**Supporting Information**

Authentication of herbal medicines from multiple botanical origins with cross-validation mebabolomics, absolute quantification and support vector machine model, a case study of Rhizoma Alismatis

Jianqing Zhanga,#, Cuicui Wanga,#, Wenyong Wua, Qinghao Jinb, Jia Wua, Lin Yanga, Yaling Ana, Changliang Yaoa, Wenlong Weia, Jingyuan Songc, Wanying Wua,\*, De-an Guoa \*

aShanghai Institute of Materia Medica, Chinese Academy of Sciences, Haike Road #501, Shanghai 201203, China.

bZhejiang Yangshengtang Institute of Natural Medication Co.,Ltd., Hangzhou 310000, China

cInstitute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, China.

# These two authors contributed equally to this work.

**\*Corresponding authors**:

E-mail addresses: [wanyingwu@simm.ac.cn](mailto:wanyingwu@simm.ac.cn) (W Wu); daguo@simm.ac.cn (D. Guo)

**Contents**

**Fig.S1** The structures of 35 authentic compounds isolated from both *A. plantago-aquatica* and *A. orientale* in our laboratory

**Fig.S2** OPLS-DA score plot, permutation, S-Plot and VIP of *A. plantago* and *A. orientale*

**Fig.S3** System suitability evaluation of SVM (50%, 100%, and 150% concentration of samples)

**Fig.S4** The base peak ion chromatograms of 7 batches inferior samples

**Table S1** Information on 31 batches of *A. orientale* and *A. plantago-aquatica* identified by morphological identification and further confirmed by DNA barcoding analysis

**Table S2** Information on 47 batches of *A. orientale* and *A. plantago-aquatica* supplied from local GAP bases

**Table S3** Information on 21 batches of *A. orientale* and *A. plantago-aquatica* purchased from the market

**Table S4** MRM parameters of internal and standard compounds

**Table S5** Investigation of intra-day precision of UPLC-LTQ-Orbitrap analysis

**Table S6** Investigation of inter-day precision of UPLC-LTQ-Orbitrap analysis

**Table S7** 41 chemical compounds for differing *A. plantago* and *A. orientale* identified by DNA barcoding analysis (VIP>4)

**Table S8** 29 chemical markers obtained by “multi-duplicated samples” (VIP>4)

**Table S9** 30 chemical markers obtained by “traceability samples” (VIP>4)

**Table S10** Pattern precognition results of 31 batches of Rhizome Alismatis with two botanical origins

**Table S11** System suitability results of different sample concentrations

**Table S12** Pattern recognition results of inferior samples

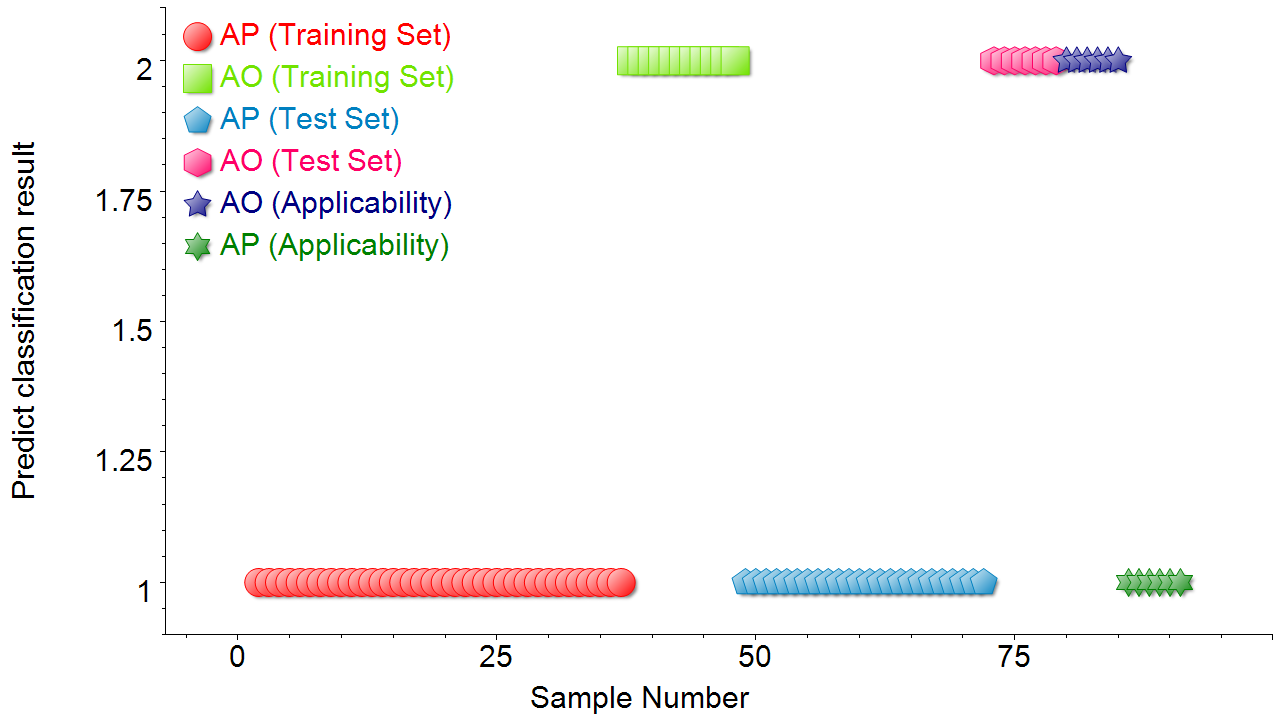
**Table S13** Pattern recognition results of 21bathches of commercial Rhizoma Alismatis

