

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 behavior, 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. Quantization 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 quantization 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 Vander Waals interaction, electrostatic interaction, dipole-dipole interaction, hydrophobic interaction, and the hydrogen bond. An efficient, bio-specific 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 ($A\beta$) 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-D-aspartate 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 **slow down the** 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 [20]. 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, $t_{1/2}$ 70 h, protein binding 96% and oral bioavailability 100% [8, 21, 22].

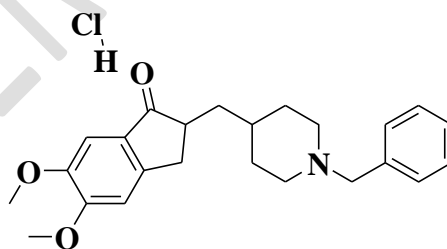


Figure 1: *Donepezil hydrochloride*

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. 2). 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, $t_{1/2}$ 1.5h, protein binding 40% and oral bioavailability 36% [8, 21, 22].

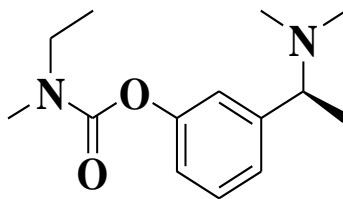


Figure 2: Rivastigmine

Galantamine

Galantamine ($C_{17}H_{21}NO_3$) is a phenanthrene alkaloid and a reversible competitive acetyl cholinesterase inhibitor preventing the hydrolysis of acetylcholine, 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 (-)-N-ethyl-3-[(1-dimethylamino)ethyl]-N-methyl phenyl-carbamate hydrogen (Fig. 3). 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, $t_{1/2}$ 7h, protein binding 18% and oral bioavailability 80-100% [8, 21, 22].

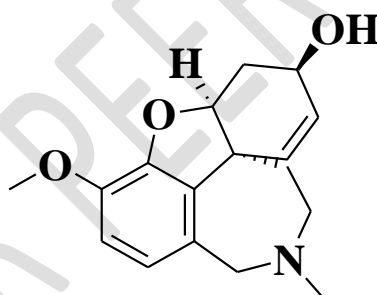


Figure 3: Galantamine

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. 4) 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, $t_{1/2}$ 2-4h, protein binding 55% and oral bioavailability 2.4–36% [23].

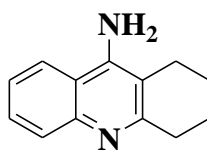


Figure 4: Tacrine

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 Tonibril 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. 5). Its molecular weight 179.3, pKa 10.7, LogP 3.5, therapeutic dose 5-10 mg/day, T_{\max} 3–7h, $t_{1/2}$ 60-100h and oral bioavailability ~100% [8, 21, 22].

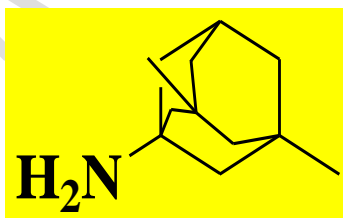


Figure 5: Memantine

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	-	KMnO ₄ 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) and Acetonitrile	230 nm	36
14	HPLC	Tablet	Inert Sustain	Acetonitrile: Water pH 3.5	230 nm	37

			Swift™ 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, 1.7 μ	(70:30) Acetonitrile: Methanol (80:20)		
21	HPLC	Tablet	Hypersil BDS (4.6 x 150 mm, 5 μ)	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 \times 2,1 mm, 5 μ m)	(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 \times 2 mm, 5 μ m)	gradient elution consists of formic acid, ammonium formate and ACN	380.1 \rightarrow 91.2 and 384.1 \rightarrow 93.2 for donepezil and donepezil-d4.	47
25	HPLC	Tablet	Eclipse plus C18 (250 \times 4.6 mm, 5 μ m)	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 μ m, 250- \times 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
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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, <i>o</i> -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 µm)	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 µm)	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	KH ₂ PO ₄ : 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_μm)	(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: H ₂ O: 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 μ m	0.01M ammonium acetate buffer: Acetonitrile [30:70 %v/v, pH 4.0]	219nm	76
5	HPLC	Capsule	Chromosil C18 (250x4.6mm, 5 μ m)	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 μ m particle size, 150mm \times 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 μ m)	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 \times 10 cm) with 250 μ m	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 μ m)	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 \times 4.6 mm; 5 μ m 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.

