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Rapid analysis of neomycin in cochlear perilymph of guinea pigs using disposable SPE cartridges and high performance liquid chromatography-tandem mass spectrometry

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ABSTRACT

Irreversible hearing loss induced by aminoglycoside in human through local or systemic administration route negatively impacts quality of life. The aim of this work was to develop and validate an analytical method suitable for the detection and quantification of neomycin in cochlear perilymph of guinea pig after local application. The SupelMIP SPE column was used for the pre-treatment of matrix. Chromatographic separation was conducted by a reversed phase ODS column (100 × 2.1 mm, 3 μm) at 40 °C in gradient mode with 0.2‰ (v/v) HFBA in water and 0.2‰ (v/v) HFBA in acetonitrile as mobile phase, at a flow rate of 0.30 mL/min, with retention time of 3.50 and 3.62 min for internal standard tobramycin and analyte neomycin, respectively. The MS was performed with positive ionization mode, with data acquisition in Multi Reaction Monitor (MRM) mode. This method was proved to be specific, accurate (97.1–115% of nominal values) and precise (CV% < 15%). Calibration curves for matrix matched standard of neomycin ranged from 1.25 to 200 μg/mL, with LOD and LLOQ of 0.625 and 1.25 μg/mL in blank matrix. The matrix effect was corrected to (−0.1) - 1.33 by adding internal standard. The relative SPE recovery values were ≥98.9% in low, medium and high QC samples. Neomycin in matrix proved to be stable under room temperature - and −20 °C, or under three freeze-thawing cycles, or under processing as well. Finally, the proposed method was successfully applied to a toxicokinetics study of neomycin in perilymph after round window membrane (RWM) administration, which was in accordance with threshold shift of auditory brainstem response (ABR) test related to hearing loss.

1. Introduction

Aminoglycoside antibiotics (AmAns) are widely used in the treatment of gram-negative bacterial infections like tuberculosis and severe hospital acquired infections [1,2]. Dose-limiting side effects include cochlear and/or vestibular toxicity or nephrotoxicity. 10% of patients receiving these drugs intravenously suffer from bilateral and irreversible hearing loss, which results in failure to return to work and reduced quality of life [3]. As AmAns are crucial agents both in the treatment of infections and Meniere's disease [4], a great effort has been made to develop new strategies to attenuate drug induced ototoxicity. Thus study about the distribution of AmAns along scala tympani (ST) perilymph after local application, may help reveal the potential toxicological mechanism, as well as improve the efficiency of local treatment.

A quantitative method of gentamicin in perilymph from pigmented guinea pigs based on a fluorescence-polarization-immunoassay (FPIA) has been established by Plontke et al. since 2007 [5]. Several sequential perilymph samples with approximately 1 μL each were further diluted with a ratio of 1:79 before quantification with LOQ of 0.27 μg/mL [6].

Abbreviations: Heptafluorobutyric acid, HFBA; Round window membrane, RWM; auditory brainstem response, ABR; Multi Reaction Monitor, MRM; Coefficient of Variation, CV; limit of detection, LOD; lower limit of quantitation, LLOQ; quality control, QC

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However, the disadvantages of FPIA containing higher-reactivity, or a limited linearity [7], or preparation of mono- or polyclonal antibody of analyte [8], limit its application in separation and analysis of biologic samples. In contrast, high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) proves to be a promising method for bioanalysis of AmAns to monitor veterinary drug residues in animal-derived foods [9,10]. Due to highly hydrophilic nature for AmAns, general chromatographic conditions such as UV chromophores for other drug classes may be not suitable, which makes it difficult for detection. Ion-pairing chromatography containing heptafluorobutyric acid (HFBA) (0.010–0.050%) as an ion-pairing agent to conjugate the analyte ions forming column retention molecules [11,12], is more commonly needed for bioanalysis of AmAns [13,14]. Furthermore, solid-phase extraction (SPE) is required for effective sample preparation, which will control the potential matrix co-extractives from complicated tissues in a cost-effective approach [15,16]. The SupelMIP SPE column consists of highly-linked polymers, which are engineered to form specific cavities or imprints complementary to the analyte of interest [17,18], allowing extraction of a class of structural specific analytes such as AmAns.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) serves as an ideal approach to detect neomycin. Veal samples containing neomycin after extraction and cleanup were separated on a Nucleosil C18, with the help of an ion-pairing agent, prior to analyses by electrospray MS/MS. The calibration curve was linear ranging from 50 to 5000 ppb, with 51% for the recovery of neomycin in kidney, which gave equivalent results to fluorescence detection [19]. Another analytical method for the determination of neomycin in animal origin products was carried out by LC-MS/MS for quantitative analysis and liquid chromatography-quadrupole-time of flight-mass spectrometry (LC-QTOF-MS) for screening purposes, with 50 and 125 ng/g for the detection limit (LOD) and quantification limit (LOQ), respectively [20]. Furthermore, neomycin in milk after intra-mammary infusion was determined by LC/MS/MS after cleanup on cation-exchange cartridge columns and separation on a TSK-gel VMak25 column, with 66.1 ± 11.4% and 66.3 ± 14.2% for the recoveries at fortification levels of 0.01 and 0.1 μg/g, respectively [21]. Another method adapted to LC-MS(n) for neomycin detection was a derivatization procedure by reacting it with phenyl isocyanate after isolating neomycin from the milk, with 9.8 ng/mL for the detection limit [22].

