Research Article

Isolation and Identification of Chemical Constituents from Zhhideke Granules by Ultra-Performance Liquid Chromatography Coupled with Mass Spectrometry

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1.Introduction

Zhideke granules are an in-hospital preparation from Ruikang Hospital affiliated to the Guangxi University of Chinese Medicine. It contains 10 kinds of traditional Chinese medicines including Scutellaria baicalensis Georgi., Belamcanda chinensis (L.) Redouté, Mentha haplocalyx Briq., Eriobotrya japonica (Thunb.), Platycodon grandiflorus (Jacq.) A. DC., Bupleurum chinense, Nepeta cataria L., Cynanchum glaucescens (Decne.) Hand-Mazz., Nervilia fordii (Hance) Schltr., and Sauropus spatulifolius Beille. The preparation that has been used in the treatment of bronchial asthma of wind-phlegm obstructing the lung in acute attack period for many years is widely used in Guangxi Zhuang Autonomous Region of China [1]. It has the efficacy of reducing fever and removing toxins, relieving cough and resolving phlegm. Moreover, it has been proven by long-term clinical practice that Zhideke granules have an effect on flu, fever, cough, bronchial asthma, etc. [2, 3]. Although it has been reported that baicalin in Scutellaria baicalensis Georgi. and tectoridin and irisflorentin in Belamcanda chinensis (L.) Redouté are used as quality control components of Zhideke granules, the material base of it is still unclear. At the same time, some literatures about baicalin [4, 5] and tectoridin [6, 7] proved that they had antiasthma effect and reduced inflammation. However, other chemical constituents and pharmacodynamic substances of Zhideke granules have not been reported.

The ultra-performance liquid chromatography coupled with hybrid quadrupole-orbitrap mass spectrometry (UPLC-Q-Orbitrap HRMS) is a new technology developed in recent years for analyzing the structure of complex chemical constituents from Zhideke granules were rapidly isolated and identified by ultra-performance liquid chromatography (UPLC) coupled with hybrid quadrupole-orbitrap mass spectrometry (MS) in positive and negative ion modes using both full scan and two-stage threshold-triggered mass modes. The secondary fragment ion information of the target compound was selected and compared with the compound reported in databases and related literatures to further confirm the possible compounds. A total of 47 chemical constituents were identified from the ethyl acetate extract of Zhideke granules, including 21 flavonoids and glycosides, 9 organic acids, 4 volatile components, 3 nitrogen-containing compounds, and 10 other compounds according to the fragmentation patterns, relevant literature, and MS data. The result provides a new method for the analysis of chemical constituents of Zhideke granules which laid the foundation for quality control and the study of pharmacodynamic materials of Zhideke granules.
traditional Chinese medicine (TCM) and its compound preparations [8–11], and it has the advantages of high efficiency, high speed, high sensitivity, and high resolution and specificity [12–14]. In this study, UPLC-Q-Orbitrap HRMS was used to rapidly isolate and identify unknown chemical components in Zhideke granules for the first time. We analysed the secondary fragment ion information of the target compound as well as relevant literature to further determine the possible chemical constituents in Zhideke granules, which has provided a reference for the quality control and its pharmacodynamics substances of Zhideke granules.

2. Materials and Methods

2.1. Chemicals and Reagents. Zhideke granules were provided by Ruikang Hospital affiliated to Guangxi University of Chinese Medicine. UPLC-grade acetonitrile was purchased from Merck (Merck, Germany). UPLC-grade ammonium acetate was obtained from Shanghai Sixin Biotechnology Co., Ltd. (Shanghai, China). Formic acid and methanol at UPLC-grade were acquired from Fisher (Fisher, USA). Analytical grade ethyl acetate was purchased from Fisher (Fisher, USA). Ultra-pure water was purified with Milli-Q synergy (Millipore, USA). Forsythoside A (no. 111810-201606), baicalin (no. 110715-201720), and wogonin (no. 111514-201706) were purchased from the National Institutes for Food and Drug Control (Beijing, China). Forsythoside A, baicalin, and wogonin purity were found to be above 97.2%, 93.5%, and 96.3%, respectively. Other chemicals were of analytical grade and their purity was above 99.5%.

2.2. Ethyl Acetate Extracts Preparation. A 0.1 g sample of Zhideke granules was weighed by using a XS205DU electronic balance (Mettler-Toledo, Switzerland) and extracted with 20 mL of water in an ultrasonic bath (40 kHz, 500 W) for 30 min. Then, the solutions were extracted twice with 20 mL ethyl acetate and combined two ethyl acetate extracts. Subsequently, these extracts were evaporated at the temperature of 80°C by water bath, and the residue was dissolved in 5 mL of methanol. Finally, the solution of the residue was filtered and was analysed.

2.3. The Sample Solution of HPLC Analysis. A 2.0 g sample of Zhideke granule was precisely weighed and extracted with 10 mL of methanol in an ultrasonic bath for 30 min. Then, the extract was centrifuged at 13000 r/min for 10 min by using a TGL-16G centrifuge (Shanghai Anting Scientific Instrument Factory, China) and the supernatant was taken as the sample solution. Finally, the solution was analysed by HPLC.

2.4. Standards Preparation. We accurately weighed appropriate amounts of baicalin and wogonin, respectively, and then dissolved with methanol to prepare a mixture solution including two standards. Forsythoside A standard solution was prepared by dissolving 11.84 mg each of accurately weighed pure compound in 5 mL methanol.

