Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print) 2521-3520 (electronic)

REVIEW ARTICLE *OPEN ACCESS*

Chemometric Techniques for Enhancing UV-Vis Spectrophotometric Determination of Metal Ions: A Review

Suhair M. Yaseen [a], Mustafa F. Mahmood [a], Noor M. Yaseen [b], and Zahraa T. Khudhair [c]

[a] *Department of Medical Instrumentation Techniques Engineering, Electrical Engineering Technical College, Middle Technical University, Baghdad, Iraq*
[b] *Department of Physics, College of Science, Al-Nahrain University, Baghdad Iraq*
[c] *Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq*

CORRESPONDENCE
Suhair M. Yaseen
suhair.yaseen@mtu.edu.iq

ARTICLE INFO
Received: Nov. 08, 2025
Revised: Feb. 24, 2026
Accepted: March. 09, 2026
Published: Mar. 30, 2026

ABSTRACT: ***Background:*** *Trace metal ions play vital roles in living organisms and, at higher concentrations, are important in industrial applications such as food supplement production and polymer manufacturing. Therefore, reliable analytical methods for quantifying metal ions in different samples are urgently needed. Spectrophotometry is a widely used technique for this purpose due to its simplicity, sensitivity, rapid analysis, and cost-effectiveness in pharmaceutical, environmental, and industrial fields.* ***Objective:*** *The simultaneous quantification of multiple analytes in a single sample hinders the classical spectrophotometric methods. To overcome issues such as matrix interference, signal noise, and the need for separation or concentration steps, chemometric techniques combined with spectrophotometry have been proposed as effective solutions. However, despite their advantages, many chemists remain partially or fully unfamiliar with these analytical approaches.* ***Methods:*** *The current review article aims to attract more attention to the concepts of chemometric statistical models and their applications in UV-Vis spectrophotometric determination of metal ions and drug contents, which have been recorded in international publications between 2015 and 2025. We summarized and compared the publications on metal ion determination using only classical UV-Vis spectrophotometry and by using a combination of classical and chemometric techniques; we also compared those of chemometric models for drug spectrophotometric determination.* ***Results:*** *During the past ten years, studies on metal ion determination using chemometric-assisted spectrophotometry represented 32.7% (17 out of 52) of the total published research. In the same period, drug-related chemometric research was about four times higher than metal studies. This difference arises because metal ion analysis usually requires complexation with organic reagents to form colored complexes, while drug determination often relies on their inherent absorption bands without complexation.* ***Conclusions:*** *It is essential for chemists to understand chemometric principles as a modern, essential, and advanced branch of analytical chemistry that opens many new windows for research with many benefits and great facilities.*

KEYWORDS: *Chemometric techniques; UV-Vis spectroscopy; Metal ions determination; Statistical treatment; Sample matrix*

INTRODUCTION

All heavy metals are toxic at concentrations above the allowable limitations [1], [2]. Many of these ions play vital roles in living organisms [3]–[5] and industrial processes, such as drug manufacturing, food supplement production, polymer fabrication, and agricultural systems. The cornerstone of metal spectrophotometric analysis is the availability of a chelate reagent that is required to react with metal ions and form a complex. The term "chelate" is derived from the Greek word "chele", which refers to a lobster claw, implying that the organic reagent should possess more than one dentate

functional group. Spectrophotometric quantification in the UV-Vis region is a cost-effective method that offers high accuracy and reproducibility through a simple procedure. Consequently, spectrophotometric quantification is widely used in all research institutes and analytical laboratories, and it has high performance in the determination of simple compounds as well as in complex mixtures [6].

A UV-Vis recorded spectrum is the sum of analyte absorbance and matrix (reagents, buffers, and accompanying compounds). The recorded band of the analyte is distorted by the background, which can be defined as the absorbance exhibited by the matrix. Isolation of an analyte from a matrix is a logical solution for increasing the selectivity, specificity, reproducibility, and sensitivity of an assay. However, every additional operation introduced into the sample preparation procedure extends the time and cost of a single analysis and increases the risk of loss or contamination of the analyte. In classical spectrophotometric organometallic analysis, a proactive step (technique) of preconcentration or separation may or may not be necessary in the analytical procedure for quantifying trace metal ions from various sample matrices [7]. Therefore, an extraction step is necessary to be performed before quantification using a chelated ligand as an extractant (in the organic phase) that reacts with metal ions, forming a complex that is spectrophotometrically determined [8], [9]. Some samples of metal ions are required to preconcentration step before performing spectrophotometric analysis to meet method quantification limits and linearity response. Separation of metal ions to be determined from other interferences to improve method selectivity and sensitivity.

In addition, undesired contents in the source spectrum can be filtered. Optical, electronic, and mathematical techniques have been used in early UV-Vis spectrophotometers; however, the latter technique is largely used for the enhancement of spectrophotometric determination without the need for time- and cost-intensive sample pretreatment. These techniques are listed under the chemometric term. Chemometrics is considered a relatively new branch of science that has been defined as "the chemical discipline that uses mathematical, statistical, and other methods employing formal logic (a) to design or select optimal measurement procedures and experiments and (b) to provide maximum relevant chemical information by analyzing chemical data" [10]. In spectroscopic mixture and signal analysis, the main obstacle is the spectral recovery of all mixture components. With spectra of pure substances, it is possible to qualify and quantify all individual components of a mixture and study the factors that affect component concentrations. Mixture samples can be determined and physiochemically studied without the need for a separation step using chemometric methods, which depend on soft modelling data [11]. Many modern analytical instruments have computers or microprocessors enriched with chemometric software, such as MATLAB, by which many complex mathematical calculations can be replaced by a single coding expression. In addition to the series of artificial neural network methods, genetic algorithms, support vectors, and wavelet transforms are used by analysts to solve problems in spectrophotometric analysis [12]. Chemometrics has been a part of analytical chemistry since the early 1970s. It has many analytical applications, such as process control analysis, food analysis, forensic science, metabolomics, clinical diagnosis, environmental monitoring, reaction monitoring, and synthesis optimization. The expected error in spectrophotometric determination is the interference of the recorded spectrum, which is the sum of the absorption of the analyte and its matrix. In the case of simple samples, uncomplicated matrix interference can be corrected by omitting the background measurements versus the blank. However, many complicated spectral-related errors have been minimized by various mathematical modifications in the spectrophotometer software, such as: removes spectral interferences caused by the scattering of light, eliminates background (matrix) absorption, and thus improves the signal-to-noise ratio [12]. Improve the capability to identify small spectral features; accordingly, multicomponent analysis can be effectively performed [12]. Increase sensitivity, specificity [13], accuracy, and precision [12]. Easily calculated and re-calculated different parameters, such as physicochemical constants and complexation or binding constants [14]. Chemometrics provides a wealth of techniques for both the exploratory analysis of multivariate data and the development of reliable calibration and classification strategies to predict quantitative and qualitative responses based on experimental profiles collected from the samples.

Chemometrics development has passed through four stages [12]: In the first stage, pre-establishment, basic concepts of statistics, such as standard deviation, least-squares regression, and confidence intervals, were used by analysts without a common name for chemometrics. In the second stage, chemometrics was born, and its definition occurred in 1970. In the third stage of the 1980s, many chemometric techniques witnessed significant development in theory and algorithms, such as partial least squares, rank annihilation factor analysis, and multivariate calibration. Moreover, chemometric research became a trend because of its publication in the professional journals "Journal of Chemometrics" (1987, Wiley) and "Chemometrics and Intelligence Laboratory Systems" (1988, Elsevier). In addition to the MATLAB software (1984, officially launched by the American MathWorks Company), which replaced many complicated mathematical calculations with a single coding expression.

In the fourth stage, during the 1990s, chemometric techniques entered many fields of practical applications, such as analytical instruments with computers or microprocessors, sensors, and medicine and pharmacy.

The current study explains the principles of different chemometric techniques and highlights their important applications in analytical chemistry research, in addition to their role in overcoming the obstacles faced by classical spectrophotometry, such as complex sample matrices, response signal interferences, and the difficulty of analyzing more than one analyte in the same sample without the need for additional steps. Understanding chemometric techniques and the analytical facilities they offer opens up diverse and new research horizons and paves the way for joint scientific collaborations between chemists and specialists in processing programs. Iraq was isolated from the world during 1990–2003 because of the economic and cultural blockade, which led to a decline in the understanding and use of chemometrics techniques. The current educational overview attempts to focus light on basic chemometrics principles and their extensive applications in the determination of metal ion and drug contents. In most cases, the spectrophotometric determination of drug constituents can be performed directly depending on their characteristic electron transport bands of the organic content, which can be reliably used analytically. However, because of the lack of characteristic absorption bands, some other drugs as well as most metal ions need to undergo a prior chemical reaction of complexation or redox reactions to obtain a product with analytically considered absorption bands. Chemometrics in both drug and metal ion determination are viewed and compared with each other as a result of the need to analyze or monitor them in vital samples, such as water, blood, urine, and pharmaceuticals. In the past 10 years, Iraqi chemists have achieved advanced rankings in publishing in Scopus-indexed journals; therefore, we chose the period 2015–2025 to bridge the gap in understanding the importance of these technologies and cover it within our research period.

CHEMOMETRICS METHODS

In spectrophotometric quantification analyses, many achievements of data treatment, such as smoothing data, removing baselines, peak recognition, peak fitting, recognizing patterns, and categorizing data, can be performed and are included under the chemometric term. There are many different chemometric techniques, such as the least-squares method (LSM) [15], partial least squares (PLS), inverse least-squares (ILS) [16], principal component analysis (PCA), multiple linear regression (MLR), derivative spectra (DS) [13], principal component analysis, rank annihilation factor analysis (RAFA), and several others [14].

The Least-Square Method (LSM)

The least-squares method (LSM) is an important statistical method used to determine a regression line or best-fit line for the given data. The best-fit result is expected to reduce the sum of squared errors (deviations) or residuals, which are the differences between the experimental value and the corresponding fitted value given in the model. This method is considered a standard approach for the approximation of sets of equations with more equations than unknowns [16]. There are two basic categories of least-squares problems: ordinary (linear) least-squares and nonlinear least-squares. As shown in Figure 1, which corresponds to linear least squares, if the given points of data are $(x_1, y_1), (x_2, y_2), ..., (x_n, y_n)$ in which all x values are independent (predictor) variables, while all y values are dependent variables. In addition, suppose that f(x) is the fitting curve and d represents the deviation from each given point. Then, the least square (also known as deviation or error) treatment is performed according to the following equations [17]:

$$d_i = x_i - f(x_i) \tag{1}$$

The least-squares method states that the curve that best fits is represented by the property that the sum of squares of all deviations from the given values must be minimum, that is,

$$S = \sum_{i=1}^{n} d_i^2 \tag{2}$$

The least-squares line equation is given by:

$$Y = a + bX \tag{3}$$

Normal equation for determining' a':

$$\sum Y = na + b\sum X \tag{4}$$

Normal equation for evaluating' b':

$$\sum XY = a\sum X + b\sum X^2 \tag{5}$$

Solving these two normal equations yields the required trend line equation. Thus, we can get the line of best fit with the formula $y = ax + b$.

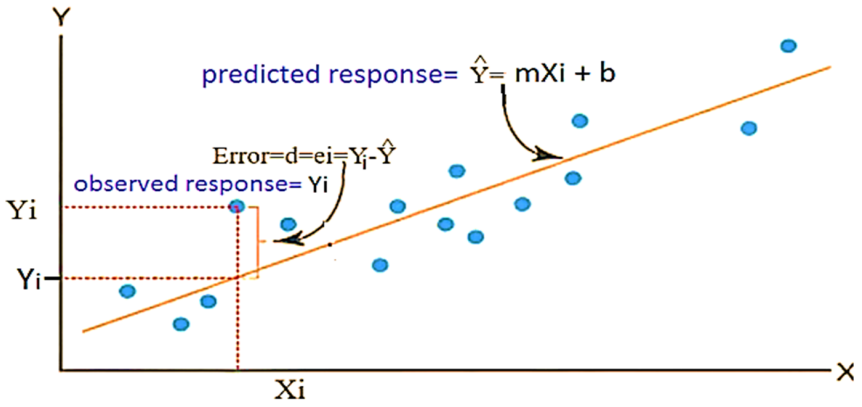


Figure 1. Representation of least square method [15]

1 Classical Least Squares (CLS)

Classical least squares (CLS), ordinary least squares (OLS), or direct least squares is the K-matrix algorithm used for data analysis and calibration curve fitness. Spectroscopists use this algorithm based on the Lambert–Beer law [17]:

$$A_i = a_i b c_i \tag{6}$$

where A_i is the total absorbance of the ith component in the sample mixture a_i is the absorptivity of the pure ith component, b is the pathlength of light passing through the sample, c_i is the concentration of the pure ith component. The proportional relationship between A_i with a_j, c_j and b is correct at each chosen wavelength of the molecule spectrum. Because b is constant, it is possible to combine it with a to get the product ab as the quantity we are measuring. This product was represented with the symbol K, the origin of the "K matrix" nomenclature. Equation 6 is suitable to apply for a single component in a sample. While in a sample of multiple absorbing components, the total absorbance is the sum of the absorbance of all the absorbing materials at the wavelength of interest.

$$A = \sum_i a_i b c_i \tag{7}$$

The light path length is a constant for the sample cell containing all sample constituents. The fixed b is multiplied by a to produce ab as the measured quantity, represented by the K symbol or K matrix. For a sample of three components and one value of b, the equation of absorbance at the j wavelength is

$$A_j = a_{1j}c_1 + a_{2j}c_2 + a_{3j}c_3 \tag{8}$$

E is the error in determination A_j value, and it is defined as:

$$E = A_j - (a_{1j}c_1 + a_{2j}c_2 + a_{3j}c_3) \tag{9}$$

Based on the "least-squares" principle, error is minimized by the sum of squared values to obtain A values for all j wavelengths. The sum of squares is expressed as follows:

$$\sum_j (E_j)^2 = \sum_j (A_j - a_{1j}c_1 - a_{2j}c_2 - a_{3j}c_3)^2 \tag{10}$$

The sum square error is error (4) of the sum-squared is minimized more by taking its derivative with considering to three concentrations (c_1, c_2, c_3) and setting ZERO derivative value. The result will be:

$$\frac{d(\sum_j E_j^2)}{dc_{(1,2,3)}} = 2(\sum_j A_j - a_{1j}c_1 - a_{2j}c_2 - a_{3j}c_3)\sum_j a_{(1,2,3)j} = 0 \tag{11}$$

By rearranging the equations, we obtain

$$\sum_j a_{(1,2,3)j}A_j = \sum_j a_{1j}a_{(1,2,3)j}c_1 + \sum_j a_{2j}a_{(1,2,3)j}c_2 + \sum_j a_{3j}a_{(1,2,3)j}c_3 \tag{12}$$

By converting the equations into matrices, the result is

$$[c][aa^T] = [a][A^T] \tag{13}$$

Solving the relationship above yields the concentration as follows:

$$[c] = [A][a]^T[aa^T]^{-1} \tag{14}$$

Equation (14) is the equation of classical or direct least-squares methods and appears to be an MLR equation. In both MLR and CLS formulations, the spectra of mixture samples are measured. In multiple linear regression (MLR), least-squares results are used to provide correlations between the spectra and reference laboratory values. In contrast, in the classical least-squares (CLS) approach, the spectra are not compared with laboratory values but with the spectra of pure components. In the case of the CLS algorithm, but not of MLR, the spectra of the pure components in the mixture must be measured, as they are used instead of the reference laboratory data obtained by conventional methods of analysis. The pure-component spectra are regarded as absolute and are therefore used directly in the computation process [18]. Hence, the basic distinction between the classical and inverse least-squares approaches does not lie in the calculation of the methods and the equations of the matrices, which are fundamentally identical, but in the meaning attached to the variables in the equations.

2 The Inverse Least Square (ILS)

Inverse least-squares (ILS) or P-matrix calibration involves the application of multiple linear regression to the inverse expression of the Beer–Lambert law for spectroscopy: $C = PA$, where P is a matrix that contains one row of coefficients for each component being predicted [18]. These coefficients are used to predict the unknown concentrations of analytes from their spectra. This technique is applied in the near-infrared (NIR) community. The [c] vector (in (14)) represents the concentrations of various sample components; in the MLR equations, these concentrations are known using other external laboratory methods. In the CLS method, the concentrations are unknown and are computed as a result of the least-squares calculation. The concentration of mixture components is computed based on the lambert beer principle of proportionality between absorbance and concentration.

3 Partial Least Squares Regression (PLS)

Regression analysis models the relationship between one, two, or more latent variables of X and the dependent variables of Y by performing suitable statistical algorithms. There are many regression methods, and the simplest one is ordinary least squares (OLS), which minimizes the sum of squared deviations and finds the best data fit. While PLS maximizes the covariance between latent variables and dependent variables, which helps in dealing with high-dimensional and multicollinearity data. The PLS regression equation is represented by the following [19].

$$Y = XM + E \tag{15}$$

where Y represents the dependent variable, X is the predictors' matrix, M is the regression coefficients matrix, and E is the residuals matrix. The requirements of PLS regression to apply are that the predictors are highly correlated, or there are more predictors than observations; in such cases, application of ordinary least-squares regression either produces coefficients with high standard errors or fails completely. PLS does not postulate that the predictors are fixed, unlike multiple regression.

The predictors can be measured with error by PLS application; therefore, PLS will be more robust to measurement uncertainty [20]. The PLS technique works by reducing the predictors to a smaller set of orthogonal factors that capture most of the variable features and performs least squares regression on these components instead of the original data. PLS regression is used in the industrial process of complex samples of chemicals, drugs [21], food, and plastics. A common application is to model the relationship between spectral measurements (in the NIR, IR, and UV regions), which include many variables that are often correlated with each other and chemical composition or other physicochemical properties. A full-spectrum method can be another description of PLS regression; therefore, it enjoys the advantages of signal averaging in comparison with other full-spectrum methods. As shown in Figure 2, PLS regression depends on finding the fewest number of orthogonal latent variables, which include the most significant information of both predictors X and response Y variables.

