Research Article

Determination of Alkaloids and Flavonoids in *Sophora flavescens* by UHPLC-Q-TOF/MS

Yaqian Dong,1 Guoxiang Jia,1 Jingwen Hu,1 Hui Liu,1 Tingting Wu,1 Shenshen Yang,1 Yubo Li,1 and Ting Cai2,3

1School of Traditional Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine, No. 10 Poyang Lake Road, Tuanbo New City, Jinghai District, Tianjin 301617, China
2Hwa Mei Hospital, University of Chinese Academy of Sciences (Ningbo No. 2 Hospital), Ningbo 315010, China
3Ningbo Institute of Life and Health Industry, University of Chinese Academy of Sciences, Ningbo 315010, China

Correspondence should be addressed to Shenshen Yang; shine2099@163.com, Yubo Li; yaowufenxi001@sina.com, and Ting Cai; caiting@ucas.ac.cn

Received 23 March 2021; Accepted 12 July 2021; Published 28 July 2021

Academic Editor: Boryana M. Nikolova Damyanova

Copyright © 2021 Yaqian Dong et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

This study is based on UHPLC-Q-TOF/MS and fragment ions to achieve classification and identification of alkaloids and flavonoids in *Sophora flavescens*. By reviewing the available and relevant literature, the mass fragmentation rules of alkaloids and flavonoids were summarized. 0.1% formic acid water (A) and acetonitrile (B) were used as mobile phases. 37 chemical constituents were identified, including 13 alkaloids and 24 flavonoids. This research method offers a complete strategy based on the fragmentation information of characteristic fragment ions and neutral loss obtained by MS/MS to characterize the chemical composition of *Sophora flavescens*.

1. Introduction

The analytical methods of traditional Chinese medicine (TCM) are not sufficient for the separation and identification of many complex chemical components, which brings challenges in terms of the quality control and clinical application of TCM [1]. Ultrahigh-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF/MS) has become the main means of component analysis of modern traditional Chinese medicine because of its high speed, high efficiency, and high resolution. It can overcome the limitation of ultraviolet detectors, so it is suitable for component analysis in the complex traditional Chinese medicine system [2–4]. The chemical components in TCM can be classified and quickly identified on the basis of secondary fragments [5, 6]. In the process of treating diseases, traditional Chinese medicine often has multiple components and multiple targets, which often lead to the problem of unclear components. Therefore, the classification and identification of chemical components in traditional Chinese medicine are very meaningful. According to differences in the chemical structure, the compounds can be divided into different parent nuclear structure types. Compounds with the same parent nuclear type will produce some ion fragments which are the same in the process of mass spectrometry collision.

The traditional Chinese medicinal herb *Sophora flavescens* comes from dried roots of *Sophora flavescens* Ait., a leguminous plant which is listed as middle grade in Shen-nong Materia Medica, and is bitter and cold in taste. Alkaloids and flavonoids are considered to be the main active components of *Sophora flavescens* [7]. Studies have shown that alkaloids in *Sophora flavescens* can reduce the secretion of inflammatory factor TNF-α by regulating the expression of BMP2, Runx2, and other proteins, so as to increase the activity of alkaline phosphatase to treat chronic osteomyelitis caused by *Staphylococcus aureus* infection [8]. Indole-amine 2-dioxygenase-1 (IDO1), a tumor cell survival factor, can lead to the escape of many kinds of cancer cells. As inhibitors of IDO1, many flavonoids in *Sophora flavescens*...
have potential uses in cancer immunotherapy [9]. In view of the good clinical efficacy and research prospects of *Sophora flavescens*, it is of great significance to establish a technique that can quickly classify and identify the chemical composition of *Sophora flavescens*.

Based on the UHPLC-Q-TOF/MS technology, this study summarized the characteristic fragments and neutral losses during the cleavage process of compounds, classified and identified the chemical components in *Sophora flavescens*, and identified 37 alkaloids and flavonoids in *Sophora flavescens*.

### 2. Methods

#### 2.1. Materials and Instruments. *Sophora flavescens* (Beijing Tongren Drug Store), matrine, oxymatrine, sophocarpine standard (Chengdu Ruifensi Biotechnology Co., Ltd., China), ethanol (Tianjin Huaihang, analytical grade), acetonitrile (Sigma, USA, HPLC grade), formic acid (Sigma, USA, HPLC grade), distilled water (Guangzhou Watsons, China), UPLC-Q-TOF-MS (Waters, Milford, MA, USA), a Waters ACQUITY UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm), and MassLynx V4.1 were used.

#### 2.2. Preparation of Samples. 5.0g of *Sophora flavescens* was precisely weighed, refluxed, and extracted twice with 8 times and 6 times of 70% ethanol for 2 hours each time. The combined extract was evaporated and concentrated to 0.1g/mL and then filtered by a 0.22 μm microporous membrane, which was the sample solution to be injected [10, 11].

1 mg of matrine, oxymatrine, and sophocarpine was precisely weighed. Then, 1 ml of 70% ethanol was added to dissolve and passed through a 0.22 μm microporous filter membrane.

