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ADVANCING PHARMACEUTICAL ANALYSIS THROUGH RAMAN SPECTROSCOPY FOR MONITORING OF LEVETIRACETAM IN COMBINATION THERAPY

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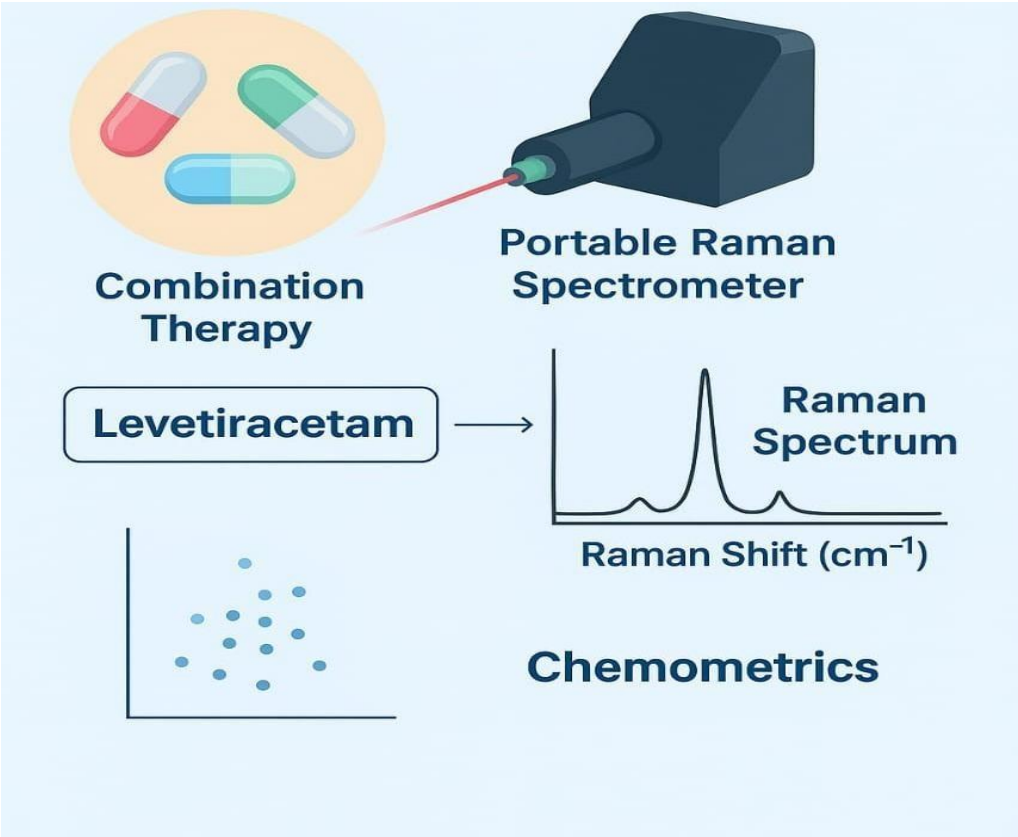
ABSTRACT

Combination therapy is a common approach in pharmaceutical treatments, where multiple active pharmaceutical ingredients (APIs) are combined to enhance efficacy and reduce side effects. However, analyzing these complex formulations can be challenging, especially when dealing with similar molecular structures. Raman spectroscopy offers a rapid and non-invasive solution for identifying and quantifying APIs in combination therapies. This study demonstrates the application of Raman spectroscopy for the analysis of levetiracetam, a widely used anticonvulsant, in combination with other APIs. To measure the Raman spectra of the samples a Peak Seeker Pro-Agiltron Raman spectrometer (USA) was used, equipped with laser light 785nm at 50mW power. The Raman spectral characteristics directly linked to the medicine and excipients vary as API concentrations increase. The spectral response was analyzed qualitatively and quantitatively using principal component analysis (PCA). The maximum variations in the data were explained by the first principle component (PC-1), and the next-highest source of variations is explained by each additional principal component. A total of 15 different formulations containing levetiracetam in combination with other APIs were prepared and analyzed using Raman spectroscopy. The spectra were collected using a portable Raman spectrometer, and the data was processed using principal component analysis (PCA) and partial least squares regression (PLS-R). The results showed excellent

discrimination between the different formulations, with accurate identification and quantification of levetiracetam in the presence of other APIs. The advantages of Raman spectroscopy in this study include rapid analysis time (less than 5 minutes per sample), non-destructive sampling, and minimal sample preparation. The technique also offers high sensitivity and specificity, allowing for the detection of levetiracetam in complex formulations. The results demonstrate the potential of Raman spectroscopy for the analysis of APIs in combination therapies, offering a valuable tool for pharmaceutical development, quality control, and regulatory compliance.

Graphical Abstract:



1. Introduction

The pharmaceutical industry is crucial to the healthcare sector since it develops drugs to fight illness and raise people's profile [1]. The pharmaceutical business implemented various effective manufacturing procedures and quality testing to ensure the superior quality and safety of its goods [2]. Curing illness preventing infection and preserving health are the primary goals of the pharmaceutical sector [3]. To achieve this, novel research methods and various approaches are used in medication development [4]. Among other things, Raman spectroscopy is seen to be a crucial method for revealing structures and has the ability to disclose highly significant structural characteristics [5]. Recent years have demonstrated that Raman spectroscopy is a highly sensitive and specific method for

diagnosing a variety of disorders and identifying biological changes in the body [6]. This innovative approach has several advantages over other traditional approaches, including as resilience, low cost, non-destructive, and label-free analysis [7]. As a result, this method is more potent and suitable for a variety of uses. This approach has been widely employed in surgical guidance , drug-target interaction, characterisation and treatment monitoring over the past 20 years. Raman spectrum analysis requires little to no sample preparation [8], [9].

The promise of Raman spectroscopy as an analytical method for drug evaluation has been shown by its rising popularity in recent years due to advancements in the field [10]. The advancement of Raman spectroscopy has been facilitated by the development of charge coupled device (CCD) detectors in Fourier Transform-Raman spectrometers advances in laser technology, the creation of various optical components of Raman spectrometers, the ease of sample preparation and handling, and the ability to solve sub-sampling issues by utilising different geometric laser irradiance pattern [11], [12]. The proof that a drug may be used safely in clinical settings is the outcome of a multi-step process that produces a pharmaceutical treatment [13]. A pharmaceutical product must show that it satisfies patients' needs for safety and care in order to be approved for distribution [14].

Levetiracetam, also known as Keppra, is an antiepileptic medication (AED) with a broad range of therapeutic efficacy, a distinct profile of activity in seizure models, and a novel mechanism of action [15], [16]. It is a distinct, water-soluble pyrrolidine derivative with the molecular formula $C_8H_{14}N_2O_2$. It is S-enantiomer of a-ethyl-2-oxo-1-pyrrolidine acetamide and a unique structure that differentiates it is from other existing AEDs [17], [18]. Its mode of action involves interaction with the synaptic vesicle protein 2A (SV2A),but it is chemically unrelated to other AEDs [19], [20]. Levetiracetam is commonly used in combination therapy for epilepsy, where it acts additively or synergistically with other antiepileptic medications (AEDs), especially those that affect glutamate neurotransmission [21], [22]. Levetiracetam with phenytoin, lamotrigine, phenobarbital, valproate, topiramate, and oxcarbazepine are common combinations [23]. In addition to having a favourable pharmacokinetic profile and fewer interactions than some other drug combinations, this strategy improves seizure control and has demonstrated promise in cases of general epilepsy and paediatric epilepsy [24]. Combining levetiracetam with other AEDs can greatly enhance their seizure-protective benefits, as it operates through a unique mechanism [25], [26]. Comparing levetiracetam combination therapy to monotherapy, studies reveal that the former results in greater rates of seizure control, including seizure freedom [27], [28]. Levetiracetam has a low risk of drug-drug interactions because it is not heavily metabolised in the liver and is less than 10% protein-bound. It is therefore a good choice for combo therapy [29], [30] .