2.5. HPLC Chromatographic Conditions. Separation was achieved on Agilent ZORBAX Eclipse Plus-C18 column (4.6 × 250 mm, 5 μm). The mobile phase was methanol (A) and 0.2% phosphoric acid (B) by gradient elution (0–30 min, 5%–20% A; 30–50 min, 20%–35% A; 50–75 min, 35%–40% A; 75–110 min, 40%–60% A; 110–150 min, 60%–90% A) with photo-diode array (PDA) detection at 254 nm at the flow rate of 0.8 mL/min. The column temperature was 30°C and the volume was 10 μL.

2.6. UPLC Chromatographic Conditions. In the UPLC system, the column was a Thermo Hypersil Gold C18 column (2.1 mm × 100 mm, 1.9 μm). The mobile phase consisted of 0.1% formic acid acetonitrile (A) and 0.1% formic acid water containing 10 mmol of ammonium acetate (B) which was programmed with a gradient elution (0–2.0 min, 5% A; 2.0 to 42.0 min, 5% to 95% A; 42.0 to 47.0 min, 95% A; 47.1 to 50 min, 95% to 5% A) at a flow rate of 0.3 mL/min. The sample injection volume was 1 μL. The column temperature was maintained at 35°C.

2.7. Instruments and MS Conditions. Chemical constituent’s analyses were performed on Thermo Fisher U3000 UPLC system (Thermo Fisher, USA), Trace Finder software (Thermo Fisher, USA), which was used for the UPLC-Q-Exactive Orbitrap MS data processing. The ion source was the heated electrospray ionization (ESI). The electrospray ionization source in both positive and negative ion modes was used in MS analysis. Spray voltages were set at 3.5 kV in a positive ion mode and 3.2 kV in a negative ion mode, respectively. The auxiliary gas temperature was 300°C, and the capillary temperature was 320°C. MS data were obtained on Full MS/dd-MS2 mode in the mass range of 100–1500 Da. The resolution of the precursor mass was 70000 FWHM, while the resolution of the product mass was 17500 FWHM. The specific ion scan mode was off. High purity nitrogen was used as the collision gas, and nitrogen was used as spray gas. The flow rates of sheath gas and auxiliary gas were at the rate of 30 and 10 μL/min, respectively.

3. Results and Discussion

We chose 50% ethyl acetate as the extraction solvent and identified chemical constituents from the ethyl acetate extract of Zhideke granules by UPLC-Q-Orbitrap MS in this paper. In the positive and negative ion modes, the total ion chromatograms of ethyl acetate extract in Zhideke granules are shown in Figure 1. The HPLC spectra of the standard of forsythoside A (A), the sample solution (B), a mixture of two standards (C), and the HPLC fingerprint of Zhideke granules (D) are shown in Figure 2.

According to MS mass, MS/MS fragmentation information, fragmentation patterns, and literature reports, we
Figure 1: The total ion current chromatogram in positive (a) and negative ions (b). Mode for ethyl acetate of Zhideke granules.
Figure 2: HPLC-UV (PDA) chromatograms obtained from the standard of forsythoside A (a), the sample solution (b), a mixture of two standards (c), and HPLC fingerprint of Zhike granules (d).
identified 47 possible chemical constituents including 21 flavonoids and glycosides, 9 organic acids, 4 volatile components, 3 nitrogen-containing compounds, and 10 other compounds from the ethyl acetate extract of Zhideke granules. The retention time, mass spectrometry information, and related literature of identified compounds are shown in Table 1.

3.1. Identification of Flavonoids and Glycosides. These compounds with a C6–C1–C6 carbon skeleton in the structure called flavonoids had two benzene rings formed by three carbon atoms. The structure of flavonoids often results in substituents such as hydroxyl, methyl, and methoxyl groups. Therefore, in the fragmentation regularity of flavonoids, these compounds easily lose neutral fragments of CO (28 Da), H2O (18 Da), CO2 (44 Da), and fragment ions of substituents [15]. In addition, the retro-Diels–Alder (RDA) fragmentation is a common fragmentation pattern in flavonoids. Taking compound 11 as an example, compound 11, with the quasimolecular ion m/z 301.0354 [M-H]− and formula of C15H10O7, was identified as wogonin by comparing with the reference standard and the literature report [15]. Based on the fragmentation patterns of glycosides, compounds 1, 2, 4, 5, 6, 7, 13, 14, and 20 were possibly identified as isoquercitin, tectoridin, diosmin, hesperidin, luteolin 7-glucuronide, iridin, linarin, diydmyin, and pnomembrin, respectively.

Compound 8 with the quasimolecular ion at m/z 447.0902 [M+H]+ and formula of C22H16O11, was identified as baicalin in the positive ion mode. The ion at m/z 447.0902 produced the ion at m/z 271.0588 [M+H-C6H6O6]− after the loss of a glucuronic acid molecule (177 Da). The fragment ion at m/z 169.0124 [M+H-C6H6O6-C6H6]− was from the ion at m/z 271.0588 by the loss of a C6H6 fragment ion (102 Da). Subsequently, the ion at m/z 225.0564 [M+H-C6H6O6-C6H6-CO2H]− originates from the ion at m/z 271.0588 by loss of a H2O molecule (18 Da) and a CO molecule (28 Da). The possible fragment pathway of compound 8 is shown in Figure 5 according to this fragmental information. Compound 8 was baicalin by comparing with the reference standard and the literature report [15].

