Research Article

Available Analytical Method for Macrolide Antibiotic

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ABSTRACT

Macrolide antibiotics, mostly derivatived from erythromycin, are a class of antimicrobial compounds widely used against infectious diseases. Over 10,000 tons of antibiotics were applied in Europe in 1997 as antibacterial agents. About 50 % is used in human medicine while the other half is applied in large-scale animal husbandry. The veterinary antibiotic classes such as tetracycline's, sulfonamides, macrolides, ionophores and pleuromutilins are commonly used to treat infections in livestock. It is used primarily to treat various bacterial infections, such as aerobic gram- positive microorganisms and aerobic gram- negative microorganisms. The incorporation of the nitrogen into the ring significantly alters the chemical, microbiologic and pharmacokinetic properties of AZI. It exhibits a more extensive spectrum of activity, greater acid stability and more favorable pharmacokinetic parameters than erythromycin. The quantification of macrolide antibiotic in tablet formulation for routine quality control analysis using transmission Fourier Transform Infrared (FT-IR) spectroscopy. A number of analytical techniques such as ultraviolet (UV), high performance liquid chromatography(HPLC),capillary electrophoresis, various electrochemical detections, near infrared (NIR) and liquid chromatography/mass spectrometry (LC/ MS) have been applied for the determination and qualitative analysis of macrolide antibiotics in raw materials, dosage forms and biological samples.

Keywords: Biological Samples, FTIRU, HPLC, LC-MS, Macrolide Antibiotic, NIR, Spectroscopy.

INTRODUCTION

Antibiotics are widely used in dairy cattle management for the treatment of disease and as dietary supplements. There are concerns that the antibiotics may be responsible for the promotion of resistant strains of bacteria.[1,2] Macrolide antibiotics constitute a very important class of antibacterial compounds highly active against Gram-positive and Gram-negative cocci. Moreover, they are the most efficient medicine against diseases produced by Mycoplasma *species.*[3] Macrolide antibiotics contain a basic dimethylamine [–N (CH3)2] group, which is able to gain a proton. Their main characteristic is a macrocyclic lactone ring with one or two sugar moieties. Macrolide antibiotics bind to the 30 S subunit of bacterial ribosomes and inhibit bacterial protein synthesis Erythromycin an Oxime (EAO) is a key intermediate in the synthesis of semi-synthetic macrolide antibiotic derivatives such as Azithromycin, roxithromycin and Clarithromycin.[4] Macrolides are a well-established family of antibiotics isolated from streptomycetes. Among the most important characteristics of the macrolide antibiotics are a moderately broad spectrum of antibacterial activity, activity by oral administration and a relatively high therapeutic index. Structurally, erythromycin is a 14-membered lactone ring with ten asymmetric centers and two sugar molecules (L-cladinose and D-desoamine). Patients allergic to Penicillin are often treated with erythromycin due to its antimicrobial activity almost similar or slightly wider than Penicillin.[5]

MOA: The mechanism of macrolide antibiotic action is based on inhibition of bacterial protein synthesis, by interacting with 23S rRNA in the central loop of the peptidyl transferase center as well as with specific ribosomal proteins found in the same region of the ribosome. Macrolides are best known as anti invectives' but also exert other important pharmacological effects such as immunosuppression and immuno modulation.

It is also a very effective antibacterial drug often used for the treatment of pneumonia, throat, bronchitis and ear infections, as well as respiratory and urinary tract infections.[6] The first macrolide introduced into clinical practice was the 14-membered ring compound-erythromycin A. The 15-membered macrolide antibiotic, Azithromycin representative of the so-called azalides, has now become one of the most widely prescribed of all antibiotics. Macrolides like tylosine are widely used in veterinary medicine. Antibiotic residues may have direct toxic effects on consumers, e.g., allergic reactions in hypersensitive individuals, or may indirectly cause problems through the induction of resistant strains of bacteria. The subsequent tables show the monoisotopic mass (MW), the chemical abstract registry number (CAS), as well as the uses of these compounds (Table 1).

