Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 3 Issue 10 October 2019

Review Article

Physicochemical Properties and Bioanalytical Methods of Therapeutic Agents Utilized in the Management of Alzheimer's Disease

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Received: September 12, 2019; **Published:** September 13, 2019

DOI: 10.31080/ASPS.2019.03.0398

Abstract

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive deterioration in cognition, function and behavior. Patients therefore suffer from confusion, disorientation, impaired judgment, memory loss and have difficulty in expressing themselves. The objective of the study was to summarize literature reports on the physicochemical properties and bioanalytical methods of drugs used to treat Alzheimer's disease. Information was obtained from published works in scientific journals, official books as well the online search. The drugs currently used clinically to treat the disorder are donepezil, galantamine, memantine, rivastigmine and tacrine. Physicochemical properties of the drugs namely dissociation constants, melting points, partition coefficients and solubility are presented. Various analytical chromatographic methods (hyphenated and non-hyphenated) used to determine the drugs in biological fluids are discussed. Sample preparations were noted to involve extraction, centrifuging, addition of internal standard.

In general, hyphenated chromatographic methods (LC-ESI-MS/MS; LC-MS/MS; LC-APCI-MS/MS respectively) were observed to be the analytical methods of interest for the quantification of Alzheimer's drugs in biological matrices. In conclusion, the review of the literature has shown that accuracy, precision, specificity, sensitivity, rapidity might be responsible for the use of hyphenated chromatographic methods as the bioanalytical methods of choice.

Keywords: Alzheimer's Disease; Chromatographic Methods; Physicochemical Properties

Abbreviations

AD: Alzheimer's Disease; APCI: Atmospheric Pressure Chemical Ionization; MRM: Multiple Reaction Monitoring; MS: Mass Spectrometry; ESI: Electrospray Ionization; NMDA: N-methyl-D-aspartate; RP: Reverse Phase; UV: Ultraviolet; SRM: Selected Reaction Monitoring; UPLC: Ultra-Performance Liquid Chromatography.

Introduction

Neurodegenerative disease is a neurologic disorder characterized by neuronal loss and accumulation of insoluble extracellular or intracellular material in certain regions of the brain. The disorder which affects mostly the elderly is progressive and of unknown etiology. It could be of inherited form, but the occurrence could be

sporadic with genetic predisposition, aging and environmental factors as risk factors. Neurodegenerative disease could be:

- **Alzheimer's disease:** Most common cause of dementia, arising from neural injury occurring primarily in the cortex and hippocampus. It accounts for about 50% of clinically diagnosed dementia [1].
- **Amyotropic lateral sclerosis:** The progressive weakness and muscle atrophy are as result of degeneration of bulbar, cortical and spinal neurons.
- **Huntington's disease:** A motor disorder caused by loss of a specific subset of striatal neurons and characterized by abnormal and excessive movements.
- **Parkinson's disease:** A disabling motor impairment disorder as a result of loss of nigrostriatal dopamine neurons.

The neurochemical changes in Alzheimer's disease form the basis for the symptomatic treatment with therapeutic agents such as donepezil, galantamine, memantine, rivastigmine and tacrine. The present study deals with Alzheimer's disease, focusing on the physicochemical properties and bioanalytical methods of therapeutic agents (drugs) employed in the treatment of the disease.

These agents are competitive and reversible cholinesterase inhibitors (AChEI), inhibiting the activity of enzyme cholmesterase and increasing the level of acetylcholine in brain. Furthermore, dysfunction of glutamatergic neurotransmission is involved in the etiology of the disease, hence the use of memantine, an N-methyl D-aspartate antagonist (NMDA) to treat moderate to severe form of the disease. Thus, these drugs are primarily used to improve motivation, anxiety level and confidence of Alzheimer's patients.

A number of analytical methods including capillary electrophoresis [2,3] and chromatographic methods have been used to determine these drugs in biological fluids however, chromatographic methods are the analytical methods mostly used. Biological fluids very often utilized are blood (whole blood, serum or plasma), urine, cerebrospinal fluid (CSF), bile and saliva.

In this context, we present the physicochemical properties as well as the bioanalytical methods of Alzheimer's drugs. They include:

Donepezil is chemically defined as 2,3-Dihydro5,6-dimethoxy-2-{[1-(phenylmethyl)-4-piperidinyl]methyl}1H-inden-1-one. It has a molecular formula and molecular weight of $C_{24}H_{29}NO_3$ and 379.49 g/mol respectively. The chemical structure is given in figure 1. It melts at 206-207 deg C. Donepezil is sparingly soluble in water, soluble in acetone, chloroform. The logarithm partition coefficient in octanol-water is 3.6 and has pKa value of 8.9.

