Supporting Information

In vivo selective capture and rapid identification of luteolin and its metabolites in rat livers by molecularly imprinted solid-phase microextraction

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Supplemental methods

1. Instrument and Chromatographic Conditions.

Analyses were carried out on an Agilent 1260 Series HPLC system (Agilent Technologies, Palo Alto, California, USA). Chromatographic separation was performed using an Agilent SB-C18 column (150×4.6 mm, particle size 5 μm). HPLC-MS/MS analysis was based on a Nexera X2 HPLC system (Shimadzu, Japan) coupled to an AB Sciex Triple 5600 tandem mass spectrometer (Applied 24 Biosystems/MDS Sciex, MA, USA). A Zorbax Eclipse plus C18 column, Rapid Resolution HD (2.1×100mm, 1.8-Micron) was used for separation. JSM-7600F Feld emission scanning electron microscope (JEOL, Japan) was used to observe morphology of MIPs. Fourier transform infrared (FT-IR) spectra were obtained via an IR Affinity-1 FT-IR spectrometer (Shimadzu, Japan). The surface-area and the pore-size distribution were obtained via Autosorb - iQ automatic specific surface and pore size distribution analyzer (Autosorb-iQ, America).

2. Surface Treatment of Stainless Steel Fibers

Firstly, stainless steel fibers were cut into 4.0 cm of segments (each about 20.0 mg). Then put the cut fibers into acetone and ultrasonic for 15 min to clean the surface. The fibers were washed with methanol for another three times and dried in oven. Secondly, the self-assembly of dopamine was conducted on fibers. The fibers were immersed into 10 mL of Tris-HCl solution containing 6 mg/mL of dopamine (pH8.5) and kept for 24 h in dark. Then, the fibers were washed with methanol for three times to obtain the modified stainless steel fibers. Thirdly, silylation was carried out on the dopamine modified fibers. The fibers were soaked in a mixture of

3-MPS/H_2O/Methanol (v/v/v, 1:1:8) and kept for 30 min, then took out the fibers and dried in a vacuum drying oven for 150 min at 150 ^oC. The dried fibers were washed with methanol for three times and dried under nitrogen.

3. Calibration curve of luteolin analyzed by HPLC

10 mg of luteolin was dissolved in 10.0 mL methanol to prepare the stock solution (1 mg/mL) and working standard solutions were diluted using 55% (v/v) methanol-water with a series of concentrations (1, 5, 10, 20, 40, 60, 80 and 100 μg/mL, respectively). The absorbance of different concentration of luteolin was measured using HPLC.

4. Preparation of liver homogenates

Rats decapitated and livers were transferred into a 5 mL beaker. Then livers were washed with saline till becoming a gray. After that, pieces of fresh livers of rats (approximately 0.5 g each group) were transferred into a microcentrifuge tube (2 mL) prior to homogenization with a tissue tearor for 30 s.

5. ***Extraction Time Profile***

The extractions by using MIPs fibers were performed at 2, 5, 10, 15 and 20 min from prepared liver homogenates spiked with 30 μg/mL of luteolin. Then the fibers were quickly rinsed for 30 s in ultrapure water and dried under nitrogen. After that, fibers were desorbed in 500 μL (minimum volume to ensure complete immersion of the coating into the solvent) of methanol/acetic acid (8:2, v/v) to elute luteolin. Finally, eluants were dried under nitrogen and dissolved in 100 μL methanol for HPLC analysis.

6. Diffusion-Based Calibration: Pre-Determined Sample In Rates

Different concentrations of target luteolin were prepared in 0.5 g prepared liver homogenates (0.1, 0.25, 0.5, 1, 5, 10, 20 and 30 μg/mL) to determine sampling rates in matrix-matched samples. On the basis of the extraction time profile (Fig.6A), MIP-SPME sampling was performed for 10 min. Adsorption by MIP-SPME fibers, rinsing and desorption steps were performed using the same conditions as described in section 2.8.2. The total concentrations in each liver homogenates were measured by the following method: after adding the different concentrations of luteolin in 0.5 g prepared liver homogenates (0.1, 0.25, 0.5, 1, 5, 10, 20 and 30 μg/mL), 1 mL ethyl acetate were added into liver homogenates to extract luteolin. Liver homogenates solutions then were centrifuged at 13000×*g* for 20 min at 4^{o}C and the supernatants were obtained. After that, the supernatants were dried under nitrogen and dissolved in 100 μL methanol for HPLC analysis.

