

Supplementary material

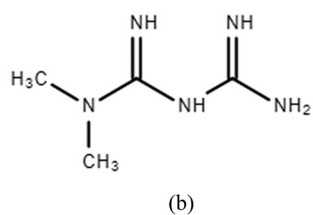
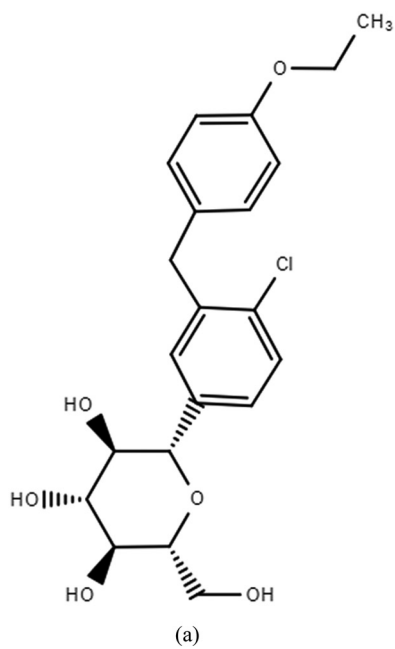


Figure S1: Chemical structure of (a) MET and (b) DAP.

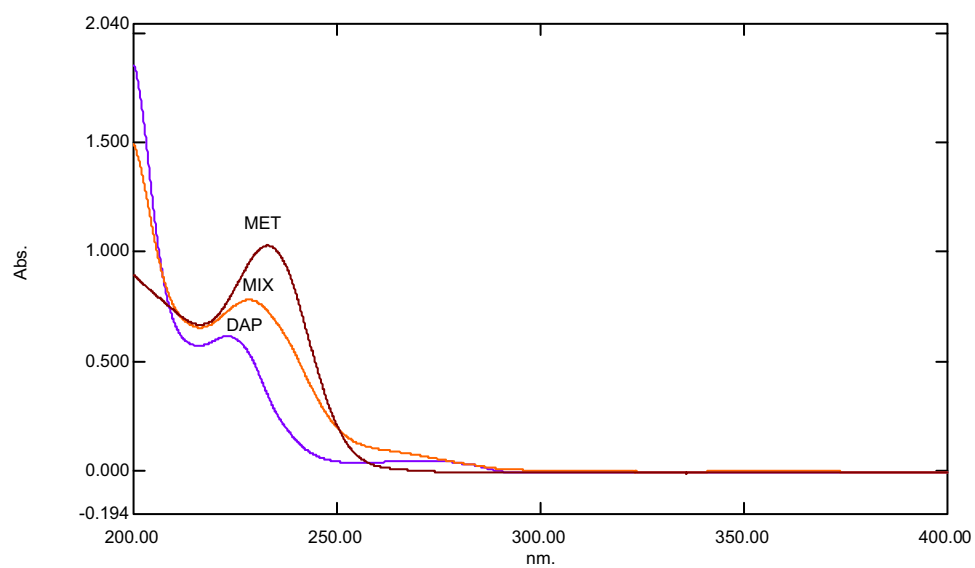
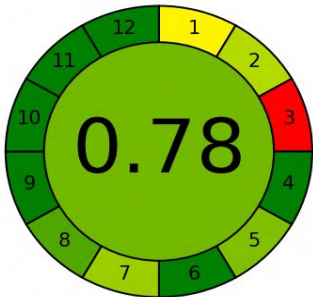


Figure S2: Zero-order absorption spectra of binary combination, MET, and DAP at $10 \mu\text{g}\cdot\text{mL}^{-1}$ with solvent used as blank.

Analytical Greenness report sheet
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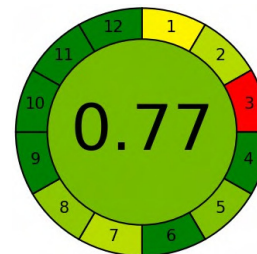
Criteria	Score	Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.48	2
2. Minimal sample size and minimal number of samples are goals.	0.65	2
3. If possible, measurements should be performed in situ.	0.0	2
4. Integration of analytical processes and operations saves 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.	0.69	2
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	0.84	2
9. The use of energy should be minimized.	1.0	2
10. Reagents obtained from renewable sources should be preferred.	1.0	2
11. Toxic reagents should be eliminated or replaced.	1.0	2
12. Operator's safety should be increased.	1.0	2

(a)

Figure S3: AGREE (a) and (b) and AGREEprep (c) and (d) applications for evaluating the UPLC and UV techniques.

