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Quantitative Estimation of L-Dopa from Polyhebal Formulation by using RP-HPLC

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ABSTRACT

Use of herbal medicines is increasing day by day. Herbal medicine has become popular form of healthcare. The consumption of plant based medicines and other botanicals in the west have increased manifold in recent years. Application of modern scientific knowledge coupled with sensitive analytical technique is important for the quality evaluation and standardization of polyherbal formulations. *Mucuna pruriens*, an important medicinal plant with wide medicinal properties, is frequently used in a large number of traditional herbal preparations. The present study deals with development and validation of RP-HPLC method for the quantitative estimation of L-DOPA from polyherbal formulations. The method involves Chromatographic separation was performed with SHIMADZU HPLC (Model no. LC- 20 AD) equipped with isocratic pump and manual injector (20µl). Spinchrom chromatographic software was used for data acquisition. RP- C18 (250 mm X 4.6 mm X 5 micron) column was used for analysis. Mobile phase comprising of methanol and water in ratio of 20:80 v/v was filtered through 0.45 micron in membrane filter (Millipore) and degassed by sonication; flow rate of 1ml/min was maintained throughout the run. Column effluent was monitored at 280 nm with variable wavelength UV detector. Thus this developed HPLC method could be further used for the determination of L-dopa in the polyherbal formulations.

Keywords: Polyherbal formulation, HPLC analytical method, *M. pruriens*, L-dopa, Validation.

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INTRODUCTION

In recent days the need of natural drugs in treatment of various life threatening diseases and disorders goes on increasing as they offer minimum side effects. The wide numbers of herbal formulations are in the market. The authenticity of such herbal formulations remains unrevealed and there is current need to develop newer analytical techniques for the estimation of active principles from such formulations. L-DOPA (L-3, 4-dihydroxyphenylalanine) is a precursor of neurotransmitter dopamine and useful in treatment neurological disorders like Parkinson's disorder.

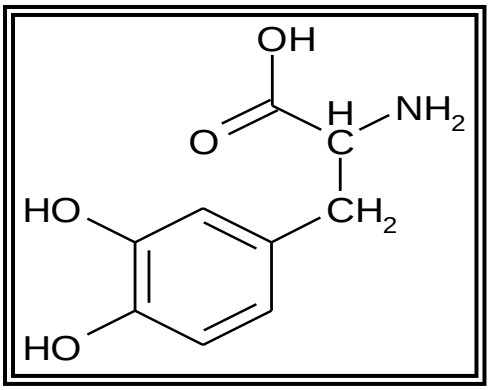

Figure 1: Structure of L-DOPA

Mucuna pruriens Linn. (*Leguminosae*) is a tropical legume known as velvet bean or cowitch**,** found in Africa, India and the Caribbean. The plant is easily identified by its extreme itchiness when it comes in contact with skin especially when it is in early stage of developments. The plant seeds contain higher concentration of levodopa which is a direct precursor of the neurotransmitter dopamine. It has long been used in traditional Ayurvedic Indian medicine for diseases including Parkinson's disease.[1-3] It has been shown to be as effective as pure levodopa/carbidopa in the treatment of Parkinson's disease, but no data on long-term efficacy and tolerability are available.[4] The various analytical methods were reported for estimation of L-DOPA from herbal formulations like non-aqueous titrimetric analysis [5], UV assay [6], HPLC [7], HPTLC[8].

MATERIALS AND METHOD

Separation of Levodopa from marketed formulations.

Major and important step in method development for the estimation of levodopa was isolation and purification of levodopa from marketed formulations. Levodopa is heat and temperature sensitive therefore it was extracted by cold maceration .The Mucuna prurience powder was treated with water: ethanol 30:70 ,kept in tightly closed container for seven days. The

supernatant was separated. The marc was further treated with fresh solvent procedure repeated till the extracted solvent showed negative test for levodopa. The collected fraction was concentrated by vacuum evaporation. The Isolated levodopa was purified by passing through column of silica (60-80 µ). Identification was done by chemical test, melting point, UV λ_{max}& by IR spectrum.

HPLC method development for L-DOPA as per ICH guidelines

Chromatographic conditions and instrumentation:

Chromatographic separation was performed with SHIMADZU HPLC (Model no. LC- 20 AD) equipped with isocratic pump and manual injector (20µl). Spinchrom chromatographic software was used for data acquisition. RP- C18 (250 mm X 4.6 mm X 5 micron) column was used for analysis. Mobile phase comprising of methanol and water in ratio of 20:80 v/v was filtered through 0.45 micron in membrane filter (Millipore) and degassed by sonication; floe rate of 1ml/min was maintained throughout the run. Column effluent was monitored at 280 nm with variable wavelength UV detector.

