**Piper methysticum Rootstock Dry Extract**

**Proposed For Development Version 0.1**

*Piper methysticum* Rootstock Dry Extract

**DEFINITION**

The article prepared from the dried rootstock (rhizome) of *Piper methysticum* G. Forst (Family Piperaceae) by extraction with hydroalcoholic mixtures. The ratio of the starting crude plant material to native extract is between 6:1 and 10:1. It contains not less than 90% and not more than 110% of the labeled amount of kavalactones, calculated as the sum of methysticin, dihydromethysticin, kawain [1], dihydrokawain, yangonin, and desmethoxyyangonin on the dried basis. It contains labeled added substances as carriers.

**POTENTIAL CONFOUNDING MATERIALS**

*Piper wichmannii* C.DC.

*Piper methysticum* var. *wichmannii* (C.DC) Lebot.

**CONSTITUENTS OF INTEREST**

**Kavalactones:** Kawain [1](kavain), dihydrokawain, methysticin, dihydromethysticin, yangonin, and demethoxyyangonin

**Chalconoides:** Flavokavain A-C

**IDENTIFICATION**

- **A. Thin-Layer Chromatography**
  
  **Standard solution A:** 0.5 mg/mL of USP Kawain RS [1] in toluene
  
  **Standard solution B:** 50 mg/mL of USP Powdered Kava Extract RS [2] in methanol. Sonicate for 10 min, centrifuge and use supernatant.
  
  **Sample solution:** Mix about 0.25 g of *Piper methysticum* Rootstock Dry Extract with 5 mL of methanol and sonicate for 10 min. Centrifuge and use supernatant.

  **Chromatographic system**
  
  (See Chromatography <621>, Thin-Layer Chromatography.)
  
  **Adsorbent:** Use a suitable chromatographic material with an average particle size of 5 µm (HPTLC plates, Si 60 F<sub>254</sub>).
  
  **Application volume:** 2 µL as 8-mm bands
  
  **Relative Humidity:** Condition the plate to a relative humidity of about 33% using a suitable device
  
  **Developing solvent system:** tert-Butyl ether and cyclohexane (70:30)
  
  **Developing distance:** 6 cm
  
  **Derivatization reagent:** Anisaldehyde reagent: a mixture of 170 mL of ice-cooled methanol with 20 mL of glacial acetic acid, 10 mL of sulfuric acid, and 1 mL of anisaldehyde

  **Analysis**
  
  **Samples:** Standard solution A, Standard solution B, and Sample solution

  Dip the plate in the Reagent for impregnation. Dry at the room temperature for 5 min, then heat at 80° for 5 min. Apply the Samples as bands to a suitable HPTLC plates. Develop the chromatogram in an unsaturated chamber, remove the plate from the chamber, and dry. Treat with Derivatization reagent, heat at 100° for 4 min, and examine under UV 366 and visible light.

  **System suitability:** Under UV 366 nm, in the lower half of the chromatogram, the Standard solution B exhibits an orange band corresponding to kawain similar in R<sub>f</sub> and color to the band observed in the chromatogram of Standard solution A; below kawain, with decreasing R<sub>f</sub>, a bright blue fluorescent band and a yellow/orange band due to yangonin and methysticin, respectively; above kawain, a pale blue band corresponding to desmethoxyyangonin; one yellowish green band above desmethoxyyangonin band and one bright blue band below methysticin band. In upper half of the chromatogram, with increasing R<sub>f</sub>, a green, a pale yellow, a black, a white, and an orange bands appear.

  Under visible light, in the lower half of the chromatogram, the Standard solution B exhibits a red band corresponding to kawain similar in R<sub>f</sub> and color to the band observed in the chromatogram of Standard solution A; below kawain, with decreasing R<sub>f</sub>, a brown band due to methysticin. Above kawain band, with increasing R<sub>f</sub>, three yellow and one purple bands appear.

  **Acceptance criteria:** Under UV 366 nm, in the lower half of the chromatogram, the Sample solution exhibits an orange band corresponding to kawain similar in R<sub>f</sub> and color to the band observed in the chromatogram of Standard solution A; below kawain, with
decreasing \( R_f \), a bright blue fluorescent band and a yellow/orange band due to yangonin and methysticin, respectively; above kawain, a pale blue band corresponding to desmethoxyyangonin; one yellowish green band above desmethoxyyangonin band and one bright blue band below methysticin band. In upper half of the chromatogram, with increasing \( R_f \), a green, a pale yellow, a black, a white, and an orange bands appear.

Under visible light, in the lower half of the chromatogram, the Standard solution B exhibits a red band corresponding to kawain similar in \( R_f \) and color to the band observed in the chromatogram of Standard solution A; below kawain, with decreasing \( R_f \), a brown band due to methysticin. Above kawain band, with increasing \( R_f \), three yellow and one purple bands appear.

**B. HPLC**

**Analysis:** Proceed as directed in the Assay for Content of Kavalactones

**Acceptance criteria:** The chromatogram of the Sample solution exhibits peaks at the retention times corresponding to the peaks due to methysticin, dihydromethysticin, kawain, dihydrokawain, yangonin, and demethoxyyangonin of the Standard solution B.