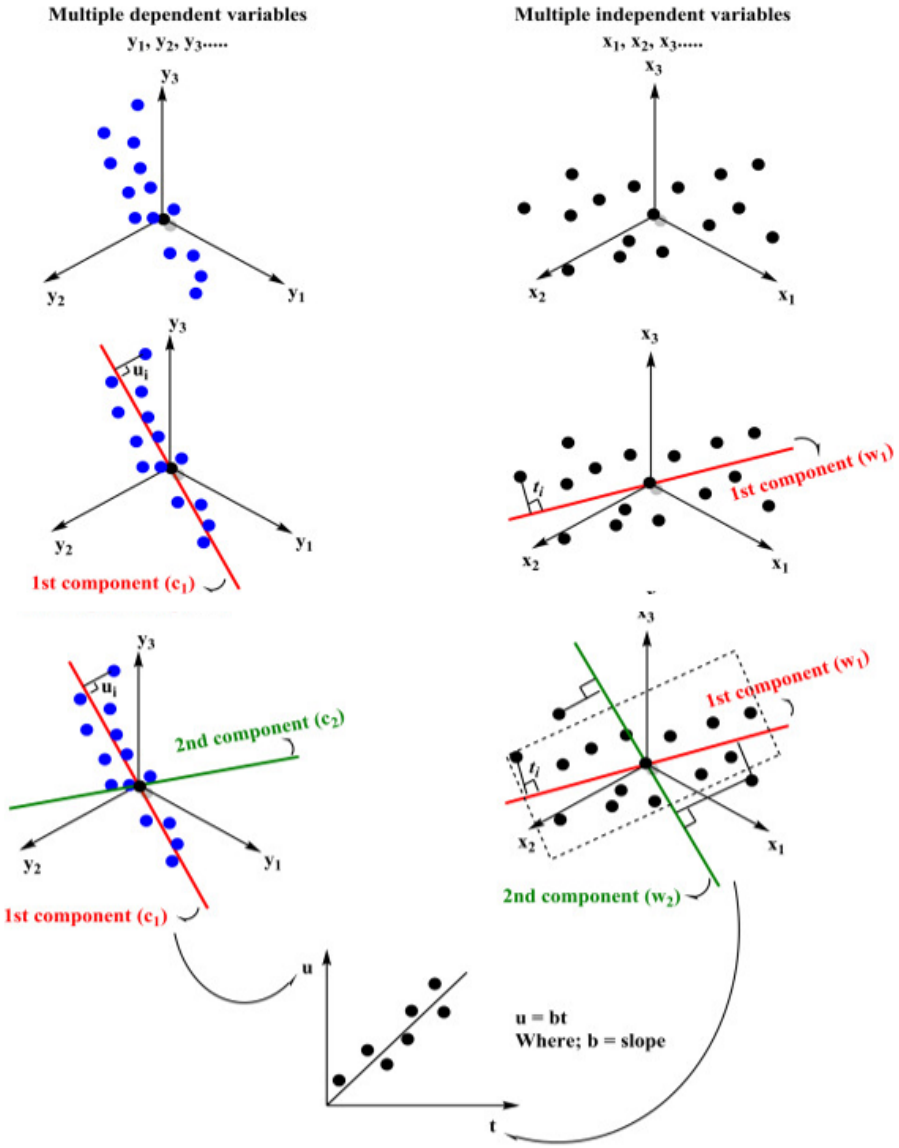


Figure 2. Representation of the PLS concept [22]

Principal Component Analysis (PCA)

Principal component analysis (PCA) is a technique that reduces the number of dimensions in large datasets to principal components that retain most of the original information. This is achieved by transforming potentially correlated variables into a smaller set of variables, known as principal components. As shown in Figure 3, PCA is based on finding the variables that are most strongly

correlated with each component; that is, which of these variables have large magnitudes and are farthest from zero in either direction. The decision to include or exclude variables must be subjective. In PCA, two matrices of a smaller dimension, known as the score and loading matrices, are generated from the PCA implementation on a given dataset. The score matrix that describes the relationship among the samples can be regressed against the analyte concentration to develop a calibration model, also known as the principal component regression (PCR) approach [23]–[25].

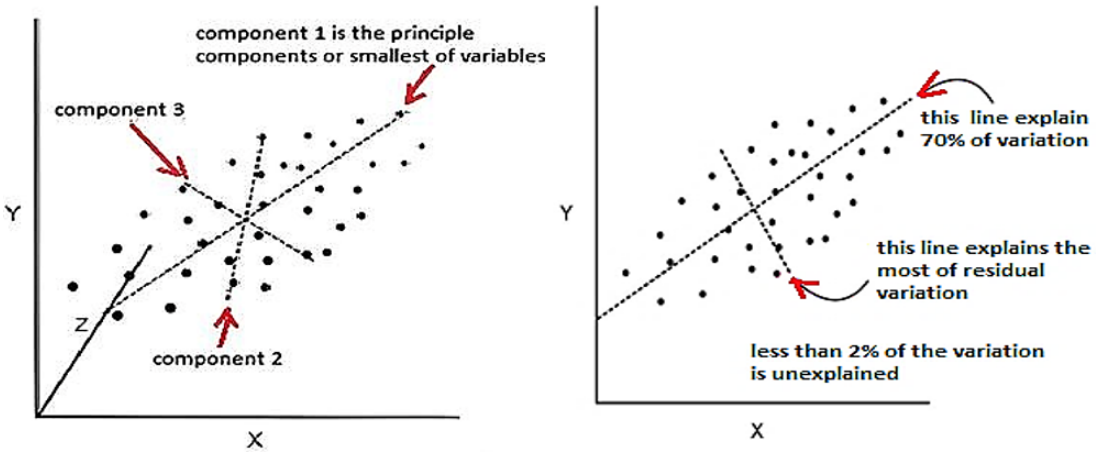


Figure 3. The basic concept of PCR technique

The Multiple Linear Regressions (MLR)

The MLR approach is similar to PCR; however, it has a few limitations, such as the relative abundance of response variables to the number of available calibration samples for the typical spectral calibration model, unstable matrix inversions, and regression outcomes resulting from the collinearity of the predictor variables [26]. Both PCA and MLR are performed based on collinearity approaches and maximizing the correlation between the predictor and predicted variables. More than two independent variables can be considered in the MLR analysis. The MLR models the relationship between independent (X) and dependent (Y) variables using a linear equation. It is suitable statistical method to use in condition of small no. of independent variables. The MLR analysis is suitable for the following cases: (i) simple data; (ii) more than two independent variables are considered; (iii) the three variables are not highly correlated; and (iv) noise sensitivity in the data is not required because there are no variable reductions [27]. Because MLR and PLS are similar regression methods, when MLR is suitable for simple dataset analysis, MLR can be replaced by PLS. However, with complex datasets, MLR fails to provide accurate results because of the absence of variable reduction. PLS is a generalization of MLR, meaning that in some cases where MLR would be suitable, PLS can also be applied [19].

Derivative spectrophotometry (DS)

Derivative spectrophotometry (DS) plays an important role in multi-component analysis. The digital algorithm method is known as Savitzky–Golay. The normal spectrum (fundamental, zeroth-order spectrum) is differentiated by converting the spectral curve into first- or higher-order derivatives by differentiating the absorbance (dA) of the sample with respect to "wavelength ($d\lambda$) or time (dt)" versus wavelength (λ) or time (t) [28], [29]. DS is very effective in the signal separation of an analytical compound from the background (analyte matrix) or other compound signals. In addition, it enhances the sharpness and interpretability of the peaks. It has a high signal-to-noise ratio, high selectivity and specificity, high precision and accuracy, robustness, good limit of detection and quantification, and high linearity with a good range [28]–[30]. DS maintains all the characteristics of classical spectrophotometry, such as obeying Lambert–Beer and additivity laws, which can be expressed in their differential forms [29], [30] as follows:

$$\frac{d^n A}{d\lambda^n} = {}^{n}D_{x,\lambda} = f(\lambda) \quad \text{or} \quad {}^{n}D_{x,\nu} = \frac{d^n A}{dV^n} = f(v) \quad \text{or} \quad {}^{n}D = \frac{d^n A}{d\lambda^n} = \frac{d^n \epsilon}{d\lambda^n} = C \tag{16}$$

where n,$^n D_{(x,\lambda)}$ are the order of derivative and derivative amplitude value respectively of analyte (x) at analytical wave lengthy (λ) or at wave number (v), A-absorbance, ϵ is molar absorptivity coefficient (L/(mol.cm)), C is analyte molar concentration (molar), l is thickness of solution layer in (cm) unit. Derivative spectrum of n-components mixture (analyte matrix) is a sum of derivative spectra all individual components [30]:

$$^n D_{mix} =^n D_1 +^n D_2 +^n D_3 + \ldots +^n D_n \tag{17}$$

The results of spectrum derivatization depend on the geometrical characteristics of the original (or starting zeroth-order) spectrum, and this dependence is considered to be a valuable feature. The shape and intensity of the derivative obtained depend on the half-height width of the peak in the basic spectrum (zeroth-order derivative):

$$^n D = P^n A_{max} L^{-1} \tag{18}$$

Here, P^n is a polynomial that describes the run of the n$^{\text{th}}$-order derivative curve, and L represents the width of half the height of the peak in the basic spectrum. Based on this property, the broad zero-order spectra are quenched with the generation of higher-order derivatives, whereas the narrow ones undergo amplification. If the zero-order derivative spectrum processes two bands, A and B, which differ in their half-height widths ($L_B > L_A$), the integration of the derivative intensities after the generation of the n$^{\text{th}}$-order derivative can be represented as:

$$\frac{^n D_A}{^n D_B} = \left(\frac{L_B}{L_A}\right)^n \tag{19}$$

Thus, increasing the selectivity and sensitivity of the assay is due to the feature of dependence that permits the use of an overlapped, narrow, or even completely hooded band by a broad band. As shown in Figure 4, the shape of the resulting derivative spectrum is more complicated than that of its parent. Spectrum derivatization leads to the appearance of new maxima and minima. The (n + 1) new signals are the results of the derivative spectrum of the n$^{\text{th}}$ order; these new (n + 1) signals have an intense main signal and weaker bands called satellite or wing signals. The positions of the minima and maxima depend on the order of the spectrum derivative.

In even-order derivatives (except for the 2, 6, and 10th-order derivatives), the main spectral extreme is located at the same wavelength as the zero-order spectrum, and vice versa for the 2, 6, and 10th-order derivatives. In odd-order derivatives, the point of inflection of the spectra is situated in the same position as the point of initial maximum of the zero-order spectrum. During the performance of consecutive derivative spectra, a narrowing of the new signals is observed. The advantageous property of narrowing bands helps to solve the problem of overlapped peaks by separating them [31]. As shown in Figure 4b, a first-order derivative starts and finishes at zero. It also passes through zero at the same wavelength as the maximum of the absorbance band. On either side of this point are positive and negative bands with maxima and minima at the same wavelengths as the inflection points in the absorbance band. While the most characteristic feature of a second-order derivative is a negative band with a minimum at the same wavelength as the maximum on the zeroth-order band. It also shows two additional positive satellite bands on either side of the main band as in Figure 4c. In the Figure 4d, the bipolar function of 1st derivative is characteristic of all odd-order derivatives. Unlike second order, spectrum 3$^{\text{rd}}$ derivative spectrum shows disperse function to that of original curve. Finally, in Figure 44e the 4$^{\text{th}}$ order derivative shows a positive band. A strong negative or positive band with minimum or maximum at the same wavelength as max of the absorbance band is characteristic of the even-order derivatives.

Derivative methods are used in analytical chemistry to perform the following:

- Spectral differentiation: This is considered a qualitative method that distinguishes between small variations in similar spectra.
- Spectral resolution improvement: The problem of overlapping spectral bands was resolved by simply distinguishing the entire number of bands and their wavelengths.
- Quantitative analysis. This makes the analysis of multicomponent samples possible and provides a simple process. In addition, it corrects for the related background absorption. The derivative spectroscopy technique is considered the beginning of the separation of overlapped

bands; the active role of the derivative process suppresses broad bands and produces relatively sharp bands. In samples quantification of multicomponent drugs and multi-ions assay, derivative spectrophotometric quantification methods improved linearity and increased recovery.

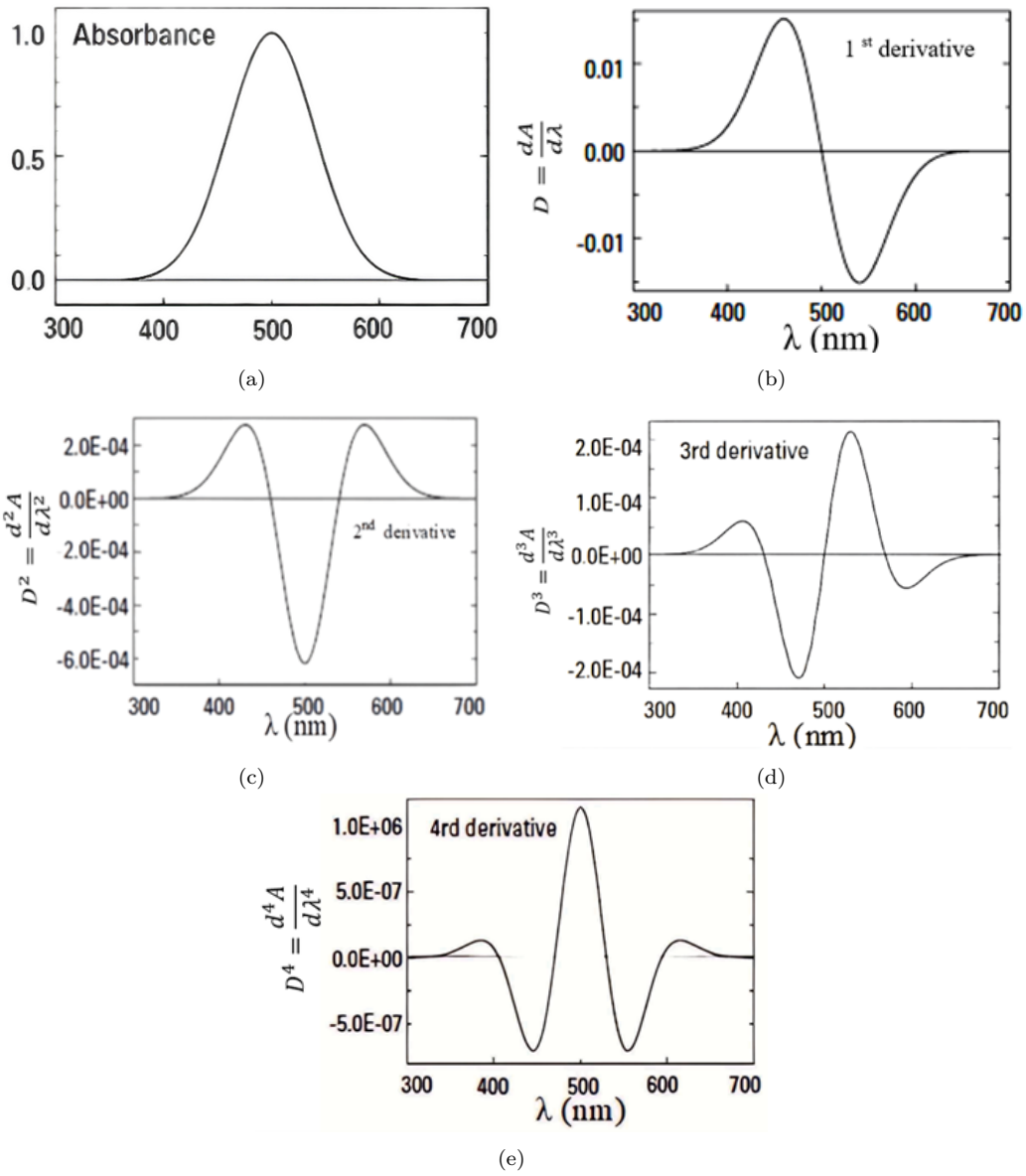


Figure 4. a) The parent band (zero derivative order) of absorption spectrum and its b) 1st derivative, c) 2nd derivative, d) 3rd derivative and e) 4th derivative [29]

1 Ratio Spectra (By Divisor or Double Divisors) and Ratio Spectra Derivative Techniques

The performance of ratio spectra (RS) followed by achieving one of the calculation techniques are procedures widely used in the quantification of multicomponent samples. Ratio or ratio spectra derivative methods are proposed for the simultaneous determination of multicomponent mixtures without the need for prior separation steps. The method is based on the conversion of normal spectra to their first, second, and third-derivative spectra. The ratio spectra derivative method is performed by dividing the mixture (of two or more components) spectrum by the standardized spectra of each analyte with a known concentration (called dividsor) and deriving the resulting ratio to obtain a spectrum that is not dependent on the analyte concentration. While ratio derivative techniques were

suggested by researchers for the quantification of binary or ternary mixtures of some drugs without the need for a separation step and performed excellent results [32]. As example to clarify:

The ratio spectra method is used to quantitatively analyze a mixture of two components, X and Y (without interaction between them). The mixture absorption obeys Lambert–Beer law, as follows [33]:

$$A_M = \alpha_X C_X + \alpha_Y C_Y \tag{20}$$

A_M is the mixture's absorbance, α_X and α_Y are the molar absorptivity of X and Y components, C_X and C_Y are concentrations of X and Y components respectively. Absorbance of prepared standard solution of Y component (with concentration equaled to its concentration in the sample mixture or not) is recorded and expressed in following equation:

$$A_{Y^\circ} = \alpha_Y C_{Y^\circ} \tag{21}$$

where $A_{Y^\circ}$is absorbance of Y standard solution which has concentration of C_{Y°.

In single divisor of Y, by dividing (20) on (21), the divisor ratio spectra method is represented by

$$\frac{A_M}{A_{Y^\circ}} = \frac{A_X}{A_{Y^\circ}} + \frac{C_Y}{C_{Y^\circ}} \tag{22}$$

The term $\frac{C_Y}{C_{Y^\circ}}$is constant and it will be eliminated when the difference in absorbance between two wave lengths (peak to peak ratio) is considered according to the following equation:

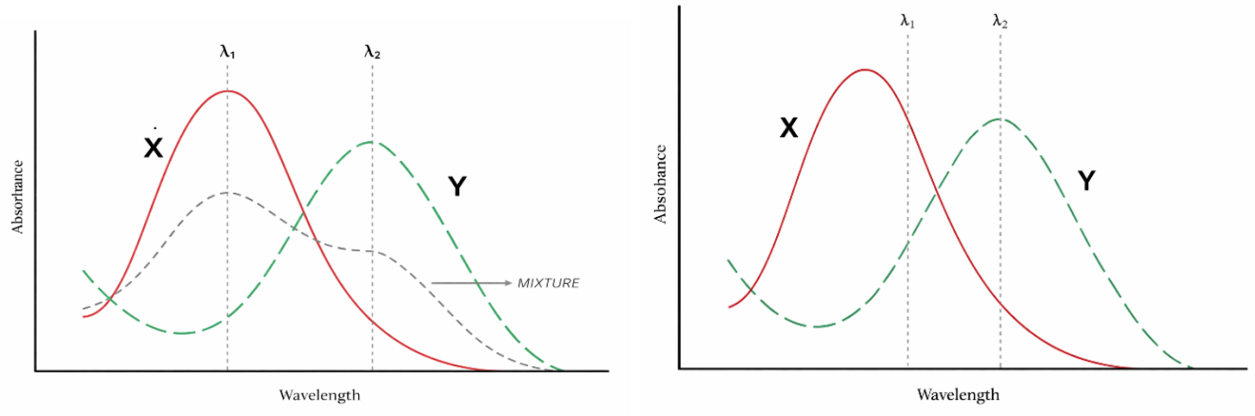
$$(\frac{A_M}{A_{Y^\circ}})_{\lambda_1} - (\frac{A_M}{A_{Y^\circ}})_{\lambda_2} = (\frac{A_X}{A_{Y^\circ}})_{\lambda_1} - (\frac{A_X}{A_{Y^\circ}})_{\lambda_2} \tag{23}$$

Equation (23) indicates that the difference in the absorbance ratio amplitudes between two wavelengths is proportional to the concentration of the X component, and this amplitude difference is constant when analyzing X alone or with the Y component as the interference (constant). A calibration curve is plotted using the amplitude difference obtained by analyzing pure X standard solutions. The same procedure is followed to quantify the other component of Y. to define the ratio spectra derivative method, the 1st derivative of ratio spectra equation of (23) is taken and the final results to determine concentration of X and Y components respectively will

$$\frac{d}{d\lambda}\frac{A_M}{A_{Y^\circ}} = \frac{d}{d\lambda}\frac{\alpha_X C_X}{\alpha_Y C_{Y^\circ}} \tag{24}$$

Therefore, by preparing pure standard solutions of X and Y and applying the steps of the ratio derivative method, the concentrations of X and Y in the sample mixture can be evaluated using the following equations [32]:

$$C_X = \frac{d}{d\lambda}\left(\frac{X absorbance}{Y Absorbance}\right) - \frac{Intercept}{Slope} \quad \text{and} \quad C_Y = \frac{d}{d\lambda}(\frac{Y absorbance}{X Absorbance}) - \frac{Intercept}{Slope} \tag{25}$$



(a) (b)

Figure 5. a) Absorption spectra of substances X, Y and mixture, b) Absorption Ratio Method (peak to peak) [34]

To analyze a mixture of (M) tertiary components (X, Y, and Z), the double divisor–ratio spectra method is performed using similar steps with a standard binary mixture (N) of Y and Z components (their concentrations are identical to their concentrations in the sample mixture or not). The absorbance of the M sample mixture and N binary mixture are represented by Equations and, respectively [35].

$$A_M = \alpha_X C_X + \alpha_Y C_Y + \alpha_Z C_Z \tag{26}$$

where A_M is absorbance of tertiary mixture.

$$A_N = \alpha_Y C_Y + \alpha_Z C_Z \tag{27}$$

A_N is absorbance of binary mixture (double divisor).

