Charged Aerosol Detection and Evaporative Light Scattering Detection – Fundamental Differences Affecting Analytical Performance

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Overview

Purpose: To evaluate and compare the analytical performance of charged aerosol and evaporative light scattering detection.

Methods: Several different isocratic and gradient HPLC methods were used to evaluate the two detectors.

Results: Charged aerosol detection (CAD) had lower limits of detection, a wider dynamic range, less inter-analyte response variability, and better precision than evaporative light scattering detection (ELSD).

Introduction

At a fundamental level, both CAD and ELSD share some similarities in that mobile phase exiting the column is first nebulized and then dried to form analyte particles. However, the mechanism by which these techniques measure analyte mass differ markedly and this has major impact on analytical performance. In CAD, charged particles are measured by an electrometer generating a signal that is proportional to particle size (i.e., the mass of analyte). For ELSD, signal is also proportional to particle size, but this relationship is much more complex, as the magnitude of scattered light varies depending on particle size, resulting in sigmoidal response curves. Unlike CAD, ELSD uses non-contiguous signal attenuation. As each attenuation setting has its own unique sensitivity, response, calibration curve and dynamic range, samples may have to be reanalyzed multiple times in order to quantify analytes occurring at different levels. In this poster the analytical performance of CAD and ELSD are evaluated and include: sensitivity, dynamic range, inter-analyte response, linearity, reproducibility and the effects of mobile phase flow rate.

Methods

Liquid Chromatography

Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system with:

- Thermo Scientific™ Dionex™ Corona™ Veo Charged Aerosol Detector
- Sedex 90LT™ Evaporative Light Scattering Detector

Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) 7.2

Reagents: Reagent-grade or better

Linear and Dynamic Range:

Column:	Thermo Scientific™ Acclaim™ 120 C18, 3 µm, 3.0 × 50 mm
Column Temp:	25°C
Flow Rate:	1.0 mL/min
Injection Vol.:	10 µL
Mobile Phase A:	water
Mobile Phase B:	methanol, Optima™ LCMS
Gradient:	Isocratic, 20%B
CAD:	35°C; PF 1.0; 2Hz; 3.6s
ELSD:	3.5 bar; 35°C; 10Hz; 4s; Gain 2 -12
Stock standards:	Theophylline and caffeine, 0.1 mg/mL in mobile phase, diluted as necessary in mobile phase

Inter-analyte Response:

Column:	Acclaim 300 C18, 3 µm, 4.6 × 150 mm
Column Temp:	30°C
Flow Rate:	0.8 mL/min
Injection Vol.:	2 µL
Mobile Phase A:	20 mM ammonium acetate pH 4.5
Mobile Phase B:	acetonitrile, Optima LCMS
Gradient Time, %B:	0.1 min, 2; 15.1, 98; 16,2; 22, 2. Inverse Gradient employed.
CAD:	35°C; PF 1.0; 2Hz; 3.6s
ELSD:	3.5 bar; 35°C; 10Hz; 4s; G12
Stock standard:	Phenylalanine, theophylline, propranolol HCl, naproxen sodium, diclofenac sodium and progesterone, 100 mg/L each as API in 50:49:1 (v/v) water:acetonitrile:2-propanol; diluted as necessary in 50:50 (v/v) water:acetonitrile

Results

1. Evaporative Light Scattering Detection And Charged Aerosol Detection Response Characteristics

The signal (S) obtained within the working range of an HPLC detector can normally be related to the analyte amount (M) by the following relationship:

$$(1) \qquad S = aM^b$$

Where the coefficient "a" represents the response intensity and the exponent "b" represents the shape of the response curve. When "b" = 1.00, the response is linear and the coefficient "a" is the slope of the line, which is often referred to as the response factor (e.g., related to molar absorbtivity with a UV detector). When "b" $\neq$ 1 the detector response is non-linear. In this case, the response factor can be thought of as changing as a function of analyte amount.

The response for LC-aerosol detectors is typically observed to be non-linear (i.e., b $\neq$1). The value of b results primarily from two processes. The first is related to nebulization-evaporation, which is common to all LC-aerosol detectors. The second is related to the particular dried aerosol detection technique (i.e., light scattering, aerosol charge). The relationship that describes the nebulization-evaporation process is given by:

$$(2) \qquad D = D_0(C/\rho)^{1/3}$$

Where D is the dried aerosol particle diameter, C is solute concentration in the initial wet aerosol droplet of diameter D_0 and ρ is the solute density. The exponent (1/3) describes a non-linear 'cube root' relationship that is common to all LC-aerosol detectors. This exponent is further modified by the dried aerosol detection process to give the value of b in equation 1 above.

