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Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 1006–1012
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Determination of tobramycin and impurities using high-performance anion exchange chromatography with integrated pulsed amperometric detection

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Received 1 April 2005; received in revised form 1 August 2005; accepted 1 August 2005
Available online 21 October 2005

Abstract

Tobramycin is one of a class of aminoglycoside antibiotics that lack a good chromophore, and is therefore difficult to determine using reversed-phase HPLC with absorbance detection. This is especially true for determining the quantity of each impurity. We show that tobramycin and its major impurities, including kanamycin B and neamine (neomycin A), can be separated on a strong anion-exchange column using a weak potassium hydroxide eluent (2.00 mM) at a column temperature of 30 °C, and directly detected by integrated pulsed amperometric detection (IPAD). The resolution (United States Pharmacopeia (USP) definition) between tobramycin and kanamycin B ranged from 5.71 and 6.06 over 7 days of consecutive analysis (5.92 ± 0.07, $n = 590$ injections). Due to the difficulty of producing weak hydroxide eluents of the required purity (i.e. carbonate-free), this method depends on automatic eluent generation to ensure method ruggedness. This method exhibited good long-term (50 days, 2368 injections) retention time stability with R.S.D.s of 0.4% and 0.3% for tobramycin and kanamycin B, respectively. Peak area R.S.D.s for tobramycin and kanamycin B (10 μM each, 20 μL injection) over 7 days (572 injections) were 2.3% and 1.9%, respectively. Method robustness was evaluated by intentionally varying the flow rate, eluent concentration, column temperature, and column. Based on the results of these evaluations, this method can be used for tobramycin identity, assay, and purity.

Keywords: HPAE-PAD; HPAE-IPAD; Tobramycin; Kanamycin B; Neamine; Neomycin A; Aminoglycoside; Antibiotic; Electrochemical detection; Anion-exchange; Chromatography

1. Introduction

Tobramycin is a water-soluble aminoglycoside antibiotic purified from the fermentation of the actinomycete *Streptomyces tenebrarius* and used in a variety of pharmaceutical applications [1], including ophthalmic suspensions and ointments, such as TobraDex® (Alcon Inc., Fort Worth, TX), inhalation solutions such as TOBI® (Chiron Corporation, Woodstock, IL), and intravenous administrations such as Tobramycin Sulfate Injection (Eli Lilly and Company, Indianapolis, IN). Kanamycin B (also known as bekanamycin), nebramine, and neamine (also known as neomycin A) are three known impurities of tobramycin [2] that result from either incomplete purification of the drug, or from its degradation. The chemical structure of tobramycin and its major impurities are shown in Fig. 1. These aminoglycosides, like most carbohydrates, lack a good chromophore, and therefore require high concentrations to be detected by UV absorbance. Many manufacturing process intermediates and ingredients of final pharmaceutical formulations are chromophoric and can interfere with the direct detection of tobramycin and its impurities by absorbance. Refractive index detection has similar limitations. For these reasons, most methods for identity, assay, or purity of tobramycin require some type of chemical derivatization to increase detection sensitivity [3].

Derivatization techniques have the associated limitations of varying derivatization efficiencies and reagent instabilities that compromise method ruggedness. These techniques also require handling and disposal of hazardous materials. Tobramycin and its major impurities may be oxidized under alkaline conditions and directly detected by pulsed amperometric detection (PAD), which has a broad linear range and very low detection limits for aminoglycoside antibiotics. The HPLC method specified by the European Pharmacopoeia (EP) procedures for tobramycin assay

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doi:10.1016/j.jpba.2005.08.009

