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Method development and validation for the simultaneous estimation of memantine and donepezil in bulk form and marketed tablet dosage forms by RP-HPLC

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Abstract
A novel very rapid, sensitive, reverse phase High-Performance Liquid Chromatography (RP-HPLC) technique was developed for the quantitative simultaneous estimation of Memantine and Donepezil in bulk and pharmaceutical dosage form. It was resolved by using a mobile phase of Buffer, Methanol and Acetonitrile in the ratio of 65:35:10% v/v/v at a flow rate of 1.0 mL/min using UV-Visible detector at the wavelength of 234 nm for quantification. Efficient separation was achieved for Memantine and Donepezil on used Altima C_{18} (4.6mm × 150mm, 5.0 µm) Column. The retention times of Memantine and Donepezil were found to be 2.088 min and 6.068minutes respectively. The calibration graphs were linear and the method showed excellent recovery for Memantine and Donepezil respectively. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness. The results presented in this report revealed the development of simple, rapid, precise and accurate RP-HPLC method for immediate determination and validation of Memantine and Donepezil in bulk form and their marketed pharmaceutical dosage forms.

Keywords: Memantine and Donepezil, RP-HPLC, Accuracy, Precision, ICH Guidelines

Introduction

Memantine is a primary aliphatic amine that is the 3, 5-dimethyl derivative of 1-aminoadamantane. A low to moderate affinity uncompetitive (open-channel); NMDA receptor antagonist which binds preferentially to the NMDA receptor-operated cation channels [1]. It has a role as a dopaminergic agent, an antiparkinson drug, an NMDA receptor antagonist, a neuroprotective agent, and an antidepressant. It is a member of adamantanes and a primary aliphatic amine. It is a conjugate base of a memantinium (1+). It derives from a hydride of an adamantane. Memantine is used to manage moderate to severe Alzheimer's dementia. A more recent systemic review and meta-analysis 6 indicates that Memantine is beneficial as a first line drug for the treatment of Alzheimer's dementia. Cholinesterase inhibitors may be added to Memantine for further beneficial effects on behavioral symptoms and other symptoms of dementia [2]. Continuous activation of the N-methyl-D-aspartate (NMDA) receptors in the central nervous system caused by glutamate is thought to cause some of the Alzheimer's disease symptoms. This over activation is thought to contribute to neurotoxicity due to the excitatory properties of glutamate 9. The pharmacological effect of Memantine likely occurs via the drug's behavior as an uncompetitive (open-channel) NMDA receptor antagonist, preventing glutamate action on this receptor [3]. Memantine has a preference for the NMDA receptor-operated cation channels. Despite these antagonist effects, Memantine has not been proven to prevent or retard the neurodegeneration seen in patients diagnosed with Alzheimer's disease. The IUPAC name of 3, 5-dimethyl adamantan-1-amine. The Chemical Structure of Memantine is shown in follows

