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Application Note SI-01227

Determination of Aminoglycoside Antibiotics by HPLC with ELSD

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Introduction

Streptomycin and neomycin are members of the family of antibiotics called aminoglycosides. Aminoglycoside antibiotics are complex organic molecules produced by streptomyces strains and are extremely important in clinical medicine, due to their efficacy and low cost. However, owing to their potential toxicity, the accurate quantification of these antibiotics in pharmaceutical and medicinal preparations is vital. Structurally, aminoglycosides have an alcoholic function and are amino substituted sugars linked together by glycosidic linkages to form pseudo-oligosaccharides, which are easily recognized. Antibiotics, such as streptomycin and neomycin, are difficult to detect by UV due to their extremely weak chromophore, and so evaporative light scattering detection should be used, which is independent of the optical properties of the analyte. The separation of streptomycin and neomycin requires the use of an ion-pair reagent in the mobile phase, which demands special attention when using ELSD. Traditional ion-pair reagents, such as hexanesulphonic acid, cannot be employed where ELSD is the detection method, because this ion-pair reagent is not volatile. Instead, a volatile ion-pair reagent, such as pentafluoropropionic acid, must be used.

Instrumentation

Column: C18 5 μ m, 150 x 4.6 mm
Detection: Varian ELSD (neb=40 °C, evap=85 °C, gas=1.2 SLM)

Materials and Reagents

Eluent A: 0.3 % Pentafluoropropionic acid (PFPA) in MeOH
Eluent B: 0.3 % PFPA in 47 mM ammonium formate

Sample Preparation

Antibacterial cream containing streptomycin and neomycin.

Conditions

Flow Rate: 1.0 mL/min
Injection Volume: 10 μ L
Gradient: 45/55 A/B

Results and Discussion

The retention time of streptomycin and neomycin can be altered by adjusting the ion-pair concentration to achieve the desired separation for sample mixtures of aminoglycosides, as shown in Figure 1.

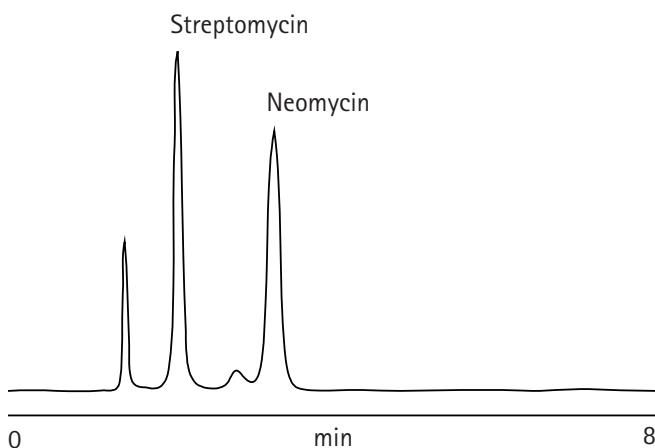


Figure 1. Efficient separation of two antibiotics using Varian ELSD.

Conclusion

Evaporative light scattering detection is universal and not dependent on the optical properties of the compound under consideration. ELSD is capable of detecting any analyte that is less volatile than the mobile phase, making it fully gradient compatible; it also removes the need for sample derivatization. ELSD is therefore the ideal choice for detection of streptomycin and neomycin, because of its high sensitivity.

These data represent typical results. For further information, contact your local Varian Sales Office.

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