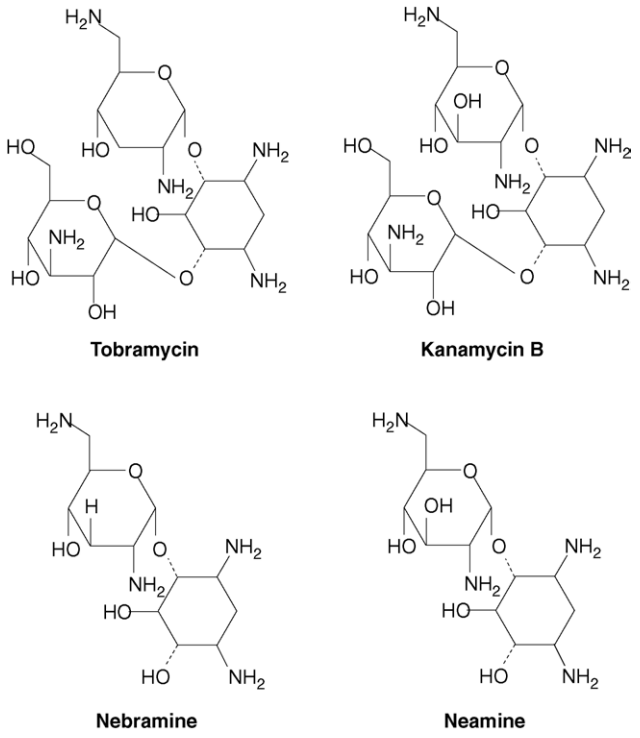

Fig. 1. Chemical structures of tobramycin and known impurities (kanamycin B, nebramine, and neamine).

and purity [2,4] uses a non-alkaline mobile phase and requires a post-column addition of NaOH for PAD. This requires an additional pump and dilutes eluting peaks, reducing method sensitivity compared to a method with a sufficiently alkaline eluent.

High-performance anion-exchange chromatography (HPAE) with PAD using alkaline eluents is an established technique for carbohydrate analysis. HPAE-PAD has been used for tobramycin determinations [5–8], and for other aminoglycoside antibiotics determinations [9–12]. All published methods require weak sodium hydroxide eluents (1–10 mM) that are difficult to prepare reproducibly without carbonate contamination. Varying amounts of carbonate adversely affect retention time precision. This problem has limited the adoption of HPAE-PAD for tobramycin determinations. To address this limitation, we added two commercially available devices to an HPAE-PAD system, an eluent generator (EG), and a continuously regenerated anion trap column (CR-ATC). The EG automatically prepares precise concentrations of KOH eluent from water and a potassium electrolyte solution using electrolysis. The electrolysis occurs without exposure to atmospheric gases, and therefore with a significant reduction of carbonate contamination. The minor amounts of carbonate, as well as borate and other contaminating anions from the supply water, are removed by a continuously regenerated anion trap column (CR-ATC), installed after the EG. Consequently, the normal variability in hydroxide concentration associated with manual eluent preparation and the variability of carbonate contamination are essentially eliminated, leading to highly reproducible retention times. We used this system to develop a fast rugged assay of tobramycin and its major known impurities. To achieve the best sensitivity and to have the best reproducibility of electrochemical response, we used a different waveform compared to typical carbohydrate analysis, and we used disposable gold working electrodes. In this paper, we evaluated the performance of this method in terms of ruggedness and the ability to measure low levels of the major known impurities of tobramycin.

2. Experimental

2.1. Standards

Solid tobramycin (Sigma–Aldrich Chemical Co., Cat# T40014), kanamycin B; Sigma–Aldrich Chemical Co., Cat# B5264), and neamine (International Chemical Reference Substances, World Health Organization; Cat# 9930354) standards were placed in plastic vials and dissolved in deionized water to a 10 mg/mL concentration. The masses of moisture, salt, and impurities, as stated on the manufacturer's Certificate of Analysis, were subtracted from the measured mass to improve solution concentration accuracy. These solutions were further diluted with water (gravimetrically) to yield the desired stock mixture concentrations. For this work, all dilutions were made gravimetrically to ensure high accuracy. Masses of 0.2, 1, 2, 20, 100, 200, 300, 400, 600, 800, 1000, 1200, 1400, 1800, 2000, 4000, 10,000, and 21,000 pmol tobramycin and kanamycin B were injected four-times each for linear range studies. Tobramycin, and to a lesser extent neamine and kanamycin B, when dissolved in water adsorbs to glass surfaces. Significant losses due to adsorption occur at dilute concentrations. Polypropylene injection vials and other labware must be used to ensure accurate results.

