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Ecofriendly Simple UV Spectrophotometric and Chemometric Methods for Simultaneous Estimation of Paracetamol Aceclofenac and Eperisone Hydrochloride in Pharmaceutical Formulation: Assessment of Greenness Profile

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Citation: Rathinam, S.; Karunanidhi Santhana, L. Ecofriendly Simple UV Spectrophotometric and Chemometric Methods for Simultaneous Estimation of Paracetamol Aceclofenac and Eperisone Hydrochloride in Pharmaceutical Formulation: Assessment of Greenness Profile. *Processes* **2021**, *9*, 1272.

<https://doi.org/10.3390/pr9081272>

Academic Editor: Alina Pyka-Pajak

Received: 1 June 2021

Accepted: 20 July 2021

Published: 23 July 2021

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Abstract: This work introduces three eco-friendly UV spectrophotometric methods for the simultaneous estimation of Paracetamol, Aceclofenac and Eperisone Hydrochloride in pharmaceutical tablet formulation. The procedures employed were simultaneous equation method and multivariate chemometric methods with phosphate buffer pH 7.80 as diluent. The simultaneous equation method encompasses absorbance measurement at three different wavelengths (λ_{\max} of the drugs). It exhibits linearity between 12–18 $\mu\text{g mL}^{-1}$ for paracetamol, 3.69–5.53 $\mu\text{g mL}^{-1}$ for Aceclofenac, and 2.76–4.15 $\mu\text{g mL}^{-1}$ Eperisone hydrochloride. The results obtained for accuracy and precision by the simultaneous equation method were within the permissible limits. Principal component regression and partial least squares were the tools used for chemometric methods. The calibration set and prediction set were constructed, and the UV spectra were recorded in zero order mode, further subjected to chemometric analysis. The % recoveries obtained for Paracetamol, Aceclofenac, and Eperisone Hydrochloride by chemometric techniques showed good accuracy, and the results obtained for analytical figures of merit were acceptable. Statistical comparison of the assay results obtained for the proposed methods showed no significant difference found among the methods using one way analysis of variance. Greenness evaluation tools revealed the greenness profile of the proposed methods and found them to be ecofriendly. The described methods were appropriate for routine quality control laboratories, facilitating eco-friendly, fast, and cost effective determination of Paracetamol, Aceclofenac, and Eperisone Hydrochloride in Acemyoset P tablets.

Keywords: paracetamol; aceclofenac; eperisone hydrochloride; chemometrics; UV spectrophotometric; eco-friendly

1. Introduction

Paracetamol (PAR) (Figure 1a) is therapeutically used as an analgesic and antipyretic, while chemically, it is N-(4-Hydroxy phenyl)-acetamide [1]. Aceclofenac (ACE) (Figure 1b) is chemically 2-[(2, 6 dichlorophenyl)-amino] benzene acetic acid carboxymethyl ester and used as an analgesic [1]. Eperisone Hydrochloride (ES) (Figure 1c) is therapeutically used as a muscle relaxant, and chemically, it is 1-(4-Ethylphenyl)-2-methyl-3-(1-piperidinyl)-1-propanone hydrochloride [1].

The fixed-dose combination of PAR, ACE and ES in Acemyoset P tablet is therapeutically indicated for muscle pain. The literature review focused on analytical methods for estimating PAR, ACE, and ES concluded that no analytical methods for estimating PAR, ACE, and ES concurrently were revealed. However, few chemometric aided UV spectrophotometric methods [2–7] were reported for PAR and ACE in combination with other drugs. The rationale of the present work aims to develop eco-friendly, simple UV spectrophotometric and chemometric methods Principal Component regression (PCR) and

array of principal components. The suitability of the chemometrics model PCR and PLS were determined by statistical parameters like predicted residual sum of squares (PRESS), root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), root mean standard error of prediction (RMSEP), and figure of merits (FOM) like sensitivity, analytical sensitivity, the limit of detection (LOD), and limit of quantitation (LOQ) [18].

The application of twelve ideologies of green analytical chemistry (GAC) is becoming more popular in analytical chemistry and, in particular, the analytical techniques employed to estimate active pharmaceutical ingredient(s) in Pharmaceutical formulations and biological fluids. GAC's concepts should be taken into account while developing analytical techniques with a view to safeguarding the environment. The use of hazardous chemicals in analytical procedures should be replaced with eco-friendly chemicals and their usage minimized. Once the method was developed, their greenness profile was evaluated.

The first greenness profile evaluation technique is the analytical eco scale [19] calculated on allotting the penalty points based on the number of pictograms with its signal words given by The Globally Harmonized System of Classification and Labelling of Chemicals (GHS), along with its quantity. Analytical eco scale methodology includes every reagent, its kind and amount, potential occupational exposure, and energy depletion, including waste. The penalty points were deducted from the base of 100.

$$\text{Analytical eco - scale} = 100 - \text{Total penalty points} \quad (4)$$

AGREE Metrics tool [20], the second evaluation technique, is a novel software used to assess the greenness profile. As shown in Figure 2, the agreeing metrics software findings form a circular diagram with numbers at the edge ranging from 1 to 12 in a clockwise motion. Figure 2 was created by randomly selecting values on the 12 GAC principles through the use of the software. Each of these numbers denotes the 12 ideologies of green analytical chemistry. The outcomes from each of these 12 principles are made on an aggregate scale ranging from 0–1, considering the provided inputs and their weightage. This aggregate scale is color coded as red, yellow, and green, where red indicates the value zero while dark green indicates a value of one or close to one and yellow in between red and dark green. The result from all the 12 principles and the core gives the score indicative of the extent of greenness.

