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An analytical review on method development and validation of drugs used for Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory defeat and impairment in behaviour, language, and visuospatial skills. Current approved drugs for the treatment of Alzheimer disease (AD) include cholinesterase inhibitors (donepezil, galantamine and rivastigmine) and the NMDA receptor antagonist memantine. These drugs can provide a symptomatic relief but they poorly affect the progression of the disease. There are several risk factors for the development of Alzheimer's disease which include factors like age, genetic factor family history, Down's syndrome, head injury and cardiovascular diseases. Cardio vascular risk factors may include blood pressure, cholesterol, diabetes, obesity and smoking. People may experience cognitive mental illness, difficulty in understanding and thinking, forgetting things easily, making things complicated, mental confusion, difficulty in concentrating, inability to create old memories, inability to do simple things, or inability to recognize common things. The main objective of this review is discussion on various analytical methods used, different solvents used as mobile phase and their retention time. This review includes method development and validation of cholinesterase inhibitors like Donepezil, Galantamine, Rivastigmine and Tacrine combination of drugs which include cholinesterase inhibitors like Donepezil and NMDA receptor antagonist Memantine. The review is a collection of data including various analytical methods used, the different columns used, mobile phase used, flow rate, different detectors and detection wavelength and retention time. This review includes discussion on method development and validation of Alzheimer's drugs and newly developed compounds which have lesser side effects and are proving more efficient for treatment of Alzheimer's disease.

Keywords: Alzheimers Disease, Cholinesterase inhibitors, Dementia, Method Development, Validation..

1. Introduction

Alzheimer's disease (AD) is also referred to as Alzheimer's, which is a chronic neurodegenerative disease that usually starts slowly and then worsens over when the disease prolongs. About 60% to 70% cases are seen, it is also known as senile dementia. This disease mostly affects the age group of more than 60-65 years of age, this disease may be genetically proven, by a family history, people with Down's syndrome are more proven to this disease, also cardiovascular diseases can be a major cause to this disease like smoking and drinking, high blood pressure, diabetic patients, high cholesterol and obesity can lead to AD and people with severe head injuries have a higher risk to AD, since it leads to dementia and cognition. Alzheimer's disease is a progressive disease that destroys memory and other important mental functions.

It is a neurological disorder in which the brain cells die and causes memory loss and cognitive decline. It is a neurodegenerative type of dementia, where the disease starts mild and gets progressively worsens as the disease prolongs. This disease can be for life long.

The most common symptom seen in AD is difficulty in remembering recent events which is called as short-term memory loss. As the disease prolongs, symptoms can include problems with speaking and language, , mood swings, loss of motivation, not managing self care, behavioural changes and issues and easily getting lost. Although the disease progression can vary, the average life span may decrease from three to nine years following diagnosis.
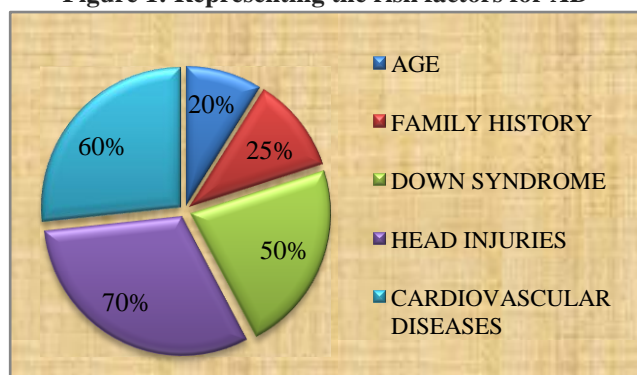
The cause of Alzheimer's disease is poorly understood. About 70% of the cause is believed to be genetically involved with many genes usually. Other risk factors may include a history of head injuries, depression or hypertension, hypercholesterolemia, obesity etc. The diagnosis is based on the history of the illness and cognitive testing with medical and blood tests to rule out other possible issues. Initial symptoms are often mistaken for normal ageing. Examination of brain cells is needed for a definite diagnosis. Mental and physical exercise, and avoiding smoking, hypertension, diabetes and obesity may decrease the risk of AD.

Chronic AD can last for years or be lifelong. Brain cell connections and the cells themselves degenerate and die, eventually destroying memory and other important mental functions. Memory loss and confusion are the main symptoms. No cure exists, but medication and management strategies may temporarily improve symptoms. The method development and validation of cholinesterase inhibitors like Donepezil, Galantamine, Rivastigmine and Tacrine. Combination drugs which include cholinesterase inhibitors like Donepezil and NMDA receptor antagonist Memantine are discussed in this review. The various analytical methods used, the different columns used, mobile phase used, flow rate, different detectors and detection wavelength and retention time are discussed in this review.

