Supplementary Information

An integrated approach of green chemistry and quality-by-design for simultaneous estimation of drugs in fixed dose combination by stability-indicating RP-HPLC method

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1. Method development:

A Quality by Design (QbD) approach using a Central Composite Design (CCD) was applied to systematically optimize chromatographic conditions for the simultaneous estimation of telmisartan, amlodipine and chlorthalidone. Four critical method attributes—flow rate (1.20-1.70 mL min^{-1}), column temperature (25.0–40.0 °C), pH (2.30–2.50) and detection wavelength were varied at three levels each, resulting in 17 different experimental runs generated through design-expert. The method was developed on a C18 column with dimension of 4.6 mm × 150 mm. under isocratic conditions with 35 % ethanol as organic phase, injection volume of 20 µL, the analytical target profile was defined in terms of resolution in-between critical peak pairs, theoretical plates, and peak asymmetry, all of which served as system suitability parameters.

Fusion QbD Software parameters details mentioned as below

Instrument Data System: Chromeleon

Table S1: **Instrument Definition**

Device Name	Category	Version
Vanquish Quaternary Pump C/CN (VC-P20-A-01 / VC-P21-A-01)	Pump	1.0.0.0
Vanquish Column Compartment C (VC-C10-A-03)	Oven	1.0.0.0
Vanquish Diode Array Detector FG/CG (VF-D11-A-01 / VC-D11-A-01)	Wavelength Detector	1.0.0.0
Vanquish Split Sampler C/CT (VC-A12-A-02 / VC-A13-A-02)	Autosampler	1.0.0.0

Table S2: **Vanquish Quaternary Pump C/CN (VC-P20-A-01 / VC-P21-A-01)**

Flow Line	Configuration
A	Reservoir
B	Reservoir
C	Reservoir
D	Reservoir

Table S3: **Vanquish Column Compartment C (VC-C10-A-03)**

Setting	Value
Mode	Combined Mode
Column Switching Valve	7 Port, 6 Position Switching Valve

Table S4: **Experiment Setup Settings**

Setting	Value
Experiment Phase	Method Development
Experiment Type	Optimization
Separation Mode	Reversed Phase (RPC)
Method Type	Gradient
Sample Preparation Mode	Online Mixing

No. of Mobile Phase Solvents	2

Table S5: **Column Settings**

Name	Valve Position	pH Upper Limit	Diameter (mm)	Length (mm)	Conditioning Time (min)
sunniest	P1	12.00	4.60	150.00	21.00

Table S6: **Experiment Constants**

Constant Name	Constant Value	Units
Column Type	sunniest	*
Injection Volume	20.00	µL
Equilibration Time	1.0	min
Initial Hold Time	1.0	min
Initial Hold % Organic	35.0	%
Gradient Time	0.0	min
Final Hold Time	1.0	min
Final Hold % Organic	35.0	%
Ramp Up to Wash Time	1.0	min
Column Wash Time	7.0	min
Column Wash % Organic	35.0	%
Ramp Down from Wash Time	1.0	min
Re-equilibration Time	3.0	min

Table S7: **Design Wizard Settings**

Setting	Value
Design Wizard Mode	Automated
Design Type	Response Surface - Central Composite Full
Design Model	Quadratic
Generate Design Option	New
Number of Design Runs	17
Blocking Strategy	None
Number of Center Points	3
Number of non-center points to be repeated	0
Axial Point Option	Face-Centered
Alpha Value	1.00
Inscribed	False

The QbD-driven approach effectively recognized key method variables and characterized a solid design space, enabling dependable and sustainable separation of the combined dosage form. As outlined in the operational matrix, multiple trials were conducted for optimization as per experiment design matrix.

Table S8: **Experiment Design Matrix**

Run No.	Pump Flow Rate (mL/min)	Oven Temperature (°C)	pH
Conditioning_Run_1	1.500	25.0	2.30
1	1.200	25.0	2.30
2	1.700	25.0	2.30
Conditioning_Run_2	1.500	25.0	2.40
3	1.500	25.0	2.40

Conditioning_Run_3	1.500	25.0	2.50
4	1.200	25.0	2.50
5	1.700	25.0	2.50
Conditioning_Run_4	1.500	30.0	2.30
6	1.500	30.0	2.30
Conditioning_Run_5	1.500	30.0	2.40
7	1.200	30.0	2.40
8	1.700	30.0	2.40
9	1.500	30.0	2.40
10	1.500	30.0	2.40
11	1.500	30.0	2.40
Conditioning_Run_6	1.500	30.0	2.50
12	1.500	30.0	2.50
Conditioning_Run_7	1.500	40.0	2.30
13	1.200	40.0	2.30
14	1.700	40.0	2.30
Conditioning_Run_8	1.500	40.0	2.40
15	1.500	40.0	2.40
Conditioning_Run_9	1.500	40.0	2.50
16	1.200	40.0	2.50
17	1.700	40.0	2.50
Conditioning_Run_10	1.500	40.0	2.50

