A REVIEW ON: ANALYTICAL TECHNIQUES DEVELOPMENT AND VALIDATION OF DRUGS USED FOR ALZHEIMER'S DISEASE

ABSTRACT

Pharmaceutical analysis plays a very prominent role in quality assurance as well as quality control of bulk drugs and pharmaceutical formulations. Rapid increase in pharmaceutical industries and production of drug in various parts of the world has brought a rise in demand for new analytical techniques in the pharmaceutical industries. As a consequence, analytical method development has become the basic activity of analysis. Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory defeat and impairment in behaviour, language and visuospatial skills. Donepezil, rivastigmine, galantamine, tacrine and memantine are the US Food and Drug Administration approved oral drugs used in the treatment of AD. These drugs can provide a symptomatic relief but they poorly affect the progression of the disease. Quantitation of these drugs in various biological matrices, dosage forms and monitoring them in long-term treatment is essential to titer the dose of these drugs and ensures patient compliance. The main objective of this review mainly focused on spectrophotometric, high-performance liquid chromatography (HPLC), HPTLC and liquid chromatography-mass spectroscopy (LC-MS) which can be used for method development and validation of different Alzheimer's drugs. 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. This review challenges to researches for development of front line drug for Alzheimer's disease.

Keywords: Pharmaceutical analysis, Analytical methods, Alzheimer's disease, Donepezil, Rivastigmine, Galantamine

INTRODUCTION

Analysis is vital in any product or service, and it is also important in drug because it involves life [1]. Analytical method development and validation for the analysis therapeutic components and associated substances play an important role in the discovery, development and manufacture of pharmaceuticals and natural medicinal compounds. Analytical instruments play a major role in the process to achieve high quality and reliable analytical data. Thus everyone in the analytical laboratory should be concerned about the quality

assurance of equipment. Analytical method could be spectral, chromatographic, electrochemical, hyphenated or miscellaneous. Analytical method development is the process of selecting an accurate assay procedure to determine the composition of a formulation. It is the process of proving that an analytical method is acceptable for use in laboratory to measure the concentration of subsequent samples. Analytical methods should be used within GMP and GLP environments and must be developed using the protocols and acceptance criteria set out in the ICH guidelines Q2(R1) [2-6]. 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 [7]. 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 is 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 [8]. 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 [9]. An LC-MS system contains an interface that efficiently transfers the separated components from the LC column into the MS ion source [10]. 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 [10]. High performance thin layer chromatography (HPTLC) is an enhanced form of thin layer chromatography (TLC). A number of enhancements can be made to the basic method of thin layer chromatography to automate the different steps, to increase the resolution achieved and to allow more accurate quantitative measurements. Automation is useful to overcome the uncertainty in droplet size and position when the sample is applied to the TLC plate by hand. Nowadays, HPTLC has become a routine analytical technique due to its advantages of reliability in quantitation of analytes at micro and even in nanogram levels and cost effectiveness [11]. This chromatographic process relies on the property of biologically active substances to form stable, specific, and reversible complexes. The formation of these complexes involves the participation of common molecular forces such as the Van der Waals interaction, electrostatic interaction, dipole-dipole interaction, hydrophobic interaction, and the hydrogen bond. An efficient, biospecific bond is formed by a simultaneous and concerted action of several of these forces in the complementary binding sites [12]. Alzheimer's disease (AD) is characterized by progressive deterioration in cognition, function and behavior or manifestations of stress [13]. AD is characterized by the deposition of β-amyloid protein (Aβ) in the form of senile plaques and intraneuronal neurofibrillary tangles, hyperphosphorylated tau protein and neuronal cell loss [14, 15]. These result in patients suffering from memory loss, confusion, impaired judgment, disorientation and trouble expressing themselves. It is estimated that there are currently about 18 million people worldwide with AD. In around 50-60% of the patients having dementia, it affects memory, thinking, language, judgment and behavior [16]. In AD neurochemical alterations such as choline esterase deficit and glutamatergic overstimulation of postsynaptic N-methyl-Dasparate receptors will occur. The neurochemical changes in AD are the basis for the symptomatic treatment of AD. Currently no drugs are available in the market that can completely cure AD [17]. Drugs which are available can only reduce the symptoms and progression of disease. Therefore the detection Alzheimer's drugs in biological fluids are critical for the evaluation of correct treatments. There are two major classifications available for the treatment of AD (approved by the US Food and Drug Administration, FDA), which are cholinesterase inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists. Donepezil, rivastigmine, galantamine and tacrine are cholinesterase inhibitors, which increase the concentration and duration of action of acetylcholine in brain [18]. The NMDA receptor antagonist is memantine, which reduces the glutamatergic overstimulation [19]. Cholinesterase inhibitors are major drugs for this AD treatment because 70% of the people obtain relief from these drugs. The main objective of these drugs is to improve motivation,

