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Suitability of a liquid chromatography assay of neomycin sulfate to replace the microbiological assay for neomycin in USP Monographs☆

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ABSTRACT

The current USP National Formulary contains 65 Monographs for drug formulations containing neomycin. All 65 Monographs prescribe a bioassay for neomycin assay. This bioassay, based on cell culture, is labor intensive, has poor precision, and cannot be adapted for purity or identification. High-performance anion-exchange chromatography with integrated pulsed amperometric detection (HPAE-IPAD), a liquid chromatography technique, has been shown to be suitable for neomycin purity analysis and neomycin assay of an over-the-counter first aid cream (Hanko and Rohrer [17]). Here we propose that an HPAE-IPAD assay can replace the bioassay in the 65 neomycin-containing Monographs. We applied the HPAE-IPAD assay to four neomycin-containing drug products representing the four classes of formulations found in the 65 Monographs, liquid, solid, suspension, and cream. Each drug was analyzed with two chromatography systems, and on 3 separate days. For all products, HPAE-IPAD measurements were precise and accurate with respect to the label concentrations. There was also high accuracy for spike recovery of neomycin from the four drug products throughout 70–150% of the labeled concentration. These results suggest that an HPAE-IPAD assay would be an accurate assay for neomycin, and would be faster and more precise than the current bioassay.

1. Introduction

Neomycin is a water-soluble complex of aminoglycosides produced from the fermentation of the actinomycete *Streptomyces fradiae* [1–4]. Neomycin B (also known as framycetin) is the principal component of the complex, and has the highest antibiotic activity. Neomycin B is purified from the fermentation complex, and the free base is coupled with sulfate counter-ions, which is then used in a variety of antibiotic pharmaceutical products, labeled as containing neomycin sulfate. These product applications include ophthalmic, topical, oral, and intravenous administrations. The current USP Monographs specify that an assay for neomycin sulfate and all neomycin sulfate-containing pharmaceutical products be performed using an antibiotics microbial assay with *Klebsiella pneumoniae* or *Staphylococcus epidermidis* as the test organism [5,6]. Microbial assays are labor intensive and drug potency is normally measured in units of activity, relative to a designated federal master standard [7]. Inter- and intra-assay variables impact reliability. Each test requires 16–24 h to prepare the inoculums, and either 16–18 h to incubate cylinder plates or 4–5 h to incubate test tubes for the turbidimetric method. No purity information can be obtained using the microbial assay, but antibiotic impurities can produce errors in the measured activity, thereby compromising method accuracy with respect to the measurement of just neomycin B activity.

In 2002, the Council of Europe revised the European Pharmapoeia (EP) official monograph for neomycin sulfate and framycetin sulfate from a bioassay to a liquid chromatographic (LC) method for

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identity, assay, and purity [8,9]. The LC methods use pulsed amperometric detection (PAD) with a gold working electrode. Neomycin B and its major impurities belong to a class of compounds, including carbohydrates, glycols, alcohols, amines, and sulfur-containing compounds, that can be oxidized and therefore directly detected by amperometry. PAD has a broad linear range and very low detection limits for aminoglycoside antibiotics [10–19]. The LC method specified by the EP requires a non-alkaline mobile phase; therefore the pH must be elevated through post-column addition of NaOH to achieve detection. The post-column addition requires an additional pump and dilutes eluting peaks through a reaction coil, causing a reduction in method sensitivity compared to a method with a sufficiently alkaline eluent. In addition to the complication of a post-column setup, there are concerns about the reproducibility of the method based on the choice of an older PAD waveform that is known to have reduced response with use, and possible issues resulting from an inadequate description of the electrochemical conditions [19]. In Ref. [19], the authors had to alter the eluent conditions to reproduce the reported chromatography, and they adapted the method to another column to improve the chromatography. Our experience with the EP method suggests that there are problems with varying quality of eluent components, some difficulty in eluent preparation, and possible issues with column lifetime.

High-performance anion-exchange chromatography (HPAE) is a technique capable of separating aminoglycoside antibiotics and their impurities [13–18]. The USP Compendial Method for streptomycin currently uses HPAE-PAD to assay this aminoglycoside antibiotic [20]. The CarboPac® PA1 anion-exchange column (USP packing L46) separates neomycin B and its impurities using an alkaline mobile phase, necessary for amperometric detection. In previous publications [15,17], we evaluated accuracy, precision, lower limits of detection, linearity, and ruggedness, in a manner consistent with the requirements of USP method validation [21]. We demonstrated the capability of HPAE-IPAD for the determination of neomycin B in three different topical over-the-counter pharmaceutical formulations also containing pramoxine HCl, Polymyxin B sulfate, and Bacitracin Zinc among the active ingredients; and emulsified wax, methylparaben, mineral oil, propylene glycol, cocoa butter, cottonseed oil, olive oil, sodium pyruvate, vitamin E, and white petrolatum among the inactive ingredients [22]. Overall, the method demonstrated good sensitivity, good sample throughput (15 min per sample), and high retention time reproducibility. Spike recovery of neomycin B from these two ointments and one cream ranged from 95 to 100%, and the measured concentrations closely agreed with their respective label concentrations. The same method can be used to evaluate the purity of neomycin sulfate. Although this previous publication demonstrated good performance for the assay of neomycin sulfate contained within what we considered to be a challenging formulation, the USP National Formulary has 65 Monographs of neomycin sulfate-containing formulations, and we did not demonstrate that HPAE-IPAD was applicable to the other classes of formulations among the 65.

