Review on: RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF LEVODOPA

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***Abstract*: High performance liquid Chromatographic method (HPLC) was developed for the analysis of levodopa. Levodopa (L-dopa) is an antiparkinsonian drug & it is used as per oral application. A simple accurate & reproducible HPLC-UV method has been developed and validated for the quantification of L-dopa are mainly based on capillary electrophoresis. i.e. HPLC (high performance liquid chromatography) coupled to ultraviolet visible or mass spectroscopy detection. This paper aims to give information on the latest developments in the chemical study of L-dopa & HPLC method. Levodopa is forerunner to dopamine. It is most effective medication to treat the motor symptoms of Parkinson's disease. L-dopa has held the attention of neurologist & pharmacologist.**

***Key words*: Levodopa, HPLC, chromatography, Parkinson's disease.**

Introduction:

L-dopa or levodopa is an amino acid analogue & it belongs to the class of catecholamine compound[1].An intermediate in the dopamine biosynthesis clinically is levodopa. Levodopa is used as antiparkinsonian drug. Chemical name of levodopa is known as 2-amino-3-(3, 4dihydroxi phenyl) propionic acid[2-5].Levodopa is considered the most effective oral dopaminergic treatment for the main motor symptoms of Parkinson's disease (PD)[1].L-dopa is a forerunner for the neurotransmitter dopamine. Parkinson's disease is encouraged by impairment of motor & non motor functions. For Parkinson's disease treatment L-dopa is observed as the gold standard. L-dopa is able to crossing a blood-brain barrier[6].The levodopa drug is chemically synthesized through a action that requires a costly metal catalyst & advanced technologies [7].Levodopa enhance the motor symptoms in patients with Parkinson's disease (PD). However many patients treated with levodopa chronically experience levodopa-induced dyskinesia (LID)[8,9,10].The success of levodopa therapy for Parkinson's disease also was the key factor in generating a new subspecialty of neurology namely movement disorders. The levodopa therapy in Parkinson's disease almost half a century back has transformed the therapeutic outcome of this challenging disease [11,12,13].

Chemical and Physical Properties :

LD(Levodopa) structure is categorized by the catechol moiety bonded to the amino acid functionality (-CH2NH2COOH) in -meta and -para positions to the hydroxyl groups in positions 3and4, respectively (fFigure1.). The main chemical and physical properties are summarized in Figure1. [14,15]

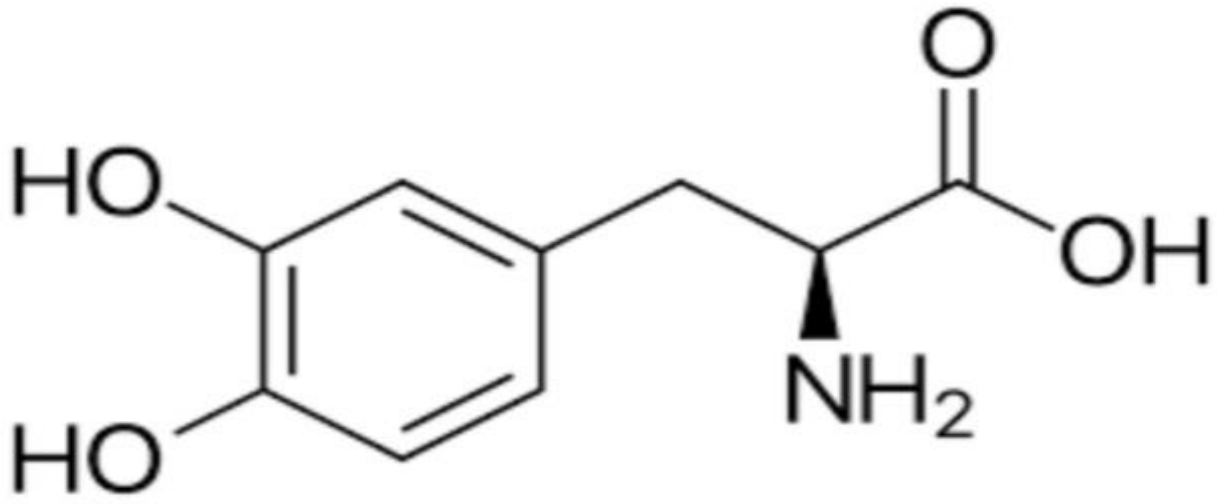

Figure [1]: Chemical Structure of Levodopa

Molecular Formula	**C9H11NO4**
Molecular Weight	**197.188**
Boiling Point	**537.89[°C]**
Melting Point	**396.26[°C]**
Log P	**0.05**
Critical Temperature	**588.32[°C]**
Critical Pressure	**59.26[Bar]**
Critical Volume	**510.5[cm3/mol]**
Gibbs Energy	**-451. 83[kJ/mol]**
Heat of Form	**-668. 94[kJ/mol]**

Mechanism of Action:

Degradation of the substantia nigra occurs in patients with Parkinson disease. This condition result in the disturbance of the nigrostriatal pathway and thus decreases the striatal dopamine levels. Unlike dopamine levodopa can cross blood brain barrier (BBB). Levodopa change to dopamine in both the CNS and periphery [16].To increase the bioavailability of levodopa and decrease it's side effects. Dopamine decarboxylase inhibitors stop the conversation of levodopa to dopamine in the periphery , allowing for more levodopa to cross the BBB[17].