References

1. Hema, Swati Reddy G. A review on new analytical method development and validation by RP-HPLC. *Int Res J Pharm Biosci* 2017; 4:41-50.
2. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Topic Q7: Good Manufacturing Practices for Pharmaceutical Ingredients, 2000.
3. Current Good Manufacturing Practices for finished Pharmaceuticals, 21 CFR, Parts 210 and 211, US Food and Drug Administration.
4. European Commission. Final Version of Annex 15 to the EU Guide to Good Manufacturing Practice: Qualification and validation 2001; 4: 1-10.
5. McDowall RD. Effective and Practical risk management options for computerized system validation, *Quality Assurance Journal* 2005; 9(3): 196-227.
6. Xiao-Wu Che, Zhi-Xu He, Zhi-Wei Zhou, Tianxin Yang, Xueji Zhang, Yin-Xue Yang, Wei Duan, Shu-Feng Zhou. Clinical pharmacology of dipeptidyl peptidase 4 inhibitors indicated for the treatment of type 2 diabetes mellitus. *Clinical and Experimental Pharmacology and Physiology*, 2015; 42(10): 999-1024.
7. Frederic Gerber, Markus Krummen, Heiko Potgeter, Alfons Roth, Christoph Siffrin, Christoph Spöndlin. Practical aspects of fast reversed-phase high-performance liquid chromatography using 3 μ m particle packed columns and monolithic columns in pharmaceutical development and production working under

- current good manufacturing practice". *Journal of Chromatography A*. 2004; 1036(2): 127–33.
8. Hemambika Sadasivuni, Narayana Rao Gundaju. A review on: Analytical techniques on drugs for Alzheimer's disease. *International Research Journal of Engineering and Technology*, 2020; 7(4): 6165-6170.
 9. Dass, Chhabil. *Fundamentals of Contemporary Mass Spectrometry || Hyphenated Separation Techniques*, John Wiley & Sons, Inc. 2007; 151–194.
 10. James J Pitt. Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry. *The Clinical Biochemist Reviews*. 2009; 30 (1): 19–34.
 11. Shuijun Li, Gangyi Liu, Jingying Jia, Xiaochuan Li, Chen Yu. Liquid chromatography-negative ion electrospray tandem mass spectrometry method for the quantification of ezetimibe in human plasma. *J Pharm Biomed Anal*. 2006; 40(4):987-92.
 12. Ravali R, Phaneendra M, Bhanu Jyothi K, Ramya Santhoshi L, Sushma K. Recent Trends in Analytical Techniques for the Development of Pharmaceutical Drugs. *J Bioanal Biomed*, 2011; S11.
 13. Waldemar G, Dubois B, Emre M, Georges J, McKeith IG, Rossor M, Scheltens P, Tariska P and Winblad B. Recommendations for the diagnosis and management of Alzheimer's disease and other disorders associated with dementia: EFNS guideline. *European Journal of Neurology* 2007; 14: e1–26.
 14. Amatsubo T, Yanagisawa D, Morikawa S, Taguchi H and Tooyama I. Amyloid imaging using high-field magnetic resonance. *Magnetic Resonance Medical Sciences* 2010; 9: 95–99.
 15. Wang JM and Sun C. Calcium and neurogenesis in Alzheimer's disease. *Frontiers in Neuroscience* 2010; 4: 194.
 16. Blennow K and Hampel H. CSF markers for incipient Alzheimer's disease. *Lancet Neurology* 2003; 2: 605–613.
 17. Berchtold NC and Cotman CW. Evolution in the conceptualization of dementia and Alzheimer's disease: Greco-roman period to the 1960s. *Neurobiology of Aging* 1998; 19: 173–189.
 18. Francis PT, Palmer AM, Snape M and Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: a review of progress. *Journal of Neurology, Neurosurgery and Psychiatry* 1999; 66: 137–147.