#### 2.3. UHPLC and MS Conditions.

**UHPLC:** Waters ACQUITY UHPLC BEH C18 Column, 2.1 × 100 mm, 1.7 μm; column temperature is set to 35°C; mobile phase: the aqueous phase is 0.1% formic acid aqueous solution (A), and the organic phase is acetonitrile solution (B); flow rate: 0.4 mL/min. The gradient elution method is used for chromatographic separation, and the gradient procedure is as follows: 0–10 min, 3–20% B; 10–15 min, 20–30% B; 15–20 min, 30–50% B; 20–25 min, 50–70% B; 25–27 min, 70–100% B; 27–30 min, 100% B; 30–32 min, 100–3% B; 32–35 min, 3% B

**TOF-MS:** electrospray ionization source (ESI), scanning mode: positive and negative ions. The MS parameters are as follows: dry gas temperature: 325°C; dry gas flow rate: 11 ml/min; desolvation gas flow rate: 800 L/h; capillary voltage: 3.0 kV; collision dissociation voltage: 6 kV; collision energy: 20–50 eV; atomizer pressure: 350 psi; auxiliary gas: N2; positive and negative reference ion calibration ([M+H]+ = 554.2615, [M-H]- = 554.2615) to ensure accuracy in spectral acquisition. The range of data acquisition is 50 to 1500.

### 3. Results and Discussion

#### 3.1. Establishment of the Method. The mass spectrometry experimental data reported in the literature were used to summarize the fragments missing from the fragment ion peaks of known chemical components in *Sophora flavescens* and summarize the fragmentation rules of different fragment ions. Subsequently, MassLynx software was used for peak matching, and the chemical composition of *Sophora flavescens* was deduced based on the retention time of its components and the fragmentation rules. Finally, 13 alkaloids and 24 flavonoids were identified, as shown in Table 1. The fragmentation rules of the chemical components in *Sophora flavescens* are shown in Figure 1, and the base peak ion (BPI) chromatogram of the *Sophora flavescens* extract in positive and negative ions is shown in Figure 2.

#### 3.2. Fragmentation Rules of Alkaloid Compounds. According to the structure type of the mother nucleus, the alkaloids in *Sophora flavescens* are mainly divided into matrine type, broom alkaloid type, anagyrine type, and lupine type [25]. Among them, matrine-type compounds easily lose H2O (18), C7H6NO (97), and C6H4NO (99) in the collision process, resulting in characteristic fragments of m/z 150 and m/z 148. Nitrogen oxides of matrine alkaloids easily lose H2O (18) and OH (17), resulting in high-abundance fragments [M+H-H2O]+ and [M+H-OH]+ [13, 14]. The cleavage of C7-C13/C9-C11 and C6-C7/C1-C10 of broom alkaloid bonds will produce characteristic fragments such as 146 [M+H-C7H6N]+ and 148[M+H-C6H4N]+ which are related to the methyl substituents at position 12 [12]. The characteristic fragments of daidzein alkaloids include 243[M+H-H2O]+, 205[M+H-H2O-C3H4]+, 123[M+H-C8H14N2]+, and 114[M+H-C6H4N]+ [17].

The molecular formula of compound 2 is C15H24N2O, and the retention time is 1.59 min. The main secondary fragments are 247.1815, 231.1959, 176.1083, 150.1298, and 148.1144. In the positive ion mode, the molecular ion peak is m/z 249.1980[M+H]+, and its parent ion removes a molecule of H2 and H2O, respectively, resulting in an ion peak of m/z 247.1815[M+H-H2] and a dehydration peak of m/z 246.1795[M+H-H2O]++. Then, the compound undergoes RDA cleavage. On this basis, characteristic matrine-type ion fragments are m/z 150.1298 [M+H-H2-C3H2NO]+, m/z 148.1152[M+H-H2-C3H2NO]+, and m/z 136.1144[M+H-C3H2NO]+. On the basis of losing a molecule of H2, the compound can continue to lose a molecule of C6H4NO and form an ion peak of m/z 176.1083[M+H-H2-C3H4NO]+. According to fragment information, standard reference substance retention time, relative molecular mass, and MS and MS information, the compound is identified as matrine. The fragmentation rules are shown in Figure 3.

