Arabian Journal of Chemistry (2023) **16**, 104527

King Saud University

**Arabian Journal of Chemistry**

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REVIEW ARTICLE

# Comprehensive chemical profiling and quantification of Shexiang Xintongning tablets by integrating liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry

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Received 5 July 2022; accepted 11 December 2022
Available online 16 December 2022

**KEYWORDS**

Shexiang Xintongning tablet;
traditional Chinese medicine prescription;
Material basis;

**Abstract** Shexiang Xintongning tablet (SXXTN) is a traditional Chinese medicine (TCM) preparation for the treatment of coronary heart disease (CHD) angina pectoris. However, due to the complexity of the compounds in SXXTN, the active chemical components responsible for the therapeutic effect are still ambiguous. The purpose of our study was to characterize the chemical profile of SXXTN and quantify the representative chemicals. The high-performance liquid chromatogra-

*Abbreviations*: CHD, coronary heart disease; ESI, electrospray ionization; GC–MS, gas chromatograph coupled with mass spectrometry; GC-QQQ MS, gas chromatograph coupled with triple-quadrupole tandem mass spectrometry; HPLC, high-performance liquid chromatography; HRMS, high resolution mass spectrometry; HPLC-QTOF MS, high-performance liquid chromatography coupled with time-of-flight mass spectrometry; HPLC-QQQ MS, high performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantitation; MRM, multiple reaction monitoring; OA, oleanane; PPD, 20(*S*)-protopanaxadiol; PPT, 20(*S*)-protopanaxatriol; RDA, Retro Diels-Alder; RSD, relative standard deviation; SXXTN, Shexiang Xintongning tablet; TCM, traditional Chinese medicine; TLC, thin-layer chromatography; TICs, typical total ion chromatograms

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.arabjc.2022.104527

Multi-component content determination;
GC–MS;
HPLC-MS

phy coupled with time-of-flight mass spectrometry (HPLC-QTOF MS) method and gas chromatograph coupled with mass spectrometry (GC–MS) method were utilized to identify the chemical constituents of SXXTN. A total of 140 compounds including alkaloids, ginsenosides, organic acids, esters, triterpenes, phthalides and amino acid were identified in accordance with their retention times, accurate masses and characteristic MS/MS fragment patterns. Forty-four volatile components were characterized by GC–MS through NIST database matching. In the further research of quantitative analysis, 40 non-volatile compounds and 17 volatile compounds were determined and successfully applied for detecting in 7 batches of SXXTN samples by high performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry (HPLC-QQQ MS) and gas chromatograph coupled with triple-quadrupole tandem mass spectrometry (GC-QQQ MS) in multiple reaction monitoring (MRM) mode, respectively. The quantitative methods were verified in linearity, precision, repeatability stability and recovery. The above results indicated that the established method was practical and reliable for synthetical quality evaluation of SXXTN. In addition, our study might supplement the chemical evidence for disclosing the material basis of its therapeutic effects.



## 1. Introduction

Preparations of TCM formulae have been extensively utilized for clinical medication owing to their therapeutic effects on various diseases and relatively low side effects (Sun et al., 2017). Shexiang Xintongning tablet (SXXTN), a newly hospital preparation which has got a wide application in China to treat coronary heart disease angina pectoris (qi stagnation and blood stasis syndrome) and reportorial clinical studies have shown its efficacy (Shen and Lu, 2005). SXXTN comprised of Artificial Musk, Corydalis Rhizoma (*Corydalis yanhusuo* W. T. Wang.), Ginseng Radix et Rhizoma (*Panax ginseng* C. A. Mey.), Chuanxiong Rhizoma (*Ligusticum chuanxiong* Hort.), Styrax (*Liquidambar orientalis* Mill.) and Borneolum Syntheticum. All of the above crude drugs have been reported to be associated with the effect of SXXTN in the treatment of CHD. Here, Musk and Corydalis Rhizoma are reported to reduce infarct size and improve cardiac function (Li et al., 2008; Ling et al., 2010). The mechanisms of Ginseng Radix et Rhizoma in preventing coronary artery disease, myocardial hypertrophy, heart failure and arrhythmia are gradually being revealed (Zheng et al., 2012). Chuanxiong Rhizoma, Styrax and Musk have been proved to have the role of anti-myocardial ischemia (Liu et al., 2016; Wang et al., 2019; Wu et al., 2011). Besides, Borneolum Syntheticum as an adjuvant has been reported to provide new possibilities for the treatment of atherosclerosis (Zhang et al., 2017). Muscone, tetrahydropalmatine, ginsenoside, tetramethylpyrazine, cinnamic acid and borneol have been reported as important bioactive components relevant to treatment of CHD. Recently, SXXTN was revealed have the function of reducing oxidative stress-mediated damage and enhancing angiogenesis, and might play an important role in the treatment of myocardial infarction (Li et al., 2020). Obviously, the identification and detection of the main components in SXXTN is the premise and key to reveal its active ingredients. However, the chemical composition of SXXTN is complicated, having both volatile small molecules and non-volatile components such as alkaloids, organic acids and ginsenosides. In previous studies, the chemical constituents of each crude drugs in SXXTN have been reported (He et al., 2018; Zheng et al., 2018; Yang et al., 2021; Gurbuz et al., 2013; Ding et al., 2022; Sun et al., 2014), but little attention was paid to the integral chemical composition of SXXTN. Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) have made powerful contributions for quality control of SXXTN (Zhang et al., 2016). Nevertheless, they were preferred to assay the limited components in SXXTN with difficult access to comprehensive chemical information. Thus, new methods for chemical profiling and quantification of SXXTN are necessary to solve the limitations of the previous techniques.

Comprehensive profiling of chemical constituents in TCM preparations is still facing great challenges on separation, detection and identification due to their structural complexities and diversities. Nowadays, various chromatographic coupled with mass spectrometry techniques, such as GC–MS (Su et al., 2008) and LC-MS (Xu et al., 2015; Luo et al., 2019), are widely used in the study of TCM preparations due to their combined superiorities of high-efficient separation and high-sensitive detection for multi-components in complex samples. On one hand, HPLC-QTOF MS and GC–MS could provide molecular weights and abundant fragment information for structure identification of compounds in TCM preparations. On the other hand, tandem mass spectrometers coupled to LC or GC systems were powerful tools for high-throughput quantitative analysis of TCM preparations owing to their high-selective simultaneous detection of multiple compounds with MRM modes. Therefore, the integration of LC-MS and GC–MS was a potentially effective approach for in-depth chemical profiling and quality control of SXXTN.

In this paper, HPLC-QTOF MS and GC–MS analysis methods were established for the global characterizations of complicated non-volatile and volatile ingredients in SXXTN. Besides, considering the representative components of all relevant single drugs in SXXTN, the abundance and activity of chemicals and the availability of reference substances, 57 compounds were further quantitatively analyzed by HPLC-QQQ MS and GC-QQQ MS method. The aims of our study were comprehensively qualitative and quantitative profiling the chemical basis of SXXTN, which were expected to improve the quality control, promote the pharmacological researches and expand the clinical applications.

## 2. Materials and methods

### 2.1. Reagents and materials

Seven batches of SXXTN were generously provided by Shandong Hongjitang Pharmaceutical Group Co., ltd. (Shandong, China) and listed in Table S1. A total of 101 reference standards and 3 internal standards were presented in Table S2. All standards were≥98 % by HPLC and 1H NMR analyses.

Ultrapure water (18.2 MΩ cm) for analysis was prepared by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Methanol and acetonitrile (HPLC grade) were provided by Merck (Darmstadt, Germany), and formic acid (HPLC grade) were purchased from ROE (Newark, New Castle, DE, USA). Ethyl alcohol

(HPLC grade) was offered by Sichuan Ruijingte Technology Co., ltd. (Sichuan, China).

### 2.2. Standard solution and samples preparations

The reference standards were solubilized by 75 % methanol-aqueous solution (*v*/*v*) to obtain 1.00 mg/mL reserve solution and diluted with appropriate solvent to a range of proper concentrations.

In qualitative analysis, for LC-MS, the SXXTN was ground into powder. SXXTN powder (0.3003 g) was accurately weighed and ultrasonic extracted (40 kHz, 500 W) with 5 mL 75 % methanol-aqueous solution (*v*/*v*) for 30 min. The extracts were centrifuged (13,000 rpm, 10 min, 4℃) before LC-MS analysis. For GC–MS, the powder (0.3000 g) was accurately weighed, then sonicated for 30 min at 40 kHz with 5 mL of ethanol. The filtrate was filtered by 0.45 μm filter membrane and centrifuged before sampling.

For quantitative analysis, to determine the non-volatile constituents, each batch of SXXTN powder (0.3 g) was weighed in three parallel times, then ultrasonic extracted (40 kHz, 500 W) with 5 mL 75 % methanol for 30 min. The filtrate was filtered by 0.45 μm filter membrane and centrifuged (13000 rpm, 10 min, 4℃). For alkaloids quantification (group A, 24 alkaloids), the supernatant was diluted after adding proper nitidine chloride (IS1, 1.11 μg/mL) as internal standard. For ginsenosides and acids quantification (group B, 14 ginsenosides, cinnamic acid and phenylalanine), the supernatant was diluted after proper saikosaponin C (IS2, 0.985 μg/mL) adding. To determine the volatile constituents, about 0.3 g the powder of SXXTN was extracted with 10 mL ethanol under ultrasonic conditions in ice-water bath for 20 min. The extraction was filtered through syringe filter (0.45 μm) and centrifuged. Isoborneol, borneol, 3-phenylpropyl cinnamate and cinnamyl cinnamate possessed significantly higher abundances in SXXTN comparing with other volatile components, indicating the large differences of contents among various compounds. Therefore, the supernatant was diluted 10 times before injecting to GC–MS for quantitative analysis of high-abundant volatile compounds (Group D), whereas directly injected for others with relatively low-abundances (Group C). And a certain amount of naphthalene (IS3, 23.2 μg/mL) was added to the supernatants as internal standard.

### 2.3. HPLC-QTOF MS analysis conditions

Agilent 1290 HPLC system (Agilent corporation, USA) was used to determine the non-volatile components of SXXTN. A ZORBAX Eclipse Plus C18 column (150 × 2.1 mm, 1.8 μm, Agilent Technologies, Santa Clara, USA) was used for sample separation. The mobile phase consisted of 0.1 % (*v*/*v*) formic acid in water (A) and acetonitrile (B) with the gradient elution set as follows: 0–3 min, 10 %-12 % B; 3–8 min, 12 %-17 % B; 8–20 min, 17 %–22 % B; 20–30 min, 22 %-35 % B; 30–45 min, 35 %-42 % B; 45–50 min, 42 %-60 % B; 50–57 min, 60 % B; 57–60 min, 60 %-80 % B; 60–68 min, 80 %-100 %. The flow rate was set at 0.4 mL /min, and the column temperature was maintained at 30 ℃. Sample volume was 1 μL for injection.

The Q-TOF mass spectrometer equipped with electrospray ionization (ESI) source was used to acquire data in positive and negative ion modes. The operation conditions were as below: drying gas ($N_2$) temperature, 300 ℃; drying gas flow, 8.0 L/min; nebulizer gas ($N_2$) pressure, 35 psig; sheath gas ($N_2$) temperature, 350 ℃; sheath gas flow, 11.0 L/min; capillary voltage positive ion mode, 4000 V; negative ion mode, 3500 V; fragmentor voltage, 120 V; skimmer voltage, 65 V. Full-scan MS and MS/MS data was collected over the *m*/*z* range of 50–1500 using extended dynamic range. Collision energy of secondary mass spectrometry was set as 15 eV, 30 eV and 45 eV.

### 2.4. GC–MS analysis conditions

Compound identification was performed by Agilent 7890B GC system combined with Agilent 5977 Mass Selection Detector. Samples were separated by Agilent HP-5MS (30 m × 0.25 m m, 0.25 μm) column. The carrier gas was high purity helium, and the flow rate was 1 mL/min. Initial column temperature was 60 ℃, and programmed to rise at 20 °C/min to 85 °C (1 min held), 5 ℃/min to 100 ℃ (5 min held), 15 ℃/min to 150 ℃ (6 min held), 5 ℃/min to 200 ℃ (4 min held), rising at 5 ℃/min to 280 ℃ (5 min held). The injection volume was 1 μL and the splitting ratio was 30:1. The temperature of injector and aux heaters was controlled at 250℃ and 280℃, respectively. MS quadrupole and ion source temperature were maintained at 150℃ and 230℃, severally. MS data were recorded at 70 eV and acquired in full scan mode over the range of *m*/*z* 40–600.

### 2.5. HPLC-QQQ MS analysis conditions

The quantitative analysis was performed on the Shimadzu LCMS-8050 triple quadrupole tandem mass spectrometry detector (Shimadzu, Kyoto, Japan) with an Agilent Zorbax Eclipse Plus C18 column (2.1 × 150 mm, 1.8 μm, Agilent Technologies, Santa Clara, USA). For group A, 0.1 % (*v*/*v*) formic acid water (A) and acetonitrile (B) were used as mobile phases, and the gradient elution procedure was as follows: 0–12 min, 19 %-20 % B; 12–14 min, 20 %-35 % B; 14–16 min, 35 %-90 % B; 16–19 min, 90 %-100 % B. For group B, the mobile phase was water (A) and acetonitrile (B), with the following gradient elution: 0–3 min, 10 %-12 % B; 3–6 min, 12 %-35 % B; 6–14 min, 35 %-36.5 % B 14–15 min, 36.5 %-90 % B; 15–19 min, 90 %-100 % B. The flow rate was maintained at 0.4 mL/min, with the injection volume 2 μL for all samples. The MS conditions were as below: capillary voltage, 4000 V; drying gas temperature, 300 °C. The flow rate of drying gas ($N_2$) and nebulizer gas ($N_2$) was 10.0 L/min and 3.0 L/min, severally. Analytes were determined in MRM modes, and the optimized parameters were shown in Table S3 and S4.