19. Farlow MR. NMDA receptor antagonists. A new therapeutic approach for Alzheimer's disease. *Geriatrics* 2004; 59: 22–27.
20. Pohanka M. Cholinesterases, a target of pharmacology and toxicology. *Biomed Papers of the Medical Faculty of the University Palacky Olomouc, Czechoslovakia Republic* 2011; 155: 219–229.
21. Avuthu Sai Sheela, Mukthinuthalapathi Mathrusri Annapurna. Analytical methods for the determination of drugs used in Alzheimer's disease- A Review. *Research J. Pharm. and Tech.* 2019; 12(11):5561-5565.
22. Suresh Ponnayyan Sulochanaa, Kuldeep Sharmab, Ramesh Mullangiband Sathesh Kumar Sukumarana. Review of the validated HPLC and LC-MS/MS methods for determination of drugs used in clinical practice for Alzheimer's disease. *Biomed. Chromatogr.* 2014, 28(11):1431-90.
23. <https://pubchem.ncbi.nlm.nih.gov/compound/Tacrine-hydrochloride>
24. Arun Shirwaikar, Sarala Devi, Rajagopal.P.L, Kiron.S.S and Sreejith.K.R. Development and validation of analytical method for determination of Donepezil hydrochloride in pure and dosage forms. *Asian J Pharm Clin Res*, 2014; 7(1): 149-153.
25. Ravi Kumar V, Chaitanya S, Sambasiva Rao A, Sreekiran M. Estimation of Donepezil hydrochloride by ion complex extractive spectrometry. *International journal of Research in pharmacy and chemistry.* 2011; 1(3): 512-518.
26. A.Tanuja, CH. Srujani, Varanasi. S. N. Murthy. Development and validation of novel UV- spectrophotometric method for the estimation of donepezil in bulk and pharmaceutical dosage form. *Indo Am. J. Pharm. Sci*, 2015; 2(8): 1147-1153.
27. J.N. Sangshetti, P.R. Mahaparale, S. Paramane and D.B. Shinde. Spectrophotometric estimation of Donepezil hydrochloride in bulk and tablet formulation. *Trends in Applied Sciences Research.* 2008; 3(1): 109-112.
28. Mohd Yasir, Uvs Sara. Development of UV spectrophotometric method for the analysis of acetylcholinesterase inhibitor. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2014; 6(9): 128-131.
29. Jayanna Bidarur Krishnegowda. A facile Spectrophotometric method for the determination of Donepezil hydrochloride in tablets formulation using potassium permanganate. *Asian Journal of Pharmaceutical and Biological Research.* 2012; 2(4): 216-218.

30. Sharmila D, Swaminathan J. Method development and validation of Donepezil hydrochloride by using UV Spectrophotometric method. International Journal of Pharmaceutical Research and Bio-Science. 2019; 8(3): 1-9.
31. V.A. Ghodake, B.A. Agarwal, A.R. Tekade. Stability-indicating densitometric method for simultaneous determination of donepezil hydrochloride and curcumin in *insitu* nasal gel. Indo American Journal of Pharmaceutical Sciences. 2018, 05 (05), 4097-4106.
32. Sharmila Dusia, Ramanamma.L, Prasad. DSVS. Method development and validation of Donepezil hydrochloride by RP-HPLC. Indo American Journal of Pharmaceutical Sciences. 2018; 05 (05): 4228- 4251.
33. Murugesan Jagadeeswarana, Natesan Gopal, Murugananthan Gandhimathi, Rajagounden Rajavel, Mani Ganesh, Thangavel Sivakumar. A validated HPTLC method for the estimation of donepezil hcl in bulk and its tablet dosage form. Eurasian J Anal Chem 6(1): 40-45, 2011.
34. Tushar G. Barot and P. K. Patel. RP-HPLC Method for the estimation of Donepezil hydrochloride dosage form. E-Journal of Chemistry. 2009; 6(2): 594-600.
35. P. Sreelatha¹, D. Vivekananda Reddy, Sripal Reddy Palavai and B. Rama Devi. Impurity Profiling for Donepezil Hydrochloride Tablet Formulations and Characterisation of Potential Degradant. Am. J. PharmTech Res. 2016; 6(6),213-229.
36. B. Anbarasi, M.S.S.K. Prasanth, N. Senthil Kumar. Analytical method development and validation of Donepezil hydrochloride tablets by RP-HPLC And UV. International Journal of Pharmacy and Technology , 2011, 3(2):1988-2000.
37. Patel Yesha K., Noolvi M.N., Raj Hasumati, Patel Meghna P. Development and validation of stability indicating reverse phase high performance liquid chromatographic method for estimation of Donepezil HCl from bulk drug. Asian Journal of Pharmaceutical Research. 2015; 5(2): 96-101.
38. R.Dharmaraj Santhosam, S. Kannan,S. Lakshmi Devi. Development and validation of RP- HPLC method for estimation of Donepezil HCl from bulk and marketed dosage forms. J.Chem. Pharm. Res. 2010; 2(6): 62-67.
39. Mohammed A. Abonassif, Mohammed M. Hefnawy, Mohamed G. Kassem, Gamal A. E. Mostafa. Determination of donepezil hydrochloride in human plasma and pharmaceutical formulations by HPLC with fluorescence detection. Acta Pharm. 61 (2011) 403–413.

