Waters™

應用手冊

Real Time Lipidomic Profiling Using Desorption Ionization with Ion Mobility MS

Jordan Krechmer, Kieran J. Neeson, Giorgis Isaac, Joseph Tice, Marc V. Gorenstein, Alan L. Millar, Michael P. Balogh, James I. Langridge, Giuseppe Astarita

Waters Corporation

Abstract

In this application note, a rapid (few seconds), real-time method using DI in combination with post-ionization ion mobility separation to analyze lipidomic profiles in food and biological samples is presented.

Benefits

The combination of real time desorption ionization and ion mobility MS offers a convenient solution for phenotypic identification and comparative lipidomic analysis.

Introduction

Lipids are major constituents of food and biological tissues. Among lipid key properties are those to determine the caloric content, texture, and taste of food. Besides their importance in food and nutrition, lipid composition affects the physiology of living cells. Alterations in lipid profiles have been implicated in a wide range of

pathologies in many types of organisms including plants and humans. Therefore, assessing lipid profiles and ratios between various lipid species can be indicative of the quality of food or health status of living organisms, as shown in Figure 1.

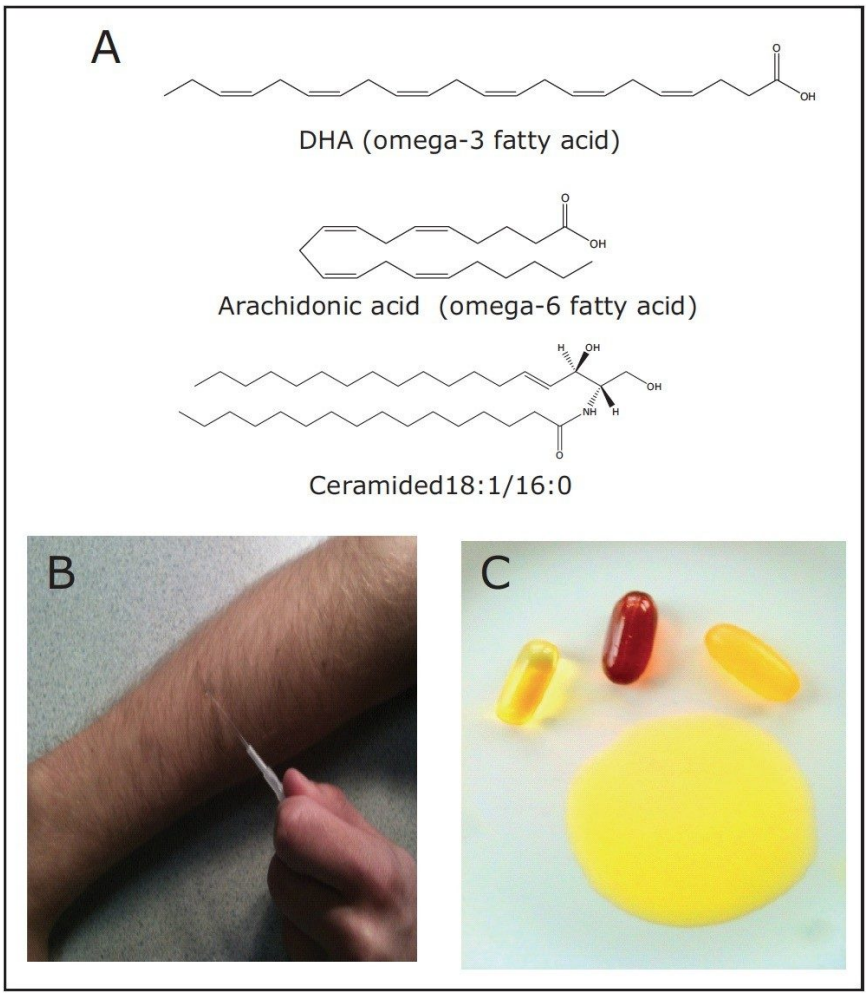

Figure 1. Representative lipid structures analyzed in the study (panel A). Lipids contained in human sebum from skin (panel B) and edible oils (panel C) have been used as representative samples for the DART-IMS-MS analyses.