### 2.6. GC-QQQ MS analysis conditions

The quantitative analysis of volatile components was operated on an Agilent 7890B gas chromatography coupling to Agilent 5977A mass spectrometry (Agilent, Santa Clara, CA, USA). For group C, the initial column temperature was 60 ℃, and programmed to rise at 20 °C/min to 85 °C, 5 ℃/min to 100 ℃ (5 min held), 15 ℃/min to 150 ℃, 5 ℃/min to 180 ℃ (1 min held), finally rising at 15 ℃/min to 280 ℃ (2 min held). For group D, the initial column temperature was set at 100 ℃,

and programmed to rise at 10 °C/min to 110 °C, 3 ℃/min to 120 ℃, 55 ℃/min to 265 ℃, finally rising at 18 ℃/min to 280 ℃ (2 min held). The injection volume was 1 μL and the splitting ratio was 10:1. The MRM parameters for all analytes are presented in Table S5 and S6. Other analytical conditions refer to *Section 2.4*.

## 3. Results and discussion

### *3.1. Qualitative analysis of SXXTN based on diagnostic ion strategy by HPLC-QTOF MS*

The HPLC-QTOF MS conditions of the mobile phase systems (methanol-aqueous, acetonitrile-aqueous, and acetonitrile-aqueous with 0.1 % formic acid), gradient program, column temperature (25 °C, 30 °C, and 35 °C) and the flow rate (0.2, 0.3 and 0.4 mL/min) were optimized in order to obtain overall constituents of SXXTN with good resolution within a short analysis. The total peak area was adopted as a criterion for optimization. Ultimately, the optimum conditions mentioned in *Section 2.3* were preferred.

Diagnostic ion strategy is regarded as a powerful approach for rapid characterization of chemicals in TCMs based on the principle that similar chemical constituents have similar cleavage rules and the fragmentation information, which is applicable for the identification of structural analogues in complex TCMs and formulae (Wang et al., 2017). In our study, by comparing with the reference standards, the known compounds were marked. On the basis of MS/MS analysis of authentic compounds, the characteristic fragmentation pathways of compounds with the same carbon skeleton were presented, the obtained rules were further applied to the structural characterization of its derivatives. For other unknown compounds, identification based on MS/MS spectra and relevant literature or online databases, including PubChem search (https://pubchem.ncbi.nlm.nih.gov/) and the Human metabolome database (https://www.hmdb.ca/). The typical total ion chromatograms (TICs) of SXXTN by HPLC-QTOF MS in both of positive and negative ion modes were displayed in Fig. 1. Totally, 140 compounds were identified based on diagnostic ion strategy, including 60 alkaloids, 34 ginsenosides, 21 organic acids, 12 phthalides, 10 triterpenes, 2 esters and 1 amino acid. The chemical structures and detailed information of compounds could be viewed in Fig. 2 and Table 1, respectively. The MS/MS spectra and fragmentation pathways of the representative chemicals were shown in Fig S1 and Fig S2.

#### *3.1.1. Identification of alkaloids in SXXTN*

Sixty alkaloids in SXXTN demonstrated quasi-molecular ions $[M + H]^+$ or $[M]^+$ in positive ion mode and listed in Table 1, mostly originated from Corydalis Rhizoma and identified as four main types, including tetrahydroproberberines, berberines, protopines and aporphines.

A total of 19 tetrahydroprotoberberine-type (**21, 22, 24, 28, 29, 33, 34, 35, 38, 39, 42, 47, 48, 50, 51, 58, 59, 60, 72**) and 3 protopine-type alkaloids (**36, 44, 69**) were tentatively identified or unambiguously characterized with the characteristic cleavage pathway of Retro Diels-Alder (RDA) reaction, which can be used to distinguish them from other types of alkaloids (Yuan et al., 2016). In MS/MS of tetrahydropalmatine (**48**) shown as Fig. S1A, the fragment ion with the strongest intensity was located at *m/z* 192.1019 $[M + H\text{-}C_{10}H_{12}O_2]^+$, and it

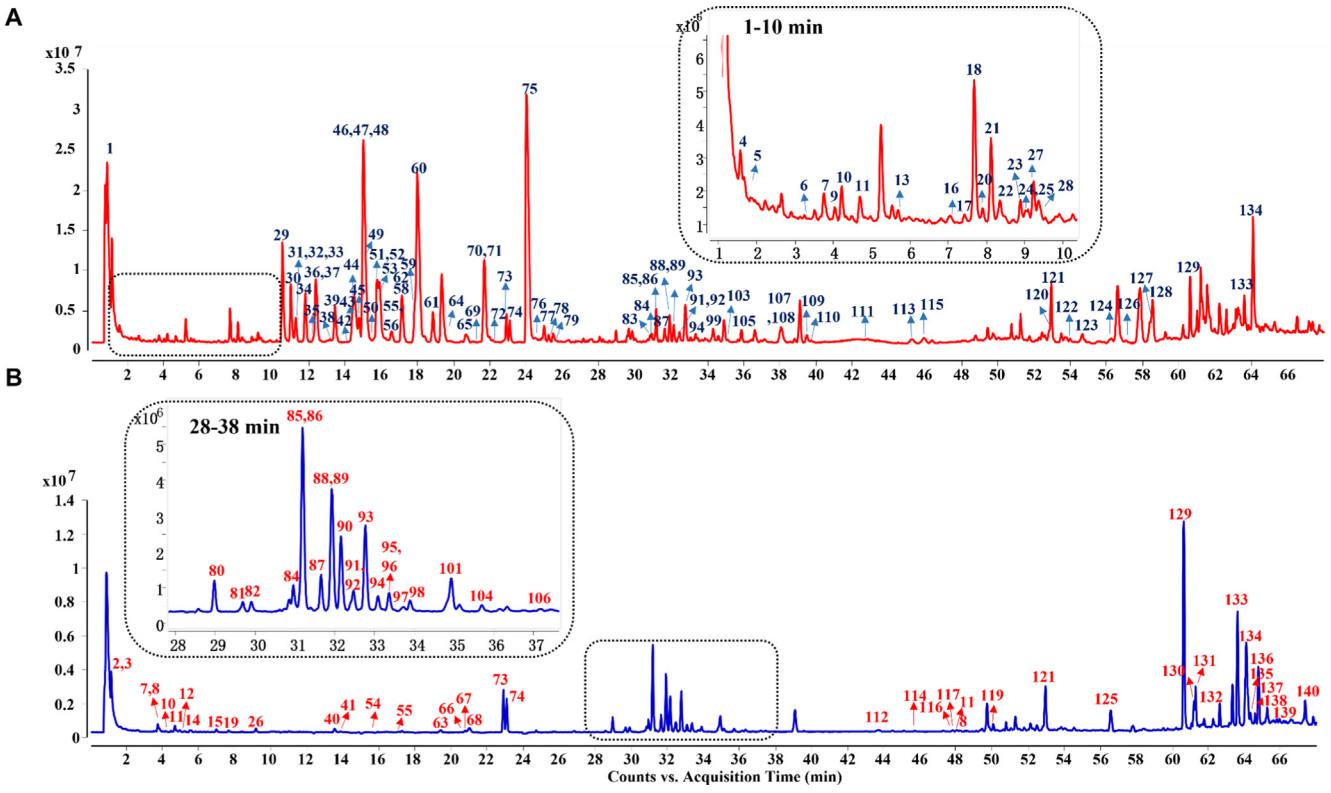


**Fig. 1** Total ion current chromatograms of SXXTN in positive ion mode (A) and negative ion mode (B) by HPLC-QTOF MS.

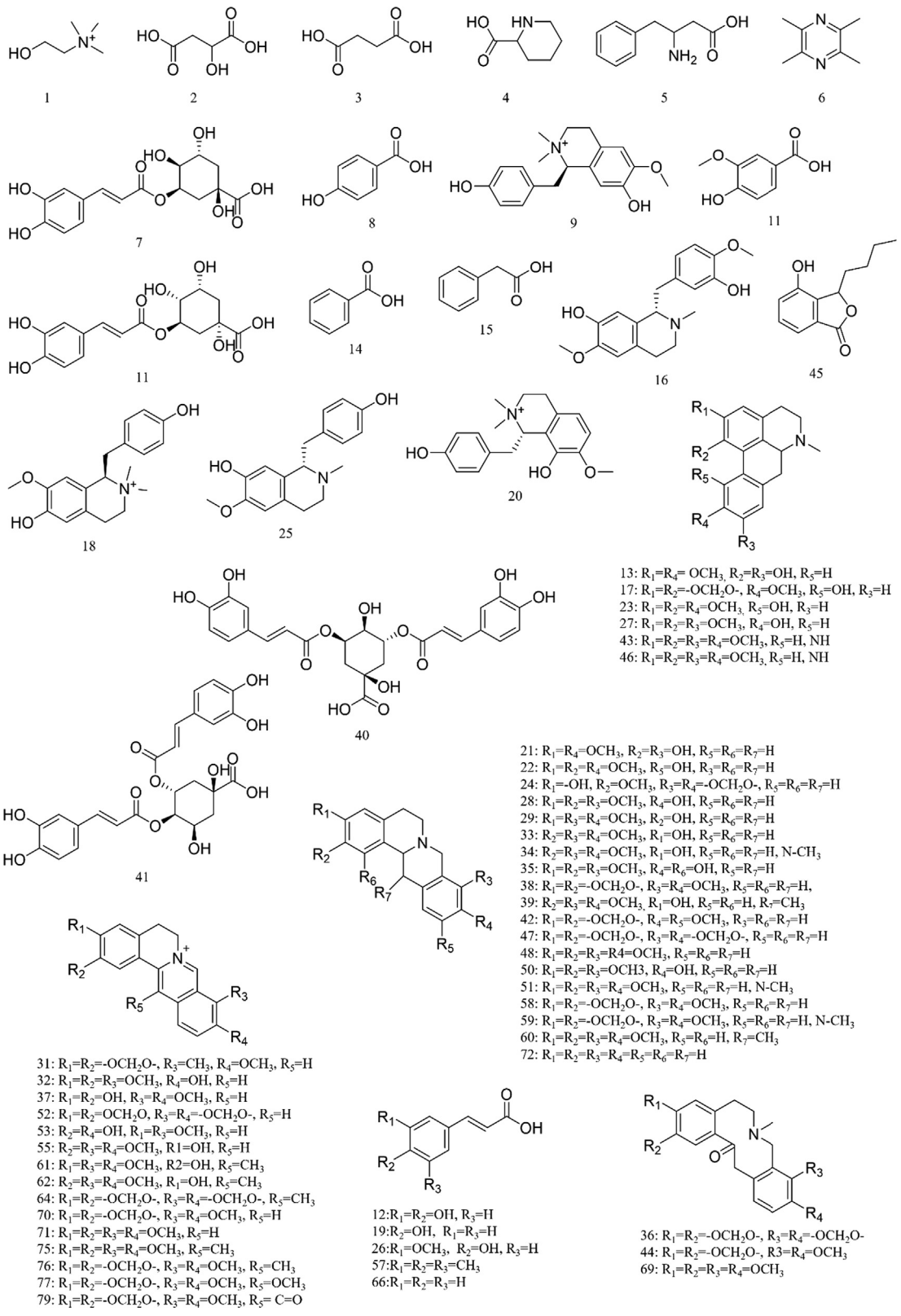


**Fig. 2** Structures of chemical constituents from SXXTN.

was found that the complementary fragment ion *m*/*z* 165.0909 $[M + H\text{-}C_{11}H_{13}NO_2]^+$ was corresponding to the RDA reaction of C ring. The detailed fragmentation pathways of tetrahydropalmatine (**48**) were displayed in Fig. S2A. For protopine-type alkaloids, C-14 position is linked to oxygen to form carbonyl, which is easy to dehydrate and forms stable fragment ions, thus distinguishing it from tetrahydroberberberine-type alkaloids (Yuan et al., 2016).