40. Ravi Kumar vejendla, G. Hemanth Kumar, G. Raveendra Babu and J.Srinivasarao. New Comprehensive HPLC assay method for Donepezil hydrochloride. Asian J. Research Chem. 4(10): Oct., 2011; Page 1508-1512.
41. Horacio Pappa, Romina Farru, Paula Otano Vilanova, Marcelo Palacios, Maria Teresa Pizzorno. A new HPLC method to determine Donepezil hydrochloride in tablets. Journal of Pharmaceutical and Biomedical Analysis. 2002; 27(1-2): 177- 182.
42. B. K. Singh, R. K. Srivastava, S. Senthil Kumar, P. K. Dutta. Stability-indicative HPLC determination of Donepezil hydrochloride in tablet dosage form. Pharmaceutical Chemistry Journal. 2012; 45(12): 766-770.
43. Mahalingam. V, Vijayabaskar. S, Kalaivani. R.A, Somanathan.T. Analytical method development and validation for the analysis of Donepezil hydrochloride and its related substances using ultra performance liquid chromatography. Research J. Pharm. and Tech. 2017; 10(8): 2743-2749.
44. Syeda Noorain Amena, S. H. Rizwan. Stability indicating analytical method development and validation of memantine hcl and donepezil hcl using RP-HPLC. Int J Pharm Pharm Sci, Vol 7, Issue 11, 204-210
45. Kamepalli Sujana, D. Gowri Sankar, K. Abbulu. Simultaneous Estimation of Donepezil and Memantine by Reverse Phase HPLC in Bulk and Pharmaceutical Dosage Form. Research J. Pharm. and Tech. 5(7): July 2012; Page 958-961
46. Maria Petrocheilou, Victoria Samanidou¹, Leda Kovatsi, Magda Tsolaki, Ioannis Papadoyannis. A simple and direct HPLC-DAD method for the simultaneous determination of galantamine, donepezil and rivastigmine in cerebrospinal fluid, blood serum and urine Journal of Applied Bioanalysis, 2017, 3(4) 59-69.
47. Meier-Davis SR, MengM, YuanW, Diehl L, Arjmand FM, Lucke RM, Huang B, Wen J, Shudo J and Nagata T. Dried blood spot analysis of donepezil in support of a GLP3-month dose-range finding study in rats. International Journal of Toxicology 2012; 31: 337–347.
48. Prakash Kumar B, Sharath H.N. Development and validation for estimation of Donepezil HCl from bulk and marketed dosage forms by using RP-HPLC. International journal of Pharmacognosy and Phytochemistry Research. 2016; 7 (3): 343-349.
49. Yihong Lu, Hongmei We n, Wei Li, Yumei Chi, and Zhengxing Zhang. Determination of Donepezil hydrochloride (e2020) in plasma by liquid chromatography–mass spectrometry and its application to pharmacokinetic studies in

