**Analysis of *Aristolochia fangchii* root Extract Using LC-QToF-MS method**

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Analysis date: December 13, 2021

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| --- | --- | --- | --- | --- |
| Common Botanical Name | CAS No. | Lot No. | Container ID | Net Weight |
| ***Aristolochia fangchii*** |  | RK-3-27-1-AF-C  RK-3-27-1-AF-D |  |  |

|  |
| --- |
| Sample storage condition until analysis |
| **2-8°C** |

**Quantitative/Targeted method:**

|  |  |
| --- | --- |
| **UHPLC-PDA Method** | |
| UHPLC conditions |  |
| System:Waters H-Class  Column: Acquity BEH Sheild RP18 (2.1 X 100mm, 1.7µm) (Waters corporation, MA, USA)  Mobile phase A: Water+0.1% formic acid  Mobile phase B: Acetonitrile +0.1% formic acid  Flow rate: 0.20 mL/min  Column temperature: 40°C  Gradient   |  |  | | --- | --- | | Time (min) | Mobile phase B (%) | | 0.0 | 30 | | 6.5 | 30 | | 34.0 | 43 | | 50.0 | 65 | | 55.0 | 100 | | |  |  | | --- | --- | | Compound | Retention time (min) | | Aristolochic acid C | 19.8 | | Aristolochic acid II | 29.3 | | Aristolochic acid I | 37.7 | |

**Quantitative results**

|  |  |  |
| --- | --- | --- |
| **Compound** | **Concentration in extract (mg/g)**  **RK-3-27-1-AF-C** | **Concentration in extract (mg/g)**  **RK-3-27-1-AF-D** |
| Aristolochic acid C | 0.6mg/g | 0.7mg/g |
| Aristolochic acid II | 3.0 mg/g | 3.0 mg/g |
| Aristolochic acid I | 76.0 mg/g | 76.0 mg/g |

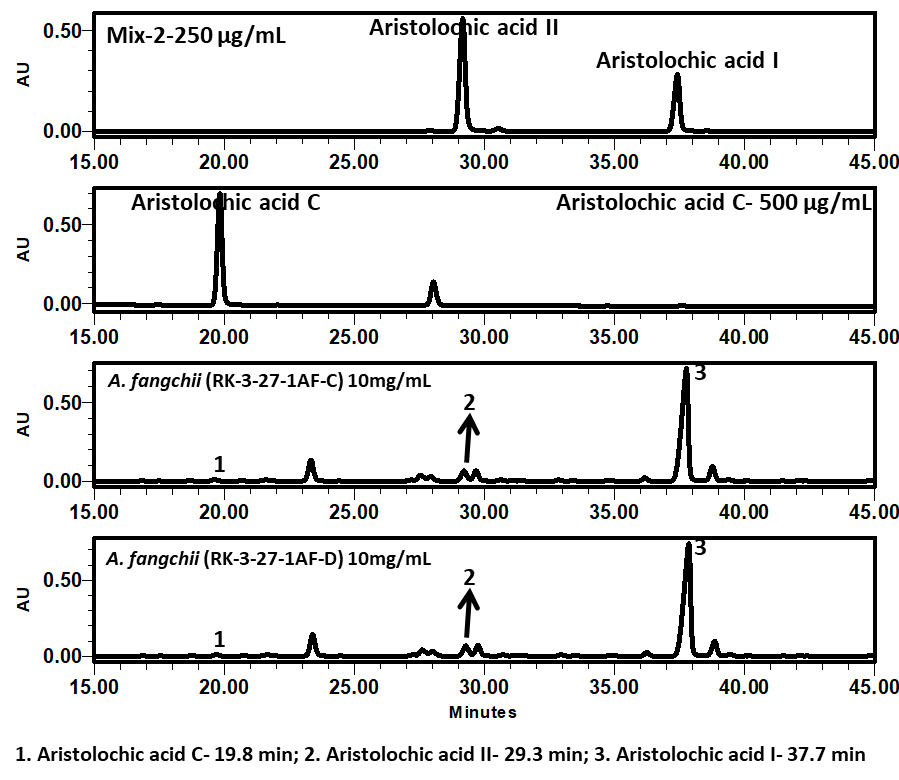
**Standards**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Supplier** | **CAS Number** | **Catalog number** | **Purity %** |
| Aristolochic acid C | Millipore Sigma | 4849-90-5 | PHL80355 | 80% |
| Aristolochic acid II | Millipore Sigma | 475-80-9 | PHL89566 | 97% |
| Aristolochic acid I | Millipore Sigma | 313-67-7 | PHL89565 | 99% |

