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# A HPTLC method for quantitative estimation of L-dopa from *Mucuna Pruriens* in polyherbal aphrodisiac formulation

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## ABSTRACT

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. L-dopa, a major bioactive was selected as a chemical marker of *M. pruriens.* A selective, precise, and accurate high-performance thin-layer chromatographic (HPTLC) analytical method has been developed for the fingerprinting of *M. pruriens* and analysis of L-dopa in *M. pruriens* seed extract and its polyherbal formulation. The purpose of the study was to develop and validate method for precision, accuracy, linearity, specificity, robustness, LOD and LOQ. The method involves densitometric evaluation of L-dopa after resolving on silica gel HPTLC 60 $F_{254}$ plates with n-butanol-water-glacial acetic acid (80:20:20, v/v/v) as the mobile phase. Densitometric analysis of L-dopa was carried out in reflection–absorption mode at 520 nm. The relationship between the concentration of L-dopa and corresponding peak areas was found to be linear in the range of 600 to 1400 ng/spot. Thus this developed HPTLC method could be further used for the determination of L-dopa in the polyherbal formulations.

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

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## INTRODUCTION

*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 [1]. It is still used to increase libido in both men and women due to its dopamine inducing properties. Its different preparations from the seeds are also used for the management of ageing, rheumatoid arthritis, diabetes, male infertility and nervous disorder [2]. *M. pruriens* has been used as an aphrodisiac (hence the species name, *pruriens*, i.e. prurient). Other parts of the plants are also medicinally used for various ailments, e.g., trichomes of pods are used for antihelmintics and decoction of root in delirium. Leaves are useful in ulcers, inflammation, cephalagia and general debility. *M. pruriens* seed in addition to levodopa, contains tryptamine, 5- hydroxytryptamine (5-HT), mucunine, mucunadine, prurienine and prurieninin [3]. It is also rich in fatty content [4]. *M. pruriens* seed powder contains high amount of L-dopa, which is a neurotransmitter precursor and effective remedy for the relief in Parkinson’s disease [5]. 3-(3, 4-dihydroxyphenyl)-L-alanine (L-DOPA) is a neurotransmitter precursor, and is an effective remedy for the relief of Parkinson’s disease[6]. 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. The British Pharmacopoeia describes a non-aqueous titration for the determination of L-dopa[7]. The United States Pharmacopoeia 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[8]. Parikh et al. described high performance liquid chromatography method for estimation of L-dopa in plant[9]. Siddhuraju et al. 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[10]. A quantitative estimation of L-dopa in tablets has been reported by high performance thin layer chromatography method[11]. Our formulation, polyherbal dispersible granules for aphrodisiac effect contains extract of seven herbal plants. One of the major plant extract was *M. pruriens.* It was necessity to develop simple, accurate, precise HPTLC method for estimation of L-dopa in polyherbal dispersible granules.

## EXPERIMENTAL

### Materials

The powdered extracts of different plants for polyherbal formulation including M. pruriens were obtained from Vedic Lifesciences, Mumbai. L-dopa was procured from Sunpharma, Dadra, Gujarat, India. The plants extract were stored in airtight containers at room temperature.

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**HPTLC procedure**

A Camag HPTLC system equipped with an automatic TLC sampler ATS4, TLC scanner 3, and integrated software win CATS version 1.4.2 was used for the analysis. HPTLC was performed on a pre-coated silica gel HPTLC 60 $F_{254}$ (20 cm × 10 cm) plate of 0.20 mm layer thickness. Chromatography was carried in developing chamber which was pre-saturated with 20 ml mobile phase n-butanol-water-glacial acetic acid (80:20:20, v/v/v) for 30 min at room temperature (25 ± 2 °C). The samples and standards were applied on the plate as 6 mm wide bands with an automatic TLC sampler (ATS4) under flow of $N_2$ gas, 10 mm from the bottom, 10 mm from the side, space between two bands was 6 mm of the plate and application speed 150 nm/s. The length of chromatogram run was 8 cm from the base. After that, TLC plates were dried in a current of air. Bands were visualized by dipping in chamber containing visualizing agent 0.5% Ninhydrin in ethanol, followed by heating on Camag HPTLC plate heater at 120 °C for 2 min. Quantitative evaluation of the plate was performed after 20 min in reflection–absorption mode at 520 nm, slit width 6 mm × 0.3 mm, scanning speed 20 mm/s and data resolution of 100 µm/step.

**Development of the optimum mobile phase**

The TLC procedure was optimized with a view to develop an assay method. The standard and the test solutions were spotted on HPTLC plates and different individual solvents as well as combination of solvents were tried to get a good separation and stable peak. Both the pure drug (L-dopa) and the drug in M. pruriens solution were spotted on the TLC plates and run in different solvent systems. The mobile phase n-butanol-water-glacial acetic acid (80:20:20, v/v/v) gave good resolution Well-defined spots (compact dense spots) were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature.