- healthy young Chinese subjects. *Journal of Chromatographic Science*. 2004; 42(5): 234- 237.
50. Andre L. M. Ruela, Mariane G. Santos, Eduardo C. Figueiredo, Gislaine R. Pereira. LC-PDA and LC-MS studies of Donepezil hydrochloride degradation behaviour in forced stress conditions. *J. Braz. Chem. Soc.* 2014; 25(11): 2094-2101.
51. Ravisankar P, G. Devala Rao and Ch. Devadasu. A novel Spectrofluorimetric method for the determination of Memantine hydrochloride in bulk and pharmaceutical formulation. *International journal of pharmaceutical sciences and Research*. 2014; 5(11): 4808-4814.
52. S.K. Naffiz, S.D. Ameen, M. M. Eswarudu, P. Srinivasa Babu, M. Raja Kumari, P. Sindhuja and P. Gouthami. Validated spectrophotometric methods for the determination of Memantine hydrochloride in pure and tablet dosage form by using different chromogenic reagents. *World Journal of Pharmaceutical Research*. 2017; 6(5): 1198-1209.
53. Karim Michail, Hoda Daabees, Youssef Beltagy, Magdi Abdel-khalek, Mona Khamis. Spectrophotometric And Spectrofluorimetric determination of memantine hydrochloride in bulk and pharmaceutical preparations. *International Journal of Pharm Pharm Sciences*. 2011; 3(3): 180–185.
54. Kaur Navneet, Mittal Karan, Nagar Rishabh, Upadhyay Ashutosh, Nepali Kunal, Thakkar Arti. Estimation of Memantine hydrochloride using Ultraviolet visible spectrophotometry in bulk drug and formulation. *Journal of Pharmaceutical Research*. 2011; 10(2): 80-82.
55. Sergio del Rio-Sancho, César E. Serna-Jiménez, M. Aracely Calatayud-Pascual, Cristina Balaguer-Fernández, Andrés Femenía-Font, Virginia Merino and Alicia López-Castellano. High-Performance Liquid Chromatographic Ultraviolet Determination of Memantine Hydrochloride after In Vitro Transdermal Diffusion Studies. *Journal of Chemistry* Volume 2013, Article ID 502652.
56. Bhavil Narola, A.S. Singh, P. Rita Santhakumar, and T.G. Chandrashekhar. A Validated stability-indicating reverse phase HPLC assay method for the determination of Memantine hydrochloride drug substance with UV-Detection using precolumn derivatization technique. *Anal Chem Insights*. 2010; 5: 37-45.
57. Patel KH, Patel SK, Karkhanis VV and Captain AD. Development and validation of analytical method for estimation of Memantine hydrochloride. *Austin J Anal Pharm Chem*. 2015; 2(4): 1047. Ayesha Anees, Asra Ali Bahazeq, MD. Muzaffar-Ur-

58. Rehman, Syed Akbar, Juveria Mehveen. Development and validation of Memantine hydrochloride by RP-HPLC method. Asian Journal of Pharmaceutical Research. 2019; 9(2): 69-74.
59. Hassan Jalalizadeh, Mahdi Raei, Razieh Fallah Tafti, Hassan Farsam, Abbas Kebriaeezadeh, and Effat Souri. A Stability indicating HPLC Method for the determination of Memantine hydrochloride in dosage forms through derivatization with 1- Fluoro-2, 4-dinitrobenzene. Sci Pharm. 2014; 82(2): 265-279.
60. Asra Ali Bahazeq, Wasfiya Noor Syeda¹, Nashra Fatima Isba¹, MD. Muzaffar-Ur-Rehman, Uzma Amatul Baqi. Assay of Memantine Hydrochloride by UV Spectrophotometer. International Journal of Pharma Sciences and Research, 2019, 10(1), 27-30.
61. Serife Evrim Kepekci Tekkeli and Sidika Erturk Toker. A New HPLC Method with Fluorescence Detection for the Determination of Memantine in Human Plasma. Journal of AOAC International Vol. 96, No. 1, 2013, 52-56.
62. Marjan Piponski, Tanja Bakovska Stoimenova, Stefan Stefov, Trajan Balkanov, Gordana Trendovska Serafimovska, Liliya Logoyda. Development of a Novel, Fast, Simple, Nonderivative HPLC Method with Direct UV Measurement for Quantification of Memantine Hydrochloride in Tablets. Journal of Separation Science, 2020, 43(17), 3482-3490.
63. Noetzli M, Ansermot N, Dobrinas M and Eap CB. Simultaneous determination of antimentia drugs in human plasma: procedure transfer from HPLC-MS to UPLC-MS/MS. Journal of Pharmaceutical and Biomedical Analysis 2012; 64–65: 16–25.
64. Patel Hitesh N, Patel Amit V., Patel Vishal J., Dave Jayant B. and Patel Chhaganbhai N. UV-Spectrophotometric method development and validation for estimation of Galantamine hydrobromide in tablet dosage form. J. Chem. Pharm. Res. 2010; 2(2): 44-49.
65. Amit V. Patel, Vishal J. Patel, Avani V. Patel, Jayant B. Dave, and Chhaganbhai N. Patel. Determination of Galantamine hydrobromide in bulk drug and pharmaceutical dosage form by spectrofluorimetry. J Pharm Bioallied Sci. 2013; 5(4): 314-317.
66. Mittal K, Dhingra T, Upadhyay A , Mashru R , Malik J , Thakkar A. Estimation of uncertainty for measuring Galantamine hydrobromide in pharmaceutical formulation using ultraviolet spectrophotometry. Journal of Pharmaceutical Research. 2013; 12(1): 34-38.

