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Greenness-sustainability metrics for assessment smart-chemometric spectrophotometric strategy for evaluation of the combination of six gastric proton-pump inhibitors with two selected impurities

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A R T I C L E I N F O

Method name:
Chemometric spectrophotometric

Keywords:
Proton-pump inhibitors
Orthogonal-partial least square
Pharmaceutical impurity
Hexagonal-tool

A B S T R A C T

Green analytical approaches are employed for the determination of active pharmaceutical ingredients, in conjunction with their impurities. Smart chemometric spectrophotometric techniques, including orthogonal partial least square (OPLS), variable selection such as genetic algorithm (GA-OPLS), and interval selection (i-OPLS), were utilized. These chemometric models were implemented for assessing six proton-pump inhibitors Omeprazole, Esomeprazole, Lansoprazole, Pantoprazole, Rabeprazole, and Dexlansoprazole along with two selected official impurities, namely 4-Desmethoxy omeprazole impurity and Rabeprazole-impurity B. Experimental design was implemented to separate impurities, in the process of multivariate calibration, a five-level eight-factor calibration design consisting of 25 samples was selected. This design was deliberately selected to guarantee that the components were mutually orthogonal to assess the model's performance and reliability, a separate validation set of 15 samples was constructed. The best-performing of the proposed techniques were identified by considering the least favorable values of the Correlation Coefficient ($R \geq 0.9995$), the Root Mean Square Error of Prediction (RMSEP) values between (0.0102–0.5622), and the Relative Error of Prediction (REP) values between (0.2961–1.1917). The proposed and reported methods' greenness-sustainability was quantitatively evaluated, and a comparative study of the greenness profile was established through a spider chart, the National Environmental Method Index tool, advanced and modified NEMI along with the Hexagon tool, and the whiteness qualities of the presented approaches were assessed by implementing the recently adopted Red-Green-Blue paradigm and White Analytical Chemistry tool. These approaches are well-suited for use in quality control laboratories due to their observed acceptance, long-term sustainability, simplicity, and affordability.

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Specifications table

Subject area:	Chemistry
More specific subject area:	Determination of pharmaceutical components with official impurities
Name of your protocol:	Greenness-Sustainability Metrics for Assessment Smart-Chemometric Strategy for Evaluation of the Combination of Six Gastric Proton-Pump Inhibitors with Two Selected Impurities
Reagents/tools:	A list of reagents: • Rabeprazole sodium salt (99.67%) • Esomeprazole magnesium trihydrate (98.0%) • Lansoprazole (98%) • Omeprazole sodium (99.98%) • Pantoprazole (99.36%) • Dexlansoprazole (99.51%) • Rabeprazole EP impurity B (>%98) • 4-Desmethoxy omeprazole impurity (>%98) • HPLC reagent-grade ethanol from Sigma-Aldrich A list of equipment: • UV–visible spectrophotometer (1800 Shimadzu, Japan) • Software: Matlab R2021a v9.10.0.1669831 and the Partial Least Squares (PLS) Toolbox
Experimental design:	Chemometric methods (OPLS, GA-PLS, iOPLS) have been implemented for assessing six proton-pump inhibitors Omeprazole, Esomeprazole, Lansoprazole, Pantoprazole, Rabeprazole, and Dexlansoprazole along with two selected official impurities, namely 4-Desmethoxy omeprazole impurity and Rabeprazole-impurity B. The Chemometric method is a greenness method by applies (Greenness and whiteness assessment.
Trial registration:	*N.A.*
Ethics:	This article does not contain any studies with human participants or animals performed by the author.
Value of the Protocol:	• The presented data established that the three Chemometric models can be applied for the determination of pharmaceutical ingredients and their impurities with great efficiency. • Experimental design consisting of five level eight factors and calibration design consisting of 25 samples was selected • The chemometric model is the Greenness and sustainable method according to the greenness and whiteness assessment tools (spider chart, NEMI, Hexagonal, RGB-12, and WAC).

Description of protocol

Introduction

Green Analytical Chemistry (GAC) concepts seek to reduce environmental contamination while improving analyst well-being. Medicinal impurities refer to compounds that coexist with the active components of a drug and emerge during its production and storage processes. Minimal amounts of such contaminants can impact the drug's effectiveness and safety. The medicine's safety is determined not only by the toxicological qualities of the active pharmaceutical ingredient as well as by the impurities it currently incorporates [1]. Detecting, separating, and assessing these contaminants is crucial in drug manufacturing and regulatory assessments. The analysis of impurities offers valuable insights into the impurity landscape of Active Pharmaceutical Ingredient (API) drugs, playing a pivotal role in quality control. Synthetically generated impurities act as standardized reference materials, facilitating the development of analytical methods and the quantification of contaminants. It is imperative to establish these impurities as regulatory standards for relevant drug authorities. Pharmaceutical Impurity Profiling (PIP) has advanced, and these impurity standards are now integral to the list of essential drugs. A comprehensive understanding of a compound's chemistry and manufacturing processes not only aids in synthesizing novel compounds but also provides valuable information about the compound's activity, impurity profile, and stability [2,3]. Stereochemistry has arisen as a critical concern in medication advancement and research considering the discovered differences in pharmacodynamics, pharmacokinetic properties, and toxicology characteristics among enantiomers. The manufacture of enantiopure medications has increased dramatically in the last decade. The FDA and other regulatory organizations approved a significant amount of developed pharmaceuticals originally advertised as racemates to be launched as single enantiomers [4].

In numerous instances, an individual enantiomeric version of a chiral medication comprises a more beneficial chemical-based organization, exhibiting improved pharmacologic as well as medicinal capabilities along with a lower negative side effect characteristic. The other stereoisomers of medicine are regarded as organic contaminants under the framework for an individual enantiomeric medication [5]. Proton pump inhibitors (PPIs) have emerged as a prominent therapeutic approach for treating gastrointestinal disease and pose a significant and widespread challenge, comprising a range of conditions such as peptic ulcer, stress ulcers, Zollinger-Elison syndrome, gastrointestinal hemorrhage, Helicobacter payroll infections, and gastroesophageal reflux disease (GERD). PPIs with an asymmetric sulfoxide core among their molecules, such as omeprazole, rabeprazole, lansoprazole, and pantoprazole, are frequently prescribed as racemic mixes [6]. Solely omeprazole (OME) was initially manufactured and distributed to be an individual enantiomer. Omeprazole occurs as a racemic mixture for its two distinct optically isomers, S-omeprazole (esomeprazole) and R-omeprazole, both of which have been shown to have stereo-selective metabolic processes. OME, depicted in Fig. 1a, is chemically identified as (5-methoxy-2-[[(4methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole). OME is an FDA-approved medication employed independently or in conjunction with other drugs to reduce acid production in the stomach. It has been utilized to treat ulcers, gastroesophageal reflux disease, and erosive esophagitis [7]. Esomeprazole magnesium trihydrate (ESO), as depicted in Fig. 1b, known as [bis(5-methoxy-2 -[(*S*)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole-1yl)magnesium trihydrate] [8].