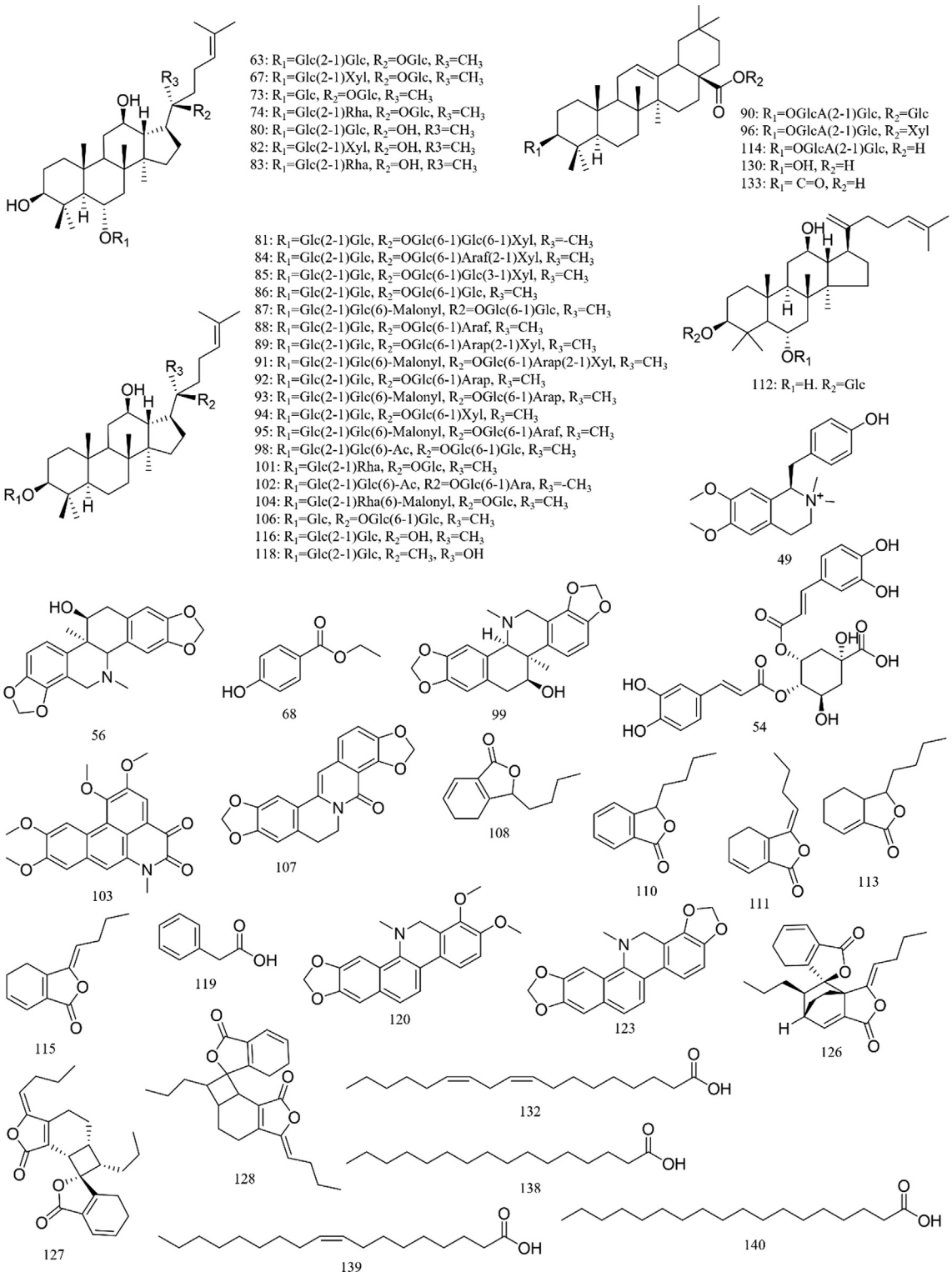


**Fig. 2** (*continued*)

Taking protopine (**36**) as an example (Fig. S1B), the product ions at *m*/*z* 336.1232 $[M + H\text{-}H_2O]^+$ and *m*/*z* 188.0709 $[M + H\text{-}C_9H_8O_2\text{-}H_2O]^+$ may be formed by neutral losses of $H_2O$ from molecular ions and *m*/*z* 206.0813 (Fig. S2B).

Fifteen protoberberine-type (**31, 32, 37, 52, 53, 55, 61, 62, 64, 70, 71, 75, 76, 77, 79**) and six aporphine-type alkaloids (**13, 17, 23, 27, 43, 46**) were identified in SXXTN with the cleavage pathway based on the fragmentation of substituents

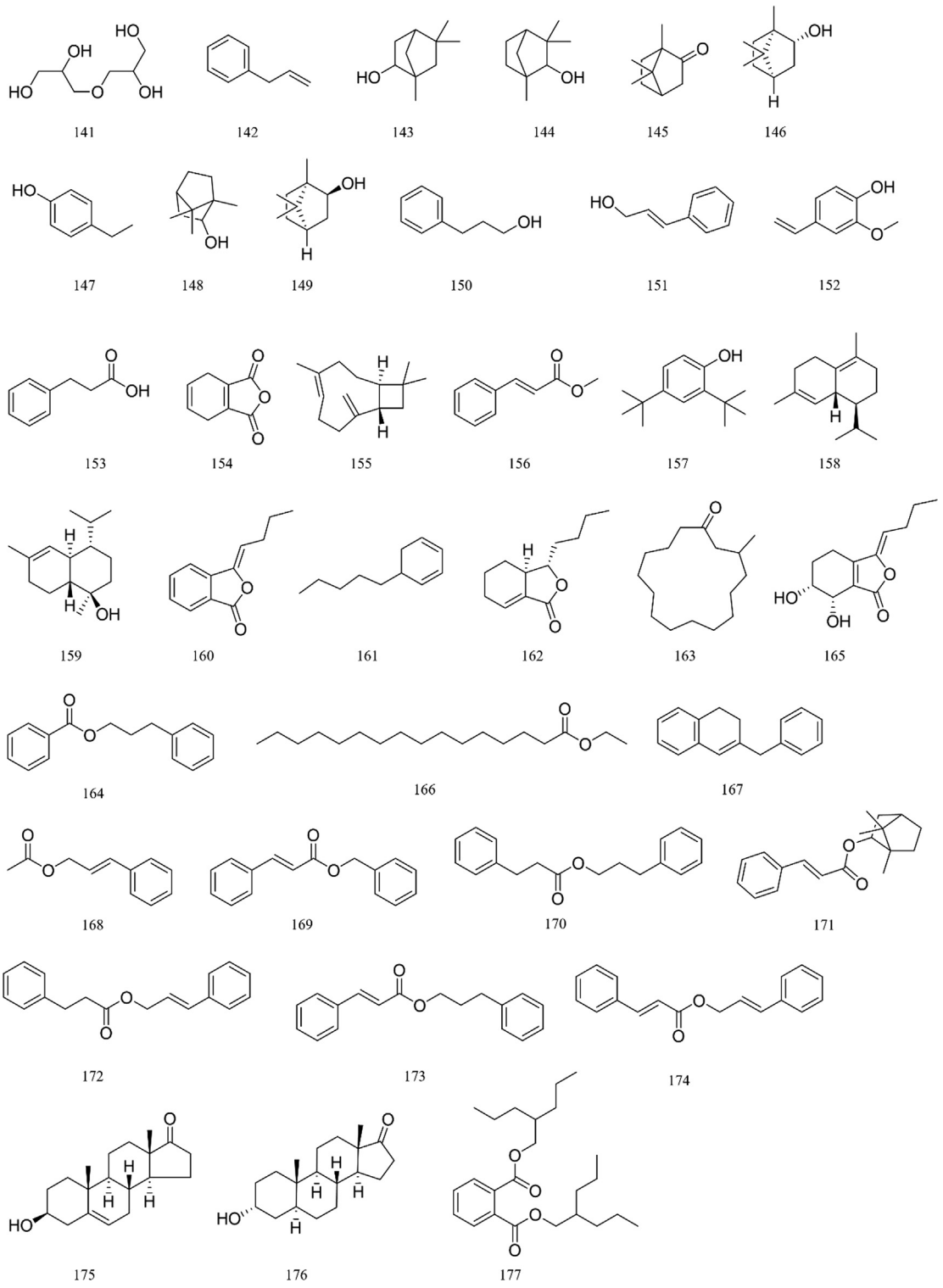


**Fig. 2** (*continued*)

(Yuan et al., 2016) as displayed in Fig. S2C. For protoberberine-type alkaloids, usually losing 15 Da (–$CH_3$) substituent as see in MS/MS spectrum (Fig. S1C) of berberine (**70**), the main product ions appeared at *m*/*z* 320.0919 $[M-CH_4]^+$ and *m*/*z* 321.0978 $[M-CH_3]^+$. In addition, the successive losses of $CH_3$ and CO were the characteristic cleavage pathway of this alkaloid. For aporphine-type alkaloids, the fragment ions with the highest relative abundance usually appear when the methoxy group at 31 Da is lost. For example, fragment ion *m*/*z* 297.1108 $[M + H\text{-}OCH_3]^+$ was found in

**Table 1** Characterization of chemical constituents of SXXTN by HPLC-QTOF MS.

| No. | $t_R$ (min) | Formula | Precursorions (*m*/*z*) | Diff (ppm) | Fragment ions (*m*/*z*) | Identification | Structural Types |
|---|---|---|---|---|---|---|---|
| 1 | 0.88 | $C_5H_{14}NO^+$ | 104.1069 $[M+H]^+$ | −0.87 | 58.0658,60.0813 | Choline | Alkaloid |
| 2* | 1.06 | $C_4H_6O_5$ | 133.0143 $[M-H]^-$ | 0.40 | 115.0036,89.0252,71.0148 | Malic acid | Organic acid |
| 3 | 1.33 | $C_4H_6O_4$ | 117.0192 $[M-H]^-$ | −1.13 | 99.9252,73.0303 | Succinic acid | Organic acid |
| 4* | 1.46 | $C_6H_{11}NO_2$ | 130.0867 $[M+H]^+$ | 3.42 | 70.0653,84.0814,56.0510 | *Dl*-pipecolinic acid | Organic acid |
| 5* | 1.56 | $C_9H_{11}NO_2$ | 166.0864 $[M+H]^+$ | 0.87 | 120.0806,103.0547 | Phenylalanine | Amino acid |
| 6* | 3.28 | $C_8H_{12}N_2$ | 137.1067 $[M+H]^+$ | −4.50 | 55.0550,80.0475 | Tetramethylpyrazine | Alkaloid |
| 7 | 3.74 | $C_{16}H_{18}O_9$ | 353.087 $[M-H]^-$ | −2.28 | 191.0562,179.0339 | Neochlorogenic acid | Organic acid |
| 8* | 3.89 | $C_7H_6O_3$ | 137.024 $[M-H]^-$ | −3.05 | 93.0348,65.0413 | 4-Hydroxybenzoic acid | Organic acid |
| 9 | 4.07 | $C_{19}H_{24}NO_3$ | 314.1755 $[M]^+$ | 1.37 | 269.1173,175.0748,107.0491 | Magnocurarine | Alkaloid |
| 10* | 4.22 | $C_{16}H_{18}O_9$ | 353.0901 $[M-H]^-$ | 6.50 | 191.0563,179.0364,173.0456 | Chlorogenic acid | Organic acid |
| 11* | 4.75 | $C_8H_8O_4$ | 167.0341 $[M-H]^-$ | −5.28 | 152.3304,123.0429,108.0130 | Vanillic acid | Organic acid |
| 12* | 5.04 | $C_9H_8O_4$ | 179.0352 $[M-H]^-$ | 1.22 | 135.0458 | Caffeic acid | Organic acid |
| 13 | 5.69 | $C_{19}H_{21}NO_4$ | 328.1547 $[M+H]^+$ | 1.11 | 265.0853,297.1108,282.0882,165.0713 | Isoboldine | Alkaloid |
| 14 | 5.71 | $C_7H_6O_2$ | 121.0291 $[M-H]^-$ | −3.33 | 92.0281,76.9491 | Benzoic acid | Organic acid |
| 15 | 7.04 | $C_8H_8O_2$ | 135.0456 $[M-H]^-$ | 3.31 | 120.0225,92.0278 | Phenylacetic acid | Organic acid |
| 16 | 7.07 | $C_{19}H_{24}NO_4$ | 330.1702 $[M]^+$ | 0.65 | 299.1269,192.1032,175.0186, 143.0016 | Reticuline | Alkaloid |
| 17 | 7.44 | $C_{19}H_{19}NO_4$ | 326.1386 $[M+H]^+$ | −0.26 | 295.0969,263.0696,235.0752 | Bulbocapnine | Alkaloid |
| 18 | 7.66 | $C_{19}H_{24}NO_3$ | 314.1759 $[M]^+$ | 2.64 | 269.1173,237.0907,175.0745, 107.0490 | Lotusine | Alkaloid |
| 19 | 7.71 | $C_9H_8O_3$ | 163.0402 $[M-H]^-$ | 0.81 | 119.0497 | 4-Hydroxycinnamic acid | Organic acid |
| 20 | 7.92 | $C_{19}H_{24}NO_3$ | 314.1743 $[M]^+$ | −2.45 | 237.0885,209.0961,107.0488 | Oblongine | Alkaloid |
| 21* | 8.13 | $C_{19}H_{21}NO_4$ | 328.1549 $[M+H]^+$ | 1.72 | 178.0862,163.0627,151.0755 | Scoulerine | Alkaloid |
| 22 | 8.39 | $C_{20}H_{23}NO_4$ | 342.1697 $[M+H]^+$ | −0.83 | 178.0856,326.1402 | Corytenchine | Alkaloid |
| 23* | 8.83 | $C_{20}H_{23}NO_4$ | 342.1699 $[M+H]^+$ | −0.25 | 279.1015,311.1278,342.1699 | Isocorydine | Alkaloid |
| 24 | 9.06 | $C_{19}H_{19}NO_4$ | 326.1393 $[M+H]^+$ | 1.89 | 178.0854,151.0730 | Cheilanthifoline | Alkaloid |
| 25 | 9.10 | $C_{18}H_{21}NO_3$ | 300.1596 $[M+H]^+$ | 0.60 | 269.1175,237.0921,192.1025 | *N*-Methylcoclaurine | Alkaloid |
| 26* | 9.19 | $C_{10}H_{10}O_4$ | 193.0505 $[M-H]^-$ | −0.69 | 178.0277,149.0594,134.0374 | Ferulic acid | Organic acid |
| 27 | 9.21 | $C_{20}H_{23}NO_4$ | 342.1698 $[M+H]^+$ | −0.54 | 192.1020,148.0753 | Lirioferine | Alkaloid |
| 28* | 9.36 | $C_{20}H_{23}NO_4$ | 342.1700 $[M+H]^+$ | −0.25 | 327.1472,165.0909,192.1016 | Corydalmine | Alkaloid |
| 29* | 10.57 | $C_{20}H_{23}NO_4$ | 342.1707 $[M+H]^+$ | 2.09 | 327.1472,326.1414,178.0866 | Tetrahydrocolumbamine | Alkaloid |
| 30 | 11.02 | $C_{21}H_{25}NO_4$ | 356.1856 $[M+H]^+$ | −0.10 | 341.1609,326.1389,308.1276, 192.1020,177.0783 | *N*-Methyltetrahydropalmatrubie | Alkaloid |
| 31 | 11.24 | $C_{19}H_{16}NO_4$ | 322.1074 $[M+H]^+$ | −2.57 | 307.0839,294.2059,279.0888 | Berberrubine | Alkaloid |
| 32 | 11.29 | $C_{21}H_{25}NO_4$ | 356.1857 $[M+H]^+$ | 0.18 | 341.1617,326.1390,192.1018, 165.0909,150.0672 | *N*-Methylcorydalmine | Alkaloid |