The final double divisor–ratio equation is

$$B = \frac{A_M}{A_N} = \frac{\alpha_X C_X}{\alpha_Y C_Y + \alpha_Z C_Z} + k \tag{28}$$

For the three-component mixture (X, Y, and Z) analysis, the maximum absorption wavelengths of X, Y, and Z are denoted as 1, 2, and 3, respectively. B is evaluated in three wavelengths, Equations (21)-(24) can be written as [36]:

$$C_1 = B_1 - B_3 = \left(\frac{a_{X1}}{a_{Z1}} - \frac{a_{X3}}{a_{Z3}}\right) C_X + \left(\frac{a_{Y1}}{a_{Z1}} - \frac{a_{Y3}}{a_{Z3}}\right) C_Y \tag{29}$$

$$C_2 = B_1 - B_2 = \left(\frac{a_{X1}}{a_{Z1}} - \frac{a_{X2}}{a_{Z2}}\right) C_X + \left(\frac{a_{Y1}}{a_{Z1}} - \frac{a_{Y2}}{a_{Z2}}\right) C_Y \tag{30}$$

$$D = \frac{c_1}{\left(\frac{a_{Y_1}}{a_{Z_1}} - \frac{a_{Y_3}}{a_{Z_3}}\right)\left(\frac{a_{Y_1}}{a_{Z_1}} - \frac{a_{Y_2}}{a_{Z_2}}\right)} = \left(\frac{\left(\frac{a_{X_1}}{a_{Z_1}} - \frac{a_{X_3}}{a_{Z_3}}\right)\left(\frac{a_{X_1}}{a_{Z_1}} - \frac{a_{X_2}}{a_{Z_2}}\right)}{\left(\frac{a_{Y_1}}{a_{Z_1}} - \frac{a_{Y_3}}{a_{Z_3}}\right)\left(\frac{a_{Y_1}}{a_{Z_1}} - \frac{a_{Y_2}}{a_{Z_2}}\right)}\right) C_X \tag{31}$$

Equation (30) is the mathematical foundation of multi-component analysis that allows the evaluation of X concentration in the sample solution without interference from other components. A calibration curve is constructed by plotting D versus X concentration in standard ternary mixture solutions. Obtaining ratio spectra followed by applying different calculation techniques is a common procedure in multicomponent analysis.

The mean centering of ratio spectra is a recent technique used to resolve severe overlapping in binary or tertiary component mixture. Other calculation techniques apply derivatives to this RS, considering the quantitative wavelength to resolve band overlapping problems. Derivative Spectroscopy is an analytical technique for extracting both qualitative and quantitative information from spectra which are composed of unresolved bands The differentiation of (29) yields

$$\frac{d}{d\lambda}\frac{A_M}{A_N} = \frac{d}{d\lambda}\frac{\alpha_X C_X}{\alpha_Y C_Y + \alpha_Z C_Z} \tag{32}$$

1 Zero-Crossing Difference Techniques

The method evaluates the derivative spectra at a specific wavelength, where the 1st and 2nd derivative crosses the point at zero-crossing wavelengths. At point of zero crossing, when absorbance of one component (A) has maximum value at λ_{max} of A, the absorbance of other component (B) equals to zero. According to this concept B interference can be removed in A component determination [29]. Figure 6 explains zero-crossing concept. Zero crossing difference method introduce a simple and reliable spectrophotometric quantification for analyte without interference of other components [32].

Rank Annihilation Factor Analysis (RAFA)

RAFA has been applied in the quantification of analytes in multicomponent samples [37], in the study of chemical reaction kinetics, in the evaluation of acid dissociation constants, stability constants, or formation constants of metal–ligand complexes [38], [39], and in the analysis of Stern–Volmer quenching constants in fluorescence, phosphorescence, or any other type of light emission

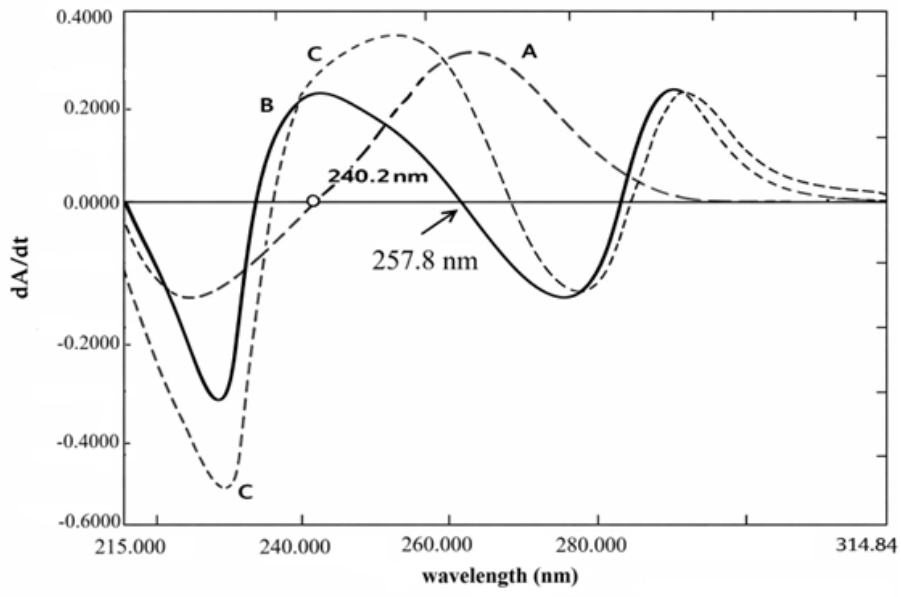


Figure 6. Representation of zero crossing difference method [32]

phenomenon [39]. Many chemometric methods have the disadvantage of interferences because they are mainly dependent on first-order techniques. Unknown interference matrices will cause inaccurate results in the analysis of real samples. Focusing on high-order data has attracted more attention to overcome this obstacle, especially in the analysis of multicomponent samples and complex matrices. Increased attention has been directed toward second-order analysis, in which single two-way matrix data results are obtained from a single sample. RAFA is an appropriate chemometric technique for the calibration and quantitative analysis of multicomponent samples with unknown interferences, depending on two-array data. In the RAFA technique, the rank of the data is reduced by subtracting the analyte contribution from the standard matrix. Owing to this treatment, a new data matrix with a rank that is one lower than that of the sample data is yielded, and the rank of the analyte of interest will be one.

The uniqueness of the decomposition of a three-way data array related to second-order data makes it applicable. The proposed RAFA technique has the 2nd-order advantage of extracting spectral profiles and individual component concentrations possible without the interference of uncalibrated absorbing components. This advantage of the 2nd order is the ability to quantitatively determine the effective substitute in a multicomponent sample without the need to remove sample matrix interferences. The third dimension in three-way array data may be a pH gradient, reaction time, or any factor that causes absorbance changes and leads to a rise in the absorbance spectra–pH data matrix.

A three-way array can be produced from these data matrices for a set of samples. RAFA has been widely used recently to determine the formation constants of 1:1 and 1:2 metal:ligand mole ratio complexes, and the equations are as follows [40]:

$$M + L \rightarrow ML(1) \tag{33}$$

$$K_f = \frac{[ML]}{[M][L]} \tag{34}$$

$$C_L = [L] + [ML] \tag{35}$$

$$C_M = [M] + [ML] \tag{36}$$

[L], [M], and [ML] are the equilibrium concentrations of the ligand, metal, and complex, respectively. Kf is formation constant of complex, CL is total concentration of ligand which remains constant while CM is total concentration of metal which is varied during mole ratio method performing. By substituting [M] and [ML] from (3) and (4) into (2) and rearranging, we obtain:

$$K_f\ [L]^2 + (K_f\ C_M - K_f\ C_L)[L] + [L] - C_L = 0 \tag{37}$$

Similar steps are derived to find the equation of K_f calculation of a complex with a 1:2 M:L mole ratio:

$$M + 2L \rightarrow ML_2 \tag{38}$$

$$K_f = \frac{[ML_2]}{[M][L]^2} \tag{39}$$

By following the same steps to express C_L and C_M and substituting them into (39), and rearranging it, the final form of the K_f calculation is

$$K_f[L]^3 + (2\ K_f\ C_M - K_f\ C_L)[L]^2 + [L] - C_L = 0 \tag{40}$$

The values of C_M and C_L are known, and the free ligand concentration [L] can be determined from the root of the associated polynomial. The equilibrium concentrations of the other species can be calculated using (3) and (4), and K_f was evaluated using RAFA. A two-way data matrix with a rank two was obtained by measuring the absorbance of different wavelengths of a series of M:L mole ratios with a constant analytical ligand concentration. The matrix rank decreases by one when the participation of one component from the original absorption matrix is removed using RAFA. Different vectors of ligand concentrations are obtained from substituting different values of Kf in Equation 40 for a given amount of CM and CL [38].

To shed more light on importance of chemometric techniques in UV-Vis spectrophotometric determination of metal ions, Table 1, and Figure 6 involved the published researches of metal ions quantification using chemometric-assisted spectrophotometric methods and by using classic UV-Vis spectrophotometric method during period 2015-2025. To demonstrate the broad application of chemometrics in the analytical analysis of drug contents, similar steps of spectrophotometric determination of metal ions were followed, and the published research in the field of drug quantification is listed in Table 2 and represented in Figure 7 for the aforementioned period. Finally, the comparison between no. Finally, a comparison between the number of published research articles in the two fields (metal ions and drug contents) of spectrophotometric determination based on chemometrics was conducted to understand the differences. The differences in published research are due to the fact that many pharmaceutical spectrophotometric determination procedures do not require the selection of a suitable reagent or the synthesis of a new one to react with the analyte and produce a sensitive-colored complex that is required for analysis, similar to that for metal ions.

Advantages and Challenges of Chemometric-Assisted UV-Vis Spectrophotometric Determination

Chemometrics principles, advantages, and limitations can be mentioned as follows [41], [42]:

- application of mathematical models to establish the connection, rationalize, and interpret the data obtained by chemical analysis.
- Include variability in the model and handle the variability using a distribution.
- Use statistical designs for planning sets of experiments when needed to alter or optimize conditions, rather than changing one factor and keeping others constant.
- Fourth, multivariate data analysis methods were used, the results were shown as plots, and the mathematical model was evaluated by determining the validation parameters.

  These achievements have been attributed to computer power, efficiency, and complexity, whereas data can be easily handled with common mathematical software packages; MATLAB is one such package. However, in analytical chemistry, chemometrics faces challenges related to preserving data integrity, transferring models from laboratories to production locations, and navigating complex information networks. Two common flaws in modern research employing proficient methodologies are small sample sizes and improper use of complex modelling tools. When using machine learning (ML) in chemistry, repeatability and data availability are crucial considerations. Calibration transfer is necessary to develop a single spectral database using all instruments: a combination of chemometric techniques and instrument technology. This is achieved by constructing multivariate calibration schemes, calibrating reference samples, and then generalizing these calibrations to other instruments. Chemometrics faces several challenges when dealing with high-dimensional data, such as feature selection, clustering, and anomaly detection.

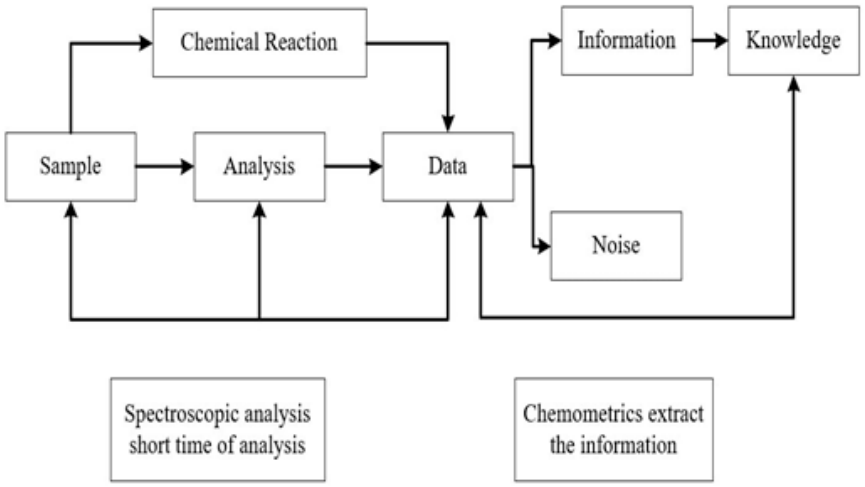


Figure 7. Illustration of the performance of the combination of spectroscopy and chemometrics

Conventional chemometric methods become more complex as dimensionality increases and result in more factors influencing the model and lower interpretability. The estimation of correlation matrices, which is an important task in both supervised and unsupervised learning, is also more challenging in high dimensions and requires sufficient samples to ensure accuracy. Many statistical algorithms fail in such situations, and new methods are required that can handle the size and complexity of high-dimensional data, as few reliable methods are currently available. Classical least squares (CLS): is the application of MLR to the conventional Lambert–Beer law equation, which is expressed as A = KC (or $\frac{dA}{d\lambda} = KC$), where A is the zeroth-order absorbance matrix, while $\frac{dA}{d\lambda}$ is the first-order derivative absorbance matrix, C is the concentration matrix, and K is the calibration coefficient matrix. The limitation of this chemometric technique is its conditional use with samples, wherein all constituents are identified and known. If any contaminant is present in the calibration mixture, the model yields incorrect results regarding the sample content concentration.

Inverse Least Squares (ILS) is known as p matrix calibration, and it also uses MLR to inverse equation of Lambert-beer law as in equation below: C = PA (or $C = P \times \frac{dA}{d\lambda}$) , where P is the calibration coefficient matrix. ILS has the advantage over CLS in that it can be used for analyte mixtures, even with unknown compositions. However, wavelength selection is a difficult and time-consuming decision. In addition, the number of wavelengths depends on the number of calibration curves because an accurate calibration requires a large number of samples. Principal component regression (PCR) is typically used for data with independent variables with a high degree of covariance. PCR depends on omitting vectors with small magnitudes to prevent collinearity problems. In PCR, data decomposition is performed using only spectral information. PCR solves collinearity, band overlap, and other interaction problems by deleting blower ranked principal components. The omitting action decreases the system noise level but reduces the sensitivity. Partial least squares regression (PLS) is a model related to both PCR and MLR, in which PCR captures the factors of most of the data variance before the regression of concentration variables. The correlation of data and their concentrations is performed using MLR. The covariance increases in this method because of the correlation of data and concentrations. In comparison with PCR, in PLS more parameters are necessary to measure large samples no. under various changing conditions. Artificial neural networks (ANNs): Under conditions of nonlinear calibration because of high noise levels, better results are obtained with the ANN approach. ANNs work by duplicating human brain functions. ANNs are a suitable choice for linear and nonlinear applications. A complex model infrastructure is considered the main disadvantage, and a large number of samples are required.

Table 1. Researches review on determination of metal ions using classical spectrophotometric analysis with or without Chemometric Techniques (2015-2025)

Ligand name	Metal ion	Analytical conditions	Technique used	Validation parameters of analytical method	Reference
2-[(4-antipyrinyl)azo] imidazol. (4- APAA)	Cu (II)	pH= 8-9, λ_{max} = 578nm for analyzing Violet complex	Classical tech.	1×10^{-5} - 1×10^{-4} M, L.O.D=1×10^{-6} M	[43]
2,2 -azino-bis(3-ethylbenzothiazoline-6-sulfonate (ABTS) by iron (III)	Fe (III)	415nm for analyzing green solution	Classical spectrophotometric Detection of nFIA and rFIA	0–4.5 mgL^{-1}, LOD= 25.5, , %RSD 0.97%,	[44]
o-vanillidine-2-amino-4-ethyl benzothiazole.	Cu (II)	pH=5.89, λ_{max} = 450nm for analyzing Brownish yellow complex	Classical tech.	0.41-3.78 $\frac{\mu g}{mL}$, Sensitivity =	[45]
Esomeprazole	Ni (II)	pH= 5 for analyzing greenish yellow	Classical tech.	0.139–1.394$\frac{\mu g}{mL}$, sensitivity= 0.0029	[46]
2-(benzothiazolyl azo)-4-nitrophenol reagent (BTANP)	Co (II)	pH= 7 and micelle-mediated extraction using Triton X-114 λ_{max} = 549nm	Classical tech.	10–300 ng/ml. RSD%= 0.03125 L.O.D(mg/L) = 1.31, L.O.Q(mg/L) = 4.37	[47]
(BPT) 2-Benzoyl pyridine thiosemi carbazone	Zn (II)	pH= 5, λ_{max} = 430nm	Classical tech.	0.1-6 μg/ml	[48]
2,6-diacetyl pyridine bis-4-Phenyl-3-thio semicarbazone 2,6-DAPBPTSC	Zn (II)	pH= 4.5, extraction with n-Butanol Yellowish Orange at λ_{max} = 490nm	Classical tech.	1.6-13.6 , L.O.D= 0.0081 , sensitivity = 0.0138	[49]
[2-(4-methoxyphenyl) azo(4, 5-dipheny imidazole)] (MPAI)	Co (II)	pH= 9, λ_{max} = 491nm	Classical tech.	IN μg/mL unit, linearity= 3-50, L.O.D=2.083, L.O.Q=6.309	[50]
3-Hydroxy-1,2-Benzoquinone	Bi(III)	pH= 4-6, λ_{max} = 510nm	Classical tech.	The constant of conditional stable of complex is logβ =5.185	[51]
Mixed ligand of mixed ligands of i. DTP-An ii. DTP-mAn iii. DTMP-An iv. DTPP-An v. DTBP-An 2, 6-dithiolphenol (DTP) 2, 6-dithiol-4-methylphenol (DTMP) 2, 6-dithiol-4-propylphenol (DTPP) 2, 6-dithiol-4-tert-buthylphenol (DTBP) amines aniline (An) N-methyl aniline(mAn)	Co (II)	λ_{max} = i. at 550nm ii. at 552nm iii. at 552nm iv. at 555nm v. at 560nm	Classical tech.	In μg/ml i. 0.5-14 ii. 0.5-80 iii. 0.5-85 iv. 0.6-80 v. 0.5-100	[52]

