Development of validated RP-HPLC method for the estimation of L-Dopa from *Mucuna pruriens*, its extracts and in Aphrodisiac formulation.

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ABSTRACT

In the present study, reversed phase HPLC method was developed for the estimation of L-Dopa from *Mucuna Prurines*, its extract and in Aphrodisiac formulation. HPLC analysis was performed on C_{18} column using a mixture of Water: Acetonitrile: Methanol containing 0.2 % Triethylamine PH adjusted to 3.3 as isocratic mobile phase at a flow rate of 1.0 ml per minute at detection wavelength of 280 nm. The method was validated for accuracy, precision, linearity, specificity and sensitivity in accordance with International conference on Harmonization guidelines. Validation revealed the method is specific, accurate, precise, reliable and reproducible. Good linear correlation coefficients ($r^2 > 0.999$) was obtained for calibration plots in the range of 10 – 80 µg/ml. Intraday and Interday RSD of retention times and peak areas were less than 2.0 %. Average Percent Recovery was 98.83 %. The method was successfully used for quantitative analysis of this marker compound in Polyherbal formulation.

KEYWORDS: HPLC, L-Dopa, *Mucuna Prurines*, Atmagupta, Polyherbal formulation.

INTRODUCTION

Mucuna pruriens Linn. (Fabaceae), commonly known as Kewanch or Kaunch, Atmagupta and Velvet bean, is a climbing annual legume, endemic in India and in other parts of the tropics including Central and South America. About fifteen species of *Mucuna* are found in the forests and plains of India. It is useful as a green manure and cover crop and is also grown for its pods and young leaves, which are used as vegetable and fodder [1]. Traditionally, the seeds of *Mucuna Pruriens* have been used for treating male sexual dysfunction in Tibb-e-Unani (Unani Medicine), the traditional system of medicine of Indo-Pakistan Subcontinent. It is also used in Ayurvedic medicine. *M. pruriens* has been shown to improve sexual function in rats [2]. *M. pruriens* seeds have also been found to have antidepressant properties in cases of depressive neurosis when consumed [3]. The roots are used against cholera, as diuretic and for dropsy [3]. Seeds are powerful aphrodiasiacs and are known to promote sexual vigour and semen [3]. Formulations of the seed powder have shown promise in the management and treatment of Parkinson's disease [4]. In Ayurvedic system, the kaunch seeds and roots are used for the treatment of diseases of central nervous system and also as an anthelmintic [5], [6]. Its different preparations from the seeds are also used for the management of ageing, rheumatoid arthritis diabetes, male infertility and nervous disorders. *M. pruriens* seed powder contains high amount of L-DOPA (L-3, 4 dihydroxy phenylalanine), which is a neurotransmitter precursor and effective remedy for the relief in Parkinson's disease. In addition seeds contain tryptamine, 5-hydroxytryptamine (5-HT), mucunine, mucunadine, prurienine and prurieninine [7], [8]. Though there is dearth information available on the variability and methods to determine L-Dopa, viz. titrimetric method [9], UV assay [10], HPTLC method [11], and the methods are laborious and time consuming. The present method has been developed for the estimation of L-dopa from *Mucuna Pruriens*, its extract and in Polyherbal (Aphrodisiac) formulation. In the present method solvents used for mobile phase are not hazardous to the column as compared to the other buffered mobile phase, resulting in the long life of the column; hence the developed validated method can be used for routine analysis of L-Dopa.

MATERIALS AND METHODS

Powdered drug and extracts were purchased from different vendors. Polyherbal (Capsule) formulation was procured from Sunrise Remedies Pvt. Ltd., Santej. Pure L-Dopa was purchased from Yucca Enterprise Pvt. Ltd., Bombay.

Preparation of Standard solution

A stock solution of 100 µg/ml was prepared by dissolving 10.0 mg of L-Dopa in 10 ml of 0.1 M HCl and diluted to 100.0 ml with HPLC grade methanol.

Preparation of Sample solution

Accurately weighed one gram of powders and extracts of *Mucuna pruriens* were refluxed with a mixture of methanol and 0.1 M HCl (70:30) for 30.0 minutes and filtered. The extracts were evaporated to dryness. The residue was redissolved in methanol, filtered through 0.45 µm membrane filter and used for HPLC analysis. For capsule average net content was determined and was refluxed with a mixture of methanol and 0.1 M HCl (70:30) for 30.0 minutes and filtered. The extract was evaporated to dryness. The residue was redissolved in methanol, filtered through 0.45 µm membrane filter and used for HPLC analysis. The analytical HPLC experiments were performed with an Agillent Technologies 1120 compact LC equipped with variable wavelength detector operating at 280 nm. Separation was carried out with C_{18} (5 µm) column with Water: Methanol: Acetonitrile (100:60:40) containing 0.2 % Triethylamine, pH adjusted to 3.3 as an eluent at a flow rate of 1.0 ml/minute. Validation of quantitative method was performed with samples for five injections of 20 µl each.

