Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 2–8

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

UPLC/Q-TOFMS/MS as a powerful technique for rapid identification of polymethoxylated flavones in *Fructus aurantii*

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ARTICLE INFO

Article history:
Received 29 October 2008
Received in revised form 9 March 2009
Accepted 11 March 2009
Available online 24 March 2009

Keywords:
UPLC
Q-TOFMS/MS
PMFs
Fructus aurantii
Rapid identification
Mass spectrometric fingerprint

ABSTRACT

Polymethoxylated flavones (PMFs), as potential cancer chemopreventive agents, are widely distributed in Citrus genus. In this study, a selected ion monitoring–tandem mass (SIM–MS/MS) method for the rapid identification of PMFs in *Fructus aurantii* (*F. aurantii*) with ultra-performance liquid chromatography (UPLC) coupled to quadrupole, hybrid orthogonal acceleration time-of-flight tandem mass spectrometer (Q-TOFMS/MS) was proposed. The MS data for candidates, containing accurate mass and isotopic patterns for both precursors and their fragment ions, were acquired selectively. Based on the MS data, the mass spectrometric fingerprint (MSFP) for candidates, consisting of chemical formula and dissociation pattern, was determined. Comparing the MSFPs of the observed compounds with the diagnostic MSFP of the species, 44 PMFs were tentatively identified. The method was validated by tangeretin and sinensetin, two representative compounds of PMFs, and was considered to be suitable for the rapid screening of PMFs in crude and partially purified samples.

1. Introduction

Dietary flavonoids and other polyphenols show great potential as cancer chemopreventive agents in cell culture studies [1,2]. This does not translate well into *in vivo* activity, because of their low bioavailability as result of conjugative metabolism [3]. However, PMFs, the flavonoid subclass in which all or almost all hydroxyls are capped by methylation, have high oral bioavailability [4,5]. PMFs belong to potentially anticancer compounds, which have been demonstrated by an epidemiological investigation recently [6]. The anticancer bioactivity has aroused the interest of the food, nutraceutical and pharmaceutical industries for the use of these compounds as specialty ingredients. As PMFs are widely distributed in Citrus genus with wide dynamic range, the rapid and sensitive characterization of these constituents in such samples is necessary.

Early reported methods for analysis of PMFs were based on high-performance liquid chromatography (HPLC) separation coupled with ultraviolet (UV) detection [7,8]. The methods are limited to the detection of a number of known compounds with purified standard. Recently, the methods based on LC–MS/MS have overcome the limitation and allow the identification of PMFs in crude and partially purified samples even without any need for purified standard [9–11] whereas, the low-resolution MS instruments used in those studies cannot do exact mass measurement of precursor and fragment ions to yield the highest confidence in structural identification. In order to make sure the correct identification, some potential PMFs detected by LC–MS/MS were isolated and identified further by NMR [9,10]. It would need not only complicated work of sample purification but also several orders of magnitude more analyte than the low nanogram quantities required for LC–MS/MS. Therefore, the throughput and sensitivity of the method based on NMR cannot fulfill the rapid identification of PMFs in abundantly natural extracts.

The accurate mass data is a key information for structural elucidation using mass spectrometry, as which can confirm the molecular formula of a compound [12]. Q-TOFMS enables automated exact mass measurement of precursor and fragment ions to yield high confidence in structural elucidation. Therefore, it provides an attractive alternative. Chromatographic resolution is a key factor for reliable accurate mass measurement. The introduction of pressure stable 1.7 μm particulate packing materials and novel low dead volume, high pressure LC equipment provided strategies to improve resolution while maintaining or even shortening run times. This technique has been termed UPLC. The combination of UPLC/Q-TOFMS/MS offers high chromatographic resolution with exact mass measurement for both MS and MS/MS, then provides significant advantages concerning flexibility, selectivity, sensitivity, accuracy and speed for rapid screening for target compounds in crude samples.

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doi:10.1016/j.jpba.2009.03.010

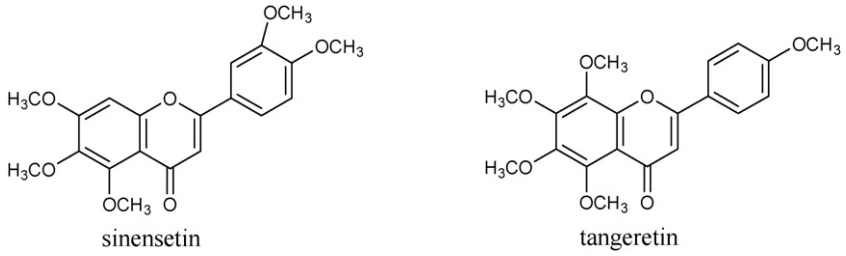

Fig. 1. Structures of SIN and tangeretin TAN.

In this study, we demonstrate the application of exact mass measurement using the UPLC/Q-TOFMS/MS in a routine high-throughput screening for PMFs in *F. aurantii* extract. Chemical formulas and dissociation patterns of candidate compounds, determined by exact mass measurements, were used to form the MSFPs. Comparing the MSFPs of the observed candidates with the diagnostic MSFP of the species, PMFs were tentatively identified.

2. Experimental

2.1. Reagents and chemicals

Methanol HPLC grade was purchased from Fisher Scientific Co. (Loughborough, UK); formic acid purchased from J&K CHEMICA Co. (Beijing, China); reverse osmosis Milli-Q water (18.2 MΩ) (Millipore, Billerica, USA) was used for all solutions and dilutions. PMFs standards (Fig. 1), sinensetin (SIN, 3′,4′,5,6,7-pentamethoxyflavone, MW 372.1209) was purchased from Meryer Co. (Shenzhen, China); tangeretin (TAN, 4′,5,6,7,8-pentamethoxyflavone, MW 372.1209) was purchased from Standardherbs Co. (Beijing, China). The standards were diluted in methanol/water (v:v, 1/1) to 0.1 mg/mL and filtered through 0.20 μm membranes before LC–MS analysis.

