Guidelines for Method Transfer and Optimization of the Corona Veo Charged Aerosol Detector

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Overview

Purpose: To exhibit the process[1] of transferring an HPLC method from a Corona ultra RS charged aerosol detector (CAD) to a Corona Veo RS detector.

Methods: An optimized HPLC method for theophylline and caffeine was created and optimized on the Corona ultra RS and transferred to the Corona Veo RS charged aerosol detector. A second method, using alkaline mobile phase, was optimized for the Corona Veo RS detector.

Results: The optimum detector settings for the Corona Veo RS detector, for the conditions used with the Corona ultra RS CAD method, were an evaporation temperature of 30 °C, and a filter setting of 3.6 seconds..

Introduction

No single liquid chromatography (LC) detector delivers ideal results. Often one analyte responds more strongly than another, or may not respond at all. What is most desired is the ability to accurately measure a wide range of analytes with consistent response, simultaneously. Charged aerosol detection is a mass sensitive technique for determining levels of any non-volatile and many semi-volatile analytes after separation by liquid chromatography. This technique provides consistent analyte response independent of chemical characteristics, and gives greater sensitivity as well as having a wider dynamic range than other nebulizer-based detectors. The response to an analyte does not depend on optical properties, as with UV-vis absorbance, or the ability to ionize, as with mass spectrometry (MS). There is no need for the presence of chromophores, radiolabels, ionizable moieties, or chemical derivatization for detection. HPLC and UHPLC methods using CAD have limits of detection of between mid-picograms to low nanograms on column (ng o.c.) and have a wide dynamic range from nanogram to microgram levels, with high reproducibility.

Since the introduction of this technology in 2004, the charged aerosol detector is now a mature, fourth-generation product. The latest product, the Thermo Scientific™ Dionex™ Corona™ Veo™ shows a number of improvements over its predecessors. A new concentric nebulizer design forms a stable aerosol, which increases both assay reproducibility and sensitivity. The use of evaporation temperature enables an expanded range of mobile phase constituents to be used, including basic mobile phases in which carbonate accumulation in the mobile phase contributes to elevated detector background noise. A linearity function can also be used for linear calibration curves. Comparative data is presented and certain guidelines for method transfer from previous generation products are highlighted. Important parameters that require consideration during method transfer include evaporation temperature, filter time constant and mobile phase quality.

To demonstrate method transfer between earlier version of CAD to the current Corona Veo RS detector, a reversed phase, isocratic HPLC method for nonvolatile theophylline and the semi-volatile caffeine is transferred from a Corona ultra RS to the Corona Veo RS, with parameters on the Veo RS optimized for detection performance. A second method, using an alkaline mobile phase is also optimized using evaporation temperature and data filter settings.

Methods

Sample Preparation– Caffeine and Theophylline

Caffeine and theophylline were dissolved in 20% methanol at 1 mg/mL stock concentration separately. In an HPLC vial, 60 µL of caffeine and 20 µL of theophylline solutions were added and diluted with 920 µL of 20% methanol. A 50 µL aliquot was then diluted with 950 µL of 20% methanol for a final concentration of 3 µg /mL of caffeine and 1 µg/mL of theophylline.

Sample Preparation– Erythromycin

Erythromycin was dissolved at a concentration of 1000 µg/mL in 20% methanol and then diluted to 200 µg / mL in 20% methanol. Solutions were prepared by serial dilution in an HPLC vial to a concentration from 200 µg/mL, with subsequent 50% dilutions to a final concentration of 0.78 µg/mL.

Liquid Chromatography using the UltiMate 3000RS UHPLC system including:

Thermo Scientific™ Dionex™ UltiMate™ 3000 DGP-3600SD, WPS-3000RS autosampler, and TCC-3000RS column oven, Corona Veo RS Charged Aerosol Detector, Corona ultra RS Charged Aerosol Detector

Liquid Chromatography conditions- Caffeine and Theophylline

Column:	Hypersil Gold C18 column, 2.6 µm, 2.1 x 100 mm
Eluent A:	Water
Eluent B:	Acetonitrile
Flow Rate:	0.5 mL/min at 10% B
Sampler Temperature:	20 °C
Injection volume:	2.00 µL
Column Temperature:	30 °C
Data Rate:	20 Hz for both detectors
Detector 1:	Corona ultra RS Charged Aerosol Detector
Filter Constant:	variable, optimized #5 (5.9 s)
Nebulizer Temp.:	20 °C
Detector 2:	Corona Veo RS Charged Aerosol Detector
Filter Constant:	variable, optimized 3.6 s
Evaporator Temp.:	variable, optimized 30 °C

