Determination of Sodium and Chloride Ions in Teicoplanin Lyophilized Vials by Nonsuppressed, Single-Column Ion Chromatography

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Two nonsuppressed, single-column ion chromatographic procedures have been developed and validated for the determination of the total sodium and chloride ion content in lyophilized vials containing teicoplanin. The assays served to determine the amount of sodium chloride added to the vials and to estimate the amount of sodium associated with the teicoplanin molecule.

KEY WORDS: assay; determination; sodium; chloride; teicoplanin; nonsuppressed ion chromatography.

INTRODUCTION

Teicoplanin (T) is a zwitterionic glycopeptide member of the vancomycin-ristocetin class of antibiotics (Fig. 1) and is produced by the fermentation of *Actinoplanes teichomyceticus* (1,2). It is active *in vitro* and *in vivo* against aerobic and anaerobic Gram-positive bacteria by inhibiting the biosynthesis of the cell wall. T is typically isolated at pH 7.5 as a partial internal salt and a partial monosodium salt (2). It is packaged as Targocid in lyophilized vials containing sodium chloride, which is added in a sufficient amount to yield an isotonic solution when the powder is reconstituted for intravenous administration. As a result, the total sodium content of the vials is from two sources: what is liberated when the partial monosodium salt is dissolved and what is added in the form of sodium chloride.

Gravimetric and titrimetric techniques have been used for determining the total amounts of sodium and chloride ions in T. However, these techniques are time-consuming and do not readily lend themselves to automation when multiple samples are analyzed. Ion chromatographic procedures amenable to automation have received increased attention lately for the routine determination of ionic species in pharmaceuticals (3). This paper describes two nonsuppressed, single-column ion chromatographic procedures which have been developed and validated for the direct determination of the total sodium and chloride ion content in lyophilized vials of T. From the individual results, the amount of sodium chloride added to the vials may be verified. In addition, the actual amount of sodium associated with the T molecule may be estimated.

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MATERIALS AND METHODS

Reagents

Teicoplanin lyophilized vials were obtained from the Pharmacy Research Department (Merrell Dow Research Institute, OH). Disodium edetate (reagent grade, Dow Chemical, USA) and 4-hydroxybenzoic acid (PHBA) (GOLD LABEL, Aldrich, USA) were used without further purification. HPLC-grade methyl alcohol (American Burdick & Jackson, USA) and 18-MΩ-cm water (NANOpure, Barnstead/Thermolyne, USA) were used throughout. A 5% (v/v) solution of nitric acid (Ultrex grade, J. T. Baker, USA) was prepared by diluting 5 ml to 100 ml with water. A standard solution of sodium chloride (crystals, Baker Analyzed Reagent grade, J. T. Baker, USA), containing ca. 9.8 μg/ml of sodium and ca. 15.2 μg/ml of chloride, was prepared by dissolving 25 mg in 1.0 l of water. A 5 *M* solution of sodium hydroxide (reagent A.C.S., MCB, USA) was prepared by dissolving 50 g in 250 ml of water. Cation (lithium, sodium, ammonium, and potassium) and anion (fluoride, chloride, nitrite, bromide, nitrate, phosphate, and sulfate) test mixtures (Wescan Instruments, USA) were used for establishing the suitability of the chromatographic systems.

Chromatographic Conditions

An ion chromatograph (Model 26650000 Ion Analyzer, Wescan Instruments, USA), equipped with a 100-μl syringe-loading sample injector (Model 7125, Rheodyne, USA), a pump (Model 110A, Beckman Instruments, USA), and a strip-chart recorder (Model BD-40, Kipp & Zonen, USA) was used. The detector range was 100 μS for the sodium assay and 10 μS for the chloride assay. The temperature was controlled at 30°C. The flow rate was 2.0 ml/min. Unless otherwise specified, single injections were made. Data acquisition was performed with a Computer Automated Laboratory System (CALS) (Beckman Instruments, USA).