**D:\1张建青\博士后\投稿\张建青ABC投稿\Fig.2.tif**

**Fig.S1** The structures of 35 authentic compounds isolated from both *A. plantago-aquatica* and *A. orientale* in our laboratory (alisma A/B (**C1/2**) , alisol A (**C3**), alisol G (**C4**), alisol A 23/24 acetate (**C5/6**), alsiol E 23 acetate (**C7**), alisol B (**C8**), alisol B 23 acetate (**C9**), alisol C (**C10**), alisol C 23 acetate (**C11**), alisolide G (**C12**), 16-oxoalisol A (**C13**), 16-oxo-11-anhydroalisol A (**C14**), alismanol B (**C15**), 16-oxo-alisol A 23/24 acetate (**C16/17**) , 16-oxo-11-anhydroalisol A 23/24 acetate (**C18/19**), alsiol F (**C20**), 11-anhdroalisol F (**C21**), alisol F 24 acetate (**C22**), alisol O(**C23**), alisol P (**C24**), alisolide A-C (**C25-27**) , neoalisol (**C28**), alisolide F (**C29**), alisolide E (**C30**), alisolide D (**C31**), 25-O-methylalisol A (**C32**), alismanol J (**C33**), alisolide H/I(**C34/35**))



**Fig.S2** OPLS-DA score plot, permutation, S-Plot and VIP of *A. plantago* and *A. orientale*



**Fig.S3** System suitability evaluation of SVM (50%, 100%, and 150% concentration of samples)



**Fig.S4** The base peak ion chromatograms of 7 batches inferior samples

**Table S1** Information on 31 batches of *A. orientale* and *A. plantago-aquatica* identified by morphological identification and further confirmed by DNA barcoding analysis

|  |  |  |  |
| --- | --- | --- | --- |
| No. | Sample label | Collection regions | Species |
| 1 | AP1 | unknown | *Alisma plantago-aquatica* |
| 2 | AP2 | unknown | *Alisma plantago-aquatica* |
| 3 | AP3 | unknown | *Alisma plantago-aquatica* |
| 4 | AP4 | unknown | *Alisma plantago-aquatica* |
| 5 | AP5 | unknown | *Alisma plantago-aquatica* |
| 6 | AP6 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 7 | AP7 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 8 | AP8 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 9 | AP9 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 10 | AP10 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 11 | AP11 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 12 | AP12 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 13 | AP13 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 14 | AP14 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 15 | AP15 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 16 | AP16 | unknown | *Alisma plantago-aquatica* |
| 17 | AP17 | unknown | *Alisma plantago-aquatica* |
| 18 | AP18 | unknown | *Alisma plantago-aquatica* |
| 19 | AP19 | unknown | *Alisma plantago-aquatica* |
| 20 | AP20 | unknown | *Alisma plantago-aquatica* |
| 21 | AP21 | unknown | *Alisma plantago-aquatica* |
| 22 | AP22 | unknown | *Alisma plantago-aquatica* |
| 23 | AP23 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 24 | AP24 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 25 | AO1 | unknown | *Alisma orientale* |
| 26 | AO2 | Fujian Province, China | *Alisma orientale* |
| 27 | AO3 | Jiangxi Province, China | *Alisma orientale* |
| 28 | AO4 | Jiangxi Province, China | *Alisma orientale* |
| 29 | AO5 | Jiangxi Province, China | *Alisma orientale* |
| 30 | AO6 | unknown | *Alisma orientale* |
| 31 | AO7 | Fujian Province, China | *Alisma orientale* |