**Chemical structures of standards used for quantitative analysis**



**Chromatograms**

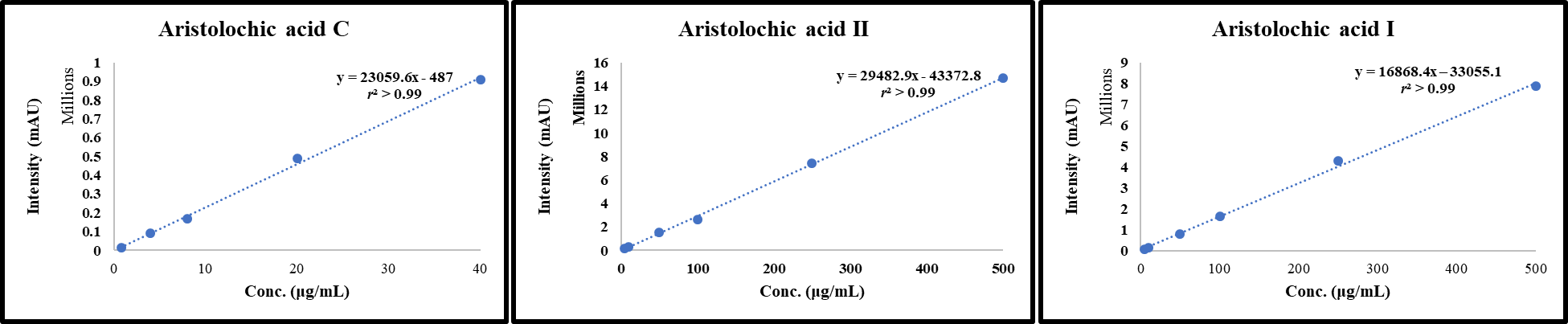
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**Ref.:** Schaneberg, B.T. and Khan, I.A., 2004. Analysis of products suspected of containing Aristolochia or Asarum species. *Journal of Ethnopharmacology*, *94*(2-3), pp.245-249.

**Calibration**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Compound | Limit of detection (LOD) ng/mL | Limit of quantitation (LOQ) ng/mL | Calibration range (mg/g) | Number of Calibration Points | *r*2 | Concentration  mg/g (n = 3)  RK-3-27-1-AF-C | Concentration  mg/g (n = 3)  RK-3-27-1-AF-D |
| Aristolochic acid C | 50 ng/mL | 100 ng/mL | 0.8 – 40μg/mL | 5 | 0.9998 | 0.6 ± 0.34 | 0.7 ± 0.40 |
| Aristolochic acid II | 50 ng/mL | 100 ng/mL | 5.0 – 500μg/mL | 5 | 0.9999 | 2.7 ± 0.21 | 2.8 ± 1.87 |
| Aristolochic acid I | 50 ng/mL | 100 ng/mL | 5.0 – 500μg/mL | 5 | 0.9999 | 75.5 ± 0.13 | 77.0 ± 3.50 |