**ASSAY**

- **Content of Kavalactones**

  **Mobile phase:** 0.1% phosphoric acid, acetonitrile, and isopropyl alcohol (64:20:16).

  **Solvent:** Methanol and water (70:30)

  **Standard solution A:** 0.2 mg/mL of USP Kawain RS [1] in methanol

  **Standard solution B:** Transfer a quantity of USP powdered Kava Extract RS [2], accurately weighed, to a suitable volumetric flask, and dilute with solvent to obtain a solution having known concentration of about 0.2 mg of kawain per mL, sonicate to dissolve. Pass through a membrane filter of 0.45-μm of pore size before injection.

  **Sample solution:** Transfer about 125 mg of *Piper methysticum* Rootstock Dry Extract, accurately weighed, to a 50-mL volumetric flask, dilute with mixture of methanol and water (70:30) to volume, and sonicate for 60 min at room temperature. Decant, and pass through a 0.45-μm nylon membrane filter.

**Chromatographic system**

(See Chromatography <621>, System Suitability.)

- **Mode:** LC
- **Detector:** UV 220 nm
- **Column:** 4.6-mm × 25-cm; L7 (similar to Waters YMCbasic S-5)
- **Flow rate:** 0.6 mL/min
- **Injection volume:** 5 μL

**System suitability**

  **Sample:** Standard solution A and Standard solution B

  **Suitability requirements**

  - **Chromatogram similarity:** The chromatogram of Standard solution B is similar to the reference chromatogram provided with the lot of USP Powdered Kava Extract RS [2] being used.
  - **Resolution:** NLT 1.8 between desmethoxyyangonin and yangonin
  - **Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** Standard solution A, Standard solution B and Sample solution

Using the chromatograms of Standard solution A and Standard solution B, and the reference chromatogram provided with the lot of USP Powdered Kava Extract RS being used, identify the retention time of the peaks corresponding to methysticin, dihydromethysticin, kawain, dihydrokawain, yangonin, and desmethoxyyangonin in the Sample solution.

Separately calculate the percentage of methysticin, dihydromethysticin, kawain, dihydrokawain, yangonin, and desmethoxyyangonin in the portion of *Piper methysticum* Rootstock Dry Extract taken:

\[
\text{Result} = (r_0/r_s) \times (C_s/W) \times 5000 \times F
\]

- \( r_0 \) = response from the Sample solution
- \( r_s \) = response from the Standard solution
- \( C_s \) = concentration of the Standard solution A (mg/mL)
- \( W \) = weight of *Piper methysticum* Powder taken to prepare the Sample solution, (mg)
- \( F \) = conversion factor for the analytes as provided in Table 1

**Table 1**
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Approximate relative retention time</th>
<th>Conversion factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methysticin</td>
<td>0.80</td>
<td>0.66</td>
</tr>
<tr>
<td>Dihydromethysticin</td>
<td>0.94</td>
<td>1.65</td>
</tr>
<tr>
<td>Kawain [1]</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Dihydrokawain</td>
<td>1.09</td>
<td>1.70</td>
</tr>
<tr>
<td>Yangonin</td>
<td>1.20</td>
<td>0.88</td>
</tr>
<tr>
<td>Desmethoxyyangonin</td>
<td>1.31</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Calculate the percentage of the labeled amount of kavalactones in the Dry Extract.

\[
\text{Result} = (P/L) \times 100
\]

\[P = \text{content of kavalactones as determined above (%)\]}
\[L = \text{labeled amount of kavalactones (%)}\]

**Acceptance criteria:** 90.0%-110.0% on the dried basis

**CONTAMINANTS**

- **Elemental Impurities—Procedures <233>**
- **Acceptance criteria**
  - Arsenic: NMT 2.0 µg/g
  - Cadmium: NMT 0.5 µg/g
  - Lead: NMT 5.0 µg/g
  - Mercury: NMT 0.1 µg/g

- **Articles of Botanical Origin, General Method for Pesticide Residues Analysis <561>:** Meets the requirements
- **Microbial Enumeration Tests <61>:** The total aerobic bacterial count does not exceed \(10^5\) cfu/g, the total combined molds and yeasts count does not exceed \(10^3\) cfu/g, and the bile-tolerant Gram-negative bacteria does not exceed \(10^3\) cfu/g.
- **Tests for Specified Microorganisms <2022>:** Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*
- **Articles of Botanical Origin, Test for Aflatoxins <561>:** Meet the requirements

**SPECIFIC TESTS**

- **Loss on Drying <731>:**
  - Analysis: Dry 1 g *Piper methysticum* Rootstock Powder at 105° for 2 h.
  - **Acceptance criteria:** NMT 7%

**ADDITIONAL REQUIREMENTS**

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling:** The label states the Latin binomial and the part(s) of the plant contained in the article.
- **USP Reference Standards <11> [3]**
  - **USP Aflatoxins RS [4]**
  - **USP Kawain RS [1]**
  - **USP Powdered Kava Extract RS [2]**

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