(2-hydroxy-5-nitrobenzylidene)-isonicotinoyl hydrazone HNBISNH 2-(4-fluoro benzylidene ami-Ne. (FBBT)	Ni (II) Ni (II)	pH= 4 at 480nm for red complex with HNBISNH, pH= 4.7 at 520nm for pale purple complex with FBBT	Classical tech.	0.81-19.7 , LOD = 0.89 0.84-19.0 , LOD= 0.82	[53]
2-((E)-(1H-benzo[d] imidazol2-yl)diazenyl)-5-((E)-benzyl ideneimino) phenol (BIADPI)	Cr (III) Fe (III)	pH=7.5 at 586nm pH=4 at 536nm	Classical tech.	1-14 , LOD=0.275 R.S.D%=0.9 1-21 , LOD=0.14R.S.D%= 0.467	[54]
4-[(4-antipyl azo)]orcinol(APAO)	Fe (II) Ni (II)	λ_{max} = 469nm for Fe (II) and λ_{max} = 461nm for Ni (II)	Classical tech.	In μg.ml (0.2-1.5), R.S.D = 1.8% and (0.4-2., R.S.D = 1.8%	[55]
tris(2 aminoethyl) amine-hexaacetic acid(TAHA)	Cu (II)	pH =10 Volumetric titration with ligand	Classical tech.	–M R.S.D%= (0.9-0.467) %	[56]
promethazine	Cr (VI)	red colored complex λ_{max} = 518 nm	Classical tech.	0.05-4.0 μg/ml	[57]
3-(2-HYDROXYPHENYLIMINO) INDOLIN -2-ONE	Ce (IV)	Extraction of Ce (IV) by reagent With pH 5.0, λ_{max} = 430nm	Classical tech.	1 - 12 ppm	[58]
5,7-Dibromo-8-hydroxyquinoline	Sn (II)	Yellow complex λ_{max} = 393nm	Classical tech.	0-9.0 μg	[59]
thoron (THO)	Pb (II) Cr (III), Ba (II)	In nm λ_{max} =539, 540 and 538nm for Pb (II)-THO, Cr (III)-THO and Ba (II)-THO respectively.	Classical tech.	In μgmL-1 :1 - 35, 1 - 70, and 1 – 45 for Pb (II), Cr (III) and Ba (II) respectively	[60]
Vilazodone Hydrochloride	Cu (II) Zn (II)	-	Classical tech.	2.40-12.0 μg/mL, L.O.D=2.93 L.O.D=13.67 μg/mL	[61]
3-(4 -Antipyriyl azo)-1-Nitroso-2- naphthol (APANN)	Cu (II)	pH 8.5 and λ_{max} = 430.5 nm	Classical tech.	0.1– 2.5 μg/mL	[62]
6-[(E)-(1,5-Dimethyl-3-Oxo-2-Phenyl-2,3-Dihydro-1H-Pyrazol-4-Yl)Diazenyl]-1H-Indole-2,3-Dione	Cr (III)	pale-red complex, 446.4	Classical tech.	0.15-1.18 ppm, L.O.D = 0.04ppm L.O.Q = 0.13 ppm	[63]
p- methylphenyl thiourea	Os (VIII)	Extraction using ligand in Chloroform and λ_{max} = 512nm	Classical tech.	Up to 60 μg/ mL, L.O.D =0.044 , RSD%= 0.096	[64]
(1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol- 3(2H)-one)	Co (II) and Pb (II)	pH = 7.5 for Co (II) purple reddish complex λ_{max} = 430 nm pH = 6 for Pb (II) red complex at λ_{max} = 417nm	Classical tech.	1 - 25 ppm, L.O.D = 15 ppm, RSD% = 0.096 1 - 33 ppm, L.O.D = 15 ppm, RSD% = 0.044	[65]

2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono) methyl)imidazo [1,2-a] pyridine (BDNHMIP)	Co (II)	λ_{max} = 530nm	Classical tech. as detection technique in flow injection	25-400 ppm, L.O.D = 2.28ppm	[66]
4-(N -(4-imino-2-oxo-thiazolidine-5-ylidene)-hydrazino)-benzoic acid	Pd (II)	pH= 7.0, λ_{max} = 450 nm	Classical tech.	0.64–10.64 , L.O.D = 0.23	[67]
desferrioxamine B (DFO)	Fe (III)	λ_{max} = 432 nm pH =3.5 - 8	Classical tech.	4.5×10−5 – 8×10−4 M, L.O.D = 0.008 mg/L, L.O.D = 0.026 mg/L	[68]
Glutaraldehydephenyl hydrazone (GPH)	Pb (II), Cr (III) Cd (II) and As (III)	pH of 6.5-7.5 and 20% (DMF) dimethylformamide solution to give stable coloured complexes.In nm λ_{max} = 387 for Cd-GPH (), 395 for As-GPH, 395 for Pb-GPH, 360 for Cr-GPH	Classical tech.	0.01 to 100 mg/L L.O.D (μg/g) = 0.3432 (As) - 0.5250 (Pb)	[69]
2-(4-biphenyl)imidazo[1,2-]pyrimidine-3-hydrazone (BIPH)	Cu (II)	pH = 4 yellowish green complex λ_{max} = 430nm	Classical tech.	0.05-500ppm, L.O.D= 0.122 L.O.Q= 0.4026 mg/L	[70]
Murexide	Zn (II) Cu (II)	pH=7 and λ_{max} = 450nm for Zn (II) complex and pH=5.5 and 470nm for Cu (II) complex,	Classical tech.	0.2-2.0 ppm, Sensitivity= 0.2982ppm, for Zn (II), 0.5-5.0 ppm, Sensitivity=0. 1017ppm for Cu (II)	[71]
2-((5-(2-hydroxy-3-methoxybenzylideneamino)–2H-1,2,4-triazole-3-ylimino)methyl)-6-methoxyphenol (HMBT)	Hg (II)	pH =10, λ_{max} = 475nm	Classical tech.	0.1–6 , L.O.D =0 0.016, LOQ= 0.051 μg/L., RSD %=0.51	[72]
2, 4-dimethyl -3H- 1, 5 benzodiazepine (DBA)	Cd (II)	Cd (II) is extracted from its alloy by n-beutanol with required pH= 8.9 red colored complex at 450 nm	Classical tech.	1 – 10mg/ml R.S.D% = 1.52	[73]
1-(4-(Phenyldiazenyl) phenyl) azo naphthalene -2-ol	Zn (II) and Cd (II)	Triton X–114 with Ligand as Extractant in cloud Point extraction. At pH is 9 and 460nm Zn (II) complex is analyzed and at 389nm Cd (II) complex is analyzed.	Classical tech.	0.25-700ppm, L.O.D = 0.035, L.O.Q = 0.116 ppm 0.25-400 ppm, L.O.D = 0.042, L.O.Q = 0.14 ppm	[74]
erythromycin	Cu (II)	pH= 5, 404nm	Classical tech.	2.5-30 , L.O.D =0.274, L.O.Q = 0.915	[75]
6-bromo-3-hydroxy-2-(5-methylfuran-2-yl)-4H-chromen-4-one (BHMF)	Sn (II)	in a slightly acidic medium (HCl) yellow colored complex at 434 nm	Classical tech.	0 - 1.3	[76]

2-[2-(5-nitrothiazolyl)azo]-4,6-dibromophenol	Ag (I)	(pH = 6) 252nm	Classical tech.	1.0 - 50.0 ppm, L.O.D = 0.139	[77]
bis(indoline-2, 3-dione) thiosemicarbazone	Cd (II)	pH = 12 290 nm	Classical tech.	L.O.D = 0.245, L.O.Q = 0.817 μg.ml ^(-1), R.S.D=5.1%	[78]
mixed organic reagents (diphenylcarbazide and 1,2-phenanthroline, diphenylcarbazide and sulphosalicylic acid	Cr (VI) and Fe (III)	the curve fitting by base line subtraction to resolve overlapped absorption spectrum	home-made Assayer and resolution software	Simultaneous Determination Correlation coefficient (R) Cr= 0.993, Fe= 0.994	[79]
2-benzyl espiro[isoindoline-1,5oxasolidine]-2,3,4 trione (BSIIOT).	Cu (II) and Pb (II)	pH =8.0	multi-component analysis method based on zero-crossing point-continuous wavelet transformation (CWT) and RAFA		[80]
Rubeanic acid	Cu (II) Co (II) Ni (II)	pH= 3.5 at 380nm pH= 9 at 470nm pH= 9 at 590nm	multi-component systems	0.65 – 2.65 , LOD=2.31ppm, sensitivity= 0.0941, RDS%= 1.16 0.50 – 2.40 , LOD= 2.58ppm, sensitivity= 0.1674 0.60 – 2.45 , L.O.D = 2.65ppm, sensitivity= 0.0764	[81]
Pyridoxal thiosemicarbazone and 2-Acetyl pyridine thiosemicarbazone. (2-APT)	Zn (II)	pH= 6 pH= 9	A first derivative spectrophotometry method is proposed for the determination of Zn(II). 400nm at 1st derivative 385nm Yellow at 2nd derivative	0.26-2.62 , R.S.D% = 0.52 0.25-2.56 , R.S.D% = 1.2	[82]
hematoxylin	Al (III) and Bi (III)	pH 5.8 400–650 nm	continuous wavelet transform (CWT), wavelet orthogonal signal correction-partial least squares (WOSC-PLS) and least squares-support vector machine (LS-SVM) (multivariate chemometrics	0.1–11.0 , 0.1–7.0 Recovery (%) = 98.4-101.6	[83]
tripodal	Co (II)		RAFA to evaluate complex stability	log Kf = 5.09 ± 0.02	[40]
dimethyl 2,2',-(ethan-1,2-diylbis1)bis(cyclopent-1-ene-1-carbodithioate	Ag (I), Cu (II) Ni (II)	pH at 7.0 and at 397 nm	principal component analysis	In0.010-2.000, L.O.D=0.013, L.O.Q=0.043 0.030-0.700, L.O.D=0.078, L.O.Q= 0.250 0.500-7.500, L.O.D=0.003, L.O.Q= 0.011	[84]
5-bromosalicylaldehyde thiosemicarbazone (5-BSAT)	Cu (II) and Co (II)	greenish yellow and brown colored of Cu (II) and Co (II) respectively. 370 and 446 nm at an interval of 4 nm.	partial least squares regression	In M (4.0– 9.6)×1 Cu (II) and 8.0×1 – 8.0×1for Co (II)	[85]

methylthymol blue	Zn (II) CuI (I), Fe(II)	600nm for Zn (II)/or CuI (I) 622nm for Fe (II)	Multivariate System and least squares (MCR-ALS) to determine acidity constants of ligand and complexes	Study of interaction between ligand and metal ions	[86]
Without complexation or Separation step	Zn (II) Ni (II) Co (II)	Zn at 303 nm Ni at 305 nm Co at 322 nm	partial least squares regression (PLSR) combined with wavelet transform (WT)	In mg/L 10–100 for Zn (II), 0.6–6.0 for Ni (II) and 0.3–3.0 for Co (II)	[87]
diethyldithiocarbamate	Cu (II) Co (II) mixture	436nm 320nm in the presence of polysorbate 20 (Tween 20)	partial least squares modeling	0.5-4.0 μg, in ppm LOD=0.1588, LOQ=0.4813 Sensitivity= 0.3345 0.5-3.0 μg., in ppm LOD=0.1574, LOQ=0.4770 Sensitivity= 0.1796	[88]
nitroso R salt	Cu (II) Ni (II) mixture	pH = 5.5 and hexadecyl trimethyl ammonium bromide as stabilizer	partial least squares method	0.3–3.0 mg/L for Both of them, relative error% = 3.27% for Cu and 4.12% for Ni	[89]
1-(2-pyridylazo)-2-naphtho	Co (II), Pd (II), Cu (II, Zn (II, Fe (II) And Ni (II)	Triton X-100 , pH = 4.5 581nm for Co-PAN 617nm for Pd -PAN 556nm for Cu -PAN 555nm for Zn -PAN 550nm for Fe -PAN 569nm for Ni-PAN	spectral ratio methods	Concentration range 0.1 - 5 μg/L RSDs under 4.21% for mixture of them	[36]
without prior complexation or separation	Cu (II) Fe (III), Ni (II)	805 nm	Single and Multivariate Regression	Cu (II) 3-5 g/L Fe (III) 0.010-0.002 g/L Ni (II) 7-11 g/L	[90]
Ammonium pyrrolidine dithiocarbamate (APDC)	Cr (III) Co (II)	Triton X-114 as extractant pH=5 576nm and 511nm	MultI-component system	LOD=0.74 ng/ml, RSD%=1.6 LOD=2.12 ng/ml, RSD%= 2.8	[91]
4-(2-pyridylazo) resorcinol	Cu (II, Ni (II), Co (II, Pb (II), Cd (II)	pH =10	Partial Least Squares – PLS and Artificial Neural Networks- ANN (MATLAB software)	In mg/L 0.1 – 1.4 for Cu (II) 0.2 0.1 – 1 for Ni (II) 0.1 – 1.2 for Co (II) 0.5 – 6 for Pb (II) 0.1 – 1.6 for Cd (II)	[92]
ammonium pyrrolidine dithiocarbamate (APDC)	Pb (II) Cu (II) Cd (II)	339.3 nm 346.2 nm 344.5 nm pH 10 and 10 minutes holding time	Principal component regression tech.	0.1-0.4ppm for Pb+2 0.1-0.4 ppm for Cu+2 0.1-0.7 ppm ranges for Cd+2	[93]

Table 2. Researches review on the determination of different mixtures of pharmaceutical preparations using Chemometric techniques without need for pre-separation step (2015-2025)

Analyte name	Chemometric techniques	Validation parameters of chemometric analytical method	Reference
octinoxate (OMC), oxybenzone (OXY), and octocrylene (OCR) in a sunscreen formulation	1-derivative ratio spectra zero crossing (DRSZ), 2-double divisor ratio spectra derivative (DDRD), 3-mean centering ratio spectra (MCR)	1- Recovery% = 100.04, 99.54, and 99.96, respectively 2-99.91, 100.10, 100.03 respectively 3-99.90, 100.62, 100.14 respectively. Linearity = 0.5-13.0, 0.3-9.0, and 0.5-9.0 respectively.	[94]
mycophenolate mofetil (MPM), and mycophenolic acid (MPA)	multivariate chemometric methods, i.e., partial least squares regression, principal component regression and principal component artificial neural networks	Linearity = 5.0-215.0 mg l-1, and 10.0-1000.0 respectively. L.O.D = 0.3 and 1.1 respectively.	[95]
Cinitapride hydrogen tartarate (CNT) and Pantoprazole sodium (PANTO)	Principal Component Regression (PCR) and Partial Least Squares (PLS)	Recovery% =99.4 – 102 for CNT, 99.6-100.47% for PANTO R.S.D% = 0.069-0.208 and 0.005-0.022% respectively.	[96]
Phenytoin	1^{st} and 2^{nd} Derivative Spectrophotometric	103.52 using D1at 267 nm 102.5 using D2 at 226 nm Linearity= 2-10 ppm, L.O.D = o.133mg/L	[97]
Chlorpheniramine maleate in pure and syrup form	1^{st} and 2^{nd} Derivative Spectrophotometric	Recovery% =95.555 - 102.916 Linearity = 5-45μg/ml, R.S.D% = 0. 039-0. 751%	[98]
Furosemide, Carbamazepine, Diazepam, and Carvedilol	partial least squares regression (PLS-1and PLS-2)	R.S.SD%=0. 1148-3. 6284%	[99]
Paracetamol (PAR) and Dantrolene Sodium (DAN)	Partial Least-Squares (PLS) and Classical Least Squares (CLS)	Recovery% = 90.09 - 95.37 for PAR 95.08 - 105.01 for DAN	[100]
Mometasone Furoate (MO) and Miconazole Nitrate (MI)	partial least-squares (PLS) and principal component regression (PCR)	Linearity = 3–33 and 60–840μg/mL for PAR and DAN respectively.	[101]
Beclomethasone dipropionate (BEC) and Salbutamol sulphate (SAL)	Multivariate analysis of Partial least squares	Recovery% = 100.199 for BEC, 100.157 for SAL, Linearity = 4-50 and 2-20 μg/ml for MF and CP, respectively	[102]

Imipenem (IMP), ciprofloxacin hydrochloride (CIPRO), dexamethasone sodium phosphate (DEX), paracetamol (PAR) and cilastatin sodium (CIL) in human	extended derivative ratio (EDR), principal component regression (PCR) and partial least-squares (PLS) methods	Linearity =3.00–45.00, 1.00–15.00, 4.00–40.00, 1.50–25.00 and 4.00–50.00 μg mL− 1 for IMP, CIPRO, DEX, PAR and CIL, respectively.	[103]
sulfanilamide and furosemide	first and second derivative spectrophotometry, with zero-crossing and peak to base line and peak area measurements	Recovery% = 98.00 - 101.60 for sulfanilamide 98.53 - 101.58 for furosemide	[104]
Metronidazole (MTZ) and Metronidazole benzoate (MTZB)	1st and 2nd Derivative order spectroscopy	Recovery% = 97.87 – 101.87 for MTZ, 98.033 – 102.39 for MTZB Linearity = 1-25 RSD% were between (0.041-0.751%) and (0.0331-0.452%) respectively. LOD between (0.051-0.231	[105]
Atorvastatin calcium (ATV) and Ezetimibe (EZT)	Principal Component Regression (PCR) and Partial Least Square (PLS)	Recovery% = 98.4 - 102.7 for ATV, 98.9 - 102.3 for EZT Linearity = 5.0- 30.0	[106]
Donepezil and rivastigmine	partial least square PLS and principal component regression PCR	Recovery% =90. 28571 – 99. 85185 for Donepezil, 98. – 100. 0278 for Rivastigmine	[107]
Paracetamol (PCM) and Tolperisone Hydrochloride (TOL)	Classical Least Square (CLS), Partial Least Square (PLS) and Principal Component Regression (PCR)	Recovery% = 97.16 – 101.24 for PCM, 97.96 - 103.61 for TOL Linearity = 5 – 25 μg/ml for PCM and 1.5 – 7.5 for TOL	[36]
Apremilast	Derivative Spectroscopy and Area Under Curve	Recovery% = 98-101 Linearity = 2–12 , accuracy% 98–101%.	[108]
Ternary mixture of guaifenesin (GUA), dextromethorphan (DMP), and diphenhydramine HCL (DPH)	double divisor ratio spectra derivative spectrophotometry	Recovery% = 100.60%, 99.95%, and 101.74%, L.O.D = 1.581, 1,167, and 1.107 , Accuracy% = 100.6, 99.95, and 101.74 for GUA, DMP, and DPH respectively.	[109]
ciprofloxacin and doxycycline hyclate	principal component regression and partial least squares	Recovery% = 97.50- 101.87 Linearity = 1-10 μg/mL for ciprofloxacin and 5-25 μg/mL, RSD < 2%	[110]
simvastatin (SIM) and nicotinic acid (NIA)	classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS)	-	[111]

doxylamine succinate (DOX) and pyridoxine hydrochloride (PYR)	partial least squares (PLS) and multivariate curve resolution-alternating least squares (MCR-ALS) methods	Recovery% = 98.43 - 100.79 for DOX, 99.18 - 102.34 for PYR	[112]
Acetylsalicylic acid, paracetamol, and ascorbic acid	Minitab and chemometric programs Of PCR and PLSR	Recovery% = 98.78 - 99.37 for Acetylsalicylic acid 98.11 - 99.00 for paracetamol 98.43 - 99.03 for ascorbic acid	[113]
Paracetamol (PAR), Propyphenazone (PRO) and Caffeine (CAF)	Principal component regression and Partial least squares regression	Recovery% = 100.04 for CAF 100.36 for PRO 100.87 for PAR RSD% = 1.57, 5.31, and 6.54% respectively	[114]
binary mixture of Ibuprofen and Paracetamol	1st method of ratio difference 2nd method of constant center 3rd method of mean centering of ratio spectra	Recovery% = 99.64 - 100.56, linearity 2-50 μg/mL for Ibuprofen and 2-20 μg/mL for Paracetamol	[115]
furosemide (FURO), carbamazepine (CARB), diazepam (DIAZ) and carvedilol (CARV)	D1, D2, D3, and D4	-	[116]
Etodolac (ET) and thiocolchicoside (TC)	partial least squares (PLS), principal component regression (PCR), and inverse least squares (ILS)	Recovery% = 98.26%, 98.16%, and 98.17%. RSD %= 3.583, 2.582, and 1.08 respectively for ET using PLS, PCR, ILS respectively and 99.14%, 98.26%, and 98.15% for TC using PLS, PCR, ILS respectively.	[117]
Paracetamol (PCT) and caffeine (CAF)	partial least-square regression (PLSR) and artificial neural network (ANN)	-	[118]
Aripiprazole in presence of its alkaline and oxidative degradation products	principal component regression (PCR) and partial least-squares regression (PLS)	RSD %= 0.58 for PCR and 0.77 for PLS	[119]
ternary mixture of carboxin, chlorpyrifos, and tebuconazole	double divisor-ratio spectra derivative of ratio spectra means centering of ratio spectra	Recovery% = 87.02 - 94.53 for carboxin, 92.32 - 108.53 for chlorpyrifos, 87.19 - 98.00 for tebuconazole RSD% = 0.62- 4.30	[120]

