Advances in Universal Detection

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ABSTRACT

Universal HPLC detectors offer the analyst many advantages over more specific detectors when used for the measurement of impurities and in degradation or mass balance studies. For the commonly used universal detectors, UV is limited to compounds that possess a chromophore, mass spectrometry (MS) to those compounds that ionize, and refractive index (RI) detector are chromatographically inferior as they are extremely temperature sensitive and cannot be used with gradients. This presentation will focus on evaporative light scattering detection (ELSD), charged aerosol detection (CAD®), and condensation nucleation light scattering detection (CNLSD).

Each of these newer evaporative detectors use common principles: pneumatic nebulization of the mobile phase containing the analyte eluting from the column to form droplets, and drying of the droplets into particles. However, the way in which analyte mass is determined from particles differs between the three detectors. ELSD uses a laser beam and measures the reflected light scattered to a sensitive photomultiplier: greater mass results in larger particles, thus greater light scattering. CAD uses a high-voltage corona needle to charge nitrogen gas, which collides with analyte particles resulting in the formation of charged particles: the greater the mass, the larger the particle and the more charge carried. These charged particles are then collected and measured using a sensitive electrometer. CNLSD is similar to ELSD but contains an additional water condensation step to grow the particle size prior to detection. Each of these technologies has certain analytical advantages and disadvantages. A brief overview of the operating principles for the newer universal detectors will be provided. Fundamental principles of these different detectors including advantages and disadvantages and figures of merit for each design will be discussed.

INTRODUCTION

- The fundamental characteristics of various nebulizer-based HPLC detectors (ELSD, CNLSD, and CAD) were compared in this study.
- These evaporative detectors use common principles: pneumatic nebulization of the chromatographic eluent to form a wet aerosol; and solvent evaporation to form dried aerosol particles. In each case, dried aerosol particle size increases with analyte concentration. However, the way in which concentration-dependent response is obtained differs between the three techniques.

- ELSD response depends on changes in light scattering intensity as a function of aerosol particle size as measured using a photodiode or photomultiplier. Because light scattering efficiency changes exponentially with particle size, ELSD response curves are typically complex and often sigmoidal.[1]
- The Corona® CAD uses a high-voltage corona to charge gas molecules which collide with analyte particles resulting in the formation of charged particles. Aggregate charge is measured using a conductive filter and sensitive electrometer. The level of charge acquired increases with particle size. CAD response curves, while nonlinear when assessed over a wide dynamic range, are typically less complex than ELSD.[2] Linear calibration fits can often be used for quantitation over a narrow concentration range.
- CNLSD is similar to ELSD but contains an additional water condensation step to increase the particle size prior to detection. Individual particles are counted using a light scattering technique. The response is typically curvilinear when assessed over a wide concentration range, but linear response can often be obtained over a narrow concentration range.[3] Detector overload can occur when higher concentrations are present.
- Each of these technologies demonstrate certain analytical advantages and disadvantages for specific types of analyses.

METHODS

All chemicals and standards were purchased from Sigma Chemicals Co., St. Louis, MO. Individual chromatographic methods and analyte concentrations are indicated on the individual panels for each application.

