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Green analytical chemistry-based spectrophotometric techniques for ternary component analysis of pain relievers

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Abstract

Background The management of pain presents a significant challenge in healthcare, particularly in cases where conventional therapies prove inadequate. In response to this need, this study aims to devise two innovative UV spectrophotometric techniques rooted in the principles of green analytical chemistry for the analysis of Aceclofenac (ACE), Paracetamol (PAR), and Tramadol (TRM) in both bulk and tablet forms.

Results Utilizing advanced mathematical methodologies such as the double divisor ratio spectra method and area under the curve, the concentrations of these drugs were accurately determined. Validation of the developed methods adhered to the guidelines outlined by the International Council for Harmonisation in the Q2 (R1), revealing linear calibration curves for ACE (8–12 µg/mL), PAR (22.75–35.75 µg/mL), and TRM (2.62–4.12 µg/mL). Furthermore, statistical analyses employing Student's *t* test and *F* test were conducted to ensure the robustness of the proposed method. The evaluation of environmental impact through green metric tools confirmed the eco-friendliness of the proposed methodologies.

Conclusion The assessment performed utilizing green metric tools has substantiated the environmental sustainability of the proposed approach. Thus, this methodology offers accurate and reliable outcomes for the determination of three drugs, as indicated by the complete overlap observed in the zero-order spectra.

Keywords Double divisor ratio spectra method, Area under the curve, Green analytical chemistry, AGREE, GAPI

Background

Pain is prevalent in today's society, serving both as a valuable warning to prevent harm and as an unwelcome aspect of our lives. Its impact on our quality of life is influenced by its intensity and duration. The opioid crisis has prompted discussions on pain treatment. While opioids are highly effective in suppressing pain signals, they also pose potential harm [1]. Nowadays treatment using a tri-drug therapy has shown a prominent effect over the treatment for pain management rather than using single and dual regimens due to the tolerance effect of the individuals. Among several combinations, two Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and one opioid have shown effective treatment in the management of pain.

Paracetamol (PAR), chemically N-(4-hydroxyphenyl) acetamide [2] (Fig. 1a), acts as a centrally and peripherally acting non-opioid analgesic and antipyretic [3]. The mechanism of action involves inhibiting prostaglandin synthesis. This inhibition is achieved by targeting COX-1 and COX-2 in conditions with low levels of arachidonic acid and peroxides. Beyond treating headaches, muscular aches, arthritis, backaches, toothaches, and fevers, PAR is frequently used as an over-the-counter medication to

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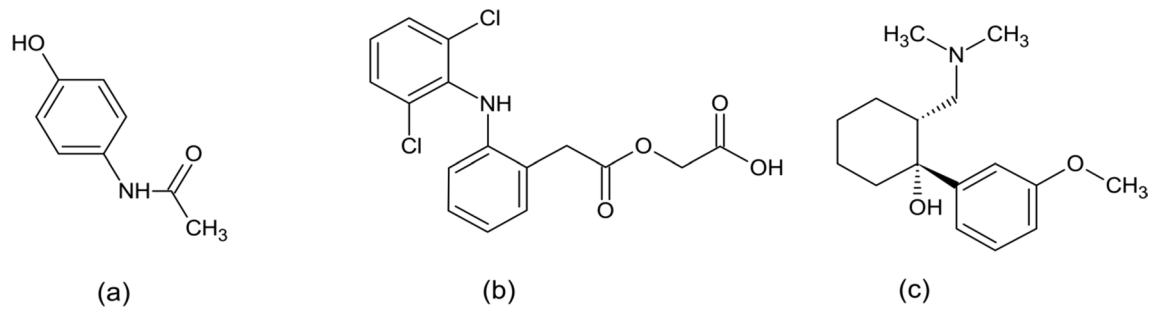


Fig. 1 Structure of **a** PAR, **b** ACE **c** TRM

alleviate pain and reduce fever. However, a PAR overdose may lead to severe side effects, such as fulminant liver necrosis [4].

Aceclofenac (ACE) is an orally administered NSAID, chemically known as 2-[2-[2-[(2,6-dichlorophenyl) amino] phenyl] acetyl] oxyacetic acid (Fig. 1b). It demonstrates a favorable tolerability profile and produces analgesic effects in various painful situations. ACE is employed in the treatment of rheumatic arthritis and soft-tissue injuries [5]. Its potent inhibitory impact on the cyclo-oxygenase (COX) enzyme disrupts the synthesis of prostaglandins, which are inflammation mediators responsible for heat, pain, edema, and inflammation [5–7].

Tramadol (TRM) is chemically (1R, 2R)-2-[(dimethyl amino) methyl]-1-(3-methoxy phenyl) cyclohexanol (Fig. 1c). It acts as an opioid agonist, effectively reducing pain without suppressing prostaglandins or inducing cardiovascular or respiratory depressive effects. Due to its complementary enantiomers, enhancing effectiveness and tolerance, it is utilized as the racemate [5]. The agonistic activity of (+)-tramadol and its main metabolite, (+)-O-desmethyl-tramadol (M1), on the μ opioid receptor (μOR), inhibiting serotonin and norepinephrine reuptake, synergizes to control pain perception and reaction [8]. TRM is efficacious for a broad spectrum of pain, ranging from moderate to severe, including pain during child birth [5].

Misuse of TRM raises concerns about overdose, opiate addiction, and physical dependence. Its connection to addiction in sports athletes is notable, as it is reportedly used in professional sports to endure discomfort and enhance performance, recognized even as a doping substance in cycling. Despite its hazards, TRM is frequently employed to treat injuries and alleviate perceived exertional pain and fatigue, particularly in cycling, as evidenced by the World Anti-Doping Agency (WADA) Monitoring Program [9]. Athletes, coaches, sports physicians, and pharmacists should familiarize themselves with WADA's 2024 Prohibited List, effective from January 1, 2024 [10]. TRM is commonly prescribed in combination with non-opioid analgesics like PAR for the treatment of moderate-to-severe pains. A fixed-dose combination of PAR, ACE, and TRM provides multimodal analgesic effects with an extended half-life and quick onset. Patients experiencing both chronic and moderate to severe pain are often prescribed and taken orally every 4–6 hr, with a maximum daily intake of 400 mg. The instructions were very clear on the label as the drug cannot be used for more than 5 days [11].

