- Electronic Supplementary Information -

Multi-response optimization of a green solid-phase extraction for the analysis of heterocyclic aromatic amines in environmental samples

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Run	A ACN (%)	B HCOOH (mM)	C Elution flow rate (mL min⁻¹)	D Elution volume (μL)	DMIP	IQ	MeIQ	MeIQx	4,8-DiMeIQx	PhIP	Trp-P1	AαC	MeAαC	Trp-P2
									ERs (%)					
1	50	0.008	0.33	750	73.1	75.9	78.3	73.7	76.6	82.4	72.3	85.9	78.9	73.1
2	60	0.016	0.15	500	93	90.8	92.5	88.04	88.4	87.9	82.7	87.5	89.9	82.5
3	60	0.004	0.15	1000	80.7	82.5	82.2	79	80.8	79.9	69.4	79.5	77.2	71.9
4	60	0.004	0.50	1000	88.9	90.8	91.3	82.3	86.9	74.7	77.9	69.9	74.2	70.1
5	40	0.016	0.50	1000	63.1	67.6	60.5	75.1	65.7	57.6	74.9	66.3	69.8	72.6
6	60	0.004	0.50	500	85.9	82.6	84.5	82.7	80.3	80.3	70.9	82.3	65.3	68.4
7	50	0.008	0.33	750	75.9	71.3	77.2	79.1	73.9	78.8	79.3	84.2	81.1	78.5
8	60	0.004	0.15	500	82.1	83.1	86.9	80.2	82.5	71	66.1	73.5	73.8	82.7
9	40	0.004	0.50	1000	58	55.7	56.8	62.5	53.8	58.6	70.7	60.1	71.2	53.5
10	40	0.004	0.50	500	56.1	53.9	42.7	54.3	56.2	50.1	59.1	56.4	47.5	69.2
11	40	0.016	0.15	500	56.9	54.9	59.2	53.8	51.8	47.1	52.8	41.7	54.8	51.8
12	60	0.016	0.50	1000	94.1	97.1	96.4	98.5	92.9	97.2	95.5	96.5	92.7	91.1
13	50	0.008	0.33	750	72.9	76.2	72.9	75.27	77.9	75.6	76.6	78.5	73.9	80.5
14	40	0.004	0.15	1000	38.1	42.9	37.8	44.02	52.7	61.4	38.2	50.4	50.5	41.9
15	40	0.016	0.15	1000	62.1	68.2	61.1	70.5	70.2	68.4	86.8	61.2	65.8	70.6
16	60	0.016	0.50	500	92	92.5	97.4	95.74	94.2	99.9	93.1	97.06	92.8	95
17	60	0.016	0.15	1000	82.1	86.5	85.9	84.4	84.1	75.1	76.1	80.8	87.9	83.7
18	40	0.016	0.50	500	60.1	63.4	59.7	58.7	66.3	69.5	81.9	72.6	70.5	72.1
19	40	0.04	0.15	500	30.2	41.1	36.7	38.4	34.1	38.5	30.5	36.7	35.58	40.7

Table S1. Experiments and responses of the HAAs in the 2^4 full factorial design.

Table S2. ANOVA test results obtained from the first (2^4) full factorial design for IQ response (model analyte).

Source	Sum of Squares	df^a	Mean Square	F_{value}^b	P_{value}^c	Prob> F
Model	4834.67	3	1611.56	78.19	<0.0001	*significant*
A	4166.70	1	4166.70	202.1	<0.0001	
B	488.41	1	488.41	23.70	<0.0002	
C	179.56	1	179.56	8.71	<0.0105	
Curvature	14.15	1	14.15	0.69	0.4212	*not significant*
Residual	288.55	14	20.61			
Lack of Fit	273.47	12	22.79	3.02	0.2754	*not significant*
Pure error	15.09	2	7.54			
Cor total	5137.38	18				

[a] Degrees of freedom; [b] Test for comparing model variance with residual (error) variance; [c] Probability of seeing the observed F value if the null hypothesis is true.