In the case of ELSD, the dried aerosol detection process involves changes between 3 different light scattering domains as particle size changes. The scattered light intensity (Q) is dependent upon the particle diameter (D) and the light source wavelength (λ):

$$(3) \qquad Q=f(D/\lambda)$$

The three scattering domains, their proportionality to D and the resultant exponents "b" from equation 1 (i.e., including the 1/3 proportionality from nebulization) are:

- Rayleigh: If (D/λ) < 0.1, scattered light is proportional to D^6 and b=6/3
- Mie: If 0.1 < (D/λ) < 1.0, scattered light is proportional to D^4 and b=4/3
- Refraction and reflection: If (D/λ) > 1.0 scattered light is proportional to D^2 and b=2/3

Since detection efficiency changes between light scattering domains, the value of b for an ELSD actually changes quite dramatically (i.e., from 6/3 to 2/3) over a fairly small dynamic range. ELSD response curves are therefore typically quite complex and often sigmoidal as shown in Figure 1.

A major consequence of ELSD sigmoidal response is that the dynamic range is small and analyte signal rapidly decreases and completely disappears as the amount of analyte decreases.

CAD is also non-linear and shows a parabolic response curve (Figure 2). As with ELSD, the shape of the response curve is partly a function of the 1/3 proportionality described above for the nebulization-evaporation process. In the case of CAD, dried aerosol detection is based on measurement of charge acquired through diffusional processes as a function of particle size. With CAD, the mean charge per particle has been shown to be nearly linear ($D^{1.1}$) with dried particle diameter (D) over a wide range of D (10 - 1000nm) and with a higher exponent for D <10nm. Importantly, the relationship between particle diameter and measured charge for CAD is much simpler than that of light scattering for ELSD. As a result, the value of b is relatively constant throughout the working range of the detector and is typically observed to be ca 2/3 for a wide range of conditions and analytes.

Unlike ELSD, CAD response does not simply disappear for the same lower levels of analytes. Subsequently charged aerosol detection performs better for measurement of lower analyte levels and is generally more sensitive and provides a wider dynamic range than ELSD.

FIGURE 1. Typical ELSD sigmoidal response curve.

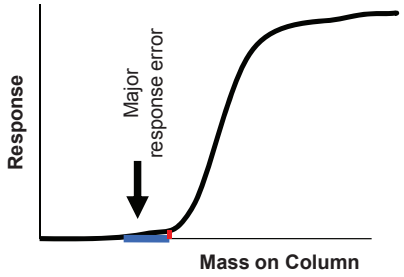
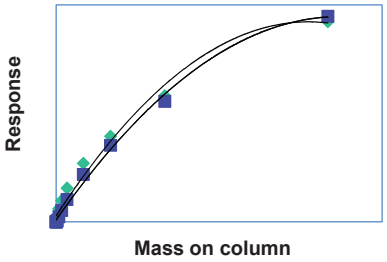


FIGURE 2. Typical CAD parabolic response curve.



2. Estimation Of LOD Or LOQ From Single High Level Calibrant

While it is common practice to estimate the limits of detection (LOD) or limits of quantification (LOQ) for a linear detector by extrapolation of signal to noise ratio from a single high level standard, the same cannot be done for non-linear detectors and any estimates derived this way are totally meaningless and misleading. For example, Figure 3 shows the response of a high level standard of caffeine (semi-volatile) and theophylline (non-volatile) by ELSD and CAD. Figure 4 shows the response of the two detectors for lower level standards. Table 1 shows the extrapolated LOD obtained if the detectors were assumed to be linear. Table 1 also shows the estimated LOD for the two detectors derived from running a calibration curve. As can be seen the estimated LODs are very different from the extrapolated values assuming linear performance.

The only way to estimate the LOD when response is non-linear is to construct a calibration curve. Often the response of the detector to a high concentration of standard is used to imply that the performance of one detector is superior to the other. Such a comparison is completely meaningless.

FIGURE 3. Analysis of higher level standards (62 ng o.c.) by ELSD and CAD.