- The Acclaim Trinity P1 column is based on innovative Nanopolymer Silica Hybrid (NSH) technology, which consists of high-purity porous spherical silica particles coated with charged nanopolymer particles. The inner-pore area of the silica particle is modified with a covalently bonded organic layer that provides both reversed-phase and anion-exchange retention. The outer surface is modified with cation-exchange functionality. This chemistry ensures spatial separation of the anion-exchange and cation-exchange regions, and allows both retention mechanisms to function simultaneously and be controlled independently.
- Analyte response on the Corona CAD detector is less dependent on chemical characteristics than ELSD or CNLSD.
- LOD for all ions measured using the Trinity-CAD method is < 10 ng (on column) (s/n = 3).
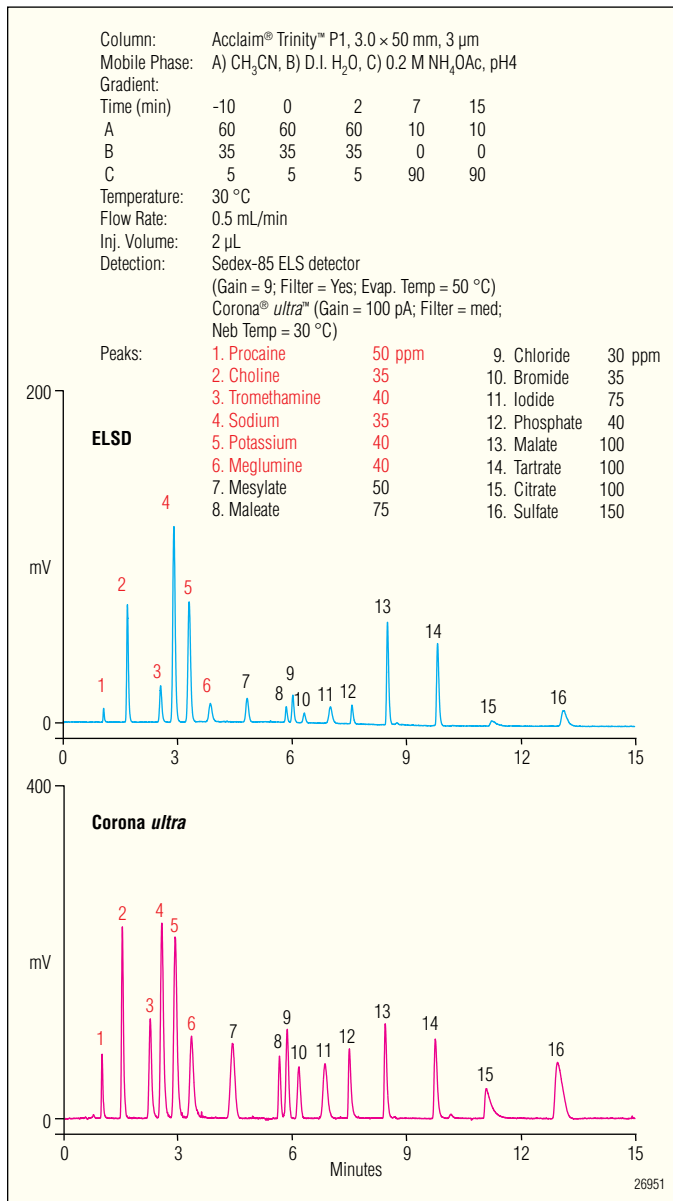
Column: Acclaim® Trinity™ P1, 3.0 × 50 mm, 3 µm
Mobile Phase: A) CH₃CN, B) D.I. H₂O, C) 0.2 M NH₄OAc, pH4
Gradient:

Time (min)	-10	0	2	7	15
A	60	60	60	10	10
B	35	35	35	0	0
C	5	5	5	90	90

Temperature: 30 °C
Flow Rate: 0.5 mL/min
Inj. Volume: 2 µL
Detection: Sedex-85 ELS detector
               (Gain = 9; Filter = Yes; Evap. Temp = 50 °C)
               Corona® ultra™ (Gain = 100 pA; Filter = med;
               Neb Temp = 30 °C)

Peaks:
1. Procaine	50 ppm	9. Chloride	30 ppm
2. Choline	35	10. Bromide	35
3. Tromethamine	40	11. Iodide	75
4. Sodium	35	12. Phosphate	40
5. Potassium	40	13. Malate	100
6. Meglumine	40	14. Tartrate	100
7. Mesylate	50	15. Citrate	100
8. Maleate	75	16. Sulfate	150

Figure 1. CAD vs. ELSD response for ion analysis.

Analyte	ELSD (height)	ultra (height)
1) Gallic acid	113997	91164
2) Protocatechuic acid	47346	92661
3) Chlorogenic acid	95604	71814
4) 4HPAC	2463	61702

Phenolics, 250 ng o.c., on Waters ACQUITY® UPLC® with ELSD. Nebulizer Temperature at 12 °C, Evaporation Tube at 80 °C, N₂ pressure at 60 psi.

