Supplementary Material

Evaluation of environmentally relevant nitrated and oxygenated polycyclic aromatic hydrocarbons in honey

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Table S1. Structures of environmentally relevant nitrated and oxygenated polycyclic aromatic hydrocarbons.

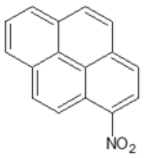
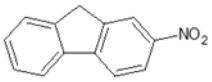
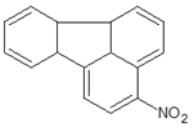
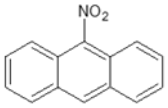
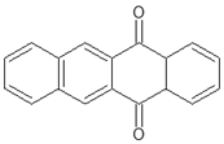
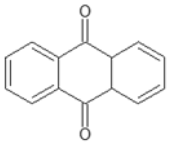
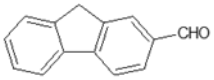
Compound	Empirical formula	Structure	Abbreviation	Molecular Weight (g mol $^{-1}$)
1-Nitropyrene	$C_{16}H_9NO_2$		1-NPYR	247.25
2-Nitrofluorene	$C_{13}H_9NO_2$		2-NFLU	211.22
3-Nitrofluoranthene	$C_{16}H_9NO_2$		3-NFLUANT	247.25
9-Nitroanthracene	$C_{14}H_9NO_2$		9-NANTHR	223.23
5,12-Naphthacenequinone	$C_{18}H_{10}O_2$		5,12-NAPHTONA	258.27
9,10-Anthracenequinone	$C_{14}H_8O_2$		9,10-ANTHRONA	208.21
2-Fluorenecarboxaldehyde	$C_{14}H_{10}O$		2-FLUCHO	194.23

Table S2. Optimized MRM scanning mode mass spectrometer conditions for NPAH and OPAH.

Compound	Cone (V)	Precursor Ion (m/z)	Collision (V)	Product Ion (m/z)
NPAH				
1-NPYR	30	248	16	218
			25	202*
			30	190
2-NFLU	30	212	12	195
			17	165*
3-NFLUANTH	19	248	17	231*
			16	218
			20	190
9-NANTHR	19	224	8	207
			30	178*
OPAH				
5,12-NAPHTONA	10	259	35	242
			21	231
			23	203*
9,10-ANTHRONA	35	209	20	181
			20	153*
2-FLUCHO	32	195	16	167*

* Transition selected for quantification

Table S3. Variables and levels used in the 2^4 Full Factorial Design.

Factor	Variable	Levels		
		-1	**0**	**+1**
A	Acetonitrile volume (mL)	1	5	9
B	Water volume (mL)	5	10	15
C	NaCl concentration (%, w/v)	20	35	50
D	Ultrasound-assisted agitation time (min)	0	15	30

Table S4. Variables and levels used in the Central Composite Design.

Factor	Variable	Levels		
		-1	**0**	**+1**
A	Acetonitrile volume (mL)	5	10	15
B	Water volume (mL)	10	20	30

Table S5. Penalty Points (PPs) considering the Analytical Eco-Scale.

Category	Sub-total PPs		Total PPs
Reagents			
Amount	<10 mL (<10 g)	1	PPs quantity x PPs denomination
	10-100 mL (10-100 g)	2	
	>100 mL (>100 g)	3	
Hazards	Nonexistent	0	
	Mild	1	
	High	2	
Instrument			
Energy	<0.1 kWh per sample		0
	<1.5 kWh per sample		1
	>1.5 kWh per sample		2
Risk	No emission of vapors into the atmosphere		0
	Emission of vapors into the atmosphere		3
Waste	Nonexistent		0
	<1 mL (<1 g)		1
	1-10 mL (1-10 g)		3
	>10 mL (>10 g)		5
	Recycled		0
	Degradation		1
	Inactivate		2
	No treatment		3

Table S6. ANOVA chart of statistical effects.

