Identification of antibiotic by chromatographic and spectrophotometric methods – A Review

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Abstract
The purpose of this article to reviwe the validation and content of antibiotics using various chromatographic and spectrophotometric methods. This review article used five analytical methods on antibiotics, namely high-performance liquid chromatography (HPLC), fourier transform infrared (FTIR), gas chromatography (GC), ultraviolet visible (UV-Vis) spectrophotometer and liquid chromatography/mass spectrophotometer (LC/MS) methods. Antibiotics are the most widely used drugs for infections caused by bacteria. Based on review of several studies that related to antibiotic analysis the general methods applied are HPLC, FTIR, GC, UV-Vis Spectrophotometry and LC-MS. Analysis of this antibiotic is a huge challenge due to its sensitivity and instability to various conditions.

Keywords: Antibiotics, Chromatography, Drugs, Spectrophotometry

Introduction
The relatively high intensity of use of antibiotics causes various problems and is a global threat to health, especially bacterial resistance to antibiotics. In addition to having an impact on morbidity and mortality, it also has a very high negative economic and social impact. Resistance initially occurred at the hospital level but gradually developed in the community, especially Streptococcus pneumoniae, Staphylococcus aureus, and Escherichia coli [1-3].

Antibiotics are the most widely used drugs for infections caused by bacteria. Various studies have found that about 40-62% of antibiotics are used inappropriately, among others, for diseases that do not actually require antibiotics. In research on the quality of antibiotic use in various parts of the hospital, it was found that 30% to 80% were not based on indications [4-6].

Pharmacokinetics and pharmacodynamics of antibiotics
Pharmacokinetics (PK) discusses the course of antibiotic levels in the body, while pharmacodynamics (PD) discusses the relationship between these levels and the effect of antibiotics. In the past, antibiotic doses were only determined by PK parameters. However, it turns out that PD also plays the same, or even more important, role. In this century of increasing antibiotic resistance, PD is even more important, because these parameters can be used to design dosing regimens that fight or prevent resistance. Thus, although clinical efficacy and safety remain the gold standard for comparing antibiotics, pharmacokinetic and pharmacodynamic measures have been increasingly used. Some measures of PK and PD are more predictive of clinical efficacy [7-10].

Factors to Consider in the Use of Antibiotics.
Factors Resistance of Microorganisms Against Antibiotics, Resistance is the ability of bacteria to
neutralize and weaken the action of antibiotics. Pharmacokinetic and Pharmacodynamic Factors. An understanding of the pharmacokinetic and pharmacodynamic properties of antibiotics is needed to determine the type and dose of antibiotics correctly. Interaction Factors and Side Effects of Drugs. Concurrent administration of antibiotics with other antibiotics, other drugs or food can cause unexpected effects. The effects of interactions that can occur are quite diverse, ranging from mild such as decreased drug absorption or delayed absorption to increased toxic effects of other drugs. For example, coadministration of ciprofloxacin with theophylline can increase theophylline levels and may increase the risk of cardiac arrest or permanent brain damage [11-15].

Likewise, administration of doxycycline along with digoxin will increase the toxic effect of digoxin which can be fatal for the patient. Cost Factors. Antibiotics available in Indonesia can be in the form of generic drugs, trademark drugs, originator drugs or drugs that are still under patent protection (patent drugs). The price of antibiotics also varies. The price of antibiotics with the same content can differ up to 100 times more expensive than the generic ones. Especially for parenteral preparations which can be 1000 times more expensive than oral preparations with the same content. Expensive antibiotic prescriptions, with prices beyond the patient's financial capacity will have an impact on patients not buying antibiotics, resulting in therapy failure. No matter how precise the antibiotics are prescribed, if they are far from the level of the patient's financial capacity, it will certainly not be useful [16-20].

Analytical Approaches

There several analytical approaches are elaborated in the review paper. In this manuscript, it will elaborate about the advantages and disadvantages in various methods.

High Performace Liquid Chromatography (HPLC)

HPLC methods are the most common method for the analysis of cephradine in formulation, and in biological fluids, the several analytical procedures have been described for the analysis of cephradine in different pharmaceutical formulations. HPLC-UV methods are fast, but it requires elevated temperature, it may cause thermal degradation of drugs, and to avoid that, it requires derivatization to improve volatility and to improve chromatographic behavior. Hence, these methods are not applicable for antibiotics. the method HPLC-UV has very low detection limits so this technique has the ability to estimate the archeological samples from pharmaceutical drugs. HPLC can be a valuable tool in the analysis and evaluation of pharmaceutical, organic, and high-volatility samples and for this reason can be used in many pharmaceutical research. Many antibiotics contain ionizable group which can be analyzed by ion exchange chromatographic methods. The high-resolving power of HPLC serves as a particularly important method for the isolation and purification of antibiotic. There are various HPLC methods reported for the analysis of a single cephradine in pharmaceutical drugs. All these methods present a unique preparatory and chromatographic protocol. Several methods have been used for the analysis of cephradine which analyzes in HPLC method that is very accurate and sensitive [21-23].