Analytical Greenness report sheet

13/06/2024 09:26:02



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.	0.64	2
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	0.72	2
9. The use of energy should be minimized.	1.0	2
10. Reagents obtained from renewable sources should be preferred.	1.0	2
11. Toxic reagents should be eliminated or replaced.	1.0	2
12. Operator's safety should be increased.	1.0	2

(b)

Figure S3: (Continued)

AGREEprep

Analytical Greenness Metric for
Sample Preparation
13/06/2024 09:30:31



#	Criterion	Score Weight	
1.	Sample preparation placement	0.0	1
	Sample preparation placement: Ex situ		
2.	Hazardous materials	1.0	5
	Mass [g] or volume [mL] of problematic materials: 0		
3.	Sustainability, renewability, and reusability of materials	1.0	2
	Only sustainable and renewable materials are used SEVERAL TIMES		
4.	Waste	0.63	4
	Mass [g] or volume [mL] of waste: 1		
5.	Size economy of the sample	0.67	2
	Mass [g] or volume [mL] of the sample: 1		
6.	Sample throughput	0.71	3
	Hourly sample throughput: 20		
7.	Integration and automation	0.5	2
	No. of sample prep. steps: 2 steps or fewer; degree if automation: Semi-automated systems		
8.	Energy consumption	1.0	4
	Approximate energy consumption per analysis [W]: 0.1		
9.	Post-sample preparation configuration for analysis	0.25	2
	Liquid chromatography, gas chromatography with quadrupole detection, etc.		
10.	Operator's safety	1.0	3
	No. of distinct hazards: No hazards or no exposure		

(c)

Figure S3: (Continued)

AGREEprep

Analytical Greenness Metric for
Sample Preparation

13/06/2024 10:10:48



#	Criterion	Score	Weight
1.	Sample preparation placement	0.0	1
	Sample preparation placement: Ex situ		
2.	Hazardous materials	1.0	5
	Mass [g] or volume [mL] of problematic materials: 0		
3.	Sustainability, renewability, and reusability of materials	1.0	2
	Only sustainable and renewable materials are used SEVERAL TIMES		
4.	Waste	0.45	4
	Mass [g] or volume [mL] of waste: 3		
5.	Size economy of the sample	0.67	2
	Mass [g] or volume [mL] of the sample: 1		
6.	Sample throughput	0.58	3
	Hourly sample throughput: 12		
7.	Integration and automation	0.5	2
	No. of sample prep. steps: 2 steps or fewer; degree if automation: Semi-automated systems		
8.	Energy consumption	1.0	4
	Approximate energy consumption per analysis [W]: 0.1		
9.	Post-sample preparation configuration for analysis	0.75	2
	Spectrophotometry, surface analysis techniques, voltammetry, potentiometry, etc.		
10.	Operator's safety	1.0	3
	No. of distinct hazards: No hazards or no exposure		

(d)

Figure S3: (Continued)

GAPI Chart Generator

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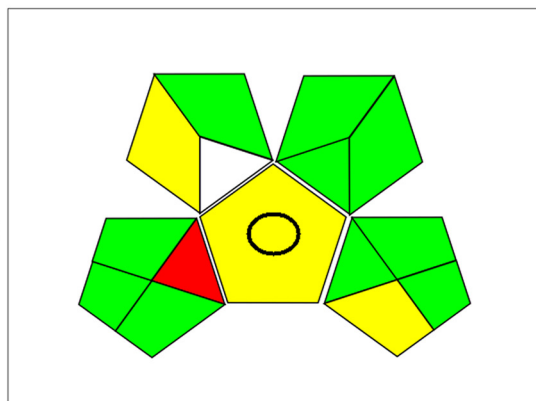


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GAPI chart generator

- For detailed description refer to DOI: 10.1016/j.talanta.2018.01.013
- Please fill in the form using the drop-down lists

Quantification Procedure for qualification and quantification	Reagents and Solvents <10 mL (< 10g) Slightly toxic, slight irritant; NFPA health hazard score of 0 or 1. No special hazards. Highest NFPA flammability or instability score of 0 or 1. No special hazards.
Sample Preparation Off-line None None None Simple procedures Scale of extraction (6) Green solvents / reagents None	Instrumentation <=0.1 kWh per sample Hermetic sealing of the analytical process <1 mL (<1 g) Degradation, passivation