Figure 1: Chemical structure of Donepezil.

Donepezil has been determined by numerous chromatographic methods, however only few will described. They include;

Human plasma

- Lordachescu., *et al.* [4] hyphenated chromatographic system (LC-MS/MS, by MRM in positive mode) consisting of chiral RP-C_{18} column, isocratic separation at room temperature using ammonium carbonate (pH 9.0) and acetonitrile as mobile phase at a flow rate of 0.3 ml/min, a total run time of about 8.0 min and a calibration range of 0.05-25 ng/ml. Internal standard was donepezil-d_7.
- Khuroo., *et al*. [5] hyphenated chromatographic system (LC-ESI-MS/MS, by MRM in positive mode) consisting of RP-C_{18} column, isocratic separation at 35 deg. C using methanol and ammonium acetate (pH 6.2) as mobile phase at a flow rate of 0.4 ml/min, a total run time of about 9.0 min and a calibration range of 0.33-51ng/ml. Internal standard was galantamine.
- Noetzli., *et al*. [6] hyphenated chromatographic system (UPLC-MS/MS, by MRM in positive mode) consisting of RP-C_{18} column, gradient separation at room temperature using ammonium acetate (pH 9.3) and acetonitrile as mobile phase at a flow rate of 0.4 ml/min, a total run time of about 4.5 min and a calibration range of 1-300 ng/ml. Internal standard was galantamine radioisotope.
- Kim., *et al*. [7] hyphenated chromatographic system (LC-MS/MS, by MRM in positive mode) consisting of RP-C_{18} column, isocratic separation at room temperature using ammonium acetate (pH 5) and acetonitrile as mobile phase at a flow rate of 0.2 ml/min, a total run time of about 5 min and a calibration range of 0.1-50 ng/ml. Internal standard was pioglitazine.

- Pilli., *et al*. [8] hyphenated chromatographic system (LC-MS/MS, by MRM in positive mode) consisting of RP-C_{18} column, isocratic separation at room temperature using ammonium formate and acetonitrile as mobile phase at a flow rate of 0.6 ml/min, a total run time of about 3 min and a calibration range of 0.09-24.2 ng/ml. Internal standard was dipyridamole.

Human serum

Petrocheilou., *et al*. [9] non-hyphenated chromatographic system (UPLC-DAD) consisting of RP-C18 column, gradient separation at room temperature using acetonitrile, methanol and buffer solution of sodium acetate/acetic acid, (0.2 M, pH 4.8) as mobile phase at a flow rate of 0.6 ml/min, a total run time of about 5 min. The same method was used to determine the drug in human cerebrospinal fluid and urine.

Galantamine, chemically is{[4aS-(4aα,6β,8aR*)]-4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro-[3a,3,2ef]2benzazepin-6-ol}. It has a molecular formula and molecular weight of $C_{17}H_{21}NO_3$ and 287.35 g/mol respectively. The chemical structure is given in figure 2. It melts at 126-127 deg C. Galantamine is fairly soluble in hot water, freely soluble in alcohol, acetone, chloroform and less soluble in benzene and ether. The logarithm partition coefficient in octanol-water is 1.8 and has pKa value of 8.91, 14.81.

Figure 2: Chemical structure of Galantamine.

Galantamine has been determined by the following chromatographic methods

Human plasma

- Park., *et al*. [10] hyphenated chromatographic system (LC-ESI-MS/MS, in positive mode) consisting of RP-C_{18} column, isocratic separation at room temperature using acetonitrile and ammonium acetate (0.01M) as mobile phase at a flow rate of 0.2 ml/min, a total run time of about 2 min and a calibration range of 4-240 ng/ml. Internal standard was glimepride.
- Nirogi., *et al*. [11] hyphenated chromatographic system (LC-MS/MS, by MRM in positive mode) consisting of RP-C_{18} column, isocratic separation at room temperature using 0.03% formic acid and acetonitrile as mobile phase at a flow rate of 1.5 ml/min, a total run time of about 3 min and a calibration range of 0.5-100 ng/ml. Internal standard was loratadine.
- Zhang., *et al*. [12] non-hyphenated chromatographic system (HPLC with fluorescence detector) consisting of RP-C_{18} column, isocratic separation at room temperature using acetonitrile, water and triethylamine (pH 7.0) as mobile phase at a flow rate of 1.0 ml/min, a total run time of about 15 min and a calibration range of 2-160 ng/ml. Internal standard was tramadol.
- Tencheva., *et al*. [13] non-hyphenated chromatographic system (HPLC with UV detector) consisting of RP-C_8 column, isocratic separation at room temperature using methanol, water and dibutylamine (pH 7.0) as mobile phase at a flow rate of 1.2 ml/min, a total run time of about 20 min and a detection limit of 0.05 µg/ml. Internal standard was codeine. The same method was used to determine the drug in human urine.
- Verhaeghe., *et al*. [14] hyphenated chromatographic system (LC-ESI-MS/MS, in positive mode) consisting of RP-C_{18} column, isocratic separation at room temperature using acetonitrile and ammonium acetate (0.01M) as mobile phase at a flow rate of 1.5 ml/min, a total run time of about 3 min and a calibration range of 1-500 ng/ml. Internal standard was galantamine radioisotope.

