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Short Communication

Determination of azithromycin in raw materials and pharmaceutical formulations by HPLC coupled with an evaporative light scattering detector

Aiguo Zeng [a], *Xia Liu* [a], *Siruo Zhang* [b], *Yan Zheng* [c], *Ping Huang* [c], *Kangli Du* [a], *Qiang Fu* [a,*]

[a] *School of Pharmacy, Xi'an Jiaotong University, Xi'an 710061, China*
[b] *Department of microbiology, Dalian Medical University, Dalian 116044, China*
[c] *Xi'an Institute of Food and Drug Control, Xi'an 710054, China*

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ABSTRACT

A simple high-performance liquid chromatography (HPLC) method coupled with an evaporative light scattering detector (ELSD) was developed for the determination of azithromycin in raw materials and pharmaceutical formulations (injections, capsules and tablets) without any pretreatment or derivatization step. Azithromycin, degradation products and formulation ingredients were separated efficiently by using the mobile phase consisted of ammonium acetate (0.05 M, pH 8.0) and acetonitrile (60:40, *v/v*) in an isocratic mode at 0.8 ml/min flow rate. Parameters of ELSD were 60 °C for evaporation temperature and 50 psi for pressure of carrier gas (air). A logarithmic calibration curve was obtained from 50.93 to 509.30 μg/ml ($r = 0.9996$) for azithromycin, with the limit of detection (LOD) of 6.75 μg/ml ($S/n = 3$) and the limit of quantification of 22.50 μg/ml ($S/n = 10$). The developed method was validated and applied with satisfactory accuracy and precision for the determination of azithromycin in raw materials and pharmaceutical formulations (recovery 99–102%, RSD <1.2%, $n = 3$). No significant difference (t-test) was found between the results of the developed HPLC–ELSD method and the HPLC–UV or microbiological method.

* *Corresponding author*. Xi'an Jiaotong University Health Science Center, No. 76, Yanta East Road, Xi'an 710061, China. Tel./fax: +86 029 82655382.
E-mail address: fuqiang@mail.xjtu.edu.cn (Q. Fu).
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1. Introduction

Azithromycin (AZM) is a novel macrolide antibiotic and a semisynthetic- erythromycin derivative. It has a methyl-substituted nitrogen at position 9a in the lactone ring to create a 15-membered-ring macrolide [1]. AZM produces an enhanced spectrum and potency against bacteria compared with other macrolides and superior stability in acid environment. Its mechanism is similar to erythromycin, appearing to bind to the same receptor, 50s ribosomal subunits of susceptible bacteria and suppresses protein synthesis. AZM has greater oral bioavailability, longer elimination half-lives and much higher tissue concentrations than erythromycin in animals and humans, which plays a leading role in the treatment or prophylaxis of several diseases such as bacterial upper and lower respiratory tract infections, urinary tract infections, skin and soft tissue infections, and sexually transmitted diseases [2,3].

A number of reports have been published regarding to the determination of AZM, in which microbiological method was the general content assay method [4] with disadvantages of time-consuming, low detectability and poor precision. In order to overcome these problems, several high-performance liquid chromatography (HPLC) methods have been developed. The official method [5–8] for the assay of AZM in pharmaceuticals is HPLC with UV detector, but AZM has only a weak UV absorbance in the wavelength range of less than 220 nm, leading to an asymmetric peak profile and low column efficiency. Other methods, including electrochemical detection [9–12], fluorescence detection by pre-column derivatization [13–15] and liquid chromatography–mass spectrometry (LC–MS) [16–18] have been used to determine AZM in routine pharmaceutical dosage forms or biological matrices. Obviously, electrochemical detectors are not widely available in many laboratories. The USP method [19] describes a high pH mobile phase (pH11) as well as a specific column "Gamma-alumina" which is quite expensive and difficult to obtain in order to assay AZM using an amperometric electrochemical detector. Moreover, pre-column derivatization is time-consuming due to the complex steps involved. Also, it would be unrealistic to use LC–MS for the routine quality control of AZM preparations. Therefore, it is necessary to develop a convenient and effective method for the quality control of AZM by using conventional materials, reagents and equipment.

Evaporative light scattering detector (ELSD) is described as a quasi-universal detection mode suitable for non-absorbing analytes [20–22] and end-absorbing analytes [23–25]. The response does not depend on the solute optical properties, but on the size, shape and surface properties of the particle formed, any compound having lower volatility than the mobile phase can be detected. ELSD operation principle mainly consists of three successive processes: (a) nebulization of chromatographic eluent using nitrogen or air, (b) evaporation of mobile phase at relatively low temperature and (c) light scattering by the non-volatile residual particles, which ideally consist of analyte molecules [26,27]. This complex mechanism leads to a non-linear empirical quantitative law described by the relation:

$$A = am^b$$

where A is the area of the chromatographic peak, m the mass of the analyte, a the response factor and b is the response index measured from the slope of the curve log $A = f(\log m)$ [i.e. $\log A = b\log m + \log a$]. The coefficients a and b depend on many parameters, such as the average size, the shape and the distribution of the particles, the nature, the volatility and the concentration of the analyte, the nature of the mobile phase and nebulizing gas, the liquid and gas flowrates, evaporation temperature, etc. In the field of pharmaceutical analysis, it has already been proposed as an effective alternative for both the determination of the aminoglycoside antibiotics, natural medicines [28], compound medicines and assessing the separation of drug combination [29].

The purpose of this study was to develop and validate a rapid and simple HPLC method with ELSD detection for the determination of AZM. The composition and flow rate of mobile phase and the column temperature of the method were optimized by using an orthogonal experimental design at four levels. The results proved the established method is precise, accurate, robust and practical, and is suitable for the routine quality control of AZM in raw materials and pharmaceutical dosage forms.

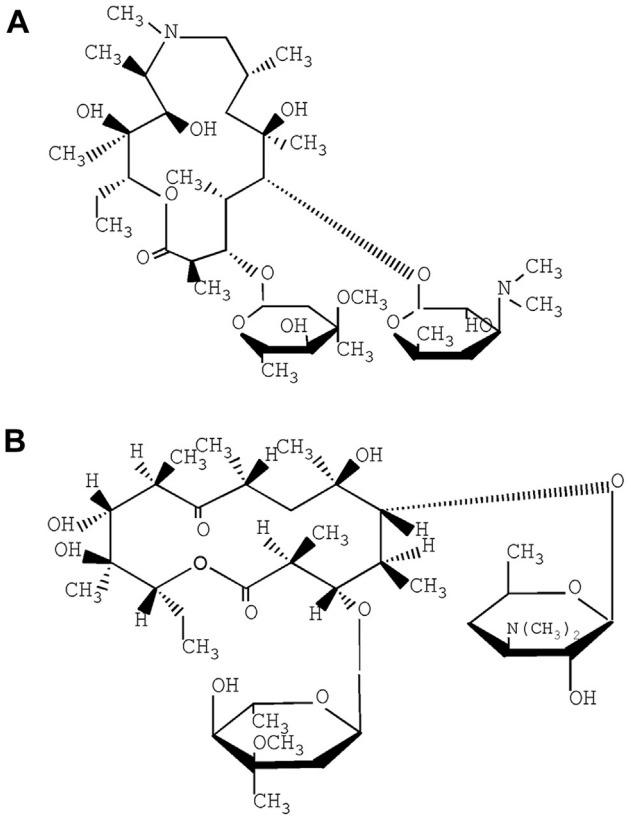

Fig. 1 – The chemical structures of AZM (A) and erythromycin (B).