Supplementary Material

Development of a method for the detection of fungicide residues in foods of animal origin based on modified QuEChERS-GC-MS/MS

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Table S1 Beef solvent profiles, matrix profiles, and matrix effects(ME)

Compound	Solvent curve equation	Matrix curve equation	ME
Tebufloquin	y=55.770x-6.292	y=110.125x-1141.703	97.46
Fluopyram	y=126.687x-31.662	y=250.545x-2849.028	97.77
Thifluzamide	y=6.746x+13.750	y=12.908x-387.373	91.32
Penflufen	y=196.181x-91.365	y=357.863x-3760.196	82.41
Penthiopyrad	y=86.368x+3.772	y=169.738x-939.274	96.53
Fluopicolide	y=104.799x-7.757	y=198.925x-827.110	89.82
Fluxapyroxad	y=140.642x-39.815	y=268.143x-7019.464	90.66
Inpyrfluxam	y=127.416x-104.018	y=223.508x-2349.380	75.42
Metrafenone	y=26.775x+81.335	y=48.405x-686.753	80.78
Mefentrifluconazole	y=80.915x-44.784	y=157.470x-554.512	94.61
Ametoctradin	y=14.632x+23.142	y=28.328x-564.517	93.60
Pydiflumetofen	y=144.374x-13.641	y=239.245x+816.159	65.71
Bixafen	y=149.093x-24.087	y=248.223x-205.358	66.49

Table S2 Chicken solvent profiles, matrix profiles, and matrix effects(ME)

Compound	Solvent curve equation	Matrix curve equation	ME
Tebufloquin	y=55.770x-6.292	y=106.639x-1053.259	91.26
Fluopyram	y=126.686x-31.662	y=246.749x-1677.312	94.77
Thifluzamide	y=6.747x+13.750	y=13.073x-114.856	93.77
Penflufen	y=196.181x-91.365	y=343.696x-1151.091	75.19
Penthiopyrad	y=86.368x+3.772	y=165.971x-628.420	92.17
Fluopicolide	y=104.799x-7.757	y=185.609x-204.068	77.11
Fluxapyroxad	y=140.642x-39.815	y=269.664x-1371.633	91.53
Inpyrfluxam	y=127.416x-104.018	y=220.681x-567.770	73.20
Metrafenone	y=26.775x+81.335	y=45.675x-515.891	70.59
Mefentrifluconazole	y=80.915x-44.784	y=150.112x-1.953	85.52
Ametoctradin	y=14.632x+23.142	y=25.806x-526.629	76.36
Pydiflumetofen	y=144.374x-13.641	y=258.979x-1505.799	79.38
Bixafen	y=149.093x-24.087	y=241.769x-279.668	62.16

Table S3 Spiked recovery experiments of 13 fungicides in beef and chicken meat(n=3)

Compound	Beef						Chicken					
	LOQ(%)	RSD(%)	2LOQ(%)	RSD(%)	10LOQ(%)	RSD(%)	LOQ(%)	RSD(%)	2LOQ(%)	RSD(%)	10LOQ(%)	RSD(%)
Tebufloquin	97.87	7.49	94.92	2.48	94.85	2.51	111.39	0.25	87.82	0.88	88.49	2.07
Fluopyram	88.40	2.30	92.03	2.06	95.07	1.66	108.90	5.39	108.41	0.66	83.49	2.43
Thifluzamide	94.32	2.93	85.25	0.50	89.42	0.47	94.74	2.83	84.98	0.18	83.90	2.04
Penflufen	91.31	2.59	89.66	1.09	86.69	1.27	99.81	4.44	87.95	0.88	84.23	0.27
Penthiopyrad	92.40	1.91	92.50	1.77	95.70	2.02	101.09	2.44	87.56	0.64	96.49	0.96
Fluopicolide	89.98	1.98	83.67	0.97	88.25	3.22	97.48	3.34	94.51	2.38	82.23	5.04
Fluxapyroxad	96.51	1.54	97.01	0.12	94.81	0.61	96.72	2.47	89.71	2.31	90.71	1.00
Inpyrfluxam	99.92	3.34	103.98	2.54	103.38	2.06	100.44	3.99	89.67	3.34	101.65	2.98
Metrafenone	98.35	2.82	100.76	6.34	95.28	2.37	90.10	3.60	96.04	7.88	85.10	6.26
Mefentrifluconazole	85.80	1.56	84.07	1.32	85.51	2.45	100.88	3.19	79.51	0.55	79.87	0.48
Ametoctradin	86.98	1.34	89.33	2.24	88.80	5.47	89.77	3.28	94.78	3.40	89.54	3.19
Pydiflumetofen	98.17	1.36	94.63	1.23	89.17	3.38	105.89	5.68	101.17	0.94	99.97	1.79
Bixafen	99.42	0.85	88.70	2.64	83.35	0.62	101.55	6.65	92.76	0.83	97.07	1.97