Compound	*Response Transformation*	*Modeling*	p[a]-value		$R^2_{adj.}$	*CV (%)*
			Modeling	*Lack of adjustment*		
1-NPYR	Natural Logarithm	Quadratic	0.0062	0.9045	0.9073	1.7
2-NFLU	Base 10 Logarithm	Linear	0.0068	0.9140	0.9585	1.8
3-NFLUANTH	Natural Logarithm	Quadratic	< 0.0001	0.9484	0.9935	0.4
9-NANTHR	Natural Logarithm	Linear	< 0.0001	0.0818	0.9493	1.5
5.12-NAPHTONE	Natural Logarithm	Quadratic	< 0.0001	0.9022	0.9805	0.6
9.10-ANTHRONE	Natural Logarithm	Quadratic	< 0.0001	0.8949	0.9905	0.5
2-FLUCHO	Natural Logarithm	Quadratic	< 0.0001	0.8523	0.9801	0.6

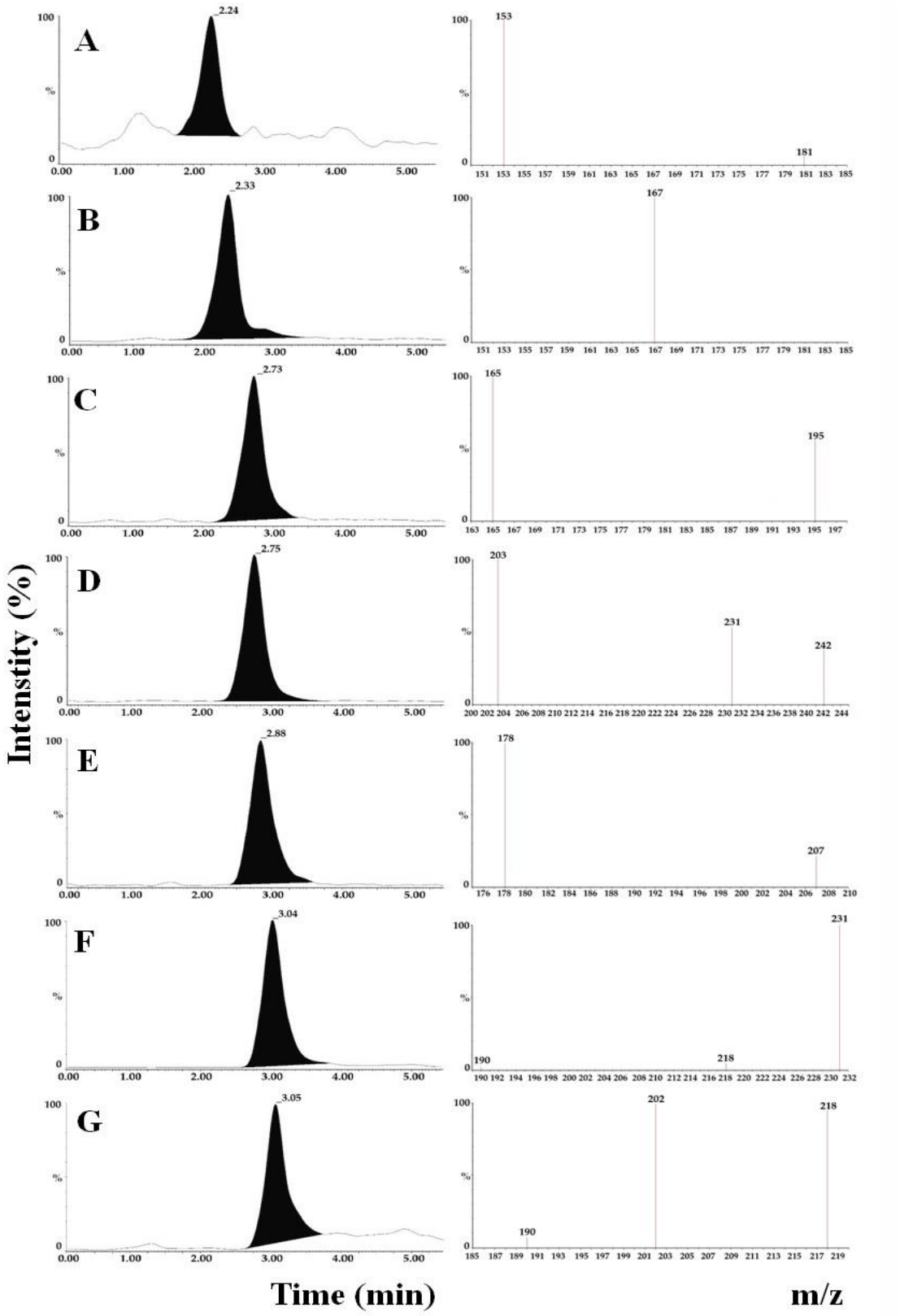


Figure S1. Chromatograms and mass spectra of products ions for NPAH and OPAH compounds for UHPLC- (+)APCI-MS/MS optimal conditions, (A) 9,10-ANTHRONA (Retention time, Rt: 2.24 min); (B) 2-FLUCHO (Rt: 2.36 min); (C) 2-NFLU (Rt: 2.73 min); (D) 5,12-NAPHTONE (Rt: 2.75 min); (E) 9-NANTHR (Rt: 2.88 min); (F) 3-NFLUANTH (Rt: 3.04 min), and (G) 1-NPYR (Rt: 3.05 min).

