

Supplementary Material

**Analytical Method Development for Multiresidue Determination in Water Using
Automated Parallel Dispersive Pipette Extraction and HPLC-DAD**

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TABLES:

Table S1: Structures and physicochemical properties of the studied analytes.

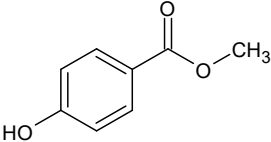
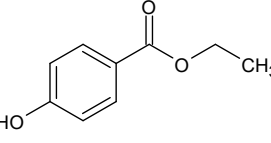
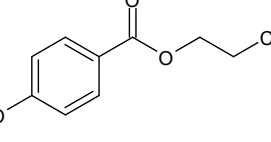
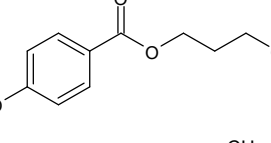
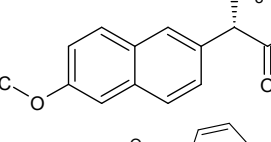
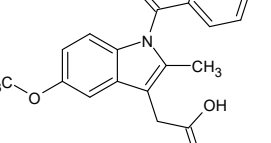
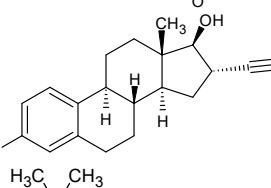
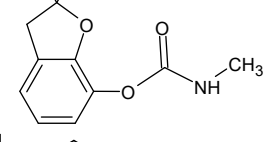
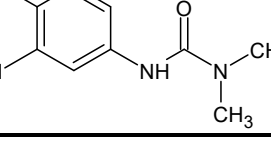
Analytes	Chemical Structure	log K _{ow}	pKa	Classification	Typical Uses	Reference
Methylparaben (MP)		1.96	8.17	Preservative	Preservative in cosmetics and pharmaceuticals	1
Ethylparaben (EtP)		2.47	8.22	Preservative	Preservative in personal care products	1
Propylparaben (PrP)		3.04	8.35	Preservative	Preservative in cosmetics	1
Butylparaben (BuP)		3.57	8.37	Preservative	Preservative in industrial products	1
Naproxen (NAP)		3.18	4.15	Pharmaceutical	NSAID used for pain and inflammation	2
Indomethacin (IND)		4.27	4.5	Pharmaceutical	NSAID used for anti-inflammatory treatment	2
17 α -ethinylestradiol (EE2)		4.12	10.46	Hormone	Synthetic hormone used in contraceptives	3
Carbofuran (CF)		1.76	10.09	Pesticide	Insecticide used in agriculture	4
Diuron (DIU)		2.68	13.55	Pesticide	Herbicide used for weed control	4

Table S2: Robustness table, conditions, variations and parameters.

Effect	Nominal Condition	Variation (+)	Variation (-)	Parameter
Extraction Cycles	9	10	8	a
Desorption Cycles	3	4	2	b
Sample Volume	1500 μ L	1525 μ L	1475 μ L	c
Desorption Solvent Volume	200 μ L	210 μ L	190 μ L	d
pH	8.0	8.2	7.8	e
Flow	1 mL/min	1.1 mL/min	0.9 mL/min	f
Extractor Phase Mass	20 mg	21 mg	19 mg	g

Table S3: Robustness table experiments.

Exp	Ext	Des	Sample Vol	Desorption Vol	pH	Flow	Extractor Phase Mass
1	10	4	1525	190	8.2	0.9	19
2	8	4	1525	210	7.8	1.1	19
3	8	2	1525	210	8.2	0.9	21
4	10	2	1475	210	8.2	1.1	19
5	8	4	1475	190	8.2	1.1	21
6	10	2	1525	190	7.8	1.1	21
7	10	4	1475	210	7.8	0.9	21
8	8	2	1475	190	7.8	0.9	19

Statistical Test Data for the Selection of Desorption Solvent

Table S4: Desorption solvent selection statistics.

ANOVA						
Model	Effect SS	df	Effect MS	F	p-value	R ²
Linear	1.22E+11	2	6.12E+10	268.69	<1E-6	0.987
Quadratic	1.16E+09	3	3.87E+08	3.57	0.125	0.996
Total	1.24E+11	9	1.38E+10			

Statistical Test Data for the Selection of Desorption Cycles

Table S5: Statistics for the selection of desorption cycles.

ANOVA						
Model	Effect SS	df	Effect MS	F	Critical F	p-value
Linear	3.23E+10	6	5.38E+09	8.836	2.848	4.18E-04
Quadratic	8.52E+09	14	6.08E+08			
Total	4.08E+10	20				

Comparison using Tukey's test		
Desorption cycles	Desorption cycles	p-value
1 Cycle	2 Cycles	0.03806
	3 Cycles	0.00026
	4 Cycles	0.04031
	5 Cycles	0.01487
	6 Cycles	0.00056
	7 Cycles	0.09532
	3 Cycles	0.13653
2 Cycles	4 Cycles	1.00000
	5 Cycles	0.99810
	6 Cycles	0.28147
	7 Cycles	0.99802
3 Cycles	4 Cycles	0.12952
	5 Cycles	0.30262
	6 Cycles	0.99893
4 Cycles	7 Cycles	0.05578
	5 Cycles	0.99735
	6 Cycles	0.26878
5 Cycles	7 Cycles	0.99861
	6 Cycles	0.54146
6 Cycles	7 Cycles	0.93561
6 Cycles	7 Cycles	0.12472

Analysis of significant groups for desorption cycles using Tukey's test		
Desorption cycles	Mean	Group
3 Cycles	476900.7813	A
6 Cycles	467496.5989	A
5 Cycles	430311.6489	A
2 Cycles	419880.6776	A
4 Cycles	419237.7321	A
7 Cycles	409375.1698	A, B
1 Cycle	348042.3954	B

Statistical Test Data for the Selection of Cleaning Cycles

Table S6: Statistics for the selection of cleaning cycles using the geometric mean of chromatographic peak areas.