Table S4 Intra and inter day precision of 13 fungicides in beef and chicken meat (20 μg/kg, n=6)

Compound	Intraday（%）		Interday（%）	
	beef	chicken	beef	chicken
Tebufloquin	4.31	4.82	5.84	8.31
Fluopyram	3.54	5.91	4.47	3.25
Thifluzamide	2.09	2.48	3.90	2.08
Penflufen	2.45	3.18	3.91	3.01
Penthiopyrad	1.83	2.19	4.21	1.89
Fluopicolide	1.84	4.20	4.37	3.75
Fluxapyroxad	1.54	2.88	4.26	2.49
Inpyrfluxam	2.52	3.99	4.80	3.30
Metrafenone	5.70	6.04	7.86	4.88
Mefentrifluconazole	8.59	2.39	7.30	2.58
Ametoctradin	1.28	3.83	2.88	3.38
Pydiflumetofen	2.35	9.04	8.93	10.51
Bixafen	1.74	5.06	2.45	4.12

Table S5 Comparison of LOQs for 13 fungicides with the maximum residue limit (MRLs) of Japan, South Korea, EU, and Codex Alimentarius

Compound	This work（μg/kg）		Standard MRLs（mg/kg）			
	Beef LOQs	Chicken LOQs	Japan	EU	Codex Alimentarius	South Korea
Tebufloquin	9.52	7.82	0.09	/	/	/
Fluopyram	6.70	9.34	Cattle muscle 0.8 Chicken muscle 0.5	Bovine muscle 0.15 Poultry muscle 0.07 Reg. (EU) 2021/1807	Meat (from mammals other than marine mammals) 1.5 Poultry meat 1.5	/
Thifluzamide	6.86	5.70	Cattle muscle 0.05 Chicken muscle 0.02	/	/	/
Penflufen	1.94	1.78	0.2	Bovine muscle 0.01 Poultry muscle 0.01 Reg. (EU) 2021/644	/	/
Penthiopyrad	1.62	2.14	Cattle muscle 0.04 Chicken muscle 0.03	Bovine muscle 0.01 Poultry muscle 0.01 Reg. (EU) 2023/173	Meat (from mammals other than marine mammals) 0.04 Poultry meat 0.03	Poultry meat 0.03 Mammalian meat 0.04
Fluopicolide	7.90	7.18	0.01	Bovine muscle 0.02 Poultry muscle 0.02 Reg.(EU) No 2021/616	Meat (from mammals other than marine mammals) 0.01 Poultry meat 0.01	Poultry meat 0.01 Mammalian meat 0.01
Fluxapyroxad	3.70	1.08	Cattle muscle 0.2 Chicken muscle 0.02	Bovine muscle 0.015 Poultry muscle 0.02 Reg. (EU) 2022/1324	Meat (from mammals other than marine mammals) 0.2 Poultry meat 0.02	Poultry meat 0.05 Mammalian meat 0.2
Inpyrfluxam	10.00	7.40	0.02	/	Meat (from mammals other than marine mammals) 0.02 Poultry meat 0.02	/
Metrafenone	10.00	9.60	0.01	Bovine muscle 0.01 Poultry muscle 0.01 Reg. (EU) 2018/687	Meat (from mammals other than marine mammals) 0.01 Poultry meat 0.01	Poultry meat 0.01 Mammalian meat 0.01

