

Application of Charged Aerosol HPLC Detection in Biopharmaceutical Analysis

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Introduction

UV/Vis detection has been the workhorse of biopharmaceutical HPLC analysis because many proteins and other active biopharmaceutical ingredients typically possess a good chromophore. However, there are many instances where, although the biotherapeutic agent itself may be UV active, it has critical non-chromophoric chemical modifications and/or is formulated in UV-transparent excipients that must be detected and measured. In contrast to UV/Vis instruments, the Charged Aerosol Detector (CAD) is a mass-based instrument that provides a sensitive, near universal response for any nonvolatile analyte independently of chemical composition. Thus it can detect substances unseen by UV/Vis absorption providing valuable orthogonal data. CAD is well suited for the label free detection of analytes without chromophores and the quantification of unknowns. By virtue of its universal response, the charged aerosol detector can be used for many analytical applications in bioPharm ranging from the label free analysis released glycans to quantification of formulation excipients for quality control. This poster demonstrates several examples where the CAD can be applied to detect and quantify substances separated by reversed phase and hydrophilic interaction chromatography. Examples include: glycans, sialic acids, surfactants, adjuvants, proteins and amino acids. In many cases, detection limits are in the low nanogram range with good peak area precision.

FIGURE 1: Charged Aerosol Detector and Principle of Operation

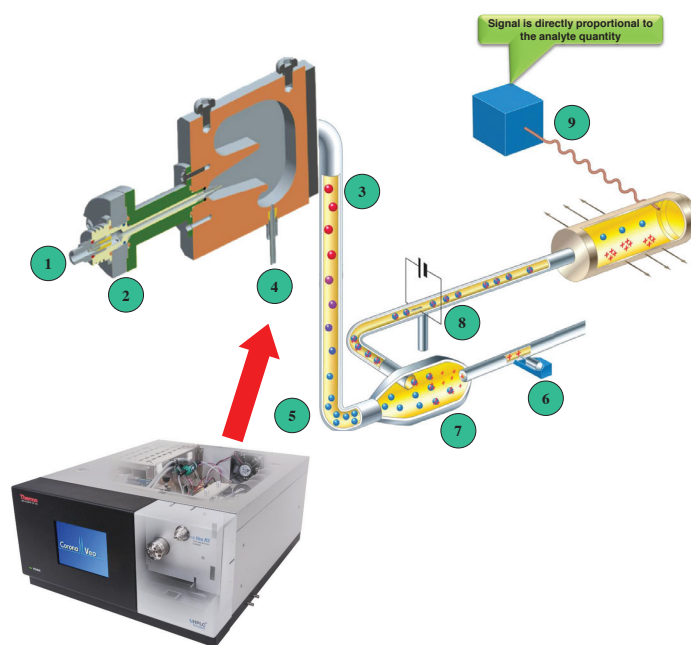


Figure 1 depicts the operation of the charged aerosol detector. At the top left (1) the mobile phase from the LC column entering the detector is nebulized by combining with a concentric stream of nitrogen gas or air (2). The fine droplets carried by bulk gas flow to the heated evaporation sector (3) are desolvated to form dry particles (5) from any nonvolatile or semivolatile species. Any remaining large droplets drain away to waste (4). The dry analyte particles combine with another gas stream that has been charged by a high voltage Corona charger (6). The charged gas transfers positive charge to the analyte particle's surface (7). The charged analyte particles pass through an ion trap (8) that removes any high mobility species and pass to a collector where they are measured by a sensitive electrometer. The signal produced (9) is directly proportional to the quantity of analyte.

Glycans, Sialic Acids and Adjuvants

FIGURE 3: Direct Charged Aerosol Detection of α_1 -Acid Glycoprotein and Bovine Fetuin N-linked Glycans

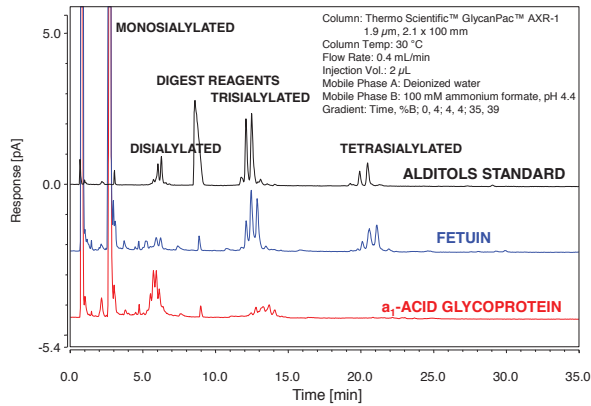


FIGURE 4: Label-free Detection of O-linked Glycans by HPLC-CAD Mucin Glycans Released by Reductive Beta Elimination

