Electronic Supplementary Information

Highly hydrophilic polyhedral oligomeric silsesquioxane (POSS)-containing aptamer-modified affinity hybrid monolith for the efficient on-column discrimination with an extremely low nonspecific adsorption†

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1. Experimental

1.1 Permeability and swelling propensity analysis

Permeability was measured by using a LC-20AD pump (Kyoto, Japan) delivering methanol (η = 0.544 mPa·s) with different flow rates and the resulting backpressure was recorded. The permeability was calculated by using the Darcy's law as below:

$$K = \frac{F\eta L}{\pi r^2 \Delta P} \qquad \text{(Equation 1)}$$

K is the permeability (m^2), F is the applied flow rate ($m^3\ s^{-1}$), η is the viscosity of the solvent (Pa·s), L is the length of the capillary (m), ΔP is the measured backpressure (Pa), and finally r is the capillary inner diameter (m).

The swelling propensity (SP) was calculated according to the following equation. Tetrahydrofuran (THF) was applied as the mobile phase for 20 min and the pressure drop was measured, the mobile phase was then changed to water and the pressure drop was measured again once the system became stable.

$$SP = \frac{p(solvent) - p(water)}{p(water)} \qquad \text{(Equation 2)}$$

where p is the mobile phase pressure drop relative to the corresponding viscosity.

1.2 Analytical procedure

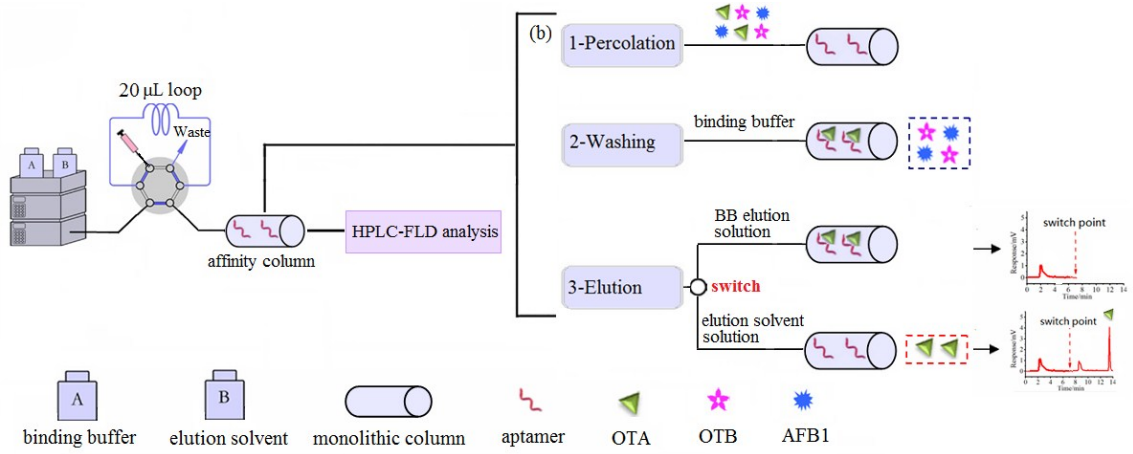

Fig.S1 HPLC system with PMAA affinity monolith for the on-line recognition of OTA.

In the step 1, the binding buffer (Tris-HCl 10 mM, NaCl 120 mM, $CaCl_2$ 20 mM, KCl 5 mM, pH 8.5) was balanced through the column and 20 μL of OTA solution solubilized with binding buffer was percolated through monolithic column. The percolation solution was detected in HPLC system.

In the step 2, a washing step was finished to remove the residual and nonspecific adsorption to ensure no obvious OTA signal was eluted by the binding buffer solution. The washing fraction was detected in HPLC system.

In the step 3, the bound OTA in affinity monolith was eluted with the binding buffer and elution solvent (30% ACN/70% Tris-base 10 mM, EDTA 2.5 mM, pH 8.0) respectively, then the obtained effluents were injected in HPLC and shown before and after the switch point.

1.3 Calculation of binding capacity

Binding capacity of OTA on aptamer-based affinity monolith was measured by dynamic frontal analysis. The calculation equation was shown as below:

$Q_{max}=C\ (V_R-V_0)$

Where, Q_{max} is the maximum binding capacity (ng), C is the analyte concentration (ng/mL), V_R is the retention volume (μL), V_0 is the void volume (μL).

The breakthrough curve was constructed by plotting the peak area of OTA versus the volume of effluent solution. V_R could be determined from the diagram of breakthrough curve, and corresponded to 0.5 of the value of maximum analyte concentration in the effluent, which was according to the previous references [*Talanta*, 80 (2009) 614-621, *J. Chromatogr. A*, 693 (1995) 217-225].

