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 1.000 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-dihydroxyphenylalanine (L-DOPA, levodopa)\(^1\) (Fig. 1), a pharmacetical 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 reported\(^6\). 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.

**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. pruriens* seeds (stock No. 55132) were obtained from the Genebank of the National Institute of Agrobiological Sciences.

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1) Formerly Chiba Prefectural Institute of Public Health
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 \(\times\) 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-\(\mu\)m 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 an ultraviolet (UV) detector (model UV-970; JASCO Corporation, Tokyo, Japan). A TSK-GEL Amide-80 column (250 \(\times\) 4.6 mm i. d.; 5 \(\mu\)m; Tosoh Co., Tokyo, Japan) was used. The mobile phase was 10 mmol/1 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 \(\mu\)L. 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 were 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 \(\times\) 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.
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. 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, 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

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

<table>
<thead>
<tr>
<th>Spiked amount (mg/g)</th>
<th>Recovery* (%)</th>
<th>Repeatability (%RSD)</th>
<th>Intermediate precision (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>100.8</td>
<td>7.23</td>
<td>7.50</td>
</tr>
</tbody>
</table>

a) Means of 10 replicates

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

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

a) Values are means (n=3)
0.2 to 0.6 g/day divided over 1, 2, or 3 doses. 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.

**References**