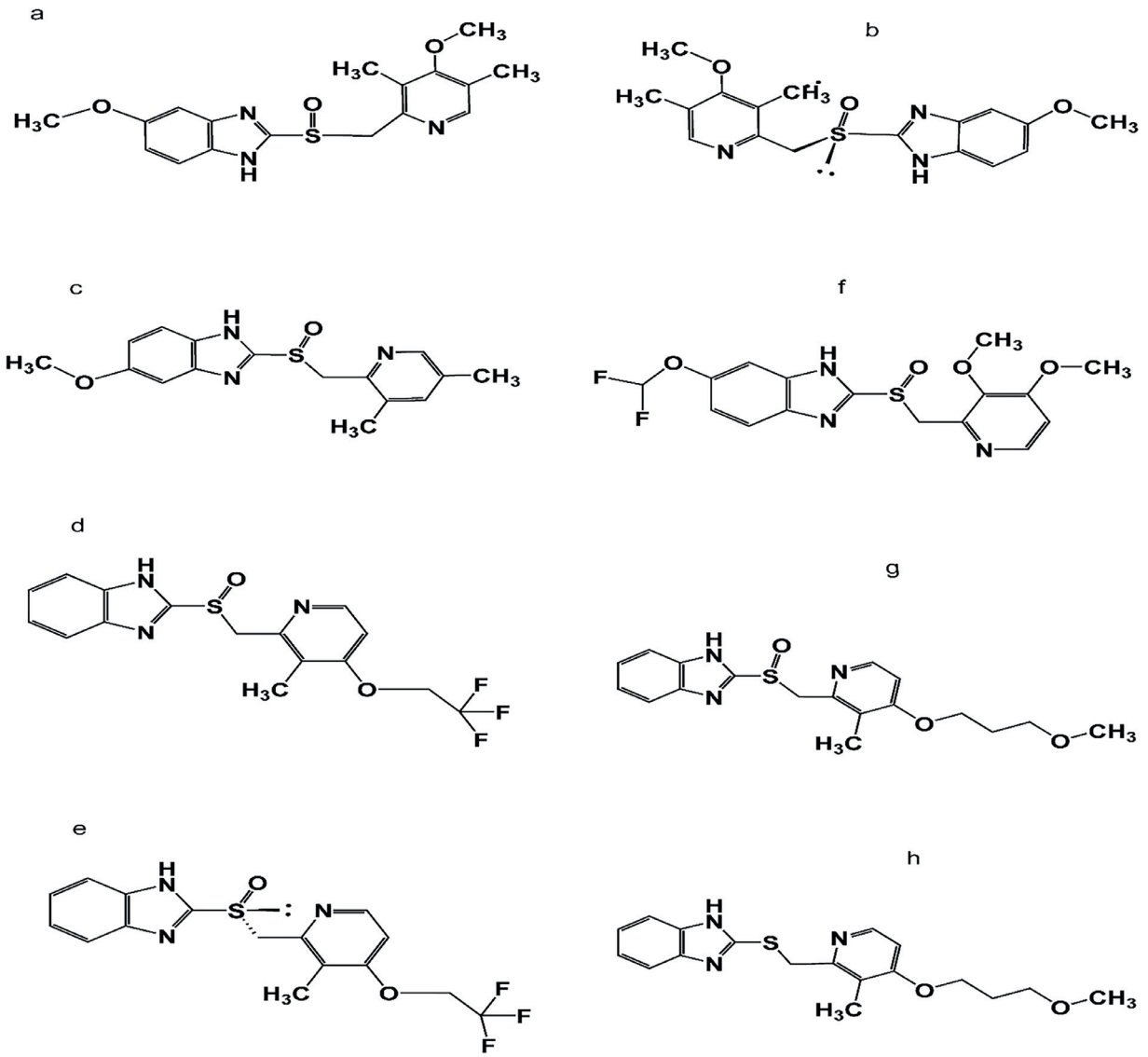


Fig. 1. Structure of (a) Omeprazole (OME), (b) Esomeprazole (ESO), (c) 4-Desmethoxy omeprazole impurity (4-DES-impurity), (d) Lansoprazole (LAN), (e) Pantoprazole (PAN), (f) Rabeprazole (RAB), (g) RAB-impurity B, and (h) Dexlansoprazole (DEX).

The (*S*)-enantiomer exhibits significantly better metabolic characteristics than the similar racemic medication OME, resulting in a greater influence on gastrointestinal problems such as ulcers of the gastric and duodenal tract. OME and ESO specify different specific official impurities, and the structure of the related substances described in the European Pharmacopoeia (EP) monograph [7]. 4-Desmethoxy omeprazole impurity (4-DES-Imp.) is a potential impurity found in commercial OME and ESO magnesium preparations, also known as OME-impurity B or ESO-impurity B and chemically identified as 2-[(RS)-[(3,5-dimethylpyridin-2-yl)methyl] sulphinyl]-5-methoxy-1H-benzimidazole [9], as depicted in Fig. 1c.

Lansoprazole (LAN), presented in Fig. 1d, is chemically identified as 1H-Benzimidazole, 2-[3-methyl-4-(2,2,2-trifluoromethoxy)-2-pyridinyl][methyl]sulfinyl]-2-[[3-methyl-4-(2,2,2,2,2-trifluoromethoxy)-2-pyridyl]-ethylSulfinyl] benzimidazole. LAN also occur as a racemic mixture, Lansoprazole's R-enantiomer is Dexlansoprazole (DEX), which is a racemic combination of the R- and S-enantiomers. LAN functions as an inhibitor of H^+/K^+-ATPase in gastric parietal cells, effectively restraining acidity in stomach production [10].

DEX, represented in Fig. 1e, is chemically identified as 2-[(R)-[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]me thyl]sulfinyl]-1H-benzimidazole. DEX is more effective in inhibiting the production of acid in the stomach. Dexlansoprazole has a distinct pharmacokinetic profile when compared to the previous generation of PPIs (that comprises Pantoprazole, Omeprazole, and Lansoprazole), as a result of its impact delayed-release and dual-delivery releasing mechanism [11].

Pantoprazole (PAN), depicted in Fig. 1f, is chemically recognized as 5-(difluoromethoxy)-2-[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl-1H-benzimidazole. It is employed in the treatment of ulcers, effectively inhibiting the secretion of

stomach acid. This medication is also utilized in addressing esophageal erosion and ulceration resulting from gastroesophageal reflux disease [12].

Rabeprazole Sodium monohydrate (RAB), illustrated in Fig. 1g, is chemically identified as 2-([[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]-methyl]sulfinyl)-1H-benzimidazole sodium salt monohydrate. It functions as an antisecretory agent and lacks anticholinergic or histamine H2-receptor antagonist properties [13]. Various impurities have been documented and are listed in its European Pharmacopeia (EP), Rabeprazole-impurity B (RAB-Imp. B) is known chemically as 2-([[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]- methyl]sulfanyl)-1H-benzimidazole [14] as depicted in Fig. 1h. In a comprehensive review of the literature, various analytical techniques have been employed for the assessment of proton pump inhibitors (PPIs), either individually or in combination. These techniques encompass UV–visible spectrophotometry [15–20], as well as HPLC, HPLC-DAD, and RP-HPLC [21–24]. Additionally, UPLC, UPLC–MS/MS [25–27], LC-MS/MS, and LC-DAD techniques were applied [28–30]. Several reports also highlight eco-friendly approaches such as derivative analysis, ratio spectra, and mathematical modeling in both binary and ternary mixtures with other drugs [31–33]. On the other hand, none of these methods possesses the capability to determine OME, ESO, LAN, PAN, RAB, and DEX along with two selected official impurities,4-DES-Imp., and RAB-Imp. B. Furthermore, there is no chemometric approach available in the literature for effective resolution of the spectra of these six medications as well as the impurities.

Chemometrics is the practice of analyzing data to obtain important information using various methodologies [34]. Multivariate calibration methods have shown to be a significant resource for interpreting data from analytical procedures. They're extremely effective when dealing with outcomes with an extensive amount of variables. Added to that, multivariate testing procedures are recognized as powerful tools in spectral analysis because they can effectively utilize multiple spectral intensities, which significantly contribute to precision [35].

The objective of this study is to improve the dependability of quantitative analysis by integrating impurity analysis through the use of multivariate linear calibrations. Consequently, the focus of the three chemometric spectrophotometric techniques OPLS, iOPLS, and GA-OPLS is to concurrently determine the concentrations of the six proton pump inhibitors (PPIs) along with two impurities in prepared-laboratory mixtures and pharmaceuticals. The hexagonal, NEMI, advanced, and Modified NEMI along with RGB and the WAC tools were used to efficiently analyze the environmental sustainability of the suggested approaches.

Theoretical background

OPLS is a relatively recent method for modeling multivariate data. It is a variant of the standard modeling approach known as Partial Least Squares (PLS), which at first is widely employed in mathematical regression modeling purposes. OPLS, introduced in the early 2000s, represents a more recent and modified algorithm that is sometimes favored for its potential to enhance model interpretation [36]. When examining systems having a similar overall amount of ingredients, a PLS and an OPLS matched with identical information are expected to have comparable predicting effectiveness in a situation comprising just one variable. One of the biggest advantages of OPLS above PLS lies in the significantly simpler comprehension of models that come from OPLS. This is due to OPLS's efficiency in splitting differentiated clarified differences among prediction as well as orthogonal base categories, making it easier to understand and interpret the model results. The Orthogonal Partial Least Squares (O-PLS) method can be understood in two ways. Firstly, it can be considered as a pure preprocessing method intended to eliminate strong systematic orthogonal variation concerning Y from a given dataset X. Alternatively, it can be integrated into regular Partial Least Squares (PLS) modeling to produce simpler models. An additional advantage of O-PLS is that it allows for the separate analysis of all orthogonal variations in X, thereby enhancing the interpretability of the model [37]. The Genetic Algorithm (GA) feature selection approach is used throughout the present study, which is based on the theory of evolution, and works as a collective problem-solving behavior in which any potential results are considered as chromosomal that undergo alterations and intersections [38]. GA can determine variables that offer adequate responses concerning both predictability as well as validity, and because specific wavelengths have less dispersion than alternative approaches, GA provides more understandable and accessible results. Besides, consisting of variable selection processes that involve the genetic algorithm (GA) encourages improvements in OPLS the efficiency of the model due to its capacity to eradicate unreliable or insignificant variables to result in more reliable predictions [39]. To improve the dependability of the approaches, the goal is to incorporate mostly contiguous wavelengths while removing uncorrelated variables. In addition, interval selection is provided as a means of visualization, the principle behind the aforementioned approach aims to split every spectrum area into equal-width intervals and periods. An algorithm is built for each interval and scale, and the method with the highest predicted performance is chosen as the final version, producing the expected result [40].

Experimental

Instrumentation and software

(SHIMADZU, Kyoto, Japan) UV-1800 PC-type spectrophotometer was used with the software SHIMADZU UV prop data. The gadget is intended as a dual-beam device that has a quartz cell with a 1.0 cm path length. Matlab R2021a v9.10.0.1669831 and the Partial Least Squares (PLS) Toolbox were used to perform chemometric studies on the collected data.

