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ORIGINAL ARTICLE

Rapid Analysis of Components in *Coptis chinensis* Franch by Ultra-Performance Liquid Chromatography with Quadrupole Time-of-Flight Mass Spectrometry

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ABSTRACT

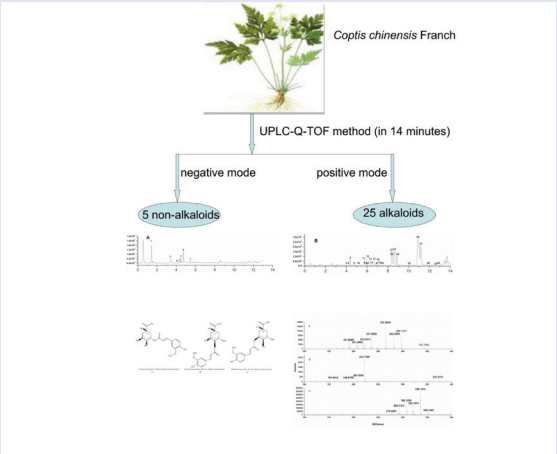
Background: *Coptis chinensis* Franch is a traditional Chinese medical herb. **Objective:** In this article, ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry was used to rapidly, qualitatively, and comprehensively identify the components in *Coptis chinensis* Franch. **Materials and Methods:** Chromatographic separation was achieved on an Agilent Zorbax RRHD Eclipse Plus C_{18} column. The mobile phase consisted of 0.1% formic acid water (A) and 0.1% formic acid acetonitrile (B) with a gradient program. Qualitative analysis was performed on an Agilent 6540 quadrupole time-of-flight mass spectrometer, which was equipped with a Dual AJS ESI source operating in negative mode. **Results:** A total of 30 alkaloid and non-alkaloid components of *Coptis chinensis* Franch were identified in only 14 min. **Conclusion:** This study helped to provide a basis for the quality control of *Coptis chinensis* Franch.

Key words: Alkaloids, Coptis chinensis Franch, non-alkaloids, Q-TOF, UPLC

SUMMARY

- Qualitative analysis method of chlorogenic alkaloids and non-alkaloids in *Coptis chinensis* Franch is developed by Ultra-performance liquid chromatography with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS/MS) method.
- Established UPLC-Q-TOF-MS/MS analysis method is validated with rapidness and accuracy.
- The developed method was successfully applied for qualitative analysis of *Coptis chinensis* Franch sample collected from cultivation place in China.

Abbreviations used: Q-TOF-MS: quadrupole time-of-flight mass spectrometry, UPLC: ultra-performance liquid chromatography, POS: positive, NEG: negative.



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INTRODUCTION

The traditional Chinese medicine, Coptis chinensis, is the dry root and stem of *Coptis chinensis* Franch, and is widely used in clinic. It is also called 'Weilian'.[1] Coptis chinensis is a common detoxification agent in traditional Chinese medicine, which can purge fire and clear heat, with a very bitter taste. Earlier assessment[2] has verified the antibacterial and anti-inflammatory functions of the active components of Coptis chinensis. Many studies[3-6] have also evaluated the pharmacodynamics effects of the active components on high blood sugar, high cholesterol, arrhythmia, cerebral ischemia, and heart failure. Liu *et al.*[7,8] studied the main active components in Coptis chinensis using high-performance liquid chromatography. However, the analysis was incomplete, and non-alkaloids were seldom reported.

Ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS/MS) has been widely used in the field of analytical chemistry and in the quality control of traditional Chinese medicine[9-12] because of its high resolution, high sensitivity, and high resolution. UPLC-Q-TOF-MS/MS can extrapolate the molecular formula and chemical structural composition of compounds according to the molecular weight and fragment ions in the secondary MS of the compound. In this study, UPLC-Q-TOF-MS/MS was used to identify the alkaloids and non-alkaloids in *Coptis chinensis* Franch.

