RESEARCH PAPER

Development and Comparative Validation of RP-HPLC and Chemometric-Assisted UV Spectrophotometric Methods for Simultaneous Estimation of Pyridoxine HCl and Nicotinamide in Pharmaceutical Formulations

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ABSTRACT

The present investigation aimed to develop and comparatively validate a reversed-phase high-performance liquid chromatographic (RP-HPLC) method and a chemometric-assisted UV spectrophotometric method for the simultaneous estimation of Pyridoxine Hydrochloride (PYR) and Nicotinamide (NIC) in pharmaceutical formulations. Chemometric analysis using Partial Least Squares (PLS) regression was employed to overcome spectral overlap limitations encountered in conventional spectrophotometric analysis. RP-HPLC separation was achieved using a C18 column under optimized chromatographic conditions with an isocratic mobile phase system. The developed analytical methods were validated according to International Conference on Harmonisation (ICH) guidelines with respect to linearity, accuracy, precision, robustness, reproducibility, specificity, limit of detection, and limit of quantitation.

The chemometric-assisted spectrophotometric method demonstrated excellent predictive capability with satisfactory calibration and validation performance. The RP-HPLC method exhibited sharp peak resolution, acceptable retention times, high theoretical plate count, and minimal tailing. Both analytical methods showed good linearity over the selected concentration ranges with correlation coefficients approaching unity. Recovery studies confirmed the accuracy of the developed methods with percentage recoveries within acceptable limits. Statistical analysis demonstrated no significant difference between the proposed RP-HPLC and chemometric-assisted methods regarding quantitative estimation of both analytes.

The comparative evaluation established that the chemometric-assisted spectrophotometric method provides a rapid, economical, and environmentally friendly alternative to chromatographic analysis, whereas RP-HPLC offers superior selectivity and sensitivity for routine quality control applications. The developed methods were successfully applied for simultaneous estimation of Pyridoxine HCl and Nicotinamide in marketed pharmaceutical formulations.

Keywords: *Pyridoxine HCl, Nicotinamide, RP-HPLC, Chemometrics, Partial Least Squares Regression, UV Spectrophotometry, Method Validation, Simultaneous Estimation.*

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1. INTRODUCTION

The pharmaceutical industry demands highly reliable analytical methodologies to ensure quality, safety, efficacy, and regulatory compliance of pharmaceutical formulations. Analytical method development and validation play a critical role in routine quality control and stability assessment of multicomponent pharmaceutical products [1-2]. Simultaneous estimation of active pharmaceutical ingredients in combined dosage forms has become increasingly important due to the widespread utilization of multivitamin and multicomponent formulations in clinical practice [3-4].

Pyridoxine Hydrochloride (Vitamin B6) and Nicotinamide (Vitamin B3) are essential water-soluble vitamins extensively incorporated into pharmaceutical and nutraceutical preparations. Pyridoxine is involved in amino acid metabolism, neurotransmitter synthesis, and haemoglobin formation, whereas Nicotinamide plays an essential role in cellular metabolism and enzymatic

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oxidation-reduction reactions [5]. Deficiency of these vitamins is associated with several pathological disorders, necessitating their incorporation into combination formulations [6].

Conventional UV spectrophotometric methods often encounter analytical limitations due to overlapping absorption spectra in multicomponent formulations. To overcome these challenges, chemometric-assisted spectrophotometric techniques have gained considerable importance owing to their ability to resolve complex spectral data using multivariate calibration algorithms [7]. Partial Least Squares (PLS) regression represents one of the most effective chemometric tools for simultaneous quantitative analysis of compounds exhibiting overlapping spectra [8].

High-performance liquid chromatography (HPLC) remains one of the most reliable and widely accepted analytical techniques for pharmaceutical analysis due to its superior selectivity, sensitivity, reproducibility, and robustness [9]. Comparative evaluation of chromatographic and chemometric-assisted spectrophotometric methods can provide valuable analytical insight regarding their suitability for routine pharmaceutical quality control [10].

Therefore, the present study aimed to develop, validate, and comparatively evaluate RP-HPLC and chemometric-assisted UV spectrophotometric methods for simultaneous estimation of Pyridoxine HCl and Nicotinamide in pharmaceutical formulations [11].

2. MATERIALS AND METHODS

2.1 Materials

The present investigation utilized a Shimadzu UV-1800 double-beam UV-Visible spectrophotometer and a Younglin 9000 gradient RP-HPLC system equipped with a UV detector for analytical measurements. Data acquisition and chemometric analysis were performed using UV-Probe 2.34, Autochro-3000, UNSCRAMBLER® X 10.3, MATLAB, and OriginPro 6.1 software on a Windows platform. Pyridoxine Hydrochloride IP and Nicotinamide IP reference standards were obtained from Symbiosis Co-operative Pharmaceutical Ltd., while analytical-grade chemicals, HPLC-grade solvents, and standard laboratory glassware were employed throughout the study.

2.2 Preparation of Standard Solutions

Standard stock solutions of Pyridoxine HCl and Nicotinamide were prepared individually using methanol-water solvent system. Working standard solutions were prepared by appropriate dilution to obtain the desired concentration ranges for calibration and validation studies.

3. CHEMOMETRIC-ASSISTED SPECTROPHOTOMETRIC METHOD

3.1 Spectral Acquisition

Distilled water was selected as the solvent system for preparation of all analytical solutions because Pyridoxine Hydrochloride (PYR) and Nicotinamide (NIC) are freely soluble in aqueous media according to pharmacopeial standards. Standard stock solutions of PYR and NIC were prepared separately by dissolving 10 mg of each drug in distilled water and diluting to 100 mL to obtain final concentrations of 100 µg/mL. The prepared solutions were evaluated for stability prior to analysis.

For spectral analysis, working standard solutions containing 1.5 µg/mL of PYR and 20 µg/mL of NIC, along with their binary mixture, were prepared by appropriate dilution of the stock solutions. The absorption spectra of individual drugs and mixed solutions were recorded using a UV-Visible spectrophotometer over the wavelength range of 190–1000 nm. Significant absorbance was observed between 210 and 370 nm, and the absorption maxima (λmax) values of both analytes were found to be consistent with reported literature values.

To establish linearity, calibration curves were constructed using standard solutions prepared in the concentration range of 1–50 µg/mL for both analytes at intervals of 1 µg/mL. The absorbance values measured at the respective λmax were plotted against concentration, and regression analysis was performed to determine correlation coefficients and evaluate Beer–Lambert's law compliance [12].

3.2 PLS Regression Model

Partial Least Squares (PLS) regression was employed for multivariate calibration and simultaneous quantitative estimation of PYR and NIC from overlapping UV spectra. Independent calibration (training) and validation (prediction) data sets were prepared to construct and evaluate the predictive chemometric model. The calibration set consisted of 121 binary mixtures prepared in different concentration combinations containing PYR in the range of 0–10 µg/mL and NIC in the range of 0–20 µg/mL. These concentration ranges were selected within Beer–Lambert's linearity limits to ensure proportionality between absorbance and concentration.

For external validation of the developed model, 25 independently prepared binary mixtures were used as the prediction set. The absorbance data obtained from spectral scanning at multiple wavelengths were arranged into an absorbance matrix, while the corresponding concentration values were organized into a concentration matrix. In the developed model, 121 calibration samples measured at 91 wavelengths generated a 121 × 91 absorbance matrix suitable for multivariate chemometric analysis.

PLS regression analysis was carried out using UNSCRAMBLER® X 10.3 software. Full cross-validation was applied to determine the optimum number of latent variables required for accurate prediction while minimizing the risk of overfitting and underfitting. During cross-validation, one calibration sample was excluded at a time, the model was recalculated using the remaining samples, and the excluded sample concentration was predicted. The residual errors generated during each cycle were combined to evaluate the predictive efficiency and robustness of the developed model.

The optimized PLS model was subsequently applied to validation samples for prediction of unknown concentrations of PYR and NIC. The predicted concentrations were compared with actual concentrations, and percentage recovery values were calculated to assess the accuracy and reliability of the developed chemometric-assisted spectrophotometric method [13].

