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Degradation and stability testing of chloramphenicol ear drops using derivative spectrophotometry combined with chemometrics

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Abstract

Background: Chloramphenicol eye drops have a lower stability than solid dosage forms. Hence, it is necessary to assess their stability. One stability test that can be conducted is the forced degradation approach, which involves applying stress conditions that are more severe than those used in accelerated stability testing.

Objective: This study used forced degradation to explore the stability profile of chloramphenicol ear drops.

Method: Stability analysis was carried out using a derivative spectrophotometric instrument combined with chemometric analysis. The forced degradation study was conducted by exposing the sample to three conditions: acidic (0.1 N HCl at 80°C for 2 hours), alkaline (0.1 N NaOH at 80°C for 2 hours), and heat (90°C for 4 hours). Principal Component Analysis (PCA) and Partial Least Squares–Discriminant Analysis (PLS-DA) were utilized for the chemometric analysis.

Results: Sequential chloramphenicol observations with a zero to third derivative show a maximum wavelength of 278, 260, 234, and 292 nm. According to stability studies with forced degradation, chloramphenicol tended to degrade under alkaline and thermal conditions compared to acidic conditions. A typical grouping pattern amongst forced degradation treatments is revealed by chemometric analysis, which characterizes chloramphenicol's stability profile under different experimental settings.

Conclusion: The UV-Vis spectrophotometric approach, both non-derivative and derivative, can describe changes in chloramphenicol degradation profiles, although the specific degradation products generated remain unknown.

Keywords: Chloramphenicol, forced degradation, spectrophotometry, chemometrics, stability testing

1. Introduction

Streptomyces venezuelae is the source of the antibiotic chloramphenicol, which has a wide range of action and works against both Gram-positive and Gram-negative bacteria (Mitchell *et al*., 2015). It works through a bacteriostatic mechanism by penetrating bacterial cells and reversibly binding to the 50s ribosomal subunit (Singhal *et al*., 2020). On the market, chloramphenicol is available in various dosage forms, including solid preparations like tablets and capsules, semi-solid forms such as creams and ointments, and liquid preparations like suspensions, eye drops, and ear drops.

The Indonesian Pharmacopoeia Edition VI (Depkes, 2020) specifies that chloramphenicol ear drops must contain between 90.0 and 130.0% of the active ingredient, chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$), relative to the amount stated on the label. Due to their liquid nature, ear drops are generally less stable than solid dosage forms, so evaluating chloramphenicol's stability in ear drops is essential. Furthermore, the amide bond in chloramphenicol is susceptible to hydrolysis when exposed to acids, bases, or heat, which can compromise its stability. Additionally, the carbonyl group (C=O) in chloramphenicol is highly reactive with nucleophiles, leading to nucleophilic substitution

reactions with chlorine atoms (Cl) and resulting in degradation products such as 2-amino-1-(4-nitrophenyl)propane-1,3-diol (Al-Rimawi & Kharoaf, 2011).

Stability testing in the pharmaceutical field plays a crucial role in ensuring that a pharmaceutical preparation remains effective over time. A preparation is considered stable when its characteristics continue to meet established specifications, even after a period of storage or use. This stability is essential for the preparation to deliver optimal therapeutic effects. Therefore, conducting stability tests is vital to confirm that the quality of the preparation remains consistent with its initial standards, ensuring its efficacy. Additionally, stability testing helps determine the shelf life, appropriate storage conditions, and the best formulation methods for a pharmaceutical preparation, thereby preserving its stability (González-González *et al*., 2022).

Stability studies typically include real-time stability tests lasting 12 months and accelerated stability tests lasting 6 months. However, this study utilized a forced degradation approach for stability testing. This method subjects the test sample to more extreme conditions than those used in accelerated stability tests to determine stability characteristics and degradation pathways. The International Conference on Harmonisation (ICH) recommends specific degradation conditions, such as a 10°C increase in temperature (from 50 to 60°C), humidity levels exceeding 75%, and exposure to acid hydrolysis, base, oxidation, and photolysis. Forced degradation studies are essential for elucidating the stability characteristics and degradation pathways of an active pharmaceutical compound or preparation (González-González *et al*., 2022).

Derivative spectrophotometry, an advancement of traditional spectrophotometric methods, offers a viable alternative for stability testing through the forced degradation of chloramphenicol. According to Chadha & Bali (2016), this technique is noted for its accuracy in stability assessments and often enhances spectral resolution, resulting in more precise results. Furthermore, using UV-Vis spectrophotometry is justified by chloramphenicol's chromophore group, which absorbs UV light (Aisha *et al*., 2018; Lisnawati *et al*., 2019). In UV-Vis spectrophotometry, the intensity of absorbed light is measured and presented as absorbance data and spectra (Pratiwi & Nandiyanto, 2022). When conducting stability analysis with forced degradation, the spectrophotometric data can be visualized to create a more distinct grouping profile among treatments using chemometric analysis methods.

Chemometrics is a scientific discipline that employs mathematical and statistical techniques to process chemical data obtained from measurements. It facilitates data visualization and the extraction of relevant information. One significant application of chemometrics is in analyzing the degradation of drugs and pharmaceuticals. By using chemometric methods, researchers can create grouping profiles of degradation samples, allowing for an evaluation of the relationships between

different treatments and the effects of degradative conditions on the samples. Common techniques used in data exploration include Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA). The results from PCA and PLS-DA indicate the percentage of variance, reflecting the extent to which the information in the data can be explained (Roberto de Alvarenga Junior & Lajarim Carneiro, 2019).

