Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Facile aptamer immobilization strategy to fabricate robust affinity monolith for highly specific in-tube solid phase microextraction

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1. Experiment

1.1 Permeability

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

$$K=\eta L^2/\Delta P t_2 \qquad \text{(Equation 1)}$$

Where, K is the permeability (m^2), 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 t_2 is the breakthrough time of mobile phase (min).

1.2 Binding capacity

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

$$Q_{max}=C\ (V_R-V_0) \qquad \text{(Equation 2)}$$

Where, Qmax 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).

In this work, a 25 ng/mL of OTA dissolved in binding buffer solution was used to saturate the aptamer binding sites. Each 20-μL of effluent liquid was collected and measured. 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. V_0 of the affinity monolith was calculated according to the previous references [*Talanta*, 80 (2009) 614-621, *J. Chromatogr. A*, 693 (1995) 217-225].

1.3 Aptamer coverage density

Aptamer coverage density was evaluated according to the method reported previously [*Talanta, 154*(2016) 555. *Analyst, 141*(16) (2016) 4961]. By using a UV-Vis spectrometer at 255 nm, the aptamer coverage density was calculating by dividing the amount of aptamer by the volume of obtained aptamer-modified monolith.The aptamer coverage density was calculated as below:

$$\rho = \frac{n_{injected} - n_{washed}}{V_{monolith}} = \frac{C_{injected} \times V_{injected} - C_{washed} \times V_{washed}}{V_{monolith}} \qquad \text{(Equation 3)}$$

Where ρ was coverage density of aptamers on the resultant monolith, $n_{injected}$ and $n_{eluated}$ were the amount of aptamer injected into Apt@EDV monolith and eluted from the monolith, respectively. $C_{eluated}$ was the concentration of aptamer solution injected into Apt@EDV monolith, $V_{injected}$ was the volume of aptamer solution injected into Apt@EDV monolith. $C_{eluated}$ was the concentration of unbound aptamer in the total eluate solution, $V_{eluated}$ was the total volume of unbound aptamer which consists of the eluate solution and the washing solution. $V_{monolith}$ was the volume of obtained aptamer-modified monolith.

1.4 Online affinity recognition of OTA by HPLC-FLD

The schematic diagram for on-line recognition by aptamer-affinity monolith and subsequent HPLC separation of target OTA was summarized in Fig. S1 with four steps.

1. Loading step, a 20 μL of OTA standard solution was loaded in the sample loop.

2. Percolation step, by using the binding buffer (BB solution) as mobile phase, the sample was injected and percolated through aptamer-based affinity monolith column at 0.10 mL/min and 250 psi back pressure. The pump system was coupled with a split-flow apparatus.

3. Washing step, a 20 μL BB solution was used to remove the residual free OTA and background substances with 0.1 mL/min flow rate and 250 psi back pressure. The resultant washing solution was collected in sample loop 2 and could be injected in HPLC if needed.

4. Elution step, the affinity monolith was further eluted with another 20 μL BB solution to ensure all the nonspecific adsorption was removed. The obtained eluent was collected in sample loop 2 and injected in HPLC for 15 s for chromatographic analysis. The flow rate for the aptamer device was 0.10 mL/min with the pressure drop of 250 psi. The flow rate of mobile phase in HPLC was 1.0 mL/min.

HPLC-FLD system(Shimadzu LC-20A, Shimadzu, Japan) include a Alltima^{TM}C$_{18}$ column (250 mm × 4.6 mm, 5 μm) and a fluorescence detector (RF-20A, Shimadzu, Japan). Samples were separated using a mobile phase composed of aqueous phase containing water (2% acetic acid) and acetonitrile (38:62,v/v) at a flow rate of 1.0 mL/min. The excitation and emission wavelengths of the fluorescence detector were set at 333 nm and 460 nm, respectively.

