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ORIGINAL

Rapid Profiling of Chemical Constituents in Qingfei Paidu Granules Using High Performance Liquid Chromatography Coupled with Q Exactive Mass Spectrometry

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Abstract

Qingfei Paidu (QFPD) granules have played a critical role during the Coronavirus Disease 2019 (COVID-19) in China. However, worldwide acceptance has been a problem because of the complex ingredients and unique theory of treatment. In this study, high-performance liquid chromatography (HPLC)-Q Exactive Orbitrap-mass spectrometry (MS) and the Orbitrap traditional Chinese medicine library (OTCML) were used to investigate the chemical constituents of QFPD granules. By comparing retention times, masses, isotope ion patterns, and MS^2 profiles, 108 compounds were putatively identified using the OTCML combined with manual verification, including 12 alkaloids, 49 flavonoids, 13 terpenoids, 14 phenylpropanoids, 4 phenolic acids, 5 phenols, and 11 other phytochemicals. Of these compounds, 17 were confirmed using reference standards. In addition, representative compounds of these different chemical types were used as examples to analyze the fragmentation pathways and characteristic product ions. Moreover, 20 herbs within the QFPD granules were also identified to establish the sources of these chemical components. This is the first rapid profiling of the chemical constituents of QFPD granules using HPLC-Q Exactive Orbitrap-MS and yields valuable information for further quality control and mechanistic studies of QFPD granules.

Keywords Chemical constituent identification · Qingfei Paidu granules · HPLC-Q Exactive Orbitrap-MS · Orbitrap traditional Chinese medicine library

Introduction

Qingfei Paidu (QFPD) granules and decoctions are effective traditional Chinese medicines (TCMs) that are included in the Guidelines for Diagnosis and Treatment of COVID-19 Pneumonia, issued by the National Heath Commission of the People's Republic of China [1]. QFPD granules and decoctions are based on the following four formulae: Maxing-Shigan-Tang, Wuling-San, Xiaocaihu-Tang, and Shegan-Mahuang-Tang [2], which are different forms of prescription QFPD. QFPD granules contain 20 herbs: *Ephedrae Herba*, *Glycyrrhizae Radix Et Rhizoma Praeparata Cum Melle*, *Armeniacae Semen Amarum*, *Cinnamomi Ramulus, Pogostemonis Herba*, *Alismatis Rhizoma*, *Polyporus*, *Atractylodis Macrocephalae Rhizoma*, *Poria*, *Bupleuri Radix*, *Scutellariae Radix, Pinelliae Rhizoma Praeparatum Cum Zingibere Et Alumine*, *Zingiberis Rhizoma Recens*, *Asteris Radix Et Rhizoma*, *Farfarae Flos*, *Belamcandae Rhizoma*, *Asari Radix Et Rhizoma*, *Dioscoreae Rhizoma*, *Aurantii Fructus Immaturus*, and *Citri Reticulatae Pericarpium*. In addition, QFPD contains the mineral *Gypsum Fibrosum*.

In China, QFPD granules and decoctions have been widely used to treat patients infected with SARS-CoV-2 owing to positive treatment results. Early treatment with prescription QFPD was associated with favorable patient outcomes and may be an effective strategy for epidemic control

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[1]. Functional network pharmacology analysis units showed that QFPD protected against COVID-19 through anti-viral and anti-inflammatory activities [2]. A systematic pharmacological study illustrated that QFPD exhibited immune regulation, anti-infection and anti-inflammatory properties, and multi-organ protection [3]. QFPD granules were, therefore, approved for market use by the National Medical Products Administration in China [4]. However, worldwide acceptance of QFPD granules is challenging because of the TCM complexity, and unique theory of treatment, in addition to quality and safety issues [5, 6]. Thus, comprehensive identification of the chemical components of QFPD granules is extremely critical for quality control, in addition to identification of the active ingredients and investigation of the mechanism-of-action.

Few analytical strategies have been applied to study the chemical constituents of QFPD decoctions, and no detailed analysis of the chemical composition of QFPD granules has been reported [7–9]. Hybrid quadrupole-Orbitrap mass spectrometry (MS) is a powerful tool for structure elucidation of TCMs due to its high resolution and high-quality MS^2 fragmentation patterns. In this study, high-performance liquid chromatography (HPLC)-Q Exactive Orbitrap-MS was used to analyze the chemical constituents of QFPD granules, with 108 compounds putatively identified, including 12 alkaloids, 49 flavonoids, 13 terpenoids, 14 phenylpropanoids, 4 phenolic acids, 5 phenols, and 11 other phytochemicals. The individual herbs within the QFPD granules were also analyzed. The aim of this study is to develop an analytical method for elucidating the chemical constituents of QFPD granules and provide valuable quality control and mechanism-of-action data.

Material and Methods

Reagents and Materials

QFPD granules were a gift from Renmin Hospital of Wuhan University. The 21 raw materials were purchased from Yifeng Pharmacy Chain Co., Ltd. (Changde, China). Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). Formic acid was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Watsons distilled water was obtained from Jingdong Mall (Beijing, China).

Authentic standards of cytosine, sucrose, citric acid, uridine, adenosine, 2-pyrrolidinecarboxylic acid, and guanosine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nicotinic acid was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Nicotinamide and tangeretin were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). Salicylic acid was acquired from Ascender Chemical Co., Ltd. (Shanghai, China). Glycyrrhizic acid, 18-β-glycyrrhetinic acid, isoliquiritigenin, baicalin, and narirutin were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Chlorogenic acid was a gift from Thermo Fisher Scientific.

Standard Solutions and Sample Preparations

The QFPD granules were ground, and the resultant powder (0.4 g) was accurately weighed, dissolved in 60% methanol (v/v; 20 mL), and sonicated for 30 min, resulting in partial precipitation of the QFPD granules. The solution was centrifuged, and the supernatant was filtered through a 0.22 μm membrane prior to HPLC-Q Exactive Orbitrap-MS.

The individual raw materials were treated using the same procedure.

The authentic standards were dissolved in 50% methanol and stored at – 80 ℃. Prior to qualitative analysis, they were mixed appropriate concentrations and filtered using a 0.22 μm membrane.

HPLC-Q Exactive Hybrid Quadrupole-Orbitrap MS

LC–MS was performed using an UltiMate 3000 UPLC system (Thermo Fisher Scientific), autosampler, a vacuum degasser, binary pump, and column compartment. A Hypersil Gold aQ C18 column (2.1 × 150 mm, 3 μm) was used at 40 ℃ for chromatography. The mobile phase consisted of acetonitrile/0.1% formic acid (A) and water/0.1% formic acid (B) at a flow rate of 0.2 mL/min. The following gradient elution program was used: 0–2 min, 0–5% (A); 2–42 min, 5–95% (A); 42–46.9 min, 95% (A); 46.9–47 min, 95–5% (A); 47–50 min, 5% (A). The total run time was 50 min, and the sample injection volume was 5 μL.

