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From HPLC to Chemometric-Assisted Spectroscopy: A Comparative Study of Analytical Approaches

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Abstract

Analytical techniques are crucial in pharmaceutical quality control, particularly in advanced drug delivery systems, where drug loading, release, and stability are critical parameters. Among the available techniques, High Performance Liquid Chromatography (HPLC) remains the established reference method for determining drug content. However, there is increasing interest in approaches that reduce analysis time and cost while maintaining compliance with validation requirements. This review compares HPLC with spectroscopic techniques, including ATR-FTIR, Raman, and UV–Vis, applied in combination with chemometric tools such as Partial Least Squares Regression (PLSR) and Principal Component Analysis (PCA). These integrated methods are non-destructive, require minimal sample preparation, and allow quantification of active ingredients, detection of impurities, and assessment of formulation consistency. This study focuses on integrating chemometric techniques with spectroscopic methods and comparing their performance with that of HPLC, given evidence that chemometric-assisted spectroscopy can match the accuracy of HPLC while improving efficiency and sustainability. The review highlights the potential of these approaches in modern pharmaceutical analysis.

Keywords: Bioactive peptides; Biomaterial; Immobilization; Polystyrene; Solid-phase peptide synthesis.

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1. Introduction

The pharmaceutical industry continually seeks rapid, accurate, and non-destructive analytical techniques to ensure the quality and efficacy of pharmaceutical products. Spectroscopic methods have emerged as essential tools in this regard, offering detailed molecular insights without the need for extensive sample preparation (1-3).

Ultraviolet spectroscopy is a widely utilized technique in analytical chemistry for determining analyte concentrations and examining biomolecules. The method involves comparing the transmitted light of a sample with that of a reference, relying on electronic transitions between energy states. Its ease of use and accuracy make it a practical tool for analyzing conjugated double bonds and aromatic conjugations. Although UV detection is the most straightforward and convenient method, its low sensitivity and selectivity are the most limiting problems, especially in pharmaceutical products (4).

High-performance liquid chromatography (HPLC) is a unique technique that remains a cornerstone in pharmaceutical analysis, renowned for its precision in separating

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and quantifying components within complex mixtures. Its versatility and reliability make it indispensable for tasks ranging from drug formulation to stability testing (5). One of the most common detectors used in HPLC is Ultraviolet-Visible (UV-VIS) spectroscopy, which, known for its simplicity and rapid analysis capabilities, plays a crucial role in quantifying active pharmaceutical ingredients and assessing drug stability (6, 7) .

Although UV detection is the most straightforward and convenient method, High-Performance Liquid Chromatography (HPLC) is essential for achieving proper separation of components. High-Performance Liquid Chromatography (HPLC) remains a cornerstone in pharmaceutical analysis, renowned for its precision in separating and quantifying components within complex mixtures. Its versatility and reliability make it indispensable for tasks ranging from drug formulation to stability testing (5).

One of the most common detectors used in HPLC is Ultraviolet-Visible (UV-Vis) spectroscopy, which, known for its simplicity and rapid analysis capabilities, plays a crucial role in quantifying active pharmaceutical ingredients and assessing drug stability (6, 7).

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy provides detailed information about molecular vibrations, enabling the identification of functional groups and assessing drug purity. Raman spectroscopy, non-invasive and compatible with aqueous samples, complements ATR-FTIR and is effective in characterizing polymorphic forms and monitoring in-process quality (8-10).

Despite the capabilities of ATR-FTIR, Raman, and UV–Vis, interpreting spectral data from complex pharmaceutical formulations can be challenging. Chemometrics is a branch of science that utilizes mathematical and statistical methods to extract useful information from physical and chemical phenomena involved in a production process (11, 12). Chemometrics is considered a valuable tool for drug discovery, development, design, and synthesis. It can also identify, classify, and predict the toxicity of various medicinal substances. Additionally, chemometric methods proved suitable for performing multidimensional calibration across spectroscopic, electrochemical, and chromatographic techniques. Their use, particularly in interpreting UV-Vis and near-infrared (NIR) spectra, as well as datasets from other analytical instruments, enables both the identification and quantitative determination of active compounds within complex mixtures. Such applications are critical in the quality control and analysis of pharmaceutical products available on the market (13).

These methods can be broadly divided into unsupervised approaches, which explore natural groupings in data such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), and supervised approaches, which build predictive models based on known outputs like Partial Least Squares Regression (PLSR), Linear Discriminant Analysis (LDA), Artificial Neural Network (ANN), and Vector Machine Support (SVM) (14-17). In pharmaceutical sciences, chemometrics is essential for quantitative analysis, the discrimination of polymorphs, and the detection of counterfeit medicines (18-20). Table 1 highlights the primary purposes, advantages, limitations, and typical applications of the aforementioned chemometric techniques .

