



**Sensitivity  
Flexibility  
Experience**

# Advantage of a New Generation of Evaporative Light-Scattering Detectors: Universality, Higher Sensitivities and Cost-Effectiveness for Multi-Element Analyses in Liquid Chromatography - An Application Review

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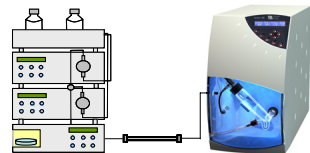
## Abstract

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 the majority of the liquid chromatography applications. Today, the power of this detection mode is further extended with a new model which proposes a genuine and efficient Low-Temperature technology (LT-ELSD™) combined with an innovative detection chamber, thus providing the highest sensitivities with all compounds including semi-volatile and thermo-labile ones.

To show the strength and the versatility of this new ELSD model, several liquid chromatography applications are thoroughly developed in this work. These applications use the most recent liquid chromatography media, such as multi-mode, HILIC and sub-two-micron particle phases, allowing outstanding separations and simultaneous analyses of a wide range of compounds belonging to the major chemical and biochemical classes. This work proposes several generic LC-ELSD methods and includes the analyses of the following groups of compounds:

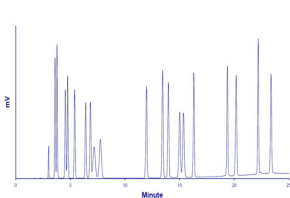
- Carbohydrates (mono-, di- and oligosaccharides) and polyols obtained with a gradient elution.
- Polar and non-polar lipids, which represents a very convenient and efficient alternative to GC analyses.
- Amino acids without any tedious derivation step before detection.
- Inorganic ions without the use of any additional equipment such as ion suppressors.
- Water-soluble and fat-soluble vitamins analyzed simultaneously.
- Organic and phenolic acids.

Validation data are provided and include low limits of detection (down to the sub-nanogram levels) to emphasize the universality and the strength of this outstanding, highly sensitive and cost-effective detection mode.



**HPLC / LT-ELSD System**

## I - Generic HPLC/LT-ELSD Method for Carbohydrates



Chromatogram of the Simultaneous HPLC/LT-ELSD Analysis of Polyols, Mono-, Di- and Oligosaccharides.

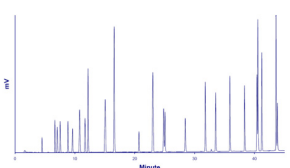
Standard mixture: 20 Compounds (see Table beside)  
Injection volume: 2µL  
Column: Intakt UK-Amino (3µm, 3.0 x 250mm), 60°C  
Flowrate: 0.7mL/min  
Eluent: A: H<sub>2</sub>O - B: ACN  
Gradient: 0-8 minutes: 10%A, 6-20 minutes: from 10%A to 25%A, 20-25 minutes: 25%A

Detector: SEDEX 90LT, 28°C, 3.5Bar

	RT		%RSD (n=6)		LOD (S/N=3)
	Minutes	RT	Response	ng (o.c.)	
1 - Glycerol	3.05	0.07	4.1	145*	
2 - Rhamnose	3.65	0.04	2.0	22.9	
3 - Erythritol	3.85	0.05	1.2	11.7	
4 - Arabinose	4.63	0.06	2.7	20.0	
5 - Xylose	4.79	0.06	1.7	20.0	
6 - Fructose	5.51	0.08	3.1	15.4	
7 - Sorbitol	6.57	0.03	1.0	23.1	
8 - Mannose	6.99	0.04	0.4	20.0	
9 - Galactose	7.37	0.18	2.5	35.3	
10 - Glucose	7.93	0.12	2.1	28.6	
11 - Inositol	12.25	0.05	1.0	8.6	
12 - Sucrose	13.66	0.08	1.7	5.1	
13 - Maltulose	14.19	0.04	1.7	7.1	
14 - Lactose	15.23	0.06	1.5	6.2	
15 - Maltose	15.54	0.04	1.4	6.5	
16 - Trehalose	16.49	0.05	1.8	34.5	
17 - Raffinose	19.54	0.04	3.4	33.7	
18 - Maltotriose	20.30	0.04	0.6	38.7	
19 - Nystose	22.28	0.03	1.4	30.9	
20 - Maltotetraose	23.45	0.03	0.7	34.9	

\* Semi-volatile compound

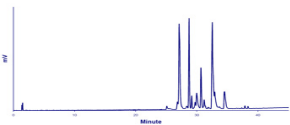
## II - Generic HPLC/LT-ELSD Method for Lipids



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/H<sub>2</sub>O/Formic acid (500:300:198:2) - B: MeOH/ACN/Formic acid (988:400:2)  
Gradient: 0-3 minutes: 100%A, 3-4.3 minutes: from 100%A to 100%B

Detector: SEDEX 90LT, 28°C, 3.5Bar



Chromatogram of the HPLC/LT-ELSD Analysis of Phospholipids.

