International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 07 Issue: 04 | Apr 2020 www.irjet.net p-ISSN: 2395-0072

A review on: Analytical techniques on drugs for Alzheimer's disease

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Abstract - *Alzheimer's disease (AD) is an irreversible, progressive brain disorder that slowly destroys memory and thinking skills causes of dementia. Abnormal deposits of proteins form amyloidal plaques and tangles, loss of connections between neurons in the brain are considered the main features of AD. The damage initially appears to take place in the hippocampus and the entorhinal cortex parts of the brain which essential in forming memories. By the final stage of Alzheimer's more neurons die, additional parts of the brain are affected and begin to shrink (atrophy) significantly. The main objective of this review mainly focused on spectrophotometric, high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectroscopy (LC-MS) which can be used for method development and validation of different Alzheimer's drugs .This review challenges to researches for development of front line drug for Alzheimer's disease.*

Key Words: Alzheimer's drugs, validation of drugs, HPLC, LC-MS, Robustness.

1.INTRODUCTION

Alzheimer's diseases (AD) is one of the most financially costly diseases in this globe.[1][2] In 2019, there were approximately 50 million people suffer with AD in worldwide. It is a chronic neurodegenerative disease that starts slowly and gradually worsens over time and results dementia. These result in patients suffering from memory loss, confusion, impaired judgment, disorientation and trouble expressing themselves. The common early stage of AD is difficult in remembering recent events. As the disease advances, symptoms can include problems with language, disorientation, mood, loss of motivation, not managing self-care, and behavioral issues.[3] Gradually, bodily functions are lost, ultimately leading to death.[4] The disease process is associated with plaques and neurofibrillary in the brain.[5] The food and drug administration (FDA) has approved two types of drugs specifically to treat symptoms of Alzheimer's disease one is cholinesterase inhibitor another one is Memantine. Cholinesterase inhibitors (Donepezil, Galantamine, Rivastigmine) to treat mild to moderate AD, boost the amount of acetylcholine available to nerve cells by preventing destruction of nerve cells. The common side effects are nausea, Memantine is approved by FDA for treatment of moderate to severe Alzheimer's. It regulates the activity of Galantamine chemical involved in brain functions. The common side effects of this is dizziness, headache, confusion, agitation. The main objective of these drugs is to improve motivation, anxiety level and confidence. Currently no drugs are available in the market that can completely cure AD.

AD effected mainly in six stages, in the stages I/II called Transentorhinal region the first area of the brain to be affected by Alzheimer's.[6] Which is part of the medial temporal lobe located deep within the brain. Neurons start dying in this area then spreads into the entorhinal cortex (EC) which acts as a central hub.[7] The EC is the main area for communication between the hippocampus, and the neocortex which is the outer portion of the brain. The stages III/IV called limbic stage; the disease then spreads into the hippocampus which is part of the limbic system. The hippocampus is the part of the brain that is involved in forming new memories, organizing them, and storing them for later recall. The stage V/VI called Isocortical stage, neurofibrillary tangles in throughout the cerebral cortex usually developed Alzheimer's diseases.

There are Several medications are approved by the U.S. Food and Drug Administration (FDA) to treat mild to moderate Alzheimer's Donepezil (Aricept®), Rivastigmine (Exelon®) and Galantamine (Razadyne®) are used. Donepezil can be used for severe Alzheimer's as well. Memantine (Namenda®), Exelon® patch and Namzaric® (a combination of Memantine and Donepezil) are used to treat moderate to severe Alzheimer's. These drugs work by regulating neurotransmitters, the chemicals that transmit messages between neurons. Alzheimer's drug might be one strategy to help temporarily manage memory loss, thinking and day to day function. These drug's don't work for everyone and can't stop its progression over time drug effects wear off.

The development and validation of drugs used in Alzheimer's disease estimated quantitatively by spectrophotometric, RP-HPLC, LC-MS method because of several aspects observed as below discussed that, spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.[8] Spectrophotometers use a monochromator containing a diffraction grating to produce the analytical spectrum. The grating can either be movable or fixed. If a single detector, such as a photomultiplier

tube or photodiode is used. HPLC has many applications in both laboratory and clinical science. It is a technique used in pharmaceutical development to ensure product purity.[9] The synthesis used by HPLC according to the European pharmacopoeia.[10] United States pharmacopoeia.[11] is 15.5% , 44% respectively. A large scale synthesis can be done by HPLC technique, it studied the increase in specificity, precision and accuracy. Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. The stationary phase is a silica with surface-modified RMe_2SiCl, where R is $C_{18}H_{37}$ or C_8H_{17}, retention time is longer for less polar (methanol, acetonitrile) while polar molecules elute more readily, retention time increases with hydrophobic (non-polar) surface area.