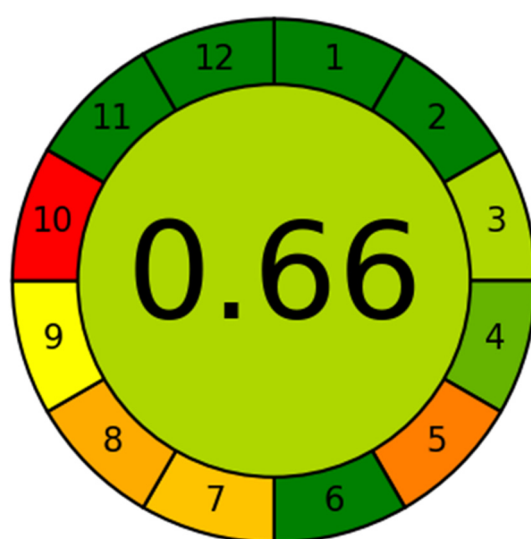


Figure 2. Example of agree metrics output including 12 principles of green chemistry with an overall score in the middle.

2. Materials and Methods

2.1. Instrumentation

Lab India double beam UV visible spectrophotometer model UV 3092 was used with 1.00 cm quartz cells. The scan was carried out in the range of 200 to 400 nm at 0.1 nm intervals. UV win5 Software v5.2.0.1104 was used.

2.2. Reference Samples

PAR, ACE, and ES active pharmaceutical ingredients with a purity of 99.72%, 99.89%, and 99.69% *w/w*, respectively, were supplied from Ideal Analytical and Research Institution, Puducherry, India.

2.3. Marketed Formulation

Acemyoset P tablets containing 325 mg of PAR, 100 mg of ACE, and 75 mg of ES per tablet (manufactured by Sparsh remedies, India) were kindly supplied by Medplus chain store, Hyderabad, India.

2.4. Software

PCR and PLS models performed through Unscrambler 11 software trial version

2.5. Chemicals and Reagents

Potassium dihydrogen phosphate and sodium hydroxide of analytical reagent grade were from m/s Rankem, India. Water obtained from Milli-Q Plus water purification system (Millipore, Milford, MA, USA).

2.6. Preparation of Diluent

Phosphate buffer pH 7 prepared as per Indian Pharmacopoeia 1996 was used as diluent.

2.7. The Standard Stock Solution of Analytes

Individual standard stock solutions of PAR, ACE, and ES ($1000 \mu\text{g mL}^{-1}$) were prepared by dissolving 100 mg in 100 mL of diluent.

2.8. Methodology for Simultaneous Equation Spectrophotometric Method

2.8.1. Overlay Spectrum Analysis and Wavelength Selection

Appropriate dilutions were from the stock solutions diluted with the diluent to prepare the solutions of $10 \mu\text{g mL}^{-1}$ for each PAR, ACE, and ES. The prepared solutions were scanned over 200 to 400 nm against phosphate buffer pH 7.80 as blank. The maximum absorptions (λ_{max}) of PAR, ACE, and ES were 243, 272, and 262 nm, respectively. All the drugs have shown ideal absorbance at the λ_{max} of others. Standard solutions of $15 \mu\text{g mL}^{-1}$ for PAR, $4.61 \mu\text{g mL}^{-1}$ for ACE, and $3.46 \mu\text{g mL}^{-1}$ for ES were prepared separately to quantify pharmaceutical formulation from the stock solution. The absorbivities were calculated for PAR (X) at λ_1 , λ_2 , and λ_3 (a_{x_1} , a_{x_2} , and a_{x_3}); ACE (Y) at λ_1 , λ_2 , and λ_3 (a_{y_1} , a_{y_2} , and a_{y_3}); and ES (Z) at λ_1 , λ_2 , and λ_3 (a_{z_1} , a_{z_2} , and a_{z_3}). The absorbance of sample solutions was measured at 243 nm (λ_1), 272 nm (λ_2), and 262 nm (λ_3).

2.8.2. Analysis of Pharmaceutical Formulation

Ten tablets of Acemyoset P were weighed accurately and then crushed to powder and mixed well. A quantity of tablet powder corresponding to 100 mg of PAR (30.77 mg of ACE and 23.08 mg of ES) was transferred to a 100 mL volumetric flask; 75 mL of diluent was added and assorted well, and the mixture was sonicated for 10 min and then completed to volume with diluent, assorted well, and flowed through a $0.45 \mu\text{m}$ PTFE syringe filter (13 mm diameter). Aliquots of the filtrate were diluted appropriately to make a final solution of $15 \mu\text{g mL}^{-1}$ of PAR, containing $4.61 \mu\text{g mL}^{-1}$ of ACE and $3.46 \mu\text{g mL}^{-1}$ of ES. The absorbance was measured at the selected wavelengths, and the concentration of three analytes was determined.

2.8.3. Solution Stability

Assessment of solution stability was performed at 25 ± 3 °C with relative humidity between $40 \pm 10\%$. The $10 \mu\text{g mL}^{-1}$ of solution of PAR, ACE, and ES were prepared separately with the diluent and were assessed for solution stability at 0, 6, and 12 h, respectively. Following that, the assay solution was prepared as stated in Section 2.8.2 was assessed for solution stability. The stability was measured by % assay value with that of freshly made standard solutions.

2.8.4. Method Validation

ICH guidelines were followed for method validation [21]. The linearity of the method was established by plotting calibration curves in the series of $12\text{--}18 \mu\text{g mL}^{-1}$ for PAR, $3.69\text{--}5.53 \mu\text{g mL}^{-1}$ for ACE, and $2.76\text{--}4.15 \mu\text{g mL}^{-1}$ for ES. Calibration curves were obtained by plotting concentration against absorbance. System precision, Intraday, and Interday precision were performed at 100% of test concentration. Accuracy was performed using the standard enrichment technique at three different levels of 80%, 100%, and 120% of test concentration. The standard deviation approach has been used to determine the LOD and LOQ.

