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Rapid screening and identification of target constituents using full scan-parent ions list-dynamic exclusion acquisition coupled to diagnostic product ions analysis on a hybrid LTQ-Orbitrap mass spectrometer

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ABSTRACT

A highly sensitive and effective strategy for rapid screening and identification of target constituents has been developed using full scan-parent ions list-dynamic exclusion (FS-PIL-DE) acquisition coupled to diagnostic product ions (DPIs) analysis on a hybrid LTQ-Orbitrap mass spectrometer. The FS-PIL-DE was adopted as a survey scan to trigger the MS/MS acquisition of all the predictable constituents contained in traditional Chinese medicines. Additionally, DPIs analysis can provide a criterion to judge the target constituents detected into certain chemical families. Results from analyzing polymethoxylated flavonoids (PMFs) in the leaves of *Citrus reticulata* Blanco demonstrated that FS-PIL-DE was capable of targeting a greater number of constituents than FS, FS-PIL and FS-DE, thereby increasing the coverage of constituent screening. As a result, 135 PMFs including 81 polymethoxyflavones, 54 polymethoxyflavanones or polymethoxychalcones were identified preliminarily. And this was the first time to systematically report the presence of PMFs in the leaves of *Citrus reticulata* Blanco, especially for polymethoxylated flavanones and chalcones, most of which were new compounds. The results indicated that the developed FS-PIL-DE coupled to DPIs analysis methodology could be employed as a rapid, effective technique to screen and identify target constituents from TCMs extracts and other organic matter mixtures whose compounds contained can also be classified into families based on the common carbon skeletons.

1. Introduction

Traditional Chinese medicines (TCMs) have been gained increasing popularity worldwide owing to the changes in the types of diseases, especially the prevalence of chronic and systematic diseases and limitations of western medicines [1,2]. However, because of the large variation in the content, physical and chemical properties of the constituents and unclear mechanisms of action, it is rather difficult to guarantee the consistency of quality and therapeutic efficacy of TCMs. It is well known that TCMs, either formed as a single herb or a group of herbs in composite formula, are a complex mixture containing hundreds of different chemical constituents responsible for their therapeutic effects. In this respect, the rapid screening and identification of the constituents, particularly the microconstituents in TCMs, is an integral part of the drug discovery and development process.

Although the separation and identification of constituents contained in TCMs with phytochemistry methods have been developed, the previous analytical results showed that numerous compounds have not been investigated yet [3,4]. HPLC–ESI-MS^n has become a powerful approach for the rapid identification of constituents in TCM extracts [5–9]. Constituent profiling of LC/MS involves detection of parent ions and subsequent structural elucidation of the detected constituents based on their molecular weights, fragmentation pathways and/or elemental compositions. Traditionally,

Abbreviations: FS-PIL-DE, full scan-parent ions list-dynamic exclusion; DPIs, diagnostic product ions diagnostic product ions; PMFs, polymethoxylated flavonoids; TCMs, traditional Chinese medicines; CID, collision induced dissociation; RDB, ring double bond; OCH_3, methoxyl group; OH, hydroxyl group; *m/z*, mass-to-charge; RDA, retro-Diels–Alder; TIC, total ion chromatogram; OH-PMFs, hydroxylated polymethoxyflavonoids

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the detection of common constituents with predictable molecular weights is accomplished by acquiring full-scan LC/MS data followed by generation of extracted ion chromatograms corresponding to their mass-to-charge(m/z) values. Over the last decade, ion trap and linear ion trap LC/MS have been extensively used for the detection and structural characterization of common constituents. These instruments employ full-scan MS analysis as a survey scan to trigger MS/MS acquisition. Therefore, both constituent detection and MS/MS spectral acquisition can be accomplished in a single LC/MS run.

Recently, a new hybrid LTQ-Orbitrap analytical platform is applied to the analysis of small molecules in biological and TCM samples [10–14]. It consists of a 2D ion trap coupled with an Orbitrap, and allows two different scan types to be acquired simultaneously. The Orbitrap mass spectrometer, otherwise defined as an electrostatic Fourier Transform mass spectrometer, provides a higher mass resolution and mass accuracy than any other mass spectrometers [15]. The ion trap can provide multi-stage MS^n mass spectra using data-dependent analysis and mass accuracies of <5 ppm can be obtained by the Orbitrap scan in an external calibration mode. A full scan mass spectrum acquired with a mass resolution of 30,000 for Orbitrap needs 0.4 s, and provides 25 data points across a peak of width at baseline of 10 s. This advantage facilitates the identification of known and novel constituents in TCMs.

However, due to the significant difference in content and occasionally poor chromatographic separation, many constituents especially microconstituents cannot be detected in the full-scan MS data or their MS/MS acquisitions cannot be triggered when coeluted with the constituents of relative higher content. Therefore, it is desired to establish a new methodology or strategy to enhance the constituent detection and identification capacities of LC–MS/MS. First, since the multiple constituents contained in a certain traditional herb are derived from one or more certain biosynthetic pathways, the constituents could usually be structurally classified into several chemical families with same carbon skeletons or substructures. So it is easily understood that their formula and molecular weights are predictable. Second, the constituents with same carbon skeletons will undergo similar fragmentation pathways in collision induced dissociation (CID) mode and thus generate similar diagnostic product ions (DPIs) from the common carbon skeletons. In other words, a series of DPIs representing a certain parent nucleus or substitution groups can be used as the characteristic peaks to select out the corresponding chemical family.

In this study, we explored a novel strategy of full scan-parent ions list-dynamic exclusion (FS-PIL-DE) acquisition coupled to DPIs analysis for screening and identification of target constituents, particularly microconstituents in TCMs on a hybrid LTQ-Orbitrap mass spectrometer, and the effectiveness of the strategy was examined by analyzing polymethoxylated flavonoids (PMFs) in the leaves of *Citrus reticulata* Blanco (Juye in Chinese). PMFs are a kind of the specific flavonoid subclasses with all or almost hydroxyls capped by methylation, and have high oral bioavailability [16–19]. Results from FS-PIL-DE acquisition were also compared with those from FS, FS-PIL and FS-DE experiments on the same LTQ-Orbitrap instrument. Additional application of FS-PIL-DE acquisition coupled to DPIs analysis for constituent detection and structural characterization were also evaluated. The results demonstrated that the strategy could greatly enhance the target constituents screening and identification capabilities of the hybrid LTQ-Orbitrap instrument.

2. Experimental

2.1. Standards and reagents

Eighteen PMF standards were provided by Professor Peng-fei Tu from Modern Research Center of Traditional Chinese Medicines, Peking University, PR China, and identified in our laboratory for qualitative analysis (shown in Fig. 1). The purities of all ingredients were determined to be no less than 95% according to HPLC-DAD analysis.

HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid was purchased from Sigma Aldrich (St. Louis, MO, USA). Deionized water used throughout the experiment was purified by a Milli-Q Gradient A 10 System (Millipore, Billerica, MA, USA). The 0.22 μm membranes were purchased from Xinjinghua Co. (Shanghai, China).

2.2. Plant material and sample preparation

The leaves of *Citrus reticulata* Blanco were collected at random from the trees in Tongzhou County, Beijing, China in October 2011. The leaves were deposited in the cool and dry place prior to analysis. It was authenticated by Professor Yan-Jiang Qiao. And its voucher specimen was deposited at Center of Scientific Experiment, Beijing University of Chinese Medicine, Beijing, China.

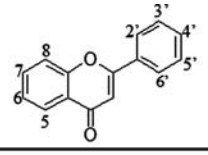
The dried leaves were powdered to a homogeneous size by a mill and sieved through a No. 40 mesh sieve. An amount of 0.5 g was extracted with 25 mL of methanol/water (70:30, v/v) in an ultrasonic bath (Eima Ultrasonics Corp., Germany) for 30 min at room temperature. The methanol solution was filtered through a 0.22 μm microporous membrane before injection to the HPLC–MS system for analysis.

2.3. Standard solutions preparation

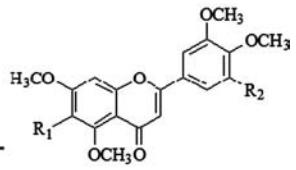
The appropriate amount of each standard was weighed and dissolved in methanol to make eighteen individual stock solutions. Then, each stock solution was mixed with methanol to prepare a final mixed standard solution.

2.4. The design of screening table

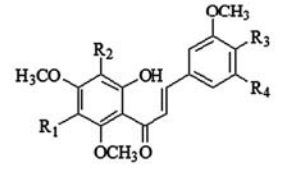
Three kinds of PMFs, i.e. polymethoxylated flavones, flavanones and chalcones have been revealed from many medicinal plants so far [20–22]. Although the phenomenon of substitution isomerism is most commonly seen, PMFs have regularity in elemental composition since they have the basic aglycone structure with substituents such as methoxyl group (OCH_3) and/or hydroxyl group (OH) on their A, B and C rings. The molecular weights of the basic aglycone structures are 222, 224 and 224 for flavones, flavanones and chalcones, which are increased by 30 or 16 when a methoxyl or hydroxyl was attached.



Compounds	-OH	-OCH3
3	-	5,7,8,3',4',5'
4	-	5,7,3',4',5'
6	-	5,6,7,3',4',5'
7	-	5,6,7,8,3',4'
8	5,3'	7,8,4',5'
9	5,4'	7,3'
10	5	6,7,3',4'
11	-	5,6,7,8,4'
12	5	7,3',4',5'
14	5	6,7,3',4',5'
13	5	6,7,8,3',4'
16	5	7,3',4'



1 R_1=OCH_3, R_2=H
5 R_1=H, R_2=OCH_3



2 R_1=H, R_2=R_3=R_4=OCH_3
15 R_1=R_2=R_3=R_4=H
17 R_1=R_2=R_3=R_4=OCH_3
18 R_1=R_2=H, R_3=R_4=OCH_3

Fig. 1. Structures of eighteen PMFs reference standards.

Hence, the screening table was designed by arranging hydroxyl and methoxyl at the two molecular weights from two to seven positions, including 42 molecular weights in which 10 data were repeated (shown in Table 1).

2.5. HPLC analysis

Thermo Scientific Accela 600 pump HPLC system used in the experiment equipped with a binary pump and an autosampler. An Agilent Zorbax Extended C_{18} ($250 \times 4.6\ mm^2$ i.d., 5 μm) was used for separation of the PMFs at room temperature. 0.1% formic acid aqueous solution (solvent A) and acetonitrile (solvent B) were used as mobile phase. The flow rate was 1.0 mL/min and elution conditions at room temperature applied with a linear gradient as follows: 0–5 min, 20–28% B; 5–70 min, 28–42% B; 70–90 min, 42–64% B; 90–95 min, 64–100% B.

Table 1
The screening table of aglycone constructed by the arrangement of hydroxyl and methoxyl from 2 to 7 positions at aglycone nucleus.