2.2. Chromatography

The chromatography system consisted of Dionex GP50 gradient pump with degas option and GM-4 gradient mixer, EG50 eluent generator with EGC II KOH eluent generator cartridge (EluGen® II hydroxide) and CR-ATC, EG40/50 vacuum degas conversion kit, ED50A electrochemical detector, AS50 autosampler, AS50TC thermal compartment, and Chromeleon® chromatography workstation (Dionex Corporation, Sunnyvale, CA, USA). Tobramycin and its impurities were separated with a CarboPac™ PA1 (4 mm × 250 mm, Dionex Corporation) anion-exchange column with its guard (4 mm × 50 mm; USP designation L46). 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 s to 0.46 s, +0.33 V from 0.47 s to 0.56 s, −1.67 V from 0.57 s to 0.58 s, +0.93 V at 0.59 s, and +0.13 V at 0.60 s, using the pH reference electrode mode with current integrated between 0.21 and 0.56 s for detection. We used *AAA-Direct*™-certified disposable gold working electrodes (replaced every 7 days) [13–15]. Tobramycin and its impurities were separated using 2.00 mM KOH, produced by the eluent generator, at a flow rate of 0.50 mL/min and a column temperature of 30 °C. This method used a 20-μL injection and had a run time of 15 min. The column set was washed once a week with 100 mM KOH for 60 min and re-equilibrated overnight to 2.00 mM to restore retention times to their initial values.

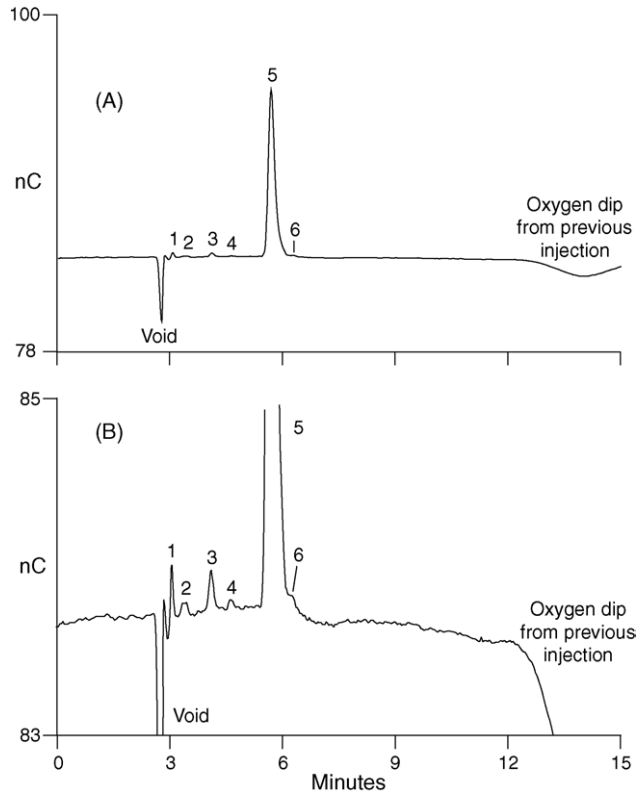

Fig. 2. Determination of 21 pmol tobramycin (1.07 μM, 20 μL injection) using a CarboPac PA1 column with a 2.00 mM KOH eluent (produced by an eluent generator) at 0.5 mL/min, and with a 30 °C column temperature, and IPAD. Full view (A), and expanded view of baseline (B). Peaks 1, 2, and 6: unknown impurities; Peak 3: kanamycin B; Peak 4: neamine; Peak 5: tobramycin; Peak 7: oxygen dip from the previous injection.

