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# Research Article
# Analysis of Ingredients of Biyuan Tongqiao Granule Absorbed into Blood in Rats Based on UPLC-QTOF-MS Technology

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## Abstract

**Background and Objective:** Biyuan Tongqiao granule is the Chinese patent medicine commonly used in the treatment of acute and chronic rhinitis and sinusitis. It is composed of 14 Chinese medicinal herbs. In this study, serum pharmacochemical methods were employed to analyze the prototype blood components of Biyuan Tongqiao granule after oral administration in rats, aiming to provide potential quality markers for the quality control of Biyuan Tongqiao granule. **Materials and Methods:** The UPLC-QTOF-MS technique was used in combination with PCDL database to analyze and investigate the difference in the chromatograms between blank plasma and administered plasma under the same detection conditions. According to mass spectrometry data including retention time, precise molecular mass and secondary fragment ion, the prototype components in the blood of rats were analyzed and identified after administration of Biyuan Tongqiao granule. **Results:** Totally 25 prototype monomers were detected, which included 11 flavonoids (like narirutin and ononin), 4 organic acids (such as protocatechuic acid and chlorogenic acid), 4 terpenoids (like glycyrrhizic acid and oleanolic acid), 2 quinones (cryptotanshinone and tanshinone IIA), 2 coumarins (scopoletin and isoimperatorin), ephedrine and 2 pseudoephedrine alkaloids. The 25 prototype components in the blood of rats after oral administration of Biyuan Tongqiao granule are derived from 13 out of 14 medicinal herbs in the compound, which can comprehensively control the quality of the compound. **Conclusion:** Results in this study lay an experimental foundation for the development of quality control methods for the compound.

Key words: Biyuan Tongqiao granule, serum pharmacochemistry, component analysis, flavonoids

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Biyuan Tongqiao granule is derived from the ancient Chinese classical formula, which is developed on the basis of "Cangerzi San" in Song Dynasty[1]. It consists of 14 medicinal herbs, including Magnolia, *Xanthium sibiricum*, *Ephedra*, Mint, *Scutellaria baicalensis*, *Forsythia suspensa*, *Chrysanthemum indicum*, Tianfen, Dihuang, Danshen, *Poria cocos*, *Angelica dahurica*, Gao ben and Licorice. It possesses the effects of "dispersing wind and clearing heat, promoting the circulation of the lungs and opening the orifices". In China, it has been extensively applied in the treatment of acute and chronic rhinitis, allergic rhinitis, sinusitis and other external evils attacking the lungs[2]. It is a commonly used traditional Chinese patent medicine and a simple preparation in clinical otorhinolaryngology.

At present, symptomatic drug application methods are frequently used in the clinical treatment of acute and chronic rhinitis, allergic rhinitis, sinusitis and other diseases. For example, antiviral drugs, antibiotics and antiallergic drugs are often used in the treatment of acute rhinitis. Glucocorticoids, antihistamines and leukotrienes are the conventional medications for allergic rhinitis. Moreover, the drugs commonly used for the treatment of chronic rhinitis and sinusitis include vasoconstrictors, corticosteroids and anti-inflammatory drugs[3]. The above-mentioned western medicine can improve the symptoms of acute and chronic rhinitis, sinusitis and other diseases, however, there are clinical problems such as disease recurrence, easy relapse after drug withdrawal, certain toxic and side effects of the drug and can not be taken for a long time[4]. Traditional Chinese medicine formulas[5], especially Biyuan Tongqiao granule, display their own unique advantages and clinical efficacy in the treatment of acute and chronic rhinitis, sinusitis and other diseases. Clinical data have shown that the use of Biyuan Tongqiao granule alone or in combination is more effective and safer for chronic sinusitis than conventional Western medicine treatment, traditional Chinese medicine preparations, or surgical treatment alone[6].

At present, studies regarding Biyuan Tongqiao granule mainly focus on its clinical evaluation and mechanism of action[1,2]. While research on the quality control of its compound only stays in determining the index components of ephedrine hydrochloride, forsythia and baicalin in the Chinese Pharmacopoeia. However, since the compound is composed of 14 traditional Chinese medicinal herbs, some of which are toxic and some are prone to confusion, the efficacy of this compound cannot be controlled with only 3 ingredients as the index, therefore, quality control methods are urgently needed.

Consequently, in response to the above issues, the research group conducted a preliminary analysis on the chemical composition of Biyuan Tongqiao granule and identified a total of 103 compounds, including 29 flavonoids, 19 terpenoids, 12 lignans, 11 coumarins, 9 quinones and 23 other compounds. Therefore, UPLC-Q-TOF-MS/MS technology was applied in combination with plasma drug chemistry methods to analyze the blood entering components of Biyuan Tongqiao granule according to the mass spectrometry fragmentation patterns of chemical components, database queries and relevant literature reviews. By combining the results of *in vitro* chemical analysis, this study aimed to lay a scientific foundation for revealing the pharmacological substances of Biyuan Tongqiao granule and establishing a quality control system.

## MATERIALS AND METHODS

**Study area:** The current work was performed at Traditional Chinese Medicine Products and Development Laboratory, Liaoning University of Traditional Chinese Medicine, China from November 2021 to April 2022.

**Reagents and materials:** Biyuan Tongqiao granule was purchased from Shandong New Times Pharmaceutical Co., Ltd. (Linyi, China). Scopoletin, narirutin, luteolin, chlorogenic acid and oleanolic acid (purities >98%) were bought from Sichuan Weikeqi Biological Technology, Co., Ltd. (Chengdu, China). Hesperidin, quercetin, wogonin and isoimperatorin (purities >98%) were provided by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The MS-grade formic acid was purchased from Thermo Fisher Scientific (China) Co., Ltd. (Shanghai, China). The MS-grade methanol was acquired from Merck (Darmstadt, Germany).

**Animals:** Sixteen male Sprague-Dawley (SD) rats (200±20 g) were purchased from Liaoning Changsheng Biotechnology Co. Ltd. [Number: SCXK (Liao) 2020-0001]. Following one week of acclimatization, rats were used in the experiments.

**Ethical consideration:** All the animal experiments were performed based on the approved animal protocols and guidelines established by the Medicine Ethics Review Committee for Animal Experiments of Liaoning University of Traditional Chinese Medicine under the approval number: 2020YS013(KT)-013-01.

**Collection of rat plasma samples:** The 16 SD rats were divided into a blank group and a treatment group, with 8 rats

in each group. To be specific, rats in the treatment group were provided with oral administration of Biyuan Tongqiao granule at 2.025 g/kg twice a day for 3 days, while those in the blank group were given the same amount of normal saline. After fasting for 12 hrs on the night of day 2, blood was collected from the orbital venous plexus at 30 min, 1, 2 and 4 hrs after intragastric administration on day 3 to extract the plasma[5]. After standing for 40 min, the supernatant was obtained by centrifugation at 3500 r/min for 5 min and set aside at -80°C.

**Treatment of plasma samples for UPLC-QTOF-MS analysis:** In brief, 150 µL plasma samples were precisely added with 1 mL ethyl acetate, followed by swirling for 2 min, ultrasonic treatment for 1 min and centrifugation for 10 min at 13000 r/min at 4°C and then 900 µL supernatant was collected for vacuum-drying. Thereafter, the residue was redissolved with 50 µL methanol, swirled for 2 min, treated with ultrasound for 1 min, refrigerated and centrifuged at 13000 r/min and 4°C for 5 min to collect the supernatant for testing[7].

**Preparation of reference solution:** An appropriate amount of each reference substance was accurately weighed and added with methanol to prepare a mixed reference solution including 0.172 mg chlorogenic acid, 0.164 mg scopoletin, 0.273 mg narirutin, 0.271 mg hesperidin, 0.124 mg quercetin, 0.162 mg luteolin, 0.139 mg wogonin and 0.489 mg isoimperatorin per 1 mL. Another 150 µL blank rat plasma was collected and the vacuum-dried substance was obtained according to the treatment method of UPLC-QTOF-MS analysis. Afterwards, the residue was added into 50 µL mixed reference solution and redissolved. After vortexing, ultrasonic treatment and centrifugation, the supernatant was obtained as the blank plasma+mixed reference solution.

