

# APPLICATION REPORT

LC004

## Analytical Report on Robustness Testing of CHIROBIOTIC T

### 1. Objective

A method for detection of teicoplanin by LC/MS with an electrospray-interface was developed (API-ES/LC-MS) using standard solutions. Specific detection of characteristic ions of teicoplanin ( $m/z$  940 and 1563.3) under the optimal MS condition was performed. Robustness testing of CHIROBIOTIC T media was then conducted.

### 2. Results

A sample solution (SR-53-77) from the testing was analyzed and repeated twice. It was found that there was no trace of teicoplanin, in any form, present.

### 3. Analytical Method

#### 3.1 Standard Solutions

A stock solution was made by dissolving the teicoplanin powder in a 50/50/0.3 (v/v/v) mixture of methanol, water and acetic acid at a concentration of 1 mg/mL. A 1.0  $\mu\text{g/mL}$  solution was made by diluting 1 mL of the stock solution to 1000 mL. This solution was the starting point for serial dilutions. Concentrations of standards were 1.0  $\mu\text{g/mL}$ , 500 ng/mL, 100 ng/mL, 10 ng/mL, 5.0 ng/mL, 2.5 ng/mL and 1.25 ng/mL. All sample solutions were stored in the freezer when not in use.

#### 3.2 Calibration

A calibration curve was established with standard solutions in the range of 1.25 to 100 ng teicoplanin/mL. Excellent linearity between concentration and intensity (peak height) was obtained. The correlation coefficient was determined to be  $>0.9999$ .

#### 3.3 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD was found to be 0.5 ng/mL, while the LOQ was 1.25 ng/mL.

### 3.4 Reagents and Materials

#### CHEMICALS

Teicoplanin  
HPLC Grade MeOH  
HPLC Grade Acetonitrile  
HPLC Grade H<sub>2</sub>O  
Reagent Grade Acetic Acid  
Reagent Grade Ammonium  
Acetate

#### SUPPLIER

Astec-Advanced Separation Technologies  
Inc.  
Fisher  
Fisher  
Fisher  
Fisher  
Sigma-Aldrich

#### GAS FOR LC/MS

Nitrogen  
Whatman USA

### 3.5 Liquid Chromatography (LC)

Column: Astec C18, 5 $\mu$ m, 150x4.6mm  
Mobile Phase: 50/50/0.3 (v/v/v): MeOH/H<sub>2</sub>O/HOAc  
Flow Rate: 0.8 mL/min.  
Autosampler Injection: 100  $\mu$ L  
Temperature: 45°C

### 3.6 Detection (LC/MS)

LC/MSD (1100 Liquid Chromatograph/Mass Selective Detector, Agilent Technologies) was used for the entire experiment. The HPLC was interfaced by an electrospray interface (ESI). The MS spectrum was scanned at the mass range of m/z 400-2000. The system was controlled by Chem Station software Rev. A.10.02 under Microsoft Windows XP Professional 2002.

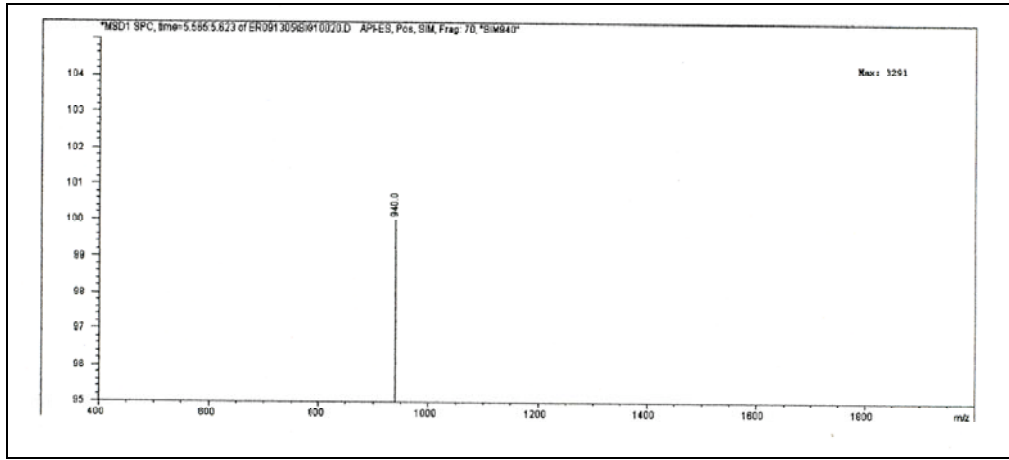
Ion: m/z=940.0  
Fragmentor: 70 V  
Capillary Voltage: 4000 V  
Drying Gas Flow: 1 1 L/min.  
Nebulizer Gas Pressure: 30 psig  
Drying Gas Temperature: 350°C