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MATERIALS AND METHODS

Chemicals and instruments

Methanol, formic acid, and acetonitrile (all LC-MS grade) were purchased from Thermo Fisher (United States). Other reagents were of analytical grade. Agilent 1290 UPLC, which was equipped with a binary pump, an online degasser, a column oven, an autosampler, and a diode array detector, was purchased from Agilent Technologies Inc. The Agilent 6540 TOF resolution mass spectrometer, which was equipped with a Dual AJS ESI ion source and a Masshunter Data Acquisition Online Workstation and Qualitative Analysis Offline Analysis Software, was purchased from Agilent Technologies Inc. A KQ-250B ultrasonic cleaner was purchased from Kunshan Ultrasonic Instrument Co., Ltd. An N-1100 rotary evaporator was purchased from Shanghai Ailang Instrument Co., Ltd. A BP211D Balance was purchased from Sartorius Scientific Instrument Co., Ltd. A DFT-50 type grinder was purchased from Wenling Linda Machinery Co., Ltd.

Sample preparation

Coptis chinensis was purchased from Chongqing Wanglong Berberine Ltd. and identified to be the root and stem of *Coptis chinensis* Franch by Dr. Wei Sun of the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences.

Coptis chinensis Franch was crushed into powder, which was filtered with a 40-mesh screen. Then, 1.0 g of powder, which was mixed with 10 mL of methanol (70%), was extracted ultrasonically 30 min before the collection of the filtrate. The remaining powder was treated with methanol (70%) twice according to the above-mentioned method. Three mixed filtrates were concentrated by evaporation using a rotary evaporator until the methanol was completely evaporated. The 160 × sample preparation was finished after the mixture of methanol (70%), and the evaporated extract was standardized to be 5 mL. Then, the 160 × sample was diluted and filtered with a 0.22 μm microporous membrane before the injection.

UPLC-Q-TOF Parameters

For the analysis, a 1290 series UPLC system coupled to a 6540 quadrupole TOF MS was used. The 6540 Q-TOF system was equipped with an Agilent JetStream ESI interface and was operated by Masshunter Workstation B.04.01 software. Precursor and production selection, and the optimization of collision energies, were performed with flow injection of single-analyte solutions using Masshunter Optimizer software. The analytical column was a ZORBAX RRHD Eclipse Plus C18 (100 × 3 mm, 1.8 μm) column from Agilent Technologies. Chromatographic separation was performed at 45°C with a flow rate of 800 μL/min. Eluent A was composed of water/formic acid (99.9:0.1, v/v), and eluent B was composed of acetonitrile/formic acid (99.9:0.1, v/v). The total time of the chromatographic run was 14 min, which comprised the following: 0–4 min, 5–15% B; 4–5 min, 15–17% B; 5–12 min, 17–24% B; and 12–14 min, 24–95% B.

The general source settings in the positive (pos.) and negative (neg.) ionization modes were as follows: gas temperature, 300°C; gas flow, 5 L/min; nebulizer, 35 psi; sheath gas temperature, 350°C; sheath gas flow, 11 L/min; capillary voltage, 4000 V (pos.) and 3500 V (neg.); nozzle voltage, 1500 V; capillary outlet voltage, 175 V; collision energy, 30 V; and reference mass, *m*/*z* 121.0509, 922.0098 (pos.), 119.0363, 1033.9881 (neg.).

RESULTS

UPLC-Q-TOF Total Ion current chromatogram of the Extract of Coptis chinensis Franch

As shown in Figure 1, the components of the sample solution were collected and analyzed qualitatively in both positive and negative modes within 14 min, and they were well separated.

Identification of alkaloids in the extract of coptis chinensis Franch by UPLC-Q-TOF

The main alkaloids identified in the extract of *Coptis chinensis* Franch included three apomorphine alkaloids, three tetrahydroprotoberberines alkaloids, and 17 protoberberine alkaloids, as shown in Figure 2. Most of the side chains of the alkaloids were connected with -O-CH_2-O-, -OCH_3, -OH, and -CH_3. There is a quasi-molecule ion peak of $M]^+$ or $[M+H]^+$ in the positive-mode ESI mass spectrum of most alkaloids. The collision-induced dissociation (CID) qualified the cleavage of the side chain and the opening and closing of the cyclic structure in the MS^2 spectra.

