**Journal of Pharmaceutical and Biomedical Analysis Letters**
**CODEN (USA): JPBAC9 | ISSN: 2347-4742**
Journal Home Page: www.pharmaresearchlibrary.com/jpbmal

**R E S E A R C H  A R T I C L E**

# RP- HPLC Method Development and Validation for the Simultaneous Estimation of Donepezil and Memantine

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**A B S T R A C T**

A new method was established for simultaneous estimation of donepezil and memantine by RP-HPLC method. The chromatographic conditions were successfully developed for th1e separation of donepezil and memantine by using Xterra C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for wa donepezil and memantine s found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was
0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively.

**Keywords**: Donepezil, Memantine, RP-HPLC, Phosphate buffer, CAN

**A R T I C L E  I N F O**

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**A R T I C L E  H I S T O R Y: Received** 03 Oct 2019, **Accepted** 31 Dec 2019, **Available Online**18 January 2020



***Citation:*** *S. Uday Venkata Sai Kumar, et al. RP- HPLC Method Development and Validation for the Simultaneous Estimation of Donepezil and Memantine. J. Pharm, Biomed. A. Lett., 2020, 8(1): 11-16*

**C O N T E N T S**

## 1. Introduction

A drug is a substance which may have medicinal, intoxicating, performance enhancing or other effects when taken or put into a human body or the body of another animal and is not considered a food or exclusively a food.

What is considered a drug rather than a food varies between cultures, and distinctions between drugs and foods and between kinds of drug are enshrined in laws which vary between jurisdictions and aim to restrict or prevent drug use. Even within a jurisdiction, however, the status of a substance may be uncertain or contested with respect to both whether it is a drug and how it should be classified if at all. There is no single, precise definition, as there are different meanings in drug control law, government regulations, medicine, and Colloquial usage.

High performance liquid chromatography is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceuticals. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity.

**Drug Profile:**

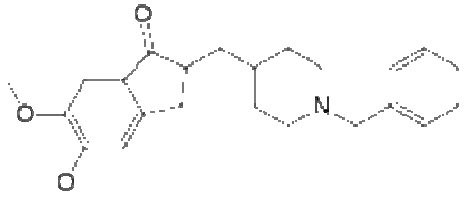


**Fig 1:** Chemical Structure of Donepezil

**Chemical Data:**

**IUPAC Name:** (*RS*)-2-[(1-Benzyl-4-piperidyl) methyl]-5,6-dimethoxy-2, 3-dihydroinden-1-one

**Chemical formula :** $C_{24}H_{29}NO_3$

**Molecular weight :** 379.492 g/mol

**Category :** antiamnestic

**Mechanism of action**

Donepezil binds and inactivates reversibly the cholinesterases, thus inhibiting hydrolysis of acetylcholine. This results in increased acetylcholine concentrations at cholinergic synapses.

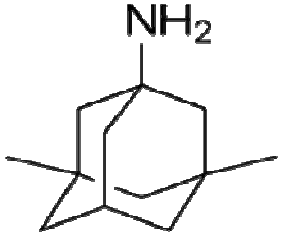


**Fig 2:** Chemical Structure of Memantine

**Chemical Data:**

**IUPAC Name:** 3,5-dimethyltricyclo[3.3.1.1$^{3,7}$]decan-1 amine or 3, 5 dimethyladamantan-1-amine

**Chemical Formula :** $C_{12}H_{21}N$

**Molecular weight :** 179.3 g/mol

**Category :** noncompetitive NMDA receptor antagonist

**Mechanism of Action:** Memantine acts as a non-competitive antagonist at different neuronal nicotinic acetylcholine receptors (nAChRs) at potencies possibly similar to the NMDA and 5-HT3 receptors, but this is difficult to ascertain with accuracy because of the rapid desensitization of nAChR responses in these experiments. It can be noted that memantine is an antagonist at Alpha-7 nAChR, which may contribute to initial worsening of cognitive function during early memantine treatment.

**Literature Review:**

**Rajgor VM et al**., simple, accurate and specific RP-HPLC method has been developed and validated for the simultaneous estimation of Memantine HCl and Donepezil HCl in bulk and pharmaceutical dosage form.

**Syeda Noorain Amena et al.,** To develop and validate stability indicating method for the analysis of Memantine HCl and Donepezil HCl.

**U.K. Chhalotiya et al.,** A stability indicating HPLC method for the estimation of donepezil hydrochloride in tablets was developed and validated.

## 2. Materials and Methods

**Method Development:**

Method development for simultaneous estimation of Donepezile and Memantine in Pharmaceutical dosage forms includes the following steps:

**1. Selection of Detection wavelength:**

10 mg of Donepezile and Memantine was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Donepezile and Dihydro artemisinin. The isobestic point was taken as detection wavelength. The overlay spectrum is shown in Fig.1.