Number	Substituents	Fomula		[M+H]$^+$	
		222	224	222	224
2	$2OCH_3$	$C_{17}H_{14}O_4$	$C_{17}H_{16}O_4$	283	285
3	$2OCH_3+OH$	$C_{17}H_{14}O_5$	$C_{17}H_{16}O_5$	299	301
	$3OCH_3$	$C_{18}H_{16}O_5$	$C_{18}H_{18}O_5$	313	**315**
4	$2OCH_3+2OH$	$C_{17}H_{14}O_6$	$C_{17}H_{16}O_6$	**315**	317
	$3OCH_3+OH$	$C_{18}H_{16}O_6$	$C_{18}H_{18}O_6$	329	**331**
	$4OCH_3$	$C_{19}H_{18}O_6$	$C_{19}H_{20}O_6$	343	**345**
5	$2OCH_3+3OH$	$C_{17}H_{14}O_7$	$C_{17}H_{16}O_7$	**331**	333
	$3OCH_3+2OH$	$C_{18}H_{16}O_7$	$C_{18}H_{18}O_7$	**345**	**347**
	$4OCH_3+OH$	$C_{19}H_{18}O_7$	$C_{19}H_{20}O_7$	359	**361**
	$5OCH_3$	$C_{20}H_{20}O_7$	$C_{20}H_{22}O_7$	373	**375**
6	$2OCH_3+4OH$	$C_{17}H_{14}O_8$	$C_{17}H_{16}O_8$	**347**	349
	$3OCH_3+3OH$	$C_{18}H_{16}O_8$	$C_{18}H_{18}O_8$	**361**	**363**
	$4OCH_3+2OH$	$C_{19}H_{18}O_8$	$C_{19}H_{20}O_8$	**375**	**377**
	$5OCH_3+OH$	$C_{20}H_{20}O_8$	$C_{20}H_{22}O_8$	389	**391**
	$6OCH_3$	$C_{21}H_{22}O_8$	$C_{21}H_{24}O_8$	403	**405**
7	$2OCH_3+5OH$	$C_{17}H_{14}O_9$	$C_{17}H_{16}O_9$	**363**	365
	$3OCH_3+4OH$	$C_{18}H_{16}O_9$	$C_{18}H_{18}O_9$	**377**	379
	$4OCH_3+3OH$	$C_{19}H_{18}O_9$	$C_{19}H_{20}O_9$	**391**	393
	$5OCH_3+2OH$	$C_{20}H_{20}O_9$	$C_{20}H_{22}O_9$	**405**	407
	$6OCH_3+OH$	$C_{21}H_{22}O_9$	$C_{21}H_{24}O_9$	419	421
	$7OCH_3$	$C_{22}H_{24}O_9$	$C_{22}H_{26}O_9$	433	435

Note: 222, 224 and 224 are molecular weights for three familiar subclasses flavonoids without substitutent groups. The ions shown in bold have the same nominal molecular weights as the others which were used to construct the screening table of PMFs.

2.6. ESI-MS/MS analysis

High-resolution MS and MS/MS spectral analysis were performed on an LTQ-Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany). The mass spectrometer was connected to the HPLC instrument via an ESI interface in a post-column splitting ratio of 1:3. Samples were analyzed in the positive ion mode with a tune method set as follows: sheath gas (nitrogen) flow rate of 30 arb, aux gas (nitrogen) flow rate of 5 arb, spray voltage of 4.0 kV, capillary temperature of 350 °C, capillary voltage of 25 V, tube lens voltage of 110 V. Accurate mass analysis were calibrated according to the manufacturer's guidelines using a standard solution mix of caffeine, sodium dodecyl sulfate, sodium taurocholate, the tetrapeptide MRFA acetate salt and Ultramark. The measured masses were within 5 ppm of the theoretical masses. Centroided mass spectra were acquired in the mass range of m/z 100–900.

In the FS experiment, resolution of the Orbitrap mass analyzer was set at 30,000 (FWHM as defined at m/z 400). Data-dependent MS/MS scanning was performed to minimize total analysis time as it can trigger fragmentation spectra of target ions. The maximum injection time was 50 ms and the number of microscans was 2. The collision energy for CID was adjusted to 35% of maximum, and the isolation width of precursor ions was m/z 2.0 Da. In the FS-PIL experiment, a parent ions list including 32 ions identical to those listed in Table 1 was added to the FS analytical method. In the FS-DE experiment based on the FS scan, the dynamic exclusion to prevent repetition was enabled, and the repeat count was set at 5 with the dynamic repeat time at 30 s and dynamic exclusion duration at 60 s. In the FS-PIL-DE experiment, the parent ions list mentioned above and dynamic exclusion were all enabled on the basis of the FS scan analytical method.

2.7. Peak selections and data processing

The Thermo Xcaliber 2.1 workstation was used for the data acquiring and processing. In order to obtain as many fragments as possible, the peaks detected with intensity over 10,000 were selected for identifications. The chemical formulas for all parent and fragment ions of the selected peaks were calculated from the accurate mass using a formula predictor by setting the parameters as follows: C [0–30], H [0–50], O [0–20] and Ring Double Bond (RDB) equivalent value [0–15]. Other elements such as N, P, S, Cl

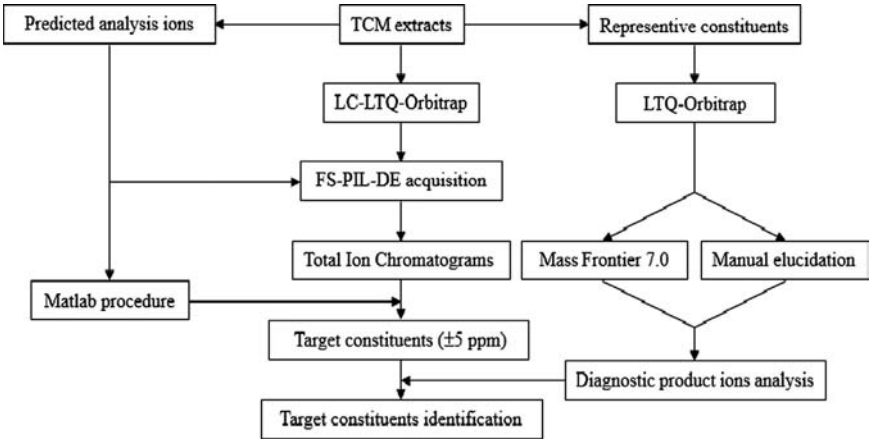


Fig. 2. Summary diagram of presently developed strategy and methodology.

Table 2
Characterizations of Eighteen PMFs Standards by CID-MS/MS.

Peak	Experimental mass (*m*/*z*)	Empirical formula	Mass error (ppm)	MS^2 (*m*/*z*)				MS^3 (*m*/*z*)			
				P-ion (%)[a]		ppm	Radical loss	P-ion(%)[a]		ppm	Radical loss
1	375.1442	$C_{20}H_{23}O_7$	1.0	211.0609[b] (100)	$C_{10}H_{11}O_5$	3.8	$^{1,3}A^{+c}$	196.0372 (100)	$C_9H_8O_5$	2.9	$CH_3^{\bullet}$
				191.0710 (42.9)	$C_{11}H_{11}O_3$	3.8	$^{1,4}B^{+c}$	178.0267 (24)	$C_9H_6O_4$	3.6	$H_2O+CH_3^{\bullet}$
				357.1348 (19.8)	$C_{20}H_{21}O_6$	4.3	H_2O	183.0659 (8.6)	$C_9H_{11}O_4$	3.9	CO
2	405.1543	$C_{21}H_{25}O_8$	−0.2	221.0812[b] (100)	$C_{12}H_{13}O_4$	1.7	$^{x}B^{+d}$	193.0862 (100)	$C_{11}H_{13}O_3$	1.5	CO
				387.1447 (32.1)	$C_{21}H_{23}O_7$	2.3	H_2O	190.0628 (52.1)	$C_{11}H_{10}O_3$	1.9	$OCH_3^{\bullet}$
				211.0608 (37.9)	$C_{10}H_{11}O_5$	3.3	$^{y}A^{+d}$	206.0578 (42.3)	$C_{11}H_{10}O_4$	2.1	$CH_3^{\bullet}$
								191.0344 (39.2)	$C_{10}H_7O_4$	2.7	$2CH_3^{\bullet}$
3	403.1391	$C_{21}H_{23}O_8$	0.9	373.0928[b] (100)	$C_{19}H_{17}O_8$	2.7	$2CH_3^{\bullet}$	345.0978 (100)	$C_{18}H_{17}O_7$	2.7	CO
				388.1164 (50.8)	$C_{20}H_{20}O_8$	2.9	$CH_3^{\bullet}$	340.0588 (55.5)	$C_{18}H_{12}O_7$	3.1	$H_2O+CH_3^{\bullet}$
				342.1112 (40.1)	$C_{19}H_{18}O_6$	4.1	$H_2O+CO+CH_3^{\bullet}$	358.0695 (25.9)	$C_{18}H_{14}O_8$	3.3	$CH_3^{\bullet}$
								312.0638 (23.1)	$C_{17}H_{12}O_6$	3.1	$H_2O+CO+CH_3^{\bullet}$
								343.0457 (18.6)	$C_{17}H_{11}O_8$	2.5	$2CH_3^{\bullet}$
4	373.1287	$C_{20}H_{21}O_7$	1.4	312.1009[b] (100)	$C_{18}H_{16}O_5$	5.4	$H_2O+CO+CH_3^{\bullet}$	284.1048 (100)	$C_{17}H_{16}O_4$	1.7	CO
				343.0829 (25.6)	$C_{18}H_{15}O_7$	4.9	$2CH_3^{\bullet}$	283.0974 (43.7)	$C_{17}H_{15}O_4$	3.2	$HCO^{\bullet}$
				358.1063 (20.3)	$C_{19}H_{18}O_7$	4.5	$CH_3^{\bullet}$	297.0766 (7.6)	$C_{17}H_{13}O_5$	2.9	$CH_3^{\bullet}$
5	375.1442	$C_{20}H_{23}O_7$	1.0	221.0813[b] (100)	$C_{12}H_{13}O_4$	2.1	$^{1,4}B^{+c}$	193.0862 (100)	$C_{11}H_{13}O_3$	1.5	CO
				181.0501 (16.9)	$C_9H_9O_4$	3.1	$^{1,3}A^{+c}$	190.0628 (54.5)	$C_{11}H_{10}O_3$	1.9	$OCH_3^{\bullet}$
				357.1343 (8.1)	$C_{20}H_{21}O_6$	2.9	H_2O	206.0578 (45.9)	$C_{11}H_{10}O_4$	2.1	$CH_3^{\bullet}$
								191.0344 (37.5)	$C_{10}H_7O_4$	2.7	$2CH_3^{\bullet}$
6	403.1391	$C_{21}H_{23}O_8$	0.9	342.1108[b] (100)	$C_{19}H_{18}O_6$	2.9	$H_2O+CO+CH_3^{\bullet}$	327.0867 (100)	$C_{18}H_{15}O_6$	1.2	$CH_3^{\bullet}$
				388.1162 (45.9)	$C_{20}H_{20}O_8$	2.4	$CH_3^{\bullet}$	281.0812 (54.8)	$C_{17}H_{13}O_4$	1.3	$H_2O+CO+CH_3^{\bullet}$
				373.0928 (28.3)	$C_{19}H_{17}O_8$	2.7	$2CH_3^{\bullet}$	309.0762 (27.7)	$C_{18}H_{13}O_5$	1.5	$H_2O+CH_3^{\bullet}$
				359.1135 (25.3)	$C_{19}H_{19}O_7$	2.7	$HCO^{\bullet}+CH_3^{\bullet}$	312.0633 (13.8)	$C_{17}H_{12}O_6$	1.5	$2CH_3^{\bullet}$
7	403.1369	$C_{21}H_{23}O_8$	−4.5	388.1143[b] (100)	$C_{20}H_{20}O_8$	−2.5	$CH_3^{\bullet}$	373.0906(100)	$C_{19}H_{17}O_8$	−3.2	$CH_3^{\bullet}$
				373.0912(72.19)	$C_{19}H_{17}O_8$	−1.6	$2CH_3^{\bullet}$	342.1087(21.50)	$C_{19}H_{18}O_6$	−3.2	$CO+H_2O$
8	375.1078	$C_{19}H_{19}O_8$	1.0	360.0852[b] (100)	$C_{18}H_{16}O_8$	3.4	$CH_3^{\bullet}$	344.0537 (100)	$C_{17}H_{12}O_8$	3.0	CH_4
				359.0776 (45.3)	$C_{18}H_{15}O_8$	4.1	CH_4	314.0797 (22.2)	$C_{17}H_{14}O_6$	3.9	$CO+H_2O$
				345.0621 (13.6)	$C_{17}H_{13}O_8$	4.7	$2CH_3^{\bullet}$	331.0826 (21.8)	$C_{17}H_{15}O_7$	4.1	$HCO^{\bullet}$
				314.0797 (12.7)	$C_{17}H_{14}O_6$	3.9	$H_2O+CO+CH_3^{\bullet}$	315.0509 (16.5)	$C_{16}H_{11}O_7$	3.1	$HCO^{\bullet}+CH_4$
9	315.0864	$C_{17}H_{15}O_6$	0.3	300.0632[b] (100)	$C_{16}H_{12}O_6$	1.2	$CH_3^{\bullet}$	272.0683 (100)	$C_{15}H_{12}O_5$	1.4	CO
10	359.1129	$C_{19}H_{19}O_7$	1.0	326.0796[b] (100)	$C_{18}H_{14}O_6$	3.4	$H_2O+CH_3^{\bullet}$	298.0843 (100)	$C_{17}H_{14}O_5$	2.4	CO
				344.0902 (71.4)	$C_{18}H_{16}O_7$	3.3	$CH_3^{\bullet}$	270.0898 (0.6)	$C_{16}H_{14}O_4$	4.2	2CO
11	373.1270	$C_{20}H_{21}O_7$	−3.1	358.1034[b] (100)	$C_{19}H_{18}O_7$	−3.6	$CH_3^{\bullet}$	343.0800(100)	$C_{18}H_{15}O_7$	−3.6	$CH_3^{\bullet}$
				343.0803(54.70)	$C_{18}H_{15}O_7$	−2.7	$2CH_3^{\bullet}$	312.0982(23.13)	$C_{18}H_{16}O_5$	−3.3	$CO+H_2O$
12	359.1129	$C_{19}H_{19}O_7$	1.0	344.0895[b] (100)	$C_{18}H_{16}O_7$	1.3	$CH_3^{\bullet}$	315.0873 (100)	$C_{17}H_{15}O_6$	3.1	$HCO^{\bullet}$
				315.0871 (16.9)	$C_{17}H_{15}O_6$	2.5	$HCO^{\bullet}+CH_3^{\bullet}$	328.0585 (64.6)	$C_{17}H_{12}O_7$	2.3	CH_4
								326.0794 (26.8)	$C_{18}H_{14}O_6$	2.8	H_2O
								299.0559 (19.8)	$C_{16}H_{11}O_6$	3.0	$H_2O+CH_3^{\bullet}$