In the MS^2 spectra of peaks 5, 8, and 11, the $[M-C_2H_7-N]^+$, $[M-C_2H_7-CH_3]$, and $[M-C_2H_7N-CH_3-CH_3OH]^+$ ions were observed, which was consistent

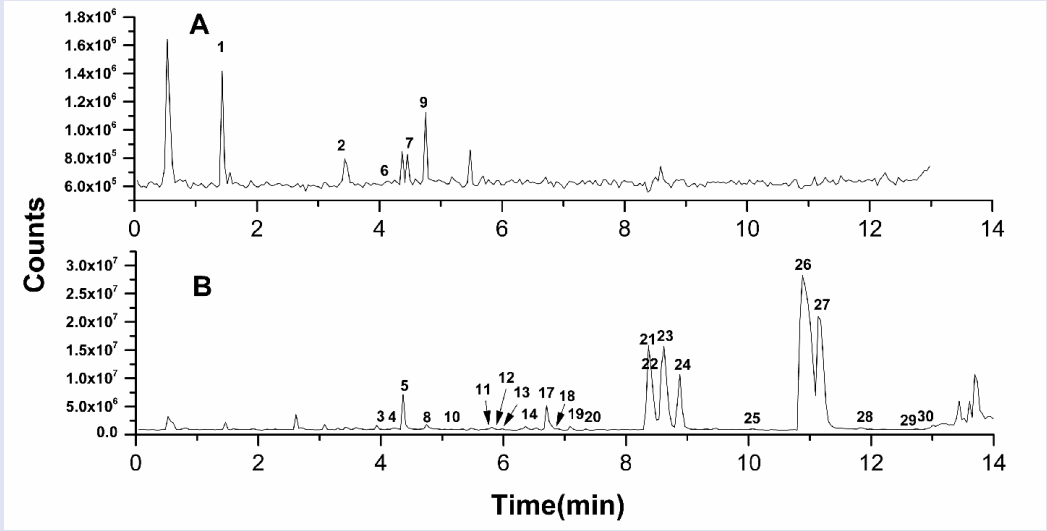

Figure 1: UHPLC-Q-TOF total ion current chromatogram of the extract of *Coptis chinensis* Franch. A: Negative mode; B: Positive mode.

with the structural assignment of the apomorphine alkaloid, suggesting that peaks 5, 8, and 11 may be this type of alkaloid.

Peak 5 shows three strong absorption bands (225--235, 270--280, and 315--335 nm) in the ultraviolet (UV) spectrum.[13] As shown in Table 1, ions of *m/z* 342.1703 ($[M]^+$) were observed, and their inferred molecular formula was $C_{20}H_{24}NO_4$. In the MS^2 spectra, *m/z* 297.11 ($[M\text{-}C_2H_7N]^+$), 282.0886 ($[M\text{-}C_2H_7N\text{-}CH_3]^+$), 265.0854 ($[M\text{-}C_2H_7N\text{-}CH_3OH]^+$), and 237.0900 ($[M\text{-}C_2H_7N\text{-}CH_3OH\text{-}CO]^+$) were observed after the energy collisions to infer that peak 11, peak 5 [Figure 3a], and peak 8 were Menisperine, Magnoflorine, and Norisocorydine, respectively.

RDA reaction, is a relatively common mass craking reaction. The six-membered ring containing endo-double bond is decomposed into a conjugated diene, alkene or alkyne under high temperature conditions. It is a concerted reaction. RDA reaction, is a relatively common mass craking reaction. The six-membered ring containing endo-double bond is decomposed into a conjugated diene, alkene or alkyne under high temperature conditions. It is a concerted reaction. RDA reaction occurs after the collision energy occurs in the tetrahydroprotoberberines alkaloids, which can cause the end chain, such as $-CH_3$, to fracture. Thus, it was inferred that peaks 13, 14, and 20 belonged to this type of alkaloid.