Numerous studies have examined the stability of chloramphenicol, employing techniques such as chromatography and spectrophotometry. Research on the stability of chloramphenicol in eye drops and capsules utilized Thin Layer Chromatography (TLC), revealing degradation percentages of 23.75% in 1N HCl, 100% in 0.1N NaOH, and 24% under thermal conditions (Musharraf *et al*., 2012). These findings indicate that chloramphenicol experiences significant degradation in alkaline environments. Other studies have employed High-Performance Liquid Chromatography (HPLC) for stability testing, where samples are injected into a system, and the resulting peaks are analyzed (AlAani & Alnukkary, 2016). Additionally, further research utilized UV-Vis spectrophotometry to assess the stability of simvastatin compounds under forced degradation, evaluating results based on absorbance and degradation percentage (Chavhan & Ghante, 2014). Furthermore, UV-Vis spectrophotometry has been integrated with chemometrics in the stability testing of cefoxitin sodium (Attia *et al*., 2018).

Previous research reviews indicate a lack of studies focusing on the stability testing of chloramphenicol through forced degradation using derivative spectrophotometry and chemometric analysis. However, there have been some studies employing derivative spectrophotometry for stability tests on raw materials and various formulations, such as trandolapril (Jaiswal & Bali, 2024), dronedarone hydrochloride (Chadha & Bali, 2016), and colistin sulfate (Mutasim Elimam *et al.*, 2015). The specific stability of chloramphenicol has not been extensively investigated. This study aims to analyze the stability of chloramphenicol in ear drop preparations subjected to forced degradation under acidic, basic, and thermal conditions, utilizing derivative spectrophotometry in conjunction with chemometric analysis for evaluation.

2. Method

2.1. Instruments and materials

The instruments used in this study included a UV-Vis spectrophotometer (Shimadzu UV-1780, Japan) equipped with a UV Probe, a water bath, an analytical balance, a micropipette, a thermometer, and various glassware. The materials comprised standard chloramphenicol (Sigma-Aldrich) and chloramphenicol ear drops from brand XX, obtained from a pharmacy in Purwokerto,

which were still within their circulation period. Additional materials included 99.9% ethanol (Merck) and pro-analysis grade substances, such as 37% hydrochloric acid (HCl), 99% sodium hydroxide (NaOH), 30% hydrogen peroxide (H_2O_2), and distilled water.

2.3. Chloramphenicol measurement

The procedures conducted in this study included standard preparation, maximum wavelength measurement, and the development of a chloramphenicol standard curve, which modified methods from previous research on stability (Yugatama *et al*., 2019). The preparation of the chloramphenicol standard began by weighing 100 mg of chloramphenicol, which was then dissolved in 50% ethanol in a 100 mL volumetric flask to achieve a final concentration of 1000 µg/mL. This 1000 µg/mL stock solution was diluted with ethanol to obtain a 10 µg/mL concentration. The absorbance of the 10 µg/mL standard solution was measured using a UV-Vis spectrophotometer across the wavelength range of 200-400 nm to generate an absorption spectrum. The 1000 µg/mL stock solution was diluted in ethanol to a 100 µg/mL concentration to create the standard curve. This 100 µg/mL solution was then transferred to a 10 mL volumetric flask, and ethanol was added to achieve a final concentration series of 4-16 µg/mL. The absorbance of each solution in this series was measured using a UV-Vis spectrophotometer, with ethanol serving as the blank.

2.4. Evaluation of analysis parameters

To confirm the parameters for spectrophotometric analysis of chloramphenicol concentrations, we evaluated linearity, accuracy, and precision according to ICH Q2(R2) guidelines (Ermer, 2025). Linearity was assessed by measuring levels across seven different concentrations and analyzing the resulting standard curve data. Each concentration was tested in triplicate, with absorbance measured at the maximum wavelength using a spectrophotometer. The linearity was determined by calculating the correlation coefficient (r) through linear regression between concentration and measured absorbance. For accuracy testing, the standard addition method was employed. We added three concentrations of standard solution, 75, 100, and 125%, to the chloramphenicol sample solution, with each measurement conducted in triplicate. Absorbance values were recorded using a spectrophotometer. Additionally, we calculated the % recovery for accuracy and the % RSD and HORRAT values for precision testing, using both underivatized and derivatized spectra. The calculations for % recovery and % RSD were performed as follows:

$$\%\ recovery = \frac{\text{measured chloramphenicol concentration in sample}}{\text{added chloramphenicol concentration in sample}} \times 100\%$$

$$\%\ RSD = \frac{\text{standard deviation of chloramphenicol measurement results in sample}}{\text{mean of chloramphenicol measurement results in sample}} \times 100\%$$

2.5. Forced degradation test

The forced degradation test method carried out in this study was adapted from Vyas *et al*. (Vyas *et al*., 2023). Initially, 0.5 mL of chloramphenicol ear drops were placed in a 50 mL measuring flask, and 50% ethanol was added to the mark. From this solution, 15 mL was transferred to another 50 mL measuring flask, and ethanol was added to the mark, resulting in a final sample concentration of 30 µg/mL. 5 mL of the sample was placed in a test tube for the degradation test. To create acidic conditions, 1 mL of 0.1 N HCl was added; for basic conditions, 1 mL of 0.1 N NaOH was added; and for oxidation conditions, 1 mL of 30% H_2O_2 was added. The samples for acidic, basic, and thermal conditions were heated at 80°C for 2 hours, while the thermal condition sample, containing only 5 mL of the sample, was heated at 90°C for 4 hours using a water bath. After heating, the samples were cooled and neutralized if they had undergone acid or base treatment before being measured with a spectrophotometer. Subsequently, all samples from the test tubes were transferred into a 10 mL measuring flask, and the volume was adjusted with 50% ethanol. The absorbance of the sample solution was then measured using a spectrophotometer over a wavelength range of 200-400 nm, with 50% ethanol serving as the blank solution.