**Calibration curve of L-dopa**

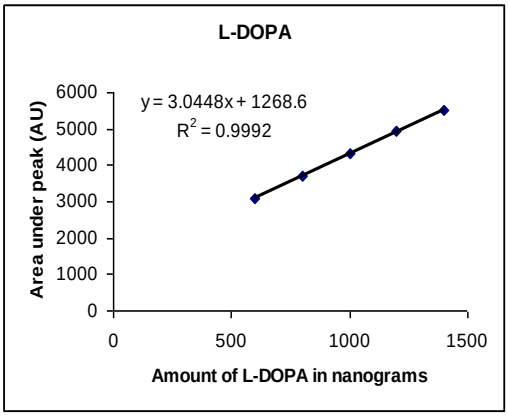


**Figure 1. Calibration plot of L-dopa by HPTLC analytical method**

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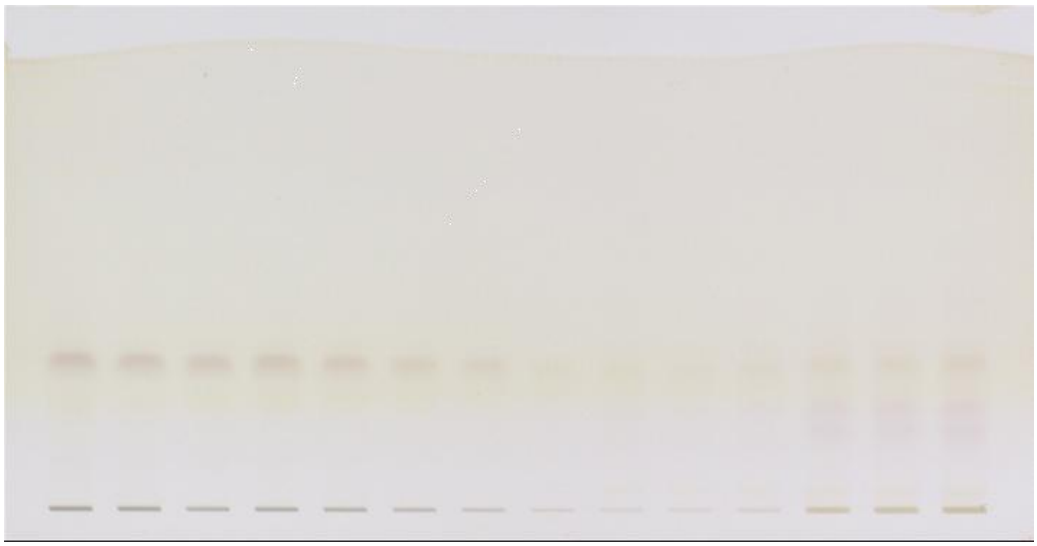
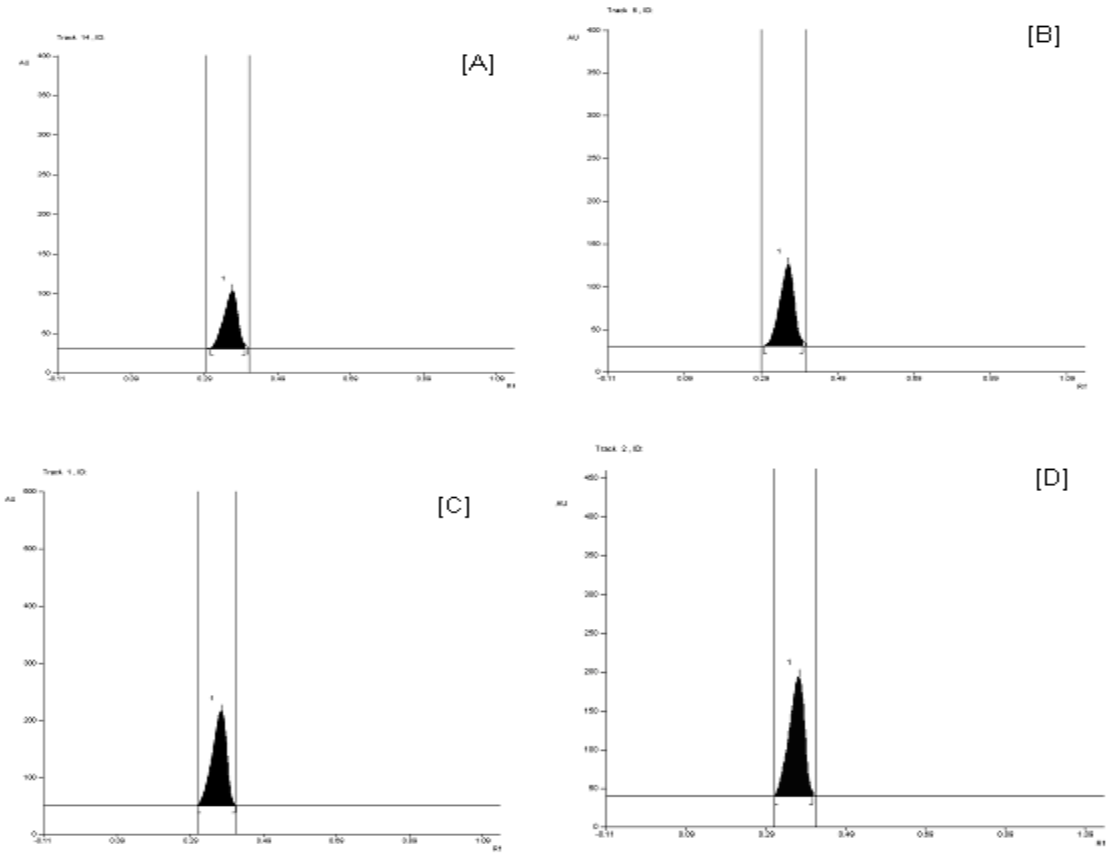