**Conditions of UPLC-QTOF-MS analysis:** Plasma samples were characterized on an Agilent-1290 UPLC/6550 QTOF-MS system (Agilent Technologies, Inc., USA). An Agilent poroshell 120 SB-C18 column (4.6×100 mm, 2.7 µm) was used for chromatographic analysis with the system of 0.1% formic acid aqueous solution (A)-methanol (B) in the positive mode, whereas water (A)-methanol (B) in the negative mode. The gradient elution conditions were as follows, 0-30 min, 5-100% B, at the flow rate of 0.4 mL/min, the column temperature of 30°C and the injection volume of 0.3 µL. Under the same conditions as those applied in our previous study, the MS analysis was performed on a dual spray ion source (Dual AJS ESI)[8].

**Analysis of components of Biyuan Tongqiao granule absorbed into the blood of rats:** The chemical components of Biyuan Tongqiao granule in the early stage were analyzed, as a result, a total of 103 chemical components, including 29 flavonoids, 19 terpenes, 12 lignans, 11 coumarins and 9 quinones, were identified. In this study, the chemical components of 14 medicinal herbs contained in Biyuan Tongqiao granule in the literature, including Magnolia, *Xanthium sibiricum*, *Ephedra*, Mint, *Scutellaria baicalensis*, *Forsythia suspensa*, *Chrysanthemum indicum*, Tianfen, Dihuang, Danshen, *Poria cocos*, *Angelica dahurica*, Gaoben and Licorice, were also integrated. By inputting the names, molecular formulas and herbs containing these chemical components into the Personal Compounds Database and Libraries (PCDLs) software (B.04.00), a Biyuan Tongqiao granule PCDL database was established and used to analyze the prototype components absorbed into blood and the attributions of medicinal materials[9].

## RESULTS

**Identification of the prototype components absorbed into the plasma:** By adopting the UPLC-QTOF-MS technology, the profiles of drug-containing plasma, blank plasma, mixed control substance and blank plasma+mixed control substance at 30 min, 1, 2 and 4 hrs after oral administration of Biyuan Tongqiao granule in rats were analyzed and compared. Through analyzing the PCDL database of the chemical composition of Biyuan Tongqiao granule, the prototype blood components and medicinal herb attributions were initially obtained. Thereafter, the structures and molecular formulas of the compounds were determined by the fragmentation regularities and relevant literature. Altogether 25 prototype components were identified and analyzed in rat plasma samples, as shown in Table 1. After analysis and identification, the 1 hr plasma sample contained all the 25 prototype components, therefore, only the baseline peak diagram (BPC) of the 1 hr plasma sample was listed. The spectra of drug-containing plasma, blank plasma, mixed control substance and blank plasma+mixed control substance under the positive and negative ion modes were presented in Fig. 1.

**Analysis of the fragmentation patterns of various blood entering compounds**

**Structural analysis of alkaloids:** The main fragmentation regularities of ephedra alkaloids are dehydration, demethylation and subsequent deamination[10]. The retention time of compound 4 was 3.427 min, the excimer ion peak m/z

Table 1: Prototype components absorbed into rat plasma

| No. | Retention time (RT) (min) | Ion mode | Measured mass (m/z) | Theoretical mass (m/z) | Mass error (ppm) | Formula | Fragment ion (m/z) | Identified compounds | Herbs |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2.922 | $[M+H]^+$ | 199.0605 | 199.0601 | 2.00 | $C_9H_{10}O_5$ | 181.0483 $[M+H-H_2O]^+$<br>137.0527 $[M+H-CH_2O_3]^+$<br>124.0518 $[M+H-COOH-CHO-H]^+$<br>111.0443 $[M+H-COOH-CHO-H-CH]^+$ | Danshensu | g |
| 2 | 3.236 | $[M-H]^-$ | 153.0199 | 153.0193 | 3.92 | $C_7H_6O_4$ | 109.0303 $[M+H-CO_2]^-$ | Protocatechuic acid | a/b/g/k |
| 3 | 3.427 | $[M+H]^+$ | 166.1221 | 166.1226 | -3.00 | $C_{10}H_{15}NO$ | 148.1121 $[M+H-H_2O]^+$<br>133.0885 $[M+H-H_2O-CH_3]^+$<br>117.0698 $[M+H-H_2O-CH_3-NH_2]^+$ | Ephedrine | b |
| 4 | 4.356 | $[M+H]^+$ | 166.1224 | 166.1226 | -1.20 | $C_{10}H_{15}NO$ | 148.1125 $[M+H-H_2O]^+$<br>133.0882 $[M+H-H_2O-CH_3]^+$<br>117.0697 $[M+H-H_2O-CH_3-NH_2]^+$ | Pseudoephedrine | b |
| 5 | 6.177 | $[M-H]^-$ | 353.0871 | 353.0878 | -1.98 | $C_{16}H_{18}O_9$ | 191.0561 $[M-H-C_9H_6O_3]^-$<br>179.0356 $[M-H-C_7H_{10}O_5]^-$<br>173.0407 $[M-H-C_9H_6O_3-H_2O]^-$<br>135.0450 $[M-H-C_9H_6O_3-CO_2]^-$ | Chlorogenic acid* | f/g |
| 6 | 9.045 | $[M+H]^+$ | 193.0493 | 193.0495 | -1.04 | $C_{10}H_8O_4$ | 178.0268 $[M+H-CH_3]^+$<br>161.0206 $[M+H-CH_3OH]^+$<br>149.0521 $[M+H-CO_2]^+$<br>133.0291 $[M+H-CH_3OH-CO]^+$ | Scopoletin* | a/i |
| 7 | 9.276 | $[M-H]^-$ | 193.0483 | 193.0506 | -1.55 | $C_{10}H_{10}O_4$ | 178.0269 $[M-H-CH_3]^-$<br>149.0611 $[M-H-CO_2]^-$<br>134.0370 $[M-H-CH_3-CO_2]^-$ | Ferulic acid | a/b/c/e/g/j/m |

Table 1: Continue

| No. | Retention time (RT) (min) | Ion mode | Measured mass (m/z) | Theoretical mass (m/z) | Mass error (ppm) | Formula | Fragment ion (m/z) | Identified compounds | Herbs |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 107.0136 $[M-H-CH_3-CO_2-C_2H_3]^-$ | | |
| 8 | 11.674 | $[M+H]^+$ | 581.1856 | 581.1865 | -1.54 | $C_{27}H_{32}O_{14}$ | 435.1285 $[M+H-C_6H_{10}O_4]^+$<br>419.1339 $[M+H-C_6H_{10}O_5]^+$<br>273.0754 $[M+H-C_6H_{10}O_4-C_6H_{10}O_5]^+$<br>153.0177 $[M+H-C_6H_{10}O_4-C_6H_{10}O_5-C_8H_8O]^+$ | Narirutin* | i |
| 9 | 11.705 | $[M+H]^+$ | 611.1602 | 611.1607 | -0.82 | $C_{27}H_{30}O_{16}$ | 465.1028 $[M+H-C_6H_{10}O_4]^+$<br>449.1078 $[M+H-C_6H_{10}O_5]^+$<br>303.0499 $[M+H-C_6H_{10}O_4-C_6H_{10}O_5]^+$<br>181.0131 $[M+H-C_6H_{10}O_4-C_6H_{10}O_5-C_7H_6O_2]^+$ | Rutin | a/b/c/e/l/m |
| 10 | 12.108 | $[M+H]^+$ | 611.1952 | 611.1970 | -2.94 | $C_{28}H_{34}O_{15}$ | 303.0831 $[M+H-C_{12}H_{20}O_9]^+$<br>288.0628 $[M+H-C_{12}H_{20}O_9-CH_3]^+$<br>272.0678 $[M+H-C_{12}H_{20}O_9-CH_3O]^+$<br>195.0652 $[M+H-C_{12}H_{20}O_9-C_6H_4O_2]^+$ | Hesperidin* | c/e |
| 11 | 12.864 | $[M+H]^+$ | 431.1329 | 431.1337 | -1.86 | $C_{22}H_{22}O_9$ | 269.0805 $[M+H-C_6H_{10}O_5]^+$<br>254.0573 $[M+H-C_6H_{10}O_5-CH_3]^+$<br>177.0546 $[M+H-C_6H_{10}O_5-C_6H_4O]^+$<br>161.0597 $[M+H-C_6H_{10}O_5-O]^+$ | Ononin | a/k |
| 12 | 12.890 | $[M+H]^+$ | 257.0806 | 257.0808 | -0.78 | $C_{15}H_{12}O_4$ | 239.0724 $[M+H-H_2O]^+$<br>137.0226 $[M+H-C_8H_8O]^+$<br>119.0828 $[M+H-C_7H_4O_3]^+$ | Liquiritigenin | k |
| 13 | 14.413 | $[M+H]^+$ | 303.0484 | 303.0499 | -4.95 | $C_{15}H_{10}O_7$ | 275.0553 $[M+H-CO]^+$<br>259.0590 $[M+H-CO_2]^+$ | Quercetin* | a/b/c/d/e/f/g |

