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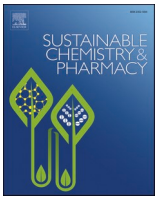

Chemometric-assisted spectrophotometric methods for simultaneous drug determination in new *Helicobacter pylori* treatment regimens - Environmental sustainability assessment

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A R T I C L E I N F O

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A B S T R A C T

The recent FDA approval of VOQUEZNA™ TRIPLE PAK™ 7-day therapy, which includes vonoprazan (VON), amoxicillin (AMO), and clarithromycin (CLA), marks a significant advancement in the treatment of *Helicobacter pylori* (*H. pylori*) infections. Accurate quantification of these active pharmaceutical ingredients (APIs) is critical for ensuring therapeutic efficacy and safety. However, conventional analytical methods often require extensive sample pretreatment and separation, which can be time-consuming and environmentally burdensome. This study presents the first simultaneous quantification of VON, AMO, and CLA using innovative chemometric-assisted spectrophotometric methods aligned with Green Analytical Chemistry (GAC) principles. Our methods eliminate the need for pretreatment or separation, thereby enhancing both analytical efficiency and environmental sustainability. We developed orthogonal partial least squares (OPLS), principal component regression (PCR), and Artificial Neural Network (ANN) models, utilizing the Design of Experiment (DoE) approach to minimize solvent use and waste.

Model validation was achieved through Orthogonal Array-based Latin Hypercube Sampling (OALHS), ensuring robust performance evaluation. The models demonstrated high precision, with recovery percentages ranging from 98.00% to 102.00%. The calibration set model fitting was assessed using the determination coefficient (R^2), and the cross-validation coefficient (Q^2), all model's R^2 and Q^2 values were close to 1.0, indicating the calibration samples' high capacity for explanation and prediction, while the root mean square error of calibration (RMSEC) values were found to be less than 0.1. The prediction of the validation set was employed by the root mean square error of prediction (RMSEP) and relative root mean square errors of prediction (RRMSEP), the values were found (0.0335–0.0613) and (0.7207–0.5287) for RMSEP and RRMSEP, respectively, while the bias-corrected mean square error of prediction (BCMSEP) was found to be between 0.0014 and 0.0001. To evaluate and enhance the sustainability of the methods, comprehensive tools were utilized: SPIDER Solvent Tool, RGB12 Algorithm, AGREE, and the Need Quality Sustainability (NQS) Index. This work supports the Sustainable Development Goals (SDGs) by demonstrating advancements in environmentally sustainable analytical methods.

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Abbreviation Indication

AMO	Amoxicillin	**OALHS**	Orthogonal Array-based Latin Hypercube Sampling
ANN	Artificial Neural Network	**OPLS**	Orthogonal Partial Least Squares
APIs	Active Pharmaceutical Ingredients	**P-CABs**	Potassium-Competitive Acid Blockers
BCMSEP	Bias Corrected Mean Square Error of Prediction	**PCR**	principal component regression
CLA	Clarithromycin	**Q^2**	Crossvalidation coefficient
FDA	Food and Drug Administration	**Q**	Quartile
FNN	Feedforward Neural Network	**R^2**	Coefficient
GAC	Green Analytical Chemistry	**REP**	Relative Error of Prediction
H. pylori	*Helicobacter pylori*	**RMSECV**	Root Mean Square Error of cross-Validation
LOO-CV	Leave-one-out cross-validation	**RMSEP**	Root Mean Square Error of Calibration
TRAINLM	Levenberg-Marquardt	**RRMSEP**	Relative Root Mean Square Error of Prediction
LHS	Latin Hypercube Sampling	**%R**	Relative Recovery
LOD	Limit of Detection	**SD**	Standard Deviation
LOQ	Limit of Quantification	**SDS**	Safety Data Sheets
LVs	Latent Variables	**SDGs**	Sustainable Development Goals
NQS	Need Quality Sustainability	**LOOCV**	Leave One Out Cross Validation
OA	Orthogonal Array	**UPLC**	Ultra PerformanceLiquid Chromatography
OALHS	Orthogonal Array-based Latin Hypercube Sampling	**VON**	Vonoprazan
		WAC	White Analytical Chemistry
		OPLS	Orthogonal Partial Least Squares
		P-CABs	Potassium-Competitive Acid Blockers

1. Introduction

Helicobacter pylori (*H. pylori*) infection is prevalent worldwide, affecting approximately 50% of the global population. This bacterium is a Gram-negative pathogen and is a leading cause of peptic ulcers. Additionally, the *H. pylori* bacteria can lead to long-term inflammation in the upper portion of the small intestine and stomachlymphoma or cancer of the stomach(Kusters et al., 2006; Zhang et al., 2022). Overall, *H. pylori* infection is crucial for preventing the development of peptic ulcers and other associated complications, highlighting the importance of timely diagnosis and appropriate treatment(Akazawa et al., 2016). Antibiotic therapy is often necessary, to combat *H. pylori* infection, the triple therapy is the standard treatment regimen, which effectively eradicates bacterial infection and reduces the risk of recurrence. The triple therapy includes a combination of two antibiotics, such as amoxicillin (AMO) and clarithromycin (CLA), along with a proton pump inhibitor (PPI)(Murakami et al., 2016; Parsonnet et al., 1991; Yang et al., 2018).

Potassium-Competitive Acid Blockers (P-CABs) such as Vonoprazan (VON), inhibit the release of stomach acid by selectively and reversibly inhibiting H+/K + -ATPase(Duan et al., 2023). P-CABs compete with potassium ions in contrast to PPIs, and their efficacy varies with dosage(Atherton, 2006). P-CABs have better acid stability,a stronger initial burst of activity, longer duration of acid suppression, and extended acid suppression, therefore P-CABs are effective and desirable alternative therapies for treating diseases associated with acids such as gastroesophageal reflux disease and H. pylori eradication treatment compared to PPIs(Boustany et al., 2023; Kajihara et al., 2017; Kinoshita et al., 2016; Sue and Maeda, 2021).

Additionally, it can be taken with medicines like (AMO and CLA)to eradicate (*H. pylori*) infections(Chey et al., 2022; Furuta et al., 2020). Voquezna Triple Pak includes VON, a potassium-competitive acid blocker, AMO, a beta-lactam antibiotic, and clarithromycin, a

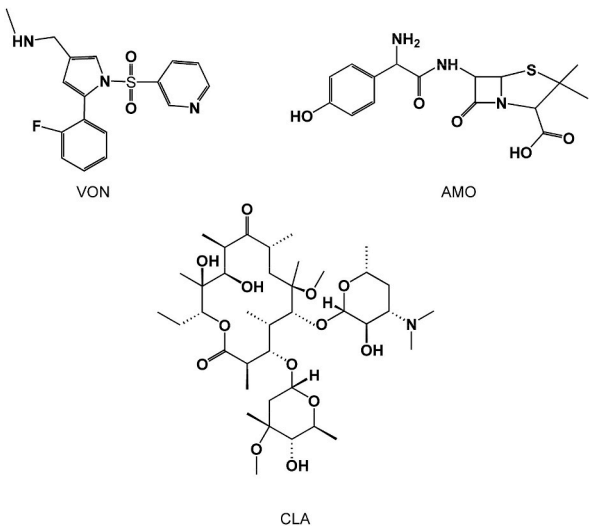


Fig. 1. Structure of vonoprazan (VON), clarithromycin (CLA), and amoxicillin (AMO).

macrolide antibiotic. Vonoprazan was initially licensed for medicinal usage in Japan in 2016(Deguchi et al., 2021), and the United States FDA granted permission in 2022 (FDA, 2022). This designation distinguishes it as a first-in-class drug, emphasizing its pioneering significance in its therapeutic field(Voquezna, 2024.).

The chemical structure of VON is given in Fig. 1, and its chemical name is1-[5-(2-fluorophenyl)-1-pyridin-3-ylsulfonylpyrrol-3-yl]-*N*-methylmethanamine monofumarate(Lin et al., 2022).

AMO, is given in Fig. 1, and its chemical name is(2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl.]amino.]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0.]heptane-2-carboxylic acid(Calhoun and Hokanson, 1993). AMO is a penicillin antibiotic, that may be degraded by bacteria's β-lactamase enzyme andis often used to treat pneumonia and, in conjunction with other antibiotics, to eradicate *H. pylori*(Aktaş and Sarıdağ, 2017).

CLA,is given in Fig. 1, and its chemical name is(2R,3S,4S,5R,6R,8R, 10R, 11 R,12S,13R)-3-(2,6-Dideoxy-3-C,3-o-dimethyl-α-L-*ribo*-hexopyranosyloxy)-11,12-dihydroxy-6-methoxy-2,4,6,8,10,12-hexamethy1-9-oxo-5-(3,4,6-trideoxy-3-dimethylarnino-β-D-xylohexo-pyranosyloxy)pentadecan-13-olide, CLAis the second generation of macrolides that is effective against a variety of bacteria, which is routinely used to treat respiratory tract infections, is also included in *H. pylori* eradication regimens(Vajdle et al., 2017).

Conserving rigorous pharmaceutical standards, reducing adverse reactions, providing assurance of quality, and enhancing treatment efficacy all depend on precise evaluation of medication concentrations in multi-component combinations. This is particularly important for improving medical research and clinical applications. Through the use of accurate and trustworthy methodologies for analysis, we can enhance multi-drug regimens while enhancing patient results(Sharma, 2015). Multi-component analysis is frequently needed in pharmaceutical formulations, particularly for complicated medicines like the Voquezna Triple Pak. Multiple APIs coexisting in a formulation more closely mimics real-world conditions when a simultaneous approach for measuring all APIs in a single measurement is used. We can expedite the analytical process and lower the time, expense, and labor involved in doing individual calibrations for every API by evaluating them together. In quality control laboratories, where minimizing sample preparation and measurement time is crucial, this strategy is quite helpful(El-Gindy and Hadad, 2012).

In the literature, numerous analytical approaches for the determination of VON have been published, either alone(Liu et al., 2016; Luo et al., 2018; Saraya et al., 2022; Yoneyama et al., 2016) or in conjunction with other drugs such as AMO(Kammoun et al., 2024), or aspirin(Abdelazim et al., 2023). A large number of analytical techniques have been published for the study of CLA and AMO, both alone and in combination with other drugs (Hassib et al., 2022; Hassib et al., 2022; Mustafah et al., 2021). These procedures are critical for accurately quantifying and controlling the quality of these drugs in a variety of pharmaceutical formulations and clinical contexts. Although there are no existing analytical methods documented for the examination of CLA, AMO, and VON in their triple co-formulated pharmaceutical formulation, only one method was applied to analyze the three drugs in separate dosage forms by (RP-UPLC)(Anusha and Sowjanya, 2024).

