Supporting Information for

pH-Responsive epitope-imprinted magnetic nanoparticles for selective separation and extraction of chlorogenic acid and caffeic acid in traditional Chinese medicines

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Contents:

Experimental

1. Preparation of standard solution

The stock solutions of chlorogenic acid and caffeic acid (2 mg/mL) were prepared in methanol. Their mixed working solution (8 μg/mL) was prepared by appropriate dilution of the stock solutions with 0.1 M phosphate buffer (pH 6.5). All solutions were stored at 4℃ protected from light.

2. Preparation of real sample solution

0.5 g of TCMs (*Taraxaci Herba*, *Lonicerae Japonicae Flos* or *Eucommiae Folium*) powder was sieved through a No.4 sieve, then put into a conical flask with a stopper, and weighed after adding 20 ml of 75% methanol. The mixture was soaked for 10 min, ultrasonically extracted for 30 min, then cooled to RT and weighed again. After the mixture was replenished, shaken, and filtered, the subsequent filtrate was collected and diluted 250-fold with 0.1 M phosphate buffer (pH 6.5). The obtained sample solutions were stored at 4℃ protected from light for later use.

3. **Determination of chlorogenic acid and caffeic acid in TCMs by the method in the Chinese Pharmacopoeia 2020 edition.**

0.5 g of *Taraxaci Herba* powder was sieved through a No.4 sieve, then put into a conical flask with a stopper, and weighed after adding 20 ml of 80% methanol. The mixture was ultrasonically treated (400W, 40kHz) for 20 min, then cooled to RT and weighed again. After the mixture was replenished with 80% methanol, shaken, and filtered, the subsequent filtrate was analyzed by HPLC-UV.

0.5 g of *Lonicerae Japonicae Flos* powder was sieved through a No.4 sieve, then put into a conical flask with a stopper, and weighed after adding 50 ml of 75% methanol. The mixture was ultrasonically treated (500W, 40kHz) for 30 min, then cooled to RT and weighed again. After the mixture was replenished with 75% methanol, shaken, and filtered, the subsequent filtrate was analyzed by HPLC-UV.

1 g of *Eucommiae Folium* powder was sieved through a No.3 sieve, then put into a conical flask with a stopper, and weighed after adding 25 ml of 50% methanol. The mixture was heated and refluxed for 30 min, then cooled to RT and weighed again. After the mixture was replenished with 50% methanol, shaken, and filtered, the subsequent filtrate was analyzed by HPLC-UV.

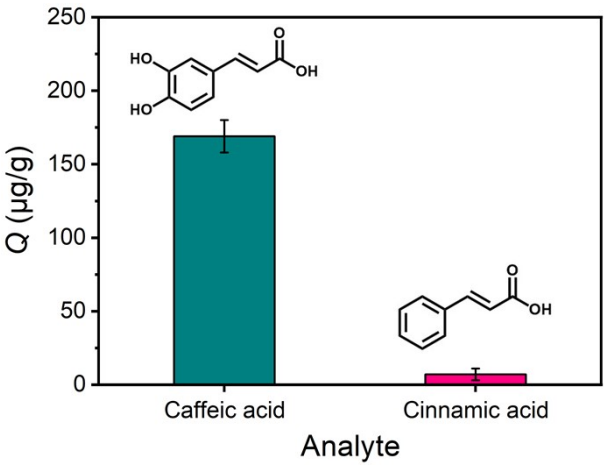

Fig. S1 The amount of caffeic acid and cinnamic acid captured by boronic acid-functionalized MNPs.

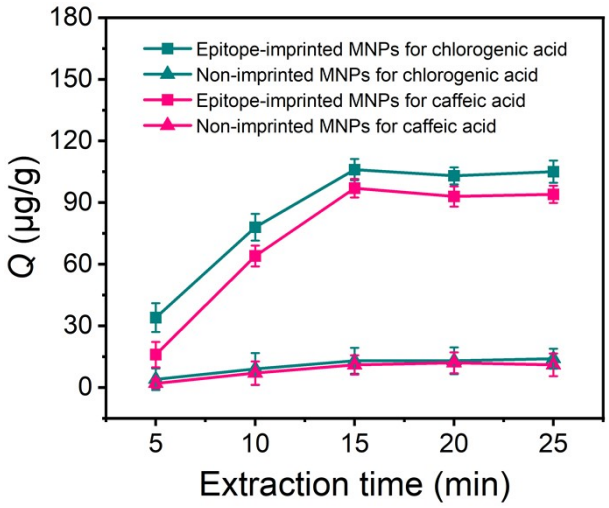

Fig. S2 The amount of chlorogenic acid and caffeic acid extracted by epitope-imprinted MNPs and non-imprinted MNPs at extraction time of 5 - 25 min.