3. Results and discussion

3.1. Chloramphenicol UV-Vis spectral profile and standard curve

The absorption spectra profile of chloramphenicol in the UV region was obtained by first measuring a standard solution. After preparing the standard solution, the next step involved measuring the maximum wavelength using a UV-Vis spectrophotometer. This measurement aimed to determine the maximum analysis sensitivity at the maximum wavelength, and the absorbance changes reached their highest value for each concentration series (Apriliyani *et al*., 2018). The maximum wavelength of the chloramphenicol standard solution was measured using a spectrophotometer over a range of 200-400 nm. The results of this measurement are presented in **Figure 1**.

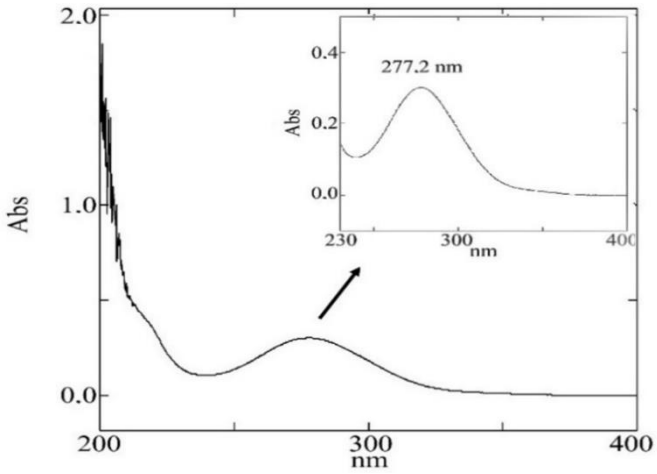

Figure 1. Absorption profile and maximum wavelength of chloramphenicol

The measured wavelength of chloramphenicol was found to be 277.20 nm. This aligns with the value stated in the Indonesian Pharmacopoeia Edition VI, which indicates that chloramphenicol has a wavelength of approximately 278 nm. The difference between the measured wavelength and the literature value falls within the acceptable tolerance limit of ±2 nm. The wavelength of 278 nm was then utilized to create a standard curve. This standard curve establishes the relationship between absorbance and the concentration of varying standard solutions at the maximum wavelength. A standard curve is deemed effective if there is a linear relationship between concentration and absorbance, indicating that an increase in concentration corresponds to an increase in absorbance. The results of the standard curve for the chloramphenicol standard solution are shown in **Figure 2**.

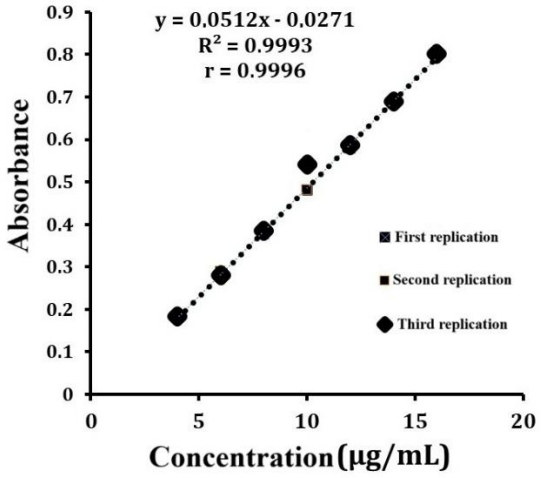

Figure 2. Standard curve of chloramphenicol standard solution
Each measurement concentration was replicated three times (n=3)

The standard curve results demonstrated the relationship between concentration and absorbance for the standard solution within the 4 to 16 µg/mL range, based on three measurements. Furthermore, the analysis of the standard curve data yielded a linear regression equation of y = 0.0512x - 0.0271, with a correlation coefficient (r) of 0.9996, confirming the linearity of the data.

3.2. Evaluation of analysis parameters

A linearity test was conducted using UV-Vis spectrophotometry to assess the detector's response to substance concentration variations and determine if the relationship is linear (Rohmah *et al*., 2021). Linearity is considered acceptable when the correlation coefficient falls between 0.995 and 1. The linearity results presented in the standard curve in Figure 2 indicate a correlation coefficient (r) of 0.9996. This result meets the established criteria, as the r value is within the range of $0.995 \leq r \leq 1$, demonstrating that the method has good linearity and can produce a linear response.

