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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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 µg mL⁻¹ for paracetamol, 3.69–5.53 µg mL⁻¹ for Aceclofenac, and 2.76–4.15 µg 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-Ethynylphenyl)-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
Partial Least Squares (PLS) to estimate PAR, ACE and ES in pharmaceutical tablet formulation, concurrently. Further, the developed methods were evaluated for their greenness profile using greenness tools such as analytical eco scale and agree metrics.

The principle of the simultaneous equation technique [8] is that when a sample solution includes three drugs (X, Y, and Z) with dissimilar λ_{max}, the concentration of all the three analytes (C_x, C_y, and C_z) is calculated using a simple simultaneous equation [9] using absorbivity of X at λ_1, λ_2, and λ_3 (ax_1, ax_2, ax_3); the absorbivity of Y at λ_1, λ_2, and λ_3 (ay_1, ay_2, ay_3); and absorbivity of Z at λ_1, λ_2, and λ_3 (az_1, az_2, az_3) and absorbance of the sample at λ_1, λ_2, and λ_3 (A_1, A_2, and A_3)

\[
C_x = \frac{A_1(ax_1az_3 - az_2ay_3) - ay_1(A_2az_3 - az_2a3) + az_1(A_2az_3 - ay_2a3)}{ax_1(ax_2az_3 - az_2ay_3) - ay_1(ax_2az_3 - az_2ax_3) + az_1(ax_2ay_3 - ay_2ax_3)}
\]

\[
C_y = \frac{ax_1(A_2az_3 - az_2A_3) - A_1(ax_2az_3 - az_2ax_3) + az_1(ax_2A_3 - az_2ax_3)}{ax_1(ax_2az_3 - az_2ay_3) - ay_1(ax_2az_3 - az_2ax_3) + az_1(ax_2ay_3 - ay_2ax_3)}
\]

\[
C_z = \frac{ax_1(ay_2A_3 - Az_2ay_3) - ay_1(ax_2A_3 - Az_2ax_3) + A_1(ax_2ay_3 - ay_2ax_3)}{ax_1(ax_2az_3 - az_2ay_3) - ay_1(ax_2az_3 - az_2ax_3) + az_1(ax_2ay_3 - ay_2ax_3)}
\]

Figure 1. Chemical structures of PAR (a), ACE (b), and ES (c).

Chemometrics is the chemical discipline that uses mathematical, statistical, and other methods employing formal logic to design or select optimal measurement procedures and experiments and to provide maximum relevant chemical information by analyzing chemical data [10]. Chemometrics approaches gather data from the entire spectrum for simultaneous analyte estimation and provide quick analysis with reasonable accuracy and precision without the need for time-consuming sample preparation [11,12].

The estimation of drugs in mixtures and multi-component pharmaceutical formulation with overlapping spectra can be estimated more accurately and free from interferences by chemometric calibration techniques. The PCR and PLS used in the present study are factor analysis methods used to launch an association among matrices of chemical data [13]. PLS regression has the advantage of faster data processing with concentration values and absorbance of analytes showing strong overlapping spectra, minimization of errors in calibration model [14–17]. PCR entails removing the least significant principal components, and the response variable is subjected to a multiple regression analysis against a reduced
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.

\[
\text{Analytical eco – scale} = 100 - \text{Total penalty points}
\] (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.

\[\text{Figure 2. Example of agree metrics output including 12 principles of green chemistry with an overall score in the middle.}\]
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 µg 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 µg 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 µg mL⁻¹ for PAR, 4.61 µg mL⁻¹ for ACE, and 3.46 µg mL⁻¹ for ES were prepared separately to quantify pharmaceutical formulation from the stock solution. The absorbivities were calculated for PAR (X) at λ₁, λ₂, and λ₃ (ax₁, ax₂, and ax₃); ACE (Y) at λ₁, λ₂, and λ₃ (ay₁, ay₂, and ay₃); and ES (Z) at λ₁, λ₂, and λ₃ (az₁, az₂, and az₃). The absorbance of sample solutions was measured at 243 nm (λ₁), 272 nm (λ₂), and 262 nm (λ₃).