**Table 1** (*continued*)

| No. | $t_R$ (min) | Formula | Precursorions (*m*/*z*) | Diff (ppm) | Fragment ions (*m*/*z*) | Identification | Structural Types |
|---|---|---|---|---|---|---|---|
| 33* | 11.35 | $C_{20}H_{23}NO_4$ | 342.1695 $[M + H]^+$ | −1.42 | 326.1387,178.0860,163.0629, 151.0725,119.0489 | Corypalmine | Alkaloid |
| 34 | 11.83 | $C_{21}H_{25}NO_4$ | 356.1860 $[M + H]^+$ | 1.03 | 341.1590,326.1380,308.1283,192.1020 | *N*-Methylcorypalmine | Alkaloid |
| 35 | 12.26 | $C_{20}H_{23}NO_5$ | 358.1652 $[M]^+$ | 0.84 | 356.1856,340.1516 | Capaurimine | Alkaloid |
| 36* | 12.41 | $C_{20}H_{19}NO_5$ | 354.1343 $[M + H]^+$ | 1.98 | 336.1229,206.0812,189.0777,275.0705 | Corydinine | Alkaloid |
| 37* | 12.45 | $C_{19}H_{18}NO_4^+$ | 324.1228 $[M]^+$ | −0.72 | 307.9500,280.0005,309.0006 | Demethyleneberberine | Alkaloid |
| 38 | 13.17 | $C_{20}H_{21}NO_4$ | 340.1538 $[M + H]^+$ | −1.57 | 324.1229,309.1100,296.1274 | Sinactine | Alkaloid |
| 39 | 13.41 | $C_{21}H_{25}NO_4$ | 356.1855 $[M + H]^+$ | −0.38 | 341.1632,340.1554,326.1415 | Corybulbine | Alkaloid |
| 40 | 13.58 | $C_{25}H_{24}O_{12}$ | 515.118 $[M-H]^-$ | −2.91 | 353.0853,191.0556 | Isochlorogenic acid A | Organic acid |
| 41 | 13.84 | $C_{25}H_{24}O_{12}$ | 515.1182 $[M-H]^-$ | −6.14 | 353.0867,191.0541 | Isochlorogenic acid B | Organic acid |
| 42 | 14.20 | $C_{20}H_{20}NO_4^+$ | 338.1390 $[M]^+$ | 0.93 | 322.1055,380.0926,294.1122,280.0937 | Tetrahydrocorysamine | Alkaloid |
| 43* | 14.40 | $C_{20}H_{23}NO_4$ | 342.1679 $[M + H]^+$ | −0.25 | 325.1432,294.1250,279.1035,251.1113 | Norglaucine | Alkaloid |
| 44* | 14.54 | $C_{21}H_{23}NO_5$ | 370.1659 $[M + H]^+$ | 2.70 | 188.0706,290.0939,321.1141,352.1548 | Allocryptopine | Alkaloid |
| 45 | 14.79 | $C_{12}H_{14}O_3$ | 207.1016 $[M + H]^+$ | 0.14 | 189.0893,175.0196,123.0433,67.0544 | 4-Hydroxy-3-butylphthalide | Phthalide |
| 46* | 14.93 | $C_{21}H_{25}NO_4$ | 356.1846 $[M + H]^+$ | −2.91 | 294.1254,310.1206,325.1436 | Glaucine | Alkaloid |
| 47* | 14.96 | $C_{19}H_{17}NO_4$ | 324.1243 $[M + H]^+$ | 2.05 | 176.0713,294.1251,149.0579, | Tetrahydrocoptisine | Alkaloid |
| 48* | 15.04 | $C_{21}H_{25}NO_4$ | 356.1879 $[M + H]^+$ | 2.71 | 192.1032,165.0918,194.1271,326.1479 | Tetrahydropalmatine | Alkaloid |
| 49 | 15.09 | $C_{20}H_{25}NO_3^+$ | 328.1916 $[M + H]^+$ | 2.68 | 283.1348,251.1070,236.0850 | 6-*O*-methylotusine | Alkaloid |
| 50* | 15.36 | $C_{21}H_{25}NO_4$ | 356.1856 $[M + H]^+$ | −0.38 | 354.1478,325.1338,194.2615 | Yuanhunine | Alkaloid |
| 51 | 15.75 | $C_{22}H_{27}NO_4$ | 370.2008 $[M + H]^+$ | −1.31 | 354.1693,206.1174,190.0871,165.0900 | *N*-Methyltetrahydropalmatine | Alkaloid |
| 52* | 15.79 | $C_{19}H_{14}NO_4^+$ | 320.0926 $[M]^+$ | 2.70 | 292.0972,262.0880,234.0919 | Coptisin | Alkaloid |
| 53* | 15.90 | $C_{20}H_{20}NO_4^+$ | 338.1395 $[M]^+$ | 2.41 | 323.1142,322.1015 | Columbamine | Alkaloid |
| 54 | 16.36 | $C_{25}H_{24}O_{12}$ | 515.1167 $[M-H]^-$ | −5.44 | 353.0848,191.0513 | Isochlorogenic acid C | Organic acid |
| 55* | 16.58 | $C_{20}H_{20}NO_4^+$ | 338.1390 $[M]^+$ | 0.93 | 323.1167,294.1143,322.1093 | Jatrorrhizine | Alkaloid |
| 56 | 16.70 | $C_{12}H_{14}O_3$ | 207.1015 $[M + H]^+$ | −0.34 | 189.0818,161.0967 | Senkyunolide F | Phthalide |
| 57 | 17.10 | $C_{12}H_{14}O_5$ | 237.0753 $[M-H]^-$ | −6.53 | 193.0849,108,0193 | Trimethoxycinnamic acid | Organic acid |
| 58* | 17.16 | $C_{20}H_{21}NO_4$ | 340.1556 $[M + H]^+$ | 3.72 | 176.0716,149.0608 | Canadine | Alkaloid |
| 59 | 17.93 | $C_{21}H_{24}NO_4^+$ | 354.1708 $[M]^+$ | 2.30 | 165.0906,190.0876 | *N*-Methylcanadine | Alkaloid |
| 60* | 18.03 | $C_{22}H_{27}NO_4$ | 370.2023 $[M + H]^+$ | 2.74 | 355.1790,192.1032,176.0731,165.0912 | Corydaline | Alkaloid |
| 61 | 18.86 | $C_{21}H_{22}NO_4^+$ | 352.1543 $[M]^+$ | −0.10 | 337.1308,322.1064,309.1345,293.1041 | 13-Methylcolumbamine | Alkaloid |
| 62 | 19.38 | $C_{21}H_{22}NO_4^+$ | 352.1550 $[M]^+$ | 1.89 | 337.1322,322.1101,336.1254 | Dehydrocorybulbine | Alkaloid |
| 63* | 19.45 | $C_{48}H_{82}O_{19}$ | 1007.538 $[M + COOH]^-$ | −5.16 | 961.5223,799.4737,637.4235,475.3723 | 20-*O*-glucoginsenoside Rf | Ginsenoside |
| 64* | 19.58 | $C_{20}H_{16}NO_4^+$ | 334.1069 $[M]^+$ | 0.35 | 291.0887,261.0785,147.0680 | Worenine | Alkaloid |
| 65 | 20.73 | $C_{20}H_{22}NO_5^+$ | 356.1503 $[M]^+$ | 2.95 | 338.1383,322.1085,308.1241,192.0662, 164.0828,149.0594 | Pseudotetrahydropalmatine | Alkaloid |
| 66* | 20.73 | $C_9H_8O_2$ | 147.0447 $[M-H]^-$ | −3.08 | 119.0485,117.0334,103.0543 | Cinnamic acid | Organic acid |
| 67* | 20.78 | $C_{47}H_{80}O_{18}$ | 977.5267 $[M + COOH]^-$ | −6.11 | 931.5112,799.4755,637.4223,475.3682 | Notoginsenoside $R_1$ | Ginsenoside |
| 68* | 21.28 | $C_9H_{10}O_3$ | 165.0562 $[M-H]^-$ | 2.92 | 137.0213,92.0268 | Ethylparaben | Ester |

(*continued on next page*)

**Table 1** (*continued*)

| No. | $t_R$ (min) | Formula | Precursorions (*m*/*z*) | Diff (ppm) | Fragment ions (*m*/*z*) | Identification | Structural Types |
|---|---|---|---|---|---|---|---|
| 69 | 21.37 | $C_{22}H_{27}NO_5$ | 386.1955 $[M + H]^+$ | −1.81 | 368.1833,190.0847,178.0980 | Muramine | Alkaloid |
| 70* | 21.58 | $C_{20}H_{18}NO_4$ | 336.1237 $[M]^+$ | 1.98 | 321.1008,306.0783,320.0931 | Berberine | Alkaloid |
| 71* | 21.96 | $C_{21}H_{22}NO_4^+$ | 352.1551 $[M]^+$ | 2.17 | 337.1324,322.1101,308.1302,294.1139, 279.0938 | Palmatine | Alkaloid |
| 72 | 22.23 | $C_{22}H_{26}NO_4^+$ | 368.1836 $[M]^+$ | −5.35 | 352.1524,338.1259,192.0987 | Tetrahydroprotoberberine | Alkaloid |
| 73* | 22.90 | $C_{42}H_{72}O_{14}$ | 845.4884 $[M + COOH]^-$ | −2.38 | 799.4690,637.4205,475.3705,161.0433 | Ginsenoside $Rg_1$ | Ginsenoside |
| 74* | 23.09 | $C_{48}H_{82}O_{18}$ | 991.545 $[M + COOH]^-$ | −3.35 | 945.5269,783.4777,637.4213,475.3705 | Ginsenoside Re | Ginsenoside |
| 75* | 24.01 | $C_{22}H_{24}NO_4$ | 366.1705 $[M]^+$ | 1.41 | 351.1488,350.1417,336.1260 | Dehydrocorydaline | Alkaloid |
| 76* | 24.47 | $C_{21}H_{20}NO_4^+$ | 350.1379 $[M]^+$ | −2.24 | 334.1062,306.1124,320.0961 | 13-Methylberberine | Alkaloid |
| 77 | 25.01 | $C_{22}H_{24}NO_4^+$ | 366.17 $[M]^+$ | −0.91 | 336.1226,351.1454 | 13-Methoxyberberine | Alkaloid |
| 78 | 25.24 | $C_{19}H_{14}NO_4^+$ | 320.0922 $[M]^+$ | 1.45 | 292.0947,262.0843,234.0893 | Coptisin isomer | Alkaloid |
| 79 | 25.51 | $C_{20}H_{18}NO_5^+$ | 352.1197 $[M]^+$ | 4.97 | 336.0877,322.0695,306.0756,292.0591 | 13-Oxoberberine | Alkaloid |
| 80* | 28.91 | $C_{42}H_{72}O_{14}$ | 845.4891 $[M + COOH]^-$ | −1.55 | 799.4723,637.4220,475.3721,161.0441 | Ginsenoside Rf | Ginsenoside |
| 81* | 29.69 | $C_{59}H_{100}O_{27}$ | 1239.6357 $[M-H]^-$ | −1.79 | 1107.5968,1077.5859 | Notoginsenoside $R_4$ | Ginsenoside |
| 82* | 29.90 | $C_{41}H_{70}O_{13}$ | 815.4785 $[M + COOH]^-$ | −1.65 | 769.4610,637.4229,475.3727, 161.0449,391.2853 | Notoginsenoside $R_2$ | Ginsenoside |
| 83* | 30.84 | $C_{42}H_{72}O_{13}$ | 829.4945 $[M + COOH]^-$ | −1.20 | 783.4794,637.4245,475.3739, 391.2800,161.0439 | 20(*S*)-Ginsenoside $Rg_2$ | Ginsenoside |
| 84* | 30.93 | $C_{58}H_{98}O_{26}$ | 1245.6046 $[M + Cl]^-$ | 0.45 | 1209.6246,1077.5795 | Ginsenoside $Ra_2$ | Ginsenoside |
| 85* | 31.18 | $C_{59}H_{100}O_{27}$ | 1239.6358 $[M-H]^-$ | −1.17 | 1107.8589,864.3445,783.5100 | Ginsenoside $Ra_3$ | Ginsenoside |
| 86* | 31.19 | $C_{54}H_{92}O_{23}$ | 1107.5962 $[M-H]^-$ | 0.49 | 945.5333,783.4762,179.0540 | Ginsenoside $Rb_1$ | Ginsenoside |
| 87 | 31.66 | $C_{57}H_{94}O_{26}$ | 1193.594 $[M-H]^-$ | −1.72 | 1159.5908,1107.5793,1089.5701, 945.5294 | Malonylginsenoside $Rb_1$ | Ginsenoside |
| 88* | 31.92 | $C_{53}H_{90}O_{22}$ | 1123.5884 $[M + COOH]^-$ | −1.94 | 1077.5685,945.5308,784.4864 | Ginsenoside Rc | Ginsenoside |
| 89* | 31.92 | $C_{58}H_{98}O_{26}$ | 1245.6046 $[M + Cl]^-$ | 0.45 | 1209.6278,945.5503 | Ginsenoside $Ra_1$ | Ginsenoside |
| 90* | 32.14 | $C_{48}H_{76}O_{19}$ | 955.4849 $[M-H]^-$ | −6.18 | 793.4271,631.3664,523.3719,455.3456 | Ginsenoside Ro | Ginsenoside |
| 91 | 32.39 | $C_{61}H_{100}O_{29}$ | 1295.6251 $[M-H]^-$ | −2.05 | 1251.6282,1209.6131,1191.6035, 1059.5610 | Malonylginsenoside $Ra_1$/$Ra_2$ | Ginsenoside |
| 92 | 32.47 | $C_{56}H_{92}O_{25}$ | 1163.5837 $[M-H]^-$ | −1.54 | 1119.5849,1077.5763,1059.5647, 927.5217 | Malonylginsenoside $Rb_2$ | Ginsenoside |
| 93* | 32.77 | $C_{53}H_{90}O_{22}$ | 1123.5874 $[M + COOH]^-$ | −2.83 | 783.4804,945.5378,149.0458 | Ginsenoside $Rb_2$ | Ginsenoside |
| 94* | 33.09 | $C_{53}H_{90}O_{22}$ | 1113.5633 $[M + Cl]^-$ | 1.37 | 1077.4828,945.5401,783.4882, 621.4349 | Ginsenoside $Rb_3$ | Ginsenoside |
| 95 | 33.37 | $C_{56}H_{92}O_{25}$ | 1163.5826 $[M-H]^-$ | −2.56 | 1119.5877,1077.5761,1059.5664 | Malonylginsenoside Rc | Ginsenoside |
| 96* | 33.50 | $C_{47}H_{74}O_{18}$ | 925.4793 $[M-H]^-$ | −1.01 | 763.4258,569.3849 | Pseudoginsenoside $RT_1$ | Ginsenoside |
| 97 | 33.72 | $C_{56}H_{92}O_{25}$ | 1163.5808 $[M-H]^-$ | −4.03 | 1119.5819,1077.5723,1059.5612, 927.5204 | Malonylginsenoside $Rb_2$/Rc isomer | Ginsenoside |
| 98 | 33.90 | $C_{56}H_{94}O_{24}$ | 1185.5829 $[M + Cl]^-$ | 0.00 | 1149.6073,1107.5942,1089.5846 | Quinquenoside $R_1$ | Ginsenoside |
| 99 | 34.23 | $C_{21}H_{22}NO_5^+$ | 368.1492 $[M]^+$ | −4.21 | 353.1250,338.1017,336.1226 | Corynoline | Alkaloid |
| 100 | 34.84 | $C_{56}H_{92}O_{25}$ | 1163.5859 $[M-H]^-$ | 0.35 | 1119.5911,1077.5854,783.4934 | Malonylginsenoside $Rb_2$/Rc isomer | Ginsenoside |
| 101* | 34.94 | $C_{48}H_{82}O_{18}$ | 991.5454 $[M + COOH]^-$ | −3.35 | 945.5265,783.4762,621.4260,459.3774 | Ginsenoside Rd | Ginsenoside |
| 102 | 35.12 | $C_{55}H_{92}O_{23}$ | 1119.5956 $[M-H]^-$ | −0.06 | 1077.5746,1059.5686,937.1230 | Ginsenoside $RS_2$ | Ginsenoside |
| 103 | 35.17 | $C_{21}H_{19}NO_6$ | 382.1282 $[M + H]^+$ | −0.82 | 336.0869,308.0974,265.0691 | Pontevedrine | Alkaloid |