Quetiapine Fumarate (QTF) with its three related compounds of Quetiapine Fumarate (QTF), lactam (LAC), N-oxide (OXD) and des-ethanol (DES) of Quetiapine Fumarate	First Derivative Zero-Crossing Coupled with Spectrum Subtraction (1D-ZC) and Ratio Difference-Isosbestic Points Coupled with Spectrum Subtraction (RD-ISO)	Recovery% = 99.10 - 101.39 for LAC 99.97 - 101.37 for QTF 99.03 - 100.36 for OXD 99.56 - 100.63 for DES	[121]
Amoxicillin, Levofloxacin and Lansoprazole Mixture	ratio - first order derivative - zero crossing	Recovery% = 100.0, 102.5, and 99.2, RSD% of 1.37, 2.04 and 2.64 for Amox, Levo and Lanso, respectively.	[122]
sulfamethoxazole (SMX) and trimethoprim (TMP) in drug formulations	RAFA	Recovery% = 90.50–109.80, RSD%= 1.71, 2.18 for SMX and TMP, respectively	[123]
Diosmine (DSM) and hesperidin (HSP)	D1 by the zero-crossing measurements and Ratio absorbance method	RSD% = 0.83	[124]
Ciprofloxacin and Isoniazid	D1 based on zero-cross, peak to baseline, peak to peak and peak area measurements	Recovery% = 99.52 – 101.51 for Ciprofloxacin, 99.99 – 100.01 for Isoniazid. RSD%= 0.24 for Ciprofloxacin and 0.01 for Isoniazid	[125]
Tenofovir and Zidovudine	partial least squares (PLS) and principal component regression (PCR)	-	[126]
valsartan (VAL) and amlodipine (AMP)	least squares calibration method and basic component regression	Recovery% = 99.9953 -1 100.0086 for VAL and 99.9930 - 100.0115 for AML SD= 0.0104 for VAL, SD= 0.0146 for AML	[127]
Paracetamol, Diphenhydramine Hydrochloride, Caffeine and Phenylephrine Hydrochloride in	Principal Component Regression method (PCR) and Partial Least Squares Regression method (PLSR)	-	[128]
cromolyn sodium (CS) and its alkaline degradation products (Deg1 and Deg2)	principal component regression (PCR), MCR and partial least square (PLS-2) methods	For CS:Recovery% = 102.96 using MCR 102.40 using PCR,101.75 using PLS linearity (μg mL−1) = 2–40, 5–40., and 10–100 for CS, Deg1, and Deg2 respectively, LOD (μg mL−1) = 0.22, 0.37, and 0.95 respectively	[129]

ambroxol hydrochloride (ABH) and doxofylline (DOX)	ratio spectra derivative method and partial least squares regression (PLS)	Recovery% =99.76- 100.2 and 99.17 - 100.91, LOD(μg/mL) = 1.28, and 2.22, Analytical sensitivity(μg/mL) = 2.56 and 1.48 respectively for ABH and DOX	[130]
Mixture of tretinoinin (TN), hydroquinone (HQ), and Fluocinolone acetonide (FA), in presence of methyl paraben preservative inSemisolid Dosage Form	derivative ratio-zero-crossing point DRSZ, derivative ratio method (DRM), and double divisor derivative ratio method (DDRD)	Recovery% = 99.8 - 101.9 98.3 - 101.8 for HQ and 98.2 - 100.8 for FA, linearity (μg/mL) = 1-10, 4-38, and 4-35 for TN, HQ, and FA, respectively	[131]
Captopril and Hydrochlorothiazide	H-point	Recovery% = 95.33–104.37% for captopril, 96.8–105% for hydrochlorothiazide, RSD% = 2.46	[132]
Timolol maleate and Brimonidine tartrate	Simultaneous equations and absorbance ratio methods	Recovery% = 98.4 - 99.2 and 96.5 - 99.0, RSD%= 0. 92-1.01, 0.88 – 1.25 for TML and BRM respectively	[133]
ketoprofen	derivative IR spectroscopy	Recovery% =97.691, RSD%= 1.15- 1.37 for ketoprofen	[134]
combinations of loratadine and dexamethasone	Ratio derivative spectrophotometry (RDM) + Ratio subtraction (RSM) coupled with extended ratio subtraction method (EXRSM)	Recovery% = 99.41 - 101.15 99.21 - 100.86, RSD%= 0.451-1.303%, and 0.88 - 1.009% for RDM and RSM respectively.	[135]
Paracetamol (Par), Caffeine (Caf) and Ibuprofen(Ibu)	1st and 2nd ratio derivative spectrum using a double devisor	Recovery% = 95.7 -104 for Par 95.9 – 104.7 for Caf, 97.7 - 103.6 for Ibu RSD% less than 2.6%	[35]
Binary Mixtures of Aniline and 2-Nitronailine in tap water	Derivative Spectrophotometry	Recovery% = 97.5-101.66 and 96.67-102.22, RSD% less than 4.7% and 4.83% for aniline and 2-nitroaniline respectively.	[136]
finasteride and tadalafil in the pharmaceutical capsules	2nd Derivative with zero crossing 1st derivative of ratio spectra	Recovery% = 99.17 - 99.37 using D2 99.56 -99.74 using D1, RSD%= 0.529- 0.615, and 0.766 -0.853 for FSD and TDL	[137]
olmesartan medoxomil and hydrochlorothiazide	partial least squares, multivariate calibration	Recovery% =98.93 - 99.85, RSD%= 0.6, 0.81 for HTZ and OLM	[138]

Beta-Antagonists	least squares (MCR-ALS) and partial least squares regression (PLSR) multivariate calibration	Recovery% =99.83% to 101.12% for MCR-ALS 99.66% to 101.54% for PLSR	[15]
Paracetamol(PAR) and tramadol hydrochloride(TRA)	partial least squares (PLS) and principal component regression (PCR) techniques	Recovery% = 97.6 - 101.4 for PAR 98.7 - 104.4 for TRA. RSD% =1.19, 1.07, and 1.46 for PCR, PLS, and RS-FDS respectively.	[139]
Lorazepam (LORA) and Clonazepam (CLON) in Ativan and Rivotril tablets	1^{st} derivative (zero cross) and 1^{st} derivative (peak area) using for LORA And 2^{nd} derivative (zero cross) Spectrophotometry for CLON	Recovery% = 98.6264 - 104.031 and 98.75 - 103.556, RSD% = 0. 1667 – 4. 7579 for LORA, 0. 5128 – 6. 7736 for CLON	[140]
Urea, creatinine, and uric acid	Ratio derivative-zero crossing and successive derivative	Recovery% = 97.10 - 101.9, 97.22–102.70 and 97.45-102.55. RSD% less than 1.56%, 3.87%, and 3.71% for urea, creatinine and uric acid respectively.	[141]
Estradiol (E2) and progesterone (PRG)	1^{st} derivative zero-crossing technique (DS) Ratio subtraction modified amplitude (RSM) modified amplitude subtraction (MAS) which performed by derivative spectroscopy and mathematical manipulations	Recovery% = 98.38 – 100.40 for E2 98.31- 100.72 for PRG, RSD%= 0.1-4.44 for E2, 0.1- 3. 26 for PRG	[142]
fluoxetine (FLX) and olanzapine (OLZ)	absorbance subtraction and absorbance correction spectrophotometric methods	Recovery% = 96.8-105.3 and 98.1-100.2 linearity = 3.12-15.62 and 3.45-17.28 μg/ml for OLZ and FLX, respectively	[143]
amlodipine / candesartan (CAN) mixture	classical least square (CLS) principal component regression (PCR) and partial least square (PLS-1)	Recovery% =99.69 - 101.94 for CAN 99.19 - 100.57 for AML RSD%= 0. 15 - 0. 75 and 0. 26- 0. 37 for CAN and AML	[144]
Abacavir sulphate (ABA), Lamivudine (LAM) and Dolutegravir sodium (DOL)	Multivariate methods, particularly Classical Least Square, Inverse Least Square (ILS), Partial Least Square and Principal Component Regression	Recovery% =99.65 - 100.12, 99.84 - 100.34 and 99.42 - 100.27, S.D = 0. 249- 0. 634, 0. 249- 0. 519, and 0. 489- 0. 634 0 for ABA, LAM, and DOL respectively.	[145]
Amlodipine (AML), valsartan (VAL), and hydrochlorothiazide (HCT)	Partial Least Squares with double divisor system	Recovery% =99.59 for AML, 101.99 for VAL ,103.60 for HCT	[146]
paracetamol	Partial least squares (PLS) and principal component regression (PCR)	Recovery% =96 - 109.2, RSD%=0.15-0.45	[147]

Nebivolol and valsartan (VAL)	PLS and PCR as multivariate methods univariate methods are derivative ratio (DD1), ratio difference (RD), constant center (CC), constant center spectrum subtraction (CC-SS), constant value coupled with amplitude difference (CV-AD), advanced concentration value (ACV), and amplitude difference (AD)	-	[148]
sitagliptin and metformin hydrochloride	partial least square (PLS) and principal component regression (PCR)	Recovery% =99.82 – 100.18 for sitagliptin 99.83 – 100.2 for metformin hydrochloride	[149]
antazoline hydrochloride (AN) and naphazoline hydrochloride (NP)	principal component regression (PCR) and partial least squares (PLS)	Recovery% =100.8 - 103.2 S.D= 0.73- 2.3 and 3.3- 3.5 FOR AN and NP respectively.	[150]
chlorzoxazone (CHZ), paracetamol (PAR), and aceclofenac (ACF)	classical least squares (CLS), partial least squares (PLS) regression, and principal component regression (PCR). in addition to feed-forward back-propagation artificial neural networks (FF-BPANN), cascade-forward back-propagation artificial neural networks (CFBP-ANN), and radial basis function artificial neural networks (RBF-ANN),	Recovery% = 97 - 104.5, S.D less than 1%	[151]
paracetamol and caffeine	partial least square regression (PLS) and principal component regression (PCR),	Recovery% =98.4 – 100.9 98.9 – 102.31, R.S.D%= 0.6 and 0.75 for paracetamol and caffeine respectively.	[152]
Caffeine (CAF), Codeine phosphate hemihydrate (COD), Paracetamol (PAR), and p-Aminophenol (PAP)	Partial Least Squares (PLS-1) and Artificial Neural Networks (ANN)	Recovery% =99.73 – 101.21, 100.28- 100.62, 99.42 – 100.83, 100.63 – 102.7 S.D = 1.19, 1.96, 0.99, and 0.64 for CAF, COD, PAR, and PAB	[153]
atenolol, losartan and hydrochlorothiazide	classical least-squares (CLS), principal component regression (PCR), and partial least-squares (PLS) along with radial basis function network (RBFN)	Linearity (μg/ mL^{-1}) 4- 20, 3.8 - 20.2, and 0.9 - 50.1 for ATN, LOS, and HCZ	[154]

RESULTS AND DISCUSSION

Chemometrics introduces the advantage of resolving overlapped spectra, as shown in Figure 7, and using chemometric tools, such as PLS, PCR, and MCR, can resolve the problem of a complex sample matrix. Other achievements of green methodology, including high sensitivity and reliability, high speed and efficiency, cost-effectiveness, and non-destructive techniques, have been performed using chemometrics.

Table 1 and Figure 8 present published research on metal ion quantification with or without (classical method) using chemometric statistical analysis methods from 2015 to 2025. The latter time period was selected to cover by our study because during the global revolution (1990s) in applying chemometrics in various practical fields, Iraq was isolated from the rest of the world and suffered from scientific and economic blockade. As a result, time gap in understanding, developing, and recognizing advantages of chemometrics in applied analytical chemistry was created. In recent ten years, Iraqi researchers achieved advanced rankings in publishing in Scopus-indexed journals in chemistry specification, the current study is attempt to shed light on chemometrics importance to open new areas of research and provide future prospects for collaboration with software specialists.

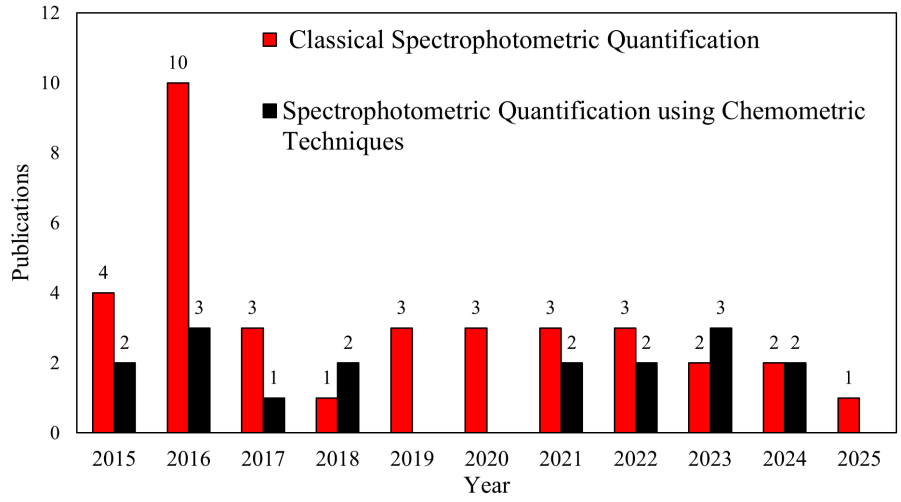


Figure 8. Publications per year of UV-Vis spectrophotometric determination of metal ions with or without chemometrics during 2015-2025

Approximately half of these published works involved chemometric techniques; in other words, 33% of all recorded research (classic methods only + chemometric-assisted classic methods) are related to metal ion spectrophotometric determination using chemometric-assisted spectrophotometric methods. For the same period, the published articles in the field of drug content determination using UV-Vis spectrophotometric determination using chemometric regression analysis methods, in addition to some AI used applications, are mentioned in Table 2 and Figure 9. While Figure 10 reveals the wide and efficient use of chemometric techniques in drug content quantification in comparison with metal ion determination at an approximate ratio of 4:1.

Figure 11 presents the participation of Iraqi journals in publications of research on classic spectrophotometric metal ion determination at 11.8%, at 0% using chemometrics, and at 9.5% in publications on spectrophotometric determination of drug contents using chemometric models. UV-Vis spectroscopy introduces a simple and inexpensive method for determining metal ions based on a complexation reaction with a chromogenic organic reagent to form a colored complex that is quantified in the visible or UV range [42]. Despite these methods' advantages, such as high reagent selectivity toward specific metal ions and good sensitivity for suggesting determination techniques depending on complex stability, there are some challenges. But there are some challenges such as: the main one related to sample is the interferences of sample matrix, other factors related to used instrument such as light source intensity, appropriate material of sample cell, ...etc., and factors related to determination technique such as possibility of light scattering by solid particles in sample solution or bubbles in sample cuvette [155]. The main error of sample matrix interference can be resolved using chemometric techniques. Chemometrics appeared for the first time in 1970 and is considered a major branch of analytical chemistry that depends on the use of statistical and mathematical techniques

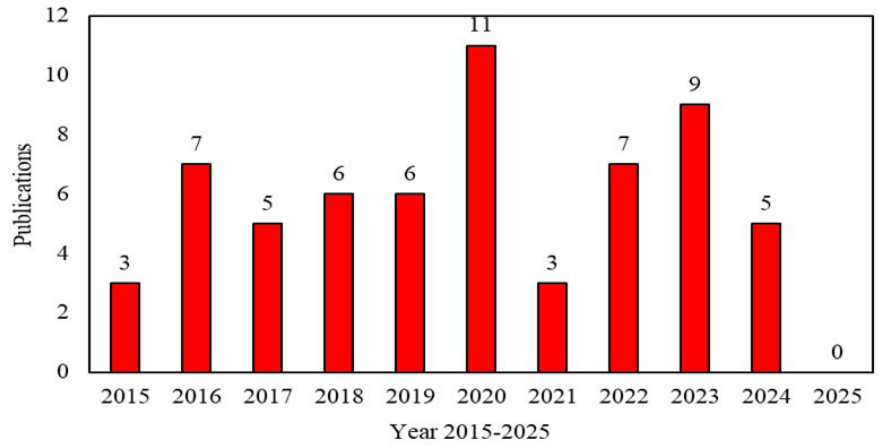


Figure 9. Publications per year of chemometric-assisted UV-Vis spectrophotometric determination of drugs contents during 2015-2025

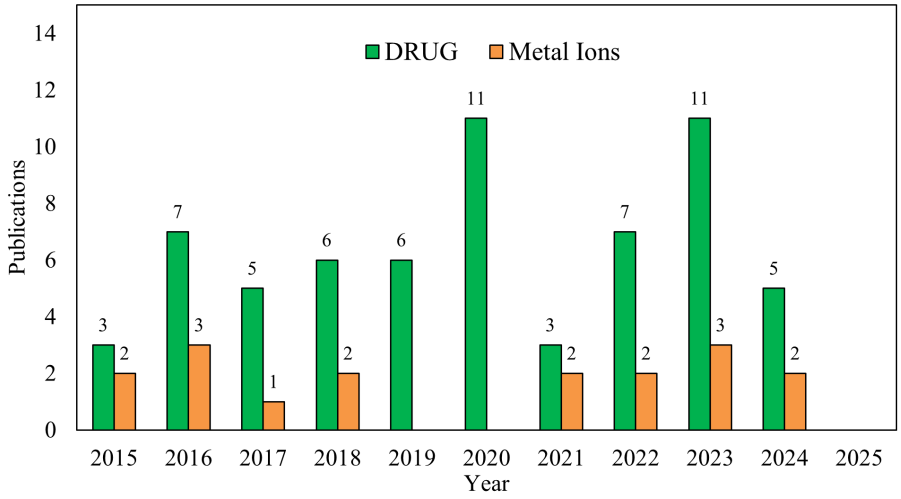


Figure 10. Publications per year of UV-Vis of chemometrics- assisted spectrophotometric determination of metal ions and drugs contents during 2015-2025

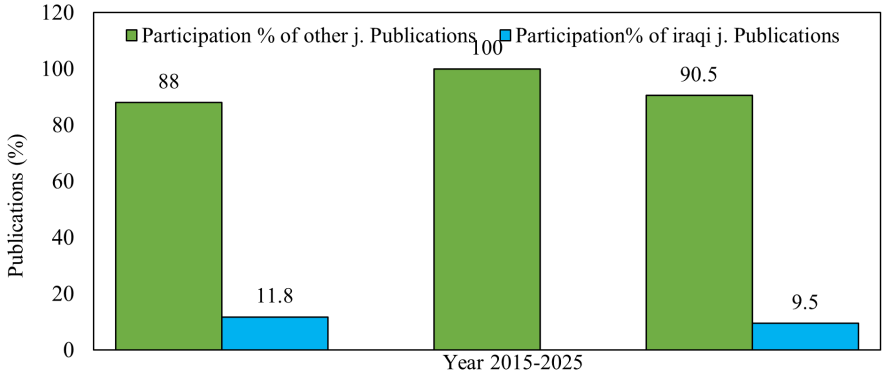


Figure 11. A participation of Iraqi journals of all publications in field of chemometric- assisted spectrophotometric analyses

to interpret chemical data and obtain useful information. Chemometrics has become necessary when

performing qualitative and quantitative analyses of complex samples [156].

Chemometric techniques have many applications in different analytical quantification methods, such as UV-visible spectrophotometry, NIR spectroscopy, and fluorescence spectroscopy. Spectroscopic analytical methods require less time to perform; however, a large amount of data is generated from each sample. The data obtained is divided into two parts: information and noise. Noise affects sample information; therefore, it is necessary to minimize noise by data treatment using suitable mathematical models. Chemometrics has witnessed rapid development, which is due to analytical instrumentation that can be depended on to obtain large reliable data and the developed computers needed to convert data into useful forms. Many of these complicated mathematical calculations of analytical data are performed using specific coding expressions of MATLAB, which were first used in 1984 [156]. In recent years, artificial intelligence (AI) techniques have been applied by researchers to model (linear or nonlinear) relationships of complicated data and solve nonlinear calibration problems. Artificial neural network (ANN) is one of the AI analyses that widely used for nonlinear relationship calibration [155], but data treatment by mathematical model introduce:

Eco-friendly quantification methods with no need for separation step of extraction with suitable solvent or ion exchange process with suitable membrane. And in some samples, there was no need for organic reagent for ions determination, because of correct selection of suitable chemometric techniques can make determination of ions mixture possible, and sensitive without pre separation step or even complexation reaction [36], [86]. Improvement data precision and accuracy depending on multivariate calibration [156]. Increasing sensitivity by considering the full spectrum, but conditional on applying advanced signal processing technology to enhance S/N ratio, remove interference, isolate overlapped peaks, extract useful hidden information in spectrum waves, and improve spectrum resolution [41]. The method described herein enables the quantification of more than one component of a mixture sample (five [36] or six [92] components) with high sensitivity and recovery% without the need for a separation step. In classical spectrophotometric determination analysis, only one ion can generally be quantified sensitively, under certain conditions of permissible concentrations of other interfering ions. Converting a nonlinear calibration to a linear one owing to a high noise level using flexible modelling of ANNs [41].