1.1 The highest risk factors which combat AD include
1) Age, 2) Family history, 3) Down's syndrome, 4) Severe Head injuries, 5) Cardiovascular disease
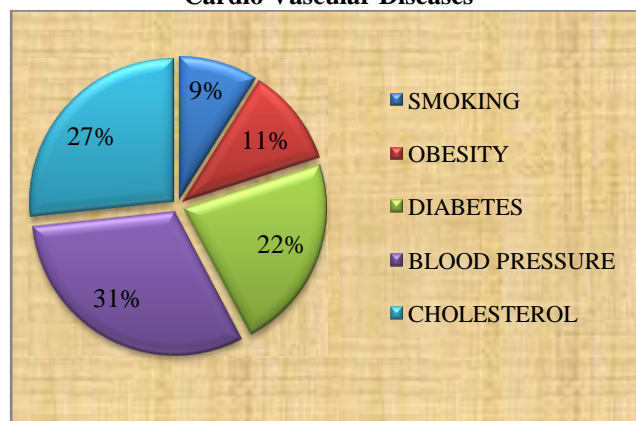
Figure 1: Representing the risk factors for AD

1.2 The five major factors in CVD include:
1) Smoking,
2) Obesity,
3) Diabetes,
4) Hypertension and High Cholesterol

Figure 2: Represents the risk factors for AD through Cardio Vascular Diseases

3. Causes of Alzheimer's disease

The total size of the brain shrinks the tissue has very few nerve cells and connections. Nerve cells (neurons) in the brain shrink. In Alzheimer's, disease there are microscopic 'plaques' and 'tangles' between and within the brain cells. Plaques are found between the dying cells in the brain from the build-up of a protein called beta-amyloidal. The tangles are within the brain neurons from a degeneration of another protein, called tau.
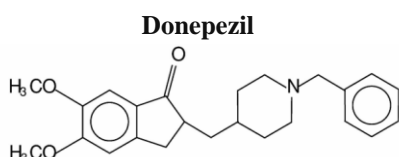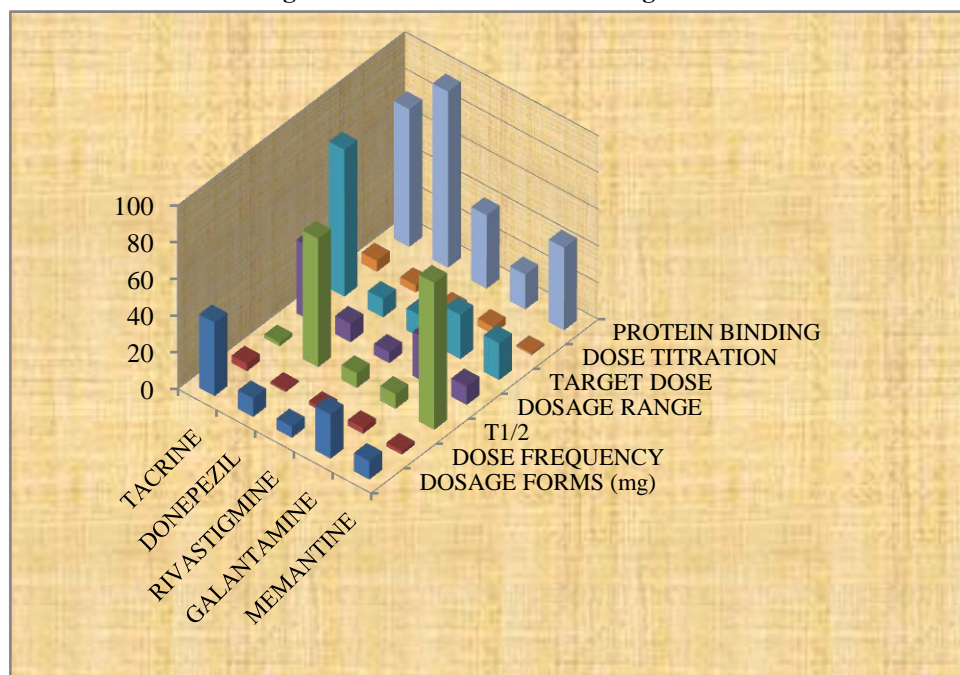
3.1 Various Stages of Alzheimer's disease

Alzheimer's can be classified into three basic stages: Preclinical, Mild cognitive impairment, Dementia.

