ELECTRONIC SUPPORTING INFORMATION

Modified QuEChERS-LC-MS/MS for the Simultaneous Detection of Eight Prohibited Dyes in Complex Food Matrices: Method Validation and Application to Real Samples

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Table S1. System suitability

Analytes	Sample	Time retention	Mean (min)	Peak area	Mean	RSD_t (%)	RSD_a (%)
Auramine O	S3-1	1.59	1.59	106450	106070	0.00	1.66
	S3-2	1.59		104358			
	S3-3	1.59		108735			
	S3-4	1.59		104825			
	S3-5	1.59		107384			
	S3-6	1.59		104665			
Rhodamine B	S3-1	1.85	1.85	128251	126406	0.00	1.48
	S3-2	1.85		124559			
	S3-3	1.85		126204			
	S3-4	1.85		124357			
	S3-5	1.85		128936			
	S3-6	1.85		126126			
Chrysoidine G	S3-1	1.38	1.38	57093	57065	0.30	1.11
	S3-2	1.38		56028			
	S3-3	1.38		56765			
	S3-4	1.38		57911			
	S3-5	1.39		57397			
	S3-6	1.38		57193			
Methylene blue	S3-1	1.43	1.42	56955	56770	0.36	1.52
	S3-2	1.42		55337			
	S3-3	1.42		56421			
	S3-4	1.42		57523			
	S3-5	1.43		57746			
	S3-6	1.42		56638			
Brilliant green	S3-1	2.00	2.00	57964	56927	0.26	1.75
	S3-2	2.01		56555			
	S3-3	2.00		57995			
	S3-4	2.00		56222			
	S3-5	2.01		57295			
	S3-6	2.00		55531			
Sudan yellow	S3-1	2.59	2.59	611193	610403	0.16	0.81
	S3-2	2.59		602164			
	S3-3	2.59		616779			
	S3-4	2.59		608857			

	S3-5	2.60		613798			
	S3-6	2.59		609629			
Toluidine red	S3-1	2.70	2.70	422913	422406	0.15	1.70
	S3-2	2.70		417985			
	S3-3	2.70		427012			
	S3-4	2.70		417457			
	S3-5	2.71		434142			
	S3-6	2.70		414926			
Sudan red G	S3-1	2.72	2.72	151358	150720	0.00	1.75
	S3-2	2.72		147921			
	S3-3	2.72		153936			
	S3-4	2.72		147271			
	S3-5	2.72		152712			
	S3-6	2.72		151120			

Table S2. Selectivity for chili sauce

Analytes	Sample	t_R	Peak area
Auramine O	M1	-	-
	M1+2LOQ	1.59	43931
	M1+5LOQ	1.59	104375
	M1+10LOQ	1.59	211469
Rhodamine B	M1	-	-
	M1+2LOQ	1.85	53823
	M1+5LOQ	1.85	123205
	M1+10LOQ	1.85	256119
Chrysoidine G	M1	-	-
	M1+2LOQ	1.38	22963
	M1+5LOQ	1.38	55455
	M1+10LOQ	1.38	110362
Methylene blue	M1	-	-
	M1+2LOQ	1.42	22662
	M1+5LOQ	1.42	54472
	M1+10LOQ	1.42	114570
Brilliant green	M1	-	-
	M1+2LOQ	2	23066
	M1+5LOQ	2	52970
	M1+10LOQ	2.01	111385
Sudan yellow	M1	-	-
	M1+2LOQ	2.59	220995
	M1+5LOQ	2.59	541161
	M1+10LOQ	2.59	1084127
Toluidine red	M1	-	-
	M1+2LOQ	2.7	170825
	M1+5LOQ	2.69	397587
	M1+10LOQ	2.7	724450
Sudan red G	M1	-	-
	M1+2LOQ	2.72	60211
	M1+5LOQ	2.72	139179
	M1+10LOQ	2.72	246683