13	389.1237	$C_{20}H_{21}O_8$	1.6	359.0778[b] (100)	$C_{18}H_{15}O_8$	4.6	$2CH_3^{\bullet}$	344.0538 (100)	$C_{17}H_{12}O_8$	3.3	$CH_3^{\bullet}$
				374.1013 (82.8)	$C_{19}H_{18}O_8$	4.5	$CH_3^{\bullet}$	331.0826 (75.2)	$C_{17}H_{15}O_8$	4.1	CO
				341.0674 (43.7)	$C_{18}H_{13}O_7$	5.3	$2CH_3^{\bullet}+H_2O$	341.0671 (72.3)	$C_{18}H_{13}O_7$	4.5	H_2O
				356.0910 (42.3)	$C_{19}H_{16}O_7$	5.5	$H_2O+CH_3^{\bullet}$	343.0463 (66.2)	$C_{17}H_{11}O_8$	4.3	CH_4
				328.0958 (15.5)	$C_{18}H_{16}O_6$	5.1	$H_2O+CO+CH_3^{\bullet}$	316.0591 (33.1)	$C_{16}H_{12}O_7$	4.3	$CO+CH_3^{\bullet}$
14	389.1237	$C_{20}H_{21}O_8$	1.6	374.1004[b] (100)	$C_{19}H_{18}O_8$	2.1	$CH_3^{\bullet}$	345.0974 (100)	$C_{18}H_{17}O_7$	1.5	$HCO^{\bullet}$
				328.0950 (95.2)	$C_{18}H_{16}O_6$	2.6	$H_2O+CO+CH_3^{\bullet}$	312.0633 (78.2)	$C_{17}H_{12}O_6$	1.5	$HCO^{\bullet}+CH_3^{\bullet}+H_2O$
				359.0772 (25.1)	$C_{18}H_{15}O_8$	3.0	$2CH_3^{\bullet}$	358.0689 (29.3)	$C_{18}H_{14}O_8$	1.6	CH_4
				345.0976 (15.2)	$C_{18}H_{17}O_7$	2.1	$HCO^{\bullet}+CH_3^{\bullet}$	356.0895 (27.6)	$C_{19}H_{16}O_7$	1.3	H_2O
				356.0901 (14.8)	$C_{19}H_{16}O_7$	2.9	$H_2O+CH_3^{\bullet}$	341.0662 (26.9)	C18H13O7	1.8	$H_2O+CH_3^{\bullet}$
								359.0766 (26.1)	$C_{18}H_{15}O_8$	1.3	$CH_3^{\bullet}$
15	331.1179	$C_{18}H_{19}O_6$	0.9	181.0500[b] (100)	$C_9H_9O_4$	2.6	$^{y}A^{+}$[d]	166.0263 (100)	$C_8H_6O_4$	1.4	$CH_3^{\bullet}$
				313.1082 (13.9)	$C_{18}H_{17}O_5$	3.7	H_2O	125.0598 (80.2)	$C_7H_9O_2$	0.8	2CO
								153.0549 (38.9)	$C_8H_9O_3$	1.8	CO
								122.0364 (30.4)	C7H6O2	1.4	$CO_2+CH_3^{\bullet}$
16	329.1023	$C_{18}H_{17}O_6$	1.0	313.0719[b] (100)	$C_{17}H_{13}O_6$	4.0	CH_4	283.0609 (100)	$C_{15}H_5O_6$	2.8	$2CH_3^{\bullet}$
				314.0796 (62.9)	$C_{17}H_{14}O_6$	3.5	$CH_3^{\bullet}$	298.0482 (33.8)	$C_{16}H_{10}O_6$	3.4	$CH_3^{\bullet}$
								270.0533 (20.9)	C15H10O5	3.8	$CO+CH_3^{\bullet}$
								267.0659 (16.6)	$C_{16}H_{11}O_4$	2.7	$CO+H_2O$
17	405.1543	$C_{21}H_{25}O_8$	−0.2	221.0815[b] (100)	$C_{12}H_{13}O_4$	3.0	$^{x}B^{+}$[d]	193.0865 (100)	$C_{11}H_{13}O_3$	3.0	CO
				387.1451 (32.5)	$C_{21}H_{23}O_8$	3.3	H_2O	190.0631 (53.6)	C11H10O3	3.4	$OCH_3^{\bullet}$
				211.0610 (25.3)	$C_{10}H_{11}O_5$	4.3	$^{y}A^{+}$[d]	206.0581 (48.3)	$C_{11}H_{10}O4$	3.6	$CH_3^{\bullet}$
								191.0347 (39.1)	$C_{10}H_7O_4$	4.3	$2CH_3^{\bullet}$
18	375.1442	$C_{20}H_{23}O_7$	1.0	221.0814[b] (100)	$C_{12}H_{13}O_4$	2.6	$^{x}B^{+}$[d]	193.0860 (100)	$C_{11}H_{13}O_3$	0.4	CO
				181.0501 (19.1)	$C_9H_9O_4$	3.1	$^{y}A^{+}$[d]	190.0626 (52.3)	$C_{11}H_{10}O_3$	0.8	$OCH_3^{\bullet}$
				357.1344 (7.7)	$C_{20}H_{21}O_7$	3.2	H_2O	206.0575 (45.7)	$C_{11}H_{10}O_4$	0.9	$CH_3^{\bullet}$
								191.0342 (37.7)	$C_{10}H_7O_4$	1.7	$2CH_3^{\bullet}$

[a] P-ion (%), the product ions and the relative intensity.
[b] Precursor-ion for next stage MS.
[c] $^{1,3}A^{+}$, $^{1,4}B^{+}$ stand for the fragment ions from the RDA cleavage from 1,3-position on the C-ring of flavanones.
[d] $^{y}A^{+}$, $^{x}B^{+}$ stand for the fragment ions from the RDA cleavage from the C-ring of chalcones.

and Br were not considered as they are rarely present in this traditional herb. All relevant data including peak number, retention time, accurate mass, the predicted chemical formula, and corresponding mass error were recorded into an Excel file.

2.8. Fragmentation mechanisms analysis

Data analysis software (Mass Frontier 7.0, Thermo Scientific) was employed to confirm manual elucidation of mechanisms and fragment ion structures. Mass Frontier predicts and displays comprehensive fragmentation pathways based on a set of general ionization, fragmentation, and rearrangement rules and by automatically extracting a decomposition mechanism for each fragmentation reaction in the fragmentation library that was operated in the positive ion electrospray mode.

2.9. Target constituents identification

The first step is to search for the $[M+H]^+$ ions from all experimentally generated ions, based on a simple program *msi.m* developed by the authors in the Matlab environment (The Mathworks, Natick). The overview and explanation of *msi.m* function was given as following: First, all potential substitutes of PMFs, such as –OH and $-OCH_3$, were supplied to *msi.m*; Second, the parent nucleus of PMFs was defined as $C_{15}H_{11}O_2$; Third, the maximum number of seven potential substitutes was defined; Last, every possible target constituent was screened according to the calculated MS table. In order to improve the search efficiency, a hash procedure was adopted, and the data stored in the desk needs to be read once. In the experiment, the maximum tolerance of mass error was set at 5 ppm when searching for quasi-molecular ions. The eligible peaks were subsequently judged whether they belong to PMFs with DPIs based strategy that has been previously well proven to be useful for rapid identification of target constituents [8,23]. The structurally characterized DPIs can be adopted as a useful "a priori" screening standard for locating the exact candidates containing such a substructure for all other constituents in this family. Then the exact structure of all constituents could be determined from these candidates by fragmentation comparisons. The general procedures of our strategy and approach are summarized into a diagram as shown in Fig. 2.

3. Results and discussion

3.1. DPIs determinations and fragmentation patterns analysis for PMFs

To perform structural identification of the PMFs in the leaves of *Citrus reticulata* Blanco, eighteen PMF standards were analyzed by HPLC–ESI-LTQ-Orbitrap. All the PMF standards, including twelve polymethoxyflavones, two polymethoxyflavanones and four polymethoxychalcones, exhibited $[M+H]^+$ ions of sufficient intensity that could be subsequently isolated automatically and subjected to CID-MS/MS analysis (shown in Table 2). The DPIs from the proposed fragmentation patterns from Mass Frontier 7.0 software and manual elucidation made for the structural identification of PMFs in the extracts. The nomenclature commonly used for mass products of flavonoids was adopted in this work [24].