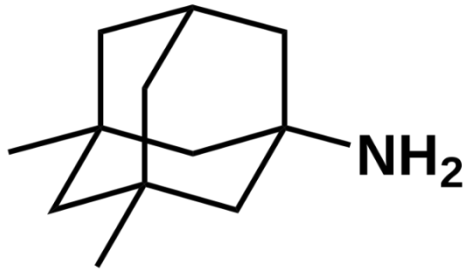

Fig 1: Chemical Structure of Memantine

Donepezil is the hydrochloride salt of a piperidine derivative with neurocognitive-enhancing activity. Donepezil reversibly inhibits acetyl cholinesterase, thereby blocking the hydrolysis of the neurotransmitter acetylcholine and, consequently, increasing its activity [4]. This agent may improve neurocognitive function in Alzheimer's disease, reduce sedation associated with opioid treatment of cancer pain, and improve neurocognitive function in patients who have received radiation therapy for primary brain tumours or brain metastases. By inhibiting the acetyl cholinesterase enzyme, donepezil improves the cognitive and behavioral signs and symptoms of Alzheimer's disease, which may include apathy, aggression, confusion, and psychosis [5]. The commonly accepted cholinergic hypothesis proposes that a portion of the cognitive and behavioral decline associated with Alzheimer's are the result of decreased cholinergic transmission in the central nervous system. Donepezil selectively and reversibly inhibits the acetyl cholinesterase enzyme, which normally breaks down acetylcholine. The main pharmacological actions of this drug are believed to occur as the result of this enzyme inhibition, enhancing cholinergic transmission, which relieves the symptoms of Alzheimer's dementia. In addition to the above, other mechanisms of action of donepezil are possible, including the opposition of glutamate-induced excitatory transmission via down regulation of NMDA receptors and the regulation of amyloid proteins, which have demonstrated significant effects on the disease process of Alzheimer's. Other possible targets for donepezil may also include the inhibition various inflammatory signaling pathways, exerting neuroprotective effects [6]. The IUPAC name of Donepezil is 2-[(1-benzylpiperidin-4-yl) methyl]-5, 6-dimethoxy-2, 3-dihydro-1H-inden-1-one. The Chemical Structure of Donepezil is shown in fig-2.

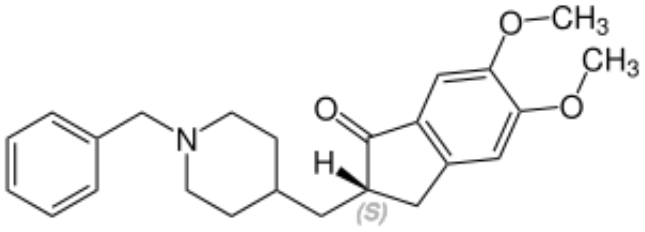

Fig 2: Chemical Structure of Donepezil

Experimental

Table 1: Instruments Used

S.No.	Instruments and Glasswares	Model
1	HPLC	WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA Detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table 2: Chemicals Used

S.No	Chemical	Brand names
1	Memantine (Pure)	Sun Pharma
2	Donepezil (Pure)	Sun Pharma
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC Method Development

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Memantine and Donepezil working standard into 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.375ml of Memantine and 0.3ml of the Donepezil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines [30, 35].

Mobile Phase Optimization

Initially, the mobile phase tried was Methanol: Orthophosphoric acid and Phosphoric acid (pH 3): Acetonitrile and Methanol: ACN with varying proportions. Finally, the mobile phase was optimized to Buffer: Methanol: ACN in proportion 65:25:10v/v respectively.

Optimization of Column

The method was performed with various columns [7, 8] like C_{18} column, ODS and Zodiac column. Altima C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Preparation of Phosphate buffer (pH-4.6)

Dissolve 0.9g of anhydrous di hydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce

1000mL. Adjust the p H 4.6 by using ortho phosphoric acid.

Preparation of Mobile Phase

Accurately measured 650 ml (65%) of Buffer and 250 ml of Methanol (25%) and 100ml (10%) of Acetonitrile were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration [9].

Diluent Preparation

The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Memantine and 10mg of Donepezil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.375ml of Memantine and 0.3ml of the Donepezil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits [10].

Specificity

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Memantine and 10mg of Donepezil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.375ml of Memantine and 0.3ml of the Donepezil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Memantine and Donepezil sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.375ml of Memantine and 0.3ml of the Donepezil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay [11] by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity

Accurately weigh and transfer 10 mg of Memantine and 10mg of Donepezil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent [12]. (Stock solution).

Preparation of Level – I (12.5ppm of Memantine&10ppm of Donepezil)

Pipette out 0.125ml of Memantine and 0.1ml of Donepezil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (25ppm of Memantine&20ppm of Donepezil)

Pipette out 0.25ml of Memantine and 0.2ml of Donepezil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (37.5ppm of Memantine &30ppm of Donepezil)

Pipette out 0.375ml of Memantine and 0.3ml of Donepezil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (50ppm of Memantine&40ppm of Donepezil)

Pipette out 0.5ml of Memantine and 0.4ml of Donepezil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (62.5ppm of Memantine&50ppm of Donepezil)

Pipette out 0.625ml of Memantine and 0.5ml of Donepezil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure

Inject each level into the chromatographic system [13] and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision

Repeatability

Preparation of Memantine and Donepezil Product Solution for Precision

Accurately weigh and transfer 10 mg of Memantine and 10mg of Donepezil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.375ml of Memantine and 0.3ml of the Donepezil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The standard solution was injected for five times and measured the area for all five injections in HPLC [14]. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

Day 1

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Day 2

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy

For Preparation of 50% Standard Stock Solution

Accurately weigh and transfer 10 mg of Memantine and 10mg of Donepezil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents [15] and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.187ml of Memantine and 0.15ml of the Donepezil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 100% Standard Stock Solution

Accurately weigh and transfer 10 mg of Memantine and 10mg of Donepezil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.375ml of Memantine and 0.3ml of the Donepezil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 150% Standard Stock Solution

Accurately weigh and transfer 10 mg of Memantine and 10mg of Donepezil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.562ml of Memantine and 0.45ml of the Donepezil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Memantine and Donepezil and calculate the individual recovery and mean recovery values [16].

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Memantine and 10mg of Donepezil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.375ml of Memantine and 0.3ml of Donepezil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition

The sample was analyzed by variation of mobile phase i.e. Buffer: Methanol: ACN was taken in the ratio and 75: 15: 10, 55:35:10 instead 65:25:10, remaining conditions [17] are same. 10µl of the above sample was injected and chromatograms were recorded.

Results and Discussion

Method Development

Optimized Chromatographic Conditions

Mobile phase: Buffer: Methanol: Acetonitrile (65:25:10% v/v/v)
Column: Altima C18 (4.6×150mm, 5.0 µm)
Flow rate: 1.0 ml/min
Wavelength: 234 nm
Column temp: 35ºC
Injection Volume: 10 µl
Run time: 14 minutes

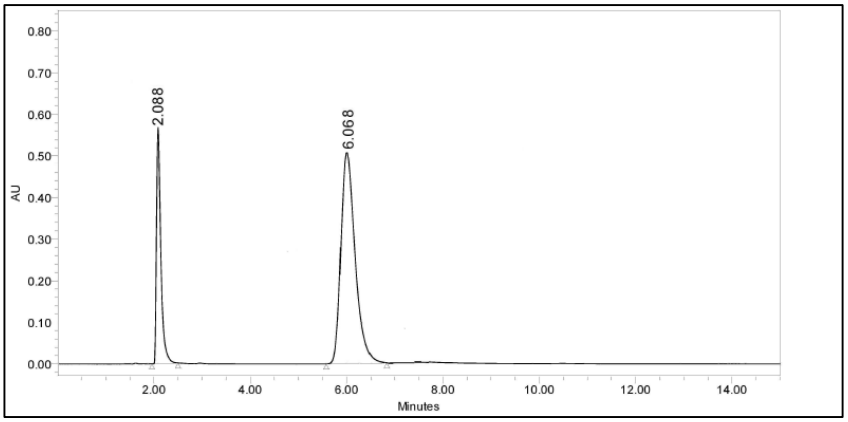

Fig 3: Optimized Chromatographic Condition

Validation of Analytical Method

The most widely applied validation characteristics are system suitability, accuracy, precision, specificity, linearity, range, robustness, and the limit of detection, limit of quantification, and limit of detection as per ICH Guidelines [30, 35].

System Suitability

Table 3: Results of system suitability for Memantine

S.No.	Name	Rt	Peak Area	Height	USP Plate Count	USP Tailing
1	Memantine	2.080	3569412	567917	5568.0	1.0
2	Memantine	2.080	3465125	517719	6359.2	1.1
3	Memantine	2.080	3598154	567933	5565.5	1.0
4	Memantine	2.081	3586491	517733	5355.2	1.1
5	Memantine	2.081	3582694	567917	6348.0	1.0
Mean			**3560375**			
Std. Dev			**54225.61**			
% RSD			**1.523031**			

Table 4: Results of Method Precision for Donepezil

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Donepezil	2.080	3582264	567917	5568.0	1.0	2.5
2	Donepezil	2.080	3586491	517719	5359.2	1.1	2.5
3	Donepezil	2.080	3598154	567933	5565.5	1.0	2.5
4	Donepezil	2.081	3564125	517733	5355.2	1.1	2.5
5	Donepezil	2.081	3569412	562173	5568.0	1.0	2.5
Mean			**3580089**				
Std. Dev			**13609.81**				
% RSD			**0.380153**				

Specificity

The ICH documents define specificity [18] as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitates MEMANTINE and Donepezil in drug product.