Based on the fragmentation patterns of glycosides, compounds 1, 2, 4, 5, 6, 7, 13, 14, and 20 were possibly identified as isoquercitin, tectoridin, diosmin, hesperidin, luteolin 7-glucuronide, iridin, linarin, diydmyin, and pnomembrin, respectively.

3.2. Identification of Organic Acids. Organic acids of Zhideke granules are mainly found in Eriobotrya japonica (Thunb.), Platycodon grandiflorus (Jacq.) A. DC., Mentha haplocalyx Briq., etc. Organic acids mainly include two types, one of which is fatty acid and the other is aromatic acid. Organic acids easily lose neutral fragments of CO (28 Da), H2O (18 Da), CO2 (44 Da), and a fragment of alkyl. Taking compound 24 as an example, compound 24, with the quasimolecular ion m/z 197.0448 [M-H]+ and formula of C8H10O5, was identified as danshensu under the negative ion mode. The fragment ion at m/z 179.0342 [M-H-H2O]− at the loss of a H2O molecule. Fragment ions at m/z 151.0401 [M-H-H2O-CO2H]− and m/z 134.0369 [M-H-H2O-CO2H]− were derived from the fragment ion at m/z 179.0343 by the loss of a CO (28 Da) molecule and a CO2 (44 Da) molecule, respectively. Based on fragment rules and literature, compound 24 was characterized as danshensu [19].

