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PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.2, pp 1033-1036, April-June 2010

Estimation of L-dopa From *Mucuna pruriens* Linn and Formulations Containing *M. pruriens* by Spectrofluorimetric Method

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Abstract: A simple, accurate and precise spectrofluorimetric method has been developed for the analysis of L-dopa in *Mucuna pruriens* seed extract and its formulations. The excitation and emission wavelength were found to be 282 and 630 nm respectively in 0.1 N formic acid. The relationship between the concentration (ng/ml) of L-dopa and corresponding fluorescence intensity (FI) was found to be linear in the range of 30-800 ng/ml. The method was validated for precision (inter and intraday), repeatability, and accuracy. Mean recovery was 99.94%. The relative standard deviation (RSD) values of the precision were found to be in the range 0.58-0.95%. The proposed method was found to be precise, specific and accurate and can be used for identification and quantitative determination of L-dopa in herbal extract and its formulations.

Key-words: L-dopa, Precision, Spectrofluorimetry, Validation.

Introduction

3-(3, 4-dihydroxyphenyl)-L-alanine (L-DOPA) is a neurotransmitter precursor, and is an effective remedy for the relief of Parkinson's disease[1]. Its relatively high demand and spiralling price in the market, demands for a suitable method to be standardized to control amount of L-dopa in the finished product. Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques.

Mucuna pruriens Linn (Leguminosae), commonly known as ''the cowhage'' or ''velvet'' bean; and ''atmagupta'' in India, is a climbing legume endemic in India and in other parts of the tropics including Central and South America. In Ayurvedic system of medicine, *M. pruriens* was used for the management of male infertility, nervous disorders and also as an aphrodisiac[2]. Its different preparations from the seeds are also used for the management of ageing, rheumatoid arthritis, diabetes, male infertility and nervous disorders[3]. Other parts of the plants are also medicinal used for various ailments, e.g., trichomes of pods are used for anthelmintics and decoction of root in delirium. Leaves are useful in ulcers, inflammation, cephalagia and general debility. *Mucuna pruriens* seed powder contains high amount of L-dopa, which is a neurotransmitter precursor and effective remedy for the relief in Parkinson's disease[4]. *Mucuna pruriens* seed in addition to levodopa, contains tryptamine, 5-hydroxytryptamine (5-HT), mucunine, mucunadine, prurienine and prurieninine[5,6]. It is also rich in fatty content[7].

The B.P. describes a non-aqueous titration for the determination of L-dopa[8]. The U.S.P. recommends a non-aqueous titrimetric procedure with potentiometric end point determination of L-dopa and extractive procedure followed by UV assay for its determination in formulations[9]. Parikh et al.[10] described high performance liquid chromatography method for estimation of L-dopa in plant. Siddhuraju et al.[11] reported a rapid reversed-phase high performance liquid chromatographic method for the quantification of L-dopa, non-methylated and methylated tetrahydroisoquinoline compounds from Mucuna beans. A quantitative estimation of L-dopa in tablets has been reported by high performance thin layer chromatography method[12]. Recently, Modi et al.[13] described HPTLC method for estimation of L-dopa

from Mucuna pruriens Linn and formulations containing M. Pruriens. The major disadvantage of HPTLC method is that, it requires time for development of TLC plate. Kim et. al.[14] reported simultaneous determination of levodopa and carbidopa by synchronous fluorescence spectrometry using double scans. We report here a simple, accurate, reproducible and cost effective spectrofluorimetric method for the estimation of L-dopa, from the seed of *M. pruriens*. L-dopa content was also estimated from two marketed herbal formulations containing *M. pruriens* as one of the ingredients.

Experimental

Materials

Standard L-dopa (assigned purity, 99.8 %) was a gift sample from Torrent Research Centre, Ahmedabad, India. All the chemicals used in the experiments are of analytical grade.

Instrumentation

Fluorescence spectra and measurements were taken on a spectrofluorometer Model RF-1501 (Shimadzu, Tokyo, Japan) equipped with a xenon lamp and 1 cm quartz cells. Excitation and emission wavelengths were set at 282 and 630 nm, respectively.