**Table S2** Information on 47 batches of *A. orientale* and *A. plantago-aquatica* supplied from local GAP bases

|  |  |  |  |
| --- | --- | --- | --- |
| No. | Sample label | Collection regions | Species |
| 1 | AP25 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 2 | AP26 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 3 | AP27 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 4 | AP28 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 5 | AP29 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 6 | AP30 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 7 | AP31 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 8 | AP32 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 9 | AP33 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 10 | AP34 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 11 | AP35 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 12 | AP36 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 13 | AP37 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 14 | AP38 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 15 | AP39 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 16 | AP40 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 17 | AP41 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 18 | AP42 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 19 | AP43 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 20 | AP44 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 21 | AP45 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 22 | AP46 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 23 | AP47 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 24 | AP48 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 25 | AP49 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 26 | AP50 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 27 | AP51 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 28 | AP52 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 29 | AP53 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 30 | AP54 | Fujian Province, China | *Alisma plantago-aquatica* |
| 31 | AP55 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 32 | AP56 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 33 | AP57 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 34 | AP58 | Jiangxi Province, China | *Alisma plantago-aquatica* |
| 35 | AP59 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 36 | AP60 | Sichuan Province, China | *Alisma plantago-aquatica* |
| 37 | AO8 | Fujian Province, China | *Alisma orientale* |
| 38 | AO9 | Fujian Province, China | *Alisma orientale* |
| 39 | AO10 | Fujian Province, China | *Alisma orientale* |
| 40 | AO11 | Fujian Province, China | *Alisma orientale* |
| 41 | AO12 | Fujian Province, China | *Alisma orientale* |
| 42 | AO13 | Fujian Province, China | *Alisma orientale* |
| 43 | AO14 | Fujian Province, China | *Alisma orientale* |
| 44 | AO15 | Fujian Province, China | *Alisma orientale* |
| 45 | AO16 | Fujian Province, China | *Alisma orientale* |
| 46 | AO17 | Fujian Province, China | *Alisma orientale* |
| 47 | AO18 | Fujian Province, China | *Alisma orientale* |

**Table S3** Information on 21 batches of *A. orientale* and *A. plantago-aquatica* purchased from the market

|  |  |  |  |
| --- | --- | --- | --- |
| No. | Sample label | Collection regions | Species |
| 1 | S1 | Fujian Province, China | unknown |
| 2 | S2 | Sichuan Province, China | unknown |
| 3 | S3 | Fujian Province, China | unknown |
| 4 | S4 | Fujian Province, China | unknown |
| 5 | S5 | Fujian Province, China | unknown |
| 6 | S6 | Fujian Province, China | unknown |
| 7 | S7 | Fujian Province, China | unknown |
| 8 | S8 | Guangxi Province, China | unknown |
| 9 | S9 | Guangxi Province, China | unknown |
| 10 | S10 | Guangxi Province, China | unknown |
| 11 | S11 | Guangxi Province, China | unknown |
| 12 | S12 | Guangxi Province, China | unknown |
| 13 | S13 | Sichuan Province, China | unknown |
| 14 | S14 | Sichuan Province, China | unknown |
| 15 | S15 | Sichuan Province, China | unknown |
| 16 | S16 | Sichuan Province, China | unknown |
| 17 | S17 | Sichuan Province, China | unknown |
| 18 | S18 | Fujian Province, China | unknown |
| 19 | S19 | Fujian Province, China | unknown |
| 20 | S20 | Fujian Province, China | unknown |
| 21 | S21 | Fujian Province, China | unknown |

**Table S4** MRM parameters of internal and standard compounds

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **Precursor** | **Product** | **DP/V** | **EP/V** | **CE/eV** | **CXP/V** |
| 16-oxo-11-anhydroalisol A | 487.3 | 397.3 | 60 | 10 | 30 | 9 |
| alisol C 23 acetate | 529.4 | 451.3 | 60 | 10 | 32 | 11 |
| alisol F 24 acetate | 513.4 | 339.2 | 50 | 10 | 21 | 7 |
| alisol A | 473.4 | 383.2 | 95 | 10 | 20 | 25 |
| alisol A 24 acetate | 515.4 | 365.3 | 80 | 13 | 26 | 24 |
| alisol G | 473.4 | 437.3 | 59 | 10 | 13 | 10 |
| alisol B | 473.3 | 365.2 | 52 | 10 | 18 | 24 |
| alisol B 23 acetate | 515.4 | 437.3 | 80 | 12 | 23 | 10 |
| internal standard | 471.3 | 317.2 | 100 | 10 | 41 | 20 |