Gallic Acid LOD s/n 3:1:
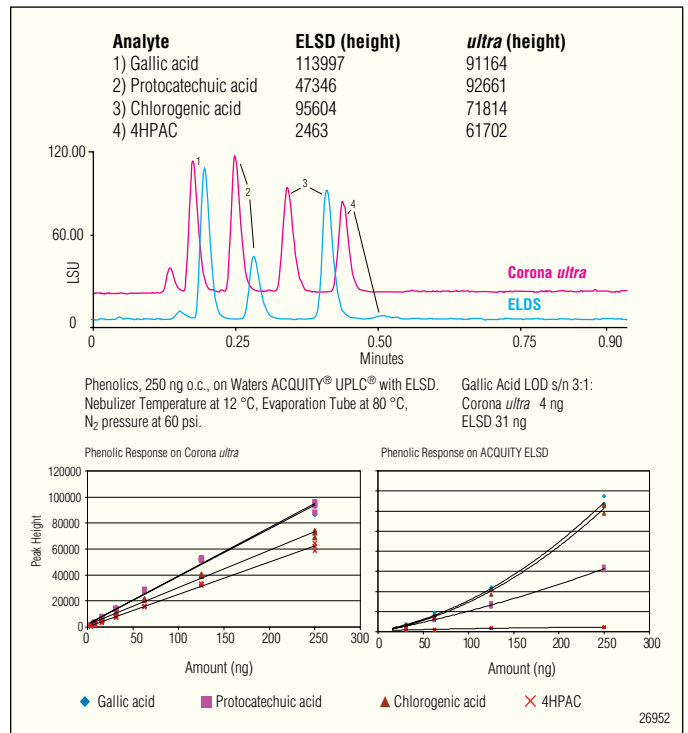Corona ultra 4 ng
ELSD 31 ng

Figure 2. CAD vs. ELSD response for phenolics.

CAD response was more consistent (uniform) among analytes than the ELSD. In this example, ELSD response for four phenolic compounds varied greatly, with 4-hydroxyphenylacetic acid (4HPAC) giving minimal signal. CAD was also more sensitive than ELSD. For example, the limit of detection (s/n = 3) for Gallic acid was 31 ng by ELSD and 4 ng (s/n = 3) by Corona ultra detector.
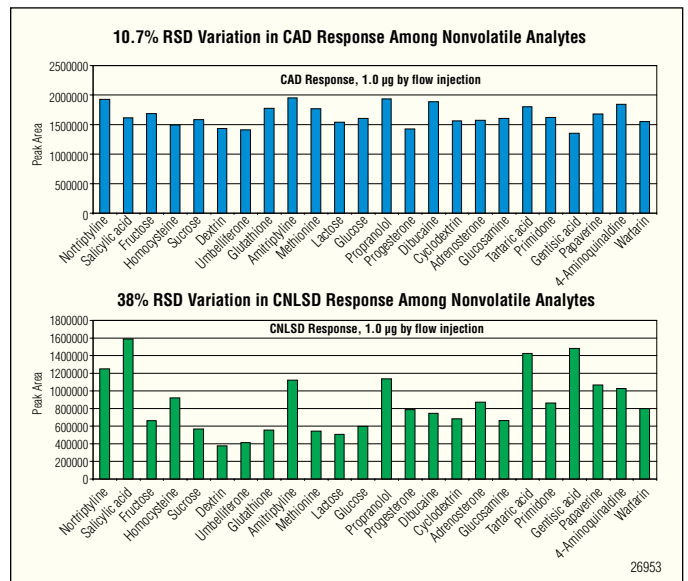

Figure 3. CAD vs. CNLSD compound response factors.

To assess the dependence of response on analyte nature, a group of 24 compounds differing in both chemical and physical properties (molecular weight, polarity, hydrophobicity, charge, and volatility) were evaluated using CAD and flow-injection analysis (FIA). The variation in response among all analytes was found to be 10.7% RSD. For comparison, the same experiment was performed using CNLSD. The variation in response with CNLSD was ~40% RSD.