4. Drug Therapy

There are four drugs in a class called cholinesterase inhibitor Include:

Donepezil (Aricept),

Galantamine (Reminyl),

Rivastigmine (Exelon),

Tacrine (Cognex),

Memantine (Namenda), an NMDA receptor antagonist, may also be used, alone or in combination with a cholinesterase inhibitor.

Figure 3: Alzheimer's disease drug scale

Donepezil

A Reverse Phase High Performance Liquid Chromatographic (RPHPLC) method was developed for the determination of Donepezil HCl tablets, C18 column 250mm x 4.6mm(l x d) in reverse phase isocratic mode of separation with mobile phase methanol : phosphate buffer : Triethylamine (60:40:0.5)% v/v were used. The flow rate was 1ml/min. [1]

A RP-HPLC method has been developed for the determination of donepezil hydrochloride in bulk and tablets. Separation was achieved with a keystone phenyl RP 250 × 4.6 mm i.d., 5 µM particle size analytical column using mixture of methanol, 0.02M phosphate Buffer (pH 7.5 ± 0.1) and triethylamine in the ratio 60: 40: 0.5 v/v as the mobile phase by Reverse phase isocratic mode. The instrumental settings are at a flow rate of 1mL/min, column temperature at 40°C and UV detection at 268 nm. The retention time was found to be 7.05 min. [2]

A Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method was developed for the determination of Donepezil HCl tablets, Xterra RP C 18, 250 x 4.6 mm, 5µ in reverse phase isocratic mode of separation with mobile phase Potassium dihydrogen orthophosphate Buffer and Acetonitrile (80:20)% v/v was used. The flow rate was 1ml/min. Detection was done at 230 nm. [3]

A new RP-HPLC method was developed and validated. Chromatography was carried out using a C18 (4.6 X 250 mm 3.5µm). A mobile phase consisting of Methanol: buffer (60:40% v/v) was pumped at an isocratic flow rate of 1ml/min. [4]

Simple and accurate methods to determine donepezil, in tablet dosage form, were developed and validated using liquid chromatography (LC). The LC separation was achieved on a Inertsil C8-3, 25 cm x 4.6-mm, 5 µ in the isocratic mode using buffer: methanol: triethylamine (550:450:5) v/v, adjusted to pH 2.50±0.05 with orthophosphoric acid, as the mobile phase at a flow rate of 1.0 mL/min. The methods were performed at 271 nm. The method was validated by determining its sensitivity, accuracy and precision. [5]

RP-HPLC method was developed and validated by assessing linearity, accuracy, selectivity, limit of quantitation, and precision for the determination of Donepezil hydrochloride. Donepezil was identified and quantitated on a C18 reversed phase column (3.9×150mm, 5.0µm), using a mobile phase composed of acetate buffer-Acetonitrile (50:50v/v) delivered at a flow rate of 1.0mL/min, and with UV detection (lexcitation = 230nm).[6]

Galantamine

$C_{18}H_{26}N_2O_8$
Mol. Wt.: 400.42

An accurate and precise RP-HPLC method for determination of Galantamine Hydrobromide has been developed. Analysis was carried out on Shimadzu HPLC system with Phenomenex C18 column (250 x 4.6 mm i.d, 5μm particle size) using 1 mM ammonium formate: acetonitrile (30:70) in Isocratic mode as mobile phase with flow rate of 0.4 ml.min-1. The detection was carried out using UV detector set at 289 nm. [7]

The reverse phase high performance liquid chromatographic RP-HPLC method has been developed to quantify galantamine HBr in raw material and capsule formulations using C18 analytical reverse phase column. Mobile phase consisted of solution A and solution B (75:25v/v) pumped at a flow rate of 1ml/min at 250c column temperature. Run time was about 8min with symmetrical peaks. Galantamine HBr was detected by PDA detector at 230 nm with no interference of excipients. [8]
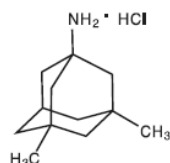
A rapid, accurate and precise RP-HPLC method was developed for the determination of Galantamine hydrobromide in pure and tablet dosage forms. Separation of the drug was achieved on a reverse phase Inertsil ODS (100 x 4.6 mm, 5). The method showed a linear response for concentration in the range of 30-180 μg/mL using phosphate buffer pH 6.3: acetonitrile as the mobile phase in the ratio of 60:40, v/v with detection at 285 nm with a flow rate of 1 mL/min and retention time was 4.1 min. [9]

