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A sensitive non-derivatization method for apramycin and impurities analysis using hydrophilic interaction liquid chromatography and charged aerosol detection

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ABSTRACT

A sensitive non-derivatization method was developed for the analysis of apramycin and impurites using hydrophilic interaction liquid chromatography (HILIC) and charged aerosol detection (CAD). Sample was pretreated with an effective SPE method (recovery >90%) to remove interference with apramycin impurities from sulfate, then analyzed with direct injection. Different chromatography modes of separation and choices of HILIC column were investigated in search of a direct analysis method. The HILIC-CAD method was optimized using a cysteine-bonded zwitterionic HILIC column and compared to the strong cation exchange-ultraviolet (SCX-UV) method with post-column derivatization recommended by the Chinese Pharmacopoeia (veterinary) 2010. The improved chromatographic resolution and peak shape with the HILIC-charged aerosol detection method allows for increase of sample load to 48.9 μg from only 2.8 μg with the SCX-UV approach. More than 16 impurities were detected with this method with improved resolution, and four were identified with MS, while only 7 impurities were detected with the SCX-UV method. Moreover, the current method has a good precision and reproducibility. The intra-day and inter-day of peak area variability was less than or equal to 4.760% RSD and 9.950%, respectively. The average limit of detection and quantization was 80 ng and 200 ng injected on the column, respectively. The overall results demonstrated that the presented method can be used as an alternative to SCX-UV method in the analysis of apramycin and impurities.

1. Introduction

Apramycin belongs to a class of compounds known as aminoglycoside antibiotics, which are widely used for treating Gram-negative and some Gram-positive bacterial infections [1]. Reliable analytical methods are required for accurate assessment of apramycin purity and minor degradants in routine quality control analysis. However, it is challenge to develop a routinely used HPLC method for quality control of apramycin. Firstly, apramycin and related substances are highly polar compounds with similar structure. They don't have sufficient retention and adequate resolution in reversed phase liquid chromatography (RPLC). Secondly, apramycin and its impurities lack of a UV-absorbing chromophore [2–4]. Thirdly, apramycin and related compounds are basic compounds which have tailing and broading peaks [5–8]. Peak broading and tailing lead to poor resolution and low signal-to-noise ratio. Thus, it is difficult to achieve high sensitivity to detect and estimate low level impurities of apramycin.

Few methods have been reported for the analysis of apramycin and related compounds including post- or pre-column derivatization strong cation exchange-ultraviolet (SCX-UV) methods [9,10]. Such methods are sensitive for some components of apramycin sample. However, they also have some drawbacks. First, derivatization is tedious and time-consuming. Furthermore, compounds lacking a specific functional group required for derivatization could not be detected. Second, non-volatile buffer solution was used as mobile phase additive for these methods. Non-volatile buffer solution are incompatible with universal detectors, such as evaporative light scattering detector (ELSD) [11,12]. and charged aerosol detector (CAD) [13,14]. Recently, reversed phase (RP)-volatile-ion-paring methods were developed to analyze

