**Analysis of Blue Cohosh Extract Using UPLC-MS/MS**

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| --- | --- | --- | --- | --- |
| Common Botanical Name | CAS No. | Lot No. | Container ID | Net Weight |
| Blue Cohosh |  |  |  |  |

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| --- |
| Sample storage condition until analysis |
| -80 ℃ |

**Quantitative/Targeted method:**

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| **UPLC-MS Method** |
| UHPLC conditions | Mass spectrometry |
| System: **Waters Acquity**Column: **Waters Acquity Ultra C18 (2.1 x 100 mm, 1.7 um)**Mobile phase A: **H2O + 0.1% FA**Mobile phase B: **CH3CN + 0.1% FA**Flow rate: **0.3 mL/min**Column temperature: **50 ℃**Gradient

|  |  |
| --- | --- |
| Time (min) | Mobile phase B (%) |
| **0** | **3** |
| **1.0** | **3** |
| **5.0** | **75** |
| **5.5** | **75** |
| **6.0** | **3** |
|  |  |
|  |  |
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 | System: **Waters TQS**Ionization: **Electrospray (ESI)**Polarity: **Positive**Main Interface: · Nebulizing gas flow: **150 L/hr** · Heating gas flow: **500 L/hr** · Interface temperature: **150 ℃** · Capillary voltage: **2.5 kV** · Cone voltage: **50 V**Ion guide: · Drying gas flow: **0.15 mL/min** · Desolvation line temperature: **500 ℃**  · Heat block temperature: SRM dwell time:

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| --- |
| Selected Reaction Monitoring (SRM) events |
| Compound | Precursor ion (*m/z*) | Product ion (*m/z*) | Collision energy (V) | Retention time (min) |
| **Baptifoline** | 261.16 | - | 45 | 0.51 |
| **Lupanine** | 249.20 | - | 45 | 0.50 |
| **Taspine** | 370.13 | - | 45 | 4.00 |
| **Anagyrine** | 245.16 | - | 45 | 0.50 |
| **Boldine** | 328.16 | - | 45 | 0.50 |
| **Magnoflorine** | 342.17 | - | 45 | 0.50 |
| **N-methylcytisine** | 205.13 | - | 45 | 0.50 |
| \*Quantifier, †Qualifier |

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**Quantitative results**

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| --- | --- |
| **Compound** | **Concentration in extract (mg/g)** |
| Baptifoline | **2.45 ± 0.31** |
| Lupanine | **6.43 ± 0.35** |
| Taspine | **0.774 ± 0.091** |
| Anagyrine | **1.257 ± 0.044** |
| Boldine | **0.275 ± 0.024** |
| Magnoflorine | **10.76 ± 0.52** |
| N-methylcytisine | **0.753 ± 0.024** |

**Standards**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Supplier** | **CAS Number** | **Catalog number** | **Purity %** | **Batch/Lot number** |
| **Baptifoline** | ALB | 732-50-3 | ALB-RS-9182 | >98% | ALB-202105 |
| **Lupanine** | ALB | 550-90-3 | ALB-RS-1465 | >98% | ALB-202105 |
| **Taspine** | ALB | 602-07-3 | ALB-RS-8444 | >98% | ALB-202105 |
| **Anagyrine** | Cayman | 486-89-5 | 31169 | >98% | 0611867-5 |
| **Boldine** | Phytolab | 476-70-0 | 80145-100mg | >98% | 88561562 |
| **Magnoflorine** | Sigma | 2141-09-5 | SMB00377-100mg | >98% | SLCH1683 |
| **N-methylcytisine** | Phytolab | 486-86-2 | 83219-10mg | >98% | 115786903 |

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



**Chromatograms**

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**Calibration**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compound | Lower limit of quantitation (LLOQ) **ug/mL** | Calibration range (**ug/mL**) | Number of Calibration Points | R2 | Concentration mg/g **in extract** (n = 3) |
| **Baptifoline** | 0.001 | 0.001 – 0.729 | 7 | 0.9994 | **2.45 ± 0.31** |
| **Lupanine** | 0.001 | 0.001 – 0.729 | 7 | 0.9990 | **6.43 ± 0.35** |
| **Taspine** | 0.001 | 0.001 – 0.729 | 7 | 0.9986 | **0.774 ± 0.091** |
| **Anagyrine** | 0.001 | 0.001 – 0.729 | 7 | 0.9994 | **1.257 ± 0.044** |
| **Boldine** | 0.001 | 0.001 – 2.187 | 8 | 0.9962 | **0.275 ± 0.024** |
| **Magnoflorine** | 0.001 | 0.001 – 0.729 | 7 | 0.9970 | **10.76 ± 0.52** |
| **N-methylcytisine** | 0.001 | 0.001 – 0.729 | 7 | 0.9990 | **0.753 ± 0.024** |

