

Optimization of A Solid-Phase Extraction Procedure for the Separation of Picrocrocin and Crocins from Saffron Extract

Panagiota-Kyriaki Revelou ^{1,2}, Eleni Kougianou ¹, Marinos Xagoraris ¹, Haralambos Evangelaras ³, George K. Papadopoulos ⁴, Charalabos D. Kanakis ¹, Irini F. Strati ², Christos S. Pappas ¹ and Petros A. Tarantilis ^{1,*}

¹ Laboratory of Chemistry, Department of Food Science and Human Nutrition, Agricultural University of Athens EU-CONEXUS European University, 11855 Athens, Greece

² Department of Food Science and Technology, University of West Attica, Ag. Spyridonos str, Egaleo, 12243 Athens, Greece

³ Department of Statistics and Insurance Science, University of Piraeus, 80 Karaoli & Dimitriou st., 18534 Piraeus, Greece

⁴ Institute for Design and Analysis of Experiments, University Research Center, Agricultural University of Athens EU-CONEXUS European University, Iera Odos 75, 11855 Athens, Greece

* Correspondence: ptara@aua.gr; Tel.: +30-2105294262

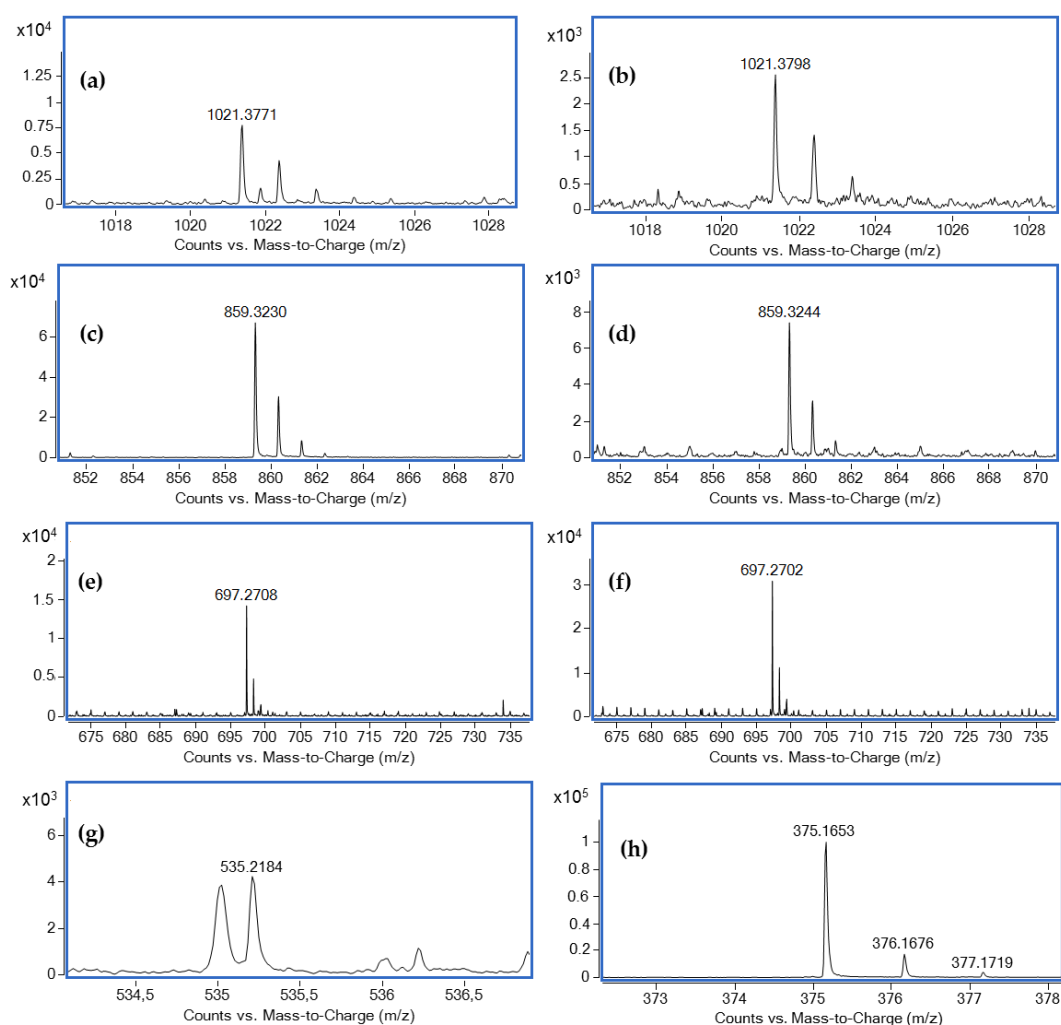


Figure S1. Mass spectra of crocins (a) trans 4GG; (b) trans 3Gg; (c) trans 2G; (d) cis 4GG; (e) cis 3Gg; (f) cis 2G; (g) cis 1g; and (h) picrocrocin.

Table S1. Rotatable central composite design for the three independent experimental factors (A, B and C) along with the experimental values of the response (absorbance of picocrocin at 257 nm).