Accuracy testing measures how closely the analysis results align with the analyte concentration, expressed as percentage recovery (% recovery). In contrast, precision testing evaluates the method's repeatability by assessing the consistency of results across multiple repetitions, indicated by the percentage of Relative Standard Deviation (% RSD) (Ehling *et al*., 2025). Both accuracy and precision are determined using the standard addition method, which involves adding standard solutions at three concentration levels: 75, 100, and 125% to the sample solution. Measurements are then taken using a spectrophotometer, with three replicates for each concentration. The results of the accuracy and precision tests, calculated as percentage recovery (% recovery) and percentage of relative standard deviation (% RSD), are presented in **Table 1**.

Table 1. Test results for accuracy and precision

Concentration level	Replication	Standard added	Measured level	*Recovery* (%)	Average	SD	RSD (%)	HORRAT
75%	1	5.20	5.56	106.92	111.72	5.11	4.57	0.37
	2	5.20	6.09	117.11				
	3	5.20	5.78	111.15				
100%	1	6.96	7.72	110.91	111.68	1.58	1.41	0.12
	2	6.96	7.90	113.50				
	3	6.96	7.70	110.63				
125%	1	8.73	9.81	112.37	111.07	2.97	2.67	0.23
	2	8.73	9.88	113.17				
	3	8.73	9.40	107.67				

The percentage recovery value for a reliable accuracy test, according to ICH guidelines, should fall within the range of 80–120%. Precision is considered acceptable if the HORRAT (Horwitz

Ratio) is calculated from the observed RSD and the predicted RSD is less than 2 (Ehling *et al.*, 2025). The results presented in **Table 1** for both accuracy and precision tests demonstrate that the applied analytical method meets these criteria. Specifically, the accuracy test results at concentration levels of 75, 100, and 125% yielded values of 111.72% ± 5.11, 111.68% ± 1.58, and 111.07% ± 2.97, respectively. These values are within the acceptable 80–120% range, indicating good accuracy. Additionally, the precision results based on the % RSD for the same concentration levels were 4.57, 1.41, and 2.67%, with corresponding HORRAT calculations of 0.37, 0.12, and 0.23, all below 2. This confirms that the method also has adequate precision (Ehling *et al*., 2025).

3.3. Chloramphenicol derivatives spectrum

The spectrophotometric analysis data will be presented as absorbance values and UV spectra. Derivatization spectra in spectrophotometric measurements enhance spectral resolution without requiring compound separation, achieved by calculating derivative data from the UV spectra (Redasani *et al*., 2018). In this study, chloramphenicol absorption spectra were derivatized by first measuring a standard chloramphenicol solution across a concentration range of 4-16 µg/mL to establish a standard curve. The derivatization process continued until the third derivative was reached. The results of this derivatization process for chloramphenicol absorption spectra are illustrated in **Figure 3** and summarized in **Table 2** below.

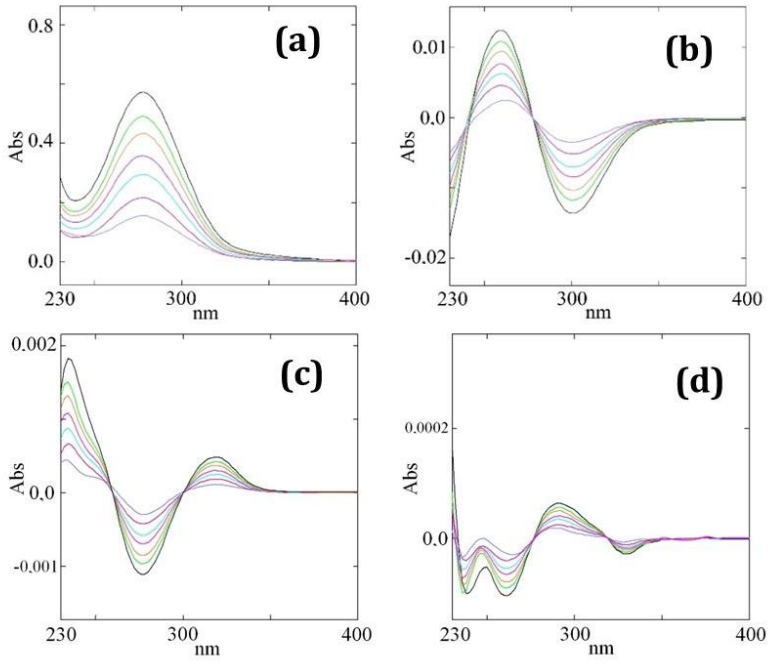

Figure 3. Spectra of chloramphenicol underivatives (a), first (b), second (c), and third (d) derivatives

Table 2. Regression analysis of data accuracy and precision in derivative spectra measurements

Parameter	D_0	D_1	D_2	D_3
λ_{max}	278 nm	260 nm	234 nm	292 nm
Slope	0.0347	0.0008	0.0001	0.000004
R	0.9993	0.9985	0.9981	0.9980
% RSD	5.661	7.488	7.820	6.926
HORRAT	0.48	0.64	0.68	0.60
% *Recovery*	111.727	123.647	131.4655	130.5077

The results presented in **Figure 3** and **Table 2** indicate that the maximum wavelengths for the zero to third derivatives were obtained sequentially: 278, 260, 234, and 292 nm. These values correspond to the sizes of the absorption peak amplitudes in the spectrum. Furthermore, the linearity met the acceptance criteria, with the correlation coefficient (r) falling within the range of reasonable linearity requirements, specifically $0.995 < r < 1$ (Ermer, 2025).

