Supporting Information

Rapid Quality Evaluation of Chinese Herbal Medicines Using a Miniature Mass Spectrometer: *Lygodium japonicum* (Thunb.) Sw. as An Example

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Outline

1 Optimization of CID energy

The miniature mass spectrometry (mini-MS) instrument used in this work supports multiple reaction monitoring (MRM) mode and therefore well supports qualitative and quantitative analysis. Each analyte precursor ion generate the corresponding product ion fragments via collision-induced dissociation (CID) in the tandem MS. For qualitative analysis, we needed to confirm that the precursor-product ion information in the MS/MS spectra of each analyte in the herbal extracts was consistent with the standards. More importantly, we use product ions for quantification to minimize the interference of isomers, so we need to try to make the quantified ions have higher peak intensities. As a result, we needed to carefully optimize the CID energy for each analyte. The process of CID optimization for the nine analytes is shown in Figure S1.

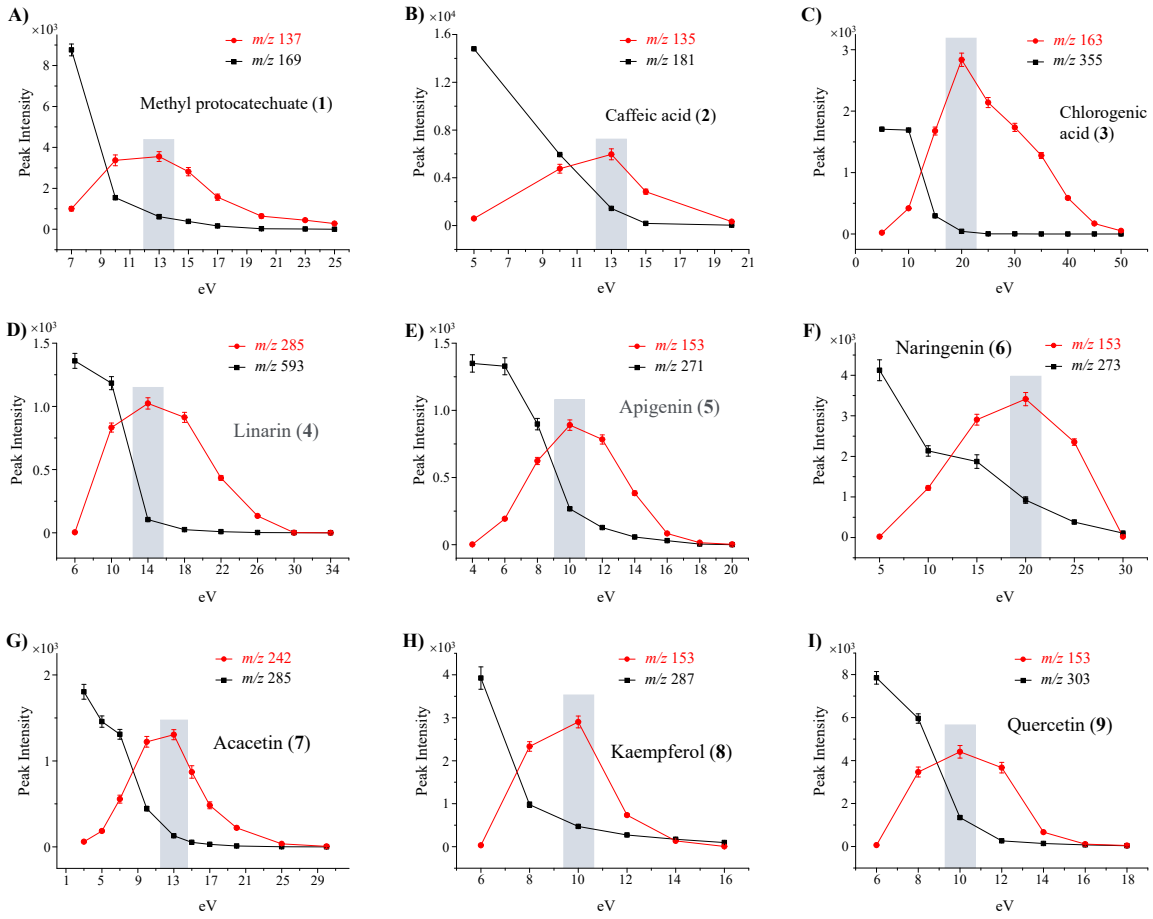

Figure S1. Optimization of CID energy for the nine analytes.

