Effect of Mobile Phase Quality on Analytical Performance of Corona Charged Aerosol Detectors

Marc Plante, Bruce Bailey, Felicity Kusinitz, and Ian Acworth
Thermo Fisher Scientific, Chelmsford, MA, USA

Technical Note 159

Key Words
Corona Charged Aerosol Detection, HPLC, Mobile Phase, Sensitivity, Signal-to-noise, Background

Goals
To demonstrate the effects of mobile phase quality on analytical performance of the Thermo Scientific™ Dionex™ Corona™ Charged Aerosol Detector

Introduction
For any analytical technique, the quality of the measurement depends upon the conditions used to make the measurement so it is quite important to properly optimize the method prior to performing any analysis. For high performance liquid chromatographic (HPLC) experiments, measurement quality for a specific detector can be optimized by following detector guidelines provided by the instrument manufacturer. When the background signal for the mobile phase is elevated it can significantly influence the quality of subsequent analytical results. Thus a mobile phase that is contaminated can lead to poor sensitivity, ghost peaks and shoulders, reduced column lifetime, and cause peak distortions.

The most common HPLC detectors are spectrophotometric detectors, however they are not universal; they require that the analyte possesses either a chromophore for ultraviolet (UV) absorbance detection or when using fluorescence detection (FLD), compounds must possess native fluorescence or be derivatized. One requirement when using mass spectrometry (MS) is that compounds must ionize prior to their detection. When compounds lack a chromophore or do not ionize, aerosol-based detection techniques, such as evaporative light scattering (ELS) or the more sensitive charged aerosol detection (CAD) are typically chosen, depending on required performance attributes such as sensitivity, reproducibility, dynamic range, and response uniformity.

Detector optimization is used to enhance the signal-to-noise ratio: the greater the difference the greater the sensitivity. For example, with UV absorbance detection, any solvent that shows significant absorbance at the analyte detection wavelength cannot be used as it increases the noise and decreases the signal-to-noise ratio.

Unlike UV or FLD spectroscopic detectors, the response of aerosol-based detectors depends upon the formation of analyte particles. If the mobile phase is contaminated with more than trace levels of particulates or dissolved, non-volatile material, this can lead to high background levels, high noise, and low sensitivity.

Thermo SCIENTIFIC

Mobile Phase Optimization for Charged Aerosol Detection

Solvents and buffers recommended for use with CAD must be volatile and clean. Since CAD uses analyte particles for detection, solvents with the least amount of non-volatile components (dissolved metals and ions, detergents, anti-polymerizating agents, undissolved residue, etc.) will provide improved chromatography and the best sensitivity. The majority of organic solvents have a specification termed "residue after evaporation," and LC/MS grade solvents and buffers have the lowest values and these are recommended for use with CAD. Freshly prepared deionized water, with a resistivity of 18.2 MΩ·cm and low total organic carbon levels, is preferred. Bottled HPLC Grade water, however, is not recommended due to the presence of leached ions from glass bottles and the potential for biological growth contamination. Storage of deionized water for later use is not recommended, as higher backgrounds can occur due to accumulated biological matter.

Filtration of the mobile phase is also not recommended, as particulates from the filtration media can contaminate the mobile phase. This is manifested an increased detector background.

Experimental Conditions

HPLC System:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC, DGP-3600RS pump, WPS-3000RS autosampler, TCC-3200 column compartment
Column:	Thermo Scientific™ Accucore™ C8, 2.6 µm, 2.1 × 150 mm
Column Temp:	40 °C
Flow Rate:	0.8 mL/min
Detector:	Thermo Scientific™ Dionex™ Corona™ Veo™ SD
Filter:	5.0 s
Evaporation Temp:	High
Data Rate:	10 Hz
Power Function:	1.00
Mobile Phase A:	Deionized Water, 0.1 v/v-% trifluoroacetic acid (TFA)*
Mobile Phase B:	Acetonitrile, 0.1 v/v-% TFA*

**Two mobile phases were prepared using the same composition, but one with TFA contaminated with polymer from the container cap[1], and another with fresh TFA.*

Gradient:	Time (min.)	%A	%B
	-3.0	70	30
	0.0	70	30
	4.0	40	60
	4.5	10	90
	7.5	10	90
	8.0	70	30

Results and Discussion

Two experiments were conducted: the first using a mobile phase that was prepared using a darkened TFA additive that had been contaminated with dissolved polymer (presumably from the cap used on the bottle),[1] and the second using a mobile phase that was prepared using clean TFA. The contaminated mobile phase used in one experiment contained non-volatile, dissolved material which contributed to elevated detector background. This contamination of the mobile phase additive can be likened to the use of a mobile phase component (aqueous or organic) that contains elevated amounts of dissolved solids or particulates.

In each experiment, injections of 2840 nanograms on column (ng o.c.) of an analyte (a glycolipid) was analyzed in triplicate, followed by amounts of 1420, 710, 355, 178, 88.8, and 44.4 ng o.c. that were analyzed until Limits of Quantitation (LOQ) and Detection (LOD) could be determined. Other metrics such as background currents and noise levels were also recorded. Generally, a mobile phase that provides a background current of <3 pA on a Corona Veo charged aerosol detector is considered suitable for use. Note that ideally background currents of <1 pA are possible with this mobile phase composition. For this specific mobile phase composition used in this evaluation, the typical peak-to-peak noise values were <40 fA.