For the determination of sodium, a Cation/R column was used (100 × 3.0-mm I.D.; Wescan, USA). The eluent was 0.1 m*M* disodium edetate in 3.2 m*M* nitric acid. For chloride, an Anion/R column was used (250 × 4.1-mm I.D.; Wescan, USA). The eluent was 4.0 m*M* PHBA (pH adjusted to 8.5). In both cases, the analytical columns were protected with stainless-steel guard cartridges (30 × 2.1-mm I.D.; Wescan, USA) containing cation or anion resins, depending on the assay.

System Suitability Tests

A system suitability test was performed with each experiment by injecting 100 μl of a cation or anion mixture. For the chromatographic system to pass the test, resolution and precision criteria had to be met. In the assay for sodium, a resolution ≥2.0 between lithium and sodium was indicative of proper performance. The precision was estimated from the peak areas for sodium from six injections of the standard solution. A relative standard deviation ≤2.0% was deemed adequate (4). Failure to meet either of these criteria was usually corrected by regenerating the column with a 100-μl injection of 5% (v/v) nitric acid (5).

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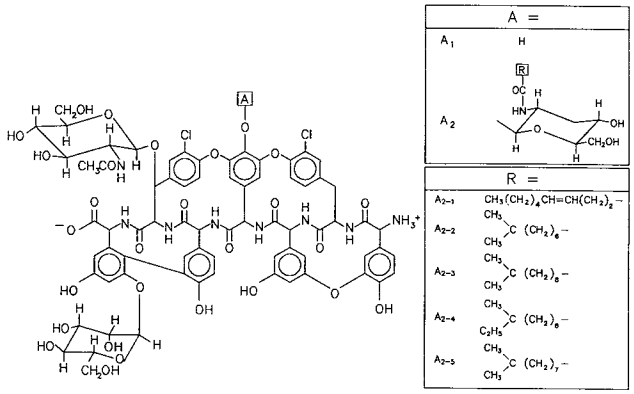

Fig. 1. Structure of teicoplanin (T).

In the assay for chloride, a resolution ≥1.5 between chloride and nitrite was indicative of proper performance. The precision was estimated from the peak areas for chloride from five injections of the standard solution. A relative standard deviation ≤2.0% was deemed adequate. Failure to meet either of these criteria was usually corrected by decreasing the pH of the mobile phase (which increased the retention of chloride and anions in general) or by regenerating the column following the manufacturer's directions.

Validation

The linearity of response for sodium and chloride was determined by preparing a 0.05 mg/ml solution of sodium chloride in water and diluting it to cover a range from 0.4 to 20 μg Na^+/ml and from 0.6 to 31 μg Cl^-/ml. The samples were analyzed using the conditions previously described.

To determine the recoveries of sodium and chloride, a spiking solution was prepared by dissolving ca. 103 mg of sodium chloride in 1.0 l of water. A 200-mg vial of lyophilized T was decapped. Sufficient water was added to dissolve the contents gently. These were transferred quantitatively into a 100-ml volumetric flask. The stopper and the vial were rinsed at least three times with water and the rinses were combined in the volumetric flask. The solution was diluted with water to just under the mark. If excessive foaming was evident, a couple of drops of methyl alcohol were added and the foaming was allowed to subside before diluting to volume with water. From this solution, aliquots of 10.0 ml were pipetted into four separate 100-ml volumetric flasks. To the first one, no spiking solution was added; to the remaining three, 5.0, 25.0, and 50.0 ml of the spiking solution were added. The mixtures were diluted to volume with water and analyzed (single injections). The experiment was repeated on at least 1 additional day using fresh vials and a second chromatographic column of each type (i.e., cation or anion). In the validation for sodium, three separate vials were used on day 1, one on day 2, and 2 on day 3. For chloride, one vial was used on each of 3 days.

Assay Procedure

To assay T lyophilized vials for sodium and chloride ion content, the above procedure is used. However, after the initial dilution to 100.0 ml with water, an aliquot of 10.0 ml is pipetted into a second 100-ml volumetric flask, diluted to volume with water, and analyzed vs a 0.025 mg/ml standard solution of sodium chloride.

RESULTS AND DISCUSSION

The ion chromatographic conditions described in this work represent modifications of existing methodology (5) adapted for a specific application and sample matrix. The concentration of T in the final solution (ca. 0.2 mg/ml) was selected so that it could be used simultaneously for the determination of sodium, chloride, and teicoplanin content by the appropriate methods.