The % RSD values for the zero to third derivatives were 5.661, 7.488, 7.820, and 6.926%, with corresponding HORRAT values of 0.48, 0.64, 0.68, and 0.60, indicating adequate precision (Ehling *et al*., 2025). The % recovery for the zero to third derivatives was recorded at 111.727, 123.647, 131.465, and 130.507%. Although there was a tendency for an increase in % recovery during derivatization (**Table 2**), ANOVA analysis revealed no significant differences among these values. Therefore, measurements from the zero to third derivative spectra yield relatively consistent results.

3.4. Forced degradation test

Samples treated with acid, base, oxidation, and thermal methods and control samples without treatment were scanned using a spectrophotometer in the wavelength range of 200-400 nm. The resulting spectra for these samples are shown in **Figure 4**, which illustrates the effects of the various treatments on chloramphenicol.

The degradation of chloramphenicol is evident from the differences in the spectra of control and treatment samples. The spectra for the acid, base, and thermal samples still show analyzable peaks. In contrast, the oxidation sample produced a noisy spectrum in the 230-300 nm wavelength range, which corresponds to the absorption area of chloramphenicol, complicating analysis. Consequently, further analysis was conducted only on the samples treated with acid, base, and thermal methods.

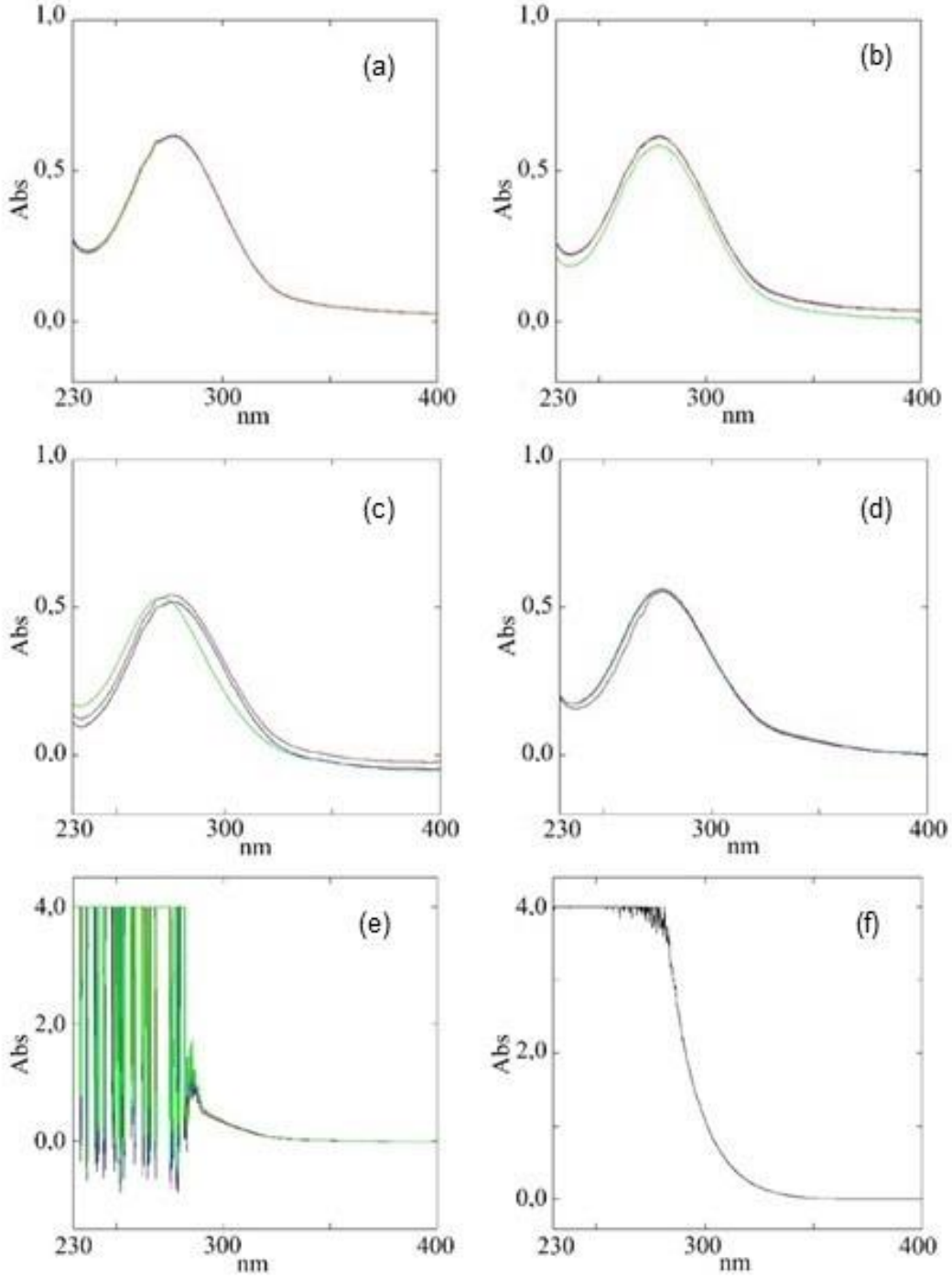

Figure 4. UV-Vis spectrum of forced degradation of chloramphenicol control sample (a), acid (0.1 N HCl; 80°C for 2 hours) (b), base (0.1 N NaOH; 80°C for 2 hours) (c), thermal (90°C for 4 hours) (d), oxidation (30% H_2O_2; 80°C for 2 hours) (e), absorbance of H_2O_2 solvent (f)
Measurements were carried out with three replications (n=3)

The degradation spectrum was analyzed using chemometric techniques, specifically PCA and PLS-DA. Chemometric analysis provides a percentage of variance that indicates how much information is retained and explained and how much is lost and cannot be accounted for. While there are no strict rules regarding variance limits, a minimum value of 50% is recommended, as it is considered sufficient to represent the data as a whole (Kamil & Hananto, 2023). Consequently, PCA and PLS-DA effectively characterize spectral data (Roberto de Alvarenga Junior & Lajarim Carneiro, 2019). The results of this analysis are presented in **Figure 5** for PCA and **Figure 6** for PLS-DA.

