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Report

Determination of Spectinomycin Dihydrochloride by Liquid Chromatography with Electrochemical Detection

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Spectinomycin dihydrochloride is determined by liquid chromatography with electrochemical detection. The drug is chromatographed on a reverse-phase Nucleosil C18 column using an eluent containing 0.02 *M* sodium citrate and 0.0015 *M* octyl sodium sulfate (pH 6.10 with perchloric acid) and acetonitrile (100:4). Detection is performed using a coulometric detector (porous carbon working electrode) at +0.85 V. The drug and primary degradation product are detectable. Detector response is linear to at least 20 μg/ml, which is four times the assay level. The procedure has relative standard deviations of ±1.21 to ±2.72% for three lots of bulk drug. Sensitivity is greater than 0.1 μg/ml of spectinomycin (5 ng on column). Repeatability at this level is ±4.94%.

KEY WORDS: spectinomycin; trobicin; liquid chromatography; electrochemical detection.

INTRODUCTION

Spectinomycin is an aminocyclitol antibiotic used primarily in humans for the treatment of infections due to *Neisseria gonorrhoeae* (1). The drug is produced as the dihydrochloride pentahydrate salt (Fig. 1). Spectinomycin is used extensively for veterinary purposes against gram-negative organisms (2). Reported analytical procedures for determining spectinomycin by microbiological, gas chromatographic (GC), and liquid chromatographic (LC) techniques all suffer to some degree from lack of either speed, specificity, and/or sensitivity.

GC procedures (3,4) for determining spectinomycin typically require silation and may produce multiple peaks for the drug and its degradation products. The use of LC is limited since the drug lacks a UV chromophore. Use of end-absorption or refractive index (RI) detection has been reported (5), but sensitivity and selectivity are poor. To overcome the lack of detectability, LC combined with postcolumn derivatization has been described (6). This technique is cumbersome since the drug is unreactive to commonly used derivatization reagents, requiring an additional oxidation step and a fairly elaborate LC setup. Precolumn derivatization of spectinomycin with 2-naphthalenesulfonyl chloride followed by normal-phase LC has been reported (7). The method requires a finely tuned normal-phase eluent and considerable sample manipulation.

In this report, we describe a procedure for determining spectinomycin hydrochloride directly using LC with electrochemical detection. This approach eliminates many of the difficulties associated with pre- or postcolumn derivatization of the drug while providing improved sensitivity. The assay is rapid and stability indicating.

MATERIALS AND METHODS

Instrumentation and Reagents

Cyclic voltammograms (CVs) were run using a BAS 100 electrochemical analyzer equipped with a Model C-1 cell stand (Bioanalytical Systems, West Lafayette, Ind.). CV measurements were made using a glassy carbon working electrode. Platinum auxillary and Ag/AgCl reference electrodes were used. The LC system used throughout this work consisted of a Constametric III pump (LDC Corp., Riviera Beach, Fla.) equipped with a Model 7125 injection valve (Rheodyne Corp., Cotati, Calif.) and a Model 5100A Coulochem detector (ESA, Bedford, Mass.). An ESA Model 5011 analytical cell was used. An ESA Model 5020 guard cell was plumbed into the system between the pump and the injection valve. Chromatograms were processed using a Chromatopac Model C-R3A integrator (Shimadzu, Kyoto, Japan). A Nucleosil C18 chromatographic column (10 μm, 4.6 mm × 25 cm) was used (Alltech Associates, Deerfield, Ill.). Chemicals and solvents were reagent grade and high-performance liquid chromatography (HPLC) grade, respectively. Bulk

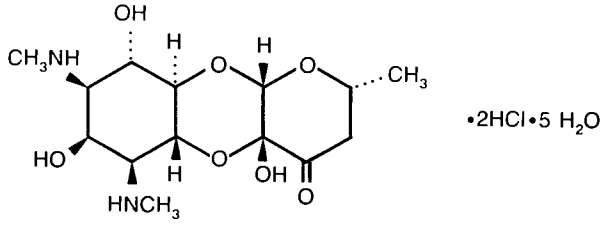

Fig. 1. Structure of spectinomycin dihydrochloride pentahydrate.

