Application Note: ANCCSLCELSDLIP

Generic HPLC-ELSD Method for Lipids

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Key Words

- Hypersil GOLD 1.9 µm
- Lipid
- Evaporative light scattering
- HPLC
- Fatty acids
- Glycerides

Abstract

This application note highlights the analysis of a mixture of twenty five fatty acids using a reversed-phase HPLC method with a 1.9 µm column and evaporative light scattering detection.

Introduction

Lipids are a diverse set of molecules, the analysis of which has come to the fore due to a greater interest in lipidomics and the drive to analyze the lipidome to provide further insight into disease states in clinical applications, as well as understanding normal physiological homeostasis.

Routine lipid analysis has historically been done by TLC. However, more recently faster technologies with better resolution have been developed including high throughput HPLC-MS and GC-MS systems. Establishing good separation, both analyte from analyte and analyte from matrix components is key to the success of these analyses. The Thermo Scientific Hypersil GOLD range of HPLC columns was developed to give reproducible and reliable chromatographic analysis with excellent peak shape. Greater resolution can be achieved through increased efficiency when using sub 2 µm particle size products. This application note demonstrates the effective separation of a number of typical compounds in the field of lipidomic analysis and their detection at low nanogram levels using a light scattering detector, the SEDEX LT-ELSD™.

Chemicals and Reagents	Part Number
Fisher Scientific HPLC grade methanol	M/4056/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Fisher Scientific HPLC grade formic acid	F/1900/PB15
Fisher Scientific HPLC grade acetone	A/0600/17
Fisher Scientific HPLC grade water	W/0106/17

Separation Conditions		Part Number
Column:	Hypersil GOLD® 1.9 µm, 200 x 2.1 mm	25002-202130

Mobile Phase			
Mobile phase:	A – 50:30:19.8:0.2 (v/v) methanol / acetonitrile / water / formic acid		
	B – 59.8:40:0.2 (v/v) methanol / acetone / formic acid		
Gradient:	Time (min)	%A	%B
	0	100	0
	3	100	0
	43	0	100
Flow rate:	0.3 mL/min		
Run time:	45 minutes		
Column temperature:	60 °C		
Injection volume:	2 µL		
Detector:	SEDEX 90LT evaporative light scattering, 28 °C, 3.5 bar pressure		

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Experimental Details

Results

Good peak shape and resolution was achieved for the analysis of twenty five fatty acids, fatty alcohols, fat soluble vitamins, mono-, di-and tri-glycerides and related compounds. Detection was by evaporative light scattering detector. RSD values for retention time were less than 0.25 % and for detector response less than 5 % indicating good levels of reproducibility and assay stability, which is essential when comparing results across different data sets. Limits of detection (defined as signal/noise ratio >3) ranged from 0.5 ng on column to 5.7 ng on column with the exception of the semi-volatile component lauric acid where the limit of detection was 16.2 ng on column.

Thermo Fisher Scientific recognize the contribution of Dr Eric Verette (SEDERE, France), for providing the experimental data from which this application note is derived.

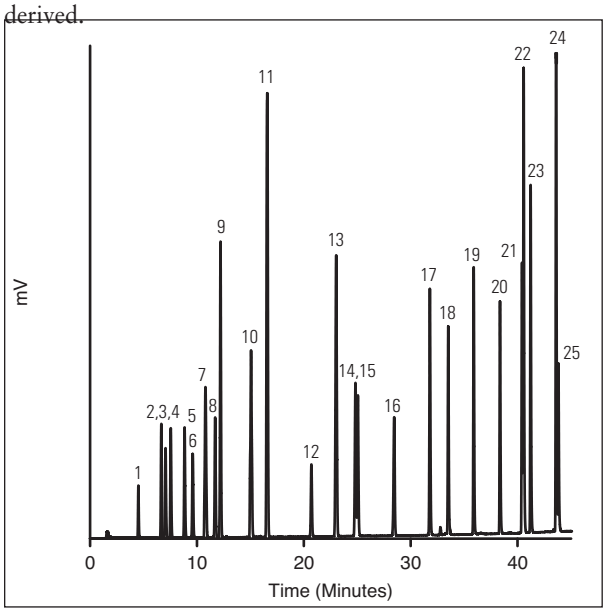

Figure 1: Chromatogram showing the analysis of twenty five lipid related compounds by HPLC-LT/ELSD. Compounds and peak numbering are provided in Table 1

Conclusions

This application note demonstrates that good chromatographic resolution can be obtained using a small particle size Hypersil GOLD 1.9 µm column. This coupled with the SEDEX LT-ELSD™ provides an effective way of analyzing and quantifying a broad range of lipid related compounds.

		t_R	% RSD (n=6)		LOD
ID	Compound	Minutes	t_R	Detector 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 (vitamin 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 (vitamin E)	25.80	0.05	2.9	3.8
16	vitamin K	29.80	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

Table 1: List of compounds analyzed showing retention time, %RSD for retention time and response, and limits of detection. ng (o.c.): amount on column (ng)

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