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Memantine and Donepezil in pharmaceutical dosage form was found to be 99.6%.

Linearity

Chromatographic Data for Linearity Study

Table 5: Chromatographic Data for Linearity Study Memantine

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	25	1010252
66.6	50	2049374
100	75	3072706
133.3	100	3921068
166.6	125	4952813

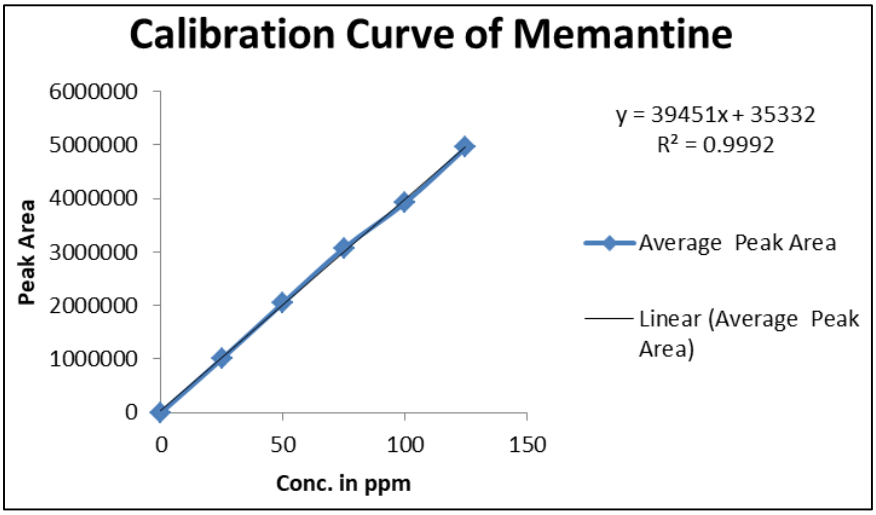

Fig 4: Calibration Graph for Memantine

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Memantine is a straight line.

Y = mx + c

Slope (m) = 39451
Intercept (c) = 35332
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity [19] is verified if the Correlation Coefficient is 0.99 or greater.
Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 35332. These values meet the validation criteria.

Table 6: Chromatographic Data for Linearity Study Donepezil

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	10	8040807
66	20	14318417
100	30	21087985
133	40	27913928
166	50	34584741

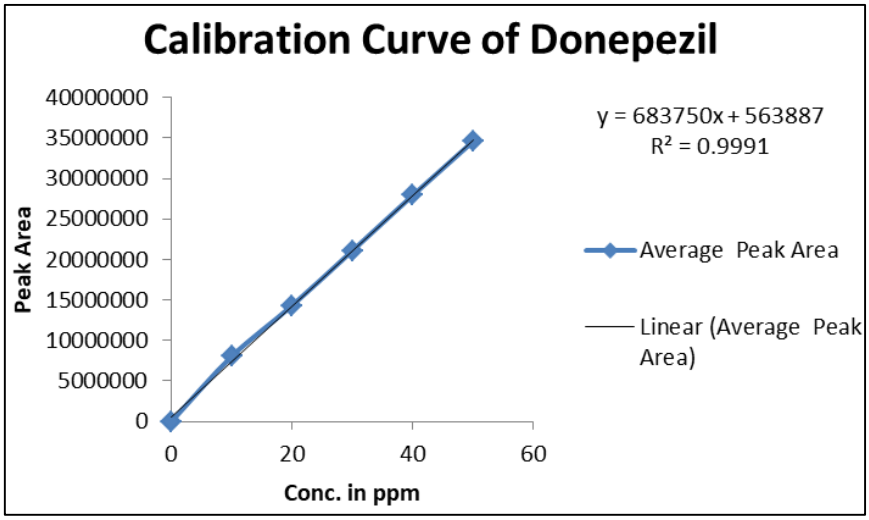

Fig 5: Calibration Graph for Donepezil

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Donepezil is a straight line.