Compound 26, with the quasimolecular ion at m/z 537.1024 [M-H]+ and formula of C27H22O12, was identified as lithospermic acid under the negative ion mode. The fragment ion at m/z 493.1024 [M-H]+ was formed due to the loss of a CO2 (28 Da) molecule of the ion at m/z 537.1024. Fragment ions at m/z 295.0602 [M-H-C6H10O5]+ and m/z 313.0711 [M-H-C6H10O5]+ were derived from the ion at m/z 493.1027 by the loss of fragment ions of C6H10O5 (198 Da) and C6H12O2 (180 Da). According to the results, compound 26 was identified as lithospermic acid [20]. The fragmentation pathway is shown in Figure 6.
| Type of compounds | No. | tR (min) | Adduct ions | Theoretical (m/z) | Measured (m/z) | Mass error (ppm) | Molecular formula | MS/MS (m/z) | Identification compounds |
|-------------------|-----|----------|-------------|------------------|---------------|-----------------|-----------------|-------------|-------------------------|
| Flavonoids and glycosides | 1 | 10.21 | M-H | 463.0882 | 463.087 | -2.81 | C_20H_16O_12 | 253.0298 [M-H-Glc-O-CHO]^+ 271.0241 [M-Glc-CHO]^+ | Isoquercitrin |
| | 2 | 10.82 | M + HCO_3^- | 461.089 | 461.078 | -0.542 | C_21H_22O_11 | 298.0472 [M-H_2CO-OH-C_6H_8O_6]^+ 284.037 [M-H_2CO-OH-C_6H_8O_6]^+ | Tectoridin |
| | 3 | 11.34 | M + H | 417.118 | 417.1159 | -4.9715 | C_16H_18O_6 | 267.0640 [M-H_2CO-OH-C_6H_8O_6]^+ 297.0743 [M-H_2CO-OH-C_6H_8O_6]^+ | Puerarin |
| | 4 | 11.65 | M-H | 607.1668 | 607.1653 | -2.564 | C_22H_20O_13 | 299.0560 [M-H-C_6H_4O_2]^+ | Diosmin |
| | 5 | 11.75 | M-H | 609.1825 | 609.1812 | -2.1958 | C_22H_20O_13 | 301.0707 [M-H-rutinoses]^- | Hesperidin |
| | 6 | 11.82 | M-H | 461.0726 | 461.0714 | -2.6024 | C_20H_16O_12 | 265.0395 [M-H-C_6H_4O_2]^+ | Luteolin 7-glucuronide |
| | 7 | 11.95 | M + H | 523.1446 | 523.1423 | -0.4864 | C_21H_16O_12 | 361.0899 [M-H-C_6H_4O_2]^+ | Inulin |
| | 8 | 12.52 | M + H | 447.0922 | 447.0901 | -0.6335 | C_20H_16O_12 | 169.0124 [M-H-C_6H_4O_2]^+ 225.0541 [M-H-C_6H_4O_2]^+ | Baicalin |
| | 9 | 12.58 | M-H | 285.0405 | 285.0395 | -3.31 | C_16H_10O_6 | 165.0185 [M-H-C_6H_4O_2]^+ | Scutellarein |
| | 10 | 12.83 | M-H | 287.0561 | 287.0532 | -0.419 | C_16H_10O_6 | 267.0290 [M-H-C_6H_4O_2]^+ 155.0460 [M-H-C_6H_4O_2]^+ | Scutellaria baicalensis Georgi. |
| | 11 | 13.91 | M-H | 301.0354 | 301.0343 | -0.6832 | C_16H_10O_6 | 273.0796 [M-H-C_6H_4O_2]^+ | Quercetin |
| | 12 | 13.94 | M-H | 315.031 | 315.035 | -3.1143 | C_16H_10O_6 | 300.0875 [M-H-C_6H_4O_2]^+ | Galangin |
| | 13 | 14.10 | M + H | 595.1865 | 593.1837 | -4.7628 | C_21H_16O_12 | 242.0599 [M-H-C_6H_4O_2]^+ 285.0742 | Linarin |
| | 14 | 14.36 | M + H | 595.2021 | 595.1993 | -0.7887 | C_20H_16O_12 | 287.1007 [M-H-C_6H_4O_2]^+ | Mentha haplocalya Briq. |
| | 15 | 15.37 | M-H | 359.0474 | 359.0469 | -3.0146 | C_16H_10O_6 | 301.0347 [M-H-C_6H_4O_2]^+ | Mentha haplocalya Briq. |
| | 16 | 16.78 | M-H | 269.0456 | 269.0446 | -3.4689 | C_16H_10O_6 | 223.0560 [M-H-C_6H_4O_2]^+ | Mentha haplocalya Briq. |
| | 17 | 19.42 | M-H | 283.0612 | 283.0602 | -0.3601 | C_16H_10O_6 | 240.0426 [M-H-C_6H_4O_2]^+ | Mentha haplocalya Briq. |
| | 18 | 19.54 | M + H | 403.1387 | 403.1368 | -0.4824 | C_20H_18O_12 | 327.0749 [M-H-C_6H_4O_2]^+ 373.0700 [M-H-C_6H_4O_2]^+ | Nobilin |
| | 19 | 19.74 | M-H | 253.0506 | 253.0498 | -1.31407 | C_12H_10O_4 | 170.0833 [M-H-C_6H_4O_2]^+ | Chrysanthemum chinesis (L.) Hand-Mazz |
| | 20 | 20.1 | M-H | 255.0663 | 255.0655 | -2.1949 | C_15H_10O_4 | 145.0652 [M-H-C_6H_4O_2]^+ | Mentha haplocalya Briq. |
| Organic acids | 21 | 20.54 | M-H | 299.0561 | 299.0551 | -3.5321 | C_17H_12O_4 | 227.0344 [M-H-C_6H_4O_2]^+ 255.0592 [M-H-C_6H_4O_2]^+ | Tectorigenin |
| | 22 | 21.5 | M-H | 169.0142 | 169.0136 | -3.8737 | C_12H_10O_4 | 69.0341 [M-H-C_6H_4O_2]^+ 97.0291 [M-H-C_6H_4O_2]^+ 145.0629 [M-H-C_6H_4O_2]^+ | Tectorigenin |
| | 23 | 1.86 | M-H | 124.047 | 124.032 | -3.892 | C_12H_10O_4 | 81.0187 [M-H-C_6H_4O_2]^+ 133.0231 [M-H-C_6H_4O_2]^+ | Tectorigenin |
| | 24 | 2.19 | M-H | 197.0436 | 197.0448 | -3.888 | C_12H_10O_4 | 154.0369 [M-H-C_6H_4O_2]^+ 151.0041 [M-H-C_6H_4O_2]^+ | Tectorigenin |
| | 25 | 8.62 | M-H | 163.0401 | 163.0395 | -3.454 | C_12H_10O_4 | 283.0602 [M-H-C_6H_4O_2]^+ 33.0711 [M-H-C_6H_4O_2]^+ | Tectorigenin |
| | 26 | 9.85 | M-H | 537.1038 | 537.1024 | -0.728 | C_20H_18O_12 | 97.0291 [M-H-C_6H_4O_2]^+ 145.0629 [M-H-C_6H_4O_2]^+ | Tectorigenin |
| | 27 | 11.68 | M-H | 187.0976 | 187.0968 | -4.1596 | C_15H_10O_4 | 69.0341 [M-H-C_6H_4O_2]^+ 83.0501 [M-H-C_6H_4O_2]^+ | Tectorigenin |
| Type of compounds | No. | tR (min) | Adduct ions | Theoretical (m/z) | Measured (m/z) | Mass error (ppm) | Molecular formula | MS/MS (m/z) | Identification compounds | Source |
|-------------------|-----|----------|-------------|-----------------|----------------|-----------------|-----------------|-------------|-------------------------|--------|
|                    | 28  | 13.37    | M-H        | 493.114         | 493.1129       | -1.789          | C_{10}H_{22}O_{10} | 185.0238295.0601 | [M-H-C_{6}H_{4}O_{3}] | Mentha haplocalyx Briq. [22] |
|                    | 29  | 13.47    | M-H        | 265.1289        | 265.128        | -3.263          | C_{6}H_{18}O_{5}  | 204.1147     | [M-H-C_{6}H_{4}O_{3}] | Abies acid |
|                    | 30  | 14.16    | M-H        | 491.0984        | 491.0971       | -3.6341         | C_{10}H_{20}O_{10} | 135.0446     | [M-H-C_{6}H_{4}O_{3}] | Mentha haplocalyx Briq. [22] |
|                    | 31  | 2.17     | M + H      | 127.039         | 127.0385       | -3.5959         | C_{6}H_{18}O_{5}  | 5.0392       | [M + H-H_{2}O-2CO] | 81.0337 | [M + H-H_{2}O-CO] | 97.0285 | [M + H-H_{2}O-CO] | 138.0656 | [M + H-H_{2}O-CO] | 153.1165 | [M + H-H_{2}O] | |
|                    | 32  | 10.98    | M + H      | 153.1274        | 153.1267       | -4.2811         | C_{10}H_{4}O_{3}  | 135.1165     | [M + H-H_{2}O] | |
|                    | 33  | 12.95    | M + H      | 191.1067        | 191.1058       | -3.3122         | C_{12}H_{22}O_{12} | 145.1066     | [M + H-H_{2}O-CO] | Bupleurnum chinense [25] |
|                    | 34  | 25.35    | M + HCO_{2} | 315.2541        | 315.2528       | -3.9393         | C_{17}H_{24}O_{2} | 127.1322     | [M + H-C_{8}H_{10}O_{3}] | Scutellaria baicalensis Georgi. [23] |
|                    | 35  | 0.32     | M + H      | 195.0876        | 195.0867       | -4.7109         | C_{9}H_{16}O_{5}  | 56.0500      | [M + H-C_{6}H_{10}O_{5}] | Methyl palmitate |
| Nitrogen-containing compounds | 36  | 0.90     | M + H      | 136.0618        | 136.0618       | -4.5036         | C_{13}H_{22}O_{4} | 92.0244      | [M + H-C_{6}H_{10}O_{4}] | Adenine |
|                    | 37  | 1.26     | M + H      | 123.0553        | 123.0549       | -3.2842         | C_{6}H_{8}N_{3}O_{3} | 80.0497      | [M + H-C_{6}H_{10}O_{4}] | Nicotinamide |
|                    | 38  | 0.85     | M-H       | 181.0718        | 181.0711       | -3.7806         | C_{4}H_{8}O_{3}  | 59.0136      | [M-H-C_{6}H_{4}O_{3}] | Saururus spatulifolius Beille. [28] |
|                    | 39  | 0.85     | M-H       | 341.1089        | 341.1078       | -3.4371         | C_{3}H_{12}O_{11} | 71.0135      | [M-H-C_{6}H_{4}O_{3}] | |
|                    | 40  | 4.56     | M+H       | 137.0244        | 137.0238       | -4.2749         | C_{4}H_{8}O_{3}  | 93.0341      | [M-H-CO] | |
|                    | 41  | 6.30     | M-H       | 177.0193        | 177.0186       | -4.0350         | C_{10}H_{16}O_{4} | 133.0290     | [M-H-CO] | |
|                    | 42  | 7.50     | M+H       | 421.0776        | 421.0766       | -3.9557         | C_{4}H_{8}O_{3}  | 258.0861     | [M-H-C_{6}H_{10}O_{4}] | |
|                    | 43  | 10.61    | M+H       | 243.0663        | 243.0655       | -3.3273         | C_{14}H_{20}O_{4} | 173.0601     | [M-H-C_{6}H_{10}O_{4}] | |
|                    | 44  | 11.07    | M-H       | 623.1981        | 623.1963       | -2.9701         | C_{26}H_{38}O_{15} | 161.0238     | [M-H-C_{6}H_{10}O_{4}] | |
|                    | 45  | 12.22    | M-H       | 191.035         | 191.0343       | -3.3107         | C_{10}H_{20}O_{4} | 163.0393     | [M-H-CO] | Gynura glaucescens (Decne.) Hand-Mazz. [25] |
|                    | 46  | 12.61    | M+HCO_{2}  | 441.1766        | 441.1754       | -2.8094         | C_{12}H_{22}O_{10} | 89.0241      | [M-H-C_{6}H_{10}O_{4}] | 159.081 | [M-H-C_{6}H_{10}O_{4}] | 179.0341 | [M-H-C_{6}H_{10}O_{4}] | 461.1656 | [M-H-C_{6}H_{10}O_{4}] | 185.0963 | [M-H-C_{6}H_{10}O_{4}] | 199.0492 | [M-H-C_{6}H_{10}O_{4}] | |
|                    | 47  | 14.86    | M+H       | 209.0808        | 209.0799       | -4.5197         | C_{6}H_{8}O_{3}  | 177.0540     | [M-H-C_{6}H_{10}O_{4}] | 134.0593 | [M-H-C_{6}H_{10}O_{4}] | 177.0540 | [M-H-C_{6}H_{10}O_{4}] | 153.1165 | [M-H-H_{2}O] | |