3.1.1. DPIs determinations for polymethoxyflavones

Tweleve polymethoxyflavone standards were subsequently analyzed first in the CID-MS/MS experiment. By comparison of their product ion spectra, some characteristic dissociation pathways could be summarized for further characterization of the other polymethoxyflavones. First, all of the $[M+H]^+$ ions could lose one or more methyl radicals ($CH_3^\bullet$) in their ESI-MS spectra, and formed the base peaks of $[M+H-15]^+$, $[M+H-30]^+$ and $[M+H-45]^+$. Second, the other dissociation pathways of $[M+H]^+$ by loss of 16 (CH_4), 18 (H_2O), 28 (CO), 29 ($HCO^\bullet$), 31 ($OCH_3^\bullet$), 33 (H_2O+CH_3), 43 ($CH_3^\bullet+CO$), 44 ($HCO^\bullet+CH_3^\bullet$), 46 (H_2O+CO), 48 ($2CH_3^\bullet+H_2O$), 59 ($2CH_3^\bullet$) and 61 ($H_2O+CO+CH_3^\bullet$) were detected as diagnostic products ions in their MS^2 and MS^3 spectra (shown in Fig. 3). These main product ions mentioned above could form the ESI-MS^n DPIs of polymethoxyflavones for rapid screening and identifying them from many complex extracts.

3.1.2. DPIs determinations for polymethoxyflavanones

In CID-MS/MS experiment, the fragmentation pathways of two polymethoxyflavanone derivatives (**1** and **5**) were similar to each other. For example, compound **5** gave the $[M+H]^+$ ion at *m/z* 375.1442 ($C_{20}H_{23}O_7$) in its ESI-MS spectrum, which further generated the prominent ion at *m/z* 221.0813 ($C_{12}H_{13}O_4$) as base peak in its MS^2 spectrum. It could be deduced that its dominating fragmentation pathway was Retro-Diels–Alder (RDA) cleavage from the 1, 4-position of C-ring. Meanwhile, the minor ion at *m/z* 181.0501 ($C_9H_9O_4$) was also detected, owing to the RDA fragmentation from the 1, 3-position of C-ring. The loss of 15 ($CH_3^\bullet$), 28 (CO), 30 ($2CH_3^\bullet$) and 31 ($OCH^\bullet$) from the base peak could also generate a series of DPIs for polymethoxylated flavanone in it MS^2 and MS^3 spectra. This kind of fragmentation pathway that the $[M+H]^+$ ions underwent RDA reaction prior to the neutral loss of $CH_3^\bullet$, H_2O, CO, etc, was strikingly different from general flavanones. Therefore, this particular pathways and DPIs could be adopted as a shortcut to rapidly distinguish polymethoxyflavanones from general flavones (shown in Fig. 3).

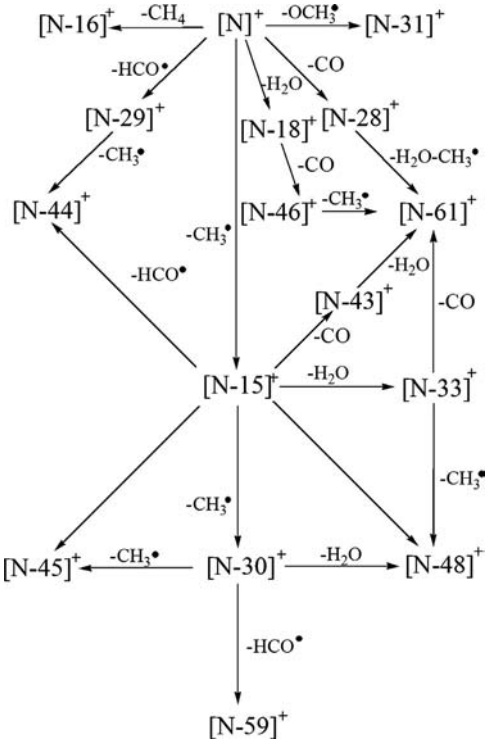


Fig. 3. Product ion formation pathways for PMFs, N stands for [M+H] of polymethoxyflavone, the RDA fragmentation ions for polymethoxyflavanone or polymethoxychalcone.

3.1.3. DPIs determinations for polymethoxychalcones

Compounds **2**, **15**, **17** and **18**, four polymethoxychalcone standards, were also analyzed by the CID-MS/MS method. Their dissociation pathways of MS spectra were similar on the whole. Taking compound **2** for example, the RDA cleavage at bond X of its $[M+H]^+$ ion (405.1543, $C_{21}H_{25}O_8$) to yield the base peak ion $^{X}B^+$

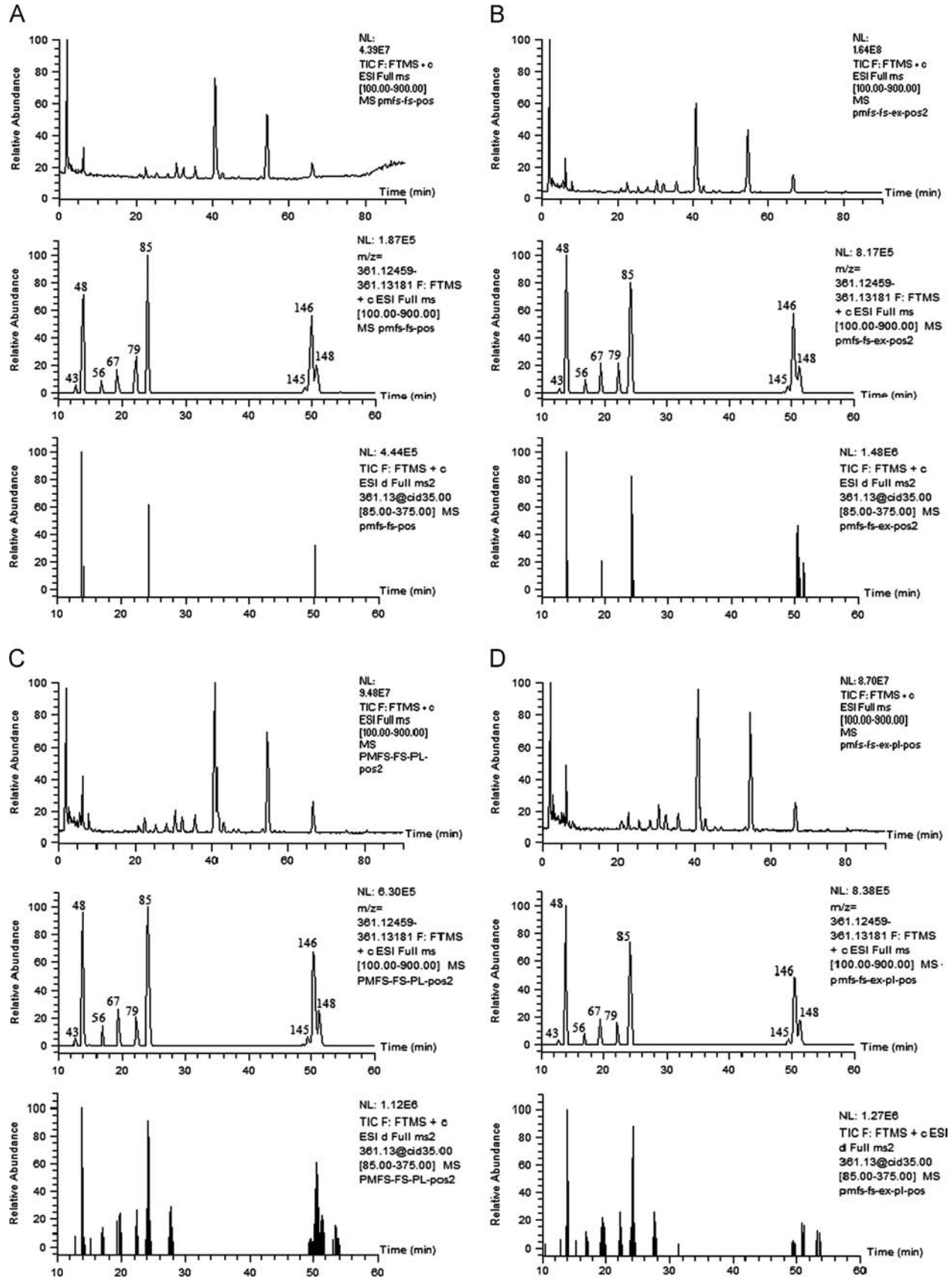


Fig. 4. TIC, EIC of *m*/*z* 361.1282, MS^2 TIC of *m*/*z* 361 from the experiments of FS (A), FS-DE (B), FS-PIL (C) and FS-PIL-DE (D).

at m/z 221.0812 ($C_{12}H_{13}O_4$) and at bond Y to yield the minor ion $^{Y}A^{+}$ at m/z 211.0608 ($C_{10}H_{11}O_5$) could also be simultaneously detected in its positive MS^2 spectrum first. The fragmentation pathway was highly similar to what happened to polymethoxylated flavanones. This is reasonable because cyclization of 6′-hydroxychalcones to flavanones has been reported in a number of studies demonstrating an intramolecular equilibrium being present between a flavanone-type and a chalcone-type molecular ion [25–26]. Meanwhile, the product ions detected from the loss of 15 ($CH_3^{\bullet}$), 16 (CH_4), 18 (H_2O), 28 (CO), 30 ($2CH_3^{\bullet}$) and 31 ($OCH_3^{\bullet}$) could be also adopted as DPIs for polymethoxylated chalcones (shown in Fig. 3).

3.2. Constituents screening by FS, FS-PIL, FS-DE and FS-PIL-DE acquisition

In order to compare the capacities to trigger the MS/MS fragmentations, PMFs in the leaves of *Citrus reticulata* Blanco were analyzed respectively using the FS, FS-PIL, FS-DE and FS-PIL-DE acquisition as survey scan. The constituent profiles of their total ion chromatogram (TIC) were similar with each other, except the intensities of detected peaks in FS-PIL, FS-DE and FS-PIL-DE were apparently higher than that in FS (shown in Fig. 4). Several $[M+H]^+$ ions whose m/z values were 315.0863, 329.1020, 333.0969, 359.1125, 361.1282, 375.1438 and 377.1231 were randomly selected and analyzed. The peaks of $[M+H]^+$ displayed in all the four ESI-MS spectra were identical to each another. However, the numbers of the peaks with MS/MS spectrum were extremely different (shown in Fig. 5). Taking m/z 361.1282 for example, nine peaks whose m/z values of $[M+H]^+$ ions within 5 ppm were detected in all the four acquisition experiments. As a result, only three MS/MS spectra were revealed in the FS experiment. In stark contrast, the MS/MS acquisitions of the peaks detected in the ESI-MS spectrum were all triggered by FS-PIL-DE, which indicated that PIL and DE could take effect to trigger the MS/MS fragmentation of microconstituents. Additionally, from the results of the other two experiments, it could also be deduced that PIL played a much more important role to obtain much more MS/MS information than DE (shown in Fig. 5). However, the comparison between the FS-PIL-DE and FS-PIL of m/z 315.0863, 333.0969 and 377.1231 demonstrated that DE was also necessary for the constituent especially microconstituents screening. Therefore, FS coupled with PIL and DE could be used as a useful survey scan to trigger MS/MS acquisition due to its superior sensitivity and selectivity in the rapid screening and characterization of constituents in complex TCM extracts.