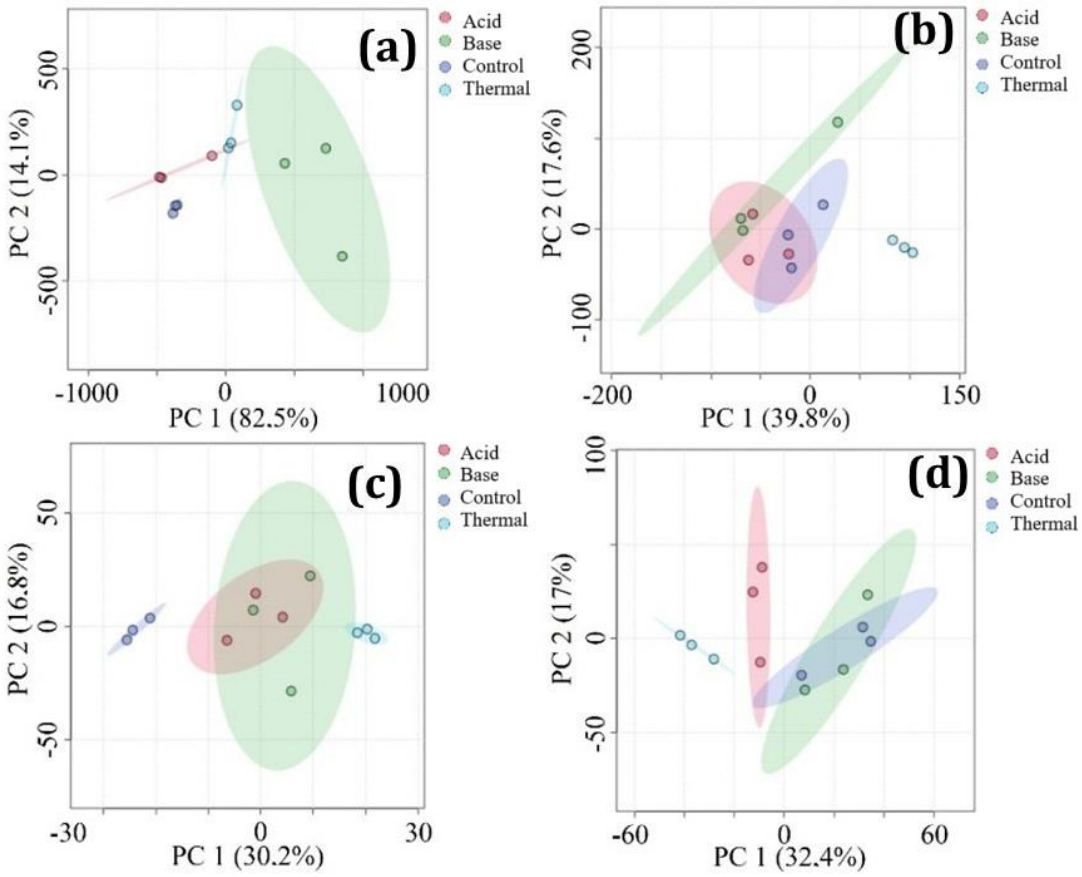

Figure 5. PCA results of the chloramphenicol spectrum of the zero (a), first (b), second (c), and third (d) derivatives

