Research Article

Bandar R. Alsehli, Ammena Y. Binsaleh, Sayed M. Saleh, Abdullah H. Alluhayb, and Mahmoud A. Mohamed*

Environmental impact of RP-HPLC strategy for detection of selected antibiotics residues in wastewater: Evaluating of quality tools

Supplementary material

Table S1: Analytical solution stability, robustness, and intra- and inter-day precision of the suggested HPLC method

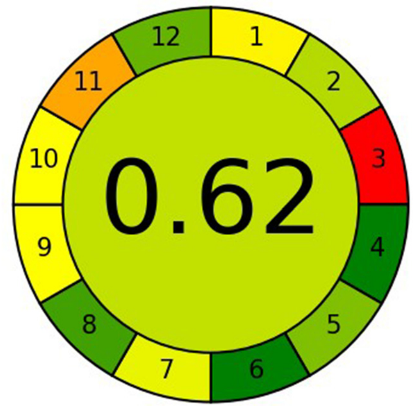
Parameter	HPLC			Limit (%)
	OXY	TET	CEP	
Day to day	0.2%	0.1%	0.1%	**RSD ≤ 2.0%**
Analyst to analyst	0.8%	0.7%	0.9%	
Column to column	0.6%	0.9%	0.8%	
Flow rate change (±0.1 mL/min)	0.7%	0.9%	0.8%	
pH changes of mobile phase (±0.05)	0.3%	0.4%	0.7%	
Organic solvent change (±2%)	0.5%	0.3%	0.2%	
Fresh sample	0.3%	0.2%	0.1%	
Stored sample in fridge	0.8%	0.9%	0.7%	
Stored sample in autosampler	0.5%	0.5%	0.4%	
Stored sample at room temperature	0.7%	0.8%	0.8%	

Table S2: Outcomes of the suggested HPLC method's system suitability parameters

Item	HPLC			Reference values
	OXY	TET	CEP	
Tailing factor	0.9	1.1	0.8	$T \leq 2.0$
Injection precision	0.2%	0.4%	0.5%	RSD ≤ 2%
Number of theoretical plates (N)	2,545	2,632	2,328	$N > 2000$
Resolution	—	3.5	4.8	$R_s > 1.5$
Retention time (R_t)	0.8%	0.6%	0.5%	RT window ≤ 10%

*** Corresponding author: Mahmoud A. Mohamed,** Hikma Pharmaceutical Company, Beni-Suef, 62511, Egypt, e-mail: ch.mahmoud88@gmail.com, mmabdelfatah@hikma.com
Bandar R. Alsehli: Department of Chemistry, Faculty of Science, Taibah University, Al-Madinah Almunawrah, 30002, Saudi Arabia, e-mail: bshle@taibahu.edu.sa
Ammena Y. Binsaleh: Department of Pharmacy Practice, College of Pharmacy, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh, 11671, Saudi Arabia
Sayed M. Saleh, Abdullah H. Alluhayb: Department of Chemistry, College of Science, Qassim University, Buraidah, 51452, Saudi Arabia, e-mail: e.saleh@qu.edu.sa, ah.alluhayb@qu.edu.sa

Analytical Greenness report sheet
20/08/2024 15:15:20



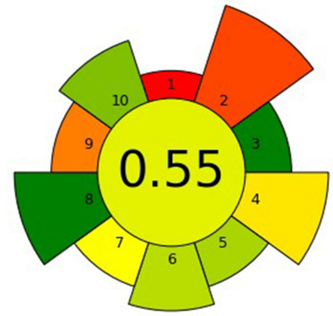
Criteria	Score	Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.48	2
2. Minimal sample size and minimal number of samples are goals.	0.65	2
3. If possible, measurements should be performed in situ.	0.0	2
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	1.0	2
5. Automated and miniaturized methods should be selected.	0.75	2
6. Derivatization should be avoided.	1.0	2
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	0.55	2
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	0.87	2
9. The use of energy should be minimized.	0.5	2
10. Reagents obtained from renewable sources should be preferred.	0.5	2
11. Toxic reagents should be eliminated or replaced.	0.33	2
12. Operator's safety should be increased.	0.8	2

(a)

Figure S1: Applications for AGREE (a) and AGREEprep (b) to assess the HPLC method.

