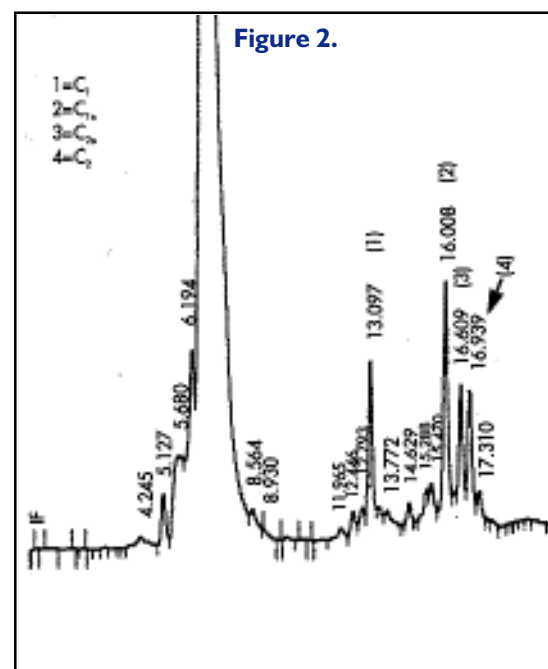
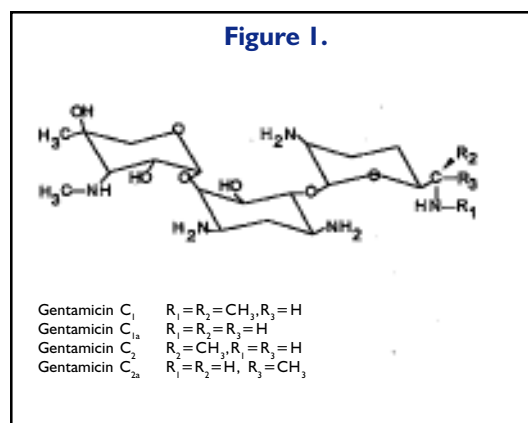


### Separation of the Gentamicin Complex

The antibiotic gentamicin complex consists of at least four closely related isomeric pseudo-oligosaccharides referred to as gentamicin  $C_1$ ,  $C_{1a}$ ,  $C_2$  and  $C_{2a}$  (see Figure 1). Gentamicin complex is produced by fermentation of *Micromonospora purpurea* l. The official Food and Drug Administration method for the determination of percent composition is thin layer paper chromatography followed by microbiological assay<sup>2</sup>.

Weinstein and co-workers<sup>3</sup> reported that the gentamicin complex consisted of three major antibiotic components, gentamicins  $C_1$ ,  $C_2$ , and  $C_{1a}$  using the technique of this layer chromatography. An attempt was also made to separate a 200 mg quantity using cellulose powder chromatography<sup>3</sup>. However, the method of separation by thin layer chromatography is extremely slow and is only feasible for the detection or separation of minute quantities. Cellulose powder chromatography is also time-consuming and irreproducible. Current USP protocol specifies an HPLC separation of pre-column derivatized components for the determination of percent composition and microbial assay for potency<sup>1</sup>.

We report here a reversed-phase HPLC method that gives results that are much more reproducible, with a higher resolution of the four gentamicin complexes:  $C_1$ ,  $C_{1a}$ ,  $C_2$ , and  $C_{2a}$ . Separation time of 18 minutes or less is achievable using conventional mobile phase and a standard analytical column. Furthermore, a very high recovery preparative column chromatographic separation is feasible with this method of analysis of the complex.



#### Chromatographic Conditions (Figure 2)

Solutes: Gentamicin complex  
 Column: SMT 0-5-100 (250x4.6mm), 0-5-100G  
 Mobile Phase: A=2.5g 1-heptane sulfonic acid sodium salt,  
 25 mL HAc, 225 mL water, dil to 500 mL MeOH  
 B-MeOH

(Linear gradient 0-5 min 75% A, 25% B; 5-15 min.  
 25% A, 75% B; 5 min hold)

Flow: 1mL/min

Detector: Fluorescence 345nm exc 445nm emi

#### Column Specifications:

Particle: Spherical silica, 5  $\mu$ m  
 Pore Size: 100Å  
 Surface Area: 340 m<sup>2</sup>/g  
 % Carbon: 14%  
 pH range: 1-12

\*SMT wishes to thank Dr. Paul Golden of U.S. Environmental Protection Agency (Washington, DC) for the method development.

1. USP XII, NF XVII, US Pharmacopeia 1989, p. 603.
2. Code of Federal Regulations, 1981, Title 2, Part 222.20a.
3. Weinstein et. al. Antimicrob Ag. Chemother. 1963, 1, 1.

**SEPARATION  
 METHODS  
 TECHNOLOGIES**