4. RP-HPLC METHOD DEVELOPMENT

4.1 Chromatographic Conditions

The RP-HPLC method was systematically optimized to achieve efficient chromatographic separation, acceptable peak symmetry, and reliable quantitative estimation of Pyridoxine Hydrochloride (PYR) and Nicotinamide (NIC). Since both analytes are hydrophilic in nature, reverse-phase chromatography was selected because of its simplicity, reproducibility, sensitivity, and suitability for routine pharmaceutical analysis. Different chromatographic parameters including mobile phase composition, stationary phase, flow rate, detection wavelength, and column temperature were evaluated during method optimization.

A reverse-phase Agilent C8 column (4.6 mm × 150 mm, 5 µm particle size) was selected as the stationary phase based on satisfactory peak shape, chromatographic resolution, and system suitability characteristics. Different combinations of organic solvents and aqueous phases were investigated, and a mobile phase consisting of methanol and water in the ratio of 70:30 (v/v) was found to provide optimum elution, better peak symmetry, and acceptable retention behavior for both analytes.

The UV absorption spectra of PYR and NIC in the optimized mobile phase were recorded over the wavelength range of 190–400 nm to determine the appropriate detection wavelength. Both analytes exhibited adequate absorbance at 264 nm; therefore, this wavelength was selected for chromatographic monitoring. The chromatographic separation was performed under isocratic elution conditions at a flow rate of 0.7 mL/min, while the column temperature was maintained at 25°C to ensure consistent chromatographic performance and reproducibility.

Under the optimized chromatographic conditions, Nicotinamide and Pyridoxine Hydrochloride were eluted at retention times of approximately 4.3 min and 6.3 min, respectively. The chromatograms showed sharp, symmetrical, and well-resolved peaks with acceptable system suitability parameters. The tailing factors for NIC and PYR were found to be 1.23 and 1.04, respectively, while theoretical plate counts exceeded 2000, confirming satisfactory column efficiency and chromatographic resolution. The developed RP-HPLC method provided rapid separation of both analytes within 7 min and was therefore considered suitable for routine pharmaceutical analysis [14].

5. METHOD VALIDATION

The developed chemometric-assisted UV spectrophotometric and RP-HPLC methods were validated according to International Conference on Harmonisation (ICH) guidelines with respect to linearity, specificity, precision, accuracy, reproducibility, robustness, limit of detection (LOD), and limit of quantitation (LOQ). Validation studies were carried out using low quality control (LQC), medium quality control (MQC), and high-quality control (HQC) samples containing 1.2, 1.5, and 1.8 µg/mL of PYR and 16, 20, and 24 µg/mL of NIC, respectively.

5.1 Specificity

Specificity of the developed RP-HPLC method was evaluated to ensure accurate measurement of analytes in the presence of excipients, impurities, degradation products, and formulation components. Blank solvent and standard drug solutions were injected separately into the chromatographic system, and no interfering peaks were observed at the retention times corresponding to PYR and NIC. The absence of co-eluting peaks confirmed the specificity and selectivity of the developed analytical method.

5.2 System Suitability

System suitability studies were performed to verify the chromatographic performance of the developed method prior to analysis. Six replicate injections of standard solutions were analyzed under optimized chromatographic conditions. Parameters including retention time, theoretical plates, tailing factor, and chromatographic resolution were evaluated. The obtained values demonstrated satisfactory chromatographic efficiency and reproducibility for routine analysis [15].

5.3 Linearity

Linearity of the developed methods was established by preparing calibration standards over suitable concentration ranges for both analytes. For RP-HPLC analysis, calibration solutions containing 1.1–2.0 µg/mL of PYR and 16–25 µg/mL of NIC were prepared from standard stock solutions. Calibration curves were generated by plotting chromatographic peak area against analyte concentration, and regression analysis was performed to determine correlation coefficients. Excellent linear relationships between concentration and analytical response were observed for both analytes within the selected concentration ranges.

Similarly, for the chemometric-assisted spectrophotometric method, calibration curves were constructed over the concentration range of 1–50 µg/mL using absorbance values measured at the respective absorption maxima. The obtained regression coefficients confirmed acceptable linearity and proportionality between absorbance and concentration [16].

5.4 Precision

Precision studies were carried out to evaluate the repeatability and intermediate precision of the developed analytical methods. Repeatability (intra-day precision) was assessed by analyzing replicate quality control samples

under identical experimental conditions over a short time interval. The obtained results were expressed as percentage relative standard deviation (%RSD), which demonstrated acceptable precision for both methods.

Intermediate precision (ruggedness) was evaluated by performing the analysis on different days, by different analysts, and using different analytical systems. Replicate quality control samples at LQC, MQC, and HQC levels were analyzed under varied laboratory conditions, and percentage recovery along with %RSD values were calculated. The low %RSD values confirmed the reproducibility and reliability of the developed analytical procedures.

5.5 Reproducibility

Reproducibility studies were performed to evaluate inter-laboratory precision of the developed methods. The analysis was repeated independently in two separate laboratories using the same analytical procedures and quality control samples. The percentage recovery and %RSD values obtained from both laboratories demonstrated consistent analytical performance and confirmed the reproducibility of the proposed methods [17].

5.6 Accuracy

Accuracy of the developed methods was evaluated by recovery studies using the standard addition technique. Pre-analyzed pharmaceutical formulation samples containing PYR and NIC were spiked with known quantities of standard drug solutions at 80%, 100%, and 120% concentration levels. The spiked samples were analyzed using the developed chemometric-assisted spectrophotometric and RP-HPLC methods, and percentage recoveries were calculated from the predicted concentrations and chromatographic peak areas. The recovery results obtained at different concentration levels confirmed the accuracy and reliability of the proposed analytical methods.

5.7 Limit of Detection and Limit of Quantitation

The sensitivity of the RP-HPLC method was evaluated by determining the limit of detection (LOD) and limit of quantitation (LOQ) for both analytes. Calibration curves prepared at lower concentration ranges were used to estimate the minimum detectable and quantifiable concentrations of PYR and NIC with acceptable accuracy and precision. The obtained LOD and LOQ values demonstrated satisfactory sensitivity of the developed chromatographic method for pharmaceutical analysis [18].

5.8 Robustness

Robustness studies were performed to examine the effect of deliberate minor variations in chromatographic parameters on analytical performance. The influence of changes in flow rate (±0.1 mL/min), mobile phase composition (±5%), and detection wavelength (±2 nm) was investigated by analyzing replicate standard solutions under modified conditions. Parameters such as retention time, relative retention time, and peak area were monitored during the study. The results indicated that small variations in chromatographic conditions did not significantly affect the analytical performance of the method, thereby confirming its robustness and reliability during routine application.

5.9 Stability Studies

Stability studies were carried out to evaluate the stability of PYR and NIC under conditions likely to be encountered during routine sample handling and storage. Quality control samples containing 1.5 µg/mL of PYR and 20 µg/mL of NIC were stored under bench-top conditions and refrigerated conditions (2–8°C). The concentrations obtained after different storage intervals were compared with freshly prepared samples, and percentage changes in analyte concentrations were calculated. The stability results demonstrated that both analytes remained stable under the investigated storage conditions [19].

5.10 Analysis of Pharmaceutical Formulation

The validated chemometric-assisted UV spectrophotometric and RP-HPLC methods were successfully applied for quantitative estimation of PYR and NIC in marketed multivitamin dry syrup formulations. Two commercial batches of New Nutrolin-B dry syrup manufactured by Cipla Ltd. were analyzed. Appropriate sample dilution, sonication, and filtration procedures were employed prior to analysis. The prepared samples containing 1.5 µg/mL of PYR and 20 µg/mL of NIC were analyzed using the developed methods in six replicates (n = 6). The assay results confirmed the applicability and suitability of the proposed analytical procedures for routine pharmaceutical quality control analysis [20].

6. RESULTS AND DISCUSSION

6.1Chemometric-Assisted Spectrophotometric Analysis

6.1.1 Spectral Analysis and Overlapping Behavior

The UV absorption spectra of Pyridoxine Hydrochloride (PYR), Nicotinamide (NIC), and their binary mixtures were recorded within the wavelength range of 190–1000 nm to evaluate their spectral characteristics and suitability for simultaneous estimation. The spectral analysis demonstrated significant absorbance for both analytes between 210 and 370 nm. Pyridoxine Hydrochloride exhibited a maximum absorbance (λmax) at 262 nm, whereas Nicotinamide showed λmax at 292 nm. However, considerable spectral overlap was observed between the two analytes within the selected wavelength region, making direct spectrophotometric estimation difficult and necessitating the application of chemometric-assisted multivariate calibration methods.

