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RESEARCH ARTICLE

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Raman spectroscopy for the quantitative analysis of Lornoxicam in solid dosage forms

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Abstract

In this study, Raman spectroscopy is used to characterize different formulations of active pharmaceutical ingredient (API) of Lornoxicam, a nonsteroidal anti-inflammatory drug for qualitative and quantitative analyses. The Raman spectral characteristic peaks associated with API and excipients are identified, which increase or decrease as their concentrations change in different formulations. The spectral response was analyzed qualitatively and quantitatively by using chemometrics tools such as principal component analysis (PCA) model and partial least squares regression (PLSR) analysis model, respectively. The characteristic Raman spectral features associated with the different formulations of API of Lornoxicam were used to differentiate different groups of Raman spectral data using PCA. By using PLSR model, root mean square error of cross validation (RMSECV) was found to be 0.76 mg during the development of prediction model and according to the (R^2) value, the goodness of the model was found to be 0.99. Furthermore, the concentration of API in the blind sample was measured. In comparison to the 15/35 mg (w/w), this concentration was projected to be 14.13/35 mg (w/w). Raman spectroscopy along with PCA and PLSR analyses is a useful tool for determining the concentration of Lornoxicam in pharmaceutical samples, both qualitatively and quantitatively.

KEYWORDS

excipients, Lornoxicam API, partial least squares regression (PLSR) model, Raman spectroscopy, solid dosage forms

1 | INTRODUCTION

Lornoxicam is an analgesic, anti-inflammatory, and antipyretic nonsteroidal pharmaceutical drug (NSAID).[1] It has a solid yellow appearance with a molecular formula ($C_{13}H_{10}ClN_3O_4S_2$) as shown in Figure 1. The molecular weight of Lornoxicam is 371.8 g, and the melting point is 225–230°C. In studies, Lornoxicam is also used for the treatment of cancer and Alzheimer's disease.[2,3] Its structure is shown in Figure 1. This drug belongs to the Oxicam nonsteroidal anti-inflammatory medicine class, which includes COX-1 and COX-2 cyclo-oxygenase inhibitors. The effectiveness of Lornoxicam is also attributed to its significant reduction of prostaglandin

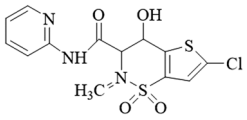

FIGURE 1 Structure of Lornoxicam

production.[4,5] It exists in three forms such as instant-release tablets (4 and 8 mg), rapid-release tablets (8 mg), and parenteral formulations (4 mg/ml) used for intravenous and intramuscular administration.[6]

According to a comprehensive literature review, different spectroscopic and chromatographic methods have been reported for the study of Lornoxicam drug, such as high performance liquid chromatography (HPLC),[7] infrared spectroscopy, Fourier transform infrared spectroscopy (FTIR),[8,9] and gas chromatography–mass spectroscopy (GC–MS).[10] These methods for determining Lornoxicam in dosage forms, raw materials, and biological samples have been found to be reliable. However, these are costly and time-consuming and involve extensive preparation of samples for determining active pharmaceutical ingredient (API) content in pharmaceutical formulations.[11] Lornoxicam in solid forms can be measured by preparation of sample or without it, a rapid, selective, nondestructive, sensitive, and reliable technique must be developed.[12] Modern spectroscopic techniques are very effective and sensitive instruments for qualitative and quantitative drug analyses, and the results are widely used in pharmaceutical quality control laboratories. FTIR is one of the quality assurance methods used in the pharmaceutical industry. Although, FTIR is used for the characterization of pharmaceutical formulations but thick samples cannot be analyzed due to signal saturation and loss of spectral information.[13] Attenuated total reflectance (ATR)-FTIR imaging is an active field of pharmaceutical applications of vibrational spectroscopy that is used to study drug release and dissolution. Unfortunately, despite extensive research on how solid formulations dissolve, there is still a lack of understanding of the mechanism that takes place within the formulation (or tablet) when it comes in contact with dissolution media.[14] Laser direct infrared (LDIR) imaging is a promising technique for rapidly characterizing the spatial distribution of active ingredients in drug formulations. The LDIR chemical imaging approach gives researchers the opportunity to get detailed domain morphology information in a fraction of the time.[15]