Table S3. ANOVA test results obtained from the first full (2^4) factorial design for Trp-P-2 response (model analyte).

Source	Sum of Squares	df^a	Mean Square	F_{value}^b	P_{value}^c	Prob> F
Model	3059.53	3	1019.84	17.52	<0.0001	*significant*
A	1870.56	1	1870.56	32.14	<0.0001	
B	915.06	1	915.06	15.72	<0.0001	
C	273.90	1	273.90	4.71	0.0478	
Curvature	142.26	1	142.26	2.44	0.1403	*not significant*
Residual	814.86	14	58.20			
Lack of Fit	785.55	12	65.46	4.47	0.1973	*not significant*
Pure error	29.31	2	14.65			
Cor total	4016.65	18				

[a] Degrees of freedom; [b] Test for comparing model variance with residual (error) variance; [c] Probability of seeing the observed F value if the null hypothesis is true.

Table S4. Experiments and responses of the HAAs in the 2^3 full factorial design.

Run	A ACN (%)	B HCOOH (mM)	C Elution flow rate (mL min^{-1})	ERs (%) DMIP	IQ	MeIQ	MeIQx	4,8-DiMeIQx	PhIP	Trp-P-1	AαC	MeAαC	Trp-P-2
1	80.00	0.06	0.80	99.337	95.38	92.29	98.04	96.79	100.21	90.32	97.25	96.1	93.5
2	60.00	0.03	0.50	80.167	83.02	80.04	89.38	86.95	91.06	80.18	88.09	86.8	81.74
3	80.00	0.00	0.80	85.558	80.75	82.89	93.32	85.87	95.65	81.8	88.09	82.21	84.53
4	40.00	0.06	0.20	72.6896	73.96	68.05	76.05	80.21	80.1	67.1	92.42	76.55	70.1
5	40.00	0.06	0.80	76.524	72.89	65.08	74.76	75.82	79.17	63.8	78.97	72.7	68.83
6	60.00	0.03	0.50	77.501	82.12	76.45	90.18	93.9	89.08	78.6	87.55	80.99	78.16
7	40.00	0.00	0.80	62.89	54.2	63.01	66.27	68.9	72.61	60.6	68.57	60.89	62.57
8	80.00	0.00	0.20	88.412	82.93	85.85	90.52	84.72	86.51	79.3	90.49	84.46	86.94
9	80.00	0.06	0.20	96.611	98.27	91.14	99.34	99.22	101.4	93.8	98.1	95.86	95.66
10	60.00	0.03	0.50	79.4029	90.58	84.48	93.87	85.2	93.57	86.6	89.08	89.8	85.1
11	60.00	0.03	0.50	76.912	76.55	84.92	91.58	94.13	92.16	87.1	87.89	78.96	86
12	40.00	0.00	0.20	63.1233	53.4	64.79	62.27	70.39	70.59	56.9	69.6	63.5	63.19
13	60.00	0.03	0.50	78.757	81.15	79.76	82.02	81.12	89.54	89.6	86.74	89.9	80.64

Source	Sum of Squares	df [a]	Mean Square	F_{value}[b]	P_{value}[c]	Prob> F
Model	1921.96	2	960.98	71.50	<0.0001	*significant*
A	1323.04	1	1323.04	98.44	<0.0001	
B	598.93	1	598.93	44.56	<0.0002	
Curvature	118.72	1	118.72	8.83	0.0157	*significant*
Residual	120.97	9	13.44			
Lack of Fit	18.21	5	3.64	0.14	0.2754	*not significant*
Pure error	102.76	4	25.69			
Cor total	2161.65	12				

Table S5. ANOVA test results for the IQ response obtained in the second (2^3) full factorial design.

[a] Degrees of freedom; [b] Test for comparing model variance with residual (error) variance; [c] Probability of seeing the observed F value if the null hypothesis is true.