Various analytical techniques, particularly spectroscopic ones, are employed to ensure this goal by analyzing drug precursors, excipients, and active pharmaceutical ingredients (APIs) [31]. The inelastic scattering causes the resultant light to have a different frequency than the incident light [32]. An analytical method for chemical examination that is non-destructive and can yield extensive information about molecular interactions, phase and polymorphy crystallinity, and chemical structure is Raman spectroscopy [31]. It operates on the basis of lights interaction with a materials chemical bonds [33]. An increasingly used method for analyzing pharmaceutical solid dosage forms is Raman spectroscopy [34]. It is employed in

the current study to guarantee the tablets' identities. This method's two main uses are the detection of counterfeit goods and the release of finished goods for quality control [35], [36].

The Raman technique is used to scatter incident light utilising a molecule and a high intensity laser light source [37]. The bulk of scattered light that has the same wavelength (or colour) as the laser source and provides no useful information is referred to be rayleigh scatter [38]. The phrase "raman scattering" refers to the tiny amount of light that, depending on the analyte's chemical structure, is scattered at different wavelengths (or colours) [39].The several peaks that comprise a Raman spectrum show the intensity and wavelength location of the dispersed Raman light [40], [41].

An Investigation into a material's chemical structure and identification is possible with Raman spectroscopy [42]. When a material or molecule is identified rapidly, or when it needs to be distinguished from other materials, its unique chemical fingerprint can be found in its Raman spectrum [43], [44]. Libraries holding thousands of spectra are quickly searched to locate a match with the spectrum of the analyte [45].

A common application of Raman spectral libraries is the identification of substances using their Raman spectra [46]. The goal of local straight line screening was to determine how well a Raman spectrometer worked for researching antibiotics and anti-diabetic medications [47]. In order to determine whether the drug is genuine or fake, the Raman spectra of pure salt's active pharmaceutical ingredients (APIs) and their various commercially available products were analyzed using a Raman spectrometer, and then local straight-line screening (LSLS) was conducted [48]. The study's results show that four anti diabetic medications and a number of incredibly accurate and responsive excipients are available. This study concludes that the Raman spectrometer when paired with Local Straight line Screening provides an immediate approved nondestructive method for examining counterfeit medications. Scattering processes that can occur when light interacts with a molecule [49], [50].

One of the essential components of a modern, compact Raman spectrometer is the laser, which serves as the excitation source to generate the Raman scattering. Raman spectrometer is properly set up and calibrated. This includes checking the laser wavelength, power, and instrument alignment. Collect Raman spectra from your sample using the spectrometer [11]. This involves exposing the sample to thsssse laser beam and recording the scattered light at various wavelengths.

This may involve comparing the spectra to reference spectra of known compounds or using spectral databases [51], [52]. If quantitative analysis is required, develop calibration curves or multivariate models based on known concentrations of the target compounds. Validate the analytical method by assessing parameters such as sensitivity, specificity, accuracy, and precision. Document findings, including the identified pharmaceutical ingredients, their concentrations and any relevant analytical parameters [53].

Pharmaceutical excipients are substances other than the active pharmaceutical ingredient (API) that are added to a finished pharmaceutical medication formulation. Any pharmaceutical formulation consists of two parts or components [54]. First, the primary ingredient, often known as the active pharmaceutical ingredients, or API [55]. As the inactive ingredient, the second is referred to as an excipient.Excipients used in pharmaceuticals were the first class of chemicals examined. These non-active components serve a variety of purposes in the formulation of

pharmaceutical products, such as coating compounds, absorbents, diluents, lubricants, and emulsifying agents. Magnesium stearate was the most common excipient that was discovered [56], [57].

The main difference in the spectra between these materials and excipients is that the Raman vibrations of the APIs fall between 1550 and 1900 cm^{-1}.Since there are no excipient interferences in this domain, they can be used to test the identity of an API in a pharmaceutical product. The differences in Raman signals between the two sets of compounds are caused by a variety of factors, including structure, functional groups, and types of bond or group vibrations [58], [59].

Spectroscopy includes Raman, mid-infrared, and near-infrared spectroscopy. Solid-state sample analysis currently frequently uses Raman techniques due to continuous instrumentation improvements [60]. Because of this, the pharmaceutical industry has used Raman spectroscopy for many different applications, including as solid-state analysis, polymorphism screening, determining the quantity of active pharmaceutical ingredient (API), and using Raman mapping to distribute API in tablets. Raman spectroscopy can also be used in a lab setting for online analysis, process analytical technology projects, and quality control applications [61], [62].

The sensitivity of Raman spectroscopy is poor; a portable device with a wavelength close to the visible range and a 785 nm excitation laser with spectra that include distinct fluorescence interference is less sensitive than a laboratory spectrometer with a 1064 nm excitation laser [63]. The first principle component (PC-1) explained the greatest degree of variation in the data, and each additional principal component explains the next highest source of variation [64]. Levetiracetam was used in 15 distinct formulations along with additional APIs, which were created and examined using Raman spectroscopy [65]. Using a portable Raman spectrometer, the spectra were obtained, and partial least squares regression (PLS-R) and principal component analysis (PCA) were used to process the data. With precise levetiracetam identification and quantification even in the presence of other APIs, the results demonstrated outstanding discrimination between the various formulations. The benefits of using Raman spectroscopy in this work include minimal sample preparation, non-destructive sampling, and quick analysis times (less than five minutes per sample).

Table 1: Raman Spectroscopy for Analysis of Pharmaceutical Research and Development (R&D).

Main Topics	Essential Points	Reference
Raman Spectroscopy in Pharmaceuticals	• Identifies prescription tablets in <1 minute. • Advantage over NIR: reveals API-specific peaks. • Classifies product family via database spectra. • Can identify formulation automatically (no prior info needed). • Techniques: Dispersive, FT, Resonance, SERS, SERRS, FT-SERS. • Applications: structural analysis, excipient interaction, detection limits, pH effects, adsorption studies. • Benefit: rapid, non-destructive, real-time process monitoring	[66], [67] [68], [69] [70] [71] [72] [73] [74]

Combination / Polytherapy	• Use of multiple drugs/modalities to enhance efficacy, applied in HIV, TB, cancers. • e.g: breast cancer treatments,surgery, chemo, radiation, drug combos. Mechanism: drugs act additively or synergistically for greater effect. • Benefits: reduces resistance, induces apoptosis, inhibits tumor growth, decreases cancer stem cells.	[75] [76] [77] [78]
Pharma R&D	• One of the most research-intensive industries • Shift from empirical ,science-driven discovery. • Strong collaboration: pharma , universities, government labs. • Regulations raise clinical trial costs (safety and efficacy requirements). • Patent protection critical for covering R a& D investment.	[79] [80] [81] [82] [83]
Other Analytical Methods	• Thin Layer Chromatography (TLC): determines chemical composition, tracks reactions. • Chemical libraries: empirical (broad) or targeted (specific molecular targets). • Virtual screening: co-crystallization and high-activity analysis.	[84] [85] [86]

2. Materials and Methods

2.1. Sample Preparation and Formulation

Principal component analysis was utilised to do both qualitative and quantitative analyses of the spectral response (PCA). The first principal component (PC^{-1}) described the greatest amount of variation in the data, and each additional principal component explains the next-highest source of variation. Using levetiracetam in combination with other APIs, 15 distinct formulations were created and examined using Raman spectroscopy. Partial least squares regression (PLS-R) and principal component analysis (PCA) were used to process the data after the spectra were obtained using a portable Raman spectrometer.Principal Componant Analysis (PCA) of raman data was done by using MATLAB R2009a.

The algorithm used for this purpose was developed in the lab.Here is a sample preparation guide for Raman spectroscopy in combination therapy analysis, along with a sample name, ACI-001.Mix 30 ml of API-1 solution, 20 ml of API-2 solution, and 10 ml of API-3 solution in a 100 mL volumetric flask. Make up the volume to 100 mL with methanol. Then Stir the mixture for 10 minutes to ensure homogenization. After that transfer 1 ml of the combination formulation to a raman compatible glass vial. Then ensure the sample is free from contaminants, bubbles, and particulate matter. Handle the sample carefully to prevent contamination, degradation, or exposure to light, heat, or moisture.Store the sample in a cool, dark place (e.g., refrigerator or freezer) to maintain stability and integrity. Verify the authenticity and purity of each API using appropriate methods.After clarify the concentration of each API in the combination formulation using appropriate methods.