Standard Calibration Curve for Teicoplanin

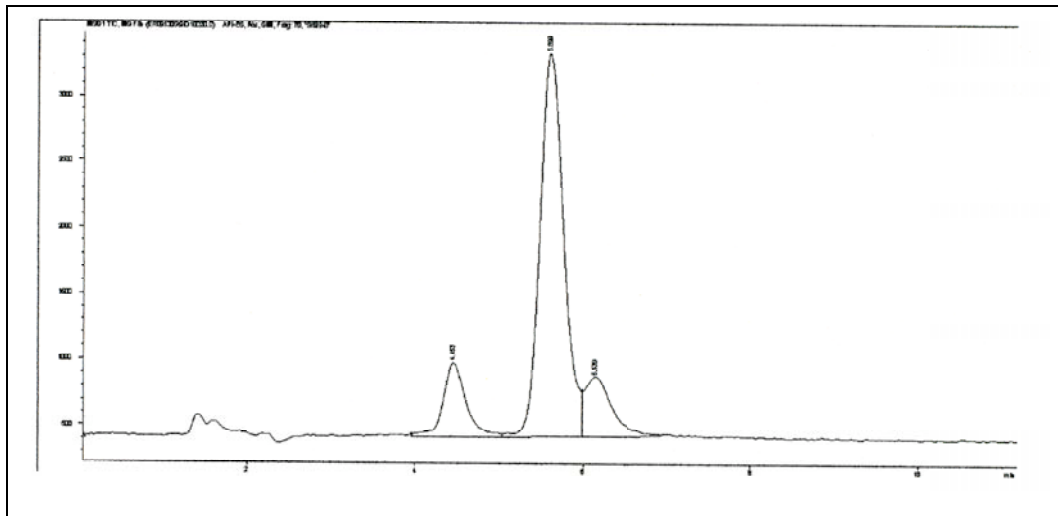
STD	Teicoplanin (ng/mL)	Peak Height
1	1.25	59.6
2	2.50	91.0
3	5.00	166.7
4	10.0	340.9
5	100.0	2903.4



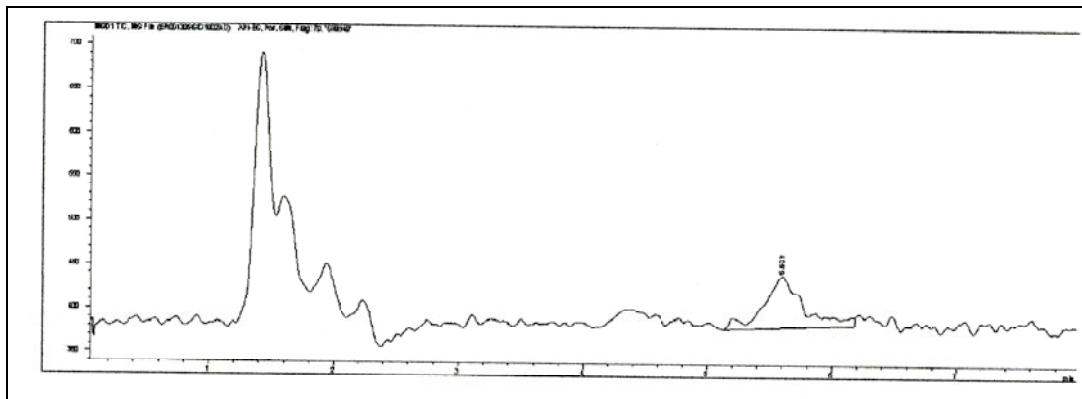
Single Ion Monitoring Spectrum of Teicoplanin Standard Solution @ 100 ng/mL