Y = mx + c

Slope (m) = 68375
Intercept (c) = 56388
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.
Conclusion: Correlation Coefficient [20] (r) is 0.99, and the intercept is 56388. These values meet the validation criteria.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [21].

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-7: Results of Repeatability for Memantine

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Memantine	2.084	3569412	567917	5568.0	1.0
2	Memantine	2.083	3465125	517719	5359.2	1.1
3	Memantine	2.082	3598154	567933	5565.5	1.0
4	Memantine	2.081	3586491	517733	5355.2	1.1
5	Memantine	2.080	3582694	567917	5568.0	1.0
Mean			**3560375**			
Std. Dev			**54225.61**			
% RSD			**1.523031**			

Table 8: Results of Method Precision for Donepezil

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Donepezil	6.056	1582264	567917	5568.0	1.0	2.5
2	Donepezil	6.057	1586491	517719	5359.2	1.1	2.5
3	Donepezil	6.058	1598154	567933	5565.5	1.0	2.5
4	Donepezil	6.059	1564125	517733	5355.2	1.1	2.5
5	Donepezil	6.060	1569412	562173	5568.0	1.0	2.5
Mean			**1580089**				
Std. Dev			**13609.81**				
% RSD			**0.861332**				

Intermediate Precision

Day 1

Table 9: Results of Intermediate Precision for Memantine

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Memantine	2.081	3481579	567917	5568.0	1.0
2	Memantine	2.082	3458121	517719	5359.2	1.1
3	Memantine	2.083	3426581	567933	5565.5	1.0
4	Memantine	2.084	3465712	517733	5355.2	1.1
5	Memantine	2.085	3451476	567917	5568.0	1.0
6	Memantine	2.085	3452106	567514	5359.2	1.1
Mean			**3455929**			
Std. Dev			**18188.92**			
% RSD			**0.5**			

Table 10: Results of Intermediate Precision for Donepezil

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Donepezil	6.061	15481579	567917	5568.0	1.0	2.5
2	Donepezil	6.062	15369852	517719	5359.2	1.1	2.5
3	Donepezil	6.063	15248454	567933	5565.5	1.0	2.5
4	Donepezil	6.064	15874692	517733	5355.2	1.1	2.5
5	Donepezil	6.064	15236547	567933	5568.0	1.0	2.5
6	Donepezil	6.064	15217547	567133	5359.2	1.1	2.5
Mean			**15404779**				
Std. Dev			**251289.4**				
% RSD			**1.6**				

Day 2

Table 11: Results of Intermediate Precision Day 2 for MEMANTINE

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Memantine	2.081	3481579	567917	5568.0	1.0
2	Memantine	2.082	3458121	517719	5359.2	1.1
3	Memantine	2.083	3426581	567933	5565.5	1.0
4	Memantine	2.084	3465712	517733	5355.2	1.1
5	Memantine	2.085	3451476	567917	5568.0	1.0
6	Memantine	2.085	3452106	567514	5359.2	1.1
Mean			**3455929**			
Std. Dev			**18188.92**			
% RSD			**0.5**			

Table 12: Results of Intermediate Precision for Donepezil

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Donepezil	6.061	15481579	567917	5568.0	1.0	2.5
2	Donepezil	6.062	15369852	517719	5359.2	1.1	2.5
3	Donepezil	6.063	15248454	567933	5565.5	1.0	2.5
4	Donepezil	6.064	15874692	517733	5355.2	1.1	2.5
5	Donepezil	6.064	15236547	567933	5568.0	1.0	2.5
6	Donepezil	6.064	15217547	567133	5359.2	1.1	2.5
Mean			**15404779**				
Std. Dev			**251289.4**				
% RSD			**1.6**				

Accuracy

Accuracy[22-24] at different concentrations (50%, 100%, and 150%) was prepared and the % recovery[25] was calculated.

