A New Generation of Evaporative Light-Scattering Detectors for Liquid Chromatography - Universality, Reliability and Cost-Effectiveness in Food Analysis

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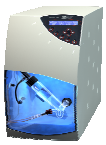


Abstract:

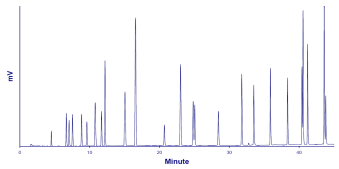
Food and beverage safety and compositional information during the full process requires increasing quantities of complete, reliable, fast and cost-effective analyses. Therefore, development of analytical methods using HPLC and U-HPLC gained an increasing importance in Food industry during the last decades. The goals of such analytical work are various, but are mainly oriented towards monitoring of the chemical and biochemical content in R&D, quality control at all stages of production and preservation, and products authenticity.

Among the detectors available in Liquid Chromatography (LC), 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, reliable and cost-effective technique, and is ideally appropriate in Food industry for a great variety of LC applications containing chromophoric and non-chromophoric compounds. Today, the power of this detection mode is further extended with the ultimate 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 ELSD model, several LC applications in Food analysis are developed in this work. These applications use the most recent LC media, such as multi-mode, HILIC and sub-two-micron particle phases, allowing outstanding separations and simultaneous analyses of a wide range of compounds. This work proposes several selected LC-ELSD methods and includes the analyses of the following groups of compounds:

- *Lipids, phospholipids.*
- *Sugars, polyols, sweeteners.*
- *Amino acids, peptides, proteins.*
- *Organic acids, phenolics.*
- *Vitamins.*
- *Inorganic ions.*
- *Food additives.*
- *Surfactants.*

Global HPLC/LT-ELSD Method for Lipids



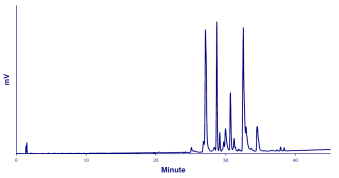
Chromatogram of the Simultaneous HPLC/LT-ELSD Analysis of Fatty Acids, Fatty Alcohols, Fat-Soluble Vitamins, Mono-, Di- and TriGlycerides and Related Compounds.

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	29.30	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**

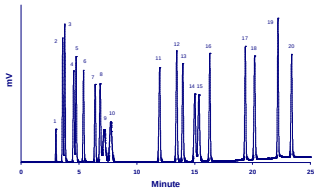


Chromatogram of the HPLC/LT-ELSD Analysis of Phospholipids.

Standard mixture: Phosphatidylcholine, Phosphatidylethanolamine, Phosphatidylinositol, Phosphatidylserine and Sphingomyelin
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, 40°C, 3.5bar

Global HPLC/LT-ELSD Method for Carbohydrates

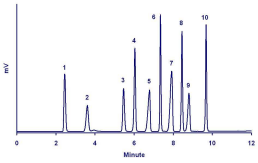


Chromatogram of the Simultaneous HPLC/LT-ELSD Analysis of Polyols, Mono-, Di- and Oligosaccharides.

Standard mixture: 1 - Glycerol, 2 - Rhamnose, 3 - Erythritol, 4 - Arabinose, 5 - Xylose, 6 - Fructose, 7 - Sorbitol, 8 - Mannose, 9 - Galactose, 10 - Glucose, 11 - Inositol, 12 - Sucrose, 13 - Maltulose, 14 - Lactose, 15 - Maltose, 16 - Trehalose, 17 - Raffinose, 18 - Maltotriose, 19 - Nystose, 20 - Maltotetraose, (Concentration: 100ppm to 500ppm).
Injection volume: 2µL
Column: Imtakt UK-Amino (3µm, 3.0 x 250mm), 60°C
Flowrate: 0.7mL/min
Eluent: A: H2O - B: ACN
Gradient: 0-6 minutes: 10%A, 6-20 minutes: from 10%A to 25%A, 20-25 minutes: 25%A

Detector: SEDEX 90LT, 28°C, 3.5bar

Global HPLC/LT-ELSD Method for Natural and Artificial Sweeteners

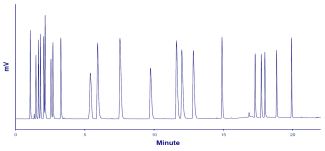


Chromatogram of the Simultaneous HILIC/LT-ELSD Analysis of Natural and Artificial Sweeteners including Polyols.