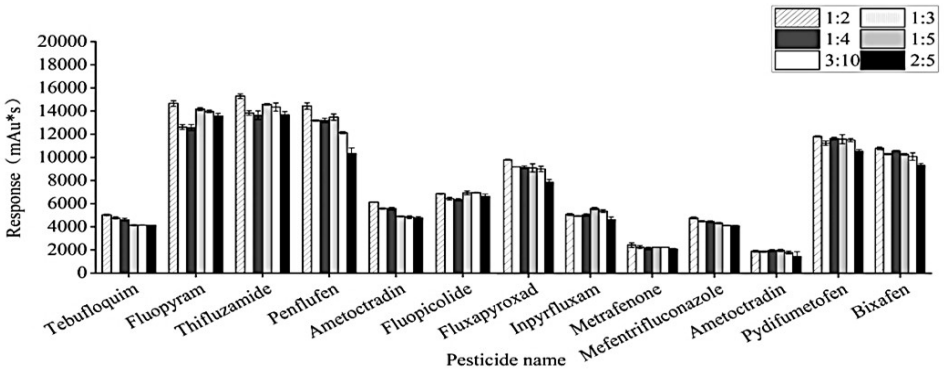
Mefentrifluconazole	9.48	2.70	Cattle muscle 0.2 Chicken muscle 0.02	Bovine muscle 0.04 Poultry muscle 0.015 Reg. (EU) 2024/1078	Meat (from mammals other than marine mammals) 0.15 Poultry meat 0.03	/
Ametoctradin	10.00	9.72	Chicken muscle 0.03	Bovine muscle 0.03 Poultry muscle 0.03 Reg. (EU) 2022/1290	Poultry meat 0.03	/
Pydiflumetofen	7.84	7.86	Cattle muscle 0.1 Chicken muscle 0.01	/	Meat (from mammals other than marine mammals) 0.1 Poultry meat 0.01	/
Bixafen	6.94	7.46	Cattle muscle 2 Chicken muscle 0.02	Bovine muscle 0.8 Poultry muscle 0.02 Reg (EU) 2023/1069	Meat (from mammals other than marine mammals) 2 Poultry meat 0.02	/

Table S6 Statistics of actual samples tested

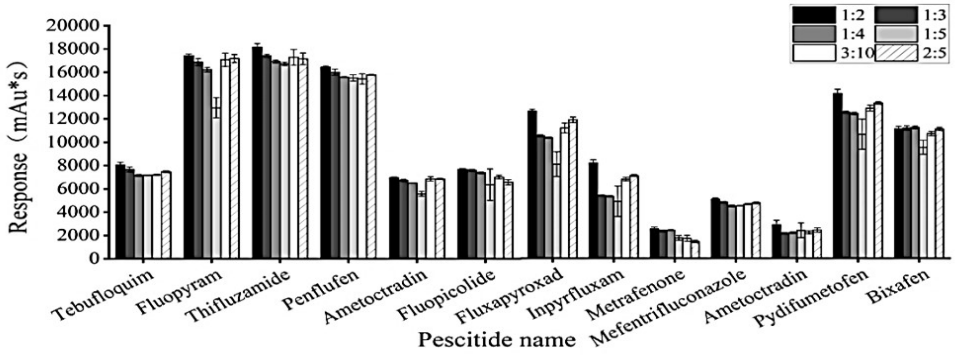
Sample name	Amount	Place of origin
Pork	32	Tibet (10), Chongqing (16), Yunnan (6)
Beef	27	Tibet(20)、Yunnan(7)
Lamb	16	Tibet(10）、Sichuan(3）、Yunnan(3)
Grass carp	23	Sichuan(8)、Chongqing(15)
Chicken	15	Chongqing(5)、Yunnan(5)、Sichuan(5)
Duck	16	Sichuan(4)、Yunnan(6)、Chongqing(6)
Bullfrog	13	Sichuan(4)、Chongqing(3)、Yunnan(6)
Eel	10	Sichuan(5)、Yunnan(5)
Total		152

Table S7 Fungicide Detection Statistics

Fungicide name	MRL(μg/kg)	Number of detections	Detection rate(%)	Residue levels(μg/kg)	Average value (μg/kg)
Tebufloquin	90	0	0	0	0
Fluopyram	1500	0	0	0	0
Thifluzamide	50	0	0	0	0
Penflufen	200	0	0	0	0
Penthiopyrad	40	1	0.66	1.72	1.72
Fluopicolide	20	0	0	0	0
Fluxapyroxad	200	0	0	0	0
Inpyrfluxam	20	0	0	0	0
Metrafenone	10	0	0	0	0
Mefentrifluconazole	200	0	0	0	0
Ametoctradin	30	0	0	0	0
Pydiflumetofen	100	0	0	0	0
Bixafen	2000	0	0	0	0