1.5 Sample pretreatment and analysis for SPE-LC-MS

1.5.1 LC-MS method

The samples were analyzed with a LC-MS system(Shimadzu, LC-MS 8040). The LC-MS

analysis conditions were based on the ref.(Analytica Chimica Acta 1165 (2021) 338517). OTA was analyzed on a reversed-phase HPLC system (Shimadzu LC-20A, Shimadzu, Japan) using a Shim-pack GIST C-18 column (150 × 2.1 mmi.d. and 5 μm particle size) and a Mass spectrometry (Shimadzu, LCMS-8040).

Samples were separated using a mobile phase composed of aqueous phase containing 5 mmol/L ammonium formate and 0.1% formic acid (A) andacetonitrile-water (containing 5 mmol/L ammonium formate and 0.1% formic acid) = 95:5 (v:v) (B) with a gradient program at a flow rate of 0.2 mL/min. Initial elution was performed by mobile phase A:B = 65:35 (v/v). After 2 min, the linear gradient reached 95% mobile phase B. After 7 min, the gradient composition returned to the initial elution and maintained for 3 min.

Mass spectrometry was operated in electrospray ionization (ESI) in negative mode with multiple reaction monitoring (MRM) and the optimized mass spectrometry parameters were shown in Table S1. The injection volume was 20 μL.Heat block temperature and DL temperature were 400 °C and 250 °C, respectively. Nebulizing gas flow and drving gas flow were 3 L/min and 15 L/min, respectively. Two transitions were followed.The precursor-to-product ion transition m/z 402.1→358.1 was selected for the quantitative calculation of OTAand the m/z 402.1→166.9 was used as confirmation transition.

Table S1 Mass parameters for the quantitative calculation of OTA

Compound	Retention time (min)	$[M-H]^-$ (m/z)	Product ion (m/z)	Collision energy (V)
OTA	4.3	402.1	358.1*	25
			166.9	47

*: Product ion of m/z 358.1 was used for the quantification.

1.5.2 Sample pretreatment with SPE for LC-MS

The C_{18} solid-phase extraction column (500 mg/3 cc, Waters Sep-pak) was treated with methanol (5 mL) and then equilibrated with Milli-Q water (5 mL). An aliquot of 10 mL of beer spiked with OTA at some concentrations ranging from 0.04 ng mL^{-1} to 0.2 ng mL^{-1} was loaded onto the C18 cartridge and washed sequentially with Milli-Q water (2 mL), then the elute solution was discarded. After air-drying the column, the elution of OTA was carried out with methanol (2 mL), and then the elute solution was filtered through 0.22 μm filter membrane before injection into LC-MS. The red-wine sample was treated similar to beer samples and spiked with OTA from 0.2 ng mL^{-1} to 1.0 ng

mL^{-1}.