anxiety level and confidence [20]. Assessment of the pharmacokinetic (PK) parameters is critical for to determine the complete pharmacodynamic effect. These PK parameters are very useful for final dosage selection and treatment. Therefore, validated analytical methods are essential for determination of drug concentration levels in PK, bioequivalence (BE) and therapeutic drug monitoring (TDM) studies. In this review we have gathered various analytical methods (UV, HPLC, HPTLC and LC-MS/MS) published on donepezil, rivastigmine, galantamine, tacrine and memantine and presented the data in a systematic table format comprising sample processing details, separation and chromatographic conditions (column, mobile phase and detection system), chosen parameters for validation and applicable conclusions. In order to make it more useful to the readers, we have provided a concise compilation of available details and applicability of the published methods for each drug through Tables 1-5.

Donepezil hydrochloride

Donepezil ($C_{24}H_{29}NO_3$) is a cholinesterase inhibitor used in the treatment of Alzheimer's disease. Its binds reversibly and inactivates the cholinesterases and thereby inhibits the hydrolysis of acetylcholine and results in increase of acetylcholine concentration at cholinergic synapses. 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. It is available with brand names Aricept, Act Donepezil, M-donepezil as tablets (Fig. 1). Its molecular weight 379.4, pKa 8.82, LogP 3.6, therapeutic dose 5-10 mg/day, T_{max} 4h, $t_{½}$ 70 h, protein binding 96% and oral bioavailability 100% [8, 21, 22].

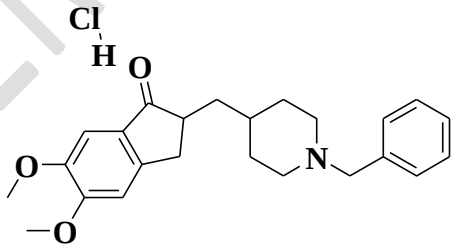

Figure 1: ***Donepezil hydrochloride***

Memantine

Memantine ($C_{12}H_{21}N$) is chemically 3, 5-dimethyladamantan-1- amine acts by blocking the current flow through channels of N-methyl-d-aspartate (NMDA) receptors-a glutamate receptor subfamily broadly involved in brain function. It is a medication used to treat moderate-to-severe Alzheimer's disease. It is less preferred than acetyl cholinesterase inhibitors such as donepezil. Treatment should only be continued if beneficial effects are

seen. Memantine was approved for medical use in the United States in 2003. 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. Memantine is available as capsule and tablet with brand names Admenta, Namenda (Fig. 2). Its molecular weight 179.3, pKa 10.7, LogP 3.5, therapeutic dose 5-10 mg/day, T_{max} 3–7h, $t_{½}$ 60-100h and oral bioavailability ~100% [8, 21, 22].

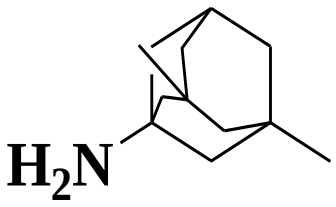

Figure 2: Memantine

Rivastigmine

Rivastigmine($C_{14}H_{22}N_2O_2$) is a acetylcholinesterase inhibitor used for the treatment of mild to moderate Alzheimer's disease and Parkinson's. It inhibits both butyryl cholinesterase and acetylcholinesterase (unlike donepezil, which selectively inhibits acetylcholinesterase) which would otherwise break down the brain neurotransmitter acetylcholine (Fig. 3). It is chemically(−)S-N-ethyl-3-[(1-dimethyl-amino) ethyl]-N-methyl phenyl-carbamate hydrogen. Its efficacy is similar to donepezil. It is available with brand names such as Exelon, Rivagem-3, Rivamer as capsule. Its molecular weight 250.3, pKa 8.85, LogP 2.3, therapeutic dose 3 mg/day, T_{max} 1h, $t_{½}$ 1.5h, protein binding 40% and oral bioavailability 36% [8, 21, 22].