In light of these considerations of the advantages of chemometric techniques, many researchers have applied variable chemometric techniques in the UV-Vis spectrophotometric determination of complex samples with variable drug contents, as shown in Table 2, and metal ions and pharmaceutical contents, or in other words, analyte quantification in any complex sample with expected large interferences. According to Table 1 and Figure 8, 31.4% of the publications are on metal ion determination using one or more chemometric techniques, whereas the remaining 68.6% are publications on classic spectrophotometric determination. In spite of the importance and effectiveness of chemometrics in the spectrophotometric determination of samples with complex matrices, chemometric methods applied to drug content quantification are four times those applied to metal ion determination, as shown in Figure 10 and listed in Tables 1 and 2. This unexpected difference may be due to: the fact that both classic and chemometric determination usually required classic steps of finding suitable organic reagent to react with one or more ion(s) to be determine, necessity of considering the optimum determination environmental such as pH, reaction time, temperature, …etc. , matrices of metal ion samples are more complicated by their broad content in comparison with drugs contents which have limited contents no. with known compounds. Finally, regarding us as Iraqi researchers, chemometric methods are a less understood branch of analytical chemistry, and therefore, students and researchers need to understand and apply them in our work in cooperation with software programmers or artificial intelligence specialists. According to Table 2, 9.5% of the publications are from Iraqi journals, and regarding Table 1, 11.8% of the publications on classic spectrophotometric metal ion determination are from Iraqi journals, as shown in Figure 11. However, participation in publications on spectrophotometric metal ion quantification using chemometric techniques is zero %. These participations increase when Iraqi authors publish in other journals.

CONCLUSION

The classic spectrophotometric analysis is simple, quick and straightforward to achieve. Additionally, it does not need complex preparation procedure of sample or sophisticated instrumentation making it accessible and cost-effective for routine analysis in laboratories. Classic qualitative and quantitative spectrophotometric analysis provide variable information of content, identity and purity. A traditional concentration determination is a univariate which depending on isolating one variable of maximum wave length after analyte scanned. The analyte quantification is achieved according to absorbance at this wave length that reflects using of 1% of data and wasting the residue. Chemometrics uses

multivariate methods instead of univariate to offer benefit of using residual data of 99%. For accurate analysis, multivariate methods introduce benefits of: looking at entire data set and removing noise and interfering data profile which differs from the analyte. The current study introduces an educational overview in explanation the basic principles of chemometrics and their potential analytical applications that makes the reader understand this important branch of chemistry.

SUPPLEMENTARY MATERIAL

Supplementary materials, including additional figures, training details, and Grad-CAM visualizations, are available upon request or accessible via the corresponding author.

AUTHOR CONTRIBUTIONS

Suhair M. Yaseen: Methodology and Writing – original draft. Mustafa F. Mahmood: Data curation and Visualization. Noor M. Yaseen: Formal analysis. Zahraa T. Khudhair: Writing – review & editing.

FUNDING

This research received no external funding.

DATA AVAILABILITY STATEMENT

Authors declare that no new data were created.

ACKNOWLEDGMENTS

This work has supported by staff of medical chemistry lab in department of Medical Instrumentation Engineering Techniques - Electrical Engineering Technical College.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DECLARATION OF GENERATIVE AI USE

During the preparation of this work, the authors used Grammarly for grammar checking and language polishing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

REFERENCES

[1] G. L. Eichhorn, "Aging, genetics, and the environment: Potential of errors introduced into genetic information transfer by metal ions," *Mechanisms of Ageing and Development*, vol. 9, no. 3–4, pp. 291–301, 1979, doi: 10.1016/0047 -6374(79)90106-4.

[2] F. Van Assche and H. Clijsters, "Effects of metals on enzyme activity in plants," *Plant, Cell & Environment*, vol. 13, no. 3, pp. 195–206, 1990, doi: 10.1111/j.1365-3040.1990.tb01304.x.

[3] J. M. O'Connor, "Trace elements and DNA damage," *Biochemical Society Transactions*, vol. 29, no. 2, pp. 354–358, 2001, doi: 10.1042/bst0290354.

[4] S. L. Marklund, "Human copper-containing superoxide dismutase of high molecular weight," *Proceedings of the National Academy of Sciences*, vol. 79, no. 24, pp. 7634–7638, 1982, doi: 10.1073/pnas.79.24.7634.

[5] W. Maret, "The metals in the biological periodic system of the elements: Concepts and conjectures," *International Journal of Molecular Sciences*, vol. 17, no. 1, Art no. 66, 2016, doi: 10.3390/ijms17010066.

[6] S. Ahmad Bhawani, S. S. Fong, and M. N. Mohamad Ibrahim, "Spectrophotometric analysis of Caffeine," *International Journal of Analytical Chemistry*, vol. 2015, no. 1, Art no. 170239, 2015, doi: 10.1155/2015/170239.

[7] L. Bai, W. Sun, M. Huang, L. Li, C. Geng, K. Liu, and D. Yan, "Study on the methods of separation and detection of chelates," *Critical Reviews in Analytical Chemistry*, vol. 50, no. 1, pp. 78–89, 2019, doi: 10.1080/10408347.2019.1573657.

[8] M. Ghaedi, K. Niknam, A. Shokrollahi, E. Niknam, H. R. Rajabi, and M. Soylak, "Flame atomic absorption spectrometric determination of trace amounts of heavy metal ions after solid phase extraction using modified sodium dodecyl sulfate coated on alumina," *Journal of Hazardous Materials*, vol. 155, no. 1–2, pp. 121–127, 2008, doi: 10.1016/j.jhazmat.2007.11.038.

[9] A. B. Tabrizi, "Development of a cloud point extraction-spectrofluorimetric method for trace copper(II) determination in water samples and parenteral solutions," *Journal of Hazardous Materials*, vol. 139, no. 2, pp. 260–264, 2007, doi: 10.1016/j.jhazmat.2006.06.024.

[10] B. Vandeginste, D. Massart, L. Buydens, S. De Jong, P. Lewi, and J. Smeyers-Verbeke, "Introduction to Part B," in *Handbook of Chemometrics and Qualimetrics: Part B*. Elsevier, 1998, pp. 1–5, doi: 10.1016/s0922-3487(98)80038-5.

[11] A. J. Kałka and A. M. Turek, "Searching for alternatives to the Savitzky–Golay filter in the spectral processing domain," *Applied Spectroscopy*, vol. 77, no. 4, pp. 426–432, 2023, doi: 10.1177/00037028231154278.

[12] X. Chu, Y. Huang, Y.-H. Yun, and X. Bian, *Chemometric methods in analytical spectroscopy technology*. Springer Nature Singapore, 2022, doi: 10.1007/978-981-19-1625-0.

[13] F. Sánchez Rojas and C. Bosch Ojeda, "Recent development in derivative ultraviolet/visible absorption spectrophotometry: 2004–2008: A review," *Analytica Chimica Acta*, vol. 635, no. 1, pp. 22–44, 2009, doi: 10.1016/j.aca.2008.12.039.

[14] J. Amador-Hernandez, A. Rojas-Hernandez, E. M. Colunga-Urbina, I. M. De La Garza-Rodriguez, M. Velazquez-Manzanares, and L. F. Medina-Vallejo, "New chemometric strategies in the spectrophotometric determination of pKa," *European Journal of Chemistry*, vol. 5, no. 1, pp. 1–5, 2014, doi: 10.5155/eurjchem.5.1.1-5.901.

[15] A. Mostafa and H. Shaaban, "Chemometric assisted UV-spectrophotometric methods using multivariate curve resolution alternating least squares and partial least squares regression for determination of beta-antagonists in formulated products: Evaluation of the ecological impact," *Molecules*, vol. 28, no. 1, Art no. 328, 2022, doi: 10.3390/molecules28010328.

[16] A. Diwan and M. Linford, "An introduction to classical least squares (cls) and multivariate curve resolution (mcr) as applied to uv-vis, ftir, and tof-sims," *Vacuum Technology & Coating*, pp. 2–9, Dec. 2014, [Online]. Available: https://www.researchgate.net/publication/268745821_An_Introduction_to_Classical_Least_Squares_CLS_and_Multivariate_Curve_Resolution_MCR_as_Applied_to_UV-VIS_FTIR_and_ToF-SIMS.

[17] J. N. Miller and J. C. Miller, *Statistics and chemometrics for analytical chemistry*, Sixth. Pearson education, 2010, [Online]. Available: https://www.africanfoodsafetynetwork.org/wp-content/uploads/2021/09/Statistics-and-Chemometrics-2010.pdf.

[18] T. G. Mayerhöfer, O. Ilchenko, A. Kutsyk, and J. Popp, "Complex-Valued chemometrics in spectroscopy: Inverse least squares regression," *Applied Spectroscopy*, vol. 80, no. 1, pp. 100–108, 2026, doi: 10.1177/00037028251358392.

[19] *Partial least squares (pls) regression in SPSS - explained, https://spssanalysis.com/partial-least-squares-regression-in-spss/*, Published: July 16, 2024. Accessed on March 16, 2026, 2024.

[20] V. Cerdà, P. Phansi, and S. Ferreira, "From mono- to multicomponent methods in UV-VIS spectrophotometric and fluorimetric quantitative analysis – A review," *TrAC Trends in Analytical Chemistry*, vol. 157, no. 1, Art no. 116772, 2022, doi: 10.1016/j.trac.2022.116772.

[21] Z. Rezaei, B. Hemmateenejad, S. Khabnadideh, and M. Gorgin, "Simultaneous spectrophotometric determination of carbamazepine and phenytoin in serum by PLS regression and comparison with HPLC," *Talanta*, vol. 65, no. 1, pp. 21–28, 2005, doi: 10.1016/j.talanta.2004.05.008.

[22] M. J. Adams and N. W. Barnett, *Chemometrics in Analytical Spectroscopy*. The Royal Society of Chemistry, Feb. 2004, doi: 10.1039/9781847550484.

[23] M. Greenacre, P. J. F. Groenen, T. Hastie, A. I. D'Enza, A. Markos, and E. Tuzhilina, "Principal component analysis," *Nature Reviews Methods Primers*, vol. 2, no. 1, Art no. 100, 2022, doi: 10.1038/s43586-022-00184-w.

[24] R. G. Brereton, "Graphical introduction to principal components analysis," *Journal of Chemometrics*, vol. 36, no. 6, Art no. e3404, 2022, doi: 10.1002/cem.3404.

[25] K. Kumar, "Partial least square (PLS) analysis: Most favorite tool in chemometrics to build a calibration model," *Resonance*, vol. 26, no. 3, pp. 429–442, 2021, doi: 10.1007/s12045-021-1140-1.

[26] G. Dumancas, G. Bello, J. Hughes, and M. Diss, "Comparison of chemometric algorithms for multicomponent analyses and signal processing: An example from 4-(2- Pyridylazo) resorcinol-metal colored complexes," *Recent Patents on Signal Processing*, vol. 4, no. 2, pp. 106–115, 2014, doi: 10.2174/2210868305666150817225812.

[27] H. Volk-Jesussek, *Linear regression: A complete guide to modeling relationships between variables, https://numiqo.com/tutorial/linear-regression*, Published: January 29, 2026. Accessed on March 16, 2026, Jan. 2026.

[28] A. J. Owen, "Uses of derivative spectroscopy application note uv-visible spectroscopy," 2000, [Online]. Available: https://www.youngin.c om/application/an-0608-0115en.pdf.

[29] V. K. Redasani, P. R. Patel, D. Y. Marathe, S. R. Chaudhari, A. A. Shirkhedkar, and S. J. Surana, "A review on derivative uv-spectrophotometry analysis of drugs in pharmaceutical formulations and biological samples review," *Journal of the Chilean Chemical Society*, vol. 63, no. 3, pp. 4126–4134, 2018, doi: 10.4067/s0717-97072018000304126.

[30] J. Karpinska, "Basic principles and analytical application of derivative spectrophotometry," in *Macro To Nano Spectroscopy*, doi: 10.5772/37673, London: IntechOpen, 2012, ch. 13.

[31] K. N. Patel, J. K. Patel, G. C. Rajput, and N. B. Rajgor, "Derivative spectrometry method for chemical analysis: A review," *Der Pharmacia Lettre*, vol. 2, no. 2, pp. 139–150, 2010, [Online]. Available: https://www.scholarsresearchlibrary.com/arti thod-for-chemical-analysis-a-review.pdf.

[32] A. R. Rote and P. D. Bari, "Ratio spectra derivative and zero-crossing difference spectrophotometric determination of olmesartan medoxomil and hydrochlorothiazide in combined pharmaceutical dosage form," *AAPS PharmSciTech*, vol. 10, no. 4, pp. 1200–1205, 2009, doi: 10.1208/s12249-009-9318-y.

[33] N. M. Bhatt, V. D. Chavada, M. Sanyal, and P. S. Shrivastav, "Manipulating ratio spectra for the spectrophotometric analysis of diclofenac sodium and pantoprazole sodium in laboratory mixtures and tablet formulation," *The Scientific World Journal*, vol. 2014, no. 1, p. 495 739, 2014, doi: 10.1155/2014/495739.

[34] S. Baghel and K. Shah, "A review on methods developed for estimation of paracetamol in combination with other drugs," *International Journal of Pharmaceutical Research And Allied Sciences*, vol. 12, no. 1, pp. 75–94, 2023, doi: 10.51847/t9gdpyn3lh.

[35] K. F. Alsamarrai and S. T. Ameen, "Simultaneous ratio derivative spectrophotometric determination of paracetamol, caffeine and ibuprofen in their ternary form," *Baghdad Science Journal*, vol. 19, no. 6, p. 1276, 2022, doi: 10.21123/bsj.2022.6422.

[36] U. Shah and A. Jasani, "Chemometric assisted spectrophotometric methods for simultaneous determination of paracetamol and tolperisone hydrochloride in pharmaceutical dosage form," *Eurasian Journal of Analytical Chemistry*, vol. 12, no. 3, pp. 211–222, 2017, doi: 10.12973/ejac.2017.00164a.

[37] A. J. Kałka, E. G. Tarka, and A. M. Turek, "A new ingenious combination of rank annihilation factor analysis (RAFA) and self-modeling to enhance the unambiguous resolution of multicomponent spectra," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 294, Art no. 122525, Jun. 2023, doi: 10.1016/j.saa.2023.122525.

[38] M. A. A. Aslani, Y. F. Kuru, and C. K. Aslani, "Application of central composite design and rank annihilation factor analysis for optimization of mixed chelate of UO22+ complex with ammonium-N,N-tetramethylenedithiocarbamate and estimation of Kf value," *Radiochimica Acta*, vol. 104, no. 1, pp. 23–31, 2015, doi: 10.1515/ract-2014-2378.

[39] A. J. Kałka and A. M. Turek, "Do spectra live in the matrix? A brief tutorial on applications of factor analysis to resolving spectral datasets of mixtures," *Journal of Fluorescence*, vol. 31, no. 6, pp. 1599–1616, 2021, doi: 10.1007/s10895-021-02753-w.

[40] R. Golbedaghi and F. Khajavi, "Application of rank annihilation factor analysis to the spectrophotometric determin- ation of the formation constant of complex of a new synthesized tripodal ligand with Co^{2+}," *Arabian Journal of Chemistry*, vol. 10, S2580–S2583, May 2013, doi: 10.1016/j.arabjc.2013.09.035.

[41] P. R. Prajapati, D. N. Rathod, V. S. Modi, and T. Basuri, "Chemometrics and its applications in UV spectrophotometry," *International Journal of Pharmaceutical Chemistry and Analysis*, vol. 3, no. 1, p. 43, 2016, doi: 10.5958/2394-2797.2016.00005.8.

[42] V. Pinninti, "Spectrophotometric determination of metal ions using chromogenic organic reagents: Techniques, advantages, and challenges," *International Journal in Management and Social Science*, vol. 9, no. 11, pp. 1076–1082, 2021, [Online]. Available: https://ijmr.net.in/current/2021/NOVEMBER,-2021/FUxKAmkoWbwBnXu.pdf.

[43] K. J. Ali, L. A. Mohammed, F. J. Ali, and H. N. Raheem, "New spectrophotometric determination of copper (II) using an organic reagent derived from imidazole and 4-aminoantypyrine and applied onto different samples," *Journal of Chemical and Pharmaceutical Sciences*, vol. 8, no. 2, pp. 201–207, 2015, [Online]. Available: https://phar.uokufa.edu.iq/wp-content/uploads/2018/04/had1.pdf.

[44] S. A. A. Elsuccary and A. A. Salem, "Novel flow injection analysis methods for the determination of total iron in blood serum and water," *Talanta*, vol. 131, pp. 108–115, Jan. 2015, doi: 10.1016/j.talanta.2014.07.068.

[45] S. Karnad and B. Rao, "Spectrophotometric determination of copper using o-vanillidine-2-amino-4-ethylbenzothiazole as a chromogenic reagent," vol. 7, pp. 281–284, Jan. 2015, [Online]. Available: https://www.scholarsresearchlibrary.com/articles/spectrophotometric-determination-of-copper-using-ovanillidine2amino4-ethylbenzothiazole-as-a-chromogenic-reagent.pdf.

[46] B. Ranganath, V. S. Basha, M. R. Jayapal, and P. V. Ramana, "Direct spectrophotometric determination of ni (ii) using esomeprazole," *International Journal of Pharmacy and Chemistry*, vol. 1, no. 1, pp. 7–11, 2015, doi: 10.11648/j.ijpc.20150101.12.

[47] R. Elsheikh, A. A. Gouda, H. A. Elsayed, and E. M. Alamin, "Cloud point extraction, preconcenration and spectrophotometric determination of cobalt in water samples," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 7, no. 9, pp. 213–221, 2015, [Online]. Available: https://www.researchgate.net/publication/282740298_Cloud_point_extraction_preconcenration_and_spectrophotometric_determination_of_cobalt_in_water_samples.

[48] M. Maarouf and H. Hallak, "An analysis spectral study to determine the zinc bilateral in some syrian rocks by using the (bpt) 2-benzoyl pyridine thiosemi carbazone," *Latakia University Journal-Basic Sciences Series*, vol. 38, no. 2, pp. 99–110, 2016, [Online]. Available: https://www.shamra-academia.com/en/show/58b6f4bd0865f.

[49] S. A. N. Reddy, K. J. Reddy, L. K. Duk, and A. V. Reddy, "Evaluation of 2,6-diacetylpyridinebis-4-phenyl-3-thiosemicarbazone as complexing reagent for zinc in food and environmental samples," *Journal of Saudi Chemical Society*, vol. 20, S271–S279, Sep. 2016, doi: 10.1016/j.jscs.2012.11.004.

[50] A. F. Hussein, R. S. Hatam, and I. M. Shaheed, "Spectrophotometric study for determination of cobalt (ii) by the reagent [2-(4-methoxyphenyl) azo (4, 5-dipheny imidazole)](mpai)," *International Journal of Pharmacy and Pharmaceutical Research*, vol. 6, no. 4, pp. 671–683, 2016, [Online]. Available: https://ijppr.humanjournals.com/wp-content/uploads/2016/08/46.Alaa-F.-Hussein-Raghad-Saad-Hatam-Ihsan-Mahdi-Shaheed.pdf.