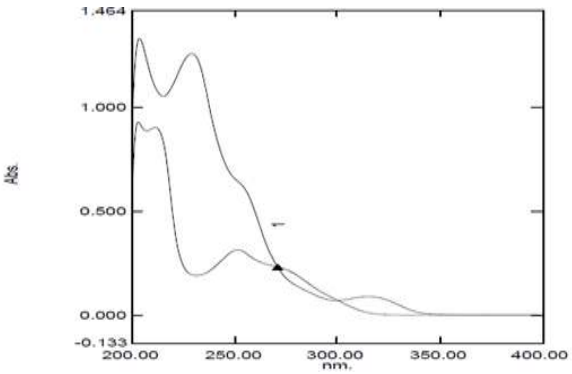


**Fig 3:** Over lay Spectrum of Donepezile and Memantine

**Selection of column:**

Column is selected based on solubility, polarity and chemical differences among Analytes [Column: Inertsil C18 (4.6 x 250mm, 5µm, Make: Waters)]

**3. Selection of mobile phase:**

- pH 3 phosphate buffer : Methanol (70 : 30% v/v)
- Buffer pH should be between 2 to 8.
- Below 2: siloxane linkages are cleaved.
- Above 8: dissolution of silica.
- pH selected: 3 ±0.05

- pH controls the elution properties by controlling the ionization characteristics.
- Reasons: To decrease the retention and improve separation. Good Response, Area, Tailing factor, Resolution.

**Selection of flow rate:**

Flow rate selected as 1ml/min

Flow rate is selected based on

1. Retention time
2. Column back pressure
3. Peak symmetry
4. Separation of impurities

**Preparations and procedures:**

**Preparation of Phosphate buffer :( PH: 4.6):**Weighed 6.8 grams of KH2PO4 was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 4.6 with ortho phosphoric acid.

**Preparation of mobile phase:**A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of MEOH (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

**Diluent Preparation:**Mobile phase is used as Diluent.

**Preparation of the individual Donepezile standard preparation:**10mg of Donepezile working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of diluent is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent.

**Preparation of the individual Memantine standard preparation:** 10mg of Memantine working standard was accurately weighed and transferred into a

10ml clean dry volumetric flask and about 2ml of diluent is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent (Stock solution). Further 1.0 ml from the above stock solution is pipette into a 10 ml volumetric flask and was diluted upto the mark with diluent.

**Preparation of Sample Solution :( Tablet)**

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Memantine and Donepezile (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluent.

**Procedure:** 10µL of the standard, sample are injected into the chromatographic system and the areas for Memantine and Donepezile peaks are measured and the %Assay are calculated by using the formulae.

**System Suitability:**

Tailing factor for the peaks due to Memantine and Donepezile in Standard solution should not be more than 2.0. Theoretical plates for the Memantine and Donepezile peaks in Standard solution should not be less than 2000

***Assay calculation:***

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where,

P = Percentage purity of working standard

Lc = LABEL CLAIM OF drug in mg/ml

**Optimized chromatogram is obtained by following conditions:**

Column : Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra)

Buffer pH : 4.6

Mobile phase : 70% MeOH : 30% Phosphate buffer pH-4.6

Flow rate : 1 ml per min

Wavelength : 273 nm

Temperature : Ambient.

Run time : 7 min.

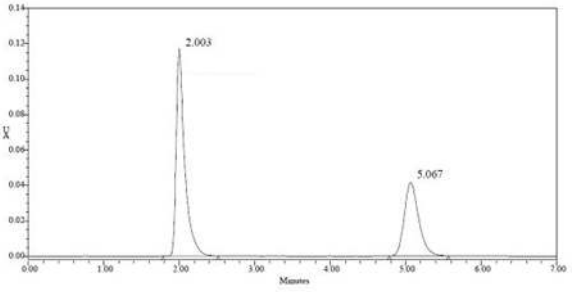


**Fig 3:**Optimized chromatogram

**Observation:** From the above chromatogram it was observed that the Donepezile and Memantine peaks are well separated.

## 3. Results and Discussion

**System Suitability:** The system suitability of the method was checked by injecting five different preparations of the donepezile and memantine standard. The parameters of system suitability were checked.

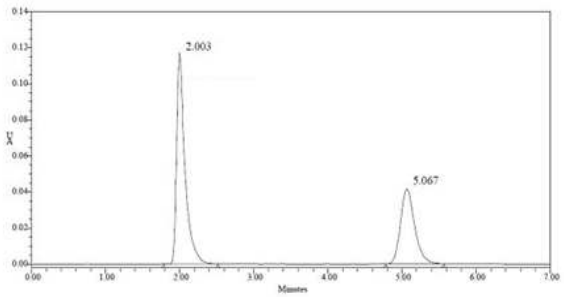


**Fig 4:** Chromatogram for system suitability

**Validation Parameters**

**Precision:** Precision of the method was carried out for both sample and standard solutions as described under experimental work. The corresponding results are shown below.