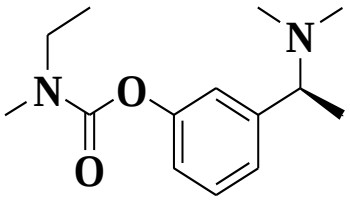

Figure 3: Rivastigmine

Galantamine

Galantamine ($C_{17}H_{21}NO_3$) is a phenanthrene alkaloid and a reversible competitive acetyl cholinesterase inhibitor preventing the hydrolysis of acetycholine, leading to an increased concentration of acetylcholine at cholinergic synapses. Galantamine is used for the treatment of cognitive decline in mild to moderate Alzheimer's disease and various other memory impairments. It is chemically (−)S-N-ethyl-3-[(1-dimethylamino)ethyl]-N-methyl phenyl-

carbamate hydrogen (Fig. 4). It is available with brand names such as Razadyne in tablet dosage form. Its molecular weight 287.3, pKa 8.91, 14.81, LogP 1.8, therapeutic dose 4 mg twice daily, T_{max} 1h, $t_{½}$ 7h, protein binding 18% and oral bioavailability 80-100% [8, 21, 22].

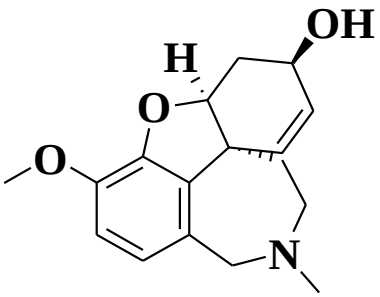

Figure 4: Galantamine

Tacrine

Tacrine ($C_{13}H_{14}N_2$) may bind reversibly to cholinesterase, acetyl cholinesterase as well as butyryl cholinesterase, thereby decreasing the breakdown of acetylcholine and prolonging synaptic actions as well as increased release of acetylcholine. In addition, this agent inhibits monoamine oxidase (MAO) and may inhibit the reuptake of catecholamines and serotonin. Finally, a novel mechanism of action studied in animal models suggests tacrine to attenuate the production of interleukin-1beta in the hippocampus and blood, thereby producing central and peripheral anti-inflammatory effects that may play a role in Alzheimer's disease. Tacrine therapy is associated with a very high rate of serum aminotransferase elevations during therapy and has been linked to several instances of clinically apparent, acute liver injury. It is chemically 1,2,3,4-tetrahydroacridin-9-amine (Fig. 5) and was marketed under the trade name Cognex. Its molecular weight 198.2, pKa 9.95, LogP 2.2, therapeutic dose 10 four times/day, T_{max} 1-2h, $t_{½}$ 2-4h, protein binding 55% and oral bioavailability 2.4–36% [23].

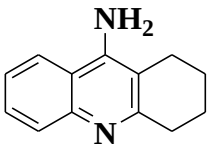

Figure 5: Tacrine

Reported analytical methods

Table 1: Analytical methods for the assay of Donepezil

Sr. No	Methods	Formulation / Biological fluid	Stationary Phase	Mobile Phase	Detection wavelength (nm)	Ref.
1	Spectrophotometry (Ion-pair complex with Bromo cresol purple in phthalate buffer)	Tablet	-	Phthalate buffer solution	410 nm	24
2	Spectrophotometry (Ion-pair complex with azo-dye in acidic medium)	Tablet	-	De-ionized water.	510 nm	25
3	Spectrophotometric method. (Derivative and AUC)	Tablet	-	Methanol: Water (30:70)	314 nm, 304-324nm	26
4	Spectrophotometry and Colorimetry	Tablet	-	Methanol	231,454 nm	27
5	Spectrophotometry	Tablet	-	Phosphate buffer (pH 7.4)	270.5 nm	28
6	Spectrophotometry	Tablet	-	KMnO4 in alkaline medium	547 nm	29
7	Spectrophotometry	Tablet	-	Acetonitrile and Water	231nm	30
8	HPTLC	in situ nasal gel	pre-coated silica gel 60 F-254 aluminium plates (10 × 10 cm, 250 µm thickness)	toluene: methanol: glacial acetic acid (8: 2: 0.1 v/v/v)	254nm	31
9	HPLC	Tablet	Unisol C18 column (150×4.6 mm, 3 µm)	Acetonitrile: Water (50:50)	268 nm	32

