

Application of high performance liquid chromatography to the determination and validation of levodopa in methanolic extract of *Mucuna utilis*

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A sensitive, precise and accurate high-performance liquid chromatographic (HPLC) method has been developed for the analysis of levodopa in methanolic extract of *Mucuna utilis*. The method utilises sample preparation step followed by separation on a Eurosphere C18, 250 × 4.0 mm, 5 µm particle size column, using methanol and 0.5% v/v acetic acid in the ratio of 70:30 v/v as the mobile phase. Analysis of levodopa was carried out in the absorbance mode at 284 nm. The method was validated in terms of linearity, precision (inter and intra day), accuracy, Limit of Detection (LOD) and Limit of Quantitation (LOQ). The proposed HPLC method was found to be precise, specific, accurate and can be used for the identification and quantitative determination of levodopa in herbal extracts.

Key words: High-performance liquid chromatography, levodopa, method development and validation, *Mucuna utilis*

INTRODUCTION

Indigenous herbs are used as remedies against various diseases in the traditional system of medicine or in ethnomedicinal practices. For the past few decades, compounds from natural sources have been gaining importance because of the vast chemical diversity that they offer, and high performance liquid chromatography (HPLC) has emerged as an important tool for qualitative, semi-quantitative and quantitative phytochemical analysis of herbal drugs and formulations.^[1-3]

Mucuna utilis (Leguminosae), commonly known as “the cowhage” or “velvet” bean and as “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. utilis* has been used for the management of male infertility and nervous disorders.^[4] Other parts of the plants also have medicinal use for various ailments, e.g. trichomes of pods are used in various anthelmintics and decoction of root in delirium. Leaves are useful in ulcers and seed powder contains high amount of levodopa, which is a neurotransmitter precursor and effective remedy for the relief in parkinson's diseases. *M. utilis* seed, in addition to levodopa, contains tryptamine, 5-hydroxytryptamine (5-HT), mucunine, mucunadine, prurienine and prurieninine. It is also rich in fatty content.^[5]

Literature survey reveals that the British Pharmacopoeia (B.P.) describes a non-aqueous titration for the determination of levodopa.^[6] The United States Pharmacopeia (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.^[7] Determination of levodopa and biogenic amines in urine samples using HPLC^[8] and determination of levodopa by capillary zone electrophoresis using an acidic phosphate buffer and its application in the analysis of beans^[9] have been reported. Studies have also been done on the rate constant of levodopa methyl ester hydrolysis by LC.^[10] Assay of tyrosine hydroxylase based on HPLC separation and quantification of L-dopa and L-tyrosine have been reported.^[11] Determination, purity assessment and chiral separation of levodopa methyl ester in bulk and formulation have been done.^[12] Studies have also been carried out on the antioxidant activities of mucuna seed (*Mucuna pruriens* var. *utilis*) extract and various non-protein amino acids through *in vitro* models.^[13] No HPLC method for the analysis of levodopa in methanolic extract of *M. utilis* has been reported so far. In the present investigation, efforts have been made to develop an accurate and precise method for the analysis of levodopa in methanolic extract of *M. utilis*.

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MATERIALS AND METHODS

Chemicals and Reagents

The authenticated sample of plant seed material of *M. utilis* Linn. Leguminosae (voucher specimen no. NISCAIR/RHMD/Consult/-2008-09/1030/61) and standard levodopa were obtained from Torrent Research Center, Ahmedabad, India. Methanol of HPLC grade was purchased from E. Merck, Mumbai. Other chemicals and reagents used were of analytical grade. The water used was of Milli-Q grade purified by a Milli-Q UV purification system (Millipore, Bedford, MA, USA).

Preparation of Standard Solutions

About 10 mg of levodopa working standard was weighed accurately into a 10-ml volumetric flask, dissolved and diluted to volume with 0.1 M hydrochloric acid to obtain a solution of 1000 µg/ml. Further dilutions were made to get the solutions in the concentration range of 10–500 µg/ml.