HPLC Method:

HPLC means High Performance Liquid Chromatography is also known as High Pressure Liquid Chromatography. It is now one of the most powerful device in analytical chemistry. This technique is used for the separation, identification and quantification of each constituent of mixture. HPLC is an modern technique of column liquid chromatography. [18,-20]Liquid chromatography was firstly discovered as an analytical technique in early twentieth century & was first used as a method of separating coloured compounds. This is where the name chromatography chroma means colour, graphy means writing was derived [21].

Advantages of HPLC :

*Simultaneous Analysis
*High Resolution
*High Sensitivity
*Good Repetability
*Moderate Analysis condition
*Small Sample Size[22].

Types Of HPLC:

A) Normal Phase Chromatography (NP-HPLC):

This method separates analytes on the premises of polarity. NP-HPLC uses the polar stationary phase &non-polar mobile phase. The molar analyte connected with & is retained by the polar stationary phase. An increase in polarity of solute molecules increases the adsorption capacity leading to an increased retention time [23,24].

B) RP-HPLC (Reversed Phase HPLC):

RP-HPLC has a non-polar or moderately polar mobile phase. RP-HPLC works on the principle of hydrophobic interaction [25]. In a mixture of constituents those analytes which are relatively less polar will be retained by the non-polar stationary phase longer than those which are relatively more polar. Therefore, the most polar constituent will be eluted first [26].

C) Size Exclusion Chromatography (SEC):

Size Exclusion Chromatography (SEC) also known as gel permeation chromatography or gel filtration chromatography mainly different particles on the basis of size. It is also useful for discover the tertiary structure & quaternary structure of proteins & amino acids. This technique is commonly used for the molecular weight determination of polysaccharides [27].

D) Ion Exchange Chromatography:

In ion-exchange chromatography retention is based on the attraction between solute ions & charged sites bound to the stationary phase. This form of chromatography is generally used in purifying water, Ligand-exchange chromatography, Ion-exchange chromatography of protein etc. [23,24].

E) Bio-affinity chromatography:

Separation based on specific reversible interaction of proteins with ligands. Ligands are covalently attached to solid support on the bio-affinity matrix, retains proteins with interaction to the column bound ligands[28].

Principle of HPLC:

The separation principle of HPLC is based on the distribution of the analyte or sample between a mobile phase eluent & a stationary phase or packing material of the column. Depending on the chemical structure of the analyte the molecules are retarded while passing the stationary phase [29].

Instrumentation of HPLC:

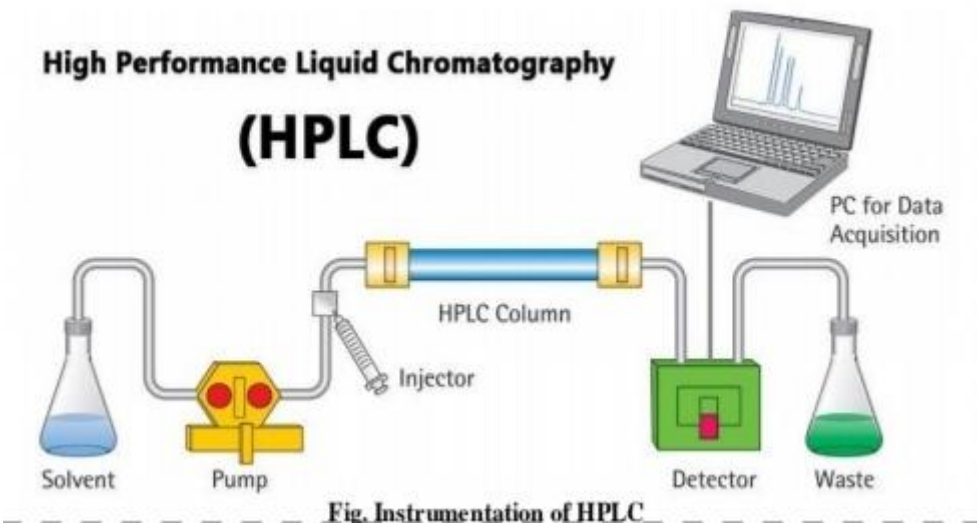

Fig. Instrumentation of HPLC

1) Mobile Phase/Solvent Reservoir:

Glass bottle reservoir hold the mobile phase. Solvent is derived from reservoir to pump by teflon tubing called inlet line to pump the reservoir in these systems allow to mobile phase to degassed and isolate in contact with air.

2) Solvent delivery system:

The solvent delivery system is described like a deliver system of continuous pulse free flow of mobile phase to the HPLC regardless of the system back pressure [30].

3) Pump:

The role of pump is force liquid through liquid chromatograph at specific rate. Normal flow rate 1-2ml/min range. Pump can reach pressure in range 6000-9000 psi. A pump can deliver constant mobile phase.

4) Injector:

Injector introduced liquid into flow stream of mobile phase. Sample volumes are 5-20micrometers.The injector must also able to stand the high pressure of liquid sample.

5) Column:

Column's stationary phase separate the sample component of interest using physical and chemical parameter. Pump must push hard to more the mobile phase through the column & this resistance cause high pressure.

6) Detector:

The detector can detect individual molecule and elute out from column. The detector provide an output to recorder or computer that result in the liquid chromatography [31].

Method of Preparation of standard and stock solution:

A stock solution is prepared by weighing out an convenient portion of a pure solid or by measuring out an convenient volume of a pure liquid placing it in a suitable flask and diluting to a known volume. Exactly how one measures reagent depends on the desired concentration unit. [32]

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