10	HPTLC	Tablet	pre-coated silica gel 60 F-254 aluminium plates (10 × 10 cm, 250 µm thickness)	Methanol: Chloroform (8:2 v/v)	254 nm	33
11	HPLC	Tablet	Inertsil C8-3 (25 cm x 4.6-mm, 5 µ)	Buffer : methanol : triethylamine (550:450:5)v/v, adjusted to pH 2.50±0.05 with orthophosphoric acid	271nm	34
12	HPLC	Tablet	Inertsil C8 3v (150mm x 4.6mm) 3µm	Solvent A (mixture of 0.1M phosphate (pH 2.8) buffer and methanol in the ratio 90: 10 (v/v); respectively) and Solvent B (mixture of 0.1 molar (M) phosphate (pH 2.8) buffer , Acetonitrile and methanol in the ratio 20:20: 60 (v/v); respectively)	215 nm	35
13	HPLC	Tablets	WakosilC-18 column 250 mm X 4.6 mm, 5 µ,	phosphate buffer (0.02 M, pH 3.67) andAcetonitrile	230 nm	36
14	HPLC	Tablet	Inert Sustain	Acetonitrile: Water pH 3.5	230 nm	37

			SwiftTM C18 (250mm×4.6 mm i.d.) 5µm	(40:60 v/v)		
15	HPLC	Tablet	*C18 column 250mm x 4.6mm(l x d)*	*methanol : 0.02m phosphate buffer : Triethylamine (60:40:0.5)% v/v*	168 nm	38
16	HPLC	Tablet, Plasma	Phenyl Hypersil C18 (125 mm ´ 4.6 mm i.d. ´ 3 mm particle diameter)	methanol, phosphate buffer (0.02 mol L–1) and triethyl amine (pH 3.5) (55 : 45 : 0.5, *V*/*V*/*V*)	290, 315nm	39
17	HPLC	Tablet	Zorbax SB C18, 150 x 4.6mm, 5µm column)	water: acetonitrile (68:32), pH adjusted to 4.5 with trifluoro acetic acid.	299 nm	40
18	HPLC	Tablet	C18 column	methanol, phosphate buffer 0.02 M and triethylamine (50:50:0.5)	268 nm	41
19	HPLC	Tablet	Kromasil C18 (250 x□4.6 mm, 5µm column.	Methanol: Potassium dihydrogen orthophosphate solution pH adjusted to 2.5 ± 0.05 with *o*-phosphoric acid (40 : 60)	268 nm	42
20	UPLC	Tablet	Waters Acquity C18 50 mm x	0.1% Tri fluoro acetic acid in water: 0.1% Tri fluoro acetic acid in	286 nm	43

			2.1mm, 1.7µ	(70:30) Acetonitrile: Methanol (80:20)		
21	HPLC	Tablet	Hypersil BDS (4.6 x 150 mm, 5µ)	Sodium dihydrogen ortho phosphate: Acetonitrile (30:70v/v)	271nm	44
22	HPLC	Tablet	Agilent C8 (150mm x 4.6mm i.d., 3.5mm particle size)	Buffer, water and Acetonitrile (50:5:45 v/v)	230 nm	45
23	HPLC	CSF, blood serum and urine	ODS Hyper-sil column (C18 classical, 250×2,1 mm, 5 µm)	(Solvent A: acetonitrile, Solvent B: methanol and Solvent C: buffer solution of sodium acetate/acetic acid, 0.2M, pH 4.8.	215, 232, 290 nm	46
24	LC-MS/MS	whole blood	SeQuant ZIC-HILIC (50 × 2 mm, 5 µm)	gradient elution consists of formic acid, ammonium formate and ACN	380.1→91.2 and 384.1→93.2 for donepezil and donepezil-d4.	47
25	HPLC	Tablet	Eclipse plus C18 (250 × 4.6 mm, 5 µm)	methanol: 0.02m phosphate buffer: Triethylamine (50:40:10)% v/v	158nm	48
26	LC-ESI- TOF-MS	Plasma	Kro m a s i l - O D S column (5 µm, 250- × 4.6-mm i.d.)	methanol–acetate b u ffer (pH 4.0) (80:20, v/v).	donepezil [M + H]+ m/z 380–	49