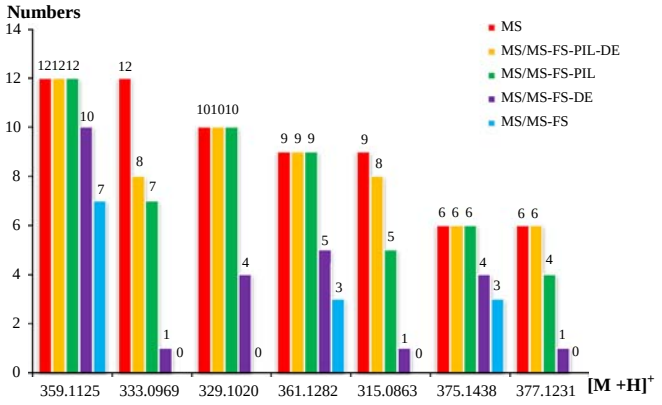


Fig. 5. The differences of PMFs detected by FS, FS-PIL, FS-DE and FS-PIL-DE acquisitions.

3.3. HPLC-LTQ-Orbitrap analysis of the PMFs in the leaves of Citrus reticulata Blanco

According to their fragmentation pathways, it was easy to tell polymethoxychalcones from polymethoxyflavones, but difficult to distinguish polymethoxychalcones from polymethoxyflavanones. Moreover, the abundances of most detected polymethoxyflavanones or chalcones were too low to obtain online UV absorption spectra. Therefore, the result currently does not differentiate between these two kinds of PMFs.

After screening the m/z values of the $[M+H]^+$ ions within ± 5 ppm mass errors with matlab procedure, 181 PMF candidates were found from the large quantity of information data beforehand (shown in Table 3 and Fig. 6). Among them, 28 candidates have no respective MS/MS spectra owing to their low contents in the TCM exact, thereby their structures could not be forecasted. The $[M+H]^+$ of 18 candidates have undergone completely different fragmentation pathways in comparison with those of PMF standards, so they could not be characterized as PMFs. As for the rest 135 candidates, they were tentatively identified as 81 polymethoxyflavones, 54 polymethoxyflavanones or polymethoxychalcones according to the respective fragmentation pathways and DPIs of the PMF standards. Since it was too popular for the phenomenon of substitution isomerism to determine the exact substitution positions of OCH_3 and/or OH on their A, B and C rings, they were preliminarily identified as M-hydroxy-N-methoxyflavone, flavanone or chalcone (M, N stand for mono, di, tri, tetra, penta, hexa or hepta) in Table 4.

Furthermore, hydroxylated polymethoxyflavonoids (OH-PMFs) have drawn more and more attention recently, because accumulating evidence has suggested that they have much stronger health-promoting biological activities compared with their permethoxylated counterparts. Additionally, OH-PMFs are even more rare PMFs existing in the medicinal plants. In this study, using the strategy of FS-PIL-DE acquisition coupled to DPIs analysis, 109 hydroxylated polymethoxyflavonoids have been screened out and identified from the TCM extract, including 70 hydroxylated polymethoxyflavones, 39 hydroxylated polymethoxyflavanones or hydroxylated polymethoxychalcones (shown in Fig. 6), which indicated that the leaves of *Citrus reticulata* Blanco could be adopted as a kind of forward-looking anticancer medicines. Our study has also provided a methodology for quick screening out important leading compounds from *Citrus* genus plants in phytochemistry study.

4. Conclusion

In this study, a highly sensitive and effective strategy for rapid screening and identification of target constituents in TCMs has been developed using FS-PIL-DE acquisition coupled to DPIs analysis on a hybrid LTQ-Orbitrap mass spectrometer for the first time. The effectiveness of FS-PIL-DE coupled to DPIs analysis was investigated by analyzing PMFs in a model medicine, the leaves of *Citrus reticulata* Blanco. The results have demonstrated that the FS-PIL-DE acquisition can target all the predictable constituents that have the same molecular weight, regardless of the site of substitution positions and fragmentations. Therefore, it can search for a greater number of potential active compounds than FS, FS-PIL and FS-DE, thereby increase the coverage of constituent screening. Additionally, DPIs analysis can provide a criterion to classify the target constituents detected into certain chemical families. In the study, eighteen PMF standards were analyzed by CID-MS/MS to obtain the respective fragmentation pathways and DPIs for polymethoxyflavones, polymethoxyflavanones and polymethoxychalcones,

Table 3
Characterization of PMFs in the leaves of *Citrus reticulata* Blanco by HPLC-DAD-ESI-MS/MS.

No.	t_R (min)	Formula $[M+H]^+$	Theoretical mass (m/z)	Experimental mass (m/z)	Mass error (ppm)	MS^2 (m/z), P-ion (%)[b]	MS^3 (m/z), P-ion (%, loss)[b]
1*	4.09	$C_{17}H_{17}O_7$	333.0969	333.0970	0.3	177.0548 (100), 183.0289 (73.7), 145.0285 (20.3)	145.0287 (100)
2	4.58	$C_{17}H_{17}O_7$	333.0969	333.0973	1.2	183.0292 (100), 177.0550 (57.4), 318.0376 (40.9)	168.0056 (100)
3^	4.62	$C_{17}H_{15}O_9$	363.0711	363.0715	1.1	–	–
4*	5.21	$C_{20}H_{23}O_9$	407.1357	407.1342	−3.7	245.0812 (100), 273.0762 (17.1), 287.0917 (16.3)	175.0394 (100), 217.0864 (31.8)
5^	5.29	$C_{17}H_{17}O_7$	333.0969	333.0974	1.5	–	–
6*	5.61	$C_{21}H_{23}O_9$	419.1336	419.1341	1.2	383.1126 (100), 401.1239 (66.1), 396.8012 (22.7)	263.0555 (100), 365.1022 (63.3), 245.0448 (27.6)
7	5.66	$C_{17}H_{17}O_7$	333.0969	333.0974	1.5	183.0290 (100), 177.0548 (71.9)	168.0058 (100)
8	5.73	$C_{18}H_{17}O_9$	377.0867	377.0872	1.3	362.0640 (100), 347.0407 (47.4), 344.0536 (16.9)	347.0404 (100), 344.0535 (59.9)
9	5.80	$C_{17}H_{15}O_8$	347.0761	347.0765	1.2	332.0537 (100)	301.0343 (100), 300.0267 (35.8), 317.0299 (35.4)
10*	6.25	$C_{17}H_{15}O_6$	315.0863	315.0866	1.0	165.0185 (100), 191.0342 (32.8)	151.71863(100), 106.93510(92.5)
11*	6.31	$C_{18}H_{17}O_7$	345.0969	345.0974	1.4	195.0291 (100), 327.0868 (11.7)	153.0185 (100), 177.0185 (49.28)
12	6.56	$C_{17}H_{15}O_8$	347.0761	347.0767	1.7	332.0532 (100)	301.0347 (100), 300.0270 (36.2), 317.0297 (32.6)
13	6.56	$C_{18}H_{17}O_9$	377.0867	377.0871	1.1	362.0640 (100), 347.0402 (45.7), 344.0532 (16.4)	347.0399 (100), 344.0528 (57.0)
14	6.59	$C_{17}H_{17}O_7$	333.0969	333.0971	0.6	183.0288 (100), 177.0547 (87.8)	168.0055 (100)
15	6.82	$C_{17}H_{15}O_6$	315.0863	315.0871	2.5	300.0636 (100), 285.0404 (4.2)	254.0584 (100), 285.0401 (83.4), 282.0532 (31.5)
16*	6.91	$C_{19}H_{21}O_9$	393.1180	393.1163	−4.3	349.1270 (100)	152.7851 (100), 185.5339 (97.2), 116.0418 (92.0)
