Amikacin analysis and aerosol particle size distribution (aPSD) determination using charged aerosol detector (CAD)

Amikacin, a broad spectrum aminoglycoside antibiotic derived from kanamycin A, is commonly used for treating severe, hospital - acquired infections caused by Gram-negative bacteria. The analysis of this molecule has always been challenging because it does not contain a UV chromophore.

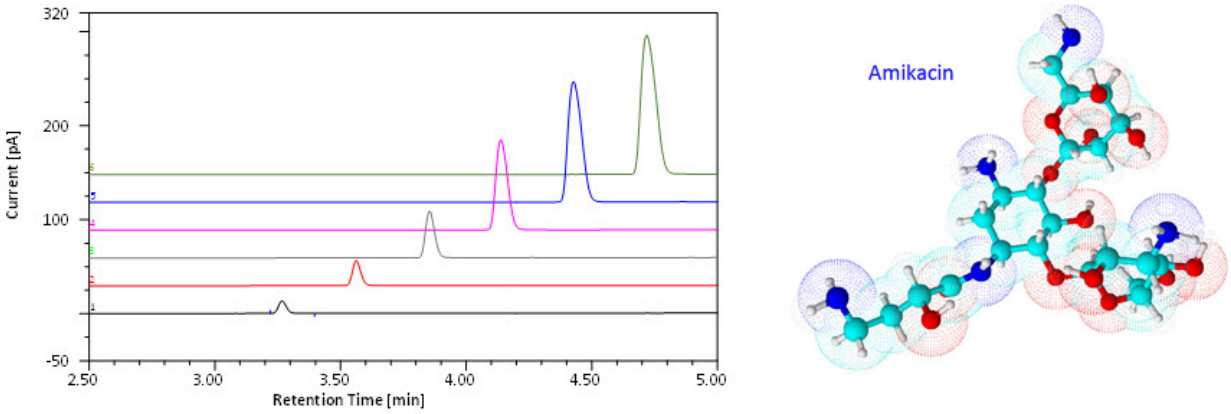

Fig. 1. HPLC stacked plot (top): Detector response as a function of retention time overlay of the 0.05, 0.1, 0.2, 0.5, 0.8, and 1 mg/mL of amikacin peak. Gradient conditions: 0-1 min (5% B), 4.5 min (95% B), 4.51-7.00 (5% B). F = 1.5 mL/min. T = 55oC. Vinj = 3 µL. 3D view of Amikacin (bottom).

A robust HPLC method for amikacin detection and aerosol particle size distribution (aPSD) determination, with performance comparable to conventional UV absorbance detection, was developed using a charged aerosol detector (CAD). HPLC-CAD chromatograms are shown in Figure 1. To select the optimal power function value (PFV) to linearize the signal, the slope and relative standard deviation (S_{rel}) of the response factor as a function of concentration were evaluated across a range of PFVs (Fig. 2).

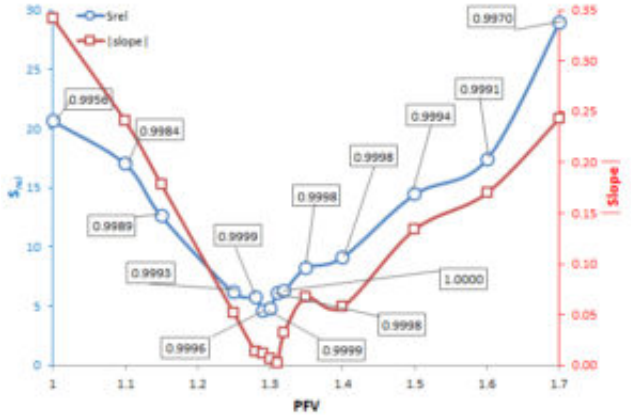

Fig. 2. Change in absolute value of the slope of response factor versus concentration plots and in Srel of the response factors as a function of PFV. The numbers reported in boxes are the correlation coefficients of the area versus concentration at each power function value. Chromatography conditions: 0 min (5% B), 8 min (40% B), 9 min (90% B), 9.1-13 (5% B). F = 1.0 mL/min. T = 55°C.

The HPLC system dwell volume inclusion in the gradient program setup reduced the variability in retention time, peak width, signal-to-noise, and resolution between amikacin and kanamycin. The incorporation of a 1.0 min isocratic hold of the initial condition allowed the method to be run on HPLC systems with 2.65 mL dwell volumes or less.

During method optimization, a power function value was carefully chosen to yield the best linearity by targeting three requirements: 1) minimum variability in response factor over concentration range (*i.e.*, lowest S_{rel}), 2) slope of response factor versus concentration as close to zero as possible, and 3) correlation coefficient of area versus concentration close to unity. Figure 2 illustrates the optimum value of the PFV in the range of 1.28 – 1.35. The influence of mobile phase grade and glassware binding of amikacin during sample preparation were also addressed. A weighed ($1/X^2$) least square regression was used for the calibration curve. The limit of quantitation (LOQ) and limit of detection (LOD) for this method were determined as 5 µg/mL and 2 µg/mL, respectively. The method was validated over a concentration range of 0.05 to 2 mg/mL. The correlation coefficient for the peak area versus concentration was 1.00 and the y-intercept was 0.2%. The recovery accuracies of triplicate preparations at 0.05, 1.0, and 2.0 mg/mL were in the range of 100 - 101%. The S_{rel} of six replicates at 1.0 mg/mL was 1%, and S_{rel} of five injections at the limit of quantitation was 4%. The method is able to support routine throughput of 200 samples per day.

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Publication

A simplified guide for charged aerosol detection of non-chromophoric compounds-Analytical method development and validation for the HPLC assay of aerosol particle size distribution for amikacin.

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