27	HPLC LC- ESI-MS	-	Phenomenex c_{18} 150.0 × 4.6 (i.d.) 4 µm	monobasic potassium phosphate buffer (0.5 mmol L^{-1}) pH 3.0 with 0.5% of triethylamine and methanol (55:45).	268nm	50

Table 2: Analytical methods for the assay of Memantine

Sr. No	Methods	Formulation / Biological fluid	Stationary Phase	Mobile Phase	Detection wavelength (nm)	Ref.
1	Spectrofluorimetry	Tablet	-	Distilled water	295,385 nm	51
2	Spectrophotometry	Tablet	-	Bromo cresol green in potassium hydrogen phthalate buffer (pH 3) Picric acid in chloroform (Ion pair complex)	420,430 nm	52
3	Spectrophotometry, Spectrofluorimetry	Tablet	-	4-chloro-7-nitro-2,1,3-benzoxadiazole in alkaline buffer and Acetone, *o*-phthalaldehyde/N-acetyl-L-cysteine	476,455,340 nm,	53
4	Spectrophotometry	Tablet	-	Dichloromethane	291 nm	54
5	HPLC	Tablet	Kromasil C18 (150 × 4.6 mm, 5 $\mu\mu$m)	hydrochloric acid water solution (0.01 M pH 2.4)-methanol (15 : 85, v/v)	218 nm	55
6	HPLC	-	Kromasil C18 (150 × 4.6 mm, 5 $\mu\mu$m)	Acetonitrile: phosphate buffer (80:20)	265 nm	56
7	HPTLC	Tablet	pre-coated with silica gel G60F254	n-Hexane: Ethyl acetate: Diethylamine (5:5:0.7 % v/v/v)	501 nm	57
8	HPLC	Tablet	Inertsil ODS 3V 250x4.6mm,5µm	KH2PO4: Acetonitrile: Methanol (30:40:30)	260nm	58

9	HPLC	-	Nova-Pak C18 column	Acetonitrile: Sodium dihydrogen phosphate (pH 2.5; 0.05 M) (70: 30)	360 nm	59
10	spectrophotometer	Tablet	-	methanol	254 nm	60
11	HPLC	Plasma	Waters (Milford, MA) Symmetry C18 column (250 × 4.6 mm id, 5 mm particle size)	acetonitrile–10 mM orthophosphoric acid containing 1 mL/L triethylamine	260,310 nm	61
12	HPLC	Tablet	Nucleosil Nucleodur C18 250 x 4.6 mm, 3 µm,	1% v/v acetonitrile and 99% v/v of 0.05% - 0.1% phosphoric acid or 2.5 – 5µM phosphate buffer	193 nm	62
13	UPLC–MS/MS	Plasma	BEH C18 column (2.1 mm × 50 mm; 1.7 m)	ammonium acetate buffer at pH 9.3 and acetonitrile	180.1→163	63

Table 3: Analytical methods for the assay of Galantamine

Sr. No	Methods	Formulation / Biological fluid	Stationary phase	Mobile phase	Detection wavelength (nm)	Ref.
1	Spectrophotometry (first order derivative)	Tablet	-	Distilled water	289, 284.8 nm	64
2	Spectrofluorimetry	Tablet	-	Distilled water	282,607 nm	65
3	Spectrophotometry	Tablet	-	Double distilled water	287 nm,	66
4	Spectrophotometry	Tablet	-	Double distilled water	287, 277.4nm	67
5	HPLC	-	SunFire C18 (150mm×2.1mm	(solvent A: 10mM ammonium acetate	290 nm	68

			, 3.5_m)	(pH 5.8): MeOH (95:5, v/v) and solvent B: 10mM ammonium acetate (pH 5.8): MeOH (5:95, v/v).		
6	HPTLC	-	silica gel 60F254 (20 × 10 cm; 0.25 mm	chloroform:methanol, 9:1 v/v)	288nm	69
7	HPLC	Biological Fluids	C18 column	methanol, acetonitrile and ammonium formate buffer, adjusted to pH 9,	212nm	70
8	HPLC	-	Phenomenex Synergi C18 column (inertsil, 150 × 4.6 mm i.d., 5 µm	40% acetonitrile and 60% 10 mM o-phosphoric acid	375,537nm	71
9	HPLC	-	chiralpak AD-H (250 · 4.6 mm)	n-hexane, 20% propionic acid in isopropanol and diethyl amine in the ratio of 80:20:0.2 (v/v)	289 nm	72