2.2. LC–MS

The LC–MS used for this study was an ACQUITYTM UPLC Q/TOF PremierTM (Waters, Milford, USA) equipped with electrospray ionization ion source (ESI). High purity nitrogen was used as the nebulizer and auxiliary gas; argon was used as the collision gas. The mass spectrometer was operated in positive ion mode with a capillary voltage of 3 kV, sampling cone voltage of 40 V, cone gas flow of 50 L/h, desolvation gas flow of 800 L/h, desolvation temperature of 300 °C, source temperature of 100 °C, collision energy of 20 V. Mass spectra were collected at the rate of 1 spectrum/s and the inter-scan delay was 0.02 s. Mass accuracy was maintained by using a lock spray with leucine enkephalin (*m/z* 556.2771, concentration: 2 ng/μL, flow rate: 5 μL/min) as reference. When the Q-TOF instrument was operated in full scan–survey mode, the full scan spectra from 150 to 600 Da were acquired. The predominant ion in each MS spectrum was selected automatically as precursor to MS/MS. MS/MS to MS switch criteria: 10 (counts/s). When the Q-TOF instrument was operated in selected ions monitoring (SIM)–MS/MS mode, the target *m/z* precursors were selected to pass through quadrupole to TOF detection for MS/MS.

The analytical column was an ACQUITY UPLC™ BEHC$_{18}$ (Waters, Milford, USA), 100 mm × 2.1 mm × 1.7 μm. The two mobile phases were phase A: water/formic acid (v:v, 100/0.1); phase B: methanol/formic acid (v:v, 100/0.1). The water was filtered prior to mixing, through a 0.2 μm membrane filter unit. A linear gradient was programmed: 0–13 min: 50% B; 13–18 min: 50–65% B; 18–20 min: 65% B. The flow rate was 0.25 mL/min. The column was held at 30 °C and the injection volume was 2 μL.

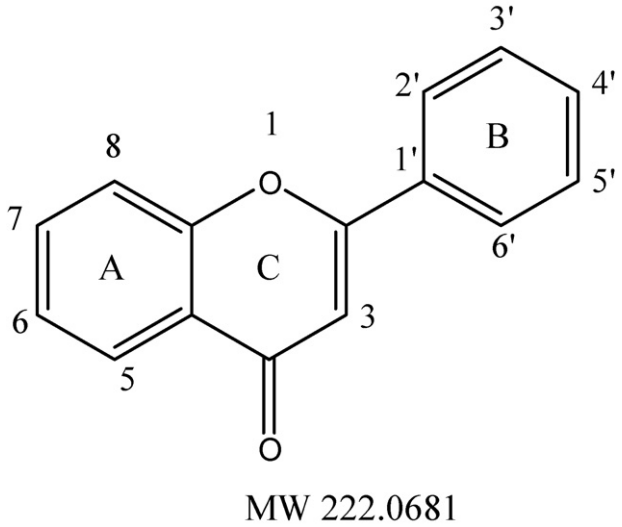

Fig. 2. Basic structure of flavone aglycones (elemental composition: $C_{15}H_{10}O_2$).

2.3. Plant material and sample preparation

F. aurantii was collected from Kai County, Chongqing city, China. A voucher specimen, identified by Da-Zhuo Shi Professor, faculty of Xiyuan Hospital of China Academy of Traditional Chinese Medicine, China, is deposited in our laboratory. The fraction containing PMFs (FP) was prepared as reported before [13]. The sample was dissolved in methanol/water (v:v, 1/1) to 0.2 mg/mL and filtered through 0.2 μm membranes before LC–MS analysis.

3. Results and discussion

PMFs have regularity in elemental composition (chemical formula). They have the basic aglycone structure with maximum seven

Table 1
Chemical formula and accurate mass of all possible PMF isomers.

	–	OH	2OH	3OH	4OH	5OH
2OCH$_3$	$C_{17}H_{14}O_4$ 282.0892	$C_{17}H_{14}O_5$ 298.0841	$C_{17}H_{14}O_6$ 314.0790	$C_{17}H_{14}O_7$ 330.0740	$C_{17}H_{14}O_8$ 346.0689	$C_{17}H_{14}O_9$ 362.0638
3OCH$_3$	$C_{18}H_{16}O_5$ 312.0998	$C_{18}H_{16}O_6$ 328.0947	$C_{18}H_{16}O_7$ 344.0896	$C_{18}H_{16}O_8$ 360.0845	$C_{18}H_{16}O_9$ 376.0794	
4OCH$_3$	$C_{19}H_{18}O_6$ 342.1103	$C_{19}H_{18}O_7$ 358.1053	$C_{19}H_{18}O_8$ 374.1002	$C_{19}H_{18}O_9$ 390.0951		
5OCH$_3$	$C_{20}H_{20}O_7$ 372.1209	$C_{20}H_{20}O_8$ 388.1158	$C_{20}H_{20}O_9$ 404.1107			
6OCH$_3$	$C_{21}H_{22}O_8$ 402.1315	$C_{21}H_{22}O_9$ 418.1264				
7OCH$_3$	$C_{22}H_{24}O_9$ 432.1420					

