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Original Article

Greenness and Whiteness Assessed Mathematically Filtered UV Spectroscopic Approaches for Quality Control of Amlodipine, Telmisartan and Metoprolol from Ternary Formulation

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ABSTRACT

Background: Latest studies highlight the effectiveness of a new combination tablet that contains Amlodipine (AML), Telmisartan (TEL), and Metoprolol (MET) for the treatment of hypertension. The present work demonstrates the establishment of efficient and environmentally friendly sensitivity-enhanced UV spectroscopic approaches for the concomitant assessment of tablet preparations of AML, TEL, and MET. **Materials and Methods:** In order to disentangle the complex ternary mixture, the double devisor spectroscopic method at two chosen wavelengths was established. The second technique measures the apex height of the ratio first derivative spectrum's at specific wavelengths among a number of maxima and minima. The ecological profile of the methods was also assessed, taking into account two factors: whiteness using RGB12 and greenness using AGREE and the hexagonal tool. **Results:** The sensitivity of the low-concentration analyte AML and low-peak amplitude analyte MET was increased by the scaling factor. AML, TEL, and MET have all been effectively analyzed using these methods, with results falling into the ranges of 1-10 µg/mL, 5-30 µg/mL, and 5-40 µg/mL, correspondingly. Additionally, we followed ICH guidelines to validate these approaches for specificity, accuracy, and precision. No statistically significant differences were seen when compared to the described HPLC approach. **Conclusion:** The recommended methods have been found to be safe, economically feasible, and sustainable based on the ecological assessment by RGB12, AGREE and hexagonal methods. Therefore, the proposed UV spectroscopic approaches could be utilized regularly for analysis of formulation comprising AML, TEL, and MET.

Keywords: Hypertension, Amlodipine, Telmisartan, Metoprolol, UV-derivative spectroscopy, Greenness.

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INTRODUCTION

One of the main causes of cardiovascular diseases such as stroke, and angina pain, is hypertension, which is a major cause of death in general, especially for people in their middle and old age.[1] Researchers are creating innovative treatment plans to improve patient outcomes and lower mortality. Combining many medications with distinct mechanisms of action into one tablet will result in a rational impact, reduced adverse effects, a low dosage, and simple administration.[2] A recently approved formulation consisting of Amlodipine besylate (AML, Figure 1A), Telmisartan (TEL, Figure 1B), and Metoprolol succinate (MET, Figure 1C) has demonstrated excellent effect in managing hypertensive patients along with angina discomfort. The FDA has approved this fixed dose combination as a convenient, more thorough, and effective method of controlling blood pressure in patients with stable coronary artery disease who have uncontrollable critical hypertension. AML is a dihydropyridine derivative (3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate) class of calcium channel blockers, used to treat angina and elevated blood pressure.[3] AML lowers blood pressure by widening the blood arteries and improve blood flow to the heart muscles in angina patients. It is one of the essential medicines according to the WHO.

TEL (4'-[(1,4'-dimethyl-2'-propyl[2,6'-bi-1H-benzimidazol]-1'-yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid) is a long-acting, antihypertensive drug. It blocks the angiotensin II receptor selectively with great affinity, thereby reducing the

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vasoconstriction effect of angiotensin II and decreases blood pressure. It also increases the blood and oxygen content of the cardiac muscles. The high lipophilic nature of TEL increases its intracellular concentration and shows good bioavailability, hence once daily dosing will help in maintaining normal blood pressure for 24 hr.[4] MET (*1-(*isopropylamino*)-3-[p-(2-*methoxyethyl*)* phenoxy*]-2-*propanol succinate) is an adrenergic receptor blocker, that selectively inhibits the action of catechol amines epinephrine and non-epinephrine on beta 1 receptor of cardiac muscle. It reduces the heart rate and contraction of heart muscles hence, utilized for the management of many cardiovascular complications for example hypertension, heart attack, cardiac infraction, and angina.[5]

There are many different analytical approaches revealed in the collection for analysis of AML,[6,7] TEL,[8,9] and MET[10,11] individually from the formulations and biological samples. AML, TEL, and MET were also analyzed in the presence of other medications.[12-19] A binary mixture of AML-TEL,[20-26] AML-MET,[27-30] and TEL-MET[31,32] were analyzed using spectroscopic method, HPLC, LC/MS, capillary electrophoresis, and HPTLC techniques. However, no spectroscopic technique has been established for the concurrent analysis of the newly developed trinary blend of AML, TEL, and MET from the solid dosage form.

Until today smart analytical procedures, employing the simple UV spectrophotometric approach have been exploited comprehensively for the estimation of medicines in the formulations.[33-37] Because the spectroscopic methods are fast, accurate, and economic, use of safer solvents, simple instrumentation, and software. Hence, the current proposal describes the efficient spectroscopic procedures for the synchronized evaluation of AML, TEL, and MET from the preparation without chemical separation using simple mathematical modification steps.

The main objective of Green Analytical Chemistry (GAC) encompasses safety and sustainability to protect the health of animals and the environment. GAC follows the 12 basic principles comprising of utilization of safer solvents and reagents, less waste generation, fewer analysis steps, and reduced energy consumption to develop environment-friendly analytical techniques.[38,39] In addition to the GAC principles, analytical method validation parameters and practical applicability were evaluated in the White analytical chemistry (WAC) to develop more efficient, and effective analytical methods.[40,41] Pharmaceutical industries are working on the expansion of green analytical procedures to protect the environment. The proposed methods follow the green-and-white analytical principles in developing safer environment-friendly analytical methods along with good qualitative, quantitative, and practical applications. Further, the proposed spectroscopic methods were evaluated for whiteness (RGB method[41]), greenness (AGREE,[42] and hexagonal[43]) and matched with the described HPLC approaches.[12,27]

MATERIALS AND METHODS

Chemicals and Reagents

An analytically authentic amlodipine besylate (Purity 99.85%), telmisartan (Purity 99.78%), and metoprolol succinate (Purity 99.05%) were bought from Biokemix India Ltd (Hyderabad, India). Analytes were dissolved in analytical-grade ethanol bought from Merck. Double distilled water arranged in our research laboratory using a Millipore water purifier (Millipore Milli-q advantage A10, USA) was used during the investigations.

