High Resolution HPLC Analysis of Streptomycin Sulfate by ELSD

Application Note

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Introduction

Streptomycin was one of the first antibiotics to be discovered. In the soluble sulfate form, it was used for a range of bacterial infections but has been largely replaced owing to the discovery of more effective antibiotics. In recent years, its popularity has increased once more because of its use in combination with other drugs for the treatment of tuberculosis. Some strains of the multi-drug resistant Mycobacterium tuberculosis are vulnerable to this treatment.

The complex synthesis of streptomycin sulfate may result in contamination of the compound with various impurities. It is therefore important to be able to identify the purity of the extract before clinical use. A quick and easy method has been developed to analyze streptomycin sulfate using a polymeric reversed-phase PLRP-S column and evaporative light scattering detector, the Agilent ELSD. PLRP-S 100Å columns are ideally suited to the analysis of low molecular weight compounds because the very small pore sizes have extremely high surface areas available to the solutes. The Agilent ELSD is renowned for its rugged design and ability to deliver high performance for demanding HPLC or GPC applications. An excellent demonstration of the high performance of PLRP-S and the Agilent ELSD is provided by the analysis of streptomycin sulfate.

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Instrumentation

Column:	PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)
Detection:	Agilent ELSD (neb=70 °C, evap=85 °C, gas=1.0 SLM)

Materials and Reagents

Eluent A:	0.1% TFA, Water
Eluent B:	0.1% TFA, ACN

Sample Preparation

Sample:	Streptomycin sulfate

Conditions

Gradient:	5-50% B in 10 min
Flow Rate:	1.0 mL/min

Results and Discussion

A short ACN/water gradient with TFA as the ion pairing agent permits detection of the main sample peak as well as two contaminants, see Figure 1.

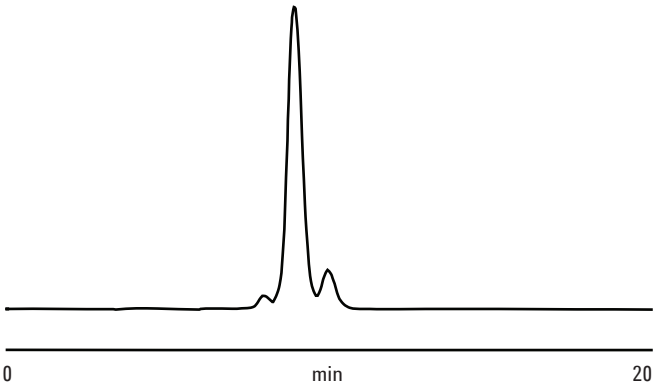

Figure 1. Chromatogram showing detection of the main sample peak and two contaminants.

Conclusion

The Agilent ELSD evaporative light scattering detector and PLRP-S column successfully revealed the presence of contaminants in a sample of streptomycin sulphate. PLRP-S columns are ideally suited to the analysis of many small molecules. These columns are more retentive for small molecules than the majority of alkyl bonded silicas. PLRP-S media possess a much greater surface area than alkyl bonded silicas and, therefore, even polar molecules, such as carboxylic acids, may be retained much longer, resulting in greater resolution. The robust design of Agilent ELSD allows the nebulizer and evaporator to operate at very high temperatures, efficiently handling the high boiling point solvents that other ELSDs simply cannot manage. PLRP-S columns used with the Agilent ELSD is an ideal combination for these challenging applications.

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