A New Generation of Evaporative Light-Scattering Detectors for Liquid Chromatography - Universality, High Performance and Robustness in Pharmaceutical Analysis

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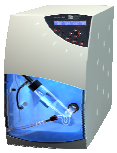


Abstract:

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 Pharmaceutical industry for a great variety of LC applications containing chromophoric and non-chromophoric compounds.

Today, an ELSD model based on a recent and unique concept is proposed which offers a genuine and efficient Low-Temperature technology (LT-ELSD™) combined with an innovative detection chamber. The overall design of this ultimate detector results in a significant increase of sensitivity providing typical limits of detection down to the very low nanogram levels for non-volatile and semi-volatile compounds. It provides an improved overall direct linearity with correlation coefficients over 0.99, consistent responses independent of the analytes chemical structure and an extended dynamic range exceeding the four orders of magnitude (from ng to µg on column). Also, this model is optimized for the recent U-HPLC technique giving peak widths of less than 1 second.

To show the strength and the versatility of this ELSD model, several relevant LC applications in Pharmaceutical 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.

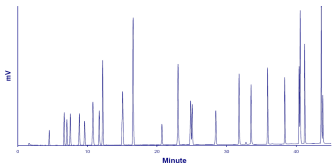
The topics that are presented and detailed here focus on:

- Sensitivity, repeatability and robustness in LC on the full pH range (acidic and basic mobile phases).
- Rapid and simultaneous separation of API, impurities and counterions.
- Relevance in the analysis of natural products or TCM, such as Ginkgo Biloba.
- Simplified and cost-effective alternative in the analysis of non-chromophoric compounds such as Aminoglycoside antibiotics, thus avoiding any tedious derivatization step.
- Response consistency in comparison with other detectors.

This work particularly emphasizes the great advancement of ELSD technology, thus offering the analysts a relevant, reliable and cost-effective solution or alternative to their chromatography challenges.

I - Sensitivity, Repeatability and Robustness

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

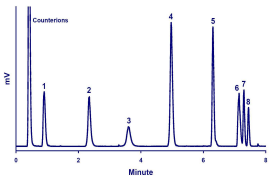
Detector: SEDEX 90LT, 28°C, 3.5bar

	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	29.20	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	40.07	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 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 semi-volatility feature with high vapour pressure), and even at the **Picogram Levels** for other semi-volatile compounds such as Fatty alcohols and some Fatty acids.

Simultaneous U-HPLC/LT-ELSD Analysis of Beta Blockers and Tricyclic Antidepressants at pH:11



Chromatogram of the Simultaneous U-HPLC/LT-ELSD Analysis of Several Beta Blockers and Tricyclic Antidepressants

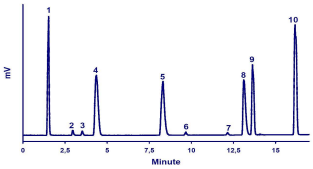
Standard Mixture: 1 - Atenolol, 2 - Pindolol, 3 - Acebutolol, 4 - Metropolol, 5 - Propanolol, 6 - Nortriptyline, 7 - Imipramine, 8 - Amitriptyline (500ppm each)
Injection volume: 1µL
Column: Zorbax Extend C18 (**1.8µm**, 2.1 x 50mm), 40°C
Flowrate: 0.3mL/min
Eluent: H2O + Triethylamine 20mM, pH:11 (A) / Methanol (B)
Gradient: 0-0.5 minute: 35%B, 0.5-4 minutes: from 35%B to 95%B, 4-8 minutes: 95%B

Detector: SEDEX 90LT, 40°C, 3.5bar

This example shows the high robustness of SEDEX 90LT on all pH range: SEDEX 90LT is not affected by very basic buffers such as 20mM triethylamine (pH:11), and provides a nice and flat baseline with no drift all along the gradient.

II - Rapid and Simultaneous Analysis of API, Counterions and Impurities

Global HPLC/LT-ELSD Method for the Simultaneous Analysis of Polar and Non-Polar, Neutral, Acidic and Basic Pharmaceutical Drugs and their Respective Counterions



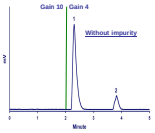
Multimodal Stationary Phase HPLC/LT-ELSD Chromatogram of the Simultaneous Analysis of Polar and Non-Polar, Neutral, Acidic and Basic Pharmaceutical Drugs and their Counterions.

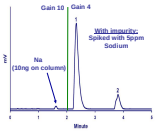
Standard mixture: 1 - Acetaminophen, 2 - Sodium, 3 - Potassium, 4 - Hydrocortisone, 5 - Procainamide, 6 - Chloride, 7 - Nitrate, 8 - Miconazole, 9 - Losartan, 10 - Dichlofenac (API 500ppm each)
Injection volume: 2µL
Column: Acclaim Trinity P1 (3µm, 2.1 x 150mm), 30°C
Flowrate: 0.35mL/min
Eluent: 80% Ammonium acetate 20mM, pH:5 + 20% ACN (A) / 30% Ammonium formate, 200mM, pH:3 + 70% ACN (B)
Gradient: 0-2 minutes: 0%B, 2-17 minutes: from 0%B to 100%B

Detector: SEDEX 90LT, 40°C, 3.5bar

This chromatogram shows that the combination of an efficient multimodal stationary phase and a single Universal ELS detector allows the quick and easy simultaneous determination of a wide range of compounds characterized by different polarities, including inorganic anions and cations.

