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Combined application of dispersive liquid–liquid microextraction based on the solidification of floating organic droplets and charged aerosol detection for the simple and sensitive quantification of macrolide antibiotics in human urine

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ABSTRACT

A novel analytical method combining dispersive liquid–liquid microextraction based on the solidification of floating organic droplets (DLLME-SFO) and liquid chromatography with charged aerosol detection (LC-CAD) was established. For the first time, CAD was applied for the detection of macrolide antibiotics lacking chromophores. Parameters influencing the microextraction efficiency were systematically investigated, and the optimized microextraction conditions yielded high enrichment factors in the range of 60–106. The combined application of DLLME-SFO and LC-CAD provided the sensitivity of the method, expressed as the limit of detection (LOD), as low as 10 to 40 ng mL^{-1} and intra-day and inter-day precisions below 8.7% and 12.6%, respectively. The measured absolute recovery values were approximately 100%, indicating that the extraction efficiency was very high. Direct comparisons of the liquid–liquid extraction and organic solvent precipitation methods demonstrated that the proposed method was more sensitive, specific, rapid, and environmentally friendly for the determination of five macrolide antibiotics in human urine. The results suggest that the combined use of DLLME-SFO and LC-CAD may be applicable to the analysis of various compounds with poor to no chromophores in complex matrices.

1. Introduction

Macrolide antibiotics are highly active against Gram-positive and Gram-negative cocci as well as non-classical pathogens and share common chemical structures, such as a macrocyclic lactone ring with one or more amino sugars (Fig. 1) [1]. These antibiotics, which are widely utilized for the treatment of both human and animal infections, are not extensively metabolized and are excreted largely unchanged in the urine [1–3]. Therefore, a number of analytical methods have been developed to determine the concentrations of unchanged macrolide antibiotics in human biological fluids such as plasma [4] and urine [1–3] and to monitor their residues in food [5].

Traditionally, microbiological assays have been used for macrolide analyses [6]. However, because of their low specificity and low sensitivity, these assays have been replaced by chromatographic techniques, especially liquid chromatography (LC). Ultraviolet (UV) detection can easily be applied for macrolides containing conjugated double bonds, including rosaramicin [7], josamycin [8], and spiramycin [9]. However, a wide variety of macrolide antibiotics, including roxithromycin, clarithromycin, azithromycin, oleandomycin, erythromycin, and dirithromycin, lack chromophores, preventing selective and sensitive UV-based detection. Mass spectrometry (MS) is a more appropriate detection method for these compounds when present at low concentrations in complex matrices, such as biological fluids and food [5]. Although MS provides useful information on the molecular weights and structures of compounds with high sensitivity and accuracy, it is relatively expensive and requires considerable technical expertise for optimal operation. Electrochemical detection (ECD) has been used as an alternative for the direct detection of macrolide antibiotics (Supplementary Table S1). Because ECD is not compatible

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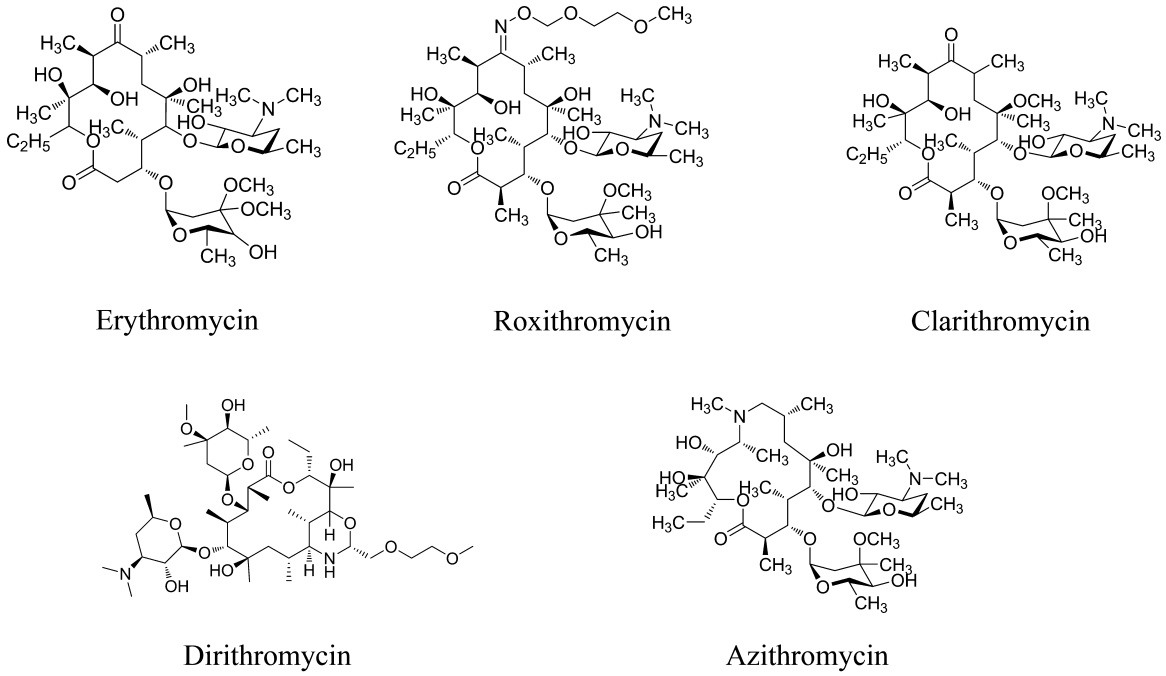