The analysis of lipid composition often requires very laborious and timeconsuming procedures. Furthermore, the detailed spatial distribution of lipid species on a surface is often missed using traditional sample preparation and

lipid extraction protocols for large-scale lipid analysis (lipidomic analysis).

The use of desorption ionization (DI) techniques in lipidomics could provide a new level of description beyond the pure measure of lipid concentration. DI-MS techniques are useful for real-time, rapid, in-situ screening of various materials including food, plant, and animal tissue.1 In particular, DI-MS spectra of biological samples feature ions corresponding mainly to lipids. By molar quantities, the most abundant ionic molecular species in biological tissue, lipids ionize well under DI conditions.

The in-situ generation of a particular profile of lipid ions has been proposed for real-time molecular fingerprinting and diagnosis. Here, a rapid(few seconds), real-time method using DI in combination with post-ionization ion mobility separation to analyze lipidomic profiles in food and biological samples is presented.

Experimental

Sample Description

No sample preparation is required. Samples were swiped on glass capillaries, which were held in the in metastable gas beam between the Direct Analysis in Real Time (DART, IonSense, MA, USA) ion source and SYNAPT G2 HDMS. Lipid standards and extracts were purchased from Avanti Polar Lipids (AL, USA). Edible oils were purchased at the local grocery store and blindly analyzed.

MS conditions

Chromatographic separation is not required. Analyses were conducted using a DART source coupled with a Waters SYNAPT G2 HDMS instrument. DART sources are designed to fit the Waters Xevo MS family of instruments. Acquisition time was 5 to 10 seconds.

Mass spectrometer:	SYNAPT G2 HDMS
Ionization:	DART +ve and –ve
Cone voltage:	20 V

Source temp.:	120 °C
DART temp.:	50 to 450 °C
Cone gas:	30 L/h
Desolvation gas:	800 L/h (Nitrogen)
IMS gas:	90 mL/min (Nitrogen)
IMS T-Wave velocity:	833 m/s
IMS T-Wave height:	40 V
Acquisition range:	50 to 1200

Results and Discussion

For a rapid lipidomic analysis, we combined two emerging technologies: DART and ion mobility separation[2] to analyze lipids extracted from biological samples.

Belonging to the DI techniques, DART is an atmospheric pressure ion source that instantaneously ionizes samples in open air under ambient conditions. DART employs an electrical discharge to create a plasma that produces helium metastables, which react with ambient water, oxygen, or other atmospheric components to produce charged water clusters. Protons are then transferred to the analytes.

Samples were swiped on glass capillaries, held in the in metastable gas beam between the DART ion source and SYNAPT G2 HDMS. Without the need for chromatographic separation, lipids were ionized by DART and guided into the mass spectrometer, where they traveled to the Ion Mobility Separation (IMS) cell. A T-Wave mobility separator used a repeating train of DC pulses to propel lipid ions through a nitrogen-filled IMS cell in a mobility dependent manner. Lipids migrated with characteristic mobility times (drift times) according to their size and

shape before TOF detection, as shown in Figure 2.