2.2 Raman Spectral Data Acquisition

Place the sample holder in the Raman spectrometer, the Acquire Raman spectra in the range of 200-2000 cm-1.Use an appropriate acquisition time (e.g.10-60 seconds) to ensure adequate signal-to-noise ratio.By placing the material on an aluminium slide at room temperature and employing a 785 nm laser as an excitation basis with a laser effect of 40 m W through a 4 objective, all spectra were exhausted using a Raman spectrometer (Peak Seeker Pro-785; Agiltron, USA).This device used an excited coupled detector to capture the signal, which decreased electrical noise.To guarantee the consistency and repeatability of the quantifiable data, three spectral peaks were acquired with a 25-second achievement time for the blind sample, and 15 spectra were collected for samples S1–S8 (eight samples).

Since the majority of the key spectrum features of combination (Levetiracetam samples) are found in the 400–1800 cm spectral range, a total of 120 Raman spectra were obtained for 8 models in this range.After being extracted as binary data, the Raman spectrum data was loaded into Matlab 7.8. The data acquisition parameters are will be used have laser power between 100-500 mW , acquisition time 10-60 seconds, spectral resolution 1-5 cm^{-1},laser wavelength 785 nm or 1064 nm,sample holder glass vial or quartz cuvette is used.So that in the data acquisition protocol acquire a background spectrum without the sample, a spectrum of the combination formulation, spectra of individual API standards and repeat steps 2-3 for multiple acquisitions to ensure reproducibility.

2.3.Vector Normalization

It is frequently utilised to produce the reference spectrum for the qualitative and quantitative characterization and identification of memory in the raw data preprocessing stage. This resolved intensity value is used in the normalisation process and is a crucial component of the Raman spectrum. Normalisation lessens the effect of varying the optical wavelength on the raw data by comparing readings for variations in strength or intensity. After the normalising process is finished, the end spectrum is mentioned normally and maintained at the same time [87],[88].

2.4 Data Analysis

Analyze the Raman spectra using chemometric methods like partial least squares (PLS) and principal component analysis (PCA). Make use of the created models to forecast API concentrations in combination formulations. PCA was used in conjunction with controller or healthy models to obtain a more comprehensive knowledge of the metabolic underpinnings of HCV growth. Principal component analysis (PCA) is a precise method that involves reducing a larger number of possibly connected variables into a smaller number of uncorrelated variables, or PC, in order to reduce the dimensionality of a fact while keeping variability. The primary source of the facts' variability is illustrated by the first principle component, and the primary cause of residual changeability is thereafter illustrated by the principal components that follow. The PC loadings can be viewed as an orthogonal biochemical difference measurement that aids in the separation of different clusters. By scoring every range of spectra along these magnitudes, Raman data spectra are able to display their variability laterally [89].

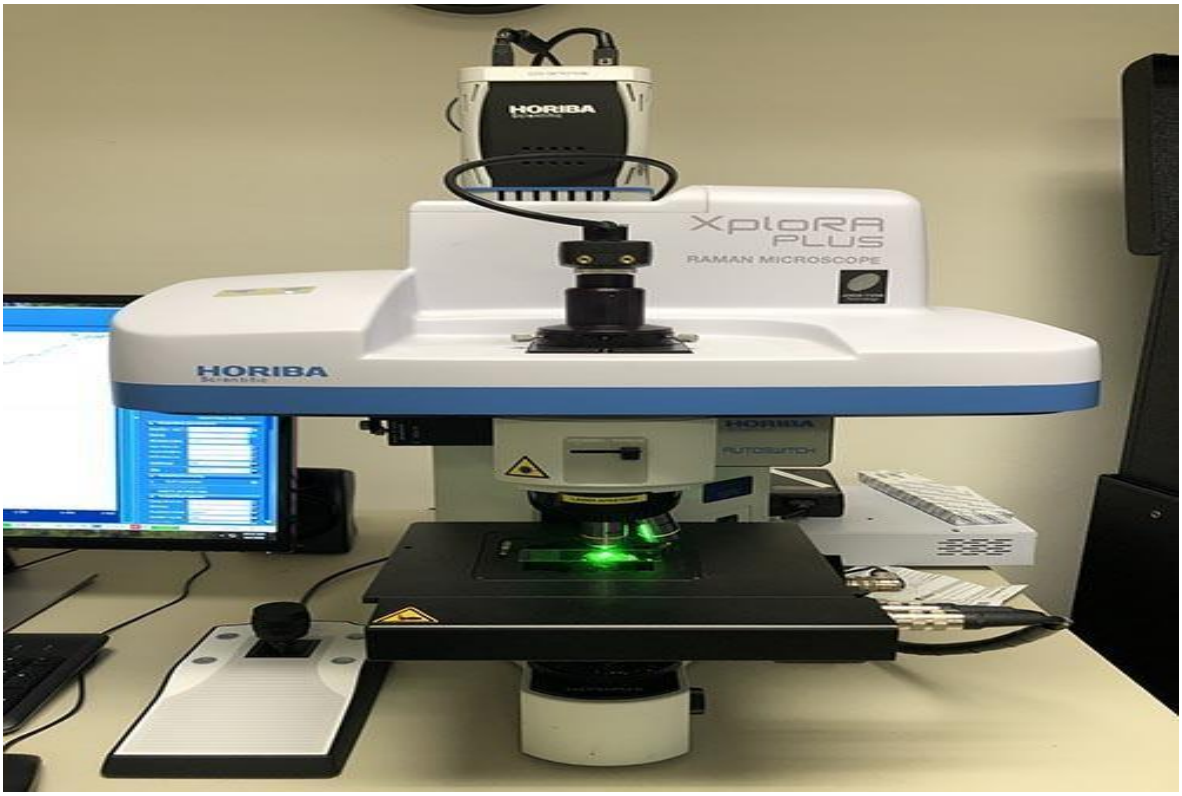


Figure 3.4: Raman Instrument [90].

2.3 Data Pre-processing

Matlab 7.8 (The Math Works Inc., Natwick, MA, USA) and its available methodologies were used for preprocessing all samples. This spectral data preprocessing includes vector normalisation, smoothing, baseline modification, and substrate eradication. In order to attain flattening with the first polynomial order and 13-point window thickness, Savitzky-Golay filtering (S-G) was utilised. Additionally, each range's substrate spectral changes were extracted, and any residual baseline was eliminated using rubber band correction. Initially charge-couple devices received high-energy photons from a light source. These photons produced a lot of electrons, which the devices utilised to interpret the data as a signal form. These look as extremely strong emission lines that affect one pixel at a time due to randomization. All extraneous spectral lines were eliminated and the substrate spectrums spectra were also acquired in order to correct the baseline. MATLAB software was used for vector normalisation, baseline correction substrate elimination smoothing baseline correction and principle component analysis (PCA) among other multivariate analysis computations and chemometric procedures [91].The vector normalisation and raw data material were smoothed using the Savitzky-Golay (13-point window, order 3) normalisation method. Every spectrum was rubber band corrected in order to eliminate the baseline. In preprocessing data analysis, Raman spectrum data enables baseline improvement, smoothing route, normalisation, and substrate consequence elimination. The complete set of protocols was utilised with MATLAB 7.8 (2009a). Raw, unprocessed spectra with the substrate present and the baseline modified, but no further processing applied [92].

2.6. Smoothing Spectrum

The smoothing process was clearly seen in such spectra. In addition, the smooth spectrum features sharp peaks that are clearly recognizable. Smooth data is represented by sharp peaks.

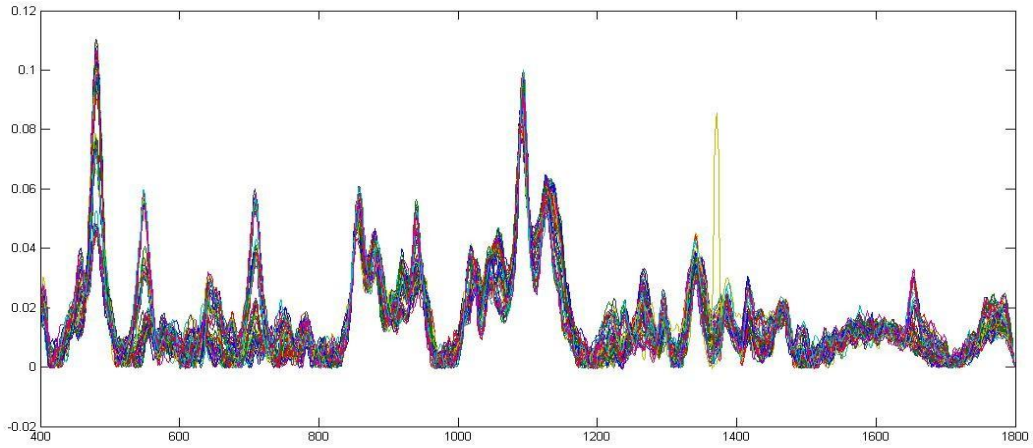


Figure 3.6: Smoothing Process.