The goal of the current research is to develop an ecologically friendly strategy for analyzing this specific medication. Green chemistry, pioneered by Paul Anastas, aims to replace harmful solvents with less or non-toxic alternatives [12, 13]. Sample preparation and separation science in analytical chemistry often involve solvents, leading to the development of the "3" approach—Reduction, Replacement, and Recycling. This has led to the redesign of instruments, the use of substitute solvents, and economic benefits in large-volume applications [14]. Additionally, environmentally friendly solvents meeting the US Environmental Protection Agency's (EPA) criteria for reducing hazardous environmental consequences have fueled the growth of green analytical chemistry (GAC). This focus addresses environmental, social, and economic objectives, emphasizing pollution control, sustainable industrial ecology, pollution prevention, and environmental safety [12].

The combination of these three drugs forms a marketed formulation categorized as a Schedule H1 drug. While the literature search indicates the detection of these three drugs as single components or in combination with other medications using UV and other techniques [15–27], this novel medication lacks a valid pharmacopeia method but is purported to effectively reduce inflammation, swelling, and discomfort in conditions such as ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis. It also aids in managing temporary discomfort, including toothache, ear, and throat pain, as well as muscular and back pain, by suppressing substances in the body that cause edema and pain [17].

This work aims to develop two simple UV spectrophotometric methods for estimating drugs in combination. The double divisor ratio spectra method (DDRSM) and area under the curve (AUC) are easy to understand, accurate, precise, and appropriate for regular QC analysis and identification in bulk and tablet pharmaceutical dosage forms.

Theoretical background

Method 1: double divisor ratio spectra method (DDRSM)

In this novel separation technique, spectra containing varying concentrations of mixture ABC are scanned separately, stored, and then subjected to mathematical operations involving standard spectra with double divisors B' and C' in case to separate and analyze A. Double divisor was prepared by the addition of standard spectra B' and C' which is divided by the mixture spectra. The resulting ratio spectra of A were further multiplied by using the same double divisor to obtain a zero-order spectrum of A which was further utilized for estimating the concentration of A in the ternary mixture; the overall process is illustrated in Eqs. 1–3.

The stored spectra are divided by the standard spectrum, which consists of double divisors such as A' and C', to find the concentration of B in the ternary mixture; it is prepared by the addition of standard spectra A' and C' which is divided by the mixture spectra yielding the ratio spectra of B, which were further multiplied by using the same double divisor to obtain a zero-order spectrum of B which were further utilized for estimating the concentration of B in the ternary mixture; the process is illustrated in Eqs. 4 and 5. Similarly, to determine the concentration of C in the ternary mixture, the spectra obtained from Eqs. 3 and 5 were combined and subtracted from the initial mixture spectra, as shown in Eqs. 6 and 7.

This approach, utilizing double divisors and ratio spectra, provides a method for analyzing and quantifying the individual components (A, B, and C) within the ternary mixture [28, 29]. The mathematical procedures involved in these calculations contribute to the accurate determination of each component's concentration in the complex mixture

$$\frac{A+B+C}{B'+C'} = \frac{A}{B'C'} + \frac{B}{B'C'} + \frac{C}{B'C'} \tag{1}$$

$$= \frac{A}{B'+C'} + \text{constant}$$

$$= \frac{A}{B'+C'} + \text{constant} - \text{constant} \tag{2}$$

$$= \frac{A}{B'+C'} \times B'+C'$$

$$= A \tag{3}$$

The same steps are used to find the standard spectrum of B, by using the double divisor $A'+C'$

$$\frac{A+B+C}{A'+C'} = \frac{A}{A'+C'} \times \frac{B}{A'+C'} \times \frac{C}{A'+C'} \tag{4}$$

$$= B \tag{5}$$

$$= A+B+C-(A+B) \tag{6}$$

$$= C \tag{7}$$

Method 2: area under the Curve (AUC)

In this method, the area under the curve (AUC) is calculated by determining the integrated absorbance value between two specified wavelengths, denoted as $\lambda 1$ and $\lambda 2$. These wavelengths serve as the starting and ending points of the curve region in the spectra. The AUC is then computed within the wavelength range of ± 20 nm, representing a specific study region. The concentration of each drug in the ternary mixture is determined solely based on its absorption characteristics within this integrated region. This method provides a straightforward approach for quantifying the amount of each drug in the mixture, relying on the cumulative absorbance values over the specified wavelength range [30]. The combination of these two methods will have an advantage over the HPLC methods because Method 1 helps in the separation of the mixture were as Method 2 helps in determining the concentration of the drug based on the area of the spectra.

Experimental

Apparatus

The JASCO UV–visible double monochromator model number V-730 is utilized, sealed, and quartz-coated with Czerny–Turner monochromator optics, providing a wavelength range of 190–900 nm. Its incredibly low stray light percentage (0.00008%) allows for precise measurements across the largest photometric range (up to 8AU). The apparatus also boasts a narrow spectral bandwidth of 0.1 nm and extended photometric linearity up to 8Abs, enabling the measurement of highly absorbing samples. The photomultiplier tube serves as the detector, and data output is facilitated using the software Spectra Manager™ version 2.5.

Materials and chemicals used

Therapeutic pharmaceutical products, namely PAR, ACE, and TRM, were acquired as gift samples from, Shree Icon laboratory, Vijayawada, India, by re-analyze the content of PAR, ACE, and TRM using an official method; the result was obtained as 99.5 ± 1.25. The same products were employed as working standards without any additional treatment. Hayman premium grade ethanol is used for diluent preparation.

Marketed formulation

The commercially available Zerodol-PT, with the batch number of JTZ013011AS containing 325 mg of PAR, 100 mg of ACE, 37.5 mg of TRM, and additional excipients in sufficient quantities, was obtained from the local pharmacy. This medicine is manufactured and marketed by IPCA Laboratories Limited.