**Accuracy:**
Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

**Intermediate Precision (Ruggedness):**
There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

**Table 1:** Results of system suitability parameters for donepezile and memantine

| S. No | Name | Retention time(min) | Area (µV sec) | Height (µV) | USP resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| 1 | Memantine | 2.003 | 920101 | 116666 | | 1.6 | 2711.8 |
| 2 | Donepezil | 5.067 | 552058 | 41531 | 11.0 | 1.3 | 3428.2 |

**Table 2:** Results of method precision for memantine

| S. No | Sample area | Standard area | Percentage purity |
|---|---|---|---|
| 1 | 983375 | 971536 | 101.04 |
| 2 | 985049 | 973007 | 101.03 |
| 3 | 982956 | 975717 | 100.54 |
| 4 | 985219 | 978909 | 100.44 |
| 5 | 994145 | 981422 | 101.09 |
| **Average** | | | **100.84** |
| **%RSD** | | | **0.304** |

**Table 3:** Results of method precision for donepezil

| S. No | Sample area | Standard area | Percentage purity |
|---|---|---|---|
| 1 | 592403 | 577531 | 101.36 |
| 2 | 592352 | 580381 | 101.85 |
| 3 | 592357 | 577723 | 102.32 |
| 4 | 592323 | 582190 | 101.44 |
| 5 | 596525 | 583378 | 101.09 |
| **Average** | | | **101.24** |
| **%RSD** | | | **0.46** |

**Table 4:** Results of Accuracy

| Sample concentration | Sample set no | Sample area | | Assay | | % Recovery | |
|---|---|---|---|---|---|---|---|
| | | ARTE | PIPE | ARTE | PIPE | ARTE | PIPE |
| 50% | 1 | 460064 | 276931 | 24.9 | 25.0 | 99.8 | 100 |
| | 2 | 460124 | 276694 | 24.6 | 24.9 | 99.6 | 99.6 |
| | 3 | 460216 | 276891 | 24.8 | 24.9 | 99.8 | 99.6 |
| | Average Recovery | | | | | 99.7% | 99.7% |
| 100% | 1 | 923429 | 554156 | 49.9 | 50.0 | 99.8 | 100 |
| | 2 | 923654 | 554897 | 49.8 | 49.9 | 99.6 | 99.8 |
| | 3 | 923742 | 556371 | 49.8 | 49.9 | 99.6 | 99.8 |
| | Average recovery | | | | | 99.6% | 99.8% |
| 150% | 1 | 1387901 | 828113 | 74.8 | 75.0 | 99.8 | 100 |
| | 2 | 1385360 | 828794 | 74.9 | 74.9 | 99.8 | 99.8 |
| | 3 | 1386984 | 828349 | 74.6 | 74.8 | 99.6 | 99.8 |
| | Average recovery | | | | | 99.7% | 99.8% |

**Acceptance criteria:** The percentage recovery at each level should be between (97-103%). The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence the method is accurate.

**Table 5:** Results of Intermediate precision for Memantine

| S. No | Sample area | Standard area | Percentage purity |
|---|---|---|---|
| 1 | 979556 | 984395 | 99.30 |
| 2 | 982467 | 984039 | 99.64 |
| 3 | 979717 | 983976 | 99.36 |
| 4 | 978909 | 984278 | 99.28 |
| 5 | 981432 | 973915 | 100.57 |
| **Average** | | | **99.63** |
| **%RSD** | | | **0.54** |

**Table 6:** Results of Intermediate precision for Donepezile

| S. No | Sample area | Standard area | Percentage purity |
|---|---|---|---|
| 1 | 583416 | 593403 | 99.12 |
| 2 | 583657 | 594352 | 99.01 |
| 3 | 584731 | 593357 | 99.52 |
| 4 | 583594 | 592673 | 99.61 |
| 5 | 597649 | 593671 | 99.12 |
| Average | | | 99.27 |
| %RSD | | | 0.27 |

**Acceptance criteria:** %RSD of five different sample solutions should not be more than 2**.** The %RSD obtained is within the limit, hence the method is rugged.

## 4. Conclusion

On the basis of experimental results, the proposed method is suitable for the quantitative determination of memantin and donepezil in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. The estimation of Memantin and Donepezil was done by RP-HPLC. The Phosphate buffer was pH 4.6 and the mobile phase was optimized which consists of MeOH : Phosphate buffer mixed in the ratio of 70:30 % v/ v. A Symmetry C18 (4.6 x 150mm, 5µm, Make XTerra) column used as stationary phase. The detection was carried out using UV detector at 273 nm. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. the linearity range of Memantin and donepezil were found to be from 25-125 µg/ml. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Memantin and donepezil LOD and LOQ was found to be within limit. The proposed method is precise, simple and accurate to determine the amount of Memantin and donepezil in formulation.High percentage of recovery shows that the method is free from the interference of excipients used in the formulation. So the method can be useful in the routine quality control of these drugs.

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