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Research Paper

# Development and Validation of Fast and Simple Fourier Transform Infrared Spectrophotometric Method for Analysis of Thiamphenicol in Capsule Dosage Form

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**Abstract**
The development of a method for identification and determination of thiamphenicol by Fourier Transform Infrared will provide convenience to developers because it is fast and easy for analysis. The research was carried out by utilizing the solubility of thiamphenicol in methanol with three stages, namely method development, sample analysis, and method validation. The method development stage showed that the specific peak of thiamphenicol was at a peak with a wavenumber of 1694.1 $cm^{-1}$; this specific peak of thiamphenicol was used for qualitative analysis and quantitative analysis of thiamphenicol in the capsule dosage form. The sample analysis showed that all analyzed thiamphenicol in capsule dosage form showed good results both qualitatively and quantitatively. Qualitatively all the samples analyzed showed a specific peak at specific positions and specific wavenumbers. These results meet the requirements for containing thiamphenicol in the dosage form. Quantitatively all the samples analyzed ranged from 97.97% to 102.24% by peak height and peak area. These results meet the requirements for active substance levels in general preparations within 90.0% to 110.0%. The method validation for peak height and peak area showed that the accuracy parameter had a recovery percentage of 100.28% and 100.41% (between 98.0% to 102.0%), the precision parameter with a relative standard deviation of 0.31% and 0.37% (not more than 2.0%), and the linearity parameter with a correlation coefficient of 0.9999 and 0.9997 (not less than 0.99). The limit of detection value was 0.2971 mg/mL and 0.5338 mg/mL, the limit of quantitation value was 0.9004 mg/mL and 1.6176 mg/mL, the range for both was 80% to 120%, and the specificity for both met the requirement. The Fourier Transform Infrared method has been successfully developed, applied, and validated for qualitative analysis and quantitative analysis of thiamphenicol in capsule dosage form.

**Keywords**
Development, Validation, Fourier Transform Infrared, Thiamphenicol, Capsule

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## 1. INTRODUCTION

Drugs have an important role in improving the quality of human life. A pharmaceutical dosage form should be tested for its quality, efficacy, and safety (Zuccari et al., 2022). In order to guarantee the quality, efficacy, and safety of a drug dosage form that has been distributed a stability study as well as a series of evaluation tests must be performed to fulfill the requirements (Gupta et al., 2020). The qualitative analysis for identification and the quantitative analysis for assay of active substances in the drug dosage form is one of the chemical evaluations that must be performed. It is a requirement that must be met to ensure the quality of a drug (Chen et al., 2018).

Thiamphenicol is a broad-spectrum antibiotic that can be used to treat infections caused by various gram-positive bacteria and gram-negative bacteria (Laconi et al., 2022). Thiamphenicol works by inhibiting the growth of bacteria (bacteriostatic) while killing the bacteria (bactericidal) which may cause infection. Thiamphenicol treats various bacterial infections, such as digestive tract infections, respiratory tract infections, and urinary tract infections (Barbieri et al., 2022).

The thiamphenicol monograph in the dosage form is not listed in the compendial monograph (United States Pharmacopoeia, Indonesian Pharmacopoeia, European Pharmacopoeia, Japanese Pharmacopoeia or Chinese Pharmacopoeia). So there is no detailed information on a drug's physical and chemical

properties, pharmacological effects, dosage forms, and analysis methods; therefore it is necessary to find and develop analytical methods for qualitative analysis and quantitative analysis of thiamphenicol. Various methods of analysis of thiamphenicol in dosage form have been developed, including ultraviolet spectrophotometric (Martins and De Oliveira, 2019) and high-performance liquid chromatography (Patyra and Kwiatek, 2019; Wu et al., 2021; Ye et al., 2022).

