



Determination of Paromomycin in a Bulk Pharmaceutical Formulation Using HPAE-IPAD

Brian De Borba, Valoran P. Hanko, and Jeffrey S. Rohrer,
Dionex Corporation

Paromomycin is an aminoglycoside antibiotic widely used in human and veterinary medicine for the treatment of various bacterial infections. In humans, paromomycin has been used to treat leishmaniasis due to its low toxicity and cost effectiveness relative to other antibiotics (1). The current U.S. Pharmacopeia compendial method for assaying paromomycin uses a microbial assay (2), which lacks specificity, accuracy, and is time-consuming. In addition, microbial assays do not identify the active ingredient(s) or yield information on the total composition of the antibiotic formulation. In this paper, we demonstrate the use of electrolytically generated potassium hydroxide eluent with a CarboPac® PA1 anion-exchange column and IPAD for determination of paromomycin in a bulk pharmaceutical formulation.

Experimental

- Dionex ICS-3000 Reagent-Free™ ion chromatography system consisting of a DP dual gradient pump, EG eluent generator with an EGC II KOH cartridge and CR-ATC trap column, and a DC detector compartment with dual temperature zones and an electrochemical detector and cell.
- CarboPac® PA1 guard (4 x 50 mm) and analytical (4 x 250 mm) columns. Eluent concentration was 1.8mM KOH, flow rate was 0.5 mL/min, and column temperature was maintained at 30 °C.

The electrochemical cell used a disposable *AAA-Direct* Au working electrode and a pH-Ag/AgCl reference electrode set in the pH mode. 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.

Results

Paromomycin exists as isomeric components (paromomycin I and II) that are well resolved on the CarboPac PA1 column in less than 10 min using an electrolytically generated 1.80 mM KOH eluent. Minor changes in the hydroxide eluent concentration produce significant changes in the retention times. Elevated concentrations of carbonate impacts retention time precision and chromatographic efficiency. Using an eluent generator for this application is advantageous because it produces an accurate, precise, carbonate-free hydroxide concentration that is difficult to obtain with manually prepared NaOH eluent. Figure 1 compares the separation of 5 μM USP grade paromomycin to paromomycin detected in a bulk pharmaceutical formulation. The between-day and intraday precisions of paromomycin were <2% for USP grade paromomycin and paromomycin prepared from the bulk formulation based on

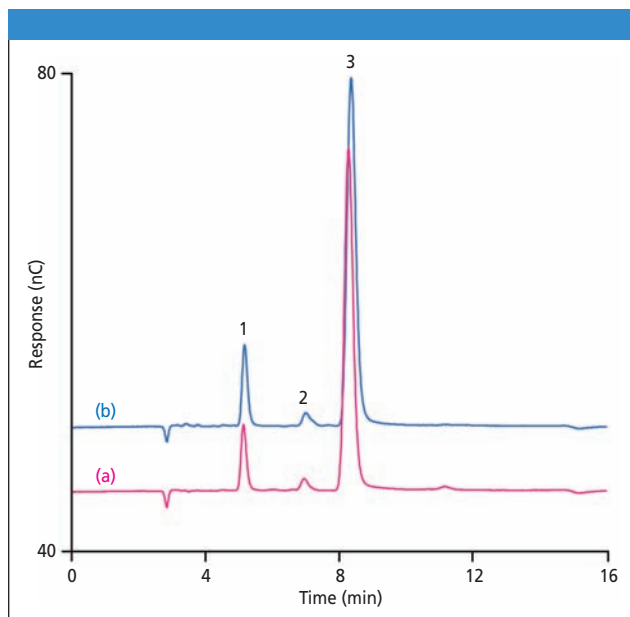


Figure 1: Comparison of (a) USP grade paromomycin to (b) paromomycin in a bulk pharmaceutical formulation. Peaks (a): 1 = paromomycin I (5.0 μM), 2 = unknown impurity, 3 = paromomycin II. Peaks (b): 1 = paromomycin I (5.3 μM), 2 = unknown impurity, 3 = paromomycin II. The specified concentrations are the combined concentrations of paromomycin I and II.

independently prepared solutions on three different days (3). The average paromomycin concentration found in the bulk pharmaceutical was 279 ± 10 mg over three trials. Although this was 11.6% above the expected value, the concentration was within 90–125% of the labeled amount according to the USP specifications. The results obtained from this study indicate the method is accurate and precise for determination of paromomycin isomers.

References

- (1) Cowley, G. Chasing Black Fever. *Newsweek Magazine*, July 2005. available at <http://www.msnbc.msn.com/id/8715762/site/newsweek>.
- (2) United States Pharmacopeia. *The National Formulary*. Paromomycin Sulfate. USP 30, NF 25, 2007, p. 2865.
- (3) Dionex Application Note 186, Dionex Corporation (Sunnyvale, California, 2007).

Dionex Corporation

1228 Titan Way, P.O. Box 3603, Sunnyvale, CA 94088-3603
tel. (408) 737-0700, fax (408) 730-9403
www.dionex.com