ANOVA						
Model	Effect SS	df	Effect MS	F	Critical F	p-value
Linear	1.65E+06	3	5.49E+05	88.301	6.59E+00	0.00041
Quadratic	2.49E+04	4	6.22E+03			
Total	1.67E+06	7				

Comparison using Tukey's test		
Cleaning cycles	Cleaning cycles	p-value
	2 Cycles	0.14534
1 Cycle	3 Cycles	0.00079
	4 Cycles	0.00074
2 Cycles	3 Cycles	0.00210
	4 Cycles	0.00196
3 Cycles	4 Cycles	0.99746

Analysis of significant groups for cleaning cycles using Tukey's test		
Desorption cycles	Mean	Group
1 Cycle	1013.71644	A
2 Cycles	790.38514	A
3 Cycles	15.4485	B
4 Cycles	1	B

Statistical Test Data for the Selection of pH

Table S7: Statistics for the selection of pH using the geometric mean of chromatographic peak areas.

ANOVA						
Model	Effect SS	df	Effect MS	F	Critical F	p-value
Linear	2.46E+10	4	6.15E+09	12.877	3.478	5.89E-04
Quadratic	4.78E+09	10	4.78E+08			
Total	2.94E+10	14				

Comparison using Tukey's test		
pH	pH	p-value
	pH 4	0.00919
pH 2	pH 6	0.00161
	pH 8	0.00044
	pH 10	0.02921
	pH 6	0.73954
pH 4	pH 8	0.24593
	pH 10	0.93709
	pH 8	0.84871
pH 6	pH 10	0.33995
	pH 10	0.08055

Analysis of significant groups for pH using Tukey's test		
pH	Mean	Group
pH 8	480893.829	A
pH 6	463012.0021	A
pH 4	441191.9738	A
pH 10	427670.7078	A
pH 2	362766.1927	B

Table S8: Statistics for the selection of pH for 17 α -ethinylestradiol.

ANOVA						
Model	Effect SS	df	Effect MS	F	Critical F	p-value
Linear	1.72E+08	4	4.30E+07	11.66	3.478	8.78E-04
Quadratic	3.69E+07	10	3.69E+06			
Total	2.94E+10	14				

Comparison using Tukey's test		
pH	pH	p-value
	pH 4	0.00062
pH 2	pH 6	0.09406
	pH 8	0.0226
	pH 10	0.6293
	pH 6	0.03794
pH 4	pH 8	0.15539
	pH 10	0.0042
	pH 8	0.88207
pH 6	pH 10	0.60691
	pH 10	0.19719

Analysis of significant groups for pH using Tukey's test		
pH	Mean	Group
pH 4	48497.33333	A
pH 8	44505	A, B
pH 6	43059.66667	B, C
pH 10	40770.33333	B, C
pH 2	38543	C

Table S9: Statistics for the selection of pH for carbofuran.

ANOVA						
Model	Effect SS	df	Effect MS	F	Critical F	p-value
Linear	3.39E+08	4	8.47E+07	5.319	3.478	0.0147
Quadratic	1.59E+08	10	1.59E+07			
Total	4.98E+08	14				

Comparison using Tukey's test		
pH	pH	p-value
pH 2	pH 4	0.0198
	pH 6	0.1227
	pH 8	0.0351
	pH 10	0.7437
pH 4	pH 6	0.7601
	pH 8	0.9953
	pH 10	0.1285
pH 6	pH 8	0.9208
	pH 10	0.5898
pH 8	pH 10	0.2205

Analysis of significant groups for pH using Tukey's test

pH	Mean	Group
pH 4	87580	A
pH 8	86369.66667	A
pH 6	83720.66667	A, B
pH 10	78862.33333	A, B
pH 2	74902.66667	B

Table S10: Statistics for the selection of pH for methylparaben.

ANOVA						
Model	Effect SS	df	Effect MS	F	Critical F	p-value
Linear	3.37E+10	4	8.42E+09	2.422	3.478	0.11695
Quadratic	3.48E+10	10	3.48E+09			
Total	6.84E+10	14				

Comparison using Tukey's test		
pH	pH	p-value
pH 2	pH 4	0.2336
	pH 6	0.3114
	pH 8	0.1953
	pH 10	0.9818
pH 4	pH 6	0.9995
	pH 8	0.9999
	pH 10	0.4594
pH 6	pH 8	0.9966
	pH 10	0.5754
pH 8	pH 10	0.3962

Analysis of significant groups for pH using Tukey's test		
pH	Mean	Group
pH 8	904402.33333	A
pH 4	898296.66667	A
pH 6	888017	A
pH 10	815028.66667	A
pH 2	789409	A

Table S11: Statistics for the selection of pH for ethylparaben.

ANOVA						
Model	Effect SS	df	Effect MS	F	Critical F	p-value
Linear	3.20E+10	4	8.00E+09	3.346	3.478	0.05523
Quadratic	2.39E+10	10	2.39E+09			
Total	5.59E+10	14				

Comparison using Tukey's test		
pH	pH	p-value
pH 2	pH 4	0.1004
	pH 6	0.238
	pH 8	0.1012
	pH 10	0.9423
pH 4	pH 6	0.9735
	pH 8	1
	pH 10	0.291
pH 6	pH 8	0.9743
	pH 10	0.5789
pH 8	pH 10	0.293

Analysis of significant groups for pH using Tukey's test		
pH	Mean	Group
pH 4	847800.66667	A
pH 8	847597.33333	A
pH 6	824237	A
pH 10	763932.33333	A
pH 2	734445.33333	A

Table S12: Statistics for the selection of pH for propylparaben.

ANOVA						
Model	Effect SS	df	Effect MS	F	Critical F	p-value
Linear	3.85E+10	4	9.63E+09	5.236	3.478	0.01545
Quadratic	1.84E+10	10	1.84E+09			
Total	5.69E+10	14				

Comparison using Tukey's test		
pH	pH	p-value
pH 2	pH 4	0.0196
	pH 6	0.1567
	pH 8	0.0381
	pH 10	0.7622
pH 4	pH 6	0.6669
	pH 8	0.9918
	pH 10	0.1209
pH 6	pH 8	0.881
	pH 10	0.6631
pH 8	pH 10	0.2261

Analysis of significant groups for pH using Tukey's test

pH	Mean	Group
pH 4	872066.66667	A
pH 8	857019.66667	A
pH 6	824639.33333	A, B
pH 10	776978.33333	A, B
pH 2	735649	B

Table S13: Statistics for the selection of pH for butylparaben.