Materials and reagent

Rabeprazole sodium salt (CAS No. 117976-90-6) with a purity level of 99.67%, Esomeprazole magnesium trihydrate (CAS No. 217087-09-7) possesses a purity of 98.0%, and Lansoprazole (CAS No. 103577-45-3) was acquired with a purity level of 98% from the same source. Omeprazole sodium (CAS No. 95510-70-6) exhibits a purity of 99.98%. Pantoprazole (CAS No. 138786-67-1) has a purity of 99.36%, and Dexlansoprazole (CAS No. 138530-94-6) boasts a purity level of 99.51%. These chemicals, characterized by their high purity, are essential for ensuring precision and reliability in analytical techniques across various research and pharmaceutical applications and were procured from GLP Pharma Standard (Hyderabad, India). Additionally, Rabeprazole EP impurity B (CAS No.117977-21-6) and 4-Desmethoxy omeprazole impurity (CAS No. 110374-16-8) were also sourced purity of >%98. HPLC reagent-grade ethanol from Sigma-Aldrich was used in the analytical procedures.

Prilosec contains 20.0 mg of Omeprazole, Esomeprazole contains 20.0 mg of Esomeprazole, Prevacid contains15.0 mg of Lansoprazole, Protonix contains 20.0 mg of Pantoprazole, AcipHex contains 20.0 mg of Rabeprazole, and Dexilant contains 30.0 mg of Dexlansoprazole were obtained from a local pharmacy in Sulaymaniyah, Iraq.

Stock and working solutions

To prepare stock standard solutions, 100 mg of each OME, ESO, LAN, PAN, RAB, and DEX, along with 4-DES-Imp., and RAB-Imp. B were individually weighed and placed into eight distinct 100-ml volumetric flasks. The volume was then adjusted to the mark with ethanol solvent, resulting in a final concentration of 1000 μg/mL for each compound. Subsequently, working standard solutions for each compound were derived from their respective stock solutions. Portions equivalent to 10.0, 25.0, 2.5, 10.0, 10.0, and 25.0 mg of OME, ESO, LAN, PAN, RAB, and DEX, respectively, were transferred into six isolated 100-ml volumetric flasks. The volume was completed to the mark with ethanol, yielding standard working solutions with concentrations of 100.0, 250.0, 25.0, 100.0, 100.0, and 250.0 μg/mL for OME, ESO, LAN, PAN, RAB, and DEX, respectively. Additionally, using their respective standard stock solutions in ethanol, working recommended solutions of 4-DES-Imp. (50.0 μg/mL) and RAB-Imp. B (25.0 μg/mL) were created.

Procedures

Characteristics of the spectrum

The wavelength range of 200.0 to 400.0 nm was used to measure the spectrum of absorption of OME, ESO, LAN, PAN, RAB, DEX, 4-DES-impurity, and RAB-impurity B. The precise wavelength measurements ranging from 230.0 to 320.0 nm were then extracted and imported to MATLAB for further investigation.

Chemometric techniques experimental design

A rigorous design of the experiment for the creation of the calibration set is critical for excellent predictions in multivariate calibration. A multiple-level multiple-factor design [35] has been employed to construct each sample in the present study. Twenty-five distinct combinations comprising OME, ESO, LAN, PAN, RAB, DEX, 4-DES-Imp., and RAB-Imp. B were developed. These mixes were made by properly transferring aliquots from the associated working standard solutions to 5.0 mL measuring glass containers and subsequently diluting them with ethanol to the mark. The linearity of each of the eight compounds was taken into account for deciding concentrations for each chemical in the 25 samples.

Specifically, 25 samples were designated for use as the calibration set, while another 15 sets of samples were allocated for use as the validation set (Table 1). This design approach ensures a robust and well-structured dataset for calibration and validation purposes.

To assess the orthogonality of factors, a correlation analysis was conducted, and the results indicated that the correlation coefficient between the factors was zero. This finding suggests that, at the outset of the analysis, there is no spurious correlation in composition that could potentially impact the outcomes of multivariate calibration. Consequently, the selected design is deemed appropriate for the execution of the experiment.

The spectra of the prepared mixtures were observed within the wavelength range of 200 to 400 nm and were acquired at 1.0 nm intervals with ethanol serving as the blank. Spectral data within the regions of 200 to 230 nm and 320 to 400 nm were excluded from analysis due to high spectral noise. These data points were used to build multivariate calibration models such as OPLS, iOPLS, and GA-OPLS. Every strategy parameter was extensively examined and improved, and utilized to assess the external validation set and calculate the drug concentrations in those samples. This procedure enabled the prediction accuracy and performance of the models to be evaluated.

Pharmaceutical formulations assay

Initially, ten tablets were ground into a fine powder. From each tablet, precise measurements of 5.0 mg of Prilosec, Esomeprazole, Prevacid, Protonix, AcipHex, and Dexilant were acquired. The powdered components were placed in 50.0 mL volumetric flasks filled with ethanol to reach the final concentrations. The following concentrations were obtained by withdrawing 5.0 mL of solution from the prior solution: 5.0 μg/mL of OME, 20.0 μg/mL of ESO, 1.0 μg/mL of LAN, 1.5 μg/mL of PAN, 4.0 μg/mL of RAB, and 20.0 μg/mL of DEX.

Table 1
Composition of Calibration and Validation Sets.

Calibration Set									Validation Set								
MixNo	OME	ESO	LAN	PAN	RAB	DEX	4-DES-Imp.	RAB-Imp.B	Mix.No	OME	ESO	LAN	PAN	RAB	DEX	4-DES- Imp.	RABImp.B
1	15	45	3.5	12	12	50	1.5	1.4	1	15	45	3.5	12	12	50	1.4	1.5
2	15	20	0.5	24	8	90	0.75	1.4	2	10	65	5.5	18	12	90	3.4	0.1
3	5	20	5.5	6	20	50	0.75	0.8	3	25	75	4.5	12	20	90	0.2	2.5
4	5	75	2	24	12	35	2.5	0.8	4	15	75	5.5	1	16	20	1.4	2.5
5	35	35	5.5	12	8	35	3.5	2.4	5	35	75	0.5	18	4	50	2.4	2.5
6	10	75	3.5	6	8	70	2.5	3.4	6	5	65	0.5	12	16	70	0.8	0.1
7	35	45	2	6	16	90	1.5	2.4	7	5	45	4.5	18	8	20	0.8	1.5
8	15	35	2	18	20	70	3.5	1.4	8	15	65	4.5	6	4	35	1.4	0.1
9	10	35	4.5	24	16	50	3.5	3.4	9	25	35	0.5	6	12	20	0.2	3.5
10	10	65	5.5	18	12	90	0.1	3.4	10	10	20	2	12	4	20	3.4	0.75
11	25	75	4.5	12	20	90	2.5	0.2	11	10	45	0.5	1	20	35	3.4	1.5
12	35	65	3.5	24	20	20	0.1	2.4	12	5	20	5.5	6	20	50	0.8	0.75
13	25	45	5.5	24	4	70	1.5	0.2	13	35	45	2	6	16	90	2.4	1.5
14	15	75	5.5	1	16	20	2.5	1.4	14	15	35	2	18	20	70	1.4	3.5
15	35	75	0.5	18	4	50	2.5	2.4	15	10	35	4.5	24	16	50	3.4	3.5
16	35	20	4.5	1	12	70	0.75	3.4									
17	5	65	0.5	12	16	70	0.1	0.8									
18	25	20	3.5	18	16	35	0.75	0.2									
19	5	45	4.5	18	8	20	1.5	0.8									
20	15	65	4.5	6	4	35	0.1	1.4									
21	25	65	2	1	8	50	0.1	0.2									
22	25	35	0.5	6	12	20	3.5	0.2									
23	10	20	2	12	4	20	0.75	3.4									
24	5	35	3.5	1	4	90	3.5	0.8									
25	10	45	0.5	1	20	35	1.5	3.4									

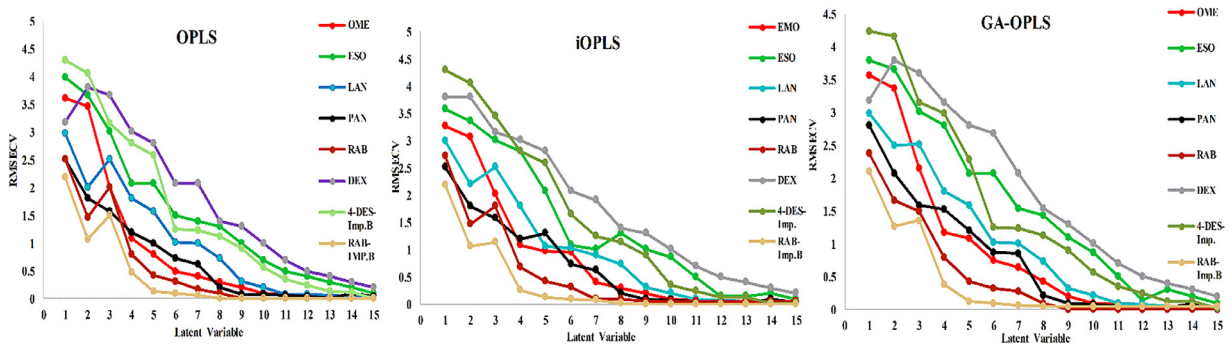


Fig. 2. Plot between RMSECV value and latent Variable OPLS, iOPLS, and GA-OPLS.

Standard addition techniques were carried out when additional quantities of standard medicines were introduced into the manufactured samples, allowing the accuracy and validity of the developed techniques to be evaluated. The entire method certified the techniques' validity and application for the precise investigation into pharmaceutical formulations.