Table S3. Effect of extraction solvent for recovery

Solvents	**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
ACN	48.8	48.4	46.8	58.3	32.2	37.9	24.4	39.2
M1-1	53.6	52.1	51.9	62.6	38.1	45.4	31.5	49.8
M1-2	44.4	44.6	38.3	55.5	26.8	31.2	16.8	28.6
M1-3	48.3	48.5	50.2	56.8	31.8	37.0	24.8	39.2
MeOH	81.7	76.1	30.8	79.9	69.3	72.8	12.3	63.6
M1-1	85.4	75.7	30.1	80.4	68.8	72.8	12.5	61.7
M1-2	81.5	76.4	30.5	81.0	70.5	73.0	12.5	62.0
M1-3	78.3	76.3	31.7	78.4	68.5	72.5	12.0	67.0
ACN:MeOH = 1:1	68.6	69.8	26.8	76.1	65.8	70.3	37.8	78.9
M1-1	69.8	70.2	27.5	78.1	65.3	71.2	38.0	79.4
M1-2	68.9	69.2	25.2	78.0	66.9	69.0	38.5	77.6
M1-3	67.2	69.9	27.5	72.3	65.0	70.6	36.9	79.6
ACN:MeOH = 4:1	86.7	79.4	68.7	80.7	70.3	82.4	64.7	95.7
M1-1	87.6	78.7	71.5	82.0	68.2	81.9	62.4	94.3
M1-2	87.2	78.6	68.7	81.0	69.8	82.1	64.1	94.4
M1-3	85.3	80.9	66.0	79.0	72.8	83.1	67.7	98.3

Table S4. Effect of extraction salt for recovery

Salts	**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
CH3COONa.3H2O:Na2SO4 = 1:4	52.6	36.3	23.2	62.7	49.5	40.2	22.8	27.0
M1-1	59.7	38.6	24.4	64.1	52.6	43.4	23.8	28.4
M1-2	55.4	39.0	24.4	67.0	49.5	41.0	24.2	27.7
M1-3	42.7	31.4	20.9	57.1	46.4	36.1	20.3	24.9
CH3COONa.3H2O:MgSO4 = 1:4	89.5	94.1	74.0	85.9	96.6	80.6	80.2	73.5
M1-1	88.7	95.6	75.5	87.8	95.9	79.2	65.3	65.6
M1-2	90.3	92.9	75.6	85.2	95.0	81.1	82.8	73.5
M1-3	89.6	93.7	71.0	84.8	98.9	81.7	92.6	81.6
NaCl:Na2SO4 = 1:4	91.1	77.4	55.4	77.8	69.0	85.3	79.9	73.1
M1-1	91.3	76.9	48.9	77.7	69.8	83.3	78.5	71.9
M1-2	88.0	77.0	55.3	74.7	68.2	85.2	80.3	73.6
M1-3	94.1	78.3	61.9	81.0	69.0	87.2	81.0	73.9
NaCl:MgSO4 = 1:4	76.5	63.9	78.9	73.4	78.3	68.5	68.1	60.4
M1-1	77.9	64.2	75.1	71.7	75.8	69.4	68.9	61.5
M1-2	75.3	63.7	79.1	72.0	77.7	68.9	69.3	60.6
M1-3	76.3	63.7	82.6	76.4	81.2	67.3	66.0	59.0

Table S5. Effect of clean up mixture for recovery

Kit d-SPE	**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
PSA:C18:Na2SO4 = 100:100:1000	95.0	81.9	82.9	98.2	85.9	80.7	63.3	73.0
M1-1	94.5	81.2	83.2	98.5	85.9	80.7	60.7	72.0
M1-2	96.1	82.3	84.1	97.5	85.9	80.3	64.3	75.9
M1-3	94.4	82.3	81.2	98.6	85.8	81.0	64.9	71.0
PSA:C18:Na2SO4 = 50:50:1000	97.3	80.1	80.1	96.3	85.6	81.5	67.2	74.8
M1-1	97.4	79.7	80.1	95.8	87.7	82.2	67.6	76.0
M1-2	96.4	80.1	79.8	96.4	83.1	81.3	66.6	74.3
M1-3	98.1	80.5	80.5	96.7	86.0	81.1	67.6	74.0
PSA:C18:MgSO4 = 100:100:1000	98.4	98.4	98.3	92.2	94.6	96.2	95.3	96.3
M1-1	98.5	101.6	100.8	92.9	96.1	95.7	95.7	96.1
M1-2	98.9	97.4	98.6	92.7	93.0	96.2	94.8	98.3
M1-3	97.8	96.1	95.5	90.9	94.7	96.8	95.4	94.5
PSA:C18:MgSO4 = 50:50:1000	97.2	97.7	98.3	87.8	95.3	94.1	78.6	87.9
M1-1	96.0	96.9	100.8	88.0	93.5	94.1	76.4	88.4
M1-2	97.1	98.2	98.6	87.5	94.9	94.4	79.3	88.5
M1-3	98.5	98.1	95.5	87.9	97.5	93.8	80.0	86.6