High-performance liquid chromatography in drug analysis is the most reliable method in drug analysis (Gupta et al., 2022). The thiamphenicol residues was analyzed in medicated feeding stuffs by a liquid chromatography with diode array detector, equipped with phenyl column, water and acetonitrile were used as the mobile phase with the gradient elution program, ultraviolet detection at 223 nm (Patyra and Kwiatek, 2019). The thiamphenicol and it metabolites in pork, beef, lamb, chicken, and their products have been determined by combination of solid-phase extraction with ultrahigh-performance liquid chromatography-tandem mass spectrometry, water and acetonitrile (gradient) as the mobile phase, and octadecyl silane column as the stationary phase (Wu et al., 2021). The thiamphenicol residues was analyzed in aquatic products by a liquid chromatography with tandem mass spectrometry detector, equipped with pentafluorophenyl propyl column as stationary phase, ammonium acetate solution and methanol as the mobile phase with the gradient elution program (Ye et al., 2022). The disadvantage of the high-performance liquid chromatography method is that it requires special analysts to prepare and operate because preparations and operations require special competency skills (Pitigoi, 2022). The materials used in HPLC are also expensive, such as reagents (which need a lot of organic solvents), spare parts, and columns (Timchenko, 2021).

Drug analysis by ultraviolet spectrophotometry is a fairly good and fast method (Patel et al., 2022). Analysis of thiamphenicol in soft capsule dosage form shows that ultraviolet spectrophotometry can analyze thiamphenicol properly without being affected by additional ingredients in the dosage form and has good validity (Martins and De Oliveira, 2019). The main disadvantage of the ultraviolet spectrophotometric method is its low level of selectivity due to interference from other components in the sample (Hladová et al., 2019). This overlapping effect increases the response value of the component to be analyzed due to the presence of other components (Ríos Reina and Azcarate, 2022). Another disadvantage of using this UV-Vis spectrophotometer instrument is that the compound to be analyzed must have a chromophore group and a wavelength in the ultraviolet or visible region (Kurzyna Szklarek et al., 2022).

Infrared is a spectrophotometric method and vibrational spectroscopy Fadlelmoula et al. (2022) with many advantages such as being selective, easy, fast, simple, environmentally friendly, and nondestructive (Estupiñán Méndez and Allscher, 2022). Infrared spectroscopy is a popular technique for analyzing various sample matrixes, namely pharmaceutical products (Siregar et al., 2018; Burela and Mandalemula, 2023), food (Sahachairungrueng et al., 2022; Mendes and Duarte, 2021), biological liquids (Kamnev et al., 2021), or environmental samples (Tkachenko and Niedzielski, 2022). Infrared spectrophotometry analysis can determine the functional groups in the compound (Enders et al., 2021) and predict the chemical reaction (Zeaiter et al., 2022). This analysis is based on studying a characteristic peak from a certain functional group at a certain wavenumber or wavelength of a sample. Fourier Transform Infrared is a powerful analytical method for qualitative analysis (identification) and quantitative analysis (assay) of several pharmaceuticals (Gosar et al., 2022).

The validated method is needed to determine the active substance content of a drug dosage form with a modified analytical method or developed analytical method (Susilo et al., 2022). A new analytical method can be used if the conditions are adjusted to the laboratory condition and validation has been carried out (Verch et al., 2022). Based on the description of the need and urgency of the research, this study is aimed at developing, applying, and validating a novel Fourier Transform Infrared spectrophotometric method for qualitative analysis (identification) and quantitative analysis (assay) of thiamphenicol in capsule dosage form.

## 2. EXPERIMENTAL SECTION

The descriptive research developed simple and fast analytical methods using Fourier Transform Infrared spectrophotometric to determine the thiamphenicol level in capsule dosage form. The developed method was validated to ensure the procedure was suitable for its intended use. Sampling was carried out purposively, and samples were taken without comparing one place with another because samples from various collection places were considered homogeneous (Vasileiou et al., 2018).

### 2.1 Materials and Tools

The materials used included a Methanol type Pro Analysis (Merck), Thiamphenicol (Sigma Aldrich), Thiamphenicol Capsule (Phapros), Thiamphenicol Capsule (Bernofarm), Thiamphenicol Capsule (Pyridam Farma), Thiamphenicol Capsule (Sanbe Farma), Thiamex® Capsule (Novapharin), Thiamycin® Capsule (Interbat), Nikolam® Capsule (Meprofarm), Zicafen® Capsule (Graha Farma), Thianicol® Capsule (Dankos Farma), and Thislacol® Capsule (Metiska Farma). The tools used included a Fourier Transform Infrared type Cary 630 (Agilent), MicroLab type Quant (Agilent), and MicroLab type Lite (Agilent), Analytical Balance type Entris 224-1S (Sartorius), Electronic Multi Dispenser Pipettes type Multipette E3X (Eppendorf), Ultrasonic Cleaner type Elmasonic SRH 4/200 (Elma), and other laboratory glassware.