Results and discussion

Impurities can form throughout the production stage or when pharmaceutical substances are inadequately preserved. Although a few techniques for determining PPIs individually in pharmaceutical formulations were earlier documented, these methods have yet to be utilized to detect or quantify all six medicines concurrently, including both 4-DES-Imp., and RAB-Imp. B. As a result, it became critical to discover precise methodologies for concurrently evaluating the active ingredients and quantifying contaminants in pharmaceutical product formulations. Multivariate calibration approaches, which rely on the concurrently processed inclusiveness of various spectral wavelengths, are successful at sorting out significantly overlapped spectra as well as offering superior consistency over techniques that operate only a particular wavelength. Multivariate calibrations are used primarily in pharmaceutical analysis, as well as in quality assurance facilities for the identification of impurities in bulk pharmaceuticals and pharmaceutical formulations. Furthermore, chemometrics, using statistical and mathematical approaches in chemistry, has a wide range of medicinal applications and may be used in metabolic profiling.

Optimization and validation

Selecting the appropriate number of factors is crucial for achieving effective quantitative analysis in OPLS. A big number may contribute to higher levels of noise, while an excessively small number might result in missing crucial data. The ideal number of latent variables (LVs) was guided by minimizing the RMSECV to reduce prediction errors and prevent model overfitting [41]. The suitable LVs for all components were identified as six factors, as illustrated in Fig. 2.

In the process of interval variable selection, following the assessment of multivariate filters employing the entire spectrum, the subsequent step involved the implementation of iOPLS to assign variable selection. The iOPLS was conducted in the optimistic direction with an interval size of 60 variables. The algorithm determined the optimal number of intervals by minimizing the RMSECV. After identifying the spectral intervals, the selected variables underwent further refinement through the application of multivariate filters, resulting in 6 intervals. Several settings have been modified to enhance the GA operations. The most significant factor was the size of the population, having exhibited a considerable impact on GA efficiency, populations with low values resulted in inadequate outcomes as the participants had a restricted chance of exploring the solution area, whilst higher numbers could search wider areas, perhaps resulting in voracious convergence to a solution. Furthermore, the mutation rate, responsible for preserving genetic population variety, had been a vital metric that needed to be fine-tuned. Furthermore, the mutation rate, which is responsible for preserving genetic population variety, had been a vital metric that needed to be fine-tuned. A mutation affected either one or more genes on the GA chromosomes, preventing early converging. Other variables, which include the number of subsets, the highest possible number of latent variables (LVs), as well as the number of iterations, were calculated, with the specified number of latent variables set to six, as shown in Fig. 2. The Genetic Algorithm (GA) was employed with a window width set to 1 and contiguity enforced through cross-validation with 15 splits.

To test linearity, calibration plots have been generated via contrasting predicted concentrations against true levels for each component. OME, ESO, LAN, PAN, RAB, DEX, 4-DES-Imp., and RAB-Imp. B linearity ranges were established at 5.0–35.0, 20.0–75.0, 0.5–5.5, 1.0–24, 4.0–20.0, 20.0–90.0, 0.1–3.5, and 0.2–3.4 μg/mL respectively.

Several statistical parameters comprising each of the three mathematical models, consisting of regression equations, slope, intercept, and correlation coefficients, were established, demonstrated in Table 2. The suggested approaches revealed relatively low RMSEP, REP, and RMSEC values, demonstrating considerable specificity when evaluating all six components in mixes along with two impurities. The standard error of prediction (SEP) was computed and reported in Table 2. The closed values of SEP and RMSEP demonstrated the absence of overfitting in the suggested calibration models.

Table 2
Statistical results for the optimized OPLS, iOPLS, and GA-OPLS.

Components	Models	Slope	Intercept	LOD	LOQ	R	RMSEP	RMSEC	REP	SEP
OME	OPLS	1.0056	−0.0318	0.994	3.013	0.9997	0.1991	0.1923	1.1095	0.1895
	iOPLS	0.9958	0.0440	0.985	2.983	0.9998	0.1645	0.1590	0.9173	0.1484
	GA-OPLS	1.0009	0.1353	1.285	3.896	0.9996	0.2157	0.2083	1.1917	0.1945
ESO	OPLS	1.0038	−0.3013	2.397	7.264	0.9997	0.3858	0.3727	0.8001	0.3780
	iOPLS	1.0049	−0.3036	2.301	6.974	0.9998	0.3181	0.3073	0.6495	0.2868
	GA-OPLS	0.9956	0.5779	2.950	8.940	0.9995	0.5622	0.5431	1.1658	0.5069
LAN	OPLS	0.9999	0.00022	0.043	0.130	0.9999	0.0542	0.0524	1.1658	0.0489
	iOPLS	0.9998	−0.0008	0.033	0.121	0.9999	0.0355	0.3433	1.0936	0.0321
	GA-OPLS	1.0013	0.0099	0.062	0.190	0.9997	0.0234	0.0226	0.7202	0.0212
PAN	OPLS	0.9946	0.0306	0.099	0.301	0.9999	0.0959	0.0926	0.8187	0.0945
	iOPLS	0.9956	0.0810	0.076	0.231	0.9997	0.1446	0.1396	1.2495	0.1304
	GA-OPLS	1.0026	0.0171	0.128	0.390	0.9998	0.0673	0.0650	0.5756	0.0607
RAB	OPLS	1.0024	−0.0378	0.659	1.997	0.9999	0.0548	0.0529	0.4279	0.0504
	iOPLS	1.0013	−0.0004	0.954	2.893	0.9998	0.0381	0.0368	0.2961	0.0344
	GA-OPLS	1.0004	0.0343	0.627	1.889	0.9995	0.1099	0.1062	0.8629	0.0991
DEX	OPLS	1.0040	−0.1575	2.815	8.531	0.9998	0.1853	0.1791	0.3538	0.1822
	iOPLS	0.9964	0.2186	3.123	9.462	0.9999	0.2418	0.2336	0.4619	0.2181
	GA-OPLS	0.9933	0.3181	3.332	10.067	0.9995	0.4751	0.4590	0.9062	0.4284
4-DES-Imp.	OPLS	0.9964	0.0025	0.033	0.099	0.9998	0.0162	0.0156	0.8667	0.0157
	iOPLS	0.9962	0.0028	0.023	0.070	0.9999	0.0135	0.0131	0.7212	0.0122
	GA-OPLS	1.0027	0.0023	0.026	0.078	0.9999	0.0102	0.0099	0.5541	0.0095
RAB-Imp. B	OPLS	0.9980	0.0019	0.020	0.059	0.9999	0.0157	0.0152	0.9201	0.0152
	iOPLS	0.9983	−0.0016	0.012	0.039	0.9999	0.0138	0.0133	0.8168	0.0125
	GA-OPLS	1.0064	0.0009	0.130	0.040	0.9997	0.0224	0.0216	1.3098	0.0211

Linearity range: OME (5.0–35.0), ESO (20.0–75.0), LAN (0.5–5.5), PAN (1.0–24), RAB (4.0–20.0), and DEX (20.0–90.0) μg/mL, while 4-DES-Imp. (0.1–3.5) μg/mL and RAB-Imp. B (0.2–3.4) μg/mL.
R: Correlation Coefficient.
RMSEP: Root Mean Square Error of Prediction.
REP: Relative Error of Prediction.
RMSEC: Root Mean Square Error of Calibration.
SEP: Standard Error of Prediction.
LOD and LOQ: Limit of Detection and Limit of Quantification in μg/mL.

Implementing an external validation set, the proposed techniques demonstrated minimal values for RMSEP, REP, and RMSEC, which suggests significant sensitivity for evaluating all six materials in mixtures along with the two impurities. The findings highlight the accuracy and level of detail of the suggested procedures, with mean recoveries of all elements within a satisfactory range, indicating the correctness of the proposed techniques (as shown in Tables 3A, 3B, and 3C).

Pharmaceutical formulations assay

The proposed chemometric methods were employed to assess the presence of OME, ESO, LAN, PAN, RAB, and DEX in commercially available tablets. Recoveries and Relative Standard Deviations (RSD%) were established according to different medication concentrations, and the results presented in Table 4 demonstrate no indication of excipient influence, alongside no impurities identified in the medication.

Standard addition techniques, detailed in Table 4, involved introducing additional quantities of standard medicines into the manufactured samples. This procedure allowed for the assessment of the accuracy and validity of the developed techniques. The overall methodology certified the validity and applicability of the techniques for the precise analysis of pharmaceutical formulation.

Statistical parameters

Table 5 shows that there is no discernible difference in reliability and precision regarding the suggested chemometric approaches with the cited procedures [42,43]. The *t*-test and F-test, both performed at $P = 0.05$, exhibited no significant differences.

Metrics for sustainability and greenness evaluation

Evaluation of greenness index through spider chart

The Greenness Index, assessed through the spider chart metric, provides a comprehensive description of the various solvent properties employed in the proposed chemometric spectrophotometric and the reported methods. This measure is based on information gathered from the relevant solvents' safety data sheets (SDSs), which include their many qualities and effects on health, safety, and the environment [44]. The major assessment methodologies are based on the Green Analytical Chemistry (GAC) principles and comprise five attribute categories (Health danger, General characteristics, Odor, Fire safety, and Stability). Each cluster is assigned a score using

Table 3A
Statistical parameters for the validation set to the optimized OPLS.