2 Quantitative methodology

2.1 Standard curves

The quantitative analysis of the analytes was performed using a standard curve method combined with the multiple reaction monitoring (MRM) strategy. The characteristic precursor/product ion pairs of the analytes were used for MRM analysis. The standard curve of each analyte was plotted with the concentration of standard solutions as the horizontal coordinate and the peak intensity of the quantitative product ion in MS/MS analysis as the vertical coordinate (Figure S2). Standard solutions were freshly made using herbal extracts without analytes as background matrices. Details of the concentration range of the standard solutions was given in Table S1.

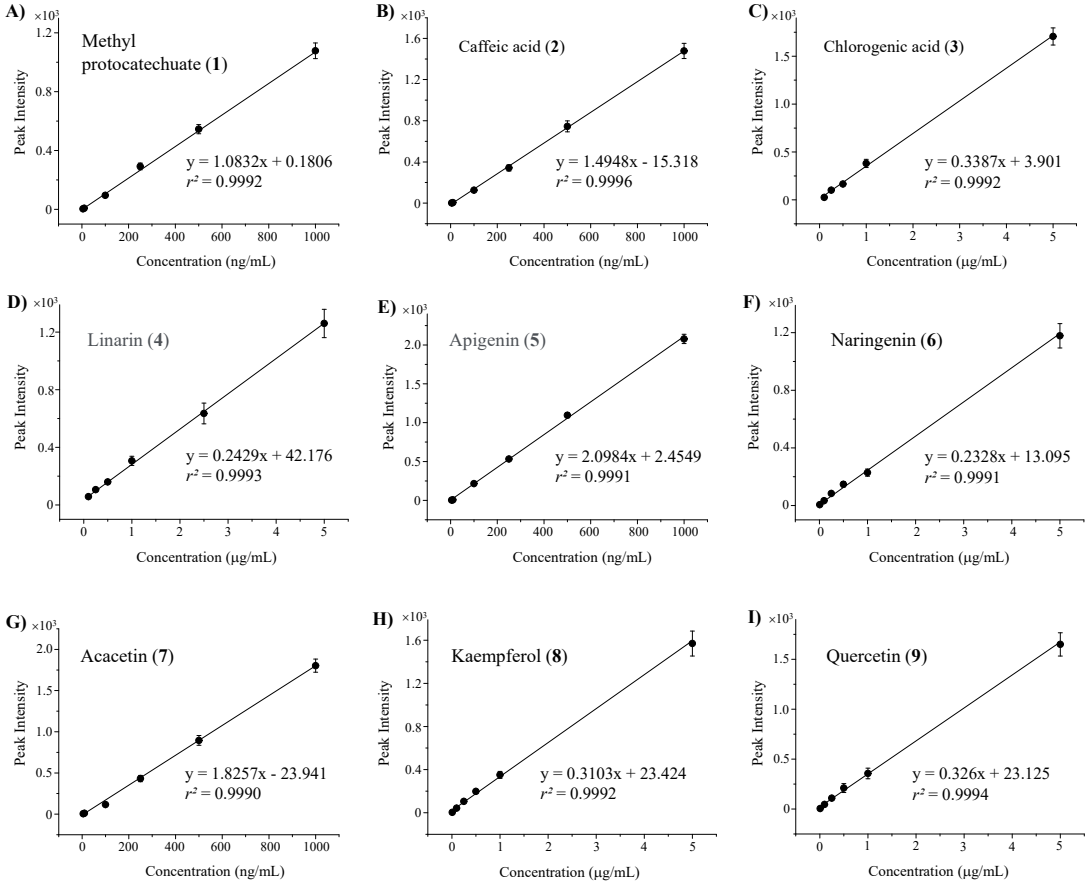

Figure S2. Standard curves for the nine analytes.

Table S1. Details of the concentration range of the standard solutions

Analyte	**Concentration range** (ng/mL)
Methyl protocatechuate (**1**)	5, 10, 100, 250, 500, 1000
Caffeic acid (**2**)	5, 10, 100, 250, 500, 1000
Chlorogenic acid (**3**)	100, 250, 500, 1000, 2500, 5000
Linarin (**4**)	100, 250, 500, 1000, 2500, 5000
Apigenin (**5**)	5, 10, 100, 250, 500, 1000
Naringenin (**6**)	10, 100, 250, 500, 1000, 5000
Acacetin (**7**)	5, 10, 100, 250, 500, 1000
Kaempferol (**8**)	10, 100, 250, 500, 1000, 5000
Quercetin (**9**)	10, 100, 250, 500, 1000, 5000

2.2 Methodology validation

The methodology of PSI-mini-MS/MS analysis was validated, including precision, accuracy and recovery rates. Quantitative control (QC) samples of nine analytes at low/high concentration were added to the background TCM extract (herbal extracts without these nine compounds) for methodology validation. The background extract without spiking analytes was used for negative samples, and each sample was detected for 5 times in 3 days as parallel detections. The results were summarized in Table S2. It has been demonstrated that the developed PSI-mini-MS/MS method is able to determine the nine analytes accurately and precisely in simulated CHM extract samples.

