Chemometric Evaluation of Ampicillin Stability using FTIR Spectroscopy and Multivariate Techniques

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2025 SBFoton International Optics and Photonics Conference (SBFoton IOPC) | 979-8-3315-9497-8/25/$31.00 ©2025 IEEE | DOI: 10.1109/SBFOTONIOPC66433.2025.11218464

***Abstract*— Antibiotic quality has always been a concern in the pharmaceutical industry and the World Health Organization (WHO), especially in the growing and emerging antimicrobial resistance crisis. FTIR spectroscopy can contribute to the monitoring of antimicrobials, especially when coupled with chemometric techniques. The present work aims to investigate spectral differences of Ampicillin samples through FTIR qualitatively and quantitatively, highlighting the spectral differences associated with different degradation conditions (acidic and thermal).**

Keywords—ATR-FTIR, antibiotic, beta-lactam, drug monitoring.

I. Introduction

In the pharmaceutical industry, the quality management of medicines is fundamental, and these standard measures must cover all aspects that ensure the efficacy and safety of medicines. The development of antimicrobial stewardship programs, which support the development of registries that track antibiotic quality and usage patterns, is crucial to combating the growing threat of antimicrobial resistance (AMR) [1], [2].

Chemometric analyses have become essential in antibiotic monitoring, allowing an exploratory analysis of complex and high-dimensional data. When associated with multivariate methods, they become robust tools for calibration and classification, essential for predicting quantitative and qualitative properties of antibiotic samples [3]. When combined with spectroscopic techniques such as Fourier Transform Infrared Spectroscopy (FTIR), chemometrics enables not only the identification but also the assurance of antibiotic quality This enhances the reliability and reproducibility of quality assessments, ultimately contributing to the efficacy and safety of antibiotic therapies [4].

The absorption of infrared light provides detailed information about the molecular structure of antibiotics since different functional groups present in these molecules vibrate at specific frequencies when excited by this radiation, making FTIR spectroscopy a powerful tool for antibiotic monitoring, effectively identifying antibiotics in clinical samples. Analyzing the obtained spectra makes it possible to identify unique absorption patterns that serve as molecular "fingerprints", enabling rapid and accurate identification [5], [6], [7].

This research explores the use of FTIR spectroscopy as a tool for drug monitoring coupled with chemometric methods, aiming to advance a more efficient methodology for assessing antibiotic quality control.

II. Methodology

A. Sample Preparation

Powder samples of Ampicillin (AMP) (Fig. 1) in its pharmaceutical ingredient (API) form (349.41 g/mol; Sigma-Aldrich, USA) were subjected to different degradation processes and divided into three groups of 50 samples each — no degradation control group, thermal and acidic.

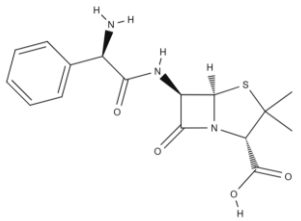

Fig. 1. Ampicillin molecule

- Acidic degradation group: 1 mL of AMP solution (286.97 mg/mL in DMSO) was mixed with 1 mL of 0.1 M HCl. The sample was stirred for 1 hour at room temperature (25 °C), achieving a concentration of 191.31 mg/mL [8].
- Thermal degradation group: 20 mg of AMP was placed in a controlled-temperature oven and heated at 150 °C for 2 hours.

B. ATR-FTIR Data Collection

All data was collected using a Thermo Scientific's Nicolet FTIR Spectrometer (model 6700, USA) equipped with an ATR diamond crystal, a Globar source, a KBr beam splitter, and MCT detector. Spectra were collected in the spectral range of 4000-400 cm^{-1}, with a spectral resolution of 4 cm^{-1}, by averaging 64 scans for each sample measurement and 128 scans for background. For each sample, powder form of AMP

was placed directly on the ATR crystal. As powder samples tend to exhibit more scatter than tablets, a swivel pressure tower was used to achieve a better signal-to-noise ratio. A FTIR purge system (Parker Hannifin, USA) monitors the humidity environment. It is equipped with four high-efficiency coalescing filters for oil and water removal positioned upstream of the PNEUDRI MiDAS adsorption dryer. Following the drying stage, the air stream passes through three additional coalescing filters before entering the spectrometer, ensuring optimal air purity. Additionally, three continuously operating ambient dehumidifiers assist in reducing residual water vapor and CO_2 levels within the laboratory environment, minimizing spectral interferences and enhancing measurement accuracy.