Untargeted Analysis

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| UHPLC conditions | Mass spectrometer conditions |
| System: Thermo VanquishColumn: Waters Acquity Ultra C18 (1.7 um, 1.0 x 150 mm)Mobile phase A: H2O w/ 0.1% FAMobile phase B: ACN w/ 0.1% FAFlow rate: 0.100 mL/minColumn temperature: 55℃Gradient:

|  |  |
| --- | --- |
| Time (min) | Mobile phase B (%) |
| 0 | 5 |
| 1 | 5 |
| 12.5 | 98 |
| 13.5 | 98 |
| 12.6 | 5 |
| 15 | 5 |

 | System: Thermo Orbitrap Fusion LumosMode: ESIPolarity: positiveMain-Interface: · Interface voltage: 3500 · Nebulizing gas flow: 25 · Heating gas flow: 5 · Interface temperature: 325 ℃ · Desolvation temperature: 350 ℃Ion guide: · Drying gas flow: 1 · DL temperature: 350 ℃ · Heat block temperature |

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| **Table 1: Proposed identification of constituents of Blue Cohosh** |
| Peak No. | RT (min) | Exp. *m/z* | Mass accuracy (ppm) | Proposed ID’s (Confirmed with Std in **green**)(Structure with correlated MS2 spectra in **orange**)(Most probable ID is **yellow**) | Molecular Formula |
| 1 | 10.47 | 221.1898 | -3.16 | Caryophyllene epoxide | C15H24O |
| 2 | 10.44 | 203.1796 | -1.48 | Alismol | C15H24O |
| 3 | 1.18 | 138.055 | -3.62 | 3-carboxy-1-methylpyridinium  | C7H8NO2+ |
| 4 | 7.23 | 453.3371 | -74.34 | DL-alpha-Tocopherol | C29H50O2 |
| 5 | 1.12 | 245.1647 | -2.86 | Anagyrine | C15H20N2O |
| 6 | 1.1 | 261.1598 | -1.91 | Baptifoline | C15H20N2O2 |
| 7 | 1.11 | 338.1385 | -2.07 | Columbamine | C20H20NO4+ |
| 8 | 1.12 | 297.1122 | -123.86 | Cryptotanshinone | C19H20O3 |
| 9 | 2.77 | 342.1703 | -0.58 | Magnoflorine | C20H24NO4+ |
| 10 | 9.55 | 274.2742 | -1.46 | N-Lauryldiethanolamine | C16H35NO2 |
| 11 | 1.12 | 249.1959 | -3.21 | Lupanine | C15H24N2O |
| 12 | 7.3 | 751.4615 | -2.26 | a-Hederin | C41H66O12 |
| 13 | 7.28 | 781.4727 | -1.41 | Saikosaponin A | C42H68O13 |
| 14 | 6.96 | 959.5229 | 1.35 | Asiaticoside | C48H78O19 |
| 15 | 1.11 | 265.1547 | -1.89 | 2-propenamide | C14H20N2O3 |
| 16 | 1.1 | 205.1337 | -1.95 | N-methylcytisine | C12H16N2O |
| 17 | 10.54 | 455.3519 | -1.32 | NCGC00380944-01 | C30H46O3 |
| 18 | 9.73 | 286.1439 | -1.40 | Piperine | C17H19NO3 |
| 19 | 5.97 | 370.1284 | -7.56 | Taspine | C20H19NO6 |
| 20 | 3.68 | 328.1544 | -7.92 | Boldine | C19H21NO4 |
| 21 |  |  |  |  |  |
| 22 |  |  |  |  |  |
| 23 |  |  |  |  |  |
| 24 |  |  |  |  |  |
| 25 |  |  |  |  |  |
| 26 |  |  |  |  |  |
| 27 |  |  |  |  |  |
| 28 |  |  |  |  |  |
| 29 |  |  |  |  |  |
| 30 |  |  |  |  |  |
| 31 |  |  |  |  |  |
| 32 |  |  |  |  |  |
| 33 |  |  |  |  |  |

