Supporting information

High-sensitivity fluorescent immunochromatographic test strips for point-of-care testing of tenuazonic acid: hapten design, monoclonal antibody preparation, and assay development

Contents

1. Reagents

Succinic anhydride, 4-dimethylaminopyridine (DMAP), anhydrous pyridine, and carboxymethoxylamine hemihydrochloride (CMO) used in the synthesis of haptens were obtained from J&K Scientific Ltd (Shanghai, China). The carrier proteins for antigen synthesis, including N, N-dimethylformamide (DMF), bovine serum albumin (BSA), keyhole-limpet haemocyanin (KLH), 2-Morpholinoethanesulphonic acid (MES), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), and N-hydroxysuccinimide (NHS), were acquired from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Reagents used for cellular experiments and Pierce Rapid ELISA Mouse mAb Isotyping Kit were supplied by Thermo Fisher Scientific Inc (Shanghai, China).

The polyvinyl chloride (PVC) base plate, nitrocellulose (NC) membrane, sample pads, conjugate pads, and absorbent pads used for assembling the FITS were sourced from Goldbio Tech Co. Ltd (Shanghai, China).

2. Equipment

The 5500 QTRAP UHPLC-Triple Quadruple Quadrupole Combined Linear Ion Trap Mass Spectrometer for hapten identification was obtained from Shanghai AB SCIEX Analytical Instruments Trading Co. Ltd (Shanghai, China). Shanghai Heal Force Biomedical Technology Holding Co. Ltd (Shanghai, China) and Beijing Dragon Laboratory Instruments Co. Ltd (Beijing, China) supplied the Eco-stir magnetic stirrer and ND100-1 nitrogen blower used for hapten synthesis, respectively. An Ultraviolet-Visible (UV-Vis) spectroscopy was provided by Agilent (Santa Clara, CA, USA) for

antigen characterization. Wuxi Determine Bio-Tech Co., Ltd. (Jiangsu, China) supplied a Fluorescent Strip Quantitative Reader for quantitative assays.

3. Antigens synthesis

First, one milligram of TEA-CMO was dissolved in 200 μL of DMF, then 1.28 mg NHS was added to activate the reaction for 15–20 min, followed by the addition of 2.12 mg of EDC, and the mixture was stirred using a magnetic stirrer at room temperature for 4 h. After the reaction was complete, the reaction solution was added drop-wise into a solution of the carrier proteins (KLH: 7.41 mg; BSA: 6.20 mg) in carbonate buffer solution (CBS) to react for 12 h, and dialyzed for three days against 0.01 M phosphate-buffered saline (PBS, pH 7.4). The dialysate was changed every 8 hours, and the coupled haptens (TEA-CMO-KLH and TEA-CMO-BSA) were stored at -20°C at the end of dialysis. The hapten TEA-HS was synthesized into the antigens TEA-HS-KLH and TEA-HS-BSA in the same way as above, except that KLH and BSA were changed to 6.73 mg and 5.64 mg.

4. Immunization details

The first immunization dose was 80 μg per mouse, followed by a first booster dose of 60 μg and second to fourth booster doses of 40 μg. After five immunizations, mice with the highest potency and sensitivity were selected for fusion experiments. Ineffective mice were executed by cervical dislocation following inhalation anesthesia with isoflurane (0.15 mL/20 g) to minimize discomfort.

5. Determination of Ka

Affinity constant (Ka) was determined according to the classical non-competitive

ELISA method. Absorbance was measured at 450 nm using different coating antigen concentrations (0.03, 0.1, and 0.3 μg/mL) from a three-fold gradient dilution of anti-TEA mAb at 0.3 μg/mL. We calculated the affinity constant (Kaff) after performing three parallel experiments. The specific formula for calculating Ka was as follows: $k_a = (n - 1)/2(n[Ab']t - [Ab]t)$, where n is the ratio of the two coating antigen concentrations, and [Ab']t and [Ab]t correspond to the anti-TEA mAb concentration that corresponds to the half of the maximum OD 450 nm in each set of coating antigen concentrations, respectively.