Table S1 Adsorption amount and precisions of chlorogenic acid and caffeic acid using the epitope-imprinted MNPs-based affinity extraction coupled with HPLC. (n = 3)

	Item	Chlorogenic acid									Caffeic acid								
		10 ng/mL			100 ng/mL			1000 ng/mL			2 ng/mL			20 ng/mL			200 ng/mL		
Intraday precision	Adsorption amount (μg/g)	0.464	0.395	0.449	4.67	4.27	4.76	45.1	48.1	46.3	0.0929	0.0952	0.0903	0.918	0.891	0.922	9.51	9.32	8.41
	Average adsorption amount (μg/g)		0.436			4.57			46.5			0.0928			0.910			9.08	
	Standard deviation		0.036			0.26			1.5			0.0025			0.017			0.59	
	RSD (%)		8.3			5.7			3.2			2.7			1.9			6.5	
Interday precision	Adsorption amount (μg/g)	0.464	0.387	0.438	4.67	4.91	4.20	45.1	42.8	46.5	0.0929	0.0946	0.0862	0.918	0.938	0.877	9.51	8.12	9.45
	Average adsorption amount (μg/g)		0.430			4.59			44.8			0.0912			0.911			9.03	
	Standard deviation		0.039			0.36			1.9			0.0044			0.031			0.79	
	RSD (%)		9.1			7.8			4.2			4.8			3.4			8.7	

Table S2 Recoveries of the epitope-imprinted MNPs-based affinity extraction coupled with HPLC for chlorogenic acid and caffeic acid in *Taraxaci Herba*. (n = 5)

Analyte	Content (mg/g)	Spiked standard (mg/g)	Observed (mg/g)	Recovery (%)	Average recovery (%)	Standard deviation	RSD (%)
	1.16	1.16	2.29	97.4			
	1.16	1.16	2.37	104.3			
Chlorogenic acid	1.16	1.16	2.26	94.8	101.4	5.5	5.4
	1.16	1.16	2.34	101.7			
	1.16	1.16	2.42	108.6			
	0.378	0.378	0.766	102.6			
	0.378	0.378	0.737	95.0			
Caffeic acid	0.378	0.378	0.747	97.6	99.1	4.9	4.9
	0.378	0.378	0.778	105.8			
	0.378	0.378	0.736	94.7			

Table S3 Determination and comparison of chlorogenic acid and caffeic acid in TCMs by the epitope-imprinted MNPs-based affinity extraction coupled with HPLC and the method in the Chinese Pharmacopoeia 2020 edition. (n = 3)

Sample	Analyte	The epitope-imprinted MNPs-based affinity extraction coupled with HPLC					The method in the Chinese Pharmacopoeia 2020 edition				
		Content (mg/g)			Average content $\overline{X} \pm S$ (mg/g)	RSD (%)	Content (mg/g)			Average content $\overline{X} \pm S$ (mg/g)	RSD (%)
Taraxaci Herba (Henan, China)	Chlorogenic acid	1.09	1.15	1.23	1.16 ± 0.070	6.0	1.19	1.16	1.05	1.13 ± 0.074	6.5
	Caffeic acid	0.371	0.401	0.363	0.378 ± 0.020	5.3	0.345	0.384	0.376	0.368 ± 0.021	5.7
Lonicerae Japonicae Flos (Shandong, China)	Chlorogenic acid	31.3	32.1	33.5	32.3 ± 1.1	3.4	31.3	30.1	33.1	31.5 ± 1.5	4.8
	Caffeic acid	0.692	0.654	0.633	0.660 ± 0.030	4.5	0.679	0.639	0.608	0.642 ± 0.036	5.6
Eucommiae Folium (Sichuan, China)	Chlorogenic acid	0.928	0.895	0.810	0.878 ± 0.061	6.9	0.865	0.921	0.962	0.916 ± 0.049	5.3
	Caffeic acid	0.0121	0.0101	0.0109	0.0110 ± 0.0010	9.1	0.0108	0.0112	0.0124	0.0115 ± 0.00083	7.2