(a)

GAPI Chart Generator

— □ ×

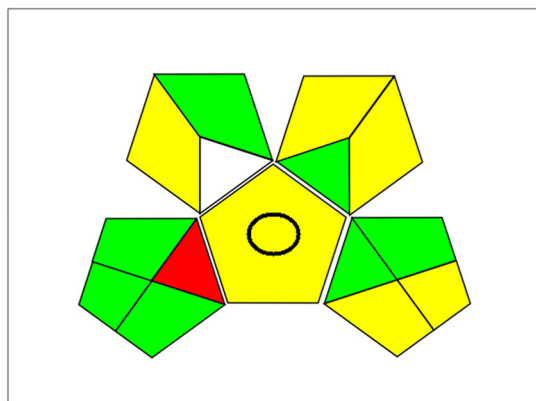


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GAPI chart generator

- For detailed description refer to DOI: 10.1016/j.talanta.2018.01.013
- Please fill in the form using the drop-down lists

Quantification Procedure for qualification and quantification	Reagents and Solvents <10 mL (< 10g) Moderately toxic; could cause temporary incapacitation; NFPA = 2 or 3. Highest NFPA flammability or instability score = 2 or 3, or a special hazard is used.
Sample Preparation Off-line None None None Simple procedures Scale of extraction (6) Green solvents / reagents None	Instrumentation <=0.1 kWh per sample Hermetic sealing of the analytical process 1-10 mL (1-10 g) Degradation, passivation



(b)

Figure S4: GAPI (a) and (b), ComplexGAPI (c) and (d), and BAGI (e) and (f) applications for evaluating the UPLC and UV techniques.

ComplexGAPI v.0.2 beta
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OF TECHNOLOGYComplexGAPI Green Analytical
Procedure Index

SAMPLE PREPARATION AND ANALYSIS		PRE-ANALYSIS PROCESSES	
Sample preparation		Yield and conditions	
1. Collection:	n.a.	I. Yield:	>89%
2. Preservation:	n.a.	II. Temperature/time:	Room temp., > 1 h, He
3. Transport:	n.a.	Relation to Green Economy	
4. Storage:	n.a.	III. Number of rules met:	5-6
5. Type of method:	n.a.	Reagents and solvents	
6. Scale of extraction:	n.a.	IVa. Health hazard:	Slightly toxic, slight irri
7. Solvents/reagents used:	n.a.	IVb. Safety hazard:	Highest NFPA flammal
8. Additional treatments:	n.a.	Instrumentation	
Reagents and solvents		Va. Technical setup:	Common setup
9. Amount:	n.a.	Vb. Energy:	≤0.1 kWh per sample
10. Health hazard:	n.a.	Vc. Occupational hazard:	Hermetization of analy
11. Safety hazard:	n.a.	Workup and purification	
Instrumentation		Via. End products workup, purification:	None or simple proces
12. Energy:	n.a.	Vib. Purity:	>98%
13. Occupational hazard:	n.a.	E-factor	
14. Waste:	n.a.	VII. E-factor input:	0 Apply
15. Waste treatment:	n.a.		
Method type			
Type of analysis:	n.a.		

