Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supplementary Material

An Integrated Framework to Develop an Efficient Valid Green (EVG) HPLC Method for Assessment of Antimicrobial Pollutants with Potential Threats for Human Health in Aquatic Systems

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Table SM1. Experimental matrix of the face-centered composite Optimization design.

Run	CMPs			CQAs		
	A- pH	B-Acetonitrile %	C- Flow rate	Rs-2	Run time	T-3
1	3.7	25	1.25	6.5	8	1.4
2	3.3	15	1.25	3	6.77	1.11
3	3.3	25	1.75	6.98	6.22	0.83
4	3.5	20	1.5	6.1	4.9	1.01
5	3.5	20	1.5	6	5.2	1.03
6	3.7	15	1.75	5.4	5.5	1.28
7	3.5	20	1.5	6.06	5	1.03
8	3.5	20	1.75	5.09	4.5	0.89
9	3.3	20	1.5	6.3	5.18	0.97
10	3.5	25	1.5	6.78	6.6	1.05
11	3.7	20	1.5	5.77	5	1.32
12	3.5	15	1.5	5.45	5	1.1
13	3.5	20	1.25	6.5	7	1.11
14	3.5	20	1.5	6.11	5.3	1.05
15	3.5	20	1.5	6.1	5.1	1.08
16	3.5	20	1.5	6.2	4.6	0.98

Table 2SM. Fractional factorial design of robustness study.

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	A: pH	**B: Flow rate**	**C: ACN**	**Rs-2**	**runtime**	**T-3**
		mL/min	%	10	10	10
1	3.45	1.55	21	5.8	5.71	1.07
2	3.55	1.6	21	5.66	5.55	1.04
3	3.45	1.6	17	5.61	5.67	0.98
4	3.55	1.55	17	5.75	5.78	1.11
RSD %				1.504	1.698	2.016
Model						
***F*- value**				3.92	21.80	44.50
***p*-value**				0.3363	0.1497	0.1054

Table 3SM. AGREE detailed analytical greenness reports.

Reported method [46]

Reported method [24]

Proposed EVG method

Analytical Greenness report sheet

Criteria	Reported method [46] Score	Reported method [46] Weight	Reported method [24] Score	Reported method [24] Weight	Proposed EVG method Score	Proposed EVG method Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.3	2	0.3	2	0.3	2
2. Minimal sample size and minimal number of samples are goals.	0	2	0	2	0	2
3. If possible, measurements should be performed in situ.	0.33	2	0.33	2	0.33	2
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	0.8	2	0.8	2	1.0	2
5. Automated and miniaturized methods should be selected.	0.5	2	0.5	2	1.0	2
6. Derivatization should be avoided.	1.0	2	1.0	2	1.0	2
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	0.29	2	0	2	0	2
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	0.68	2	0.74	2	0.82	2
9. The use of energy should be minimized.	0.5	2	0.5	2	1.0	2
10. Reagents obtained from renewable sources should be preferred.	0.0	2	0.5	2	0.0	2
11. Toxic reagents should be eliminated or replaced.	0.29	2	0.11	2	0.59	2
12. Operator's safety should be increased.	0.6	2	0.6	2	0.6	2

Table 4M. ComplexGAPI detailed analytical greenness reports.

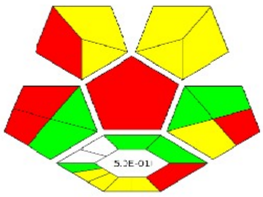
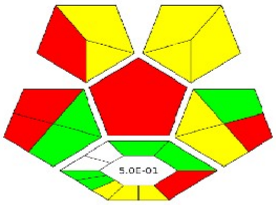
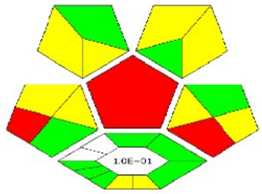
	Reported method [46]	Reported method [24]	Proposed EVG method
			