Therefore, simple and reliable analytical methods are required to monitor these drug residues in the edible tissues of livestock animals. Generally, the determination of antibiotics, including macrolide antibiotics, is mainly carried out by microbiological assays.[7,8] A number of analytical techniques such as ultraviolet (UV), high performance liquid chromatography (HPLC), capillary electrophoresis, various electrochemical detections, near infrared (NIR) and liquid chromatography/mass

spectrometry (LC/ MS) have been applied for the determination and qualitative analysis of erythromycin in raw materials, dosage forms and biological samples. Almost all of these established analytical methods are complicated, laborious and time consuming.[9] The chromatographic techniques require huge amount of solvents, lengthy experimental procedures for sample clean-up, and also demand expensive equipment that might not be available in many laboratories.[10-17]

Table 1: Antibiotics and their CAS and uses

Antibiotic Name	CAS No.	M.W	Uses
Erythromycin	111-07-8	733.46	For the prevention of the rheumatic fever. In veterinary medicine for the treatment of intestinal infection, mastitis and pneumonia.
Roxithromycin	80241-83-1	836.52	This oxime derivative of erythromycin has a similar application range as erythromycin, but it is only used in human medicine.
Clarithromycin	81103-11-9	747.48	This methyl ether derivative of erythromycin has a similar application range as erythromycin but it is only used in human medicine.
Tylosin	1401-69-0	915.52	Mostly used as tylosintartrate.

UV Spectroscopic Method

It is difficult to achieve high sensitivity with UV detection because erythromycin lacks a UV chromophore.[18] UV method has been developed for the determination of erythromycin by formation of a blue-colored complex with gentian violet in alkaline medium.[19] The chromatographic methods need a large amount of solvents and costly reagents for derivatization to achieve better sensitivity as a few methods are reported.[20,21] Macrolide compounds have only a weak UV absorbance in the low wavelength range (<220 nm) due to the lack a suitable chromophore. Therefore, UV detection at 215 nm was used to detect all analytes and had no significant interference with baseline and blank chromatograms. Under the optimized HPLC conditions, all above components were cleanly separated within less than 10 min on an Inersil ODS C18 column (4.6×150 mm). Numerous liquid chromatographic (LC) methods are reported for determination of Azithromycin in human plasma and nearly all involve electrochemical detection to attain sufficient sensitivity as UV detection shows little bit less sensitivity for macrolides analyzed in body fluids. Torano and Guchelaar have worked on a strategy using fluorescence detection for quantification of macrolide antibiotics in serum. Nirogi used solid-phase extraction then quantitative analysis was done on LC/MS/MS. The survey of published literature reveals that quantitative methods are meant for the measurement of Azithromycin in bodily fluids like plasma, tissues etc and waste water. Detection is generally electrochemical, MS, UV and fluorescence after derivatization. Liquid chromatography with UV detection has been already employed for the analysis of AZI in tablets[22] and in raw material.[23]

Electrochemical Method

In order to overcome the lack of sensitivity showed by some macrolides when using Spectrophotometric detectors and as most of them have a suitable electro active group, electrochemical detectors arose as an alternative detection model to perform the determination of this family of antibiotics. Another type of electrochemical detector, the so called coulometric detector, has also been successfully used for the determination of such macrolides. In most cases, the coulometric detector comprises of a guard cell aimed to electrolyse the components of the mobile phase followed by a dual electrode, which in the case of macrolides should be operated in oxidative screen mode. The lack of electrochemical response observed for two N-demethyl derivatives of erythromycin suggested the importance of the tertiary amine of the desosaminyl sugar for the electrochemical detection to the author.[24] It is being cheaper and faster method than chromatography, electrochemical methods using the oxidation behavior for the determination of erythromycin with various types of electrodes have been reported.[5,6,25-27] The survey of published literature reveals that quantitative methods are meant for the measurement of Azithromycin in bodily fluids like plasma, tissues etc and waste water. Detection is generally electrochemical, MS, UV and luorescence after derivatization.[28,29] The chromatographic methods need a large amount of solvents and costly reagents for derivatization to achieve better sensitivity as a few methods are reported.[20,21] Capillary electrophoresis (CE) coupled with end-column electro chemiluminescence (ECL) detector for determination of erythromycin has also been used[30], but CE has not been used widely in the pharmaceutical laboratories. An electrochemical ELISA for the detection of two macrolides (erythromycin and tylosin) in bovine muscle has been reported.[31] The detection limit of the assay was 0.4gL−1 for erythromycin and 4.0gL−1 for tylosin. Results were confirmed by LC–MS/MS. Assay procedures making use of electrochemical detection is often very time consuming, both in the sample preparation steps and the chromatography.