67. Karan Mittal, Ramni Kaushal, Rajashree Mashru, Arti Thakkar. Estimation of the Galanthamine using derivative spectrophotometry in bulk drug and formulation. J.Biomedical Science and Engineering. 2010; 3: 439-441.
68. Lygia Azevedo Marquesa, Ismail Maadaa, Frans J.J. de Kanterb, Henk Lingemana, Hubertus Irth a, Wilfried M.A. Niessena, Martin Gieraa, Stability-indicating study of the anti-Alzheimer's drug galantamine hydrobromide. Journal of Pharmaceutical and Biomedical Analysis 55 (2011) 85–92.
69. Amina H. Abou-Donia, Soad M. Toaima, Hala M. Hammoda, Eman Shawky. New Rapid Validated HPTLC Method for the Determination of Galanthamine in Amaryllidaceae Plant Extracts. Phytochem. Anal. 19: 353–358 (2008).
70. Shikha Lo.han, Rajneet Kaur, Shubham Bharti, SurinderKumar Mehta, Bhupinder Singh. QbD-Enabled Development and Validation of a LiquidChromatographic Method for Estimating GalantamineHydrobromide in Biological Fluids. Current Pharmaceutical Analysis, 2018,14(6),527-540.
71. Elif Ozdemir, Sevgi Tatar Ulu. Highly sensitive HPLC method for the Determination of Galantamine in human plasma and urine through derivatization with dansyl chloride using fluorescence detector. The Journal of Biological and chemical Luminescence. 2017; 5(4):1145-1149.
72. Ravinder, Vadde Ashok, Prasad S, Balaswamy AVSS. A validated chiral LC method for the enantiomeric separation of Galantamine. Chromatographia. 2008; 5(4): 331–334.
73. R. Kapil, S. Dhawan and Bhupinder Singh. Development and validation of a Spectrofluorimetric method for the estimation of Rivastigmine in formulations. Indian J Pharm Sci. 2009; 71 (5): 585-589.
74. Kulkarni, A.S, Chandrashekhar, V.B, Amol, N.J. Development of UV Spectrophotometric method for estimation of Rivastigmine in pharmaceutical dosage form. International Journal for Pharmaceutical Research Scholars. 2017; 6(4): 59-64.
75. Maissa Y. Salem, Amira M. El-Kosasy, Mohamed G. El- Bardicy and Mohamed K. Abd El-Rahman. Spectrophotometric and Spectrodensitometric methods for the determination of Rivastigmine hydrogen tartrate in presence of its degradation product. Drug Test. Analysis. 2010; 2(5): 225–233.
76. Kale MN. Development of validated rp-hplc method for quantitative estimation of rivastigmine hydrogen tartrate in transdermal drug delivery system. International Journal of Pharmaceutical Sciences and Research, 2014; 5(5):1892-1902.