3.8 Representative LC-MS Chromatogram of Teicoplanin Standard Solution  
A. 100 ng/mL



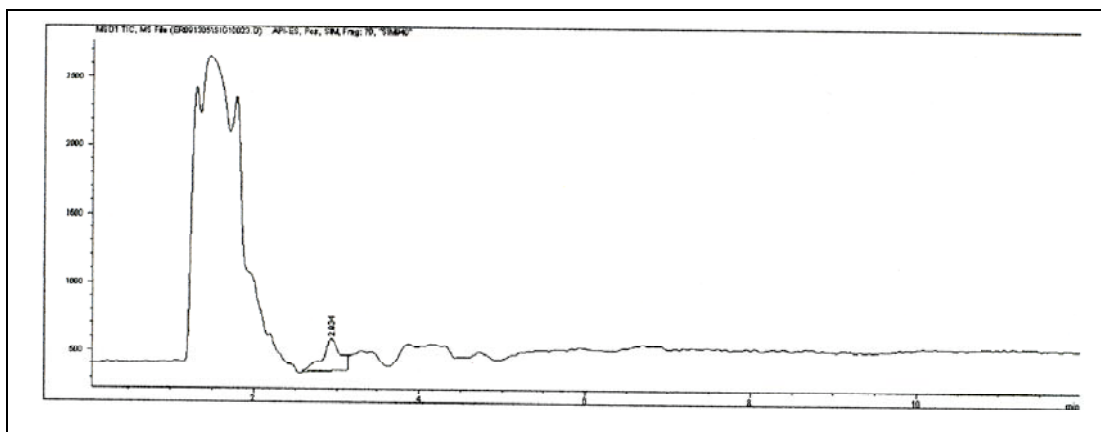
B. @1.25 ng/mL equivalent to the limit of quantification (LOQ)