Reversed phase columns are quite difficult to damage compared with normal silica columns. The components of the sample mixture are separated due to their different degrees of interaction with the adsorbent particles. Its composition and temperature play a major role in the separation .These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole and ionic, operational pressures are significantly higher, superior resolving power, quantitative analysis of the sample components. A digital RP-HPLC operates on the principle of hydrophobic interactions, Another important factor is the mobile phase pH since it can change the hydrophobic character of the analyte. For this reason most methods use a buffering agent, such as sodium phosphate, to control the pH. Microprocessor and user software control the HPLC instrument and provide data analysis. HPLC separations have theoretical parameters and equations to describe the separation of components into signal peaks when using UV detector or a mass spectrometer.

Liquid chromatography mass spectrometry (LC-MS) is an analytical chemistry technique that combines the physical separation of liquid chromatography (or HPLC) with mass spectrometry (MS).LC separates mixtures with multiple components, MS with structural identity of the individual components with high molecular specificity and detection sensitivity. LC-MS may be applied in a wide range of sectors including biotechnology, environment monitoring, and pharmaceutical, agrochemical, and cosmetic industries.[12][13] An LC-MS system contains an interface that efficiently transfers the separated components from the LC column into the MS ion source.[14] . While the mobile phase in a LC system is a pressurized liquid, the MS analyzers commonly operate under high vacuum (around 10^{-6} torr / 10^{-7} "Hg). Overall, the interface is a mechanically simple part of the LC-MS system that transfers the maximum amount of analyte, removes a significant portion of the mobile phase used in LC and preserves the chemical identity of the chromatography products. As a requirement, the interface should not interfere with the ionizing efficiency and vacuum conditions of the MS system.[14]

Results and discussion

Method development and validation of four drugs can be studied by spectrophotometric, chromatographic techniques by various authors, the results can be discussed as below.
Chlorensterase inhibitor:

Donepezil hydrochloride:

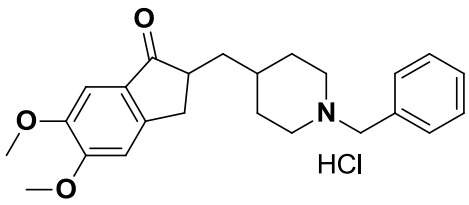

Fig.1

Donepezil hydrochloride is a cholinesterase inhibitor used in the treatment of Alzheimer's disease. Mainly it is available as its hydrochloride salt. Chemically it is 2-[(1-benzyl-4-piperidyl) methyl]-5, 6-dimethoxy-2, 3-dihydroinden-1-one hydrochloride (Fig. 1). Method development and validation of donepezil can be studied by chromatographic techniques by various authors, the results can be discussed as below.
A 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. In LC method, quantification was achieved with PDA detection over the concentration range of 20-60 µg/mL with mean recovery of 100.18±0.75%.[15]

The different conditions applied by another author using RP-HPLC 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 : 0.02m phosphate buffer : Triethylamine (60:40:0.5)% v/v were used. The flow rate was 1ml/min. Linearity for Donepezil Hcl were in the range of 50µg/ml - 150µg/ml. Amount found of Donepezil HCl in Aricept 5mg, Aricept10mg, Donepezil 5mg tablets were 5.0072mg/tab, 10.01mg/tab and 5.01mg/tab respectively. Percentage recovery obtained was 100.53%, 100.24% and 100.34%.[16]

The analyte and internal standard were extracted by liquid-liquid extraction using dichloromethane and hexane mixture and separated by isocratic elution on C18 analytical column with 0.1% formic acid and methanol in the ratio of 70:30 (flow rate of 1 ml/min) as the mobile phase in the positive ion mode. Multiple Reaction Monitoring transitions for donepezil and internal standard are 380.2/91.2 and 387.2/98.2 respectively. The lower limit of quantification was 50 pg/ml with the linearity range of 50 pg/ ml 25,000 pg/ml and the method was validated as per international regulatory guide- lines for its selectivity, stability, accuracy, precision, and recovery.[17]

