Research Article

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Impressive stability-indicating RP-HPLC method for concurrent quantification of salbutamol, guaifenesin, and sodium benzoate in cough syrup: Application of six sigma and green metrics

Supplementary material

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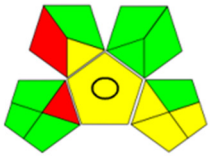
Principles	Proposed Method Chosen	Score	Figure
P1	Off-line analysis	0.48	
P2	0.01 g	1.0	
P3	Off-line	0.0	
P4	3 or fewer	1.0	
P5	Semi-automatic	0.75	
P6	75-05-8	1.0	
P7	3 mL	0.55	
P8	Three analytes, 6 samples were analyzed per hour	0.65	
P9	HPLC	0.36	
P10	Some reagents	0.5	
P11	No	1.0	
P12	2	0.6	

(a)

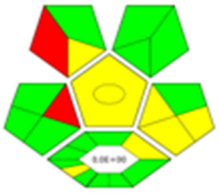
Codes	Proposed Method Chosen	Score	Figure
S1	Ex situ	0.0	
S2	None	1.0	
S3	>75% of reagents and materials are sustainable or renewable	0.75	
S4	3.0 mL	0.45	
S5	0.05 g	1.0	
S6	6	0.42	
S7	2 steps, Semi-automatic	0.5	
S8	1kWh	1.0	
S9	LC	0.25	
S10	2 hazards	0.5	

(b)

Figure S1: Assessment of the proposed method utilizing (a) AGREE and (b) AGREEprep findings.

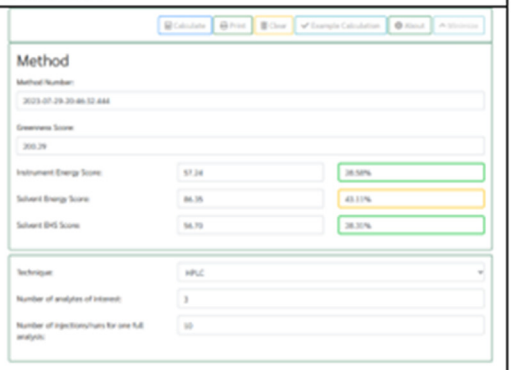
Items	Proposed Method Chosen	Figure
(A) Sample preparation		
Collection (1)	Offline (Red)	
Preservation (2)	None (Green)	
Transport (3)	None (Green)	
Storage (4)	None (Green)	
Type of method (5)	Simple procedures (Yellow)	
The scale of extraction (6)	Micro-extraction (Yellow)	
Solvents/ reagents used (7)	None- green solvents/reagents (Red)	
Additional treatments (8)	None (Green)	
(B) Reagents and Solvents		
Amount (9)	<10 mL (green)	
Health hazard (10)	NFPA=1; slightly toxic (Green)	
Safety hazard (11)	NFPA=1; highest flammability (Green)	
(C) Instrumentation		
Energy (12)	≤1.5 Kwh per sample (Yellow)	
Occupational hazard (13)	Hermetic sealing of analytical process (Green)	
Waste (14)	1 -10 mL (Yellow)	
Waste treatment (15)	Degradation (Yellow)	

(a)

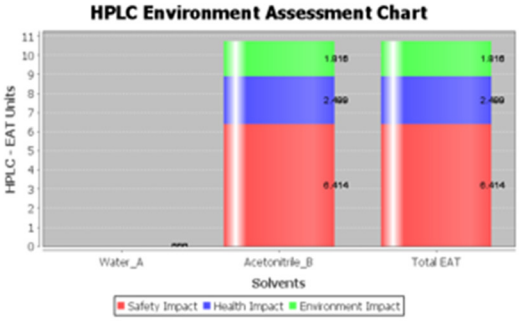
Items	Proposed Method Chosen	Figure
(A) Sample preparation		
Collection (1)	Offline (Red)	
Preservation (2)	None (Green)	
Transport (3)	None (Green)	
Storage (4)	None (Green)	
Type of method (5)	Simple procedure (Yellow)	
Scale of extraction (6)	Micro-extraction (Yellow)	
Solvents/ reagents used (7)	None-green solvents/reagents (Red)	
Additional treatments (8)	None (Green)	
(B) Reagent and Solvents		
Amount (9)	<10 mL (green)	
Health hazard (10)	NFPA=1; moderately toxic (Green)	
Safety hazard (11)	NFPA=1; highest flammability (Green)	
(C) Instrumentation		
Energy (12)	≤1.5 Kwh per sample (Yellow)	
Occupational hazard (13)	Hermetic sealing of analytical process (Green)	
Waste (14)	1-10 mL (Yellow)	
Waste treatment (15)	Degradation (Yellow)	
Pre-analysis processes		
I. Yield	> 89% (Green)	
II. Temperature/Time	Room temperature, >1h, heating (Yellow)	
(D) Relation to the green economy		
III. Number of rules met.	5-6 (Green)	
(E) Reagent and Solvents		
IVa. Health hazard	NFPA=1; Slightly toxic (Green)	
IVb. Safety hazard	NFPA=1; high flammability (Green)	
(F) Instrumentation		
Va. Technical setup	Common setup (Green)	
Vb. Energy	≤1.5kWh per sample (Yellow)	
Vc. Occupational hazard	Hermitization of analytical process (Green)	
(G) Workup and purification		
VIa. End product workup and purification.	None or simple process (Green)	
VIb. Purity	>98% (Green)	
(H) Workup and purification		
VII. E-factor input	Zero	

(b)

Figure S2: Analysis of the suggested approach using (a) GAPI and (b) ComplexGAPI.