2.9. Chemometrics Methods (PCR and PLS)

2.9.1. Designing of Experiment

A multilevel multifactor was applied to generate an experimental design [22] to construct the calibration and prediction sets. Five concentration levels coded as -2 , -1 , 0 , $+1$ and $+2$ in which level coded as (0) represents the central level PAR, ACE, and ES utilized to establish five levels three-factor calibration design [22]. The central levels for the design were $10 \mu\text{g mL}^{-1}$ of PAR, $4.61 \mu\text{g mL}^{-1}$ of ACE, and $3.46 \mu\text{g mL}^{-1}$ of ES. The measured drug concentrations were chosen based on the ratio of PAR, ACE, and ES ($10:4.61:3.46$) in their formulation and their spectral sensitivity. The concentration design matrix is illustrated in Table 1.

Table 1. Calibration set of 5 levels 3 factors and Validation set of 3 levels 3 factor experimental design shown as coding level and concentrations of the mixture components.

Standard Mixture	PAR		ACE		ES	
	Coding Level	Concentration ($\mu\text{g mL}^{-1}$)	Coding Level	Concentration ($\mu\text{g mL}^{-1}$)	Coding Level	Concentration ($\mu\text{g mL}^{-1}$)
Calibration Set						
1	0	15.00	0	4.61	0	3.46
2	0	15.00	-2	3.69	-2	2.76
3	-2	12.00	2	3.69	2	4.15
4	-2	12.00	2	5.53	-1	3.11
5	2	18.00	-1	4.15	2	4.15
6	-1	13.50	2	5.53	0	3.46
7	2	18.00	0	4.61	-1	3.11
8	0	15.00	-1	4.15	-1	3.11
9	-1	13.50	-1	4.15	1	3.80
10	-1	13.50	1	5.07	2	4.15
11	1	16.50	2	5.53	1	3.80
12	2	18.00	1	5.07	0	3.46
13	1	16.50	0	4.61	2	4.15
14	0	15.00	2	5.53	2	4.15
15	2	18.00	2	5.53	-2	2.76
16	2	18.00	-2	3.69	1	3.80
17	-2	12.00	1	5.07	-2	2.76
18	1	16.50	-2	3.69	0	3.46
19	-2	12.00	0	4.61	1	3.80
20	0	15.00	1	5.07	1	3.80
21	1	16.50	1	5.07	-1	0.05
22	1	16.50	-1	4.15	-2	2.76
23	-1	13.50	-2	3.69	-1	3.11
24	-2	12.00	-1	4.15	0	3.46
25	-1	13.50	0	4.61	-2	2.76

Table 1. Cont.

Standard Mixture	PAR		ACE		ES	
	Coding Level	Concentration ($\mu\text{g mL}^{-1}$)	Coding Level	Concentration ($\mu\text{g mL}^{-1}$)	Coding Level	Concentration ($\mu\text{g mL}^{-1}$)
Prediction Set						
26	0	15.00	0	4.61	0	3.46
27	-1	13.50	1	5.07	1	3.80
28	1	16.50	1	5.07	0	3.46
29	1	16.50	0	4.61	1	3.80
30	0	15.00	1	5.07	-1	3.11
31	1	16.50	-1	4.15	-1	3.11
32	-1	13.50	-1	4.15	0	3.46
33	-1	13.50	0	4.61	-1	3.11
34	0	15.00	-1	4.15	1	3.80

2.9.2. Constitution of the Calibration Set

Twenty-five mixtures of PAR, ACE, and ES (C_{cal}) were prepared by spreading appropriate volumes from their stock solution to attain diverse concentrations, as shown in Table 1. Calibration set solutions scanned in the range of 200 to 400 nm, and the absorbance (A_{cal}) was recorded.

2.9.3. Constitution of Prediction Set

Nine mixtures containing different PAR concentrations, ACE, and ES (C_{pre}) were prepared as shown in Table 1 to measure the predictive ability of the suggested PCR and PLS methods to study such mixtures. Prediction set solutions scanned in the range of 200 to 400 nm, and the absorbance (A_{pre}) was recorded.

2.9.4. Construction of Models

PLS and PCR models were established from the obtained spectral data of the calibration set. The absorbance data matrix was measured in the series of 221–300 nm with an interval of 1 nm. A_{cal} and C_{val} were utilized to obtain a regression equation applied to the unknown concentration of PAR, ACE, and ES. The absorbance and concentration matrix of the training set fed in the computer and calculations carried out to obtain the PCR and PLS models.

With the developed model, the correlation coefficient, PRESS, RMSEC, RMSECV, RMSEP were analyzed. The correlation coefficient assessed the extensiveness of correlation among concentration and the noticed signal.

The analytical figures of merit [23] were determined, such as sensitivity, analytical sensitivity, LOD and LOQ. The slope is the ratio of the amount of analytical signal response due to the increase in the concentration of a specific analyte at unit concentration and denotes sensitivity.

The analytical sensitivity is the proportion of sensitivity and instrumental noise, which refers to the minor concentration difference among two samples determined by the model. The LOD and LOQ were calculated using instrument noise and slope of the calibration curve by the following equations.

$$\text{LOD} = 3.3 \times \|b\| \times \|\Sigma\| \quad (5)$$

$$\text{LOQ} = 10 \times \|b\| \times \|\Sigma\| \quad (6)$$

where, $\|b\|$ = slope of the calibration curve, $\|\Sigma\|$ = noise

The solutions for the concurrent estimation of PAR, ACE, and ES in pharmaceutical formulation by PCR and PLS methods were performed as described in Section 2.8.2 and scanned in the spectral region between 221 and 300 nm.

3. Results and Discussion

The developed spectrophotometric methods is preferable to highly sophisticated instruments like HPLC, HPTLC, and LCMS, which stipulate a tedious separation process

but have the advantages of being eco-friendly, simple to use, fast, sensitive, and inexpensive. Initially, the solubility studies were made for the selected drugs in different buffers and solvents. All the three drugs were freely soluble in methanol and phosphate buffer pH 7.80. Since the objective intends to develop ecofriendly UV spectrophotometric methods, phosphate buffer pH 7.80 was used as diluent.

