Supplementary Information for

Molecularly imprinted monolith coupled on-line with high performance liquid chromatography for simultaneous quantitative determination of cyromazine and melamine

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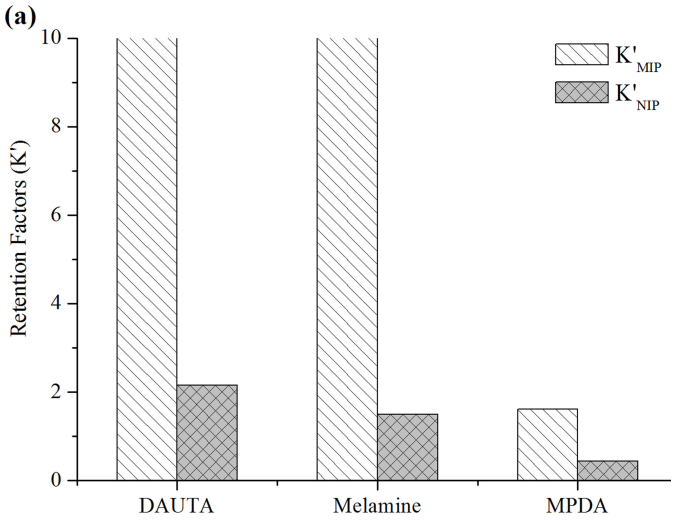
Preparation of 2,4-diamino-6-undecyl-1,3,5-triazine (DAUTA)-imprinted polymers using bulk polymerization

The template molecule 2,4-diamino-6-undecyl-1,3,5-triazine (DAUTA) (0.75 mmol), functional monomer MAA (4.5 mmol), the cross-linker EDGMA (9.6 mmol) were dissolved in chloroform (10 mL) in an 18 mm×180 mm borosilicate glass test tube. Then the mixture was sonicated for 5 min and purged with nitrogen for 20 min to remove the dissolved oxygen before the tube was sealed under reduced pressure. The polymerization was carried out in a water bath at 65 °C for 24 h and then the solid polymer was crushed, ground and extracted with methanol containing 10% acetic acid using a Soxhlet apparatus for 48 h followed by sieving to obtain particles in the size range of 32–50 μm. After repeated decanting in acetone for three times to remove the fine particles, the particles were dried under vacuum at 60 °C. The non-imprinted polymer (NIP) was prepared and treated identically except no template was present in the polymer preparation.

The retention factors (k') of DAUTA, melamine and m-phenylenediamine (MPDA) on above prepared DAUTA-imprinted MIP and corresponding NIP in different solvents were measured and the results are shown in Figure S1.



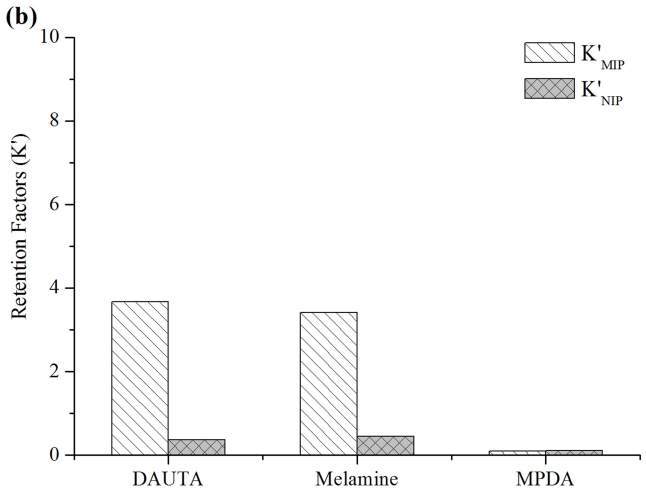


Figure S1. The retention factors (k') of DAUTA, melamine and m-phenylenediamine (MPDA) on the DAUTA-imprinted MIP and corresponding NIP prepared by bulk polymerization in different solvents. (a) acetonitrile containing 5% acetic acid (v/v); (b) methanol containing 5% acetic acid (v/v).