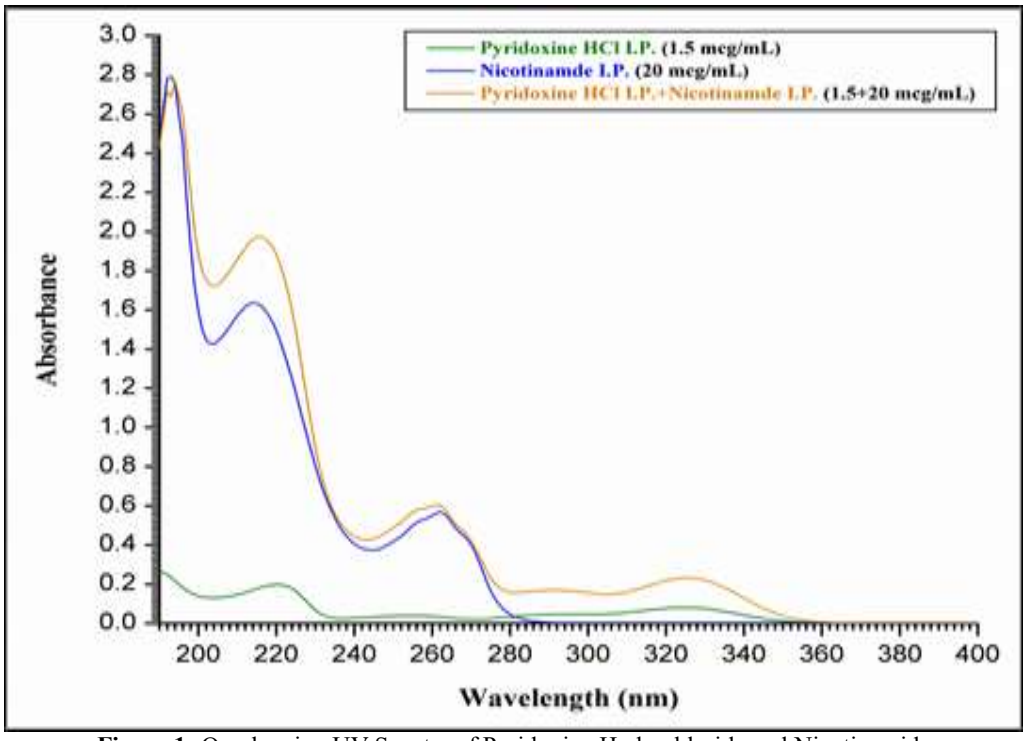

Figure 1: Overlapping UV Spectra of Pyridoxine Hydrochloride and Nicotinamide

- Pyridoxine Hydrochloride λmax: 262 nm
- Nicotinamide λmax: 292 nm
- Significant spectral overlap observed between 210–370 nm

The overlapping spectral behavior confirmed the analytical requirement for Partial Least Squares (PLS) regression to accurately resolve and quantify both analytes simultaneously in combined pharmaceutical formulations.

6.1.2 Linear Calibration Studies

Linear calibration models were developed for both PYR and NIC using standard solutions prepared within the concentration range of 1–50 µg/mL. The absorbance values recorded at the respective λmax demonstrated excellent proportionality between absorbance and concentration for both analytes. Calibration curves constructed by plotting absorbance against concentration exhibited satisfactory linearity with high correlation coefficient values. The correlation coefficient (r^2) values were found to be 0.99724 for Pyridoxine Hydrochloride and 0.99912 for Nicotinamide, indicating excellent linear analytical response across the selected concentration range.

Table 6.1. Linearity Parameters for Chemometric Calibration Model

Parameter	Pyridoxine HCl	Nicotinamide
Concentration Range	1–50 µg/mL	1–50 µg/mL
λmax	262 nm	292 nm
Correlation Coefficient (r^2)	0.99724	0.99912
Linearity	Excellent	Excellent

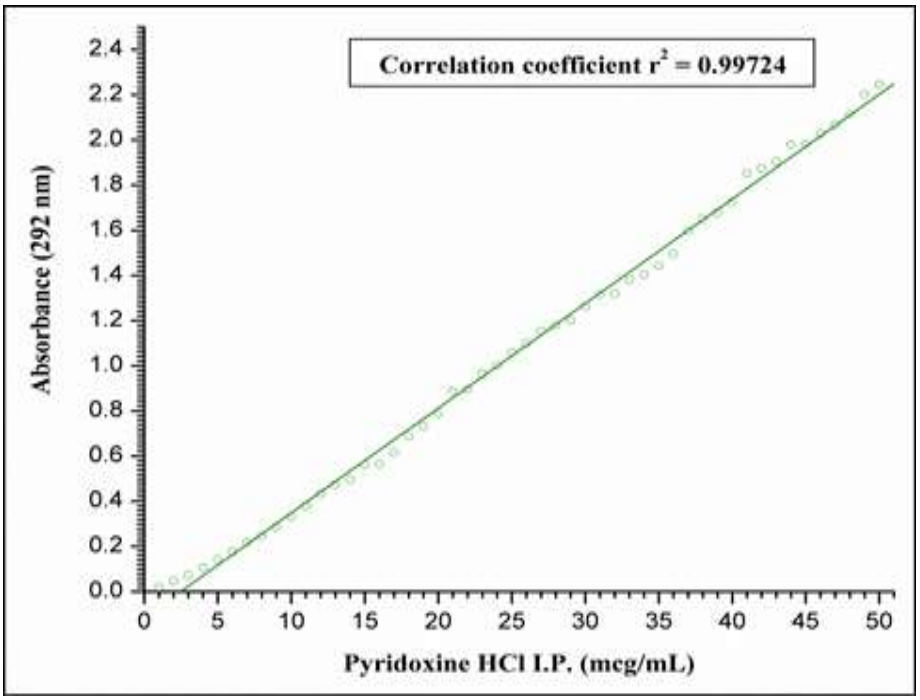

Figure 2. Linear Calibration Curve for Pyridoxine Hydrochloride

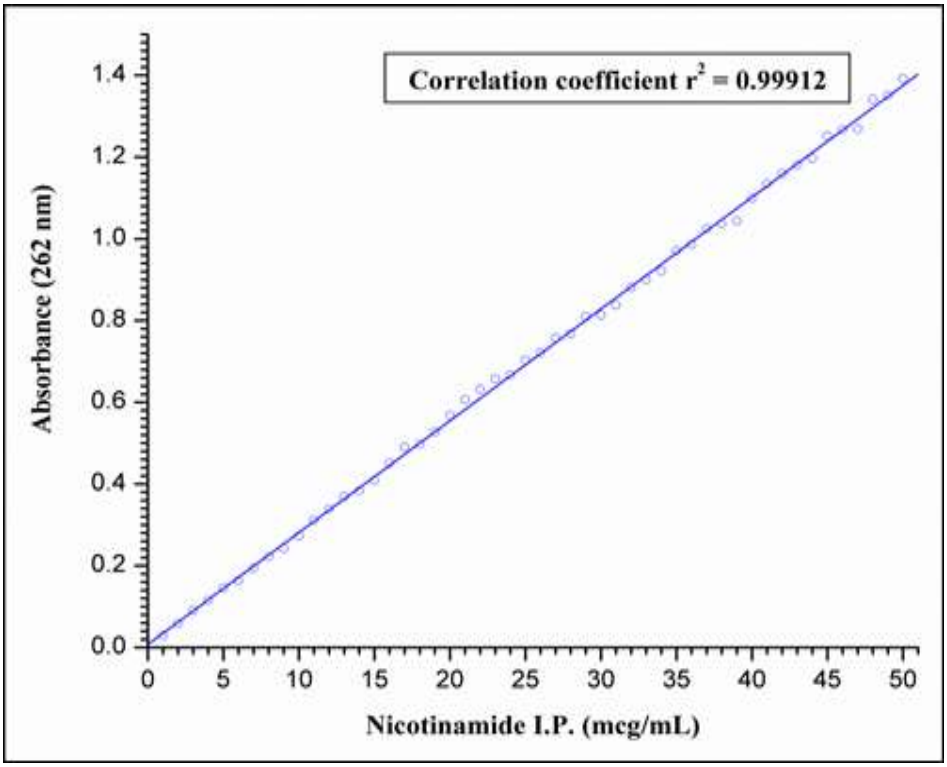

Figure 3. Linear Calibration Curve for Nicotinamide

The high correlation coefficient values confirmed the suitability of the selected concentration range for multivariate chemometric modeling and quantitative analysis.