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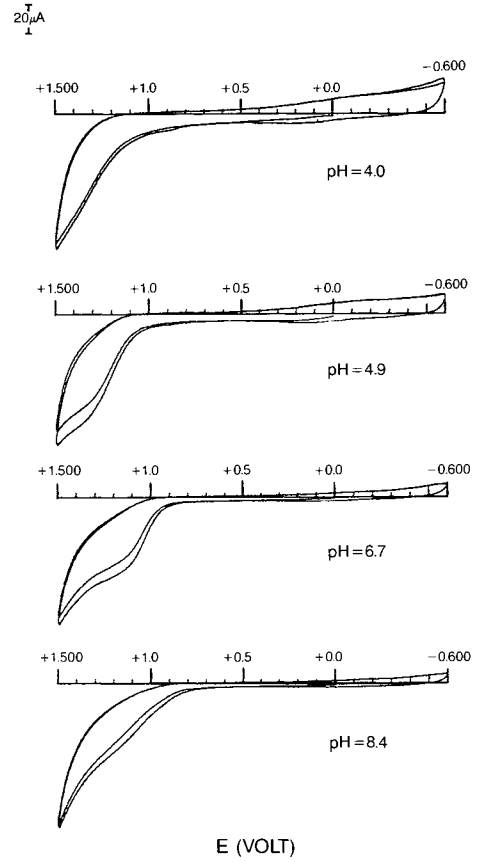

Fig. 2. Cyclic voltammograms for spectinomycin at pH 4.0 to 8.4.

spectinomycin dihydrochloride pentahydrate was produced at Abbott Laboratories, North Chicago, Ill., and the reference standard was purchased from U.S.P. Actinamine was prepared in-house by acid hydrolysis of spectinomycin.

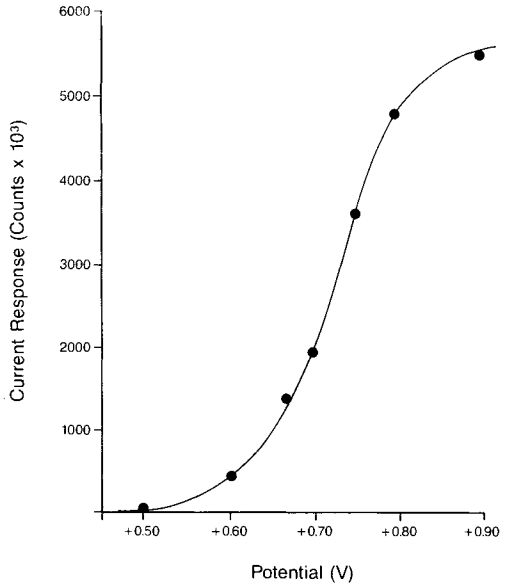

Fig. 3. Hydrodynamic voltammogram for spectinomycin prepared by chromatography of 50 ng of free base.

Chromatographic Conditions

The LC eluent was an aqueous buffer containing 0.02 *M* sodium citrate and 0.0015 *M* octyl sodium sulfate (pH adjusted to 6.1 with perchloric acid) mixed with acetonitrile at a ratio of 100 to 4. After filtering through a 0.45-μm nylon membrane (Cuno, Inc., Meriden, Conn.), the eluent was pumped at 1.5 ml/min with typical back pressures of 1800 to 2100 psi. The guard cell was operated at +0.95 V to reduce the background current at the analytical cell. A single porous carbon working electrode cell was used at +0.85 V applied potential. Background currents were typically 5–6 μA. Injection volumes of 50 μl were used.

Assay Procedure

Samples of spectinomycin dihydrochloride pentahydrate were accurately weighed and dissolved in the LC eluent. Sample solutions were diluted in the eluent to contain approximately 5 μg/ml of free base. Integrated areas of the spectinomycin peaks from the sample preparations were compared to that of a U.S.P. reference standard prepared in an identical manner, and free base content was calculated by the external standard method.

RESULTS AND DISCUSSION

In this work our purpose was to develop a procedure for determining spectinomycin in bulk drug. Additionally our interest was to provide a direct assay of the drug that could be adopted to trace-level determinations. Since the major problem in determining spectinomycin by LC is the lack of detectability, initial experiments were performed to determine if the compound showed electrochemical activity at a carbon electrode, which would allow the use of an electrochemical detector. Solutions of spectinomycin dihydrochloride pentahydrate were prepared at 1 m*M* base concentrations in Britton-Robinson buffers (8). Cyclic voltammograms were run on solutions at pH values of 4.0 to 8.4. A single oxidation wave occurred at potentials of +1.5 to +1.35 V vs Ag/AgCl in solutions at pH $\geq$ 4.9. Typical CVs are shown in Fig. 2. The oxidation wave was most pronounced in the pH range of 5 to 7.