[51] A. A. Azrak and N. A.-H. Aiosh, "Analytical study for bismuth (iii) determination using 3-hydroxy-1,2-benzoquinone reagent with spectrophotometric method and possibilityto use this indicator for complixometric titration of bismuth," *International Journal of ChemTech Research*, vol. 9, no. 3, pp. 597–608, 2016, [Online]. Available: https://www.sphinxsai.com/2016/ch_vol9_no3/2/(597-608)V9N3CT.pdf.

[52] K. A. Kuliyev, N. A. Verdizadeh, and G. S. Suleymanova, "Spectrophotometric determination of cobalt (II) with 2, 6-Dithiolphenol and its derivatives in the presence of hydrophobic amines," *International Journal of ChemTech Research*, vol. 6, no. 4, pp. 95–103, 2016, doi: 10.5923/j.chemistry.20160604.02.

[53] B. N. Kumar, S. Kanchi, M. Sabela, K. Bisetty, and N. Jyothi, "Spectrophotometric determination of nickel (II) in waters and soils: Novel chelating agents and their biological applications supported by DFT method," *Karbala International Journal of Modern Science*, vol. 2, no. 4, pp. 239–250, 2016, doi: 10.1016/j.kijoms.2016.08.003.

[54] K. Al-Adilee, S. Eassa, and H. Dakhil, "Spectrophotometric determination of chromium(III) and iron(III) by used of 2-((E)-(1H-Benzo[D]Imidazol2-Yl)Diazenyl)-5-((E)-Benzylideneimino) phenol;(BIADPI) as organic reagent," *Oriental Journal of Chemistry*, vol. 32, no. 5, pp. 2481–2491, 2016, doi: 10.13005/ojc/320518.

[55] H. J. Mohammed, W. M. Sarhan, W. Mohammed, A. Y. Muhi, and N. Sami, "Spectrophotometric determination of Fe(II)&Ni(II) as complexes with new derivative of antipyrine azo orcinol," *Der Pharma Chemica*, vol. 8, no. 19, pp. 100–108, 2016, [Online]. Available: https://www.derpharmachemica.com/abstract/spectrophotometric-determination-of-feiiniii-as-complexes-with-new-derivative-of-antipyrine-azo-orcinol-2481.html.

[56] I. A. Shqair, Z. M. Shakhshir, J. Masharqah, I. Odeh, and W. J. Jondi, "Spectrophotometric determination of Cu^{2+} metal ions via complex formation with carboxylated Tris(2-aminoethyl)amine," *Journal of Advances in Chemistry*, vol. 12, no. 3, pp. 4237–4246, 2016, [Online]. Available: https://dspace.alquds.edu/handle/20.500.12213/876.

[57] N. H. Shekho and H. A. Mahmoud, "Spectrophotometric determination of chromium using promethazine hydrochloride - application in various sample," *Ibn AL-Haitham Journal For Pure and Applied Sciences*, vol. 29, no. 2, pp. 130–143, 2017, [Online]. Available: https://jih.uobaghdad.edu.iq/index.php/j/article/view/91.

[58] V. J. Ghodvinde, S. M. Pitale, S. Janwadkar, P. P. Lohani, P. Rana, and D. Yadav, "Spectrophotometric determination of trace amount of Ce (iv) metal ion by using 3-(2-hydroxyphenylimino) indolin-2-one as an photometric reagent, HPI2O," *International Journal of Current Research*, vol. 9, no. 4, pp. 49 215–49 217, 2017, [Online]. Available: https://www.journalcra.com/sites/default/files/issue-pdf/22096.pdf.

[59] P. Bhatia, B. B. Mohan, and H. K. Sharma, "Non-extractive spectrophotometric determination of sn(ii) with 5,7-dibromo-8-hydroxyquinoline," *Chemical Science Transactions*, vol. 6, no. 4, pp. 669–674, 2017, doi: 10.7598/cst2017.1407.

[60] M. E. Mohamed, E. Y. Frag, and M. A. Mohamed, "A newly validated and characterized spectrophotometric method for determination of a three water pollutants metal ions," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 189, pp. 239–249, Jan. 2018, doi: 10.1016/j.saa.2017.08.001.

[61] C. Mazi, S. Karaderi, and F. Arioz, "Determination of UV-Vis spectrophotometric method of metal complexes stoichiometry between Cu (II) and Zn (II) with Vilazodone hydrochloride," *International Journal of Pharmaceutical Research and Allied Sciences*, vol. 7, no. 3, pp. 146–152, 2018, [Online]. Available: https://ijpras.com/storage/models/article/bxhNnxY9Yek3zTRL7Fje62oykag1rKFOPqXqpr2uMxO85liEud2R0oWunnZv/determination-of-uv-vis-spectrophotometric-method-of-metal-complexes-stoichiometry-between-cu-ii-a.pdf.

[62] S. H. Mallah, "Spectrophotometric determination of the complexation of heavy metal ion with organic reagent," *Journal of Physics: Conference Series*, vol. 1294, no. 5, Art no. 052071, 2019, doi: 10.1088/1742-6596/1294/5/052071.

[63] E. E. Chinyere, O. P. Ukoha, and C. J. Okenwa, "Spectrophotometric determination of chromium (iii) ion by 6-[(e)-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1h-pyrazol-4-yl)diazenyl]-1h-indole-2,3-dione," *International Journal of Research and Innovation in Applied Science*, vol. IV, no. I, pp. 39–44, 2019, [Online]. Available: https://rsisinternational.org/journals/ijrias/DigitalLibrary/Vol.4&Issue1/39-44.pdf.

[64] S. Kuchekar, S. Bhumkar, and H. Aher, "Extractive spectrophotometric determination of osmium (viii) using pmethylphenylthiourea as a chromogenic reagent: Mutual separation of palladium, osmium and platinum," *Journal of Materials and Environmental Sciences*, vol. 10, no. 4, pp. 316–327, 2019, [Online]. Available: https://www.jmaterenvironsci.com/Document/vol10/vol10_N4/33-JMES-Kuchekar-2019.pdf.

[65] S. M. Essa and W. H. Hoidy, "Spectrophotometric determination of cobalt(II) and lead(II) using (1,5-Dimethyl-2-Phenyl-4-((2,3,4-Trihydroxy Phenyl) Diazenyl)-1H-Pyrazol-3(2H)-One) as organic reagent: Using it as antimicrobial and antioxidants," *Nano Biomedicine and Engineering*, vol. 12, no. 2, pp. 160–166, 2020, doi: 10.5101/nbe.v12i2.p160-166.

[66] S. Mohammed Yaseen, B. Basheer Qassim, and N. Owayed Al-Lami, "Spectrophotometric determination of Co(II) in vitamin B12 using2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono)methyl) imidazo [1,2-a]pyridine as ligand by flow injection–merging zone analysis," *Al-Nahrain Journal of Science*, vol. 23, no. 3, pp. 24–38, 2020, doi: 10.22401/anjs.23.3.04.

[67] O. S. Tymoshuk, O. S. Fedyshyn, L. V. Oleksiv, P. V. Rydchuk, and V. S. Matiychuk, "Spectrophotometric determination of palladium(II) ions using a new reagent: 4-(N'-(4-Imino-2-oxo-thiazolidine-5-ylidene)-hydrazino)-benzoic Acid (p-ITYBA)," *Journal of Chemistry*, vol. 2020, pp. 1–8, May 2020, doi: 10.1155/2020/8141853.

[68] V. M. Nurchi, R. Cappai, N. Spano, and G. Sanna, "A Friendly complexing agent for spectrophotometric determination of total iron," *Molecules*, vol. 26, no. 11, Art no. 3071, 2021, doi: 10.3390/molecules26113071.

[69] S. Echioda, A. O. Ogunieye, S. Salisu, A. A. Abdulrasheed, I. Y. Chindo, and A. M. Kolo, "UV-Vis spectrophotometric determination of selected heavy metals (Pb, Cr, Cd and As) in environmental, water and biological samples with synthesized glutaraldehyde phenyl hydrazone as the chromogenic reagent," *European Journal of Advanced Chemistry Research*, vol. 2, no. 3, pp. 1–5, 2021, doi: 10.24018/ejchem.2021.2.3.59.

[70] S. Yaseen, B. Qasim, and N. Al-lame, "Spectrophotometric determination of Cu (+II) by complexation with 2-(4-biphenyl) Imidazo [1,2-] Pyrimidine-3-Hydrazone and studying characteristics of prepared complex.," *Egyptian Journal of Chemistry*, vol. 64, no. 2, pp. 673–691, 2020, doi: 10.21608/ejchem.2019.13907.1861.

[71] K. Elsherif, Q. Hadidan, and k. Alkariwi, "Spectrophotometric determination of Zn (II) and Cu (II) in analytical sample using murexide reagent," *Progress in Chemical and Biochemical Research*, vol. 5, no. 3, pp. 229–238, 2022, doi: 10.22034/pcbr.2022.338475.1222.

[72] L. Alzahrani, H. A. El-Ghamry, A. L. Saber, and G. I. Mohammed, "Spectrophotometric determination of Mercury(II) ions in laboratory and Zamzam water using bis Schiff base ligand based on 1,2,4-Triazole-3,5-diamine and o-Vaniline," *Arabian Journal of Chemistry*, vol. 16, no. 1, Art no. 104418, 2023, doi: 10.1016/j.arabjc.2022.104418.

[73] S. S. Patil, "The estimation of small metal traces of Cd (II) from the alloy and water effluents using fast and accurate extractive spectrophotometric method with newly developed DBA reagent," *Current World Environment*, vol. 17, no. 3, pp. 815–825, 2022, doi: 10.12944/cwe.17.3.26.

[74] F. Abd Wannas, E. A. Azooz, R. K. Ridha, and S. K. Jawad, "Separation and micro determination of zinc(II) and cadmium(II) in food samples using cloud point extraction method," *Iraqi Journal of Science*, vol. 64, no. 3, pp. 1049–1061, 2023, doi: 10.24996/ijs.2023.64.3.2.

[75] S. A. Kolkaila, G. S. Elasala, and A. E. Ali, "Spectrophotometric determination of copper by using erythromycin," *Austin Journal of Analytical & Pharmaceutical Chemistry*, vol. 10, no. 3, Art no. 1165, 2023, [Online]. Available: https://austinpublishinggroup.com/analytical-pharmaceutical-chemistry/fulltext/ajapc-v10-id1165.php.

[76] P. Bhatia, Virender, H. K. Sharma, G. Singh, and B. Mohan, "Extractive spectrophotometric detection of Sn(II) using 6-bromo-3-hydroxy-2-(5-methylfuran-2-yl)-4H-chromen-4-one," *Journal of Fluorescence*, vol. 34, no. 4, pp. 1877–1884, 2023, doi: 10.1007/s10895-023-03413-x.

[77] M. S. Fattah and S. M. Essa, "Spectrophotometric determination of silver ion in pharmaceutical materials using an organic reagent," *Advanced Journal of Chemistry, Section A*, vol. 7, no. 6, pp. 810–819, 2024, doi: 10.48309/ajca.2024 .465524.1579.

[78] S. Nassar, G. I. Mohammed, and T. A. Farghaly, "Direct spectrophotometric method for determination of cadmium (ii) ions using bis-thiosemicarbazone," *International Journal of Analytical Chemistry*, vol. 2025, no. 1, Art no. 3347969, 2025, doi: 10.1155/ianc/3347969.

[79] E. V. Larionova and K. A. Bulygina, "Simultaneous spectrophotometric determination of chromium (VI) and iron (III) in alloys," *IOP Conference Series: Materials Science and Engineering*, vol. 81, Art no. 012093, Apr. 2015, doi: 10.1088/1757-899x/81/1/012093.

[80] M. Abbasi Tarighat, M. Nabavi, and M. R. Mohammadizadeh, "Chemometrics-assisted spectrophotometric method for simultaneous determination of Pb 2+ and Cu 2+ ions in different foodstuffs, soil and water samples using 2-benzylspiro [isoindoline-1,5 -oxazolidine]-2 ,3,4 -trione using continuous wavelet transformation and partial least squares – Calculation of pK f of complexes with rank annihilation factor analysis," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 145, pp. 54–62, Jun. 2015, doi: 10.1016/j.saa.2015.02.048.

[81] V. Fornea, Ş. Trupină, A. V. Iosub, and L. Bulgariu, "Spectrophotometric determination of Cu(ii), Co(ii) and Ni(ii) ions in mono and multi-component systems," *Buletinul Institutului Politehnic Din Iaşi*, vol. 62, no. 66, pp. 9–20, 2016, [Online]. Available: https://www.bipcic.icpm.tuiasi.ro/pdf/2016/2/bipi_cic_2016_2_01.pdf.

[82] D. Admasu, D. N. Reddy, and K. N. Mekonnen, "Trace determination of zinc in soil and vegetable samples by spectrophotometry using pyridoxal thiosemicarbazone and 2-acetyl pyridine thiosemicarbazone," *Cogent Chemistry*, vol. 2, no. 1, Art no. 1249602, L. N. Suvarapu, Ed., 2016, doi: 10.1080/23312009.2016.1249602.

[83] F. Bagheban-Shahri and A. Niazi, "Chemometrics-enhanced simultaneous spectrophotometric determination of aluminum and bismuth with hematoxylin in vegetables and water using multivariate calibration," *Journal of Water Reuse and Desalination*, vol. 6, no. 1, pp. 137–147, 2015, doi: 10.2166/wrd.2015.047.

[84] A. Fakhraei, M. Abbasi-Tarighat, K. Mohammadi, G. Abdi, and A. Rezaei, "Chemometrics-enhanced kinetic spectrophotometric method for simultaneous determination of Ag^{+}, Cu^{2+} and Ni^{2+} ions in some medicinal plants by dimethyl 2,2'-(ethan-1,2-diylbis1)bis(cyclopent-1-ene-1-carbodithioate)," *Analytical and Bioanalytical Chemistry Research*, vol. 5, no. 2, pp. 317–330, 2018, doi: 10.22036/abcr.2018.122457.1194.

[85] L. Ngoc Tu, L. Van Tan, and N. Xuan Chien, "Simultaneous spectrophotometric determination of Cu(ii) and Co(ii) using 5-bromosalicylaldehyde thiosemicarbazone by partial least squares regression method," *Rasayan Journal of Chemistry*, vol. 11, no. 2, pp. 850–856, 2018, doi: 10.31788/rjc.2018.1122088.

[86] Z. Rasouli, M. Irani, S. Jafari, and R. Ghavami, "Study of interaction of metal ions with methylthymol blue by chemometrics and quantum chemical calculations," *Scientific Reports*, vol. 11, no. 1, Art no. 6465, 2021, doi: 10.1038/s41598-021-85940-w.

[87] F. Zhou, A. Oad, H. Zhu, and C. Li, "Quantitative analysis of polymetallic ions in industrial wastewater based on ultraviolet-visible spectroscopy," *Sustainability*, vol. 13, no. 14, Art no. 7907, 2021, doi: 10.3390/su13147907.

[88] N. T. Phuong, N. T. An, P. T. Xuan, L. T. K. Anh, T. T. P. Dung, and D. T. M. Phuong, "Simultaneous spectrophotometric determination of copper (ii) and cobalt (ii) using partial least-squares regression," *Journal of Science Technology and Food*, vol. 22, no. 3, pp. 89–95, 2022, [Online]. Available: https://scholar.dlu.edu.vn/thuviensoso/bitstream/DLU123456789/268741/1/72515-1465-178103-1-10-20221019.pdf.

[89] F.-B. Zhou, C.-G. Li, and H.-Q. Zhu, "A characteristic interval modeling method for simultaneous detection of multiple metal ions," *Frontiers in Chemistry*, vol. 10, Art no. 839633, Feb. 2022, doi: 10.3389/fchem.2022.839633.

[90] S. Suprapto, Y. L. Ni'mah, and F. A. Putrama, "Direct Cu, Fe, and Ni ions multicomponent analysis using UV-Vis spectrophotome- tric method," *IJCA (Indonesian Journal of Chemical Analysis)*, vol. 6, no. 2, pp. 106–115, 2023, doi: 10.20885/ijca. vol6.iss2.art2.

[91] H. Liu, J. Xie, and H. Qian, "Simultaneous determination of mixed heavy metal ions Cr (III) and Co (II) in wastewater by cloud point extraction dual wavelength spectrophotometry," *E3S Web of Conferences*, vol. 406, Art no. 01012, 2023, doi: 10.1051/e3sconf/202340601012.

[92] L. A. Nguyễn, B. P. Thi, D. T. N. Quyen, V. Q. Thu, N. T. Huong, K. H. Binh, K. M. Hung, N. C. Thanh, and T. T. Thao, "Machine learning approaches for simultaneous spectrophotometric determination of heavy metal ions in water samples," *Journal of Military Science and Technology*, vol. 95, pp. 47–54, May 2024, doi: 10.54939/1859-1043.j.mst.95.2024.47-54.

[93] A. H. Aktaş and İ. Gençer, "Analysis of some heavy metals level in Isparta's drinking water using UV spectrophotometry and principal component regression," *Universal Journal of Pharmaceutical Research*, vol. 9, no. 1, pp. 30–36, 2024, doi: 10.22270/ujpr.v9i1.1059.

[94] M. F. Abdel-Ghany, O. Abdel-Aziz, M. F. Ayad, and N. N. Mikawy, "Simultaneous determination of octinoxate, oxybenzone, and octocrylene in a sunscreen formulation using validated spectrophotometric and chemometric methods," *Journal of AOAC International*, vol. 98, no. 5, pp. 1215–1225, 2015, doi: 10.5740/jaoacint.14-229.

[95] T. Madrakian, M. Madadi-Shad, and M. Soleimani, "Simultaneous spectrophotometric determination of mycophenolate mofetil and its active metabolite in human plasma using chemometrics methods," *Analytical and Bioanalytical Chemistry Research*, vol. 2, no. 1, pp. 42–51, 2015, doi: 10.22036/abcr.2015.9203.

[96] J. Karanjia, "Development and validation of chemometric assisted spectrophotometric technique for simultaneous estimation of Cinitapride and pantoprazole from bulk and combined dosage form," *International Journal of Pharmaceutical Sciences and Drug Research*, vol. 7, no. 2, pp. 198–204, 2015, doi: 10.25004/ijpsdr.2015.070213.

[97] Y. K. Al-Bayati and R. R. Karabat, "Second derivative spectrophotometric determination of phenytoin in pharmaceutical preparations," *Journal of Al-Nahrain University of Science*, vol. 19, no. 1, pp. 36–42, 2016, doi: 10.22401/jnus.19.1.04.

[98] E. T. Al Samaraee, "Derivative spectrophotometric method for estimation of Chlorphinramine Maleat in pure and its formulation Phenadone," *Kerbala Journal of Pharmaceutical Sciences*, vol. 11, pp. 179–191, 2016, [Online]. Available: https://iasj.rdd.edu.iq/journals/uploads/2025/02/14/2da7e80c4a0313df79760911b71b9ea8.pdf.

[99] S. B. Dikran, A. K. Mohammed, and N. A. Alassaf, "Spectrophotometric-Assisted chemometric method for the simultaneous analysis of furosemide, carbamazepine, diazepam, and carvedilol in their bulk and marketed formulation," *Journal of Natural Sciences Research*, vol. 6, no. 6, pp. 48–59, 2016, [Online]. Available: https://www.iiste.org/Journals/index.php/JNSR/article/view/29416/30206.

[100] M. Rizk, M. Sultan, I. Habib, D. Mohamed, and R. Tony, "Simultaneous spectrophotometric determination of paracetamol and dantrolene sodium by chemometric methods," *British Journal of Pharmaceutical Research*, vol. 13, no. 3, pp. 1–8, 2016, doi: 10.9734/bjpr/2016/29078.

[101] H. A. Merey, S. S. El-Mosallamy, N. Y. Hassan, and B. A. El-Zeany, "Spectrophotometric and chemometric study for the simultaneous determination of mometasone furoate and miconazole nitrate in the presence of pharmaceutical dosage form additive," *Analytical Chemistry Letters*, vol. 6, no. 1, pp. 70–85, 2016, doi: 10.1080/22297928.2016.1153980.

