Supporting information

Contents

The purification process of mAb.

These cell lines were injected into the peritoneal cavity of experimental mice to produce ascites. 1 mL of ascites was placed in a centrifuge tube, 1 mL of sodium acetate buffer and 33 μL of n-octanoic acid were added, and the mixture was shaken on a shaker for 30 min. The centrifuge tube was centrifuged at 6000×g for 10 min at 4°C, and the supernatant was taken and an equal volume of saturated ammonium sulfate solution was added, mixed well and placed at 4°C for 4-6 h. The centrifuge tube was centrifuged at 6000×g for 10 min at 4°C, and the supernatant was discarded. The precipitate was dissolved in 0.5 mL of PBS and dialyzed at 4°C for 3 days.

Specific operating parameters in LFIA assembly.

The fluorescent immunochromatographic strip mainly consists of five parts, including a PVC backing plate, an absorbent pad, a nitrocellulose (NC) membrane, a conjugate pad, and a sample pad. Before assembly, the EFM-mAb solution was diluted and sprayed onto a glass fiber pad using a three-dimensional membrane drawing and gold spraying machine, and then dried in a hot air oven at 38℃ for 24 h to form the conjugate pad. The coated antigen and goat anti-mouse IgG antibody were fixed on the middle region of the NC membrane using a three-dimensional membrane drawing and gold spraying machine to form the test line (T line) and the control line (C line), with a spacing of 5 mm between the two lines. Then, it was placed in a hot air oven at 38℃ for more than 24 h to form the detection area of the immunochromatographic strip. Each 210 mm × 297 mm glass fiber pad was soaked in 60 mL of sample dilution solution and then dried in a hot air oven at 38℃ for 24 h to form the sample pad. During assembly,

the NC membrane was first adhered to the middle region of the PVC backing plate. Then, the absorbent pad and the conjugate pad were fixed on both sides of the PVC backing plate and overlapped with the NC membrane, with an overlap width of 1 mm. Finally, the sample pad was adhered and fixed on the side of the PVC backing plate near the conjugate pad, overlapping with the end of the conjugate pad, with an overlap width of 1 mm. The assembled large strip was cut into 3 mm wide strips using a CNC cutting machine, packaged, and stored in a cool and dry place for later use.

Production of GNP-based LFIA.

In this work, GNP with a size of 15 nm were selected. According to previous studies, purified mAb were labeled with GNP[1]. K_2CO_3 solution (0.2 M, 80 μL) was added to the GNP solution (5 mL) at pH 7.2. The mAb was diluted with carbonate buffer to 0.2 mg/mL and added to the solution at concentrations of 2 μg/mL and 6 μg/mL, respectively. After incubation at room temperature for 1 h, 0.5% BSA was added to block free GNP. Finally, the solution was centrifuged at 9000 × g for 45 min, and the precipitate was resuspended in 500 μL of GNP suspension. The GNP-labeled mAb was diluted twofold and sprayed onto the conjugate pad, followed by drying at 37°C for 12 h. The preparation methods for the T line and C line were the same as those for the EFM-based LFIA. When the GNP-based LFIA were assembled and ready for sample detection, 100 μL of sample solution was dropped onto the sample pad, and the results were detected after 8 min. The detection principle was the same as that for the EFM-based LFIA

