DETERMINATION OF ANTIDEMENTIA DRUGS BY SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC METHODS – AN OVERVIEW

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***Abstract*:** Cholinesterase inhibitors are otherwise called acetylcholinesterase inhibitors are a group of medications that can restrain the typical breakdown of acetylcholine (ACh). Acetylcholine is the principal neurotransmitter found in the human body and has capacities in both the peripheral nervous system and the central nervous system. In patients having state of Alzheimer's disease there has been irreversible degeneration of ACh -producing neurons bringing about scholarly shortcoming, cognitive decline just as other neural limits showed decay of mindfulness, learning, stress response, rest and so on. Consequently, to treat these symptoms cholinesterase inhibitors are widely used. There has been huge examination and research on wide scope of analytical techniques that could be valuable in the assessment of cholinesterase inhibitors in pure drug, pharmaceutical formulations and biological matrices. Scientific strategies, for example, HPLC, high pressure thin layer chromatography, ultraviolet (UV) spectrophotometry, high performance liquid chromatography - mass spectroscopy, ultra-performance liquid chromatography have been accounted for the assessment of cholinesterase inhibitors in single as well as in mixture with different medications or drugs. This complete review covers the greater part of the spectrophotometric and chromatographic methods depicted for the assurance of Rivastigmine, Galantamine, Donepezil in pure drug forms, in different pharmaceutical dosage forms and biological fluids. From the extensive review it tends to be found that countless chromatographic methods have been created and HPLC techniques have been generally utilized in the discovery and assessment of cholinesterase inhibitors.

Keyword: Spectrophotometric and Chromatographic methods, Cholinesterase Inhibitors, Rivastigmine, Galantamine, Donepezil.

I. INTRODUCTION

Alzheimer's Disease is portrayed as irreversible degeneration of acetylcholine-producing neurons, intellectual weakness, reformist and the amassing of neurofibrillary tangles and amyloid plaques [1]. The cholinergic framework assumes a basic part in memory, close by other significant neural capacities like consideration, learning, stress reaction, attentiveness and rest, and tactile data. Studies show that acetylcholine (ACh) is correlated to the balance of obtaining, encoding, union, reconsolidation, elimination, and recovery of memory [1]. The slow loss of cholinergic neurons in Alzheimer's Disease (AD) may, hence, add to the behavioral and cognitive decline displayed by AD patients. The generally acknowledged cholinergic speculation suggests that a segment of the psychological and behavioral decay related with Alzheimer's are the consequence of diminished cholinergic transmission in the central nervous system [2]. As acetylcholinesterase is an enzyme that is discharged by cholinergic neurons to quickly hydrolyze ACh at the synaptic cleft to deliver choline and acetate which is likewise the primary driver for lessening ACh quantity which prompts Alzheimer's disease. To treat this condition of a patient cholinesterase inhibitors are used. The principle pharmacological activities of this Cholinesterase Inhibitor drugs are accepted to happen as inhibition of cholinesterase, improving cholinergic transmission, which mitigates the indications of Alzheimer's dementia [3]. Cholinesterase (Che) Inhibitors presently utilized in the treatment of Alzheimer's Disease (AD) are the Acetylcholinesterase (AChE)- Selective Inhibitors, Donepezil and Galantamine, and The Dual Ache and Butyrylcholinesterase (BuChe) Inhibitor Rivastigmine. Notwithstanding contrasts in selectivity for Ache and BuChE, ChE inhibitors likewise vary in pharmacokinetic and pharmacodynamics properties, and these distinctions could altogether have effect on safety, resistance, and efficacy [2].

II. MECHANISM OF ACTION

Acetylcholinesterase is an enzyme produced by cholinergic neurons to quickly hydrolyse ACh at the synaptic cleft to deliver choline and acetate [1,4]. Choline is subsequently reused once again into the presynaptic cholinergic neuron by means of reuptake by the high-affinity choline transporter. There are several evidences showing the expected relationship of the acetylcholinesterase enzymes in the development of amyloid fibrils [5]. Cholinesterase inhibitor reversibly and competitively inhibits the acetylcholinesterase enzyme in the central nervous system (to be specific in the frontal cortex and hippocampal regions) by binding to the acyl-binding pocket and the choline-binding site of the enzymes dynamic site [1,4]. By impeding the breakdown of ACh, cholinesterase inhibitor improves ACh levels in the synaptic cleft. Nicotinic acetylcholine receptors (nAChR) in the central nervous system are for the most part communicated at the presynaptic neuronal membrane to control the arrival of numerous neurotransmitters, like ACh, glutamate, GABA, dopamine, serotonin, norepinephrine. Agonists of nAChRs improve execution in intellectual undertakings, while antagonists of nAChR hinder psychological cycles. A few investigations show an abatement in the articulation and action of nAChRs in patients with AD, which may clarify the decrease in central cholinergic neurotransmission in these patients. cholinesterase inhibitor binds to nAChRs at the allosteric site, prompting a conformational change of the receptor, expanded ACh discharge, and expanded action of adjoining glutaminergic and serotoninergic neurons [3,6]. The regulation of nAChRs works with both excitatory and inhibitory cholinergic transmissions in brain tissues and builds receptor sensitivity. The

tweaked arrival of different synapses by cholinesterase inhibitor may likewise add to the upregulation of nAChRs and improvement of social indications in Alzheimer's disease [6].