The molecular formula of compound 4 is C15H24N2O, and the retention time is 1.93 min. In the positive ion mode, the molecular ion peak is 247.1821[M+H]+, and the main secondary fragments are 245.1561, 179.1542, 150.1293, 148.1149, 136.1137, and 108.0833. It is conjectured that the
| No. | Identity                  | Formula        | RT    | Theoretical value | Actual value | ppm   | Main MS/MS fragments detected (arranged from large to small according to relative intensity) | Ref. |
|-----|---------------------------|----------------|-------|-------------------|--------------|-------|---------------------------------------------------------------------------------|------|
| 1   | Lamprolobine              | C_{15}H_{24}N_{2}O_{2} | 0.72  | 265.1916          | 265.1926     | 3.77  | 265.1926[M+H]^+ 150.1293[M+H-H_{2}O-C_{5}H_{7}NO]^+ [12]                           |      |
| 2   | Matrine                   | C_{15}H_{24}N_{2}O    | 1.59  | 249.1967          | 249.1980     | 5.22  | 249.1980[M+H]^+ 247.1815[M+H-H_{2}]^+ 150.1298[M+H-H_{2}O-C_{5}H_{7}NO]^+ [13, 14] |      |
| 3   | 7,11-Dehydromatrine       | C_{15}H_{22}N_{2}O    | 1.93  | 247.1810          | 247.1821     | 4.45  | 247.1821[M+H]^+ 245.1661[M+H-H_{2}O]^+ 150.1294[M+H-H_{2}O-C_{5}H_{7}NO]^+ [12] |      |
| 4   | Sophocarpine              | C_{15}H_{22}N_{2}O    | 1.93  | 247.1810          | 247.1821     | 4.45  | 247.1821[M+H]^+ 245.1667[M+H-H_{2}O]^+ 150.1294[M+H-H_{2}O-C_{5}H_{7}NO]^+ [12] |      |
| 5   | Sophoridine               | C_{15}H_{22}N_{2}O    | 1.96  | 249.1967          | 249.1983     | 6.42  | 249.1983[M+H]^+ 247.1827[M+H-H_{2}]^+ 150.1294[M+H-H_{2}O-C_{5}H_{7}NO]^+ [14] |      |
| 6   | 9α-Hydroxysophocarpine    | C_{16}H_{22}N_{2}O    | 2.14  | 263.1760          | 263.1776     | 6.08  | 263.1776[M+H]^+ 245.1667[M+H-H_{2}O]^+ 150.1299[M+H-H_{2}O-C_{5}H_{7}NO]^+ [15] |      |
| 7   | Mamanine                  | C_{15}H_{22}N_{2}O    | 2.14  | 263.1760          | 263.1776     | 6.08  | 263.1776[M+H]^+ 245.1667[M+H-H_{2}O]^+ 150.1299[M+H-H_{2}O-C_{5}H_{7}NO]^+ [12] |      |
| 8   | 9α-Hydroxymatrine         | C_{16}H_{24}N_{2}O    | 2.29  | 265.1916          | 265.1928     | 4.53  | 265.1928[M+H]^+ 150.1294[M+H-H_{2}O-C_{5}H_{7}NO]^+ 247.1824[M+H-H_{2}O]^+ [15] |      |
| 9   | Oxysophoridine            | C_{16}H_{24}N_{2}O    | 2.33  | 265.1916          | 265.1933     | 6.41  | 265.1933[M+H]^+ 247.1826[M+H-H_{2}O]^+ 148.1142[M+H-H_{2}O-C_{5}H_{7}NO]^+ [12] |      |
| 10  | Oxysophocarpine           | C_{16}H_{22}N_{2}O    | 2.65  | 263.1760          | 263.1760     | 0.00  | 263.1760[M+H]^+ 177.1402[M+H-H_{2}O-C_{5}H_{4}O]^+ 245.1662[M+H-H_{2}O]^+ [12] |      |
| No. | Identity | Formula | RT  | Theoretical value | Actual value ppm | Ref. |
|-----|----------|---------|-----|-------------------|------------------|-----|
| 11  | Oxymatrine | C_{13}H_{24}N_{2}O_{2} | 3.24 | 265.1916 | 265.1926 3.77 | 265.1926[M+H]^{+} 247.1823[M+H-H_{2}O]^{+} 205.1355[M+H-H_{2}O-C_{2}H_{4}N]^{+} 148.1139[M+H-H_{2}O-C_{2}H_{4}NO]^{+} 175.1254[M+H-C_{2}H_{4}O]^{+} 112.1129[M+H-C_{2}H_{4}NO-C_{2}H_{4}NO]^{+} [13, 14] |
| 12  | Sophoranol N-oxide | C_{13}H_{24}N_{2}O_{3} | 4.68 | 281.1865 | 281.1879 4.98 | 281.1879[M+H]^{+} 245.1696[M+H-2H_{2}O]^{+} 138.1283[M+H-H_{2}O-C_{4}H_{10}NO_{2}]^{+} 263.1678[M+H-H_{2}O-C_{2}H_{4}NO-C_{2}H_{4}NO]^{+} [15] |
| 13  | Daidzin | C_{21}H_{20}O_{9} | 6.19 | 417.1186 | 417.1184 −0.48 | 417.1184[M+H]^{+} 255.0589[M+H-Glu]^{+} 199.0759[M+H-Glu-2CO]^{+} [12, 16] |
| 14  | Baptifoline | C_{23}H_{20}N_{2}O_{2} | 6.70 | 261.1603 | 261.1595 −3.06 | 243.1435[M+H-H_{2}O]^{+} 261.1595[M+H]^{+} 137.0274[M+H-C_{2}O_{3}]^{+} 199.0759[M+H-Glu-2CO]^{+} [16] |
| 15  | Daidzein | C_{15}H_{10}O_{4} | 11.03 | 255.0657 | 255.0690 12.94 | 255.0690[M+H-Glu]^{+} 137.0274[M+H-C_{2}O_{3}]^{+} 199.0759[M+H-Glu-2CO]^{+} [12, 16] |
| 16  | Kurarinone | C_{26}H_{30}O_{6} | 19.43 | 439.2128 | 439.2128 2.98 | 439.2128[M+H]^{+} 313.0851[M+H-C_{3}H_{4}O]^{+} 253.0672[M+H-H_{2}O-C_{2}O_{3}]^{+} 351.1222[M+H-H_{2}O-C_{2}O_{3}]^{+} [17, 18] |