This article is extended to know the HPLC method. The analysis of these antibiotics is a challenge because of their sensitivity and instability to different conditions. HPLC-UV system with Zorbax 300SCX Agilent Column Ion Pac column; 5μm, 4.6x250 mm was used for the analysis of Cephradine in capsule formulation. The analytical performance that studied done by HPLC-UV method for separation and estimation of the cephradine sample, performed through some factors such as the column type, eluent, concentration, tR, and temperature effect, these factors directly affect the accuracy and precision of the results that obtained from the HPLC measurements for different concentrations of the cephradine sample. The results showed Cephradine as a capsule formulation is almost stable at room temperature for up to 2-3 days in aqueous media at a pH between 4 and 5 [24-26].

Fourier Transform Infrared (FTIR)

Infrared spectroscopy, also known as vibrational spectroscopy, is one of the standard methods used in pharmaceutical and chemical analysis, which provides images of the vibrations of atoms in compounds. One method of infrared
spectroscopy is to use the FTIR tool. FTIR is an analytical method that can be used to characterize samples in liquid, solution, and powder forms. Analysis using FTIR does not require the addition of dyes or labels for visualization of the different chemical components in the sample. FTIR measures all wavelengths simultaneously to form a transmittance or absorbance spectrum. One type of FTIR that can be used for quantification of Ceftriaxone Sodium is ATR. ATR is an analytical technique based on molecular vibrations and the bending of light when passing through different media. FTIR ATR has the advantage that it only requires a small sample to be analyzed. The spectrum produced in the analysis using FTIR is a spectrum that is in the wavelength range of 4000 – 400 cm. Ceftriaxone sodium analysis can be done using FTIR-ATR by calculating the peak area of the spectrum of the resulting carbonyl group, which is in the wavelength range of 1800 - 1700 cm⁻¹ [27-30].

Ceftriaxone is a third-generation cephalosporin-derived antibiotic. Quantitative analysis of ceftriaxone raw materials in ceftriaxone sodium preparations generally uses the HPLC method. The HPLC method relatively uses non-environmentally friendly solvents, therefore an approach using the FTIR-ATR spectroscopy method is carried out. The advantages of this method are relatively faster in preparation and sample analysis time compared to the HPLC method, low cost and using environmentally friendly solvents. The method validation is determined based on the parameters of linearity, selectivity, precision, accuracy, detection limit and quantification limit. Based on the validation results obtained linearity with a coefficient of determination of 0.9992 in the concentration range of 300-800 mg/mL, detection limit of 20.02 mg/mL, and quantification limit of 60.68 mg/mL. Thus, this method can be used as a recommendation as analytical methods in the Indonesian Pharmacopoeia [31-33].

Gas Chromatography (GC)

Static headspace gas chromatography (SH-GC) is the technique of choice due to its high sensitivity, excellent separation abilities, low limit of detection and simplicity of the instrumentation used for the technique. The static headspace (HS) sampling method has more appropriate sensitivity than the direct injection method because it can clearly separate volatile analytes from the sample matrix and effectively concentrate them. Therefore, this method results in less complex sample preparation, decreased instrument contamination, and increased GC column life. To our knowledge there is no validated SH-GC method available for analysis of residual solvents in commercially available tablets of augmentin (i.e., amoxicillin) and principen (i.e., ampicillin). The supplier claims that three common class II residual solvents e.g., methanol, dichloromethane, and toluene are existed in these tablets. In order to secure the safety and assure good manufacture practices (GMP), a precise quantification of residual solvents is essential. In the present study, we report development and full validation of a novel SH-GC analytical method with FID detector for simultaneous determination of methanol, dichloromethane, and toluene. The validation was made according to ICH guidelines in terms of several parameters e.g., specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and solution stability. Besides, the method is also applied to determine the residual solvents in both amoxicillin and ampicillin tablets obtained from local market. These solvents should be estimated and checked so that they may not exceed the amount specified by the ICH guidelines. The developed method is simple and sensitive and could be useful for rapid routine analysis of the level of residual solvents in other drug substances [34-36].

Simple and sensitive static head space gas chromatographic (SH-GC) method equipped with FID has been developed and validated for simultaneous determination of residual solvents e.g., methanol, dichloromethane and toluene in two therapeutic drugs such as amoxicillin and ampicillin. The separation was achieved with 30 m long Elite - 5 fused silica capillary column and 0.32 mm inner diameter. The developed SH-GC method offered symmetric peak shape, good resolution and reasonable retention time for all the solvents. Beer’s law was obeyed in the concentration ranges 100 – 1200, 50 – 1000 and 50 – 500 ppm for methanol, dichloromethane and toluene, respectively. The method was validated according to international conference on harmonization (ICH) guidelines in terms of specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and solution stability. The degrees of linearity of the calibration curves, the percent recoveries, relative standard deviation for the method were also
determined. All the validation parameters were within the acceptable range. The developed SHT-GC method could, therefore, be suitable for simple and rapid detection of trace levels residual solvents in other pharmaceutical products and thereby it could be used for routine analysis in any analytical laboratory [37-40].