**Linearity profiles**

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**Untargeted Analysis**

|  |  |
| --- | --- |
|  | |
| UHPLC conditions | Mass spectrometer conditions |
| System: Agilent 1290 series  Column: Poroshell 120 EC-C18 (2.1 X 150mm, 2.7µm) (Agilent technologies, Palo Alto, CA, USA)  Mobile phase A: Water+0.1% formic acid  Mobile phase B: Acetonitrile +0.1% formic acid  Flow rate: 0.20 mL/min  Column temperature: 35°C  Gradient:   |  |  | | --- | --- | | Time (min) | Mobile phase B (%) | | 0.0 | 01 | | 3.0 | 01 | | 30.0 | 45 | | 50.0 | 100 | | System: QToF-MS 6530A series (Agilent technologies, Palo Alto, CA, USA)  Ionization: ESI  Polarity: Positive mode/Negative mode  Main Interface:  · Nebulizing gas flow: 11 L/min  . Gas temperature: 325°C/300°C  . Nebulizer: 30 psig  . Sheath gas temperature: 300°C  . Sheath gas flow: 11L/min  · Capillary voltage: 3.5 kV  · Fragmentor: 100V/175V |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Table 1: Proposed identification of constituents of *Aristolochiafangchii* root extract showing RT, *m/z*, ppm, tentative compound name and molecular formula** | | | | | |
| Peak No. | RT (min) | Exp. *m/z* | Mass accuracy  (ppm) | Proposed ID’s  (Confirmed with Std in **green**)  (Most probable ID is **yellow**) | Molecular Formula |
| 1 | 15.8 | 342.1703 [M]+ | 0.6 | **Magnoflorine& Isomer** | C20H24NO4+ |
| 2 | 16.8 |
| 3 | 16.4 | 330.1703  [M]+ | 0.6 | **Sinomenine/Isosinomenine** | C19H24NO4+ |
| 4 | 18.8 | 442.1131  [M+H]+ | 0.5 | **Aristolactam-Ia-*N-β-D*-glucopyranoside/**  **Aristolactam-IIIa-*N-β-D*-glucopyranoside** | C22H19NO9 |
| 5 | 22.1 |
| 6 | 19.5 | 625.1762 | -0.2 | **Narcissin (Isorhamnetin 3-*O*-rutinoside)** | C28H32O16 |
| 7 | 21.2 | 537.1349  [M+NH4]+ | 0.4 | **Aristolochin** | C23H21NO13 |
| 8 | 22.1 | 442.1131  [M+H]+ | 0.5 | **Aristolactam-IIIa-*N-β-D*-glucopyranoside** | C22H19NO9 |
| 9 | 24.2 | 426.1183  [M+H]+ | 0.0 | **Aristolactam-II-*N-β-D*-glucopyranoside** | C22H19NO8 |
| 10 | 25.0 | 456.1285  [M+H]+ | 0.9 | **Aristolactam I-*N-β-D*-glucopyranoside** | C23H21NO9 |
| 11 | 26.2 | 340.1177  [M+H]+ | 0.6 | **Aristolactam IIIc** | C19H17NO5 |
| 12 | 27.1 | 345.0719  [M+NH4]+ | -0.6 | **Aristolochic acid IIIa/**  **Aristolochic acid Ia** | C16H9NO7 |
| 13 | 29.2 | 345.0719  [M+NH4]+ | -0.6 | **Aristolochic acid C** | C16H9NO7 |
| 14 | 28.7 | 266.0815  [M+H]+ | -1.1 | **AristolactamIIa** | C16H11NO3 |
| 15 | 28.74 | 375.0821  [M+NH4]+ | 0.5 | **Aristolochic acid D/Aristolochic acid Via** | C17H11NO8 |
| 16 | 28.9 |
| 17 | 32.7 | 264.0654  [M+H]+ | 0.4 | **Aristolactam II** | C16H9NO3 |
| 18 | 34.0 | 329.0765  [M+NH4]+ | 0.9 | **Aristolochic acid II** | C16H9NO6 |
| 19 | 34.3 | 359.0876  [M+NH4]+ | -0.6 | **Aristolochic acid III** | C17H11NO7 |
| 20 | 35.2 | 359.0876  [M+NH4]+ | -0.6 | **Aristolochic acid I** | C17H11NO7 |
| 21 | 34.6 | 389.0981  [M+NH4]+ | -0.5 | **Aristolochic acid V/**  **Aristolochic acid IV** | C18H13NO8 |
| 22 | 35.6 |