| 17  | Stamens isoflavones | C_{16}H_{12}O_{5} | 12.26 | 283.0606 | 283.0618 4.24 | 283.0616[M-H]^{−} 268.0373[M-H-CH_{3}]^{−} 211.0400[M-H-C_{2}O_{3}]^{−} 253.0472[M-H-3H_{2}O]^{−} 271.0141[M-H-H_{2}O]^{−} [12, 19] |
| 18  | (2R,3R)-8-Isopentenyl-7,4-dihydroxy-5-methoxy dihydroflavonol | C_{21}H_{22}O_{6} | 16.04 | 369.1349 | 369.1349 2.98 | 369.1349[M-H]^{−} 313.0851[M-H-C_{3}H_{4}O]^{−} 353.9778[M-H-C_{2}H_{4}O]^{−} 351.1222[M-H-H_{2}O-C_{2}O_{3}]^{−} [12] |
| 19  | Formononetin | C_{16}H_{12}O_{4} | 16.29 | 267.0657 | 267.0669 4.49 | 267.0669[M-H]^{−} 252.0430[M-H-C_{2}H_{4}O]^{−} 223.0404[M-H-CO_{2}]^{−} [20] |
| 20  | 2'-Hydroxy-isoxanthohumol | C_{21}H_{22}O_{6} | 16.58 | 369.1349 | 369.1346 2.17 | 369.1346[M-H]^{−} 207.1024[M-H-H_{2}O-C_{2}H_{4}O_{2}]^{−} 341.1357[M-H-C_{2}H_{4}O]^{−} 351.1252[M-H-H_{2}O-C_{2}H_{4}O_{2}]^{−} 354.4461[M-H-C_{2}H_{4}O]^{−} [12] |
| 21  | Kurardinol | C_{26}H_{32}O_{7} | 16.68 | 455.2070 | 455.2076 1.32 | 455.2076[M-H]^{−} 161.0246[C_{6}H_{10}O_{5}]^{−} 293.1761[M-H-C_{2}H_{4}O]^{−} 437.1793[M-H-H_{2}O-C_{2}H_{4}O_{2}]^{−} [19, 21] |
| 22  | Leachianone G | C_{20}H_{20}O_{6} | 17.37 | 355.1182 | 355.1202 5.63 | 355.1202[M-H]^{−} 161.0246[C_{6}H_{10}O_{5}]^{−} 337.1068[M-H-H_{2}O-C_{2}H_{4}O_{2}]^{−} 235.1366[M-H-H_{2}O-C_{2}H_{4}O_{2}]^{−} [17] |
| No. | Identity        | Formula       | RT  | Theoretical value | Actual value | ppm  | Main MS/MS fragments detected (arranged from large to small according to relative intensity) | Ref. |
|-----|----------------|---------------|-----|-------------------|--------------|------|--------------------------------------------------------------------------------|------|
| 23  | Norkurarinol   | C_{25}H_{30}O_{7} | 18.24 | 441.1913          | 441.1928     | 3.40 | 279.161^{1,3}A^{-}, 441.1928[M-H]^{-}, 161.0251[C_{9}H_{5}O_{3}]^{-}, 211.1704^{1,3}A^{-}C_{9}O_{2}, 162.0820^{1,4}B^{-}, 423.1707[M-H-H_{2}O]^{-} | [21]|
| 24  | Kushenol Q    | C_{25}H_{28}O_{11} | 18.39 | 441.1913          | 441.1935     | 4.99 | 279.161^{1,3}A^{-}, 441.1935[M-H]^{-}, 161.0251[C_{9}H_{5}O_{3}]^{-}, 211.1704^{1,3}A^{-}C_{9}O_{2}, 162.0820^{1,4}B^{-}, 275.1653[C_{17}H_{23}O_{3}]^{-}, 137.0245^{1,4A}-C_{6}H_{12}-CH_{3} | [22]|
| 25  | Maackiain      | C_{16}H_{12}O_{5} | 18.48 | 283.0606          | 283.0598     | –2.83| 283.0598[M-H]^{-}, 268.0368[M-H-CH_{3}]^{-}, 227.0754[M-H-2CO]^{-}, 255.0628[M-H-CO]^{-} | [16]|
| 26  | Kushenol N    | C_{26}H_{30}O_{7} | 18.76 | 453.1913          | 453.1913     | 0.00 | 453.1913[M-H]^{-}, 177.0195[C_{9}H_{5}O_{4}]^{-}, 275.1653[C_{17}H_{23}O_{3}]^{-}, 149.0249^{1,4}B^{-}, 137.0254^{1,4A}-C_{9}H_{14}O^{-} | [23]|
| 27  | Kushenol I     | C_{26}H_{30}O_{7} | 19.04 | 453.1913          | 453.1908     | –1.10| 453.1908[M-H]^{-}, 177.0195[C_{9}H_{5}O_{4}]^{-}, 149.0256^{1,4}B^{-}, 435.1866[M-H-2CO]^{-}, 425.2036[M-H-CO]^{-} | [16]|
| 28  | Sophoraflavonane B | C_{20}H_{26}O_{5} | 19.08 | 339.1232          | 339.1231     | –0.29| 219.0664^{1,4}A^{-}, 339.1231[M-H]^{-}, 119.0504^{1,4A}-C_{9}H_{14}O^{-}, 275.1648[M-H-C_{9}H_{4}O]^{-}, 321.9993[M-H-H_{2}O]^{-} | [16]|
| 29  | Noranhdrocaritin | C_{20}H_{18}O_{6} | 19.17 | 353.1025          | 353.1034     | 2.55 | 353.1034[M-H]^{-}, 298.0487[M-H-C_{9}H_{4}O]^{-}, 136.0176[M-H-C_{9}H_{5}O_{2}H]^{-}, 338.0812[M-H-2CO]^{-}, 161.0250[C_{7}H_{3}O_{3}]^{-} | [24]|
| 30  | Kushenol L     | C_{25}H_{28}O_{7} | 19.43 | 439.1757          | 439.1760     | 0.68 | 439.1760[M-H]^{-}, 275.1653[C_{9}H_{5}O_{3}]^{-}, 177.0201[C_{9}H_{5}O_{3}]^{-}, 149.0247[M-H-C_{9}H_{14}O]^{-}, 421.1667[M-H-H_{2}O]^{-} | [24]|
| 31  | Sophoraisoflavanone A | C_{21}H_{22}O_{6} | 20.18 | 369.1338          | 369.1360     | 5.96 | 161.0255^{1,4}B^{-}, 369.1360[M-H]^{-}, 135.0452^{1,4}B^{-}, 273.1674[M-H-C_{9}H_{3}O]^{-}, 288.107^{1,4}A^{-} | [12]|
| 32  | 8-Lavandulylkaempferol | C_{26}H_{30}O_{5} | 20.39 | 421.2015          | 421.2027     | 2.85 | 421.2027[M-H]^{-}, 301.1454^{1,4}A^{-}, 119.0511^{1,4A}-C_{9}H_{14}O-C_{9}H_{8}^{-}, 163.0040^{1,4A}-C_{9}H_{14}O^{-} | [12, 16]|
| 33  | Kushenol D     | C_{27}H_{32}O_{6} | 20.56 | 451.2121          | 451.2118     | –0.66| 451.2118[M-H]^{-}, 301.1442^{1,4}A^{-}, 149.0617^{1,4}B^{-}, 217.0517^{1,4A}-C_{9}H_{12}, 419.1871[M-H-C_{9}H_{2}O]^{-} | [24]|