6.1.3 Development of PLS Regression Model

The Partial Least Squares (PLS) regression model was developed using full-spectrum absorbance data acquired within the wavelength range of 210–300 nm at 1 nm intervals. Compared with conventional single-wavelength spectrophotometric methods, the PLS technique utilized multiple spectral intensities simultaneously, thereby

improving analytical precision, selectivity, and predictive performance for multicomponent analysis.

For chemometric calibration, a training set containing 121 binary mixtures of PYR and NIC was prepared within Beer–Lambert's linearity range. Pyridoxine concentrations varied from 0–10 µg/mL, whereas Nicotinamide concentrations ranged from 0–20 µg/mL. Randomized concentration combinations were selected to improve model robustness and ensure uniform distribution of calibration samples across the analytical range.

The absorbance data matrix consisted of 121 samples measured at 91 wavelengths, resulting in a 121 × 91 multivariate matrix suitable for PLS modeling. The Non-linear Iterative Partial Least Squares (NIPALS) algorithm was employed using UNSCRAMBLER® X software for data processing and model construction. Full cross-validation was performed to determine the optimum number of latent variables and minimize overfitting or underfitting of the model.

6.1.4 Evaluation of PLS Model Performance

A. Projection of Calibration Samples

The calibration and cross-validation score plots for PYR and NIC demonstrated minimal vertical variation between calibration and validation data points, indicating good agreement between predicted and actual concentrations. This confirmed the stability and predictive reliability of the developed PLS model.

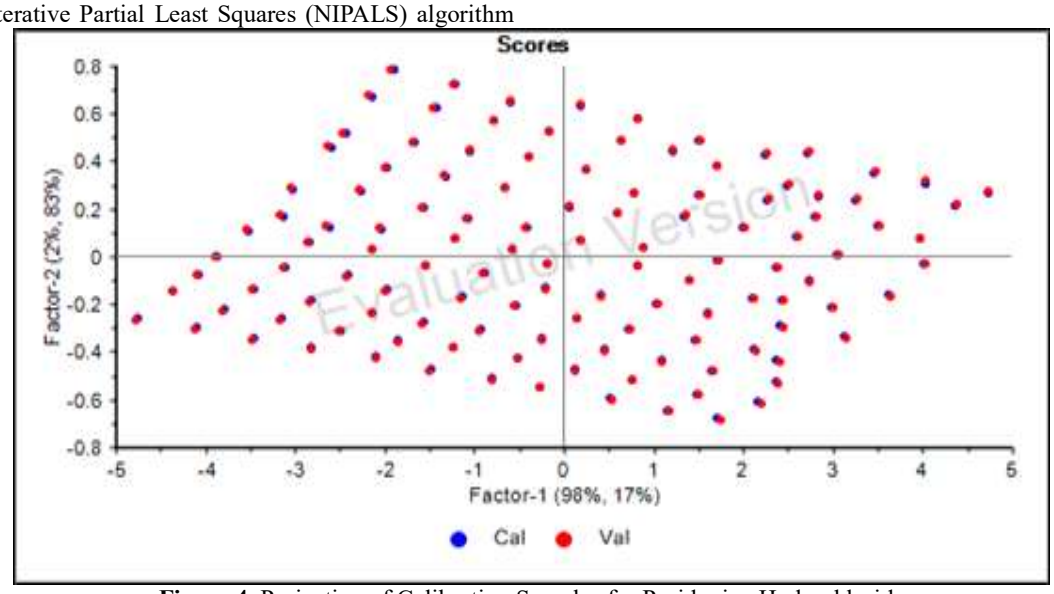

Figure 4. Projection of Calibration Samples for Pyridoxine Hydrochloride

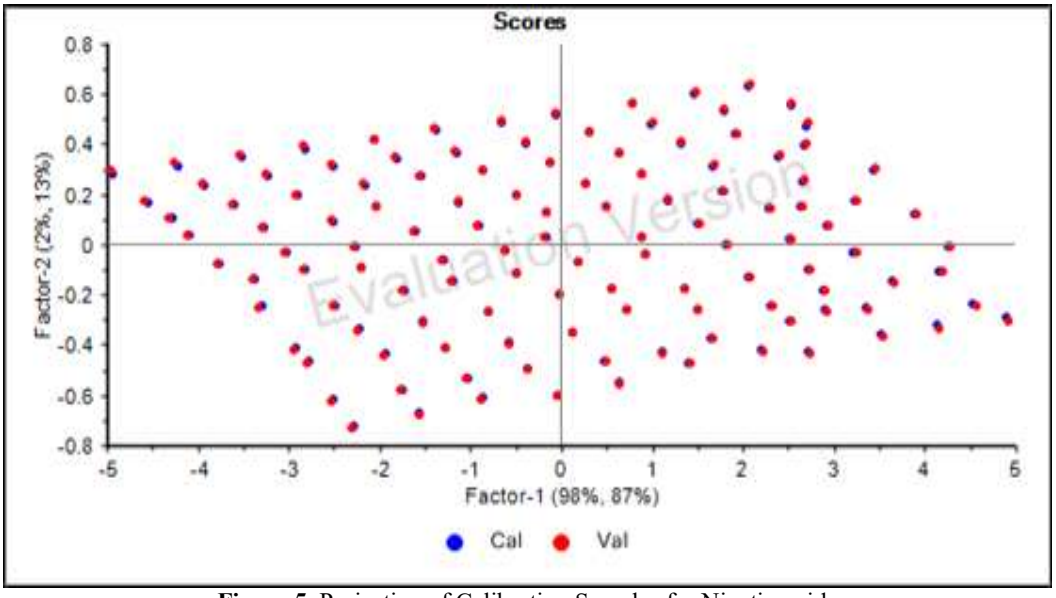

Figure 5. Projection of Calibration Samples for Nicotinamide

The close distribution of calibration and validation scores indicated that the developed chemometric model effectively captured the systematic variation in the spectral data without introducing significant prediction error.

B. Correlation Loading Analysis

Correlation loading plots were generated to evaluate the relationship between absorbance variables and analyte concentration. The plots demonstrated strong positive correlation between spectral absorbance and analyte concentration for both PYR and NIC. The positive loading values confirmed that absorbance increased proportionally with analyte concentration across the selected wavelength range.