Table 13: The Accuracy Results for Memantine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1543793	37.5	37.52	101.9	100.9%
100%	3035883	75	75.1	101.4	
150%	4451005	112.5	112.47	99.4	

Table 14: The Accuracy Results for Donepezil

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1084420	15	15.07	100.2	99.6%
100%	2096069	30	29.6	99.4	
150%	3112684	45	44.8	99.5	

Limit of Detection

The detection limit [26] of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD = 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve [27]

Result

Memantine

=4.9µg/ml

Donepezil

=8.5µg/ml

Limit of Quantitation

The quantitation limit [28] of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ = 10×σ/S

Where,

σ = Standard deviation [29] of the response

S = Slope of the calibration curve

Result

Memantine

= 14.8µg/ml

Donepezil

= 25.7µg/ml

Robustness

The robustness [31-33] was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Memantine and Donepezil. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard samples of Memantine and Donepezil were injected by changing the conditions of chromatography [35]. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 15: Results for Robustness for Memantine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical Plates	Tailing factor
Flow rate of 1.0 mL/min	3425413	2.088	5568.2	1.0
Flow rate of 0.9 mL/min	3425282	3.111	5922.2	1.2
Flow rate of 1.1 mL/min	3517879	1.880	5868.8	1.2
Less aqueous phase	3175485	3.101	5836.2	1.2
More aqueous phase	3365431	1.881	5282.6	1.1

Table 16: Results for Robustness for Donepezil

Parameter Used For Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Flow rate of 1.0 mL/min	2029854	6.068	5359.2	1.1
Flow rate of 0.9 mL/min	1738319	7.101	5999.1	1.2
Flow rate of 1.1 mL/min	1638304	5.007	5989.2	1.1
Less aqueous phase	1973724	7.108	5387.2	1.1
More aqueous phase	2102838	5.008	5938.1	1.1

Summary and Conclusion

In the present investigation the selected drug combinations were analyzed in both bulk and pharmaceutical formulations by simple, fast, precise and reliable Reverse Phase High Performance Liquid Chromatographic methods with the search for a suitable stationary and new mobile phase which were not been used until.

The chemicals and Equipment used such as HPLC is a WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector was used for the analysis of the drugs combination. This chapter also discusses the procedures used for the trials involved in the method development and validation parameters such as system suitability, selectivity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), specificity and robustness.

During method development, chromatographic parameters such as mobile phase composition, flow rate, detection wavelength, analytical column and column temperature were optimized to get improved efficiency of the chromatographic system. Two HPLC analytical columns, Symmetry C18 (150 mm × 4.6 mm; 5 µm particle size) and Zodiac C18 (250 mm × 4.6 mm; 5 µm particle size) were tested during method development but Altima C18 (4.6×150mm, 5.0 µm) column was found to be ideal as it gave good peak shape and resolution at 1.0ml/min flow rate.

The system suitability parameters like tailing factor, resolution and plate count were considered. Based on the above said parameters Altima C18 (4.6×150mm, 5.0 µm) Column was finalized. Different composition of mobile phase containing a mixture (v/v) of Phosphate Buffer and Methanol, acetonitrile, water with different ratios were evaluated so as to obtain appropriate composition of mobile phase. Finally the mixture of Buffer, Methanol and Acetonitrile were taken in the ratio of 65:25:10% (v/v/v) was selected as optimal, at a flow rate of 1.0 mL/min and with column temperature 35ºC well defined and well resolved peaks of Memantine and Donepezil. At the wavelength 234 nm, best detector response for Memantine and Donepezil was obtained. Therefore, 234 nm was selected as the analytical wavelength for the detection and quantification of Memantine and Donepezil. Under the optimized chromatographic conditions, the retention times for Memantine and Donepezil were found to be 2.088min and 6.068minutes respectively.

The developed method was validated for system suitability, selectivity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), specificity and robustness as per International Conference on Harmonization (ICH) guidelines [International Conference on Harmonization, 2005]. The studies conducted and the results obtained were summarized in summary.

Conclusion

Based on the results obtained in this study, it is concluded that the present validated method can be successfully applied for the estimation of Memantine and Donepezil in bulk form and Marketed Pharmaceutical Dosage form.

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