### 2.2 Method Development

The procedure used in the method development was modified from previous studies, and consisted of stages stock solution preparation and stages standard solution preparation (Robaina et al., 2013).

### 2.3 Stock Solution
To prepare a stock solution of thiamphenicol with a 100 mg/mL concentration, 5 g of the substance was placed in a 50 mL volumetric flask. Then, 25 mL of methanol was added to the flask, and the mixture was sonicated for 15 minutes until it dissolved completely. Methanol was added to the marked line, and the mixture was shaken well until it became homogeneous.

### 2.4 Standard Solution
To prepare a standard solution of thiamphenicol with a 50 mg/mL concentration, 5 mL of the stock solution was transferred to a 10 mL volumetric flask. Then, methanol was added to the marked line, and the mixture was shaken until it became homogeneous. The specific peak of thiamphenicol was then analyzed by measuring the blank (methanol) and the standard solution separately with a slit distance of 100 $\mu$m at wavenumbers ranging from 4000 $cm^{-1}$ to 650 $cm^{-1}$. The position and wavenumber of the specific peak were determined by overlaying the spectra of the blank and standard solution. This information was used for qualitative analysis.

### 2.5 Sample Analysis
The procedure used in the sample analysis was modified from previous studies which consisted of stages series solutions preparation, stages sample solutions preparation, stages analysis for series solutions and stages analysis for sample solutions (Robaina et al., 2013).

### 2.6 Series Solutions Preparation
To prepare a series of thiamphenicol solutions with varying concentrations, volumes of 0.0, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 mL of the stock solution were transferred to separate 10 mL volumetric flasks. Then, methanol was added to the marked line, and the mixtures were shaken until they became homogeneous. This resulted in a series of thiamphenicol solutions with 0, 30, 35, 40, 45, 50, 55, 60, 65, and 70 mg/mL concentrations.

### 2.7 Sample Solutions Preparation
To prepare a sample solution of thiamphenicol, 20 capsules were ground and homogenized. The resulting powder was weighed to obtain a quantity equivalent to 500 mg of thiamphenicol. The powder was placed in a 10 mL volumetric flask, and 5 mL of methanol was added. The mixture was sonicated for 15 minutes until the powder dissolved completely. Then, methanol was added to the marked line, and the solution was shaken until it became homogeneous. The solution was filtered using filter paper, and the first 1 mL of the filtrate was discarded. The remaining filtrate was collected to obtain a theoretical sample solution of thiamphenicol with a 50 mg/mL concentration.

### 2.8 Analysis of Series Solutions
Series solutions were executed the measurements with slit distance 100 $\mu$m for series solution separately at wavenumbers 4000 $cm^{-1}$ to 650 $cm^{-1}$, analyzed the baseline, peak height, and peak area of the specific peak (position and wavenumber) of thiamphenicol by overlay of the series solutions. The baseline, peak height, and peak area of the specific peak (position and wavenumber) of thiamphenicol was further used for quantitative analysis (each concentration was replicated six times). The study continued with plotting the calibration curve, calculating the regression equation and calculating the determination coefficient. The regression equation was used additionally to calculate the thiamphenicol concentration in the test solution. The determination coefficient was used further to analyze the degree of determination of peak height and peak area to the thiamphenicol concentration.

### 2.9 Analysis of Sample Solutions
Sample solutions were executed the measurements with a slit distance of 100 $\mu$m for sample solutions separately at wavenumbers 4000 $cm^{-1}$ to 650 $cm^{-1}$. The peak height and peak area of the specific peak (position and wavenumber) of thiamphenicol was then analyzed for further calculating the thiamphenicol concentration from sample solutions (each sample was replicated six times). The study continued by calculating the thiamphenicol level by comparing the actual thiamphenicol concentration to the theoretical thiamphenicol concentration, then the average was calculated, statistical analysis was performed, and the standard deviation was calculated.

### 2.10 Method Validation
The procedures used in the method validation have been modified from previous studies which consisted of accuracy, precision, linearity, limit of detection, limit of quantitation, range, and specificity (Wadher and Supekar, 2019).