Note. *Identification confirmed with reference compound.
Figure 3: Possible fragmentation pathway of compound 11.

Figure 4: Possible fragmentation pathway of compound 3.
Compound 27, with the quasimolecular ion at m/z 187.0968 [M-H]⁻ and formula of C₄₆H₄₆O₆₆, was identified as azelaic acid under the negative ion mode. The fragment ion at m/z 143.1070 [M-H-C₂H₄]⁻ was formed due to the loss of a CO₂ (28 Da) molecule of the ion at m/z 187.0968. The fragment ion at m/z 125.0967 [M-H-CO₂-H₂O]⁻ was derived from the ion at m/z 143.1070 by the loss of an H₂O (18 Da) molecule. Subsequently, the fragment ions at m/z 97.0654 [M-H-CO₂-H₂O-C₆H₈], m/z 83.0501 [M-H-CO₂-H₂O-C₆H₈+C₆H₆], and m/z 69.0342 [M-H-CO₂-H₂O-C₆H₈+C₆H₆+C₆H₆] were derived from the ion at m/z 125.0967 by the loss of fragment ions of C₂H₄ (28 Da), C₆H₆ (42 Da), and C₆H₈ (56), respectively. Based on MS data and relevant literature, compound 27 was identified as azelaic acid [21]. According to the fragmentation process [16], compound 29 was determined as abscisic acid.