3.1. Simultaneous Equation Method

The overlay spectrum of the standard solutions $10 \mu\text{g mL}^{-1}$ each of PAR, ACE, and ES recorded in the zero-absorbance mode, as shown in Figure 3, revealed that the simultaneous equation method was suitable for concurrent estimation of PAR, ACE, and ES. Wavelength of 243, 272, and 262 nm were selected to determine PAR, ACE, and ES as all the three-drug showed absorbance at the λ_{max} of the other two drugs.

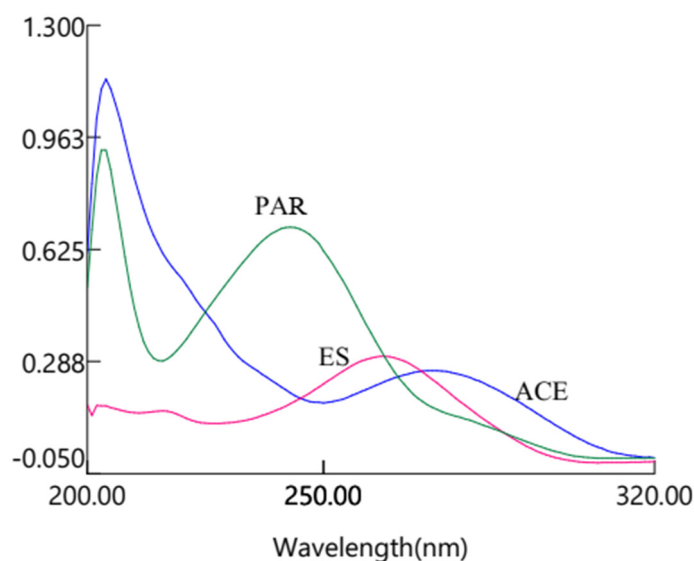


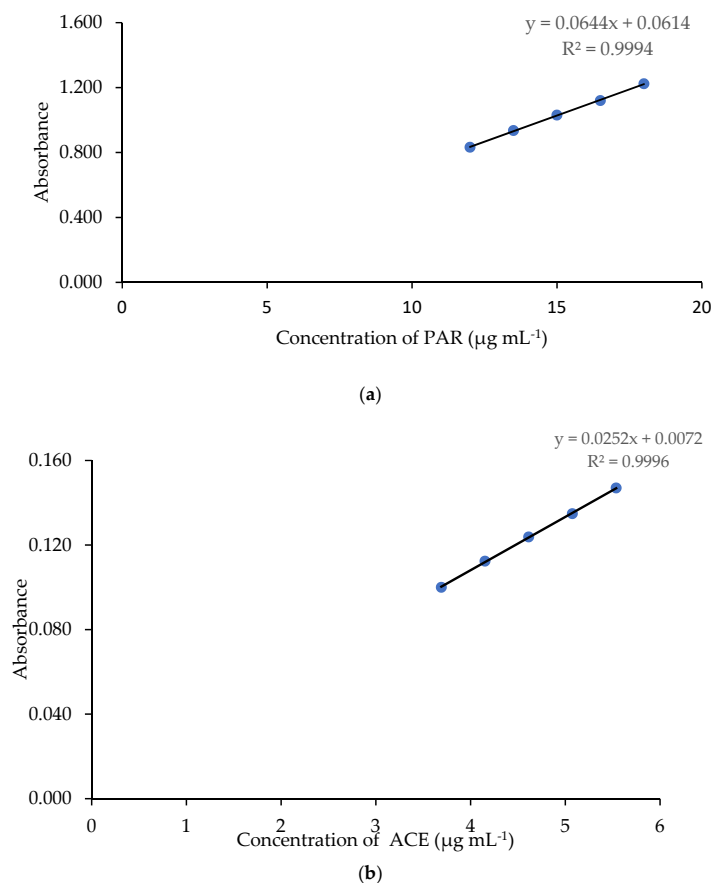
Figure 3. Zero-order overlay absorption spectrum of $10 \mu\text{g mL}^{-1}$ of PAR, $10 \mu\text{g mL}^{-1}$ of ACE, and $10 \mu\text{g mL}^{-1}$ of ES.

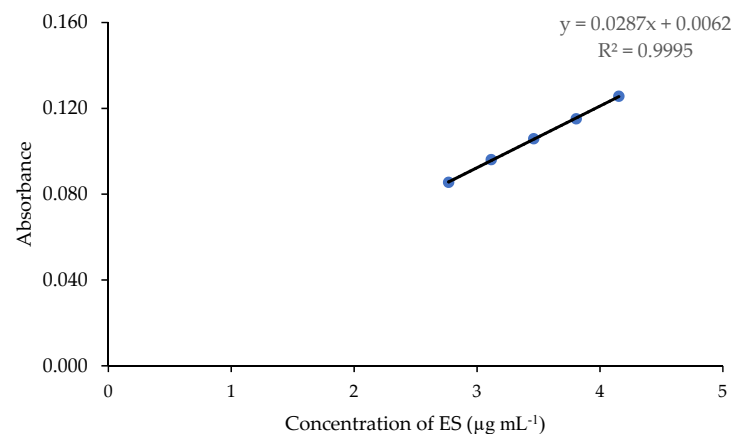
The corresponding absorbance values obtained for solution stability of standard and assay samples solutions showed no considerable variation in the absorbance for 12 hrs. The assay results found for solution stability were within $\pm 2\%$ compared with the new solution and shown in Table 2. The calibration curves of PAR, ACE, and ES were linear in the series and specified wavelength, as shown in Figure 4. The regression equation, correlation coefficient, calculated values of LOD and LOQ were presented in Table 2. The % RSD values obtained with the system, inter, and intraday precision was less than 2%, indicating good precision, while the % recoveries for accuracy were within limits. The summary of validation parameters and results obtained is presented in Table 2.