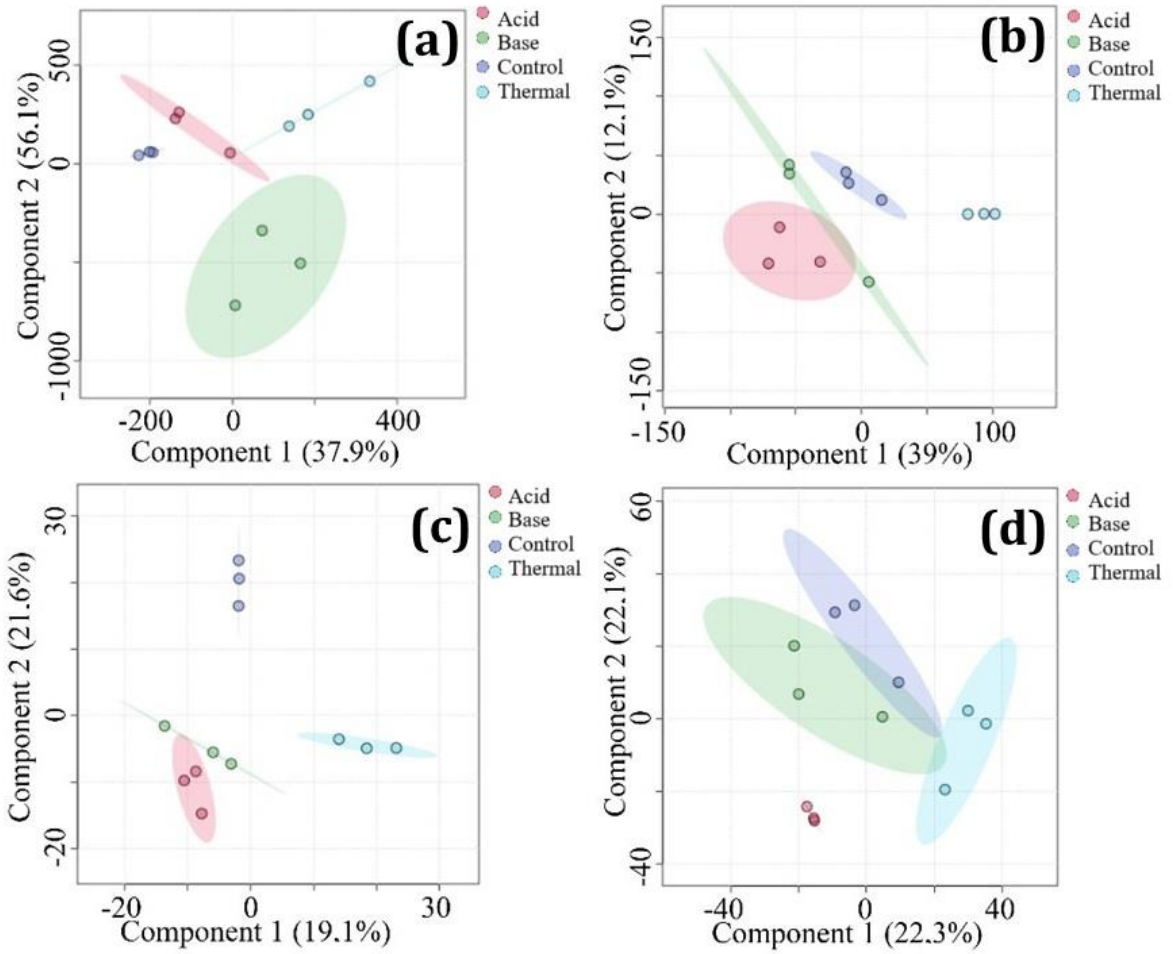

Figure 6. PLS-DA results of the zero (a), first (b), second (c), and third (d) chloramphenicol derivative spectra

In chemometrics, PCA (Principal Component Analysis) simplifies data by creating new variables, known as Principal Components (PC), which are linear combinations of the original variables. The results of this analysis are illustrated in **Figure 5**, which displays the grouping profile of treatment and control samples. Notably, the acid group tends to cluster closely with the control group. On the X-axis (PC1), the first principal component represents the direction in high-dimensional data space that accounts for the most significant variation. The Y-axis (PC2) indicates the second principal component, which captures the second most significant variation in the data, ensuring it is orthogonal to PC1. The numbers in brackets next to the axis labels denote the percentage of data variance explained by each component. The combined variance explained by the first two PCs for non-derivative data is 96.6%, which decreases during derivatization. The first derivative results yield a variance of 57.4% for the first two PCs, maintaining above 50%.

The following chemometric analysis utilizes PLS-DA, a method for discrimination and classification that optimizes the separation between sample groups (Aminu & Ahmad, 2020). The

PLS-DA results, illustrated in **Figure 6**, demonstrate that the samples are closely clustered together. The variance explained by the first two components is 94.0% for the zero derivative, which decreases upon derivatization. Notably, a variance value exceeding 50% is only achieved in the first derivative chemometric results, reaching 51.1%. Both PCA and PLS-DA analyses provide insights into the forced degradation profiles under various conditions, as shown by the visualization of spectrum data, which illustrates the sample grouping following degradative treatments such as acid, base, and thermal exposure.

Figure 7 presents the results of the degradation percentages. The data indicates that chloramphenicol degradation is minimal under acidic conditions, based on both the derivatization spectrum and non-derivatives. In contrast, degradation is more significant under basic and thermal conditions. These findings align with previous research by Musharraf *et al.* (Musharraf *et al*., 2012)**,** which demonstrated that the least degradation occurred in samples tested under acidic conditions using TLC-densitometry.

Under basic and thermal conditions related to zero and second derivatives, the basic group generally exhibits a higher percentage of degradation than the thermal group. Conversely, thermal degradation outweighs basic degradation for the first and third derivatives. These findings align with previous research (AlAani & Alnukkary, 2016), which conducted stability tests involving forced degradation on chloramphenicol using HPLC. Their results also indicated that the degradation percentage under thermal conditions was greater than under basic conditions. The degradation percentage is calculated by subtracting the sample content after degradation from the sample content before degradation and multiplying the result by one hundred percent. **Figure 7** illustrates how derivative spectrophotometry can elucidate the forced degradation analysis profile of chloramphenicol by revealing the percentage of degradation under various conditions. However, to achieve more reliable results, further research using more specific analytical methods, such as chromatography with mass spectrometry detectors, is necessary to analyze its compounds' degradation better.