Compound 30, with the quasimolecular ion at m/z 491.0971 [M-H]⁻ and formula of C₂₆H₂₉O₁₀, was identified as salvianolic acid C under the negative ion mode. The fragment ions at m/z 135.0446 [M-H-C₁₂H₁₂O₄]⁻, 179.0342 [M-H-C₁₇H₁₇O₈]⁻, m/z 197.0971 [M-H-C₁₇H₉O₈]⁻, and m/z 311.0554 [M-H-C₂₆H₂₉O₄]⁻ were from the ion at m/z 491.0970 by the loss of fragment ions C₁₀H₁₂O₈ (356 Da), C₁₇H₁₇O₆ (312 Da), C₁₇H₁₀O₅ (294 Da), and C₆H₈O₄ (180 Da), respectively. Subsequently, the fragment ion at m/z 265.0501 [M-H-C₆H₈O₄-CO₂H]⁻ was made up of the ion at m/z 311.0554 by the loss of fragment ions of the carboxyl group of the ion at m/z 265.0501. The ion at m/z 293.0448 [M-H-C₁₂H₁₀O₃]⁻ was from the cleavage of the ion at m/z 491.0970 occurring in the position of the C-O bond in the ester bond. Therefore, compound 30 was identified as salvianolic acid C by referring to the literature [22]. The fragmentation pathway of salvianolic acid C is shown in Figure 7. Based on fragment rules and literature [22], compound 28 was salvianolic acid A.

Based on the fragmentation rules of organic acids, compounds 22, 23, and 25 were identified as gallic acid, pyrogallol, and p-hydroxycinnamic acid, respectively.

3.3. Identification of Volatile Components. Volatile components were mainly found in Belamcanda chinensis (L.) Redouté, Cynanchum glaucescens (Decne.) Hand.-Mazz., and Bupleurum chinense in Zhideke granules. Taking compound 31 as an example, compound 31, with the quasimolecular ion at m/z 127.0385 [M+H]⁺ and formula of C₅H₈O₅, was identified as 5-hydroxymethylfurfural in the positive ion mode. The fragment ion at m/z 127.0385 lost the CH₂OH group (31 Da) and produced the fragment ion at m/z 97.0285 [M+H-CH₂OH]⁺. The fragment ion at m/z 81.0337 [M+H-H₂O-CO]⁺ was derived from the ion at m/z 127.0385 by the loss of a H₂O (18 Da) molecule and a CO (28 Da) molecule, successively. Then, the fragment ion at m/z 81.0337 continuously lost a CO (28 Da) molecule and yielded the fragment ion at m/z 53.0392 [M+H-H₂O-CO₂]⁺. Based on MS data and relevant literature [23], compound 31 was identified as 5-hydroxymethylfurfural.

Compound 32 showed m/z 153.1267 [M+H]⁺ ion and a formula of C₁₃H₁₆O in the positive ion mode of the first-order mass spectrum. In the MS/MS spectrum, we observed fragment ions at m/z 59.0496, 69.0701, 93.0699, 95.0854, 97.0646, 107.0854, 109.1008, and 135.1165. These results of fragmentation patterns are consistent with the relevant literature [24]. Thus, compound 32 was identified as camphor.

Compound 33, with the quasimolecular ion at m/z 191.1058 [M+H]⁺ and formula of C₁₁H₁₆O, was identified as ligustilide in the positive ion mode. Fragment ions at m/z 163.1110 [M+H-C₆H₆]⁺ and m/z 173.0953 [M+H-H₂O]⁺ were from the ion at m/z 191.1058 by the loss of C₆H₆...
(28 Da) fragment and an $H_2O$ (18 Da) molecule, respectively. Subsequently, the fragment ion at $m/z$ 173.0953 [M + H]H$_2$O$^+$ was derived from the ion at $m/z$ 173.0953 by the loss of a CO (28 Da) molecule. According to relevant literature [25], compound 33 was identified as ligustilide. The fragmentation pathway of compound 33 is given in Figure 8.

Compound 34, with the quasimolecular ion at $m/z$ 315.2528 [M + HCO$_2$]$^-$ and formula of C$_{17}$H$_{34}$O$_2$, was identified as methyl palmitate in the positive ion mode. Fragment ions at $m/z$ 127.1122 C$_9$H$_{19}$, $m/z$ 141.1279 C$_{10}$H$_{21}$, were formed because of cleavage of the alkyl group of the ester compounds. In addition, the fragment ion at $m/z$ 171.1020 [M – H – C$_7$H$_{15}$]$^-$ was derived from the [M – H]$^-$ ion. Thus, compound 34 was identified as methyl palmitate based on the fragmentation rules and related reports [23].

3.4. Identification of Nitrogen-Containing Compounds.
Nitrogen-containing compounds refer to a class of organic compounds containing a nitrogen element in the structure of the molecule and mainly include a nucleoside, an amino acid, and nicotinamide. The nitrogen-containing compounds are mainly derived from Mentha haplocalyx Briq. and Bupleurum chinense in Zhideke granules. The natural loss of H$_2$O (18 Da), CO$_2$ (44 Da), and NH$_3$ (17 Da) molecules easily takes place in the fragmentation process of nitrogen-containing compounds. Besides, amino acid molecules easily lost the carboxyl group (45 Da) and a hydroxyl group (17 Da).