This review delves into the principles and applications of ATR-FTIR, Raman, UV-Vis spectroscopy, and HPLC in pharmaceutical analysis; however, its emphasis is placed on the integration of these techniques with chemometric approaches, highlighting their collective potential in advancing pharmaceutical quality control and research.

2. Search strategy

We identified relevant literature through a structured search of PubMed, Scopus, Web of Science, and Google Scholar. Our search spanned the period from 2005 to 2025 and initially retrieved several hundred records. After removing duplicates, we screened the titles and abstracts for the required relevance. We included articles that: 1) reported the application of UV-Vis, ATR-FTIR, or Raman spectroscopy combined with chemometric methods in pharmaceutical analysis; 2) compared these approaches with HPLC; or 3) pro-

Table 1. Comparative overview of supervised and unsupervised chemometric methods.

Method	Type	Main Purpose	Strengths	Limitations	Typical Applications	Ref
PCA	Unsupervised	Dimensionality reduction	Enhanced data visualization	operational challenges posed by high-volume and high-dimensional data	Outlier detection	[21-24]
HCA	Unsupervised	Classification	Insight into nested clusters	Sensitive to noise	Identity assessment	[25-29]
PLSR	Supervised	Quantitative regression	Collinearity handling	Sensitive to outliers	API quantification	[30-35]
LDA	Supervised	Classification	Effective in separating classes	Assumes normal distribution	Dimension reduction	[36-39]
ANN	Supervised	Non-linear modeling	Parallel processing capability	Difficulty in determining proper network structure	Pattern recognition, and information processing	[40-43]
SVM	Supervised	Classification and regression	Avoiding overfitting, possibility of using high-dimensional data, high accuracy	Requires tuning	Pharmaceutical identification, and drug design	[44-48]

vided methodological or application-focused insights relevant to pharmaceutical quality control.

Exclusion criteria were studies outside the pharmaceutical field, non-English publications, and conference abstracts without full text. After conducting a full-text assessment, we compiled and analyzed the final set of studies to provide a comparative overview of HPLC and chemometric-assisted spectroscopic techniques.

3. UV-Vis analysis

3.1. Principles

UV-Vis spectrophotometry involves analyte determination by measuring the amount of UV-Vis light absorbed, taking into account standard values. In this spectrophotometry technique, analysts enhance the color of the assessing solution by adding dyes, oxidizing agents, or chelating agents. UV-Vis derivative spectrophotometry is more desirable compared to zero-order UV-Vis spectrophotometry due to its ability to determine the amount of analyte when the conventional method is ineffective. UV-Vis spectroscopy transmits light between 200 and 800 nm to the sample. It determines the amount of absorbed or transmitted light by a chromogen located between the light source and a photometer. Ultimately, a UV-Vis absorption spectrum plot displays the amount of absorbance at various wavelengths. The UV-Vis method, like other quantitative spectrophotometry techniques, applies Lambert's Law and Beer's Law (49).

3.2. Applications

One of the significant applications of UV-Vis spectroscopy involves analyzing raw or formulated pharmaceutical compounds by measuring the absorbance at a specific wavelength. In qualitative analysis, UV-Vis identifies light-absorbing molecules, while in quantitative analysis, researchers utilize Beer's law. Additionally, scientists use UV-Vis spectroscopy to study reaction kinetics, determine molecular weight, or assess the degree of conjugation (49).

3.3. Advantages

Quick and simple analysis, as well as the provision of valuable information regarding the presence of an analyte in a respective sample, are considered the advantages of UV-Vis spectroscopy (50).

3.4. Limitations

However, this technique is limited to compounds that can absorb ultraviolet or visible light. Substances lacking strong UV-

Vis absorption cannot be accurately detected or quantified. Additionally, the method often suffers from low sensitivity and interference from other sample components, which can affect accuracy. The requirement of a spectrophotometer also limits its accessibility in some settings (50).

3.5. Integrating UV-Vis spectroscopy with chemometric approaches

According to the suitability and simplicity of UV-Vis spectroscopy, to overcome its weakness, integrating compatible analysis methods has been developed. The integration of UV-Vis spectroscopy with chemometric techniques may enhance the ability to analyze complex pharmaceutical and chemical systems by enabling the resolution of overlapping spectra and the quantification of multicomponent mixtures. The methodology begins with the preparation of samples and the acquisition of spectra across an appropriate wavelength range, typically 190–800 nm. To ensure data quality, the raw spectral data undergo preprocessing steps, including baseline correction to remove background noise, normalization to standardize the spectra, and smoothing to reduce random fluctuations. To improve the resolution of overlapping peaks, researchers may apply derivative transformations (first or second derivative). After preprocessing, they employ multivariate statistical techniques such as PCA for pattern recognition and outlier detection, while they use PLSR or Multiple Linear Regression (MLR) for quantitative modeling. This chemometric integration enables the precise identification and quantification of active pharmaceutical ingredients, impurities, and degradation products, supporting quality control processes in both research and industrial settings (51).