2 Supplementary data

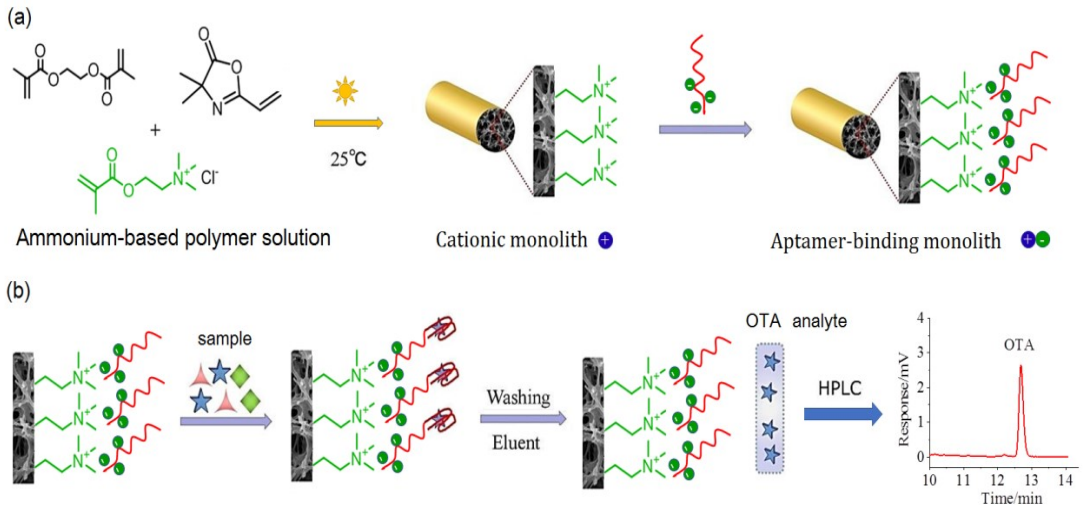

Fig. S1 Scheme of preparation of aptamer affinity monolith and affinity recognition process

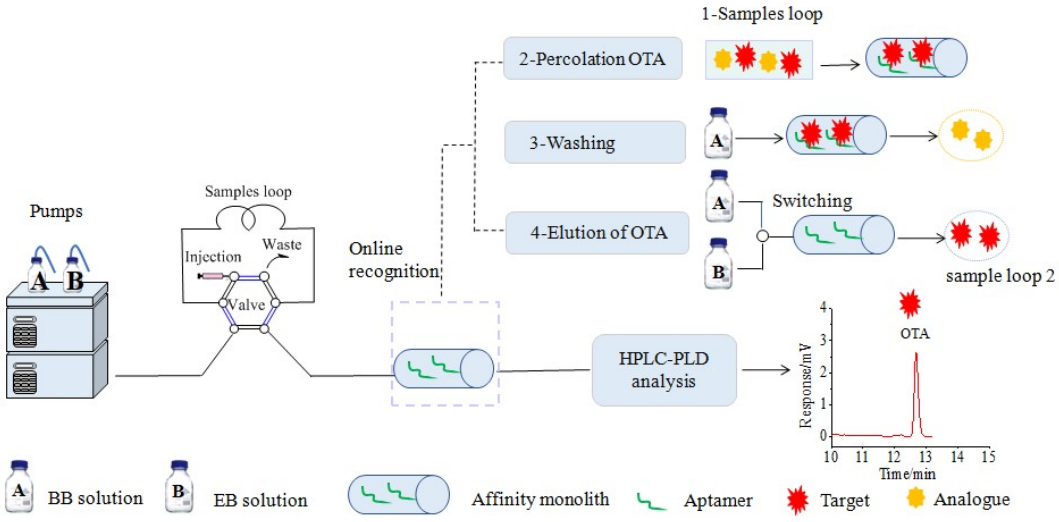

Fig. S2 Apparatus and process of selective recognition of target analyte by using the affinity monolith online coupled with HPLC system.