Raman spectroscopy was employed for a variety of analytical applications including disease diagnosis[16] in the fields of food and agricultural products,[17] such as fruits and vegetables, crops, beef, and dairy items, analysis of chemical and biological products,[18] and identification and differentiation of different bacteria.[19,20] By using Raman spectroscopy, different pharmaceuticals have been analyzed such as sitagliptin,[18] cefixime,[21] amoxicillin, and losartan potassium.[22] The aim of the current study is to develop Raman spectroscopy-based analytical method to analyze an antipyretic pharmaceutical drug qualitatively and quantitatively, Lornoxicam, which has yet to be reported using solid dosage formulations of API. In this study, Raman spectroscopy and multivariate sample analysis techniques such as principal component analysis (PCA) and partial least squares regression (PLSR) were used to examine spectral changes in solid dosage forms of Lornoxicam API and excipients prepared in different concentrations. Moreover, content of Lornoxicam in the blind sample is quantified using PLSR prediction model.[18]

2 | MATERIAL AND METHODS

2.1 | Sample preparation

Lornoxicam (API) and excipients were received from Pharmasol Pharmaceutical Private Limited, Lahore, Pakistan, with batch number SI/LXM/19/13 and were utilized without further purification. Pure Lornoxicam used as a reference sample and excipients including microcrystalline cellulose (Avicel pH 102), sodium lauryl sulfate, lactose spray dried, sodium starch glycolate, and magnesium stearate were used to prepare various concentrations of solid dosage forms, as shown in Table S1. All the abovementioned excipients are mixed in an equal concentration by measuring 2 g of each excipient and homogenized to create a homogeneous mixture of excipients for further analysis. Furthermore, by using a homogenizer (mortar pestle), excipients and API were mixed together and make a homogeneous mixture in the form of powder. Because in powdered form, exact concentrations of API and excipients can be measured, and also this can avoid heterogeneity. In this way, different samples along with a blind/unknown sample (containing 15 mg) are made by measuring and combining ingredients.

2.2 | Raman spectral acquisition

Lornoxicam samples (API and excipients) in powdered form were analyzed using Raman spectroscopy. The spectral measurements were carried out with a Raman spectrometer (Peak Seeker, Pro-Agiltron, USA) having 785 nm laser. For this, 30 mg of each solid dosage sample was put on aluminum slide, and 40 mW laser was

delivered by using a 4× objective lens, and spectra were recorded with integration time of 2 s. A total of 15 Raman spectra were recorded from different positions for each Lornoxicam sample by adjusting the focus of laser every time.

2.3 | Data preprocessing

MatLab 7.8 was used to preprocess the Raman spectral data according to specified procedures.[23] The preprocessing of data recorded from Raman spectrometer involves smoothing (Savitzky–Golay smoothing filter), removal of the aluminum substrate, and baseline correction (rubberband method). Normalization should be avoided in the PLSR model to retain the concentration to intensity relationship in the Raman spectral data.

2.4 | Data analysis

The characteristic Raman spectral peaks of API and excipients in different formulations of Lornoxicam were examined by comparing the mean Raman spectra of each sample. The peak assignments for different Raman spectral features of Lornoxicam were acquired from the literature and are given in Table S2 including relevant citations. The qualitative study of different dosage formulations of Lornoxicam was done using PCA. The first principal component (PC-1) illustrates the most variability in Raman spectral data, whereas the successive principal components explain the remaining variability. PLSR, a multivariate, quantitative data analysis approach, was used to acquire the most information regarding the covariance of spectral data (x-variables) and known concentrations (y-variables). A regression model based on changes in the Raman peaks of different formulations of Lornoxicam was used to do a quantitative investigation of spectral changes due to increase in the percentage weight of API in different formulations.[21] The fitness value of the PLSR predictive model in this study was observed using a root mean square error of cross validation (RMSECV). The optimum number of latent variables (LVs) required to build the model was examined using leave-one sample-out cross validation (LOOCV) to develop a reliable model while avoiding overfitting. The leave one sample out (15 spectra) approach was applied to make sure that the PLSR analysis is not biased by the fact that all of the spectra gathered from drug formulations are introduced into either the calibrated data set or the test data set, but not both. As a result, Raman spectral data sets of different formulations are divided at random into two groups including 60% of the spectral data set for calibration and 40% for validation.