17^	7.11	$C_{17}H_{17}O_7$	333.0969	333.0975	1.8	–	–
18	7.16	$C_{18}H_{17}O_7$	345.0969	345.0976	2.0	330.0742 (100)	284.0688 (100), 315.0508 (72.5), 312.0635 (47.1)
19^	7.24	$C_{18}H_{19}O_8$	363.1074	363.1077	0.8	–	–
20^	7.31	$C_{17}H_{15}O_6$	315.0863	315.0868	1.6	–	–
21	7.45	$C_{17}H_{15}O_8$	347.0761	347.0766	1.4	332.0533 (100)	301.0343 (100), 300.0281 (43.9), 317.0339 (32.3)
22	7.86	$C_{17}H_{17}O_6$	317.1020	317.1025	1.6	191.0705 (100), 179.0341 (95.8), 153.0184 (62.8)	163.0755 (100), 176.0469 (50.4)
23^	7.57	$C_{17}H_{17}O_7$	333.0969	333.0975	1.8	–	–
24*	7.86	$C_{17}H_{17}O_7$	333.0969	333.0975	1.8	177.0551 (100), 183.0292 (82.7), 145.0287 (20.7)	145.0287 (100)
25^	8.05	$C_{18}H_{19}O_8$	363.1074	363.1080	1.7	–	–
26	8.26	$C_{17}H_{17}O_7$	333.0969	333.0975	1.8	183.0290 (100), 177.0551 (49.1)	168.0056 (100)
27^	8.41	$C_{18}H_{19}O_8$	363.1074	363.1079	1.4	–	–
28^	8.76	$C_{17}H_{17}O_7$	333.0969	333.0974	1.5	–	–
29	9.56	$C_{17}H_{15}O_6$	315.0863	315.0871	2.5	282.0527 (100), 300.0640 (89.6), 283.0573 (13.3)	254.0580 (100), 226.0607 (10.6), 129.0780 (7.5)
30	10.04	$C_{18}H_{19}O_7$	347.1125	347.1133	2.3	197.0452 (100), 191.0710 (71.2), 183.0295 (49.0), 283.1552 (26.9), 209.0454 (16.2)	182.0215 (100), 200.0319 (27.5)
31	10.17	$C_{19}H_{19}O_7$	359.1125	359.1133	2.2	344.0896 (100), 329.0665 (6.7)	298.08401 (100), 326.0789 (47.12), 329.0659 (43.1)
32	10.29	$C_{18}H_{17}O_7$	345.0969	345.0976	2.0	312.0634 (100), 330.0738 (69.9)	284.0684 (100), 296.0331 (21.2)
33	10.29	$C_{18}H_{17}O_8$	361.0918	361.0924	1.7	346.0847 (100), 345.0931 (17.5)	303.0505 (100), 300.0900 (27.3), 331.0699 (14.9)
34^	10.34	$C_{22}H_{25}O_9$	433.1493	433.1500	1.6	–	–
35	10.79	$C_{20}H_{21}O_9$	405.1180	405.1189	2.2	390.0950 (100), 375.0727 (43.1), 391.0974 (17.9)	375.0719 (100), 372.0848 (29.9)
36^	11.02	$C_{17}H_{17}O_8$	349.0918	349.0924	1.7	–	–
37	11.27	$C_{18}H_{17}O_7$	345.0969	345.0974	1.4	330.0737 (100), 315.0502 (14.4)	315.0502 (100), 297.0396 (31.9), 312.0634 (26.8)
38^	11.68	$C_{18}H_{19}O_7$	347.1125	347.1130	1.4	–	–
39	11.98	$C_{18}H_{17}O_6$	329.1020	329.1025	1.5	314.0786 (100), 313.0712 (57.9), 315.0823 (13.9)	298.0476 (100), 299.0533 (55.9), 153.0184 (52.4)
40*	11.99	$C_{17}H_{17}O_8$	349.0918	349.0924	1.7	225.0400 (100), 197.0452 (76.4)	197.0449 (100)
41	12.07	$C_{18}H_{19}O_6$	331.1176	331.1180	1.2	301.0612 (100), 316.0847 (94.5), 197.0449 (34.6)	273.0663 (100)
42	12.37	$C_{18}H_{17}O_6$	329.1020	329.1027	2.1	299.0561 (100), 314.0796 (82.2)	271.0606 (100), 181.0136 (6.4), 229.0501 (5.7)
43	12.63	$C_{19}H_{21}O_7$	361.1282	361.1286	1.1	211.0603 (100), 177.0549 (16.9)	196.0370 (100), 178.0262 (27.5)
44*	13.05	$C_{18}H_{19}O_6$	331.1176	331.1180	1.2	167.0343 (100), 191.0707 (82.9), 313.1078 (11.4)	101.7219 (100), 115.2509 (87.1)
45	13.24	$C_{19}H_{19}O_7$	359.1125	359.1135	2.8	329.0665 (100), 344.0901 (80.6)	326.0789 (100)
46*	13.45	$C_{21}H_{23}O_9$	419.1336	419.1318	−4.3	333.0951 (100)	273.0738 (100), 161.6476 (22.8)
47^	13.60	$C_{18}H_{19}O_7$	347.1125	347.1130	1.4	–	–
48	13.77	$C_{19}H_{21}O_7$	361.1282	361.1290	2.2	211.0607 (100), 343.1186 (26.9), 177.0552 (22.3)	196.0368 (100), 178.0264 (26.0), 150.0314 (8.8)
49	13.92	$C_{18}H_{17}O_6$	329.1020	329.1025	1.5	314.0786 (100), 299.0553 (5.4)	268.0738 (100), 299.0554 (46.1), 296.0684 (41.0)
50	14.74	$C_{21}H_{23}O_9$	419.1336	419.1338	0.5	404.1115 (100), 389.0881 (40.3), 405.1156 (12.9)	389.0872 (100), 386.1003 (27.3)
51	14.76	$C_{19}H_{21}O_8$	377.1231	377.1237	1.6	227.0552 (100)	212.0320 (100)
52	15.08	$C_{19}H_{19}O_7$	359.1125	359.1132	1.9	344.0898 (100), 326.0793 (64.4)	326.0788 (100)
53	15.86	$C_{17}H_{15}O_5$	299.0914	299.0920	2.0	284.0685 (100), 285.0714 (15.8)	255.0656 (100)
54	16.22	$C_{19}H_{21}O_8$	377.1231	377.1238	1.9	241.0713 (100), 359.1120 (2.8)	226.0476 (100), 208.0372 (11.1), 211.0241 (7.2)
55	16.50	$C_{18}H_{17}O_6$	329.1020	329.1025	1.5	314.0791 (100), 299.0557 (79.5), 315.0823 (11.4)	299.0554 (100)
56	16.71	$C_{19}H_{21}O_7$	361.1282	361.1289	1.9	197.0448 (100),191.0709 (10.3)	182.0215 (100), 200.0322 (25.4), 164.0108 (14.3)
57	17.04	$C_{19}H_{19}O_7$	359.1125	359.1132	1.9	326.0773 (100), 344.0877 (58.7), 329.0645 (11.4)	298.0822 (100), 310.0458 (8.8)
58	17.38	$C_{17}H_{15}O_5$	299.0914	299.0920	2.0	284.0683 (100), 271.0608 (12.3), 285.0718 (9.2)	256.0734 (100), 268.0370 (26.7), 253.0502 (17.7)
59	17.38	$C_{18}H_{17}O_6$	329.1020	329.1026	1.8	268.0738 (100), 314.0792 (86.8), 285.0765 (27.0), 296.0688 (22.3)	240.0788 (100), 251.0709 (23.1)
60	17.90	$C_{17}H_{15}O_7$	331.0812	331.0815	0.9	316.0582 (100), 301.0348 (28.4), 298.0477 (24.0)	298.0478 (100), 301.0349 (96.9)
61	17.95	$C_{18}H_{19}O_8$	363.1074	363.1078	1.1	213.0395 (100)	198.01624(100)
62*	18.14	$C_{20}H_{23}O_9$	407.1357	407.1343	−3.4	389.1238 (100), 361.1300 (56.7), 241.0703(38.1)	361.01284 (100), 346.1029 (25.75)
63	18.36	$C_{17}H_{15}O_7$	331.0812	331.0817	1.5	316.0576 (100), 317.0609 (12.9)	288.0634 (100), 301.0347 (83.9), 273.0400 (16.3)
64	18.58	$C_{19}H_{19}O_7$	359.1125	359.1133	2.2	298.0842 (100), 344.0895 (76.7), 315.0870 (26.9)	283.0607 (100), 255.0656 (19.2), 282.0528 (15.5)
65	18.61	$C_{17}H_{17}O_5$	301.1071	301.1075	1.3	286.0476 (100)	168.0054 (100), 258.0524 (16.8)
66	19.15	$C_{19}H_{19}O_7$	359.1125	359.1133	2.2	298.0825 (100), 344.0895 (83.7), 315.0869 (29.9)	283.0605 (100), 255.0654 (23.0)
67	19.18	$C_{19}H_{21}O_7$	361.1282	361.1292	2.8	197.0449 (100), 346.0689 (71.8), 331.0457 (29.6), 191.0708 (21.8), 328.0586 (20.4)	182.0214 (100), 169.0500 (14.5)
68	19.30	$C_{18}H_{17}O_8$	361.0918	361.0928	2.8	346.0690 (100), 331.0455 (38.5), 328.0584 (27.0)	331.0454 (100), 328.0582 (92.8)
69	19.40	$C_{18}H_{19}O_8$	363.1074	363.1080	1.7	197.0448 (100), 193.0498 (52.6), 213.0398 (13.9)	182.0212 (100), 164.0105 (19.3)