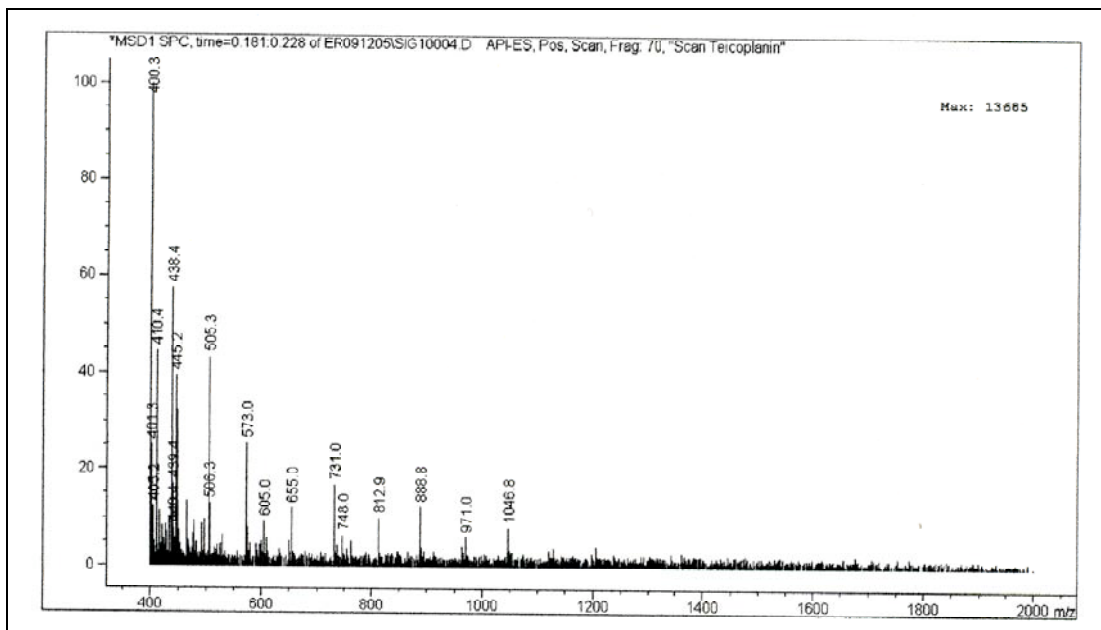
## 4. Sample Preparation

About 70g of teicoplanin media (CHIROBIOTIC T, 10  $\mu\text{m}$  silica, Batch #040614) was packed into a 250x21.2mm column (Serial No. H18-5). The column was first washed with 2 liters of solution containing 50/50 (v/v): ACN/50mM NH<sub>4</sub>OAc, pH 4.1, followed by 500 mL of HPLC grade water and 500 mL of HPLC grade methanol at 8 mL/min. This is the standard washing SOP for all new CHIROBIOTIC media. The column was then conditioned at this flow rate with mobile phase of 50/50/0.2/0.1 (v/v/v/v): MeOH/H<sub>2</sub>O/HOAc/TEA, and obtained a stable baseline after about one hour of conditioning time. Two liters of mobile phase were then collected and concentrated to 50°C under vacuum on a Rotovap to about 33 mL (60x concentration). The concentration eluate (SR-53-77) was stored in the freezer until assayed.

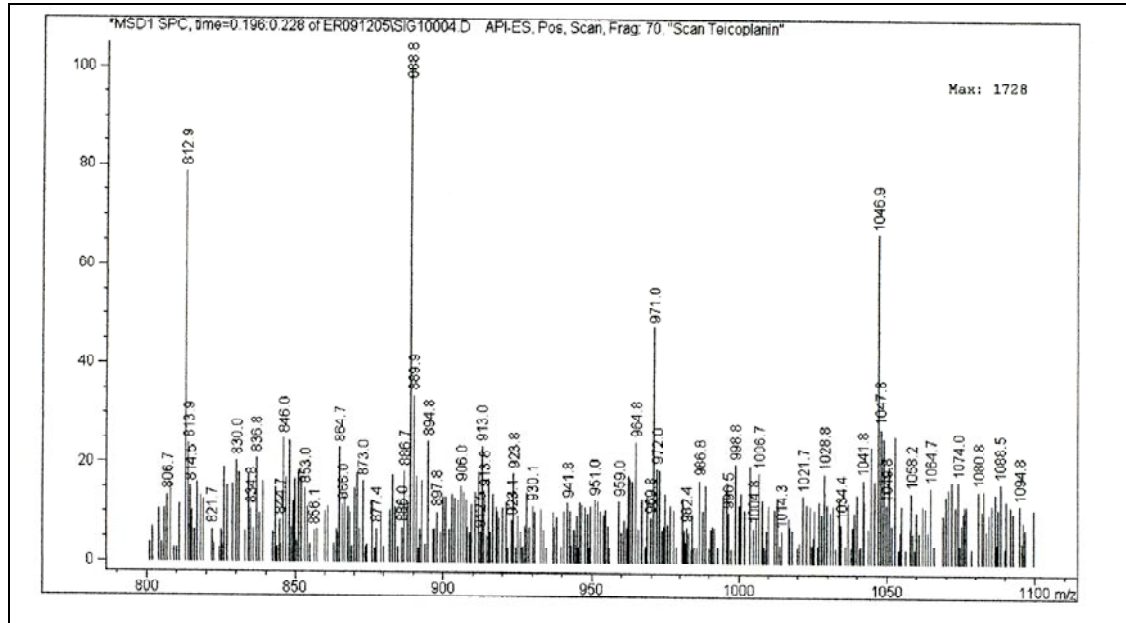
### A. Representative LC-MS chromatogram of the sample (SR-53-77)



### B. MS Spectrum of sample (SR-53-77) at scan range from 400 to 2000 m/z



### C. MS Spectrum of sample solution at scan range from 800 to 1100 m/z



### 5. Conclusion

The LC-MS chromatogram of sample (SR-53-77) showed no sign of a teicoplanin peak. Furthermore, the MS spectra of sample did not match the teicoplanin standards. No teicoplanin molecule can be identified. Since the sample has been concentrated (60x), it is concluded that the original mobile phase eluate contained <10 picogram/mL of teicoplanin.