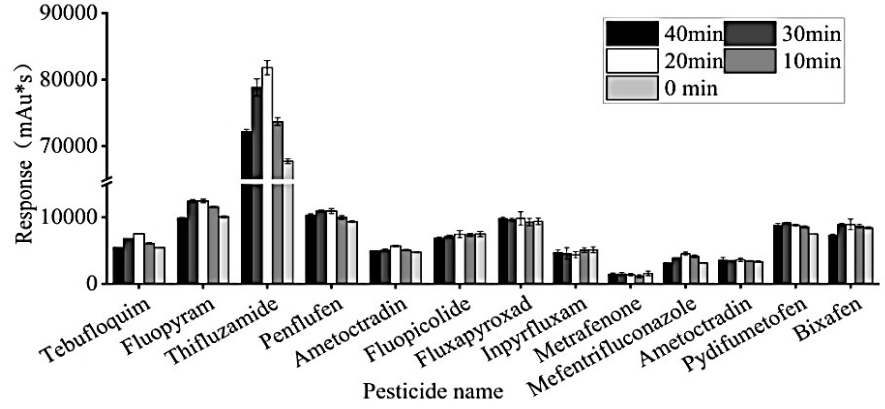


(a)

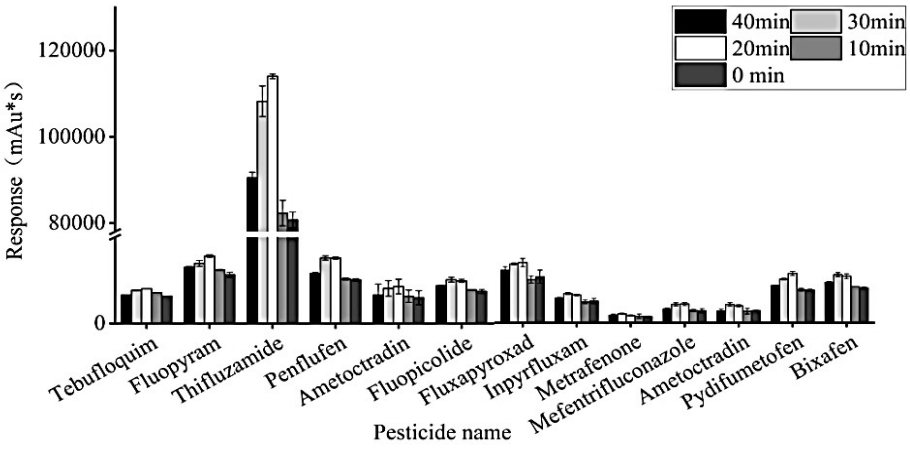


(b)

Figure S1 Optimisation of material-liquid ratio for extraction of 13 fungicides from beef (a) and chicken (b)

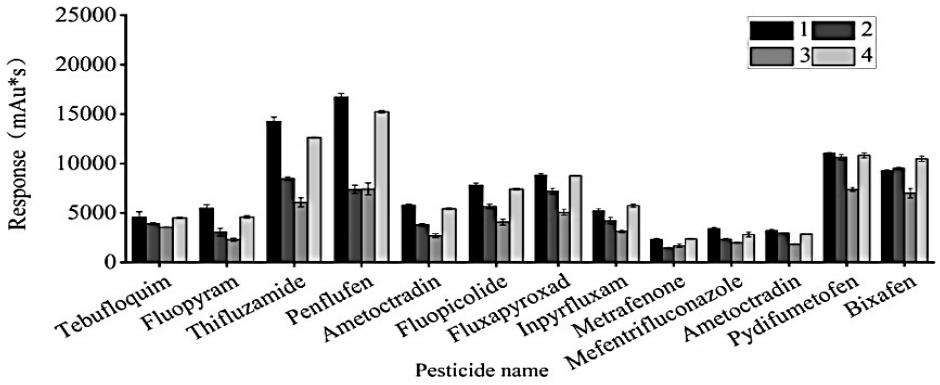


(a)