Since the detector used in this work functions with a pseudoreference, the proper operating potential of the cell was established chromatographically by injecting 1 μg/ml spectinomycin solutions at various potentials. A hydrodynamic voltammogram is generated by plotting detector response versus potential, which is presented in Fig. 3. An applied potential of +0.85 V was chosen as a compromise of having nearly diffusion-controlled conditions while maintaining reasonable background currents.

Typical chromatograms for spectinomycin standard and sample preparations are shown in Fig. 4. To determine if the assay is stability indicating, solutions were stressed in acid and base, then diluted to theoretical concentrations of 5 μg/ml in the eluent. As shown in Fig. 5, the drug was completely degraded in 1 *N* NaOH after 30 min at room temperature. When heated at steam bath temperature in 6 *N* HCl, 52% of intact drug remained after 30 min. In both solutions the primary degradation product had the same retention time as an actinamine standard.

To demonstrate linearity of the detector response, solu-

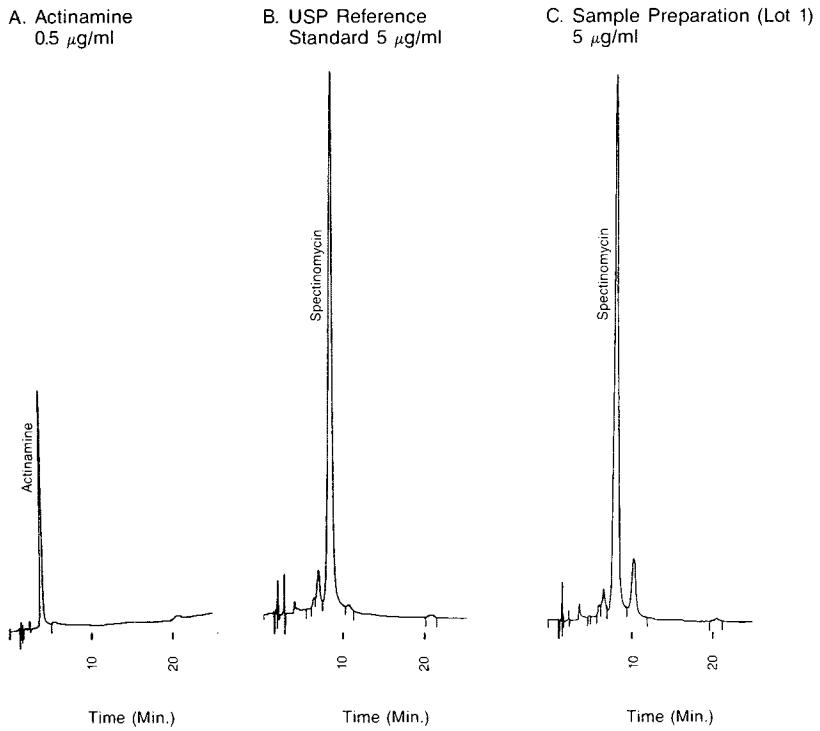

Fig. 4. Chromatograms for spectinomycin dihydrochloride pentahydrate and actinamine.

tions of spectinomycin dihydrochloride pentahydrate were chromatographed containing free base concentrations ranging from 0.10 to 20.2 μg/ml. A plot of detector response (integrator counts × 10^6 = y) versus concentration (free base, μg/ml = x) was linear and essentially intersected the origin. The regression line had a slope of 1.089, a y-intercept of −0.168, and a correlation coefficient of 0.9998.

Assay precision was determined by performing the assay for three lots of bulk drug. The measurements were made by two analysts over 3 days and the results are shown