Sample preparation			
1. Collection:	In-line	In-line	On-line or at-line
2. Preservation:	None	None	None
3. Transport:	Required	Required	Required
4. Storage:	Under special conditioi	Under special conditioi	Under normal conditio
5. Type of method:	Extraction required	Extraction required	Extraction required
6. Scale of extraction:	Micro-extraction	Micro-extraction	Micro-extraction
7. Solvents/reagents used:	Non-green solvents/re	Non-green solvents/re	Green solvents/reagent
8. Additional treatments:	Simple treatments	Simple treatments	None
Reagents and solvents			
9. Amount:	10-100 mL (10-100 g)	10-100 mL (10-100 g)	< 10 mL (< 10 g)
10. Health hazard:	Moderately toxic; coul	Moderately toxic; coul	Moderately toxic; coul
11. Safety hazard:	Highest NFPA flammal	Highest NFPA flammal	Highest NFPA flammal
instrumentation			
12. Energy:	<= 0.1 kWh per sample	<= 1.5 kWh per sample	<= 1.5 kWh per sample
13. Occupational hazard:	Hermetic sealing of the	Hermetic sealing of the	Hermetic sealing of the
14. Waste:	> 10 mL (>10 g)	> 10 mL (>10 g)	1-10 mL (1-10 g)
15. Waste treatment:	Degradation, passivatic	Degradation, passivatic	No treatment
Yield and conditions			
I. Yield:	>89%	>89%	>89%
II. Temperature/time:	Room temp., < 1 h	Room temp., < 1 h	Room temp., < 1 h
Relation to Green Economy			
III. Number of rules met:	1-2	1-2	5-6
Reagents and solvents			
IVa. Health hazard:	Moderately toxic; coul	Moderately toxic; coul	Moderately toxic; coul
IVb. Safety hazard:	Highest NFPA flammal	Highest NFPA flammal	Highest NFPA flammal
Instrumentation			
Va. Technical setup:	Additional setups/sem	Additional setups/sem	Common setup
Vb. Energy:	≤1.5 kWh per sample	≤1.5 kWh per sample	≤0.1 kWh per sample
Vc. Occupational hazard:	Hermetization of analy	Hermetization of analy	Hermetization of analy
E-factor			
VII. E-factor input:	0.5	0.5	0.1

Table 5SM. EVG radar chart evaluation criteria.

Efficiency	Evaluation criteria	3	2	1	0
A	Implementing DOE for screening / optimization	Full designs/ response surface methodology	fractional / reduced designs	one factor at a time study	No screening or optimization
B	Number of CQAs	> 3	3	2	0
C	Number of CMPs	> 3	3	2	0
D	Minimize cost (time for analyzing one sample)	≤ 1 min	1-5 min	6-15 min	> 15 min
E	Number of analyzed compounds per one experiment	> 10	4-10	2-4	1
Validation	**Evaluation criteria**	**3**	**2**	**1**	**0**
A	Type of validation	Full	Partial	cross-validation	No validation
B	Precision	Repeatability, intermediate precision & Reproducibility	Repeatability & intermediate precision	Repeatability only	No precision
C	Accuracy (SE)	> 0, ≤ 0.5	> 0.5, ≤ 1.0	> 1.0, ≤ 1.5	> 1.5, ≤ 2
D	Sensitivity (LOQ)	Ultratrace: < 0.01 ppm (10 ppb)	Trace: 0.01 ppm - 0.01 % (100 ppm)	Minor: 0.01 (100 ppm) to 1%	Major: 1-100 %
E	Robustness (no. of factors' variations)	> 4	2-4	1	0
Greenness	**Evaluation criteria**	**3**	**2**	**1**	**0**
A	Number of greenness tools	3 ≤	2	1	0
B	Sample treatment	no treatment (direct analysis)	Simple procedure	Extraction	Complex procedure / derivitization
C	Reagents & solvents (no. of GHS pictograms)	0	1-5	6-12	>12
D	Instrumentations and energy	0 kWh per sample	> 0, < 0.1 kWh per sample	0.1-1.5 kWh per sample	> 1.5 kWh per sample
E	Waste	< 1 mL	1- 10 mL	10-50 mL	> 50 mL

Fig.1SM. Map of samples of Nile River freshwater at different locations.