**Table 1** (*continued*)

| No. | $t_R$ (min) | Formula | Precursorions ($m/z$) | Diff (ppm) | Fragment ions ($m/z$) | Identification | Structural Types |
|---|---|---|---|---|---|---|---|
| 104 | 35.65 | $C_{51}H_{84}O_{21}$ | 1031.5382 $[M-H]^-$ | −4.88 | 987.5375,945.5286,927.5203, 783.4779,765.4668 | Malonyl Ginsenoside Rd | Ginsenoside |
| 105 | 35.86 | $C_{22}H_{24}NO_4^+$ | 366.1687 $[M]^+$ | −3.51 | 350.1374,336.1240,322.1413,308.1290 | Dehydrocorydaline isomer | Alkaloid |
| 106* | 37.22 | $C_{48}H_{82}O_{18}$ | 991.547 $[M + COOH]^-$ | −1.33 | 945.5397 | Gypenoside XVII | Ginsenoside |
| 107* | 37.95 | $C_{19}H_{13}NO_5$ | 336.0869 $[M + H]^+$ | 0.75 | 308.0913,293.0668,250.0864 | 8-Oxycoptisine | Alkaloid |
| 108* | 38.05 | $C_{12}H_{16}O_2$ | 193.1228 $[M + H]^+$ | 2.56 | 105.0706,137.0609,147.1169 | Senkyunolide A | Phthalide |
| 109 | 39.48 | $C_{22}H_{24}NO_4^+$ | 366.1695 $[M]^+$ | −1.32 | 350.1388,336.1237,322.1447,308.1251 | Dehydrocorydaline isomer | Alkaloid |
| 110* | 39.70 | $C_{12}H_{14}O_2$ | 191.1066 $[M + H]^+$ | −0.29 | 135.0455,145.1003 | Butylphthalide | Phthalide |
| 111* | 42.85 | $C_{12}H_{14}O_2$ | 191.1059 $[M + H]^+$ | −3.96 | 145.1010,173.0957,117.0695 | (*E*)-Ligustilide | Phthalide |
| 112 | 43.70 | $C_{36}H_{60}O_8$ | 665.4265 $[M + COOH]^-$ | −0.78 | 655.3976,569.2387,327.1338 | Ginsenoside $Rk_3/Rh_4$ | Ginsenoside |
| 113* | 45.28 | $C_{12}H_{18}O_2$ | 195.1382 $[M + H]^+$ | 1.25 | 177.1269,149.1309,125.0595 | Sedanolide | Phthalide |
| 114* | 45.71 | $C_{42}H_{66}O_{14}$ | 793.4335 $[M-H]^-$ | −5.65 | 613.3633,523.3703,455.3451 | Zingibroside $R_1$ | Ginsenoside |
| 115* | 45.95 | $C_{12}H_{14}O_2$ | 191.1073 $[M + H]^+$ | −1.34 | 145.1008,173.0960,112.9674,117.0694 | (*Z*)-Ligustilide | Phthalide |
| 116* | 47.90 | $C_{42}H_{72}O_{13}$ | 829.4918 $[M + COOH]^-$ | −4.45 | 783.4861,621.4345,113.0268, 459.3739,161.0423 | 20(*S*)-Ginsenoside $Rg_3$ | Ginsenoside |
| 117 | 48.02 | $C_{42}H_{66}O_{14}$ | 793.4377 $[M-H]^-$ | −0.35 | 613.3632,569.3760,455.3473 | Zingibroside $R_1$ isomer | Ginsenoside |
| 118* | 48.22 | $C_{42}H_{72}O_{13}$ | 819.4670 $[M + Cl]^-$ | 0.37 | 783.4903,621.4341,459.3756 | 20(*R*)-Ginsenoside $Rg_3$ | Ginsenoside |
| 119 | 50.05 | $C_{18}H_{18}O_3$ | 281.118 $[M-H]^-$ | 1.71 | 163.0350.145.0262,117.0316 | Isoeugenyl phenylacetate | Ester |
| 120* | 52.84 | $C_{21}H_{19}NO_4$ | 350.1385 $[M + H]^+$ | −0.53 | 335.1146,319.1191,334.1083 | Dihydrochelerythrine | Alkaloid |
| 121 | 52.94 | $C_{30}H_{47}O_4^-$ | 471.3487 $[M]^-$ | 1.52 | 393.3162,71.0506 | 2-Hydroxyoleanolate or isomer | Triterpene |
| 122 | 53.96 | $C_{24}H_{30}O_4$ | 383.2227 $[M + H]^+$ | 2.65 | 191.1063,149.0599 | Senkyunolide P or isomer | Phthalide |
| 123* | 54.68 | $C_{20}H_{15}NO_4$ | 334.1097 $[M + H]^+$ | 0.65 | 319.0841,304.0967,279.1013 | Dihydrosanguinarine | Alkaloid |
| 124 | 56.19 | $C_{24}H_{30}O_4$ | 383.2211 $[M + H]^+$ | −1.53 | 191.1063,149.0597 | Senkyunolide P or isomer | Phthalide |
| 125 | 56.57 | $C_{30}H_{47}O_4^-$ | 471.3480 $[M]^-$ | 0.03 | 359.2921,162.8327 | 2-Hydroxyoleanolate or isomer | Triterpene |
| 126* | 57.08 | $C_{24}H_{28}O_4$ | 381.2059 $[M + H]^+$ | −0.36 | 191.1075,173.0972,279.1508 | Tokinolide B | Phthalide |
| 127* | 58.38 | $C_{24}H_{28}O_4$ | 381.2070 $[M + H]^+$ | 2.53 | 191.107,267.1386,141.1136 | Riligustilide | Phthalide |
| 128* | 58.55 | $C_{24}H_{28}O_4$ | 381.2069 $[M + H]^+$ | 2.27 | 191.107,141.1136 | Angelicide | Phthalide |
| 129 | 60.63 | $C_{30}H_{46}O_4$ | 469.3316 $[M-H]^-$ | −1.56 | 305.1903,164.8363 | Glycyrrhetic acid or isomer | Triterpene |
| 130* | 61.18 | $C_{30}H_{48}O_3$ | 455.3528 $[M-H]^-$ | −0.59 | 410.3532 | Oleanolic acid | Triterpene |
| 131 | 61.28 | $C_{30}H_{46}O_4$ | 469.3314 $[M-H]^-$ | −1.99 | 423.3197,211.1525 | Glycyrrhetic acid or isomer | Triterpene |
| 132* | 63.34 | $C_{18}H_{32}O_2$ | 279.2327 $[M-H]^-$ | −0.91 | 261.2193,59.0146 | Linoleic acid | Organic acid |
| 133 | 63.59 | $C_{30}H_{46}O_3$ | 453.3376 $[M-H]^-$ | 0.40 | 407.3308,325.2544,100.9336 | Oleanonic acid or isomer | Triterpene |
| 134 | 64.04 | $C_{30}H_{46}O_3$ | 453.3376 $[M-H]^-$ | 0.40 | 407.3316,97.0653 | Oleanonic acid or isomer | Triterpene |
| 135 | 64.08 | $C_{30}H_{46}O_3$ | 453.3374 $[M-H]^-$ | −0.04 | 407.3316,97.0661 | Oleanonic acid or isomer | Triterpene |
| 136 | 64.31 | $C_{30}H_{46}O_3$ | 453.3380 $[M-H]^-$ | 1.28 | 407.3244,97.0693 | Oleanonic acid or isomer | Triterpene |

(*continued on next page*)

**Table 1** (*continued*)

| No. | $t_R$ (min) | Formula | Precursorions (*m*/*z*) | Diff (ppm) | Fragment ions (*m*/*z*) | Identification | Structural Types |
|---|---|---|---|---|---|---|---|
| 137 | 64.58 | $C_{30}H_{46}O_4$ | 469.3319 $[M-H]^-$ | −0.92 | 336.1460,141.8654 | Glycyrrhetic acid or isomer | Triterpene |
| 138* | 64.76 | $C_{16}H_{32}O_2$ | 255.2329 $[M-H]^-$ | −0.21 | 237.2247,116.9283 | Palmitic acid | Organic acid |
| 139* | 65.26 | $C_{18}H_{34}O_2$ | 281.2490 $[M-H]^-$ | 1.41 | 116.9279 | Oleic acid | Organic acid |
| 140* | 67.38 | $C_{18}H_{36}O_2$ | 283.2622 $[M-H]^-$ | −7.25 | 265.2464,211.6753,141.7728 | Octadecanoic acid | Organic acid |

* Compared with a reference standard.

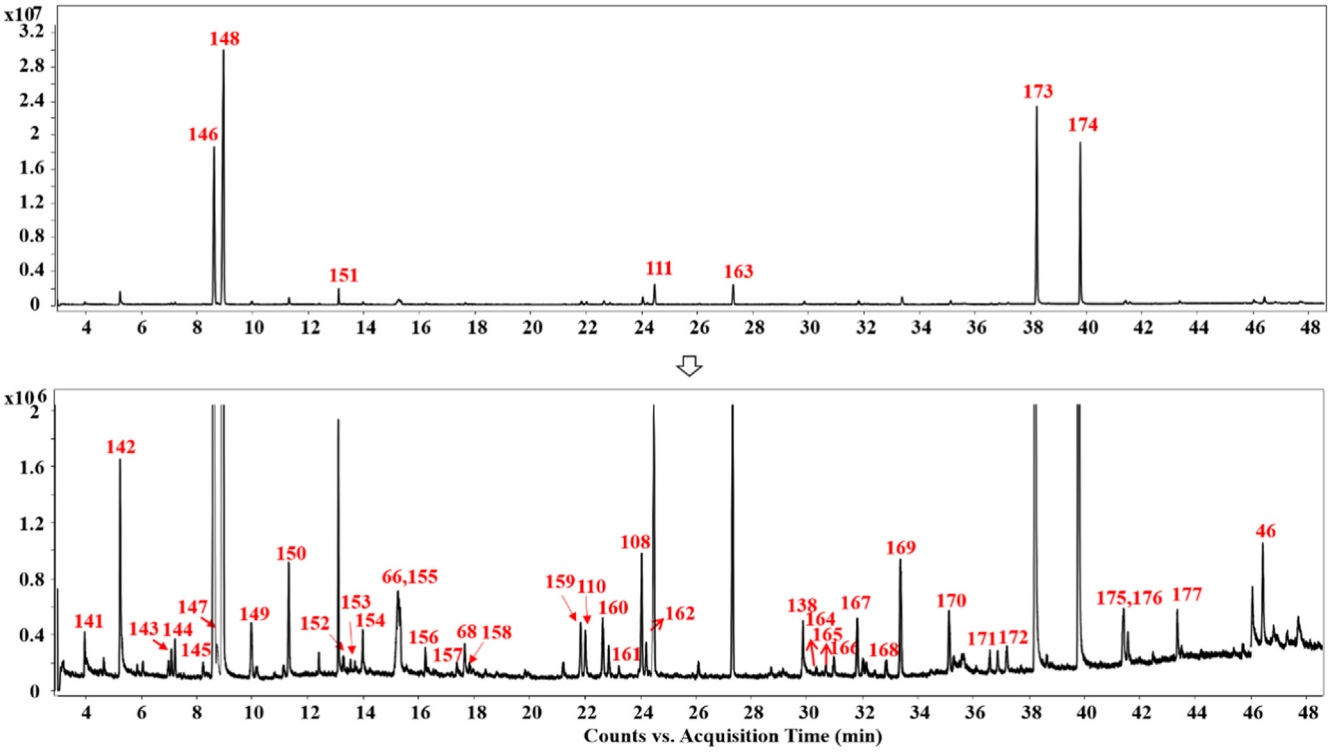


**Fig. 3** Total ion current chromatograms of SXXTN by GC–MS.

isoboldine (**13**), showing a loss of 31 Da (–$OCH_3$). Due to the loss of $NH_2CH_3$ (Fig. S2D), a crucial characteristic ion at *m*/*z* 325.1445 was obtained in glaucine (**46**) (Fig. S1D).