Table 1: Continued.
The fragmentation process of compound 4 is as follows. Firstly, the parent ions lose a molecule of C$_5$H$_7$NO (97) and C$_5$H$_9$NO (99) to produce the characteristic ion fragments of matrine type: m/z 150.1293[M+H-H$_2$-C$_5$H$_7$NO]$^+$ and m/z 148.1149[M+H-H$_2$-C$_5$H$_9$NO]$^+$. Secondly, the parent ion can lose a molecule of C$_4$H$_4$O resulting in the fragment 179.1542.

### Table 1: Continued.

| No. | Identity | Formula | RT (min) | Theoretical value | Actual value | ppm | Main MS/MS fragments detected (arranged from large to small according to relative intensity) | Ref. |
|-----|----------|---------|----------|-------------------|--------------|-----|-----------------------------------------------------------------|------|
| 34  | Norkurarinone | C$_{25}$H$_{28}$O$_6$ | 20.62 | 423.1805 | 423.1805 | -0.71 | 423.1805[M-H]$^-$ 161.0249$^{14}$A$^-$ 262.1535$^{14}$A$^-$ 138.0323$^{14}$A$^-$ 193.1601[M-H-H$_2$O-C$_{13}$H$_{15}$O]$^-$ 405.1707[M-H-H$_2$O]$^-$ 261.1497[M-H-H$_2$O-ringB-C$_{10}$H$_{15}$]$^-$ 177.0191[M-H-H$_2$O-ringB-C$_{10}$H$_{15}$]$^-$ 149.0251[M-H-C$_9$H$_{14}$O]$^-$ 439.1755[M-H]$^-$ 287.1290$^{14}$A$^-$ 109.0298[M-H-H$_2$O-ringB-C$_{10}$H$_{15}$-C$_4$H$_7$]$^-$ 152.0812$^{14}$B$^-$ | [17] |
| 35  | Kushenol X | C$_{23}$H$_{28}$O$_7$ | 21.15 | 439.1757 | 439.1755 | -0.46 | 421.1631[M-H-H$_2$O]$^-$ 287.1290$^{14}$A$^-$ 177.0191[M-H-H$_2$O-ringB-C$_{10}$H$_{15}$]$^-$ 149.0251[M-H-C$_9$H$_{14}$O]$^-$ 439.1755[M-H]$^-$ 421.1631[M-H-H$_2$O]$^-$ 287.1290$^{14}$A$^-$ 109.0298[M-H-H$_2$O-ringB-C$_{10}$H$_{15}$-C$_4$H$_7$]$^-$ 152.0812$^{14}$B$^-$ | [12, 22] |
| 36  | Kuraridin | C$_{26}$H$_{30}$O$_6$ | 23.04 | 437.1964 | 437.1984 | 4.57 | 161.0258$^{14}$B$^-$ 275.1667$^{14}$A$^-$ 437.1984[M-H]$^-$ 151.0412$^{14}$A$^-$ 161.0258$^{14}$B$^-$ 275.1667$^{14}$A$^-$ 437.1984[M-H]$^-$ 151.0412$^{14}$A$^-$ 161.0258$^{14}$B$^-$ 275.1667$^{14}$A$^-$ 437.1984[M-H]$^-$ | [23] |
| 37  | 5-Methylkushenol C | C$_{27}$H$_{32}$O$_6$ | 24.61 | 451.2121 | 451.2141 | 4.43 | 451.2141[M-H]$^-$ 301.1473$^{14}$A$^-$ 192.0471$^{14}$A$^-$ 313.0871[M-H-C$_6$H$_{12}$-C$_4$H$_6$]$^-$ 367.1223[M-H-C$_6$H$_{12}$]$^-$ | [12, 21] |