ANOVA						
Model	Effect SS	df	Effect MS	F	Critical F	p-value
Linear	2.39E+10	4	5.98E+09	7.133	3.478	0.00554
Quadratic	8.38E+09	10	8.38E+08			
Total	3.23E+10	14				

Comparison using Tukey's test		
pH	pH	p-value
pH 2	pH 4	0.0051
	pH 6	0.1562
	pH 8	0.0214
	pH 10	0.5745
pH 4	pH 6	0.237
	pH 8	0.8679
	pH 10	0.0511
pH 6	pH 8	0.7003
	pH 10	0.8383
pH 8	pH 10	0.2154

Analysis of significant groups for pH using Tukey's test

pH	Mean	Group
pH 4	643132.33333	A
pH 8	620503.66667	A
pH 6	589898	A, B
pH 10	565655.33333	A, B
pH 2	529771.66667	B

Table S14: Statistics for the selection of pH for diuron.

ANOVA						
Model	Effect SS	df	Effect MS	F	Critical F	p-value
Linear	1.14E+10	4	2.85E+09	6.196	3.478	0.00896
Quadratic	4.60E+09	10	4.60E+08			
Total	1.60E+10	14				

Comparison using Tukey's test		
pH	pH	p-value
pH 2	pH 4	0.0083
	pH 6	0.12
	pH 8	0.0213
	pH 10	0.4091
pH 4	pH 6	0.4506
	pH 8	0.9674
	pH 10	0.1359
pH 6	pH 8	0.7925
	pH 10	0.8941
pH 8	pH 10	0.3268

Analysis of significant groups for pH using Tukey's test

pH	Mean	Group
pH 4	476120.66667	A
pH 8	465153	A
pH 6	445513.33333	A, B
pH 10	429912.66667	A, B
pH 2	397852	B

Table S15: Statistics for the selection of pH for indomethacin.

ANOVA						
Model	Effect SS	df	Effect MS	F	Critical F	p-value
Linear	2.05E+11	4	5.14E+10	80.68	3.478	<0.0001
Quadratic	6.37E+09	10	6.37E+08			
Total	2.12E+11	14				

Comparison using Tukey's test		
pH	pH	p-value
pH 2	pH 4	0.0355
	pH 6	<0.0001
	pH 8	<0.0001
	pH 10	<0.0001
pH 4	pH 6	<0.0001
	pH 8	<0.0001
	pH 10	0.0007
pH 6	pH 8	0.5806
	pH 10	0.0311
pH 8	pH 10	0.0033

Analysis of significant groups for pH using Tukey's test

pH	Mean	Group
pH 8	472144.33333	A
pH 6	441093.66667	A
pH 10	366969	B
pH 4	239237	C
pH 2	166886	D

Table S16: Statistics for the selection of pH for naproxen.

ANOVA						
Model	Effect SS	df	Effect MS	F	Critical F	p-value
Linear	4.41E12	4	1.10E12	24.32	3.478	<0.0001
Quadratic	4.53E11	10	4.53E10			
Total	4.86E12	14				

Comparison using Tukey's test		
pH	pH	p-value
	pH 4	0.05399
pH 2	pH 6	0.00011
	pH 8	<0.0001
	pH 10	0.00215
	pH 6	0.00695
pH 4	pH 8	0.00256
	pH 10	0.26749
	pH 8	0.9526
pH 6	pH 10	0.18688
	pH 10	0.0655

Analysis of significant groups for pH using Tukey's test		
pH	Mean	Group
pH 8	4000316.33333	A
pH 6	3879205.33333	A
pH 10	3458619.33333	A, B
pH 4	3082646.66667	B, C
pH 2	2519261.33333	C

Statistical Test Data for the Selection of Extraction Cycles and Salt

Table S17: Statistics for the selection of extraction cycles and salt.

ANOVA					
Factor	SS	df	F	p-value	R ²
Extraction Cycles (L)	8.42E+10	1	41.11	0.0985	
Extraction Cycles (Q)	8.30E+09	1	4.05	0.2934	
Salt Percentage (L)	2.17E+11	1	105.86	0.0617	
Salt Percentage (Q)	4.04E+10	1	19.72	0.1410	
Interaction of Factors (L)	3.11E+10	1	15.21	0.1598	
Error	2.05E+09	1			
Total	3.75E+11	6			0.9945

Table S18: Experimental enrichment factor for each analyte.

Analytes	Experimental Enrichment Factor
MP	31.46
EtP	34.50
PrP	36.90
BuP	34.68
NAP	32.17
IND	28.54
EE2	27.56
CF	43.38
DIU	40.40
Average	34.05

$$EF(\text{theoretical}) = \frac{V_{\text{sample}}}{V_{\text{solvent}}} = \frac{13.5}{0.2} = 67.5$$

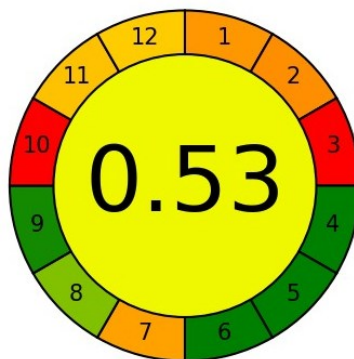
$$EF(\text{experimental}) = \frac{A_{\text{extract}}}{A_{\text{solution}}}$$

Table S19: Criteria and scores' values obtained to AGREE and AGREEprep metrics of the proposed method.

