

Advancements in Charged Aerosol Detection

Ian Acworth, Christopher Crafts, Marc Plante, and Bruce Bailey
 ESA – A Dionex Company, Chelmsford, MA



INTRODUCTION

Corona® Charged Aerosol Detector (CAD®) from ESA, a Dionex company, has become standard for many applications in a variety of industries. New sub-2 micron column technology in the field of liquid chromatography has led to the introduction of Ultrahigh Performance Liquid Chromatography (UHPLC). These changes have resulted in systems and columns that can operate at pressures up to 15,000 psi and provide unprecedented separations. This allows for faster peaks and shorter run times, which reduces the costs associated with analyst time and solvent consumption.

Universal detection of fast peaks with minimal peak broadening is required to maximize the utility of this new technology. ESA has introduced the next generation of Charged Aerosol Detection® with its ultrahigh-performance version of its award-winning Corona CAD detector. The Corona *ultra*™ detector incorporates all the features introduced in the Corona CAD detector along with faster data sampling times, nebulizer temperature control, and improved filter algorithms. These key improvements allow the Corona *ultra* to meet the detector demands required by UHPLC systems.

Evaluations were done with Rapid Resolution Liquid Chromatography (RRLC–1200–Agilent) and Ultra Performance Liquid Chromatography (UPLC®–ACQUITY® Waters) to demonstrate the new detector's capabilities. Experiments were also performed to show that the Corona *ultra* was capable of achieving or surpassing the Corona CAD for sensitivity and reproducibility of analysis under UHPLC conditions.

TECHNOLOGY AND BASIC OPERATION

The Corona *ultra* is a mass-sensitive universal detector for nonvolatile and some semivolatile analytes, and the response is independent of chemical structure, unlike absorbance, fluorescence, or electrochemical detectors.

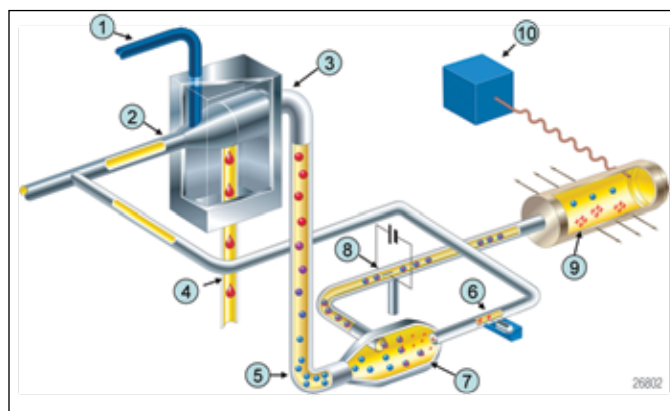


Figure 1. Illustration of how the Corona *ultra* works. The liquid eluent from the HPLC column enters the Corona (1) where it undergoes pneumatic nebulization (2). Small droplets enter the drying tube (3) and form particles while large drops exit the drain (4) to waste. Dried particles enter the mixing chamber (5). Another gas stream passes over a charged Corona Needle (6). Charged gas then mixes with the dried particles forming charged particles (7). High mobility species are removed by an ion trap (8) while the remaining charged particles pass to a collector where the charge is measured with a very sensitive electrometer (9). Signal is transferred to software (10).

Unlike other technologies (ELSD and MS), the design of Corona *ultra* allows for fewer adjustments of instrument parameters. This results in an easy-to-operate detector that is highly sensitive to a broad range of compounds. The detector requires only a clean gas input (nitrogen or air) and a gas exhaust without vacuum.

Now sold under the
 Thermo Scientific brand

Thermo
 SCIENTIFIC

IMPROVED DETECTOR DESIGN

- Output signal sample rate of 100 Hz
- Improved sensitivity under UHPLC conditions
- Improved filter algorithms for fast peaks
- Measurement of peak widths down to 1 s at base under UHPLC conditions
- USB compatible with Chromeleon® Chromatography Data System, Agilent ChemStation, Agilent EZChrom Elite™, and Waters Empower™
- Nebulizer temperature control between 5–35 °C
- New design allows the detector to be easily integrated into an existing HPLC system
- Instrument firmware is upgradeable via the USB port
- Precise internal gas pressure regulator set to 35.0 + 0.1 psi
- Factory calibrated total flow and flow ratio values to minimize inter-unit variability
- Internal log for timed preventative maintenance service and filter changes
- Color display with full touch screen capability
- On screen monitoring of chromatographic data
- Meets the EU Directive 2002/95/EC Reduction of Hazardous Materials (RoHS)

