DEVELOPMENT AND VALIDATION OF FTIR SPECTROPHOTOMETRY TO IDENTIFY AND DETERMINE CHLORAMPHENICOL IN MARKETING CAPSULES

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ABSTRACT

Chloramphenicol is an antibiotic compound that inhibits bacterial protein synthesis. All pharmaceuticals in the form of finished goods and raw materials must meet the requirements of Indonesian Pharmacopoeia or other standard books. The objectives of this study were to develop and validate a simple and fast analytic method by Fourier Transform Infrared (FTIR) spectrophotometry that allows for qualitative and quantitative analysis of chloramphenicol levels in capsule preparations. Chloramphenicol levels were determined by FTIR analysis using methanol solvents with 0.1 mm slit in the wavenumber range 4000 cm⁻¹ to 650 cm⁻¹. The specific wavenumbers of chloramphenicol solution in methanol were 1695.9 cm⁻¹, 1526.3 cm⁻¹, and 1351.2 cm⁻¹. Peak height analysis was not significantly different from peak area analysis, and all samples analyzed fulfilled the requirements of the Indonesian Pharmacopoeia, 6th Edition (2020). Analytical validation met requirements for accuracy, precision, linearity, the limit of detection, the limit of quantitation, range, and specificity. The study concludes that identification and determination of chloramphenicol can be conducted by FTIR with simple preparation and fast results.

Keywords: Development, Validation, Identification, Determination, Fourier Transform Infrared (FTIR), Chloramphenicol

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INTRODUCTION

Chloramphenicol is an antibiotic compound that was isolated from *Streptomyces venezuelae* in 1947; it inhibits bacterial protein synthesis.¹ This compound is generally bacteriostatic and bactericidal to various types of gram positive and gram negative bacteria. It is usually used in the form of capsules because it tastes very bitter.² Chloramphenicol is used in the treatment of typhoid fever because it is effective, inexpensive, and can be administered orally.³

All pharmaceutical preparations and raw materials must meet the requirements of the Indonesian Pharmacopoeia or other standard books. Quality control ensures that manufactured drugs meet quality requirements in accordance with their intended use.⁴ According to the Indonesian Pharmacopoeia (6th edition), chloramphenicol capsules should contain no less than 90.0% and no more than 120.0% of the amount stated on the label.⁵

Identification and determination of chloramphenicol in capsules are generally conducted using high-performance liquid chromatography, however, the method is expensive, time-consuming, and more complicated than other techniques.⁶ Thus, it is necessary to develop methods for chloramphenicol analysis that are cheaper, simpler, and easier to perform. FTIR is a suitable method for drug identification and determination because it provides good sensitivity, is simple to prepare, and is quickly measured.⁷

FTIR in the pharmaceutical industry is very popular as a qualitative and quantitative tool because it requires simple sample preparation and allows for non-destructive measurement. Such features are optimal for monitoring the quality and quantity of raw materials and finished goods.⁸ FTIR also minimizes environmental impacts, such as industrial chemical waste, because it does not require many solvents.⁹ This study aimed to develop a simpler, cheaper, faster, and more environmentally friendly method for qualitative and quantitative analysis of chloramphenicol in capsule preparations using the Fourier Transform Infrared spectroscopic method.

EXPERIMENTAL

Tools

In this study, the researchers used a complete Fourier Transform Infrared Spectrophotometer with Dialpath (Agilent - Cary 630), Personal Computer (Lenovo) with Microlab (Agilent) and Statistical Package for the Social Sciences (International Business Machines), Printer (Canon), Analytic Balance (Sartorius), filter paper (Whatman), Tissue Paper (Paseo), Electronic Pipette (Eppendorf - Multipette E3X), Sonicator (Krisbow), and Glassware (Iwaki).

Materials

The researchers used Methanol Analytical Grade (Merck), Chloramphenicol Working Standard (Mutuara Mukti Farma), Chloramphenicol capsules (Holi Pharma), Chloramphenicol capsules (Kimia Farma), Chloramphenicol capsules (Mutuara Mukti Farma), Etacemycin[®] capsules (Errita Pharma), Kalmicetine[®] capsules (Kalbe Farma), Colsancetine[®] capsules (Sanbe Farma), Hufamycin[®] capsules (Gratia Husada Farma), Erbacetine[®] capsules (Mutuara Mukti Farma).

Sampling

Sampling was done by purposive sampling without comparing one characteristic to another, as the sample is considered homogeneous.