**Figure 1:** Characteristic fragments and neutral loss of the chemical composition of *Sophora flavescens*. 

**Graphical Representation:**

![Graphical representation of the chemical composition of *Sophora flavescens*.](image-url)
and then directly lose a molecule of C$_2$H$_4$NO or lose C$_2$H$_4$ to yield 136.1137[M+H-C$_4$H$_4$O-$\text{C}_2\text{H}_4\text{NO}$]$^+$ and m/z 108.0833[M+H-C$_4$H$_4$O-$\text{C}_2\text{H}_4\text{NO}-\text{C}_2\text{H}_4]^+$ ion fragments. The fragment ion 245.1661 is obtained by direct loss of a molecule of H$_2$ by the parent ion. Based on the fragmentation rules and standard information, it can be inferred that the compound is sophocarpine. The fragmentation process is shown in Figure 4.

3.3. Fragmentation Rules of Flavonoid Compounds. Flavonoids in *Sophora flavescens* mainly include dihydroflavonoids, chalcones, dihydroflavonols, flavonols, and isoflavones, in which dihydroflavonoids and chalcones are easy to change. Therefore, mass spectrometry can well distinguish the two [26]. It is easy to remove neutral molecules from flavonoids such as H$_2$O, CH$_3$, CO, CO$_2$, C$_2$H$_5$O, and C$_2$O$_3$ in the negative ion mode. Most of the dihydroflavonoids in *Sophora flavescens* have fragment information such as [M-H]$^-$, [M-H-H$_2$O]$^-$, [M-H-CO]$^-$, and [M-H-CH$_3$]$^-$, and these compounds are prone to RDA cleavage at positions 1,2 and 3,4, resulting in $^{1,3}\text{A}^-$ fragment ions. Chalcone compounds form 261[C$_{16}$H$_{21}$O$_3$]$^-$ and 161[C$_9$H$_5$O$_3$]$^-$ ion fragments under the anion mode B ring, and the 1,4 cleavage occurs in different positions of dihydroflavonoids, resulting in $^{1,4}\text{A}^-$ and $^{1,4}\text{B}^-$ characteristic fragment ions [12, 22]. The main fragment of dihydroflavonols is that the C ring is rearranged by RDA to produce characteristic fragments 177[C$_9$H$_3$O$_4$]$^-$, 275[C$_{17}$H$_{25}$O$_3$]$^-$, $^{1,3}\text{A}^-$, and $^{1,3}\text{B}^-$, and the hydroxyl group at position 3 is unstable, so it is easy to eliminate the reaction and lose H$_2$O to form a double bond. Flavonol compounds undergo RDA cleavage to produce characteristic fragments $^{1,3}\text{A}^-$ and $^{1,3}\text{B}^-$ and continue to lose neutral molecules such as CO (28) and

---

**Figure 2:** The base peak ion (BPI) flow diagram of *Sophora flavescens* in the (a) positive and (b) negative mode.
Isoflavones easily lose neutral molecules such as CO, 2CO, CO₂, and C₂O₃ [23]. Based on the above mass spectrometry information combined with retention time, the chemical constituents of flavonoids in *Sophora flavescens* were identified quickly.

The molecular formula of compound 34 is C₂₆H₂₈O₆, and the retention time is 20.62 min. The main secondary fragment ions are 405.1707, 262.15351, 193.1601, 161.0249, and 138.0323. In the negative ion mode, the parent ion peak is m/z 423.1805[M-H]⁻. The parent ion 2'-OH is chemically active, which means that it easily loses a molecule of H₂O and produces dehydrated fragments m/z 405.1707[M-H-H₂O]⁻. On this basis, the neutral losing fragment C₁₅H₁₇O is lost, and the ion fragment m/z 193.1601[M-H₂O-C₁₅H₁₇O]⁻ is obtained. Due to the presence of 2'-OH, the compound does not easily produce RDA cleavage, but breaks at the 1'4 position of the C ring, resulting in ion fragments 1,4A m/z 261.1501 and 1,4B m/z 162.0281, and then the ion fragments m/z 138.0323[1,4A-C₆H₁₅]⁻ are obtained when C₆H₁₅ is lost in 1,4A. Based on the above law, it is
Sophocarpine
Rt = 1.93 min

2: TOF MS ES+
1.73e4

Figure 4: The fragmentation process of sophocarpine in the positive ion mode.
Figure 5: The fragmentation process of norkurarinone in the negative ion mode.
Figure 6: The fragmentation process of kushenol X in the negative ion mode.
inferred that the compound is norkurarinone. The fragmentation process is shown in Figure 5.

