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A Review on Method Development and Validation on Donepezil Hydrochloride by RP HPLC

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ABSTRACT

Donepezil is the leading compound for the treatment of Alzheimer's disease (AD) in more than 50 countries. As compared with other conventional acetylcholinesterase inhibitors (AChEIs), donepezil is a highly selective and reversible piperidine derivative with AChEI activity that exhibits the best pharmacological profile in terms of cognitive improvement, responders rate (40%–58%), dropout cases (5%–13%), and side-effects (6%–13%) in AD. Although donepezil represents a non cost-effective treatment, most studies convey that this drug can provide a modest benefit on cognition, behavior, and activities of the daily living in both moderate and severe AD, contributing to slow down disease progression and, to a lesser exetnt, to delay institutionalization.

The goal of this study is to describe a suitable method which is more accurate, precise, linear and adequate under the given set of laboratory condition by using the RP HPLC method.

Keywords: Donepezil Hydrochloride, Alzheimer disease, RP HPLC, Acetylcholinestearase inhibitors.

Introduction

Donepezil hydrochloride is a piperidine that is a cholinesterase inhibitor (ChE inhibitor) and it is a white or almost white, crystalline powder, which is soluble in water.Donepezil hydrochloride is a selective, reversible and non-competitive acetylcholinesterase inhibitor with a relatively high central versus peripheral specificity. Cholinesterase inhibitors can increase gastric acid secretion so the manufacturers advise monitoring of those at risk of developing ulcers. Its adverse reactions are Insomnia may occur in about 10 %, 10 % of patients suffer nausea and vomiting, Diarrhoea occurs in 10 %,Dizziness occurs in 8 % ,Nasal congestion and cold symptoms in 5 %.

Donepezil hydrochloride is a selective, reversible and non-competitive acetylcholinesterase inhibitor with a relatively high central versus peripheral specificity. Acetylcholinesterase is the predominant cholinesterase in the brain. Plasma levels of donepezil and AChE inhibition are positively correlated until a plateau of inhibition is reached at about 80 % at donepezil levels of 50 mg/mL and above the loss of cholinergic neurons in the nucleus basal is one of the hallmarks of Alzheimer's disease. Animals with nucleus basal are lesions responded to donepezil and improved on learning and memory tasks.

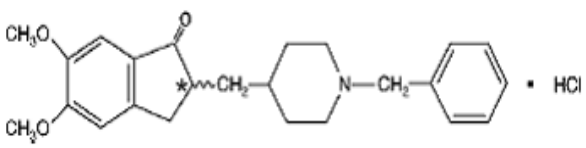

Figure 1: Chemical Structure of Donepezil Hyydrochloride.

Introduction of Chromatography: Chromatography is essentially a group of techniques for the separation of the compounds of mixtures by their continuous distribution between two phases, one of which is moving over the other.

High Performance Liquid Chromatography

HPLC is one among most useful tools, available for quantitative analysis. Reverse phase chromatography refers to the use of a polar mobile phase with a non-polar stationary phase in contrast, to normal phase being employed with a non-polar mobile phase.

Liquid chromatography is based upon the phenomenon that, under the same conditions, each component in a mixture interacts with its environment differently from other components. Since HPLC is basically a separating technique, it is always used in conjunction with another analytical tool for quantitative and qualitative analysis.

Advances in column technology, are high pressure pumping systems and sensitive detectors which have transformed liquid column chromatography into a high speed, high efficiency method of separation. This advanced technology is based upon the use of small bore (2.5 mm – internal diameter) columns and small particle size (3-50 µm) that allow fast equilibrium between stationary and mobile phases. This small particle column technology requires high pressure pumping system capable of delivering the mobile phase at high pressure, as much as 300 atmospheres, to achieve flow rates of several ml per minute. Since it is often necessary to use small amount of analyte (usually less than 20 µg) with the column packing, sensitive detectors are needed.