Table 1: Continue

| No. | Retention time (RT) (min) | Ion mode | Measured mass (m/z) | Theoretical mass (m/z) | Mass error (ppm) | Formula | Fragment ion (m/z) | Identified compounds | Herbs |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 231.0650 $[M+H-CO-CO_2]^+$ 195.0290 $[M+H-C_6H_4O_2]^+$ | | |
| 14 | 15.095 | $[M+H]^+$ | 287.0537 | 287.0550 | -4.52 | $C_{15}H_{10}O_6$ | 259.0601 $[M+H-CO]^+$ 153.0177 $[M+H-C_8H_6O_2]^+$ 135.0435 $[M+H-C_7H_4O_4]^+$ | Luteolin* | b/c/d/e/f/g |
| 15 | 15.204 | $[M+H]^+$ | 593.1843 | 593.1865 | -3.71 | $C_{28}H_{32}O_{14}$ | 447.1286 $[M+H-C_6H_{10}O_4]^+$ 285.0644 $[M+H-C_{12}H_{21}O_9]^+$ 269.0645 $[M+H-C_{12}H_{21}O_9-CH_3]^+$ 241.0495 $[M+H-C_{12}H_{21}O_9-CH_3-CO]^+$ | Linarin | c/f |
| 16 | 18.652 | $[M+H]^+$ | 285.0744 | 285.0757 | -4.56 | $C_{16}H_{12}O_5$ | 270.0520 $[M+H-CH_3]^+$ 226.0624 $[M+H-CH_3-CO-O]^+$ 242.0574 $[M+H-CH_3-CO]^+$ 241.0495 $[M+H-CH_3-CO-H]^+$ | Wogonin* | d/e |
| 17 | 18.865 | $[M+H]^+$ | 255.0645 | 255.0652 | -2.74 | $C_{15}H_{10}O_4$ | 227.0704 $[M+H-CO]^+$ 153.0182 $[M+H-C_8H_6]^+$ 132.0572 $[M+H-C_6H_3O_3]^+$ 103.0536 $[M+H-C_7H_4O_4]^+$ | Chrysin | c/d/f |
| 18 | 19.393 | $[M+H]^+$ | 285.0743 | 285.0757 | -4.91 | $C_{16}H_{12}O_5$ | 270.0515 $[M+H-CH_3]^+$ 242.0571 $[M+H-CO]^+$ | Acacetin | c/d/f |
| 19 | 21.525 | $[M+H]^+$ | 271.0961 | 271.0965 | -1.48 | $C_{16}H_{14}O_4$ | 203.0338 $[M+H-C_5H_8]^+$ 175.0333 $[M+H-C_5H_8-CO]^+$ 185.0223 $[M+H-C_5H_8-H_2O]^+$ | Isoimperatorin* | i |

Table 1: Continue

| No. | Retention time (RT) (min) | Ion mode | Measured mass (m/z) | Theoretical mass (m/z) | Mass error (ppm) | Formula | Fragment ion (m/z) | Identified compounds | Herbs |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 147.0445<br>$[M+H-C_5H_8.2CO]^+$ | | |
| 20 | 21.772 | $[M+H]^+$ | 823.4125 | 823.4111 | 1.70 | $C_{42}H_{62}O_{16}$ | 805.4005<br>$[M+H-H_2O]^+$<br>761.4107<br>$[M+H-H_2O-CO_2]^+$<br>647.3751<br>$[M+H-C_6H_8O_6]^+$<br>471.3460<br>$[M+H-2C_6H_8O_6]^+$ | Glycyrrhizic acid | k |
| 21 | 23.198 | $[M+H]^+$ | 297.1497 | 297.1485 | 4.04 | $C_{19}H_{20}O_3$ | 282.1237<br>$[M+H-CH_3]^+$<br>279.1361<br>$[M+H-H_2O]^+$<br>251.1417<br>$[M+H-H_2O-CO]^+$<br>236.1184<br>$[M+H-H_2O-CO-CH_3]^+$<br>221.0739<br>$[M+H-H_2O-CO-CH_3-CH_3]^+$ | Cryptotanshinone | g |
| 22 | 24.64 | $[M+H]^+$ | 295.1335 | 295.1329 | 2.03 | $C_{19}H_{18}O_3$ | 280.1094<br>$[M+H-CH_3]^+$<br>277.1216<br>$[M+H-H_2O]^+$<br>262.0988<br>$[M+H-CH_3-H_2O]^+$<br>249.1236<br>$[M+H-H_2O-CO]^+$ | Tanshinone IIA | g |
| 23 | 26.405 | $[M+H]^+$ | 471.3448 | 471.3469 | -4.46 | $C_{30}H_{46}O_4$ | 453.3361<br>$[M+H-H_2O]^+$ | Glycyrrhetinic acid | k |
| 24 | 27.686 | $[M-H]^-$ | 455.3523 | 455.3531 | -1.76 | $C_{30}H_{48}O_3$ | 409.3444<br>$[M-H-HCOOH]^-$<br>407.3304<br>$[M-H-HCOOH-2H]^-$<br>391.3342<br>$[M-H-HCOOH-H_2O]^-$<br>376.3179<br>$[M-H-HCOOH-H_2O-CH_3]^-$ | Oleanolic acid* | b/c/e/g/h/k |
| 25 | 27.765 | $[M-H]^-$ | 455.3514 | 455.3531 | -3.73 | $C_{30}H_{48}O_3$ | 407.3398<br>$[M-H-CH_4O_2]^-$<br>393.3593<br>$[M-H-CH_4O_2-CH_2]^-$ | Ursolic acid | b/c/e/g/k |

*Compared with the reference substance, a: *Xanthium sibiricum* (stir-fried), b: Chinese *Ephedra*, c: Mint, d: *Scutellaria baicalensis*, e: *Forsythia suspensa*, f: *Chrysanthemum indicum*, g: *Salvia miltiorrhiza*, h: *Poria cocos*, i: *Ammi majus*, j: Rhizoma ligustici, k: Licorice, l: Lily magnolia and m: *Rehmannia glutinosa*