Instrumentation

UV-vis spectrophotometer1600 (Shimadzu, Japan) allied with the workstation was useful to scan the solutions. UV Probe (Shinmadzu, Ver. 2.2) software delivered with the spectrophotometer was adopted to mathematically filter the recorded UV spectra. 1 cm quartz cuvettes were used for formulation and standard solutions. UV-vis spectrophotometer was set at fast scan speediness with a 0.1 nm sampling interval and opening breadth of 2 nm.

Preparation of stock solutions

Ethanol was used to solubilize amlodipine and telmisartan, whereas metoprolol was dissolved using deionized water. Initially, three 25 mL volumetric flasks with 25 mg of each analyte were used to produce 1 mg/mL solution. Telmisartan solution was sonicated to get a clear solution.

Preparation of working solutions

Water has been used as a dissolving agent, and calibration standard solutions for the spectrophotometric method were arranged at 0.1 mg/mL of AML, TEL, and MET by suitable attenuation.

Commercial formulation solution

A combination of AML, TEL, and MET is not available in the local market, hence AML tablet and binary combination of TEL and MET accessible in the resident marketplace were procured to make the ternary combination of AML, TEL, and MET. The 20 AML tablet powder and 20 TEL and MET tablets were thoroughly mixed and evenly 5 mg of AML, 40 mg of TEL, and 50 mg of MET were added to the 50 mL graduated container to obtain 0.1 mg/mL of AML, 0.4 mg/mL TEL and 0.5 mg/mL MET respectively. After adding 25 mL of ethanol to completely dissolve the medicines, and graduated flask was completed to the 50 mL streak using fresh ethanol. The subsequent mixture was filtered after sonication for 15 min to extract all there analytes from the formulation powder.

Procedure for constructing calibration graphs

The required quantities of authentic solutions of AML, TEL, and MET were taken in to three groups of 10 mL graduated containers and were completed using sufficient quantities of methanol to

attain 1-10 µg/mL, 5-30 µg/mL, and 5-40 µg/mL, respectively. All of the solutions within the 200-400 nm range were scanned to create the UV spectra.

Double Divisor Ratio spectra method (DDR)

UV-Absorption spectra were collected for the three binary solutions of AML(6 µg/mL) -TEL(20 µg/mL), TEL(20 µg/mL) -MET(10 µg/mL), and AML(6 µg/mL) -MET(10 µg/mL). For an establishment of the linearity curve, TEL(20 µg/mL) -MET(10 µg/mL) spectrum was utilized to divide the above collected AML spectra (1 -10 µg/mL) and to remove noise, the consequent ratio spectra were smoothed using a 2 nm scale size. The apex altitude at 288.8 nm was subtracted from 373.4 nm to find the magnitude of the peak divergence. Likewise, AML(6 µg/mL) -MET(10 µg/mL) spectrum was used to divide TEL spectra (5-30 µg/mL) and MET spectra (5-40 µg/mL) were divided using AML(6 µg/mL) -TEL(20 µg/mL) spectrum. The peak magnitude discrepancy was computed from the obtained ratio spectra by deducting the apex heights for TEL and MET at 244.2 and 242.6 nm, respectively, from 296.2 and 273.8 nm. By graphing the peak amplitude difference against the individual concentration of each analyte, the linearity relationship was investigated. Finally, the linear regression equation and correlation coefficient were computed using Microsoft Excel.

Ratio First Derivative method (RFD)

The AML, TEL, and MET ratio spectra that were generated above lie within the concentration ranges of 1-10 µg/mL, 5-30 µg/mL, and 5-40 µg/mL, respectively, the first derivative spectra were obtained using Δλ 4nm with a scaling factor of 50. Straight lines were constructed for each of the three analysts using the AML, TEL, and MET peak heights, which are, respectively, 353.8 nm, 286.8 nm, and 229.9 nm, in contrast to the corresponding concentration. The resulting calibration curves were employed for constructing the formula for the linear regression and relationship coefficient.

Quantification of laboratory tri-mixtures

By scanning in the 200-400 nm range, the absorption spectra of various ratios of tri-mixtures made in the lab were obtained. The DDR method mentioned above was used to get the ratio spectra for AML, TEL, and MET. Following the determination of the peak amplitude difference, the amount of each of the three analytes was computed using the corresponding linearity coefficients. After applying the RFD approach to convert the ratio spectra into first-order derivation spectra, the concentrations of the three analytes were determined.

Analysis of tri-mixture formulation

Absorption spectra in the 200-400 nm wavelength range were acquired for the solution of AML, TEL, and MET, which included 2, 16, and 20 µg/mL respectively. The quantity of AML, TEL, and MET in the sample solutions were ascertained by means of the congruently genarated straight line equation for individual analyte and procedure, in accordance with the previously described DDR and RFD methodologies. Further, a recovery study was carried out using a standard adding technique to show the accurateness of the recommended processes.