III. PHARMACOKINETICS

All cholinesterase inhibitors are orally ingested, while, their oral bioavailability is variable (Donepezil =100%, Galantamine =90-100%, Rivastigmine =60-72%,). Plasma t½ of these medications ranges 70 hours for Donepezil, 7 hours for Galantamine and 1.5 hour for Rivastigmine. Cholinesterase inhibitors are mainly metabolized by cyp3a4 and cyp2d6 while Rivastigmine is used by cholinesterase-mediated hydrolysis. The pharmacokinetic properties of cholinesterase inhibitors shift in each person, which may be an outcome of differential action. These drugs are significantly discharged in urine and faeces. [4,7,8,9]

Drugs included in the cholinesterase inhibitors their chemical structure with the chemical name and molecular weight is given in the Table 1. Synopsis of contrasts in the route of administration, bioavailability, metabolism, plasma protein binding and excretion between the individual compounds is depicted in Table 2. The different physicochemical properties of cholinesterase (Che) inhibitors are addressed in Table 3.

Table 1: Structural details of Drug.

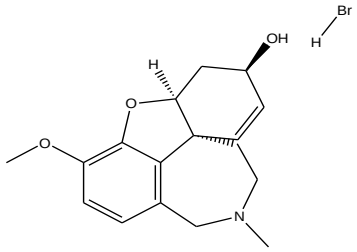
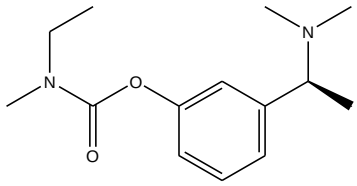
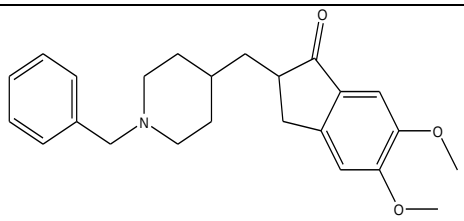
Drug	Structure	Chemical name	Molecular weight
Galantamine	 Galantamine	(4aS,6R,8aS)- 5,6,9,10,11,12-hexahydro- 3-methoxy- 11-methyl- 4aH- [1]benzofuro[3a,3,2-ef] [2] benzazepin- 6-ol	287.35 g/mol
Rivastigmine	 Rivastigmine	(S)-N-Ethyl-Nmethyl- 3-[1-(dimethylamino)ethyl]-phenyl carbamate hydrogen-(2R,3R)-tartrate.	250.337 g/mol
Donepezil	 Donepezil	2-((1-Benzylpiperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one	379.5 g/mol

Table 2: Pharmacokinetic Parameters of Drugs.

Pharmacokineticparameter	Galantamine	Rivastigmine	Donepezil
Administration route	Oral	Oral	Oral
Bioavailability %	90-100%	60 to 72%	100%
Metabolism	CYP2D6 and CYP3A4	By cholinesterase-mediated hydrolysis.	liver, primarily by CYP3A4 and CYP2D6
Elimination half-life, h	7 hours	1.5 hour	70 hours
Plasma Protein binding %	18%	40%	96%
Excretion	95% urine and 5% faeces,	97% in urine	57% in urine, and 5% in faeces.

Table 3: Pka and Solubility of Drugs

Drugs	Pka	Solubility
Galantamine	7.97	Galantamine is highly soluble in water
Rivastigmine	8.85	Rivastigmine very soluble in water, soluble in ethanol and acetonitrile, slightly soluble in n-octanol and very slightly soluble in ethyl acetate.
Donepezil	8.9	Donepezil is soluble in organic solvents such as chloroform 50 mg/ml and approximately 25 mg/ml in acetone. Donepezil is sparingly soluble in aqueous solutions.

IV. REPORTED ANALYTICAL METHODS

As there are various analytical methods that have been performed on all acetylcholinesterase inhibitors such as High-performance liquid chromatography, Gas chromatography, high performance thin layer chromatography, ultra-performance liquid chromatography, HPLC-MS-MS, ultra violet spectrophotometry etc. on the drug or pharmaceutical and finished product also on the blood, plasma, brain of human also lab animals such as Rats.

1. HPLC Method

High-performance liquid chromatography, once known as high-pressure liquid chromatography (HPLC), is a method in the analytical chemistry used to isolate, recognize and quantify each and every component in a mixture.

Chromatography includes a sample being dissolved in the mobile phase (which might be a liquid, supercritical fluid or a gas). The mobile phase is then constrained through a fixed, non-miscible stationary phase. The stages are picked with the end goal that segments of the samples will have varying solubilities in both phases. A part of segment which is imbibed in the stationary phase will take more time to go through it than a segment which isn't unexpectedly dissolvable in the fixed stage i.e., stationary phase however solvent in the mobile phase. Because of these distinctions in mobilities, test parts will get isolated from one another as they travel through the stationary phase. Chromatography is an exceptionally extraordinary partition measure for a huge number of reasons, chromatography can be utilized to isolate sensitive items since the conditions under which it is performed are not ordinarily extreme. There is an amazing expansion in the use of high-performance liquid chromatography for assurance of contemplated cholinesterase inhibitors. HPLC has been utilized routinely in all fields of cholinesterase inhibitors research. The announced strategies dependent on utilization of various stationary phase (silica c8, c18, Inertsil-ODS-3 column, Kromasil 100Å C8 column), mobile phase and utilizing UV, fluorescence, PDA or tandem mass spectrometry for detection. The different revealed HPLC and UPLC techniques (counting stability studies) for quantitative assurance of cholinesterase inhibitors either as single or in mix with different drugs in unadulterated pure drug, pharmaceutical finished forms and organic liquids such as biological fluids are shown [10-58]. HPLC method details for Rivastigmine, Galantamine and Donepezil are given in Table 4, Table 5, Table 6, respectively.