Peak 14 was one of the characteristic alkaloid absorption peaks in the UV graph. As shown in Table 1, ions of *m/z* 372.1806 ($[M]^+$) were observed in MS^1. Hence, its inferred molecular formula was $C21H_{26}NO_5$. Simultaneously, ions of $[M\text{-}C_8H_{22}O_2]^+$, $[M\text{-}C_8H_{22}O_2\text{-}CH3]^+$, and $[M\text{-}C_8H_{22}O_2\text{-}CH_3\text{-}H_2O]^+$ were also observed in MS^2, so peak 14 was inferred to be Stecepharine [Figure 3b], N-methlylcorydalmine, or its isomers.

As the important components in alkaloids, the fracture of side chains such as $-O\text{-}CH_2\text{-}O-$, $-OCH_3$, -OH, and $-CH_3$ in protoberberine alkaloids can produce the 28 Da (CO), 15 Da (CH_3), and 18 Da (H_2O) fragment ions. Thus, 17 components, such as peaks 3, 4, 12, 15, and 16, could be tentatively identified as protoberberine alkaloids based on the reported literature.[14-18]

The *m/z* of peak 27 was 352.1561 [Table 1], and peak 27 could be identified as a type of alkaloid according to the UV chromatogram

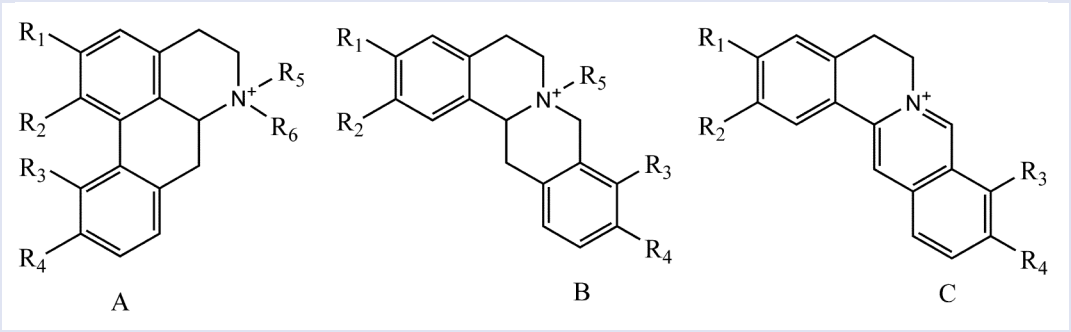

Figure 2: Chemical structures of the most reported alkaloids in the extract of *Coptis chinensis* Franch. A: Apomorphine alkaloids; B: Tetrahydroprotoberberines alkaloids; C: Protoberberine alkaloids.

