HPLC 2026
INDIANAPOLIS, INDIANA, USA
JUNE 6–11, 2026

Development and Transfer of Quantitative Analytical Methods using Universal Detectors

Jennifer Simeone, Paula Hong, and Andrew Steere
Waters Corporation, Milford, MA, USA

Waters™

Introduction

Universal detectors—such as Charged Aerosol Detection (CAD) and Evaporative Light Scattering Detection (ELSD)—offer valuable alternatives to traditional detection techniques like ultraviolet (UV), fluorescence, or mass spectrometry (MS). Fundamentally, CAD relies on charge transfer and subsequent charge measurement while ELSD is dependent on light-scattering. These differences in detection mechanism present both advantages and limitations, influencing method development and optimization. CAD and ELS detectors can be used as standalone detection techniques or in combination with other detectors to enhance the analytical information obtained from a single injection.

This presentation will explore the development and optimization of quantitative methods using both CAD and ELSD. In the context of quantitation, it is important to note that CAD and ELSD are non-linear detectors—meaning their response is not directly proportional to analyte concentration. As a result, alternative calibration models such as quadratic or log-log linear fits may be necessary. The choice of calibration model often depends on the concentration range under evaluation.

Finally, while method transfer between detectors of the same type (e.g., CAD to CAD) is generally straightforward, transferring methods between CAD and ELSD can be more complex. This presentation will address key considerations and potential modifications required when transferring methods across these detection platforms, with attention to concentration range and sensitivity requirements.

Method(s)

Sugar Content Method Conditions	
System	Arc™ HPLC System
Column	XBridge™ BEH™ Amide, 4.6 x 150 mm, 2.5 µm
Mobile Phase A	0.05% triethylamine in water
Mobile Phase B	0.05% triethylamine in ACN
Flow Rate	1.5 mL/min
Gradient	10 to 28% A over 6.5 min
Inj. Volume	10 µL
Column Temp.	55 °C

ELS Detection	
Nebulizer Mode	Cooling
Drift Tube Temp.	50 °C
Gas Pressure	40 psi
Gain	100
Data Rate	5 Hz

CAD Detection	
Power Function Value	1
Evaporator Temp.	50 °C
Data Rate	5 Hz

Deoxycholic Acid Method Conditions	
System	AQUITY™ UPLC H-Class PLUS System
Column	XBridge BEH C18, 4.6 x 150 mm, 3.5 µm
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in ACN
Flow Rate	0.85 mL/min
Gradient	25 to 45% B over 2.3 min 45 to 58% B over 14 min 58 to 100% B over 11.7 min
Inj. Volume	25 µL
Column Temp.	30 °C

CAD Detection	
Power Function Value	1
Evaporator Temp.	35 °C
Data Rate	2 Hz

Note: Method was scaled from the monograph prescribed 3 µm column to a 3.5 µm column following USP Chapter <621> guidelines

Conclusions

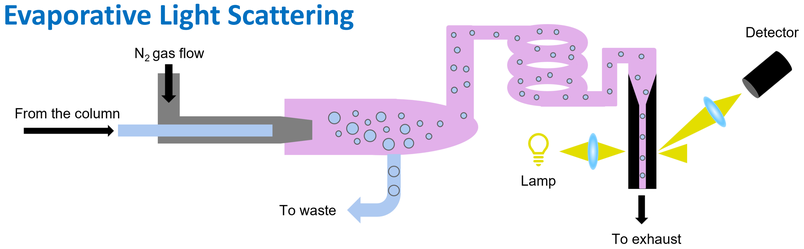
- When universal detection is required, both ELS and CAD are good alternatives to UV, MS and FLR
- A quantitative method initially developed on ELS was successfully transferred to an LC/CAD system, with only optimization of the CAD detector parameters required
- A USP monograph which specifies CAD was easily transferred from a competitive LC/CAD system to a Waters LC/CAD system while meeting all system suitability criteria and giving nearly identical %API and %impurity results

References

- USP Deoxycholic Acid: https://doi.usp.org/USPNF/USPNF_M958_05_01.html
- Analysis of Food Sugars/Saccharides in Cough Syrup Using ACQUITY UPLC BEH Amide Columns. Waters Application Brief WA60121. October 2009.
- Aubin, A., 2424 Evaporative Light Scattering Detector: Analysis of Apple Juice Sugars. Waters Application Note 720001968. December 2006.