Table 3 (*continued*)

No.	t_R (min)	Formula $[M+H]^+$	Theoretical mass (m/z)	Experimental mass (m/z)	Mass error (ppm)	MS^2 (m/z), P-ion (%)[b]	MS^3 (m/z), P-ion (%, loss)[b]
70	19.42	$C_{17}H_{17}O_7$	333.0969	333.0976	2.1	183.0291 (100), 177.0549 (49.1), 209.0449 (10.8)	168.0056 (100), 186.0163 (13.3), 155.0332 (2.9)
71	19.54	$C_{17}H_{15}O_7$	331.0812	331.0815	0.9	316.0580 (100)	301.0345 (100), 273.0396 (17.8)
72	20.43	$C_{17}H_{15}O_7$	331.0812	331.0818	1.8	316.0585 (100)	301.0347 (100), 273.0398 (20.0), 168.0057 (16.5)
73*	20.64	$C_{17}H_{17}O_5$	301.1071	301.1075	1.3	167.0341 (100), 161.0598 (18.1)	138.4497 (100)
74	20.71	$C_{20}H_{23}O_7$	375.1438	375.1443	1.3	211.0606 (100), 191.0707 (42.3), 357.1341 (20.9)	196.0369 (100), 178.0265 (24.6), 150.0314 (8.8)
75	20.79	$C_{17}H_{17}O_7$	333.0969	333.0976	2.1	197.0447 (100), 183.0290 (16.9), 163.0394 (9.7)	182.0214 (100), 200.0319 (27.5), 164.0109 (20.6)
76	21.01	$C_{20}H_{21}O_8$	389.1231	389.1231	0.0	374.1005 (100), 359.0772 (31.7), 341.0665 (9.0)	359.0772 (100), 341.0669 (30.6), 356.0906 (14.1)
77	21.19	$C_{18}H_{19}O_6$	331.1176	331.1184	2.4	211.0608 (100), 181.0502 (9.8), 177.0551 (7.6)	196.0371 (100), 168.0422 (25.5), 167.0343 (4.9)
78	21.19	$C_{20}H_{21}O_9$	405.1180	405.1185	1.2	373.0927 (100), 387.1055 (5.2)	345.0975 (100), 358.0694 (96.3), 330.0739 (23.9)
79	22.00	$C_{19}H_{21}O_7$	361.1282	361.1291	2.5	211.0607 (100)	196.0371 (100), 168.0419 (23.4)
80	22.16	$C_{20}H_{21}O_7$	373.1282	373.1284	0.5	343.0821 (100), 358.1055 (56.5), 374.1327 (15.2)	315.0868 (100), 328.0581 (10.1)
81	22.55	$C_{18}H_{17}O_6$	329.1020	329.1028	2.4	314.0791 (100), 296.0686 (51.2)	296.0687 (100)
82	23.14	$C_{18}H_{17}O_8$	361.0918	361.0927	2.5	346.0955 (100), 331.0717 (39.8), 319.0823 (31.0)	331.0719 (100), 300.0894 (21.2)
83	23.41	$C_{18}H_{19}O_8$	363.1074	363.1084	2.8	227.0557 (100), 212.0644 (4.4)	212.0323 (100), 230.0429 (38.5), 194.0221 (5.5)
84	23.51	$C_{19}H_{19}O_7$	359.1125	359.1134	2.5	344.0900(100), 329.0666 (41.0), 298.0848 (11.6)	329.0666 (100), 298.0848 (25.6), 311.0562 (5.7)
85	23.88	$C_{19}H_{21}O_7$	361.1282	361.1289	1.9	211.0607 (100), 177.0552 (2.6)	196.0364 (100), 168.0419 (24.8), 178.0262 (5.3)
86	24.02	$C_{17}H_{15}O_7$	331.0812	331.0818	1.8	316.0582 (100), 317.0623 (13.4), 287.0563 (12.9)	287.0559 (100), 298.0484 (30.5), 315.0496 (21.4)
87*	24.09	$C_{20}H_{23}O_9$	407.1357	407.1345	−2.9	375.1085 (100)	357.0974 (100), 343.0830 (52.9), 315.0826 (39.6)
88^	24.73	$C_{20}H_{21}O_9$	405.1180	405.1185	1.2	–	–
89	25.00	$C_{18}H_{17}O_6$	329.1020	329.1026	1.8	296.0688 (100), 314.0794 (81.5), 297.0731 (12.3)	268.0734 (100), 253.0505 (10.4)
90	25.02	$C_{20}H_{21}O_8$	389.1231	389.1234	0.8	374.0999 (100), 359.0769 (62.3), 328.0948 (11.7)	359.0769 (100), 328.0950 (21.9), 341.0665 (4.9)
91*	26.43	$C_{21}H_{25}O_9$	421.1493	421.1493	0.0	403.1397 (100), 375.1445 (45.7), 241.0714 (30.3)	375.0144 (100), 360.1209 (28.7)
92^	26.63	$C_{21}H_{23}O_8$	403.1388	403.1395	1.7	–	–
93	26.83	$C_{21}H_{23}O_9$	419.1336	419.1339	0.7	404.1109 (100), 389.0875 (69.4), 405.1133 (16.2)	389.0872 (100)
94	27.00	$C_{18}H_{17}O_8$	361.0918	361.0926	2.2	346.0687 (100), 331.0454 (77.6), 328.0583 (51.3), 313.0349 (33.7), 300.0638 (12.6)	331.0452 (100), 328.0579 (41.7)
95^	27.36	$C_{17}H_{15}O_5$	299.0914	299.0917	1.0	–	–
96	27.47	$C_{19}H_{19}O_6$	343.1176	343.1182	1.7	328.0946 (100), 327.0869 (62.0), 299.0919 (13.8)	299.0918 (100), 312.0632 (81.6)
97	27.56	$C_{18}H_{19}O_6$	331.1176	331.1180	1.2	197.0448 (100)	182.0214 (100), 164.0111 (19.4)
98	27.90	$C_{20}H_{23}O_8$	391.1388	391.1392	1.0	241.0715 (100)	226.0479 (100), 241.0715 (36.9), 208.0373 (14.4)
99^	28.46	$C_{20}H_{21}O_9$	405.1180	405.1183	0.7	–	–
100	28.89	$C_{18}H_{17}O_7$	345.0969	345.0973	1.2	330.0739 (100), 329.0665 (32.3), 331.0781 (12.7)	315.0502 (100), 314.0425 (31.0), 301.0718 (15.1)
101	29.69	$C_{19}H_{21}O_8$	377.1231	377.1236	1.3	197.0451 (100), 207.0655 (84.2), 213.0399 (60.9)	182.0205 (100), 152.6896 (33.1), 134.6068 (28.3)
102^	29.92	$C_{17}H_{15}O_5$	299.0914	299.0922	2.7	–	–
103	30.05	$C_{17}H_{15}O_4$	283.0965	283.0968	1.1	268.0734 (100), 267.0662 (95.0), 239.0712 (30.4)	239.0709 (100)
104	30.06	$C_{17}H_{15}O_6$	315.0863	315.0868	1.6	163.0759 (100), 153.0185 (82.0), 287.0921 (72.5), 300.0641 (50.2)	148.0520 (100)
105	30.12	$C_{20}H_{21}O_7$	373.1282	373.1285	0.8	312.1005 (100), 358.1059 (57.2), 374.1329 (48.3), 329.1034 (29.3), 343.0825 (26.2), 340.0955 (21.7)	297.0767 (100), 296.0688 (60.3), 151.0394 (45.2), 268.0739 (32.6), 269.0817 (32.6)
106^	30.43	$C_{20}H_{21}O_9$	405.1180	405.1183	0.7	–	–
107	30.98	$C_{19}H_{19}O_7$	359.1125	359.1133	2.2	344.0897 (100), 329.0661 (22.2), 326.0793 (7.0)	329.0659 (100), 311.0557 (30.5), 326.0792 (16.3)
108	31.33	$C_{19}H_{21}O_6$	345.1333	345.1338	1.4	181.0497 (100), 191.0705 (73.2), 327.1229 (14.6)	125.0598 (100), 166.0263 (55.6), 122.0364 (55.0)
109	31.48	$C_{17}H_{17}O_6$	317.1020	317.1025	1.6	197.0448 (100)	182.0216 (100), 164.0108 (27.7)
110^	31.66	$C_{20}H_{21}O_9$	405.1180	405.1183	0.7	–	–
111	31.77	$C_{17}H_{15}O_5$	299.0914	299.0921	2.3	284.0680 (100), 285.0714 (10.8)	152.0105 (100), 256.0716 (33.9), 133.0650 (27.1)
112	31.89	$C_{19}H_{19}O_6$	343.1176	343.1181	1.5	313.0715 (100), 328.0950 (59.5)	285.0757 (100), 283.0607 (4.9)
113^	32.15	$C_{18}H_{19}O_7$	347.1125	347.1130	1.4	–	–
114	32.26	$C_{19}H_{21}O_6$	345.1333	345.1325	−2.3	211.0604 (100), 315.0769 (60.8), 330.0971 (50.8)	196.0370 (100), 178.0266 (24.4)
115	32.27	$C_{17}H_{15}O_6$	315.0863	315.0869	1.9	282.0526 (100), 300.0633 (89.3), 254.0581 (3.0)	254.0578 (100)
116	32.52	$C_{17}H_{17}O_6$	317.1020	317.1025	1.6	191.0705 (100), 179 (81.8), 153 (55.0)	163.0759 (100), 176.0470 (34.3)
117	32.99	$C_{18}H_{19}O_7$	347.1125	347.1133	2.3	197.0447 (100), 177.0549 (15.5)	182.0213 (100), 200.0319 (28.2), 164.0108 (19.7)
118	33.57	$C_{19}H_{21}O_8$	377.1231	377.1237	1.6	241.0713 (100), 207.0662 (11.5)	226.0474 (100)
119	34.13	$C_{18}H_{17}O_7$	345.0969	345.0975	1.7	330.0741 (100), 312.0635 (60.4)	312.0633 (100), 315.0504 (23.3), 297.0401 (11.8)
120	34.76	$C_{18}H_{19}O_7$	347.1125	347.1132	2.0	197.0448 (100), 177.0549 (21.7), 329.1027 (9.3)	182.0214 (100), 200.0321 (26.7), 164.0109 (16.5)
121	35.30	$C_{20}H_{23}O_7$	375.1438	375.1442	1.1	211.0606 (100), 376.1482 (13.9), 191.0709 (6.3)	196.0370 (100), 168.0423 (24.4), 211.0608 (7.1)
122^	35.74	$C_{18}H_{19}O_7$	347.1125	347.1132	2.0	–	–
123^	36.32	$C_{21}H_{23}O_8$	403.1388	403.1392	1.0	–	–
124	37.39	$C_{17}H_{15}O_6$	315.0863	315.0866	1.0	300.0634 (100), 301.0671 (13.7)	271.0604 (100), 282.0525 (32.7), 297.0399 (23.3)
125	38.11	$C_{19}H_{21}O_8$	377.1231	377.1238	1.9	227.0555 (100)	212.0319 (100), 230.0426 (40.9), 194.0217 (6.3)
126	38.34	$C_{18}H_{17}O_7$	345.0969	345.0975	1.7	330.0738 (100), 331.0773 (11.7)	301.0714 (100), 314.0425 (74.9), 302.0790 (51.9)
127	39.52	$C_{18}H_{17}O_5$	313.1071	313.1074	1.0	298.0844 (100), 269.0818 (7.2)	269.0814 (100), 268.0738 (8.3)
128	39.62	$C_{18}H_{17}O_7$	345.0969	345.0973	1.2	330.0741 (100), 301.0717 (10.4), 329.0667 (10.3)	301.0713 (100), 314.0427 (90.9), 302.0791 (39.2)
129	39.68	$C_{19}H_{21}O_8$	377.1231	377.1238	1.9	227.0556 (100), 253.0714 (2.6), 335.1136 (2.5)	212.0319 (100), 194.0215 (16.3)
130	40.20	$C_{21}H_{23}O_8$	403.1388	403.1387	−0.2	388.1162 (100), 373.0928 (73.2), 342.1112 (11.78)	373.0920 (100), 342.1105 (20.6)
131	40.32	$C_{19}H_{18}O_7$	359.1125	359.1134	2.5	344.0894 (100), 343.0817 (19.3), 329.0662 (11.0)	329.0658 (100), 315.0867 (67.2), 298.0842 (29.7)
132	40.34	$C_{17}H_{15}O_5$	299.0914	299.0920	2.0	284.0692 (100), 285.0714 (7.2)	228.0792 (100), 269.0458 (66.4), 256.0745 (35.3)
133	40.91	$C_{21}H_{25}O_8$	405.1544	405.1544	0.0	241.0714 (100), 406.1590 (22.7), 226.0482 (5.4)	226.0479 (100), 208.0373 (13.8)
134	41.50	$C_{18}H_{17}O_7$	345.0969	345.0974	1.4	283.0982 (100), 329.0982 (74.5), 330.0935 (53.7), 284.0957 (52.7), 300.0960 (27.9), 315.0565 (23.5)	268.0692 (100), 255.0978 (49.3), 252.0744 (28.6), 240.0743 (20.4)
135	42.18	$C_{17}H_{15}O_5$	299.0914	299.0919	1.7	284.0688 (100), 256.0739 (16.2), 285.0723 (13.2)	256.0735 (100)
136	42.32	$C_{19}H_{19}O_6$	343.1176	343.1182	1.7	282.0895 (100), 328.0953 (59.5), 299.0925 (29.1)	267.0659 (100), 254.0948 (47.6), 266.0584 (30.8), 251.0712 (26.8), 239.0713 (22.3)
137	42.84	$C_{19}H_{19}O_9$	391.1024	391.1027	0.8	149.0236 (100), 376.0798 (69.2), 361.0566 (53.5)	–

Table 3 (*continued*)