3. Results and discussion

3.1. Specificity

3.1.1. Separation

Fig. 2 shows the separation of 1.07 μM (20 μL injection, 21.4 pmol) tobramycin (Peak 5) and five impurities (Peaks 1, 2, 3, 4, 6). Peak 3 was identified as kanamycin B and Peak 4 as neamine (neomycin A) based on the retention times of standards. The resolution (USP definition) between tobramycin and kanamycin B ranged from 5.71 and 6.06 over 7 days of consecutive analysis (5.92 ± 0.07, $n = 590$ injections). The EP method requires resolution to be greater than 3.0 between tobramycin and kanamycin B, and allows adjustment of the mobile phase concentration to achieve this required resolution.

3.1.2. Detection

The EP method specifies a gold working electrode using a 3-potential pulsed amperometric waveform with detection at +0.05 V, oxidative cleaning at +0.75 V, and gold oxide reduction at −0.15 V, but does not specify the durations of these steps. These voltages are identical to a waveform that in earlier years was used for carbohydrate determinations (Waveform B; Dionex Technical Note 21) [16]. The same waveform was also sited in reference [4], on which the EP method was based. This waveform caused the loss of gold from the surface and results in a loss in peak area response over long-term use [17]. Since the development of the EP method, the recommended waveform for carbohydrate applications has been revised [16,17] to produce a more stable long-term response. This waveform (Waveform A, Dionex Technical Note 21) [16] is a 4-potential program that uses reductive cleaning rather than oxidative cleaning, preserving the gold working electrode surface. Unlike the 3-potential waveform, the 4-potential waveform is fully compatible with disposable gold working electrodes, which have only a thin layer of gold. While we found the 4-potential waveform was suitable for use in aminoglycoside determinations [8], here we used an integrated pulsed amperometric detection (IPAD) waveform, used for amino acid determinations. An IPAD waveform detects an analyte using more than the one potential used in a PAD waveform. We chose this waveform because the signal-to-noise ratio was determined to be two to four times greater using the IPAD waveform than the 4-potential waveform. This allowed us to achieve maximum sensitivity for tobramycin and its impurities.

3.2. Linearity

3.2.1. Range

For estimation of the upper limit of the linear range, the technique of Cassidy and Janoski [18] and Snyder et al. [19] was used. The linear range was calculated using a plot that relates amount of tobramycin or kanamycin B injected to their peak area or height response factors (ratio of peak area/mass injected). The region of the plot showing the highest and constant response factor values was averaged, and then a 10% deviation from this averaged value was calculated. The upper and lower limits of the linear range were the amounts of tobramycin and kanamycin B where the observed response factors are within 10% of the average response factors. We chose a 10% deviation as our threshold for estimating the upper and lower limits. The tobramycin peak area linearity extended up to 300 pmol (15 μM for a 20 μL injection), and kanamycin B linearity extended up to 200 pmol (10 μM). The tobramycin peak height was linear up to 200 pmol (10 μM), and kanamycin B peak height was linear up to 175 pmol (8.8 μM). For both compounds, below 4 pmol (0.2 μM) the peak area response factor decreased below our 10% deviation threshold. Therefore, the calculated linear ranges for tobramycin and kanamycin B peak areas are 4–300 pmol and 4–200 pmol, respectively.

3.2.2. Linearity

The linear relationship of peak area response evaluated using 2, 20, 100, 200, and 300 pmol tobramycin produced an r^2 value of 0.9994; and 2, 20, 100, 200 pmol kanamycin B an r^2 of 0.9958. Table 1 summarizes the statistics (r^2, slope, y-intercept) for these two calibration curves. Slopes and y-intercepts for tobramycin and kanamycin B were nearly identical, an indication that an accurate measure of kanamycin B impurity can be obtained by measuring its area as a percentage of the tobramycin peak area. This eliminates the need to run separate kanamycin B standards. The known 20, 100, 200 and 300 pmol amounts were com-

Table 1
Estimated linearity for tobramycin and kanamycin B

	Tobramycin	Kanamycin B
Upper limit linearity (estimate)		
pmol	300	200
μM[a]	15	10
pg	140000	97000
μg/mL[a]	7	5
Linearity (over linear range)		
Range (pmol)	2–300	2–200
r^2	0.9994	0.9958
y-intercept (nC min)	0.336	0.380
Slope (nC min/pmol)	0.0917	0.0929

[a] 20-μL injection.