### 2.11 Accuracy and Precision
The accuracy parameter was assessed using the standard addition method to validate the method. The recovery percentage was measured in three different ranges: 80%, 100%, and 120%, where each range consisted of 70% of the analyte and 30% of the active pharmaceutical ingredient (standard). The analyte was measured both with and without the addition of a standard in each range, and the difference between the two results was compared with the actual levels to determine the percentage recovery. This process was repeated six times for each range, and the average was calculated along with the standard deviation. For the precision parameter, the relative standard deviation value was calculated from several recovery percentages obtained from the accuracy parameter.

### 2.12 Linearity
The linearity parameter for the method validation test was calculated by calculating the correlation coefficient of the peak height against the concentration or peak area against the concentration; the peak height or peak area of the various concentration of thiamphenicol concentration obtained from the measurement of series solutions of thiamphenicol concentration with six times replication for each concentration.

### 2.13 Limit of Detection and Limit of Quantitation

The limit of detection value and the limit of quantitation value were both calculated by calculating the peak height or peak area of the various concentrations of thiamphenicol concentrations obtained from the measurement of a series of solutions of different thiamphenicol concentrations with six times replication for each concentration.

### 2.14 Range and Specificity

The range parameter for the method validation and the specificity parameter for the method validation were analyzed from the entire method validation parameter, and the complete sample analysis was obtained.

## 3. RESULTS AND DISCUSSION

### 3.1 Method Development

The study began with measurements taken separately at wave numbers 4000 $cm^{-1}$ to 650 $cm^{-1}$ against methanol as a blank and thiamphenicol solutions in methanol (thiamphenicol solution with a concentration of 50 mg/mL) as a standard solution. Each spectrum obtained was analyzed for thiamphenicol's specific peak (position and wavenumber) by overlaying the blank spectra and standard solution spectra. Figure 1 shows the blank spectra (methanol) (X) and standard solution spectra (thiamphenicol in methanol) (Y) at wavenumbers 4000 $cm^{-1}$ to 650 $cm^{-1}$. Figure 2 shows the overlay blank spectra (methanol) indicated by a red line (-) and standard solution spectra (thiamphenicol in methanol) indicated by a blue line (-) at full scale wavenumbers 4000 $cm^{-1}$ to 650 $cm^{-1}$ and zoom wavenumbers 1800 $cm^{-1}$ to 1100 $cm^{-1}$.

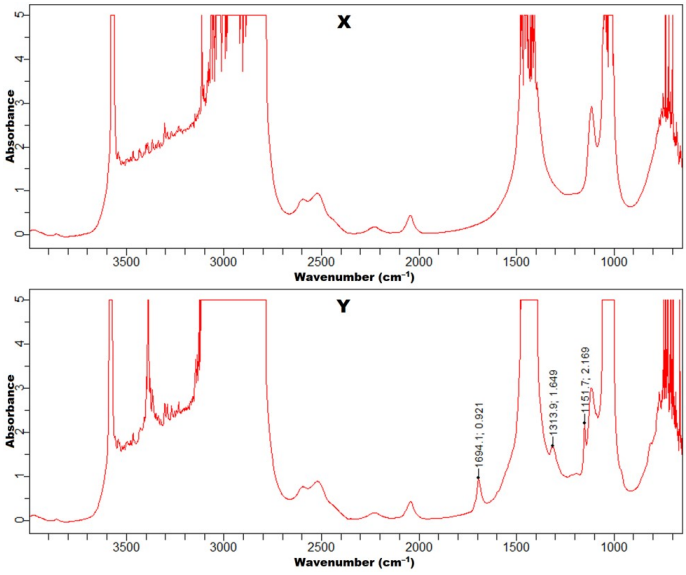


**Figure 1.** Blank Spectra (Methanol) (X) and Standard Solution Spectra (Thiamphenicol in Methanol) (Y) at Wavenumbers 4000 $cm^{-1}$ to 650 $cm^{-1}$