Diluent preparation

For analysis, a diluent is prepared by adding 20 ml ethanol (Hayman premium grade ethanol) in distilled water to reach 100 ml.

Procedure

Standard solution

Standard stock solutions for spectrophotometric analyses were prepared by accurately weighing 10 mg of pure ACE, PAR, and TRM, individually, and dissolving each in a 10-mL standard volumetric flask. To achieve complete dissolution, the solutions were prepared up to the mark using a diluent after being sonicated for three to five minutes (Dilution 1). Further, 1 mL of ACE, 3.25 mL of PAR, and 0.375 mL of TRM were taken from the above solution and diluted with 10 ml in the standard volumetric flask were made accordingly to achieve the concentration of working standards to obtain 10, 32.5, and 3.75 + 6.25 µg/mL (standard addition), respectively. Furthermore, 6.25 µg/mL of known concentration of TRM was added because TRM concentration was very less [31–34] when compared to the other two drugs ACE and PAR which is difficult to interpret in the presence of other. So, to achieve a maximum concentration equal to ACE and PAR, a 6.25 µg/mL concentration was raised by standard addition to achieve a nearer concentration of ACE (10–3.25 µg/mL = 6.25 µg/mL) (Dilution 2). From Dilution 2, 1 ml of each solution was taken and made up to 10 mL of the diluent (Dilution 3); these solutions were then scanned in a UV–visible spectrophotometer within the wavelength range of 200–400 nm. For linearity 0.5, 0.75, 1, 1.25, and 1.5 mL of each solution was taken from Dilution 2 in standard volumetric flask and was made up to mark with 10 mL diluent and these solutions were then scanned in a UV–visible spectrophotometer.

Preparation of sample solutions from marketed formulations

A total of 20 tablets from the marketed formulation were finely ground, and the powder equivalent to 100 mg, 325 mg, and 37.5 mg of ACE, PAR, and TRM, respectively, was accurately weighed. This drug was transferred into a 100 mL standard volumetric flask, and 6.25 mg of TRM was added as a standard addition technique to avoid the hindrance of the other two drugs in determining TRM. To the flask added a small amount of diluent and sonicated the mixture for approximately twenty minutes. Finally, the volume was made up to 100 mL using the diluent. The resulting solutions were further diluted to obtain final concentrations of 10, 32.5, and 3.75 + 6.25 µg/mL of ACE, PAR, and TRM, respectively, and were designated as sample solutions.

Results

After the preparation of the drug solution, UV scanning was conducted over the wavelength range of 200–400 nm. The spectra of all three drugs exhibited absorbance within the challenging range of 240–280 nm, as depicted in the zero-order spectrum (Fig. 2). To distinguish individual drugs within the ternary mixture, specific methods were applied to each D^0 spectra individually. This step is crucial for accurate identification and quantification of the individual components in the complex mixture. According to the reported method, the UV spectrophotometric methods can be divided into different windows as the methods selected in the determination of three drugs fall in Window 1 (W1), which utilizes

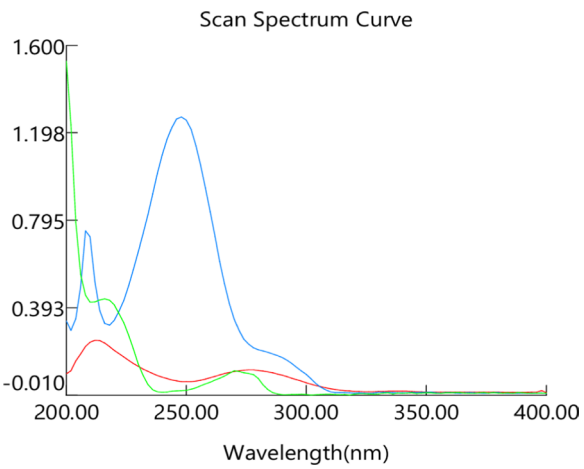


Fig. 2 Overlay of three drugs of UV spectra, ACE (10 µg/mL)—red, PAR (32.5 µg/mL)—blue, and TRM (3.75 µg/mL)—green

zero-order absorption spectra, and Window 3 (W3), which is based on ratio spectra.

Method 1: double divisor ratio spectra method (DDRSM)

Determination of ACE

In this method, the first step involves generation of double divisor by adding the standard spectra of PAR′ and TRM′ to obtain a PAR′ TRM′ followed by a constant generation, where the standard spectra of PAR and TRM are divided by the double divisor PAR′ TRM′. The resulting ternary mixture spectrum is divided by the double divisor to obtain a spectrum, which is then subtracted from the constant and subsequently multiplied by the double divisor PAR′ TRM′ to yield a D^0 spectra of ACE at a wavelength of 276 nm. The overall separation process of ACE from the ternary mixture with the help of spectrums is depicted in Fig. 3

Determination of PAR

To determine the D^0 spectra of PAR, the double devisor was prepared by adding the standard spectra of ACE′ with TRM′ to yield ACE′ TRM′ and then constant is prepared by dividing the standard spectra of ACE and TRM by the double divisor (ACE′ TRM′). The mixture spectrum is then divided by the double divisor, subtracted from the constant, and multiplied by the double divisor to obtain a D^0 spectra of PAR at a wavelength of 247 nm. The overall separation process of ACE from the ternary mixture with the help of spectrums is depicted in Fig. 4.

Determination of TRM

This is achieved using spectra manager software. The above-obtained spectra of ACE and PAR are added together which are subtracted from ternary mixture spectra to obtain D^0 spectra of TRM at a wavelength of 270 nm. The overall separation process of ACE from the ternary mixture with the help of spectrums is depicted in Fig. 5.

Method 2: Area under the curve method (AUC)

Determination of ACE

AUC is calculated for the D^0 spectra obtained from the method 1 to determine the concentration of the separated spectra. This can be performed by selecting a wavelength range of approximately 205–225 nm for the D^0 spectra (Fig. 6a).