Preparation of the Plant Extract Solution

Coarse powder of the dried seeds of *M. utilis* was extracted with methanol using a soxhlet apparatus. The methanol extract thus obtained was dried under reduced pressure at a temperature not exceeding 40°C. About 350 µg of the extract was diluted to 25 ml with 0.1 M hydrochloric acid. Further, 5 ml of this solution was diluted to 100 ml with 0.1 M hydrochloric acid and sonicated for about 60 min. The solution was then filtered and used for estimation.

Chromatographic Conditions

The following chromatographic conditions were used to quantify the levodopa.

Stationary phase: Eurosphare, C₁₈, 250 × 4.0 mm

Column oven temperature: 30°C

Mobile phase: methanol:0.5% v/v of acetic acid (70:30, v/v)

Detection wavelength: 284 nm

Flow rate: 1.2 ml/min

Injection volume: 20 µl

Assay

The percentage of levodopa present in methanolic extract of *M. utilis* was calculated by comparison of the areas measured for the standard and sample solution.

RESULTS AND DISCUSSION

The developed method was validated for the assay of levodopa as per ICH guidelines.^[14]

Specificity

Specificity was studied for the examination of the presence of interfering components. Levodopa standard solution of 100 µg/ml was injected and none of the impurities were interfering in its assay. The retention time obtained by HPLC for levodopa is about 2.44 min as shown in Figure 1.

Linearity

Linearity was studied by preparing standard solutions of levodopa at different concentration levels. The linearity was found in the range of 10–500 µg/ml. The standard calibration curve was generated using regression analysis with Microsoft excel. The assay was judged to be linear as the correlation coefficient was greater than 0.995 by the least-square method.

Accuracy

Recovery studies of the drug were carried out by the accuracy parameter at three different concentration levels, i.e. multiple level recovery studies. A known amount of levodopa standard was added into pre-analysed sample

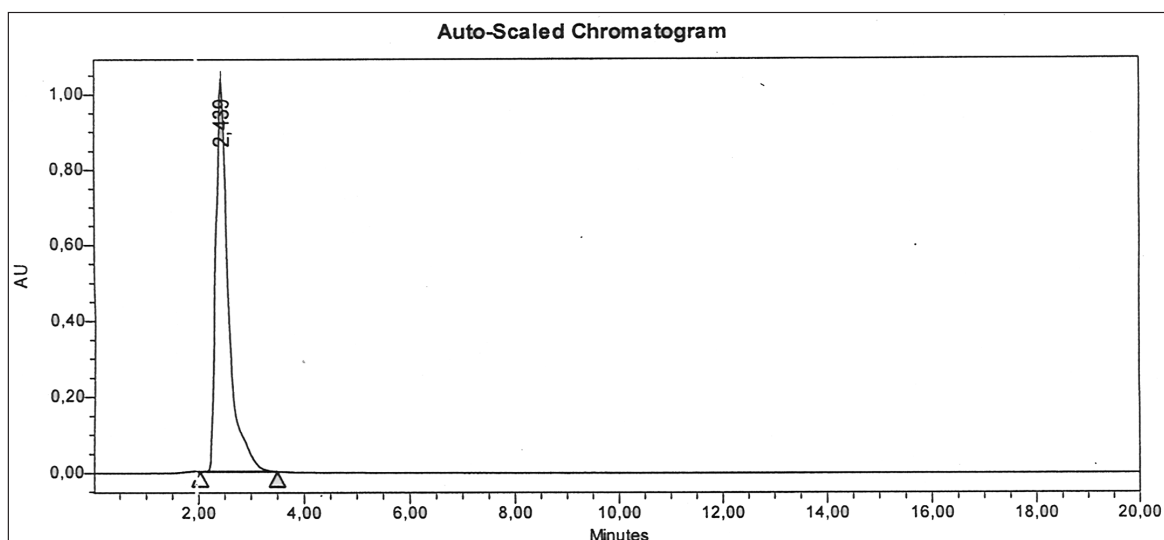


Figure 1: A typical chromatogram of levodopa standard

Table 1: Validation parameters for quantification of levodopa by HPLC

Parameters	Results
Precision (% RSD)	<2%
Linearity	10–500 µg/ml
Limit of detection	0.690 µg/ml
Limit of quantitation	2.092 µg/ml
Accuracy	98.67–100.4%
Assay	9.91% w/v

and subjected to the proposed HPLC method. Percentage recovery was found to be within the limits as listed in Table 1.