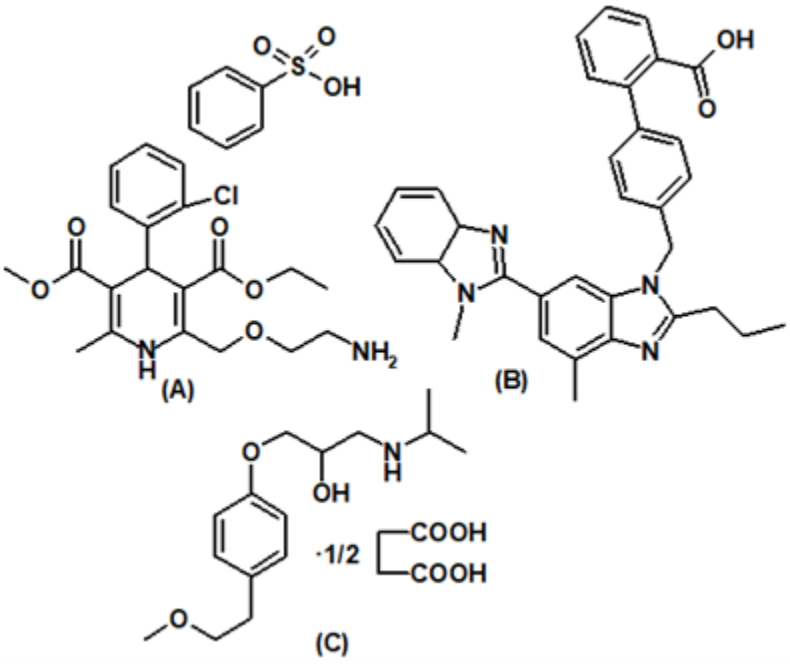

Figure 1: Structural formula of amlodipine besylate (A), telmisartan (B), and Metoprolol succinate (C).

RESULTS

Optimization

In the present work, AML, TEL, and MET showed abundant overlapping within the 200-350 nm range, as depicted in Figure 2, hence two mathematically manipulated Double Divisor Ratio spectra technique (DDR) and Ratio First Derivative technique (RFD) were established. Further, in order to develop an eco-friendly UV spectroscopic approach ethyl alcohol was preferred as a solvent to prepare the authentic stock solutions, and further dilution was made using water.

Double Divisor Ratio spectra method (DDR)

In the current work, different concentration ratios of two analytes have been investigated, using them as dividers in order to produce ratio spectra. Spectra generated using a binary mixture comprising 6 µg/ml, 10 µg/ml and 20 µg/ml of AML, TEL, and MET respectively yielded reproducible ratio spectra. The apex and trough of the ratio spectra were used to identify the two selected wavelengths. The ratio spectrum of AML showed good sensitivity and selectivity using two wavelengths of 373.4 nm and 288.8 nm. similarly, the two wavelengths indicated from TEL and MET ratio spectra were 296.2 - 244.2 nm and 273.8 -242.6 nm respectively (Figure 3A-3C). Further, ratio spectra generated using ternary mixtures and authentic analytes revealed an identical peak intensity discrepancy (Figure 3D-3F).

Ratio First Derivative method (RFD)

In the current work, the first derivative spectrum of the ratio spectrum allowed the quantification of one medicament in the existence of additional medicines despite the need for previous separation. Further, derivatization of the spectrum eliminates the effect of excipients and impurities, if any, and exhibits many maxima and minima to select. The concentration of AML is 1:8 and 1:10 compared to TEL and MET respectively. The first-order derivatization of AML exhibited low intensity. Further, the apex height of the ratio spectra of MET was low, therefore the first-order derivatization displayed less intensity. Therefore, different escalating magnitude from 10 to 50 times was envisaged and the scaling factor of 50 showed better peak amplitude for AML and MET along with good reproducibility. In the current work, first derivative spectra were generated for all three analytes from the ratio spectra by 4 nm as Δλ along with an escalating magnitude of 50. Two maxima and three minima were exhibited for the first derivative spectrum of AML, whereas three maxima and 4 minima for TEL and 2 maxima and 3 minima for MET. AML, TEL, and MET were quantified by measuring the apex height at 353.8 nm, 286.8 nm, and 229.9 nm correspondingly. These frequencies were chosen based on reproducibility, selectivity, and sensitivity. (Figure 4A-4C) Further, first derivative of ratio spectra generated using ternary mixtures and authentic analytes revealed an identical peak intensity (Figure 4D-4F). Finally, a straight line was drawn by measuring the peak height versus respective concentrations.

Validation of UV spectroscopic methods

Linearity

The direct correlation between concentration and the spectra's apex height of the defined processes was shown by testing diverse amounts of the three analytes in triplicate. The apex amplitude was directly proportional at 1-10 µg mL-1, 5-30 µg mL-1, and 5-40 µg mL-1 for AML, TEL, and MET correspondingly for both UV spectrophotometric studies. The regression outcomes are shown in Table 1. Excellent correlation coefficient (R2>0.999) readings across the ranges investigated corroborated the linear behavior of current methods.

Sensitivity

Calculating the detection limit and quantification limit involved using one of the ICH-provided formulas that took into account the slope of the measurement curve and the standard variances

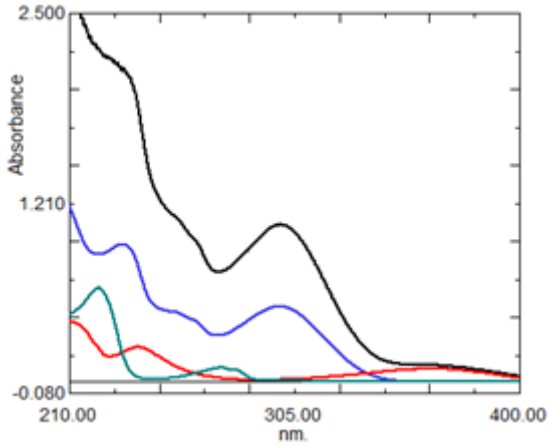

Figure 2: Parent spectra of AML (red), TEL (blue), MET (green) and blend (black).