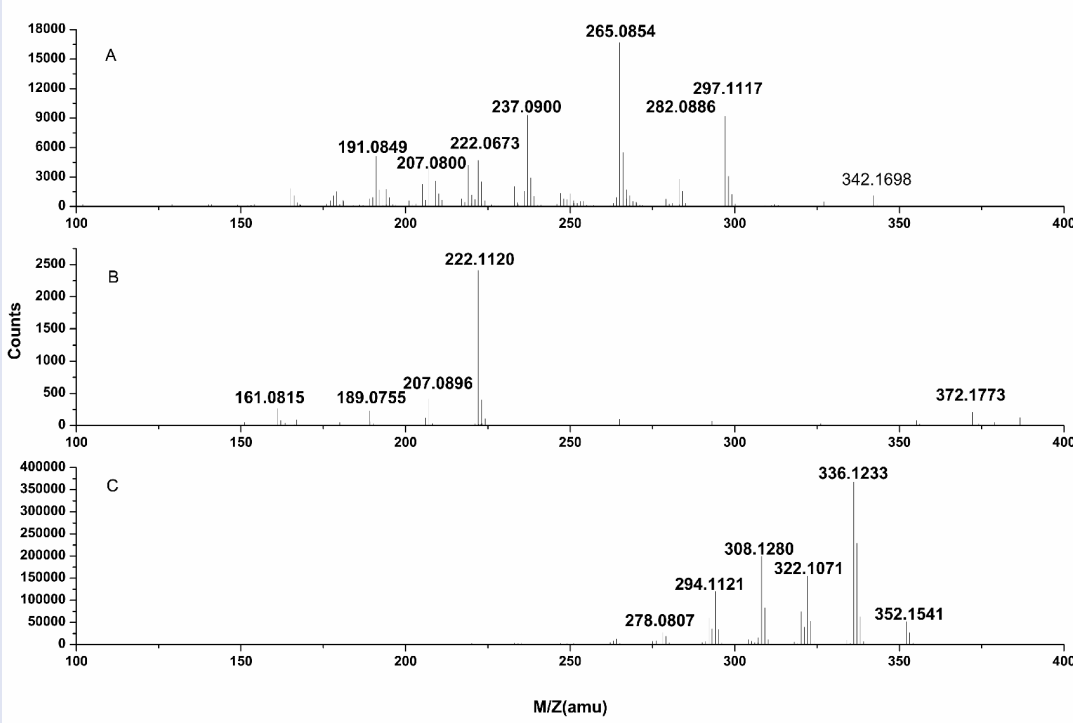

Figure 3: MS2 spectra of typical alkaloids. A: Magnoflorine; B: Stecepharine; C: Palmatine.