Table 2. Validation parameters and results obtained by the developed UV spectrophotometric method for the simultaneous determination of PAR, ACE, and ES.

Description	Observations		
	PAR	ACE	ES
Detection wavelength (nm)	243	272	262
Solution stability standard, (% RSD)	0.37	0.47	0.55
Solution stability formulation, (% RSD)	0.92	0.82	1.41
Linearity ^a ($\mu\text{g mL}^{-1}$)	12–18	3.69–5.53	2.76–4.15
LOD ($\mu\text{g mL}^{-1}$)	0.48	0.20	0.13
LOQ ($\mu\text{g mL}^{-1}$)	1.44	0.61	0.38
Slope	0.0644	0.0252	0.0287
Standard deviation of the slope	0.0006	0.0003	0.0003
Confidence limit of the slope 95%	0.0005	0.0003	0.0003
Intercept	0.0614	0.0072	0.0062
Standard deviation of the Intercept	0.0093	0.0015	0.0011
Confidence limit of the Intercept	0.0081	0.0013	0.0010
Regression coefficient (r^2)	0.9994	0.9996	0.9995
System precision ^b , (% RSD)	0.39	1.00	0.71
Confidence limit for System precision	0.0032	0.0010	0.0006
Intraday precision ^b , (% RSD)	0.32	0.31	0.15
Confidence limit for Intraday precision	0.2520	0.2436	0.1158
Interday precision ^c , (% RSD)	0.23	0.3476	0.35
Confidence limit for Interday precision	0.2252	0.1740	0.1834
Accuracy ^d , % w/w	98.25–100.43	98.16–100.33	99.23–100.07
Confidence limit for accuracy	0.5263	0.4050	0.1703

^a Mean of five replicates. ^b Mean of six determinations. ^c Mean of 18 findings in three consecutive days. ^d Mean of three findings at each level.

**Figure 4.** Cont.



(c)

Figure 4. Linear Calibration curves for PAR (a), ACE (b), and ES (c).

3.2. Chemometric Method

3.2.1. Selection of Wavelength Range for PCR and PLS

The selection of wavelengths was made so that most informative data extracted and unnecessary ones were discarded. The selected wavelength range for the chemometric model development is the region between 221 and 300 nm with 1 nm data interval; since the analytes PAR, ACE, and ES had negligible absorbance values in the spectral region 301–400 nm, they were not considered for the construction of PCR and PLS model. The spectral region 200–220 nm was removed due to noise.

3.2.2. Selection of Principal Components and Variables

The absorbance in the selected range was transferred to Unscrambler 11 evaluation version software, and the calibration model's PCR and PLS were raised by choosing the optimum factors. Prediction set were used to assess the predictive potential of PCR and PLS models by plotting known versus expected concentrations for each analyte of the established PCR and PLS model.

The predicted values obtained for the calibration set and validation set were presented in Tables 3 and 4, respectively. Values of PRESS, RMSEC, RMSECV, and RMSEP obtained are shown in Table 5.

Table 3. Outcomes of PCR and PLS chemometric methods for predicting the calibration set.

Standard Mixture	PAR				ACE				ES			
	PCR		PLS		PCR		PLS		PCR		PLS	
	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R
1	15.01	100.07	15.01	100.07	4.61	100.05	4.61	100.05	3.46	100.01	3.46	100.01
2	15.09	100.57	15.08	100.53	3.72	100.77	3.72	100.72	2.78	100.68	2.78	100.64
3	11.89	99.09	11.89	99.12	3.65	98.99	3.65	99.04	4.13	99.52	4.13	99.55
4	12.00	99.98	12.00	100.02	5.53	100.01	5.53	100.04	3.10	99.81	3.11	99.86
5	17.76	98.69	17.76	98.69	4.09	98.54	4.09	98.54	4.10	98.68	4.10	98.68
6	13.49	99.92	13.49	99.90	5.53	99.92	5.53	99.91	3.45	99.79	3.45	99.78
7	18.09	100.48	18.09	100.48	4.64	100.56	4.64	100.56	3.12	100.48	3.12	100.48
8	15.04	100.29	15.04	100.29	4.16	100.33	4.16	100.33	3.12	100.27	3.12	100.27
9	13.46	99.74	13.46	99.72	4.14	99.69	4.14	99.68	3.79	99.82	3.79	99.80
10	13.60	100.76	13.58	100.62	5.11	100.75	5.10	100.64	4.18	100.80	4.18	100.73
11	16.50	99.99	16.50	99.99	5.53	99.93	5.53	99.93	3.79	99.84	3.79	99.84
12	18.05	100.29	18.05	100.29	5.08	100.27	5.08	100.26	3.47	100.18	3.47	100.18
13	16.47	99.80	16.47	99.81	4.59	99.67	4.60	99.68	4.14	99.78	4.14	99.80
14	14.94	99.61	14.94	99.61	5.51	99.59	5.51	99.59	4.13	99.58	4.13	99.58
15	17.64	98.01	17.65	98.06	5.42	98.02	5.42	98.08	2.70	97.98	2.71	98.09
16	18.03	100.19	18.03	100.15	3.70	100.18	3.69	100.11	3.81	100.21	3.81	100.16
17	12.04	100.32	12.04	100.31	5.08	100.30	5.08	100.29	2.76	100.16	2.76	100.16
18	16.54	100.21	16.54	100.22	3.70	100.25	3.70	100.26	3.47	100.25	3.47	100.25
19	11.94	99.49	11.97	99.75	4.59	99.56	4.60	99.76	3.79	99.64	3.79	99.76

Table 3. Cont.