A sensitive, isocratic reversed-phase high performance liquid chromatographic method involving fluorescence

detection was developed for the determination of donepezil hydrochloride in tablets and in human plasma. Pindolol was used as an internal standard. The system operated at room temperature using a C18 column, mobile phase consisting of methanol, phosphate buffer (0.02 mol L–1) and triethylamine (pH 3.5) (55 : 45 : 0.5, V/V/V) at a flow rate 0.9 mL–1 min. The analyte and internal standard were extracted from human plasma via liquid-liquid extraction. The calibration curve was linear over the range of 0.005–2μg mL–1 of donepezil with detection limit of 1.5 ng mL–1. Intra- and inter-day relative standard deviations were less than 2.5 %. The method was found to be suitable for quality control of donepezil hydrochloride in bulk drug as well as in human plasma. [18]

Galantamine:

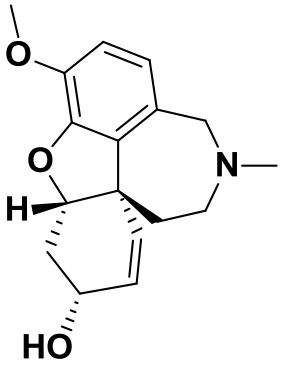

Fig. 3

Galantamine is used for the treatment of cognitive decline in mild to moderate Alzheimer's disease and various other memory impairments. Galantamine is chemically (−)S-N-ethyl-3-[(1-dimethylamino)ethyl]-N-methyl phenyl-carbamate hydrogen (Fig. 3).

Author showed the results of development of isocratic HPLC method for the simultaneous determination of mixture of Galantamine and pymadine drugs and validation of method for the analytical parameters selectivity, linearity, LOD, LOQ, accuracy and precision as per ICH requirements.The chromatographic conditions applied were column RP C18 ODC Spherisorb, column temperature 25°C, mobile phase 50 mM disodium hydrogen phosphate (99.5%): acetonitrile = 80 : 20 v/v, flow rate: 1.5 ml/min, UV-detection (λ = 280 nm). The experimental results were subjected to a linear regression analysis. The regression equations obtained y = 1.1010.x + 387106 (Galantamine hydrobromide) (LOD = 1.08.10-4 g/ml; LOQ = 3.6.10-4 g/ml); y = 9.109.x + 1.106 (Pymadine) (LOD= 1.32.10-4 g/ml; LOQ = 4.4.10-4 g/ml). Accuracy was represented by the degree of recovery, which data were included in the corresponding confidence interval: 96.66 % 98.76 % (Galantamine hydrobromide); 99.58 % 103.86 % (Pymadine). The retention time values tR 3.179 (Galantamine hydrobromide), tR 5.272 (Pymadine).The developed and validated isocratic HPLC method was appropriate for separation, and simultaneously for identification and determination of Galantamine hydrobromide and Pymadine, for which, the combination HPLC methods haven't been described.[19]

Two simple and low cost UV-spectrophotometric and first order derivative methods were developed and validated for Galantamine Hydrobromide in bulk drug and tablet dosage form. Galantamine Hydrobromide was estimated at 289 nm. In first order derivative, it showed amplitude at 284.8 nm and λmax = 290.4 nm with nm as zero crossing point (ZCP). In both the methods linearity range 20-100 μg/ml; for UV spectrophotometric method (y = 0.007x - 0.002, r^2 = 0.999) and for first order derivative spectrophotometric method (Y=0.0012 x+0.00045; r2=0.999).These methods was validated for various validation parameters according to USP guidelines. The quantitation limits were found to be 0.50 and 1.54 μg/ml, for UV-Spectrophotometric method and 3.3 and 10 μg/ml for the 1st order derivative method. The proposed methods were successfully applied for the determination of Galantamine Hydrobromide in tablet dosage forms. The results demonstrated that the procedure is simple, accurate, cost effective, precise and reproducible (relative standard deviation<2%)for the estimation Galantamine Hydrobromide in different dosage forms.[20]

Analysis of of Galantamine Hydrobromide was carried out by RP-HPLC method Shimadzu HPLC system with Phenomenex C18 column (250 x 4.6 mm id 5μ m particle size) using 1 mM ammoniumformate acetonitrile (30:70) in isocratic mode as mobile phase with flow rate is 0.4 ml.min-1. The detection at λ_{max} = 289 nm. Beer's law concentration range 100 -1000 μ g mL^{-1} of Galantamine Hydrobromide. The analysis of drug in bulk and pharmaceutical formulations, the mean percent recoveries were found to be 99.8 ± 0.13. The method was validated with respect to linearity, precision and accuracy as per the International Conference on Harmonization (ICH) guidelines.[21]