3.6. Practical applications

One study investigated the simultaneous determination of amlodipine and valsartan in combined formulations. Researchers used HPLC as a reference method and evaluated UV–Vis spectrophotometry combined with chemometric methods as an alternative. They applied feed-forward neural networks (FFNN) using the Levenberg–Marquardt (LM) algorithm and gradient descent with momentum (GDX), finding that the LM algorithm demonstrated better predictive performance. Additionally, they optimized LS-SVM with a radial basis function kernel via leave-one-out cross-validation and root mean square error (RMSE), confirming its appropriateness with coefficients of determination (R^2), relative standard deviation (RSD), and mean recovery. Both chemometric methods yielded results consistent with HPLC, demonstrating that spectrophotometric–chemometric analysis is a reliable and cost-effective alternative that reduces analysis time and simplifies operations (52).

The genetic algorithm coupled with the PLS (GA-PLS) chemometric method, combined with UV–Vis spectrophotometry, proved effective for the simultaneous quantification of paracetamol and tramadol, addressing challenges such as spectral overlap that hindered other methods. In this approach, a genetic algorithm selects specific spectral regions by encoding them as binary chromosomes, and PLS regression is applied only to the selected wavelengths. Comparison with ultra-high pressure liquid chromatography (UHPLC) data confirmed no significant difference in quantification, demonstrating that GA-PLS with UV–Vis provides a reliable, accurate, and practical alternative for routine analysis of these active pharmaceutical ingredients (APIs) (53).

After acquiring UV spectra, experts evaluated GA-PLS for the simultaneous quantification of acetaminophen and ascorbic acid, alongside PLS, Iterative Predictor Weighting (IPW), and Sub-window Permutation Analysis (SwPA). IPW iteratively rescales variables based on significance, while SwPA assesses variable importance by comparing prediction

errors before and after permutation. GA-PLS showed the best predictive performance, with the highest recovery, lowest RMSEP, and most remarkable percentage improvement (%Imp), demonstrating its effectiveness for accurate and efficient quantification (54).

Using the acquired UV spectra, GA combined with PLS and ANN was used to simultaneously quantify desloratadine, rupatadine fumarate, and montelukast sodium in Smarti-M® tablets. GA-PLS and GA-ANN effectively predicted desloratadine and rupatadine fumarate, while montelukast's broad UV absorption limited improvements in prediction accuracy. These results suggest that researchers can utilize GA-based chemometric methods as reliable alternatives to HPLC for drugs with well-defined spectral characteristics. However, the techniques may be less effective for compounds with more complex spectra (55).

Following the acquisition of UV spectra, metformin in combination with sitagliptin was quantified using PLS combined with Continuous Wavelet Transform (CWT). CWT applies a mother wavelet function to generate wavelet coefficients and performs a continuous transformation from the spatial to the temporal domains. Results were compared with HPLC data using analysis of variance (ANOVA), confirming that chemometric-assisted UV spectroscopy provides a reliable, faster, and cost-effective alternative for the simultaneous analysis of these two active agents without the need for additional solvents (56).

After obtaining UV spectra, analysts simultaneously quantified four active agents—paracetamol, phenylephrine hydrochloride, diphenhydramine hydrochloride, and caffeine—using PLSR and Principal Component Regression (PCR). We measured standard and study samples from 240 to 320 nm. They performed chemometric analysis with Unscrambler X software, which calculated R^2, root mean square error of calibration (RMSEC), and root mean square error of cross-validation (RMSECV). PCR and PLSR effectively handled spectral overlap, and we optimized principal components and latent variables via K-fold cross-validation. We confirmed the agreement between the chemometric and chromatographic results by comparing them with RT-HPLC data using one-way ANOVA (57).

Based on the recorded UV spectra, researchers applied PCR and PLS for the simultaneous analysis of metformin and glyburide. They used standard stock solutions and marketed formulation samples to construct chemometric models with selected principal components (PCs), employing multivariate calibration method 1 (MVC1) software for calibration. The team validated the models using a separate validation set and calculated the mean recovery and RMSEP. The results demonstrated that PCR and PLS offer an effective and efficient alternative to more complex, time-consuming analytical methods (58).