AGREE criteria

1. Direct analytical techniques should be applied to avoid sample treatment – Weight 2 = External sample pre- and treatment and batch analysis (reduced number of steps)
 2. Minimal sample size and minimal number of samples are goals – Weight 2 = 13.5 mL
 3. If possible, measurements should be performed in situ – Weight 2 = Off-line
 4. Integration of analytical processes and operations saves energy and reduces the use of reagents – Weight 2 = 3
 5. Automated and miniaturized methods should be selected – Weight 2 = Automatic and miniaturized
 6. Derivatization should be avoided – Weight 2 = None
 7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided – Weight 2 = 16.5 mL
 8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time – Weight 2 = 9 analytes and 3 samples/h
 9. The use of energy should be minimized – Weight 2 = 0.15 kWh
 10. Reagents obtained from renewable sources should be preferred – Weight 2 = None of the reagents are from bio-based sources
 11. Toxic reagents should be eliminated or replaced – Weight 2 = 2.5 mL
 12. Operator's safety should be increased – Weight 2 = Toxic to aquatic life, bioaccumulative, persistent
-

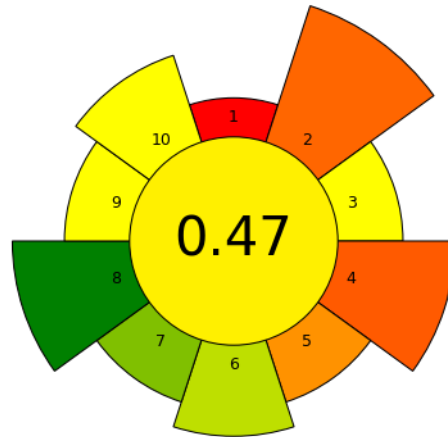
AGREE score



AGREEprep criteria

-
1. Sample preparation placement – Weight 1 = Ex situ
 2. Hazardous materials – Weight 5 = 2.5 mL
 3. Sustainability, renewability, and reusability of materials – Weight 2 = Materials are not sustainable or renewable, but are used SEVERAL TIMES
 4. Waste – Weight 4 = 16.5 mL
 5. Size economy of the sample – Weight 2 = 13.5 mL
 6. Sample throughput – Weight 3 = 14.45 samples/h
 7. Integration and automation – Weight 2 = 3 steps and fully automated systems
 8. Energy consumption – Weight 4 = 1.5 W
 9. Post-sample preparation configuration for analysis – Weight 2 = HPLC with non-MS detection
 10. Operator's safety – Weight 3 = 2 hazard
-

AGREEprep score



FIGURES:

Figure S1: Localization of the coordinates that the samples were collected (Florianópolis – SC).



Statistical Test Data for the Selection of Desorption Solvent

Figure S2: Residuals plot of observed vs. predicted for the quadratic model.

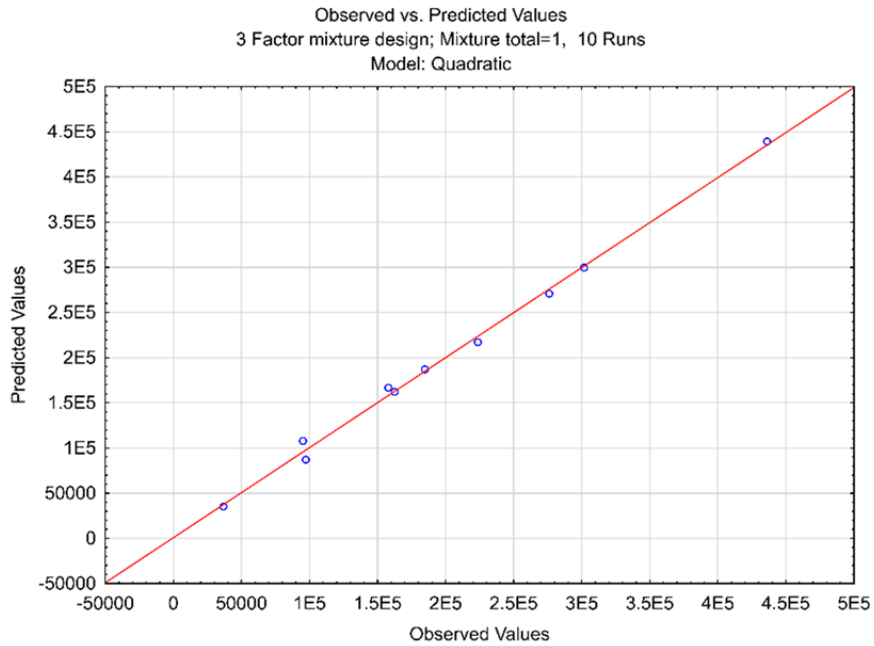


Figure S3: Pareto chart for the quadratic model of desorption solvent.

