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REVIEW ARTICLE

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Azithromycin Determination with Various Analytical Techniques: A Mini Review

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Abstract

Azithromycin (AZT) belongs to a family of macrolides with bacteriostatic activity against many Gram-positive and Gram-negative bacteria. The aforementioned drugs are of great importance due to the high rate of resistance to other antibiotics. Therefore, numerous research studies have been conducted to identify azithromycin and its metabolites as they contribute to the drug's biological activity. Liquid chromatography, gas chromatography, and other separation techniques have been used to develop, adapt, and implement analytical techniques for drug analysis. This brief overview aims to highlight the analytical techniques and new techniques implemented for Azithromycin analysis.

Keywords: Azithromycin; detection; Chromatography; electrochemical.

1. Introduction

Azithromycin (Figure1) is a broad-spectrum macrolide antibiotic with a long half-life and high tissue penetration (McMullan BJ et al., 2015). It was originally approved by the FDA in 1991 (Fohner AE et al., 2017). Azithromycin [9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin] is part of the azalide subclass of macrolides and contains a 15-membered ring containing a methyl-substituted nitrogen in place of the carbonyl group at position 9a on the aglycon ring allowing prevention of its metabolism. This distinguishes azithromycin from other types of macrolides (Fohner AE et al., 2017). It is primarily used to treat respiratory, intestinal, and genitourinary infections and can replace other macrolides in some sexually transmitted and intestinal infections (Peters DH et al., 1992). Its mechanism of action is to bind to the 23S Rrna of

the bacterial 50S ribosomal subunit. It stops bacterial protein synthesis by inhibiting the transpeptidation/translocation step of protein synthesis and inhibiting assembly of the 50S ribosomal subunit (Champney WS. et al., 2002).Metabolism takes place mainly in the liver (for inactive metabolites), with biliary excretion being an important elimination pathway. The drug he disappears in two stages, with a terminal half-life of up to 5 days (Drew RH et al., 1992). Due to its importance and the greatest need for such drugs, various types of methods such as microbiological, UV-Vis spectrophotometry and liquid chromatography combined with different detectors such as fluorescence, electrochemical, mass, etc. Multiple techniques have been employed to determine azithromycin in samples.

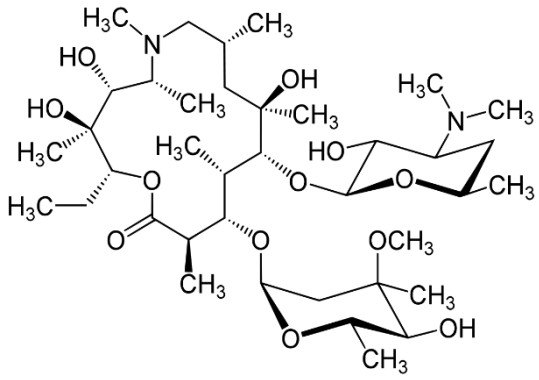

Figure 1: Structure of Azithromycin

2. Methods of Analysis

Numerous analytical methods have been reported for the estimation of AZT in bulk powder, dosage forms and biological fluids whether alone or combined with other drugs. The more recent methods for the analysis of the studied drug can be classified as follows:

2.1. Spectroscopic methods:

2.1.1. Spectrophotometry

Several spectrophotometric methods were reported for estimation of AZT in its pharmaceuticals.

- One method was based on its oxidation with potassium permanganate to produce formaldehyde, which is determined using acetyl acetone, in the presence of ammonium acetate reagent producing a yellow colored product (Suhagia B et al., 2006).
- Another method was based on the formation of an ion pair between the drug and (Mo(V)thiocyanate) followed by its extraction with dichloro ethane (Rachidi M et al., 2006).
- In another report, a charge transfer reaction between AZT and quinalizarin was conducted producing a colored product (Paula C et al., 2010).
- Second order derivative spectroscopy method was also reported for the estimation of AZT and cefixime trihydrate in combined dosage form (Shah V et al., 2012).
- Another method for analysis of AZT in bulk powders, pharmaceutical formulations and spiked biological fluids, was performed by formation of a binary complex with eosin Y in aqueous buffered medium (Walash M et al., 2007).

2.1.2. Spectrofluorometry

Synchronous fluorescence spectroscopy was adopted to assay AZT in pharmaceutical formulations that occurs when it's derivatized in a strongly acidic medium (9.0 mol. L^{-1} HCl). The effect of derivatization conditions (acid concentration, reaction time, and temperature) was investigated. Under optimized conditions, the method presented a limit of detection of 0.23 mg L^{-1} and a limit of quantification of 0.76 mg L^{-1}. The developed method has been successfully applied to the determination of azithromycin in pharmaceutical formulations (Vanessa G et al., 2012).