Table 4: Analytical methods for the assay of Rivastigmine

Sr. No	Methods	Formulation/ Biological fluid	Stationary phase	Mobile phase	Detection wavelength (nm)	Ref.
1	Spectrophotometry (Fluorescence)	Capsule	-	Triple distilled water	220, 289, nm	73
2	Spectrophotometry	Capsule	-	water + methanol (9:1)	221 nm	74
3	Derivative spectroscopy Ratio derivative Spectroscopy and TLC	Capsule	-	Methanol: Butanol: H2O: Ammonia (5:4:1:0.01)	262,263,272 nm,	75

	densitometry					
4	HPLC	Transdermal drug delivery	C18 Inertsil, 220 mm x 4.6 (i.d.) , 10 □m	0.01M ammonium acetate buffer: Acetonitrile [30:70 %v/v, pH 4.0]	219nm	76
5	HPLC	Capsule	Chromosil C18 (250x4.6mm, 5µm)	Methanol: Water: Acetonitrile (ACN) 35:25:40 v/v, (PH 4.8)	211 nm	77
6	HPTLC	Capsule	silica gel 60F25	Chloroform-methanol 4:6 (v/v)	210nm	78
7	HPLC	nanoparticle formulation	Apollo C18 column, 5 µm particle size, 150mm× 4.6mm	(20% v/v ACN in water containing 0.1% TFA,	214nm	79
8	UPLC	-	Acquity UPLC BEH Phenyl (100mm_2.1 mm, 1.7 lm)	phase-A acetonitrile-Disodium hydrogen orthophosphate (pH 7.5; 0.01 M)-Triethylamine (10:90:0.1,v/v/v), and phase-B acetonitrile-water (80:20,v/v).	210nm	80
9	HPTLC	Capsule	silica gel 60 F254, [(20 × 10 cm) with 250 µm	n- Hexane: Ethyl acetate: triethylamine (1.5: 8.5: 0.3 v/v).	213 nm	81
10	HPLC	Capsule	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)	220 nm	82
11	HPLC	Tablets	Chromatopack (250×4.6 mm; 5 µm particle size)	phosphate buffer of pH 3.2 and methanol (70:30 v/v)	220 nm	83

12	LC–ESI/MS/MS and LC–UV	-	Xterra RP-18, (250 mm × 4.6 mm, 5 µm	A, 10 mM dipotassium hydrogen phosphate adjusted to pH 7.6 ±0.05 with orthophosphoric acid–acetonitrile (90:10, v/v) and B, acetonitrile–methanol (60:40, v/v)	221nm	84
13	HPLC	-	inerstil C 18 column (250×4.6mm)	potassium di hydrogen ortho phosphate and acetonitrile(70:30v/v)	217nm	85

Table 5: Analytical methods for the assay of Tacrine

Sr. No	Methods	Formulation/ Biological fluid	Stationary phase	Mobile phase	Detection wavelength (nm)	Ref.
1	HPLC (Fluorescence)	Human plasma, urine	LiChrospher 60 RP-select B, 5 mm (25034 mm I.D.)	acetate buffer (0.2 M, pH 4.0) and acetonitrile (87:13, v/v)	330, 365, nm	86
2	LC–MS-MS	Rat Plasma	Atlantis dC18 column (50 × 4.6 mm, 3 µm	0.2% formic acid : acetonitrile (30 : 70, v/v)	199.10→171.20	87
3	HPLC (Fluorescence)	Rat plasma and brain tissue	Thermo BDS C18 Hypersil column (250 mm ×4.6 mm i.d., 5 µm	A [50 mM ammonium formate and 0.5% triethylamine (adjusted to pH 4.0 by formic acid) with 5% acetonitrile)] and eluent B [acetonitrile]	330, 365, nm	88
4	HPLC	Nanoemulsion gel	C18 column; 250 mm, 4.6 mm, 0.5µ	0.05M triethylamine: acetonitrile (80: 20,); pH 3	243nm	89

CONCLUSION

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. Currently available Alzheimer drugs are reducing the symptoms of AD, but these drugs do not cure this disease. There is plenty of research going on to find a cure for the disease, but the challenges of this are to obtain volunteers for clinical trial studies and funding for the research.

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