

Assessment of a New Generation of Evaporative Light-Scattering Detectors for Liquid Chromatography: Sensitivity, Linearity, Dynamic Range, Analyte Dispersion and Response Variation with Eluent Composition



Eric VERETTE, Ph.D.

SEDERE S.A.S., France



Abstract:

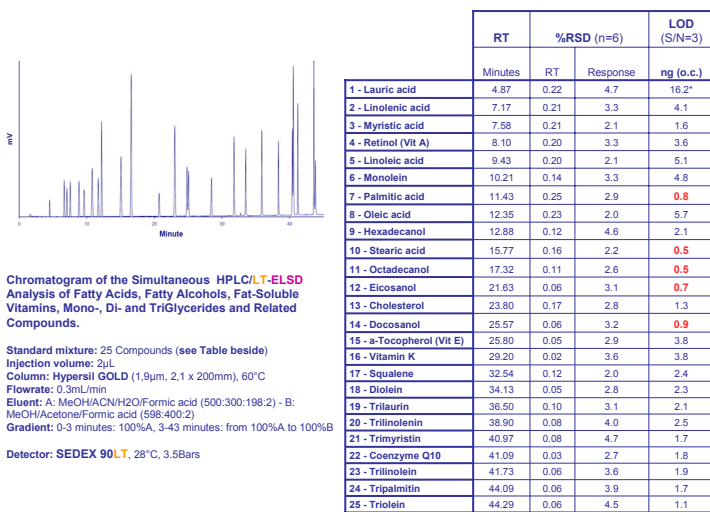
- Among the detectors available in Liquid Chromatography, Evaporative Light-Scattering Detector (ELSD) became in recent years a well established instrument thanks to several theoretical studies based on fundamental investigations and numerous applications provided during the last thirty years. Indeed, ELSD is considered as a nearly Universal, powerful and cost-effective technique, and is ideally appropriate for a great majority of liquid chromatography applications containing chromophoric and non-chromophoric compounds. However, some limitations have been attributed to this type of detection in the past, such as low sensitivities, non-linearity, reduced dynamic range, large analyte dispersion and wide response variation with the mobile phase composition.

- Today, a new ELSD model is proposed which offers a genuine and efficient Low-Temperature technology (LT-ELSD™) combined with an innovative detection chamber. The overall design of this new detector results in a significant increase of sensitivity providing limits of detection down to the sub-nanogram levels even for semi-volatile compounds. Moreover, it provides an improved direct linearity with correlation coefficients reaching 0.999 and an extended overall dynamic range exceeding the four orders of magnitude. Also, this model is optimized for the more recent U-HPLC technique giving peak widths below 1 second. Finally, a study on the response variation with the mobile phase composition showed a smaller effect compared to other aerosol-based detectors.

- In this work, several examples and data are provided on these topics to emphasize the great advancement in this technology, thus bringing to the analyst a relevant and cost-effective solution to their chromatography challenges.

I - Sensitivity

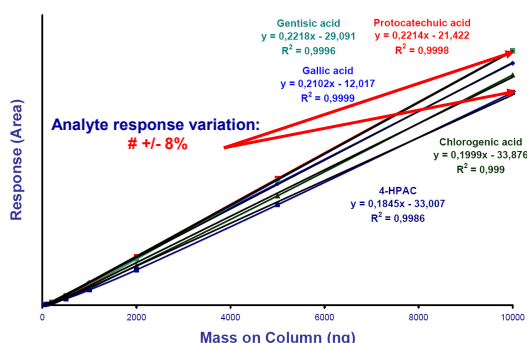
Application: Generic HPLC/LT-ELSD Method for Lipids



This example shows very Low Sensitivities obtained with a real HPLC/LT-ELSD application. LODs are much below 10ng on column for all compounds (except for Lauric acid which has got a high semi-volatility feature), and even at the Picogram Levels for other semi-volatile compounds such as Fatty alcohols and some Fatty acids.

II - Linearity

Application: HPLC/LT-ELSD Analysis of Phenolic Acids



Direct Linearity Curves of 5 Phenolic Acids Determined by HPLC/LT-ELSD

Standard mixture: Gallic acid, Protocatechuic acid, Chlorogenic acid, Gentisic acid and 4-Hydroxyphenylacetic acid (50ng, 100ng, 200ng, 500ng, 1000ng, 2000ng, 5000ng and 10000ng on column each)
 Injection volume: 10µL
 Column: Stability Basic C18 (CIL, France), 3µm, 3.0 x 150mm, 20°C
 Flowrate: 0.6mL/min
 Eluent: H2O/ACN (86:14)

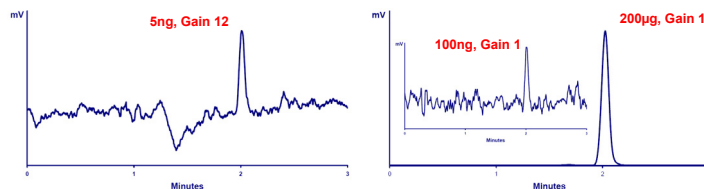
Detector: SEDEX 90LT, 40°C, Gain 5, 3.0Bars

Repeatability: %RSD (n=6) at 200ppm
 Gallic acid: 0.6% ; Protocatechuic acid: 2.2% ; Chlorogenic acid: 0.6% ; Gentisic acid: 2.5% ; 4-HPAC: 1.7%

This application shows very good Direct Linearities for the 5 Phenolic acids with concentrations from 50ng to 10000ng on column. Correlation coefficients ranged from 0.9986 to 0.9999. Additionally, repeatability in this application varied from 0.6% to 2.5%. Elsewhere, the figure also presents a small variation of the compound responses with identical concentrations and gave a relative variation percentage value of +/- 8% (e.g.: Gentisic acid and Protocatechuic acid curves are overlapping). This demonstrates that SEDEX 90LT, as an efficient and genuine mass detector, provides uniform responses.