Table S2. Precision, accuracy and recovery rates of nine analytes in background TCM extracts based on PSI-mini-MS/MS analysis (n = 5, 3 days).

Analyte	Conc. of QC sample (ng/mL)	Precision (RSD %)		Accuracy (RE %)		Recovery rate (%)
		Intra-day	Inter-day	Intra-day	Inter-day	
Methyl protocatechuate (**1**)	0.005	4.3	4.8	5.3	5.4	97.4 ± 3.2
	1	0.8	1.4	1.2	1.5	99.8 ± 1.1
Caffeic acid (**2**)	0.005	1.1	6.1	7.8	7.0	100.6 ± 1.2
	1	0.5	0.9	1.2	0.6	99.8 ± 2.4
Chlorogenic acid (**3**)	0.01	3.1	5.1	6.0	6.1	101.6 ± 0.3
	5	0.7	1.8	0.7	1.0	98.2 ± 1.7
Linarin (**4**)	0.1	3.8	6.0	4.9	-1.6	96.8 ± 3.1
	10	1.2	1.0	1.1	0.9	100.2 ± 0.9
Apigenin (**5**)	0.005	4.1	7.7	6.9	7.8	101.7 ± 1.2
	1	1.2	1.9	1.0	1.2	100.7 ± 2.1
Naringenin (**6**)	0.01	6.1	7.8	6.8	8.3	100.2 ± 0.9
	5	1.4	2.0	1.4	1.1	96.3 ± 2.2
Acacetin (**7**)	0.005	3.2	3.2	3.9	7.8	98.1 ± 2.4
	1	2.5	1.2	2.1	1.5	100.6 ± 1.5
Kaempferol (**8**)	0.01	3.7	4.1	3.2	6.2	96.3 ± 3.1
	5	1.1	1.3	1.2	2.0	99.8 ± 1.1
Quercetin (**9**)	0.01	1.9	3.5	1.3	2.9	94.2 ± 2.9
	5	0.2	1.5	0.6	1.1	95.1 ± 2.1

2.3 Representative MS/MS spectra used for quantitative analysis

PSI-mini-MS/MS was used to analyze the nine effective components in the extracts of each species of *Lygodium*. The nine target components are present in all three species of *Lygodium*, but in different amounts. Taking chlorogenic acid (**3**) as an example, Figure S3 showed the MS/MS spectra for the detection of chlorogenic acid (**3**) in *Lygodium* extracts of three different species. This showed that chlorogenic acid (**3**) is present in different species of *Lygodium* at different levels. The other eight analytes were quantified using similar methods.

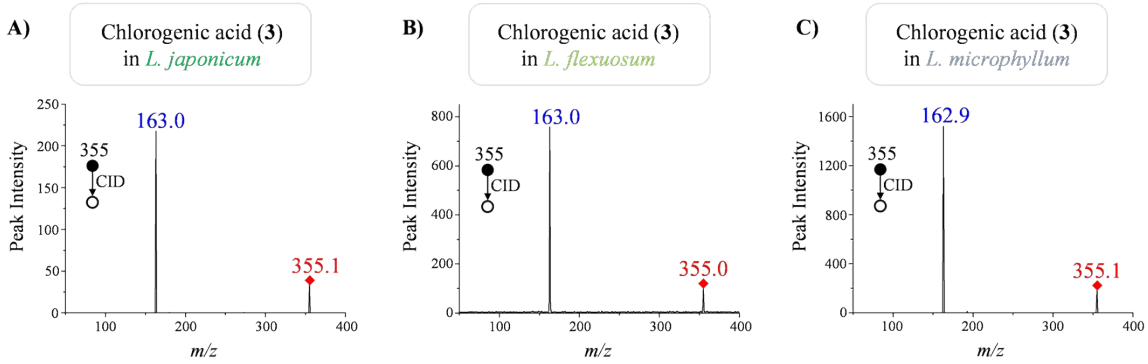

Figure S3. MS/MS spectra of chlorogenic acid (**3**) in *Lygodium* extracts of three different species.