C. Chemometric Data Analysis

Data processing and analysis were performed using Python (v. 3.11.1) in the Google Colab and Orange Data Mining (v. 3.38.1) environment, OriginLab (v. 2024b), and Jamovi (v. 2.3.28). For pre-processing signal, the spectra were cut to 2000-400 cm^{-1} (fingerprint region). The spectra's noise removal was performed by Savitzky-Golay algorithm with 11-point window, 2nd-order polynomial, and processed to second derivative. Subsequently, normalized using Standard Normal Variate (SNV) algorithm.

For exploratory data analysis, the AMP samples from each group were first analyzed using the area under the curve of the non-derivative fingerprint, and for this step, the spectra baseline was linearly corrected. Normality was assessed using the Shapiro-Wilk test, followed by an ANOVA to determine statistical significance ($\alpha = 5\%$). Afterward, second derivative data were also analyzed using Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) to evaluate group separation and identify discriminative features.

III. Results and Discussion

Fig. 2 shows the spectra of the second derivative of the signals obtained from the AMP. The application of the second derivative improves the spectral resolution, reducing the width of the bands and separating the nearby peaks, thus enabling improved distinction and qualitative analysis of the structural changes in the AMP resulting from the degradation processes.

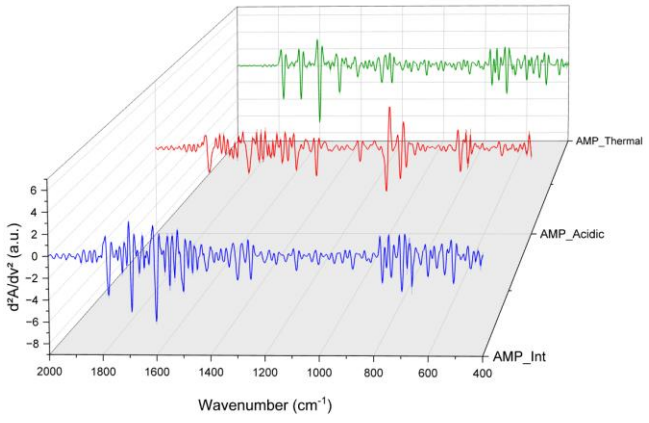

Fig. 2. ATR-FTIR Spectra of Ampicillin Samples Under Control (Int.), Acidic, and Thermal Degradation Conditions

The spectra showed notable changes in the fingerprint region, in particular, the band around 1764 cm^{-1}, attributed to the stretching of the carbonyl group (C=O) of the β-lactam ring [9], was sensitive to degradation, showing reduced intensity and frequency shifts in the degraded samples. This behavior points to bands between 1740–1770 cm^{-1} as indicative of β-lactam ring integrity in antibiotics of this group. Other relevant bands include the region of 1650 cm^{-1}, and 870–758 cm^{-1}, related to the phenyl ring out-of-plane deformations [10]. There are also relevant peaks changes in the region at 1691 cm^{-1} representing COOH, 1600 cm^{-1} the primary amine, 1498 cm^{-1} the C=C ring stretch, and C–N stretch 1413 cm^{-1} [9]. Notably, the acidic degradation group shows a pronounced change around 1002 cm^{-1}, a band related to the benzene ring (CC) [11].

A. Discrimination Based on Fingerprint's Integral

The quantitative analysis of the integral of the fingerprint region (Fig. 3) revealed a significant decrease in the mean values in the degraded samples, both acidic and thermally, compared to the control.

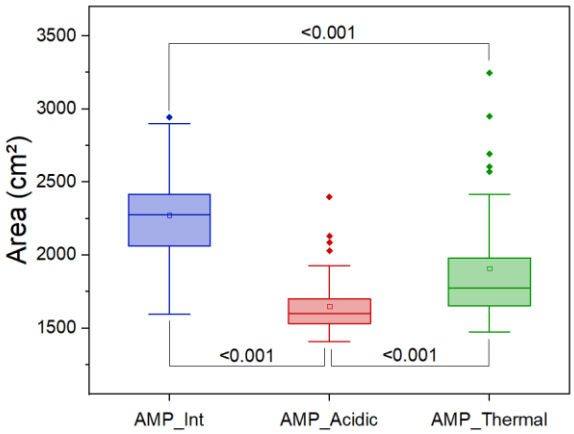

Fig. 3. Quantitative Comparison of Fingerprint Region Integrals

The β-lactam ring of ampicillin is particularly susceptible to hydrolysis, leading to ring opening and formation of degradation products such as penicilloic acid. Heating can induce decarboxylation, dehydration, or further breakdown of the ampicillin molecule, altering the functional groups.

B. Discrimination Based on PCA and LDA

The PCA analysis of the second derivative spectra (Fig. 4) showed a clear separation between the experimental groups.