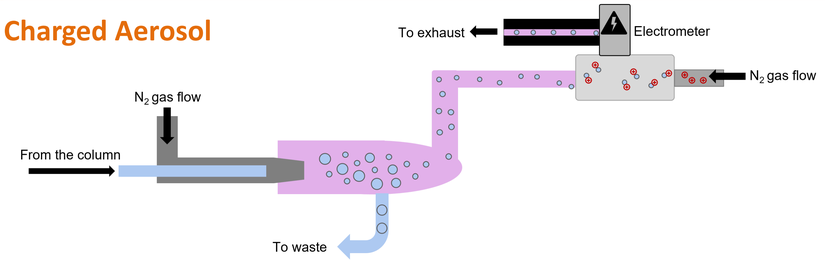
Fundamentals of Evaporative Light Scattering and Charged Aerosol Detectors

Evaporative Light Scattering



1) LC flow is mixed with N_2 to form an aerosol where larger droplets go to waste and smaller droplets enter the heated evaporation tube
2) Mobile phase is evaporated leaving dried analyte particles
3) Signal is based upon the light scattered by the solid particles remaining

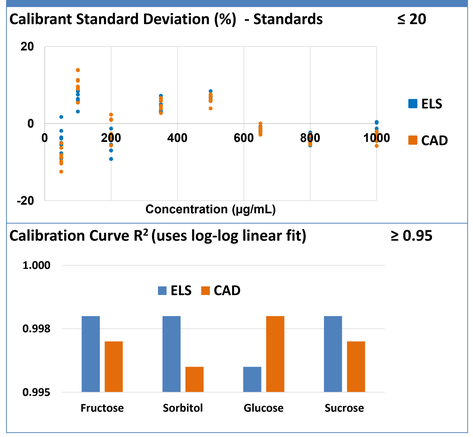
Charged Aerosol



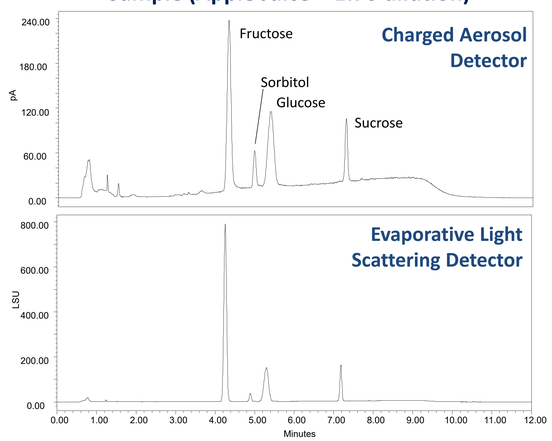
1) LC flow is mixed with N_2 to form an aerosol where larger droplets go to waste and smaller droplets enter the heated evaporation tube
2) Mobile phase is evaporated leaving dried analyte particles
3) N_2 is charged and then mixed with dried particles, transferring charge to the particles
4) Signal is based upon the measured charge of the solid particles remaining.

Sugar Content – Transfer from Evaporative Light Scattering Detector to Charged Aerosol Detector

Parameter	Acceptance
Calibrant Standard Deviation (%) - Standards	≤ 20
Calibration Curve R^2 (uses log-log linear fit)	≥ 0.95



Sample (Apple Juice – 1:70 dilution)