2. HPTLC Method

Spectro densitometric assurance of Rivastigmine after vortex assisted solid phase extraction is augmented for the assurance of Rivastigmine hydrogen tartrate in bulk and case in which MSPE was executed on human plasma to preconcentrate RIV. Co-precipitation process was applied to obtain magnetic nanoparticles and characterised by SEM, FTIR, PXRD, DLS [59].
For Rivastigmine Stability-Indicating HPTLC method was also performed for the Pharmaceutical Dosage Forms and bulk drug in which Chromatographic separation was done on the aluminium backed silica gel 60F254 HPTLC plates with chloroform–methanol 4:6 (v/v) as mobile phase [60].
For Galantamine HPTLC has been practiced for the analysis of the concentrates of Narcissus jonquilla Pipit, and TLC-densitometry has been utilized for the measurement of Galantamine in the concentrates of Galanthus elwesii Hook and Galanthus nivalis L. The developed and validated TLC-densitometric method was advisable for the simultaneous estimation of Galantamine hydrobromide and Pyridine [61]
For Donepezil stability indicating HPTLC method and validated HPTLC method was reported for bulk and pharmaceutical dosage form in which silica gel 60 F254 on aluminium sheet was used as a stationary phase [62,63]. HPTLC methods details for Rivastigmine, Galantamine and Donepezil are given in Table 7, Table 8, Table 9, respectively.

3. UV Spectrophotometric Method

The dissolvable which is utilized in spectrophotometry ought not retain UV radiation in a similar locale as the sample whose range is being resolved. By and large, the solvents which don't contain the conjugated system are generally suitable for performing Spectro photometric examination regardless of whether they shift at the most limited frequency at which they remain transparent to ultra-violet radiation.
Various methods such as second derivative spectra, first derivative ratio spectra, calibration curve method first order derivative spectra have been reported for Rivastigmine.64-67
Methods such as calibration curve method [68], first order derivative [69] have been reported for Galantamine.
and assay after solvent extraction, calibration curve method, comparison between calibration curve method and extractive ion-pair complexation method, calibration curve method with colorimetric method have been reported for Donepezil.70-76 UV Spectrophotometric methods details for Rivastigmine, Galantamine and Donepezil are given in Table 10, Table 11, Table 12, respectively.

4. HPLC MS-MS Method

For estimation of Rivastigmine only and also with its major metabolite NAP 226-90 a sensitive and rapid liquid chromatography-tandem mass spectrometry (LC-MS/MS) performed on human plasma and on rat brain and plasma. Separation was done using Surveyer HPLC with Betabasic-8 (5.0 m, 100 mm × 4.6 mm), MTBE Star RP-18 (Merck) column, Gemini C18 column (150 2.0 mm I.D.), Nucleosil CC-125/2 C18 reversed-phase column [36,37]. Mobile phase used for the separation are 0.1% formic acid in acetonitrile and 0.1% formic acid in water (70:30%, v/v), methanol–0.02 M ammonium acetate (55:45, v/v), ammonium hydroxide/methanol (50:50, v/v), acetonitrile 80% in H2O (v/v) containing 0.1% formic acid, for the proposed column respectively. flow rate used was in the range of 0.2-1.0 ml/min, and correlation of coefficient was found to be 0.99-0.9988 [77-80]

For determination of Galantamine sensitive and rapid liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed by using an internal standard such as glimepiride and loratadine on human plasma or without internal standard on human heparinised plasma water. Separation was done by using YMC Hydrosphere C18 (50 2 mm i.d., 3 mm; YMC Co., Japan), a reverse-phase C18 column, Symmetry Shield 3.5 18 mm (4.6 mm I.D.350 mm). Mobile phase used are acetonitrile –0.01M ammonium acetate (95:5, v/v), 0.03% formic acid–acetonitrile (20:80, v/v), 0.01 M ammonium acetate–acetonitrile (85:15, v/v) and flow rate was maintained between 0.2 ml/min and 1.5 ml/min. correlation of coefficient was found to be 0.99-0.996 which shows that given methods are reliable and can be used in practical. [81-83].

HPLC-MS/MS method have been developed for determination of Donepezil in rat plasma and human plasma. In rat plasma Donepezil like compound is determined by using tandem mass spectrometry on an LTQ XL system and this method can be applicable to pharmacokinetic study especially in animals [84]. In another method, enantiomers of Donepezil were determined in human plasma by API 3000 in MRM mode. As this method shown to be rapid, accurate and sensitive and is more suitable for pharmacokinetic study in human plasma [85]. HPLC MS/MS methods details for Rivastigmine, Galantamine and Donepezil are given in Table 13, Table14, Table 15, respectively

5. UPLC Method

A simple UPLC method was observed for Rivastigmine for fast separation of its potential impurities' positional isomers in pure drug and drug product. The separation was achieved using UPLC BEH Phenyl (100 mm 2.1 mm, 1.7 lm) and the mobile phase use are A) acetonitrile-Disodium hydrogen orthophosphate (pH 7.5; 0.01 M)-Triethylamine (10:90:0.1 v/v/v), B) acetonitrile-water (80:20 v/v). The flow rate was made constant at 0.4 ml/msin and correlation of coefficient was observed to be 0.999 which indicates reliability of the method and it can be applied in industry or laboratory practices [86].