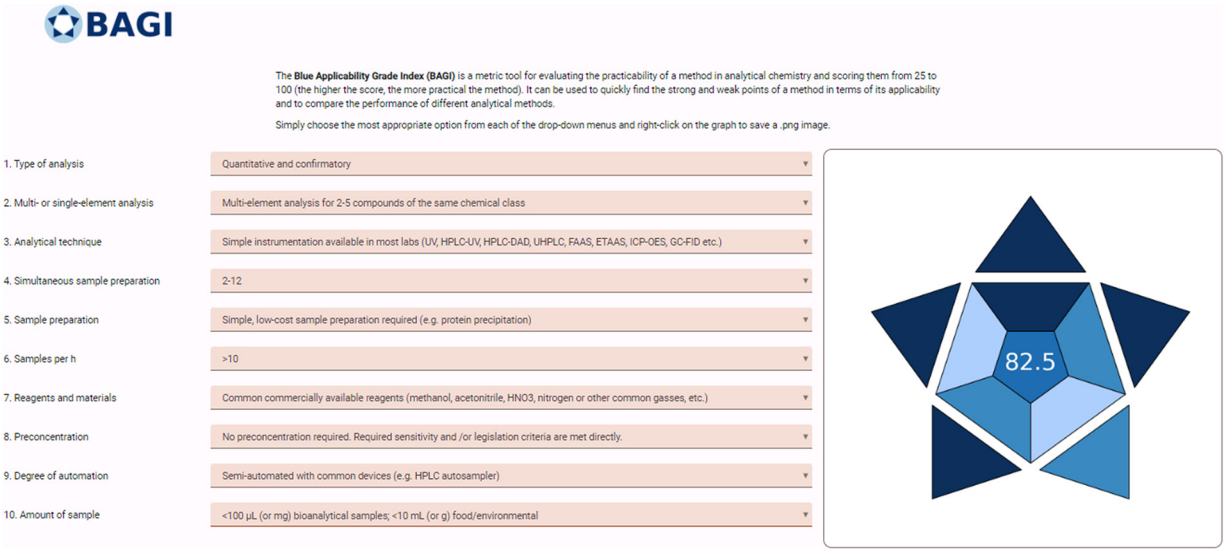
(c)

ComplexGAPI v.0.2 beta
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OF TECHNOLOGYComplexGAPI Green Analytical
Procedure Index

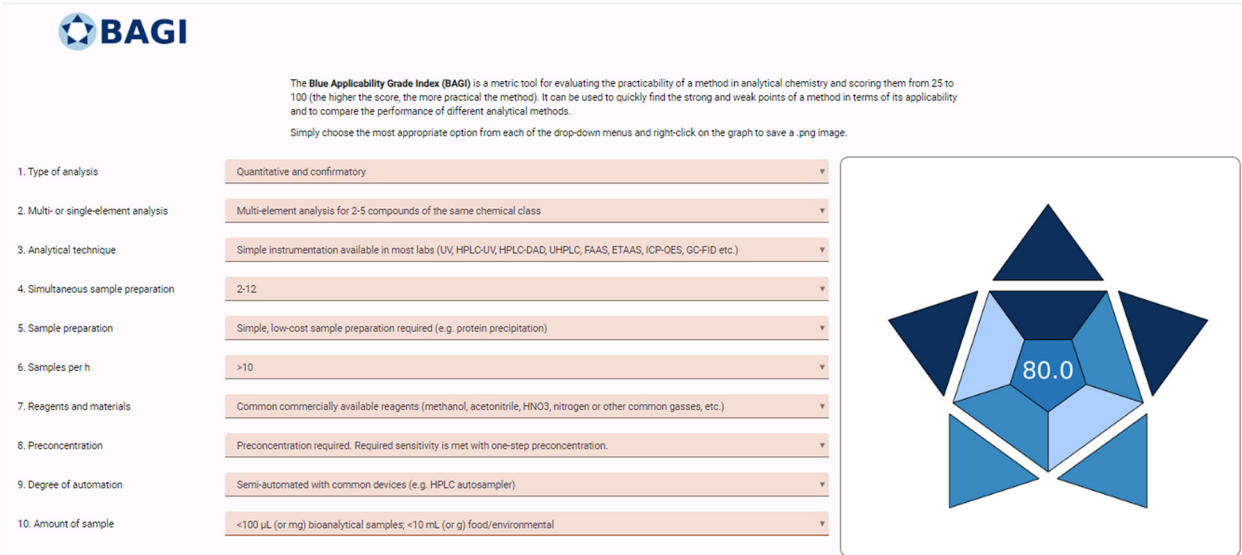
SAMPLE PREPARATION AND ANALYSIS		PRE-ANALYSIS PROCESSES	
Sample preparation		Yield and conditions	
1. Collection:	n.a.	I. Yield:	>89%
2. Preservation:	n.a.	II. Temperature/time:	Room temp., < 1 h
3. Transport:	n.a.	Relation to Green Economy	
4. Storage:	n.a.	III. Number of rules met:	5-6
5. Type of method:	n.a.	Reagents and solvents	
6. Scale of extraction:	n.a.	IVa. Health hazard:	Moderately toxic; couli
7. Solvents/reagents used:	n.a.	IVb. Safety hazard:	Highest NFPA flammal
8. Additional treatments:	n.a.	Instrumentation	
Reagents and solvents		Va. Technical setup:	Common setup
9. Amount:	n.a.	Vb. Energy:	≤0.1 kWh per sample
10. Health hazard:	n.a.	Vc. Occupational hazard:	Hermetization of analy
11. Safety hazard:	n.a.	Workup and purification	
Instrumentation		Via. End products workup, purification:	None or simple proces
12. Energy:	n.a.	Vib. Purity:	>98%
13. Occupational hazard:	n.a.	E-factor	
14. Waste:	n.a.	VII. E-factor input:	0 Apply
15. Waste treatment:	n.a.		
Method type			
Type of analysis:	n.a.		