6. Establishment of ic-ELISA

The procedure for the development of the ic-ELISA was as follows: A microplate was coated with coating antigen (100 μL/well) diluted in coating buffer (0.05 mol/L CBS, pH 9.6) and incubated 2 h at 37°C. After three washes with washing solution (0.05% (v/v) Tween-20 in 0.01 M PBS), blocking buffer (0.05 mol/L CBS containing 0.2% (m/v) gelatin) was added to the wells (200 μL/well) and incubated at 37 °C for 2 h. After another round of washing with PBS (50 μL/well), PBS (TEA negative) and TEA standard (TEA positive) 50 μl were added to the wells separately. Than the anti-TEA mAb (50 μL/well) were allowed to bind to the coated wells and incubated at 37 °C for 30 min and the unbound antibody was removed by washing a further three times. Then, HRP-labeled goat anti-mouse IgG (100 μL/well) was pipetted into each well and incubated at 37 °C for 30 min. The final washing step was then followed by the addition of TMB (100 μL/well). The plate was incubated at 37 °C for 15 min and then stopped by adding 2 M H2SO4 (50 μL/well) and the OD values were measured at 450 nm.

Experiments were performed in triplicate for each concentration of TEA standard. The absorbance values obtained at 450 nm in the TEA positive and TEA negative (maximum signal) were defined as B and B0, respectively. The inhibition ratio was then calculated as follows Inhibition rate (%) = (1− B/B0)×100%.