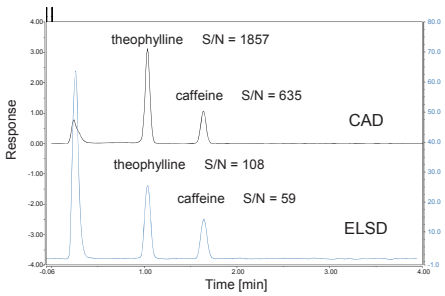


FIGURE 4. Analysis of lower level standards (8 ng o.c.) by ELSD and CAD.

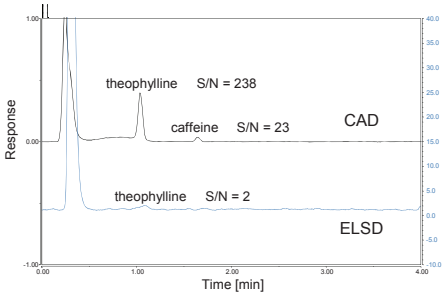


Table 1: Extrapolated LODs (Assuming Linear Response) Vs. Estimated LODs (Derived From Calibration Curves) For CAD And ELSD.

	ELSD		CAD	
	Theophylline	Caffeine	Theophylline	Caffeine
*LOD by Extrapolation (ng)	1.7	3.2	0.1	0.3
Estimated LOD S/N ≥3	8	16	0.5	4

*LOD by Extrapolation from S/N for peak area of a 62.5 ng injection

3. Dynamic Range

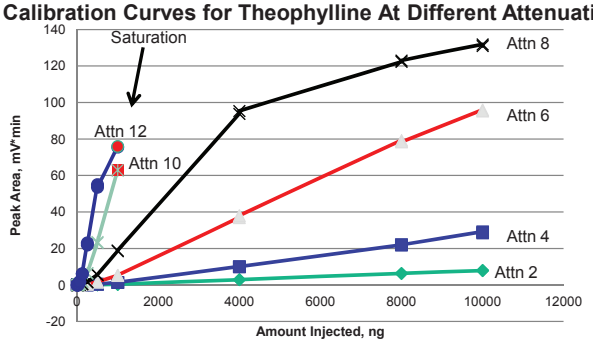
CAD has a wide dynamic range of about 4 orders of magnitude. This is important when trying to measure low levels of an analyte in the presence of another at a much higher level (e.g., for impurity testing). This can be readily accomplished without having to reanalyze the sample at different gain ranges. ELSD is very different. Rather than using contiguous gain ranges, the performance of the photomultiplier tube is attenuated. Each attenuation setting has its own unique sensitivity, noise, required filter setting, dynamic range and response saturation, as shown in Table 2 and Figure 5. The upshot of this is that any attenuation setting only has a dynamic range of 2 or so orders of magnitude. In order to cover the range required for impurity testing, samples need to be reanalyzed using at least two different attenuations. This can be time consuming.

Table 2: ELSD Attenuation Characteristics.

Attenuation	Theophylline				Caffeine			
	Estimated LOD (ng)*	S / N	Response Saturation (ng)**	Approximate Orders of Magnitude	Estimated LOD (ng)	S / N	Response Saturation (ng)*	Approximate Orders of Magnitude
2	62	4	>10,000	2	125	6	>10,000	<2
4	31	4	>10,000	<3	62	7	>10,000	2
6	16	5	>10,000	<3	31	5	>10,000	<3
8	8	5	4000	<3	16	3	4000	<3
10	8	3	1000	2	16	7	>1000	<2
12	8	3	<1000	2	16	4	<1000	<2

* LOD based on lowest amount injected with S/N ≥ 3; **Upper Concentration Limit Due to Detector Saturation

FIGURE 5. Calibration Curves for Theophylline At Different Attenuations (Attn).



4. Inter-analyte Response

The ability to obtain uniform response among analytes is an important goal of universal detectors and requires that response is largely independent of analyte nature. CAD has been shown to be little affected by a compound's physicochemical characteristics and typically shows an inter-analyte response across a broad range of compounds on the order of <11% (see Figure 6 for flow injection analysis of different analytes). Figure 7 shows the separation of six compounds using gradient chromatography with measurement by CAD and ELSD. An inverse gradient make-up flow was used to address any issues with nebulization efficiency. The corresponding normalized response variability (Figure 8) indicates higher inter-analyte response variability with ELSD. This may be related to differences in physiochemical characteristics that affect light absorption, refraction and reflection and also to the higher complexity and imprecision of light scattering response. This can severely limit the ability to obtain accurate estimation of analyte quantity in the absence of authentic standards as is required in many studies such as in mass balance, impurity determination, compound library management and lipid class analysis.