**Figure 2. Image of HPTLC plate spotted with different concentrations of L-dopa in visible light**



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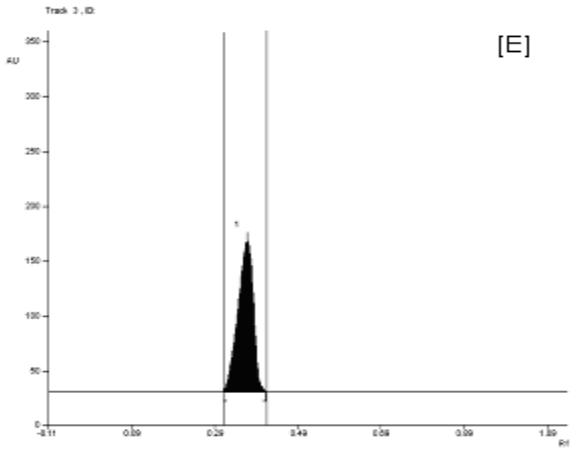


**Figure 3. HPTLC scans of L-dopa spotted with different concentrations (A) 600ng (B) 800ng (C) 1000ng (D) 1200ng (E) 1400ng**

Stock standard solution of L-dopa was prepared in methanol at the concentration of 100 µg/µl. From the stock standard solution 6, 8, 10, 12, 14 µl were applied on the TLC plates to obtain final concentration range of 600–1400 ng/spot. Each concentration was spotted six times on the HPTLC plate (Figure 1, 2 and 3).

**Analysis of L-dopa in M. pruriens powdered extract**

To determine the content of L-dopa in M. pruriens powdered extract, 50 mg powdered extract was weighed and transferred into a 100 ml volumetric flask containing 50 ml methanol and 50 ml water. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was analyzed for drug content. Twelve microlitres of the above filtered solution was applied on the TLC plate followed by development, visualization and scanning as described in HPTLC procedure. The analysis was repeated in triplicate.

**Analysis of L-dopa in developed polyherbal formulation**

Pharmaceutical formulation (Dispersible granules) equivalent to about 50 mg of *M. pruriens* powder was weighed and transferred to 100ml volumetric flask containing water:methanol mixture(1:1). The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was analyzed for drug content. Twelve microlitres of the filtered solution was applied on the TLC plate followed by development, visualization and scanning as described in HPTLC procedure. The analysis was repeated thrice.

## RESULTS AND DISCUSSION

The simple, accurate and precise HPTLC method was developed and validated for estimation of L-dopa in polyherbal dispersible granules. The optimized mobile phase for L-dopa

was n-butanol-water-glacial acetic acid (80:20:20, v/v/v). Saturation time of chamber was 30 minutes at 25°C. Visualization of spots was carried out by dipping HPTLC plates in 0.5% Ninhydrin in ethanol. Quantitative evaluation of the plate was performed after 20 minutes by heating at 120°C. The $R_f$ value of L-dopa was 0.37.

## Validation

*Linearity*

The linear regression data for the calibration curves ($n$ = 3) is shown in Table 1. It showed a good linear relationship over concentration range 600-1400 ng spot$^{-1}$ with respect to the peak area. No significant difference was observed in the slopes of standard curves.