Figure 1: Chromatograms









































Structures of compounds in Table 1





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| **Table 3: HRMS product ions for the peaks of the botanical extract** |
| Peak No. | RT (min) | *m/z* | Proposed Compounds (Confirmed with Std in **green**)(Probable ID is **yellow**)CAS Number | Product Ions*m/z* Rel. Int.. |
| N1 | 10.47 | 221.1898 | Caryophyllene oxide13877-94-6 | 55.0544 5264.7965 2667.0544 3669.0700 4479.0541 3081.0697 6691.0540 3193.0696 4693.9621 2395.0854 50105.0697 67107.0854 100119.0854 60121.1016 32131.9666 22133.1017 39189.4265 25 |
| 2 | 10.44 | 203.1796 | Alismol 87827-55-2 | 55.0541 5057.0696 1067.0540 4769.0696 3179.0539 2481.0699 4491.0545 3493.0701 3195.0855 53105.0700 100107.0861 31109.1011 18119.0857 69131.0857 11133.1015 58145.1011 12147.1169 33 |
| 3 | 1.17 | 138.055 | 3-carboxy-1-methylpyridinium 7164-65-0 | 53.0384 655.9342 10059.5012 165.0383 767.0539 478.0338 279.0414 291.0532 192.0370 192.0493 1293.0573 494.0649 3595.0492 296.0442 1096.9611 1110.0600 23110.1170 1114.9714 4138.0553 19 |
| 4 | 7.23 | 453.3371 | DL-alpha-tocopherol10191-41-0 | 69.0700 1481.0698 4893.0701 2495.0855 10097.1017 14105.0698 29106.7621 16107.0854 52109.1011 38111.0812 38119.0860 56121.1015 46123.1162 14133.1013 45135.1167 22145.1008 23147.1172 40149.1339 42159.1181 33161.1323 42163.1488 39173.1335 29175.1498 29177.1638 27178.3174 41187.1486 33189.1650 72191.1799 19201.1645 58203.1809 52213.1654 17391.3340 14 |
| 5 | 1.12 | 245.1647 | **Anagyrine****486-89-5** | 57.9729 070.0645 282.7397 083.5940 084.0801 296.0800 298.0958 10098.8100 0110.0591 1113.0349 0122.0598 0124.1109 0136.1115 1142.4957 0150.1277 1161.4449 0162.0915 0178.3302 0245.1649 10 |
| 6 | 1.10 | 261.1598 | **Baptifoline****732-50-3** | 70.0645 782.0651 094.0645 195.0492 096.0801 2498.0960 1100.0755 1110.0595 2114.0908 100122.0590 1122.0965 0134.0959 1146.0597 1146.0964 2150.0913 0152.1064 0160.0748 1162.0901 1164.1067 4166.1225 1172.0764 0214.1259 0243.1488 3261.1598 19 |
| 7 | 1.1 | 338.1385 | Columbamine3621-36-1 | 77.1201 284.2681 2184.7630 2204.1856 1205.4491 2277.1118 8279.0912 15280.0986 15293.1063 5294.1143 86306.1149 24307.0854 4308.0937 52322.1094 100323.1177 52338.1414 14 |
| 8 | 1.12 | 297.1122 | Cryptotanshinone35825-57-1 | 60.5945 470.0644 584.0802 1087.3620 3132.0811 15141.0693 13144.0571 4152.0639 6153.0696 25165.0699 82166.0774 100167.0853 18168.0569 11175.0098 4176.0621 8177.0694 38178.0776 50179.0853 184180.0929 15181.0643 33181.1008 71182.0855 6183.0821 5186.0312 5189.0698 61190.0776 90191.0853 388192.0568 6192.0940 17193.0647 43194.0724 156195.0798 29196.0876 5201.0686 13202.0773 14203.0857 15204.0564 14205.0647 173205.1075 10206.0722 34207.0803 301208.0881 141209.0529 9209.0959 145211.0754 24217.0655 30218.0726 82219.0802 211220.0885 28221.0594 91221.1017 4222.0675 335223.0750 20224.0826 9225.0921 6229.0717 6233.0593 56234.0670 18235.0754 84236.0829 124237.0908 132238.0603 5239.0701 77246.0658 7247.0754 43248.0819 8249.0544 36250.0619 36251.0705 29252.0774 20253.0858 30254.0931 11255.1007 7263.0699 60264.0780 35265.0858 101266.0943 11267.0650 62281.0819 8282.0886 147297.1116 12 |
| 9 | 2.77 | 342.1703 | **Magnoflorine****2141-09-5** | 223.0714 20238.0970 71265.0889 24266.0908 100283.0962 42298.1167 35328.4781 18 |
| 10 | 9.55 | 274.2742 | N-Lauryldiethanolamine1541-67-9 | 57.0697 6970.0650 8171.0853 2175.6440 588.0756 100102.0916 24106.0863 35113.8427 6119.5663 5129.8173 6147.8442 6242.5163 6256.2640 14274.2766 23 |