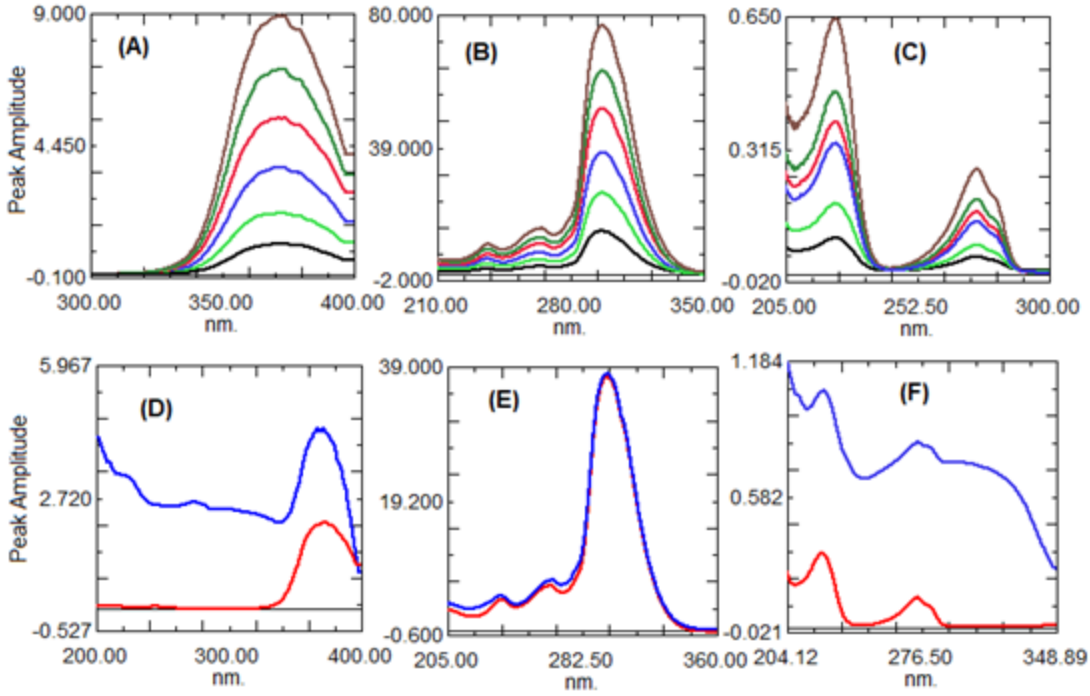

Figure 3: Double divisor ratio spectra of AML (1-10 µg/mL, A), TEL (5 -30 µg/mL, B), and MET (5 -40 µg/mL, C). Overlay presentation of double divisor ratio spectra obtained from authentic and blend having the same quantity of AML(D), TEL (E), and MET(F).

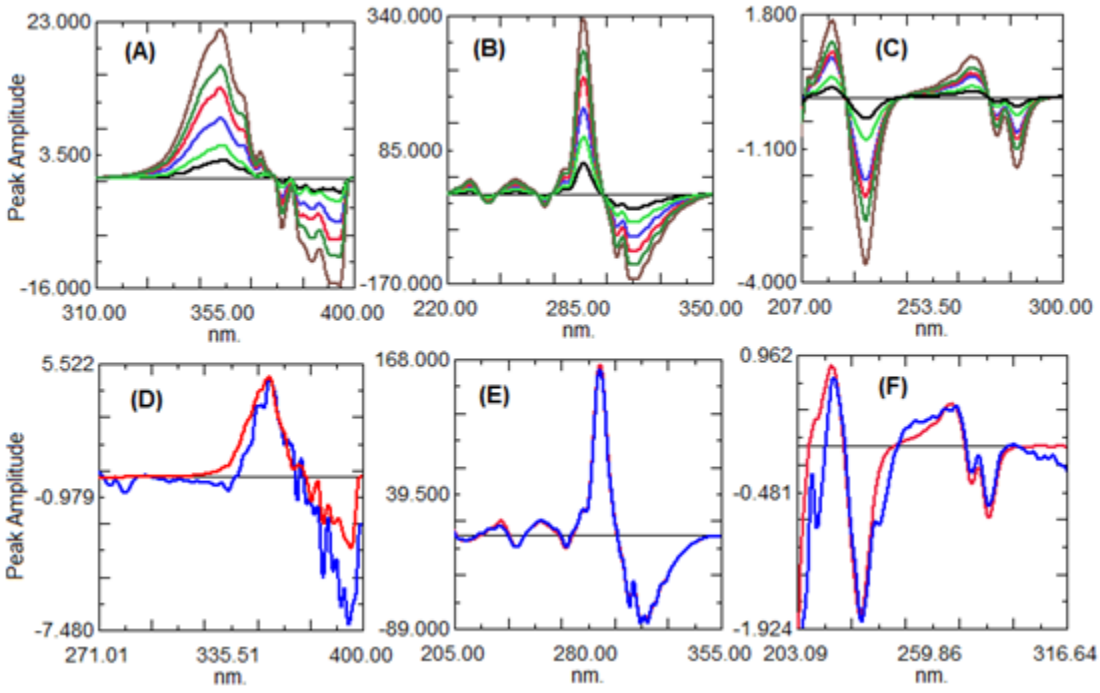

Figure 4: First derivative ratio spectra of AML (1-10 µg/mL, A), TEL (5 -30 µg/mL, B), and MET (5 -40 µg/mL, C). Overlay presentation of First derivative ratio spectra obtained from authentic and blend having the same quantity of AML(D), TEL (E), and MET(F).

Table 1: Results of Validation factors for AMD, TEL, and MET.

Factors	DDR			RFD		
Analytes	AMD	TEL	MET	AMD	TEL	MET
Wavelength [nm]	373.4 288.8	296.2 244.2	273.8 242.6	353.8	286.8	229.9
Linearity Range [µg/mL]	1 -10	5 -30	5-40	1 -10	5 -30	5-40
LOD [µg/mL]	0.151	0.124	0.645	0.237	0.186	0.893
LOQ [µg/mL]	0.454	0.369	1.867	0.782	0.614	2.947
Slop [m]	0.859	2.290	0.006	2.033	11.012	0.089
Intercept [c]	0.030	0.071	0.0035	0.684	0.796	0.0033
Relation Coefficient [r^2]	0.9998	0.9995	0.9995	0.9999	0.9994	0.9998

Table 2: Accuracy and precession results of spectroscopic methods.