3 | RESULTS AND DISCUSSION

3.1 | Mean Raman spectral characterization

The mean Raman peak intensities of solid dosage forms for different concentrations of API of Lornoxicam are shown in Figure 2. The significant differences in Raman

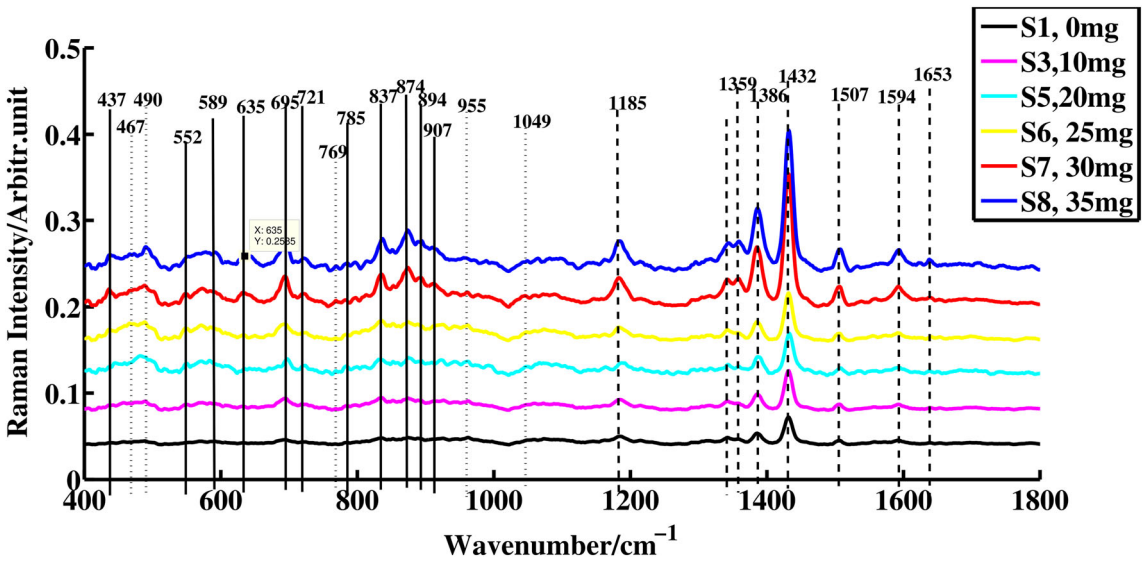

FIGURE 2 Mean Raman spectra of different solid formulations of Lornoxicam, including S1 pure excipient, S3 (10 mg), S5 (20 mg), S6 (25 mg), S7 (30 mg), and S8 (pure API) [Colour figure can be viewed at wileyonlinelibrary.com]

spectral features of the Lornoxicam drug are indicated by vertical lines, and related peak assignments from the detailed literature study are summarized in Table S2. As shown in Table S1, changes in Raman spectral peak intensities are associated with different formulations of Lornoxicam made by increasing the concentration of API and decreasing the concentrations of the excipients. Raman spectral features of the API have been shown by solid lines, and those of all excipients are labeled with dotted lines. Moreover, dashed lines are used for those features that are common in API and excipients. Notably, as these common features are found to have increase in intensities with increasing concentration of API, they are also assigned to API. The Raman spectral peaks associated with API are found at 1653, 1594, 1507, 552, 1432, 1386, 1359, 1344, 1185, 907, 874, 894, 907, 837, 785, 721, 695, and 437 cm^{-1}. The intensities of the peaks labeled with solid lines gradually increase as the concentration of API is increased. A major Raman spectral peak of Lornoxicam is at 1432 cm^{-1}, which shows the CH_2 cm^{-1} scissoring, and another strong peak of Lornoxicam is at 1386 cm^{-1}, which is assigned as the CH_3 bending. The Raman feature at 1507 cm^{-1} represents (C-C) stretching vibrations in imidazole ring. Another prominent peak at 1594 cm^{-1}shows stretching of nitrile group (C=N) and stretching of C=C in the quinoid ring. The other major peak of API is at 1185 cm^{-1}, which is assigned as C-H wagging vibrations. The strong peak of Lornoxicam is also observed at 837 cm^{-1}, which shows the bending vibrational mode of amine groups (NH_2). The medium peak intensity is at 721 cm^{-1}, which is assigned as (CH) wagging and scissoring of C-N-C (lact + dihyd). These peaks show a trend of decrease in concentration of excipient. Another peak associated with the API is observed at 894 cm^{-1} with decreasing trend and associated with backbone, C-C skeletal. The other strong peak is observed at 695 cm^{-1}, which is associated with API and assigned as C-C-C bending. In addition, the major strong Raman peak intensity of API of Lornoxicam is observed at 874 cm^{-1}, representing the stretching of alkane bond (C-C). The Raman peak at 552 cm^{-1} shows the peak intensity of the API assigned as O-S-O bending and O-N-O-S bending. Another strong peak of API is at 1344 cm^{-1}, which is assigned as the (CH_3) wagging. Raman peak of API at 907 cm^{-1} is assigned as the symmetric (COO^-) stretching mode of wavenumber cm^{-1}. The intense Raman peak of API observed at 695 cm^{-1} is assigned as CCC bending and CC scissoring. Another medium Raman peak intensity is observed at 785 cm^{-1}, which is assigned as scissoring of (O-C=O) and deformation of β lactam ring.