Central Composite Design (CCD) Statistical Analysis

Three independent variables were optimized using the CCD: the mobile phase pH (C, 2.3–2.5), the column oven temperature (B, 25–40 °C), and the pump flow rate (A, 1.2–1.7 mL/min). To assess main, quadratic, and interaction effects, a total of 17 experimental runs—including three center points—were produced. With a G-efficiency of 70.1 (>50% acceptance) and an average predicted variance of 10.2 (<17), the model showed high reliability, confirming the suitability of the design.

Table S9: Central Composite Design (CCD) statistical summary for AQbD method

Parameter	Value	Acceptance Criteria	Interpretation
Number of runs (total)	17	–	Included 3 center points, 14 non-center points
Design type	Response surface – Central Composite (Quadratic)	–	Adequate for multidimensional optimization
G-efficiency	70.1	> 50%	Indicates good design efficiency
Average predicted variance	10.2	< 17	Confirms adequate prediction capability
R^2	0.9999	> 0.90	Excellent model fit
Adjusted R^2	0.9999	> 0.90	Strong agreement between predicted and observed values
Residual mean squareerror (MSE)	0.0003	As low as possible	Negligible residual error

Standard error	±0.017	<0.05 desirable	High precision of responses
Variance inflation factor (VIF)	1.00–1.58	< 10	No multicollinearity observed
Scaled prediction variance range	3.11–14.26	–	Minimum at center points, maximum at axial runs
Significant model terms	A (Flow rate), C (pH), A^2, B^2, C^2, A×C, B×C	–	Flow rate and pH most influential
p-value (overall)	< 0.0001	< 0.05	The quadratic model is highly significant

2. Optimised method HPLC system chromatographic condition, sample preparation and methodology:

Table S10: Reagents and Equipment

Reagents and Equipment		
Water	:	Milli Q'
Triethylamine (TEA)	:	AR Grade
Orthophosphoric acid	:	AR Grade
Ethanol	:	AR Grade
Methanol	:	HPLC Grade
Filter	:	0.45 µ nylon syringe filter, 0.45 µ nylon membrane filter
HPLC system	:	Dionex Ultimate 3000 or equivalent

Table S11: Chromatographic condition:

Chromatographic condition		
Column	:	C18, 150 mm x 4.6 mm, 5 µm; e.g. Sunniest C18 or equivalent
System	:	Isocratic
Flow Rate	:	1.6 mL / min
Injection Volume	:	20 µL
Retention time	:	About 1.7 minutes for Chlorthalidone About 4.9 minutes for Telmisartan About 7.7 minutes for Amlodipine
Run Time	:	10 minutes
Wavelength	:	UV 245 nm
Buffer solution pH 2.3	:	Mix well 1 mL of TEA in 1000 mL of water. Adjust to pH 2.3 ± 0.05 with diluted orthophosphoric acid.
Mobile phase	:	Water pH 2.3: Ethanol (65: 35 v/v). Mix well. Filter through 0.45 µ membrane filter and degas.
Diluent-1	:	Ethanol: Methanol (50: 50 v/v). Mix well.

Diluent-2	:	Use mobile phase.

Table S12: Sample and standard preparations:

Preparations		
Blank	:	Use diluent-2 as blank
Telmisartan stock solution	:	Weigh and transfer accurately about 40.0 mg of Telmisartan Working Standard in to a 50 mL volumetric flask. Add 30 mL of diluent-1 & sonicate to dissolve. Cool & dilute up to the mark with diluent-1. Mix well. (Conc.: 800 ppm of Telmisartan)
Amlodipine & Chlorthalidone stock solution	:	Weigh and transfer accurately about 34.0 mg of Amlodipine Besylate Working Standard and 63 mg of Chlorthalidone Working Standard in to a 50 mL volumetric flask. Add 30 mL of diluent-1 & sonicate to dissolve. Cool & dilute up to the mark with diluent-1. Mix well. (Conc.: 700 ppm of Amlodipine Besylate equivalent to 504.7 ppm of Amlodipine and 1250 ppm of Chlorthalidone)
Reference Solution	:	Further dilute 5.0 mL of Telmisartan stock solution and 1.0 mL of Amlodipine & Chlorthalidone stock solution to 25 mL with diluent-2. Mix well and inject. (Conc.: 160 ppm of Telmisartan, 28 ppm of Amlodipine Besylate equivalent to 20 ppm of Amlodipine and 50 ppm of Chlorthalidone)
Test Solution	:	Weigh accurately 20 tablets & calculate average weight. Crush 20 tablets by suitable means to fine powder. Weigh & transfer powder equivalent to 1 tablet (about 380 mg of powder) into a 100 mL volumetric flask. Add 70 mL of diluent-1 and sonicate for 20 minutes with intermittent shaking. Cool and dilute upto the volume with diluent-1. Mix well. Centrifuge a portion of the solution at 4000 rpm for 5 minutes. Further dilute 10.0 mL of above supernatant solution to 25 mL with diluent-2. Mix well. Filter the above solution through 0.45 µm syringe filter, discarding first2 mL of the filtrate and inject. (Conc.: 160 ppm of Telmisartan, 20 ppm of Amlodipine and 50 ppm of Chlorthalidone)

Injection Procedure	:	1. Blank (diluent-2)
		2. Reference solution six times
		3. Test solution
		4. Inject reference solution after every 6 injections of test solution and at the end of the sequence

3. Method validation:

Analytical method validation performed as per Q2 ICH requirements. Observed results mentioned below:

Table S13: System suitability parameter

Sr. No.	**Chlorthalidone**	**Telmisartan**	**Amlodipine**
1	101.7	97.9	100.2
2	102.4	98.3	101.8
3	102.1	99.4	101.1
4	102.1	99.1	101.0
5	103.4	98.3	102.7
6	102.6	99.3	100.7

Mean Area (n=6)	102.4	98.7	101.3
%RSD	0.57	0.64	0.87
Mean Asymmetry (n=6)	1.11	1.16	1.10
Resolution	NA	3.15	6.58

Table S14: Results of Specificity parameter

Solution Name	Retention Time	Peak Purity (Limit:≥950)
Reference solution		
Chlorthalidone	1.6	complies
Telmisartan	4.3	complies
Amlodipine	6.2	complies
Test Solution		
Chlorthalidone	1.6	complies
Telmisartan	4.3	complies
Amlodipine	6.2	complies

Table S15: Results of Linearity parameter

Parameter	Chlorthalidone	Telmisartan	Amlodipine
Linearity Range (µg/mL)	25.23- 75.69	80.11-240.32	9.83-29..49
Slope	14.7155	37.2831	29.4184
Y-Intercept	-14.4871	-47.8569	-15.8865
Correlation Coefficient	1.000	1.000	1.000
Standard Deviation of Residuals (Sy/x)	174.6309	3654.3617	90.0375

Table S16: Accuracy results-

Level	Parameter	Chlorthalidone	Telmisartan	Amlodipine
50%	%Mean Recovery,	101.2	100.0	98.1
	%RSD	0.69	0.39	0.57
100%	%Mean Recovery	101.1	100.2	101.1
	%RSD	0.38	0.25	0.52
150%	%Mean Recovery,	101.9	100.1	101.9
	%RSD	0.64	0.65	0.66

Table S17: Precision & Intermediate precision results-

Sr. No.	Chlorthalidone	Telmisartan	Amlodipine
As such MP (n=6)	102.4	98.7	101.3
As such IP (n=6)	101	100.4	99.5
Absolute difference between MP and IP	1.4	1.7	1.8

.

Table S18: % Recoveries result for Robustness study

Condition	Chlorthalidone	Telmisartan	Amlodipine
Flow 1.4mL	100.7	100.4	100.1
Flow 1.8mL	101.0	100.6	100.2
Col Temp 23°C	100.7	100.3	99.7
Col Temp 27°C	100.6	100.3	100.1

Table S19: % Result for filter study

Sr. No.	Chlorthalidone	Telmisartan	Amlodipine
Centrifuged sample	102.7	98.1	100.1
0.45µ PVDF Filtered	103.9	97.9	100.2
0.45µ Nylon Filtered	103.4	97.6	100.9

Table S20: Summery of validation parameters.