**Table S5** Investigation of intra-day precision of UPLC-LTQ-Orbitrap analysis

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Retention time/min | | | | | | |
| injection-1 | injection-2 | injection-3 | injection-4 | injection-5 | injection-6 | RSD/% |
| 1 | 3.84 | 3.85 | 3.85 | 3.86 | 3.86 | 3.86 | 0.008 |
| 2 | 8.42 | 8.45 | 8.44 | 8.46 | 8.46 | 8.47 | 0.018 |
| 3 | 14.38 | 14.38 | 14.42 | 14.42 | 14.4 | 14.41 | 0.018 |
| 4 | 17.08 | 17.1 | 17.12 | 17.13 | 17.12 | 17.09 | 0.020 |
| 5 | 23.76 | 23.77 | 23.77 | 23.77 | 23.77 | 23.77 | 0.004 |
| 6 | 28.31 | 28.31 | 28.31 | 28.31 | 28.32 | 28.31 | 0.004 |
| 7 | 31.24 | 31.26 | 31.24 | 31.26 | 31.25 | 31.24 | 0.010 |
| 8 | 36.82 | 36.81 | 36.81 | 36.8 | 36.8 | 36.81 | 0.008 |
| No. | Peak area | | | | | | |
| injection-1 | injection-2 | injection-3 | injection-4 | injection-5 | injection-6 | RSD/% |
| 1 | 976253484 | 889918208 | 880941825 | 905113212 | 892944073 | 919965447 | 3.82 |
| 2 | 1249475658 | 1317570284 | 1322616942 | 1335437934 | 1218341036 | 1308310727 | 3.63 |
| 3 | 1763969135 | 1898128542 | 1834386816 | 1929967804 | 1758866144 | 1785607721 | 3.95 |
| 4 | 1329038075 | 1435283436 | 1466064700 | 1455205637 | 1440977329 | 1512807314 | 4.23 |
| 5 | 5951465227 | 6140626191 | 6031307433 | 6051200925 | 5767730167 | 6491043385 | 3.96 |
| 6 | 6992386809 | 7172221501 | 7131731058 | 7203241804 | 6861417324 | 7240416895 | 2.04 |
| 7 | 739900990 | 739900990 | 726127139 | 693820058 | 740106445 | 708176377 | 2.71 |
| 8 | 828309089 | 835695628 | 824258524 | 792978241 | 785580087 | 822006012 | 2.51 |

**Table S6** Investigation of inter-day precision of UPLC-LTQ-Orbitrap analysis

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Retention time/min | | | | | | | | |  |
| day-1 | | | day-2 | | | day-3 | | |  |
| 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | RSD/% |
| 1 | 3.86 | 3.86 | 3.86 | 3.85 | 3.87 | 3.86 | 3.87 | 3.88 | 3.89 | 0.012 |
| 2 | 8.46 | 8.46 | 8.47 | 8.43 | 8.45 | 8.46 | 8.46 | 8.5 | 8.48 | 0.019 |
| 3 | 14.42 | 14.40 | 14.41 | 14.4 | 14.41 | 14.4 | 14.41 | 14.42 | 14.42 | 0.009 |
| 4 | 17.13 | 17.12 | 17.09 | 17.1 | 17.1 | 17.12 | 17.13 | 17.14 | 17.17 | 0.024 |
| 5 | 23.77 | 23.77 | 23.77 | 23.77 | 23.76 | 23.76 | 23.78 | 23.8 | 23.8 | 0.015 |
| 6 | 28.31 | 28.32 | 28.31 | 28.3 | 28.28 | 28.3 | 28.3 | 28.33 | 28.32 | 0.015 |
| 7 | 31.26 | 31.25 | 31.24 | 31.23 | 31.23 | 31.22 | 31.23 | 31.26 | 31.25 | 0.014 |
| 8 | 36.8 | 36.8 | 36.81 | 36.8 | 36.81 | 36.79 | 36.79 | 36.83 | 36.82 | 0.013 |
| No. | Peak area | | | | | | | | |  |
| day-1 | | | day-2 | | | day-3 | | |  |
| 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | RSD/% |
| 1 | 97625  3484 | 88991  8208 | 88094  1825 | 93679  0025 | 90418  4330 | 90350  4529 | 86610  4743 | 88556  7383 | 89367  7700 | 3.69 |
| 2 | 12494  75658 | 13175  70284 | 13226  16942 | 12490  53120 | 12910  95231 | 12387  89423 | 12350  23306 | 12480  86007 | 12135  24070 | 3.03 |
| 3 | 17639  69135 | 18981  28542 | 18343  86816 | 18119  52925 | 18634  48772 | 18060  35512 | 18435  29599 | 17856  57466 | 17905  37891 | 2.31 |
| 4 | 13290  38075 | 14352  83436 | 14660  64700 | 14368  30454 | 14480  26389 | 14116  88038 | 14821  35558 | 14149  10074 | 14704  03674 | 3.19 |
| 5 | 59514  65227 | 61406  26191 | 60313  07433 | 60305  73585 | 63698  36431 | 61062  18114 | 61242  82785 | 61470  32963 | 61284  97793 | 1.90 |
| 6 | 69923  86809 | 71722  21501 | 71317  31058 | 69552  79209 | 70841  67794 | 72364  40599 | 69660  74661 | 69722  96029 | 70931  20479 | 1.43 |
| 7 | 73990  0990 | 73990  0990 | 72612  7139 | 80805  8193 | 81458  9337 | 74240  3955 | 69355  6370 | 75006  3585 | 66546  0608 | 6.42 |
| 8 | 82830  9089 | 83569  5628 | 82425  8524 | 76906  8547 | 81681  8134 | 85618  4039 | 80724  5951 | 78262  8057 | 79510  1922 | 3.36 |