Whereas conventional chromatographic techniques are useful for determining drug concentrations at lower concentrations, they present several difficulties. For example, the separation and quantification of numerous medicines in a single study can be challenging because of drug interactions and overlapping spectra(Luo et al., 2018). RP-UPLC provides faster, more accurate, and more sensitive analyses compared to traditional HPLCwhile developing and validating methods for UPLC can also be more expensive since it requires optimization of high-pressure settings, flow rates, and other parameters that differ from HPLC that cannot be useful for routine applications or smaller labs(Anusha and Sowjanya, 2024).

Chemometrics is better with complex datasets from methods like spectrophotometry, NMR, or chromatography, and needs to extract information without physical separation, or when looking to simultaneously quantify multiple components in overlapping spectra or chromatograms (e.g., spectrophotometric analysis)(Kalinowska et al., 2021). The chemometric-assisted spectrophotometric method has substantial benefits over traditional methodologies because they can understand both quantitative and qualitative characteristics in data, and chemometrics excels at revealing the complexities within the data matrix derived from analytical research (Miller et al., 2018). Unlike classical approaches, which evaluate one component at a time, chemometrics uses multivariate techniques to assess all important factors at the same time, resulting in models that properly match the data. In addition, chemometrics provide a powerful alternative that can tackle the drawbacks of HPLC including the overuse of poisonous and environmentally dangerous chemical solvents combined with time-consuming sample preparation procedures(Brereton, 2000)[36]. Chemometric-assisted spectrophotometric methods, provide a more practical and cost-effective solution compared to RP-UPLC for certain simultaneous drug determinations (Anusha and Sowjanya, 2024).

In experimental designs with numerous variables, it's typical for only a fraction of them to be truly influential. A possible strategy for handling this is the convey those components in to a subspace defined by the effective variables. This approach may inadvertently duplicate sample points within the effective subspace. Tang(Tang, 1993) presented Orthogonal Array-based Latin Hypercube sampling (OALHS), which is a combination of orthogonal array experimental design (OA) and Latin Hypercube Sampling (LHS), this method adds the constraint that all of the sample space must be uniformly sampled at a coarse resolution for every trial(Donovan et al., 2018). Leary et al.'s work(Leary et al., 2003) provides more insights into optimum structures utilizing this method. The utilization of OALHS in experimental design is motivated by several factors which ensure that all pairwise combinations of the chosen factors are tested, and allow the creation of a validation set with an evenly distributed representation of all pairwise combinations. In cases where compared to fractional factorial design, OALHS experimental designs are capable of accommodating factors with various levels, saving time, and generating and facilitating easy analysis. This leads to the conclusion that validation across the entire spectrum of controllable factors, reduces the likelihood of errors and realized significant savings in experimental efforts, which in turn reduces the number of runs (Burrage et al., 2015; Welch et al., 1992; Yondo et al., 2018). Implementing GAC concepts in sample preparation procedures is an important advancement in the analytical chemistry field. Analytical methods are further developed and adapted in line with global environmental and economic objectives by GAC, which prioritizes sustainability, effectiveness, and safety(Yahaya et al., 2024). These

ideas are further supported by non-destructive spectroscopy methods, which are highly beneficial in today's analytical procedures which maintain sample integrity and reduce preparatory work(Eissa and Darweish, 2024). In the field of analytical chemistry, it is crucial to develop procedures that reduce the use of solvents and eliminate waste. The present trend toward more environmentally sustainable procedures highlights the need for techniques that produce valid outcomes and adhere the environmental ideas(Saleh et al., 2024). DoE providing organized developed methods in line with the GAC concept is capable of helping laboratories improve techniques efficiency and significantly reduce their environmental impact(El-Gindy and Hadad, 2012).

Saleh et al. (2023) developed a new green tool named the Efficient Valid Green (EVG),to evaluate analytical techniques via the prisms of the effectiveness of experimental design, verification, and green chemistry implementation. Green chemistry's main objective, which is to lessen chemical-related impacts through creative design, and proved useful as the frame for the creation of new analytical techniques. Researchers strive to embody the 12 principles of GAC in their methodologies, with varying degrees of success. In response to these challenges, assessment tools like AGREE and the SPIDER solvent tool have been developed to quantify a method's alignment with GAC principles accurately(Abou-Taleb et al., 2021; Shen et al., 2016). Moreover, the advent of White Analytical Chemistry (WAC) complements GAC by emphasizing the validity and economic viability of methods alongside their environmental considerations. As proposed by Nowak(Nowak et al., 2021), the principles of WAC and the RGB12 Algorithm tool are designed to evaluate the "whiteness" of analytical methods, ensuring they do not just conform to green principles but are also economically and operationally sustainable(A. El Hamd et al., 2023).

When evaluating complicated datasets and multi-component mixtures, chemometric techniques such as Principal Component Regression (PCR), Orthogonal Partial Least Squares (OPLS), and Artificial Neural Networks (ANNs) provide considerable advantages for precise component identification and quantification since they are made to manage overlapping spectra and extract valuable information from the whole spectral range. PCR is mainly used for dimensionality reduction by selecting principal components that capture the most variation in the data, but it doesn't explicitly focus on separating predictive from non-predictive variation like OPLS. PCR is a two-step method where principal component analysis (PCA) is applied first to the independent variables (X) to reduce the dimensionality of the data. Then, linear regression is performed on the principal components (PCs) that explain most of the variance (Katamesh et al., 2024). OPLS was introduced in the early 2000s as a variant of Partial Least Squares (PLS), which is widely used in regression modeling. OPLS is often preferred for its enhanced model interpretability which refers to handling multicollinear and noisy data, especially in cases where predictive and non-predictive variations coexist. It works by maximizing the covariance between the independent variables (X) and the response variable (Y)(Trygg and Wold, 2002). While ANN is a computer model made up of layers of networked nodes, or neurons, through which input data is passed before being non-linearly transformed to anticipate results. Complex, non-linear connections between variables can be handled using ANN. Both linear and non-linear connections may be modeled using ANNs, which offer great flexibility. To prevent overfitting, big datasets and appropriate hyperparameter tuning (such as the number of hidden layers and neurons) are needed. In some tasks, ANNs are more effective than linear techniques like PCR and OPLS because they are better at modeling complicated connections and capturing non-linear patterns in data(Geramizadegan et al., 2023; Marahel et al., 2022; Tantawy and Michael, 2019).

The goals of this research topic are to explore breakthroughs and applications of chemometric methods to address new challenges and recent advances in pharmaceutical analysis. In this study, we extend the evaluation of methodological greenness and whiteness by assessing their alignment with the SDGs through the NQS index(Kiwfo et al., 2023), covering a broad spectrum including Good Health and Well-being (SDG 3), Quality Education (SDG 4), and others up to Partnerships for the Goals (SDG 17). The performance of our thermometrically optimized method is compared against the reported chromatographic RP-UPLC method(Anusha and Sowjanya, 2024), demonstrating how these advanced chemometric methods contribute to global objectives through sustainable, efficient, and inclusive practices. Chemometrics plays a crucial role in experiment design and optimization within the green approach, contributing to its effectiveness(Kalinowska et al., 2021). The chemometric analysis offers a paradigm shift in the quantification of active pharmaceutical ingredients. This research is novel in its application of advanced chemometric techniques, particularly the use of OPLS, PCR, and ANN models, to facilitate the simultaneous analysis of VON, AMO, and CLA without the need for extensive sample pretreatment or separation. By aligning our approaches with the principles of GACto address not only the analytical challenges but also the environmental concerns associated with traditional methods. This study thus fills a critical gap in the current literature by providing an efficient, reliable, and sustainable solution for drug quantification in *H. pylori* treatment regimens which is guaranteed by employing OALHS as a new useful technique for chemometric model validation that can be used for routine quality control.

2. Experimental

2.1. Apparatus and software

The UV-1800 PC-type spectrophotometer from SHIMADZU, Kyoto, Japan, was utilized in conjunction with SHIMADZU UV prop data software. For chemometric analysis of the collected data, Matlab R2021a version 9.10.0.1669831 and the Partial Least Squares (PLS) Toolbox were employed.

2.2. Materials and reagent

Pure samples of Vonoprazan(VON), CAS No. 881681-00-1, Clarithromycin (CLA) CAS No. 81103-11-9, and Amoxicillin (AMO) CAS No. 26787-78-0 were obtained from MedChemExpress (18 Wilkinson Way, Princeton, NJ 08540, USA, New Jersey, Princeton, United States, 08540). Investigating their purities were 99.52% ± 0.39, 99.90% ± 0.52, and 98.00% ± 0.49, respectively according to the

British pharmacopoeia(British Pharmacopoeia, 2016),and Analytical grade ethanol was obtained from Sigma Aldrich, based in Darmstadt, Germany.

VOQUEZNA™ TRIPLE PAK™ tablets each tablet containing VON 20.0 mg, AMO 500.0 mg, and CLA 500.0 mg)were manufactured by Phathom Pharmaceuticals, Inc., Illinois, Chicago.

2.3. Standard solutions

Stock solutions: 50.0 mg of each recommended medicine was weighed and then dissolved separately in ethanol to provide the standard stock solutions in a volumetric flask (50 mL). Following careful addition of the solvent, stock solutions of 1000.0 μg/mL of each of VON, CLA, and AMO were obtained by reaching the flask's mark.

Working solutions: by diluting the stock solutions with an adequate amount of solvent the working solutions were created. The dilution procedure was used to get all suggested medicine up to a final concentration of 100.0 μg/mL. These working solutions make it possible to achieve more precise and controlled concentrations, which guarantees accurate and trustworthy analysis throughout the experimental procedures.

Laboratory-prepared mixture: Three sets of volumetric flasks, holding 5.0 mL each, were utilized to create many standard solutions. With ethanol used to modify the volume, each set concentrated on a certain medicine concentration range: 3.0–15.0 μg/mL for VON, 2.0–10.0 μg/mL for CLA, and 2.0–16.0 μg/mL for AMO.