Since sodium and chloride were both present in the lyophilized vials, it was not possible to spike a true blank for recovery studies. Rather, the endogenous amount of sodium and chloride found in the unspiked sample of each validation set was subtracted from the rest of the spiked samples. Thus, the unspiked sample served as a "control" for all the others prepared from the same vial.

The column used in the determination of sodium is of a low capacity. It is easily poisoned by metal ions present in the dilute nitric acid eluent and in the stainless-steel parts with which the eluent comes into contact. Consequently, during the development of the method, the retention time of sodium was observed to decrease with time as the column aged. A 100-μl injection of 5% (v/v) nitric acid was used to regenerate the column (5). The effect of this rinse on the retention of the four components of a cation mixture is seen in Fig. 2. Based on these results, this injection is recommended as a column preconditioning step. Whenever large numbers of samples are analyzed, a periodic injection is recommended every few sample injections. As an extra precaution, in order to retard the saturation of the column with

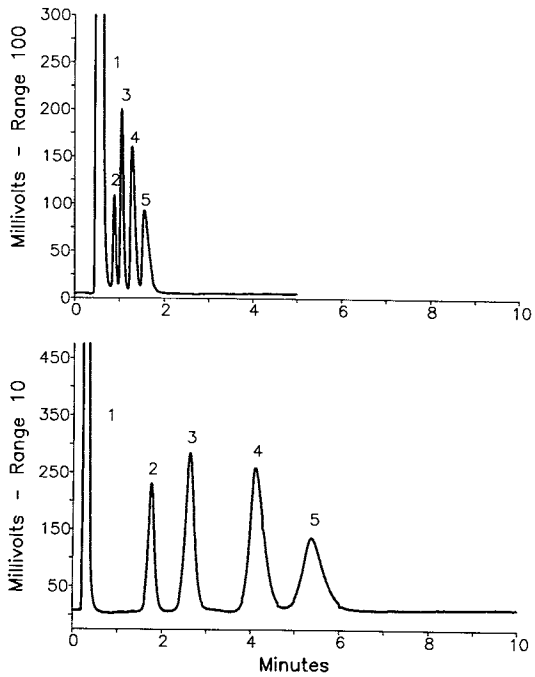

Fig. 2. Effect of 5% (v/v) nitric acid rinse on the resolution of the components of a cation mixture. Top: Before nitric acid rinse. Bottom: After nitric acid rinse. 1, System peak; 2, lithium; 3, sodium; 4, ammonium; 5, potassium.

Table I. Recoveries from Lyophilized Vials of Teicoplanin Spiked with Sodium Ion

Day No.	mg Na^+ Found[a]	mg Na^+ Recovered[b]	mg Na^+ Added	Recovery (%)
1	11.87	0.00	0.00	NA
1	13.79	1.92	1.97	97.5
1	21.48	9.61	9.84	97.7
1	30.85	19.98	19.69	96.4
1	12.19	0.00	0.00	NA
1	14.20	2.01	1.97	102.0
1	21.54	9.35	9.84	95.0
1	31.16	18.97	19.69	96.3
1	12.38	0.00	0.00	NA
1	14.33	1.95	1.97	99.0
1	21.95	9.57	9.84	97.3
1	31.73	19.35	19.69	98.3
2	12.27	0.00	0.00	NA
2	14.23	1.96	1.97	99.5
2	21.89	9.62	9.84	97.8
2	31.42	19.15	19.69	97.3
3	12.10	0.00	0.00	NA
3	14.03	1.93	1.97	98.0
3	21.71	9.61	9.84	97.7
3	31.02	18.92	19.69	96.1
3	12.05	0.00	0.00	NA
3	14.01	1.96	1.97	99.5
3	21.50	9.45	9.84	96.0
3	31.03	18.98	19.69	96.4
Mean				97.7%
SD				1.6%
Rel. SD				1.6%

[a] Uncorrected for endogenous sodium in blank.
[b] Corrected for endogenous sodium in blank.

undesired metals, a small amount (0.1 m*M*) of disodium edetate was added to the eluent. A concentration of 0.5 m*M* yielded broad peaks for ammonium and potassium. At a concentration of 1 m*M*, the background was such that these ions were not detected, and the sodium and lithium peaks were not baseline resolved.