A Q Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific) with heated electrospray ionization (ESI) was used. Source parameters were optimized with a spray voltage of 3.5 kV (+)/3.2 kV (−). The other parameters were set as follows: capillary temperature, 320 ℃; auxiliary gas temperature, 350 ℃; sheath gas, 40 Arb; auxiliary gas, 15 Arb; sweep gas, 0 Arb; S-lens RF level, 50.

The Orbitrap mass detector was operated in full scan plus data-dependent MS^2 mode. The MS resolution was set at 70,000 for the full scan and 17,500 for the MS^2 scan. The automatic gain control target and maximum injection time were 1×10^6 ions capacity and 100 ms, respectively. The top N (N: the number of most abundant ions for fragmentation) was five, while the scan range was *m/z* 100–1500. The normalized collision energies were 20%,

40%, and 60%, and the isolation window was 1.2 Da. The apex trigger was 5–15 s, and the loop count was 3. The dynamic exclusion was 5 s.

Data Analysis Using the Orbitrap Traditional Chinese Medicine Library (OTCML) and Manual Verification

The raw data were imported into the Compound Discoverer (CD) software, which is integrated into the OTCML. The molecular masses, retention times, fragments, and peak areas from both the positive and negative ESI modes were compared to the mzVault library, which was integrated into CD. The mzVault spectral library (Thermo Fisher Scientific) contained the retention times, precise mass ions, and MS^2 fragments of 1200 commercial reference standards, which were analyzed using Q Exactive Orbitrap-MS. The software identified peaks with high mass accuracy (< 10 ppm) and an isotope pattern variation within 85%. The molecular compositions adhered to the H/C ratio rules and were matched to potential compounds using ring and double-bond equivalents. The MS^2 profiles were compared with the reference spectra from the mzVault library. Compounds were identified only when the match score was > 85. In addition, compound identification accuracy was improved by comparing the obtained data and possible fragmentation patterns with those in the literature, and the corresponding individual herb pieces components were analyzed to determine the source of each compound and elucidate chemical compositions.

Results and discussion

Positive and negative ion modes were used to detect the chemical compounds within the QFPD granules. The base peak chromatograms (BPCs) of the QFPD granules are shown in Fig. 1. In total, 108 compounds are putatively identified (Table 1). The BPCs of the individual herb pieces are shown in Figs. S1 and S2. Compound identification is summarized below.

Alkaloids

Twelve alkaloids were detected. Compounds **19**, **20**, **21**, **15**, and **16** are observed in the positive BPC of QFPD, with no matching identification results after data processing using the OTCML. The mass spectra of compounds **15**, **19**, and **21** display the same fragment ions at *m/z* 117.0701 (Fig. S3). The mass spectra of compounds **19** and **20** exhibit the same $[M+H]^+$ ions at *m/z* 166.1226 ($C_{10}H_{15}NO$), with the same fragment ions also observed at *m/z* 148.1120 [M + H

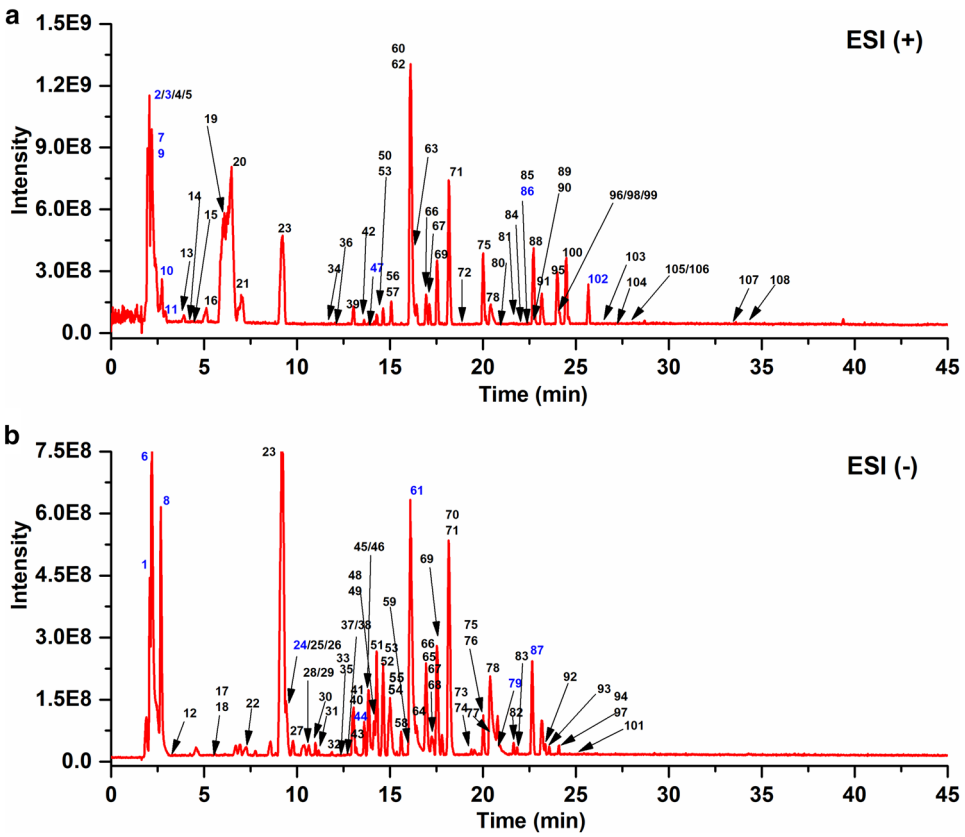

Fig. 1 Base peak chromatograms of QFPD granules obtained using high performance liquid chromatography-Q Exactive hybrid quadrupole-Orbitrap mass spectrometry. **A** Electrospray ionization in the positive mode (ESI(+)), **B** electrospray ionization in the negative mode (ESI(−))

Table 1 Identification of the chemical components of QFPD granules using high performance liquid chromatography-Q Exactive hybrid quadrupole-Orbitrap mass spectrometry combined with the Orbitrap traditional Chinese medicine library

