**Analysis of *Pausinystalia johimbe* (Yohimbe) root Extract Using LC-QToF method**

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| --- | --- | --- | --- | --- |
| Common Botanical Name | CAS No. | Lot No. | Container ID | Net Weight |
| ***Pausinystalia johimbe* (Yohimbe)** |  | RK-3-31-1-PJ-C  RK-3-31-1-PJ-D |  |  |

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

**Quantitative/Targeted method:**

|  |  |
| --- | --- |
| **UHPLC-MS/MS Method** | |
| UHPLC conditions | Mass spectrometry |
| **System**: Waters Acquity UPLC  **Column**: Waters Acquity UPLC BEH C18 (2.1 x 100 mm i.d., 1.7 μm)  **Mobile phase A**: 50 mM ammonium formate in 5% methanol aqueous (pH = 8.2)  **Mobile phase B**: methanol with 0.01% ammonium hydroxide  **Flow rate**: 0.25 mL/min  **Column temperature**: 50 °C  **Gradient**   |  |  | | --- | --- | | Time (min) | Mobile phase B (%) | | 0.0 | 32.0 | | 7.0 | 50.0 | | 11.0 | 65.0 | | 15.0 | 100.0 | | 18.0 | 100.0 | | **System**: Waters Xevo G-2 QToF  **Ionization**: Electrospray  **Polarity**: Positive Ion  **Main Interface**:  · Capillary: 3.0 kV  . Cone: 25 V  . Source temperature: 150 °C  . Desolvation temperature: 350 °C  . Desolvation gas flow: 900 L/hr  · Con gas flow: 50 L/hr  **SRM dwell time**:   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Selected Reaction Monitoring (SRM) events | | | | | | Compound | Precursor ion (*m/z*)  [M+H]+ | Product ion\* (*m/z*) | Collision energy (V) | Retention time (min) | | Yohimbic acid | 341.2 | 198.1 | 28 | 3.40 | | Corynanthine | 355.2 | 212.1 | 28 | 7.31 | | Yohimbine | 355.2 | 212.1 | 28 | 7.80 | | Rauwolscine | 355.2 | 212.1 | 28 | 11.1 | | Ajmalicine | 353.2 | 210.1 | 23 | 12.2 | | \*Quantifier, †Qualifier | | | | | |

**Quantitative results**

|  |  |  |
| --- | --- | --- |
| **Compound** | **Concentration in extract (mg/g)**  **RK-3-31-1-PJ-C** | **Concentration in extract (mg/g)**  **RK-3-31-1-PJ-D** |
| Yohimbic acid | 3.3 mg/g | 3.4 mg/g |
| Corynanthine | 8.0 mg/g | 8.5 mg/g |
| Yohimbine | 49.2 mg/g | 50.5 mg/g |
| Rauwolscine | 3.1 mg/g | 3.2 mg/g |
| Ajmalicine | 0.02 mg/g | 0.02 mg/g |

**Standards**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Supplier** | **CAS Number** | **Catalog number** | **Purity %** |
| Yohimbic acid | Chromadex | 522-87-2 | NA | 99% |
| Corynanthine | Chromadex | 483-10-3 | ASB-00003812-010 | 99% |
| Yohimbine | Millipore Sigma | 65-19-0 | Y3125 | 99% |
| Rauwolscine | Indofine | 4373-34-6 | 020622 | 99% |
| Ajmalicine | Chromadex | 4373-34-6 | ASB-00001492-050 | 99% |