Preparation of Extract

The seeds of *M. pruriens* were purchased from United Chemicals and Allied Products, Kolkata, India. It was authenticated by Dr. B. C. Patel, Botany Department, Modasa, India. A voucher specimen was retained in our laboratory for further reference. Seeds were dried in shade and powdered in a mechanical grinder. The powder of *M. pruriens* seeds was initially defatted with petroleum ether (60-80^{0}C) then aqueous extract was prepared by cold maceration method. After seven days, the extract was filtered using Whatman filter paper (No. 1) and then concentrated in vacuum and dried.

Calibration Curve of Standard L-dopa

A stock solution of L-dopa (1µg/ml) was prepared by dissolving an accurately weighed 10 mg of L-dopa standard in 5 ml of 0.1N formic acid and volume was made up to 100 ml with 0.1N formic acid in a volumetric flask. Again 1 ml of this solution was transferred in 100 ml volumetric flask and diluted up to the mark with 0.1 N formic acid. Standard working solutions were prepared by diluting stock solution with 0.1N formic acid in the concentration range 30-800 ng/ml. Fluorescence intensity was measured for all the solutions at 630 nm emission wavelength.

Estimation of L-dopa in Herbal Extract

To determine the content of L-dopa in herbal extracts, an accurately weighed 100 mg of dry aqueous extract was transferred into 100 ml volumetric flask and 10 ml of 0.1N formic acid was added, sonicated for 10 min and volume was made up to 100 ml with 0.1N formic acid. The extract was filtered on a Whatman no. 1 filter paper, from which 1.0 ml of the solution was diluted to 1000 ml with 0.1N formic acid in volumetric flask. Finally solution was diluted to get concentration of 500 ng/ml and Fluorescence intensity was measured as above. The analysis was repeated for five times.

Estimation of L-dopa in Marketed Herbal Formulations

To determine the content of L-dopa in market formulations, an accurately weighed 100 mg of powder was transferred into 100 ml volumetric flask and 5 ml of 0.1 N formic acid was added, sonicated for 10 min and volume was made up to 100 ml with 0.1 N formic acid. The extract was filtered on a Whatman no. 1 filter paper, from that 1.0 ml of the solution was diluted to 1000 ml with 0.1 N formic acid. . Finally solution was diluted to get concentration of 500 ng/ml and Fluorescence intensity was measured as above. The analysis was repeated for five times.

Results and Discussion

L-dopa shows fluorescence with high sensitivity in 0.1 N formic acid at excitation and emission wavelength 282 nm and 630 nm respectively (fig. 1). In the reported HPLC method by Siddhuraju et al.[13] the gradient elution technique was used and mobile phase was consisted of water, methanol, phosphoric acid and the pH was adjusted to 2.0. While in reported HPTLC method by Modi et al., used n-butanol-acetic acid-water (4.0 : 1.0 : 1.0) as solvent system. Thus as compared to the reported methods, our proposed method requires very simple solvent to separate the L-dopa from the extract. The peak purity was assessed by comparison of overlay spectra of standard L-dopa and *M. pruriens* seed extract at peak apex and peak base which confirm the method was selective (Fig. 2).

MethodValidation

The spectrofluorimetric method was validated in terms of Linearity, precision, accuracy and repeatability (Table 1). Linearity was evaluated by determining eight standard working solutions containing 0.03-0.8 µg/ml of L-dopa. Fluorescent intensity and concentrations were subjected to least square linear regression analysis to calculate the calibration equation and correlation co-efficient. Linearity was found over concentration range 30-800 ng/ml with a correlation coefficient (r) 0.99. The linearity of calibration graph and adherence of the system to Beer's law was validated by high value correlation co-efficient. In the reported HPTLC method by Modi et al.[13)] linearity was in the range of 100-1200 µg/spot while in our method the linearity range was 30-800 ng/ml. Hence it is comparable with the reported method. The instrumental precision was studied by repeated measuring of the same solution for seven times and % CV was found to be 0.58. Repeatability of the method

was tested by analyzing the standard solution (500 ng / ml) for five times (% CV = 0.49).