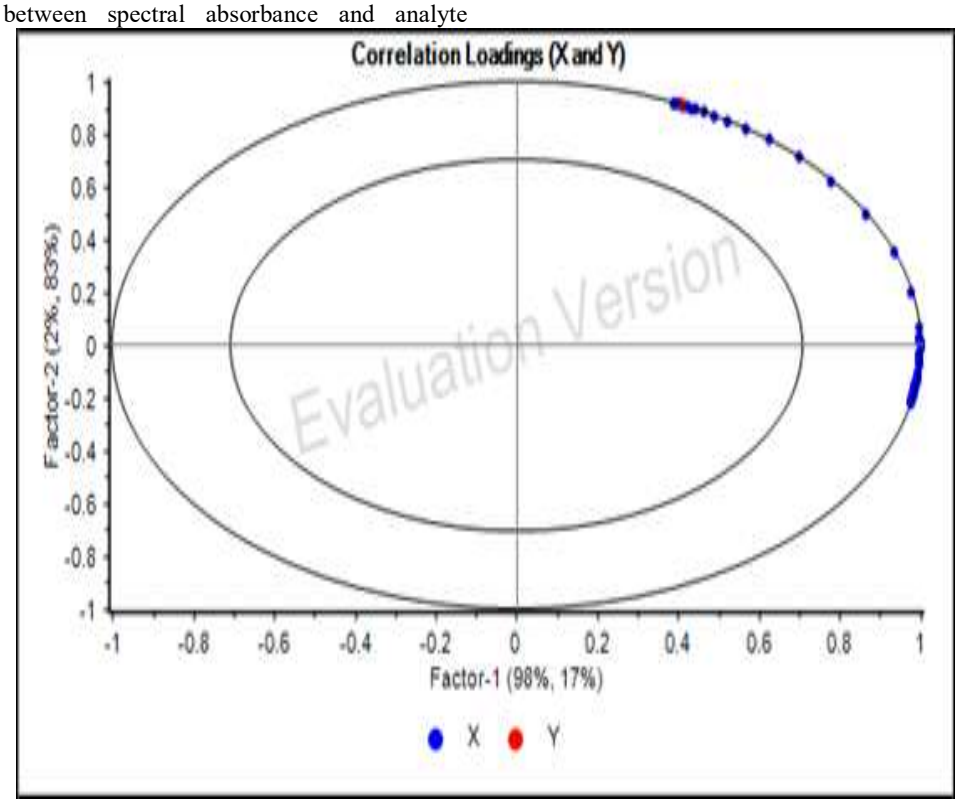

Figure 6. Correlation Loading Plot for Pyridoxine Hydrochloride

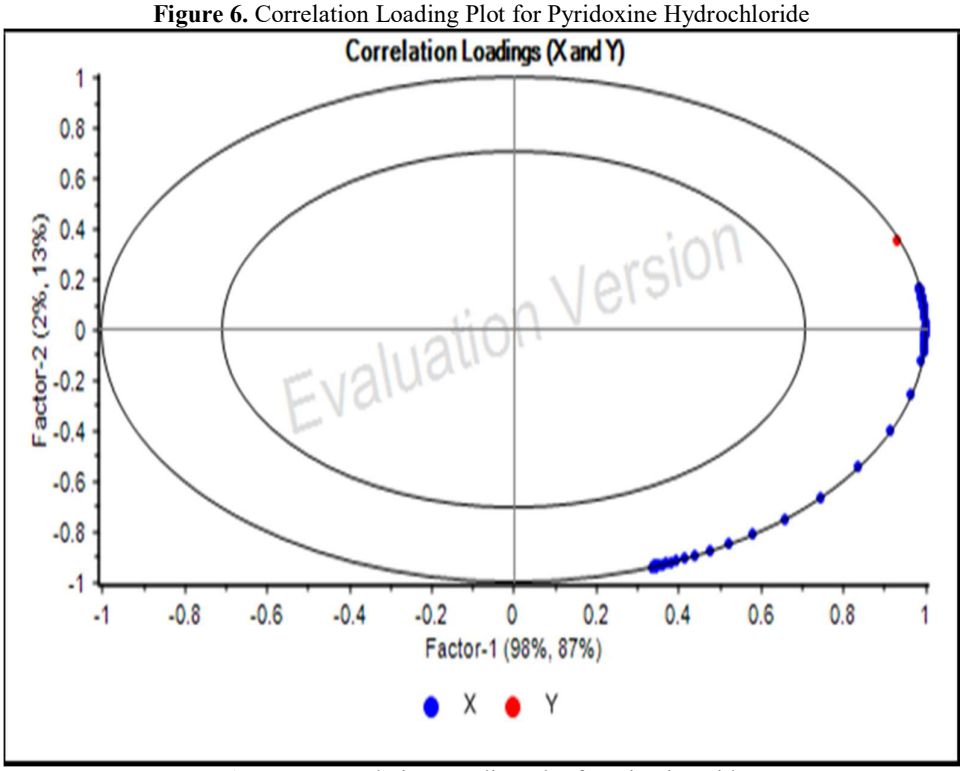

Figure 7. Correlation Loading Plot for Nicotinamide

The observed positive correlations validated the applicability of the selected spectral variables for multivariate quantitative analysis.

C. Variance Analysis

Variance projection plots demonstrated that nearly 100% of the total variance in both X-variables (absorbance values) and Y-variables (concentration values) was explained by the developed PLS model. The negligible residual variance indicated minimal analytical noise and excellent model fitting performance.

