

Supplementary Table 1. Mass spectra properties of marker chemicals.

Chemical	Formula	Calculated mass [M]	Precursor ion ^a	Collision energy ^b	Production ion ^c	Retention time ^d
Ferulic acid	C ₁₀ H ₁₀ O ₄	194.10	149.6	10	135	11.251
				20	107	
Paeonol	C ₉ H ₁₀ O ₃	166.17	150.6	10	95	12.757
				40	52	
Butylphthalide	C ₁₂ H ₁₄ O ₂	190.23	132.6	20	77	15.527
				40	51	
Z-Butylidenephthalide	C ₁₂ H ₁₂ O ₂	188.22	158.6	10	131	15.852
				20	103	
Senkyunolide A	C ₁₂ H ₁₆ O ₂	192.25	106.7	10	79	16.580
				10	77	
Z-Ligustilide	C ₁₂ H ₁₄ O ₂	190.10	147.7	20	105	16.823
				40	77	

^a The ion of relative highest intensity was used as the precursor ion for the quantification.

^b The collision energy was optimized to have the greatest product ion intensity, which was the key factor in the MRM mode.

^c Two product ions were used for the MRM analysis. The upper one was used for quantitative analysis and the lower one was for qualitative analysis, which could guarantee the precision of analytes.

^d The retention time was determined by 3 different individual analyses ($n = 3$).

Supplementary Table 2. Calibration curves, LOD and LOQ of five markers.

Chemical	Calibration curve^a	Correlation coefficient (r^2)	Linear range	LOD^b (ng/ml)	LOQ^c (ng/ml)
Z-Ligustilide	$y=0.7169x-0.0497$	0.9945	0.1-50	7.08	33.3
Ferulic acid	$y=0.7984x-0.2864$	0.9903	0.1-50	2.77	12.5
Butylphthalide	$y=14.0273x-0.1603$	0.9970	0.02-5	0.32	1.3
Z-Butylidenephthalide	$y=4.4408x-0.0611$	0.9955	0.02-5	0.47	2
Senkyunolide A	$y=2.3792x-0.0417$	0.9922	0.02-5	2.71	13

^aThe calibration curves were constructed by plotting the peak areas versus the concentration of each analyte. Each calibration curve included six data points.

^bLOD refers to the limits of detection.

^cLOQ refers to the limits of quantification.

Supplementary Table 3. Precision, repeatability and recovery of markers.

Chemical	Precision				Repeatability (n=5)		Recovery ^a (n=5)	
	Intra-day ^b (n=6)		Inter-day ^c (n=6)		Mean (ng/mL)	RSD (%)	Mean (%)	RSD (%)
	Mean (ng/mL)	RSD (%)	Mean (ng/mL)	RSD (%)				
Z-Ligustilide	0.98	1.98	0.91	3.29	17.67	2.98	96.91	3.31
Ferulic acid	1.04	0.26	1.44	1.58	1.41	1.19	97.57	1.85
Butylphthalide	1.01	1.48	0.93	1.39	0.91	1.73	99.90	1.66
Z-Butylidenephthalide	1.07	2.76	1.02	2.36	0.10	2.03	100.30	2.10
Senkyunolide A	0.96	3.01	1.04	4.05	0.89	2.61	98.75	2.21

^a Recovery (%) = $100 \times (\text{amount found} - \text{original amount}) / \text{amount spiked}$. The data were presented as average of three independent determinations, and the SD was <5% of the mean, which was not shown for clarity.

^b The intra-day analysis refers to the sample examined for six replicates within one day.

^c The inter-day analysis refers to the sample examined in duplicates over three consecutive days.