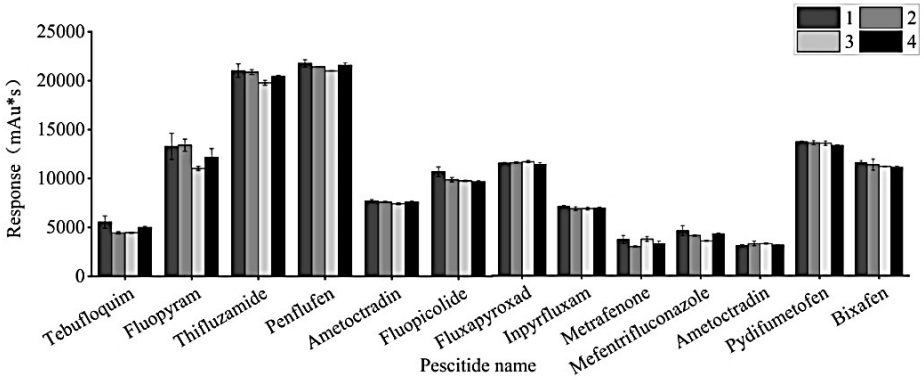


(b)

Figure S2 Optimisation of sonication time for 13 fungicides in beef (a) and chicken (b)

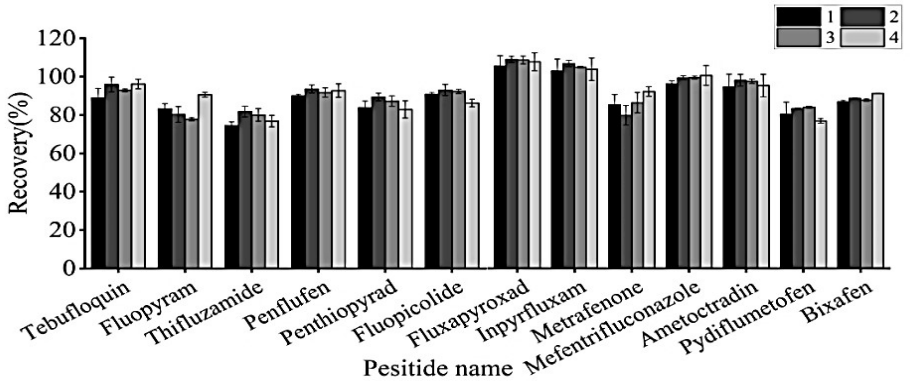


(a)

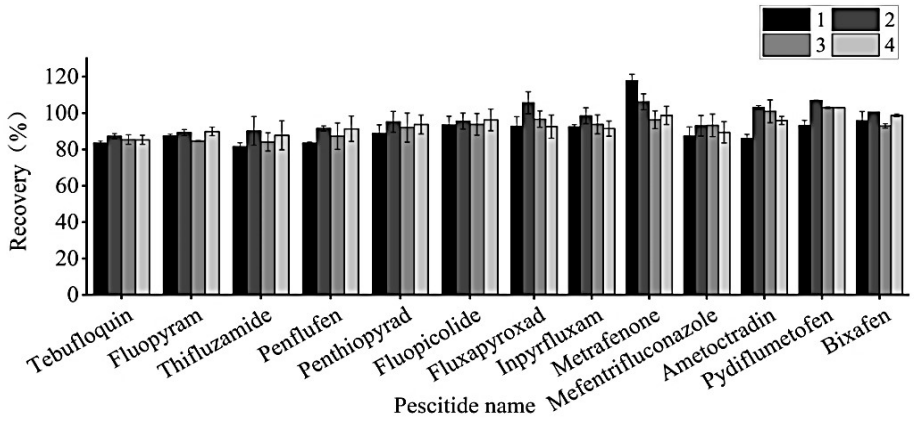


(b)

Figure S3 Optimisation of reagents for salting out of 13 fungicides in beef (a) and chicken (b) Combination 1. 3 g $MgSO_4$+1 g NaCl+1 g $C_6H_5O_7Na_3$+0.5 g $C_6H_6Na_2O_7$*1.5H_2O+0.5 g $NaHCO_3$, Combination 2. 3 g $MgSO_4$+1 g NaCl+1 g $C_6H_5O_7Na_3$+0.5 g $C_6H_6Na_2O_7$*1.5H_2O, Combination 3. 4 g $MgSO_4$+1.5 g CH_3COONa, Combination 4. 4 g $MgSO_4$+1 g CH_3COONa



(a)



(b)

Figure S4 Optimisation of 13 fungicide purification powders in beef (a) and chicken (b)

Combination 1. $MgSO_4$:PSA=6:1, Combination 2. $MgSO_4$:PSA:C18=6:1:1, Combination 3. $MgSO_4$:PSA:GCB=6:1:1, Combination 4. $MgSO_4$:C18=6:1