No.	RT (min)	Formula	Potential compound	Detected *m/z*	Characterized MS^2	Compound class	Herb	Refs
1[d]	2.11	$C_{12}H_{22}O_{11}$	Sucrose	341.1069 $[M-H]^-$	89.0235 71.1031 59.0132[b]	Miscellaneous	*PR*	[10]
2[d]	2.12	$C_5H_9NO_2$	2-Pyrrolidinecarboxylic acid	116.0709 $[M+H]^+$	116.0708 70.0658[b]	Miscellaneous	*DR*	[11]
3[d]	2.12	$C_4H_5N_3O$	Cytosine	112.0508 $[M+H]^+$	112.0508[b] 95.0244	Alkaloids	*AR*	
4	2.13	$C_5H_{11}NO_2$	Betaine	118.0865 $[M+H]^+$	118.0864[b] 59.0737	Alkaloids	*AE*	
5	2.14	$C_7H_7NO_2$	Trigonelline	138.0550 $[M+H]^+$	138.0550[b] 110.0603 94.0656	Alkaloids	*PC*	[12]
6[d]	2.2	$C_6H_8O_7$	Citric acid	191.0185 $[M-H]^-$	111.0078[b] 87.0079	Miscellaneous	*GR*	
7[d]	2.66	$C_6H_5NO_2$	Nicotinic acid	124.0395 $[M+H]^+$	124.0394[b] 96.0448 80.05	Alkaloids		
8[acd]	2.69	$C_9H_{12}N_2O_6$	Uridine	243.0608 $[M-H]^-$	200.0554 152.0339 122.0234 110.0238[b]	Miscellaneous	*PR*	[13]
9[d]	2.69	$C_6H_6N_2O$	Nicotinamide	123.0555 $[M+H]^+$	123.0554[b] 96.0448 80.0501	Alkaloids	*FF*	
10[ad]	2.73	$C_{10}H_{13}N_5O_4$	Adenosine	268.1041 $[M+H]^+$	136.0618[b]	Miscellaneous	*PC*	[14]
11[acd]	2.87	$C_{10}H_{13}N_5O_5$	Guanosine	284.0989 $[M+H]^+$	152.0567[b]	Miscellaneous	*PC*	[14]
12	3.41	$C_7H_6O_5$	Gallic acid	169.013 $[M-H]^-$	125.0233[b] 97.0285 69.0337	Phenolic acids		
13	3.9	$C_9H_{11}NO_2$	L-Phenylalanine	166.0863$[M+H]^+$	120.0809[b] 103.0546	Miscellaneous		
14	4.22	$C_6H_6O_3$	5-Hydroxymethylfurfural	127.0392 $[M+H]^+$	109.0288[b] 81.0341	Miscellaneous	*PC*	[15]
15	4.68	$C_9H_{13}NO$	L-norephedrine	152.1069 $[M+H]^+$	134.0965[b] 117.0701	Alkaloids	*EH*	[16]
16	5.12	$C_9H_{13}NO$	D-norpseudoephedrine	152.1069 $[M+H]^+$	134.0965[b] 117.0701	Alkaloids	*EH*	[16]
17	5.24	$C_{15}H_{14}O_7$	(−)-Gallocatechin	305.0651 $[M-H]^-$	219.0654 137.0232 125.0232[b]	Phenols		
18	5.32	$C_7H_6O_4$	Protocatechuic acid	153.0181 $[M-H]^-$	109.0284[b]	Phenolic acids	*GR*	[15]
19	6.09	$C_{10}H_{15}NO$	L-ephedrine	166.1226 $[M+H]^+$	148.1120[b] 133.0887 117.0701 91.0547	Alkaloids	*EH*	[16]
20	6.47	$C_{10}H_{15}NO$	D-pseudoephedrine	166.1226 $[M+H]^+$	148.1120[b] 133.0887 117.0701 91.0547	Alkaloids	*EH*	[16]
21	6.99	$C_{11}H_{17}NO$	Methylephedrine	180.1382 $[M+H]^+$	162.1276[b] 147.1041 135.0805 117.0701	Alkaloids	*EH*	[16]
22	7.26	$C_7H_6O_3$	Protocatechualdehyde	137.0233 $[M-H]^-$	137.0233[b] 119.0126 109.0285	Phenols	*CR*	[15]

Table 1 (continued)

No.	RT (min)	Formula	Potential compound	Detected *m/z*	Characterized MS^2	Compound class	Herb	Refs
23[c]	9.16	$C_{20}H_{27}NO_{11}$	Amygdalin	456.1492 $[M-H]^-$	323.0963 221.0653 161.0443 59.0132[b]	Miscellaneous	*AS*	[17]
24[cd]	9.43	$C_{16}H_{18}O_9$	Chlorogenic acid	353.0862 $[M-H]^-$	191.0548[b] 135.0441 179.0337	Phenylpropanoids	*FF*	[18]
25	9.46	$C_9H_6O_4$	Esculetin	177.0181 $[M-H]^-$	177.0180[b] 149.0236 133.0284 105.0336	Phenylpropanoids		
26	9.48	$C_7H_6O_2$	*p*-Hydroxybenzaldehyde	121.0285 $[M-H]^-$	121.0284[b] 93.0336	Phenols		
27	9.78	$C_9H_8O_4$	Caffeic acid	179.0337 $[M-H]^-$	135.0441[b]	Phenolic acids		
28[a]	10.49	$C_{15}H_{14}O_6$	Catechin hydrate	289.0703 $[M-H]^-$	245.0805 123.044 109.0284[b]	Flavonoids		
29[a]	10.63	$C_{15}H_{12}O_7$	Taxifolin	303.0494 $[M-H]^-$	177.018 125.0233[b]	Flavonoids	*SR*	[19]
30[c]	10.98	$C_{27}H_{30}O_{15}$	Vicenin II	593.1482 $[M-H]^-$	353.0648[b] 383.0753 473.1062 297.075	Flavonoids	*GR*	[8]
31	11.12	$C_{25}H_{24}O_{12}$	1,3-Dicaffeoylquinic acid	515.1168 $[M-H]^-$	353.0859 191.0547[b] 179.0336 135.044	Phenylpropanoids		
32	11.87	$C_9H_8O_3$	*p*-Coumaric acid	163.0400 $[M-H]^-$	119.0496[b] 163.0394	Phenylpropanoids		
33[ac]	11.95	$C_{26}H_{28}O_{14}$	Isoschaftoside	563.1376 $[M-H]^-$	353.0648[b] 383.0754 473.1073	Flavonoids	*GR*	[20]
34	11.96	$C_9H_{10}O_4$	3,5-Dimethoxy-4-hydroxybenzaldehyde	183.0652 $[M+H]^+$	140.0469 123.0443 95.0497[b]	Phenols	*CR* *EH* *RE*	[15]
35	12.18	$C_{21}H_{20}O_{11}$	Orientin	447.0913 $[M-H]^-$	357.06 327.0496[b] 299.0541 133.028	Alkaloids	*CP*	[21]
36	12.6	$C_{10}H_8O_4$	Scopoletin	193.0497 $[M+H]^+$	193.0496[b] 178.026 133.0285	Phenylpropanoids	*AF*	[22]
37[ac]	12.7	$C_{26}H_{30}O_{13}$	Naringenin 7-*O*-(2-β-D-apiofuranosyl)-β-D-glucopyranoside	549.1588 $[M-H]^-$	255.0649 135.0077 119.0492[b]	Flavonoids	*GR*	[20]
38	12.84	$C_{10}H_{10}O_4$	Ferulic acid	193.0492 $[M-H]^-$	178.0258 134.0362[b]	Phenylpropanoids		
39	13.03	$C_{11}H_{10}O_5$	Isofraxidin	223.0601 $[M+H]^+$	223.0601[b] 190.0261 162.0311	Phenylpropanoids		
40[c]	13.04	$C_{27}H_{32}O_{15}$	Eriocitrin	595.1638 $[M-H]^-$	459.1152 151.0025[b] 135.0441	Flavonoids	*CP* *AF*	[21] [23]
41[c]	13.04	$C_{26}H_{30}O_{13}$	Liquiritin apioside	549.1586 $[M-H]^-$	119.0491[b] 135.0077 255.0649	Flavonoids	*GR*	[20]