We reviewed all USP formulations and identified four major classes based on the methods required to prepare samples to obtain accurate measurements. We chose a commercial product to evaluate from each class. The four classes and chosen products were (1) solid (e.g., tablets and powder): Neo-Rx Neomycin Sulfate; (2) liquids (e.g., sterile injectables or irrigating solutions): Neosporin G.U. Irrigant; (3) suspensions: Cortisporin® Ophthalmic Suspension; and (4) ointments and creams: Original Neosporin Neomycin and Polymyxin B and Bacitracin Zinc First Aid Ointment. We tested these four representative pharmaceuticals for accuracy and precision using two different chromatographic systems, with assays on each conducted on 3 separate days, and found the method to be suitable for this application. Based on these results, we believe this method is suitable for assay of all 65 neomycin sulfate-containing formulations described in the USP National Formulary.

2. Experimental

2.1. Reference standard

Neomycin sulfate (782 mg standard free base per gram dry solid material, Reference Standard #45800, Lot No. L3E135; USP, Rockville, MD, USA), MW 614.65 (free base), CAS [1405–10–3].

2.2. Drug substance

Neomycin sulfate (701 mg standard free base per gram dry solid material, serving as drug substance; Cat# N5285-25G, 25 g, Lot No. 061K08921; Sigma–Aldrich Chemical Co, St. Louis, MO, USA). Formula: $C_{23}H_{46}N_6O_{13}\cdot 3H_2SO_4\cdot xH_2O$, FW 908.9, MW = 614.65 (free base), CAS [1405-10-3]. *Certificate of analysis results*: loss on drying: 3.6%, sulfate: 29.1%, residue on ignition: 0.2%.

2.3. Drug products

These four drug products were kindly provided by the USP:

Neomycin Sulfate USP, Micronized, Neo-Rx: 675 mg/g solid (labeled assay potency) as free base concentration; NDC 39822-0300-1 (Xgen Pharmaceuticals, Inc., Northport, NY, USA). Certificate of Analysis Results: assayed at 684 μg per mg, moisture: 0.2%.
Neosporin G.U. Irrigant: 40 mg neomycin base per 1 mL ampoule; NDC 61570-047-10 (Monarch Pharmaceuticals/King Pharmaceuticals, Bristol, TN, USA).
Cortisporin® Ophthalmic Suspension Sterile: 3.5 mg neomycin base per 1 mL of suspension; NDC 61570-036-75 (Pfizer Consumer Healthcare, Morris Plains, NJ, USA).
Original Neosporin® Neomycin and Polymyxin B and Bacitracin Zinc First Aid Ointment: 3.5 mg neomycin base per 1 g of ointment; NDC 0081-0730-88 (Pfizer Consumer Healthcare, Morris Plains, NJ, USA).

2.4. Apparatus

Both chromatography systems consisted of an ICS-3000 gradient pump with degas option and GM-4 gradient mixer, EG Eluent Generator with EGC II KOH eluent generator cartridge (EluGen® II Hydroxide) and CR-ATC, vacuum degas conversion kit, DC Detector Compartment, AS Autosampler, and Chromeleon® chromatography workstation (Dionex Corporation, Sunnyvale, CA, USA). Mobile phase (2.40 mM KOH) was automatically prepared by the eluent generator equipped with an EluGen Hydroxide cartridge and supplied deionized water. Neomycin, impurities, and ingredients of product formulations were separated with a CarboPac® PA1 (4 mm × 250 mm, Dionex Corporation) anion-exchange column (USP designation L46) with its guard (4 mm × 50 mm). The electrochemical waveform was +0.13 V from 0.00 to 0.04 s, +0.33 V from 0.05 to 0.21 s, +0.55 V from 0.22 to 0.46 s, +0.33 V from 0.47 to 0.56 s, −1.67 V from 0.57 to 0.58 s, +0.93 V at 0.59 s, and +0.13 V at 0.60 s, using the pH reference mode with current integrated between 0.21 and 0.56 s for detection. We used AAA-Direct™-certified disposable gold working electrodes with their specified gaskets. Solid formulations, and neomycin sulfate standards and drug substances were dried for >20 h at 0.3–0.5 Torr at 60 °C in microcentrifuge tubes with detachable caps (plastic, 1.5 mL, Sarstedt, P/N 163/204; or equivalent) using a SpeedVac Evaporator system (ThermoQuest