77. TV. Reddy and Kusumanchi Gowri. Novel RP-HPLC and Visible Spectrophotometric methods for the quantification of Rivastigmine in bulk and pharmaceutical formulations. *International Journal of Pharmaceutical and Chemical Sciences*. 2013; 2 (2): 851-857.
78. Arumugam Karthik, Ganesa Sundararajan Subramanian*, Prashant Musmade, Averineni Ranjithkumar, Mallayasamy Surulivelrajan, and Nayanabhira Udupa. Stability-Indicating HPTLC Determination of Rivastigmine in the Bulk Drug and in Pharmaceutical Dosage Forms. *Journal of Planar Chromatography* 20 (2007) 6, 457–461.
79. Naz Hasan Huda , Bhawna Gauri , Heather A. E. Benson, and Yan Chen. A Stability Indicating HPLC Assay Method for Analysis of Rivastigmine Hydrogen Tartrate in Dual-Ligand Nanoparticle Formulation Matrices and Cell Transport Medium. *Journal of Analytical Methods in Chemistry*, 2018, Article ID 1841937.
80. T. Satyanarayana Raju, L. Kalyanaraman, V. Venkat Reddy, and P. Yadagiri Swamy. Development and validation of an uplc method for the rapid separation of positional isomers and potential impurities of rivastigmine hydrogen tartrate in drug substance and drug product. *Journal of Liquid Chromatography & Related Technologies*, 35:896–911, 2012.
81. Mohammad Mojeeb Gulzar Khan, Atul Arun Shirkhedkar. Validated thin-layer chromatography/densitometry method for the analysis of anti-alzheimer drug in bulk and in capsule formulation. *J. Chil. Chem. Soc.*, 60, N° 4 (2015) ,2650-2654.
82. S. Alexandar, Rohini Diwedi, T. Ashok and M. J. N. Chandrasekhar. A validated RP-HPLC method for estimation of Rivastigmine in pharmaceutical formulations. *Der Pharmacia Lettre*. 2011; 3(3): 421-426.
83. Basavaiah K, Rajendraprasad N. Development and Validation of a New Stability-Indicating HPLC Method for the Determination of Rosiglitazone in Tablets. *Research and Reviews: A Journal of Pharmaceutical Science*. 2017; 8(1): 1–9p.
84. Saji Thomas^{a,*}, Sanjeev Shandilya^a, Amber Bharati^a, Saroj Kumar Paula^a, Ashutosh Agarwal^a, Chandra S. Mathela^b Identification, characterization and quantification of new impurities by LC–ESI/MS/MS and LC–UV methods in rivastigmine tartrate active pharmaceutical ingredient *Journal of Pharmaceutical and Biomedical Analysis* 57 (2012) 39– 51

85. S.Navaneethakrishnan, MD. Reshma Begum, P. Suresh Kumar, J. Belson David and M. Phani Kumar. HPLC method development of Rivastigmine by RP-HPLC in its bulk dosage form. *Der Pharmacia Sinica*. 2012; 3(2): 295-299.
86. Hansen LL, Larsen JT and Brosen K. Determination of tacrine and its metabolites in human plasma and urine by high performance liquid chromatography and fluorescence detection. *Journal of Chromatography B* 1998; 712: 183–191.
87. Suresh Ponnayyan Sulochana, Vishnuvardh Ravichandiran, Ramesh Mullangi, Sathesh Kumar Sukumaran. Highly Sensitive LC–MS-MS Method for the Determination of Tacrine in Rat Plasma: Application to Pharmacokinetic Studies in Rats. *Journal of Chromatographic Science*, 2016, 54 (3), 397-404.
88. Shuai Qian, Siu Kwan Wo, Zhong Zuo. Pharmacokinetics and brain dispositions of tacrine and its major bioactive monohydroxylated metabolites in rats. *Journal of Pharmaceutical and Biomedical Analysis*, 2012, 61, 57-63.
89. Sonal Setya, Bal Krishan Razdan, Sushama Talegaonkar. Development and Validation of RPHPLC method of Tacrine Hydrochloride in Nanoemulsion gel. *J. Adv. Pharm. Edu. & Res.* 2014; 4 (4): 435-439.