SHIMADZU APPLICATION NEWS

LAAN-A-LM-E009

● LIQUID CHROMATOGRAPHY MASS SPECTROMETRY

No.C51

Accurate Mass Analysis of Flavonoids by LCMS-IT-TOF

Flavonoids are a kind of polyphenol, and many are present in fruits and vegetables, grains, seeds, nuts, wine and tea. They have long been used as fragrances and pigments in food and cosmetics, and in recent years, it has become clear they impart antioxidative and hormone promotion effects. Various types of polyphenols have been discovered, extracted and developed, and many commercial products have been created as drugs and health foods, giving rise to a health boom.

A great deal of effort is required to determine the structure of a physiologically active flavonoid after discovering a minute quantity in a natural substance, and then separating and purifying it. However, using LCMS-IT-TOF to conduct accurate mass MSn analysis, high-accuracy molecular weight information and structural information from fragment ions can be obtained without actually separating and purifying the substance. Moreover, many existing TOF type mass spectrometers achieve high mass accuracy using the internal standard method. However, in the LCMS-IT-TOF, long-lasting stability of mass accuracy can be obtained using the external standard method, due to Shimadzu's unique technologies, including BIE (Ballistic Ion Extraction, Patent No. US6380666, etc.) and the internal temperature adjustment mechanism.

Here we introduce an analysis of mandarin orange methanol extract by LCMS-IT-TOF, and the structural analysis results for the included flavonoids. Fig. 1 shows the UV and MS chromatograms. Using the automatic MS/MS analysis feature, measurement up to MS^3 was conducted in one analysis while automatically selecting precursor ions. By conducting MS^n analysis, it was possible to predict the types, number and positions of the glycosides, as well as the structure of aglycon portions.

