Electronic Supplementary Material (ESI) for Analytical Methods.
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Supporting Information for

Aptamer-enhanced fluorescence determination of bisphenol A after magnetic solid-phase extraction using Fe_3O_4@SiO_2@ Aptamer

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1. O 1*s* core-level XPS spectra of Fe_3O_4@SiO_2-NH_2 and Fe_3O_4@SiO_2@Apt

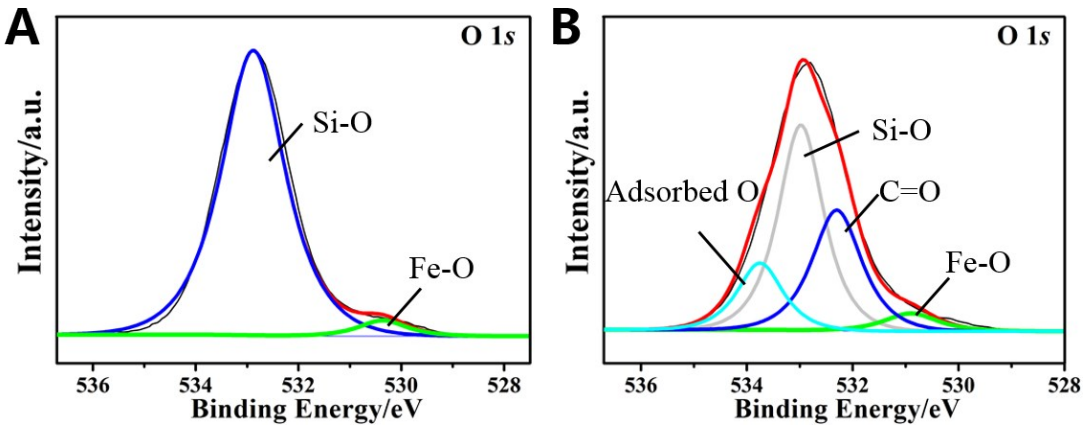

Fig. S1. O 1*s* core-level XPS spectra of (A) Fe_3O_4@SiO_2-NH_2, (B) Fe_3O_4@SiO_2@Apt.

2. Label the number of aptamers on superparamagnetic nanoparticles

The coupling rate of BPA aptamer on Fe_3O_4@SiO_2-NH_2 nanoparticles was studied using a FAM-labeled BPA aptamer (5'-carboxyl-CCG GTG GGT GGT CAG GTG GGA TAG CGT TCC GCG TAT GGC CCA GCG CAT CAC GGG TTC GCA CCA-3'-FAM). First, a set of five concentrations of FAM-labeled BPA aptamers (20 to 100 nM) was prepared as standards to draw a calibration curve of fluorimetry, as shown in Fig. S2. One milliliter of the Fe_3O_4@SiO_2-NH_2 nanoparticles (3.3×10^{-11} M) was activated by 0.3 mL of EDC (200 mM) and NHS (100 mM) for 30 min. After magnetic separation, the Fe_3O_4@SiO_2-NH_2 nanoparticles (3.3×10^{-14} mol) and 100 µL of FAM-labeled BPA aptamer (1.0×10^{-7} M) was incubated in binding buffer (Tris 40mM, NaCl 100mM, $CaCl_2$ 5mM and $MgCl_2$ 5mM, pH7.4) for 6 h at room temperature. Add 100 µL of FAM-labeled BPA aptamer (0 M) as a blank sample. Magnetic separation was applied to separate bound from unbound FAM-labeled BPA aptamers. The fluorescence absorption of supernatant was then measured by fluorescence spectroscopy (F-7000 FL spectrophotometer). Excitation and emission wavelengths were set at 492 nm and 518 nm, respectively. Related concentration was calculated by standard curve and the concentration of bound FAM-labeled BPA aptamers was extracted according to the fluorescence absorbance of samples considering Equation 1.

$$A_{bound} = [A_{initial} - A_{unbound}] - A_{blank} \qquad Eq.\ 1$$

The experiments were performed in triplicate. The results showed that the unbound FAM-labeled BPA aptamers were about 8.9×10^{-8} M (8.9×10^{-12} mol), therefore the bound FAM-labeled BPA aptamers were 1.1×10^{-8} M (1.1×10^{-12} mol). The molar ratio of the bound aptamers with Fe_3O_4@SiO_2-NH_2 was 30:1."