3. Procedure

3.1. Linearity and characteristics of spectra

To evaluate the spectral characteristics of VON, CLA, and AMO, individual UV absorption spectra were acquired throughout a 200.0–400.0 nm wavelength range. From the working solution,the solutions of VON, CLA, and AMO were prepared. Twenty-five mixtures were scanned at intervals of 1.0 nm within the range of 200.0 and 400.0 nm. The determined concentration ranges were 2.0–10.0 μg/mL for CLA, 2.0–16.0 μg/mL for AMO, and 3.0–15.0 μg/mL for VON. Fig. 2 shows overlapped spectra of the three drugs, the conventional linear ranges were used to select concentrations of 5.0 μg/mL for VON, 8.0 μg/mL for CLA, and 10.0 μg/mL for AMO (see Fig. 3).

3.2. Design of Experiment (DoE)

Multilevel multifactor experimental design(Brereton, 2000)was employed, the calibration set of 25 mixtures consisting of three factors and five different concentration levels was meticulously designed to ensure representative and relevant data acquisition. The calibration set includes different concentrationsofthree drugs (VON, CLA, and AMO), the concentration ranges that were established were 3.0–15.0 μg/mL for VON, 2.0–10.0 μg/mL for CLA, and 2.0–16.0 μg/mL for AMO. By employing the OALHS approach(Tang, 1993), the validation set was built to provide an accurate sample of the concentration level for comprehensive validation of the models. A total of sixteen equal probability strata were created from the concentration range, and chosen to be part of the validation set,as illustrated in Table 1. Either the calibration and validation designs were straightforward, sensitive, selective, economical, time- and money-efficient, and used little solvent. They also complied with GAC and WAC. The preparation of all mixtures took place in 5.0 mL volumetric flasks, where precise amounts of the working standard solutions were transferred and diluted with ethanol. Analysis was performed on the recorded absorption spectra in the 200.0–400.0 nm range; wavelengths below 205.0 nm were eliminated due to significant noise and above 300.0 nm were discarded due to inadequate analyte signals. As a result, the spectral data matrix used for the development and verification of the chemometric models included 95 data points covering the relevant range of 205.0–300.0 nm

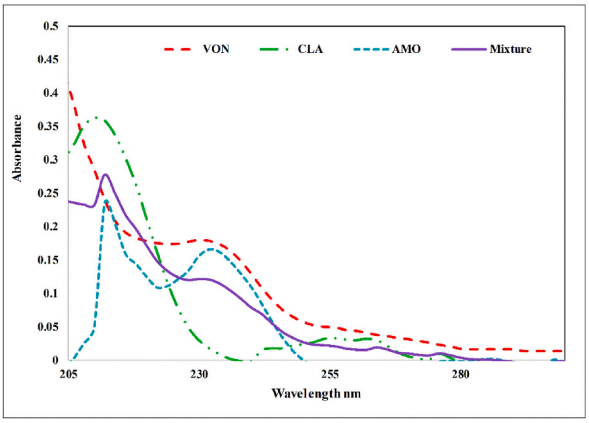


Fig. 2. Zero-order absorption spectra of 5.0 μg/mL, 8.0 μg/mL, and 10.0 μg/mL of VON, CLA, and AMO, respectively.

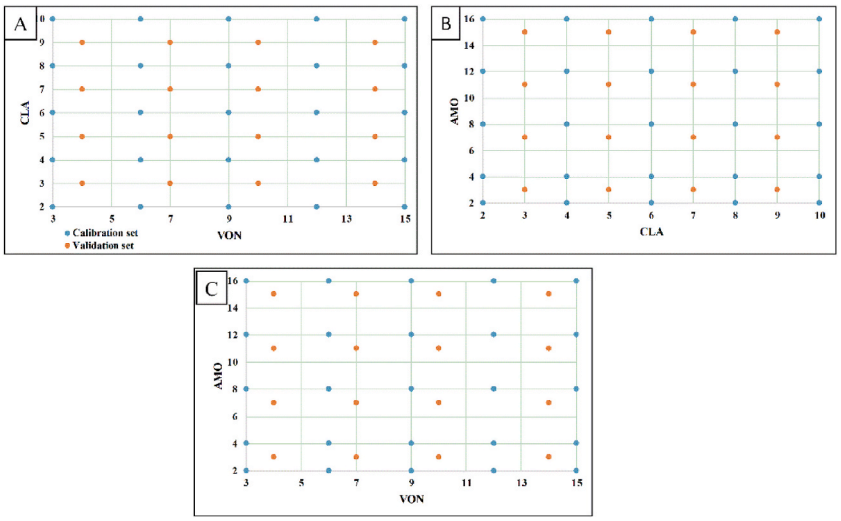


Fig. 3. Orthogonal-Array Latin Hypercube sampling design for the validation set.
(a) 2D scatter plot of VON/CLA, (b) 2D scatter plot of CLA/AMO, and (C) 2D scatter plot of VON/AMO.

Table 1
Calibration and Validation set.

Mix No.	Calibration Set (μg/mL)			Mix No.	Validation Set (μg/mL)		
	VON	CLA	AMO		VON	CLA	AMO
1	9	6	8	1	4	3	3
2	6	8	16	2	7	5	7
3	12	10	12	3	10	7	11
4	15	8	8	4	14	9	15
5	12	6	16	5	7	7	15
6	9	10	16	6	10	9	3
7	15	10	2	7	14	3	7
8	15	2	12	8	4	5	11
9	3	8	2	9	10	3	15
10	12	2	8	10	14	5	3
11	3	6	12	11	4	7	7
12	9	8	12	12	7	9	11
13	12	8	4	13	14	7	3
14	12	4	2	14	4	9	7
15	6	2	4	15	7	3	11
16	3	4	8	16	10	5	15
17	6	6	2				
18	9	2	2				
19	3	2	16				
20	3	10	4				
21	15	4	16				
22	6	10	8				
23	15	6	4				
24	9	4	4				
25	6	4	12				

and determined at optimum 1.0 nm intervals and slit width. All the absorbance and concentration data from the produced mixtures were imported into Matlab to build multivariate calibration models, then further data analysis and mean-centered before constructing the PCR, OPLS, and ANN models. Because each parameter in these models was carefully tuned before being applied to the external validation set (sixteen mixtures), they can predict drug concentrations with great accuracy. This rigorous technique allowed for a detailed examination of forecast accuracy and model performance.

3.3. Pharmaceutical formulations assay

Seven VOQUEZNA™ TRIPLE PAK™ each tablet included 20.0 mg of VON, 500.0 mg of CLA, and 500.0 mg of AMO, as stated on the package, which was pulverized into a fine powder 3.0 mg, 10.0 mg and 10.0 mg of VON, CLA, and AMO were accurately weighed in 100-ml beaker and extracted by sonication with ethanol for 15 min then filtered into a 100-mL volumetric flask. The volume was

completed to the mark with the same solvent. Appropriate dilution was made with ethanol to get a concentration claimed to contain 3.0 μg/mL for VON, 10 μg/mL for CLA and AMO, respectively. The absorption spectra of this diluted sample solution was measured between 200.0 and 400.0 nm in wavelength. The created chemometrics models were utilized to analyze the acquired spectrum and determine the concentrations of the proposed drugs in the medicinal mixture. Spiked standard adds were carried out in triplicate at three concentration levels of each drug to assess accuracy. The relative recovery (%R) and standard deviation (SD) were calculated.

4. Results and discussion

According to the literature, no reported chemometric-assisted spectrophotometric methods exist for the simultaneous determination of VON, CLA, and AMO in their mixture. Chemometric tools are extensively utilized in analytical chemistry to streamline data analysis by reducing data dimensionality, grouping variables, and processing analytical signals. Moreover, they hold promise for minimizing the environmental impact of analytical procedures.

DoE offers a superior approach compared to the traditional method of changing one variable at a time, as it provides more comprehensive insights into system response, leading to savings in materials and energy. By integrating desirability functions, it becomes feasible to incorporate variables directly related to the environmental friendliness of procedures. This integration allows for the minimization of analysis time, solvent or reagent consumption, and mobile phase usage (in liquid chromatography, for instance). Direct measurement of VON, CLA, and AMO was difficult due to high spectrum overlap, as seen in Fig. 2. Chemometric models with PCR, OPLS, and ANN procedures have proven helpful in extracting useful information from large spectrum datasets using multivariate calibration allowing reliable measurements of each component from the composite spectrophotometric data. Chemometrics-assisted spectrophotometer techniques were used to quantify laboratory-prepared mixtures and pharmaceutical formulations with optimum accuracy and precision.

4.1. Calibration and validation set design

The calibration set design is a critical step in creating reliable chemometric models for quantitative analysis. To guarantee that the calibration set design is effective and reliable, several elements must be addressed. The calibration set should cover a broad range of concentrations and fluctuations in the analyte of interest. This guarantees that the model is trained on a varied range of samples, resulting in improved prediction performance on previously unknown data. To eliminate biases, samples should be distributed evenly over the concentration range, the equal representation of low, middle, and high concentrations aids in capturing the whole dynamic range of the analyte. To avoid systematic mistakes or biases, samples for the calibration set should be selected randomly. Random sampling guarantees that the calibration set is representative of the population while reducing the danger of overfitting as shown in Table 1. To optimize the calibration set, we utilized a multilevel multifactor experimental design proposed by Brereton(Brereton, 2000), generating 25 calibration sets of mixtures with varying ratios of the three components, as shown in Table 1, the calibration set includes different concentrationsofthree drugs (VON, CLA, and AMO), the concentration ranges that were established were 3.0–15.0 μg/mL for VON, 2.0–10.0 μg/mL for CLA, and 2.0–16.0 μg/mL for AMO. A subset of the data should be reserved for validation to test the model's performance.

In this study, we designed the OALHS technique to create a validation set, to assure that the validation set is representative of the complete dataset. OALHS is a technique for designing and optimizing experiments, that combines the principles of (LHS) and (OA) designs to effectively explore the parameter space while assuring uniform coverage and minimizing the number of experimental runs necessary. In OALHS, the sample space is partitioned into equal-sized intervals along each dimension, and samples are selected so that each interval is represented precisely once in each dimension,a more consistent and complete investigation of the parameter space produced than standard sampling approaches. While preserving statistical robustness, OALHS enables the construction of sample sets with fewer runs, making it especially valuable in limited experimental resources.16OALHS-selected mixtures were found depending on the number of modified components and mixtures to construct the validation set, as shown in Table 1.