The spectral features from all excipients included in drug formulations are also identified in the mean spectra. The excipients, which are used in the current study, include lactose, microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate, and magnesium stearate; all are active excipients with different Raman features[24] as shown in Figure 2. Raman peaks showing the decrease in concentration of excipients in different solid dosage forms indicated by gradual decrease in their intensities are found at 769 cm^{-1} assigned as (out-of-plane bending mode of OCCC) 467 cm^{-1} (scissoring of C-C), 490 cm^{-1} (vibrational deformation of C-O-C), and 1049 cm^{-1} (stretching in carbonyl group [C=O] and stretching of C-N bond). The characteristic Raman spectral features of API and excipients in different concentrations of drug are successfully employed to identify API in solid dosage forms.

3.2 | PCA

PCA was employed to analyze the ability of Raman spectroscopy to distinguish solid dosage forms of Lornoxicam by using all the spectral peaks that could differentiate API of Lornoxicam and excipients. Figure 3 shows the PCA scatter plot of spectral data sets of eight solid dosage forms of Lornoxicam having different concentrations of API. It can be observed that different spectral data sets of the samples are clustered differently, each represented by a different color. Notably, each dot in the clusters in the PCA scatter plot represents one Raman spectrum. The PCA scatter plot, in particular, distinguishes all the sets of different dosage formulations of API of Lornoxicam in order of S1 to S8 with the gradual increase in the concentration of API in these formulations. PC-1 accounts for 84.53% of the data variability whereas PC-2 explains for 8.09% variability.

In order to confirm the Raman spectral features identified in Figure 2, pair-wise PCA is done between Raman spectral data sets of pure API (S8) of Lornoxicam and pure excipient (S1) as observed in the form of PCA scatter plot in Figure 4A and the loadings of PCA in Figure 4B. The Raman spectral data sets of pure excipient and API of Lornoxicam are clearly differentiated as separate clusters in the pairwise PCA scatter plot as negative and positive axes, respectively. The features responsible for this differentiation are identified as negative and positive loadings. These loadings confirm the differentiating spectral features of the mean Raman spectra (Figure 2), indicating the ability of the technique to analyze pharmaceutical samples having different concentrations of the API.

In PC-1, the spectral features of positive loadings observed at 1594 cm^{-1} indicate the (stretching of Nitrile group (C=N) and stretching of C=C in the quinoid ring),