(d)

Figure S4: (Continued)



(e)



(f)

Figure S4: (Continued)

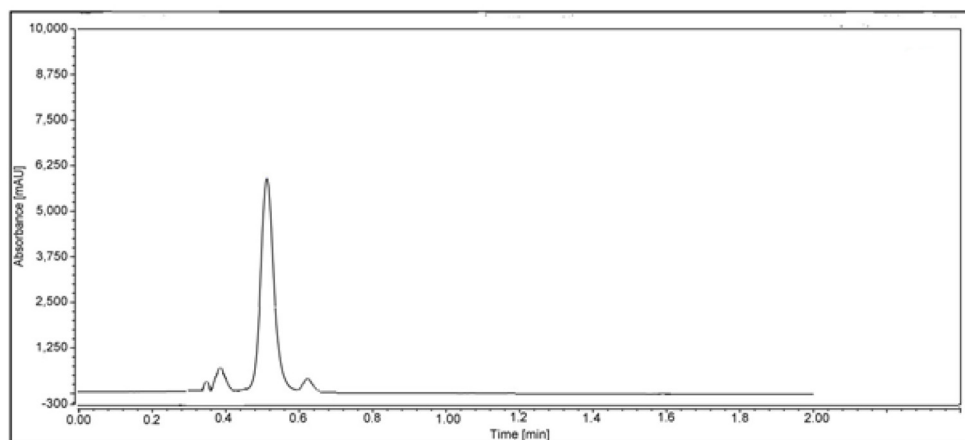
	AMGS	Proposed Method	Figure
Instrument Conditions	Technique	UPLC	Method Method Number: 2024-05-13 16:07:06.424 Greenness Score: 290.28 Instrument Energy Score: 21.447.39% Solvent Energy Score: 175.1360.33% Solvent EHS Score: 93.7132.28%
	Number of analytes of interest	2	
	Number of injections/runs for one full analysis	15	
	Flow Rate (mL/min)	0.5	
	Run time (min/injection)	2	
Solvent	Purified water and ethanol (mL)	210	
Sample	Sample prep volume (mL)	100	
	Number of sample preps	2	
Standards	Stock standard prep volume (mL)	100	
	Number of stock standard preps	1	
Standard Diluent	Working Standard prep volume (mL)	10	
	Number of stock standard preps	1	
System Suit Test (SST) volume (mL)	SST prep volume (mL)	10	
	Number of SST preps	1	
Method	Instrument Energy Score	21.44	(7.39%)
	Solvent Energy Score	175.13	(60.33%)
	Solvent EHS Score	93.71	(32.28%)
Greenness Score		290.28	

(a)