Qualitative and Quantitative Analysis of Chloramphenicol

The identification and the determination of chloramphenicol in marketed capsules was modified from methods described in Robaina et al., 2013. The analytical procedures include preparation of the stock solution, preparation of absorption spectrum, preparation of the calibration curve, and determination of chloramphenicol capsule levels.¹⁰

Preparation of Stock Solution

Chloramphenicol working standard was weighed at 2.5 g and inserted into a 50 mL volumetric flask to which 30 mL methanol was added. The result was sonicated for 15 minutes and methanol was added to the marked line. The flask was shaken until homogeneous (concentration of chloramphenicol was 50 mg/mL).

Preparation of Absorption Spectrum

5mL stock solution was pipetted into a 10 mL volumetric flask, to which methanol was added to the marked line. The flask was shaken until homogenous (concentration of chloramphenicol was 25 mg/mL). The absorbance of the solution in methanol was measured between wavenumber 4000 cm⁻¹ to 650 cm⁻¹. The spectrum was overlaid to obtain the specific peak of chloramphenicol.

Preparation of Calibration Curve

Standard solutions of chloramphenicol were prepared in a 10 mL volumetric flask to obtain a concentration series from 5 to 45 mg/mL in 5 mg intervals. This was achieved by pipetting 1 to 9 mL of chloramphenicol stock solutions in 1 mL intervals, adding methanol to the marked line, shaking until homogenous, and measuring the spectrum for 11 concentrations from 0 to 50 mg/mL. (The concentration 0 mg/mL used methanol and the 50 mg/mL used chloramphenicol stock solution). Researchers analyzed the height and area at the chloramphenicol specific peak, then calculated the determination coefficient and regression equation.

Determination of Chloramphenicol Capsule Levels

Twenty capsules were weighed, mixed until homogenous, made equivalent to 250 mg chloramphenicol, and inserted into a 10 mL volumetric flask. 6 mL methanol was added, and the result was sonicated for 15 minutes. Methanol was added to the marked line and shaken until homogenous. The mixture was filtered to remove 1 mL of the first filtrate. The following filtrate was collected, (the predicted concentration of chloramphenicol was 25 mg/mL). Researchers then measured the spectrum, calculated the height at the chloramphenicol specific peak, and calculated the chloramphenicol level in capsule preparations using the obtained regression equation.

Validation of the Method

Validation of FTIR analysis was modified from procedures by Nugrahani and Mussadah, 2016; and Sopyan et al., 2020. Method accuracy was measured by the percentage of recovery in three specific ranges (80%, 100% and 120%) with 70% of the analyzed sample and 30% of chloramphenicol working standard added (standard addition method). Precision by relative standard deviation was calculated from series of accurate data. Linearity by a correlation coefficient, detection limits, and quantitation limits was calculated from the calibration curve data series. The range was analyzed by concentration, indicating accuracy, precision, linearity, the limit of detection, and the limit of quantitation. Method specificity was analyzed by qualitative results from the sample analysis.^{11,12}

RESULTS AND DISCUSSION

Determination of Chloramphenicol Specific Wavenumber

Research began by determining the absorption spectrum of methanol. The methanol absorption spectrum can be seen in Fig.-1.

Fig.-1: Methanol Absorption Spectrum

The chloramphenicol absorption spectrum with a concentration of 25 mg/mL in methanol was then determined. The chloramphenicol absorption spectrum with a concentration of 25 mg/mL in methanol can be seen in Fig.-2.

Fig.-2: Chloramphenicol Absorption Spectrum with a Concentration of 25 mg/mL in Methanol

Infrared spectrophotometry can be used for qualitative analysis, wherein each molecule produces a different absorption spectrum.¹³ The specific absorption wavenumber of chloramphenicol from methanol is achieved by overlaying the spectra of methanol and chloramphenicol in methanol. The overlaid spectra are in Fig.-3.

Fig.-3: Display of Overlapping Spectra of Methanol and Chloramphenicol Absorption (25 mg/ml) in Methanol

There is a significant difference between the absorption spectrum of methanol and chloramphenicol in methanol, as shown at wavenumbers 1695.9 cm^{-1} , 1526.3 cm^{-1} , and 1351.2 cm^{-1} . The wavenumber is specific to chloramphenicol and not to methanol¹⁴. Thus, qualitative and/or quantitative analysis can be carried out at these wavenumbers. The peaks at wavenumbers 1526.3 cm^{-1} and 1351.2 cm^{-1} was very unique for chloramphenicol, thus they are best suited for qualitative testing. The peak at wavenumber 1695.9 cm^{-1} has the highest peak height, indicating it is best suited for quantitative determination.

Determination of Chloramphenicol Regression Equation

The absorption spectrum of chloramphenicol with a series concentration (0 mg/mL to 50 mg/mL) in methanol can be seen in Fig.-4.