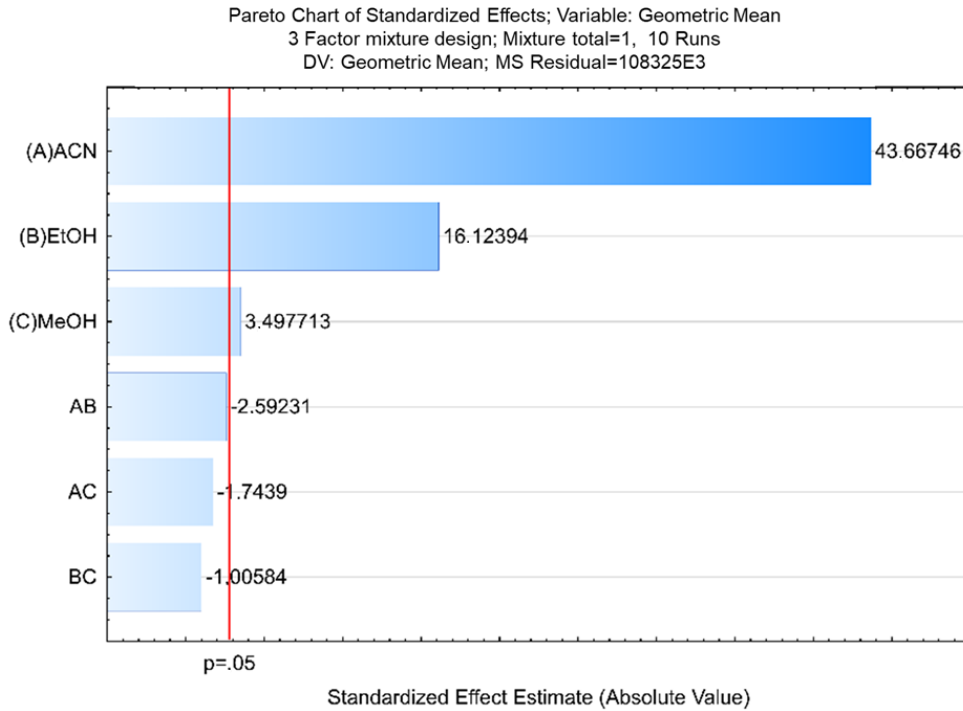
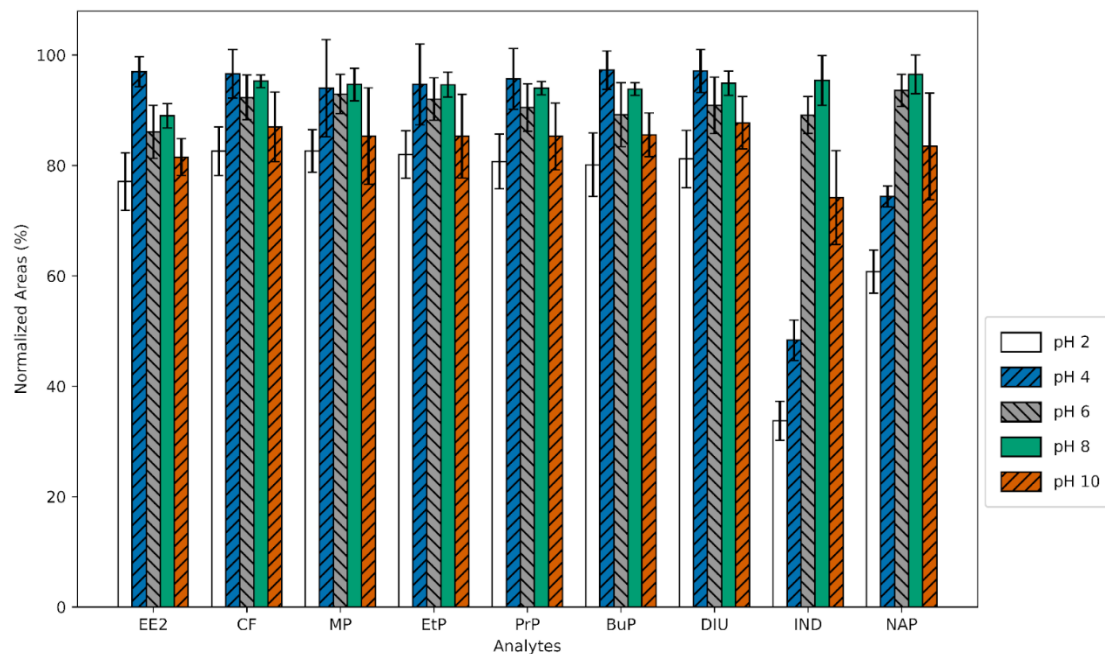


Figure S4: Optimization of pH based on the mean normalized peak areas of each analyte. Fixed Conditions: Conditioning: 2 cycles with 500 μL of MeOH:ACN (1:1 v/v) and 1 cycle with 500 μL of UPW. Extraction: 7 cycles with 1.5 mL of UPW, spiked with 300 $\mu\text{g L}^{-1}$ of the analytes. Desorption: 3 cycles with 200 μL of ACN. Evaluated pH levels: 2, 4, 6, 8 and 10. No salt added.



Statistical Test Data for the Selection of Extraction Cycles and Salting-out Effect

Figure S5: Residuals plot of observed vs. predicted for the quadratic model for extraction cycles and salting-out effect.

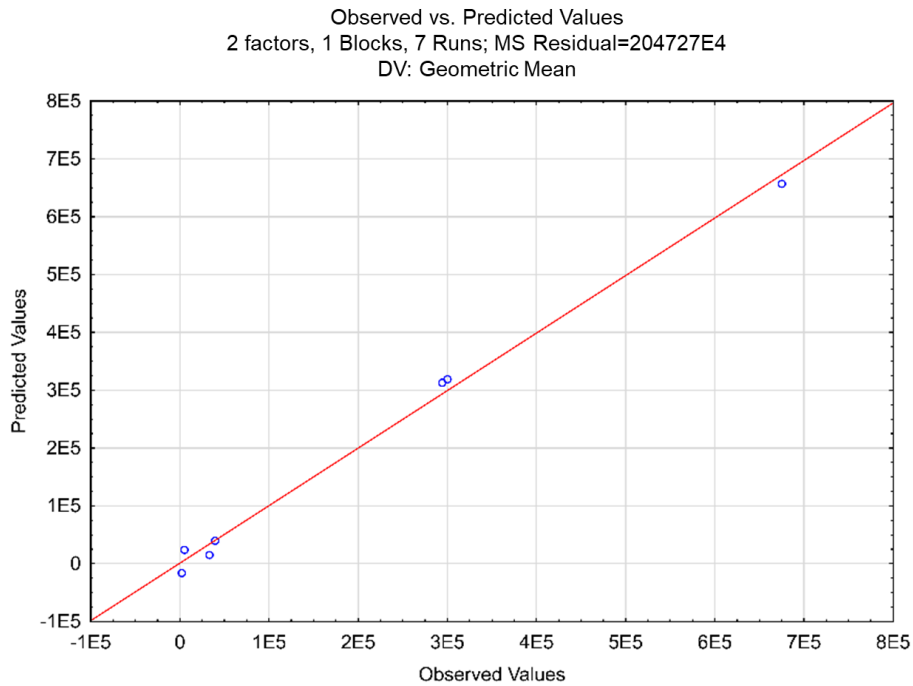


Figure S6: Pareto chart for the quadratic model for extraction cycles and salting-out effect.