[102] S. V. Gandhi, A. R. Pahade, A. S. Sutar, B. S. Kuchekar, and S. R. Tapale, "Simultaneous estimation of beclomethasone dipropionate and salbutamol sulphate in capsules by chemometric assisted UV-spectrophotometric method," *International Journal of Pharmaceutical Chemistry and Analysis*, vol. 3, no. 2, p. 84, 2016, doi: 10.5958/2394-2797.2016.00013.7.

[103] A. El-Kosasy, O. Abdel-Aziz, N. Magdy, and N. El Zahar, "Spectrophotometric and chemometric methods for determination of imipenem, ciprofloxacin hydrochloride, dexamethasone sodium phosphate, paracetamol and cilastatin sodium in human urine," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 157, pp. 26–33, Mar. 2016, doi: 10.1016/j.saa.2015.12.011.

[104] S. A. Darweesh, "Simultaneous determination of sulfanilamide and furosemide by using derivative spectrophotometry," *Ibn AL-Haitham Journal For Pure and Applied Science*, vol. 29, no. 2, pp. 240–253, 2016, [Online]. Available: https://iasj.rdd.edu.iq/journals/uploads/2024/12/24/a715241a3b67a907e61e31e40f4b0473.pdf.

[105] A. L. H. A. j. Alsamarrai and A. P. K. F. Alsamarrai, "Spectrophotometric determination of Metronidazole and Metronidazole benzoate via first and Second Derivative order spectroscopy," *Baghdad Science Journal*, vol. 14, no. 2, pp. 320–327, 2017, doi: 10.21123/bsj.2017.14.2.0320.

[106] S. V. Gandhi and A. S. Mutha, "Chemometric – assisted uv spectrophotometric method for determination of antihyperlipidemic agents in pharmaceutical formulation," *Journal of Applied Pharmaceutical Research*, vol. 5, no. 2, pp. 27–33, 2017, [Online]. Available: https://www.japtronline.com/index.php/joapr/article/view/75.

[107] G. P. Ertokuş and K. N. Çatalyürek, "Spectrophotometric and chemometric methods for simultaneous determination of alzheimer's drugs in pharmaceutical tablets," *International Journal of Pharmaceutical Research & Allied Sciences*, vol. 6, no. 4, pp. 73–79, 2017, [Online]. Available: https://ijpras.com/article/spectrophotometric-and-chemometric-methods-for-simultaneous-determination-of-alzheimers-drugs-in-pharmaceutical-tablets.

[108] S. R. Chaudhari, A. S. Patil, and A. A. Shirkhedkar, "Studies on derivative spectroscopy and area under curve UV-Spectrophotometric methods for estimation of apremilast in bulk and in-house tablets," *Asian Journal of Pharmaceutical Research*, vol. 8, no. 1, pp. 11–16, 2018, doi: 10.5958/2231-5691.2018.00003.5.

[109] F. Adriani, M. Muchlisyam, and S. M. Sinaga, "Development and validation of double divisor ratio spectra derivative spectrophotometry method for ternary mixture of guaifenesin, dextromethorphan HBr, and diphenhydramine HCl in tablet dosage form," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 11, no. 13, p. 1, 2018, doi: 10.22159/ajpcr.2018.v11s1.26550.

[110] T. Eticha, G. Kahsay, F. Asefa, T. Hailu, H. Gebretsadik, T. Gebretsadikan, and B. Thangabalan, "Chemometric-Assisted spectrophotometric method for the simultaneous determination of ciprofloxacin and doxycycline hyclate in pharmaceutical formulations," *Journal of Analytical Methods in Chemistry*, vol. 2018, pp. 1–5, Dec. 2018, doi: 10.1155/2018/9538435.

[111] S. Alahmad, H. M. Elfatatry, M. M. Mabrouk, S. F. Hammad, and F. R. Mansour, "Development and validation of chemometric spectrophotometric methods for simultaneous determination of simvastatin and nicotinic acid in binary combinations," *Current Drug Discovery Technologies*, vol. 15, no. 15, pp. 149–155, 2018, doi: 10.2174/1570163814666170810120921.

[112] P. Katsarov, G. Gergov, A. Alin, B. Pilicheva, Y. Al-Degs, V. Simeonov, and M. Kassarova, "Advanced spectrophotometric chemometric methods for resolving the binary mixture of doxylamine succinate and pyridoxine hydrochloride," *Acta Pharmaceutica*, vol. 68, no. 1, pp. 61–73, 2018, doi: 10.2478/acph-2018-0008.

[113] G. Ertokus and A. Tugrul, "Spectrophotometric determination of acetylsalicylic acid, paracetamol and ascorbic acid by chemometric methods," *Chemistry & Chemical Technology*, vol. 12, no. 3, pp. 279–284, 2018, doi: 10.23939/chcht12.03.279.

[114] A. Aktaş and H. Şahin, "Spectrophotometric determination of paracetamol, propyphenazone and caffeine in tablets by multivariate calibration approach," *Analytical Chemistry: An Indian Journal*, vol. 19, no. 1, Art no. 143, 2019, doi: 10.37532/0974-7419.2019.19(1).143.

[115] C. M. El-Maraghy and N. T. Lamie, "Three smart spectrophotometric methods for resolution of severely overlapped binary mixture of Ibuprofen and Paracetamol in pharmaceutical dosage form," *BMC Chemistry*, vol. 13, no. 1, Art no. 99, 2019, doi: 10.1186/s13065-019-0618-3.

[116] S. B. Dikran, N. A. Alassaf, F. H. Zankanah, and A. K. Mohammed, "Simultaneous determination of furosemide, carbamazepine, diazepam and carvedilol in quaternary mixture via derivative spectrophotometry," *Journal of Global Pharma Technology*, vol. 11, no. 5, pp. 128–141, 2019, [Online]. Available: https://www.scopus.com/pages/publications/85076979918.

[117] M. Albayrak, F. Demirkaya-Miloglu, O. Senol, and E. Polatdemir, "Design, optimization, and validation of chemometrics-assisted spectrophotometric methods for simultaneous determination of etodolac and thiocolchicoside in pharmaceuticals," *Journal of Analytical Science and Technology*, vol. 10, no. 1, 2019, doi: 10.1186/s40543-019-0176-2.

[118] M. Uddin, A. Mondol, M. Karim, R. Jahan, and A. Rana, "Chemometrics assisted spectrophotometric method for simultaneous determination of paracetamol and caffeine in pharmaceutical formulations," *Bangladesh Journal of Scientific and Industrial Research*, vol. 54, no. 3, pp. 215–222, 2019, doi: 10.3329/bjsir.v54i3.42673.

[119] C. M. El-Maraghy, H. Salem, S. M. Amer, and M. Nebsen, "Stability indicating spectrophotometric and chemometric methods for determination of aripiprazole in presence of its degradation products, a comparative study," *Analytical Chemistry Letters*, vol. 9, no. 2, pp. 258–272, 2019, doi: 10.1080/22297928.2019.1585286.

[120] S. A. Omer and N. A. Fakhre, "Simultaneous determination of ternary mixture of carboxin, chlorpyrifos, and tebuconazole residues in cabbage samples using three spectrophotometric methods," *Journal of Analytical Methods in Chemistry*, vol. 2020, pp. 1–16, Feb. 2020, doi: 10.1155/2020/4912762.

[121] C. M. El-Maraghy and E. H. Mohamed, "Successive stability indicating spectrophotometric technique for simultaneous determination of quetiapine fumarate and its three major related compounds," *Current Analytical Chemistry*, vol. 16, no. 4, pp. 447–455, 2020, doi: 10.2174/1573411014666180709145526.

[122] M. Gülfen, "Ratio - First order derivative - Zero crossing UV-Visible spectrophotometric method for analysis of amoxicillin, levofloxacin and lansoprazole mixture," *Pakistan Journal of Analytical & Environmental Chemistry*, vol. 21, no. 1, pp. 34–43, 2020, doi: 10.21743/pjaec/2020.06.05.

[123] M. Esteki, E. Dashtaki, Y. V. Heyden, and J. Simal-Gandara, "Application of rank annihilation factor analysis for antibacterial drugs determination by means of pH gradual change-UV spectral data," *Antibiotics*, vol. 9, no. 7, Art no. 383, 2020, doi: 10.3390/antibiotics9070383.

[124] I. Bennani, M. A. Chentoufi, I. S. E. Otmani, A. Cheikh, N. Bamou, M. E. Karbane, and M. Bouatia, "Development and validation of two spectrophotometric methods for simultaneous determination of diosmine and hesperidin in mixture and their applications," *Journal of Applied Pharmaceutical Science*, vol. 10, no. 7, pp. 100–107, 2020, doi: 10.7324/japs.2020.10713.

[125] M. A. Mohammed, S. M. Abbas, and J. M. S. Jamur, "Derivative spectrophotometric determination for simultaneous estimation of isoniazid and ciprofloxacin in mixture and pharmaceutical formulation," *Methods and Objects of Chemical Analysis*, vol. 15, no. 3, pp. 105–110, 2020, doi: 10.17721/moca.2020.105-110.

[126] B. Kumar, N. P Singh, S. Singh, and Y. Tiwari, "Synchronized spectrophotometric determination of tenofovir & zidovudine in tablets by chemometric approaches," *International Journal of Psychosocial Rehabilitation*, vol. 24, no. 3, pp. 3586–3596, May 2020, doi: 10.61841/0m3a6032.

[127] A. H. Aktas and Y. Tuna, "Simultaneous spectrophotometric determination of hypertension drug's in commercial pharmaceutical by chemometric methods," *International Journal of Scientific and Technological Research*, vol. 6, no. 12, pp. 1–8, 2020, doi: 10.7176/jstr/6-12-01.

[128] K. Palur, S. C. Archakam, and B. Koganti, "Chemometric assisted UV spectrophotometric and RP-HPLC methods for simultaneous determination of paracetamol, diphenhydramine, caffeine and phenylephrine in tablet dosage form," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 243, Art no. 118801, Dec. 2020, doi: 10.1016/j.saa.2020.118801.

[129] N. M. El Zahar, M. M. Tadros, and B. M. Ayoub, "Development of advanced chemometric-assisted spectrophotometric methods for the determination of cromolyn sodium and its alkaline degradation products," *Molecules*, vol. 25, no. 24, Art no. 5953, 2020, doi: 10.3390/molecules25245953.

[130] K. Patel, P. Shah, P. Shah, and T. Gandhi, "UV-spectrophotometry-assisted chemometric methods for simultaneous determination of ambroxol hydrochloride and doxofylline in pharmaceutical formulation," *Journal of Chemical Metrology*, vol. 14, no. 2, pp. 106–113, 2020, doi: 10.25135/jcm.47.20.05.1638.

[131] N. M. Fahmy, K. Hesham, S. M. Tawakkol, L. AbdelAziz, and M. H. Abdelrahman, "Three different approaches based on derivative ratio spectra for spectrophotometric resolution of a quaternary mixture in semisolid dosage form," *Journal of AOAC INTERNATIONAL*, vol. 104, no. 5, pp. 1223–1231, 2021, doi: 10.1093/jaoacint/qsab059.

[132] N. N. H. Asmaa Ghanim Dawood and L. S. Omer, "H-point standard addition method for the simultaneous spectrophotometric determination of captopril and hydrochlorothiazide in pharmaceutical formulations," *Jordan Journal of Chemistry (JJC)*, vol. 16, no. 2, pp. 77–85, 2021, doi: 10.47014/16.2.4.

[133] M. M. Annapurna and Y. Manishankar, "Chemo-metric assisted uv-spectrophotometric methods for the simultaneous estimation of brimonidine tartrate and timolol maleate," *Acta Scientific Pharmaceutical Sciences*, vol. 5, no. 5, pp. 60–66, 2021, [Online]. Available: https://actascientific.com/ASPS/pdf/ASPS-05-0717.pdf.

[134] A. M. Azeez and N. A. Fakhre, "Determination of ketoprofen in tablet dosage forms by derivative IR spectroscopy," *Egyptian Journal of Chemistry*, vol. 65, no. 1, pp. 215–219, 2021, doi: 10.21608/ejchem.2021.79993.3937.

[135] M. Mabrouk, S. Hammad, and N. Elzawawy, "Different spectrophotometric methods of operating ratio spectra for analyzing pharmaceutical combinations of loratadine and dexamethasone," *Journal of Advanced Medical and Pharmaceutical Research*, vol. 3, no. 1, pp. 19–28, 2022, doi: 10.21608/jampr.2022.132917.1028.

[136] S. A. Nadir and N. A. Fakhre, "Simultaneous determination of binary mixtures of aniline and 2-nitronailine in tap water samples by derivative spectrophotometry," *Eurasian Journal of Science and Engineering*, vol. 8, no. 3, pp. 12–24, 2022, doi: 10.23918/eajse.v8i3p12.

[137] A. H. Abdelazim and S. Ramzy, "Simultaneous spectrophotometric determination of finasteride and tadalafil in recently FDA approved Entadfi^{TM} capsules," *BMC Chemistry*, vol. 16, no. 1, Art no. 55, 2022, doi: 10.1186/s13065-022-00850-w.

[138] B. Mehta, H. Joshi, U. Shah, and P. Patel, "Chemometric assisted spectrophotometric method for the simultaneous determination of olmesartan medoxomil and hydrochlorothiazide in bulk and tablet dosage form," *Indian Journal of Pharmaceutical Sciences*, vol. 84, no. 3, pp. 669–675, 2022, doi: 10.36468/pharmaceutical-sciences.962.

[139] F. Selimoğlu and N. Pinarcik, "Spectrophotometric quantification of paracetamol and tramadol hydrochloride by chemometric calibration methods," *Turkish Journal of Chemistry*, vol. 47, no. 3, pp. 633–645, 2023, doi: 10.55730/1300-0527.3566.

[140] F. Zankanah, F. Al Ani, A. Shaker, A. Taha, and M. Abdulraheem, "First- and second-derivative spectrophotometry for simultaneous determination of lorazepam and clonazepam in pharmaceutical formulations," *Egyptian Journal of Chemistry*, vol. 66, no. 3, pp. 271–280, 2022, doi: 10.21608/ejchem.2022.130646.5753.

[141] D. Khasraw Ali, N. Adil Fakhre, and C. Mohameed Rasheed, "Ratio derivative-zero crossing and successive derivative of ratio spectra for simultaneous determination of urea, creatinine, and uric acid in human urine samples," *Bulletin of the Chemical Society of Ethiopia*, vol. 37, no. 4, pp. 817–829, 2023, doi: 10.4314/bcse.v37i4.2.

[142] D. K. Ali, N. A. Fakhre, and C. M. Rasheed, "Simultaneous Determination of Binary Mixture of Estradiol and Progesterone Using Different Spectrophotometric Methods," *Baghdad Science Journal*, vol. 20, no. 6, pp. 2193–2206, 2023, doi: 10.21123/bsj.2023.7822.

[143] G. Tiris, E. N. Oven, and N. Erk, "Simultaneous spectrophotmetric determination of fluoxetine and olanzapine greennes assessment," *Ankara Universitesi Eczacilik Fakultesi Dergisi*, vol. 47, no. 3, pp. 800–807, 2023, doi: 10.33483/jfpau.1294563.

[144] A. Madkour, "Chemometric assisted spectrophotometric methods for simultaneous determination of amlodipine /candesartan mixture in their pure forms and their pharmaceutical preparations," *Al-Azhar Journal of Pharmaceutical Sciences*, vol. 67, no. 1, pp. 100–111, 2023, doi: 10.21608/ajps.2023.311252.

[145] S. Rathod, P. Patel, and N. Patel, "Chemometrics assisted spectrophotometric method development and validation for simultaneous estimation of abacavir, lamivudine and dolutegravir in dosage form," *Indian Journal of Pharmaceutical Education and Research*, vol. 57, no. 2, pp. 570–582, 2023, doi: 10.5530/ijper.57.2.70.

[146] M. Bachri and S. Safarul Rizky, "UV Spectrophotometry with Chemometric methods for Concurrent Assays of Antihypertensive Components in Tablets," *Research Journal of Pharmacy and Technology*, vol. 16, no. 9, pp. 4314–4318, 2023, doi: 10.52711/0974-360x.2023.00706.

[147] M. A. Munir, A. Inayatullah, S. Ibrahim, I. R. Rimba Putri, E. Emelda, A. Fatmawati, and N. Nurhidayanti, "A modest UV spectrophotometric assisted by chemometric approach for verification of acetaminophen level in various manufactured tablets and syrups in Indonesian pharmacies," *International Journal of Applied Pharmaceutics*, vol. 15, no. 1, pp. 195–205, 2023, doi: 10.22159/ijap.2023v15i1.46290.

[148] R. Magdy, N. V. Fares, M. Farouk, and A. Hemdan, "A novel, sustainable, and Eco-friendly spectrophotometric and chemometric approach for determination of severely overlapped spectrum via unified regression equation: Greenness and whiteness assessment," *Journal of AOAC International*, vol. 106, no. 5, pp. 1348–1360, 2023, doi: 10.1093/jaoacint/qsad037.

[149] F. Boccato Payolla, A. C. Massabni, and C. Orvig, "Radiopharmaceuticals for diagnosis in nuclear medicine: a short review," *Eclética Química Journal*, vol. 44, no. 3, pp. 11–19, 2019, doi: 10.26850/1678-4618eqj.v44.3.2019.p11-19.

[150] S. M. Obeidat and I. F. Al Momani, "Spectrophotometric and chemometric methods for simultaneous determination of antazoline and naphazoline in pharmaceutical eye drops," *Spectroscopy*, Oct. 2024, doi: 10.56530/spectroscopy.rp6290m8.

[151] A. N. Mozayad, M. A. Fouad, and E. F. Elkady, "Design and validation of eco-friendly chemometric-assisted spectrophotometric methods for the simultaneous determination of chlorzoxazone, paracetamol, and aceclofenac in the presence of their main impurities," *Bulletin of Faculty of Pharmacy Cairo University*, vol. 62, no. 1, pp. 44–57, 2024, doi: 10.54634/2090-9101.1053.

[152] B. Alattab, F. A. Alarbagi, M. A. Almaqtari, E. Alhuraishi, and H. Al-Maydama, "Development and validation of a green spectrophotometric method for simultaneous determination of combined pharmaceutical dosage form (paracetamol and caffeine) using chemometrics technique in comparison with HPLC," *Ecletica Quimica*, vol. 49, pp. 69–74, Sep. 2024, doi: 10.26850/1678-4618.eq.v49.2024.e1515.

[153] K. M. Kelani, R. A. Fekry, Y. M. Fayez, and S. A. Hassan, "Advanced chemometric methods for simultaneous quantitation of caffeine, codeine, paracetamol, and p-aminophenol in their quaternary mixture," *Scientific Reports*, vol. 14, no. 1, Art no. 2085, 2024, doi: 10.1038/s41598-024-52450-4.

[154] O. B. Hassan, E. F. ELkady, A. A. El-Zaher, and R. M. Sayed, "Resolution of overlapping spectra using chemometric manipulations of UV-spectrophotometric data for the determination of Atenolol, Losartan, and Hydrochlorothiazide in pharmaceutical dosage form," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 318, Art no. 124471, Oct. 2024, doi: 10.1016/j.saa.2024.124471.

[155] R. Ríos-Reina and S. M. Azcarate, "How chemometrics revives the UV-Vis spectroscopy applications as an analytical sensor for spectralprint (Nontargeted) analysis," *Chemosensors*, vol. 11, no. 1, Art no. 8, 2022, doi: 10.3390/chemosensors11010008.

[156] O. Y. Rodionova and A. L. Pomerantsev, "Chemometrics: Achievements and prospects," *Russian Chemical Reviews*, vol. 75, no. 4, pp. 271–287, 2006, doi: 10.1070/rc2006v075n04abeh003599.