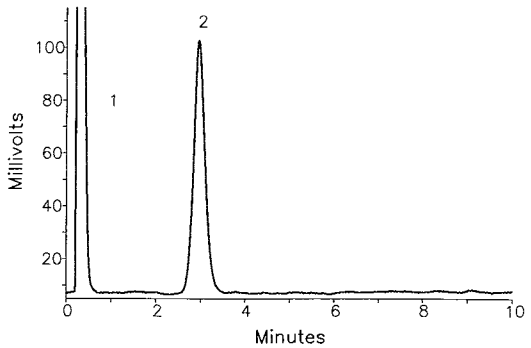

Fig. 3. Representative chromatogram for the determination of total sodium content in a lyophilized vial containing T and sodium chloride. 1, System peak; 2, sodium.

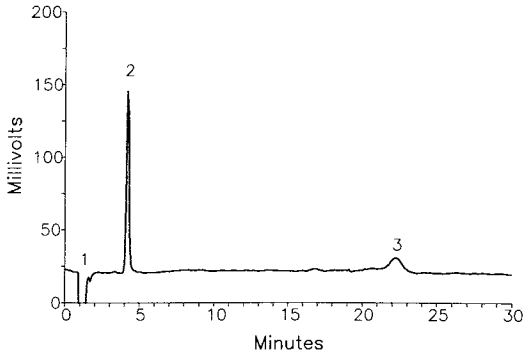

Fig. 4. Representative chromatogram for the determination of chloride content in a lyophilized vial containing T and sodium chloride. 1, 3—System peaks; 2—chloride.

Assay Validation

The linearity study for sodium yielded the following results: coefficient of correlation = 0.9994, *y* intercept = 33.1 mV · sec (peak area units), and slope = 138 mV · sec · ml/μg Na^+. The recoveries for sodium ion are shown in Table I. The mean recovery (n = 18) was 97.7% ± 1.6% (RSD) for the range of concentrations covered. While the amount of spiked sodium was within the linear range for the assay, the total amounts found at the highest spiked levels were outside of this range and may be the cause of the somewhat low recoveries. A representative chromatogram is shown in Fig. 3.

The linearity study for chloride yielded the following results: coefficient of correlation = 0.9999, *y* intercept = 4.21 mV · sec, and slope = 124 mV · sec · ml/μg Cl^-. The recoveries for chloride ion are shown in Table II. The mean recovery (n = 9) was 100% ± 1% (RSD) for the range of concentrations covered. A representative chromatogram is shown in Fig. 4.

Table II. Recoveries from Lyophilized Vials of Teicoplanin Spiked with Chloride

Day No.	mg Cl^- Found[a]	mg Cl^- Recovered[b]	mg Cl^- Added	Recovery (%)
1	14.90	0.00	0.00	NA
1	18.03	3.13	3.14	100
1	30.74	15.8	15.7	101
1	46.61	31.7	31.4	101
2	14.70	0.00	0.00	NA
2	17.83	3.13	3.14	100
2	30.39	15.7	15.7	100
2	46.59	31.9	31.4	102
3	14.66	0.00	0.00	NA
3	17.76	3.10	3.14	99
3	30.36	15.7	15.7	100
3	46.23	31.6	31.4	101
Mean				100%
SD				1%
Rel. SD				1%

[a] Uncorrected for endogenous chloride in blank.
[b] Corrected for endogenous chloride in blank.

Determination of the Sodium and Chloride Content in the Vials and Estimation of Sodium Associated with Teicoplanin

The average amount of sodium chloride in the vials was estimated from the milligrams of chloride found in the controls (Table II), assuming an equimolar amount of sodium. From these data, 24.3 mg ± 1.0% RSD (n = 3) of sodium chloride per vial was found. This result compared favorably with 24.5 mg per vial found for this batch of T vials by titrimetric analysis.

The amount of sodium associated with T was estimated from the excess sodium in the controls (Table I) not in the form of sodium chloride. This amount was found to be ca. 2.6 mg per vial, a result which agreed closely with the 2.8 mg per vial obtained by titrimetric analyses.

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