2.7 MATLAB Working

By using the MATLAB expertise line, entering commands and languages, and viewing the results displayed in the command box thus far, Matlab can be used as a statistical tool.This section describes how to change the appearance of the command window. If your approach allows you to select the typeface or lettering, such as carrier or fixedly, to guarantee sufficient graphics. The format command sets the numerical arrangement of the numbers that MATLAB displays. The command just modifies how numbers are shown; it has no impact on how MATLAB computes or stores numbers. These are the several forms and the resultant output that a route x with different-sized mechanisms can produce [93].

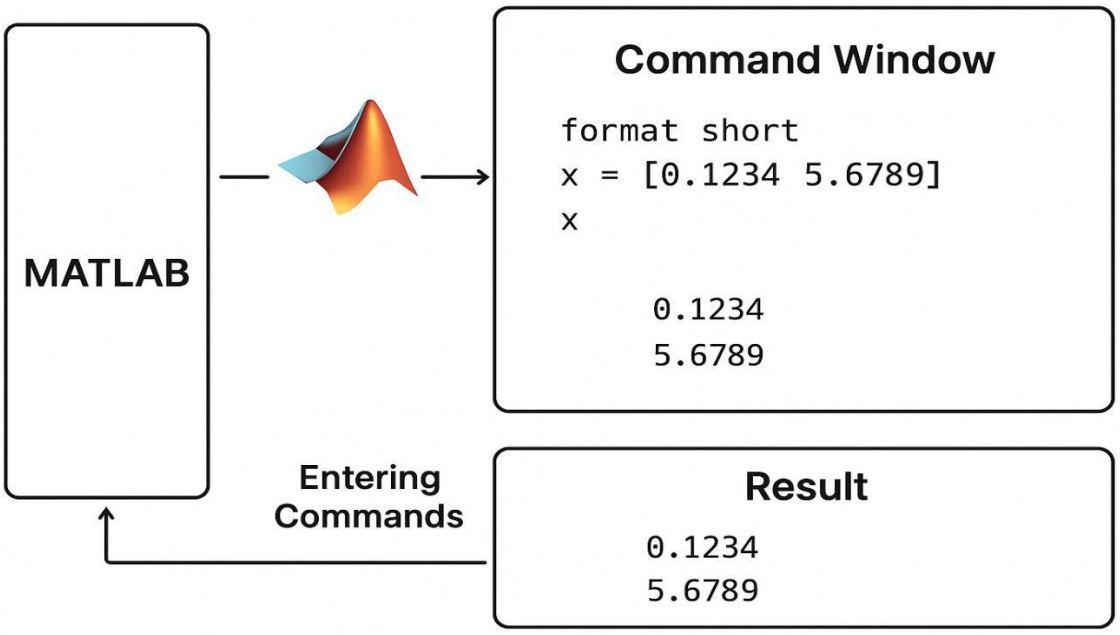


Figure 2.7: MATLAB Process.

Various figures are assigned to the charting work based on the opinions received. Plot (y) produces a piecewise linear visual that plots the origin of y against the index of its features if y is a vector.

Plot (x, y): When two vectors are specified as factors, it produces a graph of y versus x. Mesh and waves are two tasks that display three-dimensional shells in MATLAB.It constructs a surface by using the z-coordinates of facts atop a network in the x-y plane to connect neighbouring points with straight lines. In Mesh's Wireframe surfaces, just the edges connecting the defining facts are coloured. The surface's planes and connecting lines are both now coloured. Make X and Y matrices with the matching rows and columns over the desired region to represent the two-variable function z= f (x, y). With just one excitation wavelength, Raman spectroscopy is a molecular imaging technique that boasts exceptional sensitivity and the rare ability to multiplex readouts from many molecular targets. Surface enhanced Raman scattering (SERS) nanoparticles improve Raman spectroscopy. This approach has exciting promise for a range of medical applications, such as intraoperative image guidance of surgical resection and the detection and characterisation of cancer during endoscopy. Long acquisition periods,subpar spatial resolution, a short field of view and the difficulties of handling animals with current Raman spectroscopy equipment are now impeding the development of Raman molecular imaging with SERS nanoparticles [94].

2.8.Baseline Correction.

Fluorescence has a number of drawbacks when it comes to Raman spectroscopy of organic molecules.Fluorescence may make it more difficult to distinguish and interpret Raman spectra because it increases the amplitude of the Raman response in certain instances.

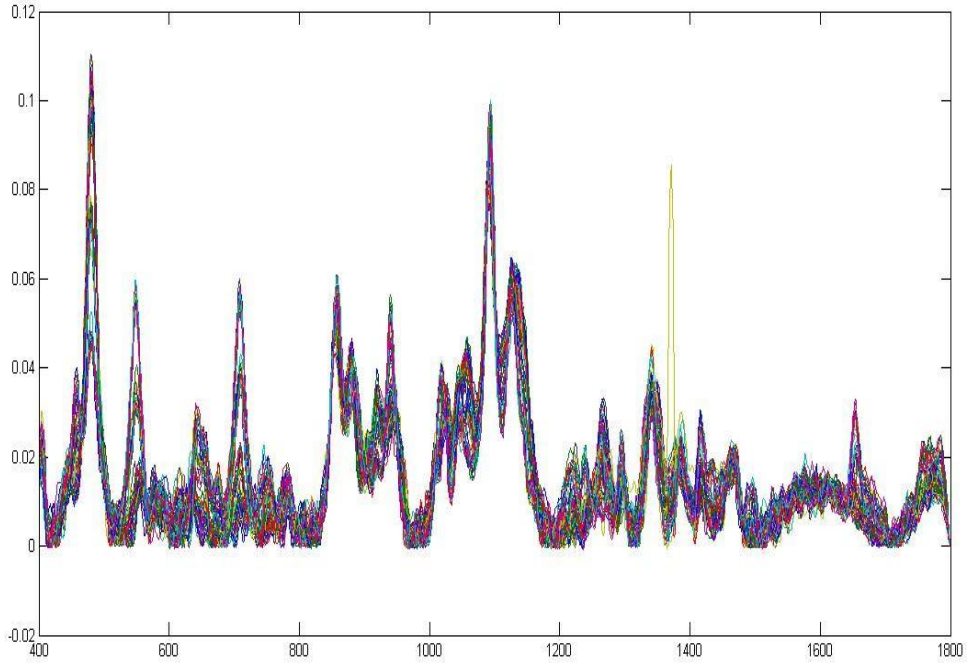


Figure 3.7: Baseline Correction During Spectra Processing

This problem was handled by keeping the laser at 750 nm and reducing the fluorescence. However, in the 750 nm laser range, several organic substances also show

fluorescence.By eliminating the background slides from the raw data, it is possible to acquire considerably clearer and finer peaks in Raman signals. Over the last several years, there has been a decrease in variability of baseline. Normalization is not ideal for vast data thus a human or automated baseline correction is required even when there is relatively small sample size.

3.9. Principal Componant Aanalysis

Principal component analysis and discriminant analysis were two multivariate figures that were used to analyse and classify the spectral data. When employing these multivariate statistical methodologies, it is imperative to have same-size information sets inside the classes since discriminant study suffers as well as PCA, which is sensitive to unfair information sets [95].

As a result,so that even non-chemo metrics can use to making this method available to everyone. But if there isn't a clear recommendation on how many machines to keep, there can be a problem. The number of PCs carrying chemically appropriate data differs from the known number of chemicals, as demonstrated by our understanding of Raman mapping ranges.This is most likelybecause of the nature of measurement errors, as Raman sound is known not to be constant (Mille, 2012).As a result, several of the basic assumptions of PCA may not be completely related to Raman data collection [96].

