Application Note

Small Molecule Pharmaceuticals, Drug Development

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Impurity Analysis of Aminoglycoside Antibiotic Using the Agilent InfinityLab Poroshell 120 HILIC-Z Column with ELSD Detection

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Abstract

Aminoglycosides represent a powerful and widely used class of antibiotics. The highly polar nature of both aminoglycosides and their impurities makes them very challenging to analyze using reversed-phase chromatography (RPLC). Hydrophilic interaction chromatography (HILIC) is a convenient and fast method for separation of these polar molecules with minimal changes to the system or solvents compared to RPLC.

Introduction

This Application Note demonstrates how a target impurity neamine and others are separated from a common aminoglycoside antibiotic ribostamycin using the Agilent InfinityLab Poroshell 120 HILIC-Z, 2.7 µm column.

ELSD was used for detection because of the weak UV signal of both compounds.

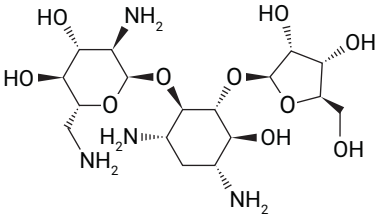

Figure 1. Ribostamycin structure.

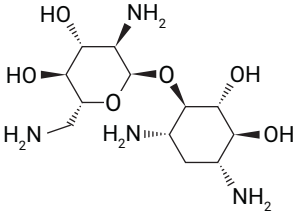

Figure 2. Neamine structure.

Experimental

Reagents and Chemicals

All reagents were HPLC grade or higher. HPLC grade acetonitrile was bought from J. T. Baker (Center Valley, PA, U.S.A.). Water was purified using an EMD Millipore Milli-Q Integral System (Darmstadt, Germany.) Ammonium acetate, ribostamycin sulfate, and neamine were from Sigma-Aldrich (St. Louis, MO, USA).

Equipment and Materials

- Agilent InfinityLab fittings
- **Column inlet:** Agilent InfinityLab Quick Connect fitting (p/n 5067-5965)
- **Column outlet:** Agilent InfinityLab Quick Turn fitting (p/n 5067-5966)
- Agilent Captiva Econofilter, PTFE membrane, 13 mm diameter, 0.2 µm pore size (p/n 5190-5265)
- Agilent Vial, screw top, amber, write-on spot, certified, 2 mL, 100/pk (p/n 5182-0716)
- Agilent bonded screw cap, PTFE/red silicone septa (p/n 5190-7024)
- Agilent InfinityLab solvent bottle, amber, 1,000 mL (p/n 9301-6526)
- Agilent InfinityLab Stay Safe cap, GL45, 3 ports, 1 vent valve (p/n 5043-1219)
- Eppendorf pipettes and repeater
- Sonicator (VWR, Radnor, PA, USA)
- Vortexer and multitube vortexers (VWR, Radnor, PA, USA)

Instrumentation

- Agilent 1260 Infinity II binary pump (G7112B)
- Agilent 1260 Infinity II vialsampler (G7129C)
- Agilent 1260 Infinity II multicolumn thermostat (G7116A)
- Agilent 1290 Infinity II ELSD (G7102A)
- Agilent OpenLAB software

Sample Preparation

- **Sample 1:** A mixture of 0.4 mg/mL ribostamycin sulfate and 0.2 mg/mL neamine was prepared in water, and diluted 50 % with acetonitrile (ACN).
- **Sample 2:** A mixture of 4 mg/mL ribostamycin sulfate was spiked with 2 % neamine (0.08 mg/mL) in water.

Mobile Phase Preparation

Ammonium acetate was weighed and diluted to a concentration of 100 mM in water. Buffers were prepared 1 L at a time, and replaced regularly to prevent degradation and microbial growth.

Instrument conditions

HPLC Conditions	
Column	Agilent InfinityLab Poroshell 120 HILIC-Z 2.1 × 100 mm (p/n 685775-924)
Mobile phase A	100 mM ammonium acetate
Mobile phase B	Acetonitrile
Flow rate	0.40 mL/min
Column temperature	25 °C
Injection volume	2 µL
Gradient	0–1 minute 65 %B 1–5 minutes 65–55 %B 5–10 minutes (stop) 55 %B 10–13 minutes (postrun) 65 %B
ELSD Conditions	
Nebulizer temperature	40 °C
Evaporator temperature	40 °C
Gas flow rate	1.6 SLM
Data rate	40 Hz

Results

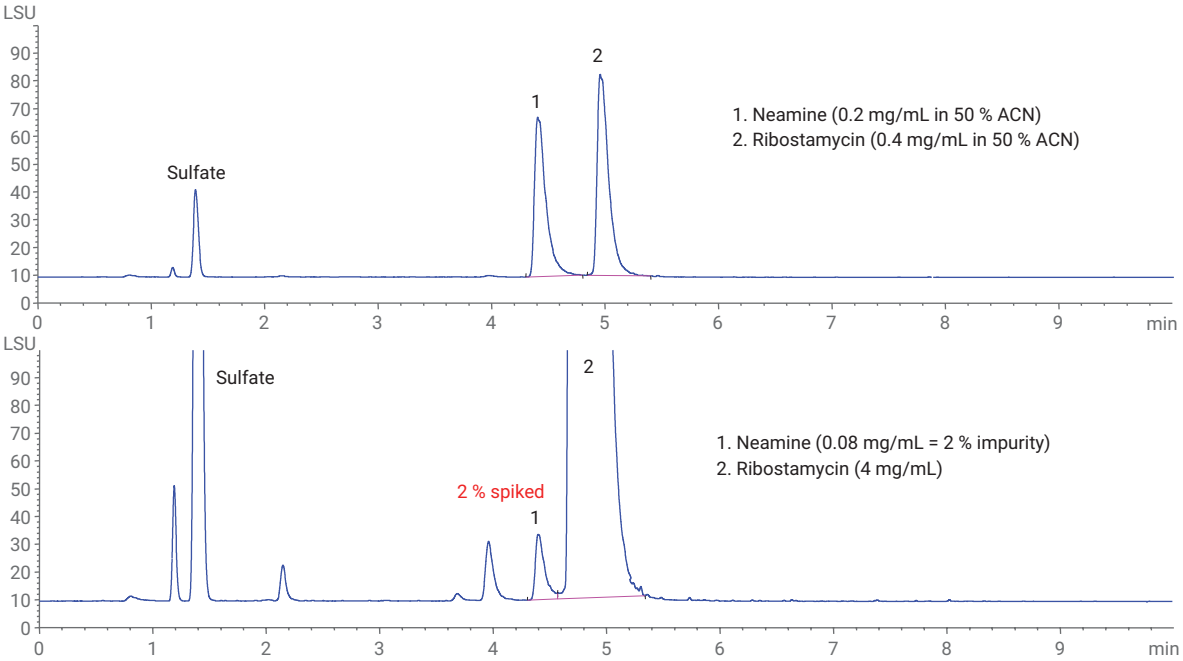

Figure 3. Separation of neamine and ribostamycin using an Agilent InfinityLab Poroshell 120 HILIC-Z LC column.

Conclusions

Impurities including neamine were all successfully separated from the main compound ribostamycin using an Agilent InfinityLab Poroshell 120 HILIC-Z column. The zwitterion-based HILIC-Z bonded phase gives both excellent resolution and peak shape.

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