No.	t_R (min)	Formula $[M+H]^+$	Theoretical mass (m/z)	Experimental mass (m/z)	Mass error (ppm)	MS^2 (m/z), P-ion (%)[b]	MS^3 (m/z), P-ion (%, loss)[b]
138	43.37	$C_{19}H_{19}O_8$	375.1074	375.1081	1.9	360.0851 (100), 345.0614 (88.8), 342.0745 (44.4), 327.0509 (38.6)	345.0617 (100), 327.0512 (29.6), 342.0747 (28.6)
139	43.85	$C_{19}H_{19}O_9$	391.1024	391.1029	1.3	376.0798 (100), 361.0567 (92.1)	361.0559 (100), 358.0697 (31.5)
140	44.85	$C_{19}H_{19}O_8$	375.1074	375.1079	1.3	360.08467 (100), 345.0611 (98.4), 342.0742 (50.1), 327.0507 (45.8), 314.0792 (16.3)	345.0609 (100), 327.0506 (32.3), 342.0741 (31.4)
141*	44.85	$C_{19}H_{19}O_9$	391.1024	391.1026	0.5	149.0236 (100), 359.0772 (43.6), 279.1603 (39.5)	138.3721 (100)
142	47.19	$C_{18}H_{19}O_5$	315.1227	315.1234	2.2	181.0498 (100), 161.0599 (16.2)	166.0264 (100), 125.0598 (49.3), 153.0549 (34.2), 111.0443 (25.9), 151.0367 (22.3)
143	47.31	$C_{22}H_{25}O_9$	433.1493	433.1499	1.4	418.1269 (100), 403.1037 (67.5), 385.0931 (11.0)	403.1026 (100), 385.0921 (15.9)
144*	48.43	$C_{17}H_{17}O_6$	317.1020	317.1026	1.9	177.0549 (100), 167.0343 (63.7), 145.0285 (17.0)	145.0289 (100)
145	48.98	$C_{19}H_{21}O_7$	361.1282	361.1289	1.9	211.0605 (100), 343.1182 (26.7), 177.0551 (23.0)	196.0371 (100), 178.0263 (26.5)
146	49.96	$C_{19}H_{21}O_7$	361.1282	361.1289	1.9	197.0450 (100), 191.0711 (35.8)	182.0215 (100), 164.0109 (18.9), 165.0188 (18.1)
147△	50.28	$C_{17}H_{17}O_6$	317.1020	317.1025	1.6	–	–
148	50.80	$C_{19}H_{21}O_7$	361.1282	361.1289	1.9	197.0447 (100), 191.0706 (34.6)	182.0213 (100), 165.0185 (26.1), 164.0107 (22.1)
149	50.87	$C_{17}H_{15}O_6$	315.0863	315.0867	1.3	300.0633 (100)	272.0684 (100)
150	52.52	$C_{19}H_{21}O_6$	345.1333	345.1341	2.3	221.0606 (100)	196.0369 (100), 168.0423 (23.8), 178.0266 (4.8)
151	52.90	$C_{19}H_{19}O_7$	359.1125	359.1130	1.4	326.0796 (100), 344.0902 (71.1), 360.1173 (24.3)	298.0833 (100)
152	53.32	$C_{21}H_{23}O_9$	419.1336	419.1343	1.7	404.1108 (100), 389.0837 (45.1), 386.1003 (11.7)	389.0871 (100), 386.1002 (28.5)
153	53.90	$C_{17}H_{15}O_4$	283.0965	283.0975	3.5	268.0734(100), 255.0658 (75.4), 265.0812 (38.7)	240.0789 (100), 253.9389 (21.3)
154△	53.94	$C_{17}H_{15}O_5$	299.0914	299.0920	2.0	–	–
155	53.97	$C_{17}H_{15}O_6$	315.0863	315.0871	2.5	300.0633 (100)	285.0399 (100), 271.0597 (16.9), 272.0696 (15.7)
156	53.99	$C_{18}H_{17}O_6$	329.1020	329.1029	2.7	314.0789 (100), 300.0635 (45.3)	285.0761 (100), 299.0553 (56.8), 296.0683 (36.0)
157	54.04	$C_{20}H_{21}O_7$	373.1282	373.1285	0.8	358.1055 (100), 343.0822 (53.6), 312.0100 (10.2)	343.0818 (100), 312.1003 (23.0)
158	54.25	$C_{20}H_{23}O_8$	391.1388	391.1388	0.0	241.0710 (100)	226.0477 (100), 208.0369 (13.3)
159△	55.27	$C_{21}H_{23}O_8$	403.1388	403.1393	1.2	–	–
160	55.98	$C_{20}H_{23}O_8$	391.1388	391.1396	2.0	227.0555 (100)	212.0322 (100)
161	58.49	$C_{20}H_{23}O_7$	375.1438	375.1444	1.6	241.0713 (100)	226.0475 (100), 208.0369 (13.8), 211.0241 (7.5)
162	60.22	$C_{19}H_{19}O_7$	359.1125	359.1136	3.1	344.0901 (100), 343.0823 (25.7), 315.0875 (15.1)	315.0874 (100), 328.0585 (66.9), 327.0500 (35.2), 326.0795 (28.3), 299.0561 (18.0)
163△	64.47	$C_{21}H_{23}O_8$	403.1388	403.1393	1.2	–	–
164	64.92	$C_{20}H_{21}O_9$	405.1180	405.1183	0.7	390.0954 (100), 375.0719 (95.1), 372.0847 (32.7)	375.0713 (100), 372.0836 (30.5)
165	65.88	$C_{20}H_{21}O_8$	389.1231	389.1233	0.5	359.0774 (100), 374.1009 (78.9), 341.0669 (43.9), 356.0906 (42.4), 328.0956 (15.7)	344.0536 (100), 331.0823 (78.9), 341.0666 (74.2), 343.0459 (69.3), 316.0585 (36.3)
166△	67.38	$C_{21}H_{23}O_8$	403.1388	403.1394	1.5	–	–
167	68.17	$C_{18}H_{19}O_6$	331.1176	331.1180	1.2	191.0705 (100), 167.0340 (92.9), 193.0498 (59.9)	163.0757 (100), 176.0460 (36.0)
168	69.21	$C_{18}H_{17}O_6$	329.1020	329.1027	2.1	296.0689 (100), 314.0795 (75.4), 268.0744 (3.8)	268.0737 (100)
169	69.73	$C_{20}H_{23}O_7$	375.1438	375.1449	2.9	211.0608 (100), 191.0709 (41.4), 357.1343 (21.3)	196.0371 (100), 178.0265 (23.9), 183.0657 (10.3)
170	70.17	$C_{18}H_{19}O_6$	331.1176	331.1178	0.6	197.0448 (100), 298.0742 (52.6), 316.0847 (44.5)	182.0214 (100), 164.0101 (27.1)
171	71.70	$C_{18}H_{17}O_6$	329.1020	329.1026	1.8	313.0713 (100), 314.0785 (80.5)	283.0609 (100), 311.0553 (71.1), 298.0479 (42.4)
172	74.27	$C_{21}H_{25}O_8$	405.1544	405.1551	1.7	241.0715 (100), 387.1451 (5.7)	226.0477 (100), 208.0371 (13.1), 211.0242 (7.5)
173	75.00	$C_{21}H_{23}O_9$	419.1336	419.1339	0.7	404.1109 (100), 389.0873 (96.2), 386.1011 (29.1)	389.0869 (100), 386.1025 (16.9)
174	80.20	$C_{19}H_{19}O_7$	359.1125	359.1132	1.9	329.0660 (100), 344.0896 (99.8), 326.0792 (54.8), 311.0557 (44.3), 360.1175 (36.2)	311.0556 (100), 301.0714 (84.7), 314.0427 (66.5), 197.0086 (37.9), 283.0608 (30.3), 286.0478 (29.8)
175	81.53	$C_{20}H_{23}O_7$	375.1438	375.1448	2.7	211.0607 (100), 191.07094 (5.8)	196.0367 (100), 168.0421 (24.8), 178.0264 (5.0)
176*	82.95	$C_{17}H_{17}O_5$	301.1071	301.1078	2.3	167.0344 (100), 161.0602 (90.4)	135.5858 (100)
177	83.09	$C_{19}H_{21}O_6$	345.1333	345.1338	1.4	211.0602 (100)	196.0371 (100), 178.0265 (26.4)
178	84.74	$C_{19}H_{21}O_6$	345.1333	345.1340	2.0	181.0498 (100),191.0709 (63.9)	166.0263 (100)
179	86.85	$C_{20}H_{23}O_7$	375.1438	375.1447	2.4	241.0711 (100)	226.0474 (100), 208.0239 (11.9), 211.0240 (6.2)
180	90.28	$C_{19}H_{21}O_6$	345.1333	345.1336	0.9	211.0606 (100)	196.0370 (100), 168.0419 (20.9)
181*	95.70	$C_{22}H_{27}O_9$	435.1650	435.1666	3.7	390.1099 (100), 267.0592 (30.2), 308.0316 (14.9)	267.0595 (100), 308.0321 (48.3), 290.0756 (10.9)

* No PMFs.
△ PMF candidates without MS/MS spetra.

which could be taken as the basis for further analysis the PMFs in the extract. As a result, 135 PMFs including 81 polymethoxyflavones, 54 polymethoxyflavanones or polymethoxychalcones were identified preliminarily. This was also the first time to systematically report the presence of PMFs in the leaves of *Citrus reticulata* Blanco, especially for polymethoxylated flavanones and chalcones, most of which were new compounds. The results indicated that the developed strategy of FS-PIL-DE acquisition coupled to DPIs analysis could be employed as a rapid, effective technique to screen and identify target constituents from the TCMs extracts. Furthermore, it is possible for the strategy to be extended to the fields of elucidating constituents from other organic matter mixtures such as substances analysis in vegetables, water quality analysis, natural organic matter analysis in soil, pesticide multi-residue analysis in food, and so on, in the view of that the compounds contained in such matrix can also be classified into families based on the common carbon skeletons.

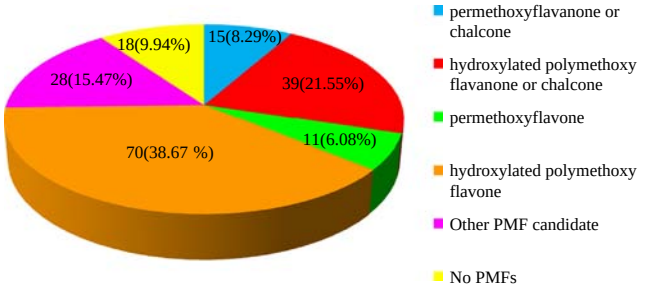


Fig. 6. The distributions of 181 PMF candidates detected.

Table 4
The structural identification of 135 PMFs detected in the leaves of *Citrus reticulata* Blanco.

Categories	Peaks	Amounts	PMFs
1	103, 153	2	Dimethoxyflavone
2	53, 58, 111, 132, 135	5	Monohydroxy-dimethoxyflavone
3	65	1	Monohydroxy-dimethoxychalcone **or** monohydroxy-dimethoxyflavanone
4	127	1	Trimethoxyflavone
5	15, 29, 104, 115, 124, 149, 155	7	Dihydroxy-dimethoxyflavone
6	142	1	Trimethoxychalcone **or** trimethoxyflavanone
7	22, 109, 116	3	Dihydroxy-dimethoxychalcone **or** dihydroxy-dimethoxyflavanone
8	39, 42, 49, 55, 59, 81, 89, 156, 168, 171	10	Monohydroxy-trimethoxyflavone
9	60, 63, 71, 72, 86	5	Trihydroxy-dimethoxyflavone
10	41, 77, 97, 167, 170	5	Monohydroxy-trimethoxychalcone **or** monohydroxy-trimethoxyflavanone
11	2, 7, 14, 26, 70, 75	6	Trihydroxy-dimethoxychalcone **or** trihydroxy-dimethoxyflavanone
12	96, 112, 136	3	Tetramethoxyflavone
13	18, 32, 37, 100, 119, 126, 128, 134	8	Dihydroxy-trimethoxyflavone
14	108, 114, 150, 177, 178, 180	6	Tetramethoxyflavanone **or** tetramethoxychalcone
15	30, 117, 120	3	Dihydroxy-trimethoxyflavanone **or** Dihydroxy-trimethoxychalcone
16	9, 12, 21	3	Tetrahydroxy-dimethoxyflavone
17	31, 45, 52, 57, 64, 66, 84, 107, 131, 151, 162, 174	12	Monohydroxy-tetramethoxyflavone
18	33, 68, 82, 94,	4	Trihydroxy-trimethoxyflavone
19	43, 48, 56, 67, 79, 85, 145, 146, 148	9	Monohydroxy-tetramethoxyflavanone **or** monohydroxy-tetramethoxychalcone
20	61, 69, 83	3	Trihydroxy-trimethoxyflavanone **or** trihydroxy-trimethoxychalcone
21	80, 105, 157	3	Pentamethoxyflavone
22	74, 121, 161, 169, 175,179	6	Pentamethoxyflavanone **or** pentamethoxychalcone
23	138, 140	2	Dihydroxy-tetramethoxyflavone
24	51, 54, 101, 118, 125, 129	6	Dihydroxy-Tetramethoxychalcone **or** dihydroxy-Tetramethoxyflavanone
25	8, 13	2	Tetrahydroxy-trimethoxyflavone
26	76, 90, 165	3	Monohydroxy-pentamethoxyflavone
27	98, 158, 160	3	Monohydroxy-pentamethoxyflavanone **or** monohydroxy-pentamethoxychalcone
28	137, 139	2	Trihydroxy-tetramethoxyflavone
29	130	1	Hexamethoxyflavone
30	35, 78, 164	3	Dihydroxy-pentamethoxyflavone
31	133, 172	2	Hexamethoxychalcone **or** hexamethoxyflavanone
32	50, 93, 152, 173	4	Monohydroxy-hexamethoxyflavone
33	143	1	Heptamethoxyflavone

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