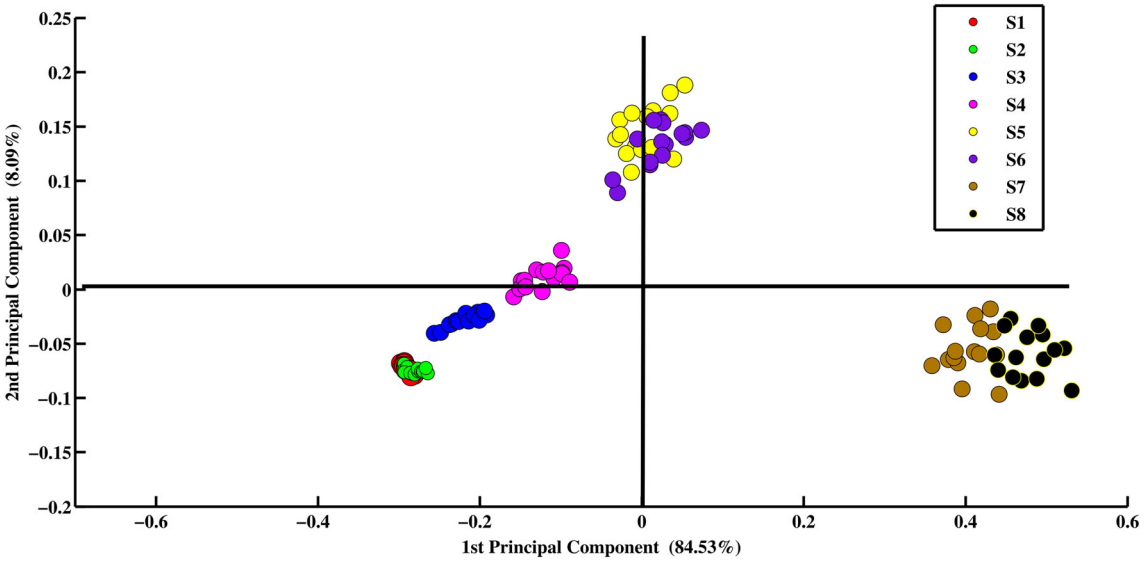

FIGURE 3 Principal component analysis (PCA) scatter plot of Raman spectral data sets of different formulations of Lornoxicam [Colour figure can be viewed at wileyonlinelibrary.com]

1507 cm^{-1} assigned as (stretching vibrational mode of C-C bond), 1432 cm^{-1} (scissoring of CH_2 bond), 1386 cm^{-1} (bending of CH_3 group), 1185 cm^{-1} (N-N stretching vibrations (tetrazole ring) + stretching vibrations of C-C (tetrazole and benzoic ring) + wagging vibrations mode of C-H (benzoic ring), 552 cm^{-1} (OSO bending, ONOS bending), 907 cm^{-1} (symmetric stretching of COO^-), 874 cm^{-1} (stretching of C-C bonding), 721 cm^{-1} (scissoring mode of C-N-C (lact + dihyd), 437 cm^{-1} (out-of-plane modes of Ph-C-Ph ring structure) are assigned to the API. On the other hand, the negative loadings in the PC-1 show the Raman spectral characteristics of excipients, which are noticed at 490 cm^{-1} (deformation of C-O-C), 769 cm^{-1} (Out-of-plane mode of OCCC), and 1049 cm^{-1} (stretching of carbonyl group [C=O], stretching of C-N bond). These observations show that PCA scatter plot is useful in identifying and differentiating different Raman spectral data sets of different pharmaceutical formulations based on distinct Raman features of API and excipients.

3.3 | PLSR analysis model

As shown by PCA, Raman spectroscopy can examine differences in spectral features associated with different sets of formulations with different concentrations of API of Lornoxicam. The PLSR model was employed using the Raman spectral data of six different formulations of Lornoxicam (S2–S7) in order to build a prediction model for measuring API in different solid formulations. The PLSR model was build using the statistically inspired modification of the partial least squares (SIMPLS) algorithm, with wavenumbers as predictors (x-variables) and concentrations of API as response (y-variables). The PLSR model was developed using spectral raw data sets from all Lornoxicam samples, with the exception of pure API (S1) and excipients (S8), which may have biased the results. To prevent data biases, all Raman spectral features of varying formulations of Lornoxicam were assembled into the matrix, and the data are randomly assigned for further modeling.[25] Notably, the total spectral data sets (120 spectra) were divided into two parts: a calibration data set of 60% (72 spectra) and a test data set of 40% (48 spectra). In the calibration data set, approach of leave one same (15 spectra) out cross-validation was employed to determine the best model complexity for test data set validation.[26]