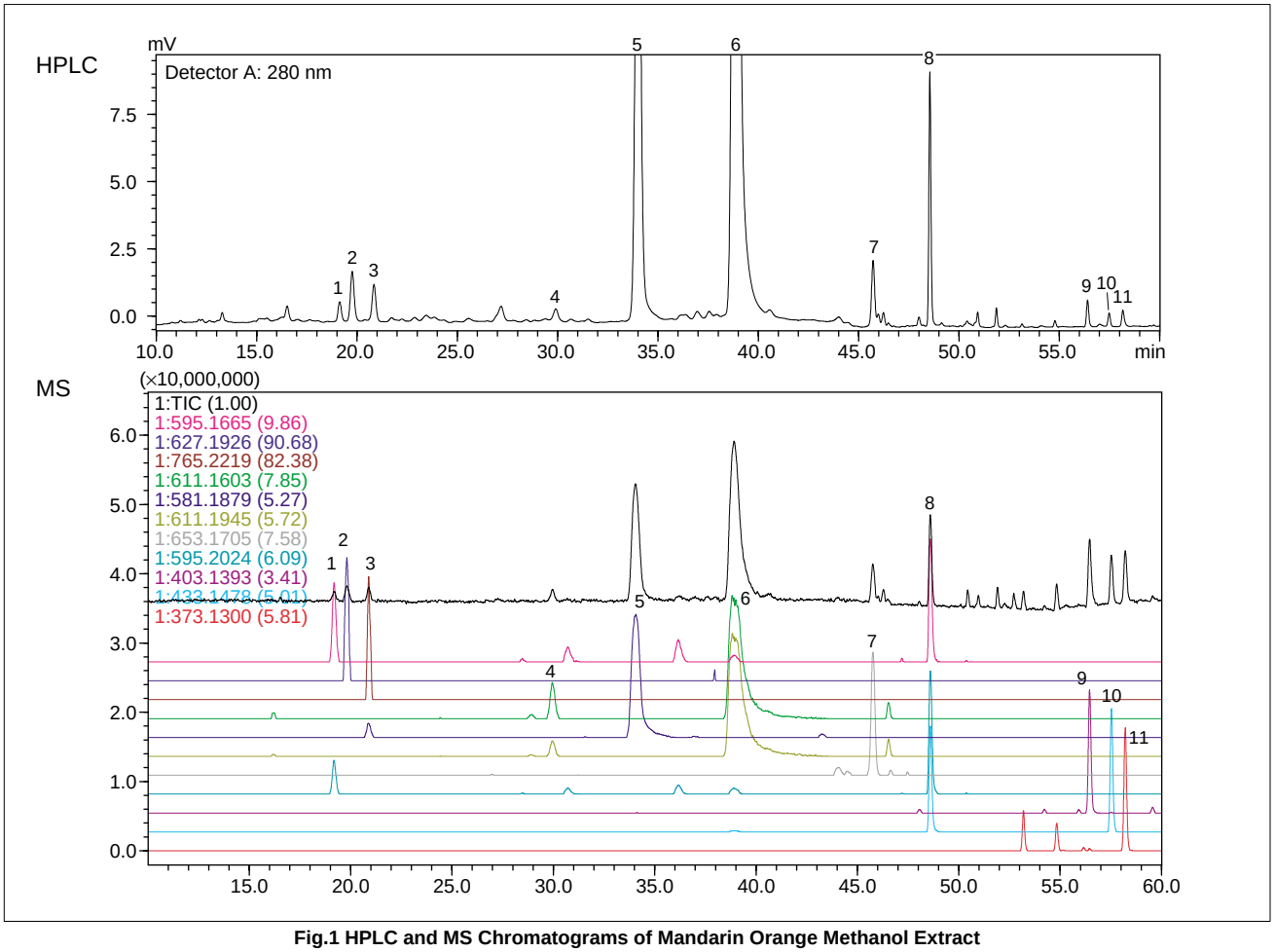


Fig.1 HPLC and MS Chromatograms of Mandarin Orange Methanol Extract

Fig.2 shows the structure of the primary constituent, hesperidin (peak 6), and Fig.3 shows the mass spectra of hesperidin. The m/z 611.1945 indicated by the red ▼1 is the protonated hesperidin molecule $[M+H]^+$, with a theoretical value of m/z 611.1976, showing a mass accuracy of -5.1 ppm. Next, selecting this m/z 611 as the precursor ion, we collected an MS/MS spectrum. Taking the differences between the precursor ion m/z 611 and fragmentation ions m/z 303 and m/z 449, respectively, yielded values of 308.1072 and 162.0532, and applying composition prediction to these values resulted in predictions of $C_{12}H_{20}O_9$ and $C_6H_{10}O_5$, respectively. These compositions correspond to the disaccharide and monosaccharide parts shown in Fig.2. Next, we collected an MS/MS spectrum, selecting m/z 303 as the precursor ion. The predicted structures of m/z 145, m/z 153 and m/z 177 are shown in Fig.2.