3. Results and Discussion

3.1. Mean Raman Spectra of Levetiracetam Samples

Figure 3.1 shows the Mean Raman spectra of Levetiracetam, containing pure (API) active pharmaceutical ingredient , pure excipient and ten other different concentrations of API combined with excipients (S1…S11), moreover these concentrations having total net weight of 100 mg. Levetiracetam's primary variations in its Raman spectral characteristics are indicated by vertical lines.The Raman spectral features attributed with the changes in the different samples of Levetiracetam. By increasing the concentration of API and decreasing the concentration of excipients. These Raman spectral features have been appeared more prominently in fig 3.1 due to changes in the peaks intensity of pure excipient (S1) to pure API (S11). The mean Raman spectra of API has shown strong Raman high intensity peaks at 457,481, 547, 640, 709, 748, 660, 885, 940, 1017, 1045, 1095, 1124, 1238, 1292, 1341, 1387, 1417, 1494 and 1654 cm^{-1} which are gradually increased in other mean spectra of Levetiracetam samples by increasing the concentration of API [97].

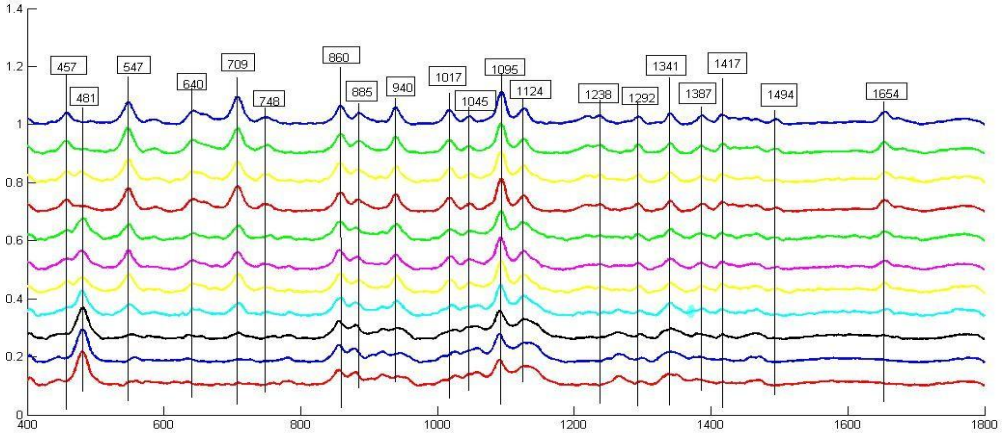


Figure 3.1: Mean Raman Spectra of Levetiracetam Containing API , Excipients and Different Concentration of API with Excipients.

Interestingly, research has shown that the strong Raman peak of Levetiracetam API at 1095 cm^{-1} represents the inplane ring deforming vibrations of aromatic carbon rings 2 and 3, the medium to weak intensity peak at 547 cm^{-1} shows the ring 1 out of plane deformation, the 709 cm^{-1} shows the ring 2 out of plane deformation, and the C-C, C-H, and O-H of COOH stretching and deformations have been assigned to 860 cm^{-1}, 1045 cm-1, 1124 cm^{-1}, and 1292 cm^{-1}.The major strong peak at 1095 cm^{-1} (C-N-C deformation) represents the spectral feature of pure binder Levetiracetam [98], has strong Raman peaks at 547cm^{-1}, 1238cm^{-1} and 1654cm^{-1} associated with the symmetric stretching vibrations of C-C bonds. The peak intensity of feature at 940cm^{-1} in Levetiracetam associated with the C-O-C asymmetric vibrations that appears more prominently [99]. Another strong Raman peak at 1045 cm^{-1} indicates the breathing of aromatic benzene ring and weak intensity peaks at 1124 cm^{-1} and 1417 cm^{-1} represents the stretching vibrations of C-H bond and asymmetric deformation of CH_3 respectively. The literature survey examined that the C-N stretching vibrations of alip1387 cm^{-1} and 1495 cm^{-1} corresponds to the stretching vibrations of C=O and C=C respectively. And it is clearly indicated in the raw data for the following samples given below that the intensity of the excipient's bands is decreasing gradually as compared to the intensity of the active pharmaceutical ingredient which are present in each sample, proving the reliability of the Raman spectroscopy.

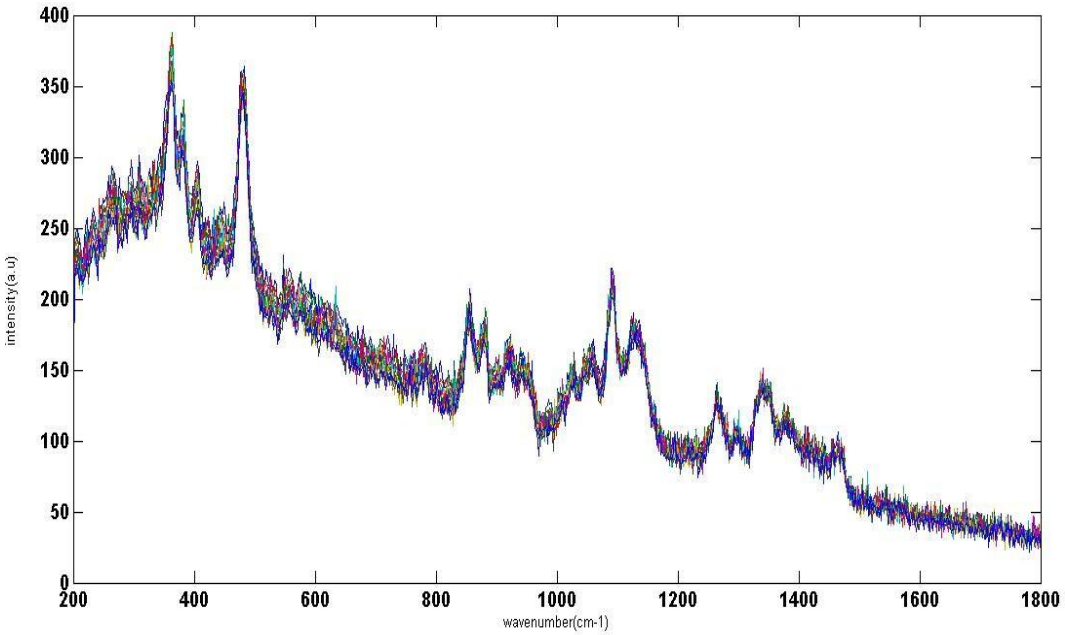


Figure 3.2: Raw Spectra of Pure excipients.

Figure 3.2 exhibits the set of 15 spectra collected from the different positions from the powdered sample of pure excipients that includes (lactulose, starch, magnesium sugar,

powder, titanium powder and talcum powder). Spectra were collected from the different positions of a single sample as to overcome the problem of heterogeneity.As from the obtained spectral image we can clearly see that the excipients are mostly inorganic in nature and are almost Raman inactive and shows a little contribution in Raman spectral acquisition.Two strong bands between 400 cm^{-1} to 600 cm^{-1} clearly indicate the presence of excipients and are characteristic features of the inorganic salts in Raman spectroscopy.

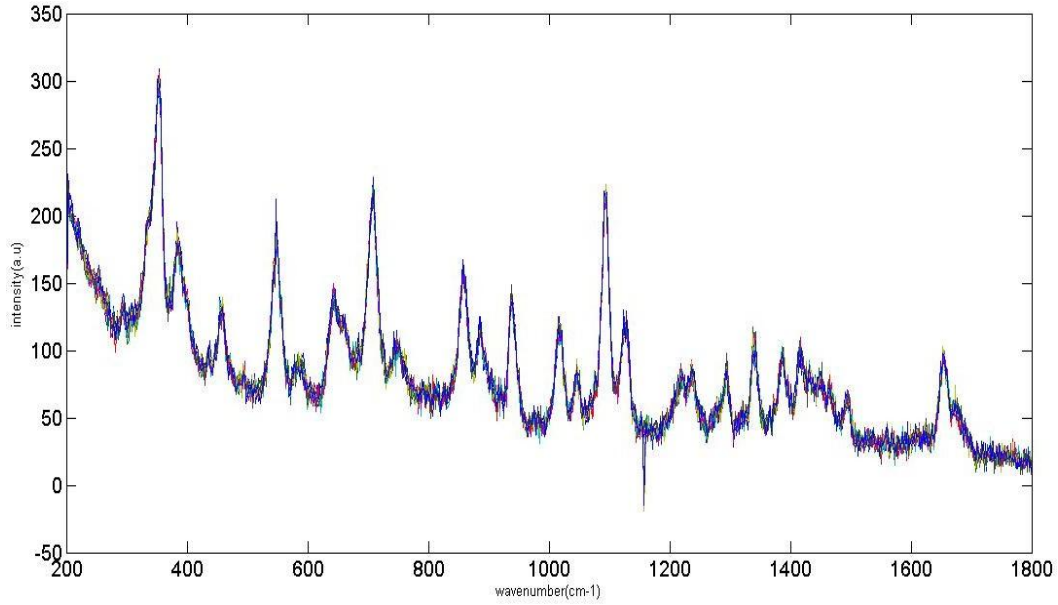


Figure 3.3: Raw Spectra of Pure API.