3.3.1 | Latent variables in PLSR

In a PLSR model, the optimum number of LVs and PLS components is significant because too many or too few LVs can affect the prediction abilities of model.[27] Root mean square error of calibration/validation of the 20 latent variables is shown in Figure S1. The optimum number of LVs is mainly chosen based on the minimum value of RMSECV in order to avoid the risk of model overfitting. By applying cross-validation, six optimal LV values were selected as shown in Figure S1. Raman spectral data from different formulations of Lornoxicam samples (API and excipients) are used to build a PLSR prediction model. It was observed useful in estimating

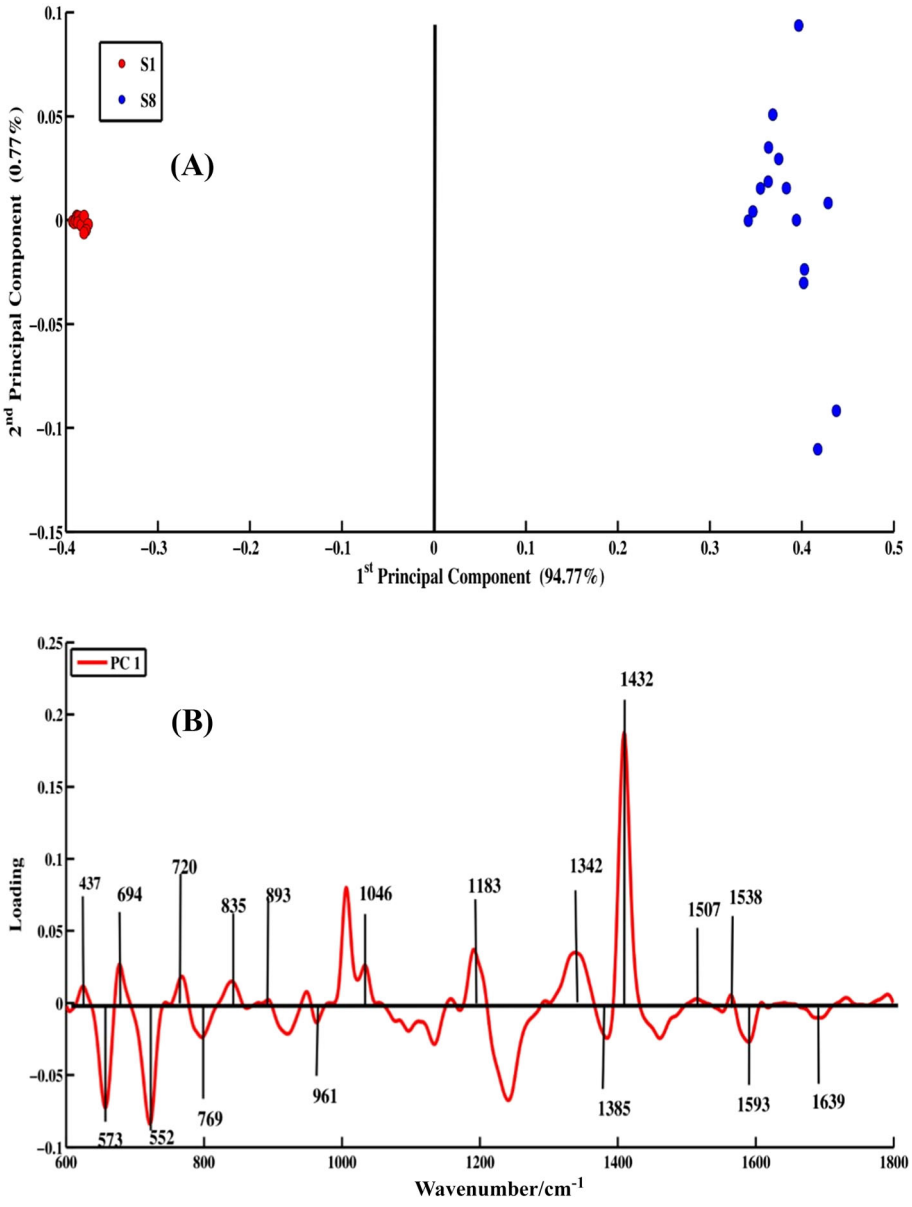

FIGURE 4 Pairwise principal component analysis (PCA) of Raman spectral data of pure API (S8) of Lornoxicam and pure excipient (S1); (A) PCA scatter plot and (B) PCA loadings of first principal component (PC-1). [Colour figure can be viewed at wileyonlinelibrary.com]

the different concentrations of API in different formulations of Lornoxicam in solid dosage forms. The goodness of fit (R^2) model value was determined 0.99, whereas the root mean square error of calibration (RMSEC) was 0.76.