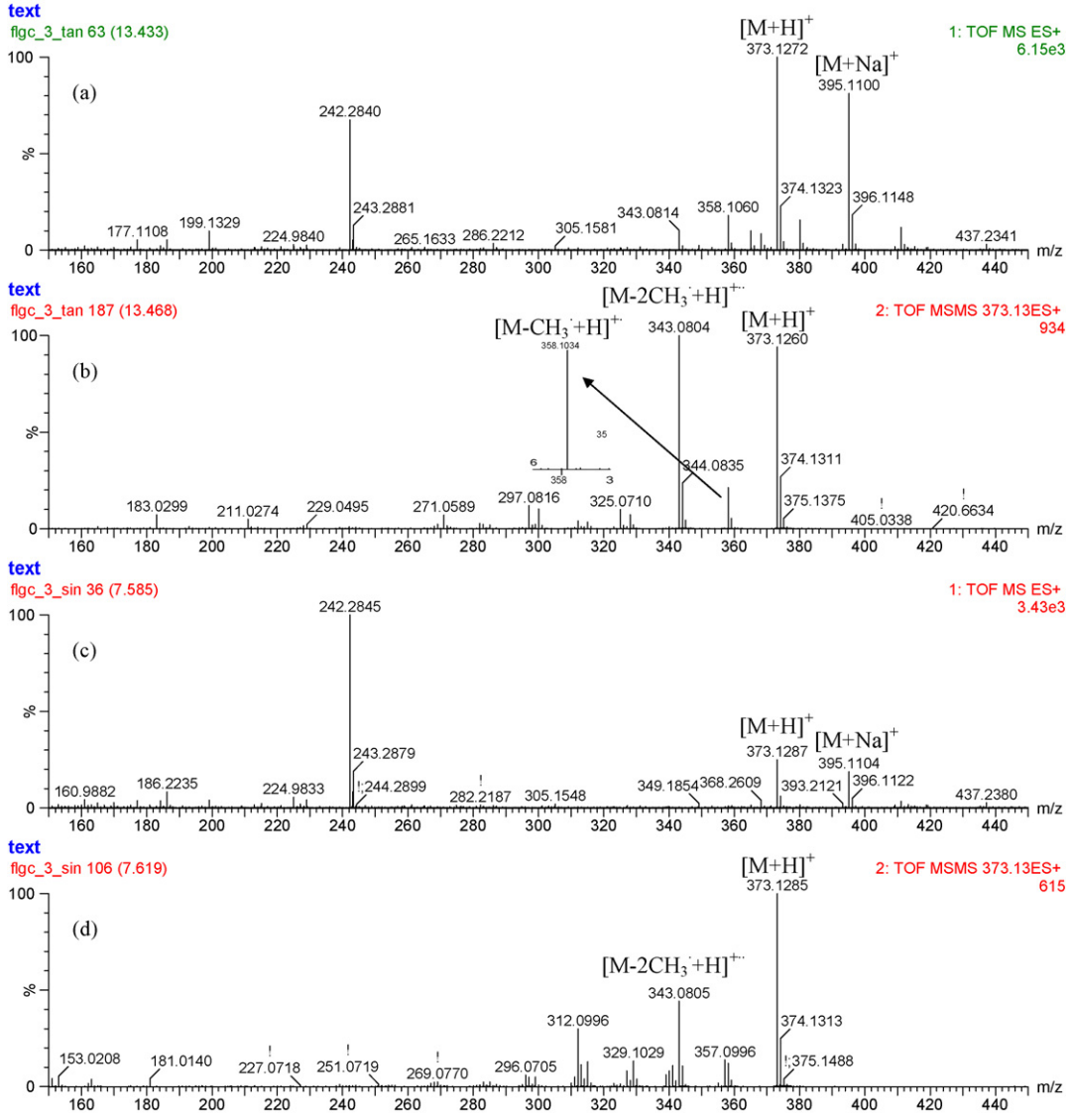

Fig. 3. UPLC/Q-TOFMS/MS analyzes TAN and SIN by using full scan–survey mode. (a) First stage MS spectrum of TAN; (b) MS/MS spectrum of TAN; (c) first stage MS spectrum of SIN; (d) MS/MS spectrum of SIN.

substituents such as methoxyl group (OCH_3) and/or hydroxyl group (OH) on its A, B and C rings (Fig. 2). Based on the numbers and the types of the substituent groups, the chemical formula and accurate mass of each possible PMF isomer can be designated (Table 1). In addition, PMFs have characteristic dissociation pattern, they can lose one or two methyl radicals ($CH_3^{\bullet}$) to produce radicals $[M+H-15.0235]^+$ or $[M+H-2\times15.0235]^+$ as predominant fragments [11]. The regular elemental composition and characteristic dissociation pattern, determined by exact mass measurements, should be used to form the diagnostic mass spectrometric fingerprint (MSFP) of the species for structural identification.

Fig. 3 shows the mass spectra acquired by using Q-TOFMS/MS in analysis of TAN and SIN. The first stage MS spectra show the protonated molecule ($[M+H]^+$) and sodium-adduct molecule ($[M+Na]^+$) of the compounds analyzed (Fig. 3a and c). Except for target ions (protonated molecule), interferences from liquid phase such as *m/z* 242.28 are shown too. In MS/MS spectra, by adjusting collision energy, the precursors (protonated molecule) and their fragment ions such as radicals $[M-CH_3^{\bullet}+H]^+$ and $[M-2\times CH_3^{\bullet}+H]^+$ can appear synchronously (Fig. 3b and d). Namely, the MS data needed to generate the MSFP for an analyte, containing accurate mass and isotopic pattern for both precursor and its fragment ions, are obtainable in the same spectrum. The accurate mass and isotopic pattern of precursors and their fragment ions observed are close to the theoretical values, which means that they can reflect the exact elemental composition (Table 2). Moreover, the diagnostic dissociation pattern of PMFs was acquired too. $[M+H-CH_3^{\bullet}]^+$ and $[M+H-2\times CH_3^{\bullet}]^+$ are the most predominant fragment ions in MS/MS spectra for both PMF references. The MS data of TAN and SIN acquired prove that the characteristic MSFP for PMFs, consisting of elemental composition and dissociation pattern, are obtainable by exact mass measurements using UPLC/Q-TOFMS/MS.