Figure 1: Chromatograms



Structures of compounds in Tables 1

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table 2: HRMS product ions for the peaks of the botanical extract (*Aristolochia fangchii* Root) showing RT, *m/z*, tentative compound name and fragment ions** | | | | |
| Peak No. | RT (min) | [M]+/[M+H]+/[M+NH4]+  *m/z* | Proposed Compounds (Confirmed with Std in **green**)  (Probable ID is **yellow**)  CAS Number | Product Ions  *m/z* |
| 1 | 15.8 | 342.1703 [M]+ | **Magnoflorine& Isomer** | 297.1117  282.0889  265.0857  237.0900  222.0670 |
| 2 | 16.8 |
| 3 | 16.4 | 330.1703  [M]+ | **Sinomenine/Isosinomenine** | 239.1010  207.0777  137.0581 |
| 4 | 18.8 | 442.1131  [M+H]+ | **Aristolactam-Ia-*N-β-D*-glucopyranoside/**  **Aristolactam-IIIa-*N-β-D*-glucopyranoside** | 424.1074  322.0695  280.0594 |
| 5 | 22.1 |
| 6 | 19.5 | 625.1762  [M+H]+ | **Narcissin (Isorhamnetin 3-*O*-rutinoside)** | 479.1173  317.0654 |
| 7 | 21.2 | 537.1349  [M+NH4]+ | **Aristolochin** | 314.0661 |
| 8 | 22.1 | 442.1131  [M+H]+ | **Aristolactam-IIIa-*N-β-D*-glucopyranoside** | 322.0695  280.0594 |
| 9 | 24.2 | 426.1183  [M+H]+ | **Aristolactam-II-*N-β-D*-glucopyranoside** | 408.1069  306.0752 |
| 10 | 25.0 | 456.1285  [M+H]+ | **Aristolactam I-*N-β-D*-glucopyranoside** | 336.0857  306.0744  294.0735  279.0557 |
| 11 | 26.2 | 340.1177  [M+H]+ | **Aristolactam IIIc** | - |
| 12 | 27.1 | 345.0719  [M+NH4]+ | **Aristolochic acid IIIa/**  **Aristolochic acid Ia** | 310.0349  284.0536 |
| 13 | 29.2 | 345.0719  [M+NH4]+ | **Aristolochic acid C** | 310.0348  284.0557  266.0523 |
| 14 | 28.7 | 266.0815  [M+H]+ | **AristolactamIIa** | 251.0550  223.0386  195.0591 |
| 15 | 28.74 | 375.0821  [M+NH4]+ | **Aristolochic acid D/Aristolochic acid Via** | 340.0423  297.0408  281.0302  253.0365  237.0425 |
| 16 | 28.9 |
| 17 | 32.7 | 264.0654  [M+H]+ | **Aristolactam II** | 206.0619 |
| 18 | 34.0 | 329.0765  [M+NH4]+ | **Aristolochic acid II** | 294.0451  268.0569  250.0466  238.0452 |
| 19 | 34.3 | 359.0876  [M+NH4]+ | **Aristolochic acid III** | 342.0592  298.0692  296.0673  281.0461 |
| 20 | 35.2 | 359.0876  [M+NH4]+ | **Aristolochic acid I** | 342.0603  324.0500  298.0710  296.0673  281.0461  268.0652 |
| 21 | 34.6 | 389.0981  [M+NH4]+ | **Aristolochic acid V/**  **Aristolochic acid IV** | 354.0603  328.0825  266.0467 |
| 22 | 35.6 | 372.0709  326.0787  311.0559  283.0604 |

**References**

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