Figures

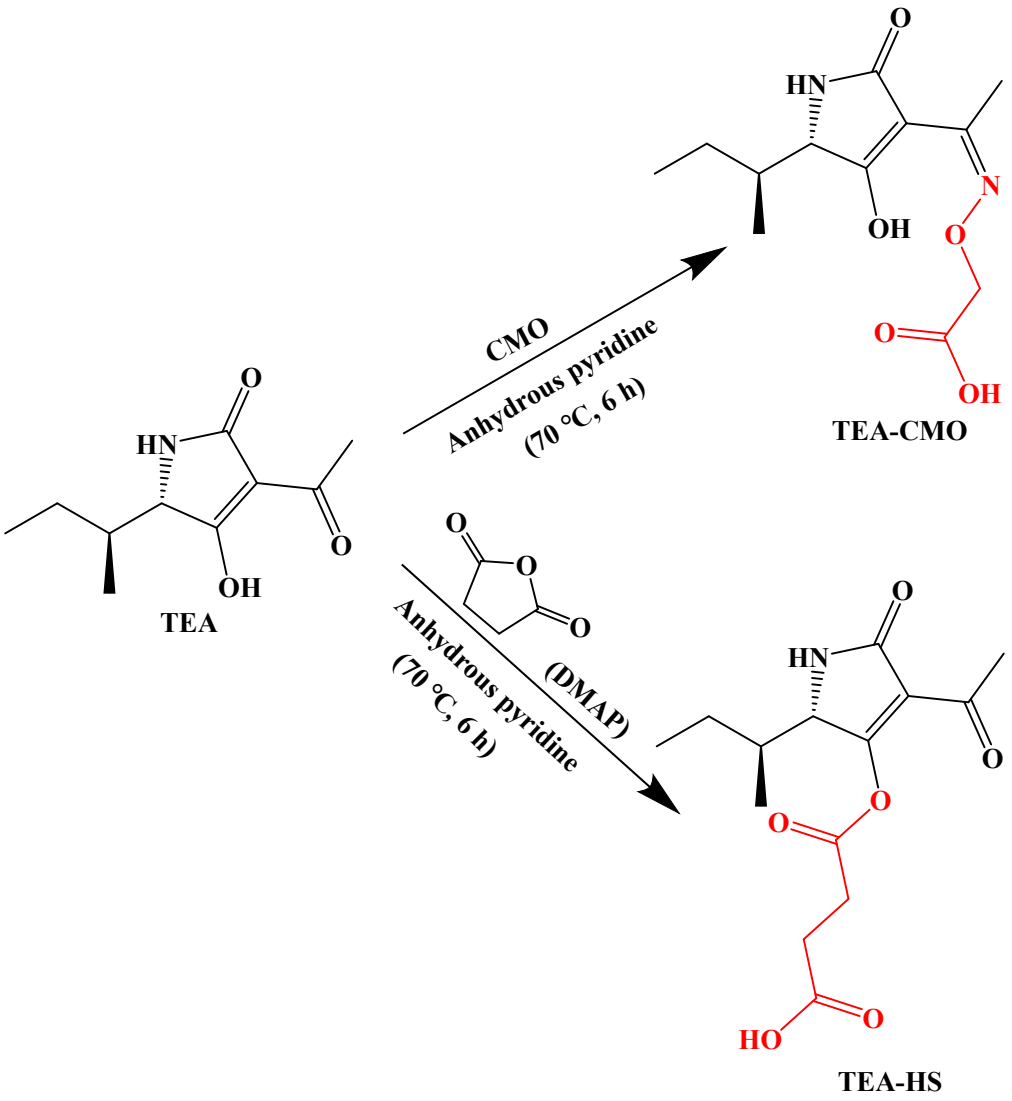

Fig. S1 Synthetic routes of TEA-CMO and TEA-HS.