The MS data of candidate PMFs in samples, including accurate mass and isotopic pattern for both precursors and their fragment ions needed to generate MFSPs, should be obtained firstly for further identification. The recognition of non-target analytes in complex mixture is a rather challenging task, considering the huge number of endogenous matrix compounds capable of producing detectable peaks. In this study, the process of MS data acquisition is illuminated by analysis of protonated pentamethoxyflavone isomers (elemental composition: $C_{20}H_{21}O_7$; theoretical mass: 373.1287) in FP using UPLC/Q-TOFMS/MS.

Table 2
The mass data of TAN and SIN acquired by using UPLC/Q-TOFMS/MS.

	Ions	Mm[a] (Da)	Mc[b] (Da)	Error (mDa)	Error (ppm)	i-FIT	Elemental composition
MS	SIN ($[M+H]^+$)	373.1287	373.1287	0	0	0.4	$C_{20}H_{21}O_7$
	TAN ($[M+H]^+$)	373.1272	373.1287	−1.5	−4.0	1.6	$C_{20}H_{21}O_7$
MS/MS	SIN ($[M+H]^+$)	373.1285	373.1287	−0.2	−0.5	0.5	$C_{20}H_{21}O_7$
	SIN ($[M-CH_3{}^{\bullet}+H]^{+\bullet}$)	358.1047	358.1053	−1.9	−5.3	1.0	$C_{19}H_{18}O_7$
	SIN ($[M-2CH_3{}^{\bullet}+H]^{+\bullet\bullet}$)	343.0823	343.0818	0.5	1.5	0.2	$C_{18}H_{15}O_7$
	TAN ($[M+H]^+$)	373.1260	373.1287	−2.7	−7.2	6.0	$C_{20}H_{21}O_7$
	TAN ($[M-CH_3{}^{\bullet}+H]^{+\bullet}$)	358.1043	358.1053	−1.9	−5.3	1.0	$C_{19}H_{18}O_7$
	TAN ($[M-2CH_3{}^{\bullet}+H]^{+\bullet\bullet}$)	343.0804	343.0818	−1.4	−4.1	2.4	$C_{18}H_{15}O_7$

[a] Mm represents mass measured.
[b] Mc represents mass calculated.

