

Supporting information for

**Dual-wavelength fluorescence polarization immunoassay for
simultaneous detection of sulfonamides and antibacterial
synergists in milk**

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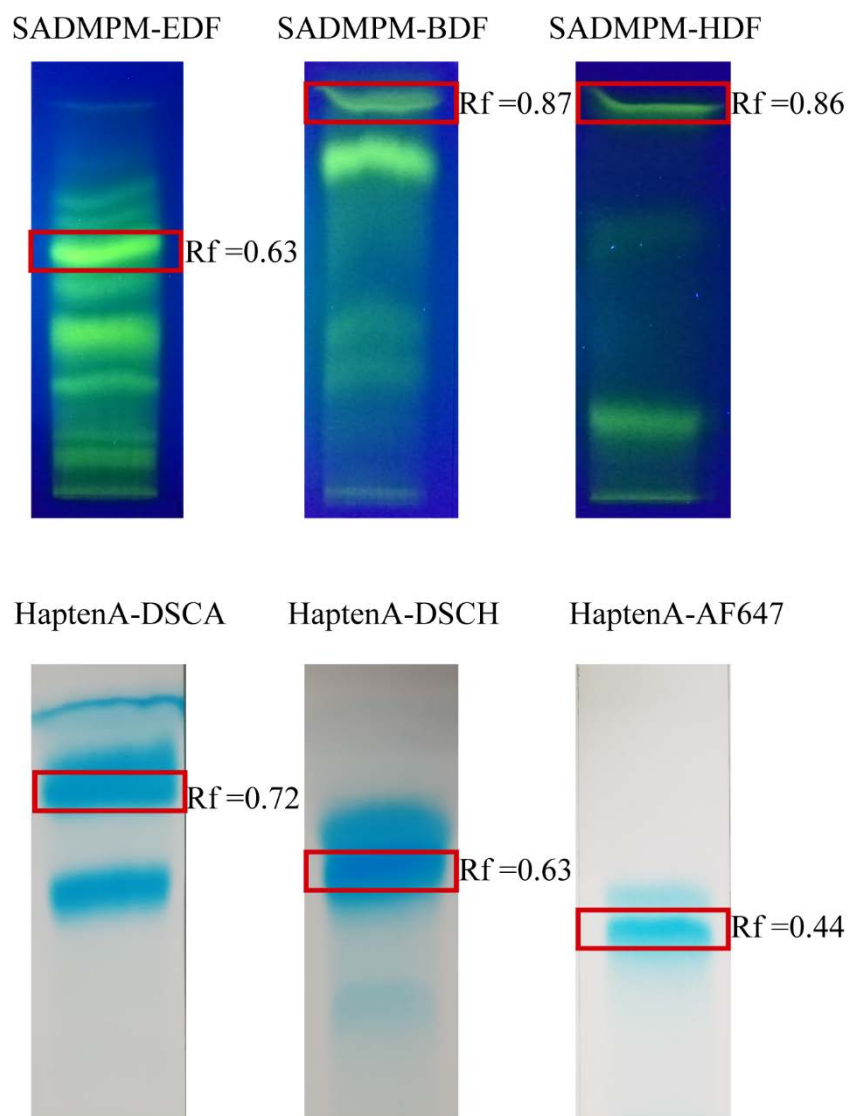


Figure S1. The TLC purification of the tracers. The target band is enclosed in a red box.