OALHS chooses precisely one sample from each of the 16 strata,to guarantee uniform and thorough coverage across all dimensions of the modeled pharmacological concentration space, since OALHS is a multidimensional sampling method, we can represent it visually in a 2D scatter plot by selecting two factors and plotting their corresponding samples. Fig.3illustrates the 2D scatter plots, which show the 16OALHS validation samples producing good uniform scattering throughout all analyte ranges with no gaps. OALHS enables a more representative and informative validation set with a lower size by increasing the efficiency of concentrating space sampling. This reduces the amount of material used, waste, and expense, improving the method's greenness.

The leave-one-out cross-validation (LOOCV) method was utilized to validate our chemometric models. The models were trained on all but one sample, and the missing sample was utilized for evaluation to guarantee every data point. Every sample is subjected to the LOOCV technique, which offers an objective evaluation of the model's performance(Haaland and Thomas, 1988).

4.2. Optimization ofthe models

4.2.1. PCR and OPLS

Spectroscopic analysis frequently employs full-spectrum computational approaches such as PCR and OPLS models. However, when noisy and uninformative wavelengths are added, the accuracy of these models might suffer. To overcome this issue, it is critical to remove noisy wavelengths before building the models. The number of components used in PCR and OPLS is crucial for generating accurate quantitative results(Katamesh et al., 2024; Trygg and Wold, 2002). If the number of factors is too great, noise may rise, while

too few factors may result in the loss of useful calibration data. LOOCV was used to determine the optimal principal components (PC) and latent variable (LV) count that would ensure the highest level of prediction accuracy while avoiding overfitting, the criteria given by Haaland and Thomas(Haaland and Thomas, 1988). When informative PCs are added, the prediction error often decreases; however, when noise-dominated higher-order PCs are added, the prediction error typically increases(Katamesh et al., 2024). As more PCs are added, the root mean square error of cross-validation (RMSECV) is monitored. The 'elbow' approach(Haaland and Thomas, 1988), is used to estimate the optimum number of latent variables (LVs),by comparing the RMSECV to the LVs graph and locating the point on the graph where the RMSECV significantly declines before beginning to level off. This represents the number of LVs that decrease the RMSECV without adequately excessive fitting. We adopted this approach to ensure that our model's complexity was appropriate and that it accurately captured the data that was necessary. As shown in Fig. 4., the optimal number of LVs for the three components was found to be four for both PCR and OPLS techniques.

4.2.2. ANN

ANN is an intelligent model which examines correlations between input and output data through employing different numbers of simple, thoroughly connected nodes or artificial neurons. Standard ANN consist of three layers: input layer, hidden layer, and output layer. Every single layer performs distinct roles in processing and converting input data to generate the desired output. The raw data are received by the input layer, sophisticated computations and transformations are performed by the hidden layer, and the final result is generated by the output layer based on the processed information from the hidden layer(El-Zeiny et al., 2021). In the current study, Levenberg-Marquardt's (TRAINLM) backpropagation algorithm was used to construct a feed-forward neural network (FNN)that is appropriate for training with fewer datasets and avoids time-consuming methods (Miller et al., 2018). The input layer consisted of 95 neurons, representing the spectral data points, while the output layer consisted of three neurons, representing the medicines under investigation. The Purelin-Purelin transfer function is used because concentration and absorbance are linearly related. A trial-and-error method was employed to experiment with various numbers of hidden neurons to optimize the process, to evaluate the performance of the model the (LOO-CV) was used, and the root mean square error of calibration (RMSEC) should be monitored as the number of hidden neurons increased. After a given amount of time, it should start to decline, but adding additional neurons might not make a big difference in performance and might even cause overfitting. Performance measures settle when there is an ideal number of hidden neurons present, and adding more neurons does not result in any further discernible improvement. A calibration set was used to train the created neural network, and 16 distinct mixes made up of a separate validation set were used to verify its performance. The mean square error (MSE) was used to track the training process for both the calibration and validation sets.

After experimenting with various hidden layer neuron counts, it was found that four hidden neurons with a purelin-purelin transfer function and 100 epochs were ideal. The performance curve of MSE vs epochs for a well-trained ANN is provided in Fig. S1 for VON, Fig. S2 for CLA, and Fig. S3 for AMO. Overfitting was not present after epochs 4, 7, and 8 for VON, CLA, and AMO, respectively, since the training MSE steadily dropped and the test and validation curves did not abruptly differ. One of the most important steps in building a neural network model is optimizing ANN characteristics. The values of the ideal ANN features for every medicine are listed in Table S1. Now that the parameters have been adjusted, learning may start.

4.3. Validation of the PCR, OPLS,and ANN models

To test linearity, calibration plots for each component were created by comparing predicted concentrations to actual values. The linearity ranges of VON, CLA, and AMO were found to be between 3.0 and 15.0, 2.0–10.0, and 2.0–16.0 μg/mL, respectively. To validate each model, the recovery and the predicted concentrations of every medication were computed in each of the sixteen validation sets, The percentage recoveries, standard deviation, and root mean square error of prediction (RMSEP) were all met with satisfaction, as indicated in Table 2.

Figures of merit (FOM) are crucial measures of method performance, reliability, and efficacy in chemometrics. They are especially helpful in the field of analytical chemistry for determining the validity of a chemometric technique. To assess the effectiveness of the upgraded models in quantifying the medication, we carried out a thorough validation procedure. The calibration set for fitting models was assessed using the determination R^2, and the Q^2, which is utilized throughout cross-validation, by comparing $Q^2$to R^2indicating how well the model predicts newly collected information(Trygg and Wold, 2002).

Table3demonstrates that all models' R^2 and Q^2 values were close to 1.0, indicating the calibration samples' high capacity for explanation and prediction. As demonstrated by the relatively small variation concerning both R^2 and Q^2, the models seem to have

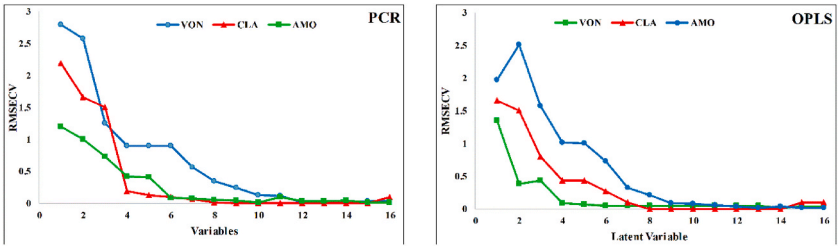


Fig. 4. RMSECV plot of the validation set against the Optimized variables in PCR and latent variables in OPLS.

Table 2
Prediction recovery results obtained by applying the proposed chemometric assisted spectrophotometric methods to the validation Set.

Added µg/mL	Recovery % VON			Added µg/mL	Recovery % CLA			Added µg/mL	Recovery % AMO		
VON	PCR	OPLS	ANN	CLA	PCR	OPLS	ANN	AMO	PCR	OPLS	ANN
4	101.88	102.33	100.50	3	98.57	98.85	101.24	3	99.70	100.19	99.70
7	101.92	101.96	102.33	5	99.62	100.44	98.50	7	98.25	99.72	99.12
10	99.82	99.94	101.96	7	98.19	101.82	101.09	11	100.66	97.52	99.69
14	98.74	101.50	99.94	9	99.21	99.68	100.72	15	100.49	99.82	100.85
7	100.29	99.55	101.50	7	99.99	99.83	99.82	15	99.03	100.23	99.92
10	100.84	99.67	99.55	9	99.82	99.99	99.95	3	97.25	102.96	100.68
14	98.96	100.70	99.67	3	99.85	99.70	99.94	7	99.87	100.14	103.17
4	99.34	101.06	100.70	5	100.31	100.92	99.70	11	100.28	100.53	100.37
10	100.90	100.80	101.06	3	100.05	100.11	99.50	15	101.19	101.06	100.85
14	99.30	99.63	100.80	5	100.95	100.35	100.25	3	100.34	99.72	100.66
4	100.18	100.47	99.64	7	99.96	100.06	100.26	7	97.61	98.52	99.82
7	99.90	100.51	100.47	9	99.94	99.94	100.04	11	101.19	100.42	101.99
14	100.24	99.43	100.51	7	98.50	100.46	99.79	3	100.56	100.16	100.18
4	100.03	99.90	99.44	9	99.84	99.68	99.64	7	100.51	100.67	100.83
7	100.20	100.13	99.90	3	99.92	99.99	100.09	11	99.87	99.58	100.52
10	99.70	100.50	100.13	5	100.42	100.40	99.93	15	99.80	99.73	99.81
Mean	100.14	100.51	100.52		99.70	100.14	100.03		99.79	100.07	100.52
SD	0.9078	0.8649	0.8649		0.7368	0.6435	0.6426		1.1828	1.1475	0.9765
%RSD	0.9064	0.8605	0.8605		0.7390	0.6426	0.6424		1.1853	1.1468	0.9715
RMSEP	0.0526	0.0613	0.0595		0.0449	0.0335	0.0379		0.0507	0.0427	0.0533

RMSEP: Root Mean Square Error of Prediction.

minimal overfitting and high prediction ability. In addition, as shown in Table 3, the RMSEC values for all models that are less than 0.1 demonstrate a strong match and minimal fluctuation between the predicted and actual concentrations for the calibration sets.

Several statistical measures were employed to evaluate the models' prediction performance on the validation set. The RMSEP and RRMSEP metrics on the external validation set were used to measure the predictive accuracy of fresh data. Moreover, the accuracy and variation of predictions were assessed using the bias-corrected mean square error of prediction (BCMSEP)(Katamesh et al., 2024).

Models with low RMSEP values were shown to be very accurate; Table 2demonstrates that the performance of the ANN model was superior when their RMSEP values fell between 0.030 and 0.05,while, PCR and OPLS were between 0.03 and 0.06. A more thorough assessment of prediction accuracy was conducted using RRMSEP, which is expressed as a percentage of the average analyte concentration. The ANN model showed the best RRMSEP values for VON, CLA, and AMO were 0.6574, 0.5955, and 0.6590, respectively, as shown in Table 3., while RRMSEP values for VON, CLA, and AMO were (0.6339, 0.7207, and 0.6526) and (0.7005, 0.5287, and 0.5519) for PCR and OPLS, respectively, as shown in Table 3. The closed values of RRMSEPto RMSEP revealed that the modified models lack overfitting. BCMSEP values justified the predictions' variance and accuracy(El-Zeiny et al., 2021; Katamesh et al., 2024). The model parameters have beneficial minimum values (BCMSEP <0.00124), signifying strong prediction accuracy and objectivity. Calculated by comparing projected and actual values, the relative error of prediction (REP) is reported as a percentage.

