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

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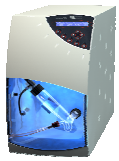


Abstract:

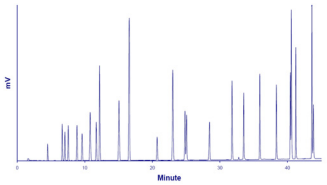
- Among the detectors available in Liquid Chromatography, Evaporative Light-Scattering Detector (ELSD) became in recent years a well established instrument thanks to several theoretical studies based on fundamental investigations and numerous applications provided during the last thirty years. Indeed, 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



Chromatogram of the Simultaneous HPLC/LT-ELSD Analysis of Fatty Acids, Fatty Alcohols, Fat-Soluble Vitamins, Mono-, Di- and TriGlycerides and Related Compounds.

Standard mixture: 25 Compounds (**see Table beside**)
Injection volume: 2µL
Column: Hypersil GOLD (1,9µm, 2,1 x 200mm), 60°C
Flowrate: 0.3mL/min
Eluent: A: MeOH/ACN/H2O/Formic acid (500:300:198:2) - B: MeOH/Acetone/Formic acid (598:400:2)
Gradient: 0-3 minutes: 100%A, 3-43 minutes: from 100%A to 100%B

Detector: SEDEX 90LT, 28°C, 3.5bar

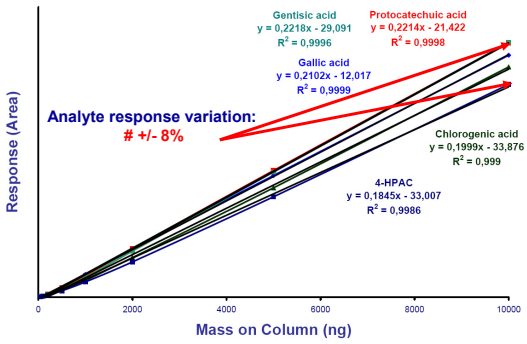
	RT	%RSD (n=6)		LOD (S/N=3)
	Minutes	RT	Response	ng (o.c.)
1 - Lauric acid	4.87	0.22	4.7	16.2*
2 - Linolenic acid	7.17	0.21	3.3	4.1
3 - Myristic acid	7.58	0.21	2.1	1.6
4 - Retinol (Vit A)	8.10	0.20	3.3	3.6
5 - Linoleic acid	9.43	0.20	2.1	5.1
6 - Monolein	10.21	0.14	3.3	4.8
7 - Palmitic acid	11.43	0.25	2.9	0.8
8 - Oleic acid	12.35	0.23	2.0	5.7
9 - Hexadecanol	12.88	0.12	4.6	2.1
10 - Stearic acid	15.77	0.16	2.2	0.5
11 - Octadecanol	17.32	0.11	2.6	0.5
12 - Eicosanol	21.63	0.06	3.1	0.7
13 - Cholesterol	23.80	0.17	2.8	1.3
14 - Docosanol	25.57	0.06	3.2	0.9
15 - a-Tocopherol (Vit E)	25.80	0.05	2.9	3.8
16 - Vitamin K	29.20	0.02	3.6	3.8
17 - Squalene	32.54	0.12	2.0	2.4
18 - Diolein	34.13	0.05	2.8	2.3
19 - Trilaurin	36.50	0.10	3.1	2.1
20 - Trilinolenin	38.90	0.08	4.0	2.5
21 - Trimyristin	40.97	0.08	4.7	1.7
22 - Coenzyme Q10	41.09	0.03	2.7	1.8
23 - Trilinolein	41.73	0.06	3.6	1.9
24 - Tripalmitin	44.09	0.06	3.9	1.7
25 - Triolein	44.29	0.06	4.5	1.1

*** Semi-volatile compound with high vapour pressure**

This example shows very High Sensitvities 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)

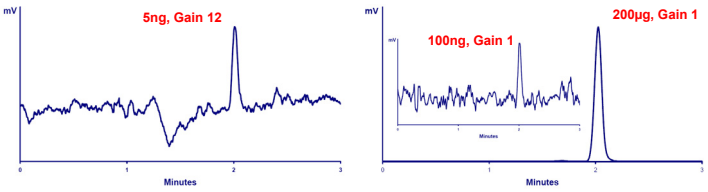
Detector: SEDEX 90LT, 40°C, Gain 5, 3.0bar

Repeatabilty: %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, repeatabilty 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

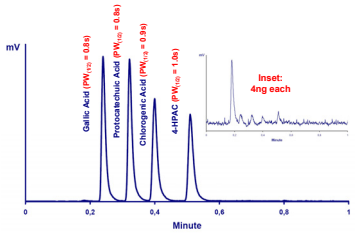
Sample: Hydrocortisone (5ng, 100ng and 200µg on column)
Injection volume: 20µL
Column: Zorbax Eclipse XDB-C18 (5µm, 4.6x150mm), 20°C
Flowrate: 1mL/min
Eluent: H2O/ACN (55:45)

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

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 minimun 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

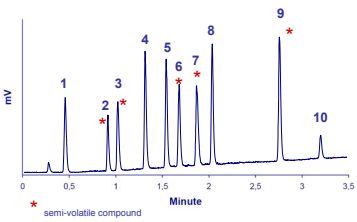


Chromatograms of 4 Phenolic Acids by U-HPLC/LT-ELSD

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), 3.5bar
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: Chloroprocaine; 4: Acebutolol; 5: Sulfamethoxazole; 6: Noscapine; 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

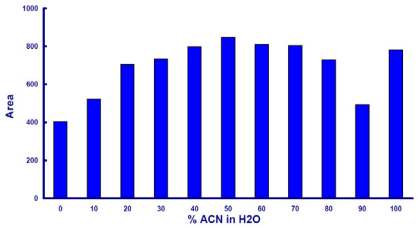
Detector: SEDEX 90LT, 50°C, Gain 10, 3.5bar
Nebulizer: U-HPLC

Courtesy of D. Guillarme, 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, 3.5bar

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 reponse 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.