UPLC method has been enforced for the estimation of Galantamine with ESI-MS, MS, DoE on human plasma, guinea pig plasma and plant alkaloids [87-90]. Jiang Wanga stated that UPLC-MS method used in guinea pig plasma can be applied for the pre-clinical bioavailability study of novel Galantamine formulations [89]. Muriel Noetzli reported a simultaneous estimation of cholinesterase inhibitor is possible on UPLC-MS/MS by using reverse phase column (BEH C18 2.1 mm × 50 mm; 1.7 m) for separation of components. Mobile phase used is buffer–acetonitrile (80:20, v/v) [90].
An UPLC method has been developed for Donepezil in combination with UV and mass spectroscopy [91-92]. In which combination with mass spectroscopy showed precise and accurate results compared to that of UPLC-UV. Although UPLC-UV method is used in biorelevant media for comparison of pharmacokinetic study [92], the UPLC-MS/MS method which is applied on dog's plasma is more practically reliable for pharmacokinetic study [91]. UPLC methods details for Rivastigmine, Galantamine and Donepezil are given in Table 16, Table17, Table 18, respectively.

Table 4: Rivastigmine HPLC Methods.

Sr. No.	Column	Mobile Phase	Wavelength	Flow Rate	Linearity Range	Linearity R^2	Reference
1	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	1.0 ml/min	1.5 -7.5 µg/ml	0.998	10
2	Silica column (250mm × 4.6mm, 5 µm)	Acetonitrile- 50 mm aqueous sodium dihydrogen phosphate (17: 83, v/v) pH 3.1	200 nm	1.3 ml/min	0.5–16 ng/ml	0.999	11
3	4.6 mm 250 mm, ODS, Xterra RP18, 5 mm	Dissolve 2.02 g of 1-octane sodium sulfonate in 1000 ml Milli – Q water sonicate to dissolve and adjust the PH of the solution 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)	217 nm	1.0 ml/min	50 - 300 mg/ml	0.9999	12
4	Grace Vydac monomeric column C18 (4.6 × 250 mm) 5 mm	20 Mmol /L phosphate buffer pH 3.0 and acetonitrile (75: 25, v/v)	Fluorometric detection with excitation (ex)and emission (em) wavelength at 220 and 293 nm, respectively	1.0 ml/min	10–3000 ng/ml	0.9998	13
5	C18 column (5mm; M/s Merk kgaa, Germany)	20mm phosphate buffer pH 3.5 and acetonitrile (75:25, v/v)	Excitation wavelength (λmax) of 220nm and emission wavelength (λmax) of 293nm	1.0 ml/min	10-800 ng/ml	0.9999	14
6	Kromasil 100Å C8 column (4.6 150 mm; 5mm)	Monobasic ammonium phosphate buffer (8.6 mg/ml; pH 7.0), acetonitrile and methanol in a (50:25:25, v/v/v)	215 nm	1.2 ml/min	20-300 mg/ml	0.999	15
7	A reverse phase monomeric column C18 (4.6 × 250 mm, 5 µm	Acetonitrile and 20 Mmol/L phosphate buffer pH 3.0 (25:75, v/v)	Excitation and emission wavelengths at 220 nm and 293 nm, respectively	1.0 ml/min	25–3000 ng/ml	0.9929	16
8	C18 (Eurospher 100−5, 250 × 4.6 mm, Knauer, Germany) column equipped with a guard column (Eurospher	20 mM acetate buffer (pH 4.0, adjusted with triethylamine, TEA)/acetonitrile (80:20, v/v	211 nm	1.2 ml/min	In rat plasma and brain were 80−3000 ng/ml and 100−3000 ng/ml, respectivel y	0.9673 &0.9911	17