Sr. No.	Parameter	Chlorthalidone	Telmisartan	Amlodipine	Acceptance Criteria
1	**System Suitability**	%RSD: 0.57; Tailing: 1.11;	%RSD: 0.64; Tailing: 1.16; Resolution: 3.15	%RSD: 0.87; Tailing: 1.10; Resolution: 6.58	%RSD ≤ 2.0% - Tailing factor ≤ 2.0 Resolution ≥ 2.0
2	**Specificity**	Complies (Purity ≥ 950)	Complies (Purity ≥ 950)	Complies (Purity ≥ 950)	No interfering peaks from blank, diluent, placebo Peak purity ≥ 950
3	**Linearity & Range**	Range: 25.23–75.69 µg/mL; r = 1.000	Range: 80.11–240.32 µg/mL; r = 1.000	Range: 9.83–29.49 µg/mL; r = 1.000	Correlation coefficient (r) ≥ 0.999 Linear range: 50% – 150% of target
4	**Accuracy**	Overall mean recovery =101.4 %	Overall mean recovery =100.1 %	Overall mean recovery =100.4 %	Mean recovery 98–102 %
5	**Method precision**	MP: 102.4%; RSD =0.57	MP: 98.7%; RSD =0.64	MP: 101.3%; RSD =0.87	Repeatability: RSD ≤ 2% (n ≥ 6)

6	**Intermediate precision**	IP: 101 %; RSD =0.29	IP: 100.4 %; RSD =0.18	IP: 99.5 %; RSD =1.54	Repeatability: RSD ≤ 2% (n ≥ 6)
7	**Difference between Method & Intermediate precision**	Δ = 1.4 %	Δ = 1.7%	Δ = 1.8%	Difference (Δ) < 2% absolute
8	**Robustness**	Flow 1.4 mL =1.7 % Flow 1.8 mL =1.4 % Col. temp. 23° c =1.7 % Col. temp. 23° c =1.8 %	Flow 1.4 mL =1.7 % Flow 1.8 mL =1.9 % Col. temp. 23° c =1.6 % Col. temp. 23° c =1.6 %	Flow 1.4 mL =1.2 % Flow 1.8 mL =1.1 % Col. temp. 23° c =1.6 % Col. temp. 23° c =1.2 %	% Change with respect to active contents ≤ 2% with respect to initial.
9	**Filter Study**	1.2 %	0.2 %	0.1 %	% Difference between filtered vs. centrifuged solution ≤ 2%
10	**Solution Stability**	Stable up to 12 hrs	Stable up to 12 hrs	Stable up to 12 hrs	% change ≤ 2% from initial results

4. Assessment of greenness by green metric tools:

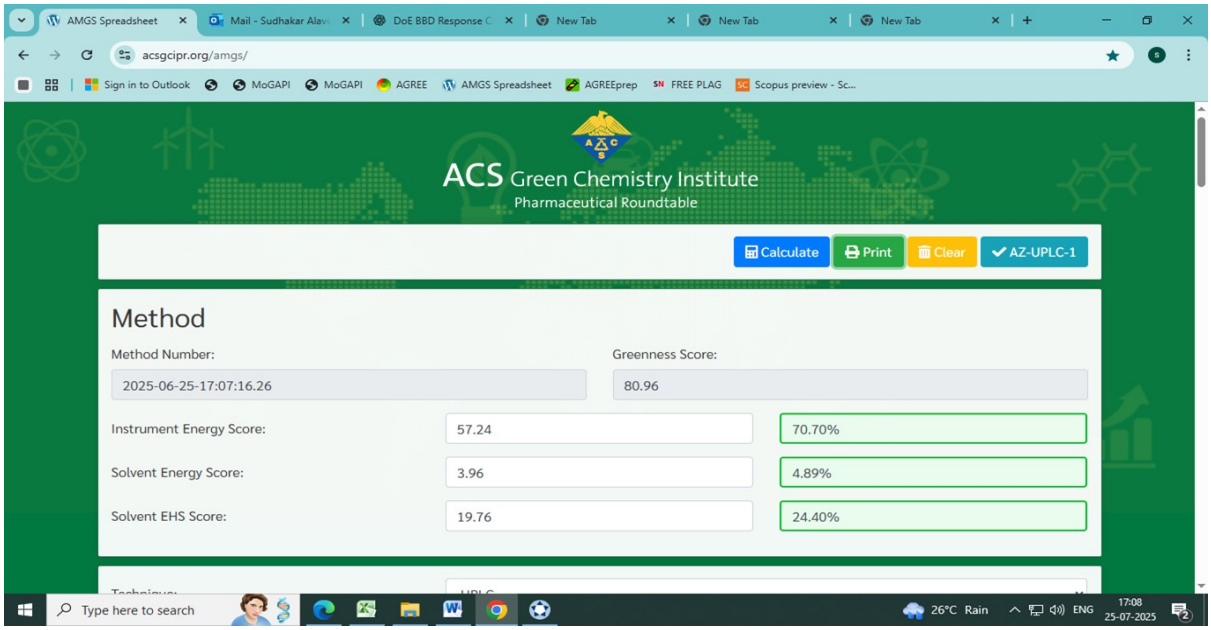

Figure S1 AMGS TOOL

Greenness Score (80.96):This is **above 75**, which typically classifies the method as **green and sustainable** in analytical chemistry.**Instrument Energy Score (57.24)**:Shows **moderate energy consumption**, possibly due to a longer run time, high temperature, or older instrument. If possible, optimize run time or use energy-efficient equipment.