Table S6. Matrix effect (ME%) values for the eight target dyes in six representative food matrices

$$\%ME = \left(\frac{slope_{matrix}}{slope_{solvent}} - 1\right) \times 100$$

M1		Auramine O	Rhodamine B	Chrysoidine G	Methylene blue	Brilliant green	Sudan yellow	Toluidine red	Sudan red G
Matrix	Calibration equation	y = 17080x + 2128	y = 19073x + 3874	y = 9268x + 989	y = 9649x + 1809	y = 8883x + 1907	y = 102048x + 6735	y = 6041x + 4591	y = 1548x + 3712
	Slope	17080	19073	9268	9649	8883	102048	6041	1548
Solvent	Calibration equation	y = 18169x - 472	y = 22037x + 598	y = 11026x + 321	y = 11019x + 874	y = 11276x - 193	y = 118095x - 2232	y = 7194x + 346	y = 1787x + 457
	Slope	18169	22037	11026	11019	11276	118095	7194	1787
%ME		-6,0	-13,5	-15,9	-12,4	-21,2	-13,6	-16,0	-13,4
M2		**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
Matrix	Calibration equation	y = 15945x + 2903	y = 17923x + 5362	y = 9337x + 528	y = 8889x + 1572	y = 10637x + 2293	y = 109700x + 4840	y = 5904x + 4227	y = 1518x + 4042
	Slope	15945	17923	9337	8889	10637	109700	5904	1518
Solvent	Calibration equation	y = 18169x - 472	y = 22037x + 598	y = 11026x + 321	y = 11019x + 874	y = 11276x - 193	y = 118095x - 2232	y = 7194x + 346	y = 1787x + 457
	Slope	18169	22037	11026	11019	11276	118095	7194	1787
%ME		-12,2	-18,7	-15,3	-19,3	-5,7	-7,1	-17,9	-15,1
M3		**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
Matrix	Calibration equation	y = 16143x - 1385	y = 18410x + 1913	y = 9022x - 482	y = 8457x + 1913	y = 9868x + 1535	y = 108851x + 4260	y = 5733x - 4206	y = 1515x - 1594
	Slope	16143	18410	9022	8457	9868	108851	5733	1515
Solvent	Calibration equation	y = 18169x - 472	y = 22037x + 598	y = 11026x + 321	y = 11019x + 874	y = 11276x - 193	y = 118095x - 2232	y = 7194x + 346	y = 1787x + 457
	Slope	18169	22037	11026	11019	11276	118095	7194	1787
%ME		-11,2	-16,5	-18,2	-23,3	-12,5	-7,8	-20,3	-15,2
M4		**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
Matrix	Calibration	y = 15517x	y = 18168x +	y = 9400x +	y = 9468x +	y = 9152x +	y = 91277x	y = 5808x -	y = 1628x +

	equation	+ 1707	2530	356	1183	432	+ 9072	2198	1538
	Slope	15517	18168	9400	9468	9152	91277	5808	1628
Solvent	Calibration equation	y = 18169x - 472	y = 22037x + 598	y = 11026x + 321	y = 11019x + 874	y = 11276x - 193	y = 118095x - 2232	y = 7194x + 346	y = 1787x + 457
	Slope	18169	22037	11026	11019	11276	118095	7194	1787
%ME		-14,6	-17,6	-14,7	-14,1	-18,8	-22,7	-19,3	-8,9
M5		**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
Matrix	Calibration equation	y = 14896x - 380	y = 18048x + 1158	y = 9172x - 942	y = 8399x + 667	y = 8966x + 1853	y = 90549x - 6937	y = 5723x - 3027	y = 1387x - 1612
	Slope	14896	18048	9172	8399	8966	90549	5723	1387
Solvent	Calibration equation	y = 18169x - 472	y = 22037x + 598	y = 11026x + 321	y = 11019x + 874	y = 11276x - 193	y = 118095x - 2232	y = 7194x + 346	y = 1787x + 457
	Slope	18169	22037	11026	11019	11276	118095	7194	1787
%ME		-18,0	-18,1	-16,8	-23,8	-20,5	-23,3	-20,4	-22,4
M6		**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
Matrix	Calibration equation	y = 16347x + 1179	y = 19042x + 2840	y = 9415x + 849	y = 8375x + 2292	y = 9512x + 1563	y = 92458x + 10847	y = 6051x + 4972	y = 1480x + 2511
	Slope	16347	19042	9415	8375	9512	92458	6051	1480
Solvent	Calibration equation	y = 18169x - 472	y = 22037x + 598	y = 11026x + 321	y = 11019x + 874	y = 11276x - 193	y = 118095x - 2232	y = 7194x + 346	y = 1787x + 457
	Slope	18169	22037	11026	11019	11276	118095	7194	1787
%ME		-10,0	-13,6	-14,6	-24,0	-15,6	-21,7	-15,9	-17,2

Table S7. Detailed recovery (%) and precision data for target analytes at three different fortification levels in all studied matrices