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 µm PTFE syringe filter (13 mm diameter). Aliquots of the filtrate were diluted appropriately to make a final solution of 15 µg mL⁻¹ of PAR, containing 4.61 µg mL⁻¹ of ACE and 3.46 µg 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 ± 10%. The 10 µg 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 12–18 µg mL⁻¹ for PAR, 3.69–5.53 µg mL⁻¹ for ACE, and 2.76–4.15 µg 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 µg mL⁻¹ of PAR, 4.61 µg mL⁻¹ of ACE, and 3.46 µg 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 (µg mL⁻¹) | Coding Level | Concentration (µg mL⁻¹) | Coding Level | Concentration (µg 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 Coding Level | Concentration (µg mL⁻¹) | ACE Coding Level | Concentration (µg mL⁻¹) | ES Coding Level | Concentration (µg 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                   | 0              | 3.46                   |
| 32               | −1               | 13.50                  | −1               | 4.15                   | −1             | 3.11                   |
| 33               | 0                | 15.00                  | 0                | 4.61                   | −1             | 3.80                   |
| 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_cal 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.

\[
LOD = 3.3 \times \|b\| \times \|\Sigma\| \quad (5)
\]

\[
LOQ = 10 \times \|b\| \times \|\Sigma\| \quad (6)
\]

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 µg 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 $\lambda_{\text{max}}$ of the other two drugs.

![Figure 3. Zero-order overlay absorption spectrum of 10 µg mL$^{-1}$ of PAR, 10 µg mL$^{-1}$ of ACE, and 10 µg mL$^{-1}$ of ES.](image)

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 ±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                        | 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 (µg mL⁻¹)              | 12–18 | 3.69–5.53 | 2.76–4.15 |
| LOD (µg mL⁻¹)                      | 0.48  | 0.20  | 0.13   |
| LOQ (µg 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 (r²)        | 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.
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      |
|------------------|---------|---------|---------|
|                  | Amount Found (µg mL⁻¹) | % R | Amount Found (µg mL⁻¹) | % R | Amount Found (µg mL⁻¹) | % R |
| 1                | 15.01   | 100.07  | 15.01   | 100.07 |
| 2                | 15.09   | 100.57  | 15.08   | 100.53 |
| 3                | 11.89   | 99.09   | 11.89   | 99.12  |
| 4                | 12.00   | 99.98   | 12.00   | 100.02 |
| 5                | 17.76   | 98.69   | 17.76   | 98.69  |
| 6                | 13.49   | 99.92   | 13.49   | 99.90  |
| 7                | 18.09   | 100.48  | 18.09   | 100.48 |
| 8                | 15.04   | 100.29  | 15.04   | 100.29 |
| 9                | 13.46   | 99.74   | 13.46   | 99.72  |
| 10               | 13.60   | 100.76  | 13.58   | 100.62 |
| 11               | 16.50   | 99.99   | 16.50   | 99.99  |
| 12               | 18.05   | 100.29  | 18.05   | 100.29 |
| 13               | 16.47   | 99.80   | 16.47   | 99.81  |
| 14               | 14.94   | 99.61   | 14.94   | 99.61  |
| 15               | 17.64   | 98.01   | 17.65   | 98.06  |
| 16               | 18.03   | 100.19  | 18.03   | 100.15 |
| 17               | 12.04   | 100.32  | 12.04   | 100.31 |
| 18               | 16.54   | 100.21  | 16.54   | 100.22 |
| 19               | 11.94   | 99.49   | 11.97   | 99.75  |

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

3.2. Chemometric Method

The selection of Wavelength Range for PCR and PLS

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. Cont.