7. HPLC analysis

For the evaluation of the adsorption capacity of MIPs and the selectivity of the MIPs toward structural analogues in section 2.7, the gradient elution process of methanol (solvent A) and 0.2% phosphoric acid aqueous solution (solvent B) was as followed: 0-10 min, 45%-55% A; 10-15 min, 55% - 60% A; 15-20 min, 60% - 80% A; 20-25 min, 80% A; 25-30 min, 80% - 45% A. The flow rate was set at 0.8 mL/min. The column temperature was maintained at 30 ^{o}C and injection volume was 10 μL. The detective wavelength was set at 350 nm.

For the analysis of luteolin in liver samples, the gradient elution process of

methanol (solvent A) and 0.2% phosphoric acid aqueous solution (solvent B) was as followed:0-50 min, 40%-42% A; 50-60 min, 42% A; 60-75 min, 42%-52% A; 75-77 min, 52%-40% A. The flow rate was set at 0.8mL/min. The column temperature was maintained at 30 ^{o}C and injection volume was 10 μL. The detective wavelength was set at 350 nm.

For the analysis of metabolites of luteolin in liver sample, high-performance liquid chromatography-tandem mass spectrometry (MS/MS) analysis was based on an Nexera X2 HPLC system (Shimadzu, Japan) coupled to an AB Sciex Triple 5600tandem mass spectrometer (Applied 24 Biosystems/MDS Sciex, MA, USA). A Zorbax Eclipse plus C18 column, Rapid Resolution HD (2.1×100mm, 1.8-Micron) was used for separation. An aqueous solution containing 0.1% formic acid (A) and (B) acetonitrile were used as the mobile phases, the gradient elution process was as followed:0-2 min, 5% B→25% B; 2-5 min, 25% B→38% B; 5-30 min, 38% B→100% B; 30-32 min, 100% B; 32-32.1 min, 100%→5% B; 32.1-35 min, 15% B→stop. The flow rate was set at 0.3 mL·min^{-1}, the column temperature was maintained at 30 ^{o}C and injection volume was 2 μL.

High resolution mass spectrometry (MS) detections were performed on AB Sciex Triple 5600tandem mass spectrometer (Applied 24 Biosystems/MDS Sciex, MA, USA), connecting to the HPLC. The conditions of MS/MS spectra were as followed: ion source: electronic electrospray ionization (ESI) source; Ionization ways: positive ion scanning; TOF - MS quality scan range (m/z): 100-2000 da. Quality of TOF MS/MS scan range (100-2000 m/z) da; auxiliary gas (GS1 andGS2), 60 and 60 psi,

respectively; Air curtain air pressure (CUR): 35 psi. (TEM) ion source temperature: 600 ℃;Ion spraying voltage (ISVF): 5500 v; To cluster voltage (DP): 100 v, the parent ion collision energy (CE): 10, the child away from the collision energy (CE): 50, sub ion collision energy difference (CES): 10.Controlof the system, data acquisition and data analysis were all carried out using the Analyst 1.5.2 software (Applied Biosystems).

8. IR analysis

As shown in **Figure S1**, MIPs (after elution) and NIPs coatings had similar IR spectra, which indicated that their backbone structure were similar. The main functional groups of MIPs coatings could be found with corresponding infrared absorption peaks. Four strong infrared absorption peaks of 3448, 2954, 1726, and 1664cm^{-1} were found, which were attributed to hydroxyl groups, methyl groups, carbonyl groups, and alkenes (C=C bonds), respectively. The characteristic peak of C=O at 1726 cm^{-1}in the MIPs spectrum showed that the MIPs were synthesized through the polymerization of EGDMA and AM. Moreover, the special peak intensity of the stretching vibration of C=C (1664 cm^{-1}) in the MIPs was crucial for the chemical bonding between MIP layer and layer during the coating procedures.