**Table S7** 41 chemical markers for differing *A. plantago* and *A. orientale* identified by DNA barcoding analysis (VIP>4)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **No.** | **tR/min** | ***m/z*** | **Adducts** | **VIP value** | **Identity** |
| 1 | 28.17 | 515.3729 | [M+H]+ | 45.5 | alisol B 23 acetate |
| 2 | 3.83 | 505.3530 | [M+H-H2O]+ | 20.2 | 16-oxoalisol A |
| 3 | 17.05 | 473.3631 | [M+H]+ | 18.1 | alisol A |
| 4 | 14.32 | 1079.6795 | [2M+Na]+ | 16.2 | alisol C 23 acetate |
| 5 | 23.68 | 455.3526 | [M+H-H2O]+ | 15.2 | alisol B |
| 6 | 22.95 | 545.3481 | [M+H]+ | 14.6 | isomer of 25-anhydroalisol P 24 acetate |
| 7 | 25.97 | 513.3583 | [M+H]+ | 11.1 | isomer of 15, l6-dihydroalisol B 23 acetate |
| 8 | 8.38 | 487.3423 | [M+H]+ | 9.9 | alisol C |
| 10 | 22.31 | 513.3583 | [M+H]+ | 7.9 | isomer of 11-deoxyalisol C 23 |
| 11 | 14.83 | 999.6911 | [2M+Na]+ | 7.8 | isomer of 13β,17β-epoxyalisol B |
| 12 | 15.45 | 469.3322 | [M+H]+ | 7.7 | alismanol B |
| 13 | 20.83 | 553.3507 | [M+Na]+ | 7.5 | isomer of 16-hydro alisol B 23 acetate |
| 14 | 2.79 | 535.3274 | [M+H]+ | 7.2 | Isomer 7-carbony-15-hydro-16-oxo-alisol A |
| 15 | 20.61 | 555.2936 | [M+Na]+ | 7.1 | isomer of alisol A 24 acetate |
| 16 | 23.62 | 469.3317 | [M+H]+ | 7.0 | isomer of alismanol B |
| 17 | 11.88 | 559.3275 | [M+H]+ | 6.8 | isomer of 16-ethoxry alisol B 23 acetate |
| 18 | 19.40 | 525.3195 | [M+Na]+ | 6.8 | isomer of alisol P |
| 19 | 6.66 | 487.3427 | [M+H]+ | 6.6 | isomer of alisol C |
| 20 | 18.87 | 579.2938 | [M+H]+ | 6.6 | C29H42O10N2 |
| 21 | 13.42 | 503.3378 | [M+H]+ | 6.1 | alisol P |
| 22 | 10.25 | 469.3321 | [M+H-H2O]+ | 5.8 | 16-oxo-11-anhydroalisolA |
| 23 | 4.58 | 431.2801 | [M+H]+ | 5.7 | isomer of alisolide C |
| 24 | 8.31 | 471.3473 | [M+H-2H2O]+ | 5.6 | isomer of 7-hydro alisol F |
| 25 | 1.11 | 471.2131 | [M+H]+ | 5.3 | C25H31O7N2 |
| 26 | 13.73 | 471.3478 | [M+H]+ | 5.3 | alisol F |
| 27 | 4.96 | 503.3377 | [M+H]+ | 5.1 | isomer of alisoline C |
| 28 | 22.78 | 527.3374 | [M+H]+ | 4.9 | isomer of alisol J 23 acetate |
| 29 | 14.99 | 473.3632 | [M+H]+ | 4.9 | isomer of alisol A |
| 30 | 10.30 | 277.2167 | [M+H]+ | 4.8 | C18H29O2 |
| 31 | 28.18 | 1083.6587 | [2M+Na]+ | 4.8 | isomer of 15,16-dihydroxyalisol B23 acetate |
| 32 | 17.94 | 515.3736 | [M+H]+ | 4.7 | isomer of alisol A 23 acetate |
| 33 | 22.62 | 455.3528 | [M+H]+ | 4.6 | alisol G |
| 34 | 26.71 | 583.3254 | [M+H]+ | 4.5 | C29H47O10N2 |
| 35 | 17.74 | 511.3423 | [M+H-H2O]+ | 4.5 | alisolide G |
| 36 | 21.41 | 511.3427 | [M+H-H2O]+ | 4.5 | isomer of alisol L 23 acetate |
| 37 | 6.05 | 469.3321 | [M+H-H2O]+ | 4.4 | isomer of 16-oxo-11-anhydro alisol A |
| 38 | 10.41 | 489.3581 | [M+H]+ | 4.3 | isomer of 13β,17β-epoxyalisol B |
| 39 | 3.30 | 503.3378 | [M+H]+ | 4.2 | isomer of alisoline C |
| 40 | 15.72 | 1115.7022 | [2M+Na]+ | 4.1 | isomer of 16-oxo-alisol A 24 acetate |