Preparation of standard solution

Standard L-DOPA was used as marker and stock solution of 100 ppm was prepared in methanol: water (20:80). From the stock solution series of different dilutions (5-12ppm) were prepared in the same mobile phase.

Preparation of stock solution of test samples

Isolated L-DOPA from 3 different formulations was used for preparation of test solution. 100ppm stock of each of A, B and C were prepared and used for analysis. Validation experiments were performed to demonstrate system suitability, linearity, precision, accuracy study, ruggedness and robustness as per ICH guidelines.

Linearity –

Working dilutions of levodopa in the range of 5-11ppm were prepared by taking suitable aliquots of working standard solutions in different 10ml of volumetric flasks and diluting upto the mark with mobile phase. 20 µl was injected each time into the column at flow rate of 1ml/min. The standard in elute was monitored at 280 nm and corresponding chromatogram were obtained, from these chromatograms peak area were calculated and plot of peak area over concentration was constructed. Regression of the plot was computed by least square regression method. The experiment was performed three times and mean was used for calculation

Precision and Accuracy –

Precision of analytical method was studied by multiple measurements of homogenous sample; 7

replicates of 10 ppm solution of L- DOPA were prepared and injected for precision at the same flow rate of 1ml/min. The intra-day and inter day precision was used to study the variability of the method. The series of dilutions prepared for linearity was used for intra-day and inter day precision. SD and RSD were calculated. Accuracy of the method was studied using the method of standard addition. Standard L-DOPA solutions were added to the unknown formulation of *M. pruriens*. The percent recovery was determined at three different levels (50%, 75%, and 100%). L-DOPA content was determined and percentage recovery was calculated.

LOD and LOQ

Limit of detection and limit of quantitation of the method was calculated by formula given below,

LOD = 3.3 X SD ÷slope

LOQ = 10 X SD ÷ slope.

HPLC Method Development Procedure for L-DOPA:

HPLC method for estimation of levodopa was developed using water: methanol (80:20) as solvent system. Validation parameters were performed as per ICH guidelines. Separation was carried out at flow rate of 1m/min. Wavelength of detection was 280nm.

RESULTS AND DISCUSSION

Method was found rugged and robust; SD and RSD of the ruggedness, robustness, inter and intraday precision are shown in table 1. As time passes percentage RSD increases; percentage RSD for inter day precision was quite higher, 4.138%, indicates instability of levodopa. Percentage recovery of sample A was found higher. Mean percent recovery is shown in table 2. The Proposed method was simple, accurate, precise and cost effective and can be used for quantitative estimation of levodopa.

Table 1: Linearity, Ruggedness, Inter and Intraday precision

Conc. (ppm)	Area in mv sec. at 280 nm			
	Flow rate 1ml/min	Flow rate1.2ml/min	Intraday 1ml/min	Inter day 1ml/min
5	124.558	128.421	121.635	118.942
6	157.336	156.518	154.833	149.574
7	181.988	180.93	178.983	176.871
8	215.433	220.883	213.673	209.318
9	240.898	242.116	238.483	235.744
10	268.532	265.209	265.387	261.18
11	304.142	307.342	300.673	298.36

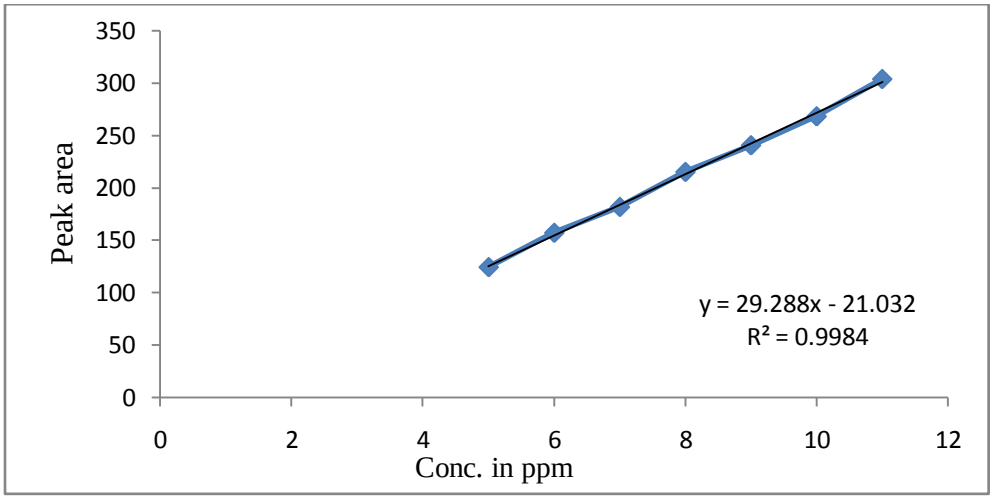

Figure 2: Graph of Peak area vs Conc. in ppm

Regression coefficient of the linearity was 0.9984; indicates linear relationship.