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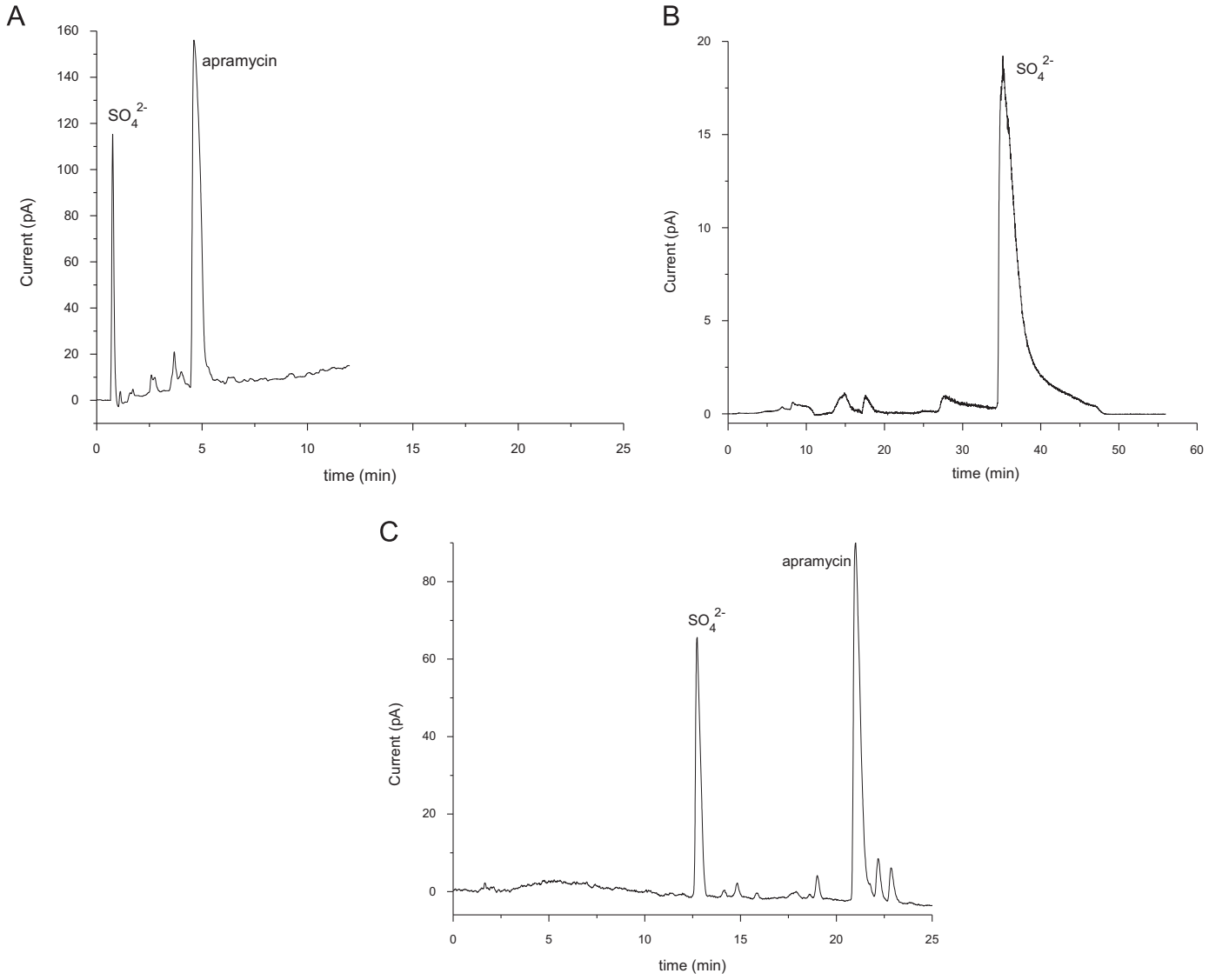

Fig. 1. The separation of apramycin and related compounds using XBridge C18 (A), ZIC-pHILIC (B) and Click TE-Cys (C).

aminoglycoside antibiotics by universal detector including ELSD, CAD and mass spectrometry (MS) [15–18]. For example, Stypulkowska and co-workers [19]. optimized a non-derivatization method for the analysis of gentamicin using CAD and trifluoroacetic acid (TFA) in the RP mode. This method is simple and it provides high sensitivity and good resolution for gentamicin and related compounds. Nevertheless, it has never been reported for the analysis of apramycin and impurities.

Hydrophilic interaction liquid chromatography (HILIC) is an alternative to RPLC [20]. HILIC provides enough retention and unique selectivity for polar compounds [21,22]. In our previous study, a cysteine-bonded zwitterionic HILIC stationary phase, Click TE-Cys [23], was introduced for the separation often aminoglycoside antibiotics in one run [24]. Symmetric peak shape and good selectivity were obtained with this type of stationary phase. Furthermore, buffer solutions used with Click TE-Cys, such as ammonium formate, are compatible with CAD and ELSD [21,23]. To the best of our knowledge, Click TE-Cys column has not yet been utilized for the separation of apramycin and impurities. In this study, a Click TE-Cys based HILIC-CAD method was developed and optimized to achieve good resolution and sensitivity for the analysis of apramycin and related impurities. The effects of sample pretreatment, chromatography mode, and mobile phase composition were investigated. Some of the impurities were identified with MS spectrometric analysis. Furthermore, the presented method was compared with a RP-volatile-ion-paring method and a post-derivatization SCX-UV method recommended by the Chinese Pharmacopoeia (veterinary) 2010 (CP2010) [10].

2. Materials and methods

2.1. Apparatus and reagents

The chromatographic system contained a LPG-3400SD pump, a WPS-3000 TSL autosampler, a TCC-3200 column oven and a CAD Veo RS detector. Data were collected and analyzed by Chromeleon version 7.2. The MS data were obtained from a TSQ Quantum mass spectrometer which is a QQQ mass spectrometer. The mass data were collected and analyzed by Xcalibur. All above instruments and workstations were from Thermofisher (Sunnyvale, CA, USA). The post-column derivatization system PCX-2500 was from Pickering Laboratories (Mountain View, CA, USA). The Click TE-Cys column (4.6 mm × 150 mm, 5 μm) for CAD analysis and the Click TE-Cys column (2.1 mm × 150 mm, 5 μm) for MS analysis were gifts from Dalian Institute of Chemical Physicals, Chinese Academy