Standard Mixture	PAR				ACE				ES			
	PCR		PLS		PCR		PLS		PCR		PLS	
	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R
20	15.31	102.06	15.31	102.06	5.17	102.01	5.17	102.01	3.88	101.99	3.88	101.99
21	16.56	100.37	16.56	100.37	5.09	100.36	5.09	100.36	3.12	100.28	3.12	100.28
22	16.60	100.62	16.60	100.61	4.18	100.79	4.18	100.78	2.78	100.73	2.78	100.72
23	13.52	100.18	13.53	100.20	3.70	100.22	3.70	100.26	3.12	100.21	3.12	100.23
24	11.97	99.77	11.97	99.77	4.14	99.79	4.14	99.79	3.45	99.85	3.45	99.85
25	13.37	99.01	13.37	99.02	4.57	99.03	4.57	99.04	2.73	98.95	2.73	98.96

% R—% Recovery.

Table 4. Outcomes of PCR and PLS chemometric methods for predicting the prediction set.

Standard Mixture	PAR				ACE				ES			
	PCR		PLS		PCR		PLS		PCR		PLS	
	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R	Amount Found ($\mu\text{g mL}^{-1}$)	% R
1	15.01	100.07	15.01	100.07	4.61	100.05	4.61	100.05	3.46	100.01	3.46	100.01
2	13.46	99.74	13.46	99.74	5.06	99.74	5.06	99.74	3.79	99.74	3.79	99.74
3	16.45	99.67	16.45	99.67	5.05	99.64	5.05	99.64	3.45	99.57	3.45	99.57
4	16.50	100.01	16.50	100.01	4.61	99.94	4.61	99.94	3.80	99.95	3.80	99.95
5	15.04	100.25	15.04	100.25	5.08	100.23	5.08	100.23	3.11	100.14	3.11	100.14
6	16.58	100.50	16.58	100.50	4.17	100.57	4.17	100.57	3.13	100.50	3.13	100.50
7	13.49	99.95	13.49	99.95	4.15	99.94	4.15	99.94	3.46	99.96	3.46	99.96
8	13.52	100.15	13.52	100.15	4.62	100.15	4.62	100.15	3.11	100.09	3.11	100.09
9	14.93	99.56	14.93	99.56	4.13	99.49	4.13	99.49	3.78	99.57	3.78	99.57

% R—% Recovery.

Table 5. Statistical parameters attained by PCR and PLS methods.

Statistical Parameters	PCR			PLS		
	PAR	ACE	ES	PAR	ACE	ES
Concentration range ($\mu\text{g mL}^{-1}$)	12–18	3.69–5.53	2.76–4.15	12–18	3.69–5.53	2.76–4.15
No. of factors	3	3	3	3	3	3
R^2	0.9978	0.9977	0.9981	0.9978	0.9977	0.9981
RMSEC	0.0976	0.0306	0.0210	0.0976	0.0306	0.0210
RMSECV	0.1188	0.0370	0.0249	0.1214	0.0379	0.0255
RMSEP	0.0439	0.0137	0.0098	0.0976	0.09770	0.0210
PRESS	0.3686	0.0361	0.0163	0.3530	0.0344	0.0156
Slope	0.9978	0.9977	0.9981	0.9978	0.9977	0.9981
Intercept	0.0318	0.0102	0.0063	0.0317	0.0102	0.0210
Calibration set Mean \pm SD	99.98 \pm 0.77	99.98 \pm 0.80	99.93 \pm 0.76	99.99 \pm 0.75	99.99 \pm 0.78	99.89 \pm 0.74
Validation set Mean \pm SD	99.99 \pm 0.30	99.97 \pm 0.33	99.98 \pm 0.77	99.99 \pm 0.32	99.97 \pm 0.33	99.95 \pm 0.30
Assay Mean \pm SD	99.85 \pm 0.10	99.83 \pm 0.10	99.98 \pm 0.77	99.96 \pm 0.10	99.83 \pm 0.10	99.79 \pm 0.18

PCR and PLS utilized the principal components (PC) and latent variables (LV) to predict components and govern the accuracy of the developed model. The number of PC's and LV's calculated derived from their loadings and coefficients. The optimal number of PC's and LV's are chosen to avoid overfitting and underfitting data. The cross-validation methodology is employed for selecting the ideal number of PC's and LV's, and the number of factors that result in minimum RMSECV was selected [24]. The explained variance plots are shown in Figures 5 and 6 which comprise dimensionality determination [25] to assess the quality of the constructed chemometric models PCR and PLS. The explained variance plot consists of calibration variance (blue in color) and validation variance (red in color). Calibration variance is based on fitting the calibration data to the model. In contrast, validation variance is computed by testing the model on data not used to build the model.

Finding out when the variance in validation reaches a plateau is the first step in determining dimensionality. The model is good if the plateau is reached with a small number of components and the explained Y-variance is large (for example, more than 80%). Figures 5 and 6 show that the calibration and validation variance curves are nearly identical, showing that the model is demonstrative. The explained variance as a function of the number of components and latent variables for PCR and PLS are shown in Figures 5 and 6, respectively. Three factors are ideal in the mixture for PCR (3 PC's) and PLS (3 LV's) techniques.

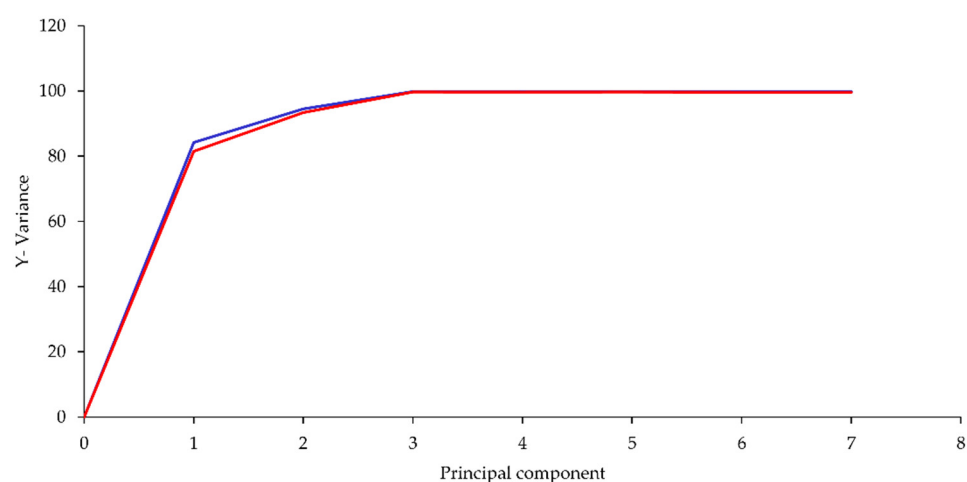


Figure 5. Explained variance plot obtained for PCR model.