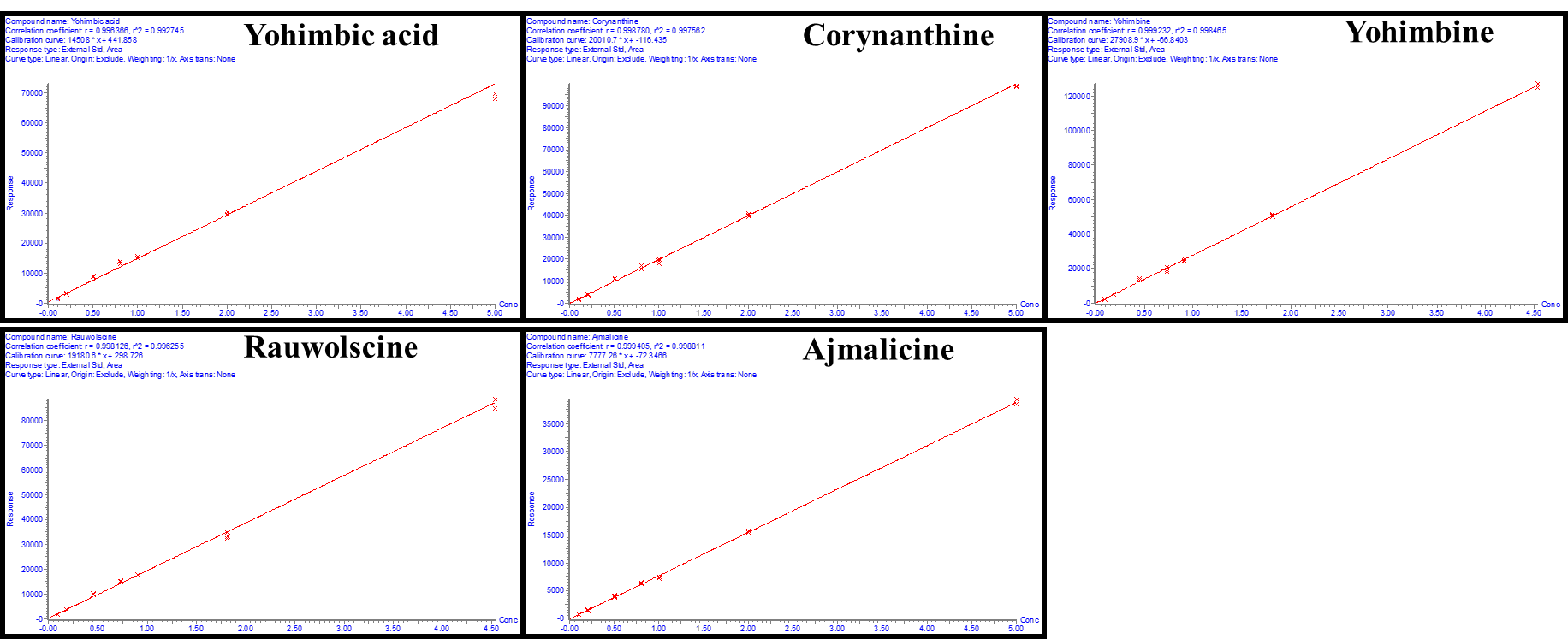
**Chemical structures of standards used for quantitative analysis**



**Calibration**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compound | Limit of detection (LOD) ng/mL | Limit of quantitation (LOQ) ng/mL | Calibration range (mg/g) | Number of Calibration Points | R2 |
| Yohimbic acid | 30 ng/mL | 100 ng/mL | 0.1 – 5.0 μg/mL | 7 | 0.993 |
| Corynanthine | 30 ng/mL | 100 ng/mL | 0.1 – 5.0 μg/mL | 7 | 0.997 |
| Yohimbine | 27 ng/mL | 90 ng/mL | 0.09 – 4.5 μg/mL | 7 | 0.998 |
| Rauwolscine | 27 ng/mL | 90 ng/mL | 0.09 – 4.5 μg/mL | 7 | 0.996 |
| Ajmalicine | 30 ng/mL | 100 ng/mL | 0.1 – 5.0 μg/mL | 7 | 0.998 |

**Linearity profiles**



**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 | 10 | | 25.0 | 33 | | 30 | 100 | | System: QToF-MS 6530A series (Agilent technologies, Palo Alto, CA, USA)  Ionization: ESI  Polarity: Positive mode  Main Interface:  · Nebulizing gas flow: 13 L/min  . Gas temperature: 270°C  . Nebulizer: 27 psig  . Sheath gas temperature: 300°C  . Sheath gas flow: 13L/min  · Capillary voltage: 3.5 kV  · Fragmentor: 150V |