	AMGS	Proposed Method		
Instrument Conditions	Technique	HPLC		
	Number of analytes of interest	1		
	Number of injections/runs for one full analysis	10		
	Flow Rate (mL/min)	1.5		
	Run time (min/injection)	10		
Solvent	Mobile phase	130		
Sample	Sample prep volume (mL)	20		
	Number of sample preps	2		
Standards	Stock standard prep volume (mL)	50		
	Number of stock standard preps	1		
Standard Diluent	Working Standard prep volume (mL)	20		
	Number of stock standard preps	1		
System Suit Test (SST) volume (mL)	SST prep volume (mL)	20		
	Number of SST preps	1		
Method	Instrument Energy Score		**57.24**	**(28.58%)**
	Solvent Energy Score		**86.35**	**(43.11%)**
	Solvent EHS Score		**56.70**	**(28.31%)**
Greenness Score		**200.29**		

(a)

Calculation SHE	Proposed Method	Figure
Sample Pre-Treatments	None	
Mobile phase A	Buffer solution (80%)	
Mobile phase B	Acetonitrile (20%)	
Flow rate	1.5 mL/min	
Run time	10 min	
Safety Impact	6.414	
Healthy Impact	2.499	
Environment Impact	1.816	
Total HPLC-EAT score	10.729	

(b)

Figure S3: Reports on evaluations of the indicated techniques utilizing (a) AMGS and (b) HPLC EAT.

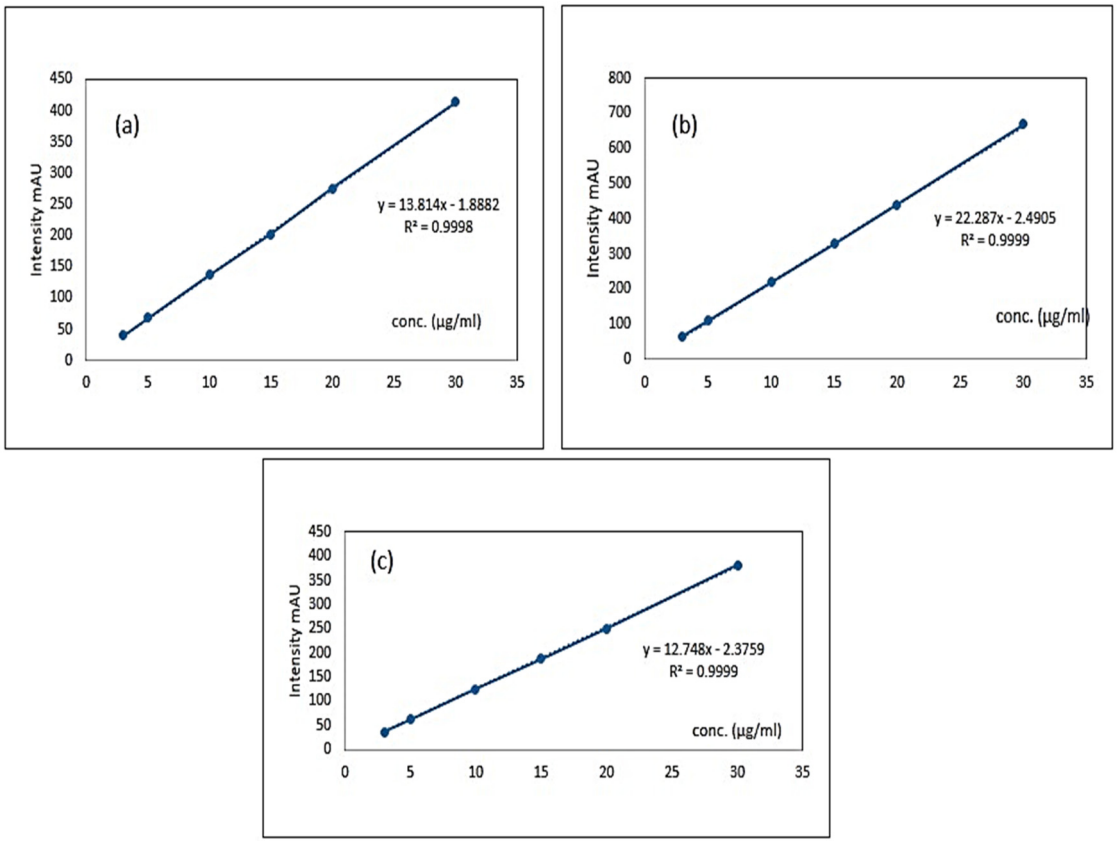


Figure S4: (a–c) Calibration curves for determination of SAL, GUA and SOB.