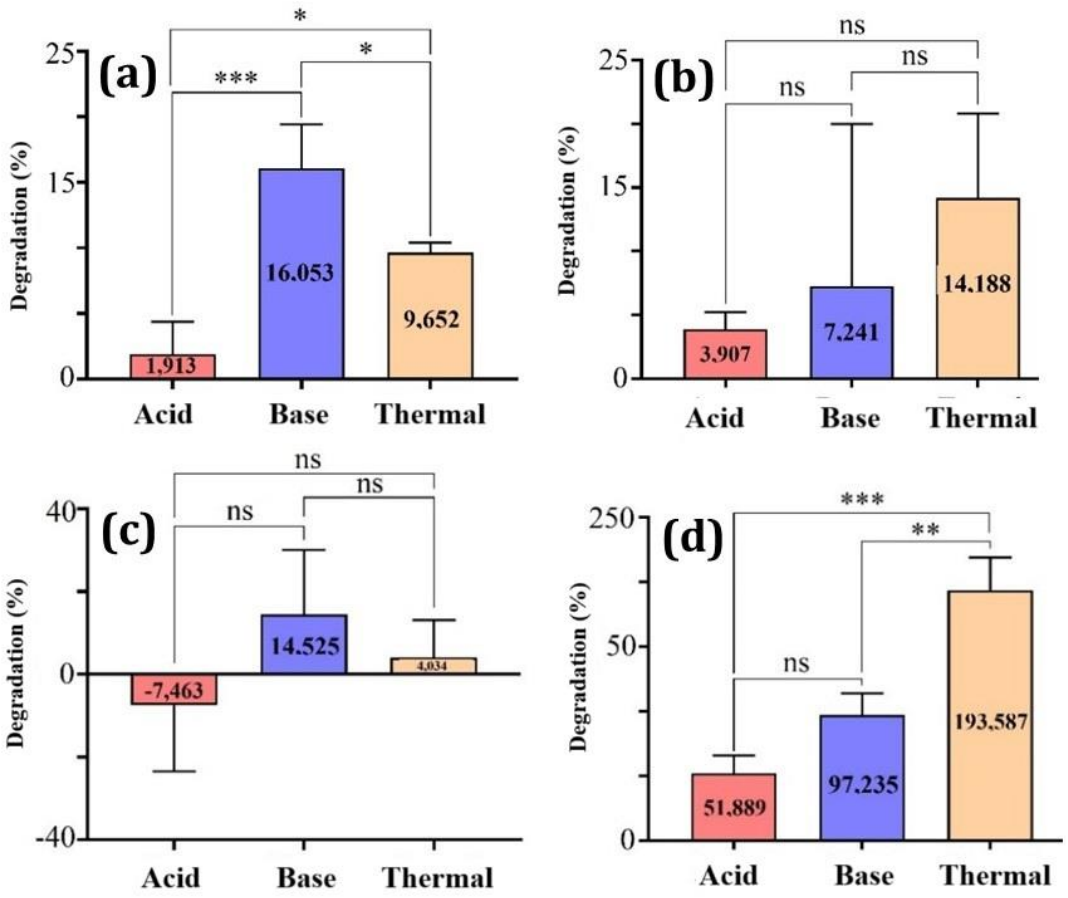

Figure 7. Degradation diagram of chloramphenicol derivatives to zero (a), first (b), second (c), third (d)

Previous studies have investigated chloramphenicol and its degradation products (AlAani & Alnukkary, 2016). The findings indicate that chloramphenicol is stable in acidic conditions, making it resistant to hydrolysis. In contrast, under basic conditions, particularly at pH levels above 10, chloramphenicol degrades to form 2-amino-1-(4-nitrophenyl)propane-1,3-diol, as these compounds are less stable in alkaline solutions. Additionally, amide compounds like chloramphenicol are thermolabile and can be easily hydrolyzed by water vapor when heated (Mitchell *et al*., 2015). The degradation of chloramphenicol is primarily attributed to the reactivity of its carbonyl carbon group (C=O) with nucleophiles, which facilitates hydrolysis reactions through nucleophilic substitution, leading to the formation of degradation products.

In line with other studies (AlAani & Alnukkary, 2016) reported that the degradation product of chloramphenicol, 2-amino-1-(4-nitrophenyl)propane-1,3-diol, has a wavelength of around 270 nm. This wavelength falls within the absorption range of chloramphenicol, which is 278 nm. Consequently, spectral overlap between the degradation products and the original chloramphenicol compound is more likely. Without a standard for the chloramphenicol degradation product, it is

challenging to assess this overlap using UV spectrophotometry accurately. The findings indicate that to effectively identify the degradation products formed, a standard for the chloramphenicol degradation product is essential, along with an analytical instrument capable of separating compounds, such as chromatography.

4. Conclusion

UV-Vis spectrophotometry methods, both non-derivative and derivative, can quantify the percentage of chloramphenicol degradation, although the formation of degradation products remains unclear. The degradation percentages obtained from the zero derivatives under acidic, basic, and thermal conditions are 1.913, 16.053, and 9.652%, respectively. The first derivative's percentages are 3.907, 7.241, and 14.188%. Additionally, the PCA and PLS-DA chemometrics analysis reveals the grouping profiles of forced degradation samples under various acidic, basic, and thermal conditions compared to the control. Notably, chloramphenicol in acidic conditions tends to cluster closer to the control sample. Furthermore, the variance from the PCA analysis for the zero and first derivatives was 96.6% and 57.4%, respectively. The PLS-DA analysis showed variances of 94.0% for the zero derivative and 51.1% for the first derivative.

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