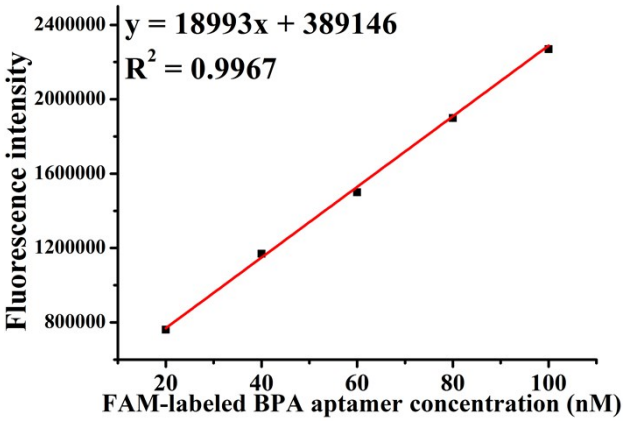

Fig. S2. Plot of fluorescence intensity versus FAM-labeled BPA aptamers concentration.

3. The dissociation constant (K_d) of the Fe_3O_4@SiO_2@Apt towards free BPA

Standard stock solutions of BPA (10 μM) were prepared with a mobile phase of methanol/H_2O (50/50, v/v). The stock solutions were further diluted with the mobile phase to obtain BPA solutions at 15 concentrations from 10 μM to 0.1 nM. One milliliter BPA solutions were added to 10 mL centrifuge tubes, and 10 mg of Fe_3O_4@SiO_2@Apt (approximately 11 nM of BPA-aptamer bound) was dispersed into the sample solutions using a homemade stirrer for 3 min. After standing for 15 min, magnetic separation was applied to separate bound from unbound BPA. An 80 μL supernatant sample was subjected to HPLC analysis to determine the concentration of unbound BPA. The experiments were performed in triplicate. The initial concentration of BPA was A_0, the unbound concentration after incubation was A_1, the fraction bound was calculated as (A_0-A_1)/A_0. The dissociation constants, or K_d, were calculated from the resulting sigmoidal curves using the built-in Hill1 sigmoidal

fit in Origin 8.5.

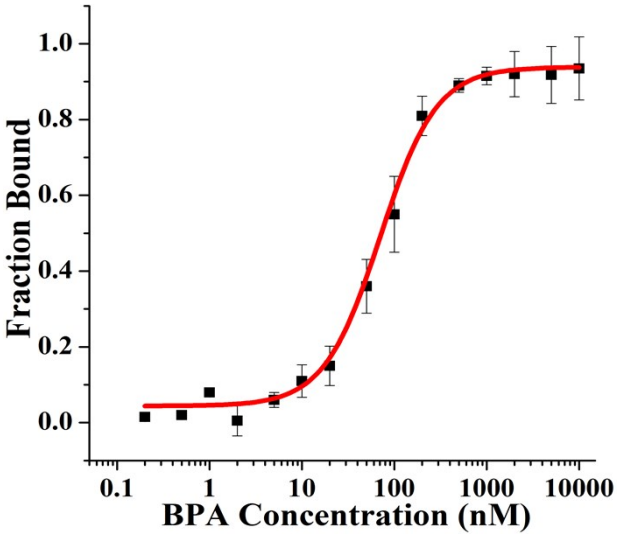

Fig. S3. Binding behavior of free BPA to the Fe_3O_4@SiO_2@Apt (K_d~70.38 nM, r^2 =0.988).

4. Calculation method of LOD and LOQ

The LOD of this method was calculated by $3\sigma_b$/slope, and the limit of quantitation (LOQ) was calculated by $10\sigma_b$/slope (σ_b, standard deviation of the blank samples).

5. Inter-day and intra-day precision of the method

Table S1 Inter-day and intra-day precision of the method

	Inter-day precision (RSD%, n=5)			Intra-day precision (RSD%, n=5)		
Analytes	Low	Medium	High	Low	Medium	High
BPA	9.5	8.1	6.3	8.6	7.9	4.9

“High”, “Medium”, and “Low” denote the concentrations of BPA in milk samples were 100, 20, and 5 ng mL^{-1}, respectively.