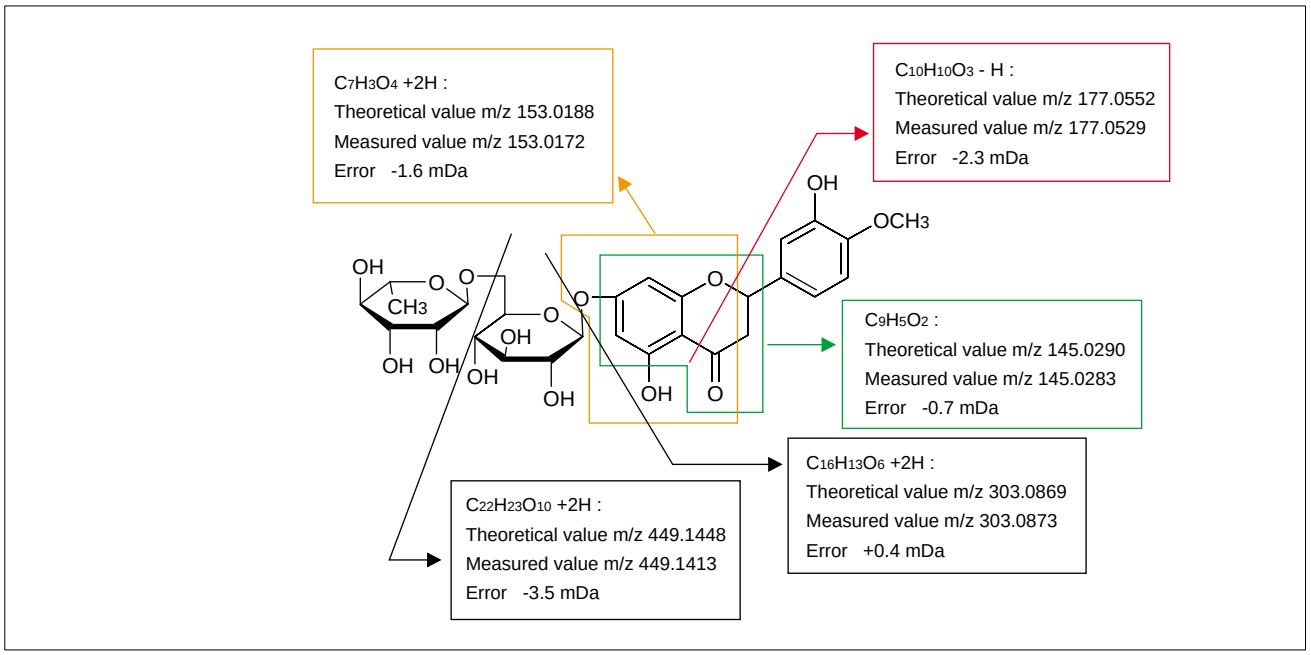


Fig.2 Structure of Hesperidin

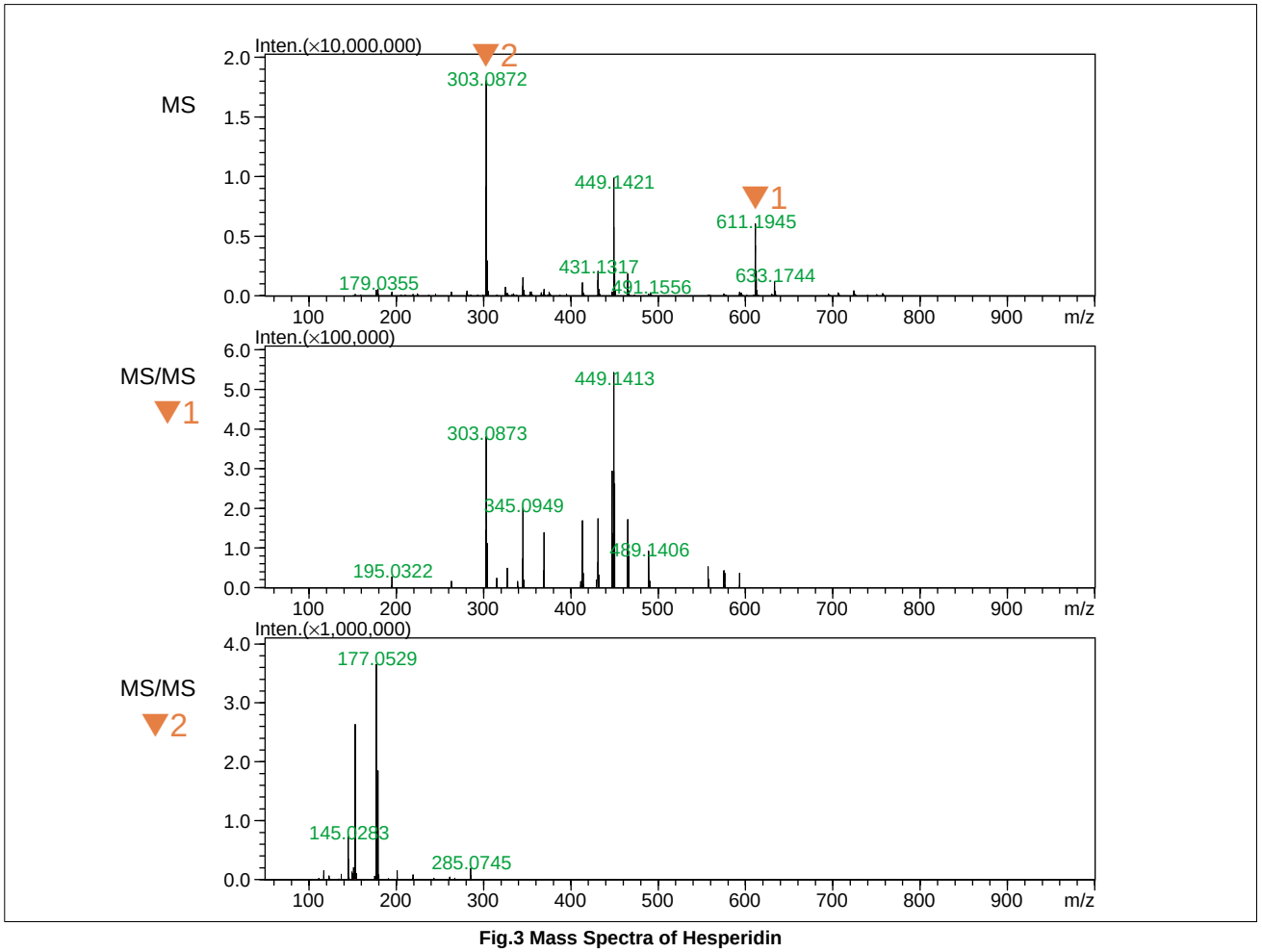


Fig.3 Mass Spectra of Hesperidin

Fig.5 shows the mass spectra of peak 8. Compared to the mass spectra of hesperidin in Fig.3, m/z 287, m/z 433 and m/z 595 were detected, each 16 mass units less than the major m/z 303, m/z 449 and m/z 611, respectively, indicating a structure in which the -OH of the hesperidin aglycon was replaced by -H. The m/z 595.2024 indicated by the red ▼1 is the protonated molecule $[M+H]^+$, with a theoretical value of m/z 595.2027, showing a mass accuracy of -0.5 ppm. Next, we collected an MS/MS spectrum, selecting m/z 287 as the precursor ion. Comparing this to the hesperidin m/z 303 MS/MS spectrum, m/z 153 is found in both, but since m/z 161, 16 mass units less than m/z 177, was detected, the structure of peak 8 was predicted as shown in Fig.4.