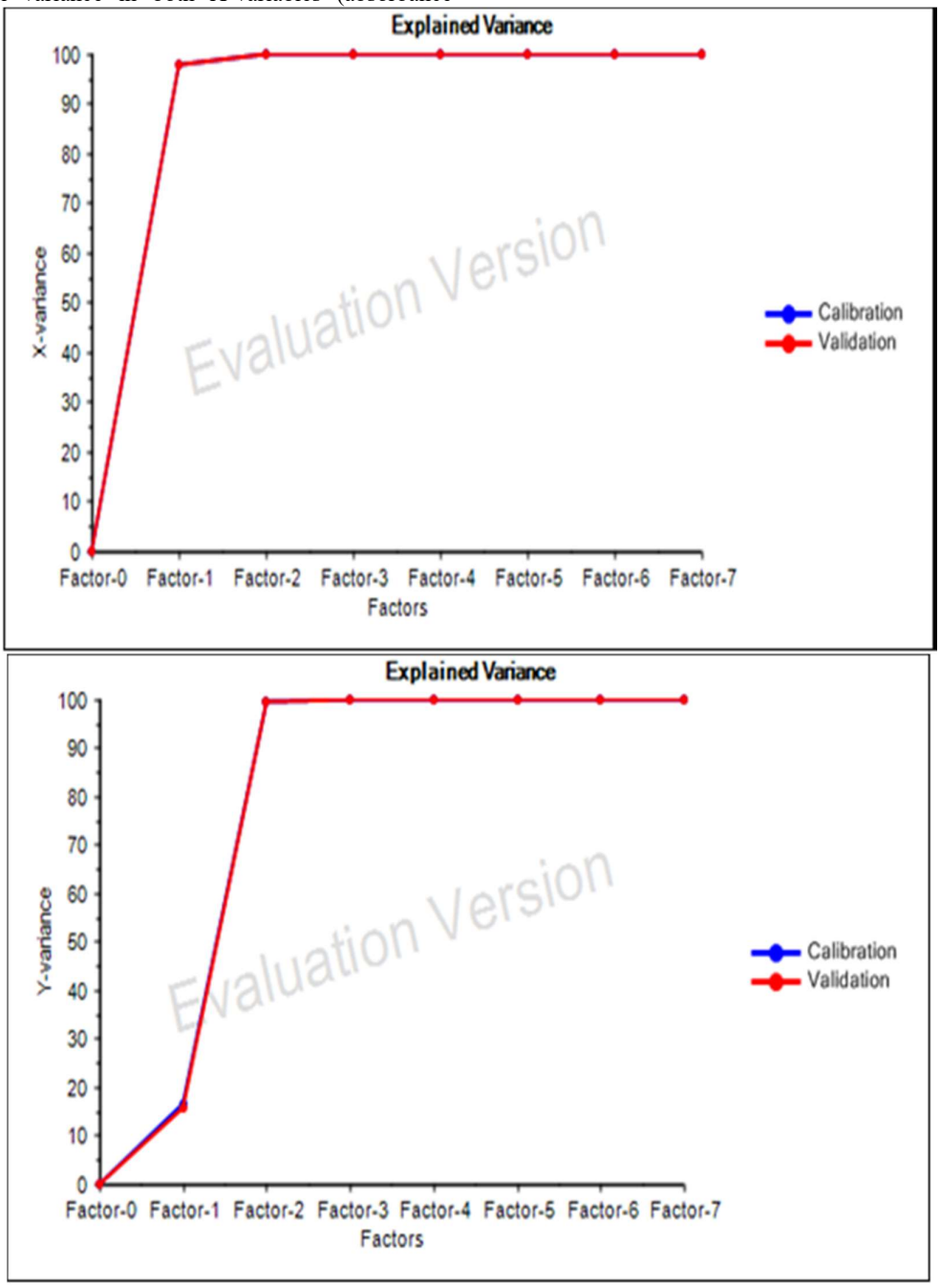

Figure 8. X- and Y-Variance Projection Plot for Pyridoxine Hydrochloride

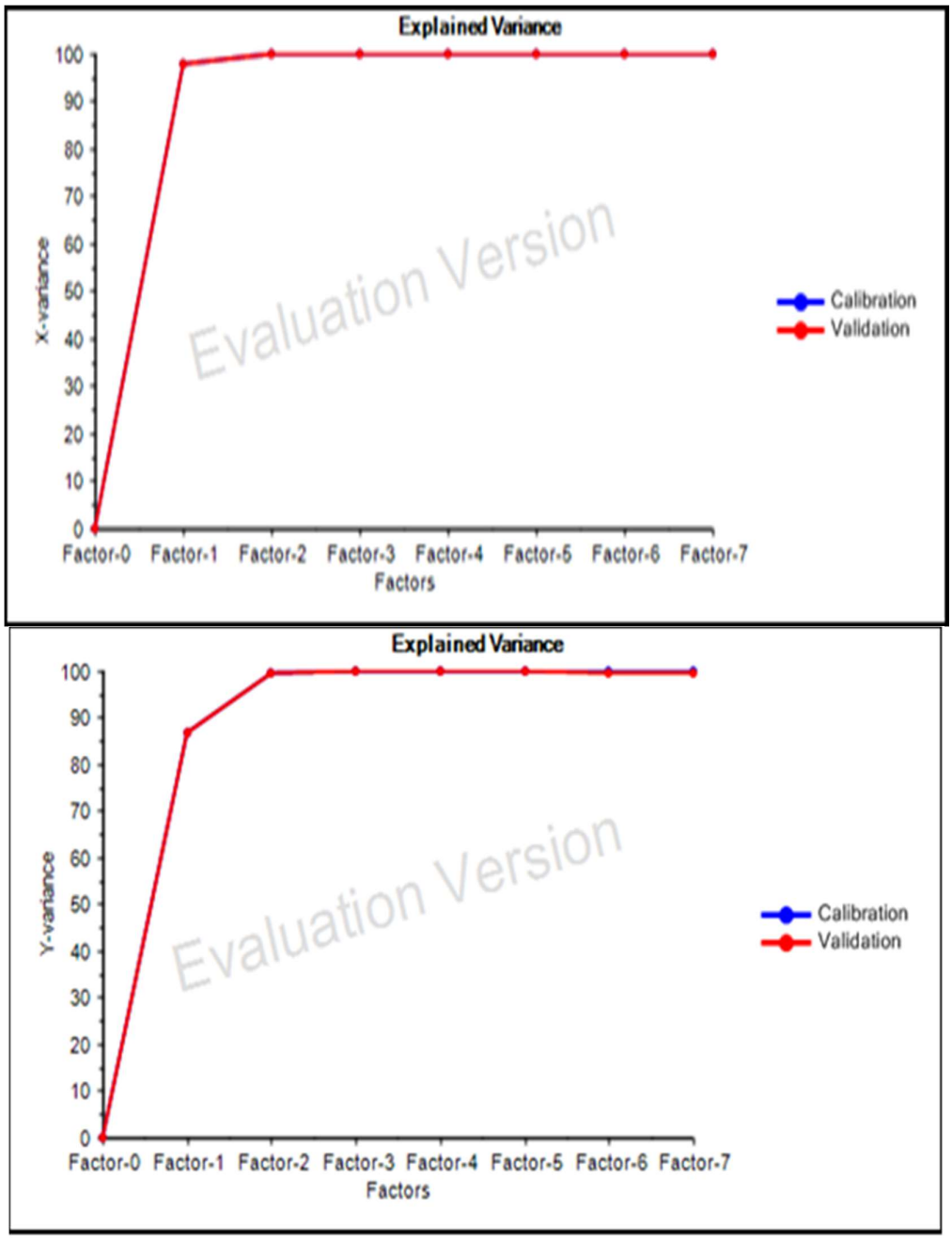

Figure 9. X- and Y-Variance Projection Plot for Nicotinamide

The explained variance analysis confirmed that the developed chemometric model effectively described the analytical data with high predictive capability and minimal residual error.

6.1.5 Predicted versus Reference Concentration Analysis

The predictive performance of the PLS model was evaluated by comparing predicted concentrations with actual reference concentrations for both analytes during calibration and validation stages. The predicted-versus-reference plots demonstrated excellent linear agreement with slope values approaching unity and minimal intercept values.

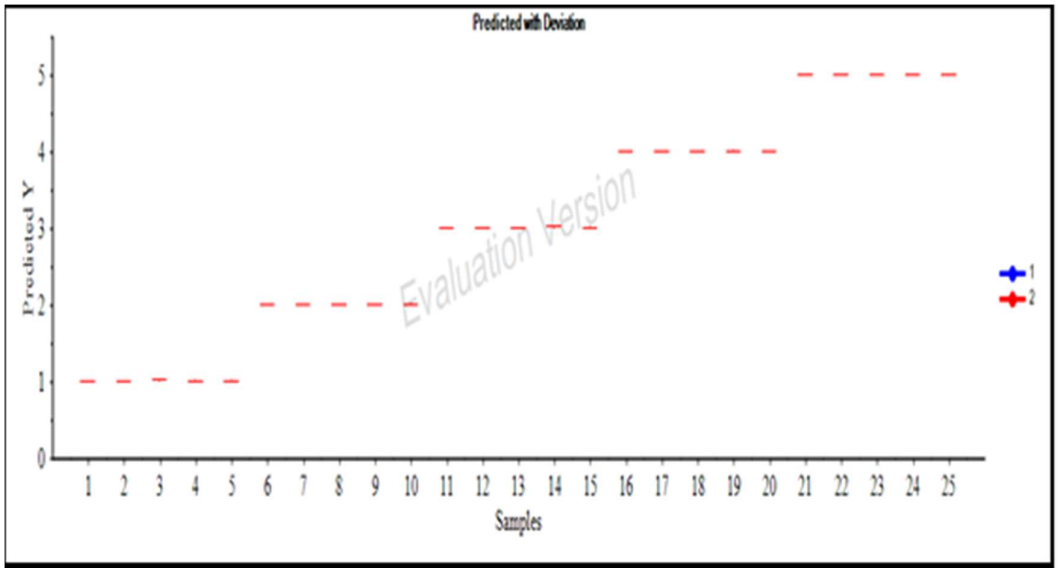

Figure 10. Predicted vs Reference Plot for Pyridoxine Hydrochloride (Calibration)

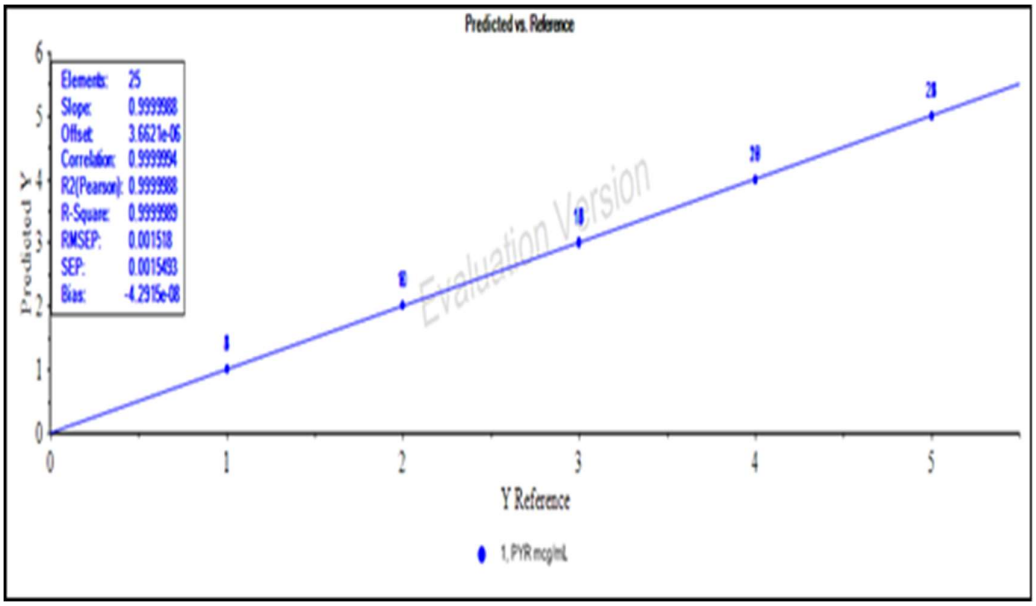

Figure 11. Predicted vs Reference Plot for Pyridoxine Hydrochloride (Validation)

Table 6.2. Multivariate Regression Parameters for Pyridoxine Hydrochloride

Parameter	Acceptance Criteria	Calibration	Validation
Slope	NLT 0.99	0.9959853	0.9956594
Offset	Approximately 0	0.0200732	0.0226631
Correlation	NLT 0.99	0.9979907	0.9978759
R^2 (Pearson)	NLT 0.99	0.9959854	0.9957563
R-Square	NLT 0.99	0.9959853	0.9958260
RMSEC	Minimum	0.2003668	0.2060054
SEC/SECV	Minimum	0.2011999	0.2068686
Bias	Near zero	-3.3497e-08	0.0009602

The obtained regression parameters satisfied the predefined acceptance criteria, confirming the excellent predictive ability and analytical reliability of the developed chemometric model for Pyridoxine Hydrochloride.