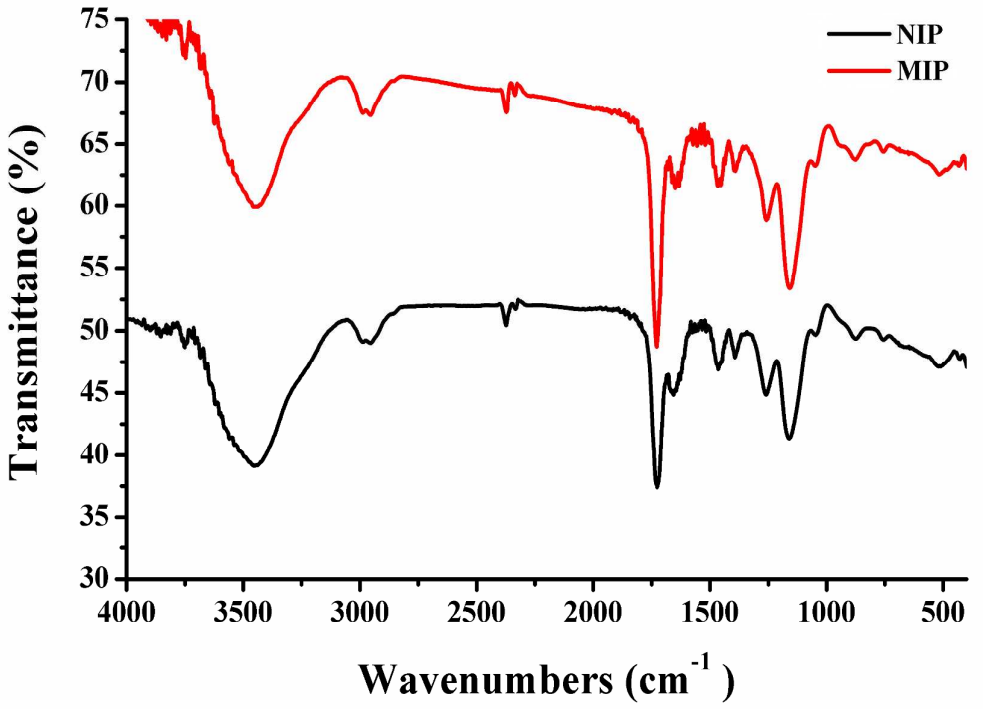

Figure S1. The FT-IR spectra of MIPs (after elution) and NIPs.

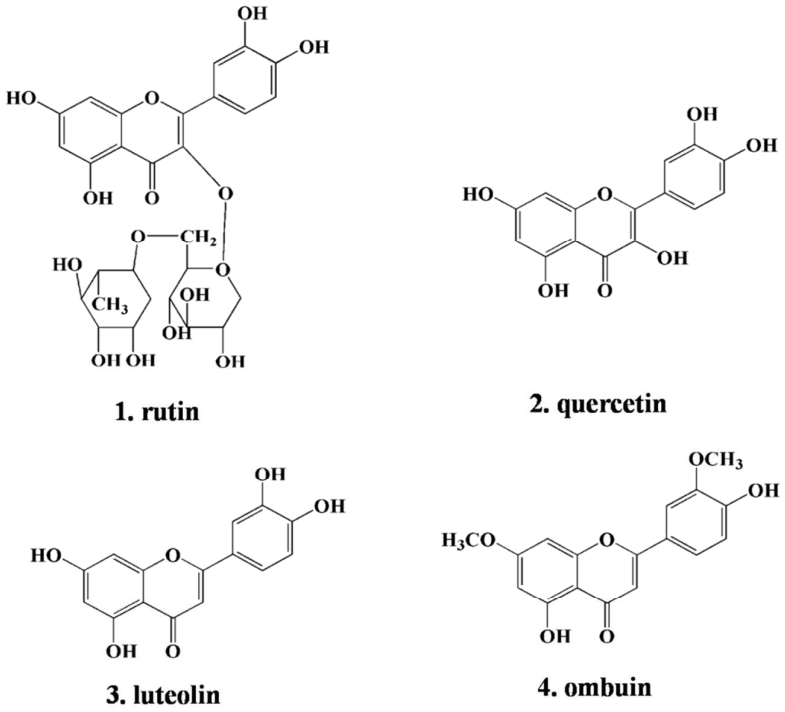

Figure S2. The structure of luteolin and its structural analogues.

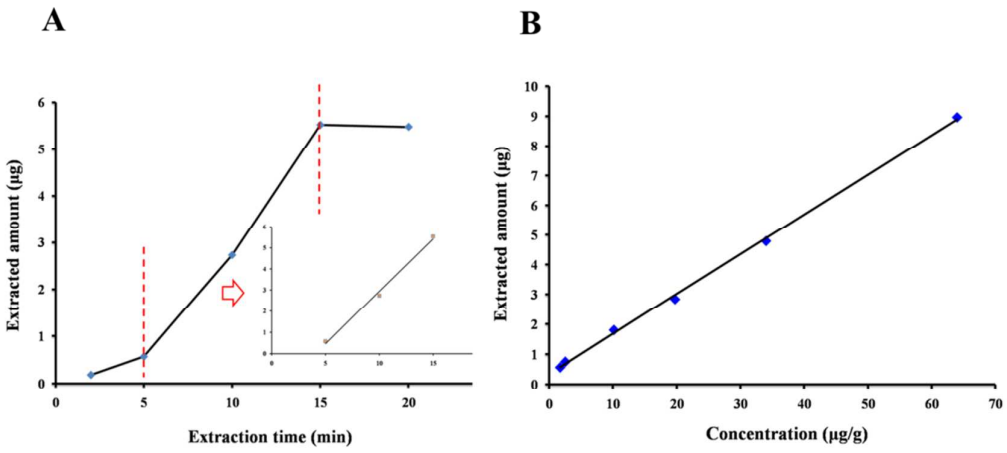

Figure S3. n-t (A) and n-Cs (B) curves of *in vitro*MIP-SPME of rat livers homogenates.