| Standard Mixture | PAR | PLS | ACE | PLS | ES | PCR | PLS | PCR | PLS | PCR | PLS |
|------------------|-----|-----|-----|-----|----|-----|-----|-----|-----|-----|-----|-----|
|                  | Amount Found (µg mL⁻¹) | % R | Amount Found (µg mL⁻¹) | % R | Amount Found (µg mL⁻¹) | % R | Amount Found (µg mL⁻¹) | % R | Amount Found (µg mL⁻¹) | % R | Amount Found (µg 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 | 100.61 | 16.60 | 100.61 | 4.18 | 100.79 | 4.18 | 100.79 | 2.78 | 100.72 | 2.78 | 100.72 |
| 23               | 13.52 | 100.20 | 13.52 | 100.20 | 3.70 | 100.22 | 3.70 | 100.22 | 3.12 | 100.21 | 3.12 | 100.21 |
| 24               | 11.97 | 99.77 | 11.97 | 99.77 | 4.14 | 99.79 | 4.14 | 99.79 | 2.78 | 99.72 | 2.78 | 99.72 |
| 25               | 13.37 | 99.01 | 13.37 | 99.01 | 4.57 | 99.04 | 4.57 | 99.04 | 2.73 | 98.95 | 2.73 | 98.95 |

% R—% Recovery.

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

| Standard Mixture | PAR | PLS | ACE | PLS | ES | PCR | PLS | PCR | PLS | PCR | PLS |
|------------------|-----|-----|-----|-----|----|-----|-----|-----|-----|-----|-----|
|                  | Amount Found (µg mL⁻¹) | % R | Amount Found (µg mL⁻¹) | % R | Amount Found (µg mL⁻¹) | % R | Amount Found (µg mL⁻¹) | % R | Amount Found (µg mL⁻¹) | % R | Amount Found (µg 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 | ACE | ES | PLS | PAR | ACE | ES |
|------------------------|-----|-----|----|-----|-----|-----|----|
| Concentration range (µg 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 |
| R² | 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 | 0.0576 | 0.0379 | 0.0210 |
| PRESS | 0.3686 | 0.0361 | 0.0163 | 0.2530 | 0.0344 | 0.0156 |
| Intercept | 0.9978 | 0.9977 | 0.9981 | 0.9978 | 0.9977 | 0.9981 |
| Calibration set Mean ± SD | 99.98 ± 0.77 | 99.98 ± 0.80 | 99.93 ± 0.76 | 99.99 ± 0.75 | 99.99 ± 0.78 | 99.89 ± 0.74 |
| Validation set Mean ± SD | 99.99 ± 0.30 | 99.97 ± 0.33 | 99.98 ± 0.77 | 99.99 ± 0.32 | 99.97 ± 0.33 | 99.95 ± 0.30 |
| Assay Mean ± SD | 99.85 ± 0.10 | 99.83 ± 0.10 | 99.96 ± 0.77 | 99.83 ± 0.10 | 99.79 ± 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.

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 PAR | PCR ACE | PCR ES | PLS PAR | PLS ACE | PLS ES |
|-----------------------------|---------|---------|--------|---------|---------|--------|
| Sensitivity (mL µg⁻¹)       | 1.0022  | 1.0023  | 1.0019 | 1.0022  | 1.0023  | 1.0019 |
| Analytical sensitivity γ⁻¹ (µg mL⁻¹) | 5.9337  | 5.5011  | 5.7088 | 5.9337  | 5.5011  | 5.7088 |
| LOD (µg mL⁻¹)               | 0.56    | 0.60    | 0.58   | 0.56    | 0.60    | 0.58   |
| LOQ (µg 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.](image)