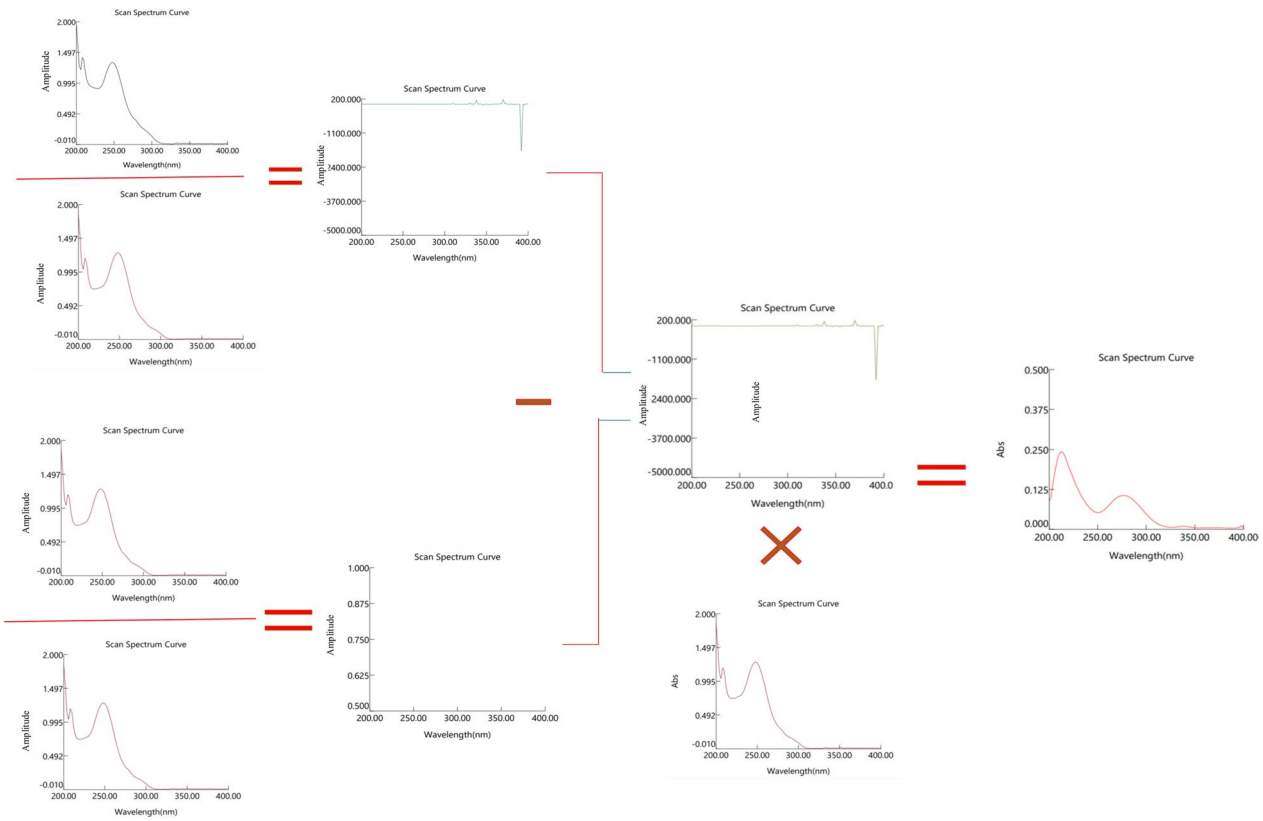


Fig. 3 The process of how the ACE (10 µg/mL) was separated from the ternary mixture

