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Analysis of macrolide antibiotics in river water by solid-phase extraction and liquid chromatography–mass spectrometry ☆

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Abstract

A liquid chromatography–mass spectrometry (LC–MS) method was developed for the determination of five macrolides in natural water samples, using kitasamycin as surrogate. The macrolides were extracted from water samples using Oasis HLB cartridges. Pre-concentration factors up to 250 were obtained. Separation was carried out in an end-capped silica-based C_{18} column and mobile phases consisting of water/acetonitrile mixtures containing ammonium acetate. Detection was performed by mass spectrometry with a single quadrupole and a triple quadrupole using an electrospray interface. The quality parameters obtained with these two approaches were compared. The detection limits of the whole process were about 1 ng l^{-1}. The recoveries from 250 ml of water samples spiked at 25–125 ng l^{-1} level were in the range 85–115%, except for azithromycin levels, which were around 70%. Erythromycin-H_2O, clarithromycin and azithromyzin were found, at the sub ng l^{-1} level, in the studied rivers.

Keywords: Water analysis; Liquid chromatography; Tandem mass spectrometry; Macrolides

1. Introduction

Since the second half of the 1990s, there has been increasing concern within the scientific community regarding the presence of different types of drugs in the environment [1–5]. This is especially true for antibiotics, which can cause adverse effects on ecological systems and also on humans, such as allergic reactions [6]. However, the main worry arises from the potential generation and/or spreading of new strains of resistant bacteria [7–9].

Significant amounts of the unmetabolised drugs used in human and veterinary medicine are excreted and thus reach the sewage treatment plants, where they are not completely removed [10–12], and finally arrive in the natural water courses. On the other hand, manure used to fertilise fields is a direct entrance pathway of veterinary drugs into soils. Subsequently, depending on the mobility of the compounds, such drugs can come into ground or surface water. However, up to now, these substances have not been included in environmental regulations such as Directive 2000/60/EC [13].

The awareness of this situation has stimulated numerous studies aiming to evaluate the behaviour of pharmaceuticals in the different environmental compartments. As a consequence, the demand for new analytical methods capable of detecting drugs at sub parts per billion levels in environmental samples has significantly increased [14–16]. Some screening methods for the analysis of antibiotics in water samples have been proposed. Among these is, a method for sulfonamides [17], based on a biosensor which provides limits of detection of 10 ng l^{-1} and an immunoassay for tetracyclines in hog lagoons [18], with detection limits about 1 μg l^{-1}. However, the most common approach for water analysis includes a preconcentration step by solid-phase extraction (SPE), and a liquid chromatographic separation coupled to a mass spectrometer, which allows a very selective and sensitive detection. Thus, SPE–LC–MS or SPE–LC–MS–MS methods have been reported for tetracyclines, sulfonamides, quinolones or macrolides, with limits of detection usually in the range of few ng l^{-1} [19,20].

The macrolide antibiotics comprise a family of antibacterial agents widely used in humane and veterinary medicine. Consequently, therapeutic activities on humans and on farming practices can lead to the presence of such drugs in the

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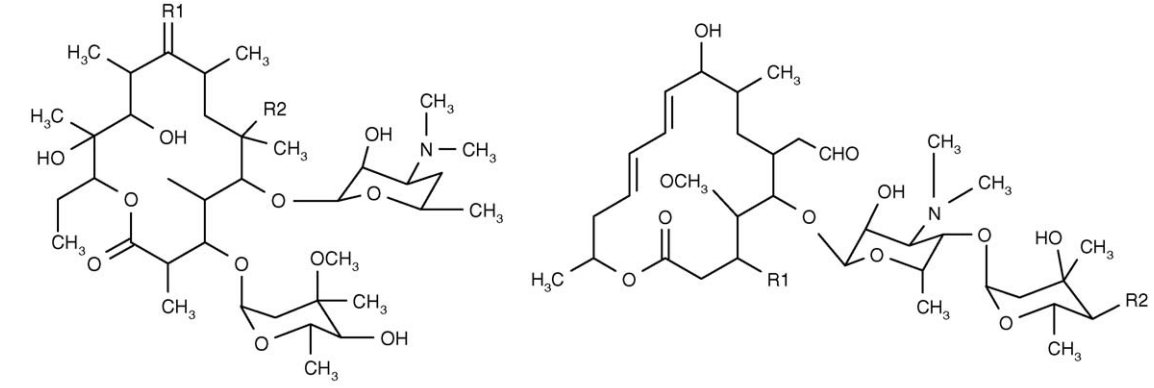
environment. Since the end of the last century, several studies have been undertaken in Germany [15,21,22], Switzerland [23,24], the United States [25,26] and Canada [27,28], aiming to detect the presence of macrolides in both river and ground waters, and in the effluents of wastewater treatment plants. In the case of Spain, few studies on the presence of antibiotics in the environment have been found in the literature. Those that exist are devoted to tetracyclines [29,30] and quinolones [29,31] in river, sea and ground water samples.

