SpyTagged Mimotope Peptide Mediated Competitive Antigen-Based Rapid Quantitative Immunoassays for Uniconazole Residue

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Figure captions:

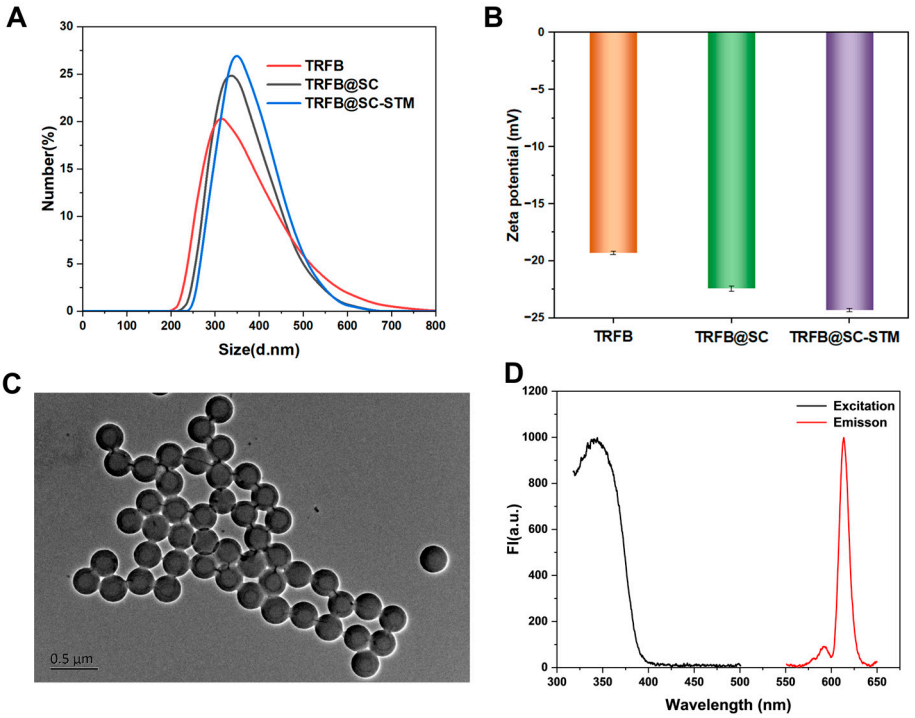

Figure S1. Characterization of TRFB@SC-STM. (**A**) D_H of TRFB, TRFB@SC, and TRFB@SC-STM. (**B**) Zeta potentials of TRFB-free, TRFB-SpyTag, and TRFB-mimotope. (**C**) TEM images of TRFBs. (**D**) Excitation and emission spectra of TRFB@SC-STM.

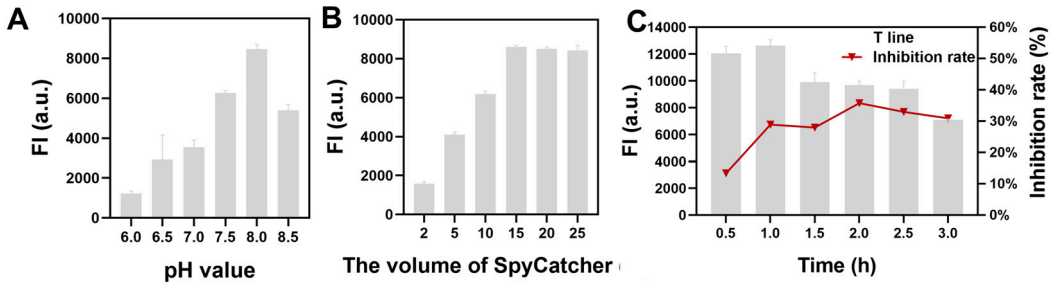

Figure S2. Parameter optimization of TRFB probes. (**A**) pH value. (**B**) The volume of SpyCatcher. (**C**) The incubation time of UCZ SpyTagged mimotope and TRFB@SC.

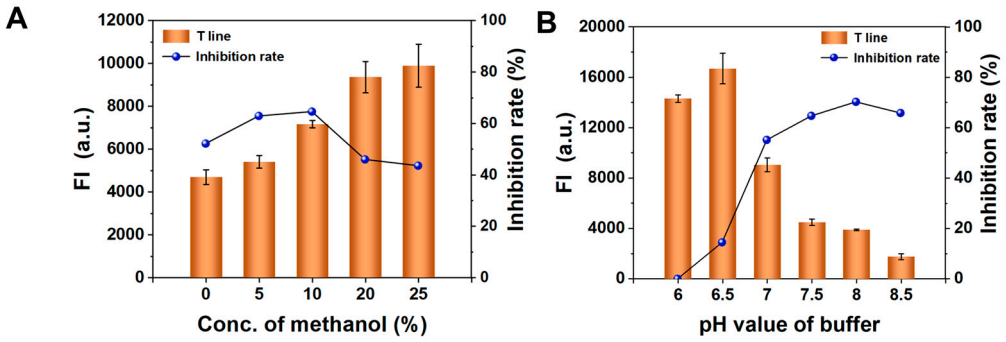

Figure S3. Optimization of LFIA buffer. (**A**) The concentration of methanol. (**B**) pH value.