**Ultraviolet-Visible (UV) Spectrophotometry**

The analysis method with ultraviolet-visible spectrophotometry that already exists is based on the absorbance value. Determination of the concentration of cefadroxil using ultraviolet spectrophotometric method using a solvent mixed with methanol and distilled water (50:50) with a maximum wavelength of 264 nm. The linearity of cefadroxil was observed in the range of 10 g/mL to 50 g/mL with a correlation coefficient of 0.9999. This method was validated for the parameters of accuracy, precision, linearity, specificity, robustness, and ruggedness. From this method obtained a valid analysis for the analysis of cefadroxil spectrophotometrically [41-43].

Research on the development and validation of cefadroxil capsule analysis method has been done by ultraviolet spectrophotometry. This research was conducted by measuring analytical solution with hydrochloric acid (0.1 N HCl) as the selected solvent. The linearity of cefadroxil was shown at concentrations of 8 – 16 ppm with coefficient values on the absorbance method and the area under the curves 0.9999 and 0.9974 respectively. The determination of sample rate by using absorbance method and the area under the curve respectively on generic cefadroxil capsule were 99.31 % and 98.11 % and cefadroxil capsule with Cefat trademark were 101.38 % and 98.54 %. The average yield of percent recovery on generic cefadroxil capsule was obtained by absorbance method and the area under the curve was 101.91 % and 98.63 % and cefadroxil capsule with Cefat trademark were 99.88 % and 100.28 %. The result of this study indicates that the levels of cefadroxil sample used in capsule preparation meet the requirement according to Indonesian Pharmacopoeia 5 th edition 2014 is 90 % - 120 %. From the data above that the absorbance method and the area under the curve is valid method [44-46].

**Liquid Chromatography-Mass Spectrometry (LC/MS)**

Liquid Chromatography Mass Spectrometry (LC/MS-MS) is an analytical technique that combines the physical separation capabilities of liquid chromatography with the detection specificity of mass spectrometry. Liquid chromatography separates the components of the sample and then charged ions are detected by a mass spectrometer. LC-MS data can be used to provide information about the molecular weight, structure, identity and quantity of certain sample components. Compounds are separated on the basis of their relative interactions with the chemical layers of the particles. The advantage of LC-MS is that it can analyze a wider range of components, such as thermal compounds. labile, high polarity or high molecular mass, even proteins. The elution component of the chromatographic column is then transmitted to the mass spectrometer through a special interface. The principle is the separation of analytes based on their polarity, the apparatus consists of a column (as the stationary phase) and a certain solution as the mobile phase and high pressure is used to push the mobile phase. The analyte mixture will separate based on its polarity and the speed to get to the detector (retention time) will be different, this will be observed in a spectrum where the peaks are separated. The liquid mobile phase pump aid is flowed through the column to the detector. The sample is introduced into the mobile phase stream by injection. In the column there is a separation of the components of the mixture, due to the difference in the strength of the interaction between the solution and the stationary phase. The solution with less strong interaction with the stationary phase will leave the column first. On the other hand, a strong solution interacts with the stationary phase so that the solution will leave the column, which is then detected by the detector and recorded in the form of a chromatogram [47-50].

Antibiotic residues in milk are a major health threat for the consumer and a hazard to the dairy industry, causing significant economic losses. This study aims to assess the presence of antibiotic residues in raw milk comparatively by a rapid screening test (BetaStar ® Combo) and Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS). A total of 445 samples were collected from 3 dairy companies of north-central Algeria (Algiers, Blida, Boumerdes),
and they were rapidly screened for β-lactams and tetracyclines; 52 samples, comprising 34 positive tanker truck milk and 18 negative bulk milk were tested by LC-MS/MS, which revealed 90.4% were contaminated (n = 47) and 55.3% exceeded the Maximum Residue Limit (MRL). The β-lactams as parent compounds and their metabolites were the most frequently detected with maximum value for cloxacin (1231 µg/kg) and penicillin G (2062 µg/kg). Under field condition, the false-positive results, particularly for tetracyclines, seems to be related to milk samples displaying extreme acidity values (≥19-D) or fat-level fluctuations (2.7 g/100 mL and 5.6–6.2 g/100 mL). Despite a relatively low prevalence (7.64%) of residues using the rapid test, the detection by LC-MS/MS of flumequine (52 µg/kg), cefaclor (maximum 220 µg/kg) and metabolites of β-lactams at high levels should lead to reflections on the control of their human and environmental toxicological effects [51-55].

Conclusions

Quantitative analysis on antibiotics was carried out using many approaches that have been elaborated above. Among all methods mentioned, HPLC was chosen because it is selective and efficient. HPLC can be performed directly on quantitative analysis of antibiotics without extraction antibiotics. This can be done because the infusion component does not have a chromophore group. So, infusion will not be detected by the Spectrophotometer as a detector. The Spectrophotometer can be used as a detector, because most antibiotics have a chromophore (aromatic compounds, carbonyl, carboxylate, and amido) which are easy to detect.

Conflicts of interest

There are no conflicts to declare.

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