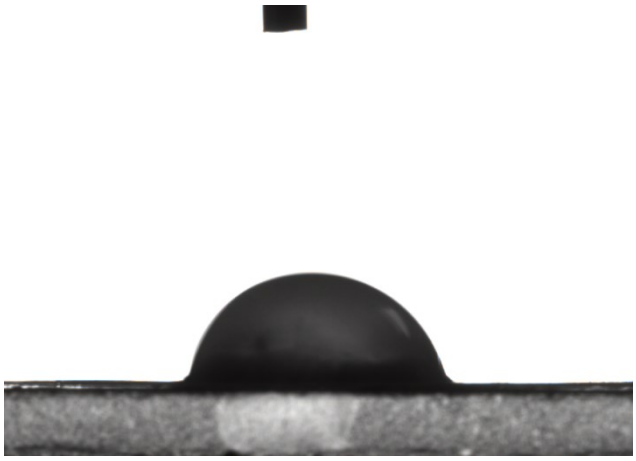

Fig. S3 The measurement of contact angle with EDV monolith

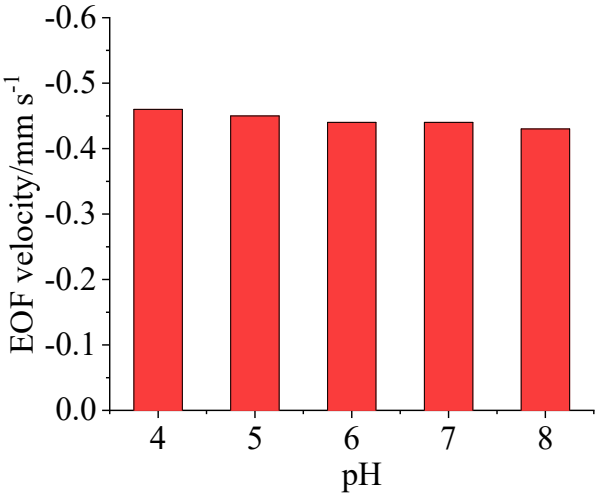

Fig. S4 EOF values of EDV monolith in different pH

Experimental conditions: mobile phase, 5mM PBS buffer containing 80% ACN at different pH; pump flow: 0.1 mL/min; applied voltage: +10 KV; applied pressure: 500 psi; detection wavelength: 214 nm.

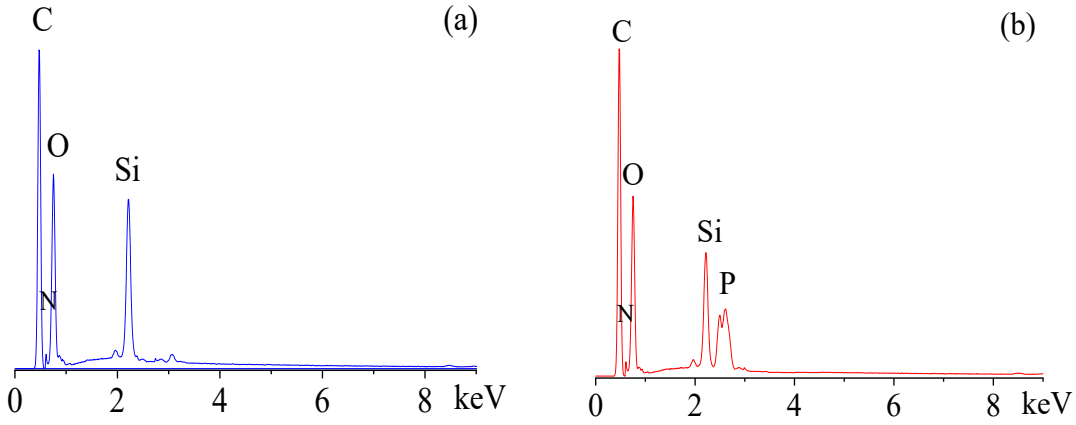

Fig. S5 EDS of monolith before and after aptamer immobilization

(a) EDV matrix monolith (b) Apt@EDV monolith

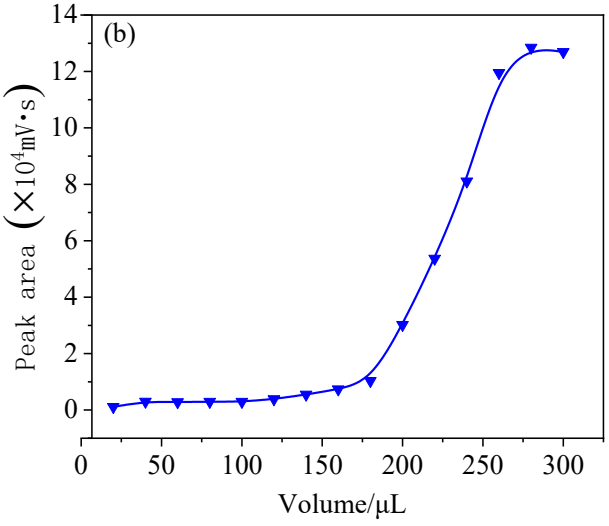

Fig. S6 Penetration curve of OTA in affinity monolithic column.

Experimental conditions: Affinity monolith: 5cm-long, 75 mm i.d, 360 mm o.d., flow rate, 0.05 mL min^{-1}, OTA: 25 ng mL^{-1}, and toluene was used to estimate the void time