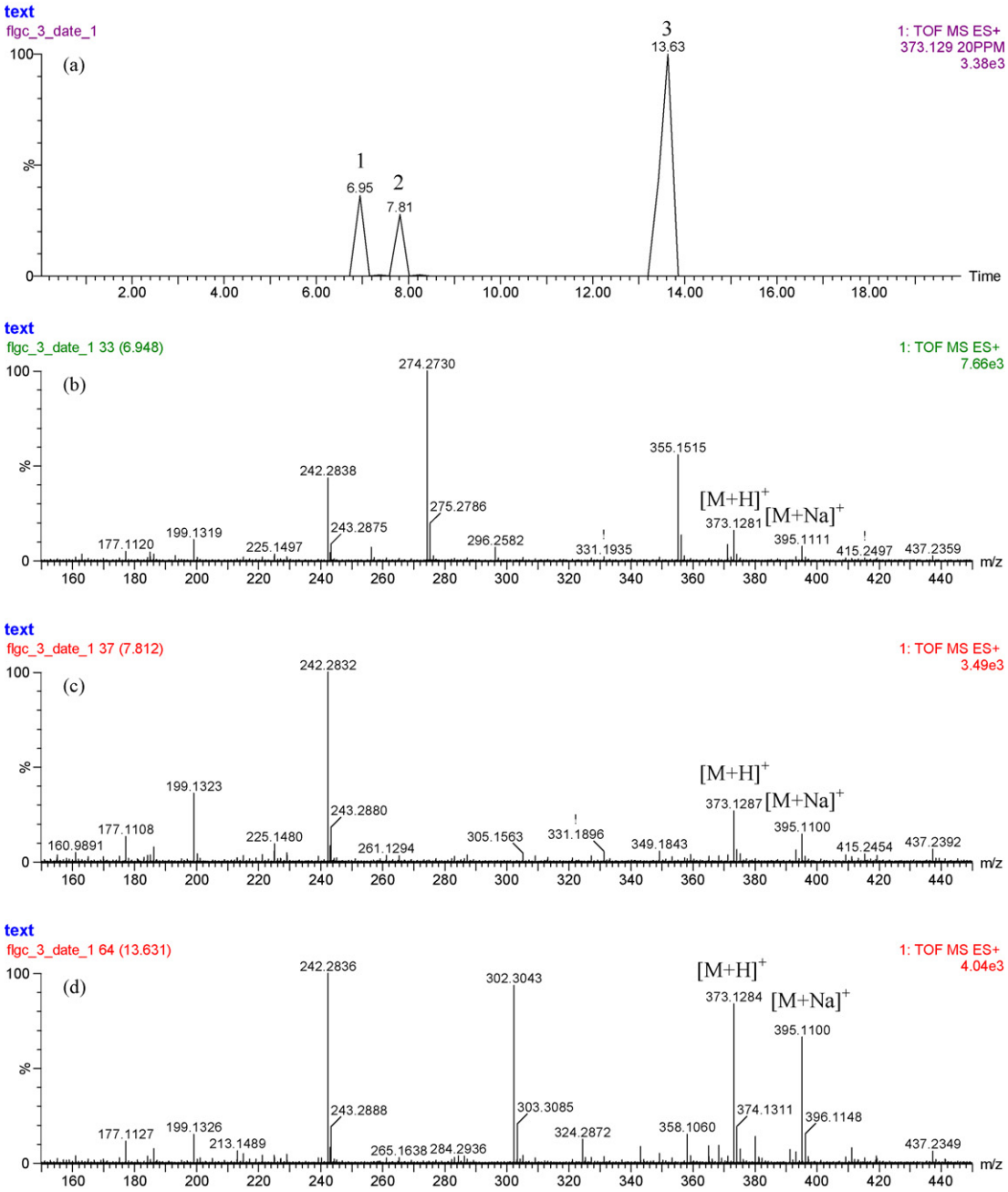

Fig. 4. UPLC/Q-TOFMS/MS analyzes FP by using full scan–survey mode. (a) EIC trace of *m/z* 373.1287 (extraction width: 0.001 Da); (b) first stage MS spectrum extracted at RT = 6.95 min in the chromatogram trace; (c) first stage MS spectrum extracted at RT = 7.81 min in the chromatogram trace; (d) first stage MS spectrum extracted at RT = 13.63 min in the chromatogram trace.

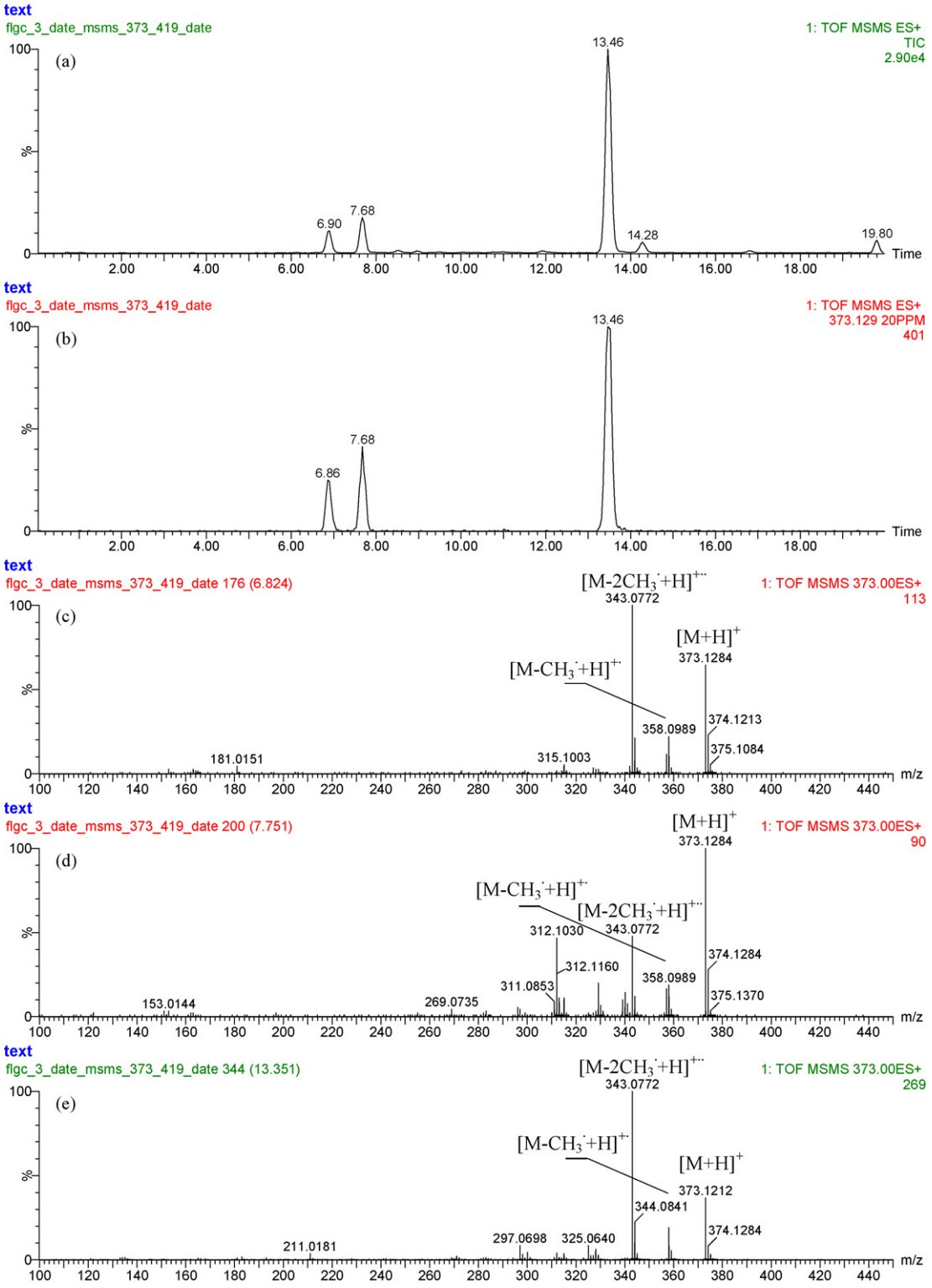

Fig. 5. UPLC/Q-TOFMS/MS analyzes FP by using SIM–MS/MS mode. (a) SIM chromatogram of *m*/*z* 373; (b) EIC trace of *m*/*z* 373.1287 (extraction width: 0.001 Da); (c) MS/MS spectrum extracted at RT = 6.82 min in the chromatogram trace; (d) MS/MS spectrum extracted at RT = 7.75 min in the chromatogram trace; (e) MS/MS spectrum extracted at RT = 13.35 min in the chromatogram trace.

