Quantitative Determination of L-DOPA in Dietary Supplements Containing *Mucuna pruriens* by High Performance Liquid Chromatography

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Summary

We have developed a simple and rapid high performance liquid chromatography (HPLC) method for the quantification of L-DOPA in dietary supplements containing *Mucuna pruriens*. Acetonitrile/water/formic acid (50:50:1) was used as the extraction solvent and the extracts obtained were analyzed by HPLC using a hydrophilic interaction chromatography (HILIC) column. The mobile phase was 10 mmol/l ammonium formate buffer (pH 3.5)/acetonitrile (3:7) and the ultraviolet (UV) detector was set at 280 nm. The recovery was 100.8%, and relative standard deviation (RSD) values of the repeatability and intermediate precision were less than 8%. The correlation coefficient was 1.0000 and the limit of quantification of L-DOPA was 100 μg/g. We used this method to determine the L-DOPA content in 14 commercial dietary supplements (capsules and tablets) containing *M. pruriens*, and found the L-DOPA content to range from 0.71 to 9.13 mg/unit.

Key words: L-DOPA, *Mucuna pruriens*, HPLC, dietary supplement, HILIC

Introduction

Mucuna pruriens (commonly known as cowhage, velvet beans, and *hassho-mame* in Japan) is an indigenous climbing legume in India and other parts of the tropics including Central and South America. *M. pruriens* seeds are used for male infertility and nervous disorders, and as an aphrodisiac in Ayurveda. *M. pruriens* seeds contain 3–6% L-3,4-dihydroxyphenyl-alanine (L-DOPA, levodopa)[1)] (Fig. 1), a pharmaceutical compound used for the treatment of Parkinson's disease[2–3)]. In fact, a powder formulation of *M. pruriens* seeds is used for the treatment of Parkinson's disease[4)].

In recent years, with the increase in health consciousness among individuals, the consumption of dietary supplements has increased. A case of sudden death associated with the ingestion of a dietary supplement containing guarana was reported[5)]. *M. pruriens* is also used in dietary supplements that appealed to have a stimulating effect. Because of the health risks associated with the intake of dietary supplements containing *M. pruriens*, it is important that the L-DOPA content in these products be determined.

A rapid reverse-phase high performance liquid chromatography (HPLC) method for the quantification of L-DOPA and non-methylated and methylated tetrahydroisoquinoline compounds present in mucuna beans was repoterd[6)]. High-performance thin-layer chromatography was used to determine the L-DOPA content in tablets[7)] and formulations containing *M. pruriens*[8)]. The HPLC determination of L-DOPA in dietary supplements has not been reported yet, as far as we know. In this study, we established a simple and rapid HPLC method for the determination of L-DOPA in dietary supplements containing *M. pruriens* and applied this method to determine the L-DOPA content in commercial dietary supplements.

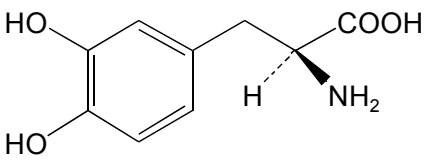

Fig. 1.　Structure of L-DOPA

Material and Methods

Standard and reagents

Standard L-DOPA was purchased from Alfa Aesar (MA, USA). HPLC-grade acetonitrile and all other reagents (analytical grade) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Standard solution

A stock standard solution (1000 μg/mL) was prepared by dissolving 20 mg of standard L-DOPA in 20 mL acetonitrile/water/formic acid (50:50:1). Working standard solutions were prepared by diluting the stock solution with acetonitrile/water/formic acid (50:50:1) in the concentration range of 0.5–100 μg/mL.

Sample

Fourteen dietary supplements that were analyzed were purchased over the internet. According to the labels, these products (tablets and capsules) contained *M. pruriens*. *M. prurien* seeds (stock No. 55132) were obtained from the Genebank of the National Institute of Agrobiological Sciences.

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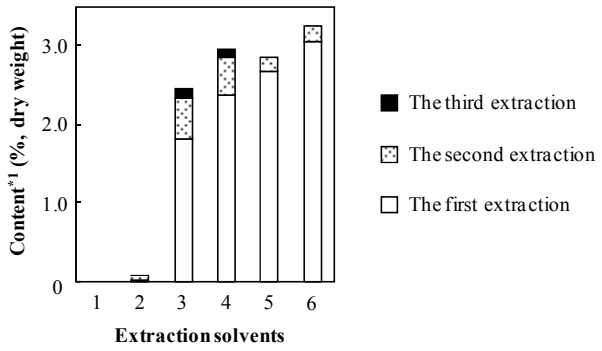