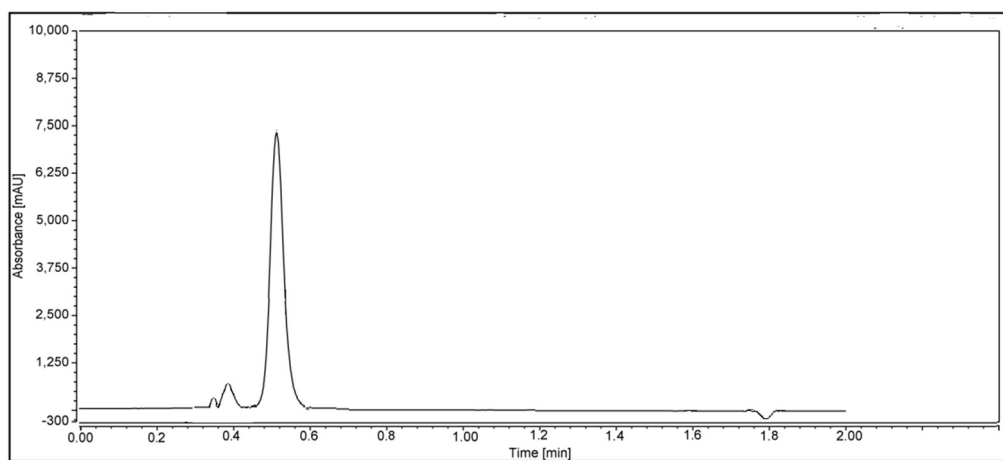
Calculation SHE	Proposed Method	Figure HPLC Environment Assessment Chart <table><tr><th>Solvents</th><th>Safety Impact</th><th>Health Impact</th><th>Environment Impact</th></tr><tr><td>Water_A</td><td>0.000</td><td>0.000</td><td>0.000</td></tr><tr><td>Ethanol_B</td><td>0.664</td><td>0.071</td><td>0.170</td></tr><tr><td>Total EAT</td><td>0.664</td><td>0.071</td><td>0.170</td></tr></table>	Solvents	Safety Impact	Health Impact	Environment Impact	Water_A	0.000	0.000	0.000	Ethanol_B	0.664	0.071	0.170	Total EAT	0.664	0.071	0.170
Solvents	Safety Impact		Health Impact	Environment Impact														
Water_A	0.000		0.000	0.000														
Ethanol_B	0.664		0.071	0.170														
Total EAT	0.664		0.071	0.170														
Sample Pre-Treatments	None																	
Mobile phase A	Phosphate buffer (55%)																	
Mobile phase B	Ethanol (45%)																	
Flow rate	0.5 mL/min																	
Run time	2 min																	
Safety Impact	0.664																	
Healthy Impact	0.071																	
Environment Impact	0.17																	
Total HPLC-EAT score	0.905																	

(b)

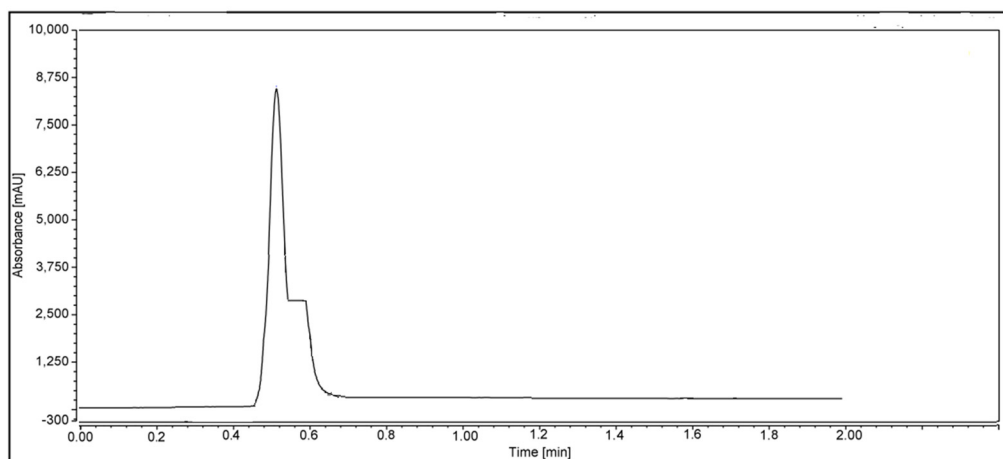
Figure S5: (a) AMGS and (b) HPLC-EAT metrics for evaluation of the UPLC approach.



(a)

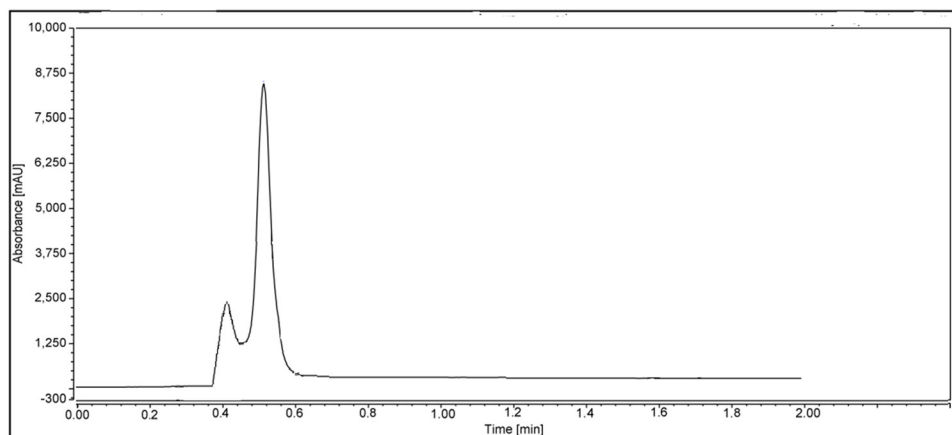


(b)