Mix No.	%Recovery							
	OPLS							
	OME	ESO	LAN	PAN	RAB	DEX	4-DES.Imp	RAB-Imp. B
1	100.58	102.14	100.15	100.07	100.13	100.44	98.81	100.58
2	102.33	100.04	99.99	100.12	100.12	99.93	99.23	100.26
3	100.62	99.19	100.12	100.08	100.07	99.98	98.62	100.56
4	101.02	100.60	100.02	99.73	99.92	99.74	99.31	100.22
5	98.03	98.50	100.09	99.44	100.08	100.26	101.67	99.21
6	99.21	100.31	99.97	98.48	100.03	100.46	100.19	98.54
7	100.46	99.66	100.05	99.09	99.30	100.10	100.62	99.30
8	99.64	99.72	102.59	99.43	99.35	99.51	99.51	100.40
9	101.47	97.29	100.15	99.98	99.83	99.99	99.54	100.53
10	100.68	99.77	99.755	100.26	100.44	100.09	98.42	100.93
11	99.97	96.07	100.02	100.01	100.30	100.06	97.62	100.85
12	101.18	100.22	99.97	99.43	98.44	99.68	102.35	97.51
13	99.83	99.42	99.98	101.45	99.18	100.02	100.25	102.21
14	101.00	100.34	100.16	100.03	100.25	99.87	99.99	99.85
15	100.76	100.04	99.64	100.42	99.98	100.27	100.14	100.81
Mean	100.45	99.55	100.18	99.87	99.83	100.03	99.75	100.12
%RSD	1.0160	1.4313	0.6818	0.6689	0.5364	0.2674	1.2232	1.1193
SD	1.0207	1.4249	0.6830	0.6680	0.5355	0.2675	1.2202	1.1207
RMSEP	0.1991	0.3858	0.5622	0.0959	0.0548	0.1853	0.0162	0.0157

Table 3B
Statistical parameters for the validation set to the optimized iOPLS.

Mix No.	%Recovery							
	iOPLS							
	OME	ESO	LAN	PAN	RAB	DEX	4-DES-Imp.	RAB-Imp. B
1	99.92	97.96	100.48	99.74	99.76	100.11	100.32	99.66
2	103.95	100.48	99.99	100.36	100.08	100.06	99.12	99.86
3	99.93	99.35	99.88	99.87	100.02	100.04	99.64	100.52
4	100.04	99.50	100.12	100.57	100.35	100.15	100.01	99.01
5	99.98	100.29	100.27	99.99	99.92	99.44	101.67	100.25
6	96.35	100.01	100.07	99.84	100.04	99.91	99.98	98.55
7	100.41	100.17	99.99	98.91	100.69	99.96	100.05	98.75
8	99.68	99.76	98.31	99.98	100.76	99.97	98.69	99.87
9	98.73	97.91	99.80	102.85	100.13	99.91	99.39	100.28
10	101.30	100.52	98.96	99.80	100.19	99.98	98.64	100.97
11	99.97	97.55	99.85	100.05	99.97	99.84	103.45	99.79
12	99.68	100.41	99.86	100.16	99.83	103.93	99.08	99.91
13	99.84	100.02	99.96	100.09	100.49	100.07	99.98	103.82
14	100.59	100.88	101.94	99.88	99.88	99.97	99.89	99.43
15	100.82	100.07	98.82	98.55	100.12	100.30	99.71	99.18
Mean	100.08	99.66	99.88	100.04	100.14	100.24	99.96	99.99
%RSD	1.5482	1.0367	0.8169	0.9263	0.3016	1.0356	1.2109	1.2494
SD	1.5494	1.0403	0.8160	0.9267	0.3026	1.0381	1.2106	1.2493
RMSEP	0.1645	0.3181	0.0355	0.1446	0.0381	0.2418	0.0135	0.0138

a preset formula, ranging from −5 (least green) to +5 (most green) [45]. The results are graphically portrayed in a primary spider chart for the five key parameters, providing a complete picture of solvent greenness. Secondary spider charts provide a more in-depth look at qualities unique to each condition. It is crucial to remember how some solvents include incomplete SDS elements [46]. Under these situations, qualities with missing data are allocated a zero score. Each of the parameters is detailed within the "Greenness Index Table," which also includes the proportion of available data needed to generate Greenness Index ratings. This proportion reflects the level of trust in the evaluation's accuracy [47]. It is worth mentioning that this is one of the first studies to apply the Greenness Index to the analytical industry. It is worth noting that this study is among the first to use the Greenness Index in the analytical sector. The spider technique used allows for the simple assessment of individual solvents or the comparison of numerous solvents. The proposed models used ethanol as a solvent, however, the previously published HPLC and LC-MS/MS approaches used acetonitrile [42,43]. Figs. 3 and 4 show the Greenness Index evaluations for ethanol and acetonitrile, respectively, utilizing a major web chart and four subordinate charts. The Greenness Index table (Table 6) displays the average scores of every parameter along with the percentage of presently available data.

Table 3C
Statistical parameters for the validation set to the optimized GA-OPLS.

Mix No.	%Recovery							
	GA-OPLS							
	OME	ESO	LAN	PAN	RAB	DEX	4-DES-Imp.	RAB-Imp. B
1	100.58	101.15	101.4	100.29	100.94	100.30	100.35	100.40
2	102.33	102.00	100.36	100.01	99.75	100.14	100.89	100.13
3	100.62	99.82	99.63	101.28	100.49	100.15	100.04	101.14
4	101.02	100.11	100.55	99.88	100.29	100.95	100.17	102.80
5	98.03	102.02	102.85	100.10	99.50	99.55	101.11	100.34
6	99.21	99.56	100.49	100.33	101.14	98.27	100.46	100.68
7	100.46	99.82	100.45	101.18	102.06	100.29	100.80	100.40
8	99.64	101.07	102.02	99.97	101.15	99.96	100.83	99.60
9	101.47	103.80	100.66	99.90	100.56	100.10	100.51	100.13
10	100.68	100.81	100.92	100.80	100.15	99.88	99.87	100.17
11	99.97	104.85	99.74	100.38	100.20	100.18	101.50	99.973
12	101.18	100.91	100.44	100.55	98.91	101.25	100.62	100.66
13	99.83	101.45	100.37	100.56	99.01	100.05	100.09	101
14	101.00	100.81	101.68	101.24	100.44	99.63	99.03	99.951
15	100.76	99.94	99.06	101.10	100.13	100.58	100.03	100.36
Mean	100.45	101.21	100.71	100.50	100.31	100.08	100.42	100.51
%RSD	1.4552	1.4716	0.9571	0.5037	0.8284	0.6690	0.5915	0.7452
SD	1.4705	1.4894	0.9639	0.5062	0.8310	0.6696	0.5940	0.7491
RMSEP	0.2157	0.5622	0.0234	0.0673	0.1099	0.4751	0.0102	0.0224

Table 4
Assay results of investigated components in pharmaceutical tablets implementing the optimized OPLS, iOPLS, and GA-OPLS.

Components µg/mL		Models	%Found* ±RSD	Standard Addition Technique %Recovery** ±RSD
OME	**5.0**	OPLS	100.44±1.4573	100.10±1.1378
		iOPLS	100.08±1.4001	100.43±1.0582
		GA-OPLS	99.92±1.3559	99.26±1.2686
ESO	**20.0**	OPLS	100.75±1.2815	100.70±1.5313
		iOPLS	100.68±0.8406	100.40±0.7243
		GA-OPLS	100.56±0.7291	100.11±0.5272
LAN	**1.0**	OPLS	99.80±1.0134	100.23±0.3669
		iOPLS	100.43±1.3468	100.65±1.3862
		GA-OPLS	99.95±0.5481	100.17±0.7823
PAN	**1.5**	OPLS	100.59±0.9211	100.04±0.1976
		iOPLS	100.06±0.5781	100.04±0.3285
		GA-OPLS	100.29±0.7262	99.72±0.4646
RAB	**4.0**	OPLS	100.23±0.6845	100.12±0.1492
		iOPLS	100.19±0.7051	99.97±0.4710
		GA-OPLS	100.55±1.6760	100.92±1.4869
DEX	**20.0**	OPLS	100.95±1.1205	100.06±0.1584
		iOPLS	99.98±1.3437	99.96±0.1545
		GA-OPLS	100.09±0.1362	99.92±0.1036

* Average of six replications.

** Average of three determination of standard added (5.0, 10.0, and 20.0 µg/mL), (5.0, 20.0, and 40.0 µg/mL), (1.0, 2.0, and 3.0 µg/mL), (5.0, 10.0, and 20.0 µg/mL), (4.0, 10.0, and 15.0 µg/mL) and (10.0, 2.0, and 40.0 µg/mL) for, OME, ESO, LAN, PAN, RAB, and DEX respectively.

The major chart (Fig. 3) shows that ethanol's total Greenness Index placed it in the greatest safety region, whereas acetonitrile's less favorable scores positioned it within a less safe area. Fig. 4 gives an extensive overview of the various features of all criteria for the solvents discussed. The spider plots and Greenness Index table data show that the adopted strategy outperforms the previously published method in terms of both human health and environmental safety.