### *3.1.2. Identification of ginsenosides in SXXTN*

A total of 34 ginsenosides in SXXTN were displayed in Table 1, mostly from Ginseng Radix et Rhizoma and demonstrated quasi-molecular ions $[M-H]^-$ or $[M + COOH]^-$ in negative ion mode due to formic acid in the mobile phase. Based on their aglycone, ginsenosides can be classified into three main categories: 20(*S*)-protopanaxadiol (PPD), 20(*S*)-protopanaxatriol (PPT) and oleanane type (OA) saponin.

19 PPD-type ginsenosides (**81, 84, 85, 86, 87, 88, 89, 91, 92, 93, 94, 95, 98, 101, 102, 104, 106, 116, 118**) were characterized and prone to produce [20(*S*)-protopanaxadiol-$H]^-$ ($C_{30}H_{51}O_3$) characteristic aglycone fragment ions at *m*/*z* 459.38 (Yang et al., 2021). For example, in MS/MS spectrometry (Fig. S1E), Compound **101** gave abundant ion at *m*/*z* 783.4762 ($[M-H-Glc]^-$), *m*/*z* 621.4260 ($[M-H-2Glc]^-$) and *m*/*z* 459.3774 ([20(*S*)-protopanaxadiol-$H]^-$, $C_{30}H_{51}O_3$), resulting from sequential eliminations of sugar residues (Fig. S2E). A total of 7 PPT-type ginsenosides (**63, 67, 73, 74, 80, 82, 83**) were tentatively identified and clearly marked with characteristic ions at *m*/*z* 475.37 ([20(*S*)-protopanaxatriol-$H]^-$, $C_{30}H_{51}O_4$) (Yang et al., 2021). As presented in Fig. S1F and Fig. S2F, ginsenoside $Rg_1$ (**73**) produced abundant characteristic ions including *m*/*z* 673.4209 ($[M-H-Glc]^-$) and *m*/*z* 475.3713 ([(20(*S*)-protopanaxatriol-$H]^-$). Three OA-type ginsenosides (**90, 96, 114**) were unambiguously elucidated as ginsenoside Ro, pseudoginsenoside $RT_1$ and zingibroside $R_1$ by comparison with reference standards with characteristic ions at *m*/*z* 455.35 ([Oleanolicacid-

**Table 2** Characterization of chemical constituents of SXXTN by GC–MS.

| No. | Rt (min) | Identification | Match | Formula | Structural Types |
|---|---|---|---|---|---|
| 141 | 4.03 | Glycerin | 91.1 | $C_3H_8O_3$ | Alcohol |
| 142 | 5.26 | Allylbenzene | 82.7 | $C_9H_{10}$ | Hydrocarbon |
| 143 | 7.06 | Bicyclo [2,2,1] heptan-2-*ol*.1,5,5-trimethyl | 85.2 | $C_{10}H_{18}O$ | Monoterpenoid |
| 144* | 7.19 | Fenchol | 93.6 | $C_{10}H_{18}O$ | Monoterpenoid |
| 145* | 8.19 | Camphor | 90.3 | $C_{10}H_{16}O$ | Monoterpenoid |
| 146* | 8.58 | Isoborneol | 98.2 | $C_{10}H_{18}O$ | Monoterpenoid |
| 147* | 8.71 | Phenol, 4-ethyl- | 90.7 | $C_8H_{10}O$ | Phenol |
| 148* | 8.92 | Borneol | 97.4 | $C_{10}H_{18}O$ | Monoterpenoid |
| 149 | 9.95 | Bicyclo [2.2.1] heptan-2-*ol*, 1,7,7-trimethyl-, (1*S*-*endo*)- | 88.3 | $C_{10}H_{18}O$ | Monoterpenoid |
| 150* | 11.31 | 3-Phenylpropanol | 96.5 | $C_9H_{12}O$ | Alcohol |
| 151* | 13.11 | Cinnamyl alcohol | 98.5 | $C_9H_{10}O$ | Alcohol |
| 152* | 13.28 | 2-Methoxy-4-vinylphenol | 91.4 | $C_9H_{10}O_2$ | Phenol |
| 153* | 13.57 | Hydrocinnamic acid | 96.1 | $C_9H_{10}O_2$ | Organic acid |
| 154 | 13.96 | 1,4-Cyclohexadiene-1,2-dicarboxylic anhydride | 86.5 | $C_8H_6O_3$ | Anhydride |
| 66* | 15.27 | Cinnamic acid | 72.1 | $C_9H_8O_2$ | Organic acid |
| 155* | 15.34 | Caryophyllene | 88.7 | $C_{15}H_{24}$ | Sesquiterpene |
| 156* | 16.25 | Ethyl cinnamate | 77.7 | $C_{11}H_{12}O_2$ | Organic acid ester |
| 157* | 17.37 | 2,4-Di-*tert*-butylphenol | 84.6 | $C_{14}H_{22}O$ | Phenol |
| 68* | 17.66 | Ethylparaben | 95.1 | $C_9H_{10}O_3$ | Organic acid ester |
| 158 | 17.84 | Δ-Cadinene | 80.7 | $C_{15}H_{24}$ | Sesquiterpene |
| 159* | 21.84 | Cadinol | 91.7 | $C_{15}H_{26}O$ | Sesquiterpene |
| 110* | 22.02 | Butylphthalide | 93.1 | $C_{12}H_{14}O_2$ | Phthalide |
| 160* | 22.66 | *Z*-Butylidenephthalide | 92.4 | $C_{12}H_{12}O_2$ | Phthalide |
| 161 | 23.23 | 5-Pentylcyclohexa-1,3-diene | 87.4 | $C_{11}H_{18}$ | Hydrocarbon |
| 108* | 24.06 | Senkyunolide A | 89.2 | $C_{12}H_{16}O_2$ | Phthalide |
| 162* | 24.22 | Neocnidilide | 92.0 | $C_{12}H_{18}O_2$ | Phthalide |
| 111* | 24.46 | (*E*)-Ligustilide | 94.9 | $C_{12}H_{14}O_2$ | Phthalide |
| 163* | 27.32 | Muscone | 95.7 | $C_{16}H_{30}O$ | Cyclic ketone |
| 138 | 29.92 | Palmitic acid | 82.1 | $C_{16}H_{32}O_2$ | Organic acid |
| 164 | 30.36 | 3-Phenylpropyl benzoate | 92.6 | $C_{16}H_{22}O_4$ | Organic acid ester |
| 165 | 30.69 | Hexadecanoic acid, ethyl ester | 88.4 | $C_{18}H_{36}O_2$ | Organic acid ester |
| 166 | 31.00 | Senkyunolide H | 84.1 | $C_{12}H_{16}O_4$ | Phthalide |
| 167 | 31.83 | 3-benzyl-1,2-dihydronaphthalene | 70.3 | $C_{17}H_{17}$ | Hydrocarbon |
| 168 | 32.86 | (*Z*)-Cinnamyl benzoate | 96.0 | $C_{16}H_{14}O_2$ | Organic acid ester |
| 169* | 33.39 | Benzyl cinnamate | 95.7 | $C_{16}H_{14}O_2$ | Organic acid ester |
| 170 | 35.15 | Benzenepropanoic acid, 3-phenylpropyl ester | 94.3 | $C_{18}H_{20}O_2$ | Organic acid ester |
| 171 | 36.62 | Borny cinnamate | 91.5 | $C_{19}H_{24}O_2$ | Organic acid ester |
| 172 | 36.89 | Benzenepropanoic acid, 3-phenyl-2-propenyl ester | 94.8 | $C_{18}H_{18}O_2$ | Organic acid ester |
| 173* | 38.26 | 3-Phenylpropyl cinnamate, (*E*)- | 97.4 | $C_{18}H_{18}O_2$ | Organic acid ester |
| 174* | 39.86 | Cinnamyl cinnamate | 97.4 | $C_{18}H_{16}O_2$ | Organic acid ester |
| 175* | 41.40 | Prasterone | 76.3 | $C_{19}H_{28}O_2$ | Steroid |
| 176* | 41.43 | Androsterone | 73.3 | $C_{19}H_{30}O_2$ | Steroid |
| 177 | 41.58 | Phthalic acid, di(2-propylpentyl) ester | 87.0 | $C_{24}H_{38}O_4$ | Organic acid ester |
| 46 | 46.06 | Glaucine | 89.7 | $C_{21}H_{25}NO_4$ | Alkaloid |

* Compared with a reference standard.

$H]^-$, $C_{30}H_{47}O_3$) (Yang et al., 2021) as shown in MS/MS spectrum (Fig. S1G) of ginsenoside Ro (**90**). The fragmentation pathways were shown in Fig. S2G.

Aglycones can be identified by discovering diagnostic ions and neutral loss can be observed to determine the number and type of glycosidic bond cleavage of ginsenosides. As shown in Fig. S1H, ginsenoside $Rb_2$ (93) appeared *m*/*z* 945.5378 ($[M-H-Ara]^-$) and *m*/*z* 783.4803 ($[M-H-Ara-Glc]^-$) in MS/MS spectrum.

### 3.1.3. Identification of organic acids in SXXTN

In total, 21 organic acids (**2, 3, 4, 7, 8, 10, 11, 12, 14, 15, 19, 26, 40, 41, 54, 57, 66, 132, 138, 139, 140**) were identified from SXXTN and shown in Table 1. Organic acids were easy to generate fragment ions in MS/MS with losing CO, $CO_2$, –COOH, $H_2O$, etc. (Yan, Wang, 2014). For example, fragment ions *m*/*z* 178.0273 $[M-H-CH_3]^-$, *m*/*z* 149.0561 $[M-H-CO_2]^-$, *m*/*z* 134.0371 $[M-H-CH_3-CO_2]^-$ and *m*/*z* 160.8423 $[M-H-CH_3-H_2O]^-$ were observed in secondary mass spectrometry of ferulic acid (**26**) (Fig. S1I) following specific cleavage pathways (Fig. S2H). In the MS/MS spectrometry of cinnamic acid (**66**) (Fig. S1J), the fragment ions with the highest abundance were observed to be *m*/*z* 103.0548 $[M-H-CO_2]^-$, which conformed to the cleavage characteristics of organic acids.

### 3.1.4. Identification of phthalides in SXXTN

Totally, 12 phthalides were tentatively identified or unambiguously authenticated, including 9 monomeric phthalides (**45, 56,**