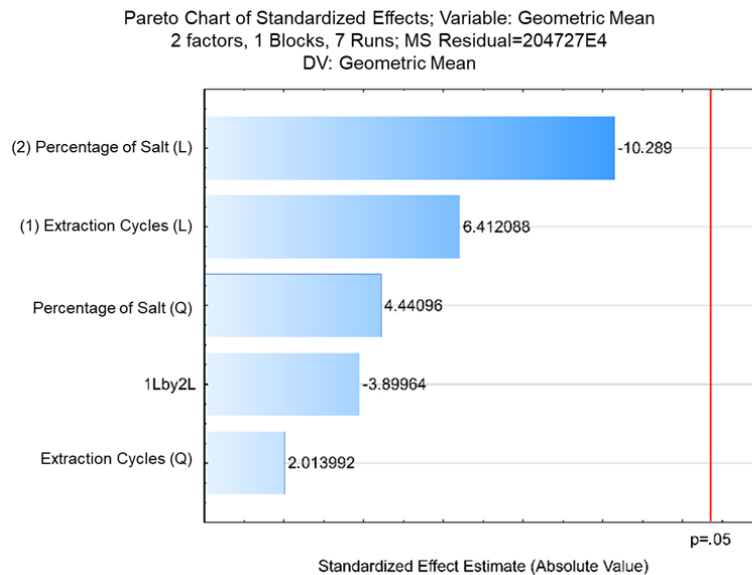
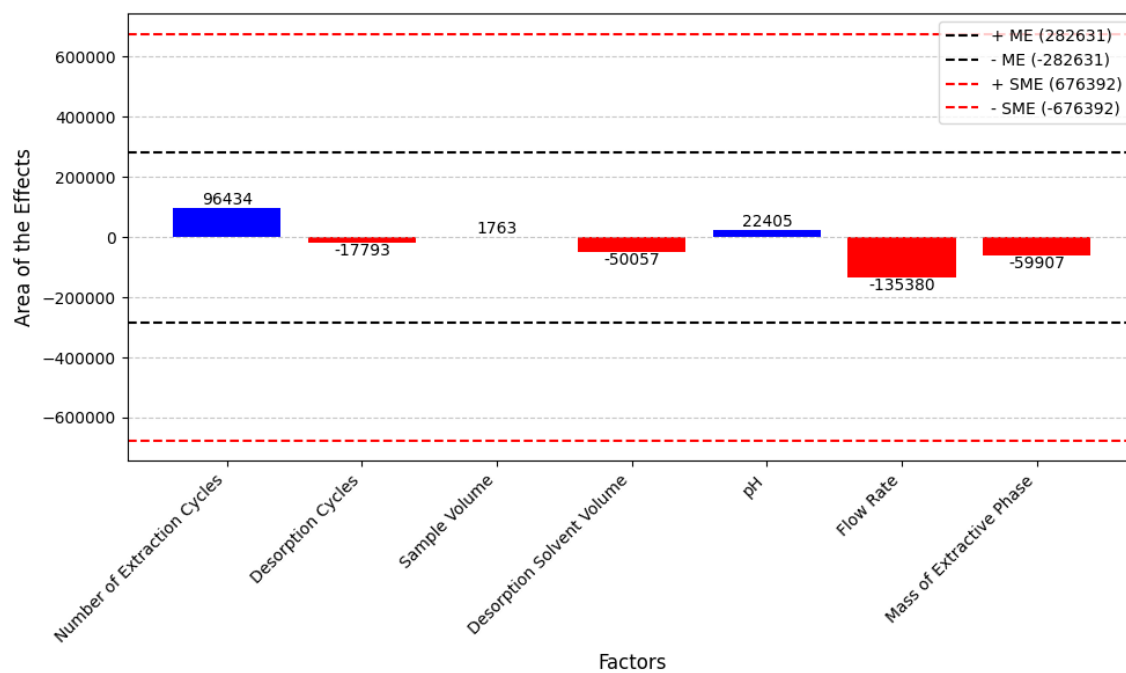


Figure S7: Robustness graph. Comparison of effects on response variables. Positive effects are shown in blue, and negative effects in red.



For the following figures, analytes are: 1- Methylparaben (MP), 2- Ethylparaben (EtP), 3- Carbofuran (CF), 4- Propylparaben (PrP), 5- Diuron (DIU), 6- Naproxen (NAP), 7- 17 α -Ethinylestradiol (EE2), 8- Butylparaben (BuP) and 9- Indomethacin (IND).

Figure S8: Chromatograms obtained at 280 nm for both the blank sample (black, sample 1) and the spiked sample (red, fortified at 10 $\mu\text{g L}^{-1}$), both subjected to the optimized extraction method.

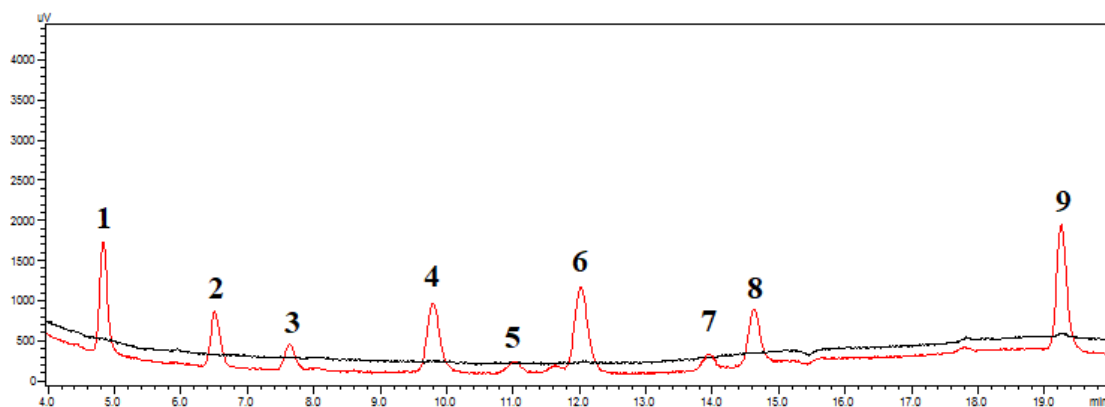


Figure S9: Chromatograms obtained at 280 nm for both the blank sample (black, sample 2) and the spiked sample (red, fortified at 10 $\mu\text{g L}^{-1}$), both subjected to the optimized extraction method.