Derived from the measured UV spectra, vitamin B9 (folic acid) and B12 (cyanocobalamin) in a commercial syrup were quantified using PLS and Net Analyte Signal (NAS) chemometric methods. NAS determines the linear domain and constructs calibration curves (200 nm for B9, 202 nm for B12), with the limit of quantification (LOQ), the limit of detection (LOD), and R^2 calculated to assess performance. Prediction Error Sum of Squares (PRESS) analysis identified two optimal components for both vitamins, confirming the accuracy and suitability of NAS. Comparison with HPLC results using one-way ANOVA showed good agreement, demonstrating that PLS and NAS provide reliable chemometric alternatives for simultaneous vitamin analysis (59).

One study analyzed CARISOMA™ tablets containing paracetamol, carisoprodol, and caffeine using UV-assisted chemometric methods, including PLS, PCR, classical least squares (CLS), and n-way PLS (NPLS). They compared the results with HPLC and the ra-

tio spectra derivative method. CLS constructs an absorptivity matrix based on Beer's law, while NPLS decomposes higher-order data to maximize covariance. Stock solutions and samples were prepared and irradiated with UV, and chemometric models were calibrated and validated by calculating RMSEC, RMSECV, RMSEP, predicted residual error sum of squares (PRESS), and R^2. ANOVA at a 95% confidence level showed no significant difference among the three methods, confirming the reliability of UV-assisted chemometric analysis (60).

Although UV spectrophotometry is a convenient and cost-effective analytical technique, it has certain limitations that can affect the accuracy and reliability of results, particularly in complex mixtures. One of the main challenges is spectral overlap, which makes it difficult to accurately distinguish and quantify individual components when their absorption bands overlap. Additionally, the presence of excipients or impurities often influences UV methods, making them less specific. Due to these limitations, HPLC is usually preferred, especially in cases requiring higher sensitivity, selectivity, and resolution. HPLC enables the precise separation and quantification of multiple components within a mixture, making it a more robust and dependable method for pharmaceutical analysis (61-63).

4. Attenuated Total Reflectance Infrared Spectroscopy (ATR-FTIR)

4.1. Principles

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy is a widely used vibrational spectroscopic technique in pharmaceutical analysis. It functions by measuring the absorption of infrared light by molecular bonds within a sample, providing a distinct fingerprint for identifying functional groups and assessing chemical composition (64).

In ATR-FTIR, researchers direct infrared light into an internal reflection element (IRE) or ATR crystal, such as diamond or zinc selenide, where it undergoes total internal reflection. This process generates an evanescent wave at the interface between the crystal and the sample, penetrating only a few micrometers into the sample. This setup allows for surface-sensitive spectral acquisition without requiring extensive sample preparation. Researchers then analyze the resulting spectrum using Fourier Transform algorithms to obtain detailed information about the sample's molecular structure (65).

4.2. Applications

Attenuated Total Reflectance Mid-Infrared (ATR-MIR) is an applicable and widely used technique in pharmaceutical sciences for different purposes such as ensuring formulation consistency, identifying and quantifying APIs, monitoring excipients, detecting degradation products, and measuring specific components within complex biological samples such as blood, urine, or tissue extracts (66, 67).

4.3. Strengths

ATR-FTIR spectroscopy offers several key advantages, including minimal sample preparation, non-destructive analysis, and rapid data acquisition. Its high spatial resolution enables detailed chemical imaging, while its effectiveness in analyzing aqueous samples reduces interference from water absorption. Additionally, ATR-FTIR's versatility allows its application across diverse fields, including pharmaceuticals, materials science, and environmental studies, making it a valuable tool for the efficient and accurate characterization of a wide range of sample types (68, 69).

4.4. Limitation

While ATR-MIR offers several advantages, such as eliminating the need for sample preparation, its major drawback lies in the complexity of interpreting data from MIR spectra. To address this issue, scientists can

combine it with other analytical or chemometric methods (66, 67).

4.5. Integrating ATR-IR spectroscopy with chemometrics

Initially, scientists perform spectral analysis using ATR-IR spectroscopy over the range of 4000–400 cm^{-1}. All measurements are conducted under controlled ambient conditions to ensure reproducibility, including consistent temperature and applied pressure on the ATR crystal. The analysis team scans each sample three times and averages the resulting spectra to minimize instrumental noise and improve signal fidelity. Subsequently, researchers preprocess the spectral data to enhance signal quality and eliminate irrelevant variations. Preprocessing techniques include baseline correction, Savitzky–Golay smoothing, and normalization (either vector or area).

Additionally, first- and second-order derivatives are applied to remove background effects and resolve overlapping spectral bands. Ultimately, the preprocessed spectral data were analyzed using multivariate chemometric techniques. Researchers use PCA for pattern recognition and to visualize inherent clustering within the dataset. They classify sample groups using PLS-DA and LDA. For quantitative analysis, they apply PLSR to model relationships between spectral features and known reference values. They conduct model validation using cross-validation methods and assess predictive performance using RMSE and R^2. They perform all chemometric modeling and data processing using software such as MATLAB R2023a, The Unscrambler X, and SIMCA.