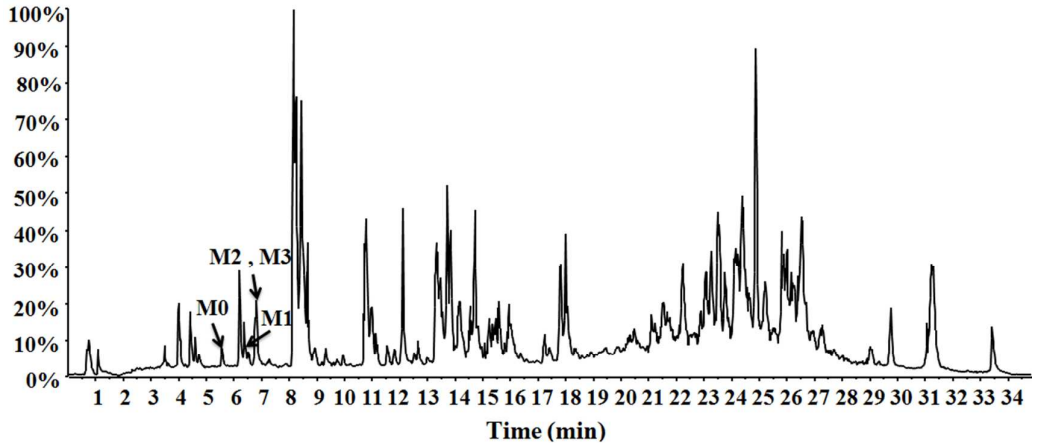

Figure S4. The total ion current of extract extracted by using MIP-SPME sampling method.

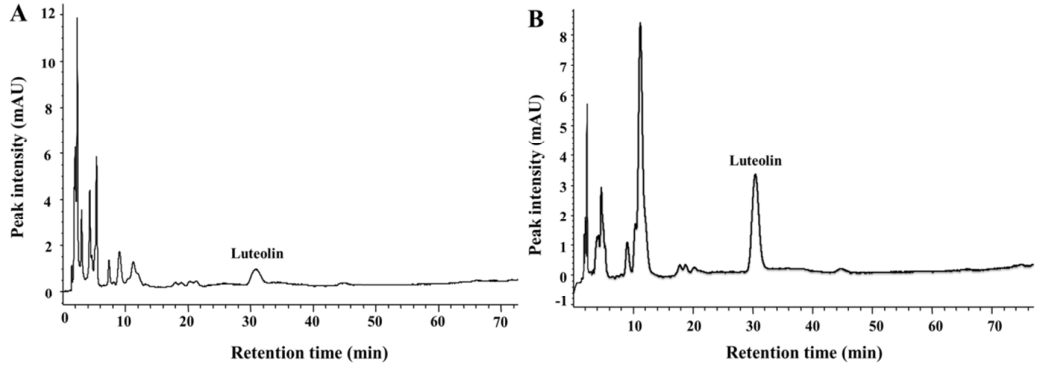

Figure S5. The chromatogram of extract in rat livers at 120 min by using commercial PDMS fiber (A) and DVB fiber.

Table S1 Specific Surface Area, Pore Structure Parameters of MIPs and NIPs Coatings

Samples	BET Surface area (m^2/g)	BJH desorption cumulative volume of pores (cm^3/g)	BJH desorption average pore radius (nm)
MIP-SPME	320.49	0.367	18.94
NIP-SPME	202.49	0.236	17.01

Table S2 Luteolin and Its Metabolites Identified in Rat Livers Using UPLC-MS/MS

Name	RT/min	$[M+H]^+$	MS/MS fragment ions
M0	5.613	287.0280	269.0195, 258.0264, 241.0240, 213.0315, 161.0029, 152.9990, 135.0262
M1	6.618	271.0339	152.9998
M2	6.860	301.0434	286.0438, 257.0195, 229.0262, 203.0120, 152.9993
M3	6.875	301.0434	286.0207, 257.0186, 229.0255, 203.0116, 152.9993