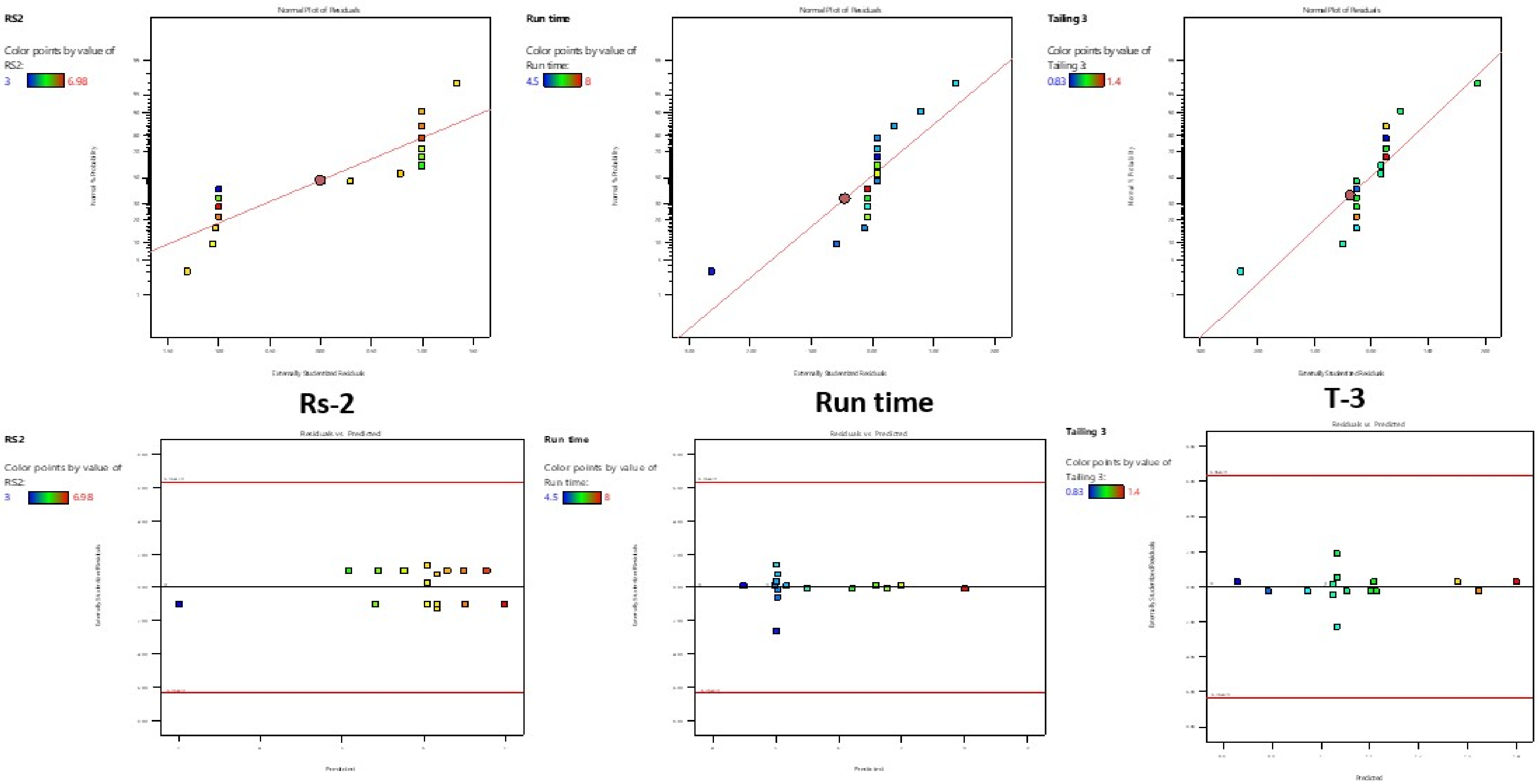


Fig. 2SM. Half normal plots and residual vs predicted plots of the FCC design.