Table 3
Statistical parameters for the developed models.

Parameters	VON			CLA			AMO		
	PCR	OPLS	ANN	PCR	OPLS	ANN	PCR	OPLS	ANN
Slope	0.9986	0.9975	1.0102	1.001	0.9994	1.0012	1.0047	0.9985	0.9961
Intercept	0.0178	0.0499	−0.082	−0.0254	0.011	−0.0063	−0.033	0.0203	0.0529
LOD	0.1156	0.0992	0.098	0.1262	0.1419	0.2076	0.1159	0.1751	0.2418
LOQ	0.3504	0.3007	0.2969	0.3826	0.4302	0.6292	0.3513	0.5307	0.7328
R^2	0.9998	0.9998	0.9999	0.9996	0.9998	0.9997	0.9999	0.9999	0.9999
Q^2	0.9997	0.9993	0.9991	0.9989	0.9992	0.9986	0.9995	0.9994	0.9997
REP	0.6201	0.6574	0.5863	0.6978	0.5251	0.5124	0.6383	0.5485	0.5258
RMSEC	0.0601	0.0620	0.0600	0.0624	0.0494	0.0612	0.0587	0.0564	0.0596
RRMSEP	0.6339	0.7005	0.6574	0.7207	0.5287	0.5955	0.6526	0.5519	0.6590
BCMSEP	0.00022	0.00043	0.00081	−0.00109	0.00050	0.00010	0.00018	0.00034	0.00124

RMSEC: Root Mean Square Error of Calibration.
RRMSEP: Relative Root Mean Square Error of Prediction.
REP: Relative Error of Prediction.
R2: Determined Variance.
Q2: Cross-validation Predictive ability.
BCMSEP: Bias-Corrected Mean Square Error of Prediction.
LOD: Limit of Detection.
LOQ: limit of Quantification.

Table 3demonstrates that forecasts with lower REP values between (0.6 and 0.5) are considered as accurate. Sensitivity was measured using the LOD and LOQ of the net analyte signals, and the findings indicated that the sensitivity was sufficient for pharmaceutical analysis, as shown in Table 3.

The comparison was made using both the calibration and validation datasets, between the predicted concentrations of each API (VON, CLA, and AMO) obtained from the PCR, OPLS, and ANN models and the corresponding experimental values, by evaluating the accuracy of the predictions through calculating the RMSEP and R^2 for the three models. Based on this comparison, it is evident that the ANN provides the most accurate prediction of the API concentrations, followed by OPLS and then PCR. These results highlight the robustness of the ANN model in handling the complexities of the data and its suitability for simultaneous quantification of VON, AMO, and CLA.

4.4. Application in pharmaceutical dose forms and statistical analysis

The suggested methods were used to quantify the examined three drugs in pharmaceutical formulations VOQUEZNA™ TRIPLE PAK™. The results from the three chemometric models created for the three indicated drugs showed excellent recoveries, the results of standard added as mentioned in the procedures are shown in Table 4.

The statistical analysis was carried out by comparing the results obtained from the proposed techniques with the reported method using the F-ratio test and the Student's t-test. Table 5 presents the results, which showed no significant difference at ($p = 0.05$). Furthermore, as shown in Table 6, a one-way ANOVA examination of the recovery data revealed no statistically significant changes. These results validated the created models' suitability for use in the production of pharmaceuticals.

F-ratio, *t*-test, and ANOVA statistical tests in Tables 5 and 6 show that the developed methods are equally as dependable as the published method. The new models' suitability for usage in pharmaceutical applications was confirmed by the absence of any noteworthy changes. The outcomes show that the methods are exact, accurate, and consistent, which qualifies them for use in industrial manufacturing.

5. Greenness, whiteness, sustainability assessment

The optimized chemometric methods have substantiated the enhanced greenness when DoE is combined with the application of evaluation tools likeAGREE,the EVG assessment, and the SPIDER solvent tool. These methods adhere closely to the 12 principles of GAC as well as the reduction of solvent use and waste, thereby outperforming traditional RP-UPLC techniques in terms of environmental impact. The developed methods comply with WAC standards through the application of the RGB12 Algorithm, and yield results from the NQS index, thereby supporting the Sustainable Development Goals through sustainable, efficient, and inclusive analytical practices.

5.1. Assessing methodological design: insights from the EVG framework

The EVG framework represents a foundational model in analytical chemistry, emphasizing the essential pillars of efficiency, validation, and greenness. This framework serves as a critical benchmark for both developed and reported analytical methods, ensuring they meet scientific rigour while adhering to sustainability principles. Within the EVG framework, each pillar is rigorously assessed on a scale from zero to three, with three indicating exemplary performance and zero reflecting non-compliance with the respective criteria. These criteria labeled A to E, encompass all dimensions of method performance and environmental impact. Utilizing a radar chart, researchers can depict the balance or lack thereof between efficiency, validation, and greenness. An ideal method demonstrates scores that are closely aligned across all pillars, indicating a balanced approach. Q1 represents the highest level of performance, whereas Quartile Q4 denotes the lowest. In practice, the EVG tool critically differentiates between the reported method (Anusha and Sowjanya, 2024)and those newly proposed. The application of this model provides a nuanced understanding of how well an analytical method balances its core objectives.

Table 4
Determination of VON, CLA, and AMO in VOQUEZNA™ TRIPLE PAK™ tablets by the suggested chemometric methods and application of standard addition technique.

Drugs		%Recovery ±%RSD		
		PCR	OPLS	ANN
VON	**Application**[a]	99.56 ± 1.35	99.83 ± 0.77	99.47 ± 1.12
	Standard Addition[b]	99.72 ± 1.03	98.58 ± 1.48	98.66 ± 1.21
CLA	**Application**[a]	98.96 ± 0.61	99.62 ± 0.52	98.55 ± 0.67
	Standard Addition[b]	98.92 ± 0.47	99.76 ± 0.38	99.12 ± 0.59
AMO	**Application**[a]	99.84 ± 0.40	99.37 ± 1.08	99.01 ± 0.69
	Standard Addition[b]	98.82 ± 1.21	99.13 ± 0.94	99.49 ± 0.42

Note: Recovery percentages for the analyzed concentrations (3.0 μg/mL for VON, 10 μg/mL for CLA and AMO) are normalized to reflect recoveries from total doses of 20 mg for VON, and 500 mg each for AMO and CLA.

[a] Average of five replicate.

[b] Average of three replicate.

Table 5
Statistical parameters of the results obtained by applying the developed chemometric models and the reported method [33].

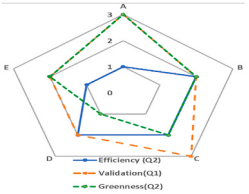
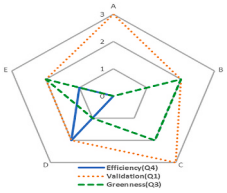
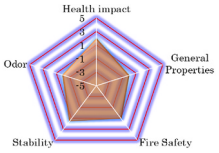
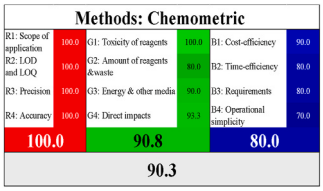
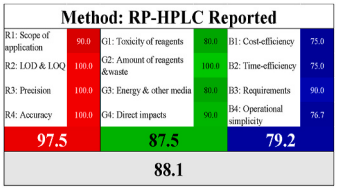
	PCR			OPLS			ANN			Reported method*		
	VON	CLA	AMO	VON	CLA	AMO	VON	CLA	AMO	VON	CLA	AMO
Mean	99.56	98.96	99.84	99.83	99.62	99.37	99.47	99.12	99.01	100.14	99.91	99.85
SD	1.35	0.61	0.40	0.77	0.52	1.08	1.12	0.59	0.69	1.6	6.3	8.9
n	5									3		
Student's t-test (2.447)[a]	1.84	0.47	1.39	2.03	0.52	1.34	1.14	1.70	1.42			
F-value (6.94)*	3.43	2.91	2.75	5.64	2.01	4.48	4.34	2.22	5.19			

[a] RP-UPLC technique with Hibar C18 (100 mm × 2.1 mm and 2 μm) column, Ammonium Acetate and Acetonitrile (60:40 v/v).

Table 6
One-way ANOVA statistical analysis of the results obtained by applying the proposed Chemometric models for the determination of VON, CLA, and AMO in the validation set.

Drugs	Source of variation	SS	df	MS	F	P-value	F-critic
VON	**Between groups**	3.8216	3	1.2738	1.8647	0.1819	3.3438
	Within groups	9.5638	14	0.6831			
	Total	13.3854	17				
CLA	**Between groups**	0.9769	3	0.3256	2.2366	0.1290	3.3438
	Within groups	2.0384	14	0.1456			
	Total	3.0154	17				
AMO	**Between groups**	1.7171	3	0.5723	1.3338	0.3031	3.3438
	Within groups	6.0076	14	0.4291			
	Total	7.7247	17				

Table 7
Comparative analysis of environmental and sustainable metrics in newly developed chemometric methods versus reported RP-UPLC method.

	Chemometric methods	RP-UPLC [33]
EVG		
AGREE		
SPIDER		
RGB12		
NQS	68% (N:50, Q: 90, S: 65)	54% (N:50, Q: 88, S: 24)

In this study, the newly developed chemometric spectrophotometric methods outperformed the reported RP-UPLC method(Anusha and Sowjanya, 2024). This enhanced performance is primarily attributed to their compliance with DoE principles, as evidenced by a good score for efficacy Q2 and an exceptional score for green criteria Q1both crucial elements of the EVG tool. Both of the chemometric-assisted spectrophotometric and RP-UPLC methods (Anusha and Sowjanya, 2024)achieved a satisfactory Q1 score for validation. However, the chemometric methods exhibit a closer alignment with the EVG criteria concerning efficiency, validation,and green. In contrast, the RP-UPLC method demonstrated comparatively weaker performance. Notably, it was not developed under the Analytical Quality by Design (AQbD) principles, leading to a reduced efficacy score in Q4. Furthermore, the omission of green tool applications notably compromised its greenness rating in Q3. Overall, the EVG radar chart and its corresponding scores, detailed in Table 7, offer a comprehensive view of method performance. This visualization not only highlights the importance of a balanced analytical method but also emphasizes the necessity for integrating efficiency, validation, and greenness in method development.