Fig. 2. Extraction behavior of L-DOPA in *Mucuna pruriens* seed sample for the 6 extraction solvents

1: acetonitrile
2: acetonitrile/water (80:20)
3: acetonitrile/water (50:50)
4: acetonitrile/formic acid (100:1)
5: acetonitrile/water/formic acid (80:20:1)
6: acetonitrile/water/formic acid (50:50:1)
*1 (n=1)

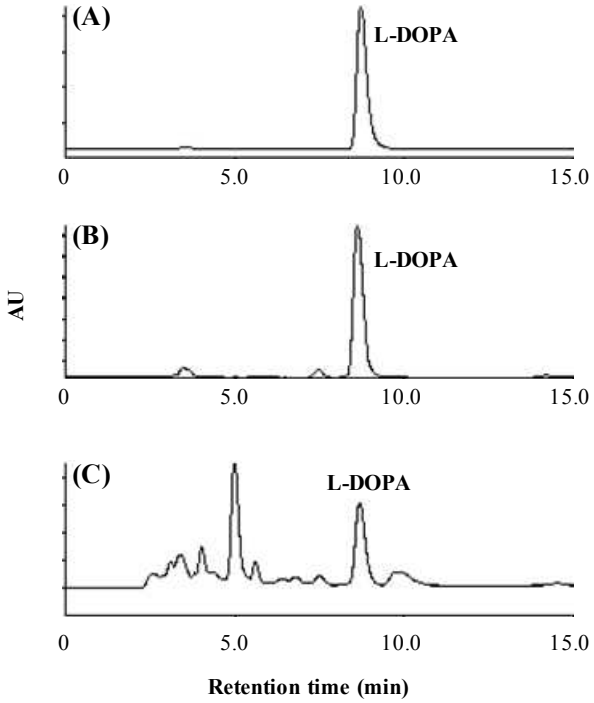

Fig. 3. Chromatograms of (A) standard solution (100 μg/ml), (B) *Mucuna pruriens* seed extract (diluted 10-fold), and (C) typical sample extract (sample No. 6)

Preparation of sample extract

The tablets, the contents of the capsules, and whole seeds of *M. pruriens* were finely powdered using a grinder. One hundred mg of this powder was transferred into a 10-mL test tube, and 5 mL acetonitrile/water/formic acid (50:50:1) was added to it. This mixture was ultrasonically extracted for 15 min. After centrifuged at 1,300 × *g* for 10 min, the supernatant was transferred to a 20-mL volumetric flask. The precipitate was reextracted with 5 mL acetonitrile/water/formic acid (50:50:1) under the same conditions and centrifuged. The supernatants collected during the extractions were combined and the volume was adjusted to 20 mL with acetonitrile/water/formic acid (50:50:1). A portion of this solution was filtered through a 0.45-μm polytetrafluoroethylene membrane filter (Toyo Roshi Kaisha, Tokyo, Japan). This filtrate was diluted 10-fold with acetonitrile/water/formic acid (50:50:1), when required.

HPLC analysis

HPLC was performed using a PU-2089 apparatus equipped with a ultraviolet (UV) detector (model UV-970; JASCO Corporation, Tokyo, Japan). A TSK-GEL Amide-80 column (250 × 4.6 mm i. d.; 5 μm; Tosoh Co., Tokyo, Japan) was used. The mobile phase was 10 mmol/l ammonium formate buffer (pH 3.5)/acetonitrile (3:7). The flow rate of the mobile phase was set at 1.0 mL/min, and the injection volume was 20 μl. The column temperature was maintained at 40°C. The UV detector was set at 280 nm.

Results and Discussion

Evaluation of the extraction method

In order to identify a suitable extraction solvent, acetonitrile, acetonitrile/water (80:20), acetonitrile/water (50:50), acetonitrile/formic acid (100:1), acetonitrile/water/ formic acid (80:20:1), and acetonitrile/water/formic acid (50:50:1) were investigated. Five milliliters of each of these solvents was added into six 10-mL test tubes; 100 mg of *M. pruriens* seed powder was transferred to each of these test tubes. The mixtures were ultrasonically extracted for 15 min. The supernatants were obtained by centrifugation (1,300 × *g* for 10 min). The precipitates were reextracted twice with 5 mL of the corresponding solvent and centrifuged. The supernatants obtained from the 3 extractions for each of the 6 different solvents were filtered, and the filtrates were analyzed by HPLC. Figure 2 shows the extraction behavior of L-DOPA in *M. pruriens* seed powder for the 6 different solvents. The amount of L-DOPA extracted with acetonitrile and acetonitrile/formic acid (100:1) was the least. Acetonitrile/water/formic acid (50:50:1) was found to be the most effective extraction solvent and L-DOPA was completely extracted after the second extraction was performed. Therefore, twice ultrasonic extraction with acetonitrile/water/formic acid (50:50:1) were applied