A simple, precise, accurate, rapid and selective liquid chromatography method coupled with tandem mass spectrometry is developed and validated for the quantification of galantamine in human plasma using a commercially available compound, glimepride, as an internal standard (IS). Following simple one-step liquid– liquid extraction by ethyl acetate, the analytes are separated using an isocratic mobile phase consisting of acetonitrile and 0.01M ammonium acetate (95/5, v/v) on a reverse-phase C18 column and analyzed by tandem mass spectrometry in the multiple reaction monitoring mode using the transitions of respective [M+H] ions, m/z 288.22 213.20 and m/z 491.17 352.30 for the quantification of galantamine and IS, respectively. The standard calibration curves show good linearity within the range of 4 to 240 ng/mL (r2 5 0.9996, 1/x2 weighting). The lower limit of quantification is 4 ng/mL. The retention times of galantamine and IS are 1.1 and 0.71 min, which shows the high through put potential of the proposed method. In addition, no significant metabolic compounds are found to interfere with the analysis. The validated method is successfully applied for pharmacokinetic

and bioequivalence studies of 24 mg of Galantamine hydrobromide capsule in 32 healthy Korean subjects. [22]

A fast simple sensitive precise, accurate and reproducible RP-HPLC method was developed and validated for the analysis of Rivastigmine bulk dosages form. The separation by using C-18 column, 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 method is validated for its precision, limit of quantification , linearity and robustness. The method was found to be linear over the concentration range 10-100 µg/ml (r2 =0.999). The retention time for Rivastigmine was found to be 3.66±25min. limit of quantitation of method is 0.196µ/ml and limit of detection 0.056µ/ml.[23]

A rapid and precise reverse phase high performance, liquid chromatographic method has been developed for the validated of Dalfampridine in its pure form as well as in tablet dosage form. Column is ODS C18 (4.6 x 250mm, 5µm) using Acetonitrile and water in the ratio of 80:20 v/v, the mobile phase at a flow rate of 1.0mL/min, The retention time obtained is 2.98 min. The concentration of Dalfampridine 20 µg/ml, RSD is 2.0%. The method is useful in the quality control of bulk and pharmaceutical formulations.[24]

Rivastigmine

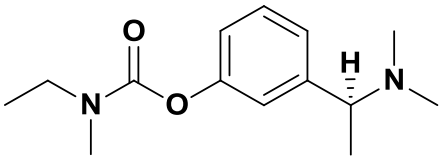

Fig. 4

Rivastigmine is a cholinesterase inhibitor used for the treatment of mild to moderate Alzheimer's disease and Parkinson's. Rivastigmine is chemically (−) S-N-ethyl-3-[(1-dimethyl- amino)ethyl]-N-methyl phenyl-carbamate hydrogen. Its efficacy is similar to donepezil.

RP- HPLC method for the estimation of Rivastigmine effect by enhancing cholinergic function, in bulk and pharmaceutical dosage form. The mobile phase used was 2.02 g of 1-octane sodiumsulfonate in 1000 ml Milli–Q water and the pH was adjusted to 3.0 with ortho phosphoric acid and filter through 0.45 mm pal pharma nylon 66 membrane filter. The mobile phase was prepared by mixing buffer and acetonitrile in the ratio of (70 : 30 % v/v). The specification of the chromatographic system, Column 250 mm, ODS, Xterra RP18, 5 mm or equivalent, flow rate 1.0 ml/min, detection 217 nm, injection volume 40 ml and run time 15 min. Only few HPLC procedures have been reported in the literature for the determination of Rivastigmine in pharmaceutical formulations and biological fluids. There are no reports for the determination of Rivastigmine by HPLC in pure form. Hence this method has made an attempt to develop a HPLC method for the determination of Rivastigmine in bulk and in pharmaceutical formulations. [25]

In isocratic RP-HPLC analytical 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. The method was validated for specificity, linearity, precision, accuracy, and limit of quantification, limit of detection, robustness, and solution stability.[26]