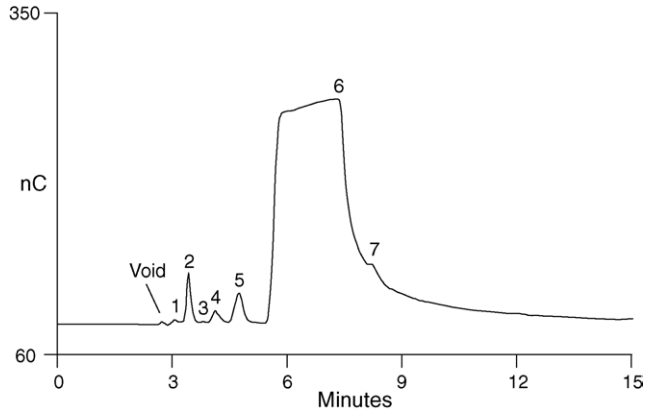

Fig. 3. Determination of impurities (Peaks 1–5, 7) when tobramycin (Peak 6) is injected outside its calculated linear range (0.50 mg/mL, 20 μL injection). Peak 4 is kanamycin B, and Peak 5 is neamine.

pared against the calculated amounts of tobramycin and errors ranged from −6.8% to +1.0%. For 20, 100, 200, and 300 pmol of kanamycin B, the calculated errors ranged from −7.5% to +8.6%. The % error increased significantly when determinations were made outside the calculated linear range. A quadratic calibration can be used to measure higher amounts. For example, at 400 or 600 pmol tobramycin, the % errors were +1.6% and +7.4%, respectively. The same quadratic fit also allowed inclusion of lower amounts of tobramycin and kanamycin B, but better results (lower error) were attained using a separate low-level quadratic calibration curve for amounts below 2 pmol.

3.3. Lower limits of detection and quantification

The concentration (or mass injected) of tobramycin at the lower limit of detection (LOD) was calculated from three times the average peak-to-peak noise (a height value), divided by the average peak height response factor for the antibiotic within its linear region. At this concentration, signal-to-noise ratio equals 3. The lower limit of quantification (LOQ) was calculated as 10 times the average peak-to-peak noise. Baseline noise ranged from 14 to 91 pC (mean 37 pC). The LOD and LOQ were 0.24 and 0.79 pmol, respectively, for a 20-μL injection of tobramycin, and 0.15 and 0.49 pmol for kanamycin B.

3.4. Determination of tobramycin purity

When tobramycin is analyzed at the upper limit of its calculated linear range (300 pmol), this method can detect kanamycin B as a 0.05 mole% impurity of tobramycin. A lower percentage can be detected by injecting tobramycin at concentrations outside its linear range. Injecting 20 μL of 1.0 mM tobramycin (21 nmol, Fig. 3) decreases the lower detectable percentage of kanamycin B impurity to 7.1×10^{-4}%; but requires a second injection of diluted tobramycin (to within its linear range) to measure the amount of tobramycin for the percent impurity calculation. Table 2 presents the measured impurities found in the tobramycin analyzed in this paper as pmol tobramycin equivalents, with their corresponding percents. Using the injection shown in Fig. 3, we determined that kanamycin B was a 0.24% impurity of tobramycin. The sum of all impurities (Peaks 1–5, 7) was 1.78%. The largest single impurity was neamine at 0.73%. The unidentified impurity (Peak 7), eluting after tobramycin, was 0.10%, and a trace impurity (Peak 3), which was only detected with 1 mM injections of tobramycin, was 0.0065%. All impurity calculations presented in Table 2 were corrected for the 52.2% recovery observed for kanamycin B spiked into tobramycin at this 1 mM tobramycin concentration (see Section 3.5 below).