The low RSD for CAD is due to the fundamental technology where charge on the analyte particle is being measured. The large variation seen with CNLSD is hypothesized to be due to complexity of the interaction (e.g., wettability and solubility) between analyte in the particle and water condensation used to grow the particle prior to detection. These data demonstrate that the Corona *ultra* CAD is ideally suited for the routine analysis of actives, impurities, and for mass balance studies, where consistent analyte response is critical.
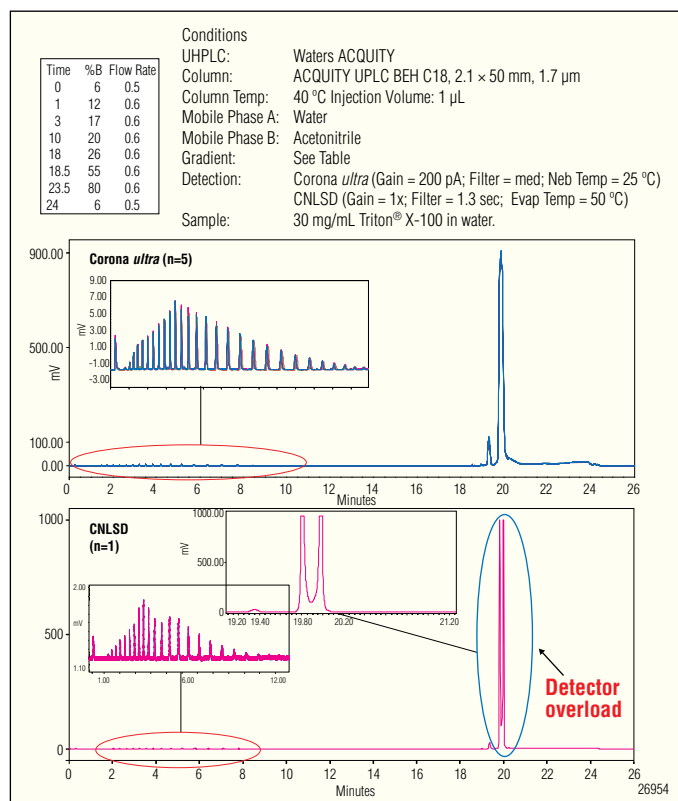
The excipient Triton X-100® was characterized using gradient HPLC with Corona *ultra* CAD. Using a single gain setting, both early eluting low-level impurities and the primary Triton peak were measured simultaneously. For comparison, the same experiment was performed using CNLSD. This approach produced poorer limits of detection, and the Triton peak overloaded the detector, necessitating dilution and reanalysis of the sample. The Corona *ultra* with its high sensitivity (high-pg), reproducibility and wide dynamic range (> four orders of magnitude) can be used for routine measurement of impurities and primary ingredients in a single injection.
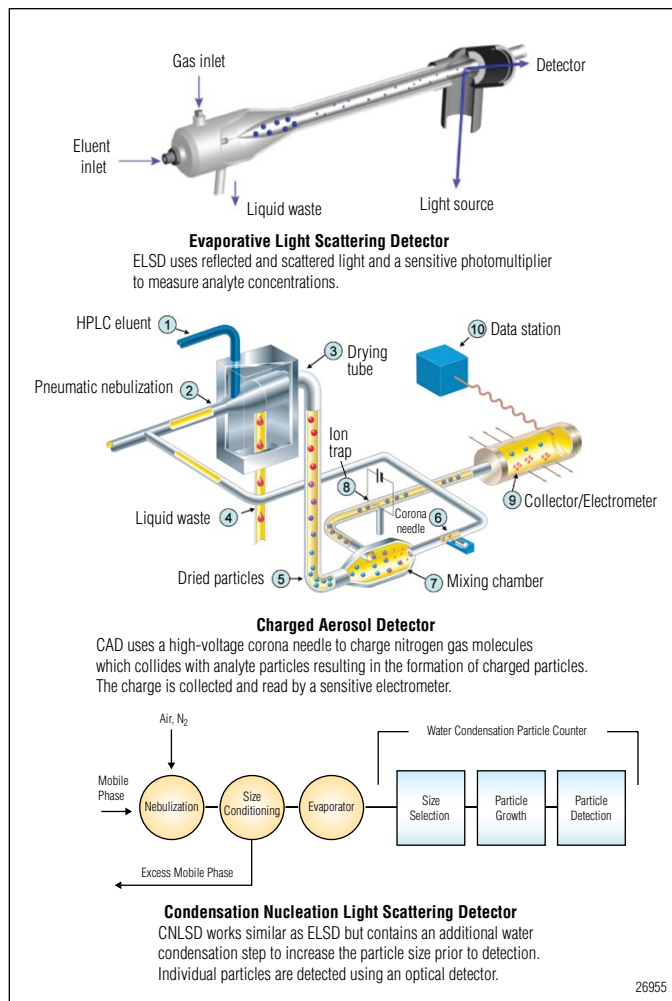