4.6. Practical applications

One study applied the partial least squares-least squares support vector machines (PLS-LS-SVM) method, combined with ATR-IR spectroscopy, to model alprazolam content in tablets, validating the predictions against HPLC data. Spectral data were preprocessed and divided into calibration and validation sets to develop the model. The resulting model achieved a high prediction level (R^2 = 0.99) and a low RMSECV (0.98), demonstrating excellent accuracy and sensitivity. Validation procedures confirmed its robustness, indicating that the approach reliably captures the quantitative relationship between spectral data and drug content. These results highlight the effectiveness of combining chemometric modeling with the ATR-IR spectroscopic technique for precise and efficient pharmaceutical analysis (66).

Another study investigated the adsorption behavior of 1-butyl mercaptan on nickel-coated carbon nanofibers (CNFs) using ATR-FTIR spectroscopy in combination with chemometric analysis. Nickel-coated CNFs were prepared and exposed to 1-butyl mercaptan under controlled conditions to evaluate the adsorption process. ATR-FTIR spectroscopy was employed to monitor the interaction in real time, capturing changes in functional groups associated with adsorption. Scientists processed the resulting spectral data using chemometric techniques, including PLS regression, to analyze adsorption kinetics and identify relevant spectral patterns. Statistical validation methods assessed model performance and reliability. This approach enabled a detailed understanding of the adsorption mechanism and demonstrated the value of combining ATR-FTIR spectroscopy with chemometrics for studying surface interactions (70).

Building on the growing use of ATR-FTIR spectroscopy combined with chemometric techniques in pharmaceutical analysis, a recent study applied this approach to quantify amikacin in amikacin sulphate injection formulations. ATR-FTIR spectra were collected and processed using multivariate calibration techniques, primarily PLSR, to model the relationship between spectral data and amikacin concentration. Chemometric preprocessing methods were employed to reduce spectral

noise and correct baseline variations, improving the accuracy of the calibration model. The approach effectively addressed spectral overlap and matrix effects inherent in complex pharmaceutical samples, enabling precise and reliable quantification. Validation of the chemometric model demonstrated strong predictive performance, confirming its suitability for routine quality control of amikacin injections. This study underscores the value of integrating ATR-FTIR spectroscopy with chemometrics as a rapid, non-destructive, and robust analytical method in pharmaceutical analysis (71).

PLSR combined with ATR-IR spectroscopy was applied for the simultaneous quantification of metformin and vildagliptin, addressing spectral overlap and improving analytical accuracy. Spectral data were preprocessed using Savitzky-Golay filtering to reduce variations. Tablets with varying amounts of metformin and vildagliptin showed distinct absorption features, which were captured by ATR-IR. PLSR modeling established a linear relationship between spectral data and predicted concentrations, which were validated against HPLC results, showing no statistically significant difference between the two methods. These findings demonstrate the effectiveness of ATR-IR coupled with chemometric modeling for accurate simultaneous determination of multiple pharmaceutical compounds (72).

Researchers have applied chemometric-based ATR-IR spectroscopy for the quantification of herbal compounds; for example, one study employed this method to determine the concentrations of phenolic compounds, including caffeic acid and rosmarinic acid, in the leaves of Thunbergia laurifolia. Due to the sophisticated sample preparation and multistep nature of the HPLC method, this study employed ATR-IR coupled with chemometric methods as an alternative. After taking ATR-IR spectra of pure caffeic acid, rosmarinic acid, and the plant extract with FTIR spectroscopy, the PLS-1 regression technique was developed and compared to the actual amounts obtained by HPLC. Like other studies, researchers utilized specific peaks in the IR spectra related to distinct structural and functional groups to construct the PLS-1 model. In addition, the PLS-1 method was validated in terms of linearity, accuracy, and precision, showing that the proposed model had acceptable validity (73).

A study has proven that integrating ATR-FTIR spectroscopy with chemometric analysis is effective for the rapid evaluation of essential oils from the Myrtaceae family. Researchers analyzed spectral data using PCA and PLSR to classify species and quantify key constituents. Chemometric preprocessing improved model accuracy by minimizing baseline variations and resolving overlapping peaks. The approach enabled fast, non-destructive profiling of essential oils, highlighting its potential for routine quality control and botanical authentication (74).