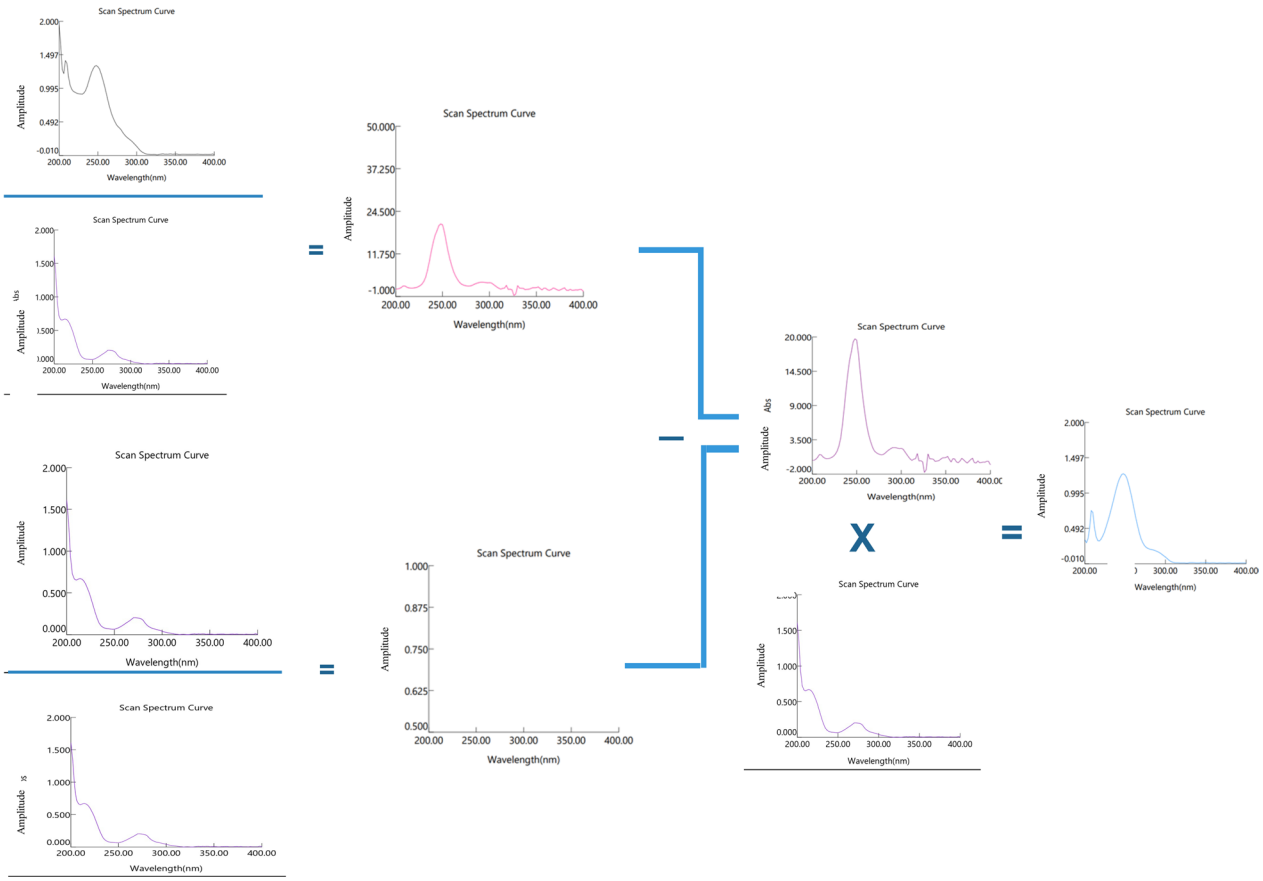


Fig. 4 The process of how the PAR (32.5 µg/mL) was separated from the ternary mixture

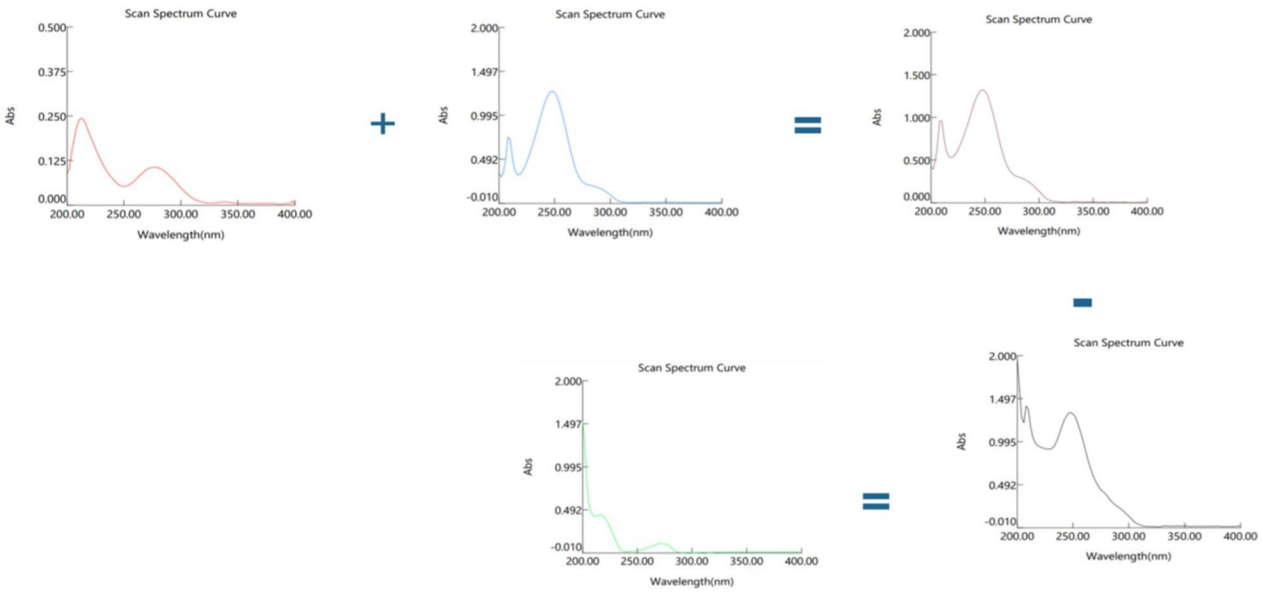


Fig. 5 The process of how the TRM (3.75 µg/mL) was separated from the ternary mixture

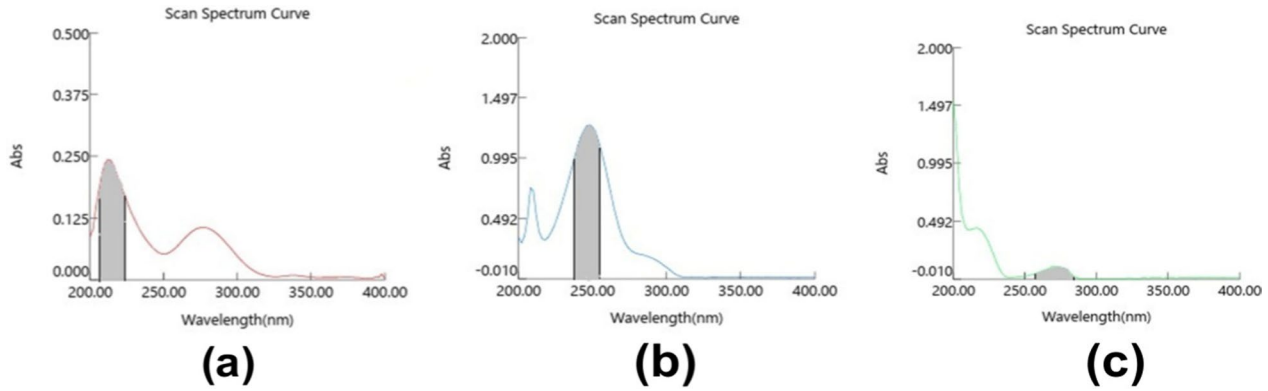


Fig. 6 Area under the curve selection for the three drugs **a** ACE (10 µg/mL), **b** PAR (32.5 µg/mL), and **c** TRM (3.75 µg/mL) at different wavelength ranges

Determination of PAR

AUC is calculated by selecting a wavelength range of approximately 235–255 nm for the AUC spectra (Fig. 6b) which is obtained in the above method. Furthermore, the spectra were utilized to calculate the marketed formulation unknown concentration.

Determination of TRM

AUC is determined for the obtained TRM spectra by selecting a wavelength range of approximately 260–280 nm (Fig. 6c).

Method validation

In accordance with ICH recommendations Q2R1, the developed methods underwent thorough validation, and specific parameters were assessed, including linearity, limit of quantification, detection, accuracy, and precision [30, 35–37].