**Table 3** Contents of 57 analytes determined in SXXTN samples by HPLC-QQQ MS and GC-QQQ MS (μg/g, Mean ± SD, n = 3).

| No. | Components | B1 | B2 | B3 | B4 | B5 | B6 | B7 |
|---|---|---|---|---|---|---|---|---|
| *non-volatile components* | | | | | | | | |
| 5 | Phenylalanine | 86.83 ± 2.32 | 86.5 ± 3.25 | 64.25 ± 6.71 | 61.17 ± 5.4 | 29.33 ± 2.88 | 20.92 ± 1.01 | 85.92 ± 7.67 |
| 6 | Tetramethylpyrazine | 1.36 ± 0.05 | 1.47 ± 0.13 | 2.28 ± 0.05 | 1.5 ± 0.08 | 2.64 ± 0.05 | 3.14 ± 0.05 | 3.19 ± 0.05 |
| 21 | Scoulerine | 65.31 ± 6.33 | 71.92 ± 2.5 | 82.53 ± 2.59 | 74.67 ± 1.67 | 103.25 ± 5.29 | 107.08 ± 6.98 | 107.58 ± 2.62 |
| 23 | Isocorydine | 17.97 ± 0.65 | 19.44 ± 0.46 | 20.53 ± 0.67 | 19.72 ± 0.61 | 28.89 ± 0.68 | 28.97 ± 0.47 | 29.72 ± 0.57 |
| 28 | Corydalmine | 47.47 ± 1.46 | 50.17 ± 1.52 | 56.75 ± 1.18 | 51.81 ± 0.77 | 51.25 ± 4.1 | 51.67 ± 2.82 | 51.53 ± 1.5 |
| 29 | Tetrahydrocolumbamie | 631.94 ± 12.95 | 621.94 ± 38.75 | 710.56 ± 17 | 602.22 ± 9.18 | 988.89 ± 26.79 | 1043.61 ± 2.55 | 1040.56 ± 44.92 |
| 33 | Corypalmine | 68.64 ± 0.6 | 73.22 ± 2.53 | 78.36 ± 1.64 | 75.42 ± 2.3 | 127.64 ± 1.77 | 137.22 ± 5.23 | 133.61 ± 3.72 |
| 36 | Protopine | 849.11 ± 12.48 | 869.92 ± 34.43 | 935.56 ± 35.06 | 860.64 ± 15.65 | 1062.14 ± 19.22 | 1125.22 ± 42.62 | 1125.03 ± 27.57 |
| 37 | Demethyleneberberine | 8.14 ± 0.05 | 8.5 ± 0.36 | 9.22 ± 0.21 | 8.58 ± 0.17 | 7.81 ± 0.13 | 8.25 ± 0.17 | 8.11 ± 0.34 |
| 43 | Norglaucine | 10063.33 ± 154.52 | 9121.39 ± 263.53 | 9767.78 ± 337.78 | 8266.94 ± 90.49 | 7842.22 ± 509.16 | 7953.89 ± 104.36 | 8121.94 ± 194.84 |
| 44 | Allocryptopine | 544.53 ± 27.26 | 534.5 ± 14.24 | 575.03 ± 20.11 | 530.03 ± 13.11 | 631.28 ± 27.8 | 672.31 ± 28.3 | 684.06 ± 22.77 |
| 46 | Glaucine | 910 ± 5.46 | 955.56 ± 31.9 | 1068.33 ± 48.18 | 929.44 ± 9.66 | 1009.17 ± 88.93 | 1036.11 ± 14.82 | 1061.11 ± 29.27 |
| 48 | Tetrahydropalmatine | 432.22 ± 14.2 | 422.78 ± 18.95 | 486.94 ± 22.12 | 418.89 ± 8.67 | 646.39 ± 57.4 | 674.17 ± 11.67 | 683.61 ± 12.48 |
| 52 | Coptisin | 513.14 ± 18.5 | 533.58 ± 19.6 | 631.47 ± 17.92 | 528.25 ± 20.18 | 683.81 ± 46.08 | 770.03 ± 47.76 | 768.31 ± 12.25 |
| 53 | Columbamine | 414.25 ± 8.33 | 426.61 ± 12.44 | 466.33 ± 14.99 | 422.61 ± 18.19 | 385.47 ± 25.7 | 417.39 ± 23.39 | 418.83 ± 8.24 |
| 55 | Jatrorrhizine | 24.75 ± 2.32 | 24.64 ± 3.56 | 28.42 ± 1.98 | 26.58 ± 1.69 | 28.64 ± 2.79 | 31.25 ± 2.35 | 32.92 ± 1.08 |
| 58 | Canadine | 103.61 ± 3.37 | 103.33 ± 3.63 | 120.28 ± 2.41 | 104.72 ± 0.48 | 171.39 ± 12.14 | 183.89 ± 2.55 | 183.89 ± 2.55 |
| 60 | Corydaline | 445.28 ± 11.71 | 455.83 ± 15.83 | 537.22 ± 17.02 | 443.89 ± 5.55 | 839.17 ± 50.26 | 885 ± 23.11 | 897.78 ± 7.74 |
| 66 | Cinnamic acid | 482.5 ± 15.52 | 529.5 ± 20.11 | 505 ± 31.83 | 515.33 ± 16.33 | 477.33 ± 14.1 | 515.25 ± 17.37 | 308.08 ± 18.32 |
| 67 | Notoginsenoside $R_1$ | 50.25 ± 0.25 | 53.58 ± 1.28 | 50.83 ± 4.94 | 49.25 ± 2.7 | 24.75 ± 2.14 | 26.25 ± 1.39 | 23.08 ± 1.01 |
| 70 | Berberine | 171.39 ± 4.86 | 176.39 ± 2.51 | 190.11 ± 4.03 | 170.92 ± 0.75 | 197.5 ± 4.01 | 203.06 ± 4.76 | 201.83 ± 2.35 |
| 71 | Palmatine | 588.44 ± 12.35 | 603.11 ± 6.88 | 638.22 ± 13.26 | 581.14 ± 4.43 | 540.64 ± 9.63 | 560.03 ± 13.97 | 555.5 ± 2.28 |
| 73 | Ginsenoside $Rg_1$ | 803.67 ± 14.68 | 831.42 ± 17.47 | 777.58 ± 34.06 | 731.5 ± 37.15 | 929.33 ± 27.19 | 915.58 ± 18.06 | 867.67 ± 54.56 |
| 74 | Ginsenoside Re | 565.17 ± 19.12 | 579.67 ± 36.71 | 547.42 ± 43.84 | 521.58 ± 32.77 | 559.42 ± 23.51 | 562.67 ± 11.2 | 826.83 ± 81.78 |
| 75 | Dehydrocorydaline | 1666.11 ± 18.15 | 1677.22 ± 49.14 | 1840.28 ± 40.79 | 1677.22 ± 25.69 | 1548.06 ± 73.42 | 1607.5 ± 28.83 | 1554.44 ± 17.8 |
| 76 | 13-Methylberberine | 11.92 ± 0.17 | 11.92 ± 0.17 | 12.89 ± 0.24 | 11.81 ± 0.05 | 8.58 ± 0.14 | 8.86 ± 0.1 | 9 ± 0.08 |
| 80 | Ginsenoside Rf | 102 ± 3.36 | 111 ± 7.15 | 102.83 ± 10.26 | 90.5 ± 6.43 | 135.25 ± 3.5 | 135.83 ± 7.52 | 108.08 ± 6.79 |
| 83 | Ginsenoside $Rg_2$ | 92.83 ± 2.74 | 98.42 ± 5.84 | 96.58 ± 7.38 | 90 ± 2.61 | 77.25 ± 1.32 | 79 ± 2.84 | 60.92 ± 3.17 |
| 84 | Ginsenoside $Ra_2$ | 71.83 ± 1.91 | 74.25 ± 2.82 | 76.33 ± 3.4 | 72 ± 2.14 | 83.67 ± 0.88 | 84.67 ± 1.91 | 80.17 ± 1.89 |
| 85 | Ginsenoside $Ra_3$ | 211 ± 0.43 | 199.25 ± 16.69 | 190.67 ± 3.39 | 188.33 ± 9.7 | 203.92 ± 3.69 | 214.67 ± 6.57 | 290.83 ± 27.19 |
| 86 | Ginsenoside $Rb_1$ | 1025.25 ± 6.51 | 1045.08 ± 54.55 | 1030.92 ± 80.79 | 960.92 ± 29.16 | 708.08 ± 22.64 | 716.33 ± 15.95 | 571.33 ± 22.92 |
| 88 | Ginsenoside Rc | 281.42 ± 4.23 | 294.17 ± 8.08 | 288.5 ± 13.56 | 267.58 ± 8.38 | 227.75 ± 0.9 | 226.5 ± 0.66 | 221.58 ± 10.04 |
| 89 | Ginsenoside $Ra_1$ | 155.58 ± 3.83 | 167.67 ± 9.87 | 169.25 ± 10.4 | 148.33 ± 3.22 | 184.5 ± 9.79 | 187.33 ± 7.04 | 180.58 ± 5.11 |
| 93 | Ginsenoside $Rb_2$ | 379.33 ± 7.69 | 398.17 ± 20.89 | 394.67 ± 31.29 | 357.75 ± 12.89 | 282.5 ± 12.67 | 281.42 ± 5.58 | 225.75 ± 5.91 |
| 94 | Ginsenoside $Rb_3$ | 37.75 ± 1 | 39.17 ± 2.01 | 41.75 ± 3.12 | 34.33 ± 2.7 | 24.5 ± 2.41 | 24.42 ± 0.95 | 227.75 ± 6.71 |
| 101 | Ginsenoside Rd | 248.33 ± 10.47 | 261.92 ± 8.61 | 261.25 ± 20.12 | 236.75 ± 9.85 | 178.5 ± 7.7 | 180.17 ± 4.69 | 142.25 ± 6.29 |
| 107 | 8-Oxycoptisine | 27.08 ± 3.18 | 25.36 ± 0.67 | 27.75 ± 0.38 | 25.06 ± 0.38 | 27.81 ± 0.42 | 28.86 ± 0.27 | 28 ± 0.38 |
| 116 | 20(*S*)-Ginsenoside $Rg_3$ | 57.67 ± 2.36 | 65.42 ± 3.15 | 64.58 ± 1.42 | 60.08 ± 2.47 | 15.83 ± 0.14 | 15.33 ± 0.38 | 14.5 ± 0.5 |
| 120 | Dihydrochelerythrine | 36.69 ± 4.46 | 34.36 ± 0.87 | 36.33 ± 0.22 | 35.44 ± 0.59 | 49.53 ± 0.63 | 49.08 ± 0.33 | 49.64 ± 0.79 |
| 123 | Dihydrosanguinarine | 31.5 ± 2.58 | 32 ± 0.58 | 34.17 ± 0.46 | 32.89 ± 0.42 | 45.86 ± 0.21 | 46.67 ± 0.38 | 45.5 ± 0.87 |

**Table 3** (*continued*)

| No. | Components | B1 | B2 | B3 | B4 | B5 | B6 | B7 |
|---|---|---|---|---|---|---|---|---|
| *volatile components* | | | | | | | | |
| 144 | Fenchol | 31.79 ± 0.72 | 24.14 ± 0.74 | 31.36 ± 0.23 | 28.89 ± 1.00 | 132.64 ± 1.08 | 233.88 ± 1.23 | 212.26 ± 0.61 |
| 145 | Camphor | 122.36 ± 0.58 | 99.94 ± 1.17 | 130.70 ± 0.35 | 115.32 ± 0.86 | 40.82 ± 0.14 | 78.08 ± 1.08 | 67.93 ± 0.08 |
| 146 | Isoborneol | 10746.58 ± 56.15 | 8297.41 ± 30.66 | 10784.14 ± 10.58 | 11041.49 ± 41.12 | 6992.38 ± 22.46 | 10192.18 ± 55.59 | 10537.26 ± 85.96 |
| 147 | 4-Ethylphenol | 111.73 ± 2.12 | 101.78 ± 2.15 | 110.57 ± 0.64 | 107.77 ± 1.56 | 92.31 ± 5.60 | 122.53 ± 3.56 | 120.49 ± 0.66 |
| 148 | Borneol | 16154.89 ± 17.28 | 12716.32 ± 71.79 | 16158.12 ± 26.98 | 16557.61 ± 73.28 | 11800.29 ± 15.21 | 15303.33 ± 71.80 | 16110.40 ± 39.41 |
| 150 | 3-Phenylpropanol | 287.24 ± 5.17 | 269.94 ± 5.95 | 295.45 ± 2.43 | 282.39 ± 4.51 | 323.49 ± 4.19 | 342.09 ± 10.25 | 332.53 ± 1.81 |
| 151 | Cinnamyl alcohol | 677.68 ± 11.97 | 659.66 ± 7.87 | 687.77 ± 9.42 | 676.31 ± 1.38 | 775.47 ± 15.66 | 782.07 ± 17.59 | 775.85 ± 9.96 |
| 155 | Caryophyllene | 99.41 ± 0.63 | 88.88 ± 0.23 | 101.31 ± 0.57 | 96.25 ± 1.05 | 68.95 ± 0.09 | 108.27 ± 0.63 | 102.55 ± 0.62 |
| 110 | Butylphthalide | 120.17 ± 0.72 | 119.28 ± 0.8 | 120.54 ± 0.52 | 118.96 ± 0.03 | 175.46 ± 0.95 | 166.65 ± 0.62 | 166.47 ± 0.78 |
| 160 | *Z*-Buthlidenephthalide | 470.46 ± 8.24 | 461.27 ± 6.60 | 469.83 ± 4.71 | 462.35 ± 5.09 | 918.91 ± 4.81 | 838.41 ± 5.49 | 834.35 ± 3.20 |
| 108 | Senkyunolide A | 445.75 ± 1.01 | 445.75 ± 5.70 | 453.88 ± 4.66 | 436.02 ± 2.77 | 1001.42 ± 2.58 | 963.05 ± 5.76 | 953.08 ± 6.39 |
| 162 | Neocnidilide | 114.94 ± 0.39 | 111.39 ± 1.46 | 112.35 ± 0.51 | 111.00 ± 0.5 | 209.89 ± 0.45 | 195.90 ± 0.17 | 196.40 ± 1.01 |
| 111 | (*E*)-Ligustilide | 708.93 ± 4.13 | 683.43 ± 5.57 | 698.54 ± 2.74 | 689.34 ± 2.44 | 1470.36 ± 15.50 | 1400.27 ± 10.26 | 1388.91 ± 5.15 |
| 163 | Muscone | 1185.77 ± 14.67 | 1092.55 ± 4.44 | 1174.19 ± 3.1 | 1145.85 ± 16.89 | 1258.91 ± 21.03 | 1206.96 ± 16.67 | 1173.77 ± 4.76 |
| 169 | Benzyl cinnamate | 663.55 ± 3.06 | 634.83 ± 5.35 | 664.60 ± 2.63 | 652.38 ± 1.63 | 694.71 ± 2.69 | 689.84 ± 4.29 | 665.64 ± 1.52 |
| 173 | 3-Phenylpropyl Cinnamate | 3532.42 ± 24.74 | 3194.93 ± 115.13 | 3503.38 ± 37.00 | 3493.68 ± 142.94 | 3855.59 ± 47.58 | 3730.66 ± 119.27 | 3828.99 ± 29.46 |
| 174 | Cinnamyl cinnamate | 4781.76 ± 48.54 | 4442.54 ± 117.11 | 4790.28 ± 38.97 | 4753.69 ± 164.10 | 5193.83 ± 65.96 | 4995.83 ± 171.5 | 5131.81 ± 42.73 |

**108, 110, 111, 113, 122, 124**) and 3 phthalide dimers (**126, 127, 128**). Monomeric phthalide compounds with a phthalide structure unit as the core, are prone to neutral loss of $H_2O$, CO, $CO_2$ and alkyl radicals or alkyl chains ($CH_3$, $C_2H_4$, $C_3H_6$, $C_4H_8$, etc.) (Yan et al, 2022) as shown in Fig. S2I. In the MS/MS spectrometry of senkyunolide A (1 0 8), the product ion *m*/*z* 175.1123 was produced by the precursor ion loss of $H_2O$. On this basis, the characteristic fragment *m*/*z* 147.1167 was produced by the successive loss of CO and *m*/*z* 137.0595 was the production of alkyl radical $C_4H_8$ lost by precursor ions (Fig. S1K). The phthalide dimer compounds are formed by the polymerization of two phthalide monomers. They are induced to dissociate into monomeric phthalide in MS/MS, and the highest intensity ions at *m*/*z* 191.11 are often produced (Zhang et al., 2018). Take Angelicide (**128**) as example, the fragment ion with highest abundance was observed at *m*/*z* 191.1066 in MS/MS spectrometry (Fig. S1L). On this basis, the cleavages of phthalide skeletons could also be observed in the MS/MS spectra of phthalide dimers, which were similar to monomeric phthalides.