The reproducibility of the method was determined by different analysis of samples from the same batch and repeatability was determined by intra-day and inter-day precision, expressed in terms of percent relative standard deviation (RSD%) or coefficient of variation (CV). Six determinations were carried out on the same sample, on the same day for intra-day and over two consecutive days for inter-day precision on five samples. The values of %RSD of intra and inter-day were found between 0.58-0.87% and 0.60-0.95%, respectively (Table 2). No significant difference was observed in the analysis of L-dopa in five different samples. The RSD% of the reproducibility of the method was found to be <2%.

The accuracy of the method was determined by multiple level recovery studies. A known but varying amount of standards from L-dopa was added to the pre analyzed sample and analyzed according to the procedure. The results are reported in Table 3. The recovery was found to be 100.77%, 98.89%, 100.15% and 99.93% at the four levels respectively and the average recovery was 99.94%.

Application of the Method

The proposed method was applied for the estimation of L-dopa content in extract *of M. pruriens* and its formulations. The obtained results are depicted in Table 4.

Conclusions

The developed spectrofluormetric method is simple, specific and accurate. Statistical analysis proves that the method is reproducible and selective. This method can be used for the quantitative determination of L-dopa in herbal extract and its formulations.

Table 1: Method validation parameters for the estimation of L-dopa by Proposed method

Sr. No.	Parameter	Values
1	Instrumental precision (CV, %) (n = 7)	0.58
2	Repeatability (CV, %) (n =5)	0.49
4	Limit of quantification (ng/ml)	30
5	Linearity (correlation coefficient)	0.9988
6	Range (ng/ml)	30 – 800

Table 2: Intra- and Inter-Day Precision of Proposed Method

Concentration (ng /ml)	Intra-day precision (RSD, %, n = 6)	Inter-day precision (RSD, %, n = 6)
200	0.63	0.60
300	0.72	0.87
400	0.58	0.95
500	0.68	0.82
600	0.87	0.76

Table 3: Recovery study of L-dopa by Spectrofluorimetric method

Sr. No.	Amount of L-dopa present in powder of *M.pruriens* (mg)	Amount of L-dopa L-dopa added (mg)	Amount of L-dopa found in the mixture (mg)	% Recovery[a]
1	7.2	1.8	9.07 ± 0.043	100.77 ± 0.035
2	7.2	3.6	10.68 ± 0.037	98.89 ± 0.058
3	7.2	5.4	12.62 ± 0.061	100.15 ± 0.019
4	7.2	7.2	14.39 ± 0.059	99.93 ± 0.022

[a] Mean ± SD, n =6.

Table 4: Percentage of L-dopa in samples analyzed

S. No.	Sample Name	% of L-dopa[a]
1	*Mucuna pruriens*	7.2 ± 0.521
2	Formulation 1	5.6 ± 0.342
3	Formulation 2	4.2 ± 0.270

[a] Mean ± SD, n = 5.

Figure 1: Fluorescence spectra of L-dopa at excitation wavelength (282 nm) and emission wavelength (630 nm).

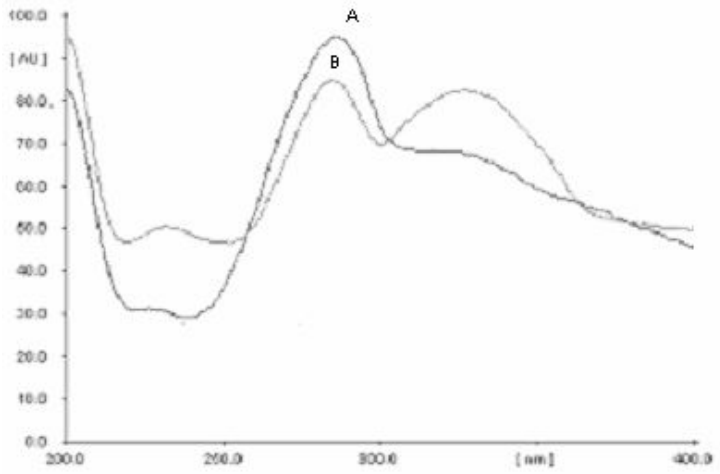

Figure 2: Overlay Spectra of (A) Standard L-dopa (B) a typical seed extract of *Mucuna pruriens*

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