**Analysis of *Symphytum officinale* (Comfrey) root Extract Using LC-QToF-MS method**

Names of investigators: Bharathi Avula, Kumar Katragunta

Affiliation: National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Analysis date: December 13, 2021

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| --- | --- | --- | --- | --- |
| Common Botanical Name | CAS No. | Lot No. | Container ID | Net Weight |
| ***Symphytum offcinale* (Comfrey)** |  | RK-3-23-1-SO-CRK-3-23-1-SO-D |  |  |

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

**Quantitative/Targeted method:**

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| **UHPLC-QToF-MS Method** |
| UHPLC conditions | Mass spectrometry |
| 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: 0.23 mL/min Column temperature: 35°CGradient

|  |  |
| --- | --- |
| Time (min) | Mobile phase B (%) |
| 0.0 | 01 |
| 3.0 | 01 |
| 40.0 | 40 |
| 45.0 | 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 . Nebulizer: 30 psig . Sheath gas temperature: 300°C . Sheath gas flow: 11L/min Capillary voltage: 3.5 kV Fragmentor: 100V/150V

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| --- | --- | --- |
| Compound | Precursor ion (*m/z*)[M+H]+ | Retention time (min) |
| Intermedine | 300.1805 | 12.3 |
| Lycopsamine | 300.1805 | 12.6 |
| Intermedine N-oxide | 316.1755 | 13.8 |
| Lycopsamine N-oxide | 316.1755 | 14.2 |
| 7-acetylintermedine | 342.1911 | 17.65 |
| 7-acetyllycosamine | 342.1911 | 17.78 |
| 7-acetylintermedine N-oxide | 358.1858 | 17.9 |
| 7-acetyllycosamine N-oxide | 358.1858 | 18.1 |
| Rosmarinic acid | DAD@330 nm | 26.6 |

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**Quantitative results**

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| **Compound** | **Concentration in extract (mg/g)****RK-3-21-1-SO-C** | **Concentration in extract (mg/g)****RK-3-21-1-SO-D** |
| Intermedine | 3 mg/g | 3 mg/g |
| Lycopsamine | 3 mg/g | 3 mg/g |
| Intermedine N-oxide | 3.7 mg/g | 3.7 mg/g |
| Lycopsamine N-oxide | 3.7 mg/g | 3.7 mg/g |
| 7-acetylintermedine | 0.2 mg/g | 0.2 mg/g |
| 7-acetyllycopsamine | 0.2 mg/g | 0.2 mg/g |
| 7-acetylintermedine N-oxide | 3 mg/g | 3 mg/g |
| 7-acetyllycopsamine N-oxide | 5 mg/g | 5 mg/g |
| Rosmarinic acid | 17 mg/g | 17 mg/g |

**Standards**

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| --- | --- | --- | --- | --- |
| **Name** | **Supplier** | **CAS Number** | **Catalog number** | **Purity %** |
| Intermedine | Millipore Sigma | 10285-06-0 | PHL82424 | 99% |
| Lycopsamine | Millipore Sigma | 10285-07-1 | PHL89726 | 99% |
| Intermedine N-oxide | Millipore Sigma | 95462-14-9 | PHL83446 | 99% |
| Lycopsamine N-oxide | Millipore Sigma | 95462-15-0 | PHL83447 | 99% |
| 7-acetylintermedine | Millipore Sigma | 74243-01-9 | PHL83759 | 99% |
| 7-acetylintermedine N-oxide | Millipore Sigma | 685132-59-6 | PHL83795 | 99% |
| 7-acetyllycopsamine N-oxide | Millipore Sigma | 685132-58-5 | PHL84275 | 99% |
| Rosamarinic acid | Millipore Sigma | 20283-92-5 | R4033 | 98% |