4.1 Memantine HCl and Donepezil HCl

To develop and validate stability indicating method for the analysis of Memantine HCl and Donepezil HCl. The chromatographic separation was performed on Hypersil BDS (4.6 x 150 mm, 5μ) using Sodium dihydrogen ortho phosphate: Acetonitrile (30:70v/v) at a flow rate of 1 ml/min and detection of both the eluents was carried out by UV Detector. The Retention time of Memantine HCl and Donepezil HCl were found to be 2.833 min. and 4.777 min respectively. [10]
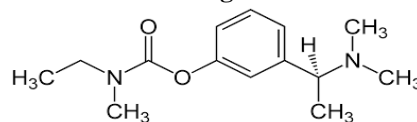
An accurate RP-HPLC method has been developed and validated for the simultaneous estimation of Memantine HCl and Donepezil HCl in bulk and pharmaceutical dosage form. Chromatographic separation was achieved on Amino, Column 250 X 4.8 Mm (5 μm) having mobile phase HPLC Grade Water (100%) at a flow rate of 1 mL/min and detection of both the eluents carried out by Waters Refractive Index Detector (RI Detectors). The retention time of MEM and DONE was found to be 3.561 min. and 4.212 min respectively. [11]

Memantine

A stability indicating RP-HPLC method has been developed and validated by using (2-Napthoxy) Acetyl chloride as derivatization agent and Amantadine as an internal standard. The separation was achieved by Inertsil ODS-3V, 250 x 4.6, 5μm column using mobile phase consisting of 0.02 M ammonium acetate buffer and methanol in the ratio (12:88) at a flow rate of 1.5 mL/min and UV detection at 226 nm. The Method was developed in isocratic mode. The retention time for Memantine and Amantadine was around 8.62 and 6.23. [12]
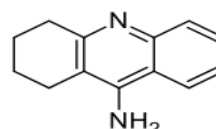
Rivastigmine

An isocratic RP-HPLC Method for analysis of Rivastigmine in pharmaceutical dosage forms has been developed and validated. Best separation was achieved on a Thermo Hypersil C4 column (25 cm X 4.6 mm, 5 μm) using a mobile phase of 0.01 M ammonium acetate buffer adjusted to pH 4.0 with orthophosphoric acid and Acetonitrile (60:40, v/v) at a flow rate of 1.0 mL min-1. UV detection was performed at 220 nm. Atrovastatin was used as an internal standard. The retention time of Rivastigmine and Atrovastatin was 4.75 and 8.83 min, respectively. [13]

A RP-HPLC method was developed and validated for the analysis of rivastigmine bulk dosages form. The separation was conducted by using C-18 RP-HPLC column which was maintained at ambient temperature. The mobile phase consist Potassium dihydrogen phosphate buffer and acetonitrile (70/30 v/v) was delivered at a rate of 1ml/min. The analysis was detected by using UV detector at the wavelength 217nm. The retention time for rivastigmine was found to be 3.66±25min. [14]

A RP-HPLC method was developed and validated for the analysis of rivastigmine hydrogen tartrate in transdermal drug delivery system. Rivastigmine tartrate is soluble in water so it was used as solvent. The separation was conducted by using C-18 RP-HPLC column which was maintained at ambient temperature. The mobile phase consist 0.01M ammonium acetate buffer and acetonitrile (70:30 v/v) was delivered at a rate of 1ml/min. The analysis was detected by using UV detector at the wavelength 219nm. The method is validated for its accuracy precision, ruggedness, linearity and range. The retention time for rivastigmine was found to be 4.40min. [15]

Tacrine

A simple RPHPLC method has been developed for determination of Tacrine hydrochloride in bulk and nanoemulsion gel using C18 column; 250 mm length, 4.6 mm internal diameter, 0.5μ particle size with UV visible detector (detection wavelength 243nm).Chromatographic separation was performed in an isocratic mode with mobile phase consisting of 0.05M triethylamine: acetonitrile (80: 20,); pH 3 using methanol as a diluent at a flow rate of 1.5 ml/minutes. Retention time was found to be 5.8 minutes. [16]