Run	Space Type	Factor 1 A: sample volume (mL)	Factor 2 B: ACN/H ₂ O (% v/v)	Factor 3 C: Aqueous ACN vol- ume (mL)	Response Absorbance 257 nm
1	Factorial	1.000	10.000	10.000	0.295
2	Axial	0.318	12.500	20.000	0.321
3	Axial	2.000	8.296	20.000	0.344
4	Factorial	3.000	15.000	30.000	1.314
5	Center	2.000	12.500	20.000	1.205
6	Factorial	1.000	15.000	30.000	0.639
7	Axial	3.682	12.500	20.000	1.851
8	Factorial	1.000	10.000	30.000	0.442
9	Center	2.000	12.500	20.000	1.164
10	Factorial	1.000	15.000	10.000	1.108
11	Factorial	3.000	10.000	10.000	1.420
12	Factorial	3.000	10.000	30.000	1.148
13	Axial	2.000	12.500	36.818	0.752
14	Center	2.000	12.500	20.000	1.187
15	Center	2.000	12.500	20.000	1.109
16	Center	2.000	12.500	20.000	1.184
17	Center	2.000	12.500	20.000	1.166
18	Factorial	3.000	15.000	10.000	0.502 ¹
19	Axial	2.000	12.500	3.182	0.897
20	Axial	2.000	16.704	20.000	1.366

¹ A dilution factor of 10 has been applied.

Table S2. Rotatable central composite design for the three independent experimental factors (A, B and C) along with the experimental values of the response (crocins absorbance at 440 nm).

Run	Space Type	Factor 1 A: Saffron extract volume (mL)	Factor 2 B: ACN/H ₂ O (%v/v)	Factor 3 C: Aqueous ACN volume (mL)	Response Absorbance 440 nm
1	Factorial	1.000	25.000	10.000	1.707
2	Factorial	3.000	25.000	10.000	0.528 ¹
3	Center	2.000	37.500	20.000	0.270 ¹
4	Factorial	1.000	50.000	10.000	0.437 ²
5	Factorial	3.000	50.000	10.000	0.712 ¹
6	Center	2.000	37.500	20.000	0.282 ¹
7	Center	2.000	37.500	20.000	0.274 ¹
8	Axial	2.000	37.500	36.818	0.339 ²
9	Axial	3.682	37.500	20.000	0.436 ¹
10	Axial	2.000	58.522	20.000	0.277 ¹
11	Factorial	3.000	50.000	30.000	0.286 ²
12	Axial	2.000	37.500	3.182	0.443 ¹
13	Axial	2.000	16.478	20.000	1.027
14	Factorial	3.000	25.000	30.000	0.411 ²
15	Factorial	1.000	50.000	30.000	1.480
16	Center	2.000	37.500	20.000	0.427 ²
17	Factorial	1.000	25.000	30.000	1.264
18	Center	2.000	37.500	20.000	0.413 ²
19	Axial	0.318	37.500	20.000	0.836
20	Center	2.000	37.500	20.000	0.243 ¹

¹ A dilution factor of 20 has been applied; ² A dilution factor of 10 has been applied.

Table S3. Analysis of variance results for the experimental design of picrocrocic model.

Source	Sum of Squares	DF ¹	Mean Square	F-value	p-value
Model	2.4100	9	0.2680	27.83	< 0.0001 ²
A-Saffron extract volume (mL)	1.2800	1	1.2800	133.28	< 0.0001
B- ACN/water (% v/v)	0.6707	1	0.6707	69.63	< 0.0001
C-Aqueous ACN volume (mL)	0.0307	1	0.0307	3.19	0.1044
AB	0.0596	1	0.0596	6.19	0.0321
AC	0.0387	1	0.0387	4.01	0.0729
BC	0.1176	1	0.1176	12.21	0.0058
A ²	0.1002	1	0.1002	10.40	0.0091
B ²	0.1276	1	0.1276	13.24	0.0045
C ²	0.0147	1	0.0147	1.53	0.2450
Residual	0.0963	10	0.0096		
Total	2.5063	19			

¹ DF: degree of freedom; ² Significant.

Table S4. Analysis of variance results for the experimental design of crocins model.

Source	Sum of Squares	DF ¹	Mean Square	F-value	p-value
Model	13.80	9	1.53	32.63	< 0.0001 ¹
A-Sample volume (mL)	6.11	1	6.11	129.99	< 0.0001
B-% v/v Aqueous ACN	1.18	1	1.18	25.17	0.0005
C-Aqueous ACN volume (mL)	4.17	1	4.17	88.67	< 0.0001
AB	0.0000	1	0.0000	0.0004	0.9846
AC	0.2996	1	0.2996	6.38	0.0301
BC	0.0906	1	0.0906	1.93	0.1951
A ²	0.1883	1	0.1883	4.01	0.0732
B ²	0.5922	1	0.5922	12.61	0.0053
C ²	0.9825	1	0.9825	20.91	0.0010
Residual	0.4698	10	0.0470		
Total	14.2698	19			

¹DF: degree of freedom; ²Significant.