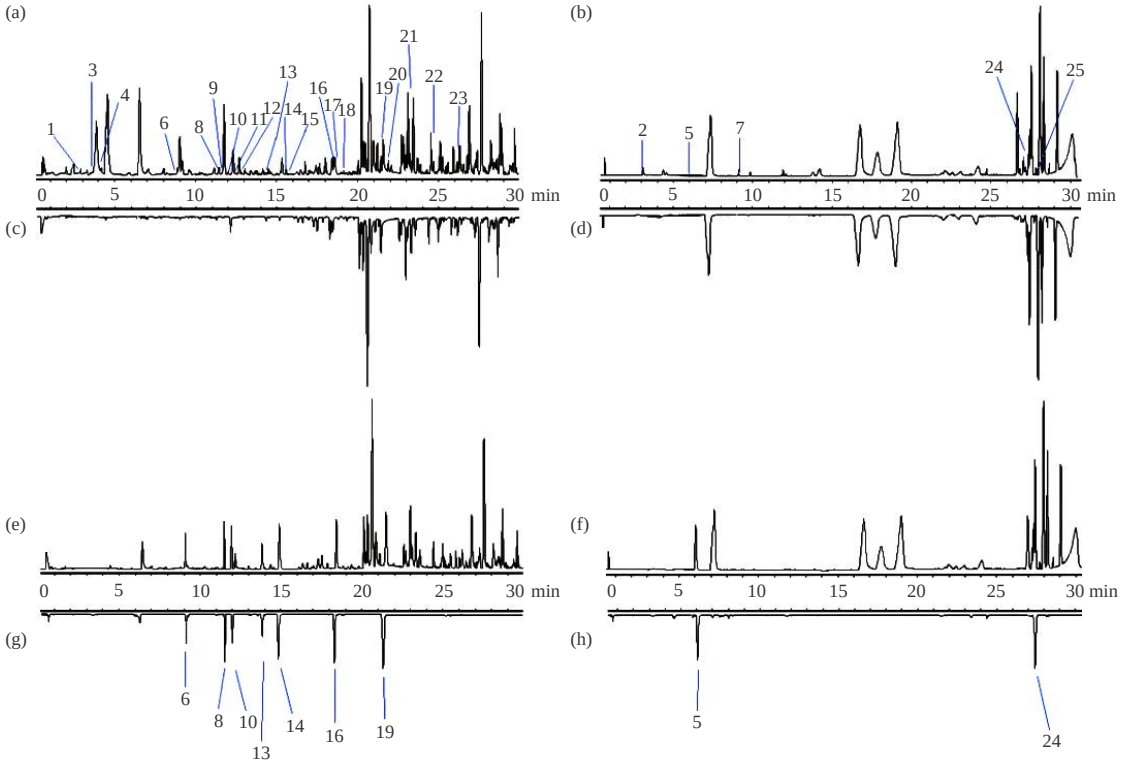


Fig. 1(a-h): BPC diagram of each plasma sample, (a) 1 hr administration plasma in positive ion mode, (b) 1 hr administration plasma in negative ion mode, (c) Blank plasma in positive ion mode, (d) Blank plasma in negative ion mode, (e) Blank plasma and mixed reference substance in positive ion mode, (f) Blank plasma and mixed reference substance in negative ion mode, (g) Mixed reference substance in positive ion mode and (h) Mixed reference substance in negative ion mode

166.1221$[M+H]^+$ was the base peak and the fragment ion m/z 148.1121$[M+H-H_2O]^+$, m/z 133.0885$[M+H-H_2O-CH_3]^+$ and m/z 117.0698$[M+H-H_2O-CH_3-NH_2]^+$ could be seen in the secondary spectrum. This suggested that it might first remove one molecule of $H_2O$ and then remove a methyl group and an amino group. According to the reference reports, the fragmentation regularities of ephedrine and pseudoephedrine were the same, therefore, it was inferred that peak 4 was ephedrine and peak 5 was pseudoephedrine[11]. The possible cleavage pathways of alkaloids were displayed in Fig. 2.

**Structural analysis of organic acids:** Organic acids are a class of organic compounds with acidic groups. Such compounds are prone to neutral molecule loss under high-energy collisions, such as $H_2O$, COOH and $CO_2$, resulting in corresponding characteristic fragment ion peaks[12]. In this study, 4 organic acid compounds, namely, protocatechuic acid, chlorogenic acid, danshensu and ferulic acid, were identified. For instance, the retention time of compound 6 was 6.177 min, the excimer ion peak m/z 353.0871$[M-H]^-$ was seen in the negative ion mode and the quinic acid m/z 191.0561 $[M-H-C_9H_6O_3]^-$ was produced due to the breaking of its acyl-oxygen bond. And fragment ion m/z 179.0356$[M-H-C_7H_{10}O_5]^-$ was seen for caffeic acid. Subsequently, the two fragment ions were further fragmented, resulting in fragment ions m/z 173.0407$[M-H-C_9H_6O_3-H_2O]^-$ and m/z 135.0450$[M-H-C_9H_6O_3-CO_2]^-$ and it was speculated that one molecule of $CO_2$ or $H_2O$ might be lost. The compound was detected as chlorogenic acid. Figure 3 presents the possible cracking pathways of organic acid compounds.

**Structural analysis of coumarins:** The mother nucleus structure of coumarin compounds is $C_{11}H_6O_3$ and its main cleavage pathway is the cleavage of the methoxy or benzyl ether bonds on the side chains of the mother nucleus[13]. In this study, 2 coumarin compounds were identified in the plasma, including scopoletin and isoimperatorin. For example, the retention time of compound 7 was 9.045 min, the excimer ion peak in the positive ion mode was m/z 193.0493$[M+H]^+$, the excimer ion peak was cracked and 1 $CH_3$ fragment group,