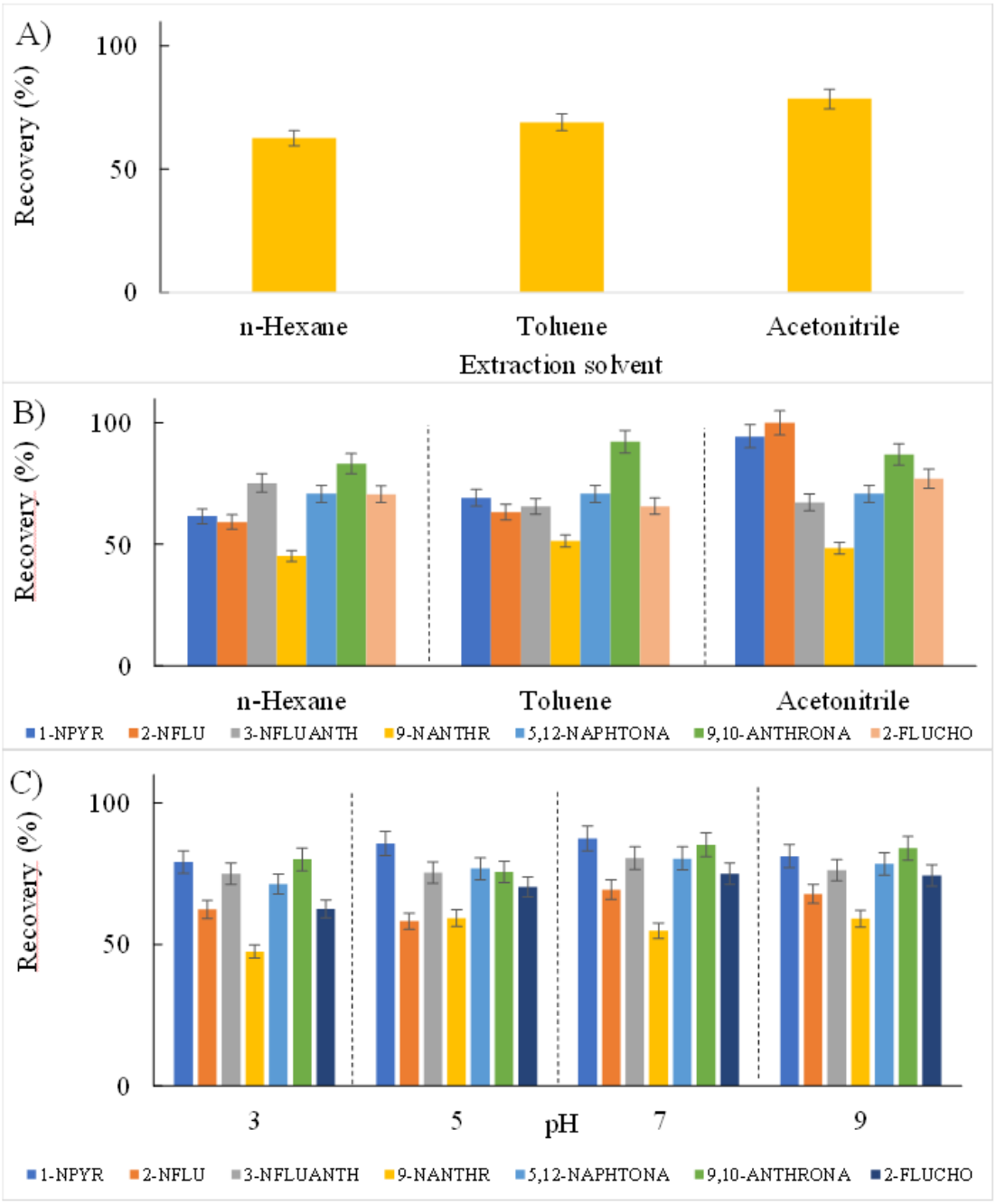


Figure S2. Optimization of the nature of the solvent (A), ionic strength effects (B), and pH (C) for the SALLE procedure.