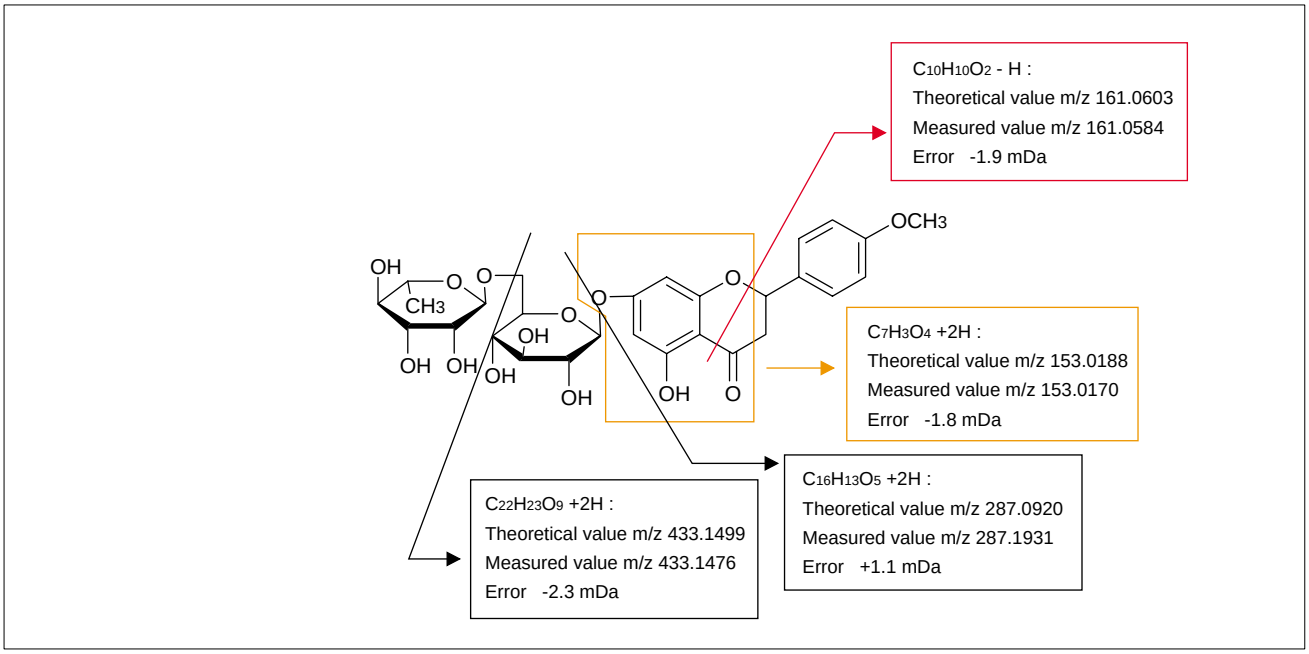


Fig.4 Structure of Dehydroxyhesperidin (peak 8)