Validation Parameters of Developed Method [12]

Validation of developed method was carried out as per ICH guidelines. Parameters such as Linearity, Accuracy, Precision, LOD and LOQ were taken up as tests for analytical method Validation.

Linearity

The linearity was evaluated by analyzing different concentration of the standard solutions of L-Dopa. The Beer-Lambert's concentration range was found to be 10-80 µg/ml.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% & 120%). Average percent recovery for was found to be 98.83 % (table 2).

Precision

The repeatability, intraday and interday variations for determination of L-Dopa was carried out three times in same day and for three consecutive days and % RSD was calculated. The method was found to be precise due to low values of %RSD.

LOD & LOQ

The LOD and LOQ of developed method were calculated by using equations:

Limit of Detection (LOD): $3.3 \times \sigma/S$

Limit of Quantification (LOQ): $10 \times \sigma /S$

Where, σ = The Standard deviation of the response,

S = Slope of calibration curve.

The results of all validation parameters obtained are shown in table no. 1.

RESULTS AND DISCUSSION

The method discussed in the present work provides a convenient and accurate way for analysis of L-Dopa. In proposed method, Linearity was observed in the concentration range of 10-80 µg/ml. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. Average percent recovery for L-Dopa was found to be 98.83 %, values of standard deviation and coefficient of variation were satisfactorily low indicating the accuracy of the method. Based on the results obtained, it is found that the proposed method is accurate, precise, reproducible & economical and can be employed for routine quality control of L-Dopa in capsule dosage form as well extracts.

Table 1: Regression Analysis Data and Summary of Validation Parameters for Proposed Method

Sr. No.	Validation Parameters	Result
1	Linearity Range	10 – 80 µg/ml
2	Linearity Equation	y = 318.3 x + 1119
3	Slope	318.3
4	Intercept	1119
5	Correlation Coefficient (R^2)	0.997
6	Precision (% RSD)	
	Repeatability (n=6)	1.12
	Intraday (n=3)	0.77
	Interday (n=3)	0.83
7	Accuracy (n=3)	98.83 %
8	LOD (µg/ml)	1.80 µg/ml
9	LOQ (µg/ml)	6.01 µg/ml
10	Theoretical Plates	12989
11	Asymmetry	1.41972
12	Capacity Factor	-0.49533

Table 2: Recovery Data of L-Dopa

Amount taken (µg)	Amount added (µg)	Total amount of L-Dopa (µg)	Amount of L-Dopa recovered (µg)	% Recovery of L-Dopa	Average % Recovery
20	16	36	35.54	98.72 %	98.83 %
20	20	40	39.65	99.13 %	
20	24	44	43.40	98.64 %	

Table 3: Data for hplc determination of L-dopa content in different extracts as well as in powder of *Mucuna pruriens* and in polyherbal formulation.

Sample	Powder		Extract		Xytone capsule (polyherbal formulation)
	P1	P2	E1	E2	F
L-Dopa	3.84 %	3.18 %	5.095 %	5.025 %	4.225 %

CALIBRATION CURVE FOR L-Dopa

Different concentration of the standard solution of L-Dopa of 10 – 80 µg were prepared from stock solution and used for HPLC analysis. The calibration data for standard L-Dopa was obtained by plotting the graph of Concentration Vs Peak area.