Human serum

Claessens., *et al*. [15] non-hyphenated chromatographic system (HPLC with UV detector) consisting of normal phase silica column, isocratic separation at room temperature using 0.1% ethanol, dibutylamine, dichloromethane and n-hexane as mobile phase at a flow rate of 1.0 ml/min, a total run time of about 7 min and a calibration range of 10-100 ng/ml. Internal standard was phenacetin. The same method was used to determine the drug in human bile and urine.

Memantine defined chemically as 3,5-Dimethyladamantan-1-amine. It has a molecular formula and molecular weight of $C_{12}H_{21}N$ and 179.3 g/mol respectively. The chemical structure is given in figure 3. It melts at 153-154 deg C. Memantine is soluble in water, freely soluble in alcohol and acetone. The logarithm partition coefficient in octanol-water is 3.28 and has pKa value of 10.7.

Figure 3: Chemical structure of Memantine.

Memantine determination is mostly by the following chromatographic methods

Human plasma

- Konda., *et al*. [16] hyphenated chromatographic system (LC-ESI-MS/MS in positive mode) consisting of RP-C_{18} column maintained at 40 deg C, isocratic separation at room temperature using acetonitrile and 0.1% formic acid as mobile phase at a flow rate of 0.6 ml/min, a total run time of about 4min and a calibration range of 50-50,000 pg/ml. Internal standard was mamantine-d_6.
- Noetzli., *et al*. [17] hyphenated chromatographic system (LC-ESI-MS/MS in positive mode) consisting of RP-C_{18} column, gradient separation at room temperature using ammonium acetate and acetonitrile as mobile phase at a flow rate of 0.8 ml/min, a total run time of about 15 min and a calibration range of 1-300 ng/ml. Internal standard was donepezil radioisotope.
- Zarghi., *et al*. [18] non- hyphenated chromatographic system (HPLC with fluorescence detector) consisting of RP-C_{18} column, isocratic separation at room temperature using acetonitrile and phosphate buffer (0.025M, pH 4.6) as mobile phase at a flow rate of 2.5 ml/min, a total run time of about 10 min and a calibration range of 2-80 ng/ml. Internal standard was amantadine.
- Pan., *et al*. [19] hyphenated chromatographic system (LC-ESI-MS/MS in positive mode) consisting of RP-C_{18} column, isocratic separation at room temperature using methanol and 0.5% formic acid as mobile phase at a flow rate of 0.45 ml/min, a total run time of about 5 min and a calibration range of 0.1-25 ng/ml. Internal standard was procainamide HCl.
- Almeida., *et al*. [20] hyphenated chromatographic system (LC-ESI-MS/MS by SRM in positive mode) consisting of RP-C_{18} column, isocratic separation at room temperature using methanol, water and formic acid as mobile phase at a flow rate of 0.15 ml/min, a total run time of about 3 min and a calibration range of 0.1-50 ng/ml. Internal standard was amantadine.

Rivastigmine, is chemically defined as [3-[(1S)-1-(dimethylamino)ethyl]phenyl] N-ethyl-N-methylcarbamate. It has a molecular formula and molecular weight of $C_{14}H_{22}N_2O_2$ and 250.34 g/mol respectively. The chemical structure is given in figure 4. It melts at 121-123 deg C. Rivastigmine is sparingly soluble in water, very soluble in anhydrous ethanol and heptane. The logarithm partition coefficient in octanol-water is 2.3 and has pKa value of 8.85.

Figure 4: Chemical structure of Rivastigmine.