Precision

The ACE, PAR, and TRM weights were measured precisely. Six repetitions of the identical process were carried out to ensure method repeatability, accounting for both intra- and interday fluctuations. Analyzing the drug solution three times in a single day allowed for the determination of intraday precision. By examining solutions with the same concentrations on three distinct days in a week, interday precision is determined in Table 1.

Accuracy

Applying the standard addition method to a medication sample containing known concentrations of ACE, PAR, and TRM standard drugs is equal to 50%, 100%, and 150% of the label claim allowed for the assessment of the method's accuracy. The experiment's results are listed in Table 1.

Stability

Working solutions of ACE, PAR, and TRM were stored in tightly sealed containers at approximately 4 °C for three weeks. Volumetric flasks containing these solutions were covered with aluminum foil to ensure protection.

Linearity

The method's linearity was determined by evaluating five different concentrations ranging from ACE (8–12 µg/mL), PAR (22.75–35.75 µg/mL), and TRM (2.62–4.12 µg/mL), respectively. The linearity spectra for each separated drugs are depicted in Fig. 7, and the results are depicted in Table 2. The detection limit (LOD) and quantification limit (LOQ) for ACE, PAR, and TRM were estimated

Table 1 Results for ACE, PAR, and TRM in terms of accuracy and precision

Parameter	Double divisor ratio spectra method			Area under the curve		
	ACE	PAR	TRM	ACE	PAR	TRM
Precision						
Interday precision (mean ± SD)	99.64 ± 1.27	99.30 ± 0.95	99.47 ± 0.87	99.33 ± 0.76	99.10 ± 0.93	99.04 ± 1.09
Intraday precision (mean ± SD)	99.62 ± 1.26	99.45 ± 1.00	99.30 ± 0.85	98.87 ± 0.79	98.60 ± 0.87	98.69 ± 1.05
Accuracy (mean ± SD)	97.76 ± 1.32	98.50 ± 0.59	97.26 ± 1.28	97.71 ± 1.00	98.01 ± 0.53	97.92 ± 1.25

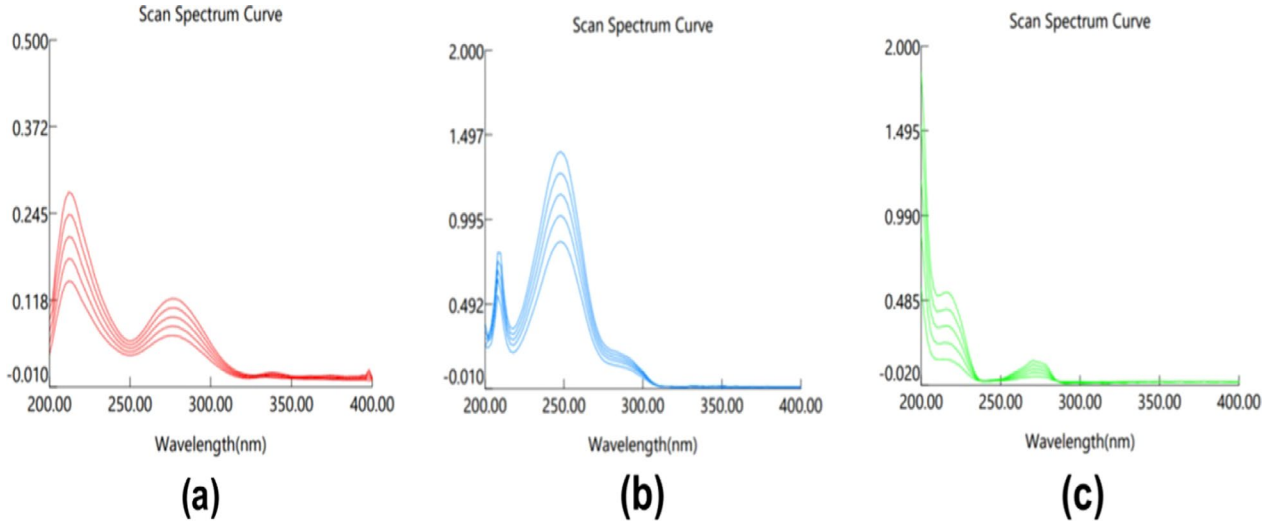


Fig. 7 Linearity spectra for the three drugs **a** ACE (8–12 µg/mL), **b** PAR (22.75–35.75 µg/mL), **c** TRM (2.62–4.12 µg/mL) at different concentration ranges

Table 2 Linearity data for the proposed method

Parameter	Double divisor ratio spectra method			Area under the curve		
	ACE	PAR	TRM	ACE	PAR	TRM
Wavelength (nm)	276	247	270	205–225	235–255	260–280
Y	0.013*x*–0.042	0.040*x*–0.049	0.062*x*–0.132	0.077*x*–0.226	0.042*x*–0.263	0.413*x*–0.883
Slope	0.013	0.04	0.062	0.077	0.042	0.413
R^2	0.999	0.998	0.999	0.999	0.998	0.999
LOD (µg/mL)	0.080	0.859	0.026	0.136	0.745	0.025
LOQ (µg/mL)	0.243	2.603	0.078	0.411	2.259	0.077
Linearity range (µg/mL)	8–12	22.75–35.75	2.62–4.12	8–12	22.75–35.75	2.62–4.12
%RSD	0.64	0.91	0.61	0.58	0.94	0.62

using the calibration curve approach. LOD represents the smallest detectable amount of an analyte, while LOQ is the smallest quantifiable amount with appropriate precision and accuracy. The results are shown in Table 2.

Assay of marketed formulation

The proposed spectrophotometric techniques were applied to analyze a commercially available tablet, Zerodol-PT. The mean drug content ranged from 99.5 ± 1.25. No interference peaks were observed in the spectra, indicating accurate approximation of the medication without considering excipients, and the results are depicted in Table 3.

Statistical analysis

A statistical comparison between the proposed method and reported method [38] is suggested for ACE, PAR, and TRM analysis in pharmaceutical dosage form with respect to assay, and the results are depicted in Table 4. The calculated Student's *t* and *F* test with a null hypothesis (H_0) stating no difference observed between the values and an alternative hypothesis (H_a) stating the maximum difference between the values is observed. The values obtained were found to be less than the tabulated ones.