Equation of regression y = 29.288x – 21.032.

Table 2: Precision of the Method

Sr. no.	Concentration in ppm	Area in mv sec	SD	RSD
1	10	268.241	1.02205	0.38279
2	10	266.542		
3	10	267.321		
4	10	265.542		
5	10	266.345		
6	10	268.342		
7	10	266.654		

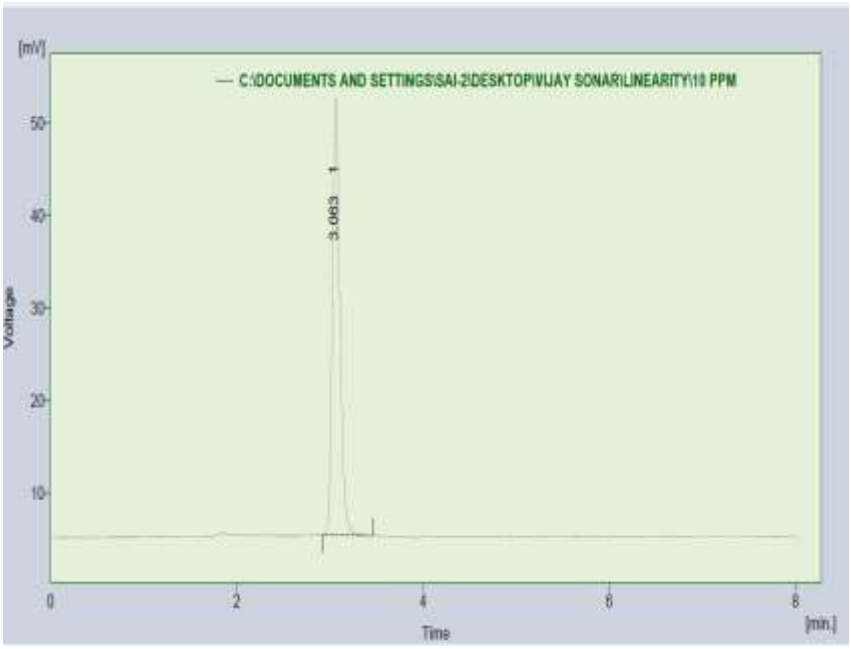

Figure 3: Peak spectrum of levodopa (10 ppm) by HPLC.

Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\SAI-2\DESKTOP\VIJAY SONAR\LINEARITY\10 PPM)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.063	265.542	47.194	100.0	100.0	0.09
	Total	265.542	47.194	100.0	100.0	

Figure 3: Shows the peak spectrum of levodopa (10 ppm), measured at 280 nm. Retention time was 3.06 min.

Table 3: HPLC Method Validation summary

Sr.no	Parameter	Standard
1	Linearity range	5-11µg/ml
2	Slope	29.288
3	Intercept	-21.032
4	Correlation coefficient	0.9984
5	LOD	0.115 µg/ml
6	LOQ	0.348µg/ml

Table 4: Robustness and Ruggedness of the Method (System Suitability Parameters)

Sr.no	Parameter	Theoretical plate	Peak area	Tailing factor	Retention time (min.)
1	Flow rate 0.8ml/min	6309	266.765	1.42	3.8
2	Flow rate 1ml/min	6421	265.542	1.38	3.06
3	Flow rate 1.2ml/min	6228	265.209	1.52	2.56
4	Analyst 1	6421	268.532	1.38	3.06
5	Analyst 2	6072	267.321	1.5	3.09

Table 5: Robustness, Ruggedness and Precision of the method

Sr. no.	Parameter	SD	%RSD
1	Precision (n = 7)	1.02205	0.38279
2	Intraday precision (n = 7)	1.9415	0.9895
3	Inter day precision (n = 7)	2.101	4.138
4	Ruggedness (change in flow rate; n =7)	1.912	0.9548
5	Robustness (change in analyst; n = 3)	0.856	0.319

Table 6: Recovery Study Results

Sr. no.	Sample	% amount recovered			Standard error of mean	SD	RSD
		50%	75%	100%			
1	A	94.45	94.17	94.73	0.1191	0.453	1.655
2	B	92.17	92.34	92.51	0.0854	0.263	0.902
3	C	89.66	91.64	90.65	0.234	0.176	0.606

CONCLUSION

The proposed method of HPLC, UV and titrimetry can be used for routine analysis of levodopa from herbal formulations of *Mucuna puriens*. HPLC is more sensitive, accurate, precise and selective one than UV and titrimetry. Percentage content of levodopa was found to be higher in sample A by all three methods of estimation; it was lowest for sample C. Amount of levodopa

found for all three samples were less than the label claim. This implicates the need of standardization of herbal formulations.

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