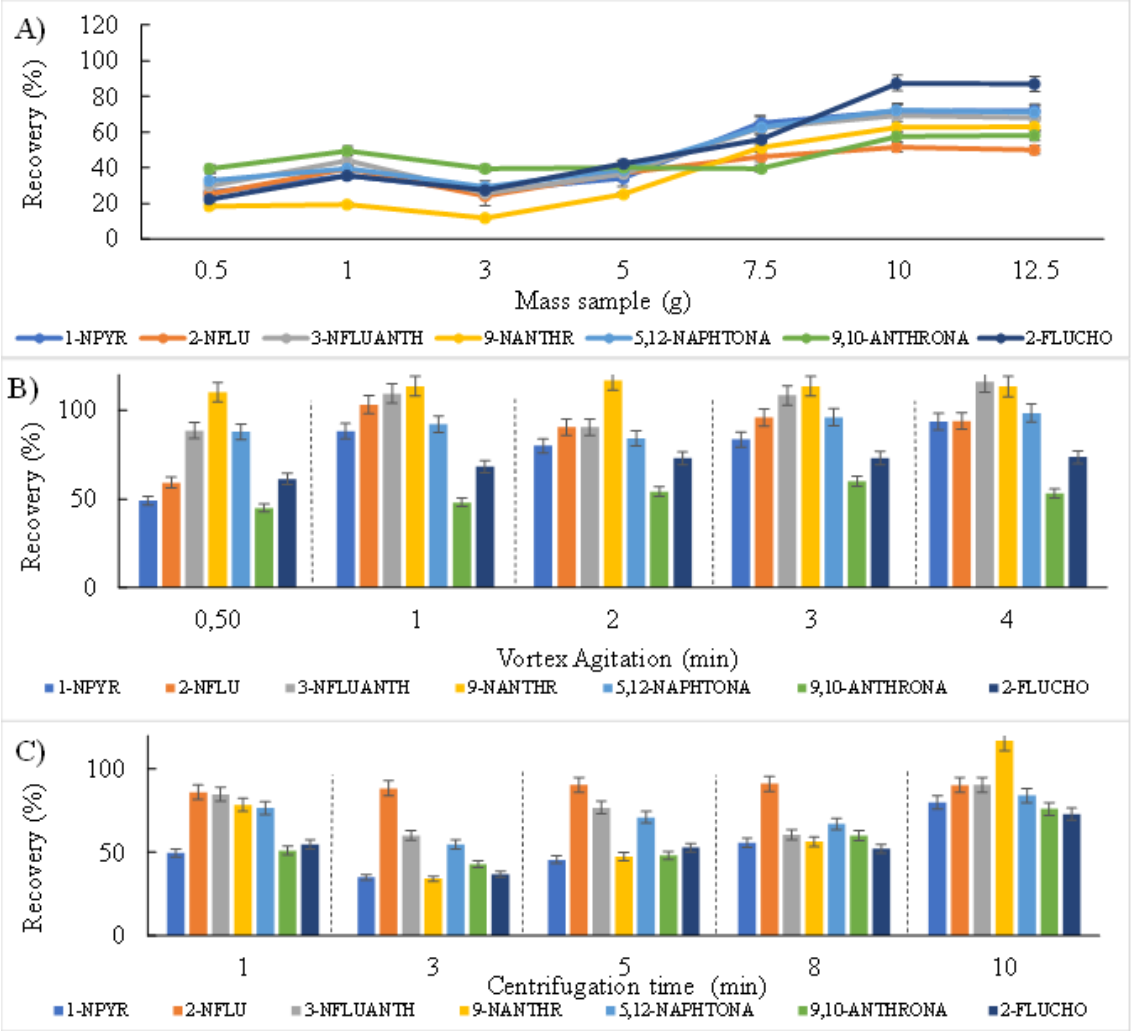


Figure S3. Optimization of vortex agitation (**A**), centrifugation time (**B**), and mass sample (**C**) for the SALLE procedure.