Optimization of the molar ratios of functional monomer to crosslinker for preparation of the imprinted monolith

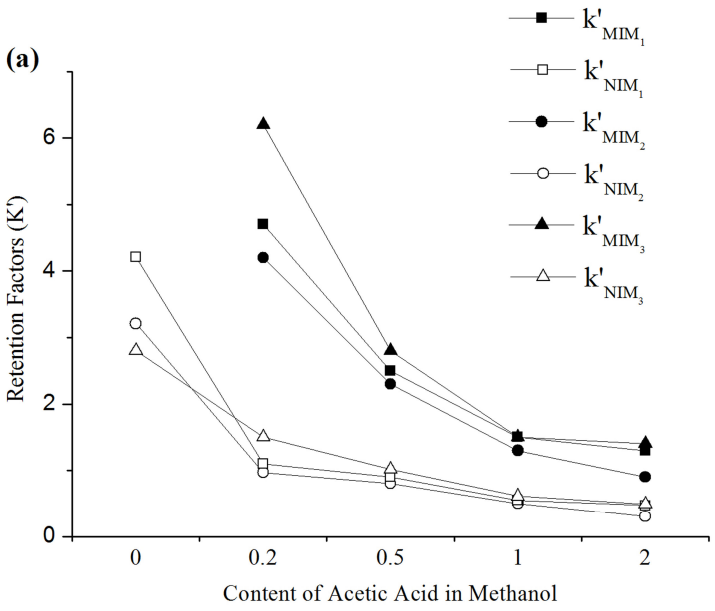


Figure S2. The retention factors (k') of melamine on MIM_1, MIM_2, MIM_3 with the crosslinker/functional monomer ratios of 1:4, 1:3.3, 1:6, respectively. Melamine was completely retained on all MIMs when pure methanol was used as the loading solvent.

Morphologies of the imprinted monolith (MIM_1) and non-imprinted monolith (NIM_1)

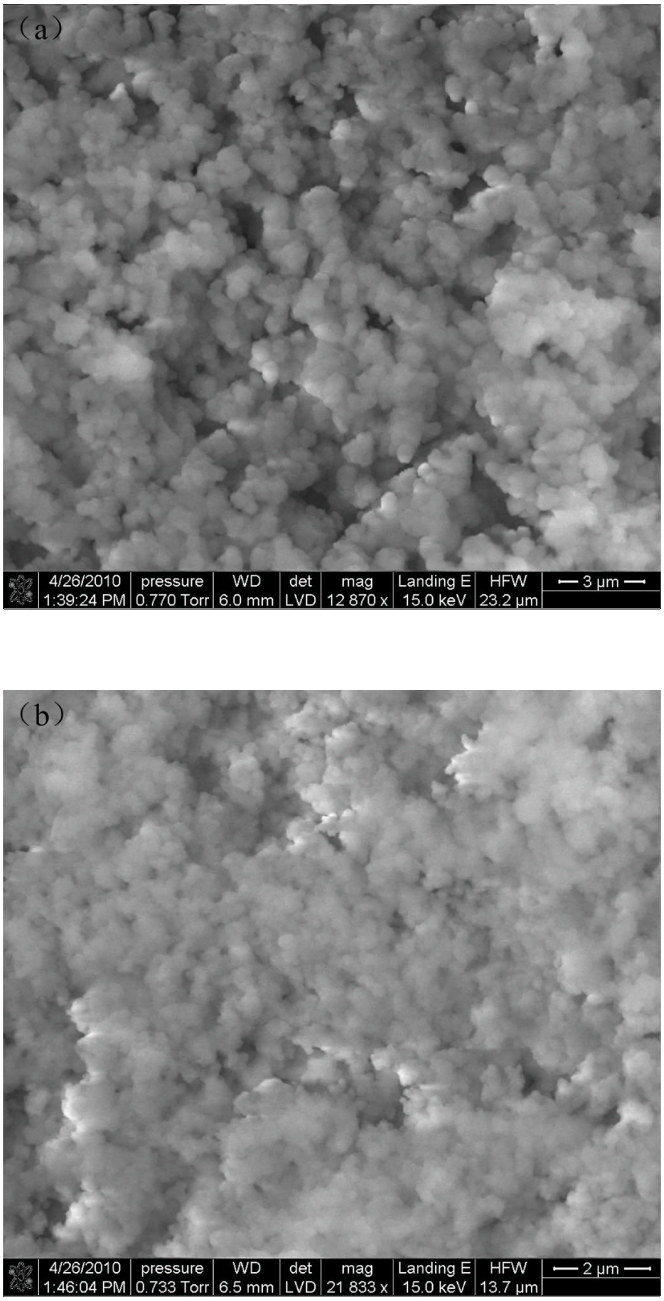


Figure S3. Scanning electron microscope (SEM) images of the imprinted and non-imprinted monoliths. (a) MIM_1; (b) NIM_1.

