

Tryptic Digest

The need for universal HPLC detection in analytical laboratories is widespread. While several detection technologies (e.g., low wavelength UV, refractive index, evaporative light scattering, chemiluminescent nitrogen detectors) are currently being used, there is significant room for improvement in performance characteristics such as sensitivity, dynamic range, consistency of response factors and gradient or solvent compatibility.

To help address the many challenges of universal detection, ESA has developed the Corona[®] CAD[®] detector. This novel technology offers many benefits to analytical scientists including:

- High Sensitivity - Low ng limits of detection.
- More Consistent Response Factors - Response magnitude does not significantly depend on analyte properties (e.g. molar absorptivity, proton affinity).
- Broad and Useful Dynamic Range - 4 orders of magnitude (ng - µg quantities).
- Excellent Reproducibility - Typically less than 2% RSD.
- Broad Applicability - Can be used with a wide variety of HPLC conditions to measure virtually any nonvolatile analyte including proteins, lipids, carbohydrates and small molecules.
- Ease of Use - Easy setup. Uses minimal bench space and requires only gas input pressure and signal output range to be set.

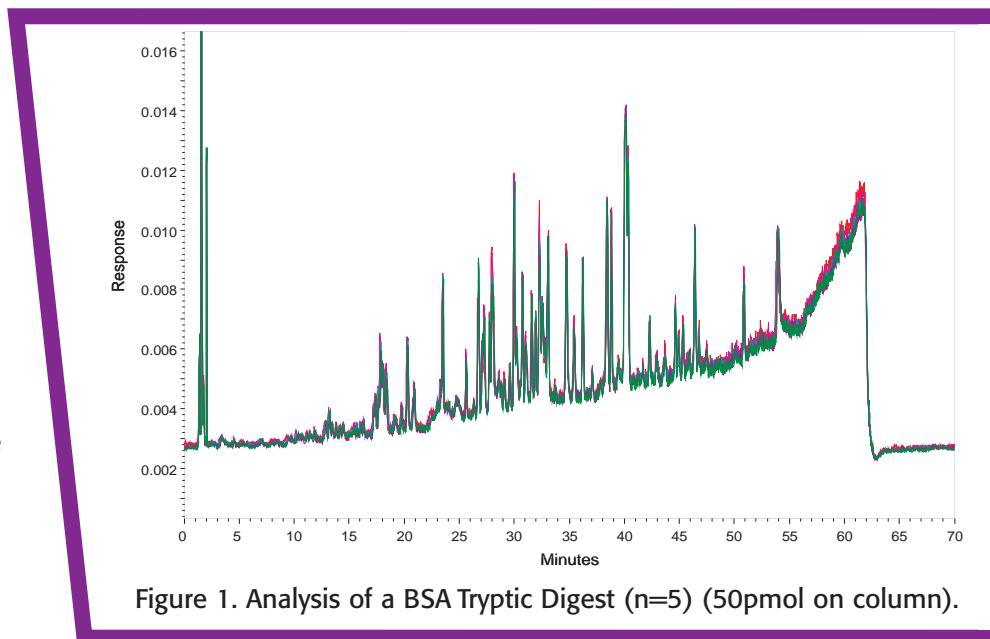


Figure 1. Analysis of a BSA Tryptic Digest (n=5) (50pmol on column).

This application note describes the use of the Corona CAD for the measurement of a BSA tryptic digest. The method had a limit of detection of ~10-20ng (on column), showed excellent precision (typical RSDs: 2.5-3.6% at 50pmol on column) (Figure 1) and had a dynamic range from ng to µg levels. The Corona CAD detector showed superior performance to UV detection (Figure 2). This is an examples of detection of analytes that possess weak chromophores.

Corona parameters

Gas: 35psi via nitrogen generator
Filter: none
Range: 100pA

HPLC Parameters:

Mobile Phase: A – 0.1% TFA in H₂O; B – 0.085% TFA in ACN
Gradient: 2% B for 5mins. 2% to 42% over 55mins.
Flow Rate: 1mL/min
Column: Zorbax Eclipse XDB-C8 4.6 x 150mm; 5µm
Column Temperature: Ambient
Injection Volume: 10µL

The Corona[®] CAD[®] Charged Aerosol Detector

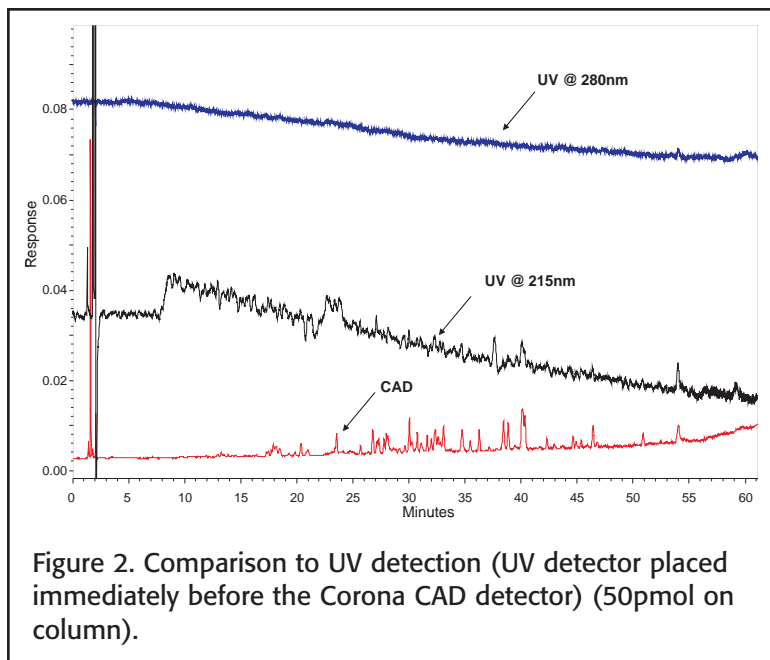


Figure 2. Comparison to UV detection (UV detector placed immediately before the Corona CAD detector) (50pmol on column).

Sample preparation

SeQuant Proteomic Calibration Kit – Bovine Serum Albumin, Cow dissolved @ 5nmol/mL in 70:30 0.1% formic acid (aq):acetonitrile.

Conclusions

The Corona CAD provides universal detection of nonvolatile analytes with response independent of chemical properties, a wide dynamic response range, high sensitivity and good precision. These characteristics, along with reliability and simple operation, make this a superior detector for a wide range of HPLC analyses.

For more information about this application, the Corona CAD, or charged aerosol detection visit www.coronacad.com. We are interested in your opinions and are available to answer any questions you may have: please contact a technical representative at 978.250.7082, or if e-mail is more convenient, send your questions to coronacad@esainc.com.

Ordering information

Description

Corona Plus
Thermal Organizer Module
Nitrogen generator
Pump, model 584
Autosampler, model 542
Elite software including PC
UV-Vis Detector

Part Number

70-7041
70-5499TA
70-6003
70-7058
70-4152
70-5073
70-6485



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