Liquid Chromatography conditions- Erythromycin

Column:	Polymer-encapsulated C18, 5.0 µm, 4.6 x 150 mm
Eluent A:	10 mM Ammonium carbonate, pH 9.0
Eluent B:	Acetonitrile
Flow Rate:	0.8 mL/min at 70 %B
Sampler Temperature:	20 °C
Injection volume:	2.00 µL
Column Temperature:	30 °C
Data Rate:	10 Hz for both detectors
Detector 1:	Corona ultra RS Charged Aerosol Detector
Filter Constant:	#6 (10.1 s)
Nebulizer Temp.:	25 °C
Detector 2:	Corona Veo RS Charged Aerosol Detector
Filter Constant:	variable, optimized 10 s
Evaporator Temp.:	variable, optimized 75 °C

Data Analysis
Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, 7.2 SR2

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Results

A method for low-level determination of theophylline and caffeine on the Corona ultra RS charged aerosol detector (specified above) was transferred and optimized on the Corona Veo RS detector.

For the Corona Veo, two main parameters that influence detector sensitivity performance are evaporation temperature and the data filter setting. The first analyte, theophylline, is a non-volatile compound whereas the second analyte, caffeine, is a semi-volatile compound and should exhibit different temperature dynamics compared to theophylline. These two parameters must be optimized from previous Corona detectors for optimal performance from Corona Veo.

Corona Veo RS: Temperature optimization

Analyses, starting with the evaporation temperature set at 30 °C, were made with an initial filter setting of 1 s. The temperature was then increased in two-degree increments, with five injections made at each temperature setting. As expected, the response of the semi-volatile compound caffeine decreased more rapidly than for the non-volatile theophylline with increasing temperature.

Both components exhibited decreasing response with increased temperature throughout the temperature range, with caffeine expectantly decreasing at a faster rate, but the noise also decreased faster than the signal in some regions (32 – 36 °C).

A bar graph showing the SNR values was made for both components at each temperature setting, as shown in Figure 1. In cases with less volatile analytes, an increase in temperature will decrease the noise more rapidly than the analyte signal, increasing the SNR. From the graph generated, a temperature setting of 30 °C is the ideal temperature for optimal conditions for the analysis of both of these analytes.

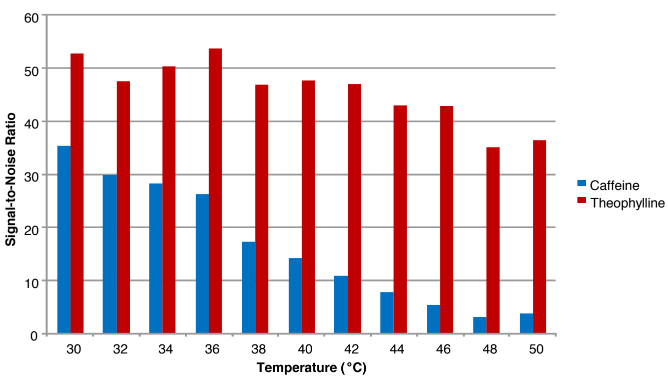

FIGURE 1. Graph of Corona Veo RS signal-to-noise ratios for caffeine and theophylline vs. evaporation temperature.

Corona Veo RS: Filter optimization

With the evaporation temperature of the Corona Veo optimized at 30 °C, the detector's filter setting was then evaluated through the eight different settings available for this instrument. As in the previous study of the Corona ultra RS, five injections were made for each filter setting. The average SNR values were then calculated and plotted as shown in Figure 2. The ideal filter setting for the Corona Veo was determined to be 3.6 s.

