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

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

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

**Quantitative/Targeted method:**

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| **UHPLC-PDA Method** |
| UHPLC conditions |  |
| System:Waters H-ClassColumn: Acquity BEH Sheild RP18 (2.1 X 100mm, 1.7µm) (Waters corporation, MA, USA)Mobile phase A: Water+0.1% formic acidMobile phase B: Acetonitrile +0.1% formic acidFlow rate: 0.20 mL/minColumn temperature: 40°CGradient

|  |  |
| --- | --- |
| Time (min) | Mobile phase B (%) |
| 0.0 | 30 |
| 6.5 | 30 |
| 34.0 | 43 |
| 50.0 | 65 |
| 55.0 | 100 |

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| Compound | Retention time (min) |
| Aristolochic acid C | 19.8 |
| Aristolochic acid II | 29.3 |
| Aristolochic acid I | 37.7 |

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**Quantitative results**

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| **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**

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| --- | --- | --- | --- | --- |
| **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**

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| --- | --- | --- | --- | --- | --- | --- | --- |
| 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**

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| 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 acidMobile phase B: Acetonitrile +0.1% formic acidFlow rate: 0.20 mL/min Column temperature: 35°CGradient:

|  |  |
| --- | --- |
| 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 modeMain 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 |

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| **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

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| **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** | 297.1117282.0889265.0857237.0900222.0670 |
| 2 | 16.8 |
| 3 | 16.4 | 330.1703[M]+ | **Sinomenine/Isosinomenine** | 239.1010207.0777137.0581 |
| 4 | 18.8 | 442.1131[M+H]+ | **Aristolactam-Ia-*N-β-D*-glucopyranoside/****Aristolactam-IIIa-*N-β-D*-glucopyranoside** | 424.1074322.0695280.0594 |
| 5 | 22.1 |
| 6 | 19.5 | 625.1762[M+H]+ | **Narcissin (Isorhamnetin 3-*O*-rutinoside)** | 479.1173317.0654 |
| 7 | 21.2 | 537.1349[M+NH4]+ | **Aristolochin** | 314.0661 |
| 8 | 22.1 | 442.1131[M+H]+ | **Aristolactam-IIIa-*N-β-D*-glucopyranoside** | 322.0695280.0594 |
| 9 | 24.2 | 426.1183[M+H]+ | **Aristolactam-II-*N-β-D*-glucopyranoside** | 408.1069306.0752 |
| 10 | 25.0 | 456.1285[M+H]+ | **Aristolactam I-*N-β-D*-glucopyranoside** | 336.0857306.0744294.0735279.0557 |
| 11 | 26.2 | 340.1177[M+H]+ | **Aristolactam IIIc** | - |
| 12 | 27.1 | 345.0719[M+NH4]+ | **Aristolochic acid IIIa/****Aristolochic acid Ia** | 310.0349284.0536 |
| 13 | 29.2 | 345.0719[M+NH4]+ | **Aristolochic acid C** | 310.0348284.0557266.0523 |
| 14 | 28.7 | 266.0815[M+H]+ | **AristolactamIIa** | 251.0550223.0386195.0591 |
| 15 | 28.74 | 375.0821[M+NH4]+ | **Aristolochic acid D/Aristolochic acid Via** | 340.0423297.0408281.0302253.0365237.0425 |
| 16 | 28.9 |
| 17 | 32.7 | 264.0654[M+H]+ | **Aristolactam II** | 206.0619 |
| 18 | 34.0 | 329.0765[M+NH4]+ | **Aristolochic acid II** | 294.0451268.0569250.0466238.0452 |
| 19 | 34.3 | 359.0876[M+NH4]+ | **Aristolochic acid III** | 342.0592298.0692296.0673281.0461 |
| 20 | 35.2 | 359.0876[M+NH4]+ | **Aristolochic acid I** | 342.0603324.0500298.0710296.0673281.0461268.0652 |
| 21 | 34.6 | 389.0981[M+NH4]+ | **Aristolochic acid V/****Aristolochic acid IV** | 354.0603328.0825266.0467 |
| 22 | 35.6 | 372.0709326.0787311.0559283.0604 |

**References**

1. Analysis of isoquinoline alkaloids from *Mahonia leschenaultia* and *Mahonia napaulensis* roots using UHPLC-Orbitrap-MSn and UHPLC-QqQLIT-MS/MS. Journal of Pharmaceutical Analysis Volume 7, Issue 2, April 2017, Pages 77-86.
2. Johanna et al., LC-MS- and 1H NMR-Based Metabolomic Analysis and in Vitro Toxicological Assessment of 43 *Aristolochia* Species. J. Nat. Prod. 2016, 79, 30−37
3. Wu et al., Aristolochic acids, aristolactam alkaloids and amides from *Aristolochia kankauensis.* Phytochemistry Volume 36, Issue 4, July 1994, Pages 1063-1068.
4. Wen-Wen et al., Characterization and quantitation of aristolochic acid analogs in different parts of *Aristolochiae fructus*, using UHPLCQ/TOF-MS and UHPLC-QqQ-MS. *Chinese Journal of Natural Medicines* 2017, 15(5): 0392-0400
5. Hee-Jung et al., Simultaneous Determination of Structurally Diverse Compounds in Different *Fangchi* Species by UHPLC-DAD and UHPLC-ESI-MS/MS. *Molecules* 2013, *18*, 5235-5250.
6. Schaneberg BT, Khan IA. Analysis of products suspected of containing Aristolochia or Asarum species. J Ethnopharmacol. 2004 Oct;94(2-3):245-9. doi: 10.1016/j.jep.2004.06.010. PMID: 15325726.