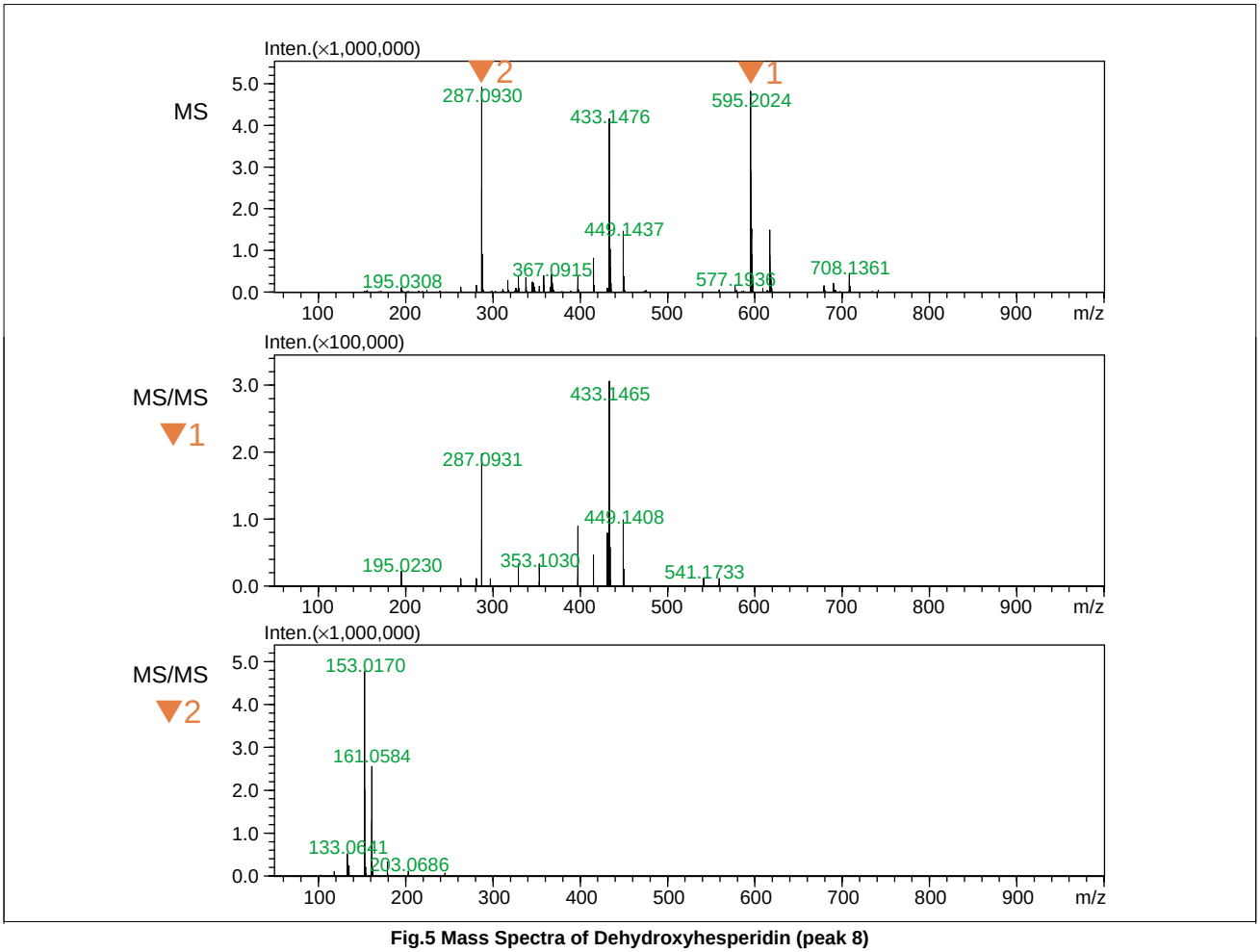


Fig.5 Mass Spectra of Dehydroxyhesperidin (peak 8)

Fig.6 shows the mass spectra of peak 4. Using an integer, it is a compound with a molecular weight of 610, the same as hesperidin. However, the protonated molecule $[M+H]^+$ accurate mass is m/z 611.1603, separated by more than 30 mDa from hesperidin, suggesting a different composition. The composition $C_{27}H_{30}O_{16}$ was obtained in the search results using composition prediction software, “Formula Predictor”, and it was identified as rutin from the retention time, etc., as shown in Fig.7.

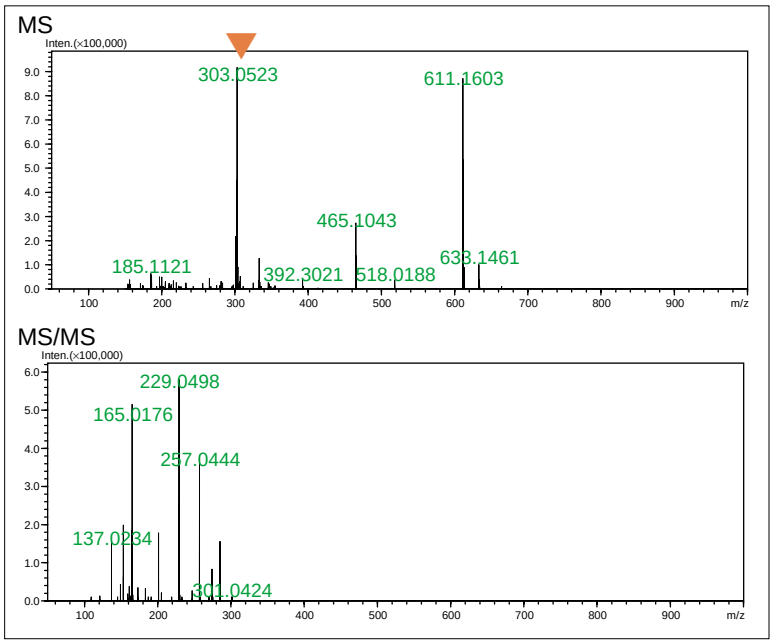
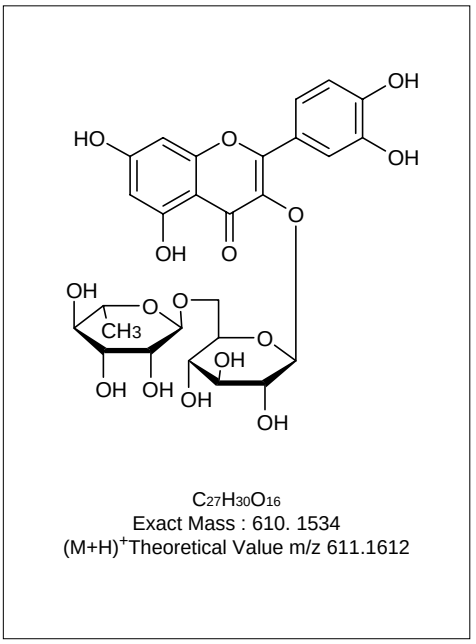