The molecular formula of compound 35 is $C_{23}H_{28}O_7$, the retention time is 21.15, and the main secondary fragment ions are 421.1631, 287.1290, 261.1497, 177.0191, 152.0812, 149.0251, and 109.0298. In the negative ion mode, the precursor ion peak is $m/z$ 439.1761[M·H]⁻, and the precursor ion removes one molecule of H₂O to obtain $m/z$ 421.1631[M·H·H₂O]⁻ ion fragment. After that, the 1,4-positions of the C ring break off one molecule of C₉H₆O₁ to obtain the ion fragment 261.1497[M·H·H₂O·C₉H₆O₁]⁻. In addition, the parent ion of the compound can also directly lose one molecule of C₁₆H₂₀O₂, generating ion fragments of $m/z$ 177.0197[M·H·O·ringB·C₁₀H₁₅], on the basis of which another molecule of C₈H₁₄ is lost, and $m/z$ 109.0298[M·H·H₂O·ringB·C₁₀H₁₅·C₄H₇]. In the case of BDA rearrangement of this compound, $m/z$ 287.1290[1,3A⁻] and $m/z$ 152.0812[1,3B⁻] ion fragments can be generated, and then the characteristic fragment 1,3A⁻ loses C₉H₁₄O to produce $m/z$ 149.0251[1,3A⁻·C₉H₁₄O]. Based on the above fragmentation rules, it can be inferred that this compound is kushenol X. The fragmentation process is shown in Figure 6.

4. Conclusion

The UHPLC-Q-TOF/MS technique combined with characteristic fragments and neutral loss was applied to the tracking and identification of alkaloids and flavonoids in Sophora flavescens, and the fragmentation rules of different parent ions were inferred. A total of 13 alkaloids and 24 flavonoids were identified. Analytical strategies for characterizing the structure of compounds by obtaining diagnostic fragment ions based on excimer ion peaks and MS/MS were summarized.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

This work was funded by the National Natural Science Foundation of China (no. 81903933), the Tianjin Science and Technology Development Fund Project (2018KJ002), and the Tianjin Talent Development Special Support Project for High-Level Innovation and Entrepreneurship.

References

[1] X.-R. He, C.-G. Li, X.-S. Zhu et al., “High-performance liquid chromatography coupled with tandem mass spectrometry technology in the analysis of Chinese medicine formulas: a bibliometric analysis (1997–2015),” Journal of Separation Science, vol. 40, no. 1, pp. 81–92, 2017.

[2] S. Yang, L. Shan, H. Luo, X. Sheng, J. Du, and Y. Li, “Rapid classification and identification of chemical components of Sophora flavescens and Sophora flavescens by UPLC-Q-TOF/MS combined with data post-processing,” Molecules, vol. 22, no. 10, p. 1778, 2017.

[3] E. E. Jones, W. Zhang, X. Zhao et al., “Tissue localization of glycosphingolipid accumulation in a gaucher disease mouse brain by LC-ESI-MS/MS and high-resolution MALDI imaging mass spectrometry,” SLAS DISCOVERY: Advancing the Science of Drug Discovery, vol. 22, no. 10, pp. 1218–1228, 2017.

[4] W.-Y. Gu, N. Li, E. L.-H. Leung et al., “Rapid identification of new minor chemical constituents from smilacis glabrae rhizoma by combined use of UHPLC-Q-TOF-MS, preparative HPLC and UHPLC-SPE-NMR-MS techniques,” Phytochemical Analysis, vol. 26, no. 6, pp. 428–435, 2015.

[5] P. Liu, P. Zhao, R. G. Cooks, and H. Chen, “Atmospheric pressure neutral reionization mass spectrometry for structural analysis,” Chemical Science, vol. 8, no. 9, pp. 6499–6507, 2017.

[6] C. Yu, Y. Xu, M. Wang, Z. Xie, and X. Gao, “Application of characteristic fragment filtering with ultra high performance liquid chromatography coupled with high-resolution mass spectrometry for comprehensive identification of components in Schisandrae chinensis fructus,” Journal of Separation Science, vol. 42, no. 7, pp. 1323–1331, 2019.

[7] Y. G. Fang, H. D. Zhu, X. Q. Liu et al., “Revision research on the quality standard of matrine and decoction pieces,” Chinese Journal of Traditional Chinese Medicine, vol. 8, no. 1, pp. 1–9, 2020, in Chinese.

[8] X. Wang, R. Zheng, X. Huang et al., “Effects of alkaloids from Sophora flavescens on osteoblasts infected with Staphylococcus aureus and osteoclasts,” Phytotherapy Research, vol. 32, no. 4, pp. 1354–1363, 2018.

[9] M. Kwon, S.-K. Ko, M. Jang et al., “Inhibitory effects of flavonoids isolated from Sophora flavescens on indoleamine 2,3-dioxygenase 1 activity,” Journal of Enzyme Inhibition and Medicinal Chemistry, vol. 34, no. 1, pp. 1481–1488, 2019.