3.3.2 | Root mean square error of prediction (RMSEP)/unknown concentrations

To measure the performance of the established model, one sample was kept blind. The PLSR model was used on that blind sample (S4), and the concentration of API in it was estimated to be about 14.13 mg, assigned in Figure 5B, whereas the actual value was 15 mg. It indicates that the established model has excellent predictive ability. Each spectrum is employed separately and projected into the prediction model for predicting the unknown/blind concentration.

As Table S3 shows, different parameters are found in the prediction performance of the model for determining the concentrations of the API in the four different unknown/blind samples. The calibration value (R^2 Cal) and prediction value (R^2 Val) of the regression analysis were determined to be 0.99 and 0.98, indicating satisfactory performance of model.

3.3.3 | Regression coefficients

The Raman spectra features found in the regression coefficient and shown in Figure 6, such as 1594 cm^{-1}

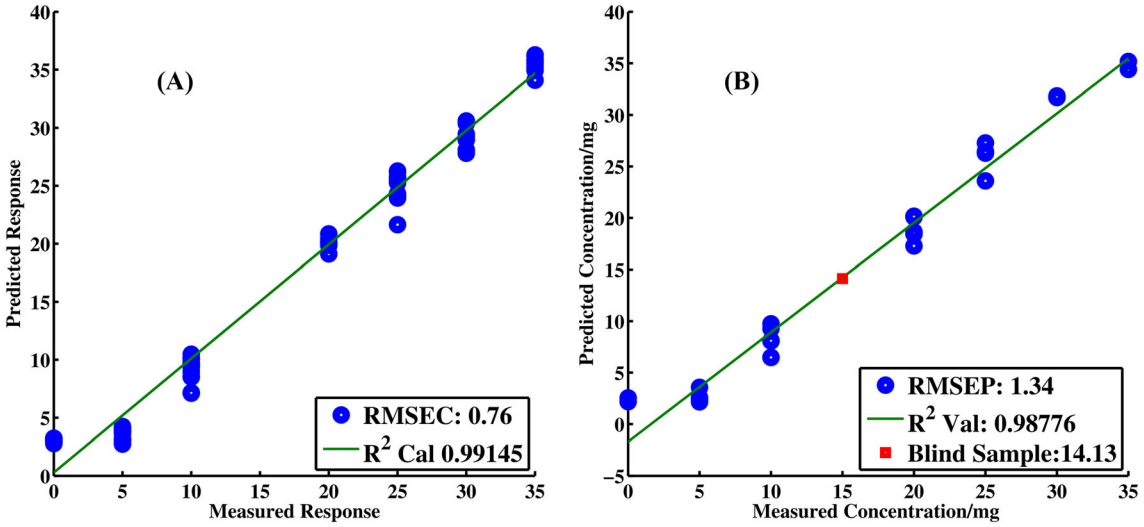

FIGURE 5 Performance of the partial least squares regression (PLSR) model for the quantitative analysis of different formulations of Lornoxicam; (A) calibration model and (B) prediction model. RMSEC, root mean square error of calibration; RMSEP, root mean square error of prediction [Colour figure can be viewed at wileyonlinelibrary.com]