AGREEprep

Analytical Greenness Metric for Sample Preparation
20/08/2024 15:27:18



#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 3.8	0.14	5
3.	**Sustainability, renewability, and reusability of materials** Only sustainable and renewable materials are used SEVERAL TIMES	1.0	2
4.	**Waste** Mass [g] or volume [mL] of waste: 3	0.45	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 1	0.67	2
6.	**Sample throughput** Hourly sample throughput: 15	0.64	3
7.	**Integration and automation** No. of sample prep. steps: 2 steps or fewer; degree if automation: Semi-automated systems	0.5	2
8.	**Energy consumption** Approximate energy consumption per analysis [W]: 1	1.0	4
9.	**Post-sample preparation configuration for analysis** Liquid chromatography, gas chromatography with quadrupole detection, etc.	0.25	2
10.	**Operator's safety** No. of distinct hazards: 1 hazard	0.75	3

(b)

Figure S1: (*Continued*)

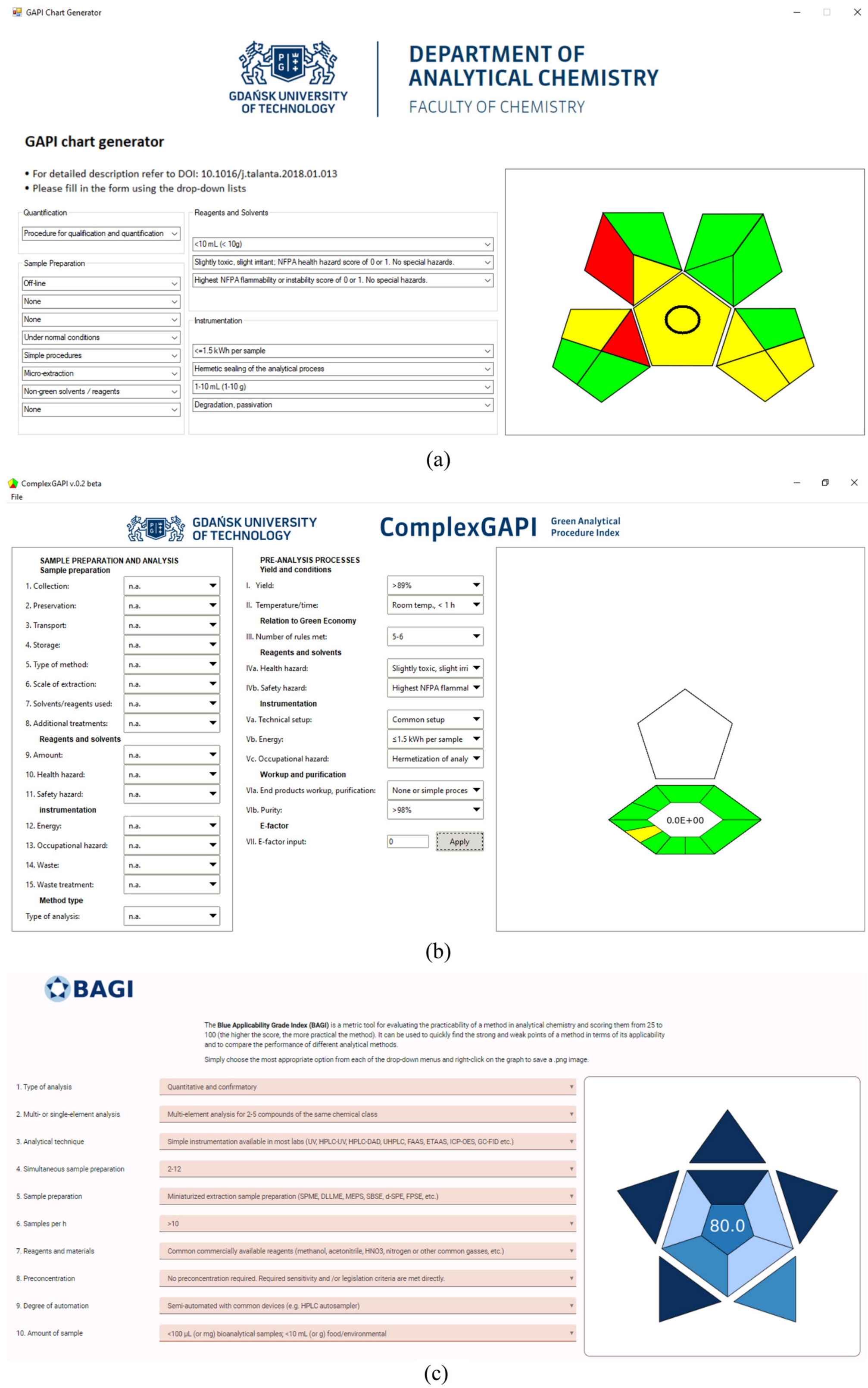
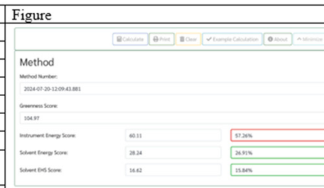
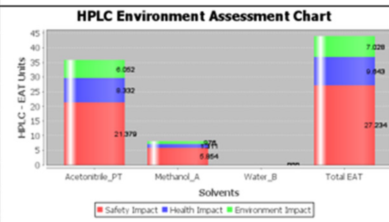