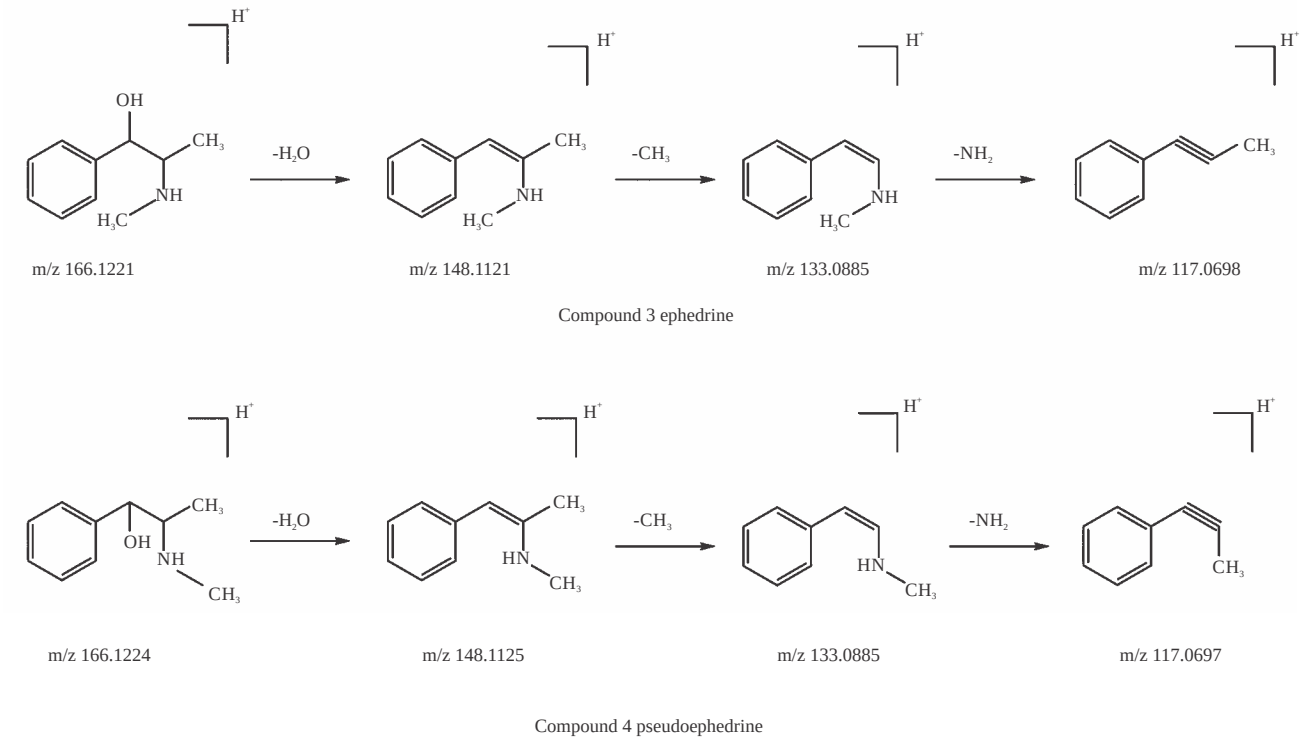


Fig. 2: Cleavage pathway of alkaloids

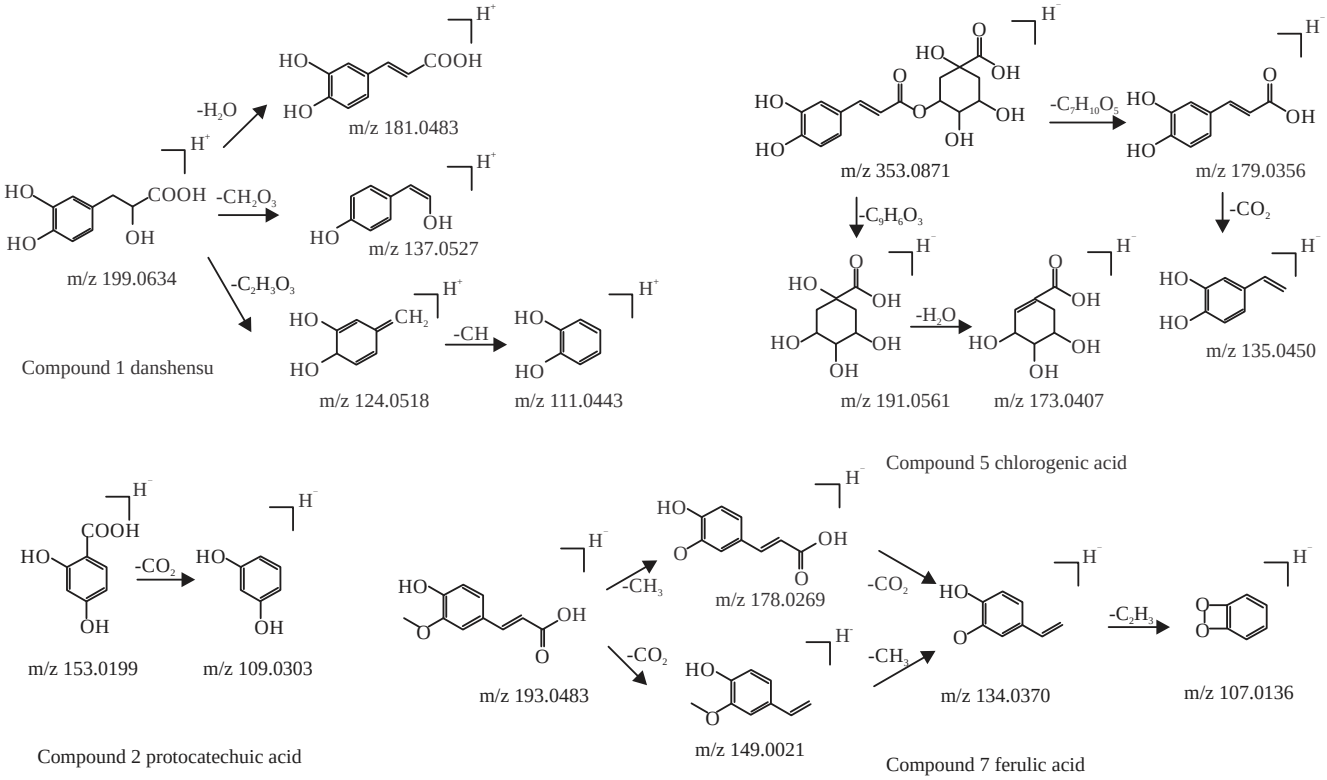


Fig. 3: Cleavage pathway of organic acids

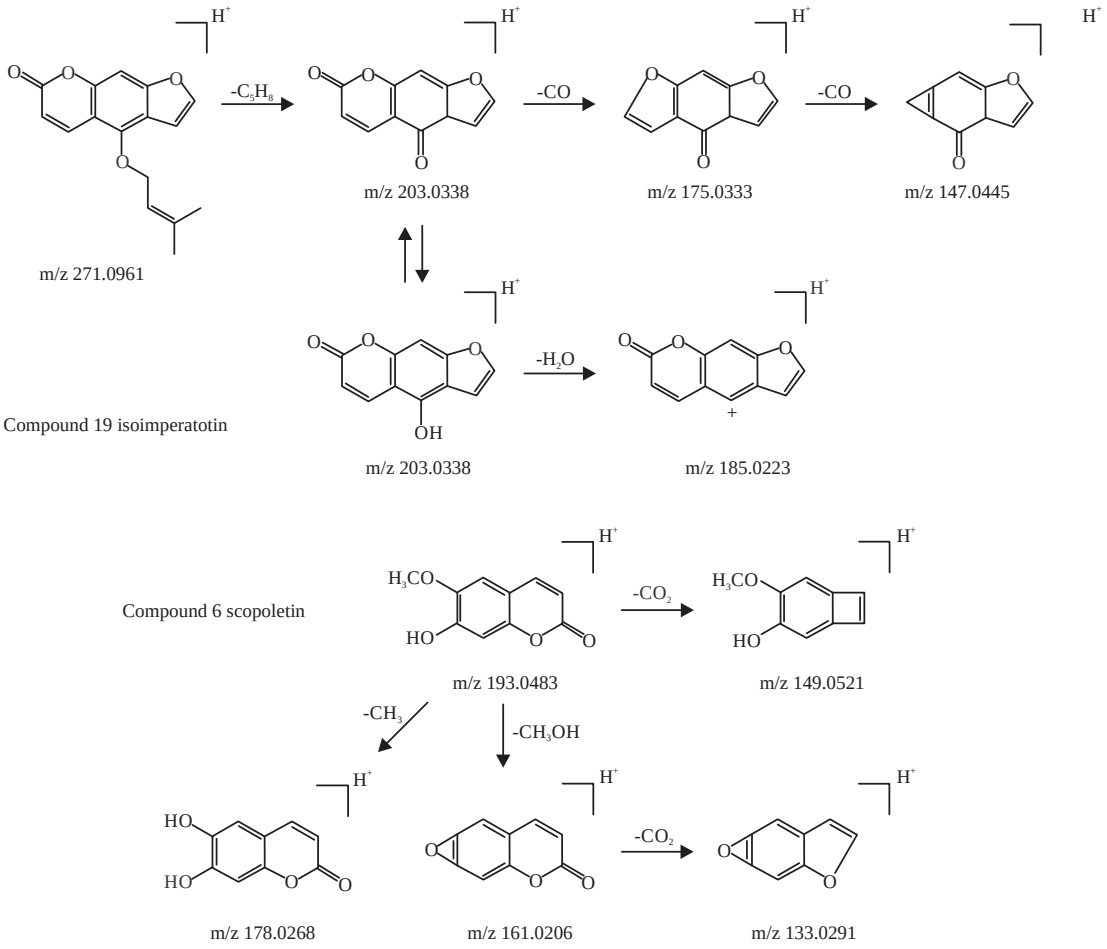


Fig. 4: Cleavage pathway of coumarins

1 neutral $CO_2$ molecule and 2 CO molecules were lost respectively. The fragment ions m/z 178.0268$[M+H-CH_3]^+$ and m/z 149.0521$[M+H-CO_2]^+$ were produced, respectively. In addition, the fragment ion peak also lost the $CH_3OH$ group first and later obtained the fragment ion m/z 161.0206$[M+H-CH_3OH]^+$. As for fragment ion m/z 133.0285$[M+H-CH_3OH-CO]^+$, it was speculated that a neutral CO molecule might be lost; according to the comparison of reference product, the compound was inferred to be scopoletin. In addition, the possible cleavage pathways of coumarins were inferred in Fig. 4.

