An Internal Communication
of Applications and Techniques

Waters
Lab Highlights ANTIBIOTICS
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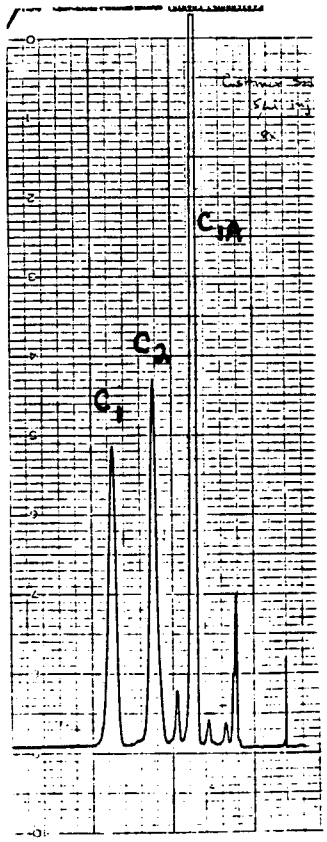
GENTAMICIN FROM FERMENTATION BROTH

Gentamicin is one of several aminoglycoside antibiotics that have been clearly established to have major value in the management of aerobic Gram-negative bacillary infections. Current production procedures for gentamicin involve fermentation of Micromonospora purpurea or M. echinospora and variants thereof. HPLC methods for the separation and post-column o-Phthalaldehyde detection of the three major components of gentamicin - C_1, C_{1A}, and C_2 - have been developed and applied to the measurement of serum levels of the antibiotic by J. P. Anhalt. Interfering serum compounds were removed by ion exchange gel chromatography utilizing CM-Sephadex (C-25). The producers of the antibiotic must also monitor the three gentamicin components, but must do so from the fermentation broth sample matrix. The application of the CM-Sephadex cleanup procedure is demonstrated on fermentation broth samples submitted by ANSA Antibiotics Co.



Standard

Column: µBondapak C_{18}

Column Eluent: 3% methanol
97% 0.2M Na_2SO_4/
0.02M pentane sulfonic acid/
0.1% (V/V) acetic acid
Flow at 1 ml/min

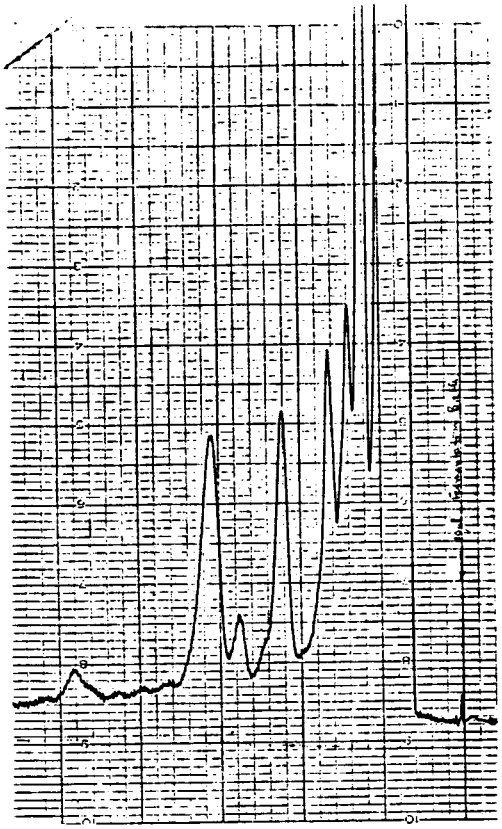
Derivatizing Reagent:
1) 15g H_3BO_3 dissolved in 500 ml H_2O
pH adjust to 10.4 with KOH pellets

2) 300 mg o-phthalaldehyde dissolved in 5 ml ethanol
+ 0.1 ml β-mercaptoethanol

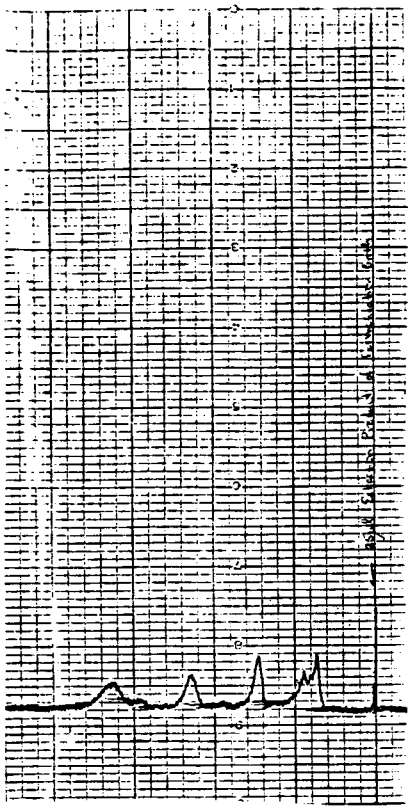
Mix solutions 1 and 2

(1) J. P. Anhalt, Antimicrobial Agents and Chemotherapy 11 (4), 651-655 (1977)

Robert Burgoyne 12/18/81



Fermentation Broth



Extraction Product
of Fermentation Broth

POST COLUMN OPA