ISSN: 2395-0536 Impact Factor- 3.50*

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

# A NEW RP HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF MEMENTINE & DONEPEZIL IN BULK AND PHARMACEUTICAL DOSAGE FORMS

**K. KranthiKiran*, K. Hema Grace, M. PavanSatya Prasad, R. Pramodh, V. Elisha Rani**
**Department of Pharmaceutical Analysis, JITS College of Pharmacy, Kalgampudi, Andhra Pradesh, India.**

## ABSTRACT

A simple and selective LC method is described for the determination of Donepezil and Memantine dosage forms. Chromatographic separation was achieved on a $C_{18}$ column using mobile phase consisting of a mixture of Methanol: Acetonitrile: Water (55: 25: 20 v/v/v), with detection of 277nm. Linearity was observed in the range 20-60 µg /ml for Donepezil ($r^2$ =0.998) and 40-120µg/ml for Memantine($r^2$ =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

**Key Words:** Donepezil, Memantine, Chromatographic separation

**Author for correspondence**
**Dr. K. KranthiKiran,**
Department of Pharmaceutical Analysis,
JITS College of Pharmacy, Kalgampudi, Andhra Pradesh, India.
Email id: drkranthikothapalli@gmail.com

## INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books.Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis. Qualitative analysis is performed to establish composition of a substance. It is done to determine the presence of a compound or substance in a given sample or not. The various qualitative tests are detection of evolved gas, limit tests, color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half life etc.Quantitative analysis techniques are mainly used to determine the amount or concentration of analyte in a sample and expressed as a numerical value in appropriate units. These techniques are based on suitable chemical reaction and either measuring the amount of reagent added to complete the reaction or measuring the amount of reaction product obtained the characteristic movement of a substance through a defined medium under controlled conditions, electrical measurement or measurement of spectroscopic properties of the compound.Chromatography is defined as a non-

destructive procedure for resolving multi-component mixture of trace, minor, or major constituents into its individual fractions. In chromatography, the sample is dissolved in the mobile phase which may be a gas, liquid, or a supercritical fluid. The principle involved in HPLC is that when a mixture containing different compounds is introduced into the mobile phase and allowed to flow over a stationary phase, the individual compounds travel at different speeds and get separated based on the relative affinities to the stationary phase and the mobile phase. The compounds are separated based on the polarity of the stationary phase and the mobile phase.Donepezil is a piperidine derivative that is a centerally active, reversible inhibitor of acetylcholinesterase. This drug is structurally unrelated to other anticholinesterase agents. Donepezil's proposed mechanism of action involves the reversible inhibition of cholinesterases (eg. acetylcholinesterase), which prevents the hydrolysis of acetycholine, and leads to an increased concentration of acetylcholine at cholinergic synapses. Evidence suggests that the anticholinesterase activity of donepezil is relatively specific for acetylcholinesterase in the brain.Memantine exerts its action through uncompetitive NMDA receptor antagonism, binding preferentially to the NMDA receptor-operated cation channels. Prolonged increased levels of glutamate in the brain of demented patients are sufficient to counter the voltage-dependent block of NMDA receptors by $Mg^{2+}$ ions and allow continuous influx of $Ca^{2+}$ ions into cells, ultimately resulting in neuronal degeneration. Studies suggest that memantine binds more effectively than $Mg^{2+}$ ions at the NMDA receptor, and thereby effectively blocks this prolonged influx of $Ca^{2+}$ ions through the NMDA channel whilst preserving the transient physiological activation of the channels by higher concentrations of synaptically released glutamate (1-4).Aim is to develop new RP HPLC method for the simultaneous estimation of donepezil &memantinein pharmaceutical dosage form.

## MATERIALS AND METHODS

### Determination of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

### Preparation of standard stock solution of donepezil

50mg ofDonepezilwas weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 0.2ml to 10ml with methanol.

### Preparation of standard stock solution ofmemantine

100mg of Memantinewas weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 0.1ml to 10ml with methanol.