#### 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 | PCR | PLS | PCR | PLS |
| PAR  | Label Claim (mg) | 325 | 325 | 325 | 325 | 325 | 325 |
|      | \( C_x \) | 14.97 | 14.96 | 14.98 | 14.96 | 14.98 | 14.98 |
|      | Amount found(mg) | 324.46 | 324.41 | 324.52 | 324.41 | 324.52 | 324.52 |
|      | % Label Claim | 99.83 | 99.82 | 99.85 | 99.82 | 99.85 | 99.85 |
| ACE  | Label Claim (mg) | 100 | 100 | 100 | 100 | 100 | 100 |
|      | \( C_y \) | 4.59 | 4.58 | 4.59 | 4.58 | 4.59 | 4.59 |
|      | Amount found(mg) | 99.66 | 99.42 | 99.69 | 99.42 | 99.69 | 99.69 |
|      | % Label Claim | 99.66 | 99.42 | 99.69 | 99.42 | 99.69 | 99.69 |
| ES   | Label Claim (mg) | 75 | 75 | 75 | 75 | 75 | 75 |
|      | \( C_z \) | 3.43 | 3.43 | 3.45 | 3.43 | 3.45 | 3.45 |
|      | Amount found(mg) | 74.32 | 74.40 | 74.78 | 74.40 | 74.78 | 74.78 |
|      | % Label Claim | 99.09 | 99.20 | 99.71 | 99.20 | 99.71 | 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 |
| Mean             | 99.83 | 99.82 | 99.66 | 99.42 | 99.09 | 99.20 |
| Mean ± S.D       | 0.04 | 0.06 | 0.12 | 0.13 | 0.38 | 0.21 |
| n                | 3 | 3 | 3 | 3 | 3 | 3 |
| F ratio          | 0.13 | 4.31 | 4.58 | 3 | 3 | 3 |
| 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.

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References
1. Williams, M. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 14th Edition. Merck Inc., Whitehouse Station/Rahway, New Jersey, October 2006. Cloth 0-911910-00X. $125. Pp. 2564. Drug Dev. Res. 2006, 67, 870. [CrossRef]
2. El-Gindy, A.; Emara, S.; Shaaban, H. Validation and Application of Chemometrics-Assisted Spectrophotometry and Liquid Chromatography for Simultaneous Determination of Two Ternary Mixtures Containing Drotaverine Hydrochloride. J. AOAC Int. 2010, 93, 536–548. [CrossRef] [PubMed]
3. Ertokuş, G.P.; Yıldız, M. Chemometric Analysis of Paracetamol and Metaclopramide In Binary Drug Combinations. Int. J. Pharm. Sci. Res. 2018, 9, 1268–1273. [CrossRef]
4. Rohman, A.; Dzulfianto, A.; Riswanto, F. The Employment of UV-Spectroscopy Combined with Multivariate Calibration for Analysis of Paracetamol, Propyphenazone and Caffeine. Indones. J. Pharm. 2017, 28, 191. [CrossRef]
5. Glavanović, S.; Glavanović, M.; Tomišić, V. Simultaneous Quantitative Determination of Paracetamol and Tramadol in Tablet Formulation Using UV Spectrophotometry and Chemometric Methods. Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 2016, 157, 258–264. [CrossRef] [PubMed]
6. Carolin Nimila, I.; Balan, P.; Yaswanth Kumar, D.; Rajasekar, S. Simultaneous Estimation of Diacerein and Aceclofenac in Bulk and Pharmaceutical Dosage Form by UV Spectroscopy Method. Int. J. Pharm. Technol. Res. 2010, 2, 2313–2318.
7. Ragupathy, V.; Arcoat, S. Simultaneous Spectrophotometric Determination of Diacerein and Aceclofenac in Tablets By Chemometric Methods. Int. Res. J. Pharm. 2013, 4, 211–214. [CrossRef]
8. Beckett, A.H.; Stenlake, J.B. Practical Pharmaceutical Chemistry, 4th ed.; CBS: London, UK, 1988.
9. Kalyani, L.; Rao, C.V.N. Simultaneous Spectrophotometric Estimation of Salbutamol, Theophylline and Ambroxol Three Component Tablet Formulation Using Simultaneous Equation Methods. *Karbala Int. J. Mod. Sci.* 2018, 4, 171–179. [CrossRef]

10. Heberger, K. Chemoinformatics-Multivariate Mathematical-Statistical Methods for Data Evaluation. In *Medical Applications of Mass Spectrometry*; Elsevier: Amsterdam, The Netherlands, 2008; pp. 141–169. [CrossRef]