**Table S8** 29 chemical markers obtained by “multi-duplicated samples” (VIP>4)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **No.** | **tR/min** | ***m/z*** | **Adducts** | **VIP value** | **Identity** |
| 1 | 28.28 | 515.3722 | [M+H]+ | 35.0 | alisol B 23 acetate |
| 2 | 14.41 | 529.3513 | [M+H]+ | 17.3 | alisol C 23 acetate |
| 3 | 3.86 | 505.3515 | [M+H]+ | 16.0 | 16-oxoalisol A |
| 4 | 17.12 | 473.3635 | [M+H]+ | 11.8 | alisol A |
| 5 | 23.04 | 545.3487 | [M+H]+ | 11.2 | 25-anhydroalisol P 24 acetate |
| 6 | 23.77 | 455.35156 | [M+H-H2O]+ | 9.7 | alisol B |
| 7 | 8.46 | 487.3427 | [M+H]+ | 8.9 | alisol C |
| 8 | 26.08 | 513.3585 | [M+H]+ | 8.1 | Isomer of 15,l6-dihydroalisol B 23 acetate |
| 9 | 22.43 | 513.3585 | [M+H]+ | 7.0 | Isomer of 11-deoxyalisol C 23 acetate |
| 10 | 15.54 | 469.3322 | [M+H]+ | 7.0 | isomer of alismanol B |
| 11 | 8.35 | 471.3477 | [M+H-2H2O]+ | 6.5 | isomer of 7-hydro alisol F |
| 12 | 6.75 | 487.3428 | [M+H]+ | 6.0 | isomer of alisol C |
| 13 | 2.81 | 535.3277 | [M+H]+ | 5.9 | Isomer of  7-carbonyl-15-hydro-16-oxo-alisol A |
| 14 | 20.46 | 555.2939 | [M+Na]+ | 5.6 | isomer of alisol A 24 acetate |
| 15 | 4.63 | 431.2801 | [M+H]+ | 5.2 | isomer of alisolide C |
| 16 | 18.81 | 579.2940 | [M+H]+ | 5.2 | C29H43O10N2 |
| 17 | 11.96 | 559.3278 | [M+H]+ | 5.0 | isomer of l6-ethoxy alisol B 23 acetate |
| 18 | 10.32 | 469.3323 | [M+H-H2O]+ | 4.8 | l6-oxo-11-anhydroalisol A |
| 19 | 5.00 | 503.3378 | [M+H-H2O]+ | 4.6 | Isomer of 7-hydroxy-16-oxo-alisol A |
| 20 | 22.72 | 455.3529 | [M+H-H2O]+ | 4.5 | alisol G |
| 21 | 15.08 | 473.3633 | [M+H-H2O]+ | 4.4 | isomer of alisol A |
| 22 | 19.49 | 1005.6683 | [2M+H]+ | 4.4 | isomer of alisol P |
| 23 | 23.78 | 855.6511 | [M+H]+ | 4.4 | C56H86O6 |
| 24 | 34.86 | 629.4782 | [M+H]+ | 4.4 | С39Н64О6 |
| 25 | 1.11 | 471.2134 | [M+H]+ | 4.3 | С25Н31О7N2 |
| 26 | 22.89 | 527.3376 | [M+H]+ | 4.2 | isomer of alisol J 23 acetate |
| 27 | 17.84 | 511.3423 | [M+H-H2O]+ | 4.2 | isomer of alisolide G |
| 28 | 19.50 | 545.3478 | [M+H-H2O]+ | 4.1 | isomer of alisol P 24 acetate |
| 29 | 21.53 | 511.3428 | [M+H-H2O]+ | 4.1 | isomer of alisol L 23 acetate |