5.2. Evaluating environmental compliance of analytical procedures via AGREE metrics

The AGREE tool, innovatively introduced by Pena-Pereira et al. (2020), quantifies adherence to green chemistry principles through a strategic, color-coded pictogram that encapsulates twelve critical dimensions of GAC. This scoring green metric, which ranges from 0, indicating significant environmental hazards, to 1, reflecting optimal environmental compatibility, is visually central within each pictogram. The color transition from deep red to deep green in the pictogram signifies the method's environmental impact.

This tool's utility in quickly assessing the environmental footprint of analytical methodologies is particularly invaluable, facilitating rapid and comprehensive evaluations essential for advancing greenness practices within the field. As illustrated in Table 7, the comparative analysis using AGREE pictograms underscores the superior environmental performance of the proposed chemometric methods, which achieves a robust score of 0.69. In contrast, the reported RP-UPLC method(Anusha and Sowjanya, 2024)registers a slightly lower score of 0.65. The deployment of multiple evaluative metrics in this study, including the AGREE metrics, allows for a detailed exploration of each method's green credentials and highlights opportunities for further refinement and enhancement of analytical procedures.

5.3. Assessing chemical safety: insights from the spider solvent tool

Evaluating the environmental and health impacts of chemicals within analytical methodologies is essential. The Greenness Index Spider Tool, commonly known as the SPIDER tool, offers a robust metric for assessing chemicals across various dimensions, including stability, fire protection, general characteristics, health effects, and odor. Utilizing data from SDS(Kayali et al., 2023) and visualizing it via spider charts, this tool assigns scores ranging from −5 for negative impacts to +5 for positive attributes, with a score of zero indicating unavailable information. This scoring system provides a detailed view of a chemical's sustainability profile.

In comparing the SPIDER scores between the proposed chemometric-assisted spectrophotometric methods and the reported UPLC method, significant differences emerge. The proposed chemometric methods, employing ethanol, achieve a high SPIDER score, as illustrated by the extensively filled areas on the spider chart, indicating excellent safety and minimal environmental impact. Conversely, the reported RP-UPLC method(Anusha and Sowjanya, 2024), which uses acetonitrile and ammonium acetate, shows a lower score. Specifically, acetonitrile's evaluation draws attention due to its environmental and safety concerns, as reflected by the sparsely filled areas on the radar chart for acetonitrile in the SPIDER, indicating potential environmental and health risks, as shown in Table 7. Additional insights are provided by secondary spider charts (Fig. S4), which offer further breakdowns of the scores for each chemical, enhancing our understanding of their specific impacts and safety profiles.

5.4. Whiteness tool for assessing analytical chemistry sustainability: the RGB12 algorithm

To facilitate a thorough adherence to WAC principles, the RGB12 sustainability algorithm has been developed. This algorithm evaluates the sustainability of analytical methods by integrating three primary colour-coded dimensions, green, and blue into a singular 'whiteness' benchmark that reflects overall method sustainability.

- **Red Dimension**: This focuses on methodological effectiveness, evaluating the scope of application, applying AQbD, detection limits, quantification thresholds, and the precision and accuracy of the analytical method.
- **Green Dimension**: This assesses environmental and ethical considerations, including the safety of the method, avoidance of animal testing, and the environmental impact of the solvents used.
- **Blue Dimension**: This examines the practical aspects of the analytical method, such as operational simplicity, economic viability, and ease of implementation.

The RGB12 algorithm provides a comprehensive assessment by combining these dimensions into an overall 'whiteness' score. A downloadable spreadsheet, categorized into red, green, and blue sections, aids in quickly assessing the sustainability of various analytical methods according to WAC guidelines(Nowak et al., 2021). Application of the revised RGB12 algorithm has proven effective in differentiating the sustainability performances of various analytical methods. Our findings indicate a disparity in performance: the developed chemometric methods scored a commendable 90.3%, demonstrating their high sustainability. In contrast, the reported RP-UPLC method scored 88.1%, as detailed in Table 7. The chemometric methods, characterized by their use of safer chemicals, affordability, and minimal power requirements, align well with WAC principles, particularly when optimized via DoE.Conversely, the

lower red score of the reported RP-UPLC method is largely due to its failure to adopt an AQbD strategy, the use of acetonitrile impacted its green score negatively, while high operational costs detracted from its blue score.

5.5. Sustainability assessment using the NQS index

This study introduces an advanced chemometric spectrophotometric method for multi-drug analysis that robustly supports the SDGs, blending scientific innovations with global sustainability efforts. As detailed in Table 8, this research closely aligns with specific SDGs, markedly advancing sustainable practices within the scientific community. The method achieved a sustainability score of 65.0%, successfully meeting 11 out of the 17 SDGs, thus representing a significant contribution to sustainability in scientific methodologies. In contrast, the reported RP-UPLC method only meets these goals (SDGs 3, 4, 5, 7) and fails to meet other goals due to the use of acetonitrile, a hazardous solvent, and reliance on high-cost equipment. Additionally, it lacks effective communication and collaboration across departments and countries, which is crucial for achieving SDG 17. This limitation contributes to its lower sustainability score of 24%.In accordance with their usefulness and resource usage, analytical methods' demand is categorized using Koel's pyramid(Koel, 2015). Simple, universally available energy-efficient tools are at the bottom, with 100% demand. Automated, high-throughput systems that are valued for their operational efficiency come in at 75%.At a 50% demand level, UPLC and chemometric methods strike an excellent compromise between specificity, solvent consumption, and resource efficiency. This highlights their usefulness for routine studies with modest resource needs. The most exacting methods, such as classic high-performance liquid chromatography, are at the top of the pyramid with a 25% demand. These methods require considerable use of chemicals and solvents and specialist handling. Despite their higher performance, they are only used in specialized applications. The industry's move toward more user-friendly and sustainable analytical techniques is reflected in this framework. "Quality," or Whiteness, is evaluated using the RGB12 Algorithm. This approach seeks to identify techniques that provide high accuracy while also being eco-conscious and cost-effective. The quality outcomes achieved by the developed chemometric method and the reported chromatographic methods were approximately 90% and 88% respectively, according to results obtained from the RGB12 Algorithm. The NQS Index calculates an average score from three essential factors: sustainability, need, and quality percentages. In this analysis, the chemometric methods scored 68 % on the NQS Index, significantly surpassing the reported chromatographic method, which achieved 54%. These outcomes highlight the chemometric method's superior performance and pivotal role in achieving the SDGs, emphasizing its sustainability, necessity, and quality in the field.

This research exemplifies how scientific innovation can be seamlessly integrated with environmental sustainability, emphasizing the critical role of aligning with the UN SDGs. It sets a standard for incorporating sustainability into scientific research and demonstrates the extensive benefits of international collaboration in progressing towards the 2030 global goals. This work not only contributes to the field of analytical chemistry but also acts as a clarion call for the scientific community to deepen its commitment to sustainable practices across all disciplines.

These chemometric methods surpass traditional RP-UPLC technique in greenness, whiteness, and compliance with sustainability standards, as demonstrated by high scores on the NQS index, affirming their alignment with the Sustainable Development Goals. Unlike classical spectrophotometric approaches, which evaluate one component at a time, chemometrics uses multivariate techniques to assess multiple factors simultaneously. This ensures the extraction of valuable information and reveals hidden interrelationships between variables(Arunagiri et al., 2024; Obaydo et al., 2021).

6. Conclusion

This study introduces a groundbreaking approach to the simultaneous quantification of VON, AMO, and CLA in newly FDA-approved VOQUEZNA™ TRIPLE PAK™ tablets through the implementation of innovative chemometric-assisted spectrophotometric methods. Moreover, our study demonstrates the potential of intelligent chemometric methods to identify medicinal drugs even in the presence of extremely overlapping spectra and interfering components without the need for any pretreatment or separation steps, our method avoids the need for more complex pretreatment steps, such as separations or additional purification processes, which are often required in traditional methods like HPLC. The effective incorporation of Artificial Neural Network models, which offer a strong substitute for traditional methods, highlights the originality of our study. The PCR, OPLS, and ANN models that were optimized showed remarkable results in terms of accuracy, precision, and environmental impact reduction. Using a statistical design method called OALHS to create the ideal validation set was a major highlight. OALHS overcame the shortcomings in chemometrics, where studies mostly employ random data splitting, by offering a thorough, objective evaluation of the models' capacity to generalize throughout the whole concentration range. By reducing solvent consumption and waste, this study not only increases analytical skills for measuring APIs in complicated formulations but also supports the concepts of sustainability in analytical chemistry, and supports routine quality control procedures and on-site inspections, especially in quality control laboratories with limited resources, this encourage the use of green and white spectrophotometric approaches over traditional chromatographic methods. The developed chemometric-assisted spectrophotometric methods surpassed reported chromatographic techniques in efficiency and environmental impact, demonstrating their superior adherence to green and white principles and contributing positively towards achieving the SDGs. In the end, our results open the door for more study in this area by demonstrating the potential of sophisticated computational tools to improve the precision and effectiveness of pharmacological analysis.

Table 8
Impact of the sustainable chemometric spectrophotometric method on UN-SDGs 3, 4, 5, 7, 10–15, and 17.

SDG	Application(s)
Good Health and Well-being (3)	Developed for multi-drug analysis crucial for health monitoring, reducing errors, and enhancing quality control accessibility in underserved areas.
Quality Education (4)	Fosters educational advancements and research, broadening the scope of knowledge in analytical chemistry and sustainability, and developing new educational modules focused on sustainable chemometric techniques.
Gender Equality (5)	Demonstrates an inclusive research approach with diverse international teams, aiming for gender balance in research collaborations.
Affordable and Clean Energy (7)	Promotes sustainable and energy-efficient analytical practices, aligning with modern energy solutions.
Reduced Inequality (10)	Offers a cost-effective, efficient, and accessible solution for quality assurance in laboratories, especially beneficial for labs in developing countries or those without access to expensive analytical equipment.
Sustainable Cities and Communities (11)	Supports urban sustainability through the use of eco-friendly solvents and minimal energy, enhancing urban lab efficiencies and reducing pollution.
Responsible Consumption and Production (12)	Operates without hazardous organic solvents, exemplifying sustainable resource usage and eliminating harmful solvent use.
Climate Action (13)	Contributes to climate action by minimizing energy usage and hazardous waste, reducing the environmental footprint of analytical practices.
Life Below Water (14)	Protects aquatic life by avoiding harmful solvents and promoting sustainable analytical processes.
Life on Land (15)	Aids in terrestrial ecosystem conservation through minimal energy and solvent use.
Partnerships for the Goals (17)	Enhances global scientific cooperation through collaborative efforts among researchers from diverse nationality (Iraq, Ejypt and Syria).