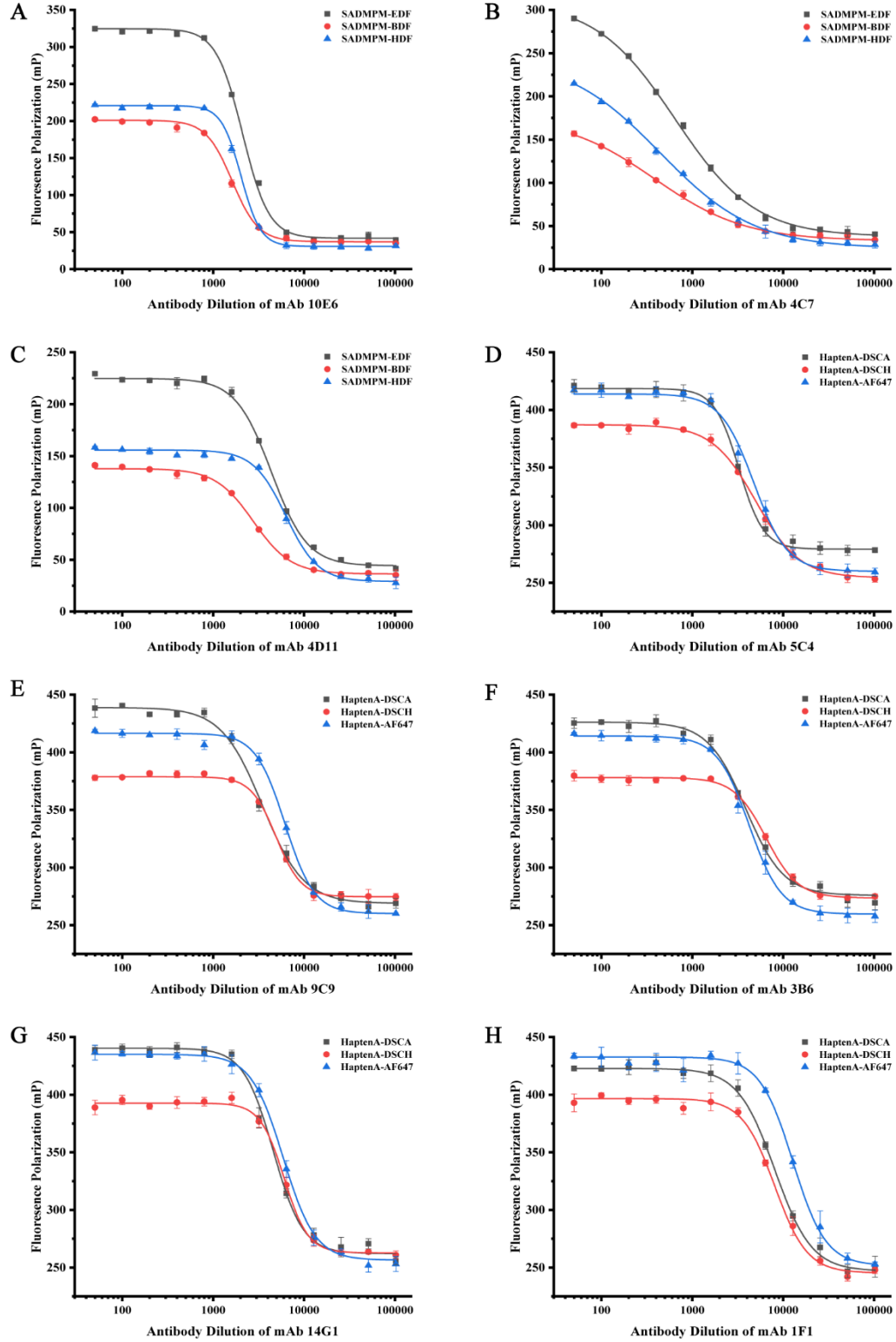


Figure S2. Antibody dilution curves of tracers (n = 3). (A) mAb 10E6 with SADMPM-EDF, SADMPM-BDF, and SADMPM-HDF. (B) mAb 4C7 with SADMPM-EDF, SADMPM-BDF, and SADMPM-HDF. (C) mAb 4D11 with

SADMPPM-EDF, SADMPPM-BDF, and SADMPPM-HDF. (D) mAb 5C4 with HaptenA-DSCA, HaptenA-DSCH, and HaptenA-AF647. (E) mAb 9C9 with HaptenA-DSCA, HaptenA-DSCH, and HaptenA-AF647. (F) mAb 3B6 with HaptenA-DSCA, HaptenA-DSCH, and HaptenA-AF647. (G) mAb 14G1 with HaptenA-DSCA, HaptenA-DSCH, and HaptenA-AF647. (H) mAb 1F1 with HaptenA-DSCA, HaptenA-DSCH, and HaptenA-AF647.

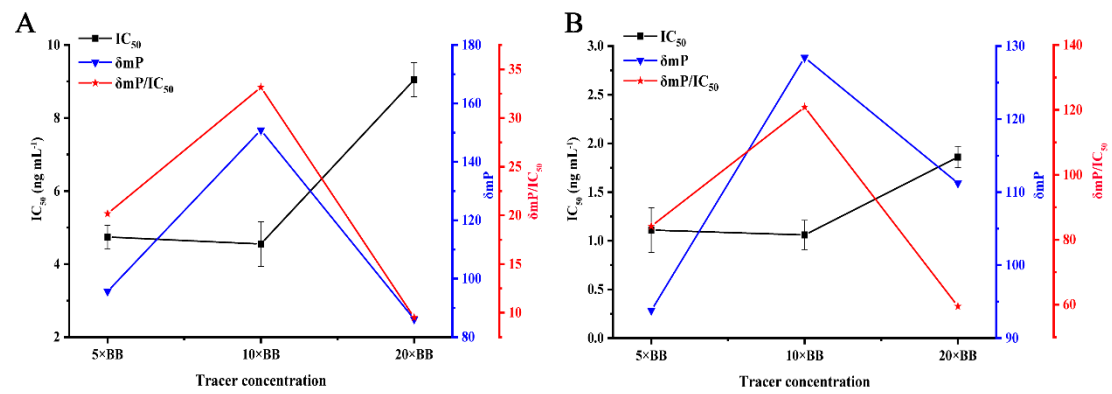


Figure S3. The effect of the tracer concentration for SMZ (A) and TMP (B).