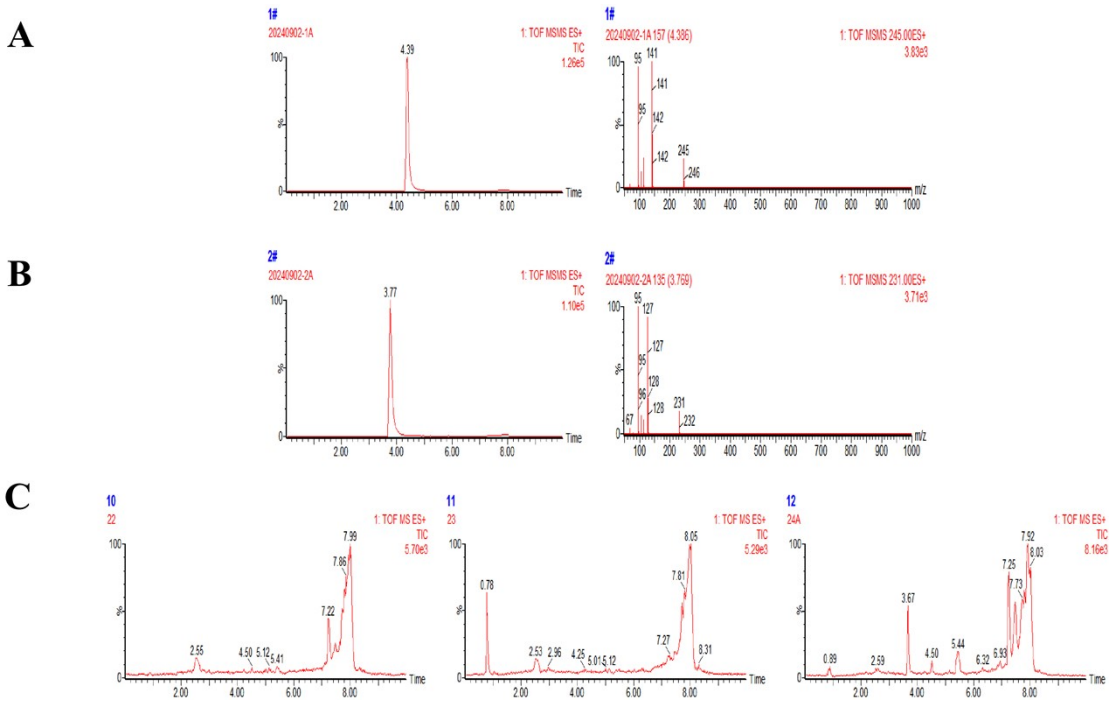

Fig. S1. The LC-MS/MS results of negative samples (C) of ET (A) and MT (B) in water (10), urine (11) and serum (12).

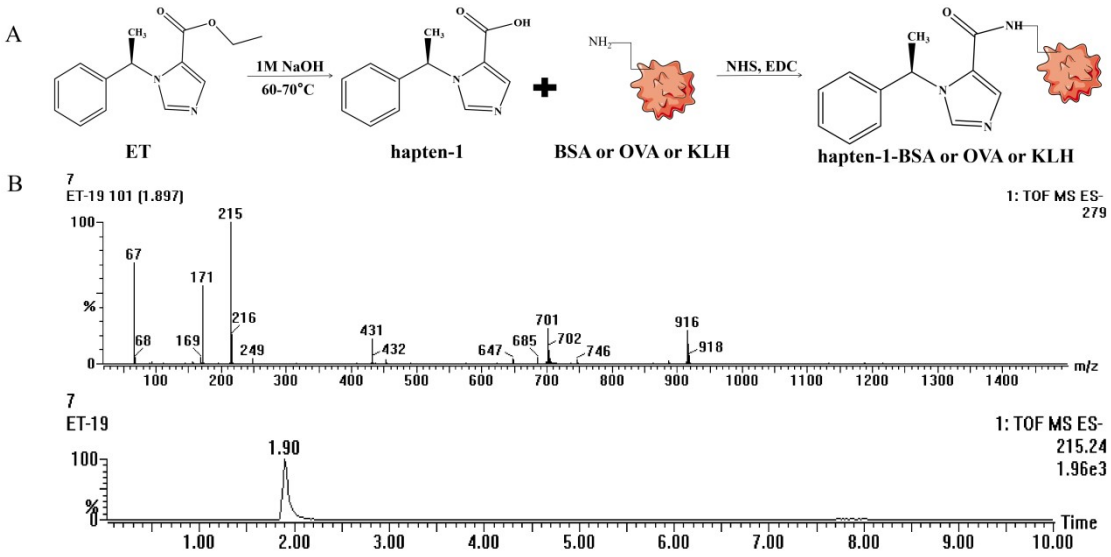

Fig. S2. The synthetic and conjugated carrier protein routes (A), LC-MS/MS (B) of hapten-1.