National environmental methods index (NEMI), advanced and modified NEMI

NEMI, an early GAC measure, employs a circle of four parts to assess an analytical method's environmental effect. If the specified criterion is satisfied the relevant section is tinted green; alternatively, it remains colorless. The following are the demands for each of the four sections of the NEMI pictogram, identification of whether the reagents utilized in the process of analysis are on the consistent, bio-concentrative, and poisonous substance (PBT) list and as toxic wastes, assessment of whether the sample is corrosive based on

Table 5
Statistical analysis of the proposed chemometric techniques and the reported HPLC method [42] and LC-MS/MS [43].

Models	Components	Mean	%RSD	n	Student's *t*-test (2.228)*	F-value (5.05)*
OPLS	OME	100.44	1.4573	6	0.9931	4.2182
	ESO	100.75	1.2815		0.4682	1.7853
	LAN	99.80	1.0134		0.5303	2.4073
	PAN	100.59	0.9211		1.1783	2.4528
	RAB	100.23	0.6845		1.1074	1.4351
	DEX	100.95	1.1205		1.5723	0.8626
iOPLS	OME	100.08	1.4001		0.3174	4.5981
	ESO	100.68	0.8406		1.6279	4.2389
	LAN	100.43	1.3468		0.5708	4.2420
	PAN	100.06	0.5781		1.7273	4.0523
	RAB	100.19	0.7051		1.1945	1.5217
	DEX	99.98	1.3437		1.2198	1.2242
GA-OPLS	OME	99.92	1.3559		0.0658	4.9252
	ESO	100.56	0.7291		1.1506	4.5156
	LAN	99.95	0.5481		1.1590	1.4161
	PAN	100.29	0.7262		0.8196	3.9685
	RAB	100.55	1.6760		1.1342	2.9432
	DEX	100.09	0.1362		1.4111	0.3198
[a]Reported methods	OME	99.80	1.1	9		
	ESO	102.50	2.4			
	LAN	98.6	1.8			
	PAN	101.10	1.2			
	RAB	98.40	2.2			
	DEX	98.99	0.22	6		

* Theoretical values of t and F at ($P = 0.05$).
[a] The reported approach for OME, ESO, LAN, PAN, and RAB: The chromatographic separation was accomplished on an InterSustain® C18 column (150 × 4.6 mm, 5 μm). The isocratic mobile phase, composed of 0.05 M potassium dihydrogen phosphate buffer (pH 4.0) and acetonitrile (65:35, v/v), was run through the column at a temperature of 30 °C and a flow rate of 1.0 mL/min.[a]DEX: LC-MS/MS employing Zorbax SB C18 column (4.6 × 100 mm, 3 μm) as a stationary phase. The mobile phase consists of (0.5 mM) Ammonium Acetate adjusted to pH 3.5: acetonitrile (30:70 V/V) at a flow rate of 0.5 mL/min.

Table 6
Greenness Index table for Ethanol and Acetonitrile solvents.

Evaluation criteria	Ethanol	Information availability (%)	Acetonitrile	Information availability (%)
Health Impact	2.63	100	1.88	100
Odor	5	100	5	100
General properties	1.61	87	−0.14	87
Fire safety	0.61	100	0.77	100
Stability	2.71	100	1.43	85
Average	2.51	97.4	1.79	97.4

a pH range of 2–12, and examining in case the created waste is less than 50 mL [48]. The proposed methods have been identified to have significant ecological sustainability when all four regions of the NEMI pictogram depicted in Table 6, are colored green. The reported methods [42,43] first and second quadrants were blank due to the usage of hazardous solvents which were listed in official lists.

NEMI serves as a straightforward tool enabling researchers to assess the environmental friendliness of an analytical assay quickly. However, it does come with certain drawbacks, NEMI is unable to provide quantitative or precise information, and its assessment method, which involves searching and symbol filling, is overdue [49]. Another metric was presented named Advanced Pictogram with four factors to enhance NEMI's statistical abilities: operator risk assessment, reagents utilized, the use of energy, and volume of waste [50]. Table 7 indicates that each of the four segments is colored green, demonstrating the fact that the proposed methods have been identified to be beneficial for environmental sustainability, whereas HPLC reported method [42] first and second quadrants were yellow due to the operational risk and energy consumed, the most noticeable change was the assessment of instrument energy and operator risk, while LC-MS/MS [43] has yellow segments representing operator risk and red segment due to its high energy consumption. Raynie and Driver also presented a modified NEMI greenness measure [50], the improved NEMI employs a pictogram determined by five concepts: health hazardous circumstances, safety potential danger, environmental risk, usage of energy, and the quantity of waste created during analytical operations. The National Fire Protection Association (NFPA) supplies health hazard and flammability ratings that influence the hue of health and safety pictograms. The following three pictograms' color-choosing method is identical to NEMI's. Only the health and safety segments of the five components have quantifiable values derived from NFPA values,

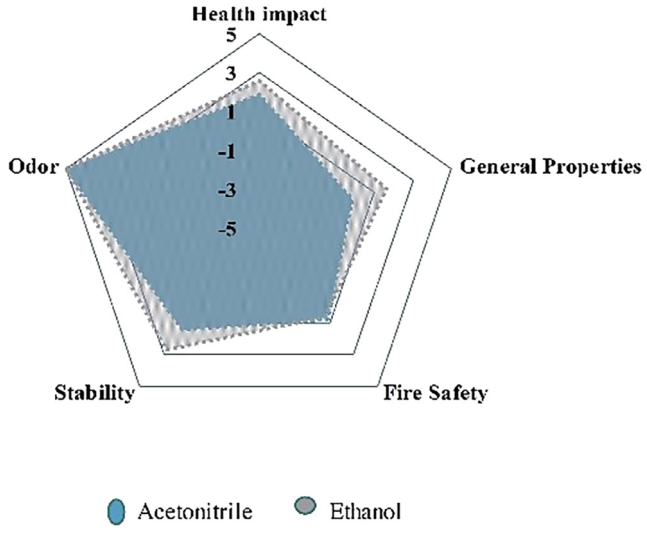


Fig. 3. Major Spider chart for Ethanol and Acetonitrile.

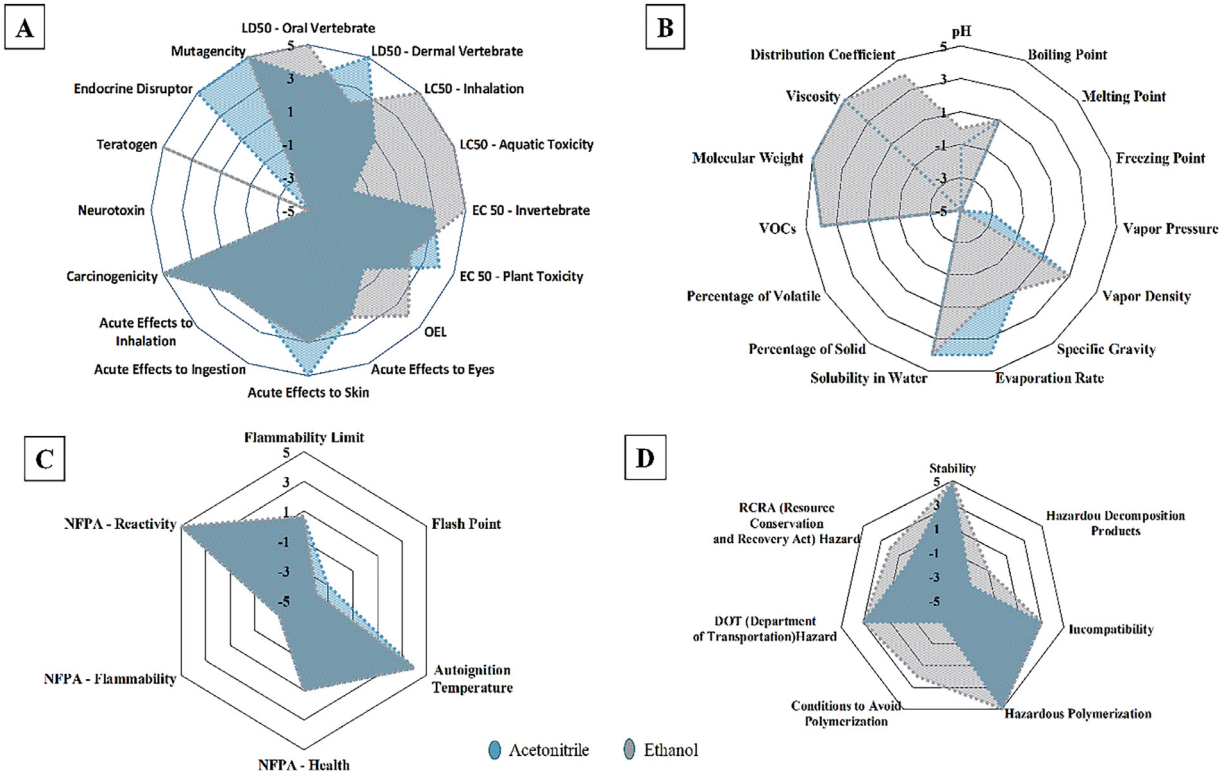
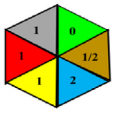
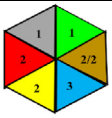
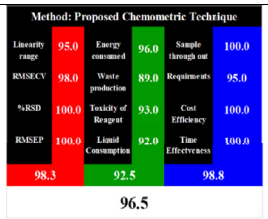
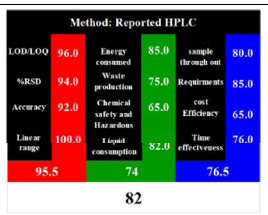
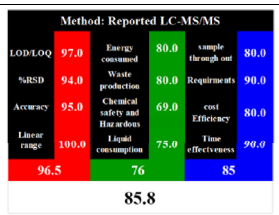


