
\( x \) = Concentration of Donepezil HCl in µg/ml.

![Fig 1: Chromatogram of standard Donepezil HCl](image3)
### Table 4: Calibration data and calibration curve

| Sr No. | Donepezil HCl (µg/ml) | Mean Area ±SD (n=6) |
|--------|------------------------|---------------------|
| 1.     | 10                     | 317958 ±620         |
| 2.     | 20                     | 533316 ±3349        |
| 3.     | 30                     | 787252 ±3677        |
| 4.     | 40                     | 1068169 ±4172       |
| 5.     | 50                     | 1326223 ±2028       |

**PRECISION**

**Intraday Precision**

| Conc. (µg/ml) | Peak Area ± SD (n=3) | %RSD | Mean % RSD |
|---------------|----------------------|------|------------|
| 10            | 318860 ± 992         | 0.31 |            |
| 30            | 787616 ± 405         | 0.05 | 0.21       |
| 50            | 1329368 ± 3805       | 0.28 |            |

**ACCUARACY**

| Conc µg/ml | %Spiking | Total Conc µg/ml | Peak Area | Recovered Amt µg/ml | %Recovery | Mean % Recovery |
|------------|----------|------------------|-----------|---------------------|-----------|-----------------|
| 10         | -        | 10               | 253049    | 9.91                | 99.18     | 99.18%          |
| 10         | 80% (8)  | 18               | 462472    | 18.12               | 100.70    | 100.70%         |
| 10         | 100% (10)| 20               | 517568    | 20.28               | 101.42    | 101.42%         |
| 10         | 120% (12)| 22               | 560704    | 21.97               | 99.89     | 100.29%         |

**LOD AND LOQ**

| Sr No. | Parameter                                                                 | Donepezil HCl |
|--------|---------------------------------------------------------------------------|---------------|
| 1.     | SD of the Y-intercepts of 6 calibration curve                             | 41169         |
| 2.     | Mean slope of the 6 calibration curves                                    | 25514         |
| 3.     | LOD = 3.3 × (SD/Slope) (µg/ml)                                           | 0.85 µg/ml    |
| 4.     | LOQ = 10 × (SD/Slope) (µg/ml)                                            | 2.59 µg/ml    |

**ASSAY**

Twenty 'AERICEP' Tablets (containing 10 mg of Donepezil HCl) were accurately weighed and ground to fine powder.

An accurately weighed quantity equivalent to 100 mg of Donepezil HCl (DONE) from the formulation fine powder was transferred to 100 mL volumetric flask and 40-50 ml Acetonitrile is added to dissolve the drug. Sonicate for 15 mins. Volume is made up to the mark with the Acetonitrile.

Filter Stock solution (100 µg/mL).
Table 9: Assay

| Sample Label | Claim (mg) | Concentration taken (µg/ml) | Concentration found (µg/ml) | % Assay  |
|--------------|------------|-----------------------------|-----------------------------|----------|
| Donepezil HCl | 10 mg      | 30                          | 31.92                       | 103.40%  |

Table 10: Robustness

| Sr No. | Factor                  | Conc  | Level | Mean(n=3) ±SD | %RSD |
|--------|-------------------------|-------|-------|---------------|------|
| 1      | Change in flow rate     | 50µg/ml | 1.2   | 1532054 ± 6104 | 0.39 |
| 2      | Change in wavelength    | 50µg/ml | 232   | 1485170±4023 | 0.27 |

Table 11: Summary of validation parameters:

| Sr No. | Parameters                  | Result                  |
|--------|-----------------------------|-------------------------|
| 1      | Wavelength (nm)             | 230 nm                  |
| 2      | Linearity range (µg/ml)     | 10-50 µg/ml             |
| 3      | Standard Regression equation| Y=25514x + 41169        |
| 4      | Correlation coefficient (R²)| 0.998                   |
| 5      | Precision (%RSD) Intraday   | 0.21                    |
| 6      | % Recovery (Accuracy, n = 3)| 100.29%                |
| 7      | LOD (µg/ml)                 | 0.85                    |
| 8      | LOQ (µg/ml)                 | 2.59                    |
| 9      | Robustness                  | 0.46                    |
| 10     | Assay (% Label claim)       | 103.46%                 |

**FORCE DEGRADATION STUDIES**

**Acid degradation**

100 mg drug dissolve in 1 ml Acetonitrile and dilute up 100 ml of 0.1N HCl. Pipette out 0.5 ml and solution neutralize with equivalent amount of 0.1 N NaOH and dilute with acetonitrile up to 10 ml. Then this solution is kept for 1 hour at 70°C and peak is recorded. Sample withdrawal at 0 min, after 10 min, 30 min, 1 hr at 70°C.

**Alkali degradation**

100 mg drug dissolve in 1 ml Acetonitrile and dilute up 100 ml of 0.1N NaOH. Pipette out 0.5 ml and solution neutralize with equivalent amount of 0.1 N HCl and dilute with acetonitrile up to 10 ml. Then this solution is kept for 1 hour at 70°C and peak is recorded. Sample withdrawal at 0 min, after 10 min, 30 min, 1 hr at 70°C.

**Oxidation**

3% H₂O₂ was taken in a 100 ml volumetric flask then accurately weighed 100 mg bulk drug was dissolved in it and then volume is made by 3% H₂O₂. Then this solution is kept for 1 hr and peak is recorded. Sample withdrawal at 0 min, after 10 min, 30 min, 1 hr.

**Neutral Condition**

First Distilled water was taken in a 100 ml volumetric flask then accurately weighed 100 mg bulk drug was dissolved in it, volume is made by Water(1000 ppm). Then this solution is kept for 1 hour at 70°C and peak is recorded. Sample withdrawal at 0 min, after 10 min, 30 min, 1 hr at 70°C.

**Photolytic Condition**

100mg of bulk drug was put into the petridish and placed under direct UV Light for 1 hr. 10mg is weighed and make upto 10 ml, using diluent Acetonitrile and chromatogram was recorded. Similar Procedure is followed for 2 hr.

**Thermal Condition**

100 mg of bulk drug was taken in a cleaned Petridish and was put it into the oven at 70°C for 1 hour. 10mg of bulk drug from the Petridish...
was weighed and dissolved in 10 ml of diluent Acetonitrile and chromatogram was recorded. Similar Procedure is followed for 2hr.

| CONDITION   | % DEGRADATION |
|-------------|---------------|
| ACIDIC      | 60.05%        |
| BASIC       | 92.31%        |
| NEUTRAL     | 13.58%        |
| OXIDATION   | 78.05%        |
| PHOTOLYTIC  | 1.62%         |
| THERMAL     | 10.76%        |

**DISCUSSIONS**
The stability of the new donepezil hydrochloride is investigated using stability indicating RP-HPLC procedure. The method permits detection and quantitation of donepezil hydrochloride in the presence of its degradation products. It was subjected to stress conditions as per ICH guidelines. The drug was found to degrade in alkaline, oxidative and neutral conditions while it was found to be stable under photolytic and dry heat conditions. The drug can be analyzed specifically in the presence of different chromophoric degradation products by using isocratic conditions and mobile phase containing Acetonitrile : Water (pH 3.5) in the ratio of 40 : 60. The method was validated for parameters like linearity, precision, accuracy, specificity and robustness.

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