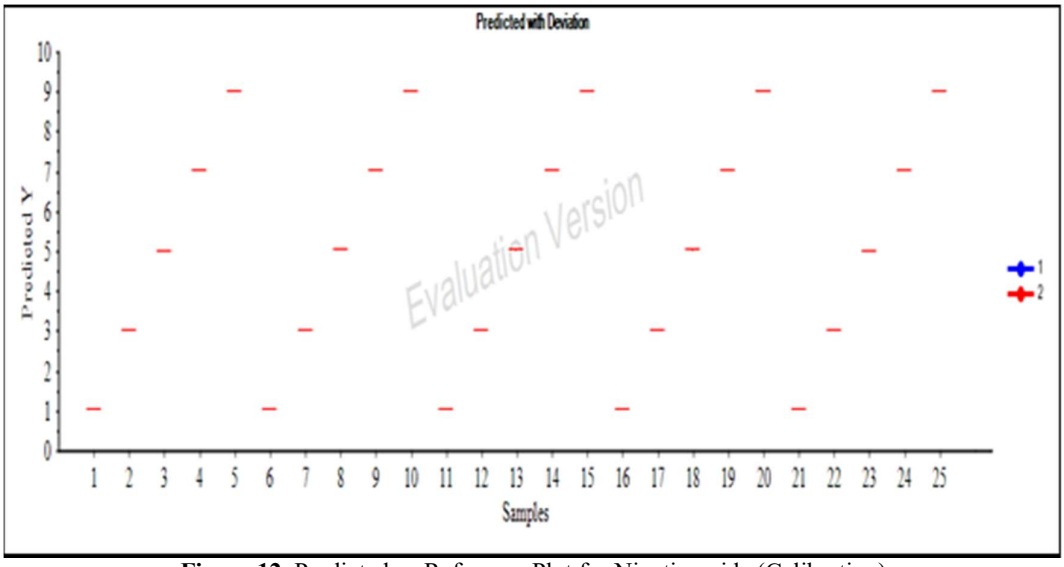

Figure 12. Predicted vs Reference Plot for Nicotinamide (Calibration)

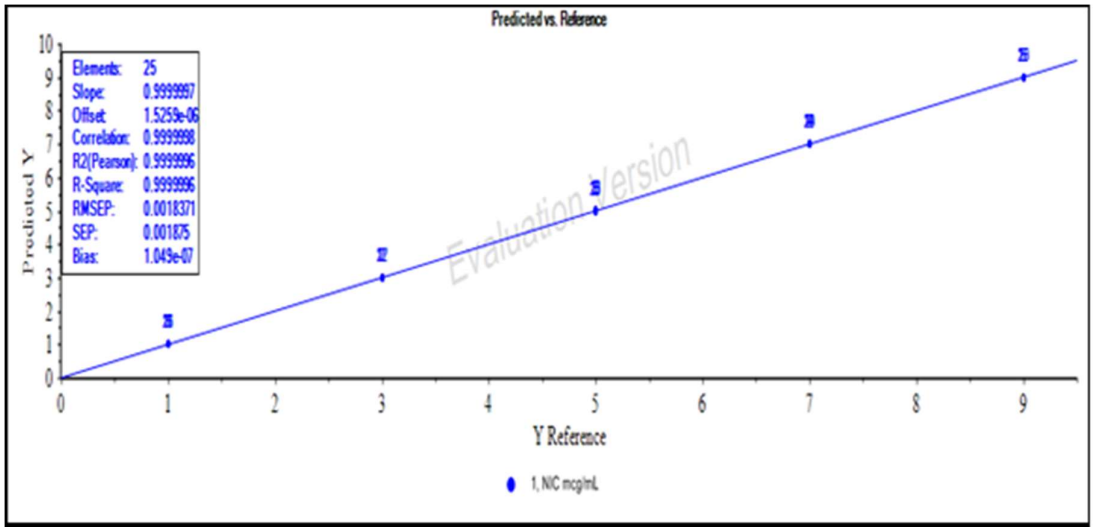

Figure 13. Predicted vs Reference Plot for Nicotinamide (Validation)

Table 6.3. Multivariate Regression Parameters for Nicotinamide

Parameter	Acceptance Criteria	Calibration	Validation
Slope	NLT 0.99	0.9972624	0.9972706
Offset	Approximately 0	0.0273760	0.0257574
Correlation	NLT 0.99	0.9986303	0.9985517
R^2 (Pearson)	NLT 0.99	0.9972624	0.9971056
R-Square	NLT 0.99	0.9972625	0.9971531
RMSEC	Minimum	0.3309101	0.3402694
SEC/SECV	Minimum	0.3322861	0.3416807
Bias	Near zero	9.0638e-08	-0.0015370

The multivariate regression parameters obtained for Nicotinamide also demonstrated excellent calibration and validation performance with negligible prediction error and high correlation coefficients.

6.1.6 Validation Set Prediction Performance

An independent validation set consisting of 25 binary mixtures was analyzed to evaluate the predictive capability of the developed PLS model. The predicted concentrations obtained for both analytes showed excellent agreement with the actual reference concentrations, confirming the robustness and predictive reliability of the chemometric model.

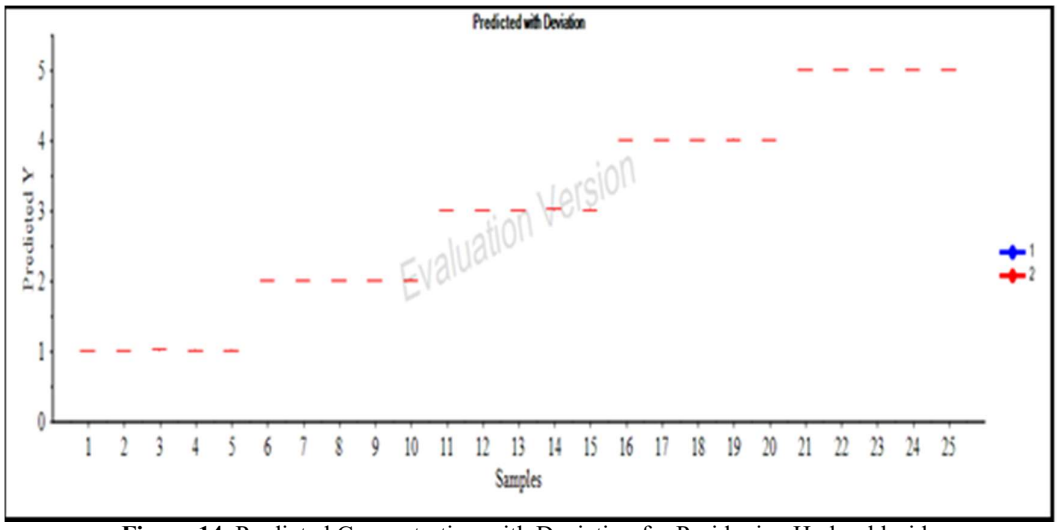

Figure 14. Predicted Concentration with Deviation for Pyridoxine Hydrochloride

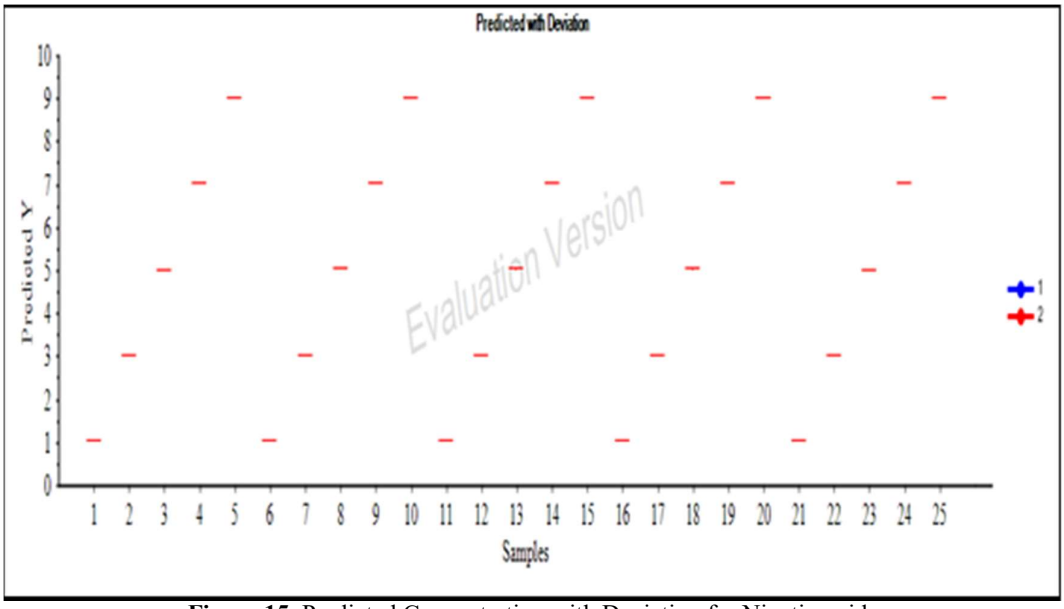

Figure 15. Predicted Concentration with Deviation for Nicotinamide

The deviation plots demonstrated minimal analytical deviation between predicted and actual concentrations, indicating accurate quantitative prediction of unknown samples.

