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Micro-scale analysis of aminoglycoside antibiotics in human plasma by capillary liquid chromatography and nanospray tandem mass spectrometry with column switching

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Abstract

A simple liquid chromatographic method combined with tandem mass spectrometry (LC–MS–MS) is described for the analysis of aminoglycoside antibiotics. Clinically these antibiotics may cause both ototoxicity and nephrotoxicity; therefore, the monitoring of aminoglycoside levels in patient plasma is required for protecting human health. In this study separation of the method is based on ion-pair chromatographic technology on a short capillary reversed-phase C18 column. The method was successfully applied to analyze amikacin in human plasma. In human plasma after deproteinisation with HFBA, an aliquot of 1 μL supernatant was injected into the chromatographic system. Only a small amount of plasma sample, 10 μL, is sufficient for the monitoring of amikacin levels in clinically therapeutic range. The relative standard deviations (RSD) of the method for intra- and inter-day analyses ($n = 5$) are less than 5.8%. Application of this method for the trace analysis of amikacin in human plasma proved simple and workable.

Keywords: Micro-scale; Aminoglycoside antibiotics; Amikacin; Capillary column

1. Introduction

The aminoglycosides (such as amikacin, gentamicin, kanamycin, neomycin and tobramycin) are broad-spectrum antibiotics and are the mainstay in the treatment of severe infections caused by gram-negative bacteria. Aminoglycosides are bactericidal antimicrobial agents and the target of aminoglycoside activity in the bacterial cell is the 30S ribosomal subunit. But the major disadvantage of the aminoglycosides is their association with nephrotoxicity and ototoxicity. These antibiotics have a narrow therapeutic range, and a very high plasma level may cause side effects of ototoxicity and nephrotoxicity [1,2]. Careful monitoring of patient serum concentration of these drugs is necessary to exclude these serious adverse effects and to control appropriate levels in serum for effective therapeutic efficacy. The therapeutic ranges in serum of amikacin, gentamicin, kanamycin, neomycin and tobramycin are between 5 and 25 μg/mL [3,4].

The aminoglycosides contain two or more aminosugars, and are very hydrophilic compounds because of many amino and hydroxyl groups in their chemical skeletons. Commercially available methods currently used for the analysis of aminoglycosides include microbiological assay, radiochemical assay, radioimmunoassay and enzyme immunoassay, etc. [5]. These methods are easily handled but are non-specific. High-performance liquid chromatographic (HPLC) methods are reliable technique, and widely used for the determination of the aminoglycoside antibiotics [3,6–14]. The major demerit in direct analysis of aminoglycosides by HPLC methods is their lack of chromophore or fluorophore. It is necessary to label these compounds with chromophore or fluorophore tags by a chemically derivatized procedure and that is complicated and time consuming.

Because aminoglycosides are very hydrophilic molecules and insoluble in water-immiscible liquids, hence liquid–liquid extraction (LLE) of aminoglycosides with organic solvent is difficult. Solid-phase extraction (SPE) is a suitable technology for isolating polar compounds, so several types of SPE columns have been commonly used for extracting aminoglycosides in biosamples [8–14]. Using SPE column target analytes could

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be cleaned up, purified and concentrated easily from a complicated matrix. Mass spectrometry (MS) is a power technology for the analysis of aminoglycosides in biological samples [15,16]. LC with tandem mass spectrometry (LC–MS–MS) coupled SPE column has become an attractive technology for identification of aminoglycosides [4,17–23].

In this paper we developed a simple micro-scale LC–MS–MS method for the analysis of aminoglycoside antibiotics in human plasma. A similar method has been developed by Keevil et al. [24]. The on-line concentration of aminoglycosides was based on ion-pair chromatographic technology. Heptafluorobutyric acid (HFBA) served as a counter ion of opposite charge and formed a neutral ion-pair with the analyte. According to this strategy the polarity of aminoglycosides decreased and was retained on the hydrophobic stationary phase of capillary column. Then the aminoglycosides extracted in the capillary column were directly eluted to quadrupole time-of-flight (Q-TOF) MS detector. The method was successfully applied to analyze amikacin in human plasma. Only a small amount of plasma sample, 10 μL, is sufficient for the monitoring of amikacin levels in the clinically therapeutic range. Application of this method for the fast trace analysis of aminoglycoside antibiotics in biological matrices proved feasible.