Full scan mode is preferred in the analysis of non-target analytes from complex mixture for which MS data for all interested compounds from one LC–MS/MS injection can be aquired. Fig. 4 shows mass spectra acquired using UPLC/Q-TOFMS/MS analysis of FP by this mode. Fig. 4a shows full scan–EIC trace of *m*/*z* 373.1287 (extraction width: 0.001 Da). Fig. 4b–d shows the first stage MS spectra extracted at retention time (RT) = 6.95 min (corresponding to peak 1), RT = 7.81 min (corresponding to peak 2) and RT = 13.63 min (corresponding to peak 3), respectively in the chromatogram trace (Fig. 4a). In full scan–survey mode, only the predominant ion in first stage MS can be selected automatically as precursor to MS/MS. In those spectra, there were several

Table 3
List of the RT, MS data and structural elucidation for protonated PMFs identified from FP by using UPLC/Q-TOFMS/MS.

Comp. no.	RT (min)	Mm[a] (Da)	Mc[b] (Da)	Error (mDa)	Error (ppm)	i-FIT	Elemental composition	Structural elucidation
1	9.84	343.1183	343.1182	0.1	0.3	2.1	$C_{19}H_{19}O_6$	Tetramethoxyflavone
2	10.95	343.1183	343.1182	0.1	0.3	2.4	$C_{19}H_{19}O_6$	Tetramethoxyflavone
3	9.13	345.0919	345.0974	−5.5	−15.9	2.4	$C_{18}H_{17}O_7$	Dihydroxy-trimethoxyflavone
4	10.52	345.0959	345.0974	−1.5	−4.3	3.2	$C_{18}H_{17}O_7$	Dihydroxy-trimethoxyflavone
5	11.28	345.0925	345.0974	−4.9	−14.2	2.9	$C_{18}H_{17}O_7$	Dihydroxy-trimethoxyflavone
6	11.64	345.0939	345.0974	−3.5	−10.1	1.3	$C_{18}H_{17}O_77$	Dihydroxy-trimethoxyflavone
7	15.42	345.0969	345.0974	−0.5	−1.4	0.8	$C_{18}H_{17}O_7$	Dihydroxy-trimethoxyflavone
8	6.69	359.1095	359.1131	−3.6	−10.0	0.1	$C_{19}H_{19}O_7$	Monohydroxy-tetramethoxyflavone
9	9.68	359.1102	359.1131	−2.9	−8.1	0.6	$C_{19}H_{19}O_7$	Monohydroxy-tetramethoxyflavone
10	14.07	359.1150	359.1131	1.9	5.3	0.9	$C_{19}H_{19}O_7$	Monohydroxy-tetramethoxyflavone
11	6.82	373.1284	373.1287	−0.3	−0.8	1.9	$C_{20}H_{21}O_7$	Pentamethoxyflavone
12	7.75	373.1284	373.1287	−0.3	−0.8	2.4	$C_{20}H_{21}O_7$	Pentamethoxyflavone
13	13.35	373.1245	373.1287	−4.2	−11.3	7.1	$C_{20}H_{21}O_7$	Pentamethoxyflavone
14	11.95	375.1051	375.1080	−2.9	−7.7	0.3	$C_{19}H_{19}O_8$	Dihydroxy-tetramethoxyflavone
15	14.34	375.1030	375.1080	−5.0	−13.3	1.7	$C_{19}H_{19}O_8$	Dihydroxy-tetramethoxyflavone
16	16.84	375.1068	375.1080	−1.2	−3.2	0.2	$C_{19}H_{19}O_8$	Dihydroxy-tetramethoxyflavone
17	7.02	389.1184	389.1236	−5.2	−13.4	1.2	$C_{20}H_{21}O_8$	Monohydroxy-pentmethoxyflavone
18	7.41	389.1188	389.1236	−4.8	−12.3	1.3	$C_{20}H_{21}O_8$	Monohydroxy-pentmethoxyflavone
19	8.41	389.1184	389.1236	−5.2	−13.4	2.7	$C_{20}H_{21}O_8$	Monohydroxy-pentmethoxyflavone
20	9.04	389.1210	389.1236	−2.6	−6.7	2.4	$C_{20}H_{21}O_8$	Monohydroxy-pentmethoxyflavone
21	10.04	389.1183	389.1236	−5.3	−13.6	1.2	$C_{20}H_{21}O_8$	Monohydroxy-pentmethoxyflavone
22	10.97	389.1203	389.1236	−3.3	−8.5	0.9	$C_{20}H_{21}O_8$	Monohydroxy-pentmethoxyflavone
23	12.99	389.1182	389.1236	−5.4	−13.9	2.8	$C_{20}H_{21}O_8$	Monohydroxy-pentmethoxyflavone
24	15.09	389.1160	389.1236	−7.6	−19.5	6.2	$C_{20}H_{21}O_8$	Monohydroxy-pentmethoxyflavone
25	15.53	389.1247	389.1236	1.1	2.8	0.7	$C_{20}H_{21}O_8$	Monohydroxy-pentmethoxyflavone
26	17.50	389.1216	389.1236	−2.0	−5.1	0.6	$C_{20}H_{21}O_8$	Monohydroxy-pentmethoxyflavone
27	14.99	391.0965	391.1029	−6.4	−16.4	0.9	$C_{19}H_{19}O_9$	Trihydroxy-tetramethoxyflavone
28	10.26	403.1391	403.1393	−0.2	−0.5	2.2	$C_{21}H_{23}O_8$	Hexamethoxyflavone
29	9.55	405.1161	405.1186	−2.5	−6.2	0.6	$C_{20}H_{21}O_9$	Dihydroxy-pentmethoxyflavone
30	10.33	405.1163	405.1186	−2.3	−5.7	0.1	$C_{20}H_{21}O_9$	Dihydroxy-pentmethoxyflavone
31	11.36	405.1125	405.1186	−6.1	−15.1	1.2	$C_{20}H_{21}O_9$	Dihydroxy-pentmethoxyflavone
32	13.39	405.1159	405.1186	−2.7	−6.7	0.1	$C_{20}H_{21}O_9$	Dihydroxy-pentmethoxyflavone
33	14.66	405.1159	405.1186	−2.7	−6.7	0.4	$C_{20}H_{21}O_9$	Dihydroxy-pentmethoxyflavone
34	15.72	405.1186	405.1186	0	0	0.9	$C_{20}H_{21}O_9$	Dihydroxy-pentmethoxyflavone
35	16.65	405.1145	405.1186	−4.1	−10.1	0.1	$C_{20}H_{21}O_9$	Dihydroxy-pentmethoxyflavone
36	4.43	419.1366	419.1342	2.4	5.7	3.5	$C_{21}H_{23}O_9$	Monohydroxy-hexamethoxyflavone
37	5.71	419.1316	419.1342	−2.6	−6.2	1.1	$C_{21}H_{23}O_9$	Monohydroxy-hexamethoxyflavone
38	7.90	419.1324	419.1342	−1.8	−4.3	1.3	$C_{21}H_{23}O_9$	Monohydroxy-hexamethoxyflavone
39	8.47	419.1308	419.1342	−3.4	−8.1	4.0	$C_{21}H_{23}O_9$	Monohydroxy-hexamethoxyflavone
40	9.45	419.1279	419.1342	−6.3	−15.0	3.2	$C_{21}H_{23}O_9$	Monohydroxy-hexamethoxyflavone
41	9.79	419.1318	419.1342	−2.4	−5.7	0.2	$C_{21}H_{23}O_9$	Monohydroxy-hexamethoxyflavone
42	13.01	419.1290	419.1342	−5.2	−12.4	0.4	$C_{21}H_{23}O_9$	Monohydroxy-hexamethoxyflavone
43	15.81	419.1291	419.1342	−5.1	−12.2	2.3	$C_{21}H_{23}O_9$	Monohydroxy-hexamethoxyflavone
44	10.86	433.1490	433.1499	−0.9	−2.1	0.1	$C_{22}H_{25}O_9$	Heptamethoxyflavone