Source	Sum of Squares	df[a]	Mean Square	F_{value}[b]	P_{value}[c]	Prob> F
Model	1269.60	2	634.80	113.08	<0.0001	*significant*
A	1150.56	1	1150.56	204.95	<0.0001	
B	119.04	1	119.04	21.21	<0.0002	
Curvature	53.32	1	53.32	9.50	0.0131	*significant*
Residual	50.52	9	5.61			
Lack of Fit	8.79	5	1.76	0.17	0.9613	*not significant*
Pure error	41.73	4	10.43			
Cor total	1373.45	12				

Table S6. ANOVA test results for Trp-P-2 response obtained of the second (2^3) full factorial design.

[a] Degrees of freedom; [b] Test for comparing model variance with residual (error) variance; [c] Probability of seeing the observed F value if the null hypothesis is true.

Table S7. Experiments and responses of the HAAs in the DCC.

A	B	ERs (%)									
ACN (%)	HCOOH (mM)	DMIP	IQ	MeIQ	MeIQx	4,8-DiMeIQx	PhIP	AαC	MeAαC	Trp-P-1	Trp-P-2
80.0	15.9	98.4	95.6	90.5	93.7	97.1	97.3	98.5	98.2	96.7	94.6
60.0	8.5	83.6	88.2	80.5	83.5	91.3	92.3	95.5	93.8	97.9	96.6
40.0	1.1	60.5	66.1	62.4	66.9	68.2	98.2	93.3	82.2	91.4	99.8
60.0	0.0	62.6	57.8	64.7	67.5	63.9	87.0	81.5	64.2	85.6	86.8
60.0	8.5	80.9	84.7	80.2	86.9	80.9	81.7	84.1	82.2	85.5	81.8
24.6	8.5	72.2	77.8	74.5	78.8	73.1	72.5	76.7	72.9	76.6	78.2
60.0	8.5	89.9	81.9	83.0	85.8	89.2	95.0	96.1	98.9	92.1	93.3
60.0	8.5	81.3	81.8	87.8	83.6	92.8	94.6	92.7	81.6	86.0	84.2
60.0	21.0	87.1	83.6	85.8	83.6	91.5	96.1	96.1	95.9	97.5	94.5
40.0	15.9	75.5	94.8	90.8	85.6	91.4	87.7	89.4	75.4	88.8	82.0
80.0	1.1	78.6	86.7	81.9	79.3	73.3	43.4	59.0	71.6	63.5	51.6
60.0	8.5	82.5	84.4	88.9	82.0	87.4	66.2	77.4	85.8	83.1	79.9

Table S8. CCD fitting models for HAAs analysis in surface water samples.

Response	Model	R^2	R^2adj	Transformation	Significant terms (x)	ANOVA p-value [a]	
						Model	Lack of fit
R1-DMIP	Quadratic	0.9235	0.8980	None*	A - B - B^2	<0.0001	0.5854
R2-IQ	*	0.8804	0.8207	*	A -B - AB - B^2	<0.0001	0.0650
R3-MeIQ	*	0.9268	0.8902	*	A -B - AB - B^2	<0.0001	0.8091
R4-MeIQx	*	0.9525	0.9367	*	A - B - B^2	<0.0001	0.4592
R5-4,8-DiMeIQx	*	0.9030	0.8707	*	A - B - B^2	<0.0001	0.6998
R6-PhIP	*	0.7670	0.6505	*	A -B - AB - A^2	<0.0120	0.7833
R7-AαC	*	0.8553	0.7830	*	A -B - AB - A^2	<0.019	0.8326
R8- MeAαC	*	0.7002	0.6002	*	A -B - AB - A^2	<0.0287	0.6070
R9-Trp-P-1	*	0.8852	0.8278	*	A -B - AB - A2	<0.0001	0.7340
R10-Trp-P-2	*	0.8716	0.8074	*	A -B - AB - A^2	<0.0012	0.8282

A: ACN (%) in ACN/H$_2$O mixture elution; B: HCOOH concentration; [a] p-values less than 0.050 indicate significance; * applied to each Response

Table S9. Analytical yields of HAAs in surface water samples by MWCNTs-SPE as clean-up and preconcentration strategy.