**Table S9** 30 chemical markers obtained by “traceability samples” (VIP>4)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **No.** | **tR/min** | | ***m/z*** | **Adducts** | **VIP value** | **Identity** |
| 1 | 28.17 | 515.3729 | | [M+H]+ | 64.6 | alisol B 23 acetate |
| 2 | 17.05 | 473.3619 | | [M+H-H2O]+ | 13.3 | alisol A |
| 3 | 31.11 | 439.3575 | | [M+H-H2O]+ | 13.2 | C30H48O3 |
| 4 | 23.68 | 455.3526 | | [M+H-H2O]+ | 11.5 | alisol B |
| 5 | 3.83 | 505.3530 | | [M+H]+ | 11.5 | 16-oxoalisol A |
| 6 | 15.72 | 1115.7022 | | [2M+Na]+ | 9.3 | isomer of 16-oxo-alisol A 24 acetate |
| 7 | 18.87 | 579.2938 | | [M+H]+ | 9.2 | C29H42O10N2 |
| 8 | 14.32 | 1079.6795 | | [2M+Na]+ | 8.3 | alisol C 23 acetate |
| 9 | 1.11 | 471.2131 | | [M+H]+ | 7.7 | C25H31O7N2 |
| 10 | 20.61 | 555.2936 | | [M+Na]+ | 7.2 | isomer of alisol A 24 acetate |
| 11 | 14.26 | 495.3475 | | [M+H-2H2O]+ | 6.7 | isomer of alisol F 24 acetate |
| 12 | 15.26 | 471.3477 | | [M+H]+ | 6.5 | isomer of 11-anhydroalisol F |
| 13 | 8.38 | 487.3423 | | [M+H]+ | 5.9 | alisol C |
| 14 | 28.18 | 1083.6587 | | [2M+Na]+ | 5.6 | isomer of  15,16-dihydroxyalisol B 23 acetate |
| 15 | 19.40 | 503.3376 | | [M+H]+ | 5.3 | isomer of alisol P |
| 16 | 14.83 | 999.6911 | | [2M+Na]+ | 5.3 | 13β,17β-epoxyalisol B |
| 17 | 14.06 | 1115.7020 | | [2M+Na]+ | 5.2 | isomer of l6-oxo-alisol A 24 acetate |
| 18 | 26.71 | 583.3254 | | [M+H]+ | 5.2 | С29Н47О10N2 |
| 19 | 22.31 | 513.3583 | | [M+H]+ | 5.1 | 11-deoxyalisol C 23 acetate |
| 20 | 12.29 | 513.3578 | | [M+H-H2O]+ | 5.1 | alisoma C |
| 21 | 28.99 | 513.3584 | | [M+H]+ | 4.9 | isomer of 15,16-dihydroxyalisol B 23 acetate |
| 22 | 14.05 | 547.3636 | | [M+H]+ | 4.8 | isomer of l6-oxo-alisol A 24 acetate |
| 23 | 31.12 | 713.5079 | | [M+H]+ | 4.8 | C39H70O10N |
| 24 | 2.79 | 535.3274 | | [M+H]+ | 4.6 | 7-carbonyl-15-hydro-16-oxo-alisol A |
| 25 | 23.56 | 531.36688 | | [M+H]+ | 4.6 | isomer of l6-hydroalisol B 23 acetate |
| 26 | 15.72 | 513.3580 | | [M+H-H2O]+ | 4.6 | isomer of l6-hydroalisol B 23 acetate |
| 27 | 23.35 | 279.2325 | | [M+H-H2O]+ | 4.5 | C18H33O3 |
| 28 | 1.59 | 455.2184 | | [M+H]+ | 4.4 | C22H33O9N |
| 29 | 1.22 | 469.1975 | | [M+H]+ | 4.3 | C27H30O2N |
| 30 | 13.93 | 513.3579 | | [M+H-H2O]+ | 4.2 | isomer of l6-hydroxy alisol B 23 acetate |