Standard mixture: 1 - Erythritol, 2 - Sucralose, 3 - Sorbitol, 4 - Neotame, 5 - Acesulfame, 6 - Neohesperidin dihydrochalcone, 7 - Saccharin, 8 - Aspartame, 9 - Cyclamate, 10 - Rebaudioside A (from *Stevia*), (Concentration: 500ppm each).
Injection volume: 2µL
Column: Hypersil GOLD HILIC (**1.9µm**, 2.1 x 150mm), 40°C
Flowrate: 0.5mL/min
Eluent: Ammonium acetate 50mM, pH5 (A) / ACN (B)
Gradient: 0-0.5 minute: 5%A, 0.5-10 minutes: from 5%A to 30%A

Detector: SEDEX 90LT, 35°C, 3.5bar

Direct HPLC/LT-ELSD Analysis of Underivatized Amino Acids



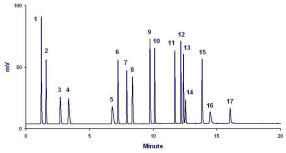
Chromatogram of the Direct HPLC/LT-ELSD Analysis of Amino Acids.

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: H2O + (0,5% TFA, 0,3% HFBA) - B: ACN
Gradient: 0-3 minutes: 100%A, 3-10 minutes from 0%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

Global HPLC/LT-ELSD Method for Amino Acids, Peptides and Proteins

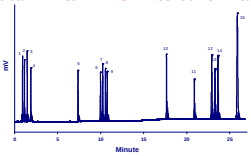


Chromatogram of the Direct and Simultaneous HPLC/LT-ELSD Analysis of Amino Acids, Peptides and Proteins.

Standard mixture: 1 - GLY, 2 - PRO, 3 - VAL, 4 - MET, 5 - LEU, 6 - TYR, 7 - GLY-TYR, 8 - PHE, 9 - TRY, 10 - VAL-TYR-VAL, 11 - MET-Enkephalin, 12 - Angiotensin II, 13 - LEU-Enkephalin, 14 - Ribonuclease A, 15 - Cytochrome C, 16 - Holo-Transferrin, 17 - Apomyoglobin (# 250ppm each)
Injection volume: 2µL
Column: Ascentis Express Peptide ES-C18 (**2.7µm**, 2.1 x 150mm), 25°C
Flowrate: 0.3mL/min
Eluent: H2O + 0.1% TFA (A) / ACN + 0.1% TFA (B)
Gradient: 0-0.5 minute: 2%B, 0.5-15 minutes from 2%B to 60%B

Detector: SEDEX 90LT, 50°C, 3.5bar

Global HPLC/LT-ELSD Method for Vitamins

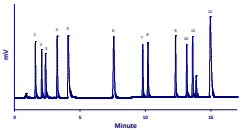


Chromatogram of the Simultaneous HPLC/LT-ELSD Analysis of Water- and Fat-Soluble Vitamins.

Standard mixture: 1 - Thiamin (B1), 2 - myo-Inositol (B8), 3 - Pyridoxine (B6), 4 - Ascorbic acid (C), 5 - Pantothenic acid (B5), 6 - Folic acid (B9), 7 - Cyanocobalamine (B12), 8 - Riboflavin (B2), 9 - Biotin (B7), 10 - Retinol (A), 11 - Menaquinone (K2), 12 - Ergocalciferol (D2), 13 - Cholecalciferol (D3), 14 - a-Tocopherol (E), 15 - Phylloquinone (K1), (Concentration: 80ppm to 200ppm).
Injection volume: 2µL
Column: Imtakt Scherzo SM-C18 (3µm, 2 x 150mm), 30°C
Flowrate: 0.3mL/min
Eluent: A: H2O + 0,3% HCOOH - B: ACN
Gradient: 0-0.1 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

HPLC/LT-ELSD Analysis of Organic Acids

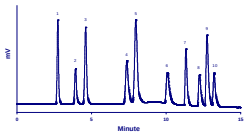


Chromatogram of the Simultaneous HPLC/LT-ELSD Analysis of Organic and Phenolic Acids.