### *3.2. GC–MS qualitative analysis of SXXTN*

In preceding reports, the volatile components in SXXTN such as Artificial Musk, Chuanxiong Rhizoma, Styrax and Borneolum Syntheticum have been revealed (Ding et al., 2022; He et al., 2018; Gurbuz et al., 2013; Sun et al., 2014), whereas little attention was paid to the volatile components in the intact SXXTN prescription. In this work, we supplemented the information of volatile chemicals and improved the global characterizations of complicated ingredients in SXXTN.

The GC–MS conditions of the temperature program, splitting ratio (10:1, 30:1 and 50:1) and the injector temperature (250℃, 280℃ and 300℃) were optimized in the direction of analyzing comprehensive volatile constituents of SXXTN with well separation performance in a short analysis. The total peak area was calculated as a criterion for optimization. The final conditions were described in *Section 2.4*.

The TICs of SXXTN by GC–MS can be viewed in Fig. 3. There were 44 volatile compounds tentatively identified from SXXTN, based on the mass spectrometric data of reference standards, the mass spectral library (NIST17) and the literature, including 12 organic acid esters, 6 monoterpenes, 6 phthalides, 3 organic acids, 3 sesquiterpenes, 3 alcohols, 3 alkanes, 3 hydrocarbons, 2 steroids, 1 macrocyclic ketone, 1 alkaloid and 1 anhydride (Table 2). The chemical structures were illustrated in Fig. 2. Among them, seven compounds (**66, 68, 110, 108, 111, 138, 46**) have been identified in the previous LC-MS analysis. According to the comparison with authentic standards, 25 components were clearly marked. Isoborneol (**146**), borneol (**148**), cinnamyl alcohol (**151**), (E)-ligustilide (**111**), muscone (**163**), 3-phenylpropyl cinnamate (**173**) and cinnamyl cinnamate (**174**) exhibited relatively high abundances during GC–MS analysis of SXXTN.

### *3.3. Quantification of 40 non-volatile compounds in SXXTN by HPLC-QQQ MS*

Forty confirmed non-volatile chemicals were further quantified by the optimized HPLC-QQQ MS method (Table S3-S4) to evaluate the quality of SXXTN. According to the difference

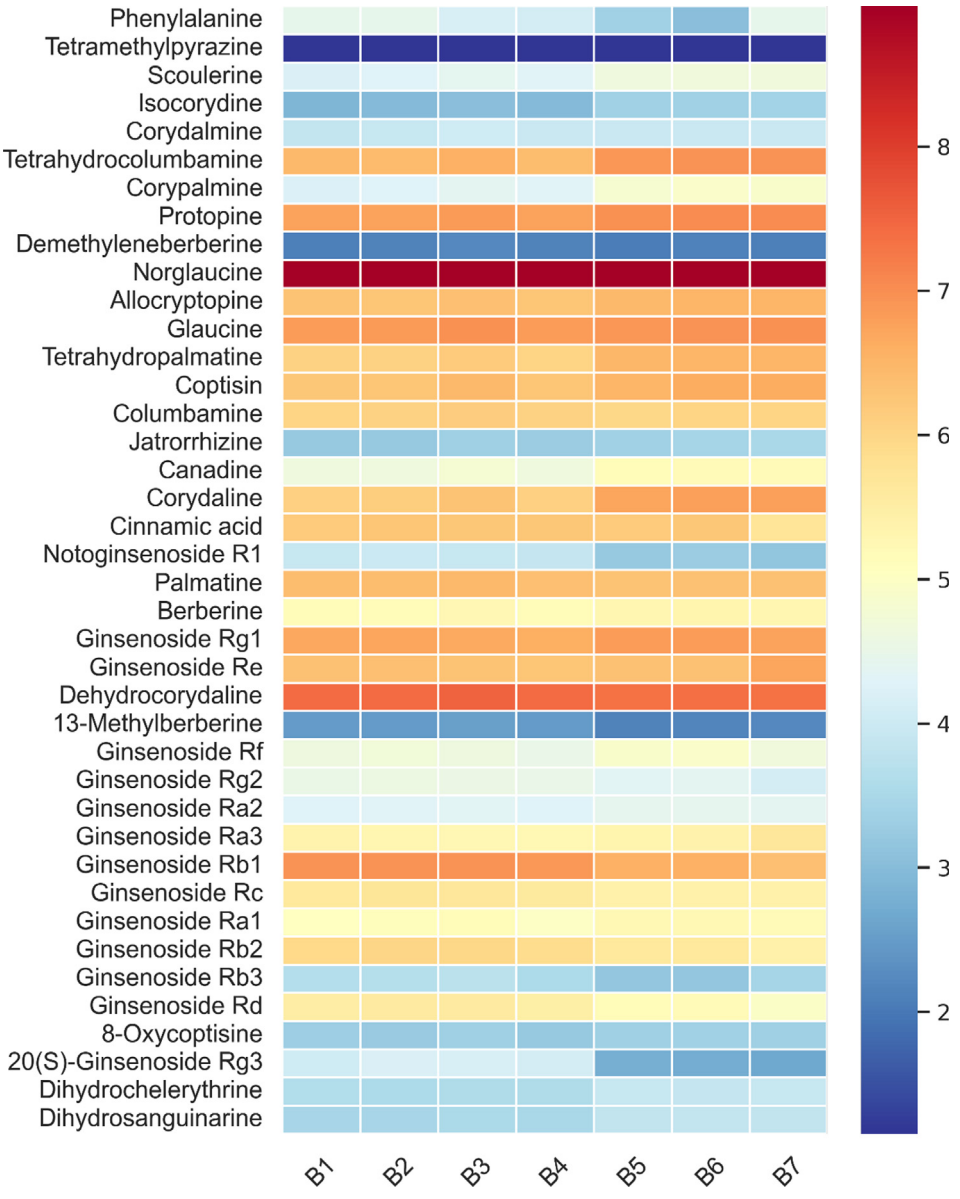


**Fig. 4** The content distribution heatmap of 40 non-volatile compounds in 7 batches of SXXTN.

in the response of the components in positive and negative ion modes, two MRM methods with different polarity were established for Quantification. The typical MRM chromatograms of analytes were illustrated in Fig S3.

Nice linearity with coefficients of determination ($R^2$ > 0.9900) were obtained for the 40 analytes. Limit of detection (LOD) and limit of quantitation (LOQ) tests were performed and listed in Table S7. As exhibited in Table S8, relative standard deviations (RSD) of repeatability, intra- and inter-day precision were 1.27 % – 4.79 %, 1.07 % – 5.41 % and 1.18 % – 9.43 %, respectively. Besides, all analytes could remain stable within 24 h under 4℃, with the RSD ranging 0.60 % – 6.14 % and recoveries of 40 compounds were ranged from 80.36 % – 117.13 % with the RSD ranging 2.38 % – 12.61 %. Consequently, the established HPLC-QQQ MS approach was proved as a sensitive, repeatable and accurate tool for the quantification of non-volatile compounds in SXXTN.

According to the established HPLC-QQQ MS quantitative analysis method, the content of 40 compounds in 7 batches of SXXTN provided by the enterprise was determined as shown in Table 3 and Fig. 4. The total content of 40 analytes in each batch was 2.03 % – 2.30 %. Among them, the components with higher content (> 1.00 mg/g) were norglaucine (**43**), glaucine (**46**), dehydrocorydaline (**75**) and ginsenoside $Rb_1$ (**86**). In the previous reported, aforementioned compounds presented promising effects for myocardial protection (Wen et al., 2022; Han et al., 2012; Zheng et al., 2017; Kong et al., 2018). For example, studies have found that intraperitoneal injection of dehydrocorydine in $ApoE^{-/-}$ mice can not only inhibit the development of atherosclerosis, but also improve aortic compliance and plaque stability (Wen et al., 2022).

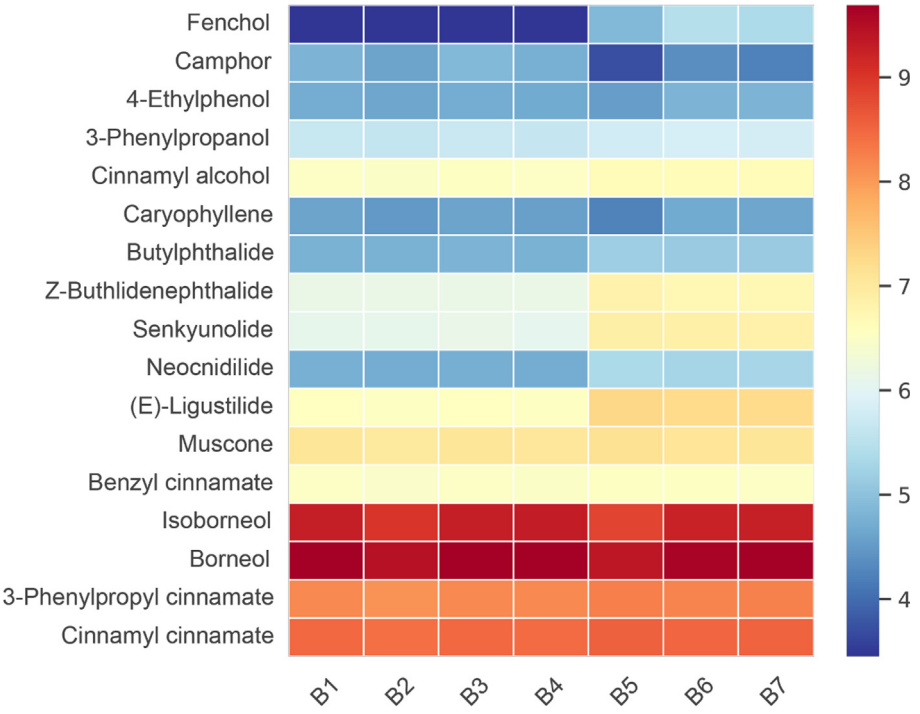


**Fig. 5** The content distribution heatmap of 17 volatile compounds in 7 batches of SXXTN.

Tetrahydropalmatine can activate PI3K/Akt/eNOS/NO pathway, increase the expression of HIF-1a and VEGF, and inhibit iNOS-derived NO production in myocardium. This effect may reduce the accumulation of inflammatory factors (including TNF-a and MPO) and reduce the degree of apoptosis (Han et al., 2012). It has also been reported that ginsenoside $Rb_1$ can improve heart failure, which may be achieved by regulating the mitochondrial membrane in cardiomyocytes (Kong et al., 2018).

### *3.4. Quantification of 17 volatile compounds in SXXTN by GC-QQQ MS*

We established rapid and accurate quantitative methods for detecting the contents of the major volatile compounds in SXXTN. Seventeen confirmed compounds including 5 phthalides, 3 organic acid esters, 3 alcohols, 3 monoterpenes, 1 sesquiterpene, 1 phenol and 1 macrocyclic ketone were determined by GC-QQQ MS with naphthalene (**IS3**) as internal standards. The optimized conditions were shown in Table S5-S6 and typical MRM chromatograms of 17 analytes were illustrated in Fig S4.

The optimized GC-QQQ MS method was validated in the aspect of linearity, LODs, LOQs, precision, repeatability, stability and recovery and the results were presented in Table S9 and Table S10. Reasonable correlation coefficient values (R2 > 0.9904) indicated good correlations between investigated standards concentrations and their peak areas within the ranges tested. The ranges of LODs and LOQs for all the analytes were 0.002 - 2.642 μg/mL, and 0.013 – 6.653 μg/mL, respectively. The RSDs of repeatability, intra- and inter-day precision were 1.63% - 9.66%, 0.44% – 9.06%, 0.80% – 8.06%, respectively. All analytes could remain stable within 24 h under 4℃, with the RSD ranging 1.14% - 7.97%. The developed method had good accuracy with the recoveries were between 90.20% and 123.51%. These results provided that the established method was accurate, reproducible, and reliable for assessing the quality of volatile compounds in SXXTN.

According to the established GC-QQQ MS quantitative analysis method, the contents of main volatile components in 7 batches of SXXTN provided by the enterprise were determined as displayed in Table 3 and Fig. 5. The total content of analytes in each batch was 3.34 %-4.26 % in SXXTN. Borneol (**146**), isoborneol (**148**), cinnamyl cinnamate (**174**), 3-phenylpropyl cinnamate (**173**) and muscone (**163**) were the predominant components and were closely related to the anti-coronary heart disease and angina pectoris effect of SXXTN (Liu et al., 2017; Wu et al., 2011; Wang et al., 2020).

Combined with the quantitative analysis of non-volatile components, the total contents of 57 main components in 7 batches of SXXTN were 5.50 % – 6.49 %. The percentages of different structural types of chemicals in the total 57 analytes were as follows: monoterpenes and sesquiterpenes accounted for the largest proportion (42 %), followed by alkaloids (26 %), organic acids and esters (18 %), ginsenosides (6 %) and phthalide (5 %). Both volatile and non-volatile components should be taken into consideration for quality evaluation of SXXTN. More batches of samples are more conducive to assessing the consistency and stability of SXXTN.

## 4. Conclusion

In view of the current deficiencies in the constituent research and quality control of SXXTN, efficient, stable and reliable LC-MS and GC–MS methods were established in our study. A total of 177 chemical components were identified from SXXTN and content of 57 components in 7 batches of SXXTN was further determined. To the best of our knowledge, this is the initial report on the comprehensive profiling

of chemical constituents in SXXTN by LC-MS and GC–MS. The evaluation approach provided much more qualitative and quantitative information of multi-components in SXXTN than other single-marker quality assessments. In all, this study provided comprehensive material basis of SXXTN, which could be beneficial to improve the quality control. Furthermore, it could facilitate the pharmacological research and clinical application of SXXTN in some degree.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The authors sincerely thank Hui-Ying Wang (State Key Laboratory of Natural Medicines, China) for the technical assistance.

## Funding

This work was financially supported by the National Natural Science Foundation of China (No. 81730104).

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2022.104527.

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