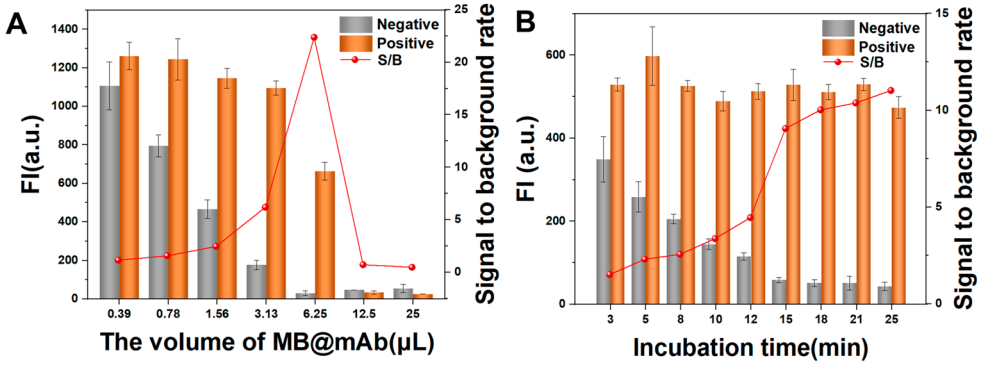

Figure S4. Optimization of MSIA. (**A**) The volume of MB@mAb. (**B**) Incubation.

Table S1. The biopanning strategy for phage displayed mimotope peptide.

Round	UCZ-mAb (μg/mL)	Input (pfu)	Blocking Reagent	PBST	Time (min)
1	100	$2x10^{11}$	3% BSA	0.1%	15
2	50	$2x10^{11}$	3% OVA	0.25%	12
3	30	$2x10^{11}$	3% BSA	0.5%	8

Table S2. Orthogonal test for parameter optimization of LFIA.

NO.	UCZ-mAb (mg/mL)	SpyTag mimotope (μg)	TRFB@SC-STM (μL)	FI_T (negative)	FI_T (positive)	Inhibition rate (%)[a]
1	0.5	10	3	2983	1509	52.67
2	0.5	15	5	5575	2299	64.77
3	0.5	20	7	8326	4608	52.11

4	0.8	10	5	4737	3092	46.11
5	0.8	15	7	8349	4428	55.89
6	0.8	20	3	5787	3475	50.62
7	1.2	10	7	9347	5697	48.44
8	1.2	15	3	5241	3488	48.77
9	1.2	20	5	8395	3229	55.40

Note: The concentration of UCZ in positive sample is 10 ng/mL.

Table S3. The precision and accuracy of LFIA.

Spiked UCZ (μg/kg)	Cabbage			Brown rice			Wheat		
	Mean (μg/kg)	Rcovery rate(%)	CV (%)	Mean (μg/kg)	Rcovery rate(%)	CV(%)	Mean (μg/kg)	Rcovery rate(%)	CV(%)
5	4.83	96.53	10.21	4.62	92.4	9.76	4.56	91.13	10.21
10	9.61	96.06	7.64	9.69	96.93	8.43	10.43	104.3	12.34
20	20.56	102.82	6.73	20.94	104.71	7.32	21.56	107.81	8.48

Table S4. The precision and accuracy of MSIA.

Sample	Spiked (μg/kg)	MSIA			LC-MS/MS		
		Detection value	Recovery (%)	CV (%)	Detection value	Recovery (%)	CV (%)
		Mean (μg/kg)			Mean (μg/kg)		
Cabbage	20	18.84	94.2	3.46	18.23	91.2	3.32
	50	48.32	96.6	4.67	49.58	99.2	5.31
	100	105.30	105.3	8.61	104.51	104.5	3.46
Brown rice	20	23.14	115.7	7.41	18.32	91.6	4.14
	50	52.11	104.2	6.34	48.83	97.7	3.94
	100	103	103.4	7.45	106.17	106.2	5.21
Wheat	20	19.41	96.1	8.31	21.34	106.7	3.74
	50	46.32	92.6	9.22	47.35	94.7	3.86
	100	94.35	94.4	7.64	93.62	93.6	4.24

Table S5. The amino acid sequence of UCZ SpyTagged mimotope, UCZ-mAb, and **SpyCatcher**.

Amino acid sequence of STMP. TrxA (cyan), SpyTag (orange), UCZ mimotope (green).

UCZ SpyTagged mimotope	MSDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPG TAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLAGSGSGHMHHHHHHSSGLVPRGS GMKETAAAKFERQHMDSPDLGTDDDDKAMADIGSAHIVMVDAYKPTKGGGSGGGSGGGSIPFKE GLGFISGGGGSGGGSGGGSLEHHHHHH*
UCZ mAb	V QVQLQQPGAELVRPGASVKLSCKASGYTFTVYYIYWVKQRPGQGLEWIGGIHPRNGGSYYNA H KFRNRATLTVDKSSNTAYMQFSSLTSEDSAVYYCTRGIYYDGKFGERAMDYWGQGTSVTVSS V DIVMTQTPNTLSVTIGQPASISCKSSQSLLYSDGKTYLHWLLQSPGQSPKLLIYLVSKLESGVPDRF L SGSGSGTDFTLKISRVEAEDLGVYYCVQAAHLPHTFGSGTKLEIK
SpyCatcher	MVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDF YLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGDAHIGGGGSGGGGSLEHHHHH H*

Table S6. Comparison of UCZ detection methods based on immunoassay.

Platform	Antigen	Antibody	Signal Materials	LOD in Buffer (ng/mL)	LOD in Matrix (μg/kg)	Detection Time (min)	Detection Sample	Ref.
ELISA	UCZ-OVA	pAb	HRP	1.82	-	-	Water，soil	[1]
ELISA	UCZ-HRP	pAb	HRP	0.022	-	75	Apple	[2]
Electrochemical Immunosensor	-	mAb	-	8	-	90	Cabbage	[3]
ICA	Mimotope peptide	mAb	TRF	50(vLod)	50(vLod)	15	Cabbage, wheat, brown rice	This work
MSIA	Mimotope peptide	mAb	TRF	2.09	2.82-3.73	20	Cabbage, wheat, brown rice	This work

References

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