Green assessment for the proposed method

The development of the method was rooted in adherence to the twelve principles of analytical green chemistry. Initially, the solvent selection was done based on the G score; it was found that ethanol has a G score of 6.6 which is near to water (7.3). To assert the eco-friendliness of an analytical approach, substantiation through assessment tools is imperative. In this case, two distinct assessment tools were employed for evaluating the developed method. The first, AES, underwent manual computation for its assessment. Conversely, the GAPI assessment

Table 3 Assay of tablet formulation

Brand	Content	Amount present (µg/mL)	Amount added (µg/mL)	Double divisor ratio spectra method		Area under the curve	
				Amt. founded (µg/mL)	Percentage recovered	Amt. founded (µg/mL)	Percentage recovered
Zerodol—PT	ACE		5	5.05	101	5.1	102
		10	10	9.95	99.5	10	100
			15	15.08	100.53	15.04	100.26
	Average:			100.75		99.45	
	PAR		16.25	16.1	99.07	16.18	99.56
		32.5	32.5	32.43	99.78	32.55	100.15
			48.75	48.6	100.3	48.7	99.89
	Average:		99.86			99.71	
	TRM		1.875	1.88	100.26	1.87	99.73
		3.75	3.75	3.72	99.2	3.8	101.33
			5.625	5.63	100.08	5.62	100.05
	Average:				99.84		100.37

Table 4 Determination by Student *t* test and *F* test for the proposed method and reported method [38]

Reported method	Proposed method		*t* test		*F* test		*t* test critical value	*F* test critical value
	DDRSM	AUC	DDRSM	AUC	DDRSM	AUC		
97.26	99.45	100.75	0.318	0.95	0.128	0.502	4.303	19
100.49	99.71	99.86						
100.59	99.84	100.37						

utilized software termed the GAPI chart generator version 1.0 which was employed for crafting the GAPI chart, while AGREE metric calculations were conducted utilizing AGREE calculator version 0.5 beta. This comprehensive approach ensured a thorough evaluation of the method's environmental sustainability [39–44]

Green analytical procedure index (GAPI)

GAPI stands as a qualitative gauge utilized to appraise the ecological soundness of an analytical methodology. Operating as a semi-quantitative instrument, it employs pictorial depictions to gauge the environmental sustainability of analytical procedures. By integrating eco-friendly ethanol solvents into the methodology, a method was developed to symbolize a green emblem, highlighting its environmentally mindful characteristics. The GAPI assessment incorporates a color-coded representation of the GAPI value within the final pictogram, offering a visual indicator of its eco-friendliness. Comprising 15 discernible stages, the GAPI evaluation framework is meticulously structured within the GAPI software interface. The proposed method underwent a comprehensive assessment via the GAPI software, with the outcomes elucidated in Table 5 showcasing its environmental viability.

Analytical eco-scale (AES)

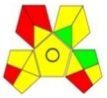
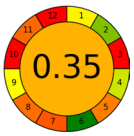
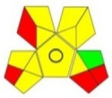
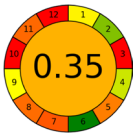
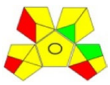
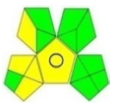
The AES assessment relies on the assignment of penalty points (PP) to chemicals involved. PP is derived through a graphical representation illustrating the chemicals employed in the process. It encompasses four primary evaluation stages, culminating in the AES computation formula: AES = 100—PP.

In the initial stage, ethanol is depicted with three pictograms, resulting in an overall PP of 3. Proceeding to the second stage, as the quantity of solvent or reagent utilized per sample is less than 10 mL, the PP is calculated as 3 × 2 (pictogram score), yielding 6.

Moving to the third stage, with the UV energy consumption per analysis falling below 0.1 kWh, the PP is set at 0. Subsequently, in the fourth stage, the method's waste of solvents, known to have environmental ramifications, is evaluated based on the employed wastage recycling approach, leading to a PP of 0.

Consequently, with the cumulative PP loss for the devised technique totaling 9, the resulting AES score for the developed method stands at 94.

Table 5 Comparison of green metric tools between developed method and proposed methods

Author name	Instrument	Solvent	GAPI	AGREE	ECO SCALE	Refs.
P. Chouhan	RP-HPLC	Methanol: 0.5% Triethanolamine			6 + 1 + 1 + 3 + 3 = 14 100–14 = 86	[38]
Preeti Chandra et al.	HPLC	40: 60 (v/v); phosphate buffer (pH 6.0): methanol			6 + 1 + 3 + 5 = 15 100–15 = 85	[5]
V.V. Vaidya et al.	RP-HPLC	0.02M potassium dihydrogen orthophosphate buffer pH 3.0: acetonitrile in the ratio (40:60) v/v			6 + 1 + 3 + 3 = 13 100–13 = 87	[46]
Proposed method	UV–visible spectrophotometer	20% ethanol; 80% distilled water			6 + 0 + 0 + 0 + 0 = 6 100–6 = 94	

Analytic GREEnness (AGREE)

AGREE metrics is an innovative tool crafted to gauge how environmentally friendly. It provides a detailed look at how these methods impact the environment. The results from AGREE are displayed as a circular graph split into 12 sections, each reflecting one of the twelve green analytical chemistry principles. Every section gets a score between 0 and 1, where 1 signifies the highest level of eco-friendliness. The average score appears at the center of the graph, and the closer it is to 1, the better it is for the environment. The software is designed to fully integrate the established methodology, ensuring that the outcomes reflect the most environmentally friendly approach possible. You can see a summary of the AGREE findings in Table 5. Application of the present method to the AGREE tool yielded a score of 0.91, indicating its strong alignment with GAC principles and highlighting its environmentally friendly analytical attributes.

The developed method underwent a thorough comparison with established HPLC techniques to evaluate its efficiency. The term "greenness" denotes not only the absence of efficiency drawbacks in the developed method but also its exceptional ecological safety profile. When scrutinized for environmental impact alongside other methods, the developed approach exhibited significantly higher greenness scores [45]. These findings, including the comparative analysis results, are detailed in Table 5.