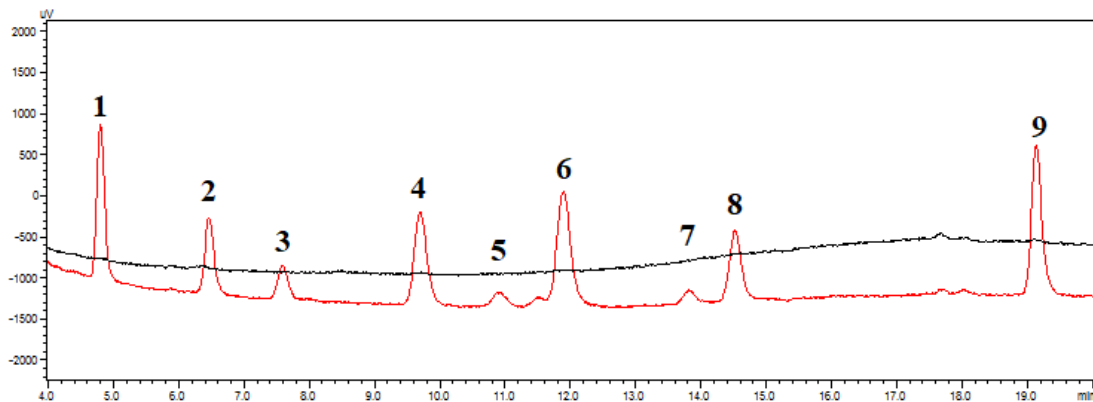


Figure S10:

A- Chromatograms obtained at 250 nm for both the blank sample (black, sample 3) and the spiked sample (red, fortified at $10 \mu\text{g L}^{-1}$), both subjected to the optimized extraction method.

B- Chromatograms obtained at 275 nm for both the blank sample (black, sample 3) and the spiked sample (red, fortified at $10 \mu\text{g L}^{-1}$), both subjected to the optimized extraction method.

C- Chromatograms obtained at 280 nm for both the blank sample (black, sample 3) and the spiked sample (red, fortified at $10 \mu\text{g L}^{-1}$), both subjected to the optimized extraction method.

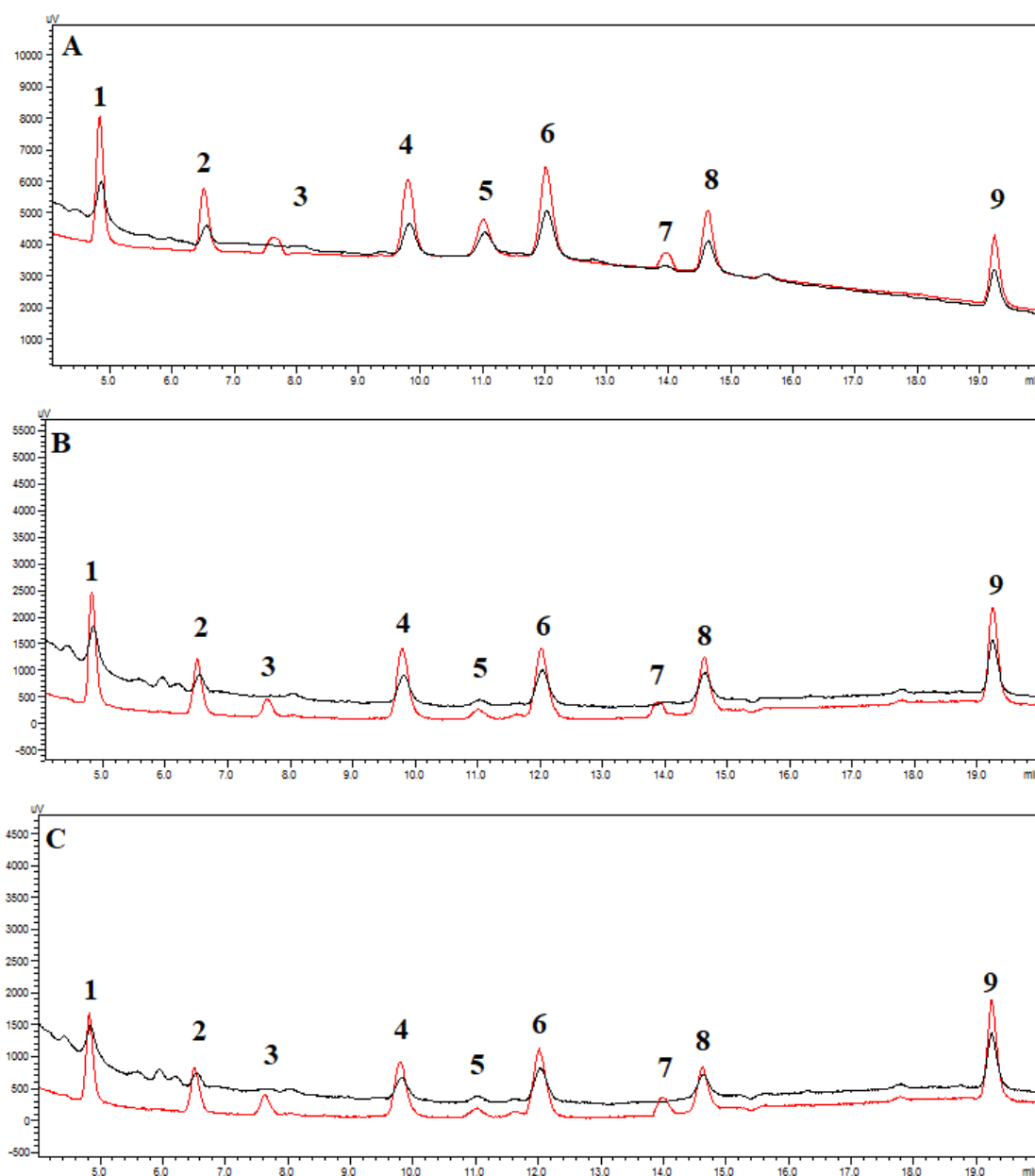


Figure S11: Chromatograms obtained at 280 nm for both the blank sample (black, sample 4) and the spiked sample (red, fortified at $10 \mu\text{g L}^{-1}$), both subjected to the optimized extraction method.