5. Raman spectroscopy

5.1. Raman Spectroscopy Principles

Researchers have identified Raman spectroscopy as one of the most versatile techniques for material analysis. Scientists base the theory behind Raman spectroscopy on the interaction of electromagnetic radiation with matter, where the amount of energy loss or gain is similar to the band gap between the initial and final energy states. We refer to the difference between the energy of the incoming photon and the energy of the outgoing photon as the "Raman Shift." Plasmonic effects in the presence of metal nanoparticles may enhance the produced signal, or "Raman signal," using a technique known as surface-enhanced Raman spectroscopy (SERS) (75). Researchers conduct quantitative SERS in three steps, initiated with the qualitative analysis of a standard solution. The second step involves providing a standard curve based on the standard solution, followed by analyzing the real sample (76).

5.2. Applications

Raman spectroscopy has become an essential analytical technique in pharmaceutical sciences, offering molecular-level information through vibrational analysis without the need for extensive sample preparation. Its applications span the identification of APIs, detection of polymorphic transitions, and characterization of excipients within complex formulations. The technique is particularly effective in solid-state analysis, allowing for differentiation between crystalline and amorphous forms. Raman mapping provides spatial distribution data, which helps evaluate the uniformity of content and coating integrity in tablets. Moreover, Raman spectroscopy is widely implemented in real-time monitoring of manufacturing processes under Process Analytical Technology (PAT) initiatives, supporting in-line and at-line quality control. Its high chemical specificity also enables detection of counterfeit or substandard products and assessment of drug–excipient compatibility, making it a versatile tool throughout drug development and production (9, 77, 78).

5.3. Strengths

Raman spectroscopy provides detailed molecular information for identifying active ingredients, excipients, and polymorphs, eliminating the need for extensive sample preparation. It is non-destructive, applicable to solids and liquids, and offers high spatial resolution for assessing content uniformity and coating in tablets. Well-suited for real-time monitoring under PAT, it enables rapid quality control during the manufacturing process. Additionally, its low interference from water makes it effective for aqueous formulations, establishing it as a versatile tool in pharmaceutical development and production (9, 77, 78).

5.4. Limitations

Raman spectroscopy faces limitations, including weak signal intensity due to low scattering efficiency and potential interference from fluorescence in specific samples. Its sensitivity for trace components is lower than that of chromatographic methods. Complex mixtures require advanced data analysis for accurate quantification. Additionally, instrument costs and sample heterogeneity can pose practical challenges in routine pharmaceutical applications (9, 77, 79).

5.5. Integrating Raman spectroscopy with chemometric approaches

Raman spectroscopy offers a powerful analytical approach for the qualitative and quantitative assessment of pharmaceutical products when integrated with chemometric techniques. The integration process begins with spectral preprocessing steps, including baseline correction, normalization, smoothing (via Savitzky–Golay filtering), and derivatization. These steps are crucial for reducing background noise, correcting for fluorescence interference, and enhancing the resolution of overlapping peaks. These steps ensure that the Raman spectral data are clean and standardized for analysis. Once preprocessed, multivariate statistical methods such as PCA, PLSR, and SVM are applied. PCA helps visualize underlying data patterns and detect outliers, while PLSR enables the prediction of active pharmaceutical ingredient concentrations in complex mixtures. In Raman imaging, chemometrics enables the practical interpretation of spatially resolved chemical data, facilitating the mapping of component distributions in tablets or formulations. This integration not only enhances data interpretability but also supports tasks like content uniformity testing, polymorphic form identification, and quality control during manufacturing (80, 81).

Combining ATR-FTIR and Raman spectroscopy with chemometrics enables quantitative monitoring of crystallization processes. This methodology collects real-time spectral data from the solid-liquid interface using ATR-FTIR to observe molecular interac-

tions and changes in functional groups. At the same time, Raman spectroscopy tracks structural and polymorphic transformations. Chemometric techniques, such as PCA and PLSR, are applied to interpret complex spectral data and quantify key parameters like nucleation and crystal growth. This integrated approach improves process understanding, control, and product quality in pharmaceutical crystallization (82). FTIR and Raman spectroscopy, combined with chemometric methods, enable the precise analysis of biomolecules in biomedical fluids. FTIR detects polar functional groups, while Raman provides information on molecular structure and non-polar bonds. Chemometric tools, such as PCA and PLSR, effectively handle complex spectral data, enabling the accurate quantification of proteins, lipids, and metabolites in fluids like blood and urine. This approach offers rapid, non-destructive, and label-free analysis, making it suitable for biomedical diagnostics (83).

5.6. Practical applications

ATR-IR and Raman spectroscopy were applied to assess piperaquine and dihydroartemisinin, with the resulting spectra analyzed using PLSR modeling. Spectral pretreatments, including SNV for Raman spectroscopy and orthogonal signal correction (OSC) for ATR-IR, were applied to remove interferences and simplify model interpretation. Researchers chose calibration and validation sets to develop predictive models that demonstrated a strong correlation with reference HPLC data. The models achieved R^2 values of approximately 0.96 for piperaquine and 0.95 for dihydroartemisinin. The models were then used to predict sample values, demonstrating that ATR-IR and Raman spectroscopy combined with chemometric analysis provide accurate and reliable quantification of both compounds, serving as effective alternatives to HPLC analysis (81).

