SHIMADZU
Excellence in Science

Application News

No.L505

High Performance Liquid Chromatography

Comprehensive 2D Separation of Lipid Species in Mussels Using the Nexera-e System Combined with LCMS-IT-TOF Detection

In metabolomics research, lipidomics is the name given to the comprehensive analysis of lipids. Lipids play a key role in living organisms, where they provide an energy source, an important structural component of biological membranes, and are involved in interorgan signaling. There are many different structural fatty acids and many combinations of degrees of lipid unsaturation, and performing a comprehensive simultaneous analysis of lipids has been regarded as difficult.

A lipidomics research workflow is shown in Fig. 1. In a typical analysis, the whole lipids extracted from a biological sample are fractionated based on lipid classes using normal-phase chromatography or hydrophilic interaction liquid chromatography (HILIC), and then LC/MS analysis is performed for each fraction obtained. One drawback of this method is that it requires a lot of time. Comprehensive two-dimensional (2D) liquid chromatography combines two independent separation modes. The different separation selectivities of the two dimensions are combined to improve the separation of components that are difficult to separate by single dimensional analysis.

This article presents an example separation of lipids in mussels using the Nexera-e, which is an effective system for the comprehensive separation of a large number of lipid molecules. A semi-micro-scale separation via the HILIC column was used for the first dimension, and an ultra-high-speed reversed-phase separation was used for the second dimension. An ion trap time-of-flight mass spectrometer (LCMS-IT-TOF) was combined with the Nexera-e to perform detection. The analytical conditions are shown in Table 1.

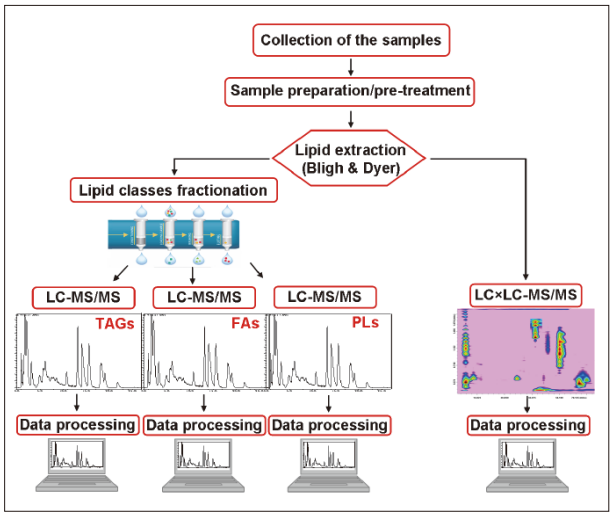

Fig. 1 Lipidomics Research Workflow

Comprehensive Separation of Lipid Species in Mussels with LCMS-IT-TOF Detection

Mussels are sedentary bivalves that grow in a wide variety of coastal waters throughout the world. Lipid molecules inside the body are known to vary in quantity dependent on the external environment. For example, there tends to be a higher quantity of triacylglycerols in spring and summer, and a higher quantity of phospholipids in autumn and winter. There is no substantial seasonal variation in cholesterol. Fatty acids are also used as a biomarker to check dietary status.

Because the lipid profile of mussels is affected by oxidization and hydrolysis caused by environmental pollutants such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs), mussels show promise as an index organism for determining environmental pollution. In this way, developing a simple analytical method that can provide an overall picture of lipids has important implications.

Table 1 Analytical Conditions

[Sample Preparation]	: Mussels tissue were pooled, homogenized and treated with Bligh/Dyer method to extract the total lipids
[Column1]	: Ascentis Express HILIC column (Supelco, 150 × 2.1 mm; 2.7 µm fused core particles)
Mobile Phase	: A; Acetonitrile: 10 mM Ammonium formate = 98:2 (v/v) B; Acetonitrile: MeOH: 10 mM Ammonium formate = 55:35:10 (v/v/v)
Time Program	: B.Conc. 0 % (0-15 min) → 100 % (40-80 min)
Flowrate	: 50 µL/min
Column Temp.	: 55 ˚C
Injection Volume	: 5 µL
Modulation Time	: 2.0 min
[Column2]	: Titan C18 column (Supelco, 50 × 4.6 mm; 1.9 µm)
Mobile Phase	: A; Acetonitrile: Methanol: 10 mM Ammonium formate = 70:25:5 B; 0.1 % Formic acid in Isopropanol
Time Program	: [0-40 min] B.Conc. 0 % (0-0.35 min) → 15 % (1 min) → 20 % (1.01 min) → 70 % (1.7 min) → 100 % (1.75-1.85 min) → 0 % (1.86-2 min) [40-62 min] B.Conc. 0 % (0-0.75 min) → 20 % (0.76-1.01 min) → 70 % (1.7 min) → 100 % (1.75-1.85 min) → 0 % (1.86-2 min) [62-80 min] B.Conc. 0 % (0-1.01 min) → 20 % (1.85 min) → 0 % (1.86-2 min)
Flowrate	: 3 mL/min was splitted to 0.3 mL/min for MS
Detector	: LCMS-IT-TOF (ESI positive/negative mode) Scan range ESI (+); *m/z* 300-1000, ESI (-); *m/z* 200-1000, for MS/MS; *m/z* 50-1000
2D Data Processing	: ChromSquare 2.0 software was used to covert raw data into 2D plot

The top image in Fig. 2 shows a comprehensive 2D plot of lipid molecular groups in mussels. The PE and PI groups are well-detected using ESI (-), and these groups have been overlaid onto the ESI (+) 2D plot.
Whole lipid molecular species extracted from mussels were separated in the first dimension HILIC column based on the polar portions of the molecules. In order of elution from the earliest, the lipid groups confirmed upon first dimension separation were [triacylglycerols (TAGs), cholesterol esters, sterols, monoacylglycerols (MGs), diacylglycerols (DGs)], phosphatidylinositol (PI), phosphatidylethanolamine (PE), lysophosphatidic acid (LysoPA), phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylcholine (PC), and lysophosphatidylcholine (LysoPC). Next, elution from the second dimension reverse-phase separation was confirmed in order of partition number (PN), which is the total carbon number minus two times the number of double bonds.