Fig. 1. The chemical structures of the macrolide antibiotics analyzed in the present study.

with gradient elution, its use is generally limited to the analysis of a small number of macrolide antibiotics in a single run, with a few exceptions (Supplementary Table S1). Though González and Rodríguez successfully separated and identified five and ten macrolide antibiotics in urine with isocratic [2] and gradient [1] elution, respectively, a mobile phase system consisting of complex buffers had to be used to maintain a constant ionic strength during the analysis, and narrow-bore columns with low flow rates were required to assist in the separation. Moreover, one of the macrolide antibiotics being analyzed had to be used as an internal standard to compensate for possible changes in the detector response under high working potentials, and the detector parameters needed to be optimized before the calibration assay.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2013.07.024.

In contrast to ECD, charged aerosol detection (CAD), a relatively recently developed universal detection method, is highly compatible with gradient elution and requires no parameter optimization for operation. It is advantageous over UV detection for compounds with poor chromophores or no chromophores at all [10]. In CAD, column eluents are nebulized by a stream of nitrogen, followed by evaporation to produce dried analyte particles. The aerosol particles are then charged by a secondary stream of nitrogen with positive charges and then detected electrically [11]. Theoretically, CAD provides a nearly uniform response independent of the chemical structures of the analyzed compounds. However, the detector response can increase with higher organic content of the mobile phase [11]. With the exception of our recent study analyzing gabapentin in serum and urine [12], the use of CAD has typically been limited to analytes existing at high concentrations in simple matrices. In the current study, CAD was used for the first time for the sensitive detection of macrolide antibiotics containing no chromophores in human urine.

The analysis of macrolide antibiotics in urine requires pre-treatment, including the clean-up and pre-concentration of urine samples, because the urine matrix is very complex, and the concentration levels of macrolide antibiotics in urine are quite low. Liquid–liquid extraction (LLE) [1,13] and simple organic solvent precipitation [2,3] are the most popular pre-treatment methods. LLE usually requires large volumes of hazardous solvents and is often very time-consuming. The organic solvent precipitation method is generally non-specific, though the procedure is very simple. Recently, a novel liquid-phase microextraction method, dispersive liquid–liquid microextraction (DLLME), has become very popular [14]. Classic DLLME uses either high-density extraction solvents such as chloroform, carbon tetrachloride, and chlorobenzene or low-density solvents such as *n*-hexanol, *n*-hexane, cyclohexane, and dibutyl ether in addition to specially shaped extraction tubes to facilitate transfer and prevent the evaporation of the extracted phase [15]. In a modified DLLME method based on the solidification of floating organic droplets (DLLME-SFO), the easy and reliable collection of the extraction phase is possible without a special extraction tube because of the use of low-density solvents with melting points close to room temperature [16]. This technique has mainly been applied to samples consisting of simple matrices, and its use with complex matrices such as biological samples, especially urine, has been very limited [17]. In this study, DLLME-SFO was applied to clean up and pre-concentrate macrolide antibiotics in human urine samples. To the best of our knowledge, this is not only the first application of CAD for macrolide detection but also the first application of DLLME-SFO combined with LC-CAD for the analysis of human urine.

2. Materials and methods

2.1. Reagents and chemicals

Erythromycin, roxithromycin, dirithromycin, clarithromycin, 1-dodecanol, 1-decanol, sodium hydroxide (NaOH), and sodium carbonate (Na_2CO_3) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ammonia solution (30%) and sodium chloride (NaCl) were obtained from Koryo Pure Chemical (Kongju, Korea) and Duksan Chemical Co. Ltd. (Ansan, Korea), respectively. Azithromycin, 2-dodecanol, and 1-undecanol were purchased from TCI (Tokyo, Japan). Acetone, water, methanol, and acetonitrile were all LC-grade and were obtained from Duksan Chemical Co. Ltd. (Ansan, Korea).