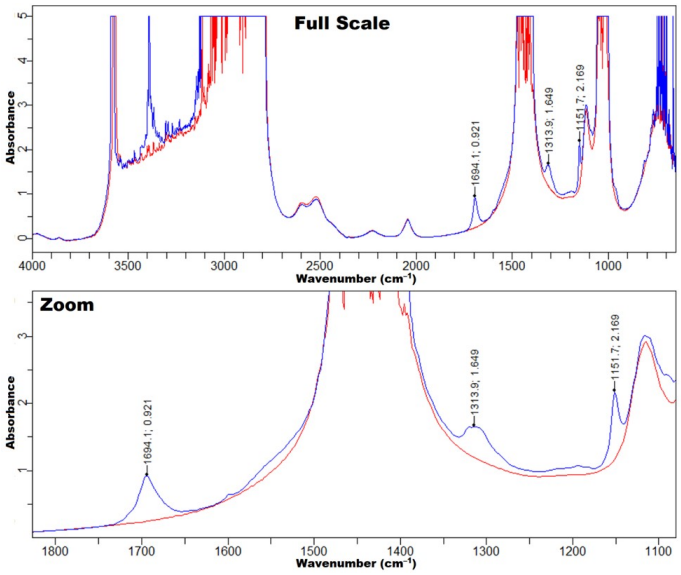


**Figure 2.** Overlay Blank Spectra (Methanol) Indicated by a Red Line (-) and Standard Solution Spectra (Thiamphenicol in Methanol) Indicated by a Blue Line (-) at Full Scale Wavenumbers 4000 $cm^{-1}$ to 650 $cm^{-1}$ and Zoom Wavenumbers 1800 $cm^{-1}$ to 1100 $cm^{-1}$

The specific peak of thiamphenicol is seen in the peak that appears in the standard solution spectra but does not appear in the blank spectra. In spectrophotometric analysis, the specific peak of the compound being analyzed is the peak that appears in the standard solution spectra but the peak does not appear in the blank spectra (Akbel et al., 2022). The overlapping analysis results show that most of the methanol absorption bands overlap with the thiamphenicol in methanol absorption bands except for the wavenumbers 1694.1 $cm^{-1}$, 1313.9 $cm^{-1}$, and 1151.7 $cm^{-1}$. The drug wavenumber at the specific peak of thiamphenicol is in accordance with the literature; the three main peaks (high peaks absorbance) of thiamphenicol is at wavenumbers around 1690 $cm^{-1}$, 1300 $cm^{-1}$, and 1140 $cm^{-1}$ (SpectraBase, 2023).

In this study, qualitative analysis and quantitative analysis of thiamphenicol were performed on the specific peaks of thiamphenicol at wavenumber 1694.1 $cm^{-1}$ because it had the greatest response (peak height and peak area). The peak with the greatest response will have the greatest sensitivity (Gardegaront et al., 2018), thus the wavenumbers used for qualitative analysis and quantitative analysis of thiamphenicol will be specific for thiamphenicol. The wavenumber 1694.1 $cm^{-1}$ represents the carbonyl group which is more specific for the amide group. These results are consistent with the thiamphenicol structure and in accordance with the literature, which states that the wavenumber for the carbonyl group is in the range of wavenumber between 1850 $cm^{-1}$ to 1650 $cm^{-1}$ and, more specifically for the amide group is at a wavenumber lower than 1700 $cm^{-1}$ (Nandiyanto et al., 2019). The peak position in

wavenumber 1694.1 $cm^{-1}$, which shows the difference between a solvent as a blank and solution of the compound being analyzed in a solvent as a standard solution, is the specific peak of the compound being examined and can be further used for qualitative analysis and quantitative analysis (Liao et al., 2022).

After it is determined that the specific peak of thiamphenicol is at the peak position with wavenumber 1694.1 $cm^{-1}$, then it can be further used for qualitative analysis. Specific peaks in the spectrophotometric method that appear at certain wavenumbers or at a certain wavelength are good markers and are used for the qualitative analysis of compounds analyzed in mixtures (Mabasa et al., 2021). The quantitative analysis of thiamphenicol using Fourier Transform Infrared was also carried out at the peak position with wavenumber 1694.1 $cm^{-1}$ using the quantitative measurement method in the form of peak height or peak area. The quantitative analysis of the spectrophotometric method can be carried out with good results and with quantitative measurements of peak height or peak area (Chrisikou et al., 2020).