Table 1: This table represents the Name of the drug, Method used, Column used, Mobile phase, Flow rate, Detection and Retention time

Drug name	Method	Column used	Mobile phase	Flow rate	Detection	Retention time
Donepezil HCl tablets	RPHPLC isocratic mode	C18 column 250mm x 4.6mm(l x d)	methanol : phosphate buffer : Triethylamine (60:40:0.5) % v/v	1ml/min		
Donepezil hydrochloride	RPHPLC isocratic mode	phenyl RP 250 × 4.6 mm i.d	methanol, 0.02M phosphate Buffer (pH 7.5 ± 0.1) and triethylamine in the ratio 60: 40: 0.5 v/v	1 ml/min	UV 268 nm	7.05 min
Donepezil HCl tablets	RPHPLC isocratic mode	Xterra RP C 18, 250 x 4.6 mm	Potassium dihydrogen orthophosphate Buffer and Acetonitrile (80:20)% v/v	1 ml/min	230 nm	
Donepezil	RPHPLC isocratic mode	C18 (4.6 X 250 mm 3.5μm).	Methanol: buffer (60:40%v/v)	1 ml/min		
Donepezil	RPHPLC isocratic mode	Inertsil C8-3, 25 cm x 4.6-mm, 5 μ	buffer: methanol: triethylamine (550:450:5) v/v	1ml/min	271 nm	
Donepezil hydrochloride	RPHPLC isocratic mode	C18 reversed phase column	acetate buffer-Acetonitrile (50:50v/v)	1 ml/min	230nm	
Galantamine Hydrobromide	RPHPLC isocratic mode	C18 column	1 mM ammonium formate: acetonitrile (30:70)	0.4 ml/min	UV detector 289 nm	
Galantamine HBr	RPHPLC isocratic mode	C18 analytical reverse phase column	solution A and solution B (75:25v/v)	1ml/min	PDA detector 230 nm	
Galantamine hydrobromide	RP-HPLC	reverse phase Inertsil ODS	phosphate buffer pH 6.3: acetonitrile as the mobile phase in the ratio of 60:40, v/v	1 ml/min	285 nm	4.1 min
Memantine HCl and Donepezil HCl	RP-HPLC	Hypersil BDS (4.6 x 150 mm, 5μ)	Sodium dihydrogen ortho phosphate: Acetonitrile (30:70v/v)	1 ml/min	UV Detector	2.833 min. and 4.777 min
Memantine HCl and Donepezil HCl	RP-HPLC	Amino, Column 250 X 4.8 Mm (5 μm)	HPLC Grade Water (100%)	1 ml/min	Refractive Index Detector	3.561 min. and 4.212 min
Memantine	RP-HPLC	Inertsil ODS-3V, 250 x 4.6, 5μm	0.02 M ammonium acetate buffer and methanol in the ratio (12:88)	1.5 ml/min	UV detection 226 nm	8.62
Rivastigmine	RP-HPLC	Thermo Hypersil C4 column (25 cm X 4.6 mm, 5 μm)	0.01 M ammonium acetate buffer adjusted to pH 4.0 with orthophosphoric acid and Acetonitrile (60:40, v/v)	1 ml/min	UV detection 220 nm	4.75
Rivastigmine bulk dosages form	RP-HPLC	C-18	Potassium dihydrogen phosphate buffer and acetonitrile (70/30 v/v)	1 ml/min	UV detector 217nm	3.66±25min
Rivastigmine tartrate	RP-HPLC	C-18 RP-HPLC column	0.01M ammonium acetate buffer and acetonitrile (70:30 v/v)	1 ml/min	UV detector 219nm	4.40min
Tacrine hydrochloride	RP-HPLC	C18 column	0.05M triethylamine: acetonitrile (80: 20,);	1.5 ml/ minutes	UV detector 243nm	5.8 minutes

5. Conclusion

Herein, an effort was made to review recent trends in AD. Well designed, independent cost effective analyses of Alzheimer's drugs are lacking. Evidence from literature review suggests that there may be cost effective treatment for AD. The new method development and validation for AD and the role of drugs, that are assumed to contribute in the significant fields for advanced research is lacking. There is significant active investigation ongoing in the analytical method development and validation as targets for treatment of AD. Thus, it is hoped that all these lines of ongoing research, combined, should lead to a deeper understanding. Thus, we conclude that these categories of drugs discussed in this review can be potentially targeted for research and development for the treatment of AD.

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