Table 1 (continued)

No.	RT (min)	Formula	Potential compound	Detected *m/z*	Characterized MS^2	Compound class	Herb	Refs
42[c]	13.18	$C_{27}H_{30}O_{16}$	Rutin	609.1431[M – H]$^-$	300.0258[b] 271.0234 255.0284	Flavonoids		
43	13.39	$C_9H_6O_4$	5,7-Dihydroxychromone	177.0180 [M – H]$^-$	177.0180[b] 135.0076	Flavonoids		
44[d]	13.75	$C_7H_6O_3$	Salicylic acid	137.0233 [M – H]$^-$	137.0233 93.0337[b]	Phenolic acids	*AE*	[15]
45	13.8	$C_{14}H_{12}O_4$	Piceatannol	243.0648 [M – H]$^-$	243.0648[b] 201.0544 159.0439	Phenols		
46	13.84	$C_{25}H_{24}O_{12}$	Isochlorogenic acid B	515.1165 [M – H]$^-$	353.0856 191.0547 179.0336 135.0440[b]	Phenylpropanoids	*FF*	[18]
47[cd]	13.89	$C_{27}H_{32}O_{14}$	Narirutin	581.1863 [M + H]$^+$	273.0755[b] 153.0181 85.0289 71.0498	Flavonoids	*CP*	[21]
48[a]	14.02	$C_{29}H_{36}O_{15}$	Verbascoside	623.1945 [M – H]$^-$	461.1639 161.0231[b] 133.0283	Phenylpropanoids	*PH*	[15]
49	14.13	$C_{25}H_{24}O_{12}$	3,5-Dicaffeoylquinic acid	515.1165 [M – H]$^-$	353.0878 191.0558[b] 179.0346 135.0448	Phenylpropanoids	*FF*	[18]
50	14.14	$C_{22}H_{22}O_{11}$	Tectoridin	463.1234 [M + H]$^+$	301.0705[b] 286.047	Flavonoids	*BH*	[24] [25]
51[ac]	14.27	$C_{27}H_{32}O_{14}$	Naringin	579.1688 [M – H]$^-$	271.0597 151.0025[b] 119.0491 107.0129	Flavonoids	*AF* *CP*	[23] [21]
52	14.57	$C_9H_{16}O_4$	Azelaic acid	187.0962[M – H]$^-$	125.0960[b] 97.0649	Miscellaneous		
53	14.62	$C_{28}H_{34}O_{15}$	Neohesperidin	609.1796 [M – H]$^-$	609.1791 301.0700[b] 286.0466	Flavonoids	*CP*	
54	14.95	$C_{25}H_{24}O_{12}$	Isochlorogenic acid C	515.1166 [M – H]$^-$	353.088 191.0558 173.0452 135.0448[b]	Phenylpropanoids	*FF*	[18]
55[c]	15	$C_{28}H_{34}O_{15}$	Hesperidin	609.1796 [M – H]$^-$	609.1791 301.0699[b] 286.0466	Flavonoids	*CP*	[26]
56	15.06	$C_{24}H_{26}O_{13}$	Iridin	523.1445 [M + H]$^+$	361.0915[b] 346.0679 331.0445	Flavonoids	*BH*	[25]
57	15.06	$C_9H_6O_2$	Coumarin	147.0440 [M + H]$^+$	147.0440[b] 103.0546 91.0547	Phenylpropanoids	*CR* *EH*	[15]
58[a]	15.63	$C_{15}H_{12}O_6$	Eriodictyol	287.0547 [M – H]$^-$	287.0547 161.0231 125.0233[b]	Flavonoids	*AF*	[23]
59[c]	15.86	$C_{26}H_{30}O_{13}$	Isoliquiritin apioside	549.1589 [M – H]$^-$	255.0649 153.0181 135.0077 119.0491[b]	Flavonoids	*GR*	[20]

Table 1 (continued)

No.	RT (min)	Formula	Potential compound	Detected *m/z*	Characterized MS^2	Compound class	Herb	Refs
60	16.06	$C_{22}H_{22}O_9$	Ononin	431.1336 $[M+H]^+$	269.0807[b] 254.0573 237.0544	Flavonoids	*GR*	[27]
61[cd]	16.08	$C_{21}H_{18}O_{11}$	Baicalin	445.0753 $[M-H]^-$	269.0439[b]	Flavonoids	*SR*	[19]
62	16.15	$C_{21}H_{20}O_{10}$	Oroxin A	433.1129 $[M+H]^+$	271.0599[b] 253.0493 123.0078	Flavonoids	*SR*	[19]
63[a]	16.23	$C_{21}H_{22}O_9$	Isoliquiritin	419.1334 $[M+H]^+$	257.0806[b] 147.0439 137.0232	Flavonoids	*GR*	[20]
64[c]	16.51	$C_{15}H_{12}O_4$	Liquiritigenin	255.0651 $[M-H]^-$	135.0078 119.0492[b] 91.0181	Flavonoids	*GR*	[20]
65	16.87	$C_{11}H_6O_4$	Bergaptol	201.0180 $[M-H]^-$	201.0192[b] 183.1012 139.1117	Phenylpropanoids		
66[c]	16.92	$C_{21}H_{18}O_{11}$	Norwogonin-8-glucuronide	445.0753 $[M-H]^-$	269.0441[b]	Flavonoids	*SR*	[19]
67	17.2	$C_{28}H_{34}O_{14}$	Poncirin	593.1842 $[M-H]^-$	593.184 285.0753[b] 151.0024	Flavonoids	*AF*	[23]
68	17.24	$C_{21}H_{18}O_{11}$	Norwogonin-7-glucuronide	445.0752 $[M-H]^-$	269.0439[b]	Flavonoids	*SR*	[19]
69[c]	17.51	$C_{22}H_{20}O_{11}$	Oroxylin A-7-*O*-β-D-glucuronide	459.0910 $[M-H]^-$	283.0595 268.0362[b]	Flavonoids	*SR*	[19]
70[c]	18.11	$C_{21}H_{18}O_{11}$	Baicalein-6-glucuronide	445.0754 $[M-H]^-$	269.0441[b]	Flavonoids	*SR*	[19]
71[c]	18.14	$C_{22}H_{20}O_{11}$	Wogonoside	459.0909 $[M-H]^-$	283.0595 268.0362[b]	Flavonoids	*SR*	[19]
72	18.58	$C_{15}H_{12}O_5$	Naringenin chalcone	273.0756 $[M+H]^+$	273.0757 153.0182[b] 147.044 119.0493	Flavonoids	*GR* *AF* *CP*	[20] [23] [21]
73[c]	19.32	$C_{16}H_{14}O_6$	Hesperetin	301.0702 $[M-H]^-$	301.0702[b] 286.044 164.0103 108.0207	Flavonoids	*CP*	[26]
74	19.38	$C_{16}H_{12}O_6$	Tectorigenin	299.0546$[M-H]^-$	284.0301[b] 240.0414	Flavonoids		
75[ac]	20.01	$C_{18}H_{16}O_8$	Irigenin	359.0756 $[M-H]^-$	344.0519 329.0286[b] 314.0054 286.0104	Flavonoids	*BH*	[24]
76	20.03	$C_{16}H_{12}O_7$	Isorhamnetin	315.0496 $[M-H]^-$	315.0496 300.0260[b] 271.0237 151.002	Flavonoids	*AE*	[28]
77[c]	20.15	$C_{17}H_{14}O_7$	Iristectorigenin B	329.0652 $[M-H]^-$	314.0417 299.0180[b] 271.0235	Flavonoids	*BH*	[24]
78	20.39	$C_{15}H_{10}O_5$	Baicalein	269.0443 $[M-H]^-$	269.0457[b] 241.0507 223.0398	Flavonoids	*SR*	[19]
79[cd]	20.97	$C_{15}H_{12}O_4$	Isoliquiritigenin	255.0650 $[M-H]^-$	135.0076 119.0491[b] 91.018	Flavonoids	*GR*	[20]
80	21.08	$C_{16}H_{12}O_4$	Formononetin	269.0807 $[M+H]^+$	269.0807[b] 254.0574	Flavonoids	*GR*	[20]