Table 2
Determination of the amounts of tobramycin impurities, and their relative percent as tobramycin equivalents

Peak identification	Measured pmol[a] amounts of tobramycin impurities						
	Unknown	Unknown	Unknown	Kanamycin B	Neamine	Unknown	Total
Peak label	1	2	3	4	5	7	
Retention time (min)	3.1	3.4	3.8	4.1	4.7	8.2	
Mean	7.24	137	1.35	50.0	151	20.7	367.4
S.D.	1.30	0.51	0.032	1.8	6.6	1.7	9.6
R.S.D. (%)	18	0.37	2.4	3.6	4.4	8.3	2.6
Percent impurity (%)	0.035	0.66	0.0065	0.24	0.73	0.10	1.78

See Fig. 3 for the chromatographic presentation of these impurity peaks. $N = 4$ injections (20 μL) of 1.034 mM tobramycin (21 nmol). All impurity data was corrected for a 52% spike recovery of kanamycin B from tobramycin observed at this concentration.

[a] pmol as tobramycin equivalents.

Table 3
Recovery of trace kanamycin B from tobramycin

Tobramycin (nmol)	Kanamycin B spiked (% of tobramycin)	Replicate #	Recovery (%)	2 mM HCl acidification recovery (%)
0.8	0.13	1	83.4	87.4
0.8	0.13	2	78.5	86.2
0.8	0.13	3	87.3	
0.8	0.13	4	83.8	
0.8	0.13	5	127.8	
0.8	0.37	1	93.1	
0.8	0.87	1	76.8	
0.8	1.4	1	73.9	
0.8	1.9	1	70.3	
0.8	2.9	1	68.8	
1	0.13	1	83.9	92.7
1	0.13	2		76.5
2	0.13	1	66.0	109.0
4	0.13	1	63.6	129.0
10	0.13	1	59.5	86.1
20	0.13	1	48.9	96.1
20	0.13	2	61.9	89.3
20	0.13	3	45.8	91.5
20	0.37	1	65.4	
20	0.87	1	72.8	
20	1.4	1	80.4	
20	1.9	1	85.8	
20	2.9	1	95.5	

The r^2 for impurity peak areas (Peaks 1, 2, 3, and 4) correlated with the amount of tobramycin injected (20–21,000 pmol) were 0.9549, 0.9997, 0.9990, and 0.9927, respectively. Although not observed in this study, any single impurity peak exceeding the upper limit of linearity (>200 pmol) would require dilution.

3.5. Accuracy

Method accuracy was determined by recovery experiments. Known quantities of kanamycin B ranging from 1 to 50 pmol were added to varying concentrations of tobramycin ranging from 0.76 to 20 nmol. The results of these experiments are summarized in Table 3. The recovery of kanamycin B from tobramycin dissolved in water ranged from 46% to 128%. For a fixed percent (0.13%) of kanamycin B relative to tobramycin, the recovery of kanamycin B generally decreased as the concentration of tobramycin increased. Recovery of kanamycin B from 0.76 nmol tobramycin generally decreased with increasing kanamycin B. The opposite trend was observed with 20 nmol tobramycin. High kanamycin B recoveries were achieved for all conditions tested when the sample was acidified with 2 mM HCl. Similar high recoveries were observed using 2 mM nitric acid (data not shown). The current USP compendial method for tobramycin chromatographic purity acidifies tobramycin with sulfuric acid prior to analysis [3]. The acidification of the tobramycin sample prior to injection yielded consistently high recovery, but decreased peak area response to 68% of the response seen in water. The varying recovery of kanamycin B under varying tobramycin conditions indicates the need for performing routine spike recovery corrections to all purity assays performed using a water sample matrix. Pre-acidification of tobramycin samples and standards apparently reduces the need for this correction. No further attempts were made to optimize the type or concentration of acid for recovery, nor was method performance evaluated using acidified samples.

The accuracy of this method for measuring impurities other than kanamycin B was not evaluated. Limited work with neamine suggests it can be determined as a tobramycin impurity in the same manner as presented for kanamycin B. The other tobramycin impurities were not commercially available.