Table 1: Identification and analysis of the extract of *Coptis chinensis* Franch

Peak number	Compound	Molecular formula	Rt (min)	ppm	Calculated m/z	m/z fragment ion
1	Unknown	-	1.462	-	359.0985	197.0454,179.0345,135.0451
2	5-O-Feruloylquinic acid	$C_{17}H_{20}O_9$	3.464	0.82	367.1032	191.0557
3	13-hydroxycolumbamine/13-hydroxyjatrorrihizine	$C_{20}H_{20}NO_5$	3.942	0.00	354.1326	339.1092, 324.0860, 296.0908, 306.0750, 278.0797
4	Tetradehydroscoulerine/ tetradehydrocheilanthifolinium	$C_{19}H_{16}NO_4$	4.198	-3.42	322.1068	307.0842, 294.0749, 279.0512
5	Magnoflorine	$C_{20}H_{24}NO_4$	4.368	-0.58	342.1703	297.1117, 282.0886; 265.0854, 237.0900
6	Unknown	-	4.378	-	340.1548	326.1364, 310.1076, 282.1126, 252.0408, 224.0468
7	3-O-Feruloylquinic acid	$C_{17}H_{20}O_9$	4.444	-1.09	367.1025	191.0551, 173.0449
8	Norisocorydine	$C_{19}H_{22}NO_4$	4.539	-1.83	328.1543	313.1335, 298.1038
9	4-O-Feruloylquinic acid	$C_{17}H_{20}O_9$	4.742	0.82	367.1032	191.0560, 173.0454
10	Lycoranine B	$C_{18}H_{13}NO_4$	5.178	-1.61	308.0918	280.0972, 265.0726, 250.0846
11	Menisperine	$C_{21}H_{26}NO_4$	5.817	-2.53	356.1853	311.3328, 296.0998
12	13-hydroxyberberine	$C_{20}H_{18}NO_5$	5.902	-1.42	352.1180	337.0859, 336.0862, 308.0889; 322.0953, 318.0731, 294.0759
13	N-methlylcorydalmine/isomer	$C_{21}H_{26}NO_4$	5.987	-1.40	356.1857	206.1170
14	Stecepharine	$C_{21}H_{26}NO_5$	6.328	-1.34	372.1806	222.1120, 207.0896, 189.0755
15	Demethyleneberberine/isomer	$C_{19}H_{18}NO_4$	6.413	-1.54	324.1231	309.0977, 294.0743, 266.0782; 280.0966
16	Demethyleneberberine/isomer	$C_{19}H_{18}NO_4$	6.498	-1.54	324.1231	309.0995, 294.0746, 266.0841; 308.0883, 280.0975
17	Berberrubine/Thalifendine	$C_{19}H_{16}NO_4$	6.711	-2.17	322.1072	307.0837, 294.1126, 279.0880
18	Lincangenine/stephabine	$C_{21}H_{21}NO_5$	6.882	3.26	368.1510	355.1073, 327.1082, 308.0946, 294.0733, 278.0828
19	13-methyljatrorrhizine/13-methylcolumbamine	$C_{20}H_{18}NO_5$	7.095	-0.28	352.1184	337.0900, 322.0704, 294.0752, 336.0868, 308.0913
20	N-methlylcorydalmine/isomer	$C_{21}H_{26}NO_4$	7.563	-0.84	356.1859	206.1169
21	Coptisine	$C_{19}H_{14}NO_4$	8.373	-2.50	320.0915	292.0963, 277.0726, 262.0857; 290.0808
22	Columbamine	$C_{20}H_{20}NO_4$	8.586	-0.30	338.1391	322.1069, 294.1120; 308.0910, 280.0957, 265.0730
23	Epiberberine	$C_{20}H_{18}NO_4$	8.628	0.30	336.1237	320.0918, 292.0966, 262.0862; 308.0913
24	Jatrorrhizine	$C_{20}H_{20}NO_4$	8.884	0.30	338.1393	322.1072, 294.1122; 308.0910, 280.0961
25	13-methylepiberberine	$C_{21}H_{20}NO_4$	10.077	-1.14	350.1388	334.1071, 306.1109; 322.1015
26	Berberine	$C_{20}H_{18}NO_4$	10.886	-1.79	336.123	321.0976, 320.0918, 292.0966; 306.0761, 304.0967, 278.0809
27	Palmatine	$C_{20}H_{18}NO_5$	11.142	-0.28	352.1561	337.1287, 336.1233, 308.1280, 322.1071, 294.1121
28	13-methylcoptisine	$C_{20}H_{16}NO_4$	11.823	-0.90	334.1076	318.0762, 290.0803; 304.0602, 276.0652
29	13-methylpalmatine	$C_{22}H_{24}NO_4$	12.675	-1.37	366.17	351.1480, 334.1406, 322.1437, 308.1265, 306.1095, 292.0922
30	13-methylberberine	$C_{21}H_{20}NO_4$	12.761	-0.57	350.139	335.1134, 306.1110; 320.0912, 292.0964

and ESI(+) MS. Thus, the inferred molecular formula of peak 27 was $C_{20}H_{18}NO_5$. In the MS^2 experiment, the predominant ions appeared at *m/z* 337.1287 $[M\text{-}CH_3]^+$, 336.1233 $[M\text{-}CH_3\text{-}H]^+$, 308.1280 $[M\text{-}CH_3\text{-}H\text{-}CO]^+$, 322.1071$[M\text{-}2CH_3]^+$, and 294.1121 $[M\text{-}2CH_3\text{-}CO]^+$, which was consistent with the reported literature.[16] Hence, we inferred that peak 27 was Palmatine [Figure 3c] shows its MS^2 chromatogram. The other 16 peaks were also observed and identified using this method.

Peaks 10 and 18 were identified as types of alkaloids according to the UV chromatogram. The *m/z* values of peaks 10 and 18 were 308.0918 and 368.1510, respectively, which was also consistent with the literature.[17,19] Thus, peaks 10 and 18 were identified as Lycoranine B and lincangenine/stephabine, respectively.