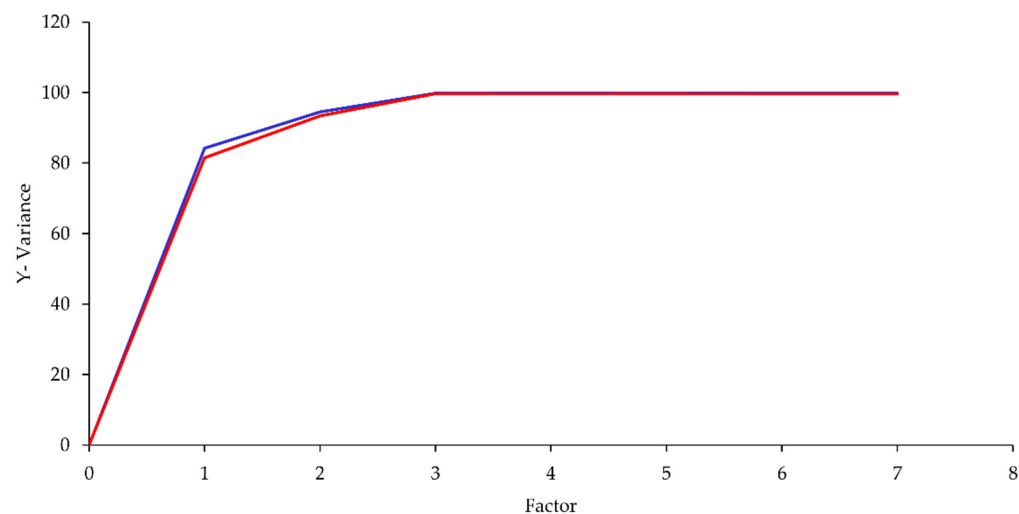


Figure 6. Explained variance plot obtained for PLS model.

The individual recoveries acquired for the Calibration set and prediction set presented in Tables 3 and 4, respectively, proved good accuracy for the developed PCR and PLS model. The overall recovery is shown in Table 5.

The correlation coefficient values obtained for each analyte by PCR and PLS models indicate an excellent linear relationship between predicted and actual values and are presented in Table 5. The values of PRESS, RMSEC, RMSECV, and RMSEP, and as shown in Table 5, obtained by optimizing the absorbance spectra's calibration matrix, establish good accuracy and precision.

Table 6 displays calculated values for analytical figures of merit such as sensitivity, analytical sensitivity, LOD, and LOQ. The assay results attained for PAR, ACE, and ES in the marketed formulation with PCR and PLSR models remained within the accepted standards.

Table 6. Analytical figures of merit.

Parameters	PCR			PLS		
	PAR	ACE	ES	PAR	ACE	ES
Sensitivity (mL μg^{-1})	1.0022	1.0023	1.0019	1.0022	1.0023	1.0019
Analytical sensitivity γ^{-1} ($\mu\text{g mL}^{-1}$)	5.9337	5.5011	5.7088	5.9337	5.5011	5.7088
LOD ($\mu\text{g mL}^{-1}$)	0.56	0.60	0.58	0.56	0.60	0.58
LOQ ($\mu\text{g mL}^{-1}$)	1.69	1.82	1.75	1.69	1.82	1.75

3.3. Assessment of Greenness of the Proposed Method

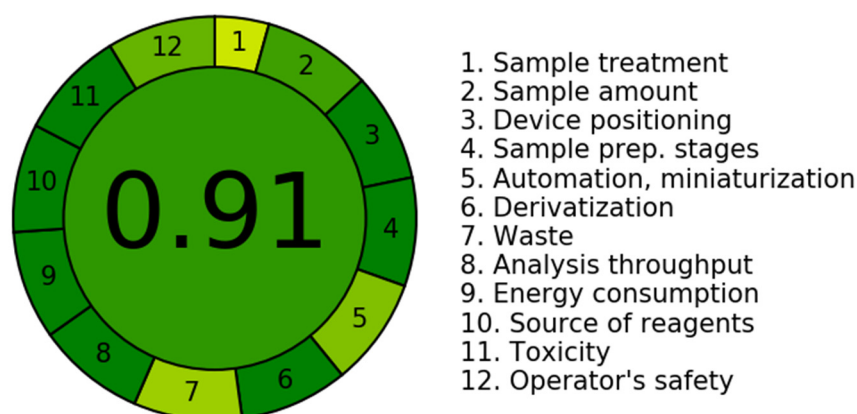
Two Green metrics, namely analytical eco-scale and the novel software-based agree metrics, engaged in assessing the green characteristics of the established UV spectrophotometric methods.

Analytical eco scale: The UV spectrophotometric methods, when subjected to evaluation by analytical eco scale, scored a value of 96, with total penalty points of 4, summarized in Table 7. The proposed simultaneous equation and chemometrics methods have the highest eco scale values since hazardous chemicals are not used.

Table 7. Summary of Eco scale penalty points for the proposed methods.