	100−5 C18, 5 × 4 mm)						
9	Inertsil, C18, 250 x 4.6mm. 5µ	Potassium phosphate mono basic buffer (pH 2.5± 0.05): Acetonitrile (70:30, v/v)	217 nm	1.0 ml/min	10-100 µg/ml	0.999	18
10	Cellulose-based chiral stationary phase Chiralcel OD-H (250 mm × 4.6 mm, 5m, Daicel make), which was safeguarded with a 1 cm long guard column Chiralcel OD-H	Hexane: isopropanol: trifluoroacetic acid (80:20:0.2, v/v/v)	210 nm	1.0 ml/min	1500–9000 ng/ml	0.998	19
11	C18 column (250 mm 4.6 mm, I.D., 5 mm, Shodex, Japan).	Acetonitrile and pH 5.5 acetate buffer solution (28:72, v/v)	224 nm	1.0 ml/min	0.1–50 µg/ml	0.9999	20
12	C18 analytical column (25cm × 4.6 I.D., 1.8 µm	1mm di ammonium hydrogen phosphate buffer (pH = 2) and acetonitrile (75:25, v/v)	217 nm	1.0 ml/min	0.15-5 µg/ml	0.999	21
13	Thermostated column with a Nucleosil CC-125/2 C18 reversedphase column (Macherey & Nagel)	Acetonitrile 80% in H2O (v/v) containing 0.1% formic acid	221 nm	0.35 ml/min	10–100 pmol/g	0.995	22
14	Inertsil-ODS-3 column (250 × 4.6mm, 5µ)	10mm ammonium acetate: methanol (35:65, v/v)	217 nm	1.0 ml/min	2-10 µg/ml	0.9999	23
15	ODS Hypersil column (C18 classical, 250×2,1 mm, 5 µm) (Thermo Fisher Scientific, Germany)	Acetonitrile: methanol: buffer (45:20:35v/v)	215 nm	0.6-0.9ml/min	5-40 ng/ml	0.9819	24
16	Inertsil-ODS-3 C18(250 × 4.6mm, 5µ).	Ammonium acetate buffer (20mm, pH4.5) &acetonitrile74:26v/v)	Excitation and emission wavelengths at 293 nm and 220 nm, respectively	1.0 ml/min	10-1000 ng/ml	0.98	25
17	Vertex Hypersil reversed phase C18 column (25 cm 4.6 mm I.D., particle size 5 µm	Acetonitrile and 0.01 M di-sodium hydrogen phosphate pH 8.4, (70:30, v/v)	214 nm	1.3 ml/min	0.400-0.772 mg/ml	0.9983	26
18	C18 column (250 × 4.6 mm, 5 µm, Shodex, Japan)	Acetonitrile and acetate buffer pH 5.5 solution (28:72, v/v)	224 nm	1.0 ml/min	100–500 µg/ml	0.99	27

Table 5: Galantamine HPLC Methods.

Sr No.	Column	Mobile Phase	Wavelength	Flow Rate	Linearity Range	Linearity R^2	Reference
1	Inertsil C18	40% acetonitrile and 60% 10 mm o-phosphoric acid	Fluorescence detector (excitation 375 nm/emission 537 nm)	1.2 ml/min	125-2000 ng/ml in water, urine and plasma.	Plasma and Urine 0.9999 0.9995	28
2	Chiralpak AD-H (250 · 4.6 mm) column	N-hexane, 20% propionic acid in isopropanol and diethyl amine in the ratio of (80:20:0.2, v/v/v)	289 nm	0.8 ml/min	0.84-15 mg/ml	0.9999	29
3	Zorbax Extend-C18 (150x4.6 mm I.D., 5µm)	0.1% triethylamine aqueous solution and acetonitrile (84:16, v/v)	288 nm	1.0 ml/min	7.1-225.0 µg/ml	0.9999	30
4	Discovery HS F5 268 J. Mal ´akov ´a et al. / J. Chromatogr. B 853 (2007) 265–274 (Supelco, Bellefonte, PA, USA) 5 m particle size (4.6 mm I.D. × 150 mm).	15% of acetonitrile and 85% of aqueous ammonium acetate (5mmol/L) pH 6.8 (v/v) to 25% of acetonitrile and 75% of aqueous ammonium acetate (5mmol/L) over 4 min	Fluorescence detector at excitation and emission wavelengths of 280 and 310 nm.	1.0 ml/min	0.50-63.47 nmol/g of galantamine, from 0.32-41.42 nmol/g of O-desmethyl-galantamine, from 0.54-569.40 nmol/g of N-desmethyl-galantamine and from 0.70-89.03 nmol/g of epigalantamine	0.999	31
5	RP-C8 column	Acetonitrile: methanol: water (containing 7.5 mm triethanolamine, pH 6.9)	292 nm	1.0 ml/min	-	0.995	32
6	Phenomenex C18 (250 4.6 mm) column)	Acetonitrile: ammonium formate buffer (2 mm, pH 9.0) (70:30, v/v)	289 nm	1.0 ml/min	-		33
7	RP C18	50 mm disodium hydrogen phosphate: acetonitrile (80: 20, v/v)	280 nm	1.0 ml/min	(5-10 mg/ml, 2.10-4 mg/ml	0.988	34
8	ODS Hypersil 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.	290 nm	0.9 ml/min	0.5-40 µg/ml	0.9989	35
9	Shim Pack CLC-ODS column (150 mm × 4.0 mm I.D., 5 µm particle size)	Acetonitrile, water, triethylamine (20:80:1, v/v/v, pH -7.0)	Fluorescence detection excitation wavelength 290 nm and emission wavelength of 320 nm.	1.0 ml/min	2.0–160 ng/ml	0.9994	36
10	Phenomenex C18 column (250 x 4.6 mm I.D - 5µm particle size)	1.0 mm ammonium formate: acetonitrile (30:70, v/v)	289 nm	0.4 ml/min	20-80 µg/ml	0.999	37
11	Luna C18 column (4.6 mm × 250 mm, 5 m,	0.2% triethylamine in 10 nm ammonium acetate (pH 8.3) and acetonitrile (65:35, v/v)	Fluorescence detector excitation and emission	0.7 ml/min	3–300 ng/ml	0.9998	38