Fig.-4: Absorption Spectrum of Chloramphenicol with Series Concentration (0 mg/mL to 50 mg/mL) in Methanol

The spectrum of chloramphenicol at various concentrations in methanol shows that concentration does not change the shape of the spectrum, but only changes the peak absorbance (peak height and peak area). The graph indicates that higher concentrations result in higher and larger peaks. Thus, peak height and area can be used as the basis for quantitative analysis.¹⁵ The chloramphenicol regression equations obtained were $Y = 0.01498 X + 0.09785$ for peak height and $Y = 0.50454 X + 13.95957$ for peak area range. The chloramphenicol determination coefficients obtained were 0.999 for peak height and 0.997 for peak area.

Qualitative Identification and Quantitative Determination of Chloramphenicol in Capsules

The qualitative identification and quantitative determination of chloramphenicol in capsules, with calculations by peak height and peak area seen in Table-1.

Table-1: Qualitative Identification and Quantitative Determination of Chloramphenicol in Capsules

Samples	Qualitative	Quantitative	
		Peak Height	Peak Area
Chloramphenicol capsules (Holi Pharma)	Passed	102.22% ± 0.92%	101.88% ± 0.99%
Chloramphenicol capsules (Kimia Farma)	Passed	100.75% ± 0.91%	100.21% ± 0.97%
Chloramphenicol capsules (Mutiar Mukti Farma)	Passed	101.38% ± 0.91%	100.67% ± 0.98%
Etagemycetin [®] capsules (Errita Pharma)	Passed	99.88% ± 0.90%	100.26% ± 0.97%
Kalmicetine [®] capsules (Kalbe Farma)	Passed	101.24% ± 0.91%	100.84% ± 0.98%
Colsancetine [®] capsules (Sanbe Farma)	Passed	102.19% ± 0.92%	101.83% ± 0.99%
Hufamycetin [®] capsules (Gratia Husada Farma)	Passed	100.56% ± 0.91%	99.93% ± 0.97%
Erbacetine [®] capsules (Mutiar Mukti Farma)	Passed	98.21% ± 0.88%	99.04% ± 0.96%

Validation of Developed Method

The average percentage of the recovery obtained was 100.06% ± 0.59% based on peak height calculation and 100.09% ± 0.63% based on peak area calculation. The results for accuracy met the requirements of the average percentage of recovery between 98% to 102%.^{16,17} The relative standard deviation of chloramphenicol obtained was 0.59% based on peak height calculation and 0.63% based on peak area calculation. Results for precision met requirements for a relative standard deviation less than 2%.^{18,19}

The chloramphenicol correlation coefficient obtained was 0.999 based on peak height calculation and 0.999 based on peak area calculation. The correlation coefficient value of the r table was 0.847, calculated with a confidence level of 99.9% with 11 series concentrations (at 9 degrees of freedom. The correlation coefficient value was not less than 0.847, showing that there was a linear correlation between chloramphenicol concentration and response (peak height or peak area).²⁰ The correlation coefficient value obtained indicated a very strong correlation with a value greater than 0.99.^{21,22}

The limit of detection for chloramphenicol was 0.663 mg/mL based on peak height calculation and 0.980 mg/mL based on peak area calculation. The limit of quantitation for chloramphenicol was 2.010 mg/mL based on peak height calculation and 2.969 mg/mL based on peak area calculation. The concentration used for the analysis was 25 mg/mL, which is much higher than the detection limit value and quantitation limit value. Thus, it can be concluded that chloramphenicol can be detected with this method at a concentration of 25 mg/mL. Furthermore, chloramphenicol can be quantitated at a concentration 25 mg/mL; the method has a low enough detection limit value for qualitative analysis and has a low enough quantitation limit value for quantitative analysis.^{23,24}

The range of analysis - accuracy, precision, linearity, the limit of detection, and the limit of quantitation - ranged from 20 mg/mL (80%) to 30 mg/mL (120%), which covers the literature requirement.^{25,26} Via FTIR,

the specific peaks of chloramphenicol were detected from various chloramphenicol capsules and good qualitative results with different matrices were achieved. This indicates appropriate specificity using the FTIR analytical method.^{27,28}

CONCLUSION

Identification qualitative testing and determination by quantitative testing of chloramphenicol capsules can be achieved by Fourier Transform Infrared spectrophotometry. The method uses simple sample preparation and achieves fast results. The qualitative and quantitative tests of chloramphenicol samples in marketed capsule preparations correctly identified and determined the presence of chloramphenicol, fulfilling the requirements in the Indonesian Pharmacopoeia for chloramphenicol content in capsule preparations. The developed method has been validated and shows appropriate results for the accuracy, precision, linearity, limit of detection, the limit of quantitation, range, and specificity required.

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