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



**Chromatograms**



**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-3-21-1-SO-C | Concentration mg/g (n = 3)RK-3-21-1-SO-D |
| Intermedine | 0.5 ng/mL | 1.0 ng/mL | 0.1 – 2.5 μg/mL | 5 | 0.9993 | 3.4 ± 0.28 | 3.4 ± 0.71 |
| Lycopsamine | 0.5 ng/mL | 1.0 ng/mL | 0.1 – 2.5 μg/mL | 5 | 0.9995 | 3.1 ± 0.32 | 3.2 ± 1.66 |
| Intermedine N-oxide | 1.0 ng/mL | 2.5 ng/mL | 0.1 – 2.5 μg/mL | 5 | 0.9995 | 3.7 ± 2.30 | 3.7 ± 0.51 |
| Lycopsamine N-oxide | 0.5 ng/mL | 1.0 ng/mL | 0.1 – 2.5 μg/mL | 5 | 0.9995 | 3.7 ± 0.33 | 3.7 ± 0.53 |
| 7-acetylintermedine | 0.5 ng/mL | 1.0 ng/mL | 0.1 – 2.5 μg/mL | 5 | 0.9994 | 0.2 ± 1.38 | 0.2 ± 2.22 |
| 7-acetyllycopsamine | 1.0 ng/mL | 2.5 ng/mL | 0.1 – 2.5 μg/mL | 5 | 0.9994 | 0.2 ± 1.61 | 0.2 ± 2.12 |
| 7-acetylintermedine N-oxide | 0.5 ng/mL | 1.0 ng/mL | 0.1 – 2.5 μg/mL | 5 | 0.9995 | 3.5 ± 0.62 | 3.5 ± 0.61 |
| 7-acetyllycopsamine N-oxide | 1.0 ng/mL | 2.5 ng/mL | 0.1 – 2.5 μg/mL | 5 | 0.9995 | 4.8 ± 0.35 | 4.7 ± 1.25 |
| Rosamarinic acid | 10 ng/mL | 50 ng/mL | 0.5 – 25 μg/mL | 5 | 0.9996 | 16.8 ± 0.10 | 16.9 ± 0.24 |

**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: 0.23 mL/min Column temperature: 35°CGradient:

|  |  |
| --- | --- |
| Time (min) | Mobile phase B (%) |
| 0.0 | 01 |
| 3.0 | 01 |
| 40.0 | 40 |
| 45.0 | 100 |

 | System: QToF-MS 6530A series (Agilent technologies, Palo Alto, CA, USA)Ionization: ESI Polarity: Positive modeMain Interface: · Nebulizing gas flow: 11 L/min . Gas temperature: 325°C . Nebulizer: 30 psig . Sheath gas temperature: 300°C . Sheath gas flow: 11L/min Capillary voltage: 3.5 kV Fragmentor: 100V/150V |

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| **Table 1: Proposed identification of constituents of *Symphytum officinale* 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 |
| **Phenolic compounds [M-H]-** |
| 1 | 8.5 | 599.1615 | -0.5 | **Hydroxybenzoic acid derivative** | C26H32O16 |
| 2 | 13.5 | 161.0818 | -0.6 | **Viridifloric acid** | C7H14O4 |
| 3 | 16.3 | 179.0347 | -1.7 | **Caffeic acid** | C9H8O4 |
| 4 | 21.9 | 537.1036 | -0.4 | **Globoidnan B** | C27H22O12 |
| 5 | 24.58 | 717.1459 | -0.3 | **Rabdosin** | C36H30O16 |
| 6 | 26.6 | 359.0770 | -0.6 | **Rosmarinic acid** | C18H16O8 |
| 7 | 26.7 | 719.1619 | 0.1 | **Dihydrorabdosin** | C36H32O16 |
| 8 | 26.9 | 493.1143 | 0.6 | **Dihydrogloboidnan A** | C26H22O10 |
| 9 | 30.6 | 491.0989 | 1.0 | **Globoidnan A/Eritrichin** | C26H20O10 |
| **Pyrrolizidine alkaloids (PAs) [M+H]+** |
| 10 | 12.3 | 300.1807 | 0.7 | **Intermedine** | C15H25NO5 |
| 11 | 12.6 | 300.1807 | 0.7 | **Lycopasamine** | C15H25NO5 |
| 12 | 13.8 | 316.1757 | 0.6 | **Intermedine-N-oxide** | C15H25NO6 |
| 13 | 14.2 | 316.1757 | 0.6 | **Lycopasamine N-oxide** | C15H25NO6 |
| 14 | 17.65 | 342.1912 | 0.3 | **7-Acetylintermedine** | C17H27NO6 |
| 15 | 17.78 | 342.1912 | 0.3 | **7-Acetyllycopsamine** | C17H27NO6 |
| 16 | 17.9 | 358.1858 | -0.6 | **7-Acetylintermedine *N*-oxide** | C17H27NO7 |
| 17 | 18.1 | 358.1858 | -0.6 | **7-Acetyllycopsamine *N*-oxide** | C17H27NO7 |
| 18 | 19.2 | 414.2123 | 0.2 | **7-Sarracinyl-9- trachelantyl-retronecine-*N*-oxide/ 7-Sarracinyl-9- viridiflorylretronecine-*N*-oxide/Echimidine-*N*-Oxide/****Heliosupine-*N*-oxide** | C20H31NO8 |
| 19 | 19.9 |
| 20 | 20.5 |
| 21 | 22.0 |
| 22 | 26.4 | 382.2223 | -0.3 | **Symphytine/ Symviridine/****Anadoline/Symlandine** | C₂₀H₃₁NO₆ |
| 23 | 26.7 |
| 24 | 26.9 |
| 25 | 27.2 |
| 26 | 26.5 | 398.2175 | 0.5 | **Echimidine/ Heliosupine/Symphytine *N*-oxide/ Symviridine *N*-oxide/ Anadoline-*N*-Oxide/ Symlandine *N*-oxide** | C20H31NO7 |
| 27 | 26.9 |
| 28 | 27.2 |
| 29 | 27.5 |