		Within-day			Between-day		
	Amount of Drug [µg /mL]	Amount found Mean [*n*=3] ± SD	%RSD	%RE	Amount found Mean [*n*=9] ± SD	%RSD	%RE
Double Divisor Ratio spectra method							
AMD	1	1.01±0.02	1.98	1.00	0.99±0.01	1.01	-1.00
	5	4.91±0.06	1.22	-1.80	4.90±0.09	1.85	-2.00
	10	9.88±0.11	1.11	-1.20	9.81±0.12	1.22	-1.90
TEL	5	5.01±0.08	1.60	0.20	4.93±0.06	1.22	-1.40
	15	14.82±0.24	1.62	-1.20	14.88±0.22	1.48	-0.80
	30	29.86±0.38	1.27	-0.47	29.47±0.25	0.85	-1.77
MET	5	4.93±0.07	1.42	-1.40	4.92±0.07	1.42	-1.60
	20	20.04±0.31	1.55	0.20	19.95±0.31	1.55	-0.25
	40	39.47±0.67	1.70	-1.33	39.27±0.68	1.73	-1.82
Ratio First Derivative method							
AMD	1	0.98±0.01	1.02	-2.00	1.01±0.01	0.99	1.00
	5	5.04±0.07	1.39	0.80	4.97±0.04	0.80	-0.60
	10	9.83±0.09	0.92	-1.70	9.93±0.12	1.21	-0.70
TEL	5	4.96±0.06	1.21	-0.80	4.95±0.08	1.62	-1.00
	15	15.12±0.12	0.79	0.80	15.23±0.24	1.58	1.53
	30	29.58±0.34	1.15	-1.40	29.31±0.39	1.33	-2.30
MET	5	4.97±0.04	0.80	-0.60	5.02±0.07	1.39	0.40
	20	19.9±0.28	1.41	-0.50	20.19±0.37	1.83	0.95
	40	39.47±0.59	1.49	-1.33	39.31±0.28	0.71	-1.72

of the straight-line intercept. Table 1 presents the computed detection and quantification limits for AML, TEL, and MET, ensuring a high degree of sensitivity for the suggested techniques.

Accuracy and precision

Three concentrations of each pure component were selected for triply analysis for these criteria. For the spectrophotometric methods, the concentrations that were used were 1, 5, and 10 µg mL-1 for AML, 5, 15, and 30 µg mL-1 for TEL, and 5, 20, and 40 µg mL-1 for MET. For the within-day precision %RSD was determined from the three assay results performed on the same day, whereas for between-day precision, %RSD was calculated from the 9 assay results performed over the course of three days. The calculated %RSD fell within an unobjectionable range. The accuracy of the suggested spectroscopic techniques was quantified in terms of relative standard deviation and percentage recovery, both of which were close to 100%, demonstrating the high level of accurateness of the developed protocols. Excipients did not

Table 3: Analysis of AMD, TEL, and MET in manually-prepared blends by anticipated techniques.

Ratio[a]	Amlodipine (recovery±SD)		Telmisartan (recovory±SD)		Metoprolol (recovory±SD)	
	DDR	RFD	DDR	RFD	DDR	RFD
5:10:20	101±0.04	99±0.75	99.2±0.66	99.0±0.19	98.6±0.27	98.4±0.44
3:15:30	98.2±0.12	99.2±0.86	100.8±0.34	101.53±0.27	100.2±0.65	99.75±0.52
3:20:40	98.8±0.24	101.04±0.18	98.6±0.5	97.7±0.43	98.675±0.84	98.175±0.91
7.5:30:40	100.2±0.07	98.6±0.67	99.4±0.43	100.4±0.51	98±0.45	101±0.38
5:20:30	98.8±0.34	99.2±0.38	99.5±0.67	100.95±0.63	100.8±0.77	99.4±0.49
7.5:20:30	99.53±0.85	98.23±0.76	98.675±0.49	98.275±0.72	98.3±0.39	99.3±0.73

[a]AMD:TEL: MET in µg/mL; SD: Standard Deviation; DDR: Double Devisor Ratio Spectra; RFD: Ratio First derivative method.

Table 4: Assay results and comparison of statistical outcomes amongst suggested and reference techniques for evaluation of AMD, TEL, and MET.

Parameters	DDR Method			RFD Method			Ref. technique		
	AML	TEL	MET	AML	TEL	MET	AML[f]	TEL[f]	MET[g]
Label Claim [a]	5	40	50	5	40	50	5	40	50
Amount taken [b]	2	16	20	2	16	20	2	16	20
The amount found [bc] ±SD	2.02 ±0.01	15.89 ±0.22	19.89 ±0.29	1.98 ±0.01	15.87 ±0.17	19.91 ±0.23	1.99 ±0.02	15.91 ±0.31	19.96 ±0.35
% Label Claim	100.83	99.31	99.45	99.16	99.3	99.56	99.48	99.41	99.81
N	6	6	6	6	6	6	6	6	6
Student's *t*-test [d]	0.165	0.179	0.534	0.575	0.197	0.365			
p-value	0.871	0.860	0.605	0.579	0.847	0.722			
F-test[e]	1.615	1.170	2.452	1.401	1.751	2.206			
p-value	0.305	0.433	0.173	0.359	0.276	0.202			

[a] mg/tablet; b µg/ml; c mean of six determinations; critical value 2.228 for [d] t-test and 5.050 for [e] F-test (at P=0.05). [f] Ref. technique[12] is RP HPLC using Zorbax C_{18} HPLC Column (150 mm X4.6 mm, 5 µm), Mobile phase Phosphate buffer (20mM, pH 3.5) : acetonitrile : methanol (35:45:20 v/v), flow rate 1.5 mL/min, wavelength:230 nm. [g27]Zorbax C18 HPLC Column (250 mm X4.6 mm, 5 µm), Mobile phase phosphate buffer (10 mM, pH 3.0): acetonitrile (50:50 v/v), flow-rate 1 ml/min, wavelength:235 nm.SD: Standard Deviation; DDR: Double Divisor Ratio spectra; RFD: Ratio First Derivative method.