FILTER SETTINGS EFFECT ON SENSITIVITY AND PEAK BROADENING

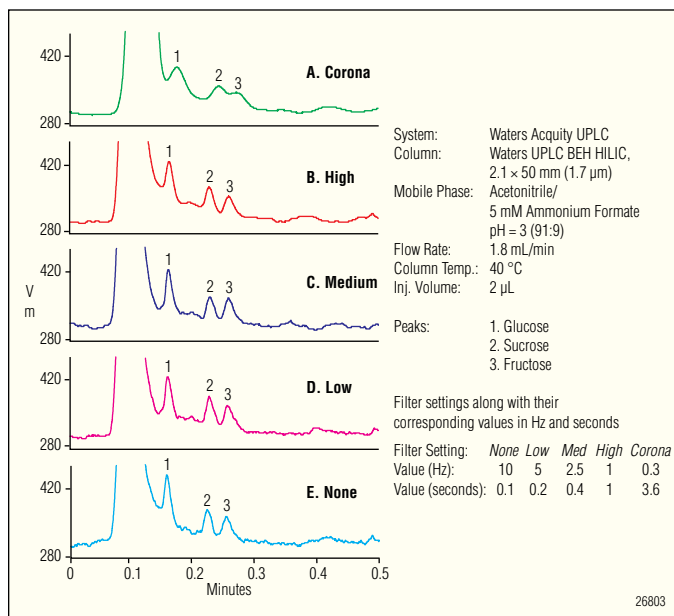


Figure 2. UPLC chromatograms of glucose (1), sucrose (2), and fructose (3) at 1 ng on column (on column), using the specific Corona ultra filter settings listed on the figure.

2 Advancements in Charged Aerosol Detection

Table 1. EP Signal to Noise Calculated Using Empower Software for Each Compound at Filter Settings (Yellow=Optimal)					
	Corona	High	Med	Low	None
Glucose	5.2	6.5	8.5	8.6	7.7
Sucrose	N/A	4.3	4	5.5	4.6
Lactose	N/A	3.2	4.1	4.1	3.7

RESPONSE FACTORS

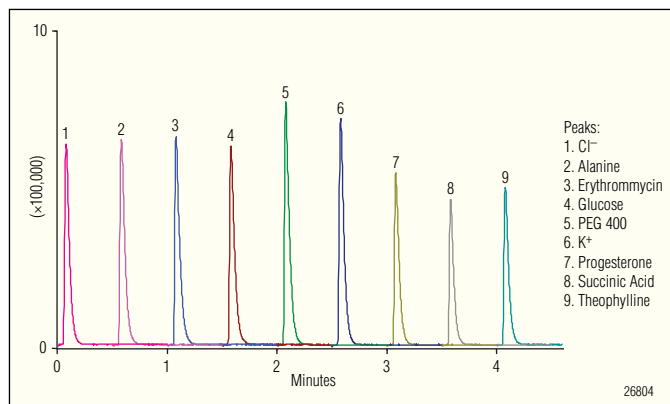


Figure 3. Overlay of flow injections for nine compounds using a 0.5 min delay for each 10 μL injection of each compound with concentrations from 48–52 μg/mL. Mobile phase and dilution solvent was 50 mM ammonium formate pH = 3, acetonitrile, methanol, isopropyl alcohol, THF (40, 30, 20, 5, 5). Interanalyte response variability ~12%.

RANGE AND REPRODUCIBILITY

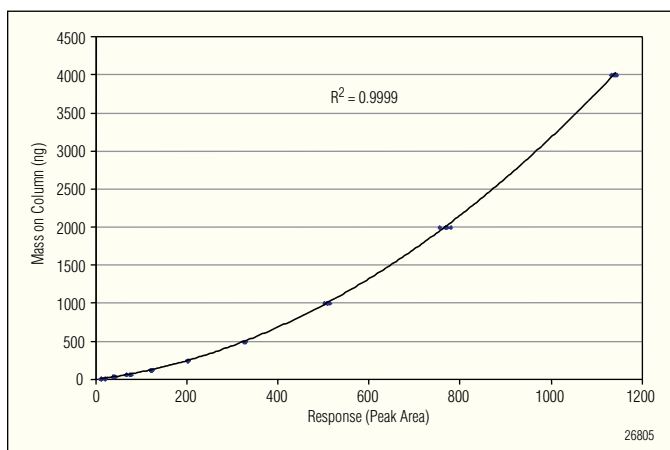


Figure 4. Calibration curve for sucrose (8 to 4000 ng on column) with five replicates at 10 levels using an inverted second-order polynomial fit with the same method as shown in Figure 2.