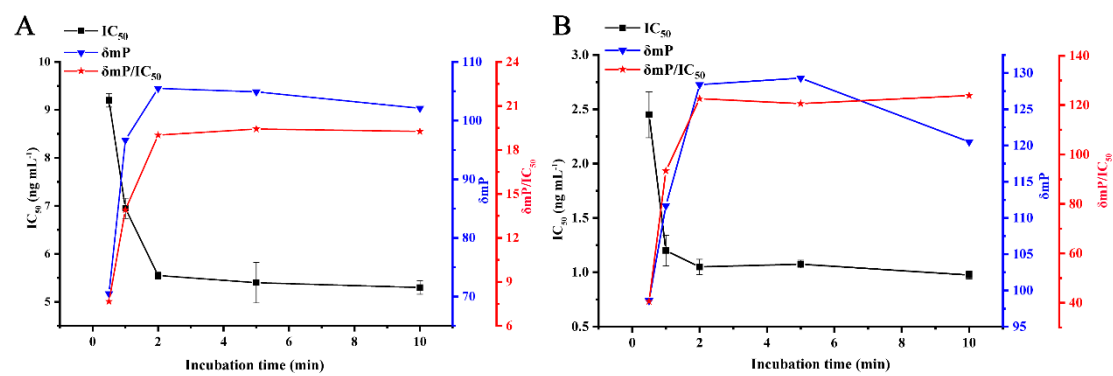


Figure S4. Influence of incubation time on DWFPIA for SMZ (A) and TMP (B)

Table S1. R_f values of the short-wavelength tracers of SAs.

Names	Volume (μl)	Developing solvent	R _f values
SADMPM-EDF	20	trichloromethane/methanol/acetic acid (6:1:0.07, v/v/v)	0.63
SADMPM-BDF	20	trichloromethane/methanol/ acetic acid (6:1:0.07, v/v/v)	0.87
SADMPM-HDF	20	trichloromethane/methanol/ acetic acid (6:1:0.07, v/v/v)	0.86

Table S2. R_f values the long-wavelength tracers of ASGs.

Names	Volume(μl)	Developing solvent	R _f values
HaptenA-DSCA	20	trichloromethane/methanol/ acetic acid (6:1:0.07, v/v/v)	0.72
HaptenA-DSCH	20	trichloromethane/methanol/ acetic acid (6:1:0.07, v/v/v)	0.63
HaptenA-AF647	20	trichloromethane/methanol/ acetic acid (6:1:0.07, v/v/v)	0.44

Table S3. The IC₅₀ values and CRs in DWFPIA of SAs

Name	IC ₅₀ (ng/mL)	CRs (%)
SMZ	4.7	100
SCP	69.2	6.8
SPA	2.5	191.7
SIZ	1.4	328.6
STZ	1.7	270.6
SMD	4.0	117.9
SPY	8.4	56.1
SMM	5.7	82.1
SQX	2.9	164.3
SDZ/SD	3.3	143.8
SMX	3.7	127.8
SDM	6.2	75.4
SMP	4.3	109.5
SCY	5.1	92.0
SMR	2.5	191.7
SDM'	32.6	14.4
SXL	3.8	124.3
SMT	2.1	219.0
SBA	3.4	139.4
SIM	6.1	76.7
SEP	19.2	24.5
SBM	6.2	75.4
SLE	3.1	153.3

Table S4. The IC₅₀ values and CRs in DWFPIA of ASGs

Name	IC ₅₀ (ng/mL)	CRs (%)
TMP	1.1	100
OMP	1.8	61.1
BQP	0.9	122.2
DVD	0.8	137.5
BOP	1.2	91.7

Table S5. Comparisons with reported simultaneous analytical methods for SAs and ASGs.

methods	analytes number	assay time (from added samples)	LOD ($\mu\text{g L}^{-1}$ or $\mu\text{g kg}^{-1}$)	references
GC-MS	1 SAs	— ^a	SMZ 1140 ^b	[11]
	1 ASGs		TMP 860 ^b	
MIP-SBSE-HPLC	3 SAs	— ^a	SMZ 4.8	[12]
	1 ASGs		TMP 1.6	
UHPLC-QE HF HRMS	27SAs	10 min	SMZ 0.01	[49]
	6ASGs		TMP 0.02	
SPE-HPLC	3 SAs	20 min	SMZ 10	[50]
	1 ASGs		TMP 10	
DWFPIA	23 SAs	2 min	SMZ 3.3	this study
	5 ASGs		TMP 0.7	

^a No report

^b positive chemical ionization (selected ion monitoring) method detection limits

Gas chromatography-mass spectral (GC-MS)

Molecularly imprinted polymer-coated stir bar sorptive extraction coupled with high performance liquid chromatography (MIP-SBSE-HPLC)

Ultra-high performance liquid chromatography coupled to quadrupole-high-field orbitrap high resolution MS (UHPLC-QE HF HRMS)

Solid phase extraction high-performance liquid chromatography (SPE-HPLC)