Figure 3.3 exhibits the set of spectra collected from the different positions from the powdered sample of pure API.Spectra were collected from the different positions of a single sample as to overcome the problem of heterogeneity.As from the obtained spectral image we can clearly see that the excipients are mostly inorganic in nature and are almost Raman inactive and shows a little contribution in Raman spectral acquisition.And it is clearly indicated in the raw data for the following samples given below that the intensity of the excipient's bands is decreasing gradually as compared to the intensity of "active pharmaceutical ingredient" present in each sample, proving the reliability of the Raman spectroscopy.

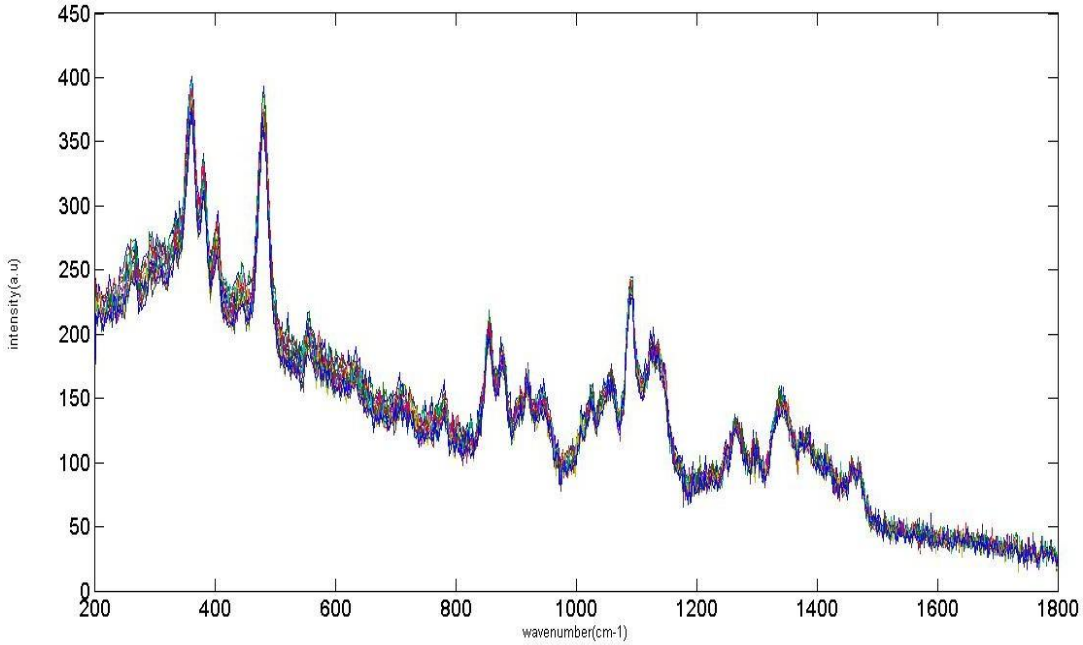


Figure 4.4: Raw Spectra of with different concentration of API and excipients.

3.2. Principal Component Analysis

The Fig.3.2 depicts the PCA scatter plot of Raman spectral data of Levetiracetam API with different concentrations in which each cluster of Levetiracetam samples show clear differentiation. In order to identify spectral features,PCA is performed by using all spectral data that could differentiate the each sample of Levetiracetam data [100]. There is a reasonably good separation of five different concentrations of Levetiracetam data is indicated according to the 1st principal component (PC^{-1} 85.74 %). The PCA loading attributed with the PC^{-1} is shown in fig.3.2 in which the loading of different concentrations associated with the Raman spectral data that is clustered in both positive axis and negative axis of PC^{-1}[101].

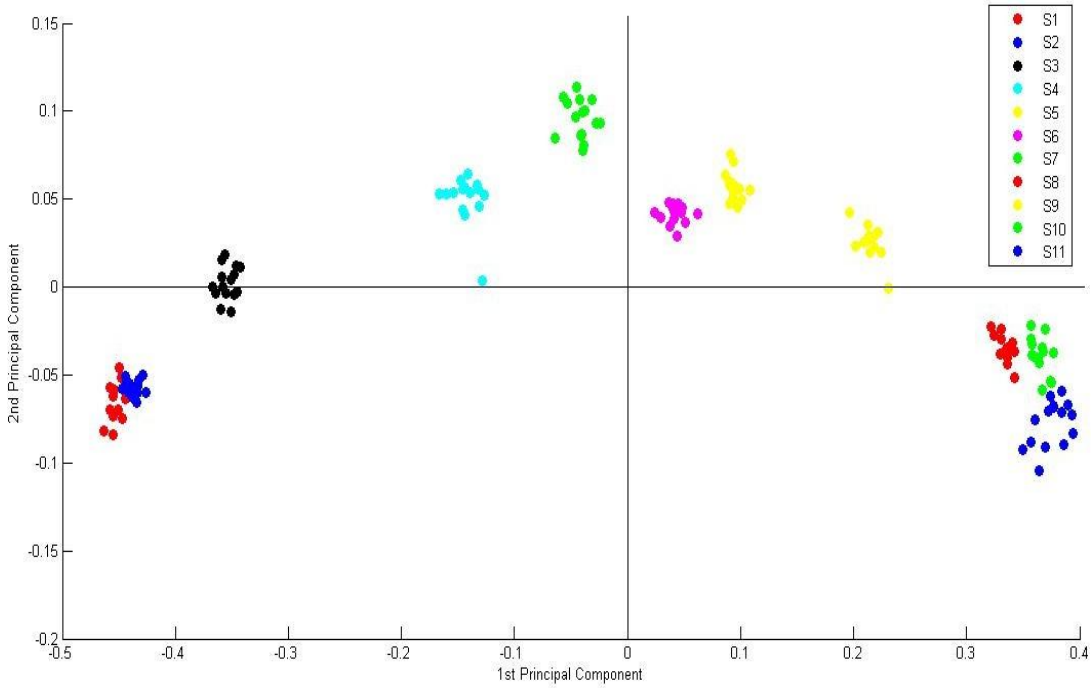


Figure 3.5: PCA Scatter Plot of Levetiracetam.

In the spectral features of mean Raman spectra are same as differences observed in the PCA loading plot of Levetiracetam data.Those spectral features have been identified successfully in Fig.3.5 and are labeled with solid lines in PCA loading as well as in mean Raman spectra of Levetiracetam. PCA proves the significance of the spectral features observed as positive loading at 457, 457, 547, 644, 709, 749, 890, 940, 1018, 1097, 1238, 1293, 1417, 1495 and 1655cm^{-1} which are associated with the C-C bending vibration of aliphatic chain, C-N-C deformation, stretching vibrations of C-F bonds, aromatic benzene ring breathing, C=C stretching vibrations of aromatic ring, stretching vibrations of C=O and C=C respectively. Whereas, some spectral features observed in PC^{-1} as negative loading at 481 cm^{-1}(C-N-C deformation) which represent as strong peak of binder and 1059 cm^{-1} assigned as C-N stretching.