Table 1 (continued)

No.	RT (min)	Formula	Potential compound	Detected *m/z*	Characterized MS^2	Compound class	Herb	Refs
81[ac]	21.35	$C_{15}H_{16}O_4$	Isomeranzin	261.1119 $[M+H]^+$	189.0546[b] 159.0439 131.0492	Phenylpropanoids		
82[ac]	21.65	$C_{42}H_{62}O_{17}$	Licorice-saponin G2	837.3869 $[M-H]^-$	837.386 351.0552 193.0341 113.0235[b]	Terpenoids	*GR*	[20]
83[c]	21.83	$C_{26}H_{30}O_8$	Limonin	469.1846 $[M-H]^-$	469.1831[b] 249.0909 229.1214	Terpenoids	*AF*	[22]
84	21.83	$C_{15}H_{22}O_2$	Curcumenol	235.1693 $[M+H]^+$	235.169 217.1588 199.1482[b]	Terpenoids		
85	22.15	$C_{20}H_{20}O_7$	Isosinensetin	373.1283 $[M+H]^+$	373.1281[b] 343.0812	Flavonoids	*CP*	[26]
86[cd]	22.64	$C_{30}H_{46}O_4$	18 β-Glycyrrhetinic acid	471.3469 $[M+H]^+$	417.3471[b] 453.3362	Terpenoids	*GR*	[29]
87[cd]	22.65	$C_{42}H_{62}O_{16}$	Glycyrrhizic acid	821.3926 $[M-H]^-$	821.3915[b] 351.056 113.0234	Terpenoids	*GR*	[20]
88	22.72	$C_{20}H_{18}O_8$	Irisflorentin	387.1073 $[M+H]^+$	387.1073[b] 372.0843 357.0603 329.0654	Flavonoids	*BH*	[25]
89	22.84	$C_{20}H_{20}O_7$	Sinensetin	373.1282 $[M+H]^+$	373.1281[b] 343.0809	Flavonoids	*CP*	[26]
90	22.98	$C_{18}H_{14}O_8$	Dichotomitin	359.0762 $[M+H]^+$	359.0761[b] 344.0526 326.0421 299.0549	Flavonoids	*BH*	[24]
91[a]	23.16	$C_{16}H_{12}O_5$	Wogonin	285.0757 $[M+H]^+$	285.0756 270.0521[b]	Flavonoids	*SR*	[19]
92	23.34	$C_{42}H_{62}O_{16}$	isomer of Glycyrrhizic acid	821.3922 $[M-H]^-$	821.3919[b] 351.0551 113.0235	Terpenoids	*GR*	
93[c]	23.57	$C_{42}H_{68}O_{13}$	Saikosaponin A	825.4599 $[M+COOH]^-$	779.4534[b] 617.40106 59.0132	Terpenoids	*BR*	[26]
94	23.91	$C_{17}H_{14}O_6$	Pectolinarigenin	313.0703 $[M-H]^-$	313.0701 283.0233[b] 255.0286	Flavonoids	*SR*	[19]
95	23.99	$C_{21}H_{22}O_8$	Nobiletin	403.1388 $[M+H]^+$	403.1388 373.0917[b] 211.0238 183.0288	Flavonoids	*CP*	[26]
96[a]	24.04	$C_{19}H_{18}O_6$	6-Demethoxytangeretin	343.1174 $[M+H]^+$	343.1173 313.0705[b] 285.0756	Flavonoids	*CP*	[26]
97[c]	24.09	$C_{42}H_{68}O_{13}$	Saikosaponin B1	825.4599 $[M+COOH]^-$	779.4542[b] 617.4028 59.0132	Terpenoids	*BR*	
98[a]	24.13	$C_{16}H_{12}O_5$	Oroxylin A	285.0758 $[M+H]^+$	285.0757 270.0523[b] 168.0054	Flavonoids	*SR*	[19]

Table 1 (continued)

No.	RT (min)	Formula	Potential compound	Detected *m/z*	Characterized MS^2	Compound class	Herb	Refs
99	24.21	$C_{15}H_{20}O_3$	Atractylenolide III	249.1486 $[M+H]^+$	231.1379[b] 249.1481 213.1276 163.0752	Terpenoids	*AM*	[30]
100	24.49	$C_{22}H_{24}O_9$	Heptamethoxyflavone	433.1493 $[M+H]^+$	403.1021 433.1492[b] 165.0546	Flavonoids	*CP*	[26]
101[c]	24.7	$C_{42}H_{68}O_{13}$	Saikosaponin D	825.4594 $[M+COOH]^-$	779.4537[b] 617.4034 59.0132	Terpenoids	*BR*	
102[d]	25.67	$C_{20}H_{20}O_7$	Tangeretin	373.1280 $[M+H]^+$	373.1278 358.1043 343.0808[b] 328.0573	Flavonoids	*CP*	[26]
103	26.41	$C_{20}H_{20}O_8$	5-*O*-Demethylnobiletin	389.1230 $[M+H]^+$	389.1227[b] 359.076 341.0652	Flavonoids	*CP*	[21]
104	26.68	$C_{32}H_{48}O_6$	Alisol C 23-acetate	529.3526 $[M+H]^+$	529.3521[b] 469.3314 451.3204 415.2842	Terpenoids	*AR*	[31]
105	27.85	$C_{15}H_{20}O_2$	Atractylenolide II	233.1536 $[M+H]^+$	233.1536[b] 215.1432 187.1482 151.0753	Terpenoids	*AM*	[30]
106	27.94	$C_{12}H_{16}O_4$	Pogostone	225.1122 $[M+H]^+$	207.1015 139.039 81.0705[b]	Miscellaneous	*PH*	[32]
107	34.7	$C_{32}H_{50}O_5$	Alisol B 23-acetate	515.3733 $[M+H]^+$	437.3412 339.2679 419.3305 97.0653[b]	Terpenoids	*AR*	[31]
108	35.79	$C_{18}H_{30}O_2$	α-Linolenic acid	279.2318 $[M+H]^+$	95.086 81.0705 67.055[b]	Alkaloids		