Identification of non-alkaloids in the extract of coptis chinensis franch by UPLC-Q-TOF

Most of the non-alkaloids that were identified in the extract of *Coptis chinensis* Franch belonged to feruloylquinic acid in positive mode. According to the accurately measured *m/z* of peaks 2, 7 and 9, they had almost the same *m/z* (367.10) as the fragment ion of $[M–H]^-$. Thus, it was inferred that their molecular formula was $C_{17}H_{20}O_9$. In addition, both fragment ions with *m/z* 191.0551 [M-Feruloyl H] and 173.0449 [M-Feruloyl H-H_2O] were observed in the three peaks, from which we can infer that peaks 2, 7, and 9 may be isomers. Peaks 2, 7, and 9 were inferred to be 5-O-feruloylquinic acid, 3-O-feruloylquinic acid, and 4-O-feruloylquinic acid, respectively, because of the difference in retention time, which is caused by different polarities, according to previous reports.[19,20] The chemical structure of them is shown in Figure 4.

CONCLUSION

UPLC-Q-TOF-MS/MS was used to identify the alkaloids and non-alkaloids in the extract of *Coptis chinensis* Franch. The *m/z* value was accurately measured, and the probable molecular composition and formula were analyzed according to the fragment ions of the extract. In total, 30 components were identified (including 25 alkaloids in positive

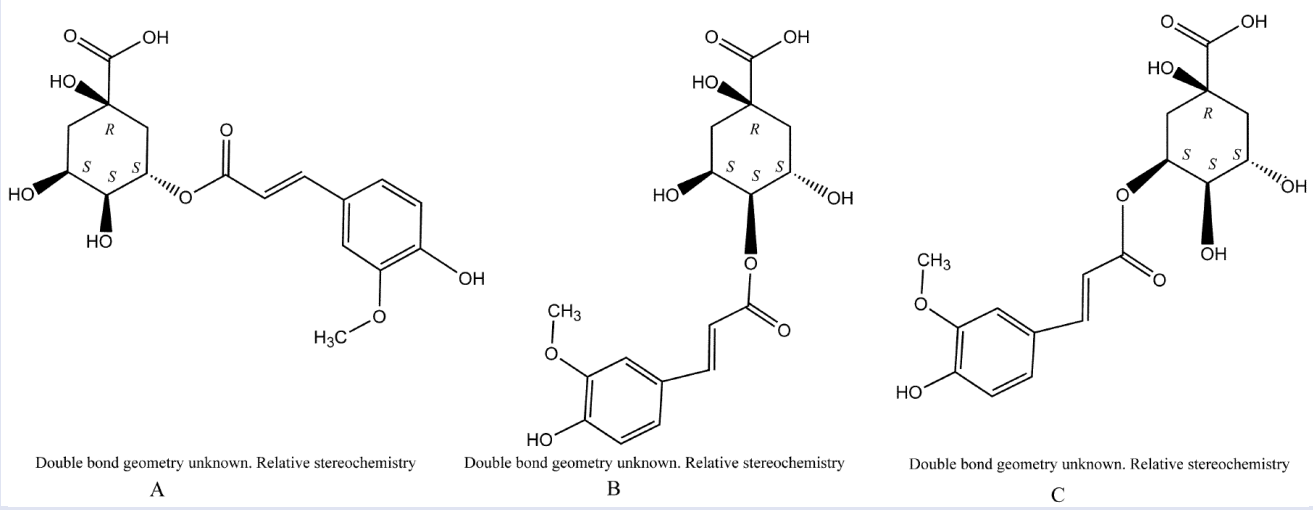

Figure 4: Chemical structures of three non-alkaloids identified in the extract of Coptis chinensis Franch. A: 3-O-Feruloylquinic acid; B: 4-O-Feruloylquinic acid; C: 5-O-Feruloylquinic acid.

mode and five non-alkaloids in negative mode) in 14 min according to the fragment ions in MS^2 and accurate *m/z* in MS^1 of the extract. The method developed in this article may provide a reference for the identification and analysis of compound used traditional Chinese medicine, and other samples, because of the rapid and comprehensive qualitative analysis of the extract of *Coptis chinensis* Franch. Two unknown components were not reported, and it remains to be determined if they are new components in the extract of *Coptis chinensis* Franch.

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Conflicts of interest

There are no conflicts of interest.

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