A long blank capillary column was used to connect with the affinity monolith (10 cm-length), and V_0 of the affinity monolith was calculated as V_{total}-$V_{capillary}$ (shown as below), where $V_{total} = \mu \times t_{total}$, where μ is the flow rate (μL/min) of mobile phase in the capillary, t is the breakthrough time of mobile phase (min). Toluene was employed as unretained compound to calculate the void volume.

2. Supplementary data:

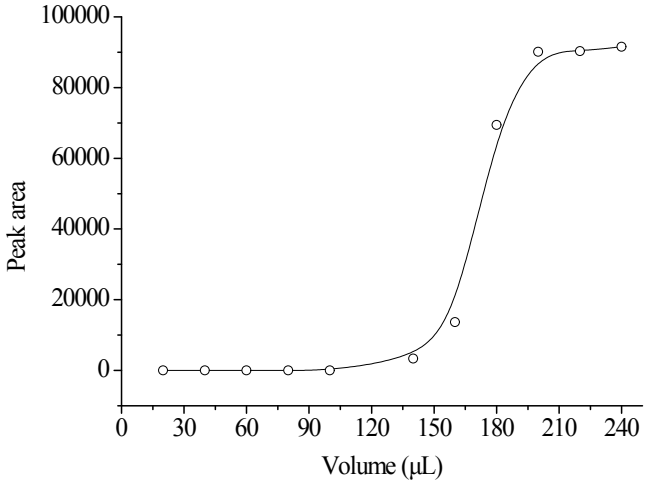

Fig.S2 Breakthrough curve of OTA for the PMAA monolith

PMAA monolithic column: 10 cm-length, 100 μm i.d. × 360 μm o.d.; The concentration of OTA was 25 ng/mL.