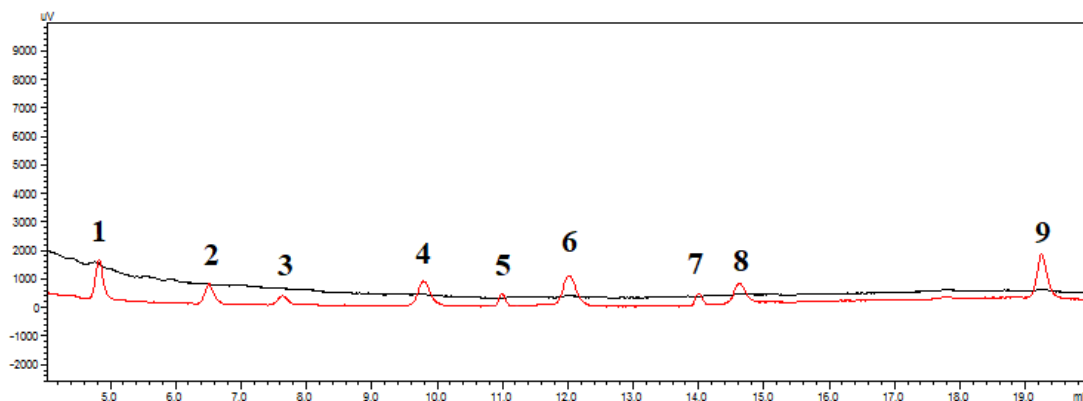


Figure S12: Chromatograms obtained at 280 nm for both the blank sample (black, sample 5) and the spiked sample (red, fortified at $10 \mu\text{g L}^{-1}$), both subjected to the optimized extraction method.

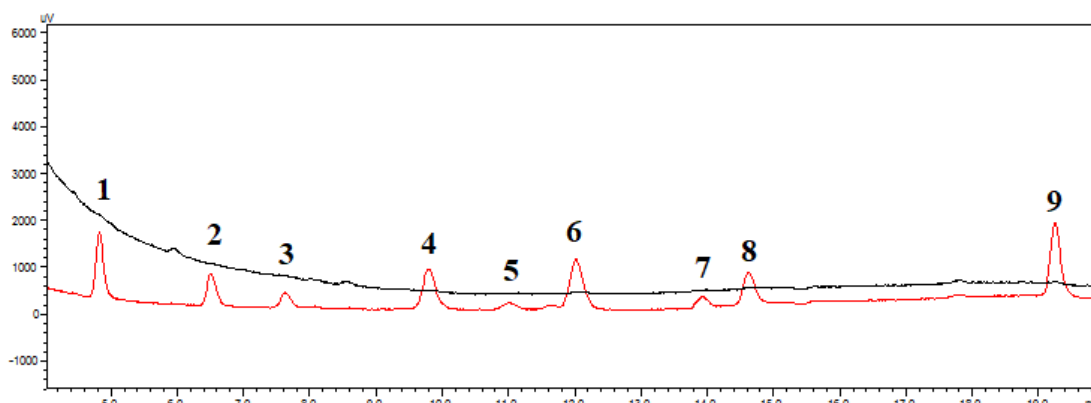


Figure S13: Chromatograms obtained at 280 nm for both the blank sample (black, sample 6) and the spiked sample (red, fortified at $10 \mu\text{g L}^{-1}$), both subjected to the optimized extraction method.

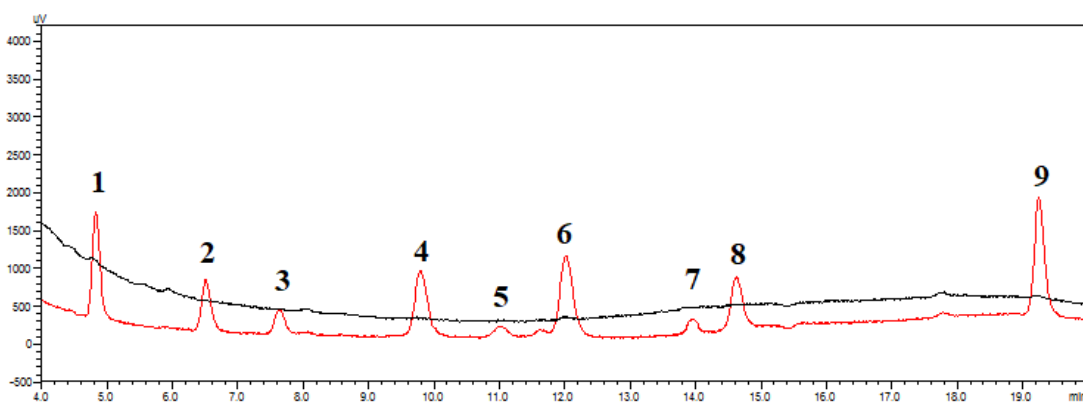
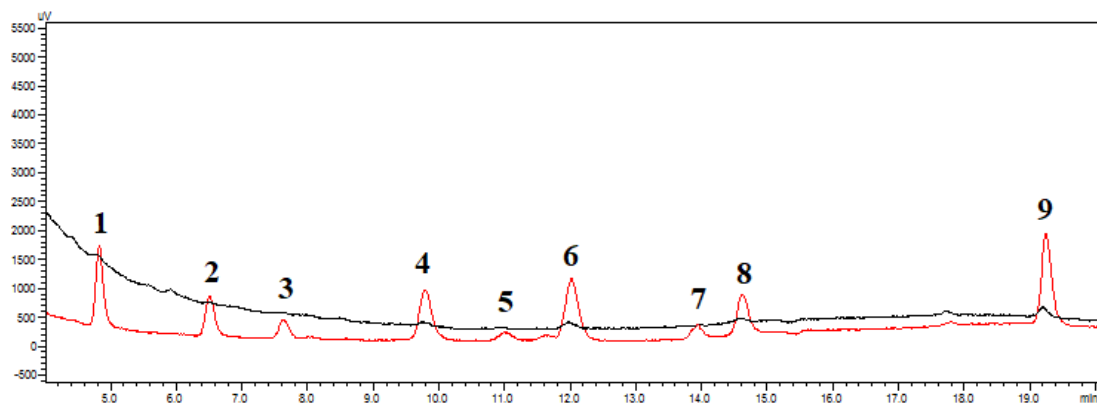


Figure S14: Chromatograms obtained at 280 nm for both the blank sample (black, sample 7) and the spiked sample (red, fortified at $10 \mu\text{g L}^{-1}$), both subjected to the optimized extraction method.



References

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