RT retention time

[a]Representative retention time, as more than one peak was identified for this compound

[b]Base fragment ion

[c]Compounds detected using both the positive and negative electrospray ionization modes. *m/z*: mass-to-charge ratio

[d]Compounds identified by comparison with reference standards. Herb: Compound detected within herb experimentally and also the reference reported the source of the compound. Ref.: The references that reported the sources of the compounds. *EH (Ephedrae Herba)*, *GR (Glycyrrhizae Radix Et Rhizoma Praeparata Cum Melle)*, *AS (Armeniacae Semen Amarum)*, *CR (Cinnamomi Ramulus)*, *PH (Pogostemonis Herba)*, *AR (Alismatis Rhizoma)*, *PP (Polyporus)*, *AM (Atractylodis Macrocephalae Rhizoma)*, *PR (Poria)*, *BR (Bupleuri Radix)*, *SR (*Scutellariae Radix); *PC (Pinelliae Rhizoma Praeparatum Cum Zingibere Et Alumine)*, *ZR (Zingiberis Rhizoma Recens)*, *AE (Asteris Radix Et Rhizoma)*, *FF (Farfarae Flos)*, *BH (Belamcandae Rhizoma)*, *RE (Asari Radix Et Rhizoma)*, *DR (Dioscoreae Rhizoma)*, *AF (Aurantii Fructus Immaturus)*; *CP (Citri Reticulatae Pericarpium)*

– $H_2O]^+$ and 133.0887 $[M+H-H_2O-CH_3]^+$. According to the literature [16], they are identified as L-ephedrine (**19**) and D-pseudoephedrine (**20**). The mass spectrum of compound **21** (methylephedrine) reveals a peak representing the protonated molecule $[M+H]^+$, at *m/z* 180.1382, and fragment ion peaks at *m/z* 162.1276 $[M+H-H_2O]^+$ and 147.1041 $[M+H-H_2O-CH_3]^+$. The mass spectra of compounds **15** (L-norephedrine) and **16** (D-norpseudoephedrine) reveal the same peak at *m/z* 152.1069, and MS^2 peaks at *m/z* 134.0965 $[M+H-H_2O]^+$ and 117.0701 $[M+H-H_2O-NH_3]^+$. However, they exhibit different retention times. These compounds are phytochemicals present in *Ephedrae Herba*.

Compounds 3 (cytosine), **7** (nicotinic acid), and **9** (nicotinamide) were identified by comparing the retention times and MS^2 fragmentation patterns with those of reference

standards. Nicotinic acid and nicotinamide exhibit the same structural skeleton, and fragment ion peaks at *m/z* 96.0448 $[M+H-CO]^+$ are observed in the MS^2 profiles. Their possible fragmentation pathways and library match results are shown in Fig. S4. The MS^2 profile of compound **5** reveals a peak representing a protonated molecule, $[M+H]^+$, at *m/z* 138.0550 and peaks at *m/z* 110.0603 $[M+H-CO]^+$ and 94.0656 $[M+H-CO-O]^+$. Therefore, compound **5** is deduced to be trigonelline.

Flavonoids

Forty-nine compounds were identified as flavonoids. Compounds **47** and **51** were identified as narirutin and naringin, respectively, by comparison with the OTCML. Furthermore, compound **47** was confirmed using a reference standard. They were detected in both the positive and negative ESI modes, displaying similar MS and MS^2 profiles that revealed peaks representing $[M-H]^-$ ions at *m/z* 579.1688. Fragment ions were represented by peaks at *m/z* 271.0615, owing to the loss of glucose (Glc) and rhamnose moieties [21]. Characterized fragment ions represented by peaks at *m/z* 151.0034 and 119.0499 were generated by retro-Diels–Alder cleavage. Narirutin and naringin are flavonoid *O*-glycoside isomers distinguished by their different retention times. Compounds **87** (isosinensetin), **89** (sinensetin), **96** (6-demethoxytangeretin), **95** (nobiletin), and **102** (tangeretin) are polymethoxyflavones, bearing numerous methoxyl and/or hydroxyl groups on the basic structure. The mass spectra of these compounds show peaks representing $[M+H]^+$ ions and characterized fragment ions due to continuous CH_3 loss [26]. The MS^2 profiles and library match results are shown in Fig. S5. As examples, the mass spectra of compounds **85** and **89** reveal peaks representing $[M+H]^+$ ions at *m/z* 373.1283 and characterized fragment ions at *m/z* 343.08 $[M+H-2CH_3]^+$. The spectra are very similar, and the compounds were identified using the OTCML by the different retention times and slight differences in the spectra. Compound **102** (tangeretin) was further confirmed using a reference standard. Compounds **30** and **33** showed similar MS^2 patterns, but the molecular ions were different, indicating the same basic structure. These compounds were assigned as vicenin II [8] and isoschaftoside [20, 33], respectively. For example, the mass spectrum of compound **30** revealed peaks representing the $[M-H]^-$ ion at *m/z* 593.1482 and fragment ions at *m/z* 297.0750 $[M-H-Glc-Glc]^-$, *m/z* 473.1062 $[M-H-120]^-$, *m/z* 383.0753 $[M-H-210]^-$, and *m/z* 353.0648 $[M-H-240]^-$. These are characterized fragment ions of the hexose ring-opening reaction [33]. The similarities of the MS and MS^2 profiles of compounds **37**, **41**, and **59** indicated isomers. By comparing the data in the OTCML combined with literature data [20], they were deduced as naringenin 7-*O*-(2-β-D-apiofuranosyl)-β-D-glucopyranoside (**37**), liquiritin apioside (**41**), and isoliquiritin apioside (**59**). The mass spectra of compounds **61**, **68**, **66**, and **70** revealed peaks representing $[M-H]^-$ ions at *m/z* 445.07 and dominant fragment ions at *m/z* 269.04, along with $[M+H]^+$ ions at *m/z* 447.09 and dominant fragment ions at *m/z* 271.05. Individual herb pieces component mass spectra showed that these compounds, baicalin (**61**), norwogonin-7-glucuronide (**68**), norwogonin-8-glucuronide (**66**) and baicalein-6-glucuronide (**70**), were chemical components of *Scutellariae Radix* [19], and baicalin (**61**) was identified using a reference standard. Based on the literature [26], compounds **73** and **100** were assigned as hesperetin and heptamethoxyflavone, respectively. Compound **79** (isoliquiritigenin) was identified using a reference standard.