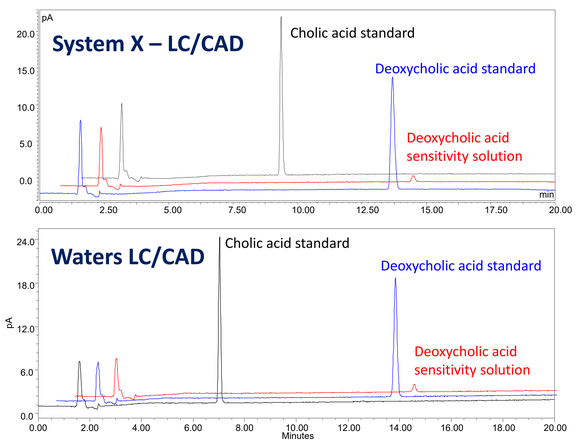


Area RSD (500 µg/mL)	ELS	CAD
Fructose	1.3	1.1
Sorbitol	0.8	0.8
Glucose	1.1	1.3
Sucrose	1.4	1.3

Calculated Concentrations (mg/mL)	ELS	CAD
Fructose	62.4	62.7
Sorbitol	6.3	6.8
Glucose	30.2	32.0
Sucrose	12.4	12.9

A HILIC method was initially developed for quantitation of sugars in fruit juice using an evaporative light scattering detector. The LC method conditions were transferred directly, but the detector settings were optimized for use with a charged aerosol detector. The method consists of a calibration curve over the range of 50 – 1000 µg/mL, with a log-log linear fit used for both detectors. The results demonstrate good precision was achieved using both detectors, with area RSDs below 1.5%. Calibration curve R^2 values were also comparable between detector, all easily meeting the requirement of **≥** 0.95. Residuals for all calibration standards were below 20%, with a similar pattern observed for both detectors. Finally, the quantitative results obtained using both ELS and CAD are in good agreement, highlighting an overall successful method migration between universal detectors.

Deoxycholic Acid – Transfer Across Different Vendor CAD Modules

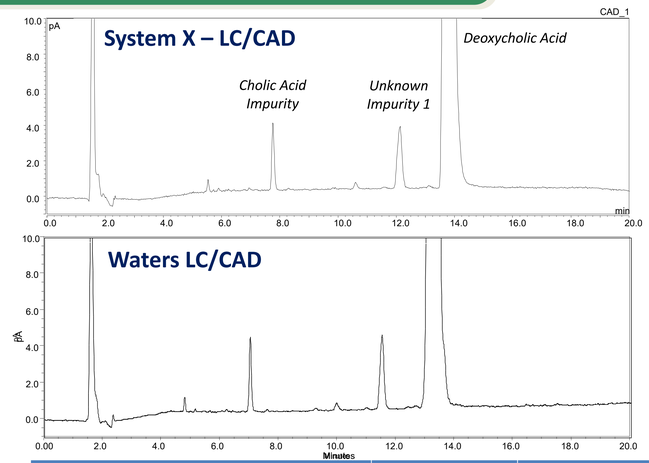


USP System Suitability	Acceptance	Sys X LC/CAD	Waters LC/CAD
Standard Solution	RSD NMT 3.0 (n = 6)	✓	✓
Sensitivity Solution	S/N NLT 10	✓	✓

Left: Chromatographic comparison of standard and sensitivity solutions required by Deoxycholic Assay & Organic Impurities USP Monograph between the Originator System (System X - LC/CAD) and the Receiving System (Waters LC/CAD). Similar results for Area RSD and USP Signal to Noise were achieved on both systems, easily meeting the criteria set forth in the monograph. Differences in retention times are due to the different dwell volume and mixing characteristics of the two different LC systems.

Right: Chromatographic comparison of a deoxycholic acid sample between the Originator System (System X – LC/CAD) and the Receiving System (Waters LC/CAD). Nearly identical results were achieved for % deoxycholic acid (98.0 vs 98.6%) and the two impurities (cholic acid impurity: 0.13 vs 0.14% and unknown impurity 1: 0.24% on both systems.)

This clearly demonstrates easy migration of a quantitative USP method between different vendor LC/CAD systems.



Sample Results	System X LC/CAD	Waters LC/CAD
% Deoxycholic Acid (Sample Solution)	98.0	98.6
Cholic Acid Impurity (Sample Stock Solution)	0.13	0.14
Unknown Impurity 1 (Sample Stock Solution)	0.24	0.24

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WATERS.COM/POSTERS
720009493EN