	Phenomenex, Torrance, CA, USA)		wavelengths 280 and 310 nm,				
12	Kinetex EVO C18 15 x 4.6 mm 5 µm, Scherzo SM-C18 150 x 4.6 mm, 3 µm (Imtakt, USA) or Luna SCX 150 x 4.6 mm, 5 µm (Phenomenex, USA).	5% Acetonitrile, 20% acetate buffer at 3.5 and 0.025 M/L diethyl amine was used as mobile phases on C18 and SM C18 columns. On SCX column mobile phase containing 8% of acetonitrile and phosphate buffer at pH 2.5	240 nm	1.0 ml/min	0.004-2 mg/ml	0.9990	39
13	RP C18 ODC Spherisorb (250 mm × 4.6 mm × 5 mm)	50 mm disodium hydrogen phosphate: acetonitrile (80:20, v/v)	280 nm,	1.5 ml/min	-	-	40
14	Supelcosil LC18 column (250 ×4.6 mm I.D.; 5 µm; Supelco, Belleforte, PA, USA)	Ammonium carbonate, water solution and acetonitrile (HPLC grade 99.93 % purity) (85:15, v/v)	292 nm	1.0 ml/min	0.025-0.4 mg/ml	0.995	41
15	Silica gel column (4.6 mm × 250 mm	Methanol –20 mm ammonium formate aqueous solution (pH 3.2) (65:35, v/v)	282 nm	1.0 ml/min	0.080–0.800 mg/ml	0.99	42
16	Zorbax Eclipse XDB-C18 4.6 × 75 mm (3.5 m particle	Ammonium acetate/acetonitrile (94:6, v/v)	210 nm	2.2 ml/ min	20-150 ng/ml	0.999	43

Table 6: Donepezil HPLC Methods.

Sr No.	Column	Mobile Phase	Wavelength	Flow Rate	Linearity Range	Linearity R^2	Reference
1	A reversed-phase TRACER EXCEL ODS-A	Acetonitrile-10 mm acetate buffer (pH 6.0)-THF (60: 35: 5, v/v/v)	Fluorescence detection at 264 nm (ex) and 313 nm (em).	1.0 ml/min	1-100 ng/ml	0.998	44
2	A keystone Ph RP 250 × 4.6 mm I.D., 5 µm particle size anal. Column	Mixt. Of methanol, 0.02 M phosphate buffer (pH 7.5 ± 0.1) and triethylamine in the ratio (60:40:0.5 v/v/v)	268 nm	1.0 ml/min	50-150 µg/ml	0.9970	45
3	A Wakosil C18 column 250 mm × 4.6 mm, 5 µ,	Phosphate buffer (0.02 M, pH 3.67) and Acetonitrile	230 nm	1.2 ml/min	20-150 µg/ml	0.99934 by HPLC 0.99927 By UV	46
4	Poroshell 120 EC-C18 column (7.5 × 4.6 mm, 2.7 µm)	Phosphate buffer (10 mm, pH 2.5)-acetonitrile (75/25, v/v)	Fluorescence detection at 325 nm (ex) and 390 nm (em).	1.2 ml/min	0.5-25 ng/ml	0.997	47
5	C18 column 250 mm × 4.6 mm	Methanol:0.02 m phosphate buffer: triethylamine (60:40:0.5, v/v/v)	168 nm	1.0 ml/min	50-150 mcg/ml	0.9970	48
6	Xterra RP C18, 250 × 4.6 mm, 5µ	Potassium dihydrogen orthophosphate Buffer and Acetonitrile (80:20, v/v)	230 nm	1.0 ml/min	25- 75 µg/ml	0.998	49
7	Zorbax Eclipse Plus C18 rapid resolution Column (4.6 × 100 mm, 3.5 µm)	72.5% acetate buffer pH 5.5 and 27.5% ethanol	315 nm	1.0 ml/min	2-28 µg/ml	0.9999	50
8	C-18 Cosmosil packed column (5 C18- MS-II, 250 mm x 4.6 mm x 5.0 µm)	0.02 M phosphate buffer (pH 7.4), methanol and Acetonitrile (ACN) (40:50:10 v/v/v)	280 nm	-	0.5-80 µg/ml		51
9	C18 column (150 × 4.6 mm)	Dihydrogen orthophosphate buffer (pH 3.5 ± 0.05), acetonitrile and methanol in a ratio of (15:20:65 v/v)	225 nm	1.0 ml/min	80-160 µg/ml	0.998	52
10	Uptisphere ODB C18 column 250 mm × 4.6 mm, 5 µm	Phosphate buffer (0.005M, pH 3.67) and methanol (38:62)	270 nm	1.0 ml/min	50-200 µg/ml	0.9980	53
11	C18 (4.6 × 250 mm 3.5µm)	Methanol: buffer (60:40% v/v)	270 nm	1.0 ml/min	200-400 µg/ml	0.999	54
12	Inertsil C8-3, 25 cm × 4.6-mm, 5 µ	Buffer: methanol: triethylamine (55:45:5, v/v/v). pH 2.50 ± 0.05	271 nm	1.0 ml/min	20-60 µg/ml	0.9999	55
13	Hypersil BDS (4.6 x 150 mm, 5µ)	Sodium dihydrogen ortho phosphate: Acetonitrile (30:70, v/v)	-	1.0 ml/min	20-60 µg/ml	-	56
14	Hypersil column (250 × 4.6 mm, 5 µm)	Acetonitrile and 0.025 M potassium dihydrogen phosphate buffer of pH 3.5 (80:20)	210 nm	1.0 ml/min	0.5-100 µg/ml	0.9998	57

15	Kromasil C18 (250 4.6 mm, 5 (m) columns.	Methanol and buffer solution (40: 60, v/v)	268 nm	1.2 ml/min	50-150 µg/ml	0.999	58

Table 7: Rivastigmine HPTLC Methods.