III - Dynamic Range

Application: HPLC/LT-ELSD Analysis of Hydrocortisone



Chromatograms of Hydrocortisone by HPLC/LT-ELSD

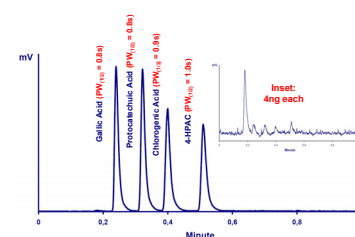
Sample: Hydrocortisone (5ng, 100ng and 200µg on column)
 Injection volume: 20µL
 Column: Zorbax Eclipse XDB-C18 (5µm, 4.6x150mm), 20°C
 Flowrate: 1mL/min
 Eluent: H2O/ACN (55:45)

Detector: SEDEX 90LT, 45°C, Gain 1 and 12, 3.5Bars

With these chromatography conditions, a minimum response value of Hydrocortisone was obtained at Gain 12 with 5ng (on column) and a maximum response was 200µg at Gain 1, which shows an overall dynamic range of 4 orders of magnitude at least for SEDEX 90LT. At Gain 1, the minimum response value was 100ng (on column), which shows a dynamic range of 3 orders of magnitude at least within a single Gain setting. These results are particularly useful for impurity assessment in drug discovery. Indeed, thanks to SEDEX 90LT impurities of 0.1% can be easily assessed within a single gain, and impurities of 0.01% can be determined using a gain change (which can be automatically set in the method with drivers).

IV - Efficiency, Peak Widths

Application: U-HPLC/LT-ELSD Analysis of Phenolic Acids

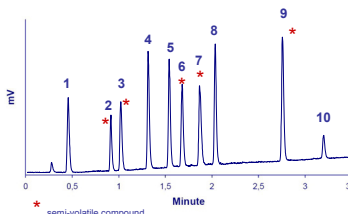


Chromatograms of 4 Phenolic Acids by U-HPLC/LT-ELSD

Standard mixture: Gallic acid, Protocatechuic acid, Chlorogenic acid and 4-Hydroxyphenylacetic acid (250ppm each and 4ng o.c. each in the inset)
 Injection volume: 1µL
 Column: Zorbax SB-C18 (1.8µm, 2.1x50mm), 60°C
 Flowrate: 0.7mL/min
 Eluent: 50mM Ammonium formate, pH:3/ACN (86:14)

Detector: SEDEX 90LT, 37°C, Gain 10 (inset: 12), 3.5Bars
 Nebulizer: U-HPLC

Application: U-HPLC/LT-ELSD Analysis of Drug Mixture



Chromatogram of 10 Pharmaceutical Compounds by U-HPLC/LT-ELSD

Standard mixture: 1: Sulfanilamide; 2: Caffeine; 3: Chlorprocaine; 4: Acetabulol; 5: Sulfamethoxazole; 6: Noscaphine; 7: Propranolol; 8: Hydrocortisone; 9: Ketoprofen; 10: Artesunate (10ppm each)
 Injection volume: 5µL
 Column: Waters Acquity BEH C18 (1.7µm, 2.1 x 50mm), 20°C
 Flowrate: 0.5mL/min
 Eluent: H2O/ACN/Formic acid (0.1%) ; 6%ACN to 56%ACN in 3.1 minutes

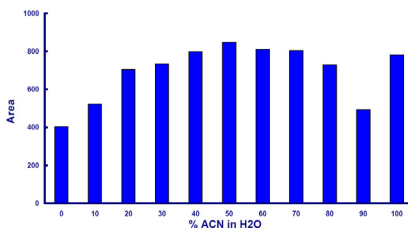
Detector: SEDEX 90LT, 50°C, Gain 10, 3.5Bars
 Nebulizer: U-HPLC

Courtesy of D. Guillema, University of Geneva, Switzerland.

These 2 applications demonstrate the good suitability of SEDEX 90LT for U-HPLC. In the application on Phenolic acids, peak widths (1/2 height) ranged from 0.8 to 1.0 second.

V - Response Variation with H2O/ACN Composition

Application: HPLC/LT-ELSD Analysis of Hydrocortisone



Histogram of Hydrocortisone Responses obtained by HPLC/LT-ELSD with Several H2O/ACN Percentages

Sample: Hydrocortisone 50ppm
 Injection volume: 10µL
 Column: No column
 Flowrate: 1mL/min
 Eluent: H2O/ACN (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100)

Detector: SEDEX 90LT, 45°C, Gain 7, 3.5Bars

Hydrocortisone area responses at different concentrations of Acetonitrile and Water ranged from 404mV/s (0% ACN) to 848mV/s (50% ACN), which means that the largest response is 2.1 times higher than the smallest one. This result clearly shows that SEDEX 90LT presents the lowest response variation with H2O/ACN composition among all other aerosol-based detectors, even compared to the one who has got a specific compensation device to control the solvent gradient effects.

Conclusion:

The applications developed above clearly show the advantages of the new SEDEX 90LT ELSD and particularly in regards to:

- Sensitivity with low nanogram and even sub-nanogram levels,
- Linearity with correlation coefficients reaching 0.999,
- Dynamic range of 4 orders of magnitude,
- Excellent suitability for U-HPLC with peak widths below 1 second,
- Low response variation compared to all other aerosol-based detectors.

This work also demonstrates the significant advancements of the new SEDEX Evaporative Light-Scattering Detector resulting from the combination of an efficient and genuine Low Temperature technology and an innovative detection chamber. These outstanding new features offer now to the analyst a Universal, powerful, versatile and cost-effective solution to their separation and quantification challenges.