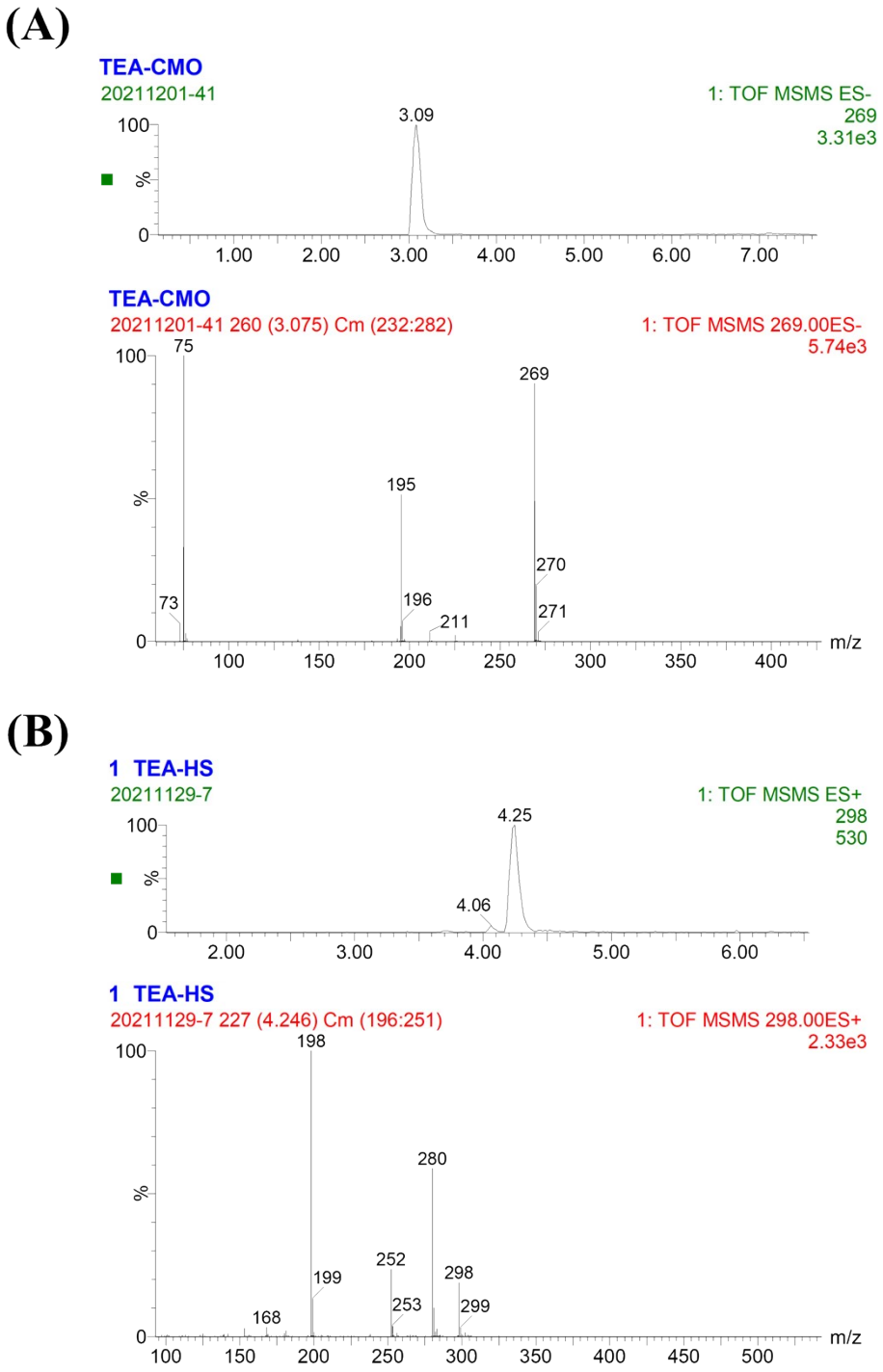

Fig. S2 LC-MS/MS characterization of TEA-CMO(A) and TEA-HS (B).

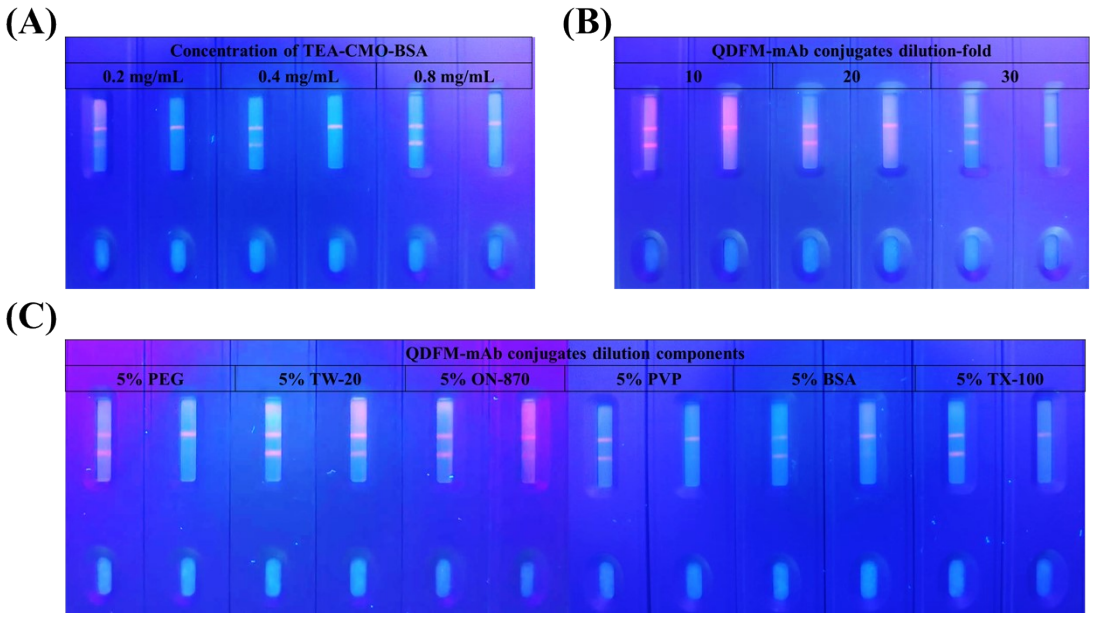

Fig. S3 (A) Optimization of the concentration of coating antigen (TEA-CMO-BSA). (B) Optimization of the QDFM-mAb conjugates dilution-fold. (C) Optimization of QDFM-mAb conjugates dilution components

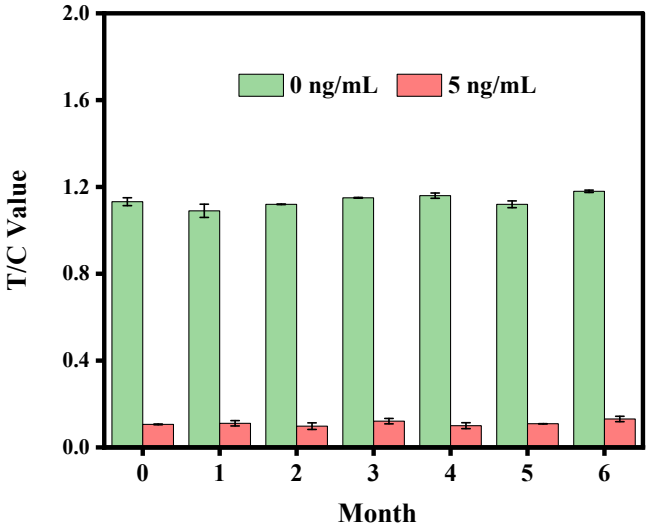

Fig. S4 Stability of FITS.