Table 5: Assay and standard adding technique results.

Amount Added (µg/mL)			DDR method (Mean %recovery)			RFD method (Mean %recovery)		
AML	TEL	MET	AML	TEL	MET	AML	TEL	MET
1	8	10	100.20	98.60	99.21	98.40	98.25	101.00
1.5	12	15	98.80	99.20	100.20	99.75	100.80	99.40
2	16	20	99.53	98.23	98.68	98.18	98.30	99.30
Across Mean			99.51	98.68	99.36	98.78	99.12	99.90
%RSD			0.70	0.49	0.90	0.85	1.54	0.95

%RSD: Percent relative Standard Deviation; Acceptable %RSD: ±2% and % recovery : 98.00%-102.00%

show any intervention with analytes of the formulations, as was ascertained by a systematic examination of the accurateness by means of the authentic drug-adding technique as shown in Table 2.

Specificity and Selectivity

To establish the specificity of the approaches, a range of laboratory-prepared blends with different concentrations of AML, TEL, and MET were subjected to spectrophotometric techniques. Satisfactory findings were observed in terms of percentage retrieval and relative mistakes as represented in Table 3. Furthermore, the results of using these techniques on commercial tablets containing AML, TEL, and MET (Table 3) verified the methodologies' selectivity and the lack of excipient impact.

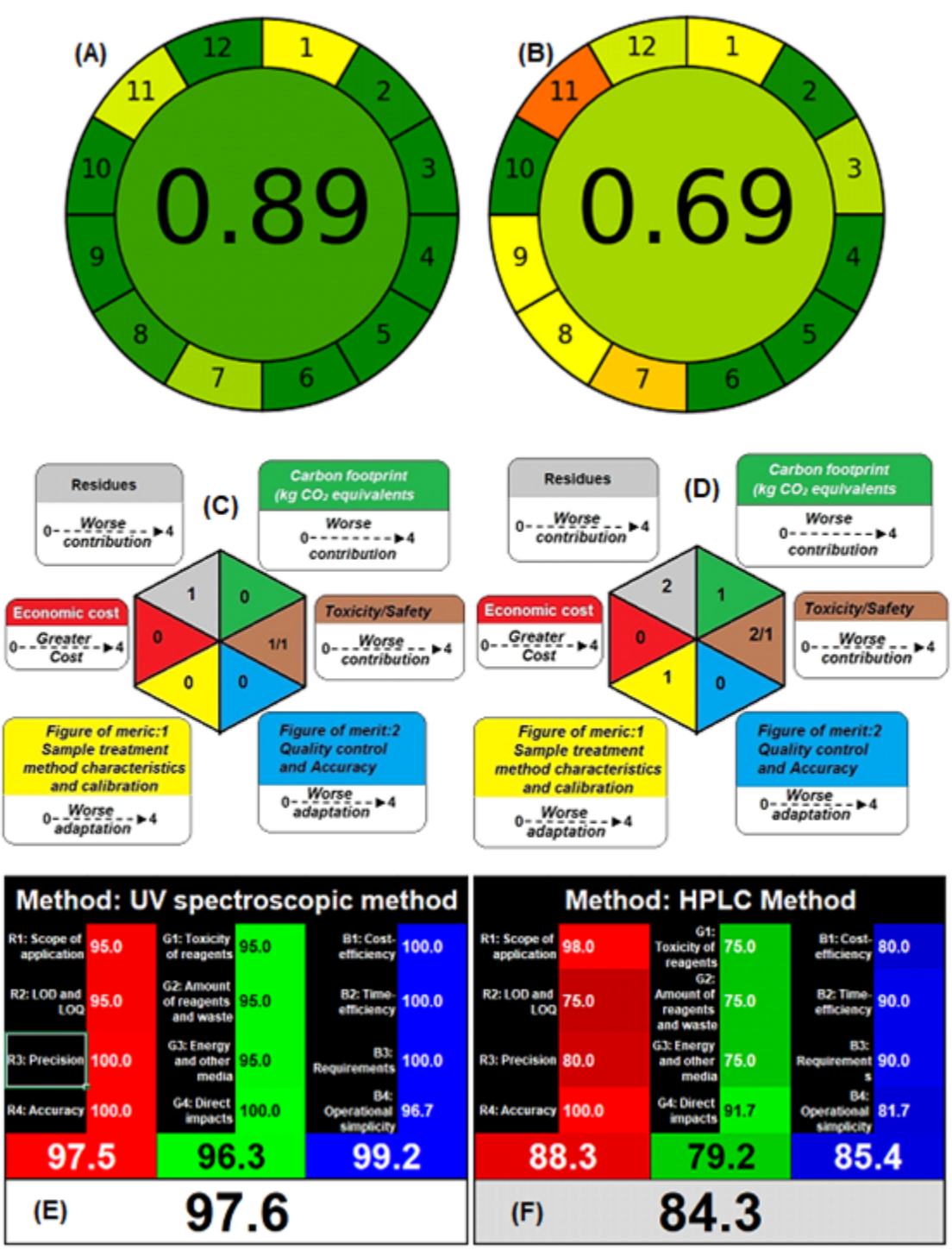

Figure 5: Greenness and whiteness results of UV spectroscopic (A, C, and E) and HPLC (B, D and F) methods by AGREE (A and B) Hexagonal (C and D) and RGB additive color method (E and F).

Application to pharmaceutical formulations

The proposed UV spectrophotometric procedures were utilized to ascertain the concentrations of AML, TEL, and MET in the formulation. The results were expressed as percentage recoveries and were within the acceptable range of 98%-102% (Table 4). The standard addition method's results (Table 5) further confirmed the applicability of the strategies developed and showed that the excipients in the tablets didn't affect the assay's desired results of analytes under investigation.