### 3.2 Sample Analysis

The study was continued with measurements of series solutions (thiamphenicol solution with concentrations 0, 30, 35, 40, 45, 50, 55, 60, 65, and 70 mg/mL). The results were used to analyze the baseline, peak height, and peak area of the specific peak (position and wavenumber) of thiamphenicol for quantitative analysis. Figure 3 shows the overlay spectra of series solutions (thiamphenicol in methanol with various concentrations) at full scale wavenumbers 4000 $cm^{-1}$ to 650 $cm^{-1}$ and zoom wavenumbers 1800 $cm^{-1}$ to 1600 $cm^{-1}$.

The obtained baseline is in the range of 1733.21 $cm^{-1}$ to 1638.16 $cm^{-1}$, with a maximum position of 1694.08 $cm^{-1}$; peak height and peak area from the series solutions measurement results were further calculated to find the determination coefficient and regression equation. The determination coefficient was 0.9998 for peak height with the regression equation $Y = 0.013005 \times X + 0.078751$, and the determination coefficient was 0.9994 for peak area with the regression equation $Y = 0.404536 \times X + 8.677541$. The determination coefficient obtained was not less than 0.99, which meets the requirements for a good coefficient of determination (Sonawane et al., 2019). A well-defined determination coefficient indicates that the peak height and peak area can be used for concentration determination using the regression equation (Asthana et al., 2019).

The study revealed that for peak height and peak area, the intercept values were 0.078751 and 8.677541, while the slope values were 0.013005 and 0.404536. The intercept values obtained were higher than the slope values in the regression equation for both peak height and peak area. However, the relative magnitudes of intercept and slope do not necessarily indicate any issue with the model. In a linear regression equation, the intercept denotes the dependent variable's value when the independent variable(s) is equal to zero. At the same time, the slope indicates the change in the dependent variable for a one-unit increase in the independent variable. Therefore, the intercept and slope have different units of measurement and do not necessarily have a direct relationship (Chen and Chen, 2022).

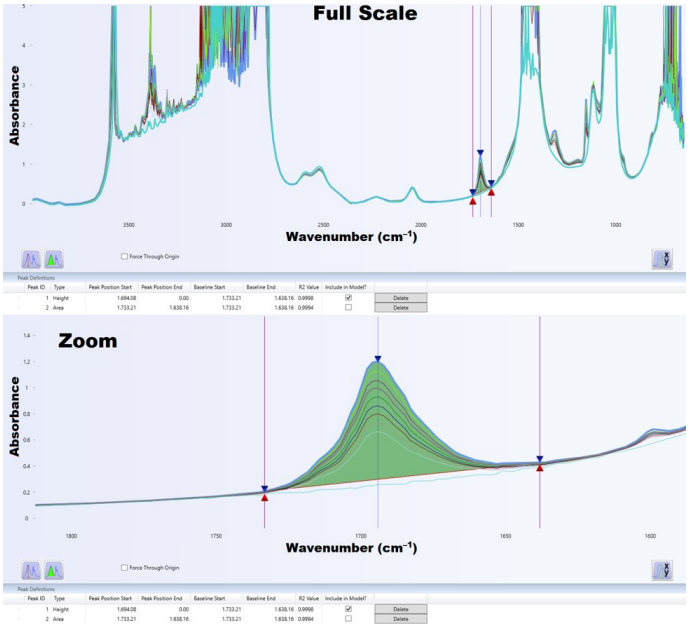


**Figure 3.** Overlay Spectra of Series Solutions (Thiamphenicol in Methanol with Various Concentrations) at Full Scale Wavenumbers 4000 $cm^{-1}$ to 650 $cm^{-1}$ and Zoom Wavenumbers 1800 $cm^{-1}$ to 1600 $cm^{-1}$

The quantitative analysis of the spectrophotometric method by peak height and peak area has a response that is proportional to the concentration; a greater concentration will give a greater peak height and peak area (Sadat and Joye, 2020). The developed method was applied for qualitative analysis and quantitative analysis of thiamphenicol in the capsule dosage form. The samples analyzed were thiamphenicol capsule dosage forms consisting of four generic names and six trade names. Table 1 shows the sample analysis results for qualitative analysis and quantitative analysis of the thiamphenicol capsule dosage form based on peak height and peak area.