*ACE and PAR dissolve readily in organic solvents and completely insoluble in water. Based on this aspect, any method which utilized water as a solvent to dissolve these drugs was not considered into account because it is practically not possible [47]. Pharmaceutical formulations typically use organic solvents such as ethanol, methanol, chloroform, and acetone to dissolve active ingredients. Both compounds are more soluble in organic solvents than in water; however, this might vary depending on the solvent and other conditions such as temperature [48].

Discussion

UV spectrophotometry, a widely employed and efficient analytical tool in both industry and academia, forms the basis of this study. The primary focus is on the Multicomponent Analytical Technique, showcasing its ease of transferability. To mitigate environmental and safety concerns associated with the typical solvent methanol, a 20% concentration of biodegradable and environmentally friendly ethanol in water is employed. This approach aims to reduce solvent consumption and enhance affordability for responsible consumption of the solvents which is an US SDG goal 12 [49]. Notably, all three medications demonstrated full solubility in the 20% ethanol solution without any issues.

The application of DDRSM facilitated the accurate determination of individual drug components within the mixture. By carefully manipulating the spectra and utilizing specific wavelengths, we isolated the D^0 spectra of each drug, enabling their quantification. Additionally, AUC provided another avenue for determining the concentration of separated spectra. By selecting appropriate wavelength ranges, we could accurately calculate the

concentration of each drug component. One significant advantage of these methods is their ability to estimate overlapping spectra, particularly when utilizing environmentally friendly solvents. This ensures accurate analysis of the ternary mixture in tablet dosage form, contributing to both analytical efficiency and environmental responsibility.

In terms of method validation, precision results were found to be accurate for both the proposed methods at various time intervals. Research has demonstrated that the low percentage RSD value and recovered concentration work synergistically, with %RSD below 2%. There was no significant variation in the accuracy results between the drug's various concentrations, despite six determinations being conducted at each level. Stability studies over a period of three weeks revealed no spectrophotometric alterations in the drug solution. The linearity assessment of the proposed method demonstrates its ability to accurately measure concentrations of ACE, PAR, and TRM over their respective linear ranges. The high correlation coefficients (R^2 values close to 1) indicate excellent linearity between concentration and response for each drug. LOD and LOQ values indicate the method's sensitivity, with low values suggesting its capability to detect and quantify trace amounts of the analytes with good precision and accuracy. The %RSD values, representing the relative standard deviation, reflect the method's precision, with low %RSD values obtained indicating good repeatability and reproducibility of the method. Overall, the linearity results validate the suitability of the proposed method for accurate and precise quantification of ACE, PAR, and TRM in pharmaceutical formulations. Statistical comparison between the proposed methods shows no significant difference in terms of the assay, as evidenced by calculated student *t* and *F* tests with H_0 (no difference observed between the values) accepted. Furthermore, there was no discernible difference seen when comparing the dosage form analysis results using the reported methodologies, hence supporting the acceptance of H_0.

The results of the green assessment for the proposed method demonstrate its eco-friendliness and adherence to GAC principles. The utilization of ethanol solvents and the implementation of efficient waste recycling approaches contributed to the high scores obtained from GAPI, AES, and AGREE assessments. The developed method's AES score of 94 indicates minimal environmental impact, further supported by its AGREE score of 0.91. These scores signify strong alignment with green analytical chemistry principles and highlight the method's environmentally friendly attributes. Comparative analysis with established HPLC techniques reaffirms the superiority of the proposed method in terms of greenness. The method not only offers efficient analytical performance but also ensures exceptional ecological safety, making it a promising choice for environmentally conscious analytical practices.

Conclusion

After dwelling on the above research, it becomes apparent that the proposed techniques are straightforward and uncomplicated to implement, requiring no specialized methods or tools. These analytical methods were very specific, exact, linear, accurate, and consistent in detecting ACE, PAR and TRM in tablet formulation. In contrast to the organic solvents typically utilized in UV spectroscopy, this approach is environmentally benign. UV–visible spectroscopy proves to be superior, cost-effective, and eco-friendly for determining the three medications. Ethanol serves as the solvent, and both methods have been validated according to ICH requirements, even with varying concentrations of the three drugs without preseparation. The %RSD value, determined from the results and discussions, is less than 2%. Notably, this method poses no negative environmental impact, making it easily adaptable for the determination of ACE, PAR, and TRM medications. The method underwent additional evaluation utilizing environmentally conscious assessment techniques, yielding notably eco-friendly outcomes, particularly evident when employing ethanol and water as solvents. With negligible adverse environmental impacts, the energy demand for UV–visible spectroscopy instrumentation remains low. Its versatility extends to the analysis of ACE, PAR, and TRM, along with other drug compounds, owing to their solubility in ethanol and water. Consequently, this technique is highly adaptable and favored by pharmaceutical industries and quality control departments for routine research and sustainable development endeavors.

Abbreviations

GAC	Green analytical chemistry
ACE	Aceclofenac
PAR	Paracetamol
TRM	Tramadol
COX	Cyclo-oxygenase
μOR	μ Opioid receptor
WADA	World anti-doping agency
EPA	Environmental Protection Agency's
DDRSM	Double divisor ratio spectra method
AUC	Area under the curve
ICH	International Council for Harmonization
LOD	Limit of detection
LOQ	Limit of quantification
μg/mL	Microgram per mL
RSD	Relative standard deviation
SDGs	Sustainable development goals
AGREE	Analytic GREEnness
GAPI	Green analytical procedure index
AES	Analytical eco-scale

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Author contributions
All authors have read and approved the manuscript. Thirumalai Arunagiri contributed to method development, Alagammai Ganesan performed manuscript draft and writing, Vamsi Ravi Kumaran performed manuscript draft writing, Bharathraj Masilamani performed experiments and writing, Kanaka Parvathi Kannaiah* contributed to supervision, manuscript correction, method development, and software detailing, and Damodharan Narayanasamy was involved in supervision and manuscript corrections.

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