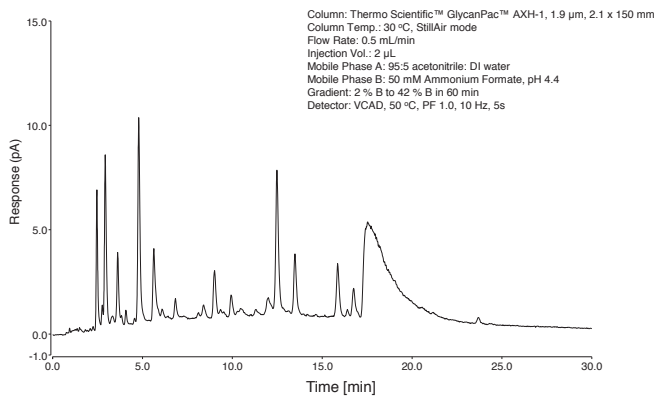


FIGURE 5: Label-free Fetuin Glycan Analysis using Charged Aerosol Detection and MS

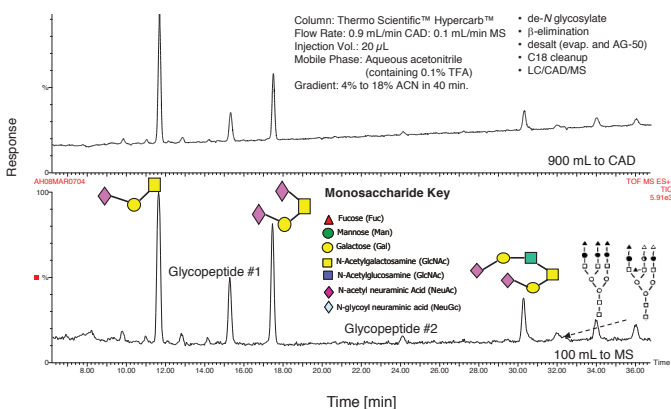


FIGURE 6: Direct Detection of Released Sialic Acids from Human and Bovine Transferrin Using HPLC with Charged Aerosol Detection.

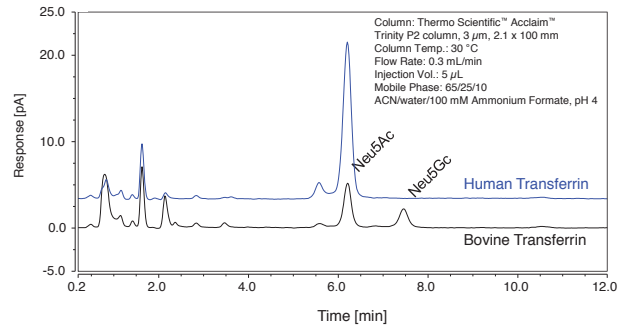
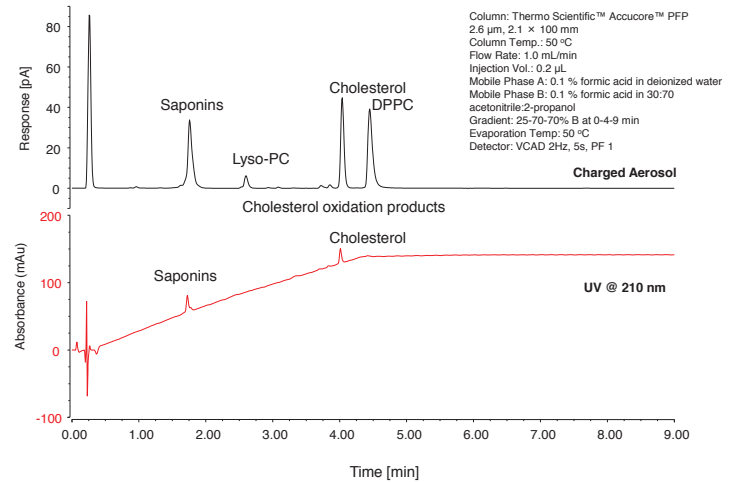


FIGURE 7: Vaccine Adjuvants by CAD and UV



Excipients

FIGURE 8: Surfactants - Quantitative