FIGURE 6. Inter-analyte Response For CAD With Flow Injection Analysis.

➢**10.7% RSD** variation in CAD response among non-volatile analytes

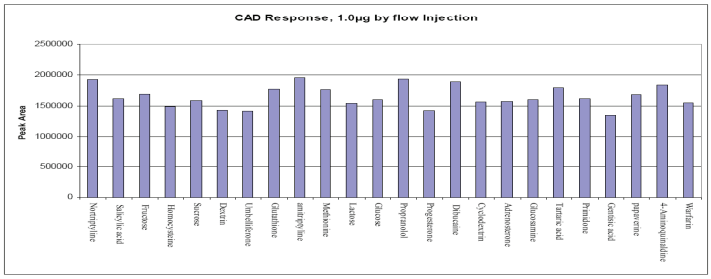


FIGURE 7. Differences In Analyte Response Using Gradient HPLC With Inverse Gradient Compensation.

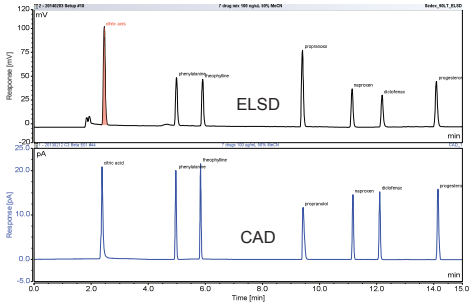
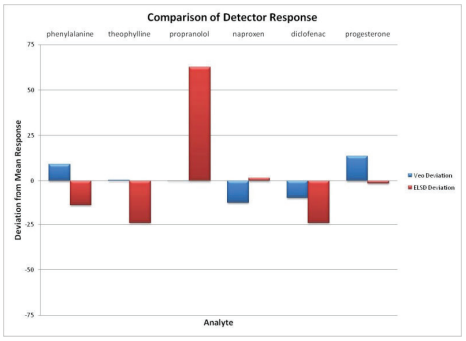


FIGURE 8. Normalized Analyte Response Variability.



5. Precision

Table 3 shows the area precision for six analytes (n=7). CAD has superior performance for measurement of both low and high level standards.

Table 3: Area Precision.

Analyte	Precision High Level 200 ng		Precision Low Level 20 ng	
	CAD	ELSD	CAD	ELSD
Phenylalanine	1.07	6.46	1.74	19.7
Theophylline	1.28	4.78	1.64	15.5
Propranolol	1.58	3.50	10.5	11.5
Naproxen	0.99	7.80	13.2	13.9
Diclofenac	1.24	2.42	6.25	18.9
Progesterone	0.85	3.95	11.1	11.9

5. Flow Rate

The charged aerosol detector uses a single nebulizer to handle flow rates from 0.1 to 2.0 mL/min. The evaporative light scattering detector required different nebulizers to achieve a similar flow rate range (Table 4).

Table 4: Flow-rate Ranges And Nebulizer Requirements.

Usable Flow Rate Range, mL/min			
Corona™ Veo™ RS	Corona™ Ultra RS™	Sedex 90LT	Nebulizer
0.010 – 2.0 HPLC/UHPLC with one nebulizer	0.200 – 2.0 HPLC/UHPLC with one nebulizer	0.005 – 0.04	1
		0.040 – 1.2	2
		0.200 – 2.5	3
		1.0 – 4.0	4
		0.200 – 1.4 (UHPLC)	5

Conclusion

- Both CAD and ELSD are non-linear. LODs cannot be extrapolated from the response of high levels of analyte but can only be determined through the generation of calibration curves.
- The sigmoidal response of ELSD results in a small dynamic range. The analyte signal rapidly decreases and completely disappears as the amount of analyte decreases.
- CAD performs better for the measurement of low levels of analytes, and has a wide dynamic range of four orders of magnitude. Furthermore, CAD is affected much less by an analyte's physicochemical properties.
- CAD uses a single nebulizer to address a wide flow rate range. ELSD requires multiple nebulizers adding to expense and downtime.

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