11. Tawakkol, S.M.; El-Zenay, M.B.; Hemdan, A. Full Spectrum and Selected Spectrum Based Chemometric Methods for the Simultaneous Determination of Cinnarizine and Dimenhydrinate in Laboratory Prepared Mixtures and Pharmaceutical Dosage Form. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* 2017, 173, 892–896. [CrossRef] [PubMed]

12. Ghasemi, J.; Niazi, A. Simultaneous Determination of Cobalt and Nickel. Comparison of Prediction Ability of PCR and PLS Using Original, First and Second Derivative Spectra. *Microchem. J.* 2001, 68, 1–11. [CrossRef]

13. Hadad, G.M.; El-Gindy, A.; Mahmoud, W.M.M. HPLC and Chemometrics-Assisted UV-Spectroscopy Methods for the Simultaneous Determination of Ambroxol and Doxycycline in Capsule. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* 2008, 70, 655–663. [CrossRef]

14. Wold, S.; Ruhe, A.; Wold, H.; Dunn Iii, W.J. The Collinearity Problem in Linear Regression. The Partial Least Squares (PLS) Approach to Generalized Inverses. *SIAM J. Sci. Stat. Comput.* 1984, 5, 735–743. [CrossRef]

15. Sjöström, M.; Wold, S.; Lindberg, W.; Persson, J.A.; Martens, H. A Multivariate Calibration Problem in Analytical Chemistry Solved by Partial Least-Squares Models in Latent Variables. *Anal. Chim. Acta* 1983, 150, 61–70. [CrossRef]

16. Karstang, T.V.; Eastgate, R.J. Multivariate Calibration of an X-Ray Diffractometer by Partial Least Squares Regression. *Chemom. Intell. Lab. Syst.* 1987, 2, 209–219. [CrossRef]

17. Esbensen, K.H.; Martens, H. Predicting Oil-Well Permeability and Porosity from Wire-Line Petrophysical Logs—A Feasibility Study Using Partial Least Squares Regression. *Chemom. Intell. Lab. Syst.* 1987, 2, 221–232. [CrossRef]

18. Oliveri, A.J.; Faber, N.M. Uncertainty Estimation and Figure of Merit for Multivariate Calibration (IUPAC Technical Report). *Int. Union Pure Appl. Chem.* 2006, 78, 633–661. [CrossRef]

19. Galusza, A.; Migaszewski, Z.M.; Konieczka, P.; Namieśnik, J. Analytical Eco-Scale for Assessing the Greenness of Analytical Procedures. In *TrAC—Trends in Analytical Chemistry*; Elsevier: Amsterdam, The Netherlands, 2012; pp. 61–72. [CrossRef]

20. Pena-Pereira, F.; Wojnowski, W.; Tobiszewski, M. AGREE—Analytical GREEnness Metric Approach and Software. *Anal. Chem.* 2020, 92, 10076–10082. [CrossRef] [PubMed]

21. ICH. Validation of Analytical Procedures: Text and Methodology Q2(R1) Current Step 4 Version. In *Guideline on Validation of Analytical Procedures: Methodology Developed to Complement the Parent Guideline*; International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Geneva, Switzerland, 2005; Volume Q2(R1).

22. Brereton, R.G. Multilevel Multifactor Designs for Multivariate Calibration. *Analyst* 1997, 122, 1521–1529. [CrossRef]

23. Valderrama, P.; Braga, J.W.B.; P hippi, R.J. Variable Selection, Outlier Detection, and Figures of Merit Estimation in a Partial Least-Squares Regression Multivariate Calibration Model. A Case Study for the Determination of Quality Parameters in the Alcohol Industry by near-Infrared Spectroscopy. *J. Agric. Food Chem.* 2007, 55, 8331–8338. [CrossRef] [PubMed]

24. Haaland, D.M.; Thomas, E.V. Partial Least-Squares Methods for Spectral Analyses. 1. Relation to Other Quantitative Calibration Methods and the Extraction of Qualitative Information. *Anal. Chem.* 2002, 60, 1193–1202. [CrossRef]

25. Camo. *Workshop on Industrial Perspectives in Analytical Techniques & Training on Chemometrics Software*; Camo: Bangalore, India, 2017.