Nano Quantity Analyte Detector (NQAD™) A New Alternative In Sensitive HPLC Detection

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Figure 1 – The NQAD™ Detector

High performance liquid chromatography (HPLC) is a common and well-established technology for a host of quantitative techniques employed by analytical chemists to separate compounds (analytes) in solutions.

The most common type of detector used in HPLC today is the UV absorbance type. Use of UV detectors is limited to analytes that contain a chromophore. To detect analytes with a weakly responding or no chromophore, chemists must rely on alternate detection systems. These systems include refractive index, conductivity, mass spectrometry, and aerosol-based detectors.

To address a broad range of issues affecting the performance of alternative detection systems, Quant Technologies of Blaine, MN (www.quanttechnologies.com) have introduced the NQAD™, or Nano Quantity Analyte Detector (Figure 1).

Background

The NQAD™'s operation is partly based on original techniques from early work on Condensation Nucleation Light Scattering Detection (CNLSD) by Dr. J. A. Koropchak and colleagues at Southern Illinois University Carbondale and a number of papers have been published detailing its development [1,2,3,4,5,6,7].

Aerosol-based HPLC detectors all initiate their detection methods on similar principles. They begin by continuously nebulizing the column eluent. The mobile phase is evaporated from the droplets, which leave particles suspended in air that consist of chemicals in the eluent that have a lower volatility than that of the mobile phase. When a non-volatile analyte elutes from the column, the size of the particle increases.

At this point, in an Evaporative Light Scattering Detector (ELSD), a photometric measurement of the aerosol cloud occurs. As the size of the residue particles increases, the level of light reflected by (or absorbed by) the aerosol changes. These detectors convert the light scattering levels to an analog output [4]. The sensitivity of this detection technique is limited by the amount of background photodetector noise [8,9].

Another type of aerosol-based detector uses Charged Aerosol Detection (CAD™). Rather than utilizing light scattering measurements with the aerosol cloud, this type of detector measures the increase in particle size by the ability of the aerosol to carry an electrical charge. The resulting electrical charge is measured using an electrometer and converted to an analog output signal. The sensitivity of this technique can be limited by background noise in the electrometer and can also be susceptible to drift [9]. A rather significant limitation that has been seen with charged aerosol detection methodology is increased noise when sampling mobile phases with high pH [9].

NQAD™

The NQAD™ employs a similar nebulization and evaporation phase to these detectors. However, measurements of the analyte are not taken at this point. Rather, the dry aerosol moves into another chamber and the size of the aerosol distribution is measured by the increase in the number of particles counted using a Water-based Condensation Particle Counter (WCPC). The WCPC condenses water vapor onto particles and grows them to a size that can be detected individually using a laser-based optical sensor (Figure 2).

The WCPC detector in the NQAD™ will only condense vapor onto particles that are above a certain size - particles below this size are not counted. As the size of the particles

increase due to an eluting analyte, the number that is large enough to grow due to water vapor condensation increases. The number of particles counted by the WCPC detector is then converted to an analog output signal.

One of the primary benefits with the NQAD™ is its sensitivity [8]. Rather than measuring a cloud of particles, this instrument counts individual particle droplets. It is minimally affected by baseline drift, and pH levels have no effect on measurement accuracy [8,9].

Performance

As with other aerosol based HPLC detectors, the sensitivity of the NQAD™ is limited by the level of background non-volatile residue that co-elutes with the analyte. However, the NQAD™ is able to measure smaller changes in the size shift of the dry aerosol distribution than any of the other current aerosol-based detectors [8]. The dynamic range of the detector spans from greater than 1 part per thousand to below 1 part per billion [7]. The residue left after evaporation of the mobile phase effectively sets the detection limit and it is therefore important to use high purity solvents and a clean HPLC system.

The NQAD™ has a linear response over several orders of magnitude.

An example of linearity of the NQAD™ response to anions and cations is shown in Figure 3.