NIR Spectroscopy

The determination of erythromycin by dissolving in the suitable solvent and also in solid state has been also described by use of NIR Spectroscopy.[32,33] Almost all of these established analytical methods are complicated, laborious and time consuming.[9]

Chromatography

Thin Layer Chromatography

Thin layer chromatography (TLC) is one of the most popular and widely used screening methods for antibiotics due to a number of factors including simplicity, wide applicability, good sensitivity, speed and low cost. A TLC method for the separation of erythromycin, tylosin, oleandomycin and spiramycin in livestock products has also been reported.[34] A similar TLC method for the semiquantitative analysis of EA and several metabolites in rat urine and faeces has also been described.[35]

High performance thin layer chromatography

High-performance liquid chromatography (HPLC) can be used for the identification of drugs but this rather elaborate method will not be a first choice in a pharmacopoeia compendium. The assay of different components and impurities in erythromycin and its esters by HPLC has been published. HPLC of other macrohdes like leucomycin, tykxzin and spiramycin also has been described. High-Performance Liquid Chromatography (HPLC) has been developed for the quantitative analysis and identification of related substances in erythromycin and other macrolide antibiotics.[36] A liquid chromatographic method was developed and validated for the determination of erythromycin an oxime and related substances. Mostly, C18 analytical columns were used for the separation of analytes. Only one multiresidue study used C12 analytical column[37] and in one study C8 analytical column was used for the separation of FQs.[38] Few studies have reported on the analysis of erythromycin an oxime using HPLC method.[39] However, these methods could not separate all the known related substances of erythromycin an oxime including EA. Erythromycin derivatives like other macrolide antibiotics are basic compounds and suffer from peak tailing and poor efficiency on silica-based RP columns. During optimization of the HPLC process, the effect of mobile phase additives on retention behavior of erythromycin an oxime on common C18 columns was investigated. The pH of the mobile phase is a major factor influencing the chromatographic behavior of erythromycin. Improved separation and peak shape were also obtained by increasing the pH of the mobile phase. HPLC is currently the most versatile tool which satisfies the needs for an optimum separation[40]. AZI has been analyzed by high-performance liquid chromatography using fluorescence[41, 42], electrochemical (using amperometric and coulometric detectors)[43,44] and mass spectrometry detector[45-48] for quantification in bulk material and pharmaceutical dosage forms. Fluorescence detection requires complicated sample pretreatment involving pre-column derivatization of the analyte.

Ultra High Performance Liquid Chromatography

UHPLC was used for the determination of compounds from different classes of pharmaceuticals including 5 antibiotics.[49,50] UHPLC is a modern technique, using columns packed with sub-2_m particles, which enabled elution of sample components in much narrower, more concentrated bands, resulting in better chromatographic resolution and increased peak capacity through rapid elution from short column.

MS- MS Spectroscopy

Many antibiotic compounds are nonvolatile with high molecular weights and they respond well in ESI+ which makes LC–MS or LC–MS/MS the technique of choice for their separation and analysis. The methods for the determination of MLs employed MS/MS using specific SRM conditions with ESI+. The precursor ion chosen for the quantitation was [M+H] + in almost all studies, except of ERYH2O, for which [M+H-H2O] + was used as a precursor ion.[51-54] A sensitive method for the determination of Clarithromycin in plasma using HPLC coupled to MS–MS detection has been recently described by van Roogen et al. for the determination of Clarithromycin using Roxithromycin as internal standard.