6.1.7 Recovery Study of Validation Set

Percentage recovery studies were performed using the independent validation set to further evaluate the quantitative accuracy of the developed PLS model. The recovery values obtained for both analytes were found to be within acceptable analytical limits.

Table 6.4. Percentage Recovery Study of Validation Set

Parameter	Pyridoxine HCl	Nicotinamide
Mean Recovery (%)	99.9996	99.9972
Recovery Range (%)	99.87–100.17	99.84–100.10
Predictive Accuracy	Excellent	Excellent

The mean recovery values close to 100% indicated excellent analytical accuracy and confirmed the suitability of the developed chemometric-assisted spectrophotometric method for simultaneous quantitative estimation of Pyridoxine Hydrochloride and Nicotinamide in pharmaceutical formulations.

7. DISCUSSION

The present study successfully established the effectiveness of chemometric-assisted UV spectrophotometry integrated with Partial Least Squares (PLS) regression for the simultaneous quantitative estimation of Pyridoxine Hydrochloride (PYR) and

Nicotinamide (NIC) in combined pharmaceutical dosage forms. Simultaneous analysis of multicomponent formulations using conventional UV spectrophotometry is often associated with substantial analytical challenges because of overlapping absorption spectra and interference between analytes. In the present investigation, both PYR and NIC exhibited considerable spectral overlap within the wavelength region of 210–370 nm, thereby limiting the applicability of direct absorbance measurement methods for accurate quantification. The overlapping nature of the spectra clearly justified the application of multivariate chemometric techniques capable of extracting relevant analytical information from complex spectral datasets.

The developed chemometric-assisted method effectively utilized the spectral information obtained over the entire wavelength range rather than relying on a single analytical wavelength. This approach significantly enhanced analytical selectivity and minimized spectral interference between the analytes. The use of PLS regression enabled simultaneous processing of multiple absorbance variables and established a reliable mathematical relationship between spectral responses and analyte concentrations. Consequently, the developed model demonstrated excellent capability for resolving the overlapping spectral behavior of Pyridoxine Hydrochloride and Nicotinamide without the need for prior physical separation.

The linear calibration studies performed for both analytes demonstrated excellent proportionality between absorbance and concentration over the selected analytical range of 1–50 µg/mL. The correlation coefficient values obtained for PYR ($r^2 = 0.99724$) and NIC ($r^2 = 0.99912$) confirmed strong linear relationships and compliance with Beer–Lambert's law within the investigated concentration range. These findings indicate that the selected concentration range was appropriate for chemometric modeling and provided sufficient analytical sensitivity for quantitative estimation. The high linearity obtained for both analytes further established the suitability of the proposed spectrophotometric method for routine pharmaceutical analysis.

The PLS regression model developed in this investigation demonstrated excellent predictive and calibration performance. The multivariate calibration matrix generated from 121 calibration samples measured across 91 wavelengths provided a robust dataset for model construction. The use of the NIPALS algorithm combined with full cross-validation significantly improved the reliability and stability of the chemometric model. Cross-validation played a critical role in selecting the optimum number of latent variables while minimizing the possibility of overfitting or underfitting. The calibration and validation score plots demonstrated close clustering and minimal variation between predicted and reference values, indicating strong agreement between experimental and predicted concentrations.

The correlation loading plots generated during model evaluation demonstrated a strong positive relationship between spectral absorbance and analyte concentration for both Pyridoxine Hydrochloride and Nicotinamide. Positive loading values observed across the selected wavelength region confirmed that increases in analyte concentration directly contributed to proportional increases in absorbance intensity. This observation validated the suitability of the selected spectral variables for multivariate quantitative analysis and confirmed that the developed model effectively captured systematic spectral variations associated with concentration changes.

Variance projection analysis further confirmed the robustness and efficiency of the developed PLS model. Nearly 100% of the variance present in both absorbance variables (X-matrix) and concentration variables (Y-matrix) was successfully explained by the developed chemometric model. The negligible residual variance indicated the presence of minimal analytical noise and demonstrated excellent model fitting performance. Such high explained variance suggests that the selected spectral region and calibration strategy were highly appropriate for simultaneous estimation of the selected analytes.

The predictive capability of the developed model was further confirmed through predicted-versus-reference concentration studies performed during both calibration and validation stages. The regression slopes obtained for Pyridoxine Hydrochloride and Nicotinamide were found to be very close to unity, while intercept values remained close to zero. These results indicated excellent agreement between predicted and actual analyte concentrations. Additionally, the high correlation coefficients and R-square values obtained for both analytes demonstrated outstanding predictive accuracy and model reliability. The low values of Root Mean Square Error of Calibration (RMSEC) and Standard Error of Calibration/Validation (SEC/SECV) indicated minimal prediction error and high analytical precision. Furthermore, the near-zero bias values confirmed the absence of systematic analytical deviation within the developed model.

The independent validation study performed using 25 binary mixtures further established the predictive reliability of the proposed chemometric-assisted method. The predicted concentrations obtained for both analytes showed excellent agreement with the corresponding reference concentrations, indicating that the model maintained consistent predictive performance even for unknown samples outside the calibration dataset. The deviation plots demonstrated minimal analytical deviation between actual and predicted values, confirming the accuracy and robustness of the developed multivariate calibration approach.

Recovery studies carried out using independently prepared validation samples demonstrated excellent analytical accuracy of the developed method. Mean percentage recoveries of 99.9996% for Pyridoxine Hydrochloride and 99.9972% for Nicotinamide were obtained, with recovery ranges remaining well within acceptable pharmaceutical analytical limits. The recovery results confirmed that the

developed chemometric-assisted spectrophotometric method was capable of accurately quantifying both analytes without interference from spectral overlap or matrix effects. These findings further validated the reliability and practical applicability of the proposed analytical approach for routine pharmaceutical quality control analysis.

Compared with conventional chromatographic methods, the proposed chemometric-assisted spectrophotometric method offers several analytical advantages, including reduced solvent consumption, shorter analysis time, lower operational cost, and environmentally friendly analytical performance. The method eliminates the requirement for extensive sample preparation and chromatographic separation while still maintaining satisfactory analytical accuracy and precision. Therefore, the developed method may serve as an efficient alternative analytical tool for routine estimation of Pyridoxine Hydrochloride and Nicotinamide in combined pharmaceutical formulations, particularly in laboratories where rapid and cost-effective analysis is required.

The present investigation confirmed that chemometric-assisted UV spectrophotometry coupled with PLS regression is a highly effective, accurate, and reliable analytical strategy for simultaneous multicomponent pharmaceutical analysis. The excellent calibration performance, strong predictive capability, negligible analytical error, and high recovery values collectively establish the suitability of the developed method for routine pharmaceutical quality control applications involving Pyridoxine Hydrochloride and Nicotinamide combined dosage forms.

8. CONCLUSION

The present study successfully developed and validated RP-HPLC and chemometric-assisted UV spectrophotometric methods for simultaneous estimation of Pyridoxine HCl and Nicotinamide in pharmaceutical formulations. Both methods demonstrated satisfactory analytical performance according to ICH validation requirements. The chemometric-assisted spectrophotometric method effectively resolved overlapping spectral interference using Partial Least Squares regression and provided a rapid and economical alternative for routine pharmaceutical analysis. The RP-HPLC method exhibited excellent chromatographic separation, specificity, and reproducibility suitable for advanced quality control applications. The comparative evaluation established that both methods can be effectively utilized for simultaneous pharmaceutical analysis depending on analytical requirements, instrument availability, and regulatory expectations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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