Linearity And Dynamic Range

Several factors influence the linearity and dynamic range of all aerosol based detectors. It is important to consider that for analyses that have a high background of non-volatile residue the overall dynamic range will be reduced and the linearity can be affected. Figure 4 shows an example of hydrocortisone linearity and dynamic range for the NQAD™ [10].

Sensitivity

The most profound feature of the NQAD™ is its ultra-sensitivity.

Figures 5 and 6 show an example of the sensitivity differences between the NQAD™, UV detector and charged aerosol detector for different applications [10,11].

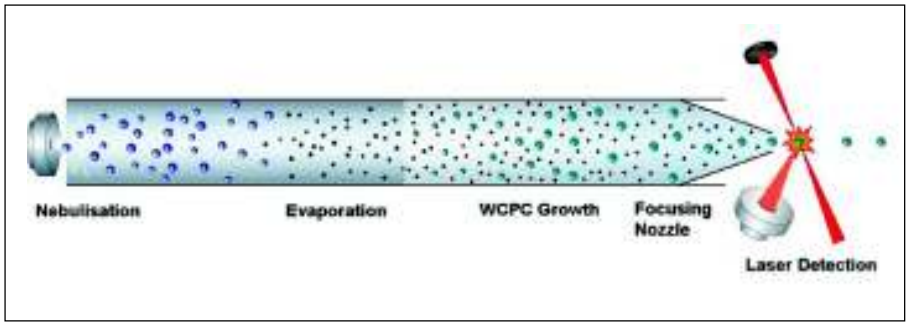

Figure 2 : Schematic of NQAD™ Operation

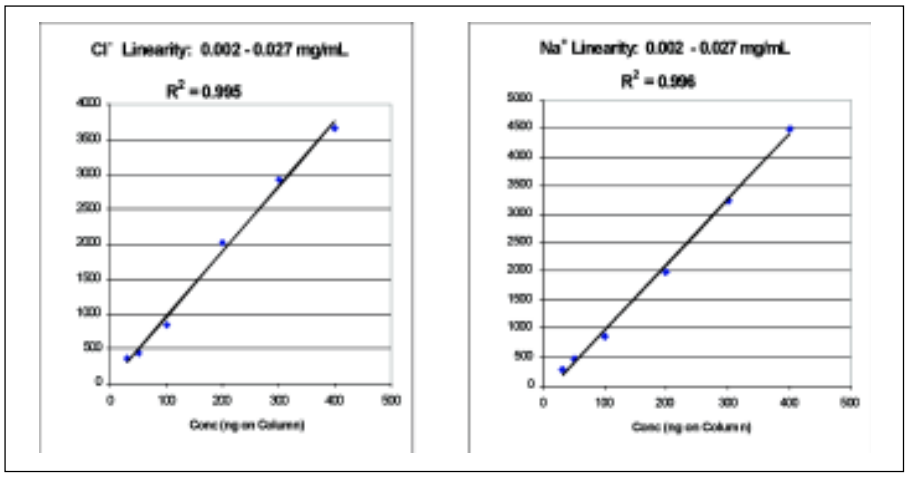

Figure 3 : Linearity of NQAD™ Response to Anions and Cations

HPLC System: HP 1100 (Agilent Technologies, Palo Alto, CA, USA)
Column: ZIC®-HILIC, 5µm, 4.6 x 150 mm (Merck SeQuant AB, Umea, Sweden)
Mobile Phase A= 85% Acetonitrile: 15% 0.05mM NH4Ac
B= 10% Acetonitrile: 90% 0.05mM NH4Ac

Time (min)	A	B
0	85	15
8	0	100
8.1	85	15
14	85	15

Flow rate: 1.4 mL/min
Detector NQAD™: Evaporator temp. 38°C, Gain 1X, Filter 5 Sec