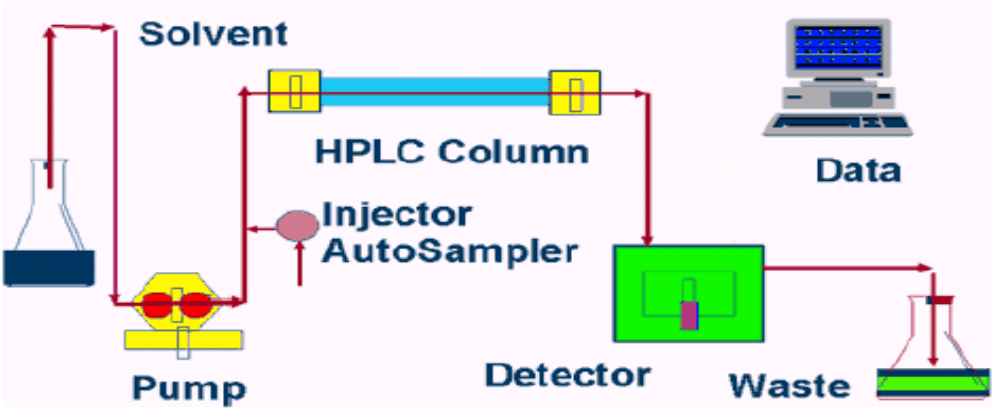

Figure 2: Schematic Diagram of HPLC

Reverse Phase High Performance Liquid Chromatography: Reverse phase chromatography refers to the use of a polar eluent with a non-polar stationary phase in contrast to normal phase chromatography, where a polar stationary phase is employed with a non-polar mobile phase.

Reverse phase chromatography is widely in use due to the following advantages.

- Many compounds such as biologically active substances, have limited solubility in non polar solvents that are employed in normal phase chromatography.
- Ionic or highly polar compounds have high heats of adsorption on straight silica or alumina columns and therefore can elute as a tailing peaks.
- Column deactivation from polar modifiers is a problem in liquid-solid chromatography which frequently can lead to irreducibility in chromatography systems.
- Ionic compounds can be chromatograph via ion exchange chromatography. This mode of

chromatography is tedious because precise control of variables such as pH and ionic strength is required for reproducible chromatography.

- Some of the advantages are:

➢ Speed (analysis can be accomplished in 20 minutes or less),
➢ Greater sensitivity (various detectors can be employed),
➢ Improved resolution (wide variety of stationary phases),
➢ Reusable columns (expensive columns but can be used for many analysis),
➢ Ideal for the substances of low volatility,
➢ Easy sample recovery, handling and maintenance,
➢ Instrumentation tends itself to automation and quantitation,
➢ Precise and reproducible,
➢ Calculations are done by integrator itself,
➢ Suitable for preparative liquid chromatography on a much larger scale

Components of high pressure liquid chromatography:

- Mobile phase reservoirs
- Pumping system
- Sample injection system
- Columns
- Detectors

The process is influenced by the nature of the analytes and general steps are as followed:

- Step 1 - Selection of the HPLC method and initial system
- Step 2 - Selection of initial conditions
- Step 3 - Selectivity optimization
- Step 4 - System optimization
- Step 5 - Method validation

Mobile phase reservoir

Equipped with glass or stainless steel reservoirs with capacity of 500 mL or more. Filter or degasser like provisions are included to avoid presence of dust or dissolved gases. They may result in band spreading and affects detectors too.
Elution with single solvent composition is named as isocratic. In gradient elution, two solvent systems that differ significantly in polarity are employed, with change of composition with respect to time.

Pumping system

Pumps should provide pressure up to 5000 psi, pulse free output, flow rates from 0.1 - 10 mL/min and 0.5 % flow reproducibility. Pumps should be free from corrosion by solvents.
Two types of mechanical pumps are employed generally:

1. A screw driven syringe: It is free from pulse and flow rate is controlled easily.
2. A reciprocating pump: It is the most widely used pump, has higher output pressure 10,000 psi, and provides gradient elution and constant flow rate.