**Structural analysis of flavonoids:** Flavonoids possess a C6-C3-C6 basic skeleton. The main characteristics of this class of compounds are the RDA cleavage of the ring and the loss of fragments of neutral small molecules including CO and $CO_2$[14]. In this study, we identified 11 flavonoids, including narirutin, rutin, hesperidin, ononin, liquiritigenin, quercetin, luteolin, linarin, wogonin, chrysin and acacetin. For instance, the retention time of compound 9 was 11.674 min in the positive ion mode and 11.665 min in the mixed control. The high-resolution excimer ion peak was m/z 581.1856$[M+H]^+$ and the molecular formula was $C_{27}H_{32}O_{14}$. The excimer ion peaks were cleaved to produce m/z 419.1339$[M+H-C_6H_{10}O_5]^+$ and m/z 435.1285$[M+H-C_6H_{10}O_4]^+$ fragment ions and it was speculated that a $C_6H_{10}O_5$ or $C_6H_{10}O_4$ fragment group might be lost. Subsequently, the fragment ion was further decomposed, losing a $C_6H_{10}O_5$ fragment group to produce fragment ion m/z 273.0754$[M+H-C_6H_{10}O_4-C_6H_{10}O_5]^+$, which later lost a $C_8H_8O$ fragment group. Finally, the fragment ion 153.0177$[M+H-C_6H_{10}O_4-C_6H_{10}O_5-C_8H_8O]^+$ was obtained. Combining with the secondary fragment ion of the reference product, the compound was inferred to be naringin. The possible pathways of flavonoid cracking were displayed in Fig. 5(a-c).

**Structural analysis of quinones:** Quinones are a kind of chemical components with quinone structure in traditional Chinese medicine, mainly including anthraquinones and most