Simultaneous HPLC/LT-ELSD Analysis of Imipramine, its Counterion and an Impurity





Chromatograms of the Simultaneous HPLC/LT-ELSD Analysis of Imipramine and its Counterion, with and without an Impurity (Sodium, 5ppm)

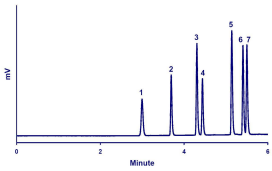
Standard mixture: 1 - Imipramine (API: 10 000ppm), 2 - Cl (Counterion)
Injection volume: 2µL (20µg API)
Column: Acclaim Trinity P1 (3µm, 2.1 x 150mm), 35°C
Flowrate: 0.5mL/min
Eluent: Ammonium acetate 50mM, pH:5 / ACN (60:40)

Detector: SEDEX 90LT, 40°C, at 0 minute: Gain 10, at 2 minutes: Gain 4, 3.5bar

In this example, SEDEX 90LT detects an impurity (sodium) at a level of **0.05%** of the major compound (Imipramine). With this detector, there are 12 gains available, which means that this percentage could typically go down to **less than 0.01%** (more than 4 orders of magnitude for the overall dynamic range).

III - Analysis of Natural Products (e.g.: TCM)

Simultaneous U-HPLC/LT-ELSD Analysis of Terpenic Lactones and Flavonoids Contained in Ginkgo Biloba



Chromatogram of 4 Terpenic Lactones and 3 Flavonoids by U-HPLC/LT-ELSD

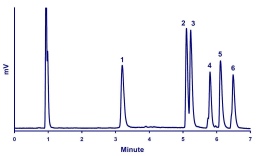
Standard mixture: 1- Bilobalide, 2 - Ginkgolide C, 3 - Ginkgolide A, 4 - Ginkgolide B, 5 - Quercetin, 6 - Isorhamnetin, 7 - Kaempferol (250ppm each)
Injection volume: 1µL
Column: Hypersil GOLD (**1.9µm**, 2.1 x 50mm), 30°C
Flowrate: 0.6mL/min
Eluent: H2O + 0.1% formic acid / Acetone + 0.1% formic acid
Gradient: 0-0.5 minute: 5%B, 0.5-4 minutes: from 5%B to 50%B, 4-6 minutes: 50%B

Detector: SEDEX 90LT, 50°C, 3.5bar

SEDEX 90LT allows the simultaneous determination of both chromophoric (flavonoids) and non-chromophoric (terpenic lactones) compounds, which shows the great advantage of this single, easy to operate and Universal detection mode. Elsewhere, it allows the use of acetone which is less toxic and cheaper than many other organic eluents (such as acetonitrile), and which possesses excellent physical and chromatography properties.

IV - Analysis of Non-Chromophoric Antibiotics

Direct HILIC/LT-ELSD Analysis of Aminoglycoside Antibiotics



Chromatogram of 6 Aminoglycoside Antibiotics by HILIC/LT-ELSD

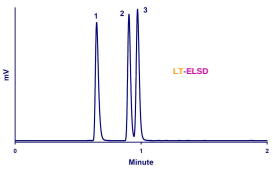
Standard mixture: 1 - Streptomycin, 2 - Amikacin, 3 - Kanamycin, 4 - Paromomycin, 5 - Tobramycin, 6 - Neomycin (1000ppm each)
Injection volume: 2µL
Column: Ascentis Express HILIC (**2.7µm**, 2.1 x 150mm), 30°C
Flowrate: 0.5mL/min
Eluent: Ammonium acetate 150mM, pH:5 (A) / ACN (B)
Gradient: 0-1 minute: 60%B, 1-5 minutes: from 60%B to 5%B, 5-7 minutes: 5%B

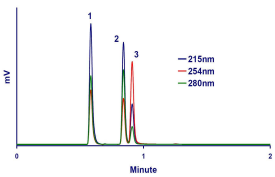
Detector: SEDEX 90LT, 60°C, 3.5bar

SEDEX 90LT allows the direct determination of aminoglycoside antibiotics. With these chromatography conditions, Gentamicin congeners (not shown here) elute after 7 minutes.

V - Response Consistency: ELSD vs. DAD

Fast HPLC/LT-ELSD/DAD Analysis of Non-Volatile Compounds with Different Chemical Structures





Chromatograms of the HPLC/LT-ELSD/DAD Analysis of 5-Fluorocytosine, Theophylline and Acetaminophen

Standard Mixture: 1 - 5-Fluorocytosine, 2 - Theophylline and 3 - Acetaminophen (500ppm each)
Injection volume: 2µL
Column: Halo C18 (**2.7µm**, 2.1 x 150mm), 30°C
Flowrate: 0.5mL/min
Eluent: H_2O / ACN (85:15)

Detector: SEDEX 90LT, 50°C, 3.5bar

Conclusion:

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

- Sensitivity with low nanogram and even sub-nanogram levels.
- Robustness at all pH.
- Simultaneous analysis of both chromophoric and non-chromophoric solutes, using just a single Universal detector.
- Wide dynamic range allowing a sensitive impurity assessment.
- Very small response variation between different compounds with identical concentrations, compared to UV detectors.
- Use of acetone in the mobile phase, which cannot be selected with UV detectors due to the high cutoff.

This work also demonstrates the significant advancement of the new SEDEX Evaporative Light-Scattering Detector resulting from the combination of an efficient and genuine Low-Temperature technology and an innovative detection device based on a high-performance laser. These outstanding new features offer now to the analyst a Universal, powerful, reliable, versatile and cost-effective solution to their separation and quantification challenges in Pharmaceutical area.