2.2. Chromatographic methods:

2.2.1. High performance liquid Chromatography

The British Pharmacopoeia (Electronic version 2013) determined AZT pure form using a mobile phase consisting of acetonitrile: dipotassium hydrogen phosphate (60:40, v/v) with UV detection at 210 nm. On the other hand, the USP (Rockville, M., 2007) assayed AZT in its pure form using amperometric electrochemical detection with dual glassy carbon

According to USP, AZT can be assayed by:

A selective and molecularly imprinted polymer (MIP)-primarily based totally electrochemical sensor become fabricated for the detection of azithromycin, a broad-spectrum macrolide antibiotic, from numerous organic samples (urine, tears, plasma). The reversible boronate ester bond-mediated, thin (75 nm) MIP-primarily based totally biomimetic popularity layer become electrodeposited in non-aqueous media onto the surface of a glassy carbon electrode (GCE). The surface morphology and the analytical performances of the advanced sensor had been assessed via way of means of scanning electron (SEM) and atomic force microscopy (AFM), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). By using an oblique electrochemical detection withinside the presence of 10 mM ferro/ferricyanide as redox probe, the sensor exhibited a completely huge dynamic range (13.33 nM-66.67 μM), with an expected detection restrict withinside the subnanomolar range (0.85 nM azithromycin). The easy to assemble sensor demonstrates reusability and suitable shelf-life, showing great selectivity over a huge quantity of structurally associated and non-associated antibiotics, usually related tablets and endogenous compounds (Stoian IA et al., 2020).

2.2.2. Ultra-performance liquid chromatography

UPLC-tandem mass spectrometry (UPLCMS/MS) method was reported for the quantitative estimation of azithromycin in human plasma. The analysis was performed using gradient elution. The solvents used in the mobile phase were acetonitrile and 50 mM ammonium acetate (Chen L et al., 2007).

2.2.3. Gas Chromatography

A gas chromatographic -mass spectrometry (GC-MS) method for the determination of azithromycin

monohydrate residues in biological fluids is described. This method enables the detection of azithromycin residues in biological fluids using single ion monitoring (SIM). Residual levels above 5 µg/mL in samples can be confirmed by full-scan electron impact (EI) mass spectra. AZM is extracted from the sample with chloroform, washed with n-hexane, and then partitioned between chloroform and phosphate buffer for purification. The purified extract was a mixture of acetic anhydride and pyridine (1:2) At room temperature (Thangadurai et al., 2015).

Table 1: Other reported HPLC methods for the assay of AZT

Sample	Mobile Phase	Detection
AZT and its related substance in dosage forms (Yang Z et al., 2009).	0.045 M ammonium dihydrogen phosphate containing 0.002 M sodium heptane sulfonate (pH 3) : acetonitrile, (47:15, v/v)	UV detector (210nm)
AZT and its related Compounds in dosage forms (El-Gindy A et al., 2011).	Methanol: acetonitrile: 14 mM disodium hydrogen phosphate: tetrahydrofuran, (30: 30: 40: 0.1, v/v/v/v)	UV detector (215 nm)
AZT in bulk and pharmaceutical dosage forms (Ghari T et al., 2013).	Methanol: 0.02 M phosphate buffer (pH 8), (90:10,v/v)	UV detector (210 nm)
AZT in human plasma (Choemunng A et al., 2010).	Acetonitrile: methanol: 20 mM ammonium acetate buffer (pH 5.2), (40:10: 50, v/v/v)	Tandem mass spectrometry
AZT in rabbit conjunctiva tissues (Shen Y et al., 2010).	Methanol: 10 mM ammonium acetate (pH 5.2), (82:18, v/v)	Tandem mass spectrometry
AZT in tablets and capsules (Kulikov A et al., 2004).	Water: 1-butanol: phosphate buffer solution (pH 6.86), (60:15:25, v/v) containing 0.1 M sodium dodecyl sulfate.	UV detector (215 nm)

3. Conclusion

Macrolide antibiotics are an important class of antibiotics effective against a wide range of bacterial infections. Azithromycin is a semisynthetic macrolide antibiotic widely used for the treatment of mild to moderate bacterial infections caused by susceptible pathogens. It is characterized by bacteriostatic properties and is used in the treatment of respiratory, intestinal, and urogenital infections. Primary metabolism of drugs occurs in the liver, resulting in inactive metabolites that contribute to the drug's biological activity.

Therefore, various analytical techniques have been developed and adopted in numerous studies and publications to determine and analyze azithromycin and its metabolites. Many studies have been performed to analyze dosage forms and formulations. While other studies have served the purpose of analyzing biofluids and matrices for the drug in question, some work has been done to adapt and optimize complex analytical techniques using azithromycin as the target analyte.

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