Comparison of the optimal chromatograms of theopylline and caffeine for both the Corona ultra RS and Veo RS detectors is illustrated in Figure 3. The sensitivity limits were calculated, based on the (International Conference on Harmonisation)ICH-defined SNR values for limits of detection (LOD) and quantitation (LOQ) of 3 and 10, respectively. LOD and LOQ values for caffeine and theophylline for both detectors are provided in Table 1. Between the two instruments, the Veo RS was shown to be approximately 5-fold more sensitive than the Corona ultra RS, both with optimized conditions for these two analytes.

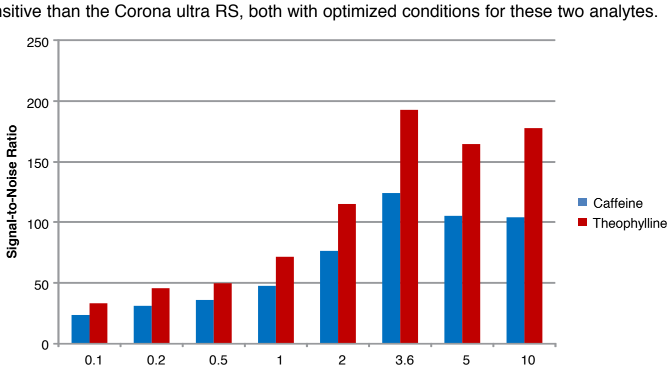

FIGURE 2. Graph of Corona Veo RS average (n=5) signal-to-noise ratios with different filter settings and evaporation temperature of 30 °C.

Caffeine and Theophylline: Corona ultra RS results

For the Corona ultra RS detector, the parameter that influences the signal to noise data the most is the filter setting. Therefore, caffeine and theophylline were first analyzed using the Corona ultra RS charged aerosol detector, with levels of caffeine at 6 ng on column (o.c.) and theophylline at 2 ng o.c. Increasing the filter value contributed to peak widening and signal attenuation, but it also decreased detector noise more aggressively, which provides a better SNR for sensitivity. A filter setting of #5 (5.9 s) on the Corona ultra RS proved to be the optimum value for SNR. For each filter setting there were five injections made to provide replicate data.

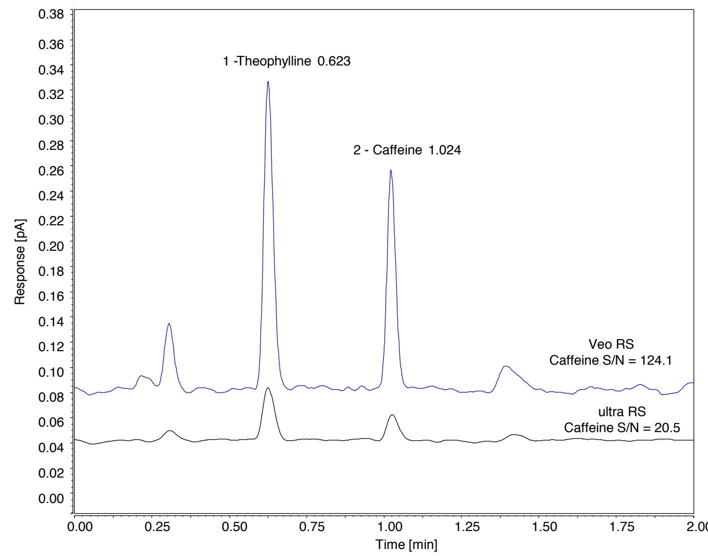

FIGURE 3. Overlaid, HPLC chromatograms of caffeine and theophylline with two detectors, Corona ultra RS and Corona Veo RS (at 30 °C evaporation temperature), with optimized conditions.

Table 1. Sensitivity limits for caffeine and theophylline with optimized parameters for Corona ultra RS and Corona Veo RS detectors.

Sensitivity Limit	Theophylline		Caffeine	
	ultra RS	Veo RS	ultra RS	Veo RS
LOD (ng o.c.)	0.14	0.03	0.88	0.15
LOQ (ng o.c.)	0.48	0.10	2.95	0.48

Another parameter, the power function (available on both instruments), was used at the default setting of 1.00. This parameter is used to produce linear calibration results from the detector, when it is required (by Quality Assurance or for complex analyte peaks such as polymers). Both analytes for these experiments produce nearly linear calibration curves (not shown) with the default power function at 1.00, so there was no need for further optimization. This parameter does not influence sensitivity, and the default power function of 1.00 will provide equivalent calibration properties.