Table 2. Percent RSD for the Highest, Middle, and Lowest Mass on Column (Nanograms) from Figure 3

Standard	High	Med	Low
Mass on Column (ng)	4000	250	7.8
%RSD	0.43	0.35	4.9

LINEARITY (PHENOLICS)

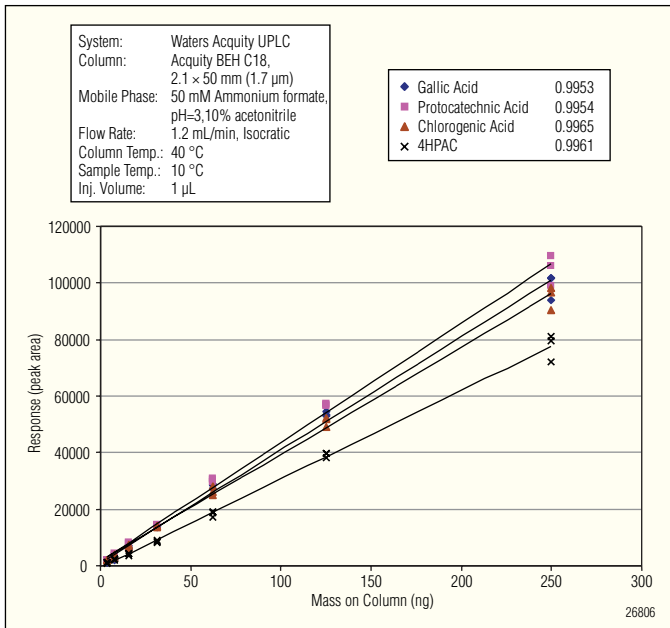


Figure 5. Linear fit of three replicates at seven concentration levels from 4 to 250 ng for the four phenolic compounds listed. Linear regression correlation coefficients are listed for each compound.

GRADIENT COMPATIBILITY

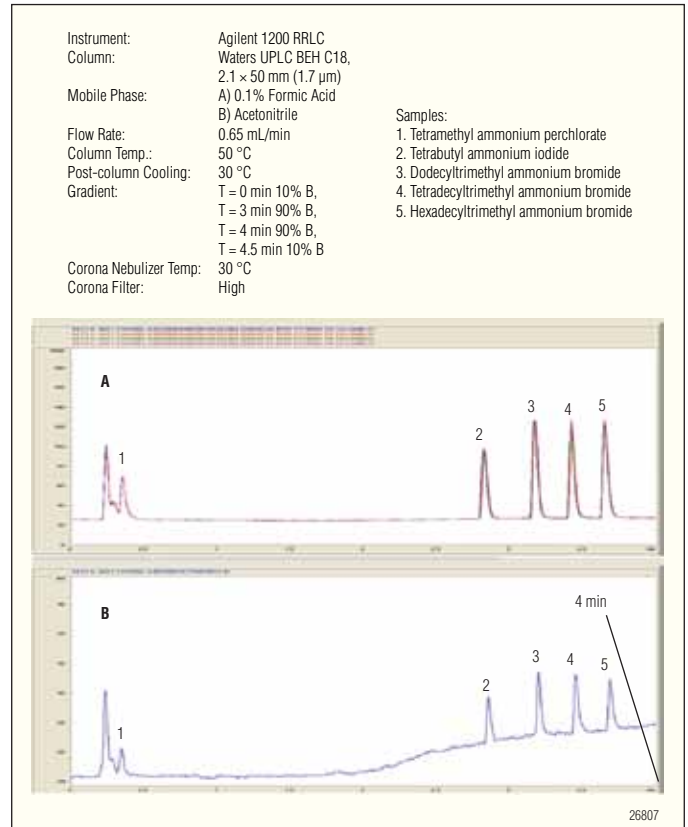


Figure 6. A) Overlay of four injections (compound 1 at ~144 ng on column, compounds 2–5 at ~72 ng on column); B) Single injection (compound 1 at ~7.2 ng on column, compounds 2–5 at ~3.5 ng on column.).

SOLVENT COMPATIBILITY

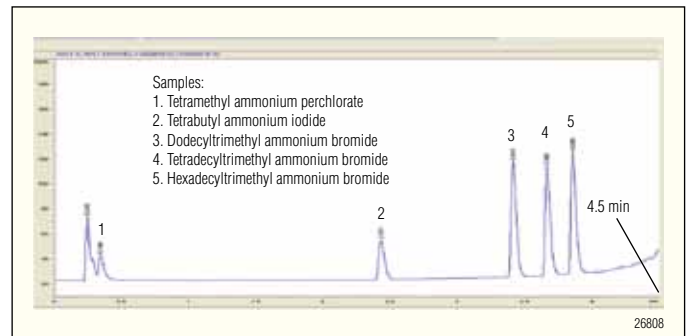


Figure 7. Samples and method described in Figure 6A but with replacing acetonitrile in mobile phase B with acetone and changing postcolumn cooling temperature from 50 to 55 °C.