M1

Fortification levels	**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
LOQ	87.0 - 100.0	95.2 - 105.5	95.4 - 106.5	92.9 - 98.3	84.6 - 110.5	97.7 - 102.4	82.7 - 94.8	83.8 - 99.2
2LOQ	97.9 - 104.2	95.2 - 105.1	93.9 - 99.8	89.7 - 106.1	95.1 - 105.4	90.6 - 100.7	95.8 - 106.0	97.7 - 107.0
5LOQ	91.5 - 99.3	95.0 - 99.9	92.7 - 104.8	92.6 - 101.8	90.9 - 99.0	92.6 - 96.5	95.9 - 103.1	93.1 - 102.9

M2

Fortification levels	**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
LOQ	95.6 - 105.3	92.9 - 103.3	94.8 - 113.1	93.6 - 112.1	94.1 - 104.0	87.3 - 91.1	89.4 - 107.1	91.0 - 107.9
2LOQ	89.9 - 97.5	90.9 - 99.5	93.3 - 97.5	90.0 - 97.9	88.1 - 98.1	89.1 - 94.8	92.2 - 99.9	91.8 - 105.0
5LOQ	97.4 - 103.7	98.5 - 104.9	92.0 - 98.1	93.2 - 102.1	92.6 - 99.5	97.1 - 101.2	98.9 - 105.0	96.7 - 102.5

M3

Fortification levels	**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
LOQ	93.5 - 106.1	86.7 - 100.9	85.7 - 99.3	93.3 - 108.1	90.4 - 101.8	98.3 - 106.6	82.1 - 101.2	94.4 - 108.2
2LOQ	87.0 - 104.7	90.6 - 103.0	89.7 - 100.3	95.4 - 105.3	96.1 - 103.8	87.5 - 103.3	85.8 - 106.2	87.7 - 102.3
5LOQ	98.6 - 107.7	95.9 - 104.8	87.2 - 97.4	96.9 - 104.3	90.4 - 97.1	89.9 - 107.6	87.0 - 110.0	82.3 - 107.6

M4

Fortification levels	**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
LOQ	93.4 - 105.6	90.9 - 108.9	86.2 - 109.3	97.2 - 106.7	99.1 - 111.2	82.7 - 108.2	82.1 - 103.8	80.4 - 100.0
2LOQ	97.0 - 105.5	97.8 - 105.3	90.6 - 103.0	98.1 - 103.3	97.9 - 105.2	85.8 - 103.7	84.3 - 106.1	81.1 - 109.1
5LOQ	98.0 - 101.4	98.1 - 102.2	88.0 - 94.7	96.9 - 101.1	94.3 - 104.2	97.0 - 98.3	101.2 - 106.9	98.5 - 106.6

M5

Fortification levels	**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
LOQ	87.2 - 99.9	89.0 - 96.4	79.0 - 104.4	76.8 - 91.1	83.5 - 98.7	97.5 - 108.3	101.8 - 108.0	89.8 - 107.4
2LOQ	89.5 - 95.3	91.4 - 100.9	89.4 - 101.7	85.3 - 92.1	88.0 - 97.2	89.6 - 99.1	91.3 - 99.1	86.8 - 97.7

5LOQ	95.0 - 100.4	96.3 - 103.3	97.0 - 104.4	95.0 - 104.9	94.9 - 99.9	94.9 - 97.2	96.3 - 98.8	93.8 - 102.3

M6

Fortification levels	**Auramine O**	**Rhodamine B**	**Chrysoidine G**	**Methylene blue**	**Brilliant green**	**Sudan yellow**	**Toluidine red**	**Sudan red G**
LOQ	90.2 - 102.8	81.9 - 104.7	81.4 - 96.5	80.7 - 92.3	88.0 - 96.3	82.9 - 101.8	85.0 - 99.8	80.9 - 107.9
2LOQ	86.3 - 108.4	85.7 - 108.8	92.7 - 106.8	85.1 - 105.2	87.1 - 107.1	87.9 - 106.7	85.9 - 107.5	81.4 - 108.4
5LOQ	92.9 - 108.6	93.9 - 96.5	92.5 - 101.5	93.7 - 99.5	92.0 - 96.1	95.4 - 103.3	85.5 - 99.0	94.6 - 107.6

Table S8. Proficiency testing results for Auramine O analysis

Parameter			**Value**
Assigned value (x)			0.520
Standard deviation for proficiency assessment			0.057
Standard uncertainty of the assigned value			0.034
Unit			**mg/kg**
Laboratory Code	**Result (mg/kg)**	**Z-score**	**Conclusion**
L-11	0.54	0.35	Satisfactory