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 (*Symphytum officinale* root) showing RT, *m/z*, tentative compound name and fragment ions** |
| Peak No. | RT (min) | [M-H]-/[M+H]+*m/z* | Proposed Compounds (Confirmed with Std in **green**)(Probable ID is **yellow**)CAS Number | Product Ions*m/z*  |
| **Phenolic compounds [M-H]-** |
| 1 | 8.5 | 599.1615 | **Hydroxybenzoic acid derivative** | 137.025093.0354 |
| 2 | 13.5 | 161.0818 | Viridifloric acid | 135.0571117.0556 |
| 3 | 16.3 | 179.0347 | Caffeic acid | 135.0448 |
| 4 | 21.9 | 537.1036 | Globoidnan B | 493.1141339.0505197.0451135.0452 |
| 5 | 24.58 | 717.1459 | Rabdosin | 537.1006519.0916475.1019339.0502197.0456 |
| 6 | 26.6 | 359.0770 | Rosmarinic acid | 197.0461161.0231 |
| 7 | 26.9 | 493.1143 | Dihydrogloboidnan A | 359.0746295.0597185.0234161.0239 |
| 8 | 30.6 | 491.0989 | Globoidnan A/Eritrichin | 311.0563179.0348135.0450 |
| 9 | 26.7 | 719.1619 | Dihydrorabdosin | 359.0765197.0456161.0246 |
| **Pyrrolizidine alkaloids (PAs) [M+H]+** |
| 10 | 12.3 | 300.1807 | Intermedine | 156.1006 |
| 11 | 12.6 | 300.1807 | Lycopsamine | 156.1006 |
| 12 | 13.8 | 316.1757 | Intermedine-*N*-oxide | 172.0841 |
| 13 | 14.2 | 316.1757 | Lycopasamine N-oxide | 172.0841 |
| 14 | 17.65 | 342.1912 | 7-Acetylintermedine | 120.0629 |
| 15 | 17.78 | 342.1912 | 7-Acetyllycopsamine | 120.0629 |
| 16 | 17.9 | 358.1858 | 7-Acetylintermedine *N*-oxide | 214.1067180.1007120.0802 |
| 17 | 18.1 | 358.1858 | 7-Acetyllycopsamine *N*-oxide | 214.1067180.1007120.0802 |
| 18 | 19.2 | 414.2123 | 7-Sarracinyl-9- trachelantyl-retronecine-*N*-oxide/ 7-Sarracinyl-9- viridiflorylretronecine-*N*-oxide/Echimidine-*N*-Oxide/Heliosupine-*N*-oxide  | 298.1575270.1307236.1311214.1048154.0832137.0810120.0800 |
| 19 | 19.9 |
| 20 | 20.5 |
| 21 | 22.0 |
| 22 | 26.4 | 382.2223 | Symphytine/ Symviridine/Anadoline/Symlandine | 138.0893120.0809 |
| 23 | 26.7 |
| 24 | 26.9 |
| 25 | 27.2 |
| 26 | 26.5 | 398.2175 | Echimidine/ Heliosupine/Symphytine *N*-oxide/Symviridine *N*-oxide/ Anadoline-*N*-Oxide/ Symlandine *N*-oxide | 254.1282138.0893120.0809 |
| 27 | 26.9 |
| 28 | 27.2 |
| 29 | 27.5 |

**References:**

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