### Preparation of samples for Assay

### Preparation of mixed standard solution

Weigh accurately 50mg of Donepeziland100 mg of Memantine in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase from above stock solution 40µg/ml of Donepezil and 80 µg/ml of Memantine is prepared by diluting 0.8ml to 10ml with mobile phase. This solution is used for recording chromatogram.

### Preparation of sample solution

5tablets (each tablet contains 5mg of Donepezil and 10mg of Memantine) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Donepezil (40µg/ml) and Memantine(80µg/ml) were prepared by dissolving weight equivalent to 50mg of Donepeziland 100 mg of Memantineand dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 40µg/ml ofDonepeziland 80µg/ml of Memantine was made by adding 0.8ml of stock solution to 10 ml of mobile phase (5-7).

## RESULTS AND DISCUSSION

The wavelength of maximum absorption ($\lambda_{max}$) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within

the wavelength region of 200–400 nm against methanol as blank.The absorption curve shows characteristic absorption maxima at nm for Donepezil and 277 nm for Memantinenm for the combination.The amount of Donepezil and Memantinepresent in the taken dosage form was found to be 98.63% and 98.49% respectively (Table-1 and fig-1).

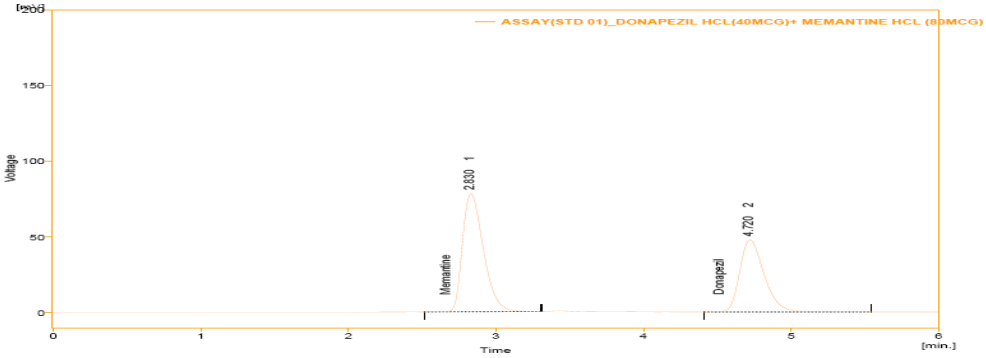


**Fig- 1 Chromatogram of Assay**

**Table -1 Assay Results**

| **MEMANTINE** | | | **DONEPEZIL** | |
|---|---|---|---|---|
| | **Standard Area** | **Sample Area** | **Standard Area** | **Sample Area** |
| **Injection-1** | 768.820 | 781.374 | 546.817 | 538.641 |
| **Injection-2** | 767.589 | 767.612 | 543.171 | 541.972 |
| **Injection-3** | 764.499 | 764.499 | 537.068 | 537.068 |
| **Injection-4** | 767.339 | 767.339 | 542.561 | 542.561 |
| **Injection-5** | 761.946 | 761.946 | 546.817 | 546.817 |
| **Average Area** | 766.9693 | 764.566 | 542.352 | 541.4118 |
| **Tablet average weight** | 18.76 | | 18.76 | |
| **Standard weight** | 100 | | 50 | |
| **Sample weight** | 187.6 | | 187.6 | |
| **Label amount** | 5mg | | 10mg | |
| **std. purity** | 99.8 | | 99.8 | |
| **Amount found in mg** | **9.85** | | **4.93** | |
| **Assay(%purity)** | **98.49** | | **98.63** | |

The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparations of Donepezil and Memantine is 0.99 and 0.99. The relationship between the concentration of Donepezil and Memantineand area of Donepezil and Memantineis linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.The % recovery of Donepezil and Memantineshould lie between 98% and 102% (Table-2 and 3).