Comparison of statistical results

The outcomes that were obtained by applying the reported HPLC techniques and the suggested UV approaches were compared using the F-test and student's t-test. According to these tests,

the percentage recovery of the analytes, there are no appreciable differences between the compared methodologies. The obtained F and student's t-test results for UV spectroscopic approaches are lower compared to the critical values and the p-values are more than 0.05 and the findings confirmed that no noticeable discrimination could be found among UV and HPLC procedures for examining AML, TEL, and MET in tablet formulations. (Table 4).

Appraisal of Greenness and whiteness

Analytical GREEnness Metric Approach (AGREE)

AGREE metric methodology is developed for gauging the greenness of the analytical technique using the 12 philosophies of GAC.[42] It utilizes both qualitative and quantitative parameters such as sample preparation steps, generation, and management of analytical waste, multicomponent or multi-parameter analysis, use of safer reagents, amount of reagents used, power consumption, automation of the procedures, and use of renewable resources. Depending upon the influence of different parameters the importance of each parameter can be varied. The effect of each parameter is scored from zero to 1 from dangerous effect to safety correspondingly. The overall influence of the investigative technique on the environment is presented as a circular representation showing the effect of each parameter at the border of the circle, along with the total score at the middle with color coding. The total value of the environment-friendly analytical procedure will be close to 1 with dark green color. Thanks to Franciso *et al.*,[42] for developing an easy-to-use, freely available software, that automatically generates the greenness report along with an easy-to-read circular pictogram. In the current work, the suggested manipulated UV spectrophotometric processes were complemented with the documented HPLC procedure (Figure 5A and 5B). The total greenness score of the UV method (0.89) confirmed the eco-friendly feature compared to the HPLC method (0.69), because of the safer solvents and the creation of a small quantity of remains. Further, the spectrophotometer requires less energy and time compared to the HPLC instrument.

Hexagonal Technique

The hexagonal technique is a quantitative assessment means used to match the analytical procedures regarding sustainability, health risks, analytical parameters, and economy.[43] The parameters assessed in the hexagonal model are divided into 5 blocks, comprising facts of metric, harmfulness and safety, remainders, carbon footmark, and annual cost. The Facts of Merit (FM) were further distributed into two: FM-1 and FM2. FM-1 evaluates the parameters related to the preparation of the sample (storage, amount, number, treatment), method features (operation mode, time of analysis, method categories), and calibration parameters (frequency, linearity, range, LOD, LOQ precision). FM-2 evaluates the quality control parameters and accurateness of the methods under study. The 2nd fraction is the toxicity and safety of the reagents and solvents used based on the globally harmonized system. It is evaluated by giving penalty points for the health, environment, and physical hazards based on the intensity of the effect on animal health. The third block signifies the quantity of waste produced and the recycling or discarding of the waste generated during the analysis, in terms of penalty points. The amount of carbon dioxide released during the process has a high impact on the environment. The fourth block represents the amount of CO_2 in Kg known as the corban footprint based on the energy consumption by the instrument and analysis time. The annual total cost of the complete procedure was evaluated by calculating the number of samples, time, equipment cost, reagents, and other consumables costs. The total penalty points for each block are converted into overall quantification starting from zero for high environmental sustainability, and low operation cost to four for high penalty points (worst analytical parameters). Analysis of the AML-TEL and AML-MET mixture was carried out using toxic solvents in the HPLC method and the quantity of waste generated is more compared to the UV spectroscopic methods. HPLC instruments consume more electricity compared to UV spectrometers and hence emit more CO_2 (Figure 5C and 5D).

White Analytical Chemistry

White Analytical Chemistry (WAC) is a modified version of Red-Green-Blue (RGB) additive color prototypical for quantitative evaluation created on the 12 philosophies of WAC.[41] The WAC philosophies are formulated from validation parameters to evaluate the performance of the analytical technique (Red), the eco-friendly feature of the technique by 12 GAC principles (Green), and the productivity, efficiency, and economy of the method (Blue). Color scores of 0-100% quantitatively measure the redness, greenness, and blueness. Mixing these primary colors produces white color; hence, the analytical technique is considered white, if all three-color parameters contribute in a positive means. For calculating the whiteness score, red, green, and blue parameter responses have to be entered into the freely available spreadsheet of Microsoft Excel. The score of zero for the worst results and 100 for the completely positive response meeting the intended applications.

The proposed UV spectroscopic method is white (Figure 5E), due to good validation parameters, use of safer ethanol as solvent, a small amount of waste generation, use of less energy, economic, rapid, less practical requirements, and simple procedure. The reported HPLC methods (Figure 5F) utilize toxic solvents, a large amount of waste formation, high energy utilization, and slow analysis due to long analysis time compared to proposed spectroscopic techniques.

DISCUSSION

The UV spectroscopic approach has been used extensively for systematic quality control of medicinal products due to its ease of use, speed, accuracy, and repeatability. However, the majority of medications are aromatic/heterocyclic molecules with strong UV absorption. As a result, the UV absorption spectra in the multicomponent formulation exhibit significant overlap, making simultaneous quantification difficult without separation. However, a number of spectral resolution methods are designed to determine these multicomponent formulations simultaneously.[35-39] In the present work, AML, TEL, and MET showed abundant overlapping within the 200-350 nm range, hence two mathematically manipulated Double Divisor Ratio spectra technique (DDR) and Ratio First Derivative technique (RFD) were established.

Double Divisor Ratio spectra method (DDR)

The UV absorption of two analytes (T and M) and three analytes A, T, and M at wavelength λ according to Beer's law is represented below.

$$X(\lambda) = \mathcal{E}_T C'_T + \mathcal{E}_M C'_M \quad (1)$$

$$Y(\lambda) = \mathcal{E}_A C_A + \mathcal{E}_T C_T + \mathcal{E}_M C_M \quad (2)$$

Where X_1 and Y are absorptions of two and three analytes at wavelength λ, $\mathcal{E}_A$, $\mathcal{E}_T$, and $\mathcal{E}_M$ are molar absorptivity of three analytes A, T, and M respectively. C_A, C_T, and C_M represent the concentration of analytes A, T, and M respectively. C'_T and C'_M also represents the different concentrations of T and M.