Surface water sample			*Cosquin River*				*San Roque Reservoir*			
HAAs	Sample Concentration (µg L-1)	Added (µg L^{-1})	Found (µg L^{-1})	Recovery (%)	RSD (%) *n=3*	Sample Concentration (µg L^{-1})	Added (µg L^{-1})	Found (µg L^{-1})	Recovery (%)	RSD (%) *n=3*
DMIP	N.D.*	0.0	-	-	-	*	0.0	-	-	-
		0.5	0.51	102.0	2.6		0.5	0.53	106.0	3.7
		1.0	1.05	105.0	3.8		1.0	0.98	98.0	3.1
		2.0	1.95	97.5	5.2		2.0	2.00	100.0	3.4
IQ	*	0.0	-	-	-	0.48[a]	0.0	0.48[a]	-	-
		0.5	0.48[a]	96.0	6.7		0.5	0.49	98.9	8.3
		1.0	0.97	97.0	8.9		1.0	1.40	94.6	4.3
		2.0	2.02	101.0	6.0		2.0	2.47	99.6	6.2
MeIQ	0.56	0.0	0.56	-	-	0.92	0.0	0.92	-	-
		0.5	1.03	97.2	5.1		0.5	1.43	100.7	2.9
		1.0	1.46	93.6	5.8		1.0	1.92	100.0	6.4
		2.0	1.85	92.5	1.8		2.0	1.97	98.5	4.2
MeIQx	0.24	0.0	0.24	-	-	0.71	0.0	0.71	-	-
		0.5	0.71	95.9	6.9		0.5	1.19	98.3	3.0
		1.0	1.20	96.8	2.5		1.0	1.70	99.4	6.1
		2.0	1.97	98.5	5.6		2.0	1.99	99.5	5.0
4,8-DiMeIQx	0.16[a]	0.0	0.16	-	-	0.62	0.0	0.62	-	-
		0.3	0.63	94.7	5.3		0.5	1.12	100.0	5.9
		1.0	1.11	95.8	2.6		1.0	1.64	101.2	6.2
		5.0	4.96	99.2	3.7		5.0	4.99	99.8	4.8
PhIP	*	0.0	-	-	-	0.56	0.0	0.56	-	-
		0.5	0.49	98.0	2.82		0.5	0.96	90.6	6.3
		1.0	0.99	99.0	7.20		1.0	1.45	92.9	5.1
		2.0	1.97	98.5	3.00		2.0	1.99	99.5	8.1
Trp-P-1	0.32	0.0	0.32	-	-	0.93	0.0	0.93	-	-
		0.5	0.81	98.8	6.8		0.5	1.46	102.1	4.8
		1.0	1.30	98.5	3.4		1.0	2.02	104.7	2.6
		5.0	5.03	100.6	2.7		5.0	5.29	105.8	7.7
AαC	0.21	0.0	0.21	-	-	0.37	0.0	0.37	-	-
		0.5	0.69	97.2	5.2		0.5	0.88	101.1	6.4
		1.0	1.18	97.5	6.9		1.0	1.34	97.8	4.0
		2.0	1.99	99.5	7.4		2.0	1.97	98.5	4.9
Trp-P-2	0.51	0.0	0.51	-	-	0.76	0.0	0.76	-	-
		0.5	0.98	97.0	5.3		0.5	1.20	95.2	4.8
		1.0	1.48	98.0	4.3		1.0	1.74	98.8	2.3
		2.0	2.03	101.5	2.4		2.0	1.95	97.5	4.8
MeAαC	0.35[a]	0.0	0.35	-	-	0.56	0.0	0.56	-	-
		0.5	0.86	101.2	5.9		0.5	1.04	98.1	4.7
		1.0	1.42	105.2	4.8		1.0	1.59	101.9	6.3
		2.0	1.98	99.0	3.7		2.0	1.98	99.0	2.9

N.D.*: not detected; [a] <LOQ.