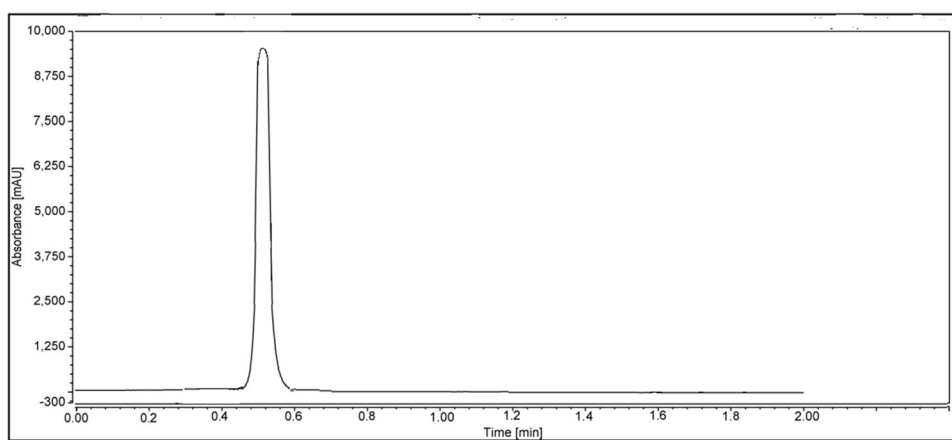


(c)

Figure S6: UPLC charts of (a) placebo and blanks for (b) diluent, (c) acid, (d) base, and (e) oxidation.



(d)



(e)

Figure S6: (Continued)

Table S1: Intra- and inter-day precision, robustness, and stability of the analytical solution of the recommended UPLC and UV methods

Parameter	UPLC		UV		Limit (%)
	MET	DAP	MET	DAP	
Day to Day	0.7	0.5	0.3	0.4	RSD
Analyst to Analyst	1.1	0.9	0.5	0.8	≤ 2.0%
Column to Column	0.9	0.8	—	—	
Flow rate change (±0.1 mL·min ⁻¹)	0.4	0.6	—	—	
pH changes of mobile phase (±0.05)	0.8	0.9	—	—	
Organic solvent change (±2%)	0.3	0.4	—	—	
Fresh sample	0.2	0.1	0.3	0.4	
Stored sample in fridge	0.7	0.5	0.8	0.6	
Stored sample in autosampler	0.6	0.7	—	—	
Stored Sample at room temperature	0.8	0.9	1.2	0.9	

Table S2: Assay of MET and DAP in the film-coated tablet employing the recommended methods

Dosage form	UPLC		UV		Limit
	Assay (%)				
Forminodab XR	MET	DAP	MET	DAP	90–110%
10/1,000 mg FCT	99.89	100.58	99.28	101.43	
	100.43	101.47	100.95	100.37	
	98.98	99.78	102.27	102.76	
	97.87	98.93	100.69	100.86	
	101.12	99.28	101.37	99.49	
	102.09	101.27	103.87	100.57	
Mean ± RSD	100.06	100.22	101.41	100.91	
	± 1.51	± 1.05	± 1.53	± 1.10	

Table S3: Results of system suitability parameters of the recommended UPLC method

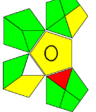
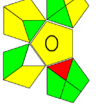
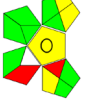
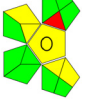
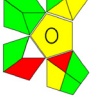
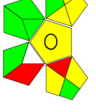
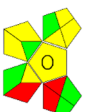
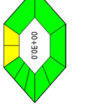
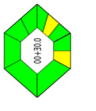
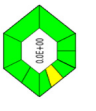
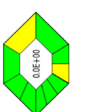
Item	UPLC		Reference values
	MET	DAP	
Tailing factor	1.4	1.2	$T \leq 2.0$
Injection precision	0.4	0.5	RSD ≤ 2%
Number of theoretical plates (<i>N</i>)	3,442	2,594	$N > 2,000$
Resolution	4.8	—	$R_s > 1.5$
Retention time (<i>R_t</i>)	0.5	0.8	RT window ≤ 10%