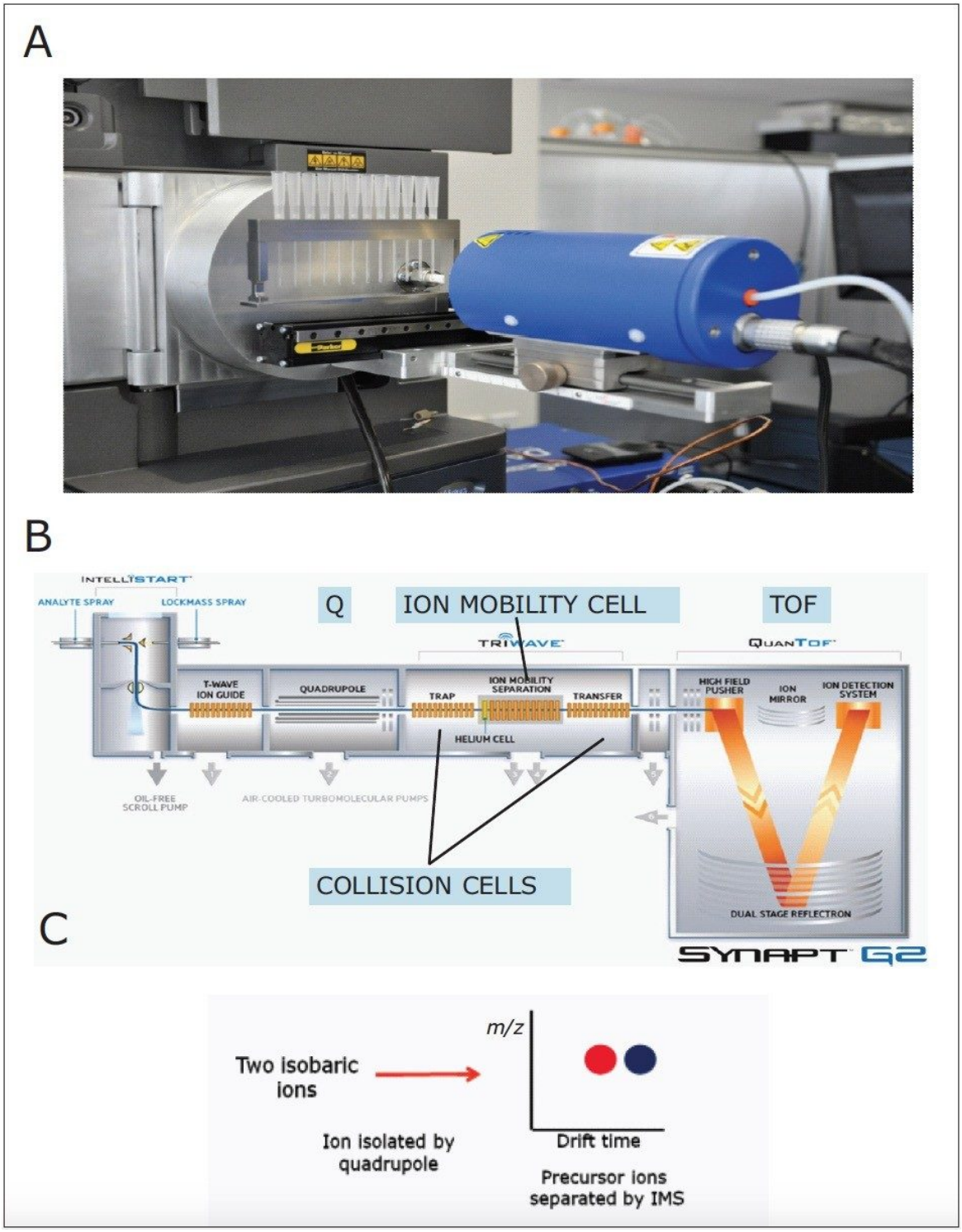

Figure 2. The DART ion source can be installed on Waters instruments (panel A). The ability to

couple DART with a SYNAPT HDMS instrument (schematic in panel B) eliminates sample preparation and chromatographic steps because of the post-ionization separation by ion mobility (C).

As an example of the power of such an approach, lipid profiles of edible oils (fish oil and olive oil), and lipids extracted from biological samples and human sebum, which is the oily matter that lubricates and waterproofs human skin, were analyzed, as shown in Figure 1. Lipid molecules with different acyl chain length or number of double bonds resulted in characteristic drift times. This enabled the separation and detection of key lipids, such as fatty acids and ceramides, on the millisecond time-scale without the need for prior derivatization or chromatography, as shown in Figures 1, 3, and 4. Ion mobility enabled the separation of the entire lipid profile of a sample on the millisecond time-scale, and a complete DART-IMS-TOF analysis required just a few seconds (0.1 min), as shown in Figures 3 and 4.