Precision

Precision was studied to find out intra and inter day variations in the test methods of levodopa in the concentration range of 10–500 µg/ml for three times on the same day and on different days. Precision was determined by analysing corresponding standard daily for a period of 3 days. The inter and intra-day precision obtained was given as %RSD. A value of <2 indicates that the proposed method is quite precise and reproducible.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) was calculated based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ, $LOD = 3.3 (SD/S)$ and $LOQ = 10 (SD/S)$ is shown in Table 1.

CONCLUSION

An analytical reverse-phase HPLC method was developed and validated for the determination of purity and assay of levodopa. The developed method is sensitive, simple, and accurate, and can be employed for monitoring the purity of

levodopa in herbal extracts. The method has been proved to be selective and stable.

REFERENCES

1. Modi KP, Patel NM, Goyal RK. Estimation of L-Dopa from *Mucuna pruriens* LINN and formulations containing *M. pruriens* by HPTLC method. *Chem Pharm Bull* 2008;56:357-9.
2. Rajeshwar Y, Gupta M, Mazumadar UK. In vitro lipid peroxidation and antimicrobial activity of *Mucuna pruriens* seeds. *Iranian J Pharmacol Ther* 2005;4:32-5.
3. Salau A, Odeleye. Antimicrobial activity of *Mucuna pruriens* on selected bacteria. *Afr J Biotechnol* 2007;6:2091-2.
4. Sathiyarayanan L, Arulmozhi S. A comprehensive review of *Mucuna pruriens*. *Pharmacogn Rev* 2007;1:157-62.
5. Anonymous. The wealth of India. New Delhi: PID, CSIR; 1962. p. 439-44.
6. Anonymous. British Pharmacopoeia. Vol. 1 and 2. London: HMSO Publication Center; 1980. p. 254, 535, 781.
7. Anonymous. The United State Pharmacopoeia, XXI, 21st Revision. Rockville: The US Pharmaceutical Convention Inc.; 1987. p. 585-6.
8. Baranowska I, Płonka J. Determination of levodopa and biogenic amines in urine samples using high-performance liquid chromatography. *J Chromatogr Sci* 2008;46:30-4.
9. Chen, Jiyong Z, Honglin Z, Zhidong H. Determination of levodopa by capillary zone electrophoresis using an acidic phosphate buffer and its application in the analysis of beans. *J Food Chem* 2005;92:381-6.
10. Wang J, Zhou Y, Fang YZ. Studies of the rate constant of levodopa methyl ester hydrolysis by LC. *J Chromatographia* 2005;62:423-8.
11. Olsovska J, Novotna J, Flieger M, Spizek J. Assay of tyrosine hydroxylase based on HPLC separation and quantification of L-Dopa and L-Tyrosine. *J Biomed Chromatogr* 2007;21:1252-8.
12. Wang J, Fang Y. Determination, purity assessment and chiral separation of levodopa methyl ester in bulk and formulation pharmaceuticals. *J Biomed Chromatogr* 2006;20:904-10.
13. Siddhuraju P, Becker K. Studies on antioxidant activities of *Mucuna* seed (*Mucuna pruriens* var *utilis*) extract and various non-protein amino acids through in vitro models. *J Sci Food Agric* 2003;83: 1517-24.
14. Validation of Analytical Procedures, Methodology, ICH harmonized tripartite guidelines, 1996. p. 1.

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