Figure S2: Utilization possibilities for assessing the HPLC method include GAPI (a), ComplexGAPI (b), and BAGI (c).

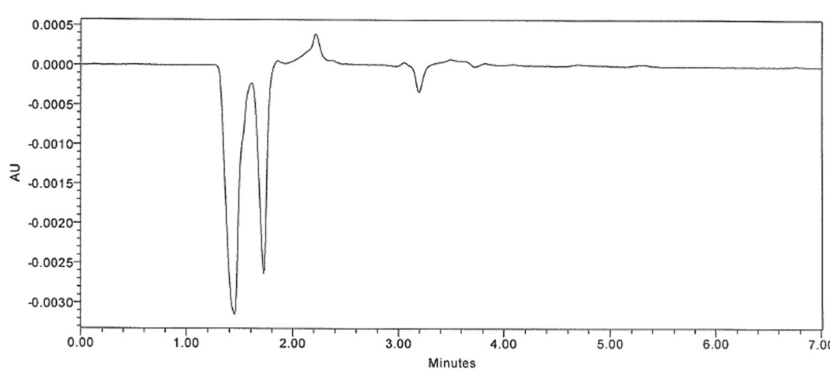
	AMGS	Proposed Method		Figure
Instrument conditions	Technique	HPLC		
	Number of analytes	3		
	Flow rate (mL/min)	1.0		
	Run time (min/injection)	7		
Solvent	Purified water	180		
Sample	Sample prep volume (mL)	10		
	Number of sample prep	3		
Standards	Stock standard prep volume (mL)	10		
	Number of stock standard preps	1		
Working standard	Working standard prep volume (mL)	10		
	Number of working standard preps	2		
System Suit Test (SST)	SST prep volume (mL)	10		
	Number of SST prep	1		
Method	Instrument Energy Score	**60.11**	**(57.26%)**	
	Solvent Energy Score	**28.24**	**(26.91%)**	
	Solvent EHS Score	**16.62**	**(15.84%)**	
Greenness score		**104.97**		

(a)

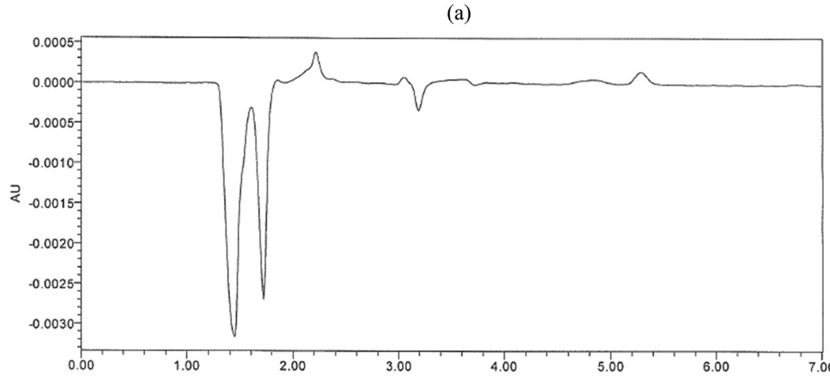
Calculation SHE	Proposed HPLC Method	Figure
Sample Pre-Treatments	Acetonitrile (10 mL)	
Mobile phase A	Methanol 55%	
Mobile phase B	Acidic water 45%	
Flow rate	1.0 mL/min	
Run time	7.0 min	
Safety Impact	27.234	
Healthy Impact	9.643	
Environment Impact	7.028	
Total HPLC-EAT Score	43.904	

(b)

Figure S3: (a) AMGS and (b) HPLC-EAT parameters for assessing the HPLC method.



(a)



(b)

Figure S4: Selectivity of HPLC chromatograms of (a) diluent and (b) placebo.