A LCMS-IT-TOF system capable of accurate mass measurements was then used as the detector for the second dimension separation, which allowed detailed qualitative analysis of multiple components eluted. The peak portion inside the white oval in Fig. 2 contains two triacylglycerols with the same PN, and the MS/MS spectrum of each is shown below in Fig. 2. Based on the structural information obtained from the peak of a diacylglycerol with one side chain removed, the structures of the two triacylglycerols were identified as OOP and OOO.
P: Palmitic acid
O: Oleic acid
As described in this article, comprehensive 2D separation combined with LCMS-IT-TOF can provide total lipid profiles that change depending on differences in external environment, and can be used to analyze a marine organism and changes in its lipid species as a biomarker.

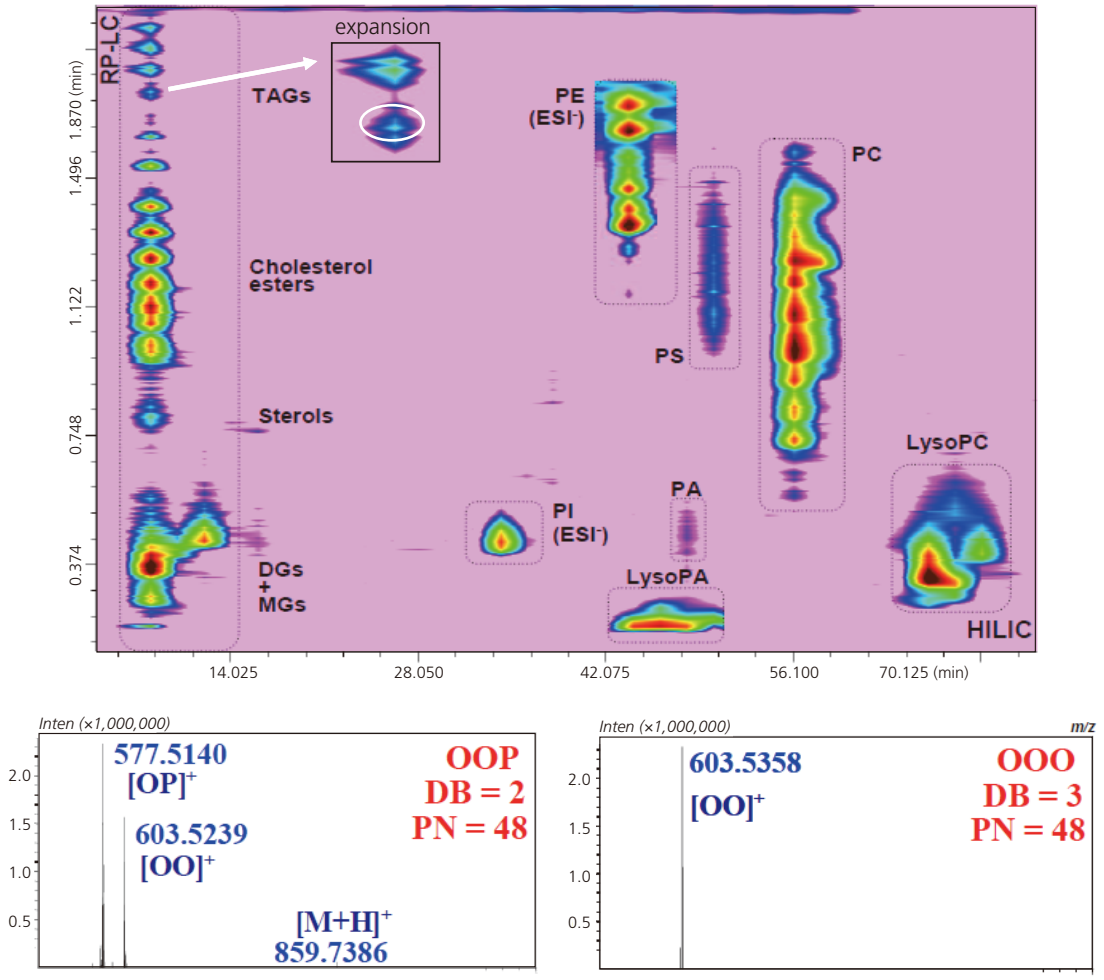

Fig. 2 Comprehensive 2D Plot Obtained from Analysis of Whole Lipids of Mussels with LCMS-IT-TOF, and MS/MS-spectra of Specific Areas

Data provided by Prof. Luigi Mondello of University of Messina and Chromaleont S.r.l.

First Edition: Jun. 2016

Shimadzu Corporation
www.shimadzu.com/an/