

Antibiotics

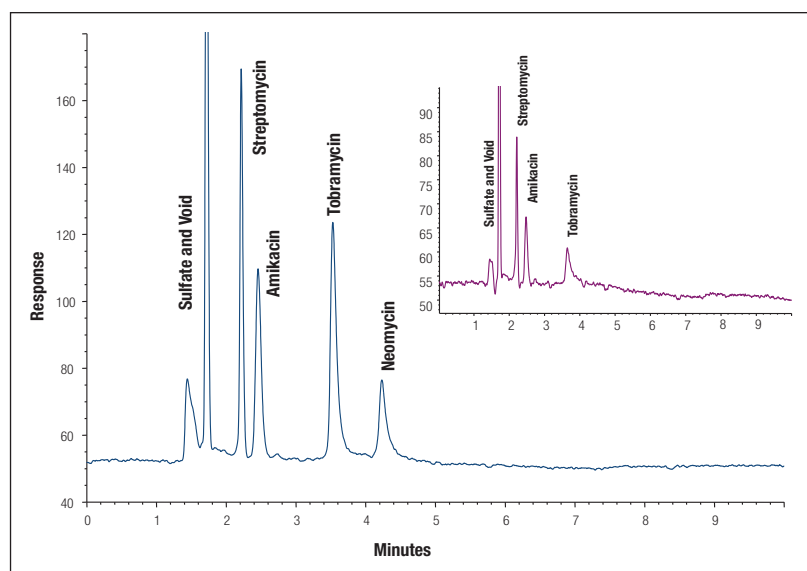


Figure 1. Antibiotic standards (200 ng each on column). Inset: 50 ng each.

The need for universal high-performance liquid chromatography (HPLC) detection in analytical laboratories is widespread. While several detection technologies (e.g., low wavelength UV, refractive index, evaporative light scattering, chemiluminescent nitrogen detectors) are currently being used, there is significant room for improvement in performance characteristics such as sensitivity, dynamic range, consistency of response factors, and gradient or solvent compatibility.

To help address the many challenges of universal detection, the Thermo Scientific Dionex Corona Charged Aerosol Detector was developed. This novel technology offers many benefits to analytical scientists including:

- High Sensitivity—Low ng limits of detection.
- Consistent Response Factors—Response magnitude does not significantly depend on analyte properties (e.g., molar absorptivity, proton affinity).
- Broad and Useful Dynamic Range—Four orders of magnitude (ng to μg quantities).

- Excellent Reproducibility—Typically less than 2% relative standard deviation (RSD).
- Broad Applicability—Can be used with a wide variety of HPLC conditions to measure virtually any nonvolatile analyte including proteins, lipids, carbohydrates, and small molecules.
- Ease of Use—Easy setup. Uses minimal bench space and requires only gas input pressure and signal output range to be set.

This application note describes the use of the CADTM Charged Aerosol Detector for the measurement of antibiotics. The method has a limit of detection of <50 ng on column (Figure 1), is reproducible (Figure 2), and has a dynamic range that covers ng to μg levels (Figure 3). This application note demonstrates the use of charged aerosol detector with binary gradient, for detection of compounds lacking a sufficient UV chromophore.

Corona Parameters

Gas:	35 psi via nitrogen generator
Filter:	None
Range:	100 pA

HPLC Parameters

Mobile Phase A:	0.2% pentafluoropropionic acid		
Mobile Phase B:	Methanol		
Gradient:	Time	%A	%B
	0.00	55	45
	10.00	35	65
	10.01	55	45
15.00	55	45	
Flow Rate:	1.0 mL/min		
Column:	4.6 × 150 mm; 5 μm		
Column Temperature:	Ambient		
Injection Volume:	10 μL		

Sample Preparation

Stock—1 mg/mL of each standard dissolved in mobile phase (initial conditions). Dilutions in mobile phase (initial conditions).

Conclusion

The Corona charged aerosol detector provides universal detection of nonvolatile analytes with response independent of chemical properties, a wide dynamic response range, high sensitivity, and good precision. These characteristics, along with reliability and simple operation, make the Corona charged aerosol detector a superior detector for a wide range of HPLC analyses.

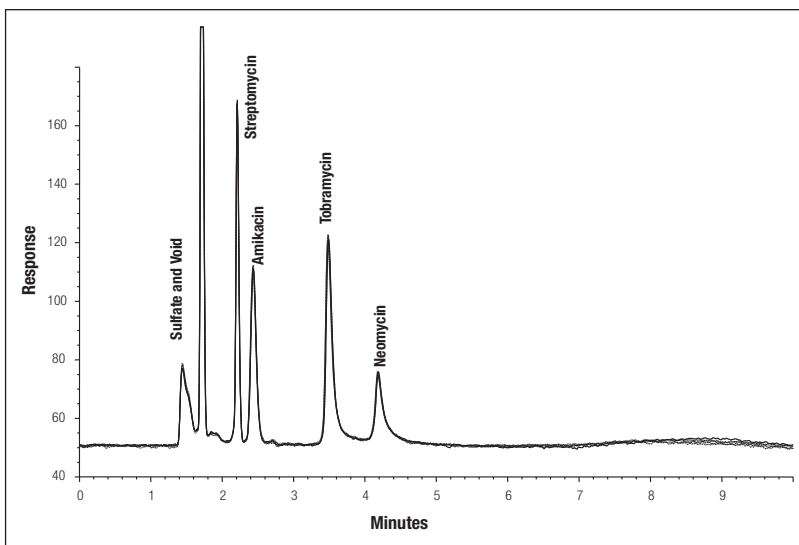


Figure 2. Reproducibility (5 replicates of 200 ng standard).

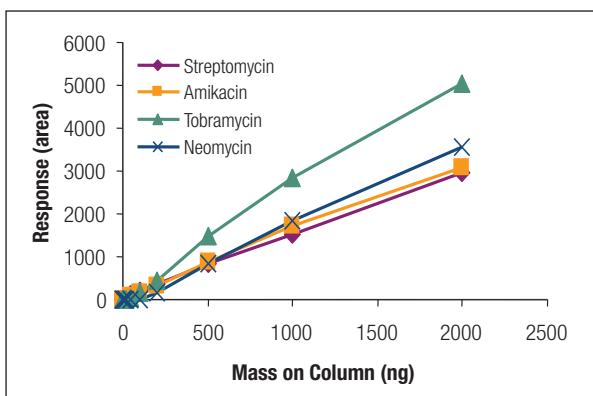


Figure 3. Antibiotic response curves.

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Australia +61 3 9757 4486
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Benelux +31 20 683 9768
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Brazil +55 11 3731 5140

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Denmark +45 36 36 90 90
France +33 1 39 30 01 10
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India +91 22 2764 2735

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Korea +82 2 3420 8600
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Taiwan +886 2 8751 6655
UK +44 1276 691722
USA and Canada +847 295 7500

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