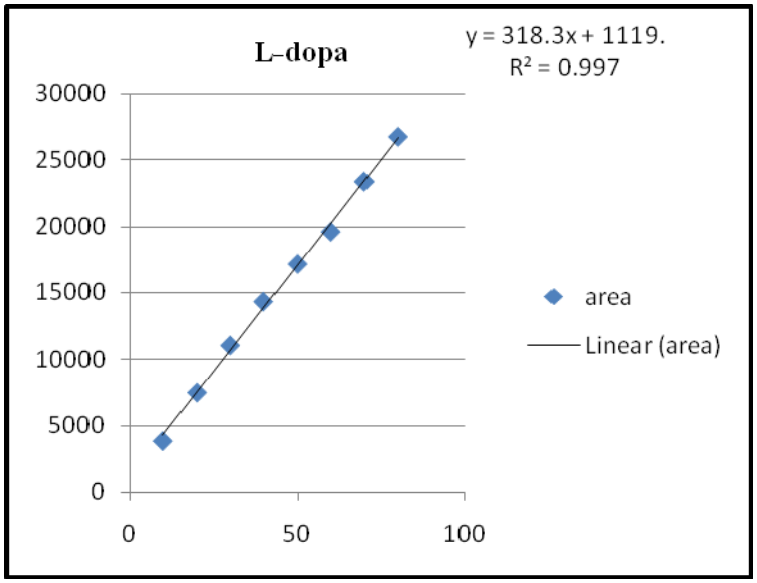

Fig.1: Calibration Curve for L-Dopa

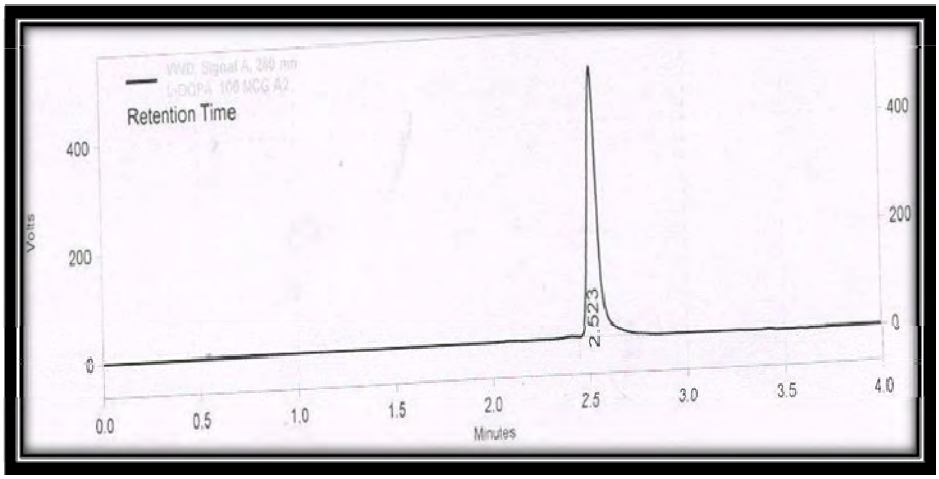

Fig.2: Chromatogram of std L-dopa

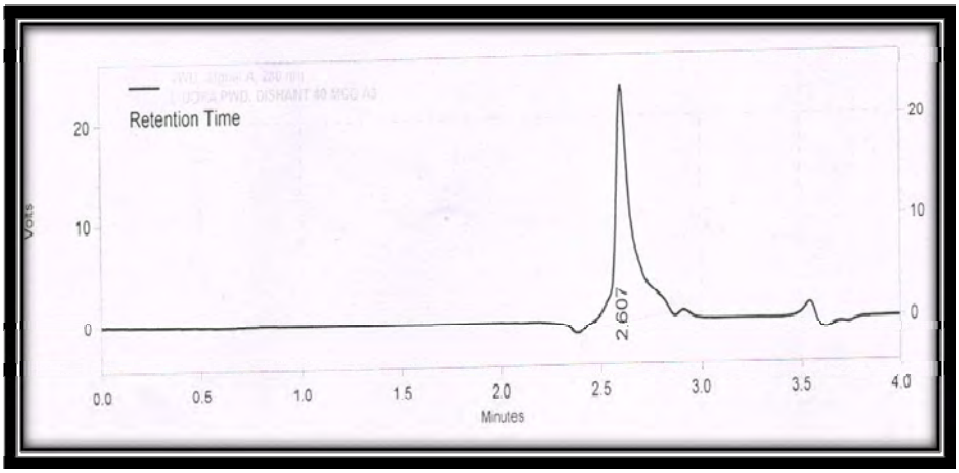

Fig.3: Chromatogram of L-dopa (Powder1)

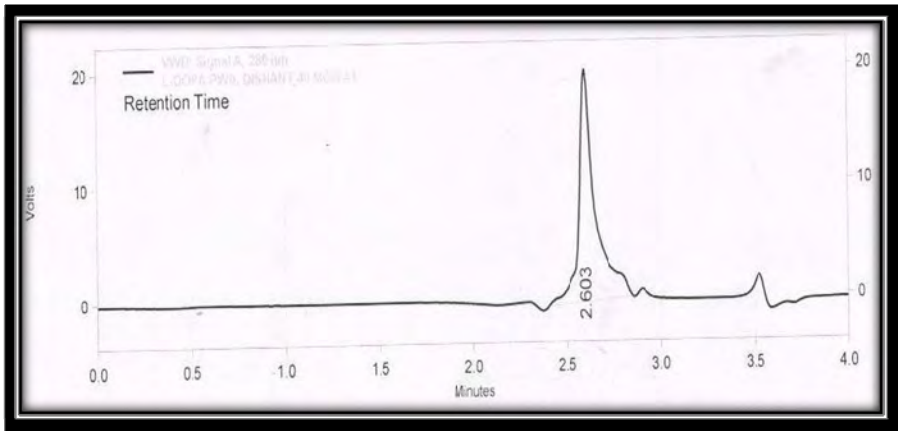

Fig.4: Chromatogram of L-dopa (Powder2)