Fig. 4. Ethanol and Acetonitrile primary charts for (A) Health Impact, (B) General properties, (C) Fire safety, (D) Stability.

whereas the other three do not. This approach, like NEMI, is simple to implement, but it lacks specialist software for obtaining quantification values for waste quality, environmental effect, and energy use. The Advanced pictogram and modified NEMI both use a color model with red, yellow, and green hues to signify rare, fair, and benign-ecologically friendly analysis, respectively [51]. The proposed chemometric method has two yellow triangles, which are represented in its pentagram, due to consuming ethanol which was listed in NFPA as a health hazard and flammable hazard. The reported HPLC [42] has three yellow triangles due to health and safety hazards and energy consumed while LC-MS/MS [43] has a red triangle representing its high energy consumption due to the use of

Table 7
Greenness-Sustainability Assessment comparison between the proposed Chemometric and reported methods.

Metrics	Proposed Technique	Reported HPLC [42]	Reported LC-MS/MS [43]
NEMI			
Advanced NEMI			
Modified NEMI			
Hexagonal Tool	 S_{av} 1.0	 S_{av} 1.625	 S_{av} 1.75
RGB12			
WAC	R (%) 98.3; G (%) 92.5; B (%) 98.8; Whiteness (%) 96.5	R (%) 95.5; G (%) 74.0; B (%) 76.5; Whiteness (%) 82.0	R (%) 96.5; G (%) 76.0; B (%) 85.0; Whiteness (%) 85.5

the mass detector, the green color indicates ecologically beneficial techniques, the new chemometric spectrophotometric approaches are more ecologically friendly than previously reported ones as shown in Table 7.

The NEMI tool gives a visual inspection of a strategy's impact on the environment along with sustainability issues. This suggests changing greener reagents to enhance the method's greenness profile. Drawbacks have been identified in assessing the greenness of numerous techniques due to NEMI's simple pass/fail methodology based on restricted criteria. In this study, we use NEMI as a first screen along with other two modified metrics and combined it with quantitative greenness criteria for a more trustworthy comparison to previous methodologies.

Hexagon tool

The Hexagon tool, designed for quantifying the sustainability of analytical procedures, integrates Green Chemistry principles along with considerations for environmental and financial impacts [52]. This quantitative tool employs a hexagon framework, assessing five variables within a method by assigning penalty points (PPs). These characteristics can be grouped into five distinct groups: merit figures, hazard characteristics, generation of waste, carbon footprint, and yearly costs to the economy. On a scale of 0 to 4, every single group is granted a final score. The representation is in the shape of a hexagon with six equilateral triangles, each representing a method variable. The resulting pictogram facilitates a convenient evaluation between various testing methods, featuring a greater number of zeros signifying a more environmentally sensitive and ecologically responsible methodology [53]. The Global Harmonized System (GHS) is a globally standardized system that assesses chemical toxicity, hazards, and safety concerns [54].

In Fig 5, the hexagonal pictogram for the proposed chemometric procedure gets an overall score of 1.0 in the FM1 triangle, which comprises the treatment of the samples, characterization of the techniques, and calibration. From the perspective of calibration, the proposed technique accrues 6.0 penalty points, taking into account factors such as the working range of concentrations, linearity, required time, precision, and linear adjacent R2. As for the method's characteristics, the proposed approach receives 3.0 penalty points, credited for being nondestructive, multi-component, rapid, and robust. In contrast, described procedures employing HPLC [42] and LC-MS/MS [43] were partially automated and incurred penalty points for analysis time, yielding total penalty point values of 21.0 and 19.0, respectively. These cumulative penalty points were subsequently assigned to the scales, yielding a total result of 2.0 for the two reported techniques, as shown in Fig. (6A and 6B).

Accuracy and quality control measurements are covered in Figures of Merit 2 (FM2), and the suggested technique earns 5.0 penalty points for quality control parameters. Accuracy is attributed to considerations such as the number of standards and concentration levels, resulting in 5.0 penalty points. The total penalty points for FM2 was ten, yielding an overall score of 2.0. In comparison,

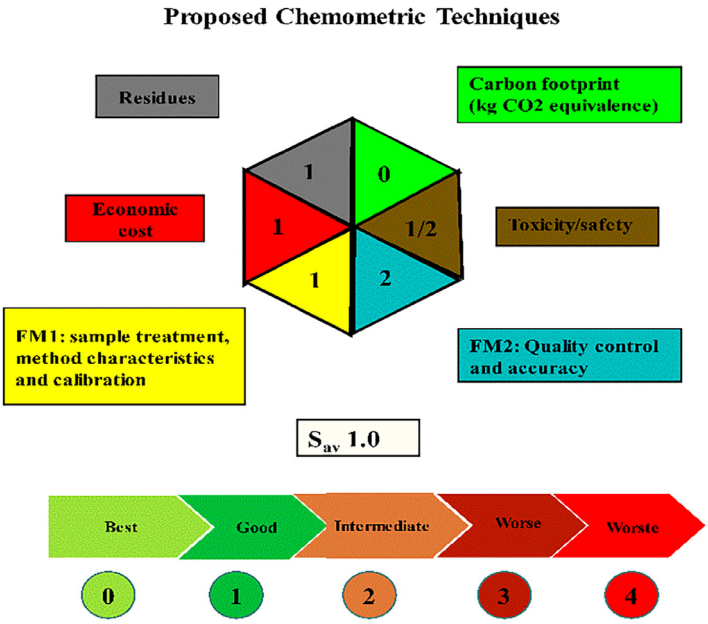


Fig. 5. Hexagon Pictogram for the proposed Chemometric techniques and arithmetic Sav.

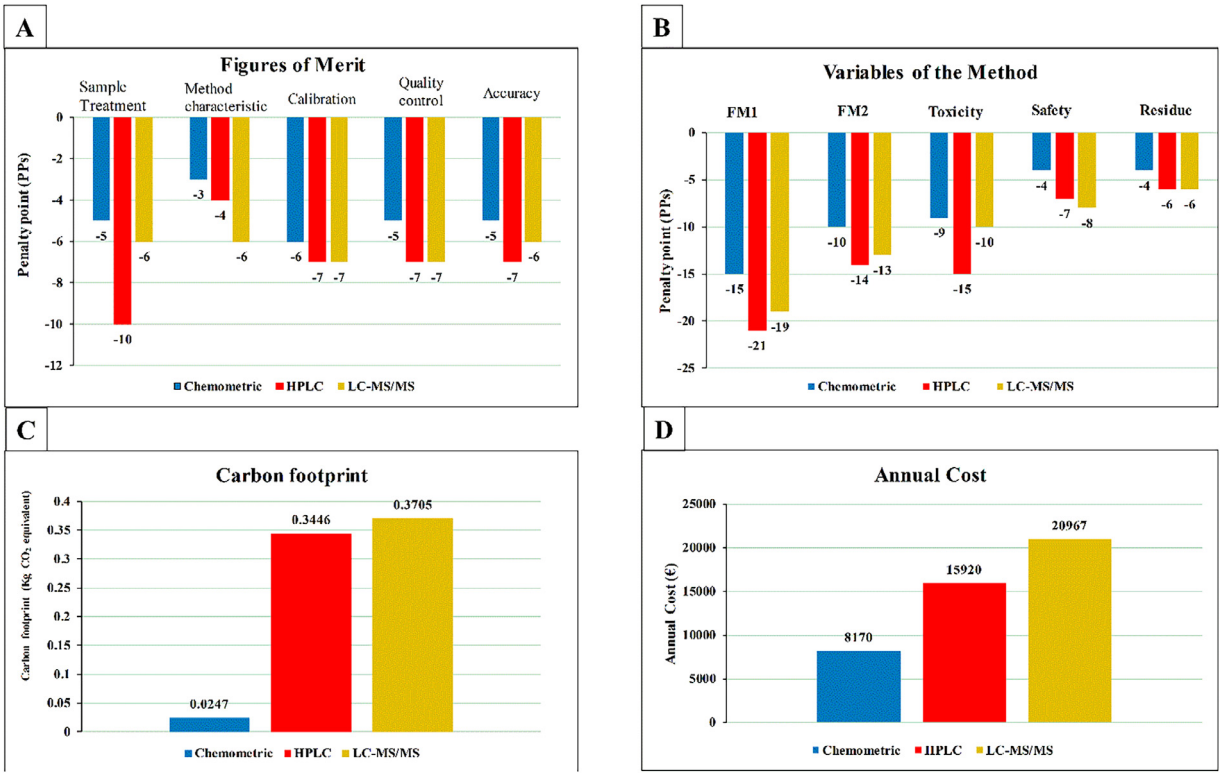


Fig. 6. (A) Penalty points of parameters of evaluation tool related to the figures of merit 1 and 2. (B) Penalty points assessment for figures of merit 1 and 2, toxicity, safety, and residues, (C) carbon footprint expressed as kg CO2 equivalent, and (D) annual economic cost for the proposed Chemometric and reported HPLC and LC-MS/MS techniques.