The ternary mixture spectrum (2) is divided by the binary mixture consisting of any two analytes of ternary mixture spectrum (1), and the yielded ratio spectrum is represented by equation 3.

$$Y(\lambda)/X(\lambda) = \mathcal{E}_A C_A / \mathcal{E}_T C'_T + \mathcal{E}_M C'_M + \mathcal{E}_T C_T + \mathcal{E}_M C_M / \mathcal{E}_T C'_T + \mathcal{E}_M C'_M \quad (3)$$

The ratio of the binary mixture spectrum of M and T at two different concentrations is constant δ at a particular wavelength (λ). This particular wavelength is selected for the purpose of quantifying compounds using the tertiary combination, incorporating the constant (δ) in the above equation (3) yields equation (4).

$$Y(\lambda)/X(\lambda) = (\mathcal{E}_A C_A / X) + \delta \quad (4)$$

It is possible to omit the constant (δ) in the preceding equation by estimating the absorbance discrepancy of the resulting spectrum at two distinct wavelengths (λ_1 and λ_2).

$$Y\lambda_1/X\lambda_1 - Y\lambda_2/X\lambda_2 = (\mathcal{E}_A C_A / X)\lambda_1 - (\mathcal{E}_A C_A / X)\lambda_2$$

$$Y\lambda_1 - Y\lambda_2 / X\lambda_1 - X\lambda_2 = (\mathcal{E}_A C_A)\lambda_1 - (\mathcal{E}_A C_A)\lambda_2 / X\lambda_1 - X\lambda_2$$

Simplifying the above equation gives equation (5).

$$\Delta Y = Y\lambda_1 - Y\lambda_2 = (\mathcal{E}_A C_A)\lambda_1 - (\mathcal{E}_A C_A)\lambda_2$$

Where ΔY is the absorbance divergences of the ratio spectrum at two distinct wavelengths (λ_1 and λ_2), signifying for a single analyte (A), suggesting that the detection of one analyte in the presence of two other analytes can be achieved by measuring the magnitude of the peak divergences at two distinct wavelengths of the ratio spectrum.

Ratio First Derivative method (RFD)

The constant (δ) from equation 4 can also be eliminated by derivation, because the derivation of any constant value is zero and the resulting derivative spectra represent one analyte.[28,33,37]

Appraisal of Greenness and whiteness

Green and white analytical chemistry helps chemists to contemplate the environmental safety and harmful effects on the animals during the process. The development of environmentally friendly analytical methods have several benefits, such as minimizing waste, using less energy, and avoiding or using fewer dangerous chemicals. Different qualitative, quantitative, and semi-quantitative greenness evaluation tools are established to measure the greenness of analytical procedures. The established techniques were rigorously ensured to meet the criteria of green and white chemistry and the importance of pollution prevention by the application of two greenness and one whiteness scalar appraisal systems.

Further, in order to develop an eco-friendly UV spectroscopic approach ethyl alcohol was preferred as a solvent to prepare the authentic stock solutions, and further dilution was made using water.

CONCLUSION

A green, affordable, novel, and easy-to-use spectrophotometric approach for the quantification of AML, TEL, and MET in tablet dosage form was developed with the use of a mathematically modified UV spectrophotometric strategy. In addition, the manipulation of spectra was simple, less complicated, and carried out with the software that was included with the UV spectrophotometer. The developed DDR and RFD spectrophotometric techniques provided acceptable validation results that were in compliance with ICH guidelines. The greenness and whiteness of the proposed spectroscopic methods were evaluated by AGREE, hexagonal, and GRB techniques. Based on the manipulation procedure, evaluation results, and pictograms, it can be concluded that the suggested and validated method is simple, accurate, and environment-friendly. Moreover, the statistical analysis of the assessment findings verified that there was no discernible difference between the reported HPLC process and the anticipated UV spectroscopic process in terms

of accuracy and precision. The suggested UV spectroscopic methods, which are rapid and environmentally friendly, can take the place of the previously documented HPLC methods for the detection of AML, TEL, and MET in pharmaceutical dosage forms. Hence, pharmaceutical labs and drug testing laboratories can employ the proposed spectroscopic methods on a daily basis for the systematic quality assurance of medicines comprising AML, TEL, and MET.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study does not include any animals or human volunteers.

ABBREVIATIONS

HPLC: High Performance Liquid Chromatography; **UV:** Ultra violet; **CVD:** Cardiovascular disease; **^{0}D:** zero-order absorption spectra; **RSD:** Relative Standard deviation; **RE:** Relative error; **GAC:** Green Analytical Chemistry; **WAC:** White Analytical Chemistry; **WHO:** World Health Organization; **GCMS:** Gas Chromatography Mass spectrometry.

SUMMARY

The present work demonstrates the establishment of two efficient and environmentally friendly sensitivity-enhanced UV spectroscopic approaches the double devisor spectroscopic and ratio first derivative spectrum's method for the concomitant assessment of tablet preparations of AML, TEL, and MET. The sensitivity of the low-concentration analyte AML and low-peak amplitude analyte MET was increased by the scaling factor. AML, TEL, and MET have all been effectively analyzed using these methods, with results falling into the ranges of 1-10 µg/mL, 5-30 µg/mL, and 5-40 µg/mL, correspondingly. Additionally, we followed ICH guidelines to validate these approaches for specificity, accuracy, and precision. No statistically significant differences were seen when compared to the described HPLC approach. The recommended methods have been found to be safe, economically feasible, and sustainable based on the ecological assessment by RGB12, AGREE and hexagonal methods. Therefore, the proposed UV spectroscopic approaches could be utilized regularly for analysis of formulation comprising AML, TEL, and MET.

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