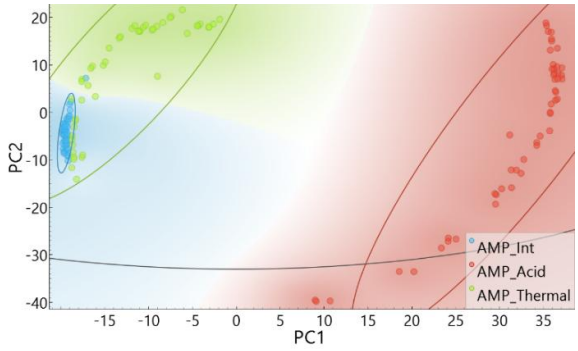

Fig. 4. PCA Score Plot of Second-Derivative ATR-FTIR Spectra

The first two principal components explained most of the total variance of the data (PC1: 69,3% and PC2: 20,3%), excelling in discriminating degraded samples relative to control. However, under the thermal conditions applied in this study, the thermal degradation group shows partial overlap with the control group.

The projection of the LDA results (Fig.5) demonstrate a clear and distinct separation between all sample groups, it was possible after a dimensionality reduction.

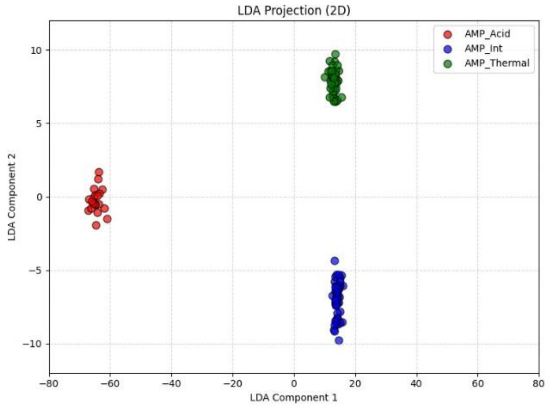

Fig. 5. LDA Score Plot of Second-Derivative ATR-FTIR Spectra

Notably, LDA component 1 accounts for the primary separation between acidic degradation and the other groups, while component 2 contributes to the finer distinction between degradation types. The corresponding loading plots (Fig. 6) reveal that the most relevant wavenumbers for discrimination are located within the fingerprint region, particularly around ~660, ~750, ~1000, ~1300, ~1550, and ~1830 cm^{-1}. These spectral bands likely reflect structural alterations induced by thermal and acidic degradation.

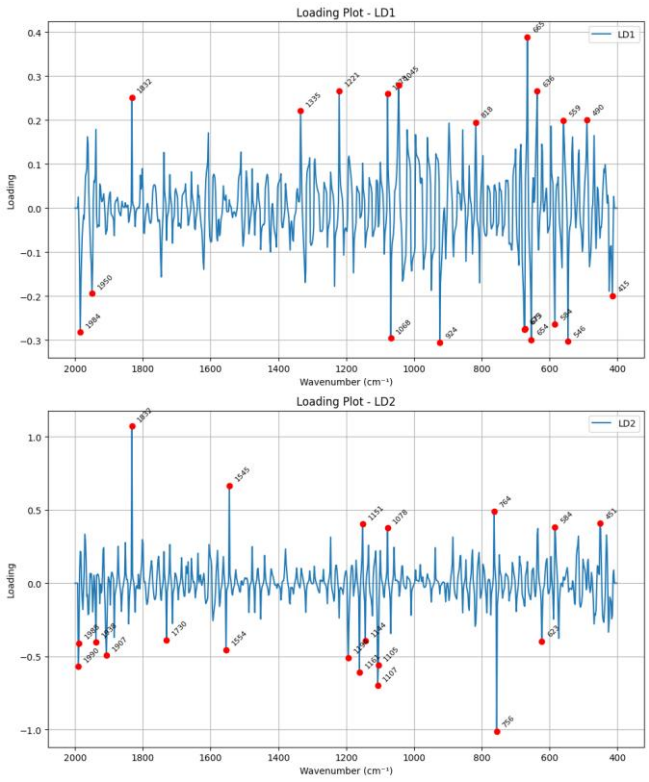

Fig. 6. LDA Loadings

IV. Conclusion

The FTIR enabled the observation of spectral changes being observed, especially in the region of the β-lactam ring, which associates acid and thermal degradation with the opening of this ring and the modification of other structural regions of the molecule. In conclusion, this study demonstrates that this methodology provides both qualitative and quantitative insights to structural degradation, making it a robust method for monitoring antibiotic integrity.

Acknowledgment

This research was supported by CNPq (INCT-INTERAS 406761/2022-1; Sisfoton 440228/2021-2; PQ: 314517/2021-9); FAPESP (21/00633-0, 2020/07065-4, 2025/03135-1); and CAPES-finance code 001.

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