The aim of the present paper is to develop a method based on solid-phase extraction and LC–MS for the determination of five macrolide antibiotics (azithromycin, erythromycin, clarithromycin, roxithromycin and josamycin) in river water samples. All these compounds are used in medicine, and erythromycin and josamycin are also used as veterinary drugs. After a solid-phase extraction step, separation is performed by using an end-capped silica-based C18 column and water-acetonitrile mixtures, containing acetate buffer, as mobile phases. Detection is done by MS (single quadrupole) and MS–MS (triple quadrupole) coupled to liquid chromatography using electrospray interfaces. The quality parameters obtained with these two different LC–MS approaches are compared.

2. Experimental

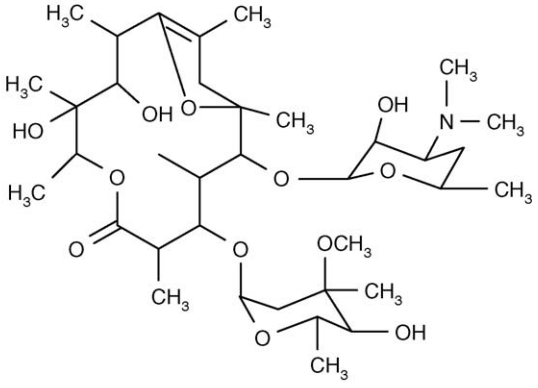
2.1. Chemicals and solutions

Roxithromycin (ROX) and erythromycin (ERY) were supplied by Sigma (St. Louis, MO, USA). Kitasamycin (KIT), josamycin (JOS), azithromycin (AZI) and clarithromycin (CLAR) were kindly supplied by Esteve (Barcelona, Spain), Virbac (Esplugues Llobregat, Spain), Pfizer (Groton, CT, USA) and Abbott (Illinois, USA), respectively. Since in the aquatic environment ERY easily degrades to erythromycin-H_2O (ERY-H_2O), it is always detected as this metabolite [21,26]. Thus, in order to prepare an ERY-H_2O standard solution, the pH of an erythromycin solution was adjusted to 3.0 with 3 M H_2SO_4. After 4 h stirring at room temperature complete conversion was

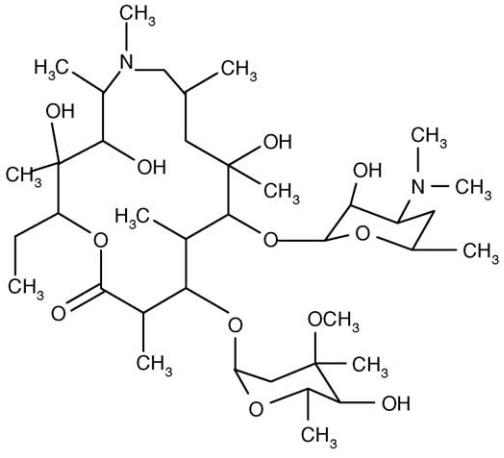


Erythromycin: R1 = O; R2 = OH
Clarithromycin: R1 = O; R2 = OCH_3
Roxithromycin: R1 = $NOCH_2OCH_2CH_2OCH_3$; R2 = OH

Kitasamycin: R1 = OH; R2 = $OCOCH_2CH_2CH_3$
Josamycin: R1 = $OCOCH_3$; R2 = $OCOCH_2CH(CH_3)_2$



Erythromycin-H_2O



Azithromycin

Fig. 1. Structures of the macrolides studied.