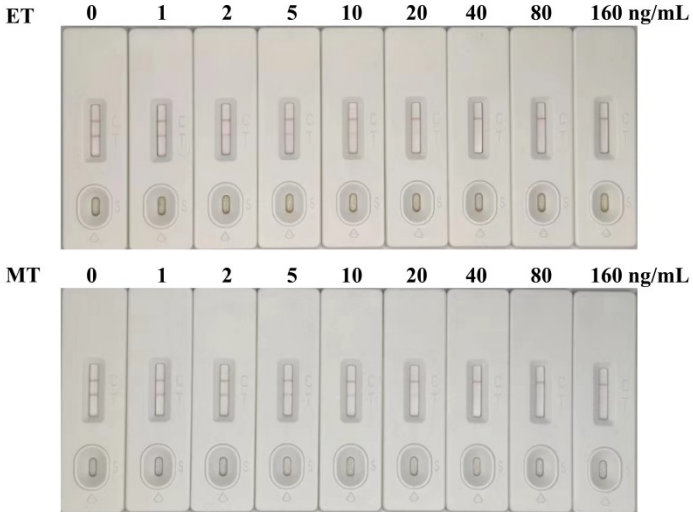

Fig. S3. The test images of GNP-LFIA for the determination of ET and MT spiked in water.

Tab. S1 Comparison of the immunochromatographic assay with other reporting methods.

Method	Samples	Linearity and range	LOD	Reference
Immunochromatographic Assay	Water	ET: 0.08 to 1.31 μg/kg MT: 0.08 to 2.21 μg/kg	ET and MT: 0.3μg/kg	This work
	Urine	ET: 0.12 to 3.13 μg/kg MT: 0.09 to 2.98 μg/kg	ET: 0.3μg/kg MT: 1μg/kg	
	Serum	ET: 0.15 to 21.2 μg/kg MT: 0.08 to 13.8 μg/kg	ET and MT: 3μg/kg	
UPLC-MS/MS	Human hairs	ET: 1-500(μg/kg) MT: 1-500(μg/kg)	ET: 0.5μg/kg MT: 1μg/kg	[2]
LC-MS/MS	Serum	ET: 10-500(μg/kg) MT: 1-50(μg/kg)	ET: 5μg/kg MT: 0.5μg/kg	[3]
LC-MS/MS	Human hairs	ET: 0.25 to 50 μg/kg	ET: 0.05 μg/kg	[4]
LC-MS/MS	Urine	ET: 0.4-120 μg/kg	ET: 0.4μg/kg	[5]

References

1. J. Y. Li, L. M. Jiang, Y. Shu, S. S. Song, L. G. Xu, H. Kuang, C. L. Xu and L. L. Guo, *Journal of Hazardous Materials*, 2024, **469**.
2. Y. Y. Dai, X. L. Zhang, K. D. Zhao, Y. Wang, J. L. Liu, J. Gu, H. R. Bai, K. Hasegawa and A. Wurita, *Forensic Toxicology*, 2024, **42**, 232-241.
3. H. Yum, S. Jeong, M. Jang, S. Moon, M. Kang, B. Kim, D. Kim, S. Choe, W. Yang, J. Kim and S. B. Han, *Journal of Forensic Sciences*, 2021, **66**, 2532-2538.
4. Y. J. Park, E. Cho, S. H. Kim, H. Lee, H. Jegal, M. Park, S. Choe, Y. E. Sim, S. H. Baek, K. M. Kim and J. Pyo, *Journal of Forensic Sciences*, 2022, **67**, 2479-2486.
5. Y. K. Jung, S. Y. You, S. Y. Kim, J. Y. Kim and K. J. Paeng, *Molecules*, 2019, **24**.