3.6. Precision and reproducibility

3.6.1. Peak retention time

The average retention times for tobramycin and kanamycin B (10 μM each in a 20 μL injection) over 7 days (572 injections) were 5.74 ± 0.02 and 4.12 ± 0.01 min, respectively. Long-term (50 days, 2368 injections) retention time R.S.D.s were 0.4% and 0.3% for tobramycin and kanamycin B, with no upward or downward trend. These high retention time precisions were attributed to the automated production of KOH. When analyzing a relatively pure preparation of tobramycin, this method can be used without column regeneration for at least 7 days.

3.6.2. Peak area and height

The mean peak area for tobramycin (10 μM, 20 μL injection) injected for 7 days (572 injections) was 18.52 ± 0.42 nC min (2.3%). Peak height was 76.34 ± 1.20 nC (1.6%). Peak area for kanamycin B (10 μM, 20 μL injection) injected for 7 days (572 injections) was 17.81 ± 0.33 nC min (1.9%). Peak height was 116.7 ± 1.7 nC (1.5%). Daily (24 h) peak area R.S.D.s ranged from 1.1% to 2.3% for tobramycin and 0.8% to 1.7% for kanamycin B. The high retention time and response reproducibility indicate that this method is suitably rugged for this application.

3.7. Robustness

3.7.1. Eluent concentration

A 10% increase in KOH (2.20 mM) produced a retention time decrease to 4.7 min (−18% change from 2.00 mM) for tobramycin; while a 10% decrease in KOH (1.80 mM) produced a retention time increase to 7.8 min (+36% change). Kanamycin B retention time decreased by 9.2% with 10% increase in eluent concentration, and increased 17% with a 10% eluent concentration increase. With either change, tobramycin and kanamycin B were still resolved (Rs = 4.18 ± 0.04 and 7.82 ± 0.02 for a 10% eluent concentration increase and decrease, respectively). The large % change in retention time and resolution for a relatively small change in KOH eluent concentration demonstrated the importance of producing a consistent eluent concentration, which was achieved with automated eluent generation. Poor retention time reproducibility was observed when attempting to use manually prepared 2 mM NaOH.

3.7.2. Eluent generation cartridges and water

Two eluent generation cartridges were compared for their influence on retention time. No statistically significant differences in retention times were observed between the end of the first cartridge and the beginning of the new cartridge.

A change of deionized water to supply the eluent generator before and after routine maintenance of the lab's water-purification system did not cause a statistically significant change in tobramycin retention time.

3.7.3. Disposable gold working electrode response

Using three electrodes from the same manufacturing lot, mean tobramycin peak area response factors ranged from 83.6 to 94.8 pC min/pmol (based on the mean of four 200 pmol injections for each electrode approximately a day after installation); mean of means ± standard deviation of 90.1 ± 5.8 (6.5%). Mean kanamycin B peak area response factors ranged from 83.3 to 92.9 pC min/pmol (based on 200 pmol); mean of means ± standard deviation of 88.5 ± 4.8 (5.5%). The mean ratio of tobramycin to kanamycin B peak area response factors was 1.02 ± 0.01 (1.3%). Using four electrodes from different lots, mean tobramycin response factors ranged from 73.7 to 90.1 pC min/pmol; mean of means ± standard deviation of 83.8 ± 7.1 (8.5%). Mean kanamycin B response factors ranged from 75.5 to 88.5 pC min/pmol; mean of means ± standard deviation of 83.6 ± 5.7 (6.8%). The R.S.D. of tobramycin and kanamycin B peak area response factors between different lots of electrodes correspond closely to the R.S.D. of amino acids previously reported using disposable electrodes [15]. The mean ratio of tobramycin to kanamycin B peak area response factors was 1.00 ± 0.02 (1.7%).

3.7.4. Column temperature

At the recommended operating temperature of 30 °C, the retention times for tobramycin and kanamycin B were 5.68 and 4.11 min, respectively. At 33 °C (10% increase), the retention times for tobramycin and kanamycin B were 5.97 (+5.1% change) and 4.19 min (+1.9%), respectively, while at 27 °C (10% decrease), they were 5.50 (−3.2%) and 4.05 min (−1.5%), respectively. These changes did not impact separation. A 10% increase in temperature increased peak areas 7–8% and a 10% decrease in temperature decreased peak areas 4%. A 10% increase in temperature increased background 6% and a 10% decrease in temperature decreased background 5%. Noise was unaffected by 10% temperature changes. The temperature of the electrochemical cell was not intentionally altered.

