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Letter to the Editor

Determination of tobramycin using high-performance liquid chromatography with pulsed amperometric detection

Sir,

Tobramycin (Fig. 1), or factor 6 of the nebramycin complex, is an aminoglycoside antibiotic produced by *Streptomyces tenebrarius*. It may be administered systemically, as is Nebcin (Eli Lilly), or topically, as is Tobrex (Alcon). Tobramycin and other nebramycin factors usually are chromatographed by reversed-phase high-performance liquid chromatography (HPLC) [1,2]. Because these and other aminoglycosides have no strong UV chromophore ($\lambda_{max} > 220$ nm), direct optical detection is limited to refractive index. For this reason, aminoglycosides are often derivatized to form fluorescent or UV-absorbing compounds. A recent report of the pulsed amperometric detection of the nebramycin factors [2] demonstrated that direct amperometric detection of underivatized tobramycin is a viable alternative. However, reversed-phase chromatography was not suitable for such hydrophilic analytes and resulted in poor efficiency. We have now developed a high-performance anion-exchange

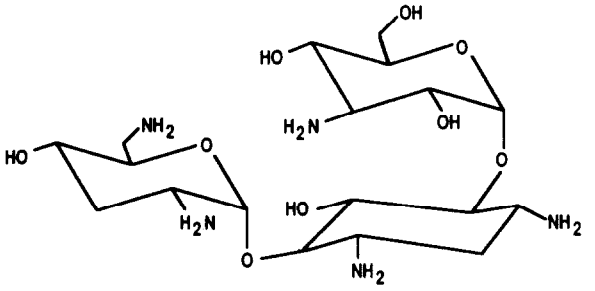

Fig. 1. Structure of tobramycin.

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(HPAE) separation under alkaline conditions which, when coupled with pulsed amperometric detection, provides an analytical method with sensitivity equal to or better than current HPLC methods [1] without the need to form derivatives.

Sodium hydroxide solution (50%, w/w, low carbonate) was purchased from Fisher (Pittsburgh, PA, U.S.A.). 2-Deoxystreptamine, apramycin and nebramycin factors 4 and 5 were obtained from Dr. Dennis Johnson, Iowa State University (Ames, IA, U.S.A.). Tobramycin was purchased from Sigma (St. Louis, MO, U.S.A.). A sample of sterile injectable tobramycin was obtained from Elkins-Sinn (Cherry Hill, NJ, U.S.A.) and Tobrex from Alcon Labs. (Fort Worth, TX, U.S.A.). All analyses were performed on a Dionex 4500i chromatograph equipped with a basic postcolumn delivery system and a pulsed amperometric detector with a gold working electrode. The separations were accomplished with a Dionex CarboPac PA1 polymeric anion-exchange column. A Dionex AI-450 automation system and an IBM AT computer were used for data acquisition and reduction.

Tobramycin was determined by HPAE with pulsed amperometric detection, the technique commonly used for carbohydrate determination [3–6]. The samples were chromatographed with a CarboPac PA1 analytical column using a sodium hydroxide gradient (0.5–5.0 m*M*, 10 min) (see Fig. 2). Aminoglyco-

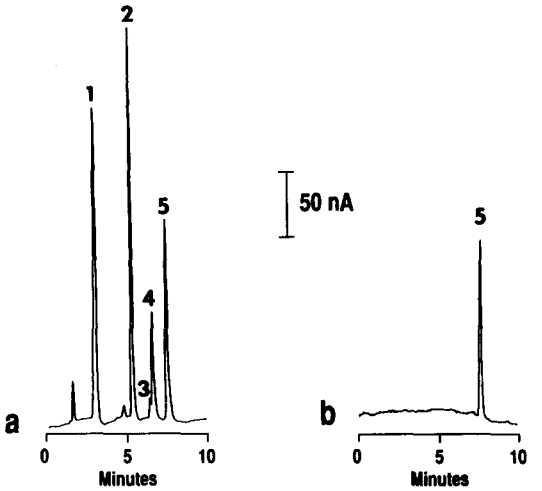

Fig. 2. (a) Chromatogram of several common factors of the nebramycin complex (20.0 μg/ml each). (b) Chromatogram of Tobrex, diluted to 18.0 μg/ml. Conditions: column, CarboPac PA1; injection loop, 10 μl; mobile phase, 0.5–5.0 m*M* sodium hydroxide solution (10 min, 1.0 ml/min); post-column reagent, 0.5 *M* sodium hydroxide solution (0.5 ml/min); detection, pulsed amperometry (gold electrode); detector settings, E1 = 0.1 V, E2 = 0.6 V, E3 = −0.8 V, t1 = t3 = 300 ms, t2 = 120 ms. Peaks: 1 = 2-deoxystreptamine; 2 = apramycin; 3 = nebramycin F4; 4 = nebramycin F5; 5 = tobramycin.

sides, which exhibit chromatographic behavior similar to aminosugars [5,6], are anionic at high pH and thus are retained, albeit weakly, by the anion-exchange column. During amperometric detection, the gold electrode oxidizes tobramycin at a pH of about 13. Therefore, additional sodium hydroxide was added post-separation and mixed with the eluate in a beaded reaction coil in order to raise the pH and ionic strength to levels required for sensitive detection. The minimum detection limit, determined at a signal-to-noise ratio of 3, was 2 ng (4 pmol) for a tobramycin standard. The response (y) was linear ($r > 0.999$) with respect to mass (x) over the range 10 ng to 1.0 μg on-column: $y = (19.4\ \mathrm{C/g})x - 5.25 \cdot 10^{-8}$ C. Fifteen replicate injections of a standard (100 ng) established the reproducibilities of retention (coefficient of variation, C.V. = 0.2%) and peak-area response (C.V. = 2.9%). Samples of injectable tobramycin and Tobrex were prepared by diluting with deionized water to concentrations within the linear range for a 10-μl injection. Concentrations of tobramycin in the injectable solution and in Tobrex were determined to be 10.0 and 3.0 mg/ml, respectively, which are precisely the concentrations reported by the manufacturers. Ordinarily during pulsed amperometric detection, only a fraction (ca. 1–15%) of the total quantity of a carbohydrate is oxidized by passing over the electrode [7]. Hence, minor differences in extents of oxidation among a set of analytes may result in large proportional variations in response, as seen in Fig. 2a.

HPAE with pulsed amperometric detection proved applicable to standards and preparations of tobramycin. The simplicity of sample preparation, lack of analyte derivatization and sensitivity make this method well suited to the analysis of such pharmaceutical samples of tobramycin.

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