Sr. No.	Drug Name	Plate	Mobile Phase	Linearity Range	Linearity R^2	Reference
1	Rivastigmine	20 cm × 10 cm	Benzene: acetone: ammonia (6.5:3:0.1, v/v/v)	55–720 ng/ spot	0.9986	59
2	Rivastigmine	10 cm × 20 cm	Chloroform–methanol (4:6, v/v)	200–1600 ng/spot	0.9916	60

Table 8: Galantamine HPTLC Methods.

Sr No.	Plate	Mobile Phase	Wavelength	Linearity Range	Linearity R^2	Reference
1	Silicagel G60F254	Chloroform: acetone: ethyl acetate: methanol (20:10:5:5 v/v/v/v)	UV detection at λ = 282 nm,	$1*10^{-3}$– $3*10^{-2}$ g/ml	0.995.	61

Table 9: Donepezil HPTLC Methods.

Sr No	Plate	M0bile Phase	Wavelength	Linearity range	Linearity R^2	Reference
1	Silica gel 60 F254 on aluminium sheet	Methanol: chloroform (8:2 vol./vol.) As mobile phase	254 nm	200-1000 ng/spot	0.998	62
2	Silica gel 60 F254 on aluminium sheet (20 × 10 cm)	Butanol-water-glacial acetic acid (5:4:1, vol./vol./v)	260 nm	50-1000 ng/spot	0.9908-0.9995	63

Table 10: Rivastigmine U.V. Spectrophotometric Methods.

Sr. No.	Method	Wavelength	Solvent	Linearity Range	Linearity R2	Reference
1	1. First method is a second derivative (D2) 2. First derivative of the ratio spectra (DD1)	1. 262 nm 2. 272 nm	1. Distilled water 2. Distilled water	1.50–500 µg/ml 2. 50–500 µg/ml	-	64
2	Calibration curve	224 nm	Methanol	100–700 mg/ml	0.9996	65
3	1.zero order 2. First derivative 3.Direct absorbance measurement	1. 263.6nm 2. A.265.6nm B.272.8nm 3. A. 263.6nm B. 267.3nm C.270.2nm	1.Methanol: Water (60:40, v/v) 2.Methanol: Water (60:40, v/v) 3.Methanol: Water (60:40, v/v)	1. 0.2- 1mm 2. 0.2-1mm 3. 0.2-1mm	1. 0.999 2. A. 0.998 B. 0.999 3. A. 0.998 B. 0.998 C. 0.999	66
4	Calibration curve	224 nm	Methanol	100–500 µg/ml	0.99	67

Table 11: Galantamine U.V. spectroscopic Methods.

Sr No.	Method	Wavelength	Solvent	Linearity Range	Linearity R^2	Reference
1	Calibration curve method	214 nm	Distilled Water.	1.0–120.0 ng/ml	0.998	68
2	First order derivative spectroscopy	289 nm	Distilled Water.	20-100 µg/ml	0.999	69

Table 12: Donepezil U.V. Spectrophotometric Methods.

Sr No.	Method	Wavelength	Mobile Phase	Linearity Range	Linearity R2	Reference
1	Assay after solvent extraction spectrophotometric method of sample	410 nm	Phthalate buffer solution	2-14 µg/ml	0.9999	70
2	Calibration curve method	270.5 nm	pH 7.4 phosphate buffer	2-20 mg/ml	0.999	71
3	Calibration curve method	231 nm	Mixt. Of Acetonitrile and water	4-20 µg/ml	0.9983	72
4	Comparison of calibration curve method of sample with ion pair complexation reaction	420, 413, 415 and 409 nm for DNP-BCG, DNP-BTB, DNPBPB and DNP-BCP complexes,	Dichloromethane	1.0-12 µg/ml	0.999	73
5	Colorimetric method and calibration curve method	Method A and method B λmax was 231 and 454 nm, resp.	2,4-dinitrophenyl hydrazine in dil. Sulfuric acid.	5-40 and 10-60 µg/ml for method A and B, resp.	0.999	74
6	Calibration curve method.	547 nm	Potassium permanganate in alkaline. Medium.	2 - 35 µg/ml	0.997	75
7	Assay after solvent extraction spectrophotometric method	411 nm	Chloroform	2-12 µg/ml	0.999	76

Table 13: Rivastigmine HPLC MS/MS Methods.

Sr. No.	Column	Mobile Phase	Linearity Range	Flow Rate	Linearity R^2	Reference
1	Surveyer HPLC with Betabasic-8 (5.0 m, 100 mm × 4.6 mm	0.1% formic acid in acetonitrile and 0.1% formic acid in water (70:30, v/v)	0.2–20.0 ng/m	1.0 ml/min	0.9988	77
2	3-mm Purospher compounds were extracted from plasma using MTBE Star RP-18 (Merck) column (55×2 mm I.D.	Methanol– 0.02 M ammonium acetate (55:45, v/v)	0.200–30.0 ng/ml	0.2 ml/min	0.9998	78
3	Gemini C18 column (15×2.0 mm I.D., particle size 5 mm; Phenomenex, Torrance, CA, USA)	10 m ammonium hydroxide/methanol (50:50, v/v)	0.25 to 50.0 ng/ml	0.2 ml/min	0.99	79
4	Nucleosil CC-125/2 C18 reversed-phase column (Macherey & Nagel	Acetonitrile 80% in H2O (v/v) containing 0.1% formic acid	10–100 pmol/ml or pmol/g	0.35 ml/min	0.995	80

Table 14: Galantamine HPLC-MS/MS Method.