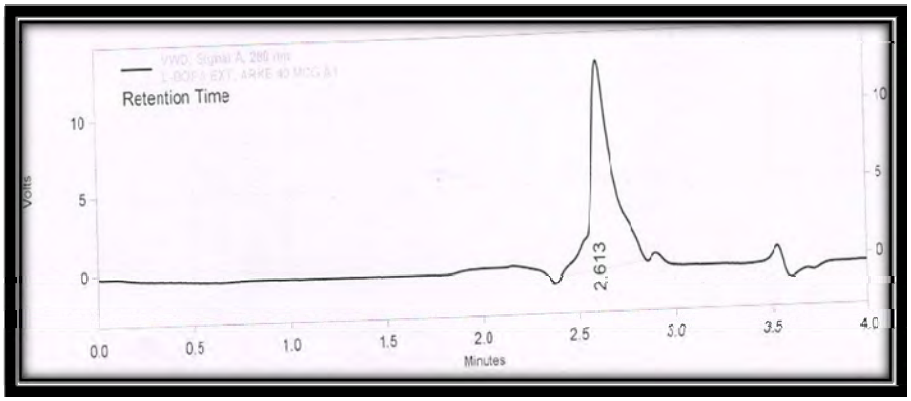

Fig.5: Chromatogram of L-dopa (Extract1)

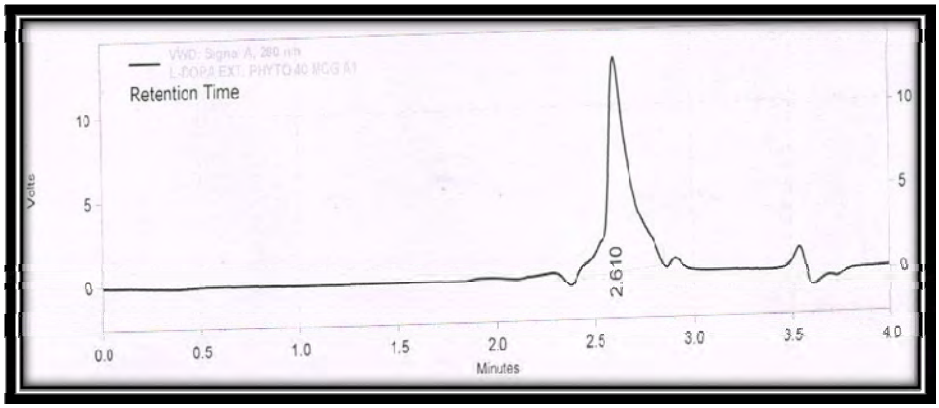

Fig.6: Chromatogram of L-dopa (Extract2)

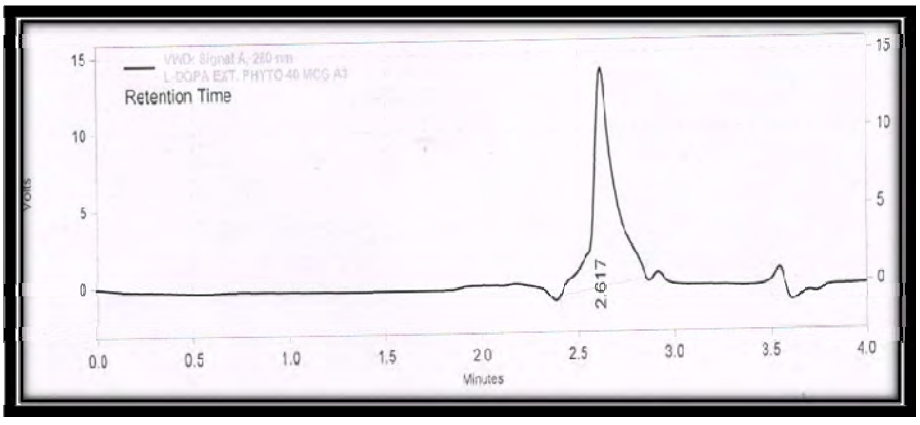

Fig.7: Chromatogram of L-dopa (Capsule)

CONCLUSION

A validated method has been developed for the estimation of L-Dopa in Capsule dosage form. Proposed method is simple, accurate and precise. The method is suitable for routine analysis of L-Dopa in Capsule and Extracts. The simplicity of this method allows for application in laboratories that lack sophisticated analytical instruments such as HPTLC, LC–MS. Detection and quantification limit achieved, describe the method is very sensitive. High recoveries and acceptable % RSD values confirm established method is accurate and precise. Hence, the method is recommended for routine quality control analysis of L-Dopa.

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