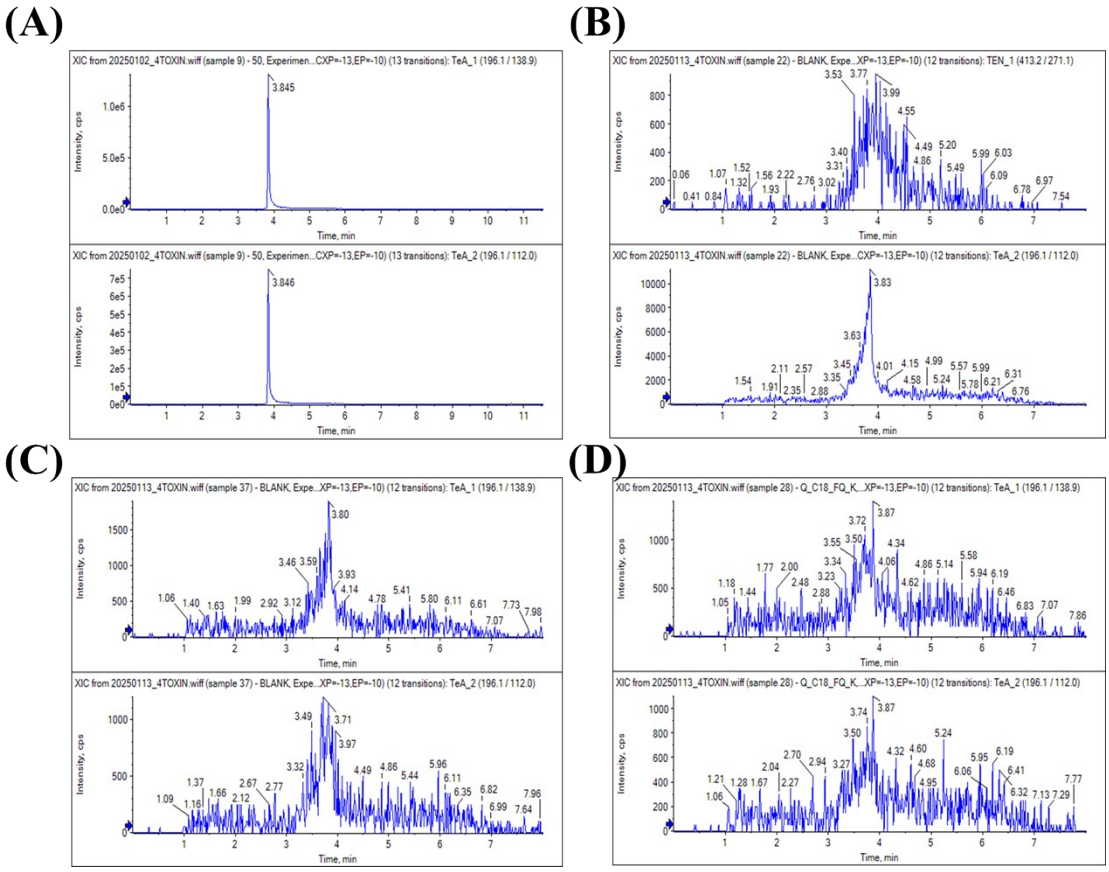

Fig. S5 The LC-MS/MS analysis of TEA standard solution (A), wheat flour sample (B), tomato sauce sample (C), and apple juice sample (D).