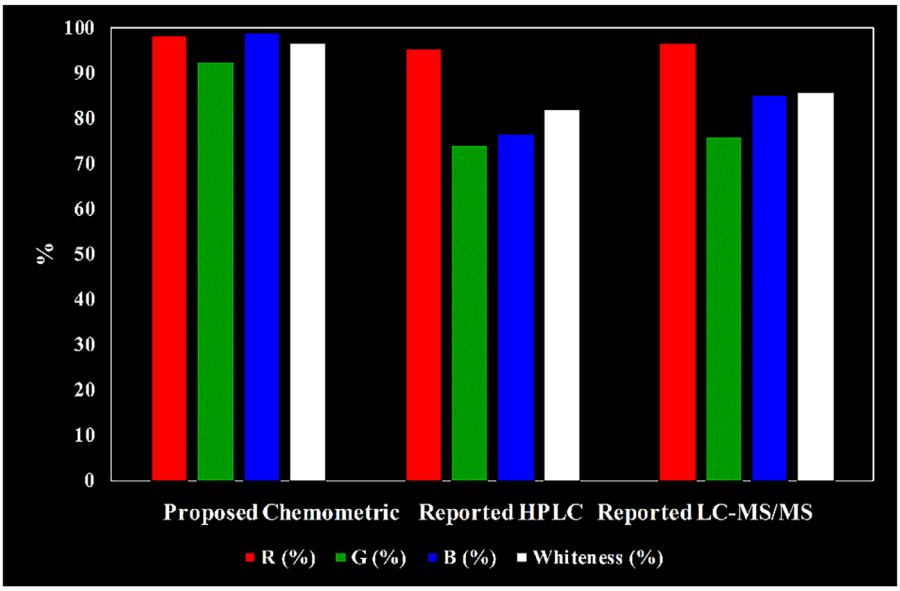


Fig. 7. The RGB 12 assessment of comparison revealed significant assessments for both the proposed Chemometric and reported HPLC and LC-MS/MS techniques.

reported HPLC [42] and LC-MS/MS [43] methods are assigned 14 and 13 penalty points for FM2, respectively, culminating in overall scores of 3.0 for both approaches, as shown in Fig. (6A and 6B).

Concerning safety as well as hazardous toxicity measurement, the suggested chemometric methods and the reported HPLC [42] and LC-MS/MS [43] methods accumulate 12, 15, and 17 penalty points for toxicity assessment, resulting in a total rating equal to 1, 2, and 2, respectively, as a result of solvent's different levels of toxicity. Additionally, as a result of the flammability of the solvent, the suggested technique obtains one penalty point for safety evaluation.

The solvents involved in the reported techniques, on the other hand, are flammable and cause burns incurring an additional three penalty points. The total quantity of reagent needed will be given three penalty points for each of the suggested chemometric, published HPLC [42], and LC-MS/MS [43] techniques. As a result, the total ratings for safety are 1, 2, and 3, respectively, as shown in Fig 6B.

The green procedure for analysis in the residue study is decided through the lowest total of penalty points, taking into account criteria such as the quantity of waste created, ineffective handling, and non-disposable substance produced [49]. For the suggested chemometric method's residue appraisal, 4 penalty points were allocated for the quantity of waste created (varying from 1 to 10 mL) alongside no treatment carried out for the resulting waste. In contrast, both the reported HPLC and LC-MS/MS procedures earned 6 penalty points for producing more waste (between 10 and 100 mL) and non-waste treatment. As a result, the suggested chemometric technique received a 0 total score, while it was 1 for both the HPLC [42] and LC-MS/MS [43] methods in terms of residue evaluation, as shown in Fig. 6B.

A yearly cost evaluation was performed, taking into account aspects such as reagents, materials, power usage, and the labor cost necessary to accomplish the analytical determination over one year. The calculation estimated an all-encompassing collection of tests throughout an entire year, including competent individuals earning a set wage of 15 euros per hour, which was kept constant for both the suggested technique according to research and the stated methods [53], as shown in Fig. 6C.

The proposed chemometric procedure exhibits a remarkably low carbon footprint, as depicted in Fig. 6D, attributed to the sample treatment stage and analysis time, and additionally, the generated waste quantity is notably lower compared to the reported methods. These parameters reflect the adverse environmental impact of carbon footprint observations, which encompass the energy use of the devices and the time necessary for the investigation. It's widely acknowledged that the LC-MS/MS method's detector consumes significantly more energy than HPLC equipment. Consequently, the LC-MS/MS technique's carbon footprint assessment and yearly costing are greater than those of the HPLC approach.

The overall hexagon pictogram values given within the HEXAGON tool, when combined with the average mathematical (Sav) estimation, provide a simple technique for comparison via easy visual assessment [52]. As presented in Table 7, the Sav values for the suggested chemometric, reported HPLC [42], and LC-MS/MS [43] techniques were 1.0, 1.635, and 1.75, respectively. The tool efficiently assesses three key aspects of sustainability: method validity, green solvent scores, and financial implications, providing a powerful tool for evaluating analytical procedures. The hexagon tool incorporates GAC and WAC criteria, providing an extensive evaluation of the analysis strategies' environmental sustainability. It is possible to conclude that the suggested chemometric process is a potentially environmentally friendly and sustainable alternative to prevalent techniques for identifying six components with two contaminants in routine pharmaceutical studies. Notably, improvements in waste products, figures of merit, safety, toxicity, and carbon footprint have been found.

RGB12 and whiteness assessment

White Analytical Chemistry (WAC) evolved as the assessment aspect of a technique based on the RGB tool. WAC solves the RGB dilemma by functioning as a sequel and augmentation to GAC's concepts as well. In the context of WAC, a white analytical method signifies the consistency as well as the convergence of analytical, environmental, with economical features, drawing inspiration from the RGB color model, in which the intersection of the three colors of light rays (red, green and blue) forms a representation of white.

The degree of whiteness may be measured, providing a useful metric for comparisons and selecting the best approach. WAC is more closely associated with sustainable development by taking a holistic approach, avoiding an unqualified rise of "greenery" at the expense of performance [55–57]. The proposed approaches acquire an overall score of 96.5%, as shown in Fig. 7, confirming their efficiency and greenness. The suggested chemometric approaches were assessed against other published strategies in terms of whiteness, and the results are displayed in Table 7. In the three categories of the degree of Redness (evaluation accuracy), Greenness (health and environmental friendliness), and Blueness (beneficial efficacy), our proposed technique outperforms. The overall whiteness score outperforms the previously reported HPLC [42] and LC-MS/MS [43] methods. The RGB method is used to sum the markings of three unique areas or colors, resulting in the final value of "whiteness," which measures how well previously published and planned techniques comply with WAC ideas. As seen in Fig. 7. The recommended procedures have a high whiteness score of 96.5 and provide several benefits, including greenness, sustainability, analytical efficacy, and economic and practical concerns.

Conclusion

Sensitive, robust, green, and sustainable chemometric-assisted techniques including OPLS with variable selection procedures including a genetic algorithm (GA-OPLS) and interval iOPLS approaches for the simultaneous evaluation of the six regularly specified proton pump inhibitor medications in their both prepared mixture and tablets forms have been devised and thoroughly refined. Furthermore, these approaches were invented to identify possible impurities, with a focus on 4-Desmethoxy omeprazole and Rabeprazole impurity B. The suggested approaches demonstrated impressive performance in resolving a variety of combinations with extensively overlapped spectra while sustaining an excellent degree of precision and accuracy. These procedures have been validated and proved to be capable of evaluating medications under investigation while simultaneously quantifying impurities with minimal levels. As a result, they are frequently employed for regular drug analysis in both bulk and various dosage forms. Furthermore, these strategies were statistically compared to previously published methods. Several methodologies have been effectively implemented to measure and analyze both the greenness and whiteness of analytical chemometric approaches. NEMI, Advanced NEMI, Modified NEMI, the hexagon tool, WAC, and the RGB12 model are the best at covering all 12 Green Analytical Chemistry (GAC) ideas, additionally, the degree of greenness rating along with spider charts were employed to evaluate solvent sustainability, this multi-tool method provides a more reliable integrative sustainable evaluation by assessing key characteristics such as ecological sustainability, waste products, health and safety, convenience, and cost. One method to overcome the drawback of individual techniques is the employment of numerous complementary instruments. The method's consistently excellent results for greenness, and whiteness, highlight its sustainability benefits from several angles.

These Greenness metrics make good use of chemometrics and statistics to reduce the number of samples, displaying higher rates of environmentally friendly development ecological sustainability, and whiteness when compared to previously described methodologies. The assessments and validation criteria support the conclusion that the offered procedures are now appropriate for routine usage in quality assurance processes.

Declaration of competing interest

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.

CRediT authorship contribution statement

Khanda F.M. Amin: Conceptualization, Methodology, Software, Validation, Data curation, Writing – original draft, Investigation, Writing – review & editing.

Data availability

Data will be made available on request.

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