3.3. PCA Loadings

The spectral features of mean Raman spectra are same as differences observed in the PCA loading plot of Levetiracetam data.Those spectral features have been identified successfully and are labeled with solid lines in PCA loading as well as in mean Raman spectra of Levetiracetam. PCA proves the significance of the spectral features observed as positive loading at 457, 547, 644, 709, 749, 890, 940, 1018, 1097, 1238, 1293,1417, 1495 and 1655cm^{-1} which are associated with the C-C bending vibration of aliphatic chain, C-N-C deformation, stretching vibrations of C-F bonds, aromatic benzene ring breathing, C=C stretching vibrations of aromatic ring, stretching vibrations of C=O and C=C respectively. Whereas, some spectral features observed in PC^{-1} as negative loading at 457 cm^{-1}(C-N-C deformation) which represent as strong peak of binder and 1097Cm^{-1} assigned as C-N stretching.

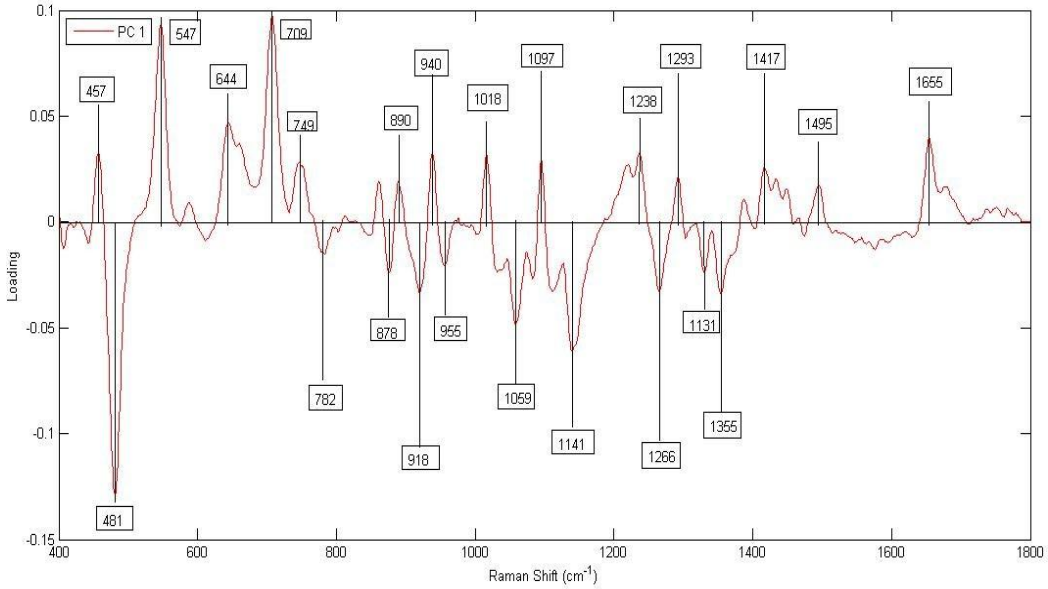


Figure 3.6:PCA Loadings of Raman Spectral Data of API vs Excipients Correlated with First Principal Component (PC^{-1}).

3.4. Partial Least Square Regression Analysis (PLSR)

Figure 3.4 represents the PLSR model of Levetiracetam data along with different concentrations. PCA has been used to detect the specific spectral features which are correlated with the different concentrations of Levetiracetam. Here, the PLSR model can be employed for the prediction of concentrations of drug based on response of Raman spectroscopy. Once predictive model established, such model could be helpful to evaluate and monitor the different unknown concentrations of Levetiracetam drug [102].

PLSR model of Raman spectral data acquired from the different concentrations of Levetiracetam were constructed to identify the ability of Raman spectral data to predict the level of concerations. Raman spectral data of nine different concentrations of Levetiracetam drug were assembled in matrix and different unknown concentrations randomly selected for modeling [103].

To determine the complexity of optimal model for testing, cross validation with the calibration was used. The randomization of data matrix was used for this process and splitting data to prevent the data bias. This process involves data set of cross validation with the calibration for the selection of optimal number of LV to maintain within the model of PLSR. On the basis of number, the optimal number were selected which provides the lowest root mean squared error.

Figure 3.7 represents the development of PLSR model and optimal numbers of LV (20) which shows the clear prediction of different concentrations of drug with an accuracy of 99.7% and root mean square error of prediction is 0.991% as represented in fig.3.7. Figure 4.17 and 3.8 shows the 20 LV that were used to build up the model, indicates minimum prediction error. Every circle in Fig. 3.7 represents the predictive response verses fit response of training spectrum. Moreover, the concentration of a sample can be predicted sufficiently with an accuracy of 99.7%, according to spectral response. For PLSR model, the values of reliability (R2) and predictivity (Q2) were found to be 0.99 and 0.99 respectively [104].

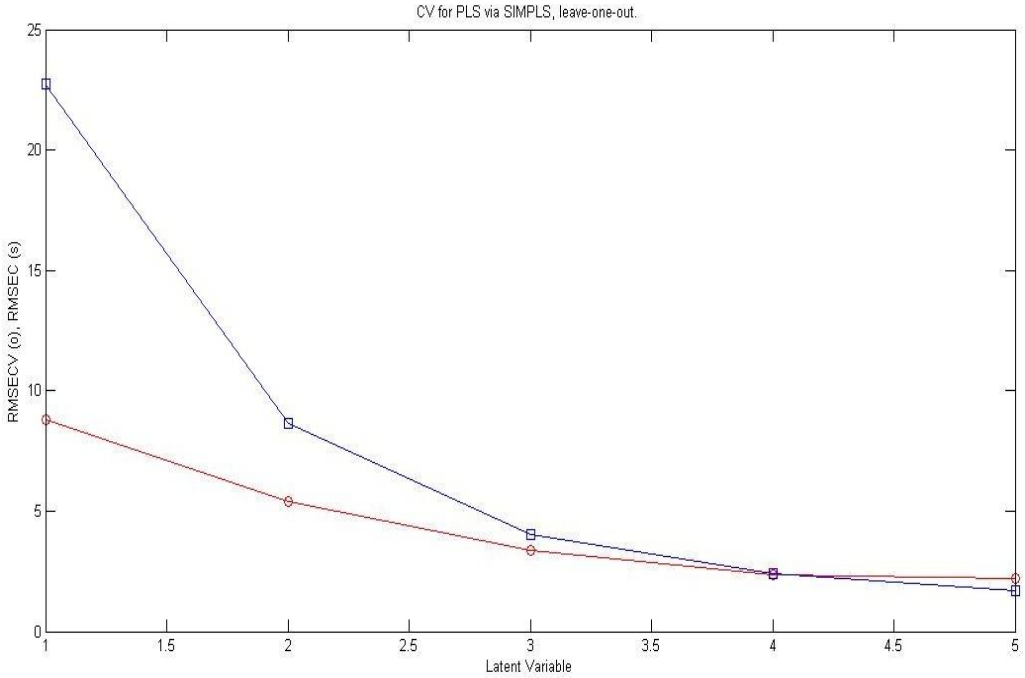


Figure 3.7: The Raman spectral figure of different formulations of Levetiracetam for optimum latent variable latent prediction (PLSR Model).

The model has been conditioned on the calibration framework and used an experimental validation dataset to evaluate the efficiency of the developed model for prediction of unknown samples. The PLSR model with 5 optimal LVs was seen in Fig. 3.7 and it has been found beneficial in predicting levels of varying amounts of Levetiracetam in solid dosing types. Calibration coefficient (R_2 cal) and projection coefficient (R_2 val) were found to be 0.99 and 0.99, respectively, indicating the reasonable and excellent efficiency of the regression model. The low standard errors for the calibration data set (RMSEC=0.2) and the evaluation data set (RMSEP=3.62) have demonstrated the outstanding efficiency and robustness of the model.