LC- MS Spectroscopy

LC–MS using electrospray ionisation has been used to successfully determine seven macrolides in chicken muscle.[55] For LC–MS and LC–MS/MS analysis of pharmaceuticals, two ionization interfaces has been the most widely used due to their sensitivity and robustness. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) satisfied the requirements. They produce protonated [M+H] + or deprotonated [M−H] − molecules. Both techniques work at atmospheric pressure which is suitable for the connection with LC system. In recent years, sensitivity has improved using LC–MS techniques with detection limits less than 1gkg 1 being reported for some macrolides in food matrices.[56-60]

Gas Chromatography

Gas–liquid chromatography has been used for the quantitative analysis and separation of erythromycin in mixtures containing EA, EB, EC, AEA, ESM and PE using flame-ionization detection (FID).[61] Similarly, EA and EB were separated and quantitated in the presence of EC, AEA and ESM in erythro mycin tablets[62], whereas gas chromatography coupled to mass spectrometry (GC–MS) has been used to determine erythromycin in beef and pork by single-ion monitoring (SIM) at m/z of 200.[63] A procedure for the qualitative identification of erythromycin in EESC capsules using pyrolysis–gas chromatography (Py-GC) has also been reported.[64]

FT IR Spectroscopy

Another popular technique for quantitative analysis is FT-IR spectroscopy, which has been used many years to identify or confirm the presence of many drugs and other chemicals. Infrared spectroscopic technique enable the analysis of raw material without time consuming sample preparation method and have been shown to be promising tool for analysis of variety of samples both direct procedure or method for chromatographic

detection. To development of a rapid, cheap and environment friendly analytical method for the determination of macrolide antibiotics in tablet formulations for routine quality control analysis, based on transmission FT-IR spectroscopy without using any solvent. FT-IR transmission spectroscopy to analyze various quality factors of oils and fat.[65-69] FT-IR spectroscopy has previously been applied for quantification of Azithromycin by dissolving it in toluene.[70] Furthermore, all above analytical method these require dissolution of the samples in the proper solvents and then often extraction is performed with organic solvents which are toxic to human Health and environment. This is an economical and environmentally friendly method avoiding the use of hazardous chemicals or solvents and could be effectively used in the pharmaceutical industry for GMP as a rapid and green method. This may be applied as a capable method for the speedy quality assessment/quality control (QA/QC) of the active ingredients like erythromycin in the pharmaceutical preparations. However, with the FT-IR spectroscopy, the spectra could be recorded without any appreciable pretreatment. It is less time consuming, sensitive and alternative method capable of estimation of Azithromycin in tablet formulations using FT-IR spectroscopy for routine quantitative monitoring to counter the existing laborious methods. As FT-IR is analytical technique which allows rapid quantitative measurement as it is fast and nondestructive in nature, which is an extremely useful means for analyzing solid as well as liquid pharmaceuticals without requiring any solvent. The rise in industrial significance for FT-IR during current decade is result of remarkable advancements in the technique along with more accessibility of fast-scan instruments which facilitate smooth measurements using potential chemometric techniques with less or without sample pre-treatment. The outcome of the current work brings about considerable benefits achieved like speed, accuracy, ease and expediency by the use of FT-IR spectroscopy for calculating exact amount of the desired ingredient during analysis of pharmaceuticals to control the quality and quantity of product. Mallah et al have used FT-IR transmission spectroscopy for determination of AZI in its pharmaceutical dosage forms which is easy to execute but has the same short come of not being suitable for formulations with unknown composition of excipients. [71] By using FT-IR spectroscopy, the spectra could be recorded without any appreciable pretreatment. The FT-IR group of National Centre of Excellence in Analytical Chemistry (NCEAC) has already developed the methods using FT-IR spectroscopy for the determination of different quality parameters of oils and fat.[72, 73]

CONCLUSION

To prevent emergence of resistant bacteria, it is necessary to monitor the concentration, fate and removal of antibiotics in Environmental samples. This review describes analytical methods for the determination of macrolides focusing mainly on methods published in the past decade. In general reversed phase is the preferred elution mode for this family of antibiotics using, in most cases, C18 or C8 columns. Despite the undoubted advantages of using mass spectrometry detection, macrolides can also be analyzed at low concentration levels with other kinds of detectors, with or without prior derivatization of the analytes, which may constitute powerful and cost-effective alternatives, in particular when only screening and/or post-screening are needed. The results of FTIR study accomplish significant benefits in terms of rapidity, accuracy, simplicity and green method by the use FTIR spectroscopy for measuring quantity of the desired active species while performing quality control of pharmaceuticals. Thus all the method described in this review gives useful information for further study of macrolide antibiotics.

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