| 11 | 1.12 | 249.1959 | Lupanine486-87-3 | 58.0646 1569.0330 1470.0645 4877.3171 980.0488 1284.0802 10084.6111 1085.0647 2087.0440 1796.0802 5297.0270 1298.0592 2098.0958 12899.6445 11101.0072 14112.0753 11114.0908 255115.1669 10129.0027 15129.0698 17134.0961 28136.1115 380150.1274 65166.1222 35206.1514 18231.1863 22247.1797 13249.1962 1657 |
| 12 | 7.30 | 751.4615 | a-Hederin27013-91-8 | 85.0282 0115.0396 1129.0533 1144.7982 1166.8288 1174.1212 1205.9828 1207.1751 1279.1080 3399.2415 1409.3452 2437.3444 8455.3527 12583.4149 1601.4205 1619.4366 1751.4638 1115.1669 1129.0027 1129.0698 1134.0961 2136.1115 23150.1274 4166.1222 2206.1514 1231.1863 1247.1797 1249.1962 100 |
| 13 | 7.28 | 781.4727 | Saikosaponin A20736-09-8 | 85.0277 7100.5207 3129.0534 13160.9071 4197.6016 4207.1744 7309.1187 35397.3084 6409.3487 16437.3436 100455.3534 95601.4177 9619.4238 10781.4708 7 |
| 14 | 6.96 | 959.5229 | Asiaticoside16830-15-2 | 129.0542 12131.0180 7147.0656 8156.9923 7165.6242 8170.9757 7179.9275 9184.0413 8207.1756 12273.0966 13309.1175 62310.1227 19408.3279 11426.3463 14436.3269 14453.3390 54454.3430 78471.3497 50472.3524 100600.3837 15618.4042 23636.4207 28652.4166 17798.4795 17 |
| 15 | 1.11 | 265.1547 | 2-propenamide501-13-3 | 64.0008 072.0802 875.1916 180.1905 186.0596 189.0381 489.1072 198.0958 598.9753 1104.0613 1105.3945 1110.9755 1112.0749 1117.0331 28134.0349 2135.0442 1145.0281 100149.0594 13163.0395 1165.0700 1166.0773 1177.0546 47177.5520 1178.2809 1179.0860 1189.0688 1190.0769 2191.0854 6194.0728 3195.0783 1205.0659 1207.0813 1208.0896 1209.0981 1219.0821 2222.0683 5247.1819 1265.1914 7 |
| 16 | 1.10 | 205.1337 | N-methylcytisine63699-79-6 | 55.9338 056.0490 058.0646 10079.2350 082.0649 084.0807 094.0651 097.3501 0108.0655 0108.0802 2110.0608 0110.0959 1118.0645 0118.0823 0122.0602 0125.7762 0130.0652 0132.0818 0133.0523 0136.4594 0139.8265 0144.0804 0146.0596 1147.0666 0160.0753 0162.0906 0164.0939 0205.1335 6 |
| 17 | 10.54 | 455.3519 | NCGC00380944-01 | 67.0544 969.0698 1379.0544 681.0697 3983.0851 886.3485 391.0537 793.0698 2295.0854 7297.0989 4105.0700 31107.0855 48109.0656 6109.1012 44111.0804 66119.0857 46121.1012 46123.1166 17131.0858 10133.1015 48135.1170 31137.1326 4139.1124 5145.1012 24147.1171 53149.1329 45151.1126 5153.0912 6159.1176 27161.1328 44163.1487 39173.1330 22175.1489 35177.1641 27178.2791 11183.8857 4185.1308 5187.1494 24189.1645 100191.1796 10199.1482 6201.1644 47203.1802 62207.1755 7213.1646 7215.1807 20219.1752 7221.1917 3227.1813 6229.1945 5243.2109 10255.2098 8269.2270 6351.3017 4409.3434 9 |
| 18 | 9.73 | 286.1439 | Piperine94-62-2 | 64.7003 1269.0696 2176.0397 13115.0543 52125.0967 11133.4080 12135.0441 55143.0500 53171.0447 35173.0614 19201.0554 100 |
| 19 | 5.97 | 370.1284 | **Taspine** | 80.9558 20131.0990 22325.0716 100 |
| 20 | 3.68 | 328.1544 | **Boldine** | 65.7076 085.0292 0139.0606 0150.0509 0165.0725 1166.0788 0167.0868 0176.0657 0177.0708 10178.0792 0179.0867 0181.0669 0181.1024 0183.0821 0187.0759 0189.0729 0190.0761 0191.0864 1193.0662 0194.0738 2195.0817 0201.0585 0204.0578 0205.0659 43206.0737 1207.0814 1208.0893 0209.0597 0209.0972 2210.0685 0211.0764 1218.0725 0219.0813 1220.0907 0221.0609 0222.0688 12223.0759 0224.0856 0225.0922 3226.1000 1227.0720 0233.0608 14234.0686 2235.0764 0236.0847 1237.0921 100237.9525 0239.0715 2240.0790 0247.0760 0248.0851 0250.0636 5251.0717 3253.0872 1254.0950 5255.1039 0264.0797 0265.0873 44266.0951 6267.0665 7268.0734 0269.1176 0270.0886 0279.1013 0281.0746 0282.0901 12297.1136 5 |