The Corona *ultra* can be operated in both reversed and normal phase applications. Flow rates can range from 0.2 to 2.0 mL/min, and the mobile phase composition can range from 0% to 100% aqueous. The sensitivity of the instrument requires that all solvents used be HPLC-grade or better, with minimum residue. The absorbance properties of the solvent are not a factor with charged aerosol detection. Therefore, solvents such as acetone and methyl alkyl ketones are also acceptable. Figure 7 shows that for the analysis of quaternary ammonium salts, changing solvents from acetonitrile to acetone has only a minor impact on the baseline of the Corona detector. Only slight changes in chromatographic selectivity were observed.

Volatile mobile phase modifiers must be used with the Corona detector. These include: ammonium acetate, ammonium formate, trifluoroacetic acid, acetic acid, pentafluoropropionic acid, and formic acid.

DISCUSSION

The data presented in this poster show that the Corona *ultra* is fully compatible with UHPLC.

Evaluation of the filter algorithms' effects on chromatographic peak shape and response was evaluated using the UPLC analysis of glucose, sucrose, and lactose at low levels (~1 ng on column). Figure 1 illustrates the difference in the Corona *ultra* filter settings on both chromatographic peak shape and response. The baseline noise decreased as the filter was adjusted to increasingly higher settings, whereas the corresponding signal intensity decreased only slightly. Changing the filter to the highest Corona setting resulted in a larger decrease in signal intensity and a loss of resolution, as expected. For UHPLC analysis of these compounds, the medium or low filter settings are most appropriate. The data presented in Table 2 show that the greatest signal to noise for all three peaks was with a filter setting of low. When analyzing peaks ≥ 6 s at base, the Corona filter setting is best.

By using UHPLC conditions, improved sensitivity is observed with faster and/or more concentrated peaks entering the Corona *ultra* detector. Detector sensitivity for various analytes is extremely important in UPLC analysis, since the column loading capacity is typically decreased. For example, if sensitivity was inadequate using traditional HPLC conditions with 10 μ L injections, then it may be at or below the LOD when only 2 μ L of sample is injected. The Corona *ultra* routinely achieves detection limits in low nanogram amounts for nonvolatile compounds. The chromatogram shown in Figure 2 illustrates that for glucose an LOD ($s/n > 3$) of 0.5 ng (on column) and an LOQ ($s/n = 10$) at or below 5 ng (on column) can be obtained. This can be important for impurity analysis of non or weak chromophore compounds.

The Corona *ultra* shows consistent interanalyte response factors independent of chemical structure. This detector can effectively analyze a wide diversity of chemical structures and important classes of molecules—from small organic molecules, proteins, and peptides to ions, carbohydrates, lipids, and polymers as shown in Figure 3. The dynamic range is over four orders of magnitude (Figure 4), with a correlation coefficient in this example of 0.9999. The use of the Corona *ultra* as an analytical tool for an assay of purity can be done using a linear fit for most compounds and a narrower dynamic range, as shown in Figure 5. This illustrates the linear response for phenolic compounds run under UPLC conditions at seven different concentrations ranging from 4 to 250 ng on column. The correlation coefficient was ≥ 0.995 for all analytes.

There was only a minor impact of the gradient on detector baseline, as shown in Figures 6 and 7. The Corona *ultra* can be used with a variety of organic solvents. Acetone is a viable replacement for acetonitrile. This is important due to the commercial scarcity of the latter.

CONCLUSIONS

- UHPLC resolution, reproducibility, reliability and simplicity all in one detector.
- The Corona *ultra* is fully compatible with UHPLC approaches and is capable of measuring fast peaks (~1 s width at base).
- The Corona *ultra* is capable of measuring any nonvolatile and some semivolatile analyte, often with an LOD at the low ng to mid pg level.
- The Corona *ultra* is compatible with both isocratic and gradient conditions.
- The Corona *ultra* offers a wide dynamic range and analyte response independent of chemical structure.
- The Corona *ultra* can also be used with traditional HPLC systems.
- Nonvolatile analytes with weak or non-chromophore structures can be easily detected.

CAD, Charged Aerosol Detection, Chromeleon, and Corona are registered trademarks and *ultra* is a trademark of Dionex Corporation. ACQUITY, UPLC, and UHPLC are registered trademarks and Empower is a trademark of Waters Corporation.

EZChrom Elite is a trademark of Agilent.

Passion. Power. Productivity.



Dionex Corporation

1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America

U.S./Canada (847) 295-7500

South America

Brazil (55) 11 3731 5140

Europe

Austria (43) 1 616 51 25 Benelux (31) 20 683 9768 (32) 3 353 4294
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0
Ireland (353) 1 644 0064 Italy (39) 02 51 62 1267 Sweden (46) 8 473 3380
Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

Asia Pacific

Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190
Taiwan (886) 2 8751 6655

www.dionex.com



LPN 2400-01 3/10
©2010 Dionex Corporation