Table S4: The distinction between the recovery outcomes of the published procedures and the recommended approaches for quantitative DAP and MET drugs

Method	Application	Mobile phase/Solvent	Flow rate	Run time	Waste ^a	Recovery (%)
UPLC and MCR (The suggested methods)	Green and White appraisal for UPLC and MCR techniques	Mobile Phase: Phosphate buffer and ethanol (55:45, v/v) adjusted to pH 7.0. Solvent: Purified water and ethanol at a ratio of (70:30, v/v) for UPLC. Ultra-pure water for UV	0.5 mL·min ⁻¹	2.0 min	1.0 g/ sample	99.5–100.9% for DAP and 99.9–100.3% for MET
[1]	First Derivative Spectrophotometric method for determining DAP in the presence of MET	Solvent: 0.1 N HCl for UV.	Not	Not	3.0 g/ sample	98.9–100.4% for DAP
[2]	Ratio difference spectroscopy Derivative	Solvent: distilled water for UV.	Not	Not	3.0 g/ sample	98.4–100.9% for DAP and 97–101% for MET
[3]	Ratio Spectrum-Zero crossing	Solvent: Methanol: water (80:20, v/v) for UV	Not	Not	3.0 g/ sample	98.7–99.1% for DAP and 98.7–99.4% for MET
[4]	Simultaneous equation method	Solvent: Methanol: water (80:20, v/v) for UV	Not	Not	3.0 g/ sample	98.7–99.1% for DAP and 98.7–99.4% for MET
	UPLC	Mobile Phase: Orthophosphoric acid (pH 3.00) and acetonitrile (55:45%, v/v)	0.2 mL·min ⁻¹	5.0 min	1.0 g/ sample	98.77–99.57% and 99.15–101.2% for DAP and MET
		Solvent: water: acetonitrile (50:50%, v/v) for UPLC				
[5]	UHPLC	Mobile Phase: A mixture of potassium dihydrogen phosphate buffer, pH (3.5)—acetonitrile (50:50, v/v). Solvent: Methanol for UPLC	0.4 mL·min ⁻¹	10 min	4.0 g/ sample	98.77–99.57% and 99.15–101.2% for DAP and MET

^aWaste = (run time × flow rate) for UPLC methods or Size economy of the sample in UV methods.

Table S5: Enhancing comparative analysis with existing methods for ecological appraisal

Method	Proposed UPLC method	Proposed UV method	Reported method [1]	Reported method [2]	Reported method [3]	Reported method [4]	Reported method [5]
AGREE							
AGREEprep							
GAPI							
ComplexGAPI							
BAGI							

References

- [1] Antakli S, Kabbani R, Labban R. Analytical spectrometric study for determining dapagliflozin propanediol monohydrate individually or in presence of metformin hydrochloride in Tablets formulation. *J Adv Chem.* 2020;17:80–7. doi: 10.24297/jac.v17i.8812.
- [2] Sen DB, Jatu S, Maheshwari RA, Zanzwar AS, Velmurugan R, Sen AK. New Eco-friendly UV-spectroscopic Methods for Simultaneous Assessment of Dapagliflozin, Saxagliptin and Metformin in Ternary Mixture. *Indian J Pharm Educ Res.* 2023;57(2):559–69. doi: 10.5530/ijper.57.2.69.
- [3] Suman SS, Chaubey R. Simultaneous spectrophotometric estimation of metformin, saxagliptin and dapagliflozin in marketed formulations. *J Pharm Res Int.* 2021;33(61B):167–73. doi: 10.9734/jpri/2021/v33i61B35495.
- [4] Vyas AJ, Jadav CD, Patel KJ, Patel R, Vadile HM, Patel AB, et al. Diode array detector based RP-UPLC method for simultaneous estimation of dapagliflozin propanediol monohydrate and metformin. *Res J Pharm Technol.* 2024;17(3):991–6. doi: 10.52711/0974-360X.2024.00153.
- [5] Zagahary WA, Mowaka S, Hendy MS. Kinetic degradation study of dapagliflozin coupled with UHPLC separation in the presence of major degradation product and metformin. *Chromatographia.* 2019;82:777–89. doi: 10.1007/s10337-019-03702-3.