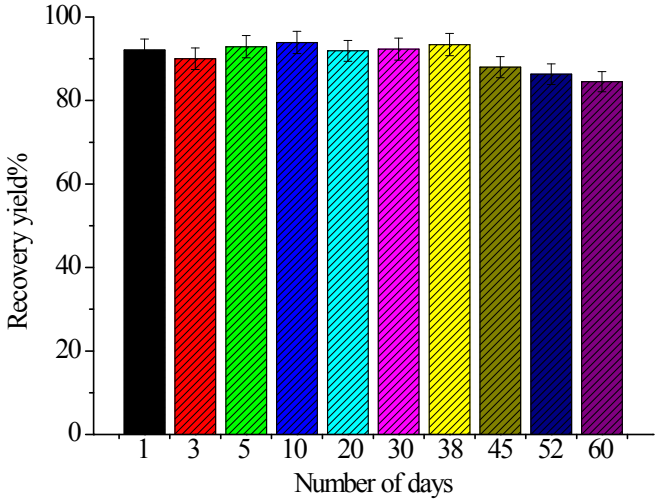

Fig.S3 The lifetime of the PMAA monolithic column

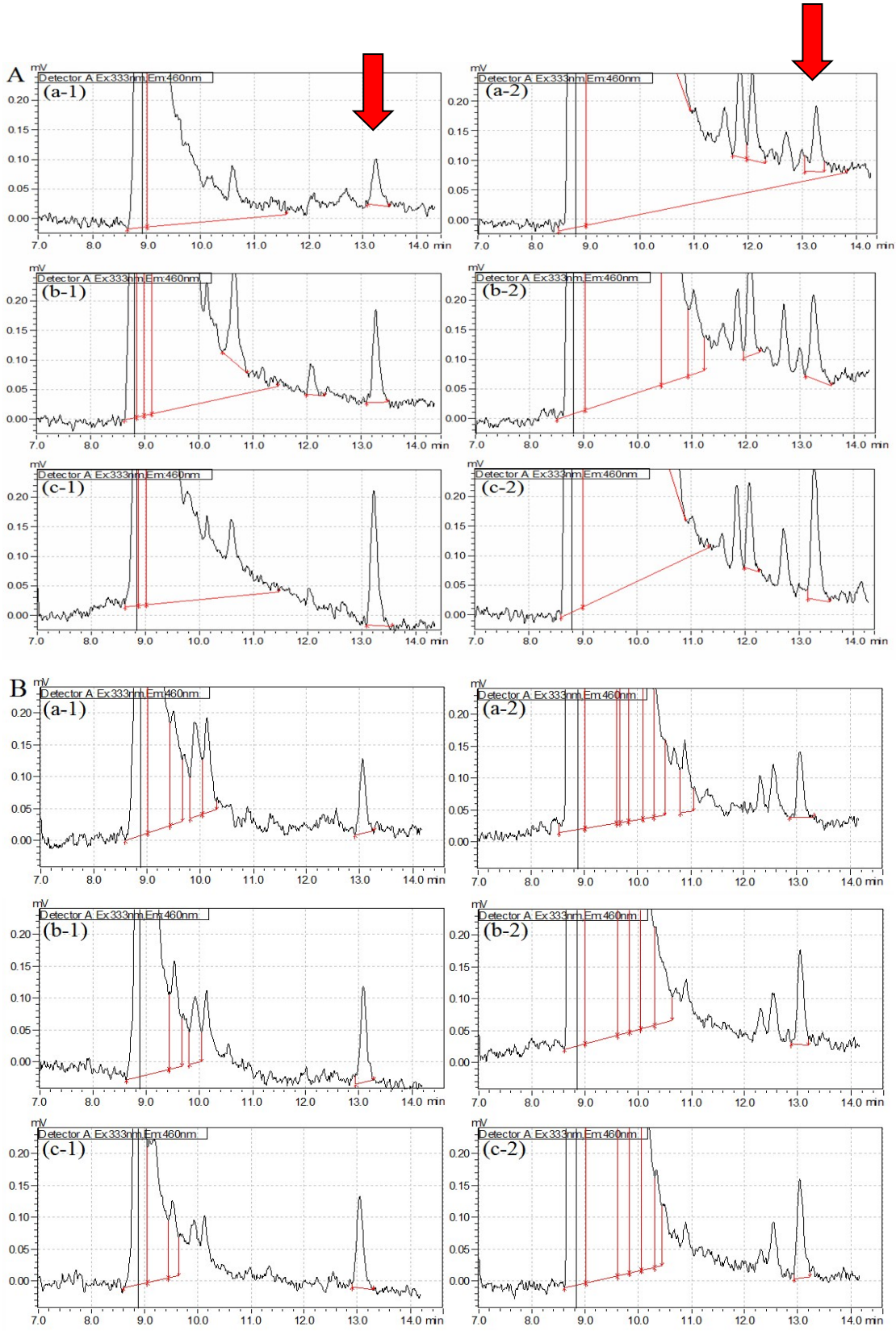

Fig.S4 Chromatograms of elution fractions of OTA in red wine (A) and wheat (B) samples on PMAA monolithic column and PEAA monolithic column.

(A) the concentrations of spiked OTA in red wine samples were (a) 0.25 ng/mL; (b) 0.5 ng/mL; (c) 0.8 ng/mL; (B) the concentrations of spiked OTA in wheat samples were (a) 2.5 μg/kg; (b) 4.5 μg/kg; (c) 5.0 μg/kg. a-1 ~ c-1 were corresponded to PMAA monolithic column; a-2 ~ c-2 were corresponded to PEAA monolithic column.

Table S1 Recipes for fabricating POSS-containing aptamer affinity hybrid monoliths

No.	Monomer-to-solvent ratio	POSS-MA (wt%) [a]	MBA (wt%) [b]	AMPS (wt%) [c]	DMF (wt%) [d]	PEG (wt%)[e]	Water (containing aptamer) (wt%) [f]	Permeability [g] K ($\times 10^{-14}$ m^2)
1	18:82	5.0%	70.0%	25.0%	75.0%	20.0%	5.0%	8.53
2	18:82	5.0%	70.0%	25.0%	74.0%	20.0%	6.0%	5.55
3	18:82	5.0%	70.0%	25.0%	72.5%	20.0%	7.5%	3.95
4	18:82	5.0%	70.0%	25.0%	70.0%	20.0%	10.0%	1.23
5	18:82	2.5%	72.5%	25.0%	72.0%	20.0%	8.0%	3.78
6	18:82	7.5%	67.5%	25.0%	75.0%	20.0%	5.0%	3.73

a/b/c. Percentage of POSS-MA/ MBA /AMPS in the monomer mixture.

d/e/f. Percentage of DMF/PEG/water in porogenic solvents.

f. A constant content of aptamer (7.749 nmol) was used.

g. The permeability was measured by using methanol. The viscosity of methanol was 0.544.

Table S2 Mechanical stability of the PMAA monolith column

mobile phase	linear equation	correlation coefficient (R^2)
methanol	Y = 1910X - 1.22	0.9976
elution solvent	Y = 3000X - 0.78	0.9975
water	Y = 2930X + 0.34	0.9995
binding buffer	Y = 3020X + 0.48	0.9995
acetonitrile	Y = 1160X - 0.7	0.9973