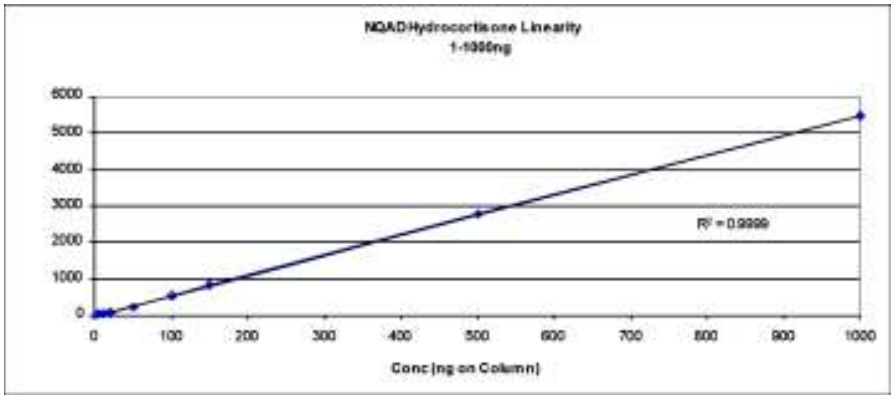

Figure 4 : Hydrocortisone Linearity with NQAD™
HPLC System: HP 1100 (Agilent Technologies, Palo Alto, CA, USA)
Column: Gemini® 5µm C18 110Å, 150 x 4.6 mm, (Phenomenex, Torrance, CA, USA)
Mobile Phase: 55% Acetonitrile: 45% Water
Flow rate: 1.0 mL/min
Detector: NQAD™ - Evaporator Temp: 60°C, Gain: 1X, Filter: 5 sec

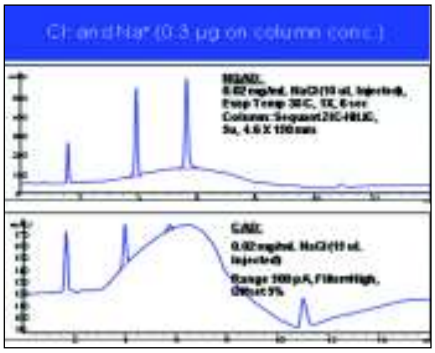

Figure 5 : Anions & Cations by NQAD™ and CAD™ Operating conditions as for Figure 3 above (CAD™ Range 500pA, Filter High, Offset 5%)

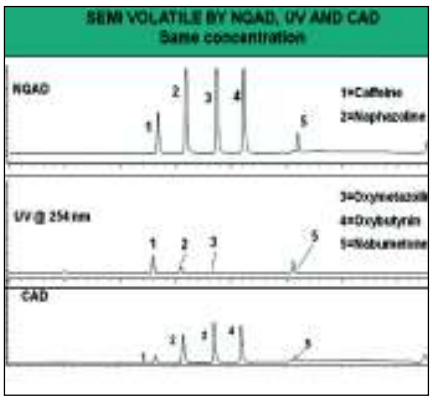

Figure 6 : Semi- Volatiles by NQAD™, UV and CAD™

Fig 6 - Semi-Volatiles Operating Conditions:

HPLC System: HP 1100 (Agilent Technologies, Palo Alto, CA, USA)
Column: Alltima™ C18, 3µm, 100 x 4.6 mm (Grace Davison Discovery Sciences, Columbia, USA)
Mobile Phase:

A= 0.05% TFA in Water, B=0.05% ACN

Time (min)	A	B
0.0	5	95
5.0	95	5
6.0	95	5
6.1	5	95
8.5	5	95

Flow rate: 1.4 mL/min
NQAD™:
Evaporator Temp: 35°C, Gain: 1X, Filter: 5 sec
CAD™:
Range 500 pA, Filter=High, Offset 5%
UV: 254nm

Conclusions

The NQAD™ is a new HPLC detector demonstrating improved sensitivity and is well suited for analyzing non-volatile and semi-volatile analytes. Since it only relies upon the analyte being less volatile than the mobile phase it is applicable to a very wide range of sample types and components.

Compared to other aerosol-based detectors, the NQAD™ shows less baseline drift and background detector noise whilst offering superior sensitivity for most analytes.

Whilst the excellent sensitivity of the detector is one of its main advantages it places more rigorous demands on the analyst to ensure that their system, solvents and operating procedures are of a high standard.

Queries about this article should be directed to David Crowshaw *(david.crowshaw@grace.com)*

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