Conditions

Time	%B	Flow Rate
0	6	0.5
1	12	0.6
3	17	0.6
10	20	0.6
18	26	0.6
18.5	55	0.6
23.5	80	0.6
24	6	0.5

UHPLC: Waters ACQUITY
Column: ACQUITY UPLC BEH C18, 2.1 × 50 mm, 1.7 µm
Column Temp: 40 °C Injection Volume: 1 µL
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Gradient: See Table
Detection: Corona *ultra* (Gain = 200 pA; Filter = med; Neb Temp = 25 °C)
                 CNLSD (Gain = 1x; Filter = 1.3 sec; Evap Temp = 50 °C)
Sample: 30 mg/mL Triton® X-100 in water.

Figure 4. CAD vs. CNLSD response for Triton X-100.

Evaporative Light Scattering Detector
ELSD uses reflected and scattered light and a sensitive photomultiplier to measure analyte concentrations.

Charged Aerosol Detector
CAD uses a high-voltage corona needle to charge nitrogen gas molecules which collides with analyte particles resulting in the formation of charged particles. The charge is collected and read by a sensitive electrometer.

Condensation Nucleation Light Scattering Detector
CNLSD works similar as ELSD but contains an additional water condensation step to increase the particle size prior to detection. Individual particles are detected using an optical detector.

Figure 5. Evaporative universal detectors.

CONCLUSION

- ELSD requires optimization of several instrument parameters, which can complicate method development. The complex nature of ELSD response curves can adversely effect quantitation including reproducibility and accuracy (i.e., underestimation at lower analyte concentrations and overestimation at higher concentrations). These factors significantly limit the ability to validate and transfer ELSD-based methods among laboratories.

- CNLSD response was determined to be more dependent on analyte nature than CAD, which limits its usefulness as a universal detector. This may be attributed to differences in the hygroscopic nature and/or water solubility among analytes. CNLSD response saturated at much lower concentrations than CAD, and peak distortion due to detector overloading was observed sporadically.

- CAD provided the broadest dynamic range, excellent sensitivity, and uniformity of response independent of chemical structure while also being the easiest to operate.

The table below summarizes the distinct differences between these detectors in analytical development.

REFERENCES

1. Allgeier, M.; Nussbaum, M.; Risley, D. *LCGC* **2003,** *21*(4), 376.

2. Gamache, P.; McCarthy, R.; Freeto, S.; Asa, D.; Woodcock, M.; Laws, K.; Cole, R. *LCGC* **2005,** *23* (2), 151–161.

3. Koropchak, J.; Magnusson, L.E.; Heybroek, M.; Sadain, S.; Yang, X.; Anisimov, M. *Adv. Chromatogr.* **2000,** *40*, 275–314.

Table 1. Differences Between Detectors Used in This Study			
	CAD *ultra*	**ELSD**	**CNLSD**
Response	Curvilinear (small change in low level = large change in response) linear Over 1–2 orders of magnitude	Sigmoidal (large change in low level = small change in response)	Curvilinear response over a wide dynamic range; linear over shorter ranges
Dynamic Range	>4 orders	2–3 orders	2–3 orders
Sensitivity (LOD)	<1 ng	>10 ng	<1 ng
Semivolatility Range	Similar	Similar	Similar
Analyte Response	Independent of structure	Variable	Dependent on compound wettability/water solubility
Ease of Operation	Simple	Can be complex and analyte dependent	Can be complex and analyte dependent

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