[a] Mm represents mass measured.
[b] Mc represents mass calculated.

interferences which have higher signal intensity than target ion (protonated molecule, *m*/*z* 373.1287), which cause the MS/MS data needed not obtainable. To overcome this problem, the inlet amount of sample has to be augmented to form more intense signal aimed, which will contaminate ion source and augment error of observed mass. To sum up, Q-TOFMS/MS in full scan mode cannot meet the analytical requirement in this study due to its low selectivity.

To improve selectivity, selected ions monitoring (SIM) mode was used to eliminate the non-target background in TOF detection and provide improved MS/MS detection capabilities. This mode has a limited screening capacity because each LC–MS/MS procedure can only be used for the detection of a small group of preselected compounds with known mass. As the mass of analytes can be designated beforehand in this study, the mode is suitable for use. Fig. 5 shows the spectra acquired in the UPLC/Q-TOF with SIM–MS/MS mode to analyze FP (target ions selected: *m*/*z* 373). Fig. 5a shows the SIM chromatogram acquired by scanning *m*/*z* 373, there were more than five obviously visible peaks in it. To eliminate the interfering peaks and alleviate the needless work of data processing, EIC trace of 373.1287 (extraction width: 0.001 Da) was extracted (Fig. 5b). The MS/MS spectra of compounds corresponding to the three peaks in the EIC trace are shown in Fig. 5c–e. In the spectra, the precursors and their product ions appear simultaneously, which offer the MS data needed for MSFP. According to the procedure, MSFPs of all candidate PMFs were obtained by multiple injections.

In each injection, five different masses listed in Table 1 were preselected for monitoring. Through retention time windows, the number of analyte peaks to be monitored could be beyond 30, but it is not suitable for analytes with unknown RT in this study. Because of the low dead volume of the whole system, UPLC allows short equilibration times (1 min between the end of the gradient and the next injection). Table 1 shows there are 21 masses for all possible PMF isomers, which need no more than five times injection with whole run time of 105 min. Among those candidates observed, 44 compounds possessing characteristic MSFP of PMFs were tentatively identified (Table 3 and Table 4). Q-TOF in SIM mode accomplished a very high selectivity and it can acquire the MS data for every one of co-eluting compounds. For example, compound 2 (*m*/*z* 343.1183, RT = 10.95 min) and compound 22 (*m*/*z* 389.1203, RT = 10.97 min) in Table 3 nearly have the same RT (ΔRT = 0.02 min), the MS data of these two compounds were obtained with high quality. Even so, the extra resolution of UPLC is still critical especially under special condition. For instance, compound 5 (*m*/*z* 345.0974,

Table 4
List of the RT, MS data and structural elucidation for main product ions of protonated PMFs identified from FP by using UPLC/Q-TOFMS/MS.