Table S1: Process capability six-pack data description for the suggested approach

Parameters	**Results**		
Descriptive statistic	**SAL**	**GUA**	**SOB**
Minimum	96.6	96.3	97.1
Maximum	101.8	101.8	101.5
Mean	99.557	99.546	99.256
Limit	90–110%	90–110%	90–110%
Sum	9955.7	9954.6	9925.6
Count	100	100	100
Standard error	0.12	0.12	0.12
Median	100.45	100.1	99.2
Mode	98.3	100	99
Standard deviation	1.23	1.22	1.16
Variance	1.52	1.48	1.34
Kurtosis	−0.41	−0.43	−0.85
Skewness	−0.05	−0.19	0.02
Range	5.2	5.5	4.4

Table S2: Method robustness for the proposed technique

Analyte	Chromatographic parameters	Wavelength (nm)		Acetonitrile ratio		Flow rate	
SAL		274	278	19.00%	21.00%	1.4 min/mL	1.6 min/mL
	Assay (%)	100.11%	100.14%	100.13%	100.01%	100.10%	100.06%
	Retention time (Rt)	1.88	1.88	1.92	1.88	2.02	1.77
	Tailing factor	1.52	1.51	1.54	1.53	1.54	1.53
	Resolution	8.21	8.22	8.11	8.04	8.14	8.07
GUA	Assay (%)	100.35%	100.30%	100.24%	100.33%	100.40%	100.26%
	Retention time (Rt)	3.40	3.40	3.47	3.38	3.63	3.19
	Tailing factor	1.30	1.30	1.30	1.30	1.31	1.31
	Resolution	16.55	16.52	16.71	16.59	16.51	16.21
SOB	Assay (%)	100.65%	100.52%	100.71%	100.58%	100.84%	100.62%
	Retention time (Rt)	6.27	6.27	6.43	6.24	6.69	5.85
	Tailing factor	1.23	1.23	1.22	1.22	1.22	1.23
	Resolution	N/A	N/A	N/A	N/A	N/A	N/A

*N/A (Not Applicable).

Table S3: Results of system suitability parameters for SAL, GUA, and SOB

Suitability parameter	SAL	GUA	SOB	Limit
Retention time	1.887	3.294	0.2	Rt ± 10%
Resolution	9.32	17.22	N/A	NLT 2.0
Theoretical plates	7497.17	11655.17	14133.33	NLT 2000
Tailing factor	1.52	1.29	1.20	NMT 2.0%

Table S4: Results of forced degradation studies of SAL, GUA, and SOB in API form

Forced degradation tests	SAL			GUA			SOB		
	% Recovered	% Degradation	Peak purity (Purity threshold)	% Recovered	% Degradation	Peak purity (Purity threshold)	% Recovered	% Degradation	Peak purity (Purity threshold)
Acid hydrolysis using 5 mL of 2 N HCl for 24 h at 25°C	90.73	9.27	999.607	96.11	3.89	999.399	98.06	1.94	999.989
Basic hydrolysis using 5 mL of 2 N NaOH for 1 h at 95°C	82.10	17.90	998.855	93.65	6.35	999.390	99.77	0.23	999.348
Oxidation using 5 mL 10% H_2O_2 for 1 h at 95°C	95.16	4.84	999.653	98.50	1.50	999.530	97.24	2.76	999.952
Stress testing by heat at 95°C for 17 h	97.80	2.20	999.932	99.31	0.69	999.436	96.19	3.81	999.991
Stress testing using sunlight (Photodegradation) for 6 h	98.49	1.51	999.930	97.93	2.07	999.470	98.92	1.08	999.790

Table S5: Comparison of advantages of the proposed method versus existing methods for analyzing cough syrup components

	Linearity range (µg/mL)	Accuracy (% Recovery)	Precision (RSD%)	LOD (µg/mL)	LOQ (µg/mL)	Published methods
SAL	—	—	—	—	—	[1]
GUA	2–100 µg/mL	100.10	0.76	—	—	
SOB	5–100 µg/mL	100.24	0.95	—	—	
SAL	—	—	—	—	—	[2]
GUA	250–400 µg/mL	99.68	0.18	0.18	0.54	
SOB	125–200 µg/mL	99.27	0.31	—	—	
SAL	25–150%	99.90	0.60	1.3 ng	4.0 ng	[3]
GUA	25–150%	99.80	0.30	7.7 ng	10.2 ng	
SOB	—	—	—	—	—	
SAL	2–50 µg/mL	99.09	1.27	0.51	1.53	[4]
GUA	2–50 µg/mL	99.77	1.42	0.56	1.70	
SOB	—	—	—	—	—	
SAL	0.5–20 µg/mL	99.62	0.003	—	0.28	[5]
GUA	0.5–30 µg/mL	100.45	0.01	—	0.30	
SOB	—	—	—	—	—	
SAL	2–50 µg/mL	100.15	0.11	0.49	1.49	Our proposed method
GUA	2–50 µg/mL	100.47	0.15	0.44	1.34	
SOB	2–50 µg/mL	100.92	0.17	0.38	1.17	