In the first experiment, the baseline current for the Corona Veo SD charged aerosol detector was approximately 20 pA, which is considered very high for any experiment requiring sensitivity. The peak to peak background noise was approximately 600 fA, again a high value for this mobile phase composition. In the second experiment, both mobile phase solutions were prepared using freshly obtained TFA, and the analysis was repeated under identical conditions. With fresh TFA, the baseline current dropped to <2 pA, and the baseline noise decreased to 36 fA.

The analysis of analyte standards at 2840 ng o.c. from each experiment is shown in Figure 1. The blue trace illustrates the lack of sensitivity and baseline artifacts due to poor mobile phase quality. The black trace was obtained using the better quality mobile phase and this illustrates the vastly improved sensitivity and lack of artifacts. As can be seen from Figure 1B, the chromatogram (black trace) for the 2840 ng o.c. analysis has a typical, low-noise baseline, the analyte peak is much better defined (both in height and in shape), and the large peaks at the end of the gradient elution are mostly gone. Interestingly, other analytes can be seen near the main analyte peak, demonstrating greater sensitivity: these were not visible in the chromatograms produced in the first experiment (Figure 1A, blue trace).

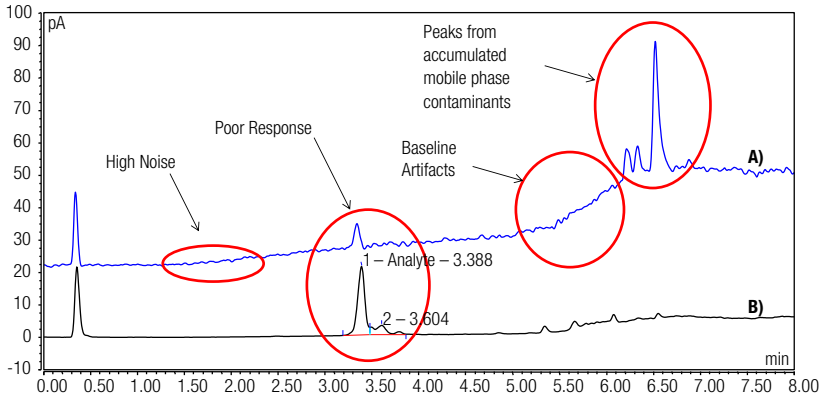

Figure 1. HPLC-CAD analysis of an analyte (2840 ng o.c. A: blue trace using poor quality phase, B: black trace using good quality phase).

Calibration plots for each experiment are shown in Figures 2A and 2B. The data points shown in triplicate were plotted on inverted axes and fit to a second-order polynomial. The calibration plot obtained using poor quality mobile phase ingredients was characterized by poor correlation and poor precision, as shown in Figure 2A since the correlation coefficient value, r^2, had a value of 0.951. Precision for peak area data was also poor with the percent relative standard deviation (%RSD) of 11% at the 2840 ng o.c. amount. The peak area imprecision for replicate injections resulted from baseline artifacts interfering with the analyte peak. In the second experiment, using the better quality mobile phase, the same standard solutions, ranging from 2840 to 44.4 ng o.c., n = 3, were used for the calibration curve shown in Figure 2B. In this case calibration analysis resulted in an improved correlation with r^2 = 0.9999. Precision for triplicate injections also improved with peak area %RSD values ranging from the lowest at 0.34 (1420 ng o.c.) to the highest value of 5.39 (88.8 ng o.c.).

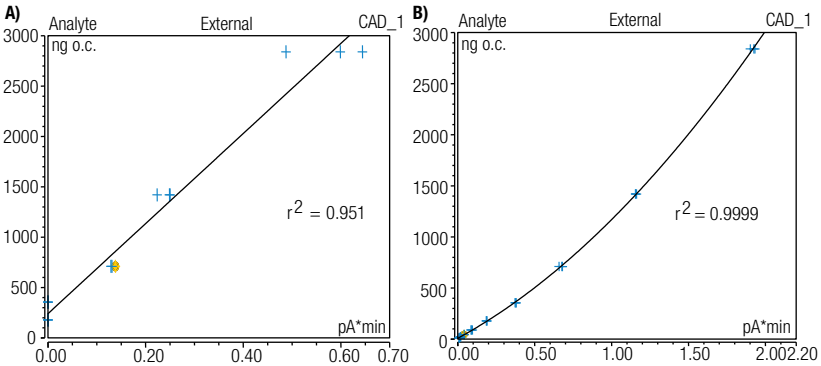

Figure 2. Calibration plots of the analyte standards. 2A) using poor-quality mobile phase, (2840 to 710 ng, in triplicate and data fitted to a linear equation on inverted axes) and 2B) using good quality mobile phase (2840 to 44.4 ng, in triplicate and data fitted to a polynomial equation on inverted axes).