Description	Penalty Points	Total Penalty Points	Score
Phosphate buffer	1	4	96
Instrument	0		
Occupational hazard	0		
Waste	3		

AGREE metrics: The novel evaluation software-based tool for assessing greenness is agree metrics, which is highly suitable since its results are more quantitative when competed to the analytical eco scale. The analytical eco scale is semi-quantitative, while in contrast, agree metrics system is entirely quantitative. The score by agree software for simultaneous equation and chemometrics methods presented in Figure 7 demonstrates that the proposed method is greener.

**Figure 7.** Results of agree metrics to analysis for the proposed methods.

3.4. Application of the Developed Methods in Pharmaceutical Formulation

Triplicate analysis of the proposed methods were performed to estimate PAR, ACE, and ES in pharmaceutical formulation, and their results were shown in Table 8. The established methods were applied to pharmaceutical formulations and replicated three times to

determine PAR, ACE, and ES concentration. The spectrum obtained for the formulation is presented in Figure 8. The concentration of PAR (C_x), ACE (C_y), and ES (C_z) obtained by simultaneous equation method as well as chemometrics models PCR and PLS along with dilution factor were used to calculate the amount of each analyte present in their tablet formulation. The results attained are shown in Table 8 and agreed with the label claim for the analytes.

Table 8. Results attained with pharmaceutical formulation.

Drug	Description	Simultaneous Equation Method	Chemometrics Method	
			PCR	PLS
PAR	Label Claim (mg)	325	325	325
	C_x	14.97	14.96	14.98
	Amount found(mg)	324.46	324.41	324.52
	% Label Claim	99.83	99.82	99.85
ACE	Label Claim (mg)	100	100	100
	C_y	4.59	4.58	4.59
	Amount found(mg)	99.66	99.42	99.69
	% Label Claim	99.66	99.42	99.69
ES	Label Claim (mg)	75	75	75
	C_z	3.43	3.43	3.45
	Amount found(mg)	74.32	74.40	74.78
	% Label Claim	99.09	99.20	99.71

C_x , C_y , and C_z are the concentrations obtained for PAR, ACE, and ES, respectively.

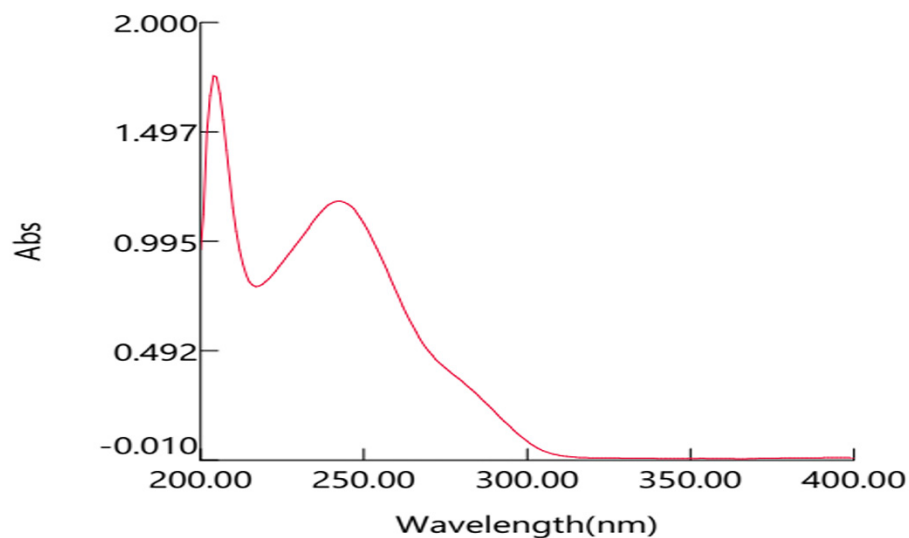


Figure 8. Assay spectrum obtained tablet formulation.

3.5. Statistical Comparison of the Developed Methods Using One-Way Analysis of Variance (ANOVA)

The statistical results obtained by comparison of proposed methods with One-way analysis of Variance (ANOVA) established that there is no noteworthy difference between the proposed methods (Table 9).

Table 9. Statistical analysis using One-way ANOVA.

Statistical Term	PAR			ACE			ES		
	Simultaneous Equation Method	Chemometrics Method		Simultaneous Equation Method	Chemometrics Method		Simultaneous Equation Method	Chemometrics Method	
		PCR	PLS		PCR	PLS		PCR	PLS
Mean	99.83	99.82	99.85	99.66	99.42	99.69	99.09	99.20	99.71
Mean \pm S.D	0.04	0.06	0.12	0.13	0.12	0.12	0.38	0.21	0.16
n	3	3	3	3	3	3	3	3	3
F ratio		0.13			4.31			4.58	
Theoretical F values at ($p = 0.05$)		5.14			5.14			5.14	

4. Conclusions

The present work accomplished the simultaneous determination of PAR, ACE, and ES in their pharmaceutical formulation. The proposed simultaneous equation and chemometric methods exhibit good accuracy, precision, and significantly less time for estimating selected analytes. Statistical assessment among the proposed methods showed that there was no noteworthy difference amongst the proposed methods. The proposed methods are advantageous in terms of cost of analysis using eco-friendly chemicals. They shall be adopted in the routine quality control to estimate PAR, ACE, and ES in commercial pharmaceutical preparations.

Author Contributions: Conceptualization, S.R. and L.K.S.; methodology, S.R.; software, S.R.; validation, S.R.; formal analysis, S.R. and L.K.S.; investigation, S.R.; resources, S.R.; data curation, S.R.; writing—original draft preparation, S.R.; writing—review and editing, S.R. and L.K.S.; visualization, S.R. and L.K.S.; supervision, L.K.S.; project administration, S.R.; funding acquisition, S.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not Applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not Applicable.

Acknowledgments: The authors are genuinely thankful to management of SRM College of Pharmacy for providing the necessary laboratory facilities to carry out the proposed work.

Conflicts of Interest: The authors declare no conflict of interest.

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