Handheld Raman spectroscopy can be less practical than NIR spectroscopy for drug analysis. In one study, artemether-lumefantrine, paracetamol, and ibuprofen were assessed using chemometric methods including hit quality index (HQI), data-driven soft independent modeling of class analogy (DD-SIMCA), and HCA. HQI evaluates spectral similarity, while DD-SIMCA provides one-class classification. Despite preprocessing, Raman-based HQI and DD-SIMCA results were insufficient for detecting drug combinations or excipient variations. These findings indicate that NIR spectroscopy, although more complex to interpret, is generally more suitable for identification purposes (84).

Although NIR spectroscopy has many advantages, it struggles to analyze pharmaceutically active compounds in aqueous environments. In a recent study, researchers employed chemometric-assisted Raman spectroscopy to quantify anthracycline agents using a handheld Raman spectrometer. They utilized PLS-DA for qualitative analysis and combined it with leave-one-out cross-validation to develop calibration models. The team evaluated predictive performance through RMSECV, RMSEP, and R^2, selecting the optimal model based on the lowest errors and highest correlation. While their models demonstrated reasonable predictive capacity, they fell short in quantifying anthracyclines at all concentrations. The researchers anticipate that improvements, such as increased signal strength, reduced noise, and additional repetitions, will boost performance. Researchers have applied chemometric-assisted SERS with gold nanoparticle substrates to quantify metformin. SERS enhances signal intensity, enabling rapid analysis compared with conventional chromatographic methods. Raman spectra obtained from metformin in a citrate-stabilized gold nanoparticle matrix were analyzed using PLS and PCA regressions. PLS modeling established a linear relationship between spectral data and metformin concentration, with pre-processing and RMSECV used to optimize the model. The optimal number of latent variables was select-

ed based on minimum RMSECV, and the most informative spectral region (1765–456 cm^{-1}) was used for model development and validation. This approach offers a reliable alternative for the rapid quantification of metformin in pharmaceutical formulations (85).

SERS, combined with multivariate analysis, has shown the ability to differentiate structurally similar fluoroquinolones. In this study, silver nanoparticles modified with 11-mercapto-1-undecanol (MUO@Ag NPs) served as SERS substrates for Norfloxacin, Ofloxacin, and Ciprofloxacin. Systematic cluster analysis (SCA) of the SERS spectra enabled feature extraction and similarity assessment via Euclidean distance. The analysis clearly distinguished the compounds: Ofloxacin overlapped with formulations containing it, while Norfloxacin and Ciprofloxacin formed separate clusters despite being in the same family. These results demonstrate the effectiveness of SERS combined with SCA and MUO@Ag NPs for accurate identification of fluoroquinolone compounds (86).

6. High-pressure liquid chromatography

6.1. Principles

High-pressure liquid chromatography (HPLC) was introduced in the 1960s with the development of early columns and other instrumental components, generating high pressures for effective separations that can be utilized for the analysis of any material soluble in a mobile phase, serving as the solvent. According to sample injection through the injector, the pump circulates the mobile phase within the system at a precise, accurate, and controllable flow rate through the column, which contains silica-based packing material with a determined particle size and pore diameter to produce the highest possible surface area. In the next step, the detector processes the concentrated data and translates it into an electric signal. UV-Vis detectors, which quantify the UV-Vis absorption of radiation, are the most commonly used and are classified into three types: fixed-wavelength detectors, variable-wavelength detectors, and diode array detectors. While fixed-wavelength detectors use a wavelength-isolating filter, variable-wavelength detectors utilize a rotatable monochromator that can emit light at various wavelengths. Diode array detectors are the most useful UV-Vis detectors since they emit a light spectrum of different wavelengths from a deuterium lamp at once and record the absorbances simultaneously. Other HPLC detectors, such as fluorescent detectors, refractive index detectors, and electrochemical detectors, serve specific conditions (74, 87-90).

6.2. Applications

HPLC plays a crucial role in the qualitative and quantitative assessment of pharmaceutical compounds, including APIs, impurities, and degradation products. HPLC is routinely employed in drug formulation development, stability testing, and quality control to ensure the safety and efficacy of products. Furthermore, its compatibility with various detectors, such as UV-Vis and mass spectrometry (MS), enhances its versatility in analyzing complex samples (91).