Sample injection system

A rheodyne injector is most widely used, having capacity of 10 µL, 20 µL etc. An auto injector, septum, stop flow injection systems, loop injectors with sample capacity of 5 - 500 µL are also available.

Columns

All columns and tubing's used in HPLC are made up of stainless steel and heavy walled glass tubing's in order to withstand high pressures.
Columns are divided as preparative, analytical and guard columns.
Guard columns are employed to remove any particulate matter and contaminants from solvents and also they serve saturation of mobile phase and stationary phase. It increases column life and minimizes loss of stationary phase.

Analytical columns

They have length of 10 - 30 cm and 4 - 10 mm i.d, column packing particle size between 3 to 10 µm. This type of column offers 40,000 - 60,000 plates/meter.
Also column with 1 - 4.6 cm i.d. and 3 - 7.5 cm length are available which have 1,00,000 plates/meter. They consume less solvent and time (speedy).
Preparative columns are larger in size and used for more amount of sample. When these columns are used, after analysis of drug sample, it can be recovered by using fractional collector.

Types of HPLC

(i) High pressure adsorption chromatography

Stationary Phase: Finely divided silica and alumina

Application: For the separation of non-polar water insoluble organic compounds with molecular mass less than 5000 Da.

(ii) High pressure ion-exchange chromatography

Stationary phase: Ion-exchange resins which are cationic or anionic for specific functional group exchange.

Application: To separate anions and cations. Currently used ion exchange packing's are suppressor-based and single column ion chromatography.

(iii) High pressure size exclusion chromatography

Stationary Phase: Consist of small (10 µm) silica or polymer particles containing a network of uniform pores. Based on hydrophilic packing's, it is called as gel filtration and gel permeation.

Application: To separate molecules with molecular weight more than 2000 Da. Hydrophilic pack can separate molecules with molecular weight more than 1000 Da.

(iv) High pressure affinity chromatography

Stationary Phase: Gels as Sephadex G - 75 (in lecithin affinity chromatography) act as stationary phase. A supporting material like agarose, cellulose, glass beads and polyacrylamide can also be used e.g. for Affinity column - lecithin, antibody and enzyme substrate inhibitor or cofactor.

Application: For separation of antibodies, hormones and other proteins.

Choice of mobile phase

Successful chromatographic separation requires a proper balance of intermolecular force among three participants in separation process, which are the analyte, mobile phase and the stationary phase. Polarity plays major role in separation.

The polarity of organic functional groups in increasing order are aliphatic hydrocarbons < olefins < aromatic hydrocarbons < halides < sulfides < ethers < nitro compounds < esters = aldehyde = ketones < alcohols = amines < sulfones < sulfoxides < amides < carboxylic acids< H_20.

1. Type of Detectors

Table 1: Variety of Detectors

Detector	Analyte	Solvent requirement	Comment
UV- visible	Any analyte with chromophores	AR grade, Non UV absorbing solvent	Has a greater degree of selectivity & is useful for many HPLC separations. PDA provides simultaneous UV scan.
Florescence	Florescent compound	UV grade, Non UV absorbing solvent	Highly selective and sensitive Often used to analyze derivatized compounds.
Refractive index (RI)	Compound with different RI to mobile phase	Cannot run mobile phase gradients	Virtually universal detector but has limited sensitivity and can't use gradient.
Conductivity	Charged or polar compound	Mobile phase must be conducting	Excellent for ion-exchange method, only for charged species

Electrochemical	Readily oxidized or reduced compound especially biological sample	Mobile phase must be conducting	Very selective and sensitive. Analyte must be able to undergo redox reaction
ELSD - Evaporative Light Scattering Detection	Low mol. wt. analyte	Mobile phase must be volatile	Universal and Highly selective. Require volatile buffer
MS - Mass Spectrometry	Biological & Non-biological species	Mobile phase must be volatile	Highly selective, hyphenated technically excellent for confirmation Require volatile buffer

Method development by HPLC:

Analytical method development plays an important role in the discovery, development and manufacture of pharmaceuticals. Quality control laboratories to ensure the identity, purity,
Potency and performance of drugs products use the official test methods that result from these processes.