Phenylpropanoids

Fourteen compounds were identified as phenylpropanoids. Compounds **31** (1,3-dicaffeoylquinic acid), **46** (isochlorogenic acid B), **49** (3,5-dicaffeoylquinic acid) and **53** (isochlorogenic acid C) were identified using the OTCML. Compound **24** (chlorogenic acid) was identified using a reference standard. Compounds **31**, **46**, **49** and **53** were isomers with skeletons similar to those of quinic and caffeic acid, generating similar MS and MS^2 profiles and distinguished by their retention times. For example, the MS^2 profile of compound **46** revealed peaks representing fragment ions at *m/z* 191.0547 [quinic acid $-H]^-$, 179.0336 [caffeic acid $-H]^-$ and 135.0440 [caffeic acid $-CO_2-H]^-$. The mass spectrum of compound **38** (ferulic acid) showed peaks representing a $[M-H]^-$ ion at *m/z* 193.0492 and the main fragment ions at *m/z* 134.0362 $[M-H-CH_3-CO_2]^-$ and 178.0258 $[M-H-CH_3]^-$. Compounds **32** (*p*-coumaric acid), **57** (coumarin), **65** (bergaptol) and **25** (esculetin) were assigned using the OTCML.

Phenolic Acids and Phenols

Four phenolic acids were identified, and they exhibited the same fragmentation pattern. The MS^2 profile of compound **12** (gallic acid) revealed peaks representing $[M-H]^-$ at

m/z 169.0130 and ions at *m/z* 125.0233 $[M - H - CO_2]^-$, 97.0285 $[M - H - CO_2 - CO]^-$ and 69.0337 $[M - H - CO_2 - CO - CO]^-$. The mass spectrum of compound **18** (protocatechuic acid) revealed a peak representing a base fragment ion at *m/z* 109.0284 $[M - H - CO_2]^-$. Compound **44** (salicylic acid) was identified by comparison with a reference standard. All of these compounds exhibited successive losses of H_2O, CO and CO_2 during fragmentation [34, 35].

Five phenols were identified. Compound **26** (*p*-hydroxybenzaldehyde) produced several clear fragment ions at high collision energies. Compound **22** (protocatechualdehyde) was identified using the OTCML. The phenols also showed neutral losses of CO, CH_3 and H_2O in the MS^2 profiles.

Terpenoids

Thirteen terpenoids are identified. The mass spectra of compounds **82** and **87** reveal peaks representing $[M+H]^+$ ions at *m/z* 839.4061 and 823.4108, respectively. The mass spectrum of compound **82** (licorice-saponin G2) reveals peaks representing fragment ions at *m/z* 469.3314 [Aglycone + H $- H_2O]^+$, 487.3412 [Aglycone + H]$^+$ and 451.3212 [Aglycone + H $- 2H_2O]^+$ [36]. Compound **87** displays a similar fragmentation pattern, yet is 16 Da smaller than compound **82**. Compound **87** was then confirmed as glycyrrhizic acid through a comparison between the negative ESI mode data, a reference standard, and literature data [20]. These spectra are shown in Fig.S6. The mass spectrum of compound **86**, 18 β-glycyrrhetinic acid, reveals a peak representing $[M+H]^+$ at *m/z* 471.3469. These are triterpenic acids. Compound **86** (18 β-glycyrrhetinic acid) was also identified using a reference standard.

The MS^2 profile of compound **104** showed peaks representing a protonated molecule, $[M+H]^+$, at *m/z* 529.3526 and dominant fragment ions at *m/z* 529.3521 $[M+H]^+$, 469.3314 [M + H − HAc]$^+$, 451.3204 [M + H − HAc $- H_2O]^+$ and 415.2842 $[M+H - C_4H_8O - H_2O]^+$ [31]. This compound was identified as alisol C 23-acetate using the OTCML. The mass spectrum of compound **107**, alisol B 23-acetate, revealed a peak representing $[M+H]^+$ at *m/z* 515.3733.

The mass spectrum of compound **93** revealed peaks representing a $[M+H]^+$ ion at *m/z* 781.4732 and fragment ions at *m/z* 455.3518 [M + H $- H_2O$ − Fuc (fucose) Glc]$^+$ and 437.3412 [M + H $- 2H_2O$ − FucGlc]$^+$. This compound was identified as saikosaponin A by comparison with data obtained from the OTCML. The mass spectrum of compound **83** exhibited peaks representing $[M+H]^+$ at *m/z* 471.2016 and fragment ions at *m/z* 425.1957 [M + H − 46]$^+$ and 161.0597. According to the literature [23] and the data in the OTCML, it was limonin.

The mass spectra of compounds **99** and **105** revealed peaks representing $[M+H]^+$ ions at *m/z* 249.1486 and 233.1536, respectively. They were identified as atractylenolide III and atractylenolide II, respectively, using the OTCML. The MS^2 profile of atractylenolide III revealed peaks representing fragment ions at *m/z* 249.1481 $[M+H]^+$, 231.1379 $[M+H - H_2O]^+$, 213.1276 $[M+H - 2H_2O]^+$ and 203.1430 $[M+H - H_2O - CO]^+$ [30].

Other Phytochemicals

Eleven compounds were identified by comparing the obtained data to the information in the OTCML, including the hydrophilic compounds **1** (sucrose), **2** (2-pyrrolidinecarboxylic acid), **6** (citric acid), **8** (uridine), **10** (adenosine) and **11** (guanosine). These compounds were also confirmed using reference standards.

Quantification Analysis

The extracted ion chromatograms (EICs) of 17 authentic standards compared with those of their corresponding detected compounds within QFPD granules are shown in Fig. 2. The HPLC-Q Exactive hybrid quadrupole-Orbitrap MS method was also used for quantification analysis of these 17 constituents within QFPD granules. The concentration of each constituent was obtained using the respective calibration curve and their contents within the QFPD granules are listed in Table 2.

Compounds from Individual Herbs Within QFPD Granules

In total, 265 compounds were putatively identified using the OTCML combined with manual verification from 20 herbs that are components of QFPD granules (Table S1), including 33 alkaloids, 106 flavonoids, 28 terpenoids, 41 phenylpropanoids, 10 phenolic acids, 18 phenols and 29 other phytochemicals. Of these, 163 compounds were from only one herb, and 102 compounds were from more than two herbs. Within the QFPD granules, 59 compounds were from only one herb and 49 compounds were from more than two herbs.