6.3 Strengths

Over the last few years, researchers and industry professionals have recognized liquid chromatography as the gold standard method in pharmaceutical research; however, it is also used as an essential analytical technique in biomedical, forensic, environmental, and agricultural fields (92, 93). In fact, the HPLC's distinctive advantages, including sensitivity, wide-range applicability, and versatility, have made it a ubiquitous technique in various industries (94). Moreover, HPLC eliminates the need for derivatization, allowing the analysis of compounds with limited volatility (87).

6.4. Limitations

Although HPLC is a powerful ana-

Table 2. Comparison chart: HPLC vs Spectroscopic Methods.

	HPLC	Spectroscopic Methods
Speed	Slower	Rapid
Sample preparation	Extensive	Minimal
Solvent usage	High	Low
Cost	Higher	Lower
Suitability for Real-time analysis	No	Yes

lytical tool, it faces several challenges. These include the potential degradation of analytes during analysis, interference between analytes and the mobile or stationary phases, and concerns about the reliability and accuracy of results under certain conditions. Additionally, HPLC is associated with high operational costs, time-consuming procedures, and the use of expensive instrumentation and materials, including high-quality solvents and filters, which can limit its accessibility in some settings. One of the most critical and challenging areas involves applying stringent standards in quality control testing to ensure consistent performance (5, 91, 95, 96).

Table 2 Key differences in operational parameters between HPLC and chemometric-assisted spectroscopic techniques.

7. Discussion

The integration of chemometric approaches with vibrational and electronic spectroscopies has substantially expanded the analytical toolbox for pharmaceutical quality assessment (97). Conventional HPLC remains indispensable for regulatory analysis owing to its unrivaled sensitivity and separation efficiency; however, its dependence on labor-intensive sample preparation, long run times, and extensive sample preparation limits its suitability for high-throughput and in-process monitoring (5, 98-103). By contrast, spectroscopic techniques such as ART-FTIR, Raman, and UV-Vis are inherently rapid and non-destructive, and achieve quantitative performance comparable to chromatographic assays when they are supported by chemometric modeling (11, 104, 105).

A central challenge in spectroscopic analysis is the overlap of spectral bands, particularly in multicomponent pharmaceutical matrices (106, 107). Multivariate calibration techniques address this problem by extracting latent variables that describe the systematic variation in spectral data (108). PLS and PCA are the most widely applied methods, with a demonstrated ability to quantify APIs and excipients simultaneously (109, 110). Nonlinear models, including ANN, provide additional flexibility when linear models fail to capture complex spectral relationships (111). The reliability of these calibrations is strongly influenced by spectral pretreatment (112, 113). Techniques such as baseline correction, derivatives, SNV, and multiplicative effects correction reduce sources of variance not related to chemical composition, thereby improving model robustness (114, 115). Model validation remains a critical step to ensure predictive reliability (116, 117). Cross-validation strategies, particularly K-fold and leave-one-out approaches, provide objective estimates of model complexity and guard against overfitting (118, 119). Statistical figures of merit, including R^2, RMSECV, and RMSEP, are typically reported to assess model quality (120, 121). In several case studies, chemometric predictions showed no significant difference from HPLC values, as confirmed by inferential statistics such as one-way ANOVA, underscoring their analytical equivalence (60).

Beyond classical regression, optimization algorithms further enhance model performance. GA-PLS is increasingly employed to select informative spectral intervals, improving accuracy by eliminating irrelevant vari-

ables (122, 123). These combined chemometric-assisted spectroscopic approaches have been effectively utilized in the quantitative analysis of multi-component pharmaceutical formulations, yielding results statistically comparable to those of chromatographic assays while substantially reducing analysis time, solvent consumption, and sample preparation requirements (57).

These advances align with the broader implementation of PAT and Quality by Design (QbD) frameworks in the pharmaceutical industry (124, 125). The capacity of chemometric-enhanced spectroscopy to deliver real-time, environmentally suitable, and cost-efficient measurements positions it as a practical complement to conventional HPLC, particularly for routine monitoring, in-line process control, and counterfeit drug detection (126-128).

8. Conclusion

In summary, chemometric-enhanced spectroscopy enables the pharmaceutical industry to conduct rapid, non-destructive, and accurate analysis across various stages of drug development. These models not only reduce reliance on solvents and consumables but also enable for the assessment of complex formulations and detection of adulterants or substandard products. As highlighted across several recent studies, chemometric modeling is no longer just a supportive tool—it is a core component of modern pharmaceutical analytical science.

Authors contributions

Conceptualization: Dr. Shohreh Alipour; Literature search: Dr. Sheida Jahanbekam; Data analysis and interpretation: Dr. Shohreh Alipour; Writing the original draft: Dr. Sheida Jahanbekam; Review and Editing: Dr. Shohreh Alipour and Dr. Sheida Jahanbekam; Supervision: Dr. Shohreh Alipour

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Conflict of Interest

The authors declare that they have no conflict of interest.

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