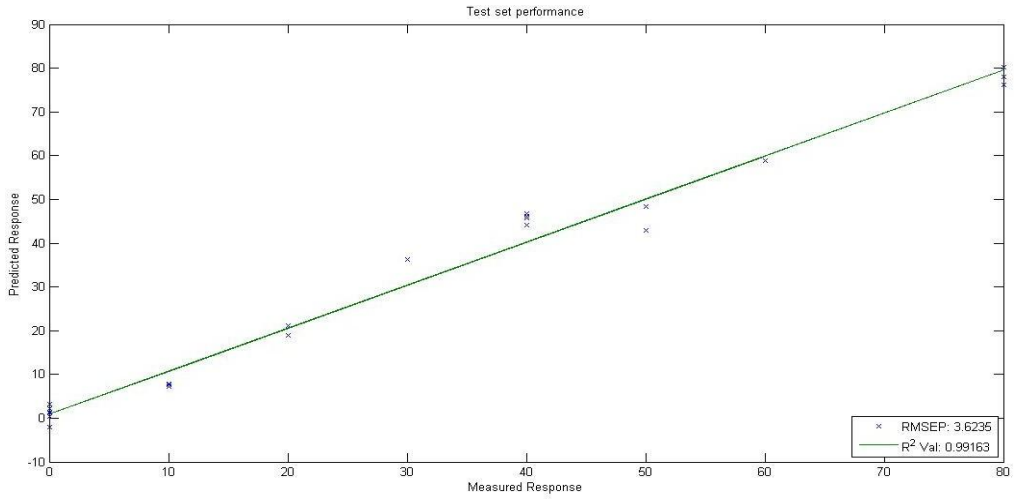


Figure 3.8: Performance prediction of PLSR model for Raman spectral data for the direct quantification of solid dosage forms of Levetiracetam.

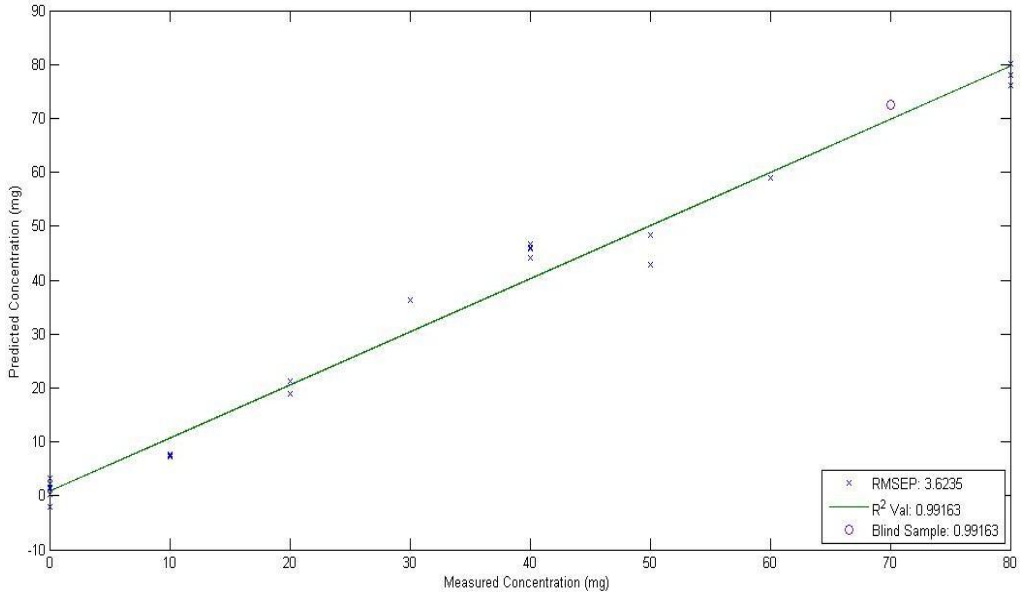


Figure 3.9: Unknown sample prediction of PLSR model for Raman spectral data for the direct quantification of solid dosage forms of Levetiracetam.

In Figure 3.9 One sample was held blind to test the efficiency of our developed model. PLSR was conducted on the unknown sample and was estimated to be approximately 70 mg as shown in Fig. 3.7(b), which is the API concentration in it, while 72 mg was its predicted value. This demonstrates the strong ability of the model developed to predict the unknown sample.

3.5. Root Mean Square Error of Cross Validation.

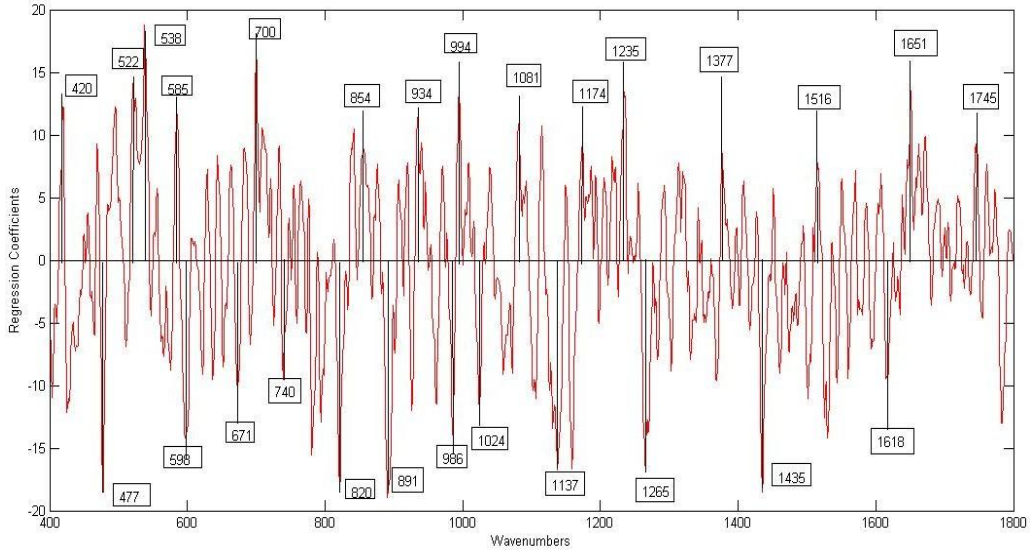


Figure 3.10: Regression co-efficient for spectral data of solid dosage forms of different concentrations of Levetiracetam.

Figure 3.10 displays the regression coefficients obtained from the PLSR analysis. The good traits that have been thoroughly examined previously 420, 522, 538, 585, 700, 854, 934, 994, 1081, 1174, 1235, 1377, 1516, 1651, and 1745 have a correlation with the API. The negative features observed here can be demonstrated to be related to excipients. Specifically, it is noticed that the intensities of certain negative characteristics such as 477, 598, 671, 740, 820, 891, 986, 1024, 1137, 1265, 1435, and 1618 cm^{-1} enlarge on the negative side, indicating a decrease in their concentrations in the examined samples and formulations.It is demonstrated that there is a correlation between excipients and the unfavourable traits observed here. It is seen that the intensities of certain negative characteristics, such as 477, 598, 671, 740, 820, 891, 986, 1024, 1137, 1265, 1435, and 1618 $cm^{-1,}$ are increasing on the negative side. This suggests that the concentrations of these characteristics are declining in the examined samples or formulations. These good attributes 420, 522, 538, 585, 700, 854, 934, 994, 1081, 1174, 1235, 1377, 1516, 1651, and 1745 cm^{-1} have been thoroughly examined before and are connected with the API.

4. Conculsion.

A method for observing a systems low frequency, rotational, and vibrational modes is called Raman spectroscopy. Vibrational spectroscopy, an effective technique that provides vibrational spectrum, physical, and chemical information of any medium in one state of matter, is used to detect compounds by providing a single fingerprint spectra.Excipients and API are the two primary parts of a medication. Pharmacological medications are prepared using API, which are derived from natural sources. While excipients are the last portion of a pharmacological product and are chemically inert or inactive components, APIs are chemically active substances that are used to produce the desired effect in the human body.They are used for the formulation of specific purpose like appearance, function, improvement of ethics or preservation of quality of drug. Raman spectroscopy has low sensitivity, a portable instrument has wavelength near to visible range with 785 nm laser for excitation and spectra tempt different fluorescence interference as compared to laboratory spectrometer with an excitation laser of 1064 nm. However, AS prevents greater fluorescence or make it challenging to get definite Raman spectra of AS sample by using portable Truscan unit. In any case, for unapproved samples with insignificant fluorescence that contained different wrong ingredients active (Levetiracetam) or no dynamic fixing, the outcomes got by the portable and research center laboratory spectrometers with consistent.

Highlights

Demonstrated the use of Raman spectroscopy for rapid, non-invasive monitoring of levetiracetam in complex drug formulations.

Fifteen different levetiracetam-based combination therapies were analyzed using portable Raman instrumentation.

Principal component analysis (PCA) and partial least squares regression (PLS-R) enabled accurate discrimination and quantification.

Achieved high sensitivity and specificity with analysis time under with results in under five minutes per sample.

Offers a robust tool for pharmaceutical development, quality control, and regulatory compliance.

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