The results showed that the thiamphenicol capsule dosage form contains thiamphenicol as the active pharmaceutical ingredient with a specific peak of thiamphenicol that appears at a specific position, namely at a wavenumber of around 1694.08 $cm^{-1}$. Qualitative analysis of the pharmaceutical dosage form is very important as an initial screening of pharmaceutical preparations before carrying out quantitative analysis to ensure quality, safety, and efficacy (Li et al., 2019; Srebro et al., 2022). The quantitative analysis of the thiamphenicol capsule dosage form shows thiamphenicol levels are in the range of 97.97% to 102.06% for the determination by using the peak height and the range of 98.08% to 102.24% for the determination by using the peak area. The level requirements for thiamphenicol in the thiamphenicol capsule dosage form are not listed in the compendial monograph, but the general requirement of a phar-

**Table 1.** Sample Analysis Results for Qualitative Analysis and Quantitative Analysis of the Thiamphenicol Capsule Dosage Form Based on Peak Height and Peak Area

| Sample | Qualitative Results | Quantitative Results | |
|---|---|---|---|
| | | Peak Height Results | Peak Area Results |
| Thiamphenicol Capsule (Phapros) | Pass | 100.01% ± 0.22% | 100.09% ± 0.24% |
| Thiamphenicol Capsule (Bernofarm) | Pass | 98.18% ± 0.42% | 98.29% ± 0.36% |
| Thiamphenicol Capsule (Pyridam Farma) | Pass | 102.06% ± 0.45% | 102.20% ± 0.34% |
| Thiamphenicol Capsule (Sanbe Farma) | Pass | 102.01% ± 0.30% | 102.13% ± 0.24% |
| Thiamex® Capsule (Novapharin) | Pass | 102.06% ± 0.40% | 102.24% ± 0.28% |
| Thiamycin® Capsule (Interbat) | Pass | 97.97% ± 0.43% | 98.08% ± 0.34% |
| Nikolam® Capsule (Meprofarm) | Pass | 100.96% ± 0.43% | 101.07% ± 0.37% |
| Zicafen® Capsule (Graha Farma) | Pass | 100.39% ± 0.33% | 100.42% ± 0.27% |
| Thianicol® Capsule (Dankos Farma) | Pass | 101.00% ± 0.31% | 101.11% ± 0.27% |
| Thislacol® Capsule (Metiska Farma) | Pass | 99.01% ± 0.45% | 99.11% ± 0.40% |

maceutical dosage form for the assay cannot be less than 90.0% and no more than 110.0% of the amount stated on the label (Canada, 2018).

### 3.3 Method Validation

From the results, it can be seen that all of the thiamphenicol capsule dosage forms met the general requirements for the assay. The spectrophotometric Fourier Transform Infrared has been successfully developed and has been successfully applied for qualitative analysis and quantitative analysis of thiamphenicol from thiamphenicol capsule dosage form, followed by the stages of validation of the analytical method. Analytical method validation is an effort to prove through laboratory experiments a series of parameters to ensure that the analytical method that has been developed or modified is suitable for its purpose (Shrivastava et al., 2018). Table 2 shows the method validation results for thiamphenicol analysis based on peak height and peak area.

Validation of the analytical method was carried out on a series of parameters. The accuracy parameter obtained a recovery percentage value of 100.28% for peak height and 100.41% for peak area. The recovery percentage value obtained met the requirements for accuracy parameters, which is between 98.0% to 102.0% (Sudarman and Haris, 2023). The precision parameter obtained a relative standard deviation value of 0.31% for peak height and 0.37% for peak area. The relative standard deviation value obtained met the requirement for the precision parameter, which is not more than 2.0% (Bui et al., 2021). The linearity parameter obtained a correlation coefficient value of 0.9999 for peak height and 0.9997 for peak area. The correlation coefficient value obtained met the requirements for the linearity parameter, which is not less than 0.99 (Matraszek Żuchowska et al., 2022).

Specifically, the limit of detection values was 0.9396 mg/mL and 1.6880 mg/mL for peak height and peak area, respectively. These values were approximately 30-54 times lower than the 50 mg/mL target concentration. On the other hand, the limit of quantitation values was 2.8472 mg/mL and 5.1153 mg/mL for peak height and peak area, respectively. These values were around 10-18 times lower than the 50 mg/mL target concentration. The method was found to have a high sensitivity, as evidenced by the very low limit of detection and limit of quantitation values obtained Namegabe et al. (2022) this suggests that the Fourier Transform Infrared method used in the study had a high sensitivity.