**Table-2 Recovery results for Donepezil**

| Recovery level | **Accuracy Donepezil** | | | | | Average % Recovery |
|---|---|---|---|---|---|---|
| | Amount taken (mcg/ml) | Area | Average area | Amount recovered (mcg/ml) | %Recovery | |
| 75% | 30 | 399.107 | **400.700** | **29.91** | **99.71** | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 30 | 401.497 | | | | |
| | 30 | 401.493 | | | | |
| 100% | 40 | 549.519 | **545.988** | **39.78** | **99.46** | |
| | 40 | 543.545 | | | | 99.45% |
| | 40 | 544.900 | | | | |
| 125% | 50 | 693.052 | **692.725** | **49.59** | **99.18** | |

**Table-3** Recovery results for Memantine

| Recovery level | **Accuracy Memantine** | | | | | Average % Recovery |
|---|---|---|---|---|---|---|
| | Amount taken(mcg/ml) | Area | Average area | Amount recovered(mcg/ml) | %Recovery | |
| 75% | 60 | 568.486 | **567.449** | **59.91** | **99.84** | |
| | 60 | 566.931 | | | | |
| | 60 | 566.935 | | | | |
| 100% | 80 | 765.196 | **762.650** | **79.32** | **99.15** | 99.62% |
| | 80 | 760.287 | | | | |
| | 80 | 762.468 | | | | |
| 125% | 100 | 971.131 | **970.872** | **99.89** | **99.89** | |

From the observation it was found that the system suitability parameters were within limit at all variable conditions (Fig-2).

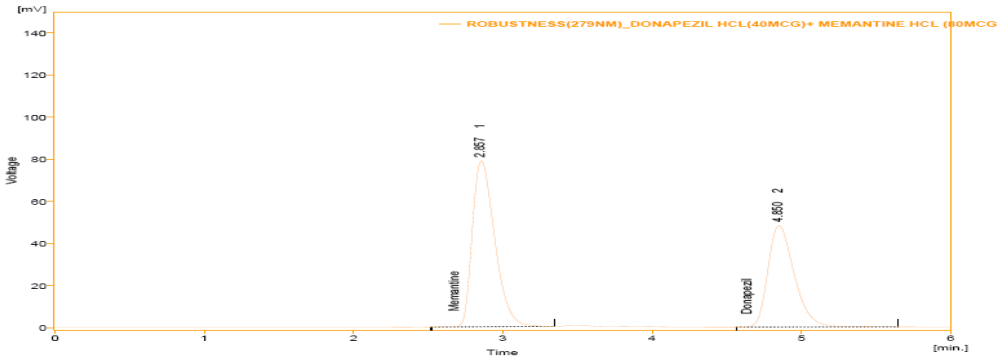


**Fig-2 Chromatogram of Donepezil and Memantine Robustness (279nm)**

## CONCLUSION

Experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation ofDonepezil and Memantinewas found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

## REFERENCES

1. Douglas, A.; Skoog, F.; James, H.; Stanley, R. C. Liquid Chromatography. In *Instrumental Analysis*, 9th ed.; Cengage Learning India Pvt. Ltd.: New Delhi, 2007; 893 - 934.
2. Skoog**;** Holler**;** Crouch**;** Liquid Chromatography**.** In *Instrumental Analysis***,** Cengage Learning India**.:**New Delhi**.** 2011**;** 893**.**
3. Chatwal**,** R**.** G**.;** Anand**,** K**.** S**.** High Performance Liquid Chromatography**.** In *Instrumental Methods Of Chemical Analysis***,** 5th ed**.;** Himalaya Publishers**.:**Mumbai, 2010**;** 2.570 - 2.629**.**
4. Sharma**,** B**.** K**.** High Performance Liquid Chromatography**.** In *Instrumental Methods Of Chemical Analysis***,** 24th ed**.;** Goel Publishers**.:** Meerut**,** 2005**;**295 - 300**.**
5. Michael Swartz, E.; Ira Krull, S, Analytical Method development. In Analytical Method Development and Validation, 1st ed.; Marcel Dekker, Inc: New York, 2009; 17-80.
6. Particle Sciences Drug Development Services. Analytic Method Development and Validation. *Technical Brief*. **2009**, *5*, 1-2.
7. Ghulam, A. S. PLC Method Development and Validation for Pharmaceutical Analysis. *Pharmaceutical Technology Europe*. **2004**, 7, 55 -63.