RP-HPLC estimation of Rivastigmine in pure as well as in pharmaceutical dosage forms, it was carried out coliumn with C-18, 250 x 4.6 mm. 5 µ using a mixture of Phosphate buffer and Acetonitrile (70 : 30 v/v) as the mobile phase at a flow rate of 1.0 mL/min the detection was done by UV at 217nm. The retention time of the drug was 3.66 ± 0.25 min. The method produced linear responses in the concentration range of 10-100 µ g/mL of Rivastigmine. The method was found to be reproducible.[27]

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 217 nm. The method is validated for its precision, limit of quantitation (LOQ) linearity and robustness. The method was found to be linear over the concentration range 10-100 µg/ml (r2 =0.999), retention time 3.66±25min. limit of quantitation of method is 0.196µ/ml and limit of detection 0.056µ/ml.[28]

Memantine

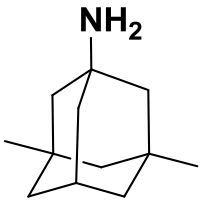

Fig. 2

Memantine is a medication used to treat moderate-to-severe Alzheimer's disease.[29] It is less preferred than acetyl cholinesterase inhibitors such as donepezil. Treatment should only be continued if beneficial effects are seen. It is taken by mouth.[30] Memantine was approved for medical use in the United States in 2003. Memantine HCl chemically is 1-amino-3,5- dimethyladamantane hydrochloride (Fig. 2). It was marketed in some countries as a combination drug with donepezil under the brands Namzaric, Neuroplus Dual, and Tonibral MD. Memantine appears to be generally well tolerated by children with autism spectrum disorder. HPTLC

analytical method for estimation of Memantine Hydrochloride was established and validated. [29]

Memantine Hydrochloride is an NMDA receptor antagonist and widely use for Alzheimer's disease. The HPTLC method was developed using aluminium plates pre-coated with silica gel G60F254 as a stationary phase and n-Hexane: Ethyl acetate: Diethylamine (5:5:0.7 % v/v/v) as mobile phase. The separated spots were visualized as orange spots after dipping with Dragendorff's reagent solution. The method was found to be Linear, Accurate, Precise, and Robust according to ICH Guideline. Linearity was found to be 5000-30000 ng/band for Memantine HCl. The LOD and LOQ were found to be 80.07ng/band and 242.637ng/band for Memantine HCl. So, developed method is applicable for the estimation of Memantine Hydrochloride. [30]

To develop and validate a chromatographic method to determine the amount of drug (assay) in the tablets of Memantine hydrochloride (MEM) using HPLC with refractive index (RI) detector, the separation was achieved on C18 (250 × 4.5 mm, 5µ) column using isocratic mobile phase comprises of buffer (pH-6.0): Methanol (45:55 v/v) flow rate 1.0 ml/min. The results were simple, commercial, precise, accurate and robust. [31]

Two different indicators are used in spectrophotometric method for the determination of Memantine hydrochloride (MTH) in bulk and tablet dosage forms.MTH undergo ion pain complex reactions with anionic dyes like bromothymol blue and solochrome black T on primary amine group presented on the drug structure which are extracted into chloroform at room temperature and have absorption maxima at 415 nm (BTB) and 510 nm (SBT). Regression analysis of the Beer's plots showed good correlation in the concentration ranges 2–20 and 5–25 µg/mL for BTB and SBT, respectively. The proposed methods were successfully applied to the tablet dosage forms containing the MTH. No interference from common recipients was observed. [32]

The simultaneous spectrophotometric-chemometric methods were developed for the Alzheimer's drugs donepezil and Rivastigmine in pharmaceutical tablets. The used chemo metric methods are partial least squares regression (PLS) and principal component regression (PCR)for the two drugs taken in synthetic mixtures and pharmaceutical tablets. A concentration set including binary mixtures of donepezil and Rivastigmine formed to 10 different combinations were randomly prepared in 0.1 M HCl. As a result of the determination, high recoveries and low standard deviations were found. Absorbance and concentration values were used in Minitab and other chemometric programs to calculate estimated concentrations with PCR and PLS. [33]

A method to determine Memantine hydrochloride in tablet formulation which is simple, sensitive, precise and economical as per ICH guidelines. The method was validated at λ max of 254nm. Beer-Lambert's was obeyed between the concentration ranges of 0.2-0.6µg/ml. A good linear relationship with correlation co- efficient of 0.998 was obtained and the LOD and LOQ values were 0.48µg/ml and 1.48µg/ml respectively. The method was also employed on the tablet formulation (Namenda) and the recovery was found to be 99.8%.[34]

3. CONCLUSIONS

In conclusion, Author 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. 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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