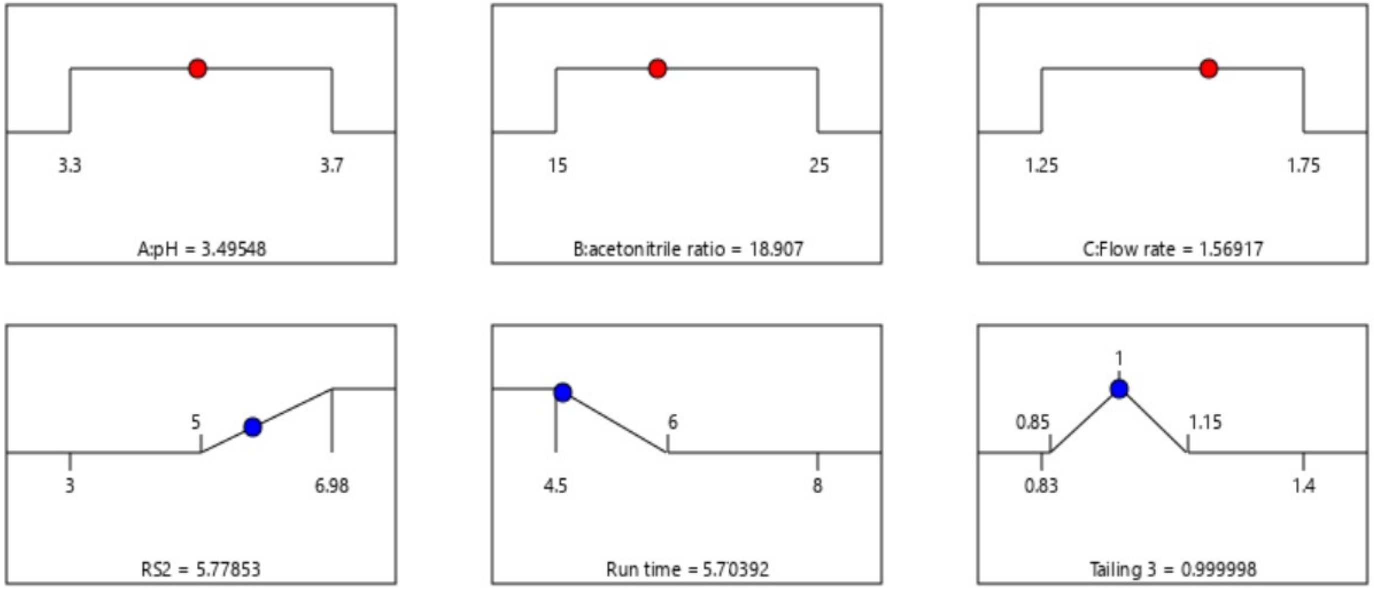
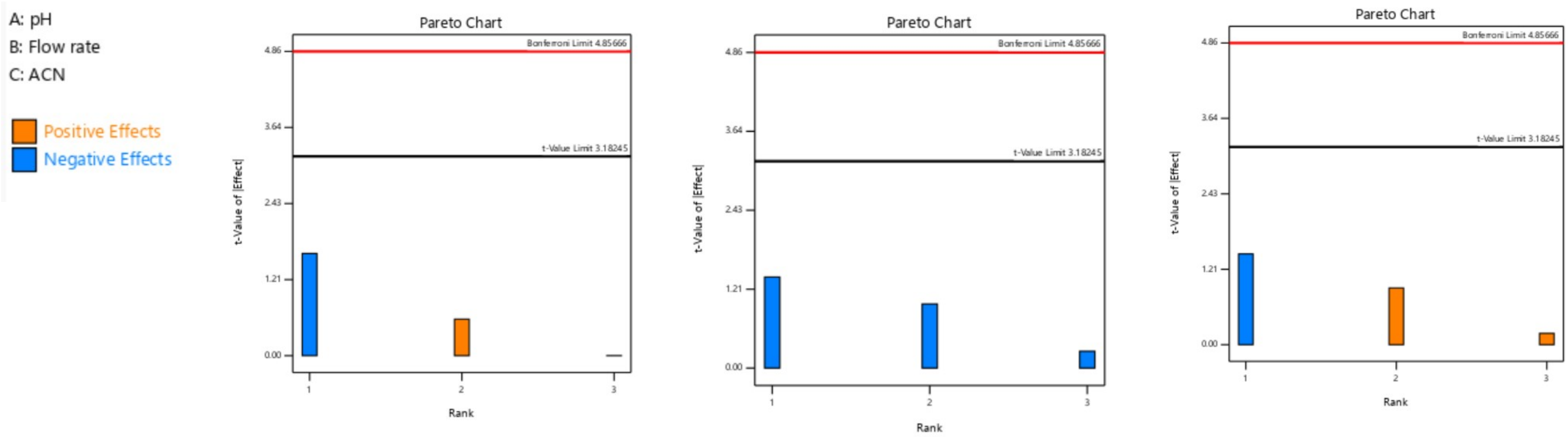


Fig. 3SM. Solution ramps for optimum chromatographic conditions with a desirability index of 0.764.



(a) **(b)** **(c)**

Fig. 4SM. Pareto chart of robustness study for the CQAs (a) Rs-2, (b) runtime, and (c) T-3.