**Table 1. Linear regression data for calibration curve of L-dopa by HPTLC analysis (n = 3)**

| | |
|---|---|
| Linearity range (ng) | 600–1400 |
| Correlation coefficient ($r^2$ ± S.D.) | 0.9992 ± 0.0084 |
| Slope ± S.D. | 3.0448 ± 0.54 |
| Confidence limit of slope (95% confidence limit) | 2.478-3.612 |
| Intercept ± S.D. | 1268.6 ± 19.8 |
| Confidence limit of intercept (95% confidence limit) | 1247.8-1289.4 |

*Precision*

The relative standard deviations (RSD) for repeatability of sample application (1000 ng spot$^{-1}$) and repeatability of measurement of peak area were found to be 0.034 and 0.018 respectively. The measurement of the peak area at three different concentration levels of 600, 1000 and 1400 ng spot$^{-1}$, showed very low values of the % RSD (<0.05%) for inter- and intra-day variation suggesting an excellent precision of the method (Table 2).

**Table 2. Intra- and inter-day precision of HPTLC method for estimation of L-dopa (n = 6)**

| Amount (ng spot$^{-1}$) | Intra-day precision | | | Inter-day precision | | |
|---|---|---|---|---|---|---|
| | Mean area | S.D. (±) | %RSD | Mean area | S.D. (±) | % RSD |
| 600 | 3076.9 | 15.3 | 0.034 | 3071.4 | 12.5 | 0.046 |
| 1000 | 4331.7 | 9.87 | 0.021 | 4338.4 | 10.7 | 0.018 |
| 1400 | 5498.6 | 11.6 | 0.037 | 5485.3 | 14.9 | 0.027 |

*Robustness*

**Table 3. Robustness of the HPTLC method (n = 6, 1000 ng spot$^{-1}$)**

| Parameters | SD of peak area | % RSD | $R_f$ |
|---|---|---|---|
| Mobile phase composition ( ± 0.1 ml) | 0.77 | 0.0284 | 0.37 |
| Mobile phase volume ( ± 5%) | 1.13 | 0.0194 | 0.372 |
| Duration of saturation (10, 15 and 20 min) | 1.70 | 0.0213 | 0.369, 0.37, 0.371 |
| Time from spotting to chromatography ( ± 20 min) | 1.24 | 0.0412 | 0.37 |
| Time from chromatography to scanning ( ± 20 min) | 0.73 | 0.0327 | 0.371 |

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The standard deviation of peak areas was calculated for each parameter and RSD was found to be less than 2%. The low values of standard deviation (SD) and % RSD along with unchanged $R_f$ values of L-dopa (0.37 ± 0.021) obtained after introducing small deliberate changes in the method indicated the robustness of the developed HPTLC method (Table 3).

*LOD and LOQ*

The signal/noise ratios 3:1 and 10:1 were considered as LOD and LOQ, respectively. The LOD and LOQ were found to be 145 ng/spot and 400 ng/spot, respectively. This indicates the adequate sensitivity of the method.

*Specificity*

Comparing the spectra at peak start, peak apex and peak end positions of the spot assessed the peak purity of L-dopa. Good correlation ($r^2$ = 0.9992) was also obtained between standard and sample spectra of L-dopa.

*Accuracy*

The proposed method when used for estimation of L-dopa from pharmaceutical dosage form after spiking with 50, 100 and 150% of additional drug afforded recovery of 99–101% as listed in Table 4.

**Table 4. Results of % recovery and % RSD for accuracy testing of HPTLC analytical method for L-dopa (n = 6)**

| Excess drug added to the analyte (%) | Theoretical content (ng) | Recovery (%) | RSD (%) |
|---|---|---|---|
| 0 | 500 | 99.18 | 0.041 |
| 50 | 750 | 98.20 | 0.035 |
| 100 | 1000 | 98.92 | 0.028 |
| 150 | 1250 | 98.21 | 1.56 |

**Table 5. Results of validation parameters for estimation of L-dopa by HPTLC**

| Parameter | Data |
|---|---|
| Linearity range | 600-1400 ng/spot |
| Correlation coefficient (± SD) | 0.9992 ± 0.0084 |
| Limit of detection | 145 ng/spot |
| Limit of quantitation | 400 ng/spot |
| Accuracy (%, n = 6) | 98.60 ± 0.27 |
| Precision (%RSD) | |
| Repeatability of application (n = 7) | 0.034 |
| Repeatability of measurement (n = 7) | 0.018 |
| Inter-day (n = 6) | 0.0354 |
| Intra-day (n = 6) | 0.0183 |
| Robustness | Robust |
| Specificity | 0.9992 |

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## CONCLUSIONS

In conclusion, the proposed HPTLC method was found to be precise, specific, accurate and robust and can be used for identification and quantitative determination of L-dopa in herbal extract and its formulations. HPTLC method is especially suitable for the fingerprinting and high throughput analysis of botanical samples and herbal formulations.

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