[10] Q. Yu, N. Cheng, and X. Ni, “Identifying 2-prenylflavanones as potential hepatotoxic compounds in the ethanol extract of Sophora flavescens,” Journal of Food Science, vol. 78, no. 11, pp. T1830–T1834, 2013.

[11] G. Wu, Optimization of the Extraction Process of a Compound Chinese Medicine and Preparation of Granules, Heilongjiang Bayi Land Reclamation University, Mishan, China, 2014.

[12] X. N. Li, X. Dong, B. Q. Bao et al., “Quick analysis and identification of the chemical constituents of Sophora flavescens based on Q-exactive high-resolution mass spectrometry,” Chinese Medicinal Materials, vol. 42, no. 1, pp. 103–109, 2019, (in Chinese).

[13] L. Sabatino, M. Scarangella, F. Lazzaro et al., “Matrine and oxymatrine in corioborant plant extracts and fertilizers: HPLC/MS/MS method development and single-laboratory validation,” Journal of Environmental Science and Health, Part B, vol. 50, no. 12, pp. 862–870, 2015.

[14] Z. J. Wu, D.-M. Sun, D.-M. Fang, J.-Z. Chen, P. Cheng, and G.-L. Zhang, “Analysis of matrine-type alkaloids using ESI-QTOF,” International Journal of Mass Spectrometry, vol. 341-342, pp. 28–33, 2013.

[15] Z. Zeng, Z. Guo, B. Peng et al., “Comparative study on the alkaloids of Sophora flavescens and Sophora flavescens by UPLC/Q-TOF MS–E,” Natural Product Research and Development, vol. 27, no. 5, pp. 804–808, 2015.

[16] X. Li, X. Dong, N. Li et al., “Quick analysis and identification of chemical constituents of siweiu tumuxiang powder by HPLC-Q-exactive-MS/MS high resolution mass spectrometry,” Chinese Journal of Experimental Traditional Medical Formulae, vol. 26, no. 6, pp. 121–131, 2020.
[17] Z. Yang, W. Zhang, X. Li, B. Shan, J. Liu, and W. Deng, “Determination of sophoradiflavanone G and kurarinone in rat plasma by UHPLC-MS/MS and its application to a pharmacokinetic study,” Journal of Separation Science, vol. 39, no. 22, pp. 4344–4353, 2016.

[18] L. Zhang, L. Xu, S.-S. Xiao et al., “Characterization of flavonoids in the extract of Sophora flavescens ait. by high-performance liquid chromatography coupled with diode-array detector and electrospray ionization mass spectrometry,” Journal of Pharmaceutical and Biomedical Analysis, vol. 44, no. 5, pp. 1019–1028, 2007.

[19] W.-Z. Yang, G. Ye, A.-H. Meng et al., “Rapid characterisation of flavonoids from Sophora alopecuroides L. by HPLC/DAD/ESI-MSn,” Natural Product Research, vol. 27, no. 4-5, pp. 323–330, 2013.

[20] P. Guo, L. Dong, W. Yan, J. Wei, C. Wang, and Z. Zhang, “Simultaneous determination of linarin, naringenin and formononetin in rat plasma by LC-MS/MS and its application to a pharmacokinetic study after oral administration of bushen guchi pill,” Biomedical Chromatography, vol. 29, no. 2, pp. 246–253, 2015.

[21] N. Fabre, I. Rustan, E. Hoffmann, and J. Quetin-Leclercq, “Determination of flavone, flavonol, and flavanone aglycones by negative ion liquid chromatography electrospray ion trap mass spectrometry,” Journal of the American Society for Mass Spectrometry, vol. 12, no. 6, pp. 707–715, 2001.

[22] J. Qi, D. Xu, Y.-F. Zhou, M.-J. Qin, and B.-Y. Yu, “New features on the fragmentation patterns of homoisoflavonoids in Ophiopogon japonicus by high-performance liquid chromatography/diode-array detection/electrospray ionization with multi-stage tandem mass spectrometry,” Rapid Communications in Mass Spectrometry, vol. 24, no. 15, pp. 2193–2206, 2010.

[23] Y. Liu, L. Chen, W. Cai, L.-L. Zhao, and Z.-X. Mo, “Use of an UHPLC-MS/MS method for determination of kuraridin and characterization of its metabolites in rat plasma after oral administration,” Molecules, vol. 23, no. 2, p. 132, 2018.

[24] F. Zhao, Study on the Difference of Chemical Composition and Content in Different Parts of Sophora flavescens, China Academy of Chinese Medical Sciences, Beijing, China, 2015.

[25] H. U. Rashid, Y. Xu, Y. Muhammad, L. Wang, and J. Jiang, “Research advances on anticancer activities of matrine and its derivatives: an updated overview,” European Journal of Medicinal Chemistry, vol. 161, pp. 205–238, 2019.

[26] Z. Weng, F. Zeng, Z. Zhu et al., “Comparative analysis of sixteen flavonoids from different parts of Sophora flavescens ait. by ultra high-performance liquid chromatography-tandem mass spectrometry,” Journal of Pharmaceutical and Biomedical Analysis, vol. 156, pp. 214–220, 2018.