Compound 35, with the quasimolecule ion at $m/z$ 195.0867 [M + H]$^+$ and formula of C$_8$H$_{10}$N$_4$O$_2$, was identified as caffeine in the positive ion mode. The fragment ions at $m/z$ 138.0656 [M + H – C$_7$H$_{15}$]$^+$, $m/z$ 110.0711 [M + H – C$_{10}$H$_{19}$]$^+$, $m/z$ 69.0451 [M + H – C$_6$H$_{19}$N$_2$O]$^+$, and $m/z$ 56.0500 [M + H – C$_6$H$_{10}$N$_2$O$_2$]$^+$ were derived from the fragment ion at $m/z$ 195.0867 by the loss of the fragment ions of C$_7$H$_{15}$ (56 Da), C$_{10}$H$_{19}$ (86 Da), C$_6$H$_{19}$N$_2$O (128 Da), and C$_6$H$_{10}$N$_2$O$_2$ (138 Da), respectively. The fragmentation patterns are basically consistent with the literature [26]. Thus, compound 35 was identified as caffeine. According to the fragmentation pattern, compound 36 was identified as adenine [27].
Compound 37, with the quasimolecular ion at \( m/z \) 123.0548 \([M+H]^+\) and formula of \( \text{C}_6\text{H}_6\text{N}_2\text{O} \), was identified as nicotinamide in the positive ion mode. The fragment ions at \( m/z \) 105.0445 \([M+H-\text{NH}_3]^+\) and \( m/z \) 95.0606 \([M+\text{CO}]^+\) were from the ion at \( m/z \) 123.0548 by the loss of a \( \text{NH}_3 \) (17 Da) molecule and a \( \text{CO} \) (28 Da) molecule, respectively. Besides, the fragment ion at \( m/z \) 80.0497 \([M+H-\text{CO}-\text{NH}_2]^+\) was formed owing to the loss of the \( \text{NH}_2 \) (16 Da) fragment. Therefore, compound 37 was identified as nicotinamide [28].

3.5. Identification of Other Compounds. There were some other compounds in the Zhidke granules, except constituents mentioned in the previous discussion such as sugar, coumarin, and phenol. Taking 38 as an example, compound 38, with the quasimolecular ion at \( m/z \) 181.0711 \([M+H]^+\) and a formula of \( \text{C}_6\text{H}_{14}\text{O}_6 \), was identified as mannitol in the positive ion mode. The fragment ions at \( m/z \) 163.0670 by the loss of the fragment ions of \( \text{C}_2\text{H}_6\text{O}_2 \) (62 Da), \( \text{C}_4\text{H}_8\text{O}_3 \) (104 Da), and \( \text{C}_3\text{H}_6\text{O}_2 \) (74 Da), respectively. Subsequently, the fragment ion at \( m/z \) 89.0241 \([M+\text{H}-\text{H}_2\text{O}-\text{CO}]^+\) lost a \( \text{H}_2\text{O} \) (18 Da) molecule and yielded \( m/z \) 71.0135 \([M+\text{H}-2\text{H}_2\text{O}-\text{C}_3\text{H}_6\text{O}_2]^-\). Taking the literature into account [29], compound 38 was identified as mannitol. The fragmentation pathway of mannitol is shown in Figure 9.

Compound 39 showed \( m/z \) 341.1078 \([M-H]^–\) ion and a formula of \( \text{C}_{12}\text{H}_{22}\text{O}_{11} \) in the negative ion mode of the first-order mass spectrum. In the MS/MS spectrum, the fragment ions at \( m/z \) 135.0446 \([M-H-C_9\text{H}_{10}\text{O}_5]^–\), \( m/z \) 197.0971 \([M-H-C_9\text{H}_{10}\text{O}_5]^–\), \( m/z \) 293.0448 \([M-H-C_9\text{H}_{10}\text{O}_5]^–\), and \( m/z \) 311.0554 \([M-H-C_4\text{H}_8\text{O}_3]^–\) were obtained. These fragmentation patterns were consistent with the literature report [25]. Therefore, compound 39 was recognized as sucrose.

Compound 40, with the qusimolecular ion at \( m/z \) 137.0238 \([M-H]^–\) and formula of \( \text{C}_7\text{H}_6\text{O}_3 \), was identified as
protocatechualdehyde in the negative ion mode. The characteristic fragment ion at \( m/z \) 93.0341 \([\text{M}-\text{CO}_2]^-\) originates from the ion at \( m/z \) 137.0238 by the loss of a \( \text{CO}_2 \) (44 Da) molecule. Based on the reported literature [30], compound 40 was identified as protocatechualdehyde.

Compound 41, with the quasimolecular ion at \( m/z \) 177.0186 \([\text{M}-\text{H}]^-\) and formula of \( \text{C}_9\text{H}_6\text{O}_4 \), was identified as esculetin in the negative ion mode. Fragment ions at \( m/z \) 133.0290 \([\text{M}-\text{H}-\text{CO}_2]^-\) and \( m/z \) 149.0237 \([\text{M}-\text{H}-\text{CO}]^-\) were derived from the fragment ion at \( m/z \) 177.0186 by the loss of a \( \text{CO}_2 \) (44 Da) molecule and a \( \text{CO} \) (28 Da) molecule, respectively. Therefore, compound 41 was identified as esculetin [31]. Meanwhile, compound 45 was identified as 5,7-dihydroxy-4-methyl coumarin according to the fragmentation pattern [32].

Compound 42, with the quasimolecular ion at \( m/z \) 421.0766 \([\text{M}-\text{H}]^-\) and formula of \( \text{C}_{19}\text{H}_{18}\text{O}_{11} \), was identified as isomangiferin in the negative ion mode. The fragment ion at \( m/z \) 258.0161 \([\text{M}-\text{H}-\text{C}_6\text{H}_{10}\text{O}_5]^-\) was formed from the cleavage of the ion at \( m/z \) 421.0766 occurring in the position of the glucoside bond by the loss of the \( \text{C}_6\text{H}_{10}\text{O}_5 \) (162 Da) fragment ion. Therefore, compound 42 was identified as isomangiferin [33].