Selective extraction of melamine from the milk samples by the imprinted monolith

To verify the capability of the imprinted monolithic column to selectively extract melamine from complex matrix, 100 μL of the extractions of milk samples spiked with 0.4 μg/mL of melamine were applied to the imprinted monolithic column in an off-line mode. Methanol was used to wash out the nonspecific retained interferences. Then the retained components were eluted with 100 μL of 8.5% H_3PO_4. The washing out solution and the elution solution were separately collected, evaporated to dryness and then reconstituted with 0.5 mL of the mobile phase (Buffer A-acetonitrile (9:1, v/v)) for further HPLC analysis. The typical chromatograms of the extractions of spiked milk sample purified by the imprinted and non-imprinted monolithic columns are compared in Figure S4. The non-imprinted monolithic column was also investigated under the same experimental conditions for comparison.

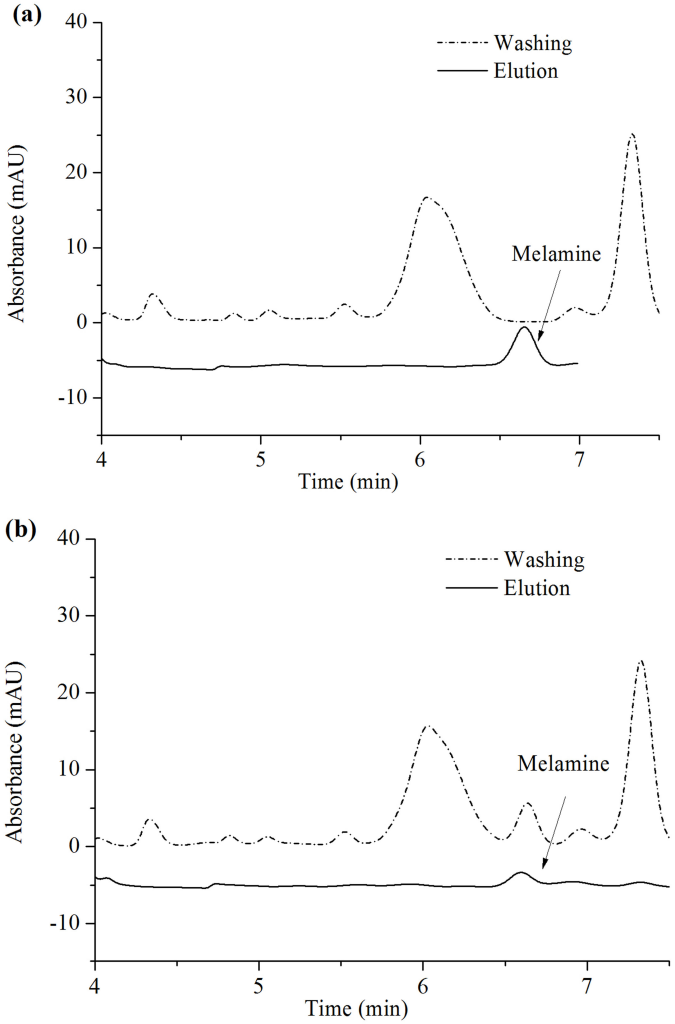


Figure S4. HPLC chromatograms of the extractions of milk samples spiked with 0.4 μg/mL of melamine purified by the off-line imprinted monolith (a) and non-imprinted monolith (b). The dash line and soild line correspond to the washing out solution and elution solution, respectively.

As shown in Figure S4a, no melamine was observed in the solution directly flowing through the imprinted monolith. In the elution solution, the concentration of melamine could be quantified without suffering from any significant interference from the matrix. From Figure S4b, melamine was not efficiently retained by the non-imprinted columns and directly flew out of the column. While large quantities of interferences were observed in the elution solution. These data demonstrated the capability of the obtained imprinted monolith to specifically bind melamine and remove most of the interferences, which enabled specific purification and concentration of melamine.