Figure S2 AGREE tools

AGREE is based on the 12 GAC principles, including:
1.Minimal sample preparation
2.Reduced solvent and reagent consumption
3.Use of safer and renewable solvents
4.Energy efficiency
5.Waste minimization
6.Operator safety

Agree tool Indicates strong compliance with the 12 principles of Green Analytical Chemistry (GAC). A score of 0.75 reflects that the method uses safer solvents, generates lower waste, avoids hazardous reagents, and employs energy-efficient conditions. Overall, the method demonstrates high sustainability with only minor aspects requiring refinement

Figure S3 Mo GAPI tool

Each stage is assessed using predefined criteria, such as:
1.Amount and toxicity of solvents and reagents
2.Number of sample preparation steps
3.Use of derivatization
4.Energy consumption and analysis time
5.Quantity and hazard of generated waste

GAPI uses a **three-color code**:
Green – environmentally friendly
Yellow – moderate environmental impact
Red – high environmental impact

(RP-HPLC method)
Ethanol–water mobile phase → greener solvent use
Short run time → lower energy consumption
Minimal sample preparation → reduced reagent usage
HPLC waste generation → depending on volume

Mo GAPI tool Reflects good overall greenness across multiple analytical stages, including sample preparation, instrumentation, solvent choice, and waste management. The score of 79 suggests that the method is environmentally responsible, with minor opportunities to further optimize solvent consumption and energy usage.

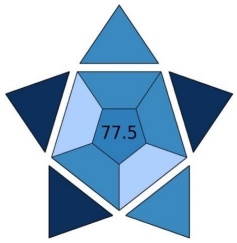

Figure S4 BAGI tools

The method is evaluated based on:
1.Instrumentation availability
2.Operational simplicity
3.Sample throughput
4.Analysis time
5.Need for skilled operators
6.Method robustness and reproducibility

BAGI uses a **blue-scale color code**:
Dark blue – excellent applicability
Light blue – acceptable applicability
White / pale – limited applicability

BAGI tools Demonstrates a strong balance between analytical performance (precision, robustness, accuracy) and environmental responsibility. A score of 77.5 shows that the method successfully integrates operational efficiency with greener practices, making it suitable for routine laboratories seeking sustainable workflows.

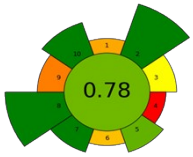

Figure S5.AGREE Prep tools

AGREE-Prep evaluated with the **10 GSP principles**, which include:

1. 1.Minimal sample amount
2. Reduction or elimination of sample pretreatment
3. Use of safe and renewable solvents
4. Miniaturization of sample-preparation steps
5. Low energy consumption
6. Reduced waste generation
7. Operator safety

RP-HPLC sample preparation score calculated based on below parameters

1. Simple dilution → high score
2. No derivatization → high score
3. Ethanol or water as solvent → high score
4. Minimal waste → improved greenness

AGREE Prep tools Shows good adherence to green principles specifically related to sample preparation. A score of 0.78 indicates reduced sample-handling steps, minimized reagent use, and lower generation of preparation-related waste, supporting overall method greenness.

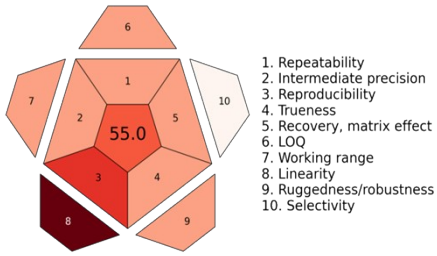

Figure S6.RAPI

RAPI assessesed whether an analytical method meets **analytical and regulatory expectations**, including:

1. Accuracy
2. Precision
3. Linearity
4. Specificity
5. Sensitivity
6. Robustness
7. System suitability

RAPI uses a **red-scale color code**:

1. **Red** – unacceptable / critical performance
2. **Orange** – marginal / needs improvement
3. **White / pale** – acceptable performance