The aim of this work is to develop and validate an analytical method for neomycin in cochlear perilymph of guinea pigs. Trace analysis in tiny tissue samples with only a few microliters was developed, followed by sufficient quantitative method validation including specificity, linearity, sensitivity, recovery, accuracy, and stability. Finally, the proposed HPLC-MS/MS was applied to determine the toxicokinetics profiles of neomycin in perilymph by round window membrane (RWM) permeation in an animal model of drug-induced hearing loss.

2. Materials and methods

2.1. Chemicals and reagents

Reference standard neomycin sulfate (N109019) and Neomycin sulfate (N109017) with USP grade were obtained from Aladdin Industrial Corporation (Shanghai, China), while tobramycin (130527–200,402) as the internal standard (IS) was from National Institutes for Food and Drug Control (Beijing, China) (Fig. 1). The stock solution of neomycin of 320 μg/mL in water was prepared and stored in fluoroethylene propylene volumetric flask, considering that AmAns prone to sticking to glass surface. For internal standard (tobramycin), a separate 400 μg/mL aqueous solution was prepared. All the above standard solutions were stored in dark place and were frozen at −20 °C.

Methanol (MeOH) and acetonitrile (MeCN) were LC-grade from Burdick &Jackson. Heptafluorobutyric acid (H106256-5 mL) used for ion-pairing chromatography was obtained from Aladdin. House-deionized water was further purified through Milli-Q lab water purification system. Sodium dihydrogen phosphate (NaH_2PO_4), ethylene diaminetetraacetic acid, disodium salt, dehydrate ($Na_2EDTA{\cdot}2H_2O$) was from XiLONG SCIENTIFIC (Guangdong, China). Trichloroacetic acid (TCA), Formic acid, and dichloromethane were obtained from Beijing Chemical Works. 1 M HCl and NaOH in H_2O were used to adjust pH of extraction solution containing 10 mM NaH_2PO_4, 0.4 mM Na_2EDTA and 2% TCA at pH 4.0. SupelMIP SPE (52777-U) special for aminoglycosides with 3 mL volume and 50 mg was obtained from Sigma. 20-position manually-operated level arm extractor was made for cleanup in SPE.

2.2. Animal preparation

Albino adult guinea pigs (Hartley strain, from Beijing Vital River Animal Technology Co., Ltd.) of both genders, weighting between 350 and 400 g were used in the study. The animals were anaesthetized with 40 mg/kg pentobarbital sodium administered intraperitoneally and were injected subcutaneously with lidocaine hydrochloride for local analgesia before surgery. All procedures of animal experiments were approved by the Institutional Animal Care and Use Committee (ACUC) and conform to the Policy on Humane Care and Use of Laboratory Animals at PLA General Hospital (process no. 2017-X13-62).

2.3. Sample preparation

For perilymph sampling, the round window near the cochlea basal turn was exposed by a dorsolateral posterior-auricular surgical approach, after the bulla of one ear was opened using a 1.5 mm diameter cutting burr under the operating microscope [23]. Then the tip of polypropylene micro-capillary was inserted into scala tympani (ST) through the round window membrane (RWM), and perilymph from the

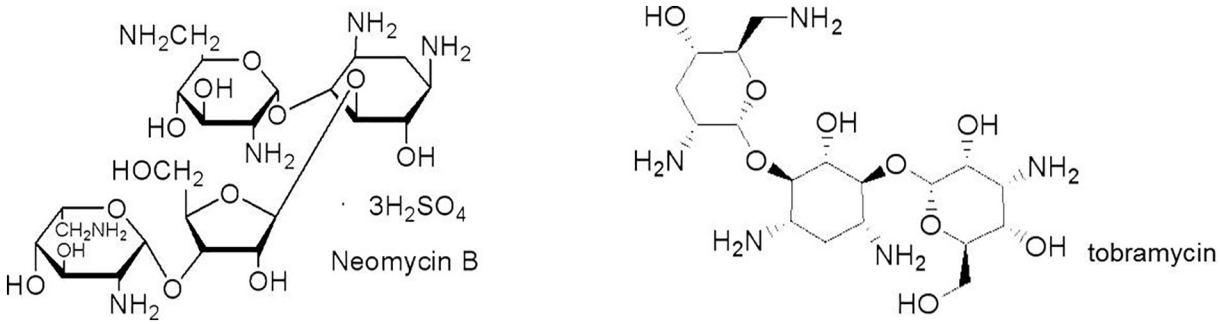

Fig. 1. The chemical structures of neomycin and tobramycin.