Metrics using International Conference on Harmonization (ICH) guidelines were calculated for limits of detection (LOD: SNR = 3.0) and limits for quantitation (LOQ: SNR = 10). Experiments using poor quality mobile phase are shown Figure 3A (blue trace). The average SNR value for the 710 ng o.c. standard was 4.6, indicating that the LOD value is 465 ng o.c., while the LOQ value was calculated to be 1540 ng o.c. Experiments using better quality mobile phase, the average SNR value for the 710 ng o.c. standard was 350, or an improvement of 76-fold. The lowest amount analyzed was 44.4 ng o.c. (Figure 3B – black trace). The average SNR value (n = 3) was 31.2, reflecting much improved sensitivity when clean mobile phase is used. The LOQ and LOD values were determined to be 15 and 5 ng o.c., respectively, or a sensitivity improvement of over 90-fold compared to that determined using the poor-quality mobile phase.

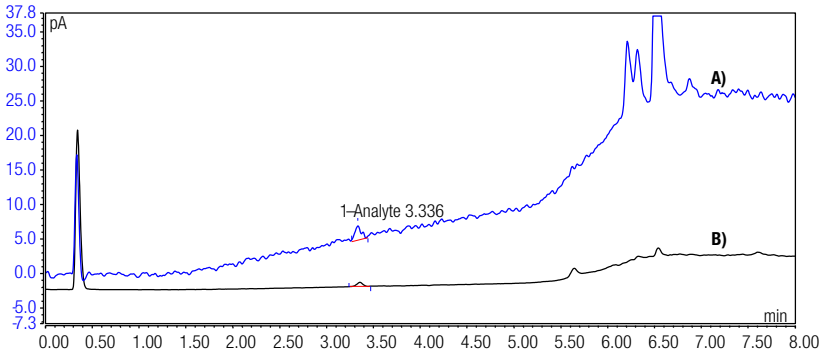

Figure 3. HPLC-CAD analysis of analyte standards; blue trace represents 710 ng o.c. using poor quality mobile phase and the black trace represents 44.4 ng o.c. using good-quality mobile phase, showing improved chromatographic performance.

Table 1: Metrics indicating important performance criteria based on mobile phase quality.

Metric	Poor Mobile Phase Quality	Good Mobile Phase Quality
Detector Background Currents	20 pA	2 pA
Detector Noise	600 fA	36 fA
Assay LOQ (S/N = 10)	1540 ng o.c.	15 ng o.c.
Assay LOD (S/N = 3)	465 ng o.c.	5 ng o.c.
Average (n = 3) SNR for 710 ng o.c.	4.6	350
R^2 Value for Calibration Curve	0.9507	0.9999
Precision (%RSD) Range	6.2–11%	0.34–5.39%

Comparative metrics are summarized and presented in Table 1. Such data can help the user to determine whether the instrument conditions, mobile phase preparation, and detector performance has been properly optimized prior to the start of an experiment.

Conclusions

Significant improvements in data quality can be achieved when mobile phase preparation has been optimized (see Table 1). For high quality analytical results, attention to the quality of solvents used in mobile phase preparation is essential. In particular, the amount of particulates, typically measured as residue after evaporation for organic solvents must be minimal. LC/MS grade solvents and volatile buffers with minimal residue are preferred. For water, only freshly prepared 18.2 MΩ·cm deionized water should be used.

Another factor that is important is the glassware that is used to prepare the mobile phases, including graduated cylinders, bottles, volumetric flasks, etc. Detergent residue and poorly rinsed glassware exposed to buffer salts can easily introduce non-volatile residues into a mobile phase preparation. For washing glassware, it is recommended to eliminate automatic dishwashers, and to simply rinse the glassware with DI water (or isopropanol for normal phase) after use.

For pH-buffered mobile phases, the thorough rinsing of the pH probe eliminates the potential for contamination of the mobile phase with non-volatile probe storage buffer salts. If mobile phases are prepared with clean glassware, good quality solvents and buffer salts, then there is no need for filtration, which can add some material to the mobile phase.

With a high quality mobile phase, improved Corona charged aerosol detector performance can be expected. If high backgrounds are seen, check that:

- The solvents are appropriate for the detector (for CAD, same solvents used in MS are appropriate)
- The solvents are of the best quality (lowest particulates available)
- The buffers are volatile and of the best quality (lowest particulates/metals)
- The glassware used in the preparation and storage is properly clean

References

1. Email conversation with Ron Epstein (Sales Director), Halocarbon Products Corporation (River Edge, NJ, USA)

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Brazil +55 11 3731 5140
Canada +1 800 530 8447
China 800 810 5118 (free call domestic) 400 650 5118
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland +358 9 3291 0200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591
Japan +81 6 6885 1213
Korea +82 2 3420 8600
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Norway +46 8 556 468 00
Russia/CIS +43 1 333 50 34 0
Singapore +65 6289 1190
Sweden +46 8 556 468 00
Switzerland +41 61 716 77 00
Taiwan +886 2 8751 6655
UK/Ireland +44 1442 233555
USA +1 800 532 4752

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

TN71390-EN 0716M