2. Experimental

2.1. Materials

Amikacin sulfate, gentamicin sulfate (major product is gentamicin C2), kanamycin (major product is kanamycin A) sulfate, neomycin sulfate and tobramycin sulfate (as internal standard, IS) were purchased from Sigma (St. Louis, MO, USA). HPLC grade methanol was supplied by E. Merck (Darmstadt, Germany). Heptafluorobutyric acid (HFBA) was obtained from Aldrich (Milwaukee, WI, USA). Deionized water from a Milli-Q system (Millipore, Bedford, MA) was used at all times. Reference solutions of aminoglycosides at various concentrations and HFBA solution were prepared by dissolving the appropriate amounts of the respective compounds in deionized water. The protein-precipitating solution was prepared by adding HFBA to water to give a concentration of 100 g/L. Fused-silica capillaries (150 μm inner diameter, 375 μm outer diameter) were purchased from Polymicro Technologies (Phoenix, AZ, USA). The 3 μm reversed-phase C18 particle was obtained from Vydac (Hesperia, CA, USA). Drug-free human plasma samples were obtained from normal volunteers.

2.2. Apparatus

The experiments were carried out using an Agilent (Palo Alto, CA) 1100 Series CapLC pump, a binary pump, an autosampler and a switch valve (Vici, Schenkon, Switzerland). The MS–MS system consisted of a Micromass Q-TOF mass spectrometer (model Q-Tof-2, Massachusetts, USA) with a nanospray ESI source.

2.3. LC conditions

A 1 cm short reversed-phase C18 capillary column was prepared in-house laboratory and this method was established in our previous study [17]. Briefly, the C18 stationary phase in the capillary was immobilized with one frit. Before packing with stationary phase the outlet frit was created and then the 3 μm C18 stationary phase was suspended in methanol and poured into an 1 mL syringe. The fused-silica capillary was cut and sealed at one end using bare-silica material and then sintered by pocket microtorch. The needle of the syringe and the fused-silica capillary was connected by a piece of PTFE tubing. The C18 stationary phase suspension was then transferred into the capillary by sedimentation with gravity and hand-push. After slurry packing procedure, 10 cm × 150 μm fused-silica capillary was finally filled with 1 cm reversed-phase C18 stationary phase. The short capillary column was used and provided the stationary phase to retain analytes.

The modified micro-scale on-line concentration system applied in this work was shown in Fig. 1. For amikacin analysis in plasma the mobile phase of pump 1 (binary pump, for sample loading) was 40 mM HFBA and pump 2 (CapLC pump, for sample analysis) was methanol: 40 mM HFBA = 70: 30 (v/v) at a flow rate of 3 and 1 μL/min, respectively. The sample loading time was 2 min (switching valve at the initial position (Fig. 1A)). After sample loading the valve was switched and mobile from pump 2 eluting the analyte of interest to the nanospray source (Fig. 1B). After analyte eluting, the valve was switched to the initial position and pump 1 delivered 40 mM HFBA to the col-

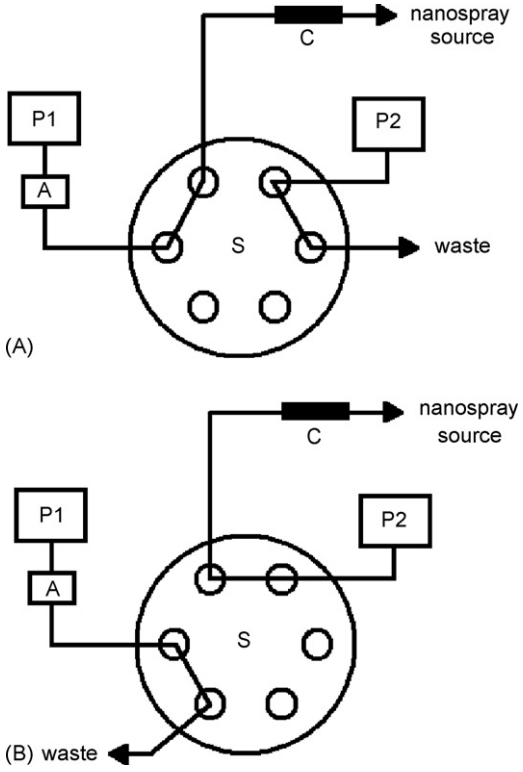

Fig. 1. The micro-scale on-line concentration system: A = autosampler; C = 1 cm capillary column; P1 = pump 1; P2 = pump 2 and S = switch valve. (A) and (B) represented the loaded and eluted positions of the switch valve.