Sr No.	Column	Mobile Phase	Linearity Range	Flow Rate	Linearity R^2	Reference
1	Waters (Milford, MA, USA) Symmetry Shield 3.5 18 mm (4.6 mm I.D.350 mm).	0.01 M ammonium acetate–acetonitrile (85:15, v/v)	1.00–500 ng/ml	1.5 ml/min	-	81
2	YMC Hydrosphere C18 (50 2 mm i.d., 3 mm; YMC Co., Japan)	Acetonitrile – 0.01M ammonium acetate (95:5, v/v)	4-300 ng/ml	0.2 ml/min	0.996	82
3	A reverse-phase C18 column	0.03% formic acid–acetonitrile (20:80, v/v),	0.5–100 ng/ml	1.5 ml/min	0.99	83

Table 15: Donepezil HPLC-MS/MS Methods.

Sr No.	Column	Mobile Phase	Linearity Range	Flow Rate	Linearity R^2	Reference
1	RP-Amide column (75 mm × 2.1 mm, 2.7 µm)	0.05 M aq. formic acid and acetonitrile	0.1-1000 ng/ml	0.4 ml/min	0.999	84
2	CHIRALCEL OJ-H (250 mm*4.6 mm, 5µm)	n-hexane: n-propanol: diethyl amine (60: 40: 0.1, V/V/V)	0.051-7.596 ng/ml	-	≥ 0.99	85

Table 16: Rivastigmine UPLC Method.

Sr. No.	Column	Mobile Phase	Linearity Range	Flow Rate	Linearity R^2	Reference
1	UPLC BEH Phenyl (100 mm 2.1 mm, 1.7 lm)	A) acetonitrile-Disodium hydrogen orthophosphate (pH 7.5; 0.01 M)-Triethylamine (10:90:0.1, v/v/v) B) acetonitrile-water (80:20, v/v)	25–75 mg/ml	0.4 ml/min	0.999	86

Table 17: Galantamine UPLC Methods.

Sr No.	Column	Mobile Phase	Linearity Range	Flow Rate	Linearity R^2	Reference
1	Shim pack XR ODS-II length 100 mm, internal diameter 3 mm with 2.2 lm particle size	Water and methanol in the ratio of 1:1 (v/v)	-	0.5 ml/min	0.999	87
2	BEH C18 column (150 mm × 2.1 mm, 1.7 µm	0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B)	10 µg ml-1 - 2000 ng ml-1	0.4 ml/min	0.9968	88
3	Acquity UPLC BEH C18 column	Solution-A acetonitrile-water (5:95, v/v) and solution-B acetonitrile water (90:10, v/v), which both contain 2 mm ammonium formate and 0.2% formic acid	2–2000 ng/ml	0.6 ml/min.	0.999	89
4	Reverse phase column (BEH C18 2.1 mm × 50 mm; 1.7 m) w	(buffer–acetonitrile 80:20, v/v),	0.2–50 ng/ml	0.4 ml/min	-	90

Table 18: Donepezil UPLC Methods.

Sr. No	Column	Mobile Phase	Linearity Range	Flow Rate	Linearity R^2	Reference
1	BEH C18 column	acetonitrile and 0.3% formic acid in water.	0.2-20 ng/ml	0.65 ml/min	0.99	91
2	C18 column (50 2.1 mm; 1.9 mm) (Hypersil Gold, Thermo Fisher, Waltham, MA)	water, acetonitrile, and perchloric acid (65:35:0.1, v/v/v)	2–14 µg/ml	0.4 ml/min	0.9995	92

Conclusion:

A thorough survey on various spectrophotometric and chromatographic methods revealed for the assurance of three acetylcholinesterase inhibitors viz. Rivastigmine, Galantamine, Donepezil in pure bulk drug, pharmaceutical dosage forms such as tablets, capsules, transdermal patches and biological fluids has been introduced. Some LC- MS/MS methods alms great sensitivity and short investigation time have likewise been created and approved for validated quantification of cholinesterase inhibitors in human plasma. The investigation of the accessible information uncovered that HPLC was extensively utilized for the quantitative assessment of cholinesterase inhibitors since particularity of HPLC method is exemplary and adequate precision is also feasible. In any case, it must be affirmed that the confound specificity, precision and accuracy are feasible just if extensive system suitability tests are done prior to the HPLC examination. We suggest the HPLC-MS/MS technique for the assurance of cholinesterase inhibitors since it associates the HPLC separation capability with MS sensitivity and selectivity that permits the clear-cut distinguishing of cholinesterase inhibitors and their metabolites. For assurance of cholinesterase inhibitors in pharmaceutical dosage form, HPLC with UV and PDA detection can be utilized in light of precision and accuracy in the outcomes and minimal expense when contrasted with further advanced detection techniques.

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