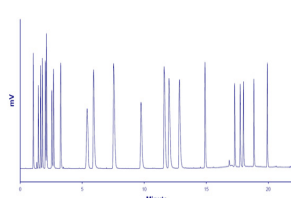
Standard mixture: Phosphatidylcholine, Phosphatidylethanolamine, Phosphatidylinositol, Phosphatidylserine and Sphingomyelin  
Chromatography: same conditions as above

Detector: SEDEX 90LT, 40°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 - α-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 - Diolenin	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

## III - HPLC/LT-ELSD Analysis of Underivatized Amino Acids



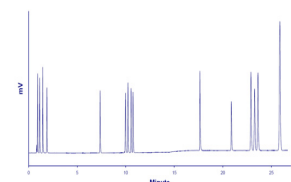
Chromatogram of the Direct HPLC/LT-ELSD Analysis of Amino Acids.

Standard mixture: 22 Compounds (see Table beside)  
Injection volume: 2µL  
Column: Zorbax SB-C18 (1.8µm, 2.1 x 150mm), 40°C  
Flowrate: 0.3mL/min  
Eluent: A: H<sub>2</sub>O + (0.5% TFA, 0.3% HFBA) - B: ACN  
Gradient: 0-3 minutes: 100%A, 3-10 minutes from 9%B to 5%B, 10-20 minutes: from 5%B to 35%B, 20-22 minutes: 35%B

Detector: SEDEX 90LT, 50°C, 3.5Bar

	RT		%RSD (n=6)		LOD (S/N=3)
	Minutes	RT	Response	ng (o.c.)	
1 - Taurine	1.06	0.05	1.7	2.2	
2 - Glycine	1.47	0.04	2.1	2.8	
3 - Aspartic acid	1.64	0.05	1.4	2.3	
4 - Glutamine	1.77	0.04	3.2	3.2	
5 - Glutamic acid	2.01	0.06	1.4	2.5	
6 - Threonine	2.10	0.04	1.0	2.3	
7 - GABA	2.50	0.06	6.1	2.0	
8 - Cysteine	2.65	0.03	3.2	4.3	
9 - Ornithine	3.23	0.05	1.1	2.4	
10 - Lysine	5.17	0.19	1.4	5.2	
11 - Proline	5.65	0.21	1.0	7.5	
12 - Histidine	7.23	0.14	1.6	3.8	
13 - Theanine	9.37	0.14	1.7	6.7	
14 - Arginine	11.29	0.17	1.5	4.5	
15 - Valine	11.77	0.12	1.9	6.3	
16 - Methionine	12.53	0.14	1.9	6.4	
17 - Tyrosine	14.67	0.05	2.4	2.9	
18 - Isoleucine	17.13	0.07	1.8	1.7	
19 - Leucine	17.60	0.03	1.7	1.6	
20 - Norleucine	17.86	0.07	1.3	1.5	
21 - Phenylalanine	18.75	0.03	0.9	1.6	
22 - Tryptophane	19.88	0.06	1.3	1.4	

## IV - Generic HPLC/LT-ELSD Method for Vitamins



Chromatogram of the Simultaneous HPLC/LT-ELSD Analysis of Water- and Fat-Soluble Vitamins.

Standard mixture: 15 Compounds (see Table beside)  
Injection volume: 2µL  
Column: Intakt Scherzo SM-C18 (3µm, 2 x 150mm), 30°C  
Flowrate: 0.3mL/min  
Eluent: A: H<sub>2</sub>O + 0.3% HCOOH - B: ACN  
Gradient: 0-1 minute: 100%A, 0.1-10 minutes: from 0%B to 30%B, 10-11 minutes: 30%B, 11-12 minutes: from 30%B to 100%B, 12-26 minutes: 100%B

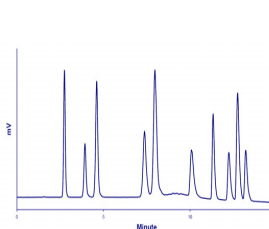
Detector: SEDEX 90LT, 40°C, 3.5Bar

	RT		%RSD (n=6)		LOD (S/N=3)
	Minutes	RT	Response	ng (o.c.)	
1 - B1	0.92	0.11	2.6	5.7	
2 - B8	1.14	0.09	6.2	6.3	
3 - B6	1.44	0.16	2.7	1.8	
4 - C	1.94	0.25	2.4	9.6	
5 - B5	7.32	0.15	6.8	6.9	
6 - B9	9.92	0.09	3.1	8.0	
7 - B12	10.17	0.11	4.1	4.6	
8 - B2	10.49	0.11	3.4	5.0	
9 - B7	10.70	0.09	4.2	4.8	
10 - A	17.64	0.06	6.7	1.7	
11 - K2	20.87	0.07	2.1	14.2	
12 - D2	22.89	0.06	1.5	47.6	
13 - D3	23.26	0.06	2.6	52.8	
14 - E	23.61	0.07	1.6	45.0	
15 - K1	25.86	0.07	1.7	41.7	