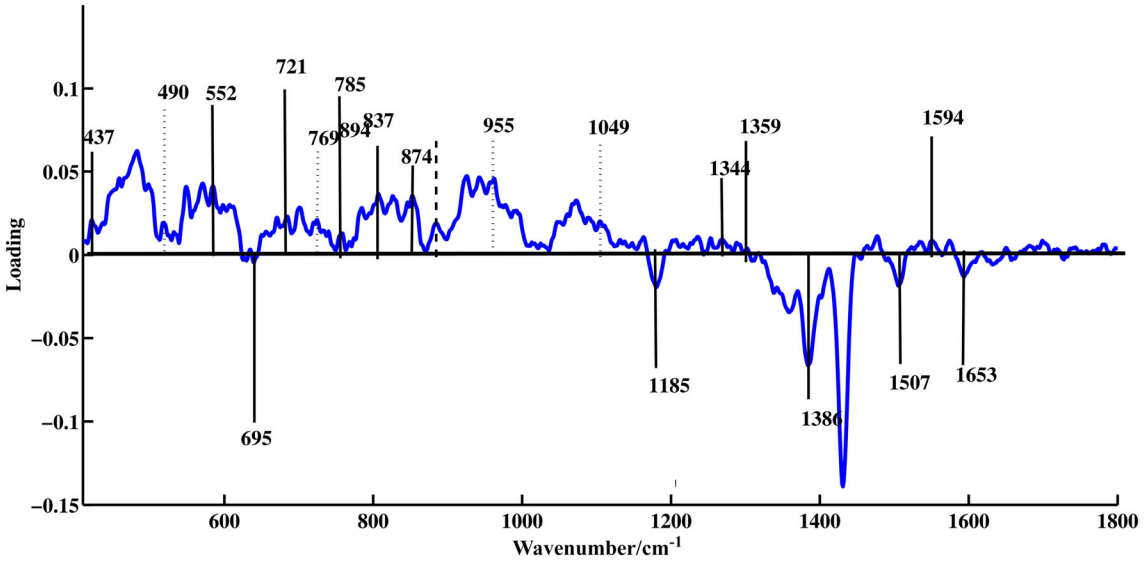

FIGURE 6 Regression coefficients for the partial least squares regression (PLSR) model for the quantification of Raman spectral features of various formulations of Lornoxicam in solid dosage forms [Colour figure can be viewed at wileyonlinelibrary.com]

(stretching of nitrile group [C=N] and stretching of C=C in the quinoid ring), 1359 cm^{-1} (out-of-plane modes of Ph-C-Ph ring), 1344 cm^{-1} (CH_3, CH_2 wagging), 874 cm^{-1} (stretching of alkane bond [C-C]), 837 cm^{-1} (deformative vibrational mode of amine groups -NH_2), 785 cm^{-1} (O-C=O scissoring, β lactam ring deformation), 721 cm^{-1} (CH wagging, scissoring C-N-C [lact + dihyd]), 1653 cm^{-1} (carbonyl stretching of bond [C=O]), 1507 cm^{-1} (stretching vibrational mode of C-C bond [imidazole ring]), 1386 cm^{-1} (bending of CH $group_3$), 1185 cm-1 (N-N stretching vibrations [tetrazole ring] + stretching vibrations of C-C [tetrazole and benzoic ring] + wagging vibration mode of C-H [benzoic ring]), and 695 cm^{-1} (CCC bending and CC scissoring), are associated to the peak intensities of API, as shown in Figure 2. Notably, the intensities of excipients showing Raman features at 490 cm^{-1} (deformation of C-O-C), 769 cm^{-1} (out-of-plane mode of OCCC), and 1049 cm^{-1} (stretching of carbonyl group [C=O] and stretching of C-N bond) indicate that their concentrations in the examined formulations are decreasing. Thus, the Raman spectral intensities assigned in the regression coefficients of

the PLSR model correspond to the Raman spectral features of Lornoxicam found in mean spectra are at 1653, 1594, 1507, 1432, 1386, 1359, 1344, 1185, 1049, 894, 874, 837, 785, 769, 721, 695, 552, 490 and 437 cm^{-1}.

4 | CONCLUSION

Raman spectroscopy can be used for the qualitative and quantitative analyses of solid dosage forms of pharmaceuticals. This research indicates that the proposed technique for quantifying pharmaceutical drugs in different formulations is simple, reliable, and quick. The quantification of Lornoxicam (API) was done in this study by forming drug formulations with different concentrations of excipients that were gradually decreased as the concentration of API increases. The spectral features of the Lornoxicam drug were identified using Raman spectroscopy coupled with a PCA model and PLSR model. The Raman spectral features of API become more prominent when concentrations of API increase and concentrations of excipient decrease. The PLSR model was successfully developed in this regard, with RMSEC and RMSEP having values of 0.76 and 1.34, respectively. This technique can also be used to find the concentration of the unknown samples to monitor the quality and quantity of the pharmaceutical samples.

CONFLICT OF INTEREST

The authors have no competing interests to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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