High pH Mobile Phase: Erythromycin

With earlier versions of the Corona charged aerosol detectors, the use of high-pH mobile phases required the use of a post-column acidification of the column eluent to decrease the background noise caused by non-volatile carbonate accumulation in the mobile phase.

The Corona Veo RS charged aerosol detector can reduce this background by using elevated evaporation temperatures. Following a similar optimization process, as performed above, the evaporation temperature and data filter setting were optimized. A plot of the SNR for erythromycin, at 40 ng o.c., over the temperature range of 50 – 90 °C is shown in Figure 4. From the plot, the SNR improved from 25 to approximately 37 from increasing the evaporation temperature from 50 °C to 75 °C. Further increases resulted in more loss in analyte signal than in background noise.

With the evaporation temperature optimized at 75 °C, the filter setting was then optimized, with three comparative chromatograms shown in Figure 5. The chromatogram using a filter setting of 10 s provided the best SNR value of 68.

With the optimization of both the evaporation temperature and the data filter value, the sensitivity of the method increased by more than 2.5-times, and this method did not require any post-column addition of acid, simplifying method operation.

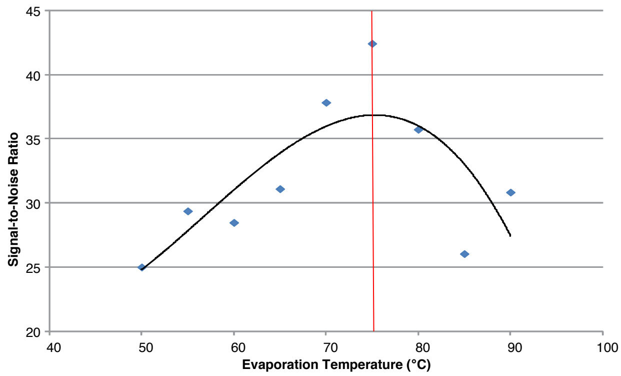

Figure 4. Average SNR values (n=3) for erythromycin with Corona Veo RS (filter at 3.6 s) evaporation temperature values, fit to a third-order polynomial

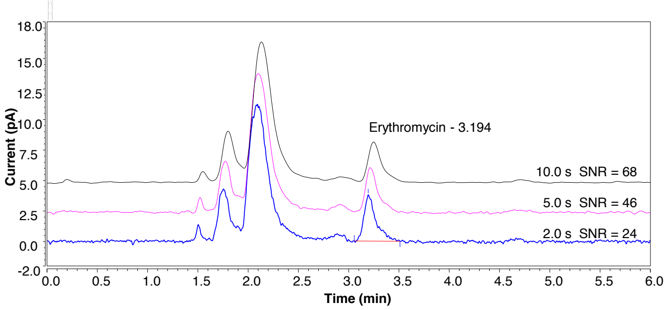

Figure 5. Overlaid HPLC-CAD chromatograms of 40 ng o.c. erythromycin at ET 75 °C with filter values of 2.0, 5.0, and 10.0 s applied.

Conclusions

An HPLC method using the Corona ultra RS detector was transferred to one using the Corona Veo RS detector. Effects of evaporation temperature and filter setting were evaluated.

- The Corona Veo detectors should have at least the evaporation temperature and filter setting optimized, using near-LOQ amounts of analytes with focus on SNR for the evaluation of detector performance.
- Both Corona Veo parameters of temperature and filter setting should be optimized for each chromatographic condition since buffers, flow rates, analytes, organic solvents, and solvent quality are all important factors that can affect detector performance.
- Sensitivity for erythromycin was improved by at least 2.5-fold when the filter setting was optimized for this experiment. Also the evaporation temperature was studied, often further improvements will be exemplified.
- The evaporation temperature of the Corona Veo RS adds an additional optimization parameter and the flexibility of using high-pH mobile phases to universal detection.

References

1. Technical Note 157, “Guidelines for Method Transfer and Optimization – From Earlier Model Corona Detectors (i.e., Corona CAD, CAD Plus, ultra, ultra RS) to Corona Veo Detectors” http://www.thermoscientific.com/en/product/dionex-corona-veo-rs-charged-aerosol-detector.html#sthash.XYXOTBge.dpuf (accessed 03 June 2015)

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