Table 1. Recovery and precision of L-DOPA from spiked sample

Spiked amount (mg/g)	Recovery[a] (%)	Repeatability (%RSD)	Intermediate precision (%RSD)
2	100.8	7.23	7.50

a) Means of 10 replicates

Table 2. L-DOPA content in commercial dietary supplements containing *M. pruriens*

Sample No.	Dosage form	Content[a] (mg/unit)	Indicated maximum dosage (unit/day)	Calculated maximum intake (mg/day)
1	Capsule	2.79	5	13.9
2	Tablet	4.53	4	18.1
3	Softgel	0.71	3	2.12
4	Tablet	6.44	8	51.5
5	Tablet	9.13	6	54.8
6	Tablet	0.91	8	7.29
7	Tablet	3.12	6	18.7
8	Capsule	2.49	5	12.4
9	Tablet	0.82	6	4.94
10	Tablet	3.34	2	6.69
11	Tablet	1.27	8	10.2
12	Capsule	5.80	6	34.8
13	Capsule	3.00	2	5.99
14	Capsule	5.88	2	11.8

a) Values are means (n=3)

in further analysis.

HPLC analysis

An octadecylsilyl column was used for L-DOPA analysis according to a previously described HPLC method for the quantification of L-DOPA in *Mucuna* beans[6]. From the result of peak purity analysis with diode array detector, L-DOPA was co-eluted with interfering components from the sample solution (data not shown). Therefore, a hydrophilic interaction chromatography (HILIC) column was used for the analysis. The chromatograms of the standard solution and *M. pruriens* seed sample, and a typical chromatogram of sample extract (sample No. 6) are shown in Fig. 3. L-DOPA was eluted at approximately 8.5 min, and interference on the chromatogram for the *M. pruriens* seed sample and the sample extracts was not observed. The standard calibration curve of L-DOPA was good in the range of 0.5–100 μg/mL. The correlation coefficient was 1.0000. The limit of quantification of L-DOPA was 100 μg/g (S/N=10).

Recovery and precision

According to the Japanese method validation guideline[9], the validation of this quantification method was evaluated by analyzing a known amount of standard L-DOPA (2 mg/g) spiked to 100 mg of a pre-analyzed sample in duplicate on 5 different days. The recovery was found to be 100.8%, and relative standard deviation (RSD) values of the repeatability and intermediate precision were less than 8% (Table 1). These results suggest that good accuracy and precision can be obtained using this method.

Determination of L-DOPA content in *M. pruriens* seeds and commercial dietary supplements

The L-DOPA content in *M. pruriens* seeds was 3.26% (dry weight), and the L-DOPA content in the 14 commercial dietary supplements ranged from 0.71 to 9.13 mg/unit (Table 2). The maximum intake of L-DOPA per day was calculated from the maximum dosage indicated on the package of each product. The maximum intake was found to range from 2.12 to 54.8 mg/day. The initial dosage of levodopa (L-DOPA) ranges from

0.2 to 0.6 g/day divided over 1, 2, or 3 doses[10]. As per the dosage mentioned on the package, if an individual took 6 tablets of sample No. 5, the amount of L-DOPA ingested would be one-fourth of the minimum L-DOPA dosage. Thus, there are health risks associated with the intake of dietary supplements containing *M. pruriens*. Therefore, it is important that the L-DOPA content in dietary supplements be monitored.

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高速液体クロマトグラフィーによるムクナ含有健康食品中の L-DOPA 定量分析

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要 旨

高速液体クロマトグラフィー（HPLC）によるムクナ含有健康食品中の L-DOPA 定量法を構築した。抽出溶媒にはアセトニトリル/水/ギ酸（50:50:1）を用い抽出を行い HPLC で分析を行った。カラムは親水性相互作用クロマトグラフィー（HILIC）カラムを用い、移動相は 10 mmol/l ギ酸アンモニウム緩衝液（pH 3.5）/アセトニトリル（3:7）を用い、UV 検出器の定量波長は 280 nm を用いた。添加回収試験の結果、回収率は 100.8%であり、併行精度及び室内再現精度は 8%以下であった。また、検量線の相関係数は 1.0000 であり、定量下限は 100 μg/g であった。本分析法を市販の健康食品 14 製品に適用した結果、1 カプセル又は錠あたりの L-DOPA 含有量は 0.71－9.13 mg であった。

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