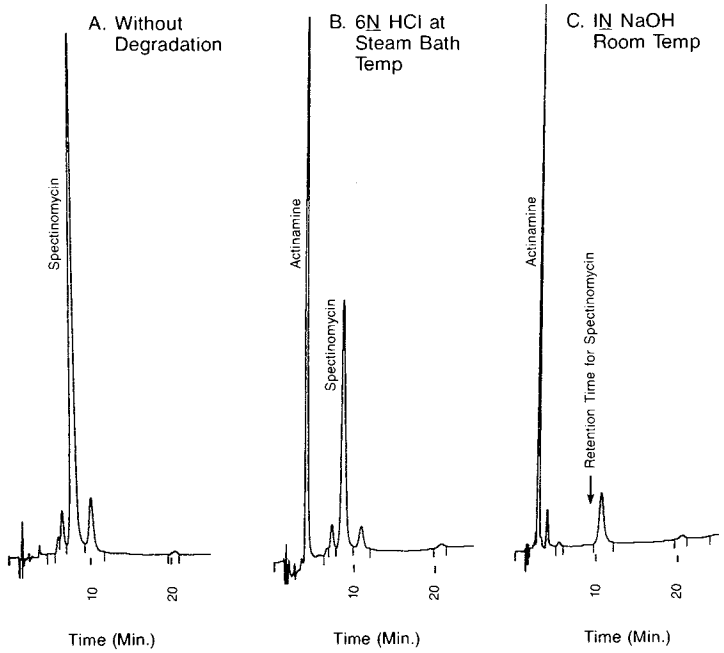

Fig. 5. Chromatograms for spectinomycin dihydrochloride pentahydrate (Lot 2) stressed in acid and base for 30 min. Concentrations were 5 μg/ml of free base.

Table I. Precision Data for Spectinomycin Dihydrochloride Pentahydrate by Liquid Chromatography with Electrochemical Detection

	Free base content (μg/mg) "as is"		
	Lot 1[a]	Lot 2[b]	Lot 3[c]
	632	—	—
	608	576	—
	608	573	625
	618	594	592
	645	580	591
	620	574	622
	620	579	625
	635	580	623
Mean	623	579	613
SD	±13.1	±7.02	±16.7
RSD	±2.10%	±1.21%	±2.72%

[a] Microbiological assay = 628 μg/mg.
[b] Microbiological assay = 612 μg/mg.
[c] Microbiological assay = 638 μg/mg.

in Table I. As shown, relative standard deviation values range from ±1.21 to ±2.72%. Results of this method generally agreed with the microbiological assay (turbidometric).

Solution stability of the drug in the sample preparation was demonstrated by chromatographing sample preparations after 24 and 48 hr at room temperature. On standing, the potency values calculated remained unchanged within the precision of the measurement, allowing automation of the chromatographic finish. The repeatability of the chromatographic finish was determined by making replicate injections of a standard preparation containing 0.101 μg/ml of spectinomycin. Shown in Fig. 6 are typical chromatograms

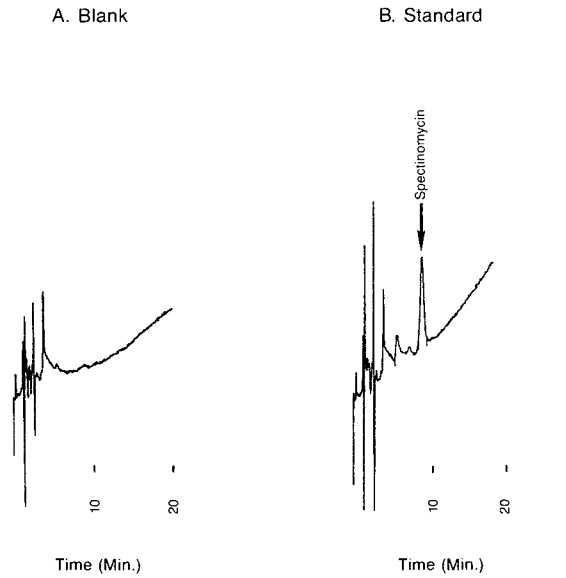

Fig. 6. Chromatograms for spectinomycin at 0.101 μg/ml (5 ng on column).

Table II. Repeatability of Spectinomycin Standard at 0.101 μg/ml

	Peak area × 10^3
	71.22
	76.78
	71.28
	80.02
	78.45
	77.57
Mean	75.89
SD	±3.75
RSD	±4.94%

of the sample preparation and blank. At this level, 5 ng of drug is injected on the column with $s/n > 20$. Presented in Table II are peak area data for six injections. As shown, the relative standard deviation was ±4.94% at this level.

The present investigation focused on the determination of bulk spectinomycin dihydrochloride. However, the performance of the described technique makes it well suited for trace-level determinations of the drug. In more complex matrices, a dual-series cell arrangement may be advantageous. By operating the upstream electrode at +0.60 to +0.70 V, improved specificity of the drug may be realized by eliminating more easily oxidized components detected at the downstream electrode operated at +0.85 V. Using these conditions, the sensitivity for spectinomycin was comparable to that described in the text using a single working electrode at +0.85 V. This configuration combined with the relatively large surface area used in the coulometric cell design greatly reduces the risk of declining detector response which can arise from chromatographing complex samples. This approach has been used successfully for determining erythromycin in urine and plasma at comparable applied potentials (9,10).

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