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| --- | --- | --- | --- | --- | --- |
| **Table 1: Proposed identification of constituents of *Pausinystalia johimbe* root extract showing RT, *m/z*, ppm, tentative compound name and molecular formula** | | | | | |
| **Peak No.** | **RT (min)** | **Exp. *m/z***  **[M+H]+** | **Mass accuracy  (ppm)** | **Proposed ID’s  (Confirmed with Std in green)**  **(Most probable ID is yellow)** | **Molecular Formula** |
| 1 | 10.14 | 341.1858 | 0.6 | **Yohimbic acid** | C20H24N2O3 |
| 2 | 11.60 | 355.2013 | 0.8 | **Rauwolscine** | C21H26N2O3 |
| 3 | 13.81 | 355.2013 | 0.8 | **Yohimbine** | C21H26N2O3 |
| 4 | 14.37 | 355.2019 | -0.8 | **Corynanthine** | C21H26N2O3 |
| 5 | 12.93 | 355.2015 | 0.3 | **β-Yohimbine/ Pseudoyohimbine/ Alloyohimbine/ Methyl (3β,16β,17α,20α)-17-hydroxyyohimban-16-carboxylate/ Yohimban-16-carboxylic acid, 17-hydroxy-, methyl ester/ Epialloyohimbine** | C21H26N2O3 |
| 6 | 13.16 |
| 7 | 13.46 |
| 8 | 14.35 |
| 9 | 15.85 |
| 10 | 16.14 |
| 11 | 17.75 |
| 12 | 18.17 |
| 13 | 18.47 |
| 14 | 19.0 |
| 15 | 12.96 | 353.1857 | 0.8 | **Ajmalicine/Tetrahydroalstonine/Akuammigine/19-Epiajmalicine/Rauniticine/Isoajmalicine/3-Iso-19-epiajmalicine** | C21H24N2O3 |
| 16 | 13.58 |
| 17 | 14.10 |
| 18 | 14.50 |
| 19 | 14.77 |
| 20 | 15.20 |
| 21 | 17.45 |
| 22 | 17.74 |
| 23 | 18.72 |
| 24 | 19.37 |
| 25 | 20.83 |
| 26 | 14.34 | 357.2170 | 0.8 | **Dihydrositsirikine epimers** | C21H28N2O3 |
| 27 | 15.03 |
| 28 | 16.78 |
| 29 | 18.86 |
| 30 | 20.50 |
| 31 | 17.74 | 369.2170 | 0.8 | ***O*-Methylyohimbine epimers** | C22H28N2O3 |
| 32 | 22.0 |
| 33 | 23.30 |
| 34 | 21.90 | 367.2014 | 0.5 | **Ajamalinine/Corynantheine epimers** | C22H26N2O3 |
| 35 | 25.14 |
| 36 | 25.80 |

Figure 1: Chromatograms



Structures of compounds in Tables 1

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table 2: HRMS product ions for the peaks of the botanical extract (*Pausinystalia johimbe* root) showing RT, *m/z*, tentative compound name and fragment ions** | | | | |
| **Peak No.** | **RT (min)** | **[M+H]+**  ***m/z*** | **Proposed Compounds  (Confirmed with Std in green)**  **(Probable ID is yellow)**  **CAS Number** | **Product Ions**  ***m/z*** |
| 1 | 10.14 | 341.1858 | **Yohimbic acid** | 144.0804 |
| 2 | 11.60 | 355.2013 | **Rauwolscine** | 144.0809 |
| 3 | 13.81 | 355.2013 | **Yohimbine** | 212.1279  144.0805 |
| 4 | 14.37 | 355.2019 | **Corynanthine** | 144.0803 |
| 5 | 12.93 | 355.2015 | **β-Yohimbine/ Pseudoyohimbine/ Alloyohimbine/ Methyl (3β,16β,17α,20α)-17-hydroxyyohimban-16-carboxylate/ Yohimban-16-carboxylic acid, 17-hydroxy-, methyl ester/ Epialloyohimbine** | 144.0808 |
| 6 | 13.16 |
| 7 | 13.46 |
| 8 | 14.35 |
| 9 | 15.85 |
| 10 | 16.14 |
| 11 | 17.75 |
| 12 | 18.17 |
| 13 | 18.47 |
| 14 | 19.0 |
| 15 | 12.96 | 353.1857 | **Ajmalicine/Tetrahydroalstonine/Akuammigine/19-Epiajmalicine/Rauniticine/Isoajmalicine/3-Iso-19-epiajmalicine** | 144.0806  117.0696 |
| 16 | 13.58 |
| 17 | 14.10 |
| 18 | 14.50 |
| 19 | 14.77 |
| 20 | 15.20 |
| 21 | 17.45 |
| 22 | 17.74 |
| 23 | 18.72 |
| 24 | 19.37 |
| 25 | 20.83 |
| 26 | 14.34 | 357.2170 | **Dihydrositsirikine epimers** | 144.0808 |
| 27 | 15.03 |
| 28 | 16.78 |
| 29 | 18.86 |
| 30 | 20.50 |
| 31 | 17.74 | 369.2170 | ***O*-Methylyohimbine epimers** | 144.0803 |
| 32 | 22.0 |
| 33 | 23.30 |
| 34 | 21.90 | 367.2014 | **Ajamalinine/Corynantheine epimers** | 144.0810 |
| 35 | 25.14 |
| 36 | 25.80 |

**References**

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