CRediT authorship contribution statement

Khanda F.M. Amin: Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Reem H. Obaydo:** Writing – review & editing, Visualization, Project administration, Methodology, Formal analysis. **Hayam M. Lotfy:** Writing – review & editing, Visualization, Supervision, Methodology, Conceptualization.

Ethics approval and consent to participate

All ethical guidelines have been followed, and there is no work on animals or humans. Hence, ethics approval is not applicable.

Availability of data and materials

Detailed data are included in the result and discussion section; other samples and information are available.

Authors' information

The authors declare that this manuscript is original, has not been published before, and is not currently being considered for publication elsewhere.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT to improve and check the language. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scp.2024.101849.

Data availability

Data will be made available on request.

References

A. El Hamd, M., Albalawi, M., Mohammad, S., Abdul-Kareem, R., Obaydo, R., Alsaggaf, W., Saleh, S., Alossaimi, M., Abdel-Lateef, M., 2023. Ziziphus spina-christi leaf-derived carbon dots as a fluorescence nanosensor to evaluate rifaximin antibacterial via inner filter effect: greenness and whiteness studies. Chemosensors 11, 275. https://doi.org/10.3390/chemosensors11050275.

Abdelazim, A.H., Abdel-Fattah, A., Osman, A.O.E., Abdel-Kareem, R.F., Ramzy, S., 2023. Spectrophotometric quantitative analysis of aspirin and vonoprazan fumarate in recently approved fixed-dose combination tablets using ratio spectra manipulating tools. J. AOAC Int. 106, 490–495. https://doi.org/10.1093/jaoacint/qsac128.

Abou-Taleb, N.H., El-Enany, N.M., El-Sherbiny, D.T., El-Subbagh, H.I., 2021. Spider diagram and Analytical GREEnness metric approach for assessing the greenness of quantitative 1H-NMR determination of lamotrigine: taguchi method based optimization. Chemometr. Intell. Lab. Syst. 209, 104198. https://doi.org/10.1016/j.chemolab.2020.104198.

Akazawa, Y., Fukuda, D., Fukuda, Y., 2016. Vonoprazan-based therapy for Helicobacter pylori eradication: experience and clinical evidence. Therap. Adv. Gastroenterol 9, 845–852. https://doi.org/10.1177/1756283X16668093.

Aktaş, A.H., Sarıdağ, A.M., 2017. Liquid chromatographic–chemometric techniques for the simultaneous HPLC determination of lansoprazole, amoxicillin and clarithromycin in commercial preparation. J. Chromatogr. Sci. 55, 798–804. https://doi.org/10.1093/chromsci/bmx039.

Anusha, K., Sowjanya, G., 2024. A reliable RP-UPLC-UV method for simultaneous estimation of Clarithromycin, Amoxicillin, and Vonoprazan in co-packed pharmaceutical dosage forms: method development and validation with stability indicating properties. Int. j. Appl. Pharmaceu 16, 351–357. https://doi.org/10.22159/ijap.2024v16i2.50059.

Arunagiri, T., Ganesan, A., Ravi Kumaran, V., Masilamani, B., Kannaiah, K.P., Narayanasamy, D., 2024. Green analytical chemistry-based spectrophotometric techniques for ternary component analysis of pain relievers. Futur. J. Pharm. Sci. 10, 75. https://doi.org/10.1186/s43094-024-00648-8.

Atherton, J.C., 2006. The pathogenesis of Helicobacter pylori-induced gastro-duodenal diseases. Annu. Rev. Pathol. 1, 63–96. https://doi.org/10.1146/annurev.pathol.1.110304.100125.

Boustany, A., Onwuzo, S., Almomani, A., Asaad, I., 2023. Epidemiology and risk of colorectal cancer in patients with a history of Helicobacter pylori infection: a population-based study. Ann. Gastroenterol. 36, 203–207. https://doi.org/10.20524/aog.2023.0783.

Brereton, R., 2000. Introduction to multivariate calibration in analytical chemistry. Analyst 125, 2125–2154. https://doi.org/10.1039/b003805i.

British Pharmacopoeia, 2016. Stationary Office, Appendix 1D, Buckingham Palace Road, p. n.d.. London, UK.

Burrage, K., Burrage, P., Donovan, D., Thompson, B., 2015. Populations of models, experimental designs and coverage of parameter space by Latin hypercube and orthogonal sampling. In: Procedia Comput. Sci., International Conference On Computational Science, ICCS, pp. 1762–1771. https://doi.org/10.1016/j.procs.2015.05.383, 2015.

Calhoun, K.H., Hokanson, J.A., 1993. Multicenter comparison of clarithromycin and amoxicillin in the treatment of acute maxillary sinusitis. Arch. Fam. Med. 2, 837–840. https://doi.org/10.1001/archfami.2.8.837.

Chey, W.D., Mégraud, F., Laine, L., López, L.J., Hunt, B.J., Howden, C.W., 2022. Vonoprazan triple and dual therapy for Helicobacter pylori infection in the United States and europe: randomized clinical trial. Gastroenterology 163, 608–619. https://doi.org/10.1053/j.gastro.2022.05.055.

Deguchi, H., Yamazaki, H., Kamitani, T., Yamamoto, Y., Fukuhara, S., 2021. Impact of vonoprazan triple-drug blister packs on H. pylori eradication rates in Japan: interrupted time series analysis. Adv. Ther. 38, 3937–3947. https://doi.org/10.1007/s12325-021-01784-w.

Donovan, D., Burrage, K., Burrage, P., McCourt, T.A., Thompson, B., Yazici, E.Ş., 2018. Estimates of the coverage of parameter space by Latin Hypercube and Orthogonal Array-based sampling. Appl. Math. Model. 57, 553–564. https://doi.org/10.1016/j.apm.2017.11.036.

Duan, M., Li, Yueyue, Liu, J., Zhang, W., Dong, Y., Han, Z., Wan, M., Lin, M., Lin, B., Kong, Q., Ding, Y., Yang, X., Zuo, X., Li, Yanqing, 2023. Transmission routes and patterns of helicobacter pylori. Helicobacter 28, e12945. https://doi.org/10.1111/hel.12945.

Eissa, M.S., Darweish, E., 2024. Insights on ecological spectroscopic techniques recently adopted for pharmaceutical analysis: a comprehensive review from the perspective of greenness assessment metrics systems application. TrAC Trends Anal. Chem. (Reference Ed.) 170, 117435. https://doi.org/10.1016/j.trac.2023.117435.

El-Gindy, A., Hadad, G.M., 2012. Chemometrics in pharmaceutical analysis: an introduction, review, and future perspectives. J. AOAC Int. 95, 609–623. https://doi.org/10.5740/jaoacint.sge_el-gindy.

El-Zeiny, M.B., Zawbaa, H.M., Serag, A., 2021. An evaluation of different bio-inspired feature selection techniques on multivariate calibration models in spectroscopy. Sens. Actuators, A 246, 119042. https://doi.org/10.1016/j.saa.2020.119042.

FDA, 2022. New Drug Therapy Approvals 2022. FDA. URL. https://www.fda.gov/drugs/novel-drug-approvals-fda/new-drug-therapy-approvals-2022, 10.18.24.

Furuta, T., Yamade, M., Kagami, T., Uotani, T., Suzuki, T., Higuchi, T., Tani, S., Hamaya, Y., Iwaizumi, M., Miyajima, H., Umemura, K., Osawa, S., Sugimoto, K., 2020. Dual therapy with vonoprazan and amoxicillin is as effective as triple therapy with vonoprazan, amoxicillin and clarithromycin for eradication of Helicobacter pylori. Digestion 101, 743–751. https://doi.org/10.1159/000502287.

Geramizadegan, A., Niknam, L., Pournamdari, E., 2023. Molecularly imprinted polymers for selective extraction and determination of toxic herbicide bentazon in water samples using liquid chromatography and assessment of mean square error using artficial neural network model. J. Anal. Chem. 78, 572–581. https://doi.org/10.1134/S1061934823050052.

Haaland, D.M., Thomas, E.V., 1988. Partial least-squares methods for spectral analyses. Relation to other quantitative calibration methods and the extraction of qualitative information. Anal. Chem. 60, 1193–1202. https://doi.org/10.1021/ac00162a020.

Hassib, Sonia T., Moffid, M.A., Aly, N.A., Mostafa, E.A., 2022. Development of a multivariate model with desirability-based optimization for simultaneous determination of five co-administrated drugs for the management of COVID-19 infection in their dosage forms via validated RP-HPLC method. J. Liq. Chromatogr. Relat. Technol. 45, 191–203. https://doi.org/10.1080/10826076.2023.2168691.

Hassib, Sonia T., Taha, E.A., Sharf, M.G., Mostafa, E.A., 2022. RP-HPLC–DAD method development and validation for simultaneous determination of lansoprazole, tinidazole, amoxicillin, and naproxen in their raw materials and combined dosage form: DOE approach for optimization of the proposed method. J. AOAC Int. 105, 675–687. https://doi.org/10.1093/jaoacint/qsab159.

Kajihara, Y., Shimoyama, T., Mizuki, I., 2017. Analysis of the cost-effectiveness of using vonoprazan-amoxicillin-clarithromycin triple therapy for first-line Helicobacter pylori eradication. Scand. J. Gastroenterol. 52, 238–241. https://doi.org/10.1080/00365521.2016.1250157.

Kalinowska, K., Bystrzanowska, M., Tobiszewski, M., 2021. Chemometrics approaches to green analytical chemistry procedure development. Curr. Opin. Green. Sust. 30, 100498. https://doi.org/10.1016/j.cogsc.2021.100498.

Kammoun, A.K., Hafez, H.M., Kamel, E.B., Fawzy, M.G., 2024. A comparative analysis of univariate versus multivariate eco-friendly spectrophotometric manipulations for resolving severely overlapped spectra of vonoprazan and amoxicillin new combination. Anal. Biochem. 689, 115501. https://doi.org/10.1016/j.ab.2024.115501.

Katamesh, N.S., Abbas, A.E.F., Mahmoud, S.A., 2024. Four chemometric models enhanced by Latin hypercube sampling design for quantification of anti-COVID drugs: sustainability profiling through multiple greenness, carbon footprint, blueness, and whiteness metrics. BMC Chem. 18, 54. https://doi.org/10.1186/s13065-024-01158-7.