3.7.5. Flow rate

At the recommended flow rate of 0.50 mL/min, the retention times for tobramycin and kanamycin B were 5.68 and 4.11 min, respectively. At 0.55 mL/min (10% increase), the retention times were 5.38 (−5.3%) and 3.80 min (−7.5%), respectively, and at 0.45 mL/min (10% decrease), 6.50 (+14%) and 4.62 min (+12%), respectively. At 10% higher flow rate, no significant change in peak areas were observed; and at 10% lower flow rate, a 12–13% increase in peak areas was observed. Noise was unaffected by a 10% change in flow rate. A resolution factor of >4 were observed with either a 10% increase or decrease in flow rate.

3.7.6. Column reproducibility

Table 4 compares the retention times for four separate columns. These columns were produced over a period of 4 years.

Table 4
Retention time reproducibility of separate analytical columns

CarboPac PA1 column serial number[a]	Retention time (min)					
	Tobramycin		Kanamycin B		Neamine	
	Mean	R.S.D. (%)	Mean	R.S.D. (%)	Mean	R.S.D. (%)
10964 (Mfg. January 2003)	5.78	0.1	4.15	0.0	4.73	0.1
10154 (Mfg. December 2001)	5.51	0.2	4.00	0.3	4.55	0.2
11553 (Mfg. June 2003)	5.63	0.2	4.09	0.3	4.63	0.1
9270 (Mfg. October 2000)	4.90	0.1	3.87	0.3	4.12	0.1
Mean	5.45		4.03		4.43	
S.D.	0.38		0.12		0.27	
R.S.D. (%)	7.0		3.1		6.1	

[a] Column 11553 was used in this paper. Columns S/N 10964 and 10154 were previously used for other applications prior to this work. Column S/N 9270 was previously unused. $N = 8$ injections/column.

Little variation was observed between columns. The tobramycin retention time R.S.D. for four different columns was 7%, while the neamine and kanamycin B retention time R.S.D.s were 6% and 3%, respectively. High analyte resolution was achieved on each column.

3.8. Potential applications

For this study we were unable to obtain and evaluate any source of tobramycin-formulated pharmaceuticals containing other active or inactive ingredients. Excipients, their concentrations, and availability of a suitable sample preparation technique will determine if the method described here can be used for applications other than testing purity, identity, and assay of the purified antibiotic. Adding NaCl to the tobramycin and kanamycin B samples showed that their peak shapes and retention times were affected at >5 mM NaCl. Other salts would presumably have the same effect. An Internet search for tobramycin-containing pharmaceutical products revealed that TobraDex and Tobrex® (Alcon Inc.), and Tobramycin Ophthalmic Solution USP (Bausch & Lomb) each contain 3 mg/mL tobramycin (6.4 mM), TOBI (Chiron Corporation) contains 60 mg/mL (128 mM) tobramycin, and tobramycin sulfate for injection (Eli Lilly and Company) has from 1 to 8 mg/mL (2.1–17 mM) tobramycin. All these formulations must be diluted at least 1000-fold to ~3–6 μM tobramycin, making their saline concentrations much less than 5 mM NaCl, suggesting these formulations can be assayed with our method. The four tobramycin products contain other ingredients. We believe that the dilution required for assay of these formulations will eliminate most, if not all, interferences possible from the other ingredients.

4. Conclusion

HPAE-IPAD with eluent generation was used to determine tobramycin and its impurities. This method was fast (<15 min per analysis) and demonstrated high retention time and peak area precision. Electrolytic eluent generation ensures reproducibility and ruggedness with respect to retention time and peak resolution. By intentionally varying method parameters, the method was shown to be rugged for the intended application of tobramycin identity, purity, and assay.

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