## Conclusion

The examples developed in this work demonstrate the Universal applicability of LT-ELSD in HPLC with high sensitivities for various types of compounds such as mono-, di- and oligosaccharides, polyols, fatty acids, fatty alcohols, mono-, di- and triglycerides, phospholipids and other compounds related to lipids, fat- and water-soluble vitamins, amino acids, organic acids, phenolic acids, inorganic anions and cations. These applications using a single detector are straightforward to operate and do not require any additional equipment. The New SEDEX 90LT benefits from both the Low-Temperature technology and a new optical head design based on a laser, which results in an outstanding sensitivity increase with LOD (S/N=3) down to the low-nanogram and even to the sub-nanogram levels (e.g. 500pg for Stearic acid and Octadecanol, 600pg for Mg, 700pg for Eicosanol,...). As a conclusion, the association of the most recent chromatography media and the New generation of high-performance ELSDs provides the analysts with a quite relevant and cost-effective solution to their separation and quantification challenges.

## V - Generic HPLC/LT-ELSD Method for Inorganic Ions



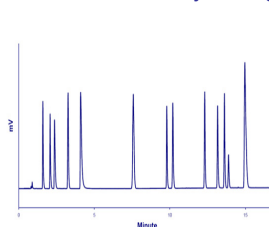
Chromatogram of the Simultaneous HPLC/LT-ELSD Analysis of Inorganic Anions and Cations.

Standard mixture: 10 Compounds (see Table beside)  
Injection volume: 2µL  
Column: ZIC-HILIC (3.5µm, 2.1 x 150mm), 40°C  
Flowrate: 0.3mL/min  
Eluent: A: Ammonium formate 20mM, pH 3 - B: ACN  
Gradient: 0-3 minutes: 20%A, 3-10 minutes: from 20%A to 80%A, 10-15 minutes: 80%A

Detector: SEDEX 90LT, 40°C, 3.5Bar

	RT		%RSD (n=10)		LOD (S/N=3)
	Minutes	RT	Response	ng (o.c.)	
1 - NO3	2.76	0.1	2.2	5.8	
2 - Br	3.93	0.07	3.1	6.7	
3 - Cl	4.60	0.09	4.0	8.1	
4 - K	7.21	0.13	1.9	9.5	
5 - Na	7.80	0.12	1.7	3.5	
6 - PO4	10.05	0.06	3.1	15.4	
7 - SO4	11.22	0.04	2.8	10.3	
8 - Zn	12.05	0.07	2.9	6.7	
9 - Mg	12.59	0.09	3.2	0.6	
10 - Ca	13.06	0.17	2.5	1.3	

## VI - HPLC/LT-ELSD Analysis of Organic Acids



Chromatogram of the Simultaneous HPLC/LT-ELSD Analysis of Organic and Phenolic Acids.

Standard mixture: 12 Compounds (see Table beside)  
Injection volume: 2µL  
Column: Intakt Scherzo SM-C18 (3µm, 2 x 150mm), 30°C  
Flowrate: 0.3mL/min  
Eluent: A: H<sub>2</sub>O + 0.3% HCOOH - B: ACN + 1% HCOOH  
Gradient: 0-2 minutes: 100%A, 2-10 minutes: from 9%B to 20%B, 10-15 minutes: 20%B

Detector: SEDEX 90LT, 30°C, 3.5Bar

	RT		%RSD (n=8)		LOD (S/N=3)
	Minutes	RT	Response	ng (o.c.)	
1 - Quinic acid	1.60	0.09	2.1	4.5	
2 - Malic acid	2.08	0.09	1.9	7.7	
3 - Tartaric acid	2.37	0.11	2.7	20.9	
4 - Succinic acid	3.27	0.20	2.2	9.0	
5 - Citric acid	4.11	0.25	1.3	25.3	
6 - Gallic acid	7.60	0.31	1.9	8.0	
7 - Lactic acid	9.81	0.18	2.4	55.9*	
8 - Protocatechuic acid	10.21	0.14	2.4	3.7	
9 - 4-HPAC	12.32	0.09	1.7	3.4	
10 - Chlorogenic acid	13.16	0.07	2.2	4.0	
11 - Syringic acid	13.62	0.07	3.0	2.3	
12 - Genisteic acid	14.97	0.06	1.9	6.2	

\* Semi-volatile compound