Comp. No.	RT (min)	Precursor (*m*/*z*)	Product ion ([M−CH_3•+H]$^{•+}$)				Product ion ([M−$2CH_3$•+H]$^{••+}$)			
			Mm[a] (Da)	Mc[b] (Da)	Error (mDa)	Error (ppm)	Mm[a] (Da)	Mc[b] (Da)	Error (mDa)	Error (ppm)
1	9.84	343.1183	328.0760	328.0947	18.7	57.0	313.0700	313.0712	1.2	3.8
2	10.95	343.1183	328.0961	328.0947	−1.4	−4.3	313.0635	313.0712	7.7	24.6
3	9.13	345.0919	330.0711	330.0739	2.8	8.5	315.0567	315.0504	−6.3	−20.0
4	10.52	345.0959	330.0683	330.0739	5.6	17.0				
5	11.28	345.0925	330.0671	330.0739	6.8	20.6				
6	11.64	345.0939	330.0771	330.0739	−3.2	−9.7				
7	15.42	345.0969	330.0810	330.0739	−7.1	−21.5	315.0458	315.0504	4.6	14.6
8	6.69	359.1095	344.0867	344.0896	2.9	8.4	329.0591	329.0661	7.0	21.3
9	9.68	359.1102	344.0848	344.0896	4.8	13.9	329.0600	329.0661	6.1	18.5
10	14.07	359.1150	344.0910	344.0896	−1.4	−4.1	329.0596	329.0661	6.5	19.8
11	6.82	373.1284	358.0989	358.1052	6.3	17.6	343.0772	343.0817	4.5	13.1
12	7.75	373.1284	358.0989	358.1052	6.3	17.6	343.0772	343.0817	4.5	13.1
13	13.35	373.1245	358.0982	358.1052	7.0	19.5	343.0756	343.0817	6.1	17.8
14	11.95	375.1051	360.0876	360.0845	−3.1	−8.6	345.0644	345.061	−3.4	−9.9
15	14.34	375.1030	360.0800	360.0845	4.5	12.5	345.0593	345.061	1.7	4.9
16	16.84	375.1068	360.0776	360.0845	6.9	19.2				
17	7.02	389.1184	374.1044	374.1001	−4.3	−11.5	359.0717	359.0766	4.9	13.6
18	7.41	389.1188	374.0999	374.1001	0.2	0.5	359.0715	359.0766	5.1	14.2
19	8.41	389.1184	374.0984	374.1001	1.7	4.5	359.0719	359.0766	4.7	13.1
20	9.04	389.1210	374.0980	374.1001	2.1	5.6	359.0709	359.0766	5.7	15.9
21	10.04	389.1183					359.0702	359.0766	6.4	17.8
22	10.97	389.1203	374.0963	374.1001	3.8	10.2				
23	12.99	389.1182	374.0710	374.1001	29.1	77.8				
24	15.09	389.1160	374.1004	374.1001	−0.3	−0.8	359.0706	359.0766	6.0	16.7
25	15.53	389.1247	374.0855	374.1001	14.6	39.0	359.0765	359.0766	0.1	0.3
26	17.5	389.1216	374.0930	374.1001	7.1	19.0	359.0718	359.0766	4.8	13.4
27	14.99	391.0965	376.0819	376.0794	−2.5	−6.6	361.0526	361.0559	3.3	9.1
28	10.26	403.1391	388.1180	388.1158	−2.2	−5.7	373.0929	373.0923	−0.6	−1.6
29	9.55	405.1161	390.0811	390.0951	14	35.9	375.0774	375.0716	−5.8	−15.5
30	10.33	405.1163	390.0956	390.0951	−0.5	−1.3	375.0700	375.0716	1.6	4.3
31	11.36	405.1125	390.0902	390.0951	4.9	12.6	375.0660	375.0716	5.6	14.9
32	13.39	405.1159	390.0923	390.0951	2.8	7.2	375.0743	375.0716	−2.7	−7.2
33	14.66	405.1159	390.0928	390.0951	2.3	5.9	375.0642	375.0716	7.4	19.7
34	15.72	405.1186	390.1003	390.0951	−5.2	−13.3	375.0637	375.0716	7.9	21.1
35	16.65	405.1145	390.0858	390.0951	9.3	23.8	375.0656	375.0716	6.0	16.0
36	4.43	419.1366	404.1026	404.1107	8.1	20.0	389.0872	389.0872	0.0	0.0
37	5.71	419.1316	404.0918	404.1107	18.9	46.8	389.0861	389.0872	1.1	2.8
38	7.9	419.1324	404.0989	404.1107	11.8	29.2	389.0790	389.0872	8.2	21.1
39	8.47	419.1308	404.1118	404.1107	−1.1	−2.7	389.0813	389.0872	5.9	15.2
40	9.45	419.1279	404.1012	404.1107	9.5	23.5	389.0826	389.0872	4.6	11.8
41	9.79	419.1318	404.0942	404.1107	16.5	40.8	389.0818	389.0872	5.4	13.9
42	13.01	419.1290	404.1040	404.1107	6.7	16.6	389.0827	389.0872	4.5	11.6
43	15.81	419.1291	404.1047	404.1107	6	14.8	389.0817	389.0872	5.5	14.1
44	10.86	433.1490	418.1241	418.1264	2.3	5.5	403.1012	403.1029	1.7	4.2

[a] Mm represents mass measured.
[b] Mc represents mass calculated.

RT = 11.28 min) and compound 6 (*m*/*z* 345.0974, RT = 11.64 min) are isomers with close RT (ΔRT = 0.36 min), their MS data can be acquired apart as they have enough chromatographic resolution. By contrast, our preliminary publication of a HPLC–MS method for analysis of PMFs in *F. aurantii* extract required 40 min run time and produced a peak width of 0.5–0.8 min [11].

4. Conclusions

UPLC/Q-TOFMS/MS is an interesting hyphenated technique for the rapid identification of PMFs in crude samples. UPLC provides high chromatographic resolution that exact mass measurement needed and high throughput that rapid structural screening needed. Q-TOF permits the extraction of any high-resolution MS traces after acquiring data. This is extremely valuable if commercially not available analytes are to be monitored. The MSFP for a candidate, consisting of chemical formula and fragment pattern, was determined by exact mass measurements for structural identification. Compared the MSFP of the observed candidates with the diagnostic MSFP of the species, 44 PMFs were tentatively identified from *F. aurantii* extract by a total 105 min run time method.

Acknowledgement

This work was supported by "Project of International Cooperation Plan from Ministry of Science and Technology of China (2007DFC30550)".

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