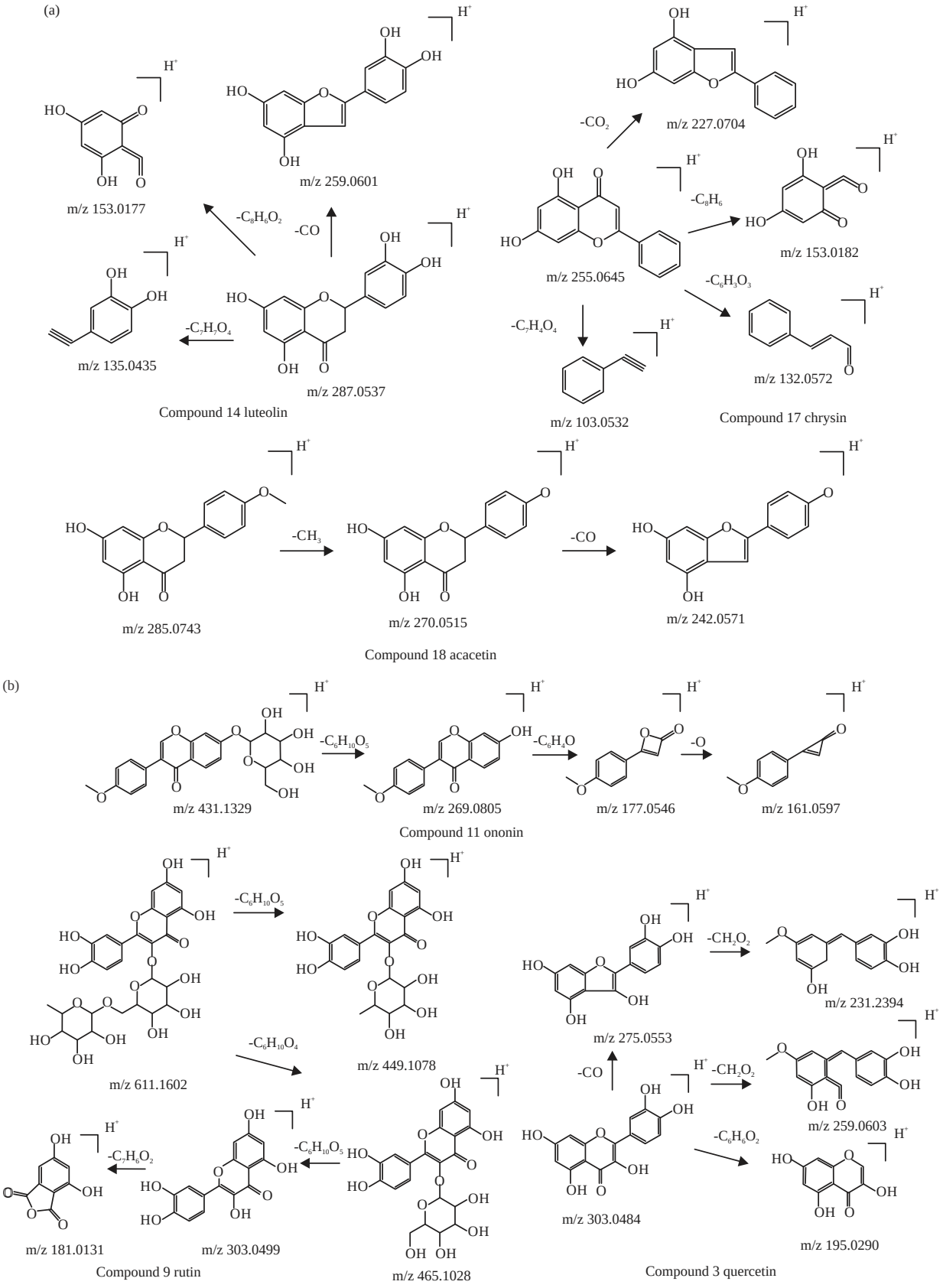


Fig. 5(a-c): Continue

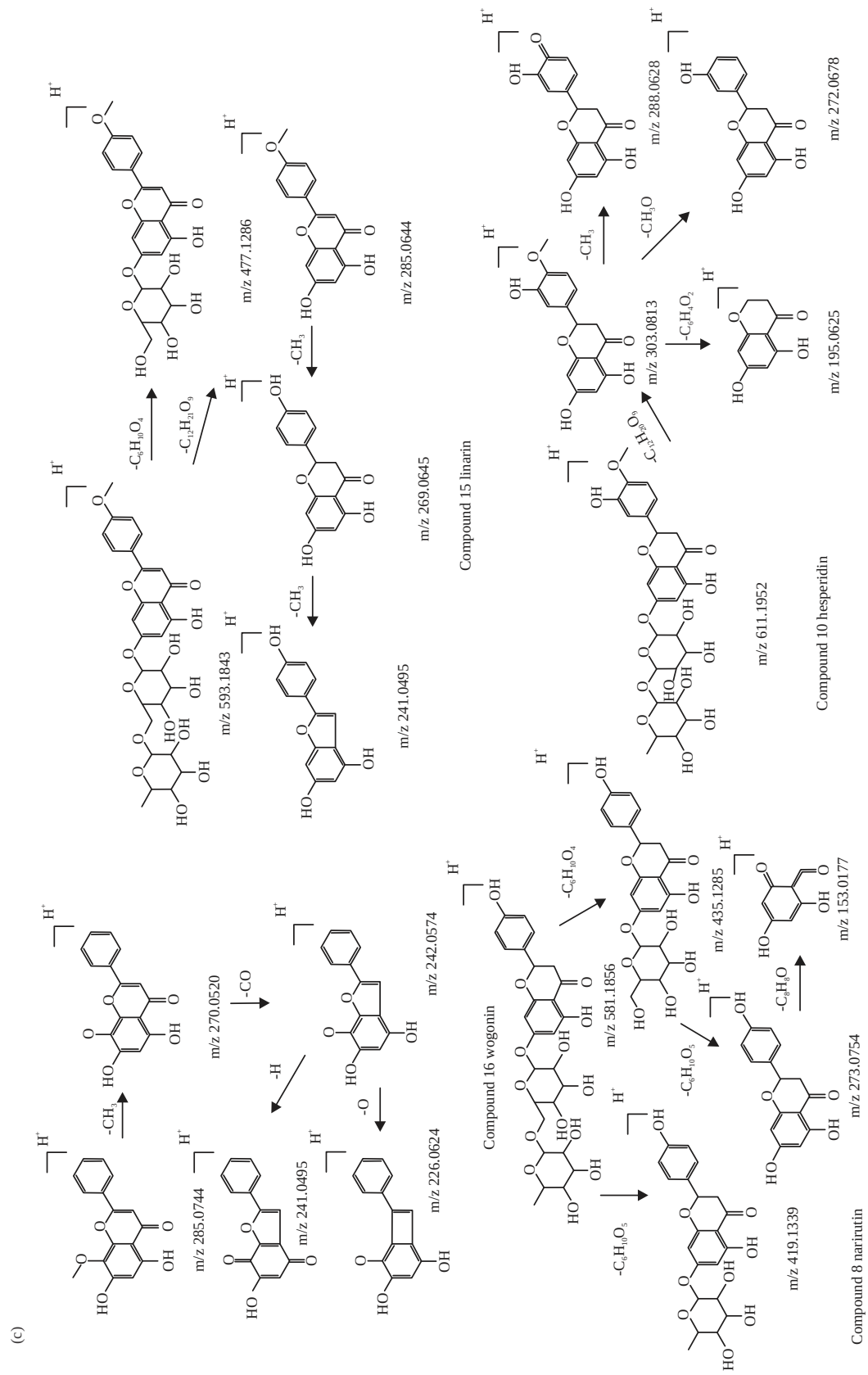


Fig. 5(a-c): Cleavage pathway of flavonoids, (a) Flavonoids, (b) Flavonols and (c) Dihydroflavonoids