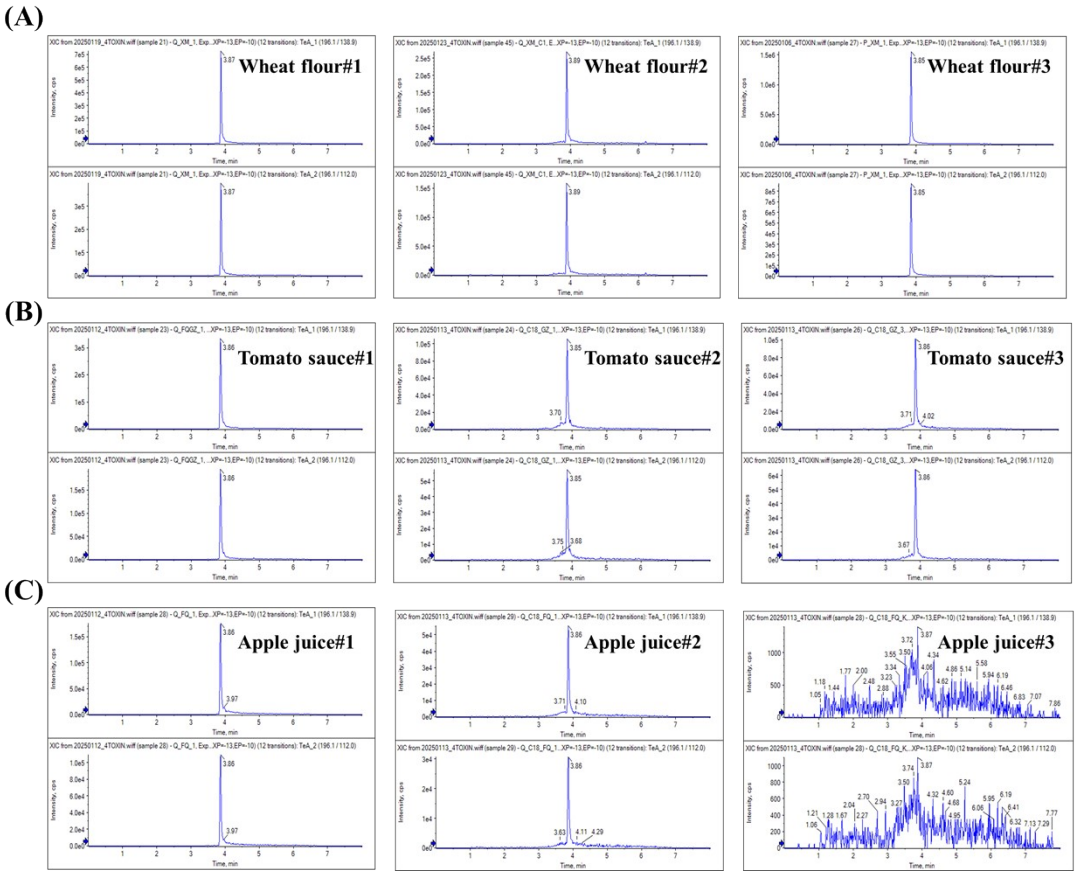

Fig. S6 Results of LC-MS/MS detection of TEA levels in real samples. LC-MS/MS analysis of samples of wheat flour (B), tomato sauce (C) and apple juice (D).

Table

Table S1. Recoveries and CVs of the FITS method for detecting TEA spiked in wheat flour, tomato sauce and apple juice (n=5).

Samples	Spiked (μg/kg)	HPLC-MS/MS		FITS	
		Recovery rate ± SD (%)	CV (%)	Recovery rate ± SD (%)	CV (%)
Wheat flour	0	NC	NC	NC	NC
	1	96.20±0.75	0.78	96.00±1.41	1.47
	5	98.72±1.85	1.88	103.84±4.45	4.28
	10	101.62±4.26	4.19	104.02±5.95	5.72
Tomato sauce	0	NC	NC	NC	NC
	1.5	97.20±1.72	1.77	101.87±5.81	5.70
	3	104.33±3.16	3.03	98.67±5.02	5.09
	6	102.23±5.50	5.38	103.10±7.42	7.20
Apple juice	0	NC	NC	NC	NC
	0.5	95.20±1.02	3.7	91.60±1.17	1.27
	1	98.40±5.08	5.17	107.00±2.61	2.44
	2	96.70±1.74	1.80	92.70±2.42	2.61