Standard mixture: 1 - Quinic acid, 2 - Malic acid, 3 - Tartaric acid, 4 - Succinic acid, 5 - Citric acid, 6 - Gallic acid, 7 - Lactic acid, 8 - Protocatechuic acid, 9 - 4-Hydroxyphenylacetic acid, 10 - Chlorogenic acid, 11 - Syringic acid, 12 - Gentisic acid, (Concentration: 80ppm to 150ppm).
Injection volume: 2µL
Column: Imtakt Scherzo SM-C18 (3µm, 2 x 150mm), 30°C
Flowrate: 0.3mL/min
Eluent: A: H2O + 0,3% HCOOH - B: ACN + 1% HCOOH
Gradient: 0-2 minutes: 100%A, 2-10 minutes: from 0%B to 20%B, 10-15 minutes: 20%B

Detector: SEDEX 90LT, 30°C, 3.5bar

Global HPLC/LT-ELSD Method for Inorganic Ions

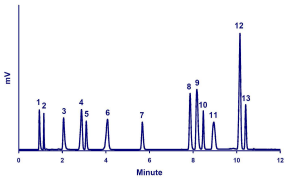


Chromatogram of the Simultaneous HPLC/LT-ELSD Analysis of Inorganic Anions and Cations.

Standard mixture: 1 - NO_3, 2 - **Br**, 3 - **Cl**, 4 - **K**, 5 - **Na**, 6 - PO_4, 7 - SO_4, 8 - **Zn**, 9 - **Mg**, 10 - **Ca**, (Concentration: 80ppm to 200ppm).
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

Global HPLC/LT-ELSD Method for Food Additives and Supplements

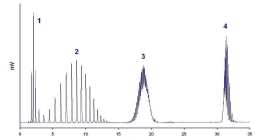


Chromatogram of the Simultaneous HILIC/LT-ELSD Analysis of Several Food Additives and Supplements.

Standard mixture: 1 - Caffeine, 2 - Vitamin B3, 3 - Vitamin B6, 4 - Chloride, 5 - Erythritol, 6 - Vitamin C, 7 - Xylitol, 8 - Potassium, 9 - Sodium, 10 - Rebaudioside A (from *Stevia*), 11 - Taurine, 12 - Magnesium, 13 - Glutamic acid, (Concentration: 500ppm to 1000ppm).
Injection volume: 1µL
Column: Ascentis Express HILIC (**2.7µm**, 2.1 x 150mm), 30°C
Flowrate: 0.5mL/min
Eluent: Ammonium formate 200mM, pH3 (A) / ACN (B)
Gradient: 0-1 minute: 8%A, 1-8 minutes: from 8%A to 25%A, 8-12 minutes: 25%A

Detector: SEDEX 90LT, 40°C, 3.5bar

Simultaneous HPLC/LT-ELSD Analysis of Several Surfactants



HPLC/LT-ELSD Chromatogram of four Surfactants

Standard mixture: 1 - PEG 200, 2 - PEG 600, 3 - PEG 2000, 4 - Triton X100
Injection volume: 2µL
Column: Acclaim Surfactant Plus (3µm, 3 x 150mm), 30°C
Flowrate: 0.6mL/min
Eluent: Ammonium acetate 100mM, pH5 (A) / Acetonitrile (B)
Gradient: 0-0.1 minute: 2%B, 0.1-20 minutes: from 2%B to 20%B, 20-30 minutes: from 20%B to 50%B

Detector: SEDEX 90LT, 35°C, 3.5bar

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 fatty acids, fatty alcohols, mono-, di- and triglycerides, phospholipids and other compounds related to lipids, mono-, di- and oligosaccharides, polyols, natural and artificial sweeteners, fat- and water-soluble vitamins, amino acids, peptides, proteins, organic acids, phenolic acids, inorganic anions and cations, surfactants… These applications using a **single Universal 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, **700pg** for Eicosanol).*

. As a conclusion, the association of the most recent chromatography media and the New generation of high-performance LT-ELSDs provides the analysts with a quite relevant, reliable and cost-effective solution to their separation and quantification challenges.