Compound 43, with the quasimolecular ion at \( m/z \) 243.0655 \([\text{M}-\text{H}]^-\) and formula of \( \text{C}_{14}\text{H}_{12}\text{O}_4 \), was identified as piceatannol in the negative ion mode. The fragment ions at \( m/z \) 71.0135 \([\text{M}-\text{H}-2\text{H}_2\text{O}-\text{C}_6\text{H}_10\text{O}_5]^-\), \( m/z \) 59.0136 \([\text{M}-\text{H}-\text{H}_2\text{O}-\text{C}_4\text{H}_8\text{O}_3]^-\), \( m/z \) 89.0241 \([\text{M}-\text{H}-\text{H}_2\text{O}-\text{C}_3\text{H}_6\text{O}_2]^-\), and \( m/z \) 71.0135 \([\text{M}-\text{H}-2\text{H}_2\text{O}-\text{C}_3\text{H}_6\text{O}_2]^-\) originated from the ion at \( m/z \) 243.0655 by the loss of a \( \text{H}_2\text{O} \) (18 Da) molecule,
a $C_4H_4O_2$ (84 Da) fragment, and a $C_2H_2O$ (42 Da) fragment. Subsequently, the fragment ion $m/z$ 173.0601 [$M-H-C_2H_2O-CO$]$^-$ was formed from the ion at $m/z$ 201.0549 [$M-H-C_2H_2O$]$^-$ due to a CO (28 Da) molecule. Compound 43 was identified as piceatannol [34].

Compound 44 showed $m/z$ 623.1963 [$M-H$]$^-$ ion and a formula of $C_{29}H_36O_{15}$ in the negative ion mode of the first-order mass spectrum. Fragment ions at $m/z$ 461.1656 [$M-H-C_9H_6O_3$]$^-$ and $m/z$ 179.0341 [$M-H-C_9H_6O_3$]$^-$ were derived from the fragment ion at $m/z$ 623.1966 by the loss of the fragments $C_9H_6O_3$ (162 Da) and $C_7H_5O_2$ (144 Da), respectively. Furthermore, the fragment ion at $m/z$ 179.0341 lost a $H_2O$ (18 Da) molecule and yielded the fragment ion at $m/z$ 161.0238 [$M-H-C_9H_6O_3-H_2O$]$^-$. Based on relevant literature [35], compound 44 may be forsythoside A, isoacteoside, or verbascoside. Then, compound 44 was identified as forsythoside A by comparing with the reference standard.

Compound 46, with the quasimolecular ion at $m/z$ 441.1754 [$M+HCOOH$]$^+$ and formula of $C_{20}H_{24}O_8$, was identified as lobetyolin in the negative ion mode. The fragment ion at $m/z$ 185.0963 [$M-H-C_9H_6O_3-CH_2O$]$^-$ was derived from the ion at $m/z$ 441.1754 by the loss of a fragment of the $C_6H_{10}O_6$ (178 Da) and $CH_2O$ (30 Da) groups. Subsequently, the fragment ion at $m/z$ 159.0811 [$M-H-C_7H_10O_6-CH_2O-C_3H_6O$]$^-$ originated from the ion at $m/z$ 185.0963 by the loss of $C_1H_3O$ (58 Da) fragment. In addition, the fragment ion at $m/z$ 89.0241 [$M-C_13H_22O_8$]$^-$ was formed from the ion at $m/z$ 441.1754 by the loss of $C_13H_22O_8$ fragment. Thus, compound 46 was identified as lobetyolin [36]. The fragmentation pathway of lobetyolin is shown in Figure 10.

![Figure 10: Possible fragmentation pathway of compound 46.](image)

![Figure 11: Possible fragmentation pathway of compound 47.](image)
Compound 47, with the quasimolecular ion at m/z 209.0799 [M + H]$^+$ and formula of C$_{11}$H$_{12}$O$_4$, was identified as methyl 4-hydroxy-3-methoxycinnamate in the positive ion mode. Fragment ions at m/z 177.0540 [M-H-CH$_2$O]$^+$ and m/z 149.0492 [M-H-C$_2$H$_4$O$_2$]$^+$ were derived from the ion at m/z 209.0799 by the loss of fragments of CH$_3$ (32 Da) and C$_2$H$_4$O$_2$ (60 Da). Subsequently, the fragment ion at m/z 134.0593 [M-H-C$_2$H$_4$O-CH$_3$]$^+$ was formed from the ion at m/z 149.0492 by loss of the CH$_3$ (15 Da) fragment. Therefore, compound 47 was identified as ferulic acid methyl ester [12]. The fragmentation pathway of ferulic acid methyl ester is shown in Figure 11.

4. Conclusions

The paper provided a new analysis method for the chemical constituents research of Zhideke granules. At the same time, this study rapidly and systematically analysed the chemical constituents of Zhideke granules by UPLC coupled with hybrid quadrupole-orbitrap MS. 47 chemical constituents were identified by UPLC-Q-Orbitrap HRMS including flavonoids and glycosides, organic acids, volatile components, nitrogen-containing compounds, sugars, and coumarins, which will provide a reference for quality control of Zhideke granules, and further reveal the pharmacodynamic substances of Zhideke granules.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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