Fig.6 Mass Spectra of Peak 4



$C_{27}H_{30}O_{16}$
Exact Mass : 610. 1534
$(M+H)^+$Theoretical Value m/z 611.1612

Fig.7 Structure of Rutin (peak 4)

Table 1 summarizes the qualitative results for each of the peaks, showing the theoretical and measured value of the ion related to the molecular weight associated with each peak. Good results were obtained, with the margin of error kept to within ±5 ppm using the external standard method.

Table 1 Qualitative Results

	Retention Time (min)	Compound Name	Molecular Weight	Molecular Formula	Theoretical Value $(M+H)^+$ m/z	Measured Value $(M+H)^+$ m/z	Error (ppm)
#1	19.20		594.1585	$C_{27}H_{30}O_{15}$	595.1663	595.1665	+0.34
#2	19.82	3’-hydroxyhesperidin	626.1847	$C_{28}H_{34}O_{16}$	627.1925	627.1926	+0.16
#3	20.90	narirutin-4’-b-D-Glu	742.2320	$C_{33}H_{42}O_{19}$	765.2218 $(M+Na)^+$	765.2219 $(M+Na)^+$	+0.13
#4	29.96	rutin	610.1534	$C_{27}H_{30}O_{16}$	611.1612	611.1603	-1.47
#5	34.06	narirutin	580.1792	$C_{27}H_{32}O_{14}$	581.1870	581.1879	+1.55
#6	38.93	hesperidin	610.1898	$C_{28}H_{34}O_{15}$	611.1976	611.1945	-5.07
#7	45.76		652.1640	$C_{29}H_{32}O_{17}$	653.1718	653.1705	-1.99
#8	48.60	5’-dehydroxyhesperidin	594.1949	$C_{28}H_{34}O_{14}$	595.2027	595.2024	-0.50
#9	56.46	nobiretin	402.1315	$C_{21}H_{22}O_8$	403.1393	403.1393	±0
#10	57.53	3,5,6,7,8,3’,4’- heptamethoxyflavone	432.1420	$C_{22}H_{24}O_9$	433.1499	433.1478	-4.85
#11	58.22	tangeretin	372.1209	$C_{20}H_{20}O_7$	373.1287	373.1300	+3.48

Table 2 Analytical Conditions

Column	: Phenomenex Gemini 5u C18 110A (2.0 mm I.D. × 150 mmL.)		
Mobile phase A	: 0.1 % (w/v) acetic acid-water		
Mobile phase B	: 0.1 % (w/v) acetic acid-acetonitrile		
Gradient program	: 0 %B (0 min) → 10 %B (5 min) → 20 %B (40 min) → 100 %B (75 to 90 min) → 0 %B (90.01 to 100 min)		
Flow rate	: 0.2 mL/min		
Injection volume	: 0.2 µL	Column temperature	: 40 ˚C
Probe voltage	: +4.5 kV (ESI-Positive mode)		
CDL temperature	: 200 ˚C	Block Heater temperature	: 200 ˚C
Nebulizing gas flow	: 1.5 L/min	Drying gas pressure	: 0.1 MPa

NOTES:
✻This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.

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