The results of the analytical method validation for the range parameter obtained a value ranging from 80% to 120%. The parameter range is the lowest concentration limit and highest concentration limit, which has good accuracy, precision, and linearity, as well as proposed based on the intended use, which is determining drug levels in finished products that are 80% to 120% (Lavanya et al., 2020). Regarding the specificity parameter, the results obtained are stated as passed, and these results are inferred from various validation parameters (accuracy, precision, linearity, limit of detection, limit of quantitation, and range). The specificity parameter of an analytical method is the ability to specifically measure the analyte in the presence of other components that might be expected to be present in the sample medium, thus producing a response for only a single analyte. In the assay, the specificity parameter was used to provide accurate and precise results on the levels or potency of the analyte in the sample (Chavan and Desai, 2022).

The thiamphenicol analysis in drug dosage form by ultraviolet spectrophotometry shows recovery percentage 99.91%, relative standard deviation 0.65%, correlation coefficient 0.9975, limit of detection 0.59 $\mu$g/mL and limit of quantitation 1.99 $\mu$g/mL (Martins and De Oliveira, 2019). The thiamphenicol analysis by high-performance liquid chromatography shows recovery percentage > 95%, relative standard deviation value < 10%, determination coefficient > 0.99, limit of detection value 0.01 $\mu$g/kg, limit of quantitation value 0.02 $\mu$g/kg (Ye et al., 2022). The validation results of the analytical method of the Fourier transform infrared method that has been developed in this study are compared to the ultraviolet spectrophotometry method and high-performance liquid-chromatography

**Table 2.** Method Validation Results for Thiamphenicol Analysis Based on Peak Height and Peak Area

| Parameter | Validation Results | |
|---|---|---|
| | Peak Height | Peak Area |
| Average Specific Range 80% | 100.64% ± 0.22% | 100.84% ± 0.17% |
| Recovery Specific Range 100% | 99.99% ± 0.27% | 100.01% ± 0.23% |
| Percentage Specific Range 120% | 100.21% ± 0.26% | 100.38% ± 0.20% |
| Accuracy (Recovery Percentage) | 100.28% | 100.41% |
| Precision (Relative Standard Deviation) | 0.31% | 0.37% |
| Linearity (Correlation Coefficient) | 0.9999 | 0.9997 |
| Limit of Detection (mg/mL) | 0.9396 | 1.6880 |
| Limit of Quantitation (mg/mL) | 2.8472 | 5.1153 |
| Range | 80% to 120% | 80% to 120% |
| Specificity | Passed | Passed |

method that previous researchers have reported. The results of the comparison show that the Fourier transform infrared method has equivalent accuracy, precision, and linearity compared to the ultraviolet spectrophotometry method and the high-performance liquid-chromatography method.

## 4. CONCLUSION

The Fourier Transform Infrared method has been successfully developed for thiamphenicol analysis. The specific peak of thiamphenicol was obtained at a peak maximum of 1694.08 $cm^{-1}$ with a baseline between 1733.21 $cm^{-1}$ to 1638.16 $cm^{-1}$. The developed method has also been successfully applied for qualitative analysis and quantitative analysis of thiamphenicol in the capsule dosage form, ranging from 97.97% to 102.24%. All the thiamphenicol in the capsule dosage form with a generic name and trade name met the general requirements of a pharmaceutical dosage form for the assay, namely not less than 90.0% and not more than 110.0% of the amount stated on the label. The Fourier Transform Infrared method has been successfully validated for thiamphenicol analysis. Respectively for peak height and peak area, the accuracy parameter resulted in a recovery percentage of 100.28% and 100.41%, the precision parameter resulted in a relative standard deviation of 0.31% and 0.37%, and the linearity parameter resulted in a correlation coefficient of 0.9999 and 0.9997. Respectively for peak height and peak area, the limit of detection value obtained was 0.2971 mg/mL and 0.5338 mg/mL, the limit of quantitation value obtained was 0.9004 mg/mL and 1.6176 mg/mL, the range for both was 80% to 120%, and the specificity for both met the requirements.

## 5. ACKNOWLEDGMENT

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