Conclusions

In this study, HPLC-Q Exactive hybrid quadrupole-Orbitrap MS coupled with the OTCML which is an automatic data analysis platform, was used to study the chemical profile of QFPD granules, an effective TCM prescribed to treat the symptoms of SARS-CoV-2 infections. Furthermore, manual

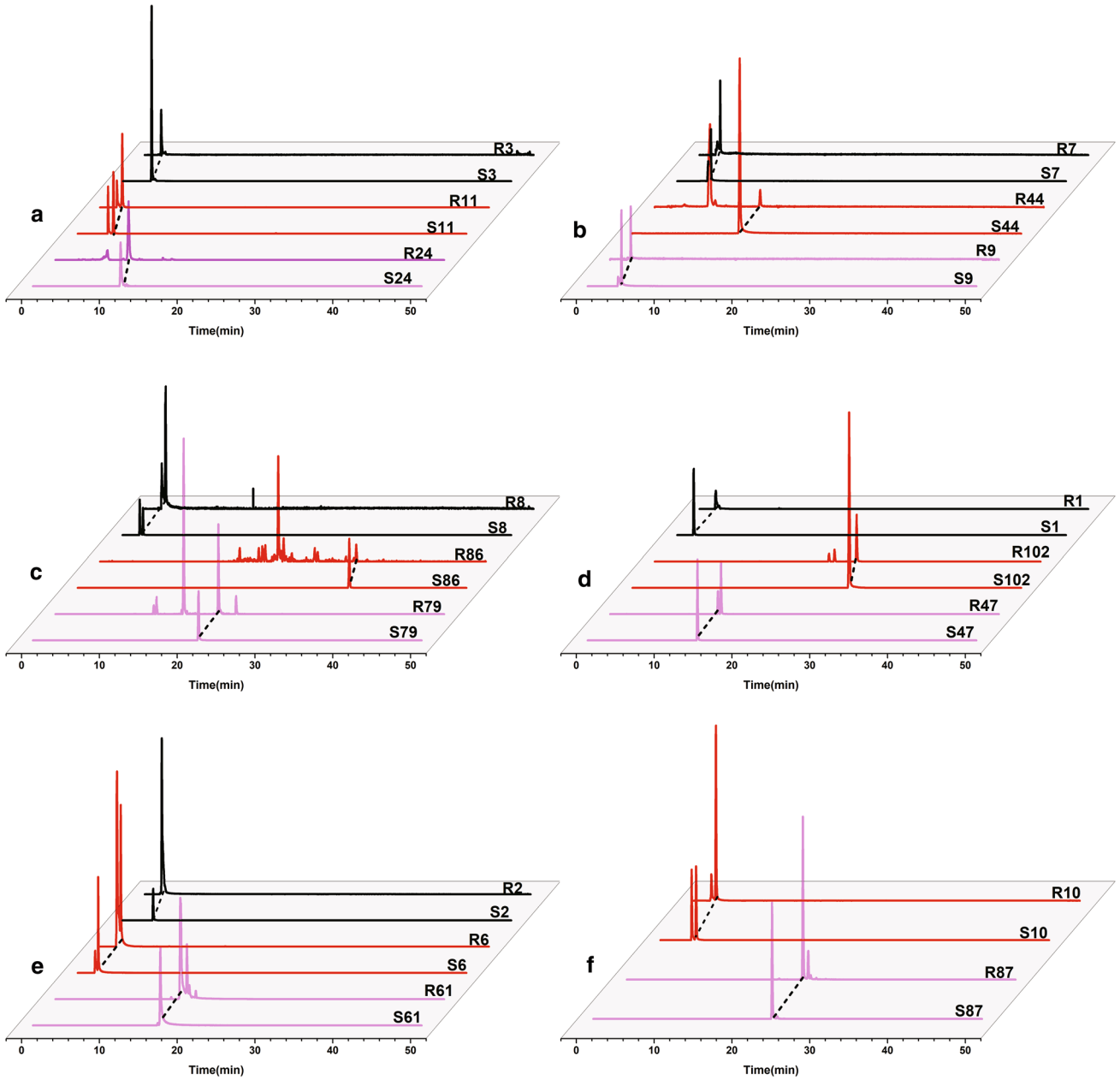

Fig. 2 Extracted ion chromatograms (EICs) of 17 authentic standards compared with those of the corresponding compounds detected within QFPD granules. **A** EICs of compounds **3**, **11**, and **24**; **B** EICs of compounds **7**, **44**, and **9**; **C** EICs of compounds **8**, **86**, and **79**; **D** EICs of compounds **1**, **102**, and **47**; **E** EICs of compounds **2**, **6**, and **61**; **F** EICs of compounds **10** and **87**. *R* sample from the QFPD granules, *S* authentic standards

verification ensured compound identification. A total of 108 compounds were putatively identified from QFPD granules, including alkaloids, flavonoids, phenylpropanoids, phenolic acids, phenols, terpenoids and other phytochemicals. This allowed rapid chemical composition screening of QFPD granules, providing potentially valuable information for quality control and further clinical application.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10337-021-04085-0.

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Table 2 Contents of the constituents within QFPD granules

No	Compound	Conc.(mg/g)
1	Sucrose	0.683 ± 0.185
2	2-Pyrrolidinecarboxylic acid	0.803 ± 0.017
3	Cytosine	0.009 ± 0.0008
6	Citric acid	4.309 ± 0.352
7	Nicotinic acid	0.011 ± 0.0007
8	Uridine	0.029 ± 0.005
9	Nicotinamide	0.006 ± 0.0003
10	Adenosine	0.164 ± 0.009
11	Guanosine	0.135 ± 0.004
24	Chlorogenic acid	0.854 ± 0.015
44	Salicylic acid	0.016 ± 0.002
47	Narirutin	0.699 ± 0.119
61	Baicalin	4.383 ± 1.107
79	Isoliquiritigenin	0.007 ± 0.00005
86	18 β-Glycyrrhetinic Acid	0.0005 ± 0.000007
87	Glycyrrhizic acid	2.199 ± 0.127
102	Tangeretin	0.003 ± 0.0003

Data present the (average ± standard deviation) of three replicates. Conc. (mg/g): mg of the constituent/ g of QFPD granules

Declarations

Conflict of Interest The authors declare that there are no conflicts of interest.

Ethical Statement We certify that this manuscript is original, has not been previously published and will not be submitted elsewhere for publication while under consideration by *Chromatographia*. This study is not split into several parts and submitted to various journals. Results are presented clearly, honestly and without fabrication. No data, text, or theories by others are presented as our own.

Human and Animal Rights Explicit permission to submit has been received from all co-authors. All the authors whose name appear on the submission have contributed sufficiently to this study.

This manuscript does not contain any studies involving humans or animals.

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