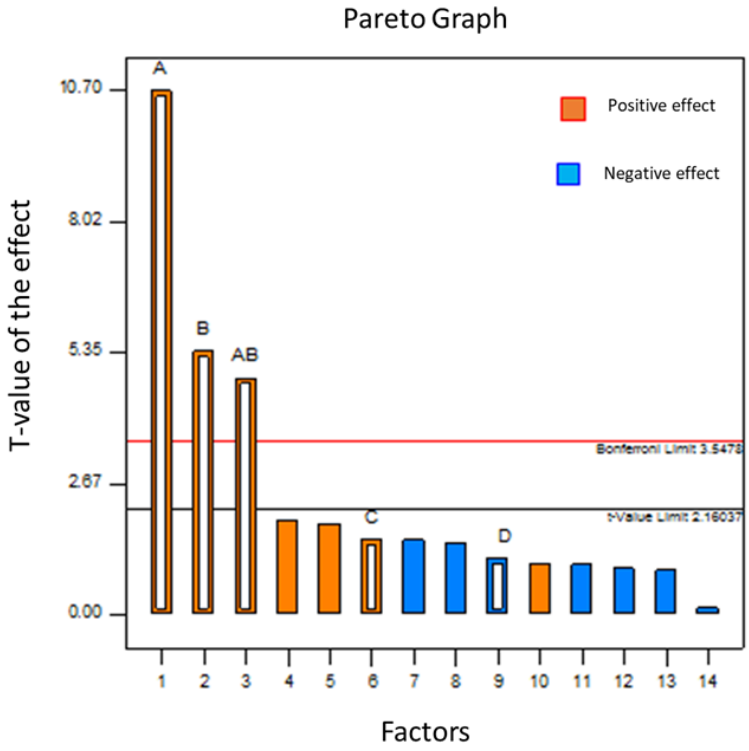


Figure S4. Pareto Chart of the main effects in the SALLE procedure for the simultaneous extraction of NPAHs and OPAHs. A: Volume of water. B: Volume of acetonitrile. AB: interaction between water and acetonitrile. C: NaCl concentration. D: Ultrasound extraction time

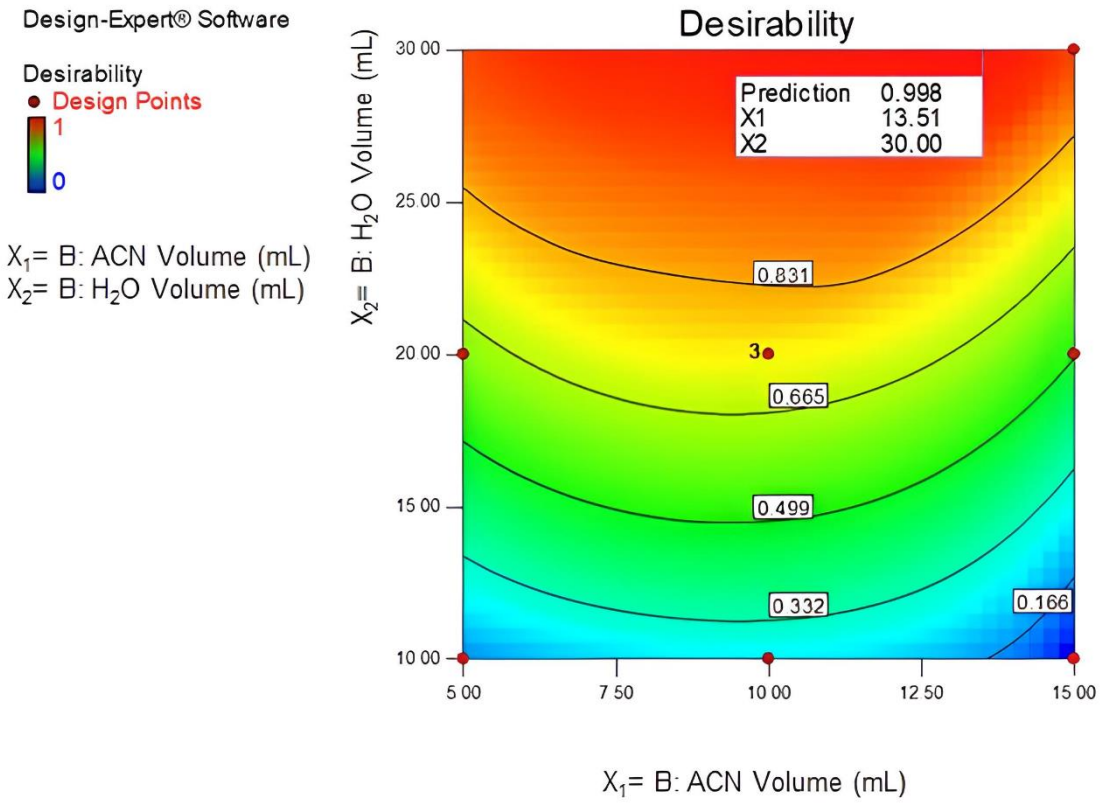


Figure S5. Representation of the desirability function for the optimized variables.