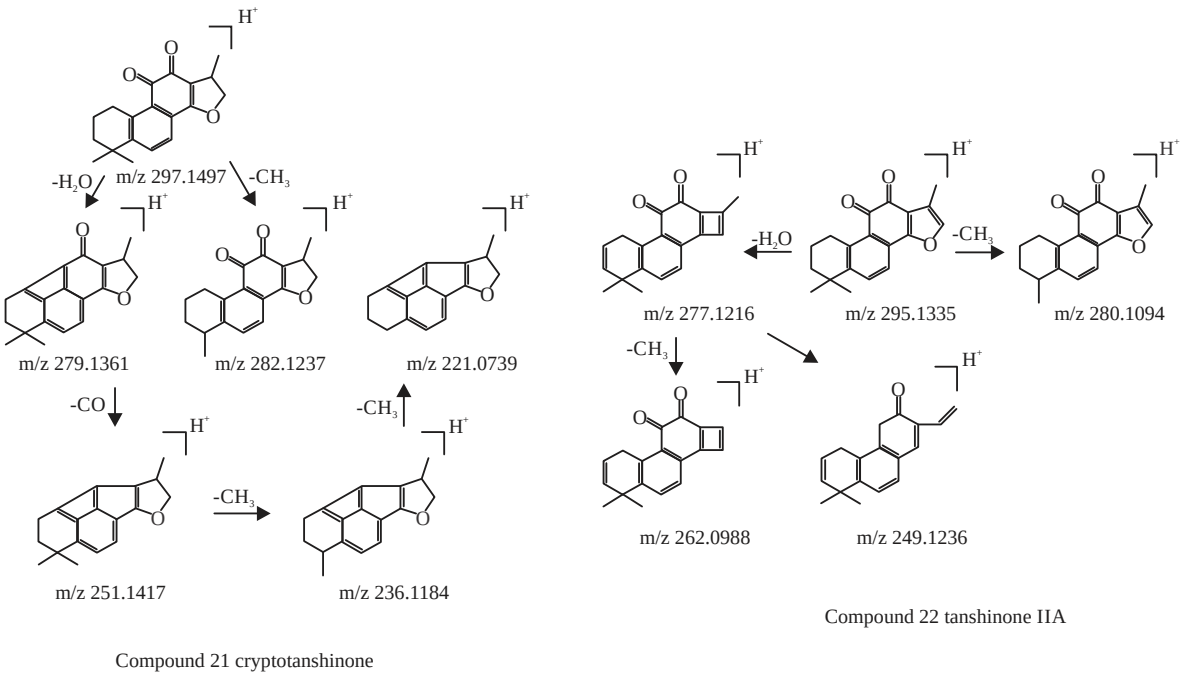


Fig. 6: Cleavage pathway of quinones

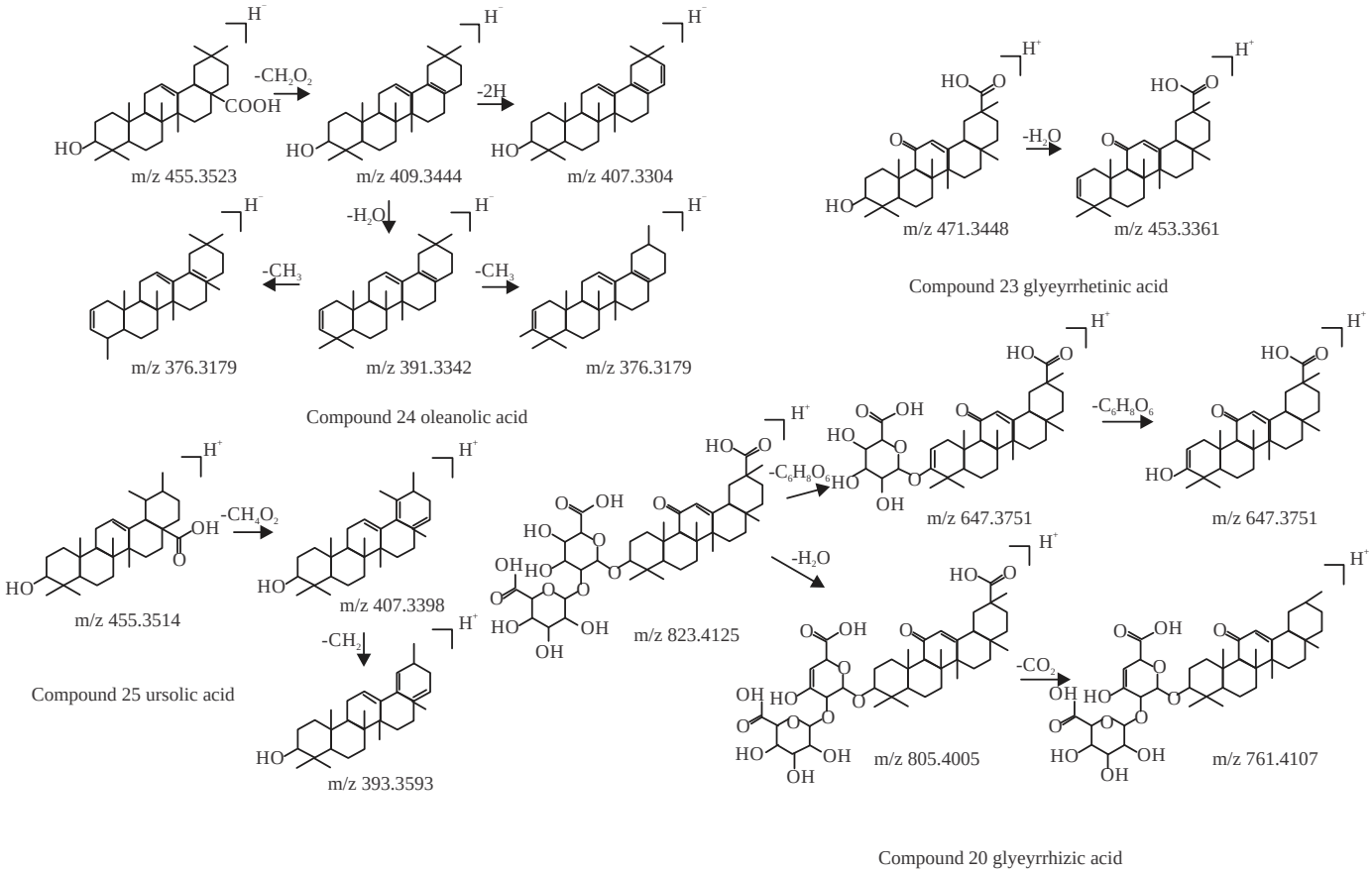


Fig. 7: Cleavage pathway of terpenoids

of them have important biological activities. Under high-energy collisions, CO is gradually lost, accompanied by the loss of $CO_2$[15]. Two quinone compounds were identified in this experiment, namely, cryptotanshinone and tanshinone IIA. For instance, the retention time of compound 22 in the positive ion mode was 23.198 min and that in the mixed reference solution was 23.196 min. The primary MS data revealed a quasi molecular ion peak of m/z 297.1497$[M+H]^+$, indicating a molecular formula of $C_{19}H_{20}O_3$. The excimer ion peaks were cleaved and one $CH_3$ fragment group and one $H_2O$ molecule were lost to generate fragment ions m/z 282.1237$[M+H-CH_3]^+$ and m/z 279.1361$[M+H-H_2O]^+$, respectively. In addition, the fragment ion m/z 279.1361$[M+H-H_2O]^+$ also lost a neutral CO molecule and a $CH_3$ fragment group successively to produce the fragment ions m/z 251.1417$[M+H-H_2O-CO]^+$ and m/z 236.1184$[M+H-H_2O-CO-CH_3]^+$. Immediately after the loss of a $CH_3$ fragment group, the fragment ion m/z 221.0739$[M+H-H_2O-CO-CH_3-CH_3]^+$ was obtained. Through comparing the retention time and secondary fragment ions with the reference product, the compound was inferred to be cryptotanshinone. Figure 6 shows the cleavage pathways of quinones.

**Structural analysis of terpenoids:** Terpenoids, especially triterpenoids, exist in the free form or as glycosides or esters combined with sugars. The cleavage mode of these compounds under MS conditions mainly shows that the sugar end of the glycoside bond is broken and one or more $H_2O$ molecules may be lost during the cleavage process[16]. In this study, 4 terpenoids were identified, which were glycyrrhizic acid, glycyrrhetinic acid, oleanolic acid and ursolic acid. For example, the retention time of compound 25 in the negative ion mode was 27.686 min and that in the mixed control product was 27.678 min. The high-resolution quasi molecular ion peak was m/z 455.3523$[M-H]^-$ and its speculated molecular formula was $C_{30}H_{48}O_3$. The quasi molecular ion was cleaved, losing one HCOOH fragment group to produce the fragment ion m/z 409.3444$[M-H-HCOOH]^-$. In addition, the fragment ions lost 2 H and 1 $H_2O$ molecules, thereby producing fragment ions m/z 407.3304 $[M-H-HCOOH-2H]^-$ and m/z 391.3042$[M-H-HCOOH-H_2O]^-$, respectively. Subsequently, a $CH_3$ fragment group was further lost to obtain m/z 376.3179$[M-H-HCOOH-H_2O-CH_3]^-$. Combined with the secondary fragment ions of the reference substance, it was inferred that the compound was oleanolic acid. The cleavage pathway of terpenoids was displayed in Fig. 7.

## DISCUSSION

In this experiment, UPLC-QTOF-MS technology was applied in combination with plasma pharmacochemical method to analyze the active monomer components of Biyuan Tongqiao granule entering into blood through clarifying the chemical composition of Biyuan Tongqiao granule. In total, 102 chemical components were analyzed and a PCDL database was established according to molecular weights, structural formulas and information of medicinal materials. By conducting differential analysis on the MS data of drug-containing plasma, blank plasma and mixed control samples after the intragastric administration of Biyuan Tongqiao granule to rats, combined with the PCDL database, chemical composition fragmentation patterns and other information, altogether 25 prototype blood components were identified, including flavonoids, organic acids, terpenes, quinones, coumarins and alkaloids. The above-mentioned blood entering prototype components were derived from 13 medicinal herbs, namely, lily magnolia, *Xanthium sibiricum* (stir-fried), Chinese *Ephedra*, *Ammi majus*, mint, rhizoma ligustici, *Scutellaria baicalensis*, *Forsythia suspensa*, *Chrysanthemum indicum*, *Rehmannia glutinosa*, *Salvia miltiorrhiza*, *Poria cocos* and licorice. These monomer chemical components provide new indexes for controlling the quality of Biyuan Tongqiao granule.

As discovered through literature review, currently, with regard to the quality control of Biyuan Tongqiao granule, the 2020 Edition of the Chinese Pharmacopoeia only uses ephedrine hydrochloride and pseudoephedrine hydrochloride as the content determination indicators. Other experimental research reports only use components such as baicalin and forsythrin as the single indicators for detection, while there is no comprehensive quality evaluation system for the compound formula of Biyuan Tongqiao granule. The 25 prototype blood components identified in this study are the potential indicator components for controlling the quality of compound prescriptions, which provide key indicators for controlling compound prescriptions. Meanwhile, the research group focused on the pharmacological effects and mechanisms of action in diseases such as acute and chronic rhinitis and sinusitis and conducted partial literature research on relevant indicators. As reported, in the blood components of Biyuan Tongqiao granule, ferulic acid regulates some inflammatory factors and exerts anti-inflammatory effects[17], whereas cryptotanshinone inhibits the inflammatory response by lowering the levels of IL-4, IL-5 and IL-13 and elevating the

level of Th1 cytokine IFN-γ[18]. In the study on drugs for the treatment of other sinusitis, chemical components such as isoimperatorin and oleanolic acid have been reported to exert therapeutic effects at varying degrees. In line with the plasma pharmacochemistry theory that "drugs must first be absorbed into the blood to play a therapeutic role"[19], the research team will construct the quality control system of Biyuan Tongqiao granule on the basis of qualitative and quantitative index methodology, by combining the discovery mechanism of disease network pharmacology with the discovered prototype blood components in the future. This research provides experimental data to ensure the effectiveness, safety and controllability of traditional Chinese patent medicines and simple preparations of Biyuan Tongqiao granule.

## CONCLUSION

In the current work, UPLC-QTOF-MS technology combined with PCDL database is adopted to analyze the blood entering components of Biyuan Tongqiao granule after oral administration in rats. A total of 25 prototype monomers are identified, including 11 flavonoids, 4 organic acids, 4 terpenoids, 2 quinones, 2 coumarins and 2 alkaloid components, which come from 13 medicinal herbs in the compound. This study lays an experimental foundation for the selection of quality control indicators for the compound.

## SIGNIFICANCE STATEMENT

Biyuan Tongqiao granule has been widely applied in the treatment of acute and chronic rhinitis, sinusitis and other external evils attacking the lungs. Clinical data have suggested that the use of Biyuan Tongqiao granule alone or in combination is more effective and safer than conventional Western medicine treatment, traditional Chinese medicine preparations, or surgical treatment alone. Although it consists of 14 traditional Chinese medicinal herbs, its current quality control indicators are limited and its therapeutic effect cannot be better guaranteed. The 25 prototype blood components identified in this study are the potential indicator components for controlling the quality of compound prescriptions, which will provide key indicators for controlling compound prescriptions and lay a foundation for their rational clinical application.

## ACKNOWLEDGMENTS

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