Kayali, Z., Obaydo, R.H., Alhaj Sakur, A., 2023. Spider diagram and sustainability evaluation of UV-methods strategy for quantification of aspirin and sildenafil citrate in the presence of salicylic acid in their bulk and formulation. Heliyon 9, e15260. https://doi.org/10.1016/j.heliyon.2023.e15260.

Kinoshita, Y., Sakurai, Y., Shiino, M., Kudou, K., Nishimura, A., Miyagi, T., Iwakiri, K., Umegaki, E., Ashida, K., 2016. Evaluation of the efficacy and safety of vonoprazan in patients with nonerosive gastroesophageal reflux disease: a phase III, randomized, double-blind, placebo-controlled, multicenter study. Curr. Ther. Res. Clin. Exp. 81–82, 1–7. https://doi.org/10.1016/j.curtheres.2016.12.001.

Kiwfo, K., Suteerapataranon, S., McKelvie, I.D., Meng Woi, P., Kolev, S.D., Saenjum, C., Christian, G.D., Grudpan, K., 2023. A new need, quality, and sustainability (NQS) index for evaluating chemical analysis procedures using natural reagents. Microchem. J. 193, 109026. https://doi.org/10.1016/j.microc.2023.109026.

Koel, M., 2015. Do we need green analytical chemistry? Green Chem. 18. https://doi.org/10.1039/C5GC02156A.

Kusters, J.G., van Vliet, A.H., Kuipers, E.J., 2006. Pathogenesis of Helicobacter pylori infection. Clin. Microbiol. Rev. 19, 449. https://doi.org/10.1128/CMR.00054-05.

Leary, S., Bhaskar, A., Keane, A., 2003. Optimal orthogonal-array-based Latin hypercubes. J. Appl. Stat. 30, 585–598.

Lin, B., Kou, J., Xiao, Q., Wu, S., Hu, J., Li, J., Zhu, Z., Zhou, X., Xin, L., Li, Y., Wang, Z., 2022. Identification, characterization, synthesis of major metabolites biotransformed from vonoprazan fumarate. Tetrahedron 108, 132669. https://doi.org/10.1016/j.tet.2022.132669.

Liu, L., Cao, N., Ma, X., Xiong, K., Sun, L., Zou, Q., 2016. Identification, characterization, and high-performance liquid chromatography quantification of process-related impurities in vonoprazan fumarate. J. Separ. Sci. 39, 1232–1241. https://doi.org/10.1002/jssc.201501154.

Luo, Z., Liu, A., Liu, Y., Wang, G., Chen, X., Wang, H., Li, M., Zhang, H., Qiu, Y., Zhai, H., 2018. Development of a stability- indicating HPLC method for simultaneous determination of ten related substances in vonoprazan fumarate drug substance. J. Pharm. Biomed. Anal. 149, 133–142. https://doi.org/10.1016/j.jpba.2017.11.011.

Marahel, F., Goodajdar, B.M., Basri, N., Niknam, L., Ghazali, 2022. Applying neural network model for adsorption methyl paraben (MP) dye using ricinus communis-capeed Fe3O4 NPs synthesized from aqueous solution. Iran. J. Chem. Chemi. Engin. 41, 2358–2377. https://doi.org/10.30492/ijcce.2021.527025.4636.

Miller, J.N., Miller, J.C., Miller, R.D., 2018. Statistics and Chemometrics for Analytical Chemistry, vol. 7. Pearson, Harlow, England.

Murakami, K., Sakurai, Y., Shiino, M., Funao, N., Nishimura, A., Asaka, M., 2016. Vonoprazan, a novel potassium-competitive acid blocker, as a component of first-line and second-line triple therapy for Helicobacter pylori eradication: a phase III, randomised, double-blind study. Gut 65, 1439–1446. https://doi.org/10.1136/gutjnl-2015-311304.

Mustafah, A., Bibi, R., Hassan, A., Khan, S., Khan, S.A., 2021. Simultaneous determination of amoxicillin, clarithromycin and esomeprazole in mice plasma after oral administration by reverse phase HPLC method. J. Chromatogr. Separ. Tech. 12, 1–7. https://doi.org/10.35248/2157-7064.21.12.439.

Nowak, P.M., Wietecha-Posłuszny, R., Pawliszyn, J., 2021. White analytical chemistry: an approach to reconcile the principles of green analytical chemistry and functionality. TrAC Trends Anal. Chem. (Reference Ed.) 138, 116223. https://doi.org/10.1016/j.trac.2021.116223.

Obaydo, R.H., Al Zakri, D.J., Sakur, A.A., Lotfy, H.M., 2021. Ultraviolet spectrophotometric methods for the determination of the minor component presented in fixed-dose pharmaceutical combinations through the last two decades (2000–2020). Futur. J. Pharm. Sci. 7, 44. https://doi.org/10.1186/s43094-021-00192-9.

Parsonnet, J., Friedman, G.D., Vandersteen, D.P., Chang, Y., Vogelman, J.H., Orentreich, N., Sibley, R.K., 1991. Helicobacter pylori infection and the risk of gastric carcinoma. N. Engl. J. Med. 325, 1127–1131. https://doi.org/10.1056/NEJM199110173251603.

Pena-Pereira, F., Wojnowski, W., Tobiszewski, M., 2020. AGREE-analytical GREEnness metric approach and software. Anal. Chem. 92, 10076–10082. https://doi.org/10.1021/acs.analchem.0c01887.

Saleh, S.S., Lotfy, H.M., Elbalkiny, H.T., 2023. An integrated framework to develop an efficient valid green (EVG) HPLC method for the assessment of antimicrobial pollutants with potential threats to human health in aquatic systems. Environ. Sci.: Process. Impacts 25, 2125–2138. https://doi.org/10.1039/D3EM00339F.

Saleh, S.S., Obaydo, R.H., El Hamd, M.A., Rostom, Y., Mohamed, D., Lotfy, H.M., 2024. Guidelines for accurate application of green and white analytical concepts: merits versus demerits with insights of significant milestones of assessment tools applied for antiviral drugs. Microchem. J. 199, 109917. https://doi.org/10.1016/j.microc.2024.109917.

Saraya, R.E., Hassan, Y.F., Eltukhi, W.E., Salman, B.I., 2022. Ultra-sensitive fluorimetric method for the first estimation of vonoprazan in real human plasma and content uniformity test. J. Fluoresc. 32, 1725–1732. https://doi.org/10.1007/s10895-022-02979-2.

Sharma, M.D., 2015. Potential for combination of dipeptidyl peptidase-4 inhibitors and sodium-glucose co-transporter-2 inhibitors for the treatment of type 2 diabetes. Diabetes Obes. Metabol. 17, 616–621. https://doi.org/10.1111/dom.12451.

Shen, Y., Lo, C., Nagaraj, D.R., Farinato, R., Essenfeld, A., Somasundaran, P., 2016. Development of *greenness index* as an evaluation tool to assess reagents: evaluation based on SDS (safety data sheet) information. Miner. Eng. 94, 1–9. https://doi.org/10.1016/j.mineng.2016.04.015.

Sue, S., Maeda, S., 2021. Is a potassium-competitive acid blocker truly superior to proton pump inhibitors in terms of Helicobacter pylori eradication? Gut. Liver 15, 799. https://doi.org/10.5009/gnl20242.

Tang, B., 1993. Orthogonal array-based Latin hypercubes. J. Am. Stat. Assoc. 88, 1392–1397. https://doi.org/10.2307/2291282.

Tantawy, M., Michael, A., 2019. Artificial neural networks versus partial least squares and multivariate resolution-alternating least squares approaches for the assay of ascorbic acid, rutin, and hesperidin in an antioxidant formulation. Spectrosc. Lett. 52, 1–7. https://doi.org/10.1080/00387010.2019.1639760.

Trygg, J., Wold, S., 2002. Orthogonal projections to latent structures (O-PLS). J. Chemometr. 16, 119–128. https://doi.org/10.1002/cem.695.

Vajdle, O., Guzsvány, V., Škorić, D., Csanádi, J., Petković, M., Avramov-Ivić, M., Kónya, Z., Petrović, S., Bobrowski, A., 2017. Voltammetric behavior and determination of the macrolide antibiotics azithromycin, clarithromycin and roxithromycin at a renewable silver – amalgam film electrode. Electrochim. Acta 229, 334–344. https://doi.org/10.1016/j.electacta.2017.01.146.

Voquezna (vonoprazan), 2024. FDA approval history - drugs.com. https://www.drugs.com/history/voquezna.html, 10.18.24.

Welch, W.J., Buck, RobertJ., Sacks, J., Wynn, H.P., Mitchell, T.J., Morris, M.D., 1992. Screening, predicting, and computer experiments. Technometrics 34, 15–25. https://doi.org/10.2307/1269548.

Yahaya, N., Mohamed, A.H., Miskam, M., Abdul Keyon, A.S., Loh, S.H., Mohamad Zain, N.N., Sajid, M., 2024. Green analytical chemistry metrics for evaluation of microextraction methods: fascinating or essential tools in real-world applications? TrAC Trends Anal. Chem. (Reference Ed.) 172, 117587. https://doi.org/10.1016/j.trac.2024.117587.

Yang, X., Li, Y., Sun, Y., Zhang, M., Guo, C., Mirza, I.A., Li, Y.-Q., 2018. Vonoprazan: a novel and potent alternative in the treatment of acid-related diseases. Dig. Dis. Sci. 63, 302–311. https://doi.org/10.1007/s10620-017-4866-6.

Yondo, R., Andrés, E., Valero, E., 2018. A review on design of experiments and surrogate models in aircraft real-time and many-query aerodynamic analyses. Prog. Aero. Sci. 96, 23–61. https://doi.org/10.1016/j.paerosci.2017.11.003.

Yoneyama, T., Teshima, K., Jinno, F., Kondo, T., Asahi, S., 2016. A validated simultaneous quantification method for vonoprazan (TAK-438F) and its 4 metabolites in human plasma by the liquid chromatography-tandem mass spectrometry. J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 1015–1016, 42–49. https://doi.org/10.1016/j.jchromb.2016.01.051.

Zhang, Mengran, Pang, M., Zhang, Mei, 2022. Efficacy and safety of potassium-competitive acid blockers versus proton pump inhibitors as Helicobacter pylori eradication therapy: a meta-analysis of randomized clinical trials. Clinics 77, 100058. https://doi.org/10.1016/j.clinsp.2022.100058.