Rivastigmine has been determined by the following chromatographic methods

Human plasma

- Amini and Ahmadiani [21] non-hyphenated chromatographic system (HPLC with UV detector) consisting of silica column maintained at 50 deg C, isocratic separation at room temperature using acetonitrile and sodium dihydrogen phosphate (pH 3.1) as mobile phase at a flow rate of 1.3 ml/min, a total run time of about 10 min and a calibration range of 0.5-16 ng/ml. Internal standard was donepezil
- Bhatt., *et al*. [22] hyphenated chromatographic system (LC-MS/MS by SRM in positive mode) consisting of RP-C_8 column maintained at 45 deg C, isocratic separation at room temperature using 0.1% formic acid and acetonitrile as mobile phase at a flow rate of 1 ml/min, a total run time of about 2 min and a calibration range of 0.2-20 ng/ml. Internal standard was zolpidem..
- Frankfort., *et al*. [23] hyphenated chromatographic system (LC-MS/MS by MRM in positive mode) consisting of RP-C_{18} column, gradient separation at room temperature using ammonium hydroxide and methanol as mobile phase at a flow rate of 0.2 ml/min, a total run time of about 14 min and a calibration range of 0.25-25 ng/ml. There was no internal standard.
- Pommier and Frigola [24] hyphenated chromatographic system (LC-APCI-MS/MS by MRM in positive mode) consisting of RP-C_8 column maintained at 45 deg C, isocratic separation at room temperature using methanol and ammonium acetate (0.02 M) as mobile phase at a flow rate of 0.2 ml/min, a total run time of about 5 min and a calibration range of 0.2-30 ng/ml. Internal standard was rivastigmine d_6.

Tacrine, is chemically defined as 1,2,3.4-Tetrahydroacridin-9-amine. It has a molecular formula and molecular weight of $C_{13}H_{14}N_2$ and 198.26 g/mol respectively. The chemical structure is given in figure 5. It melts at 183-184 deg C. Tacrine is sparingly soluble in water, soluble in alcohol. The logarithm partition coefficient in octanol-water is 2.2 or 2.71 and has pKa value of 9.95.

Figure 5: Chemical structure of Tacrine.

Tacrine has been determined by the following chromatographic methods

Human plasma

- Han., *et al*. [25] hyphenated chromatographic system (LC-MS/MS by MRM in positive mode) consisting of RP C_{18} maintained at 30 degree C, isocratic separation at room temperature using ammonium acetate (10 mM), 1% formic acid and acetonitrile as mobile phase at a flow rate of 1 ml/min, a total run time of about 3 min and a calibration range of 0.01-10 ng/ml. Internal standard was tramadol.
- Hansen., *et al*. [26] non-hyphenated chromatographic system (HPLC with fluorescence detector) consisting of RP C_8, isocratic separation at room temperature using ammonium acetate (0.2 M) and acetonitrile as mobile phase at a flow rate of 1.3 ml/min, a total run time of about 40 min and a calibration range of 6-60 nM. Internal standard was 1,2,3,4-Tetrahydro-9-acridanone. The method was also used to determine the drug in human urine.
- Ekman., *et al*. [27] non-hyphenated chromatographic system (HPLC with UV detector) consisting of RP C_{18} maintained, isocratic separation at room temperature using phosphate buffer and acetonitrile as mobile phase at a flow rate of 1.1 ml/min, a total run time of about 14 min and limit of detection of 0.3ng/ml. There was no internal standard.

Human serum

Forsyth., *et al*. [28] non-hyphenated chromatographic system (HPLC with fluorescence detector) consisting of RP C_{18}, isocratic separation at room temperature using methanol, water, 1% triethylamine (pH 5) as mobile phase at a flow rate of 1.5 ml/min, a total run time of about 8 min and a calibration range of 1-20 ng/ml. Internal standard was 1,2,3,4-Tetrahydro-9-acridanone.

The study has shown that in the chromatographic analyses, the biological fluids containing the drugs would be treated to free the drugs from interferences prior to injecting into the chromatograph. Treatment may involve cloud-point extraction, liquid–liquid extraction, microextraction, protein precipitation, solid-phase extraction. Due to the fact that biological fluids contain proteins in significant amounts, protein precipitation is very often carried out using solvents such acetonitrile and/or methanol prior to other treatments.

Conclusion

Alzheimer's therapeutic agents (drugs) are cholinesterase inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists and act by increasing the concentration and duration of action of acetylcholine or reducing the glutamatergic overstimulation in brain. The physicochemical data have shown that these drugs do not have good aqueous solubility but possess very favourable partition coefficients. Accurate determination of these drugs in biological fluids, are very vital because it assists clinicians to make better decision with regards to dosage selection and dosage regimens respectively. Amongst the hyphenated analytical methods, LC-MS/MS or LC-ESI-MS/MS method has proven to be the bioanalytical method of choice. Only few non-hyphenated bioanalytical methods have been used to determine Alzheimer's disease drugs. Finally, the study has shown that most of these bioanalytical methods have adequate accuracy, precision, specificity, sensitivity and rapidity to allow the estimation of Alzheimer's drugs from whole blood, plasma, serum, urine and other biological matrices.

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