**Table S10** Pattern precognition results of 31 batches of Rhizome Alismatis with two botanical origins

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ID** | **Sample** | **AP** | **AO** | **Predicted Classification** |
| 48 | AP1 | 1.05 | -1.05 | AP |
| 49 | AP2 | 1.11 | -1.11 | AP |
| 50 | AP3 | 1.14 | -1.14 | AP |
| 51 | AP4 | 1.12 | -1.12 | AP |
| 52 | AP5 | 1.06 | -1.06 | AP |
| 53 | AP6 | 0.89 | -0.89 | AP |
| 54 | AP7 | 0.85 | -0.85 | AP |
| 55 | AP8 | 0.94 | -0.94 | AP |
| 56 | AP9 | 0.44 | -0.44 | AP |
| 57 | AP10 | 0.88 | -0.88 | AP |
| 58 | AP11 | 0.95 | -0.95 | AP |
| 59 | AP12 | 0.94 | -0.94 | AP |
| 60 | AP13 | 0.93 | -0.93 | AP |
| 61 | AP14 | 0.92 | -0.92 | AP |
| 62 | AP15 | 0.86 | -0.86 | AP |
| 63 | AP16 | 1.00 | -1.00 | AP |
| 64 | AP17 | 1.00 | -1.00 | AP |
| 65 | AP18 | 0.99 | -0.99 | AP |
| 66 | AP19 | 1.09 | -1.09 | AP |
| 67 | AP20 | 1.05 | -1.05 | AP |
| 68 | AP21 | 1.09 | -1.09 | AP |
| 69 | AP22 | 1.04 | -1.04 | AP |
| 70 | AP23 | 1.11 | -1.11 | AP |
| 71 | AP24 | 1.12 | -1.12 | AP |
| 72 | AO1 | -0.50 | 0.50 | AO |
| 73 | AO2 | -0.70 | 0.70 | AO |
| 74 | AO3 | -0.64 | 0.64 | AO |
| 75 | AO4 | -0.47 | 0.47 | AO |
| 76 | AO5 | -0.54 | 0.54 | AO |
| 77 | AO6 | -0.90 | 0.90 | AO |
| 78 | AO7 | -0.51 | 0.51 | AO |

**Table 11** System suitability results of different sample concentrations

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ID** | **Sample** | **AP** | **AO** | **Predicted Classification** |
| 79 | 50%AO4 | -0.58 | 0.58 | AO |
| 80 | 50%AO3 | -0.78 | 0.78 | AO |
| 81 | 50%AO5 | -0.64 | 0.64 | AO |
| 82 | 50%AP10 | 0.87 | 0.87 | AP |
| 83 | 50%AP12 | 0.90 | 0.90 | AP |
| 84 | 50%AP15 | 0.75 | 0.75 | AP |
| 85 | 150% AO4 | -0.49 | 0.49 | AO |
| 86 | 150% AO3 | -0.68 | 0.68 | AO |
| 87 | 150% AO5 | -0.56 | 0.56 | AO |
| 88 | 150% AP10 | 0.96 | -0.96 | AP |
| 89 | 150% AP12 | 0.95 | -0.95 | AP |
| 90 | 150% AP15 | 0.89 | -0.89 | AP |

**Table S12** Pattern recognition results of inferior samples

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ID** | **Sample** | **AP** | **AO** | **Predicted Classification** |
| 79 | N1 | 0.34 | -0.14 | AP |
| 80 | N2 | 0.32 | -0.14 | AP |
| 81 | N3 | 0.32 | -0.14 | AP |
| 82 | N4 | 0.14 | -0.14 | AP |
| 83 | N5 | 0.25 | -0.25 | AP |
| 84 | N6 | 0.14 | -0.14 | AP |
| 85 | N7 | 0.14 | -0.14 | AP |

**Table S13** Pattern recognition results of 21bathches of commercial Rhizoma Alismatis

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **NO.** | **Sample** | **AP** | **AO** | **Predicted Classification** |
| 79 | S1 | 1.03 | -1.03 | AP |
| 80 | S2 | 1.14 | -1.14 | AP |
| 81 | S3 | 1.08 | -1.08 | AP |
| 82 | S4 | 1.06 | -1.06 | AP |
| 83 | S5 | 0.98 | -0.98 | AP |
| 84 | S6 | 1.07 | -1.07 | AP |
| 85 | S7 | 1.08 | -1.08 | AP |
| 86 | S8 | 1.06 | -1.06 | AP |
| 87 | S9 | 1.05 | -1.05 | AP |
| 88 | S10 | 0.98 | -0.98 | AP |
| 89 | S11 | 1.15 | -1.15 | AP |
| 90 | S12 | 1.02 | -1.02 | AP |
| 91 | S13 | 1.12 | -1.12 | AP |
| 92 | S14 | 0.96 | -0.96 | AP |
| 93 | S15 | 1.01 | -1.01 | AP |
| 94 | S16 | 0.97 | -0.97 | AP |
| 95 | S17 | 1.09 | -1.09 | AP |
| 96 | S18 | 1.08 | -1.08 | AP |
| 97 | S19 | 0.97 | -0.97 | AP |
| 98 | S21 | 0.92 | -0.92 | AP |
| 99 | S20 | -0.47 | 0.47 | AO |