The main objective of method development is to obtain a good separation with minimum time, effort and should economical. Based on the goal of separation, method development is preceded.

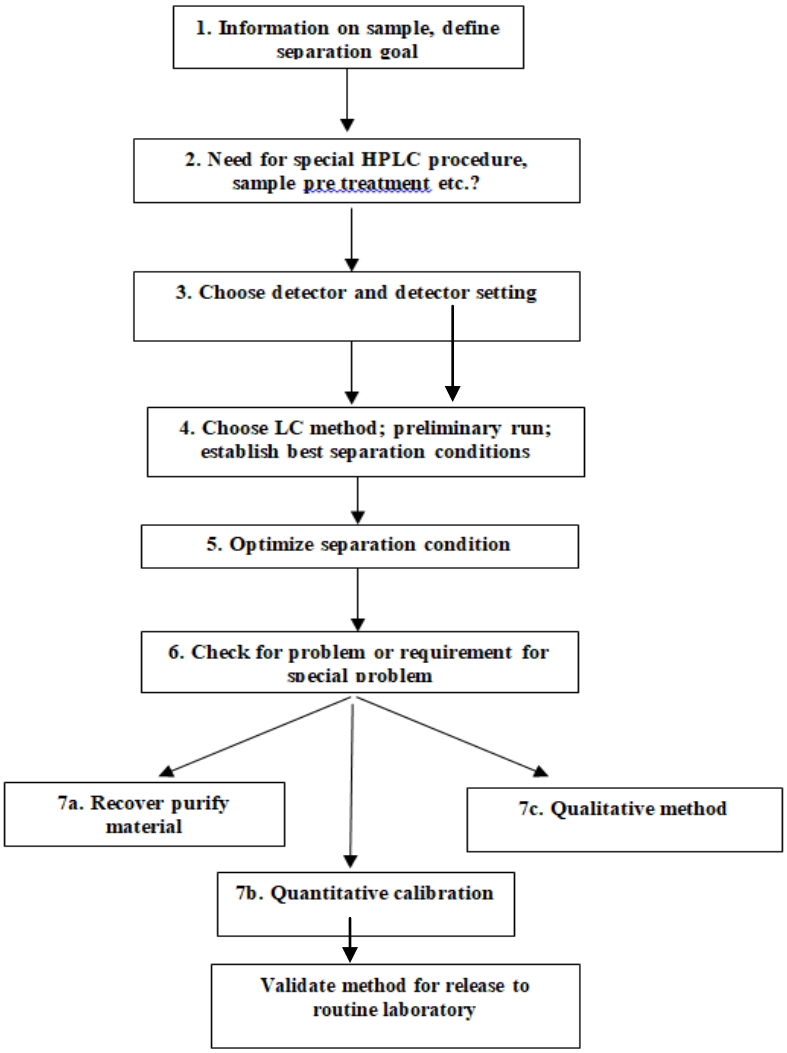

Figure 1.3: Flowchart of method development for HPLC

Method Validation: Validation is a key process for effective Quality Assurance "Validation is establishing documented evidence which provides a high degree of assurance that a specific process or equipment will consistently produce a product or result meeting its predetermined specifications and quality attributes.

The validation guidelines recommended from ICH (International Council for Harmonization) consists characteristics for consideration during the validation of analytical procedures included as part of registration applications with in EC, Japan and USA.

Validation Parameter:

Specificity
System suitability
Precision
Linearity
Robustness
Ruggedness
Limit of detection
Limit of quantification

Estimation of Donepezil Hydrochloride by RP HPLC:

Reversed-phase HPLC (RP-HPLC) separates molecules on the basis of differences in their hydrophobicity. The components of the analyte mixture pass over stationary-phase particles bearing pores large enough for them to enter, where interactions with the hydrophobic surface removes them from the flowing mobile-phase stream. The strength and nature of the interaction between the sample particles and the stationary phase depends on both hydrophobic interactions and polar interactions. As the concentration of organic solvent in the eluant increases, it reaches a critical value for each analyte which desorbs it from the hydrophobic stationary-phase surface and allows it to elute from the column in the flowing mobile phase. Since this elution depends on the precise distribution of hydrophobic residues in each species, each analyte elutes from the column at a characteristic time, and the resulting peak can be used to confirm its identity and quantify it.

A brief description of research work for Estimation of Donepezil Hydrochloride:

1. A simple and sensitive high-performance liquid chromatographic (HPLC) method with UV absorbance detection is described for the quantification of donepezil, in human plasma. The organic phase was back-extracted with 75 µL of HCl (0.1 M) and 50 µL of the acid solution was injected into a C_{18} STR ODS-II analytical column (5 µm, 150×4.6 mm i.d.). The mobile phase consisted of phosphate buffer (0.02 M, pH 4.6), perchloric acid (6 M) and acetonitrile (59.5: 0.5: 40, v/v) and was delivered at a flow-rate of 1.0 mL/min at 40 °C. The peak was detected using a UV detector set at 315 nm, and the total time for a chromatographic separation was ~8 min. The method was validated for the concentration range 3–90 ng/mL. Mean recoveries were 89–98 %.

2. This investigation describes a new precise, sensitive and accurate stereoselective HPLC method for the simultaneous determination of donepezil enantiomers in tablets and plasma with enough sensitivity to follow its pharmacokinetics in rats up to 12 hr after single oral dosing. Enantiomeric resolution was achieved on a cellulose (3, 5-dimethylphenyl carbamate) column known as Chiralcel OD, with UV detection at 268 nm, and the mobile phase consisted of *n*-hexane, isopropanol and triethylamine (87 : 12.9 : 0.1). Using the chromatographic conditions described, donepezil enantiomers were well resolved with mean retention times of 12.8 and 16.3 min, respectively. Linear response ($r > 0.994$) was observed over the range of 0.05 – 2 g/mL of donepezil enantiomers, with detection limit of 20 ng/mL. The mean relative standard deviation (R.S.D. %) of the results of within-day precision and accuracy of the drug were = 10 %. There was no significant difference ($p > 0.05$) between inter and intra-day studies for each enantiomers which confirmed the reproducibility of the assay method. The mean extraction efficiency was 92.6 – 93.2 % of the enantiomers.

3. Two simple, accurate, rapid and sensitive methods were developed for the estimation of Donepezil Hydrochloride (DH) in bulk and tablet formulation. Method A and B describes

simple UV spectrophotometric and colorimetric method in methanol, respectively. For method A and method B λ_{max} was found to be at 231 and 454 nm, respectively. In method B orange coloured complex was observed due to reaction of keto group of donepezil with 2, 4-dinitrophenyl hydrazine in dilute sulphuric acid. The Beer Lambert`s law was obeyed in the concentration range of 5 - 40 and 10 - 60 λ_{max} for method A and B, respectively.

4. A new gradient HPLC method has been developed and validated for the determination of both assay and related substances of donepezil hydrochloride in oral pharmaceutical formulation. Different kinds of columns and gradient elution programs were tested in order to achieve satisfactory separation between the active substance, four impurities and an interfering excipient used in the formulation. The best results were obtained using an uptisphere ODB C-18 column 250 mm × 4.6 mm, 5 µm, UV detection at 270 nm and a gradient elution of phosphate buffer (0.005 M, pH 3.67) and methanol as the mobile phase. The method was validated with respect to linearity, precision, accuracy, specificity and robustness. It was also found to be stability indicating, and therefore suitable for the routine analysis of donepezil hydrochloride and related substances in the pharmaceutical formulation.

Conclusion

The purpose of this review article is to provide a succinct elaboration of the RP HPLC for the determination of Donepezil Hydrochloride. The literature data indicated that certain method development and validation have been reported for Donepezil Hydrochloride . The analysis of Donepezil Hydrochloride in pure and solid dosage forms presently demanding the implementation in terms of sensitive, accurate, effective and consistent methodologies.

References

1. Adunsky A, Chesnin V, Ravona R, et al. Plasma lipid levels in Alzheimer's disease patients treated with donepezil hydrochloride: a cross-sectional study. *Arch Gerontol Geriatr.* 2004;**38**:61–8.

2. Aerssens J, Raeymaekers P, Lilienfeld S, et al. APOE genotype: no influence on galantamine treatment efficacy nor on rate of decline in Alzheimer's disease. *Dement Geriatr Cogn Disord.* 2001;**2**:69–77.

3. Holler, F. James; Skoog, Douglas A.; West, Donald M. (1996). Fundamentals of analytical chemistry. Philadelphia: Saunders College Pub. ISBN 0-03-005938-0.

4. Nieman, Timothy A.; Skoog, Douglas A.; Holler, F. James (1998). Principles of instrumental analysis. Pacific Grove, CA: Brooks/Cole. ISBN 0-03-002078-6.

5. H. Beckett, J. B. Stenlake, UV-visible Spectrophotometry: Practical Pharmaceutical Chemistry, 4th edition, Part-II, 2001, C.B.S. Publishers, New Delhi, 285-97.

6. Jeffrey, et. al., Introduction: Vogel Textbook of Quantitative Chemical Analysis, 5th edition, 1997, ELBS, Longman, 3-8.

7. R. D. Braun, Introduction to Instrument Analysis, 2005, Pharma Book Syndicate, Hyderabad, 261.

8. H. H. Willard, L. L. Merrit, J. A. Dean, F. A. Settle, HPLC Methods and applications in: Instrumental Methods of analysis, 7th Edition, 1986, C. B. S. Publishers, New Delhi, 626-627.

9. L. R. Snyder, J. J. Kirkland, J. L. Glajch, Practical HPLC method development, 2nd edition, 2000, A Wiley-Interscince Publication, 697- 709.

10. P. D. Sethi, Introduction in HPLC: Quantitative Analysis for Pharmaceutical Formulation, 1st edition., 2001, CBS Publishers and Distributors, New Delhi.

11. S. Fronk, Introduction: Handbook of Instrumental Techniques for Analytical Chemistry, 1st edition, 2004, Pearson Education,
7.www.forumsci.co.il/hplc/program.html accessed on 21/09/2010.

12. T. H. Stout, J. G. Dorsey, High Performance Liquid Chromatography. In, L. Ohannesian, A.J. Streeter editors.

Handbook of Pharmaceutical Analysis, NY: Marcel Dekker Inc., 2002, 127-40.

13. Paresh P. Chothe, Ramaa C. Subramanian, Vilasrao J. Kadam, Assessment of Donepezil Hydrochloride Using Validated RP-HPLC Method, Research Journal Of Pharmaceutical, Biological And Chemical Sciences, issn: 0275-8585.
14. Mahasen A. Radwan, Heba H. Abdine, Bushra T. Al-Quadeb, Hassan Y. Aboul-Enein Kenichiro Nakashima "Steroselective HPLC assay of donepezil enantiomers with UV detection and its application to Pharmacokinetics in rats" J. Chromatography B, 830:114-119 (2006).
15. J. N. Sangshetti, P.R. Mahaparale, S. Paramane & D. B. Shinde. Spectrophotometric Estimation of Donepezil Hydrochloride in Bulk and tablet Formulation Trends in Applied Sciences Research; 3(1), 2008, 71-75
16. Stella Kafkala, Stella Matthaiou, Pandora Alexaki, Morfis Abatzis, Antonios Bartzeliotis and Maria Katsiabani. Journal of Chromatography A, Volume 1189, Issues 1-2, 2 May 2008, Pages 392-397
17. L.R. Snyder, J.J. Kirkland, J.I. Glajchl. Practical HPLC Method Development. John Wiley and Sons, New York.1988; 3: 2 – 21.
18. Validation of Chromatographic Methods, Reviewer Guidance, Center for Drug Evaluation and Research (CDER), November,1994; 17.
19. Text on validation of analytical procedure, Q2R1 in ICH Harmonized Triplicate Guidelines.