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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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ABSTRACT

Amikacin, an aminoglycoside antibiotic lacking a UV chromophore, was developed into a drug product for delivery by inhalation. A robust method for amikacin assay analysis and aerosol particle size distribution (aPSD) determination, with comparable performance to the conventional UV detector was developed using a charged aerosol detector (CAD). The CAD approach involved more parameters for optimization than UV detection due to its sensitivity to trace impurities, non-linear response and narrow dynamic range of signal versus concentration. Through careful selection of the power transformation function value and evaporation temperature, a wider linear dynamic range, improved signal-to-noise ratio and high repeatability were obtained. The influences of mobile phase grade and glassware binding of amikacin during sample preparation were 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 to be 5 μg/mL and 2 μg/mL, respectively. The method was validated over a concentration range of 0.05–2 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 relative standard deviation (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%. A robust HPLC-CAD method was developed and validated for the determination of the aPSD for amikacin. The CAD method development produced a simplified procedure with minimal variability in results during: routine operation, transfer from one instrument to another, and between different analysts.

1. Introduction

Amikacin, (2*S*)-4-amino-*N*-{(1*R*,2*S*,3*S*,4*R*,5*S*)-5-amino-2-[(3-amino-3-deoxy-α-D-glucopyranosyl)oxy]-4-[(6-amino-6-deoxy-α-D-glucopyranosyl)oxy]-3-hydroxycyclohexyl}-2-hydroxybutanamide, a broad spectrum aminoglycoside antibiotic derived from kanamycin A, is commonly used for treating severe, hospital-acquired infections caused by Gram-negative bacteria. Due to the molecule's lack of a UV chromophore, its analysis has always been challenging; therefore, pre- and post-column derivatization and non-UV detection techniques have been developed to monitor its content in pharmaceutical formulations.

Aminosugar analytical methods using pre-column [1] derivatization in liquid chromatography (LC), capillary electrophoresis (CE) [2]. and micellar electrokinetic chromatography (MEKC) or post-column [3] derivatization or complexation in LC prior to UV or fluorescence detection have been reported [4]. Both detection techniques have pros and cons; the choice of one or the other depends on the analyte, its derivatization site(s) or, in many cases, the preference or expertise of the analyst. However, due to the reactivity of multiple functional groups, post-column derivatization is recommended [5]. The drawbacks associated with derivatization techniques are that they can be time-consuming, labor intensive, and difficult to quantitate, can demonstrate a larger overall variability due to extra sample preparation steps, and the reactions can

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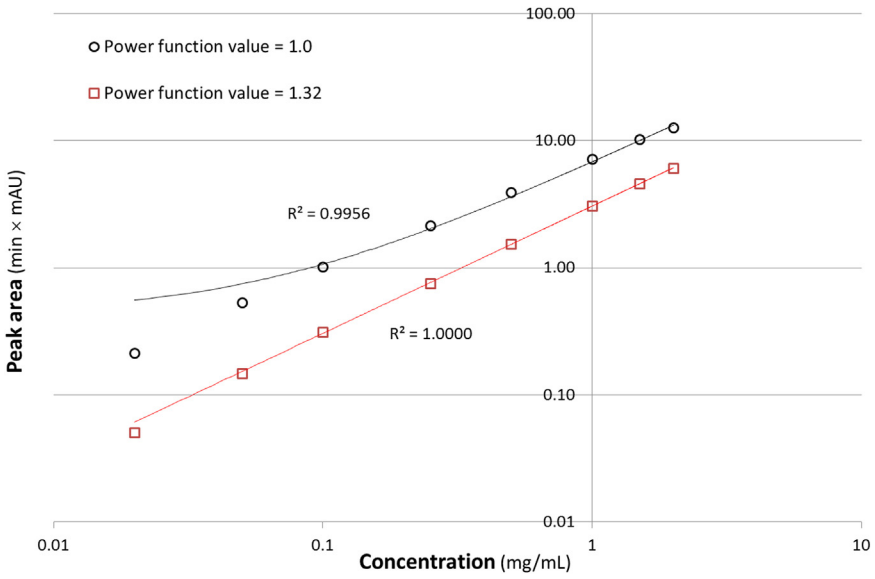

Fig. 1. Plot of peak area as a function of concentration for power function values of 1.00 (open circles) and 1.32 (open squares) at evaporative temperature of 35 °C. Both axes are on logarithmic scale to show the variability of the best fit line at low concentration.

often be difficult to control. Other methods use resonance Rayleigh scattering [6], chemiluminescence [7], cyclic voltammetry [8], or even colorimetric methods based on gentamicin-induced collapse of an Au–lipid capsule [9]. For a quality control (QC)–friendly method it is always preferable to have a direct detection mode; evaporative light scattering detection (ELSD) has therefore been used for the determination of amikacin in drug products [10]. There also exist LC methods that push the UV detection limits to 191 nm [11], or MEKC methods at 200 nm [12], but these approaches are not viable for stability-indicating methods. The ion chromatography (IC) technique with pulsed amperometric detection (PAD) became more appealing to the analytical community as the instrumentation became more robust [13]. The United States Pharmacopeia (USP) monograph and the European Pharmacopeia (Ph. Eur.) monographs for amikacin use ion chromatography with electrochemical detection in integrated amperometric mode [14]. Pre-column derivatization with 2,4,6-trinitrobenzene sulfonic acid with UV detection has also been reported [15].

Existing analytical methods including the USP and Ph. Eur. methods, are still being used; however, more robust methods with better precision are needed. In the pharmaceutical industry, advances were implemented to improve existing separations and aid method development. The corona charged aerosol detector (CAD) was developed as a direct detection mode for non-chromophoric compounds as an alternative to ELSD and was first commercially released in 2004 [16]. This mode of detection is mass sensitive, in contrast to the UV concentration dependent detector, and can detect molecules with weak or no chromophores [17]. Since its development, this detection technique has been implemented in industries, such as the pharmaceutical industry, that require rugged and robust quantitative methods [18].

CAD approaches have been criticized for their limited linear working dynamic detection range [18,19]. This narrow dynamic range resulted from the difficulty in distinguishing between tiny charged spherical particulates with smaller masses and larger charged particulates with higher masses, since both follow a non-linear signal versus charge relationship. The main disadvantage of this detector was thus its non-linear response, which complicated quantitation [20]. However, a log–log transformation of the peak area response for specific concentrations, followed by linear regression was suitable for calibration purposes [20].

The linear dynamic range of CAD detectors has been gradually extended through exploitation of a power transformation before signal output. For example, the manipulation of raw data utilizing a power transformation resulted in increased peak intensity and, decreased peak width, while peak asymmetry remained constant [21,22]. Numerous raw data sets, raised to different power functions, were shown to practically improve 1DLC, 2DLC, and post-column derivatization separations [22], demonstrating the benefit of embedding the power transformation into the instrumentation's firmware [21]. Despite the foregoing, the use of caution and working within an experimentally-determined calibration curve were highly recommended by the manufacturers; extrapolation was not advised [23]. Optimization based upon an empirical approach has been recommended as a result of a critical evaluation of the use of CAD in the pharmaceutical industry [19].

CAD detection requires more parameters for optimization when compared to UV and fewer when compared to mass spectrometry (MS) detection techniques. The reluctance associated with utilizing CAD is diminished by understanding both the non-linear detection response and the sensitivity of the detector to all non-volatile compounds. Additionally, method development is tightly coupled to the volatility of the compound of interest; hence CAD method optimization is compound specific [19]. In developing a practical CAD method, one should consider that: (1) stationary phase bleeding effect is more pronounced with CAD, and (2) the column must be selected carefully to ensure that the peak of interest is not obstructed [19]. The use of LCMS grade solvents is required to achieve ultimate sensitivity as trace particulates and impurities in HPLC grade solvents contribute to a higher background signal. Any mobile phase additives (e.g. pH buffer, ion-pair reagents) must also be selected for higher volatility than the target compound(s). An alternative approach to minimize the influence of the mobile phase volatility on the response, utilizes a T-piece and second identical pump in the workflow to deliver a reverse gradient with respect to the chromatographic separation gradient. However, this involves a more complicated workflow set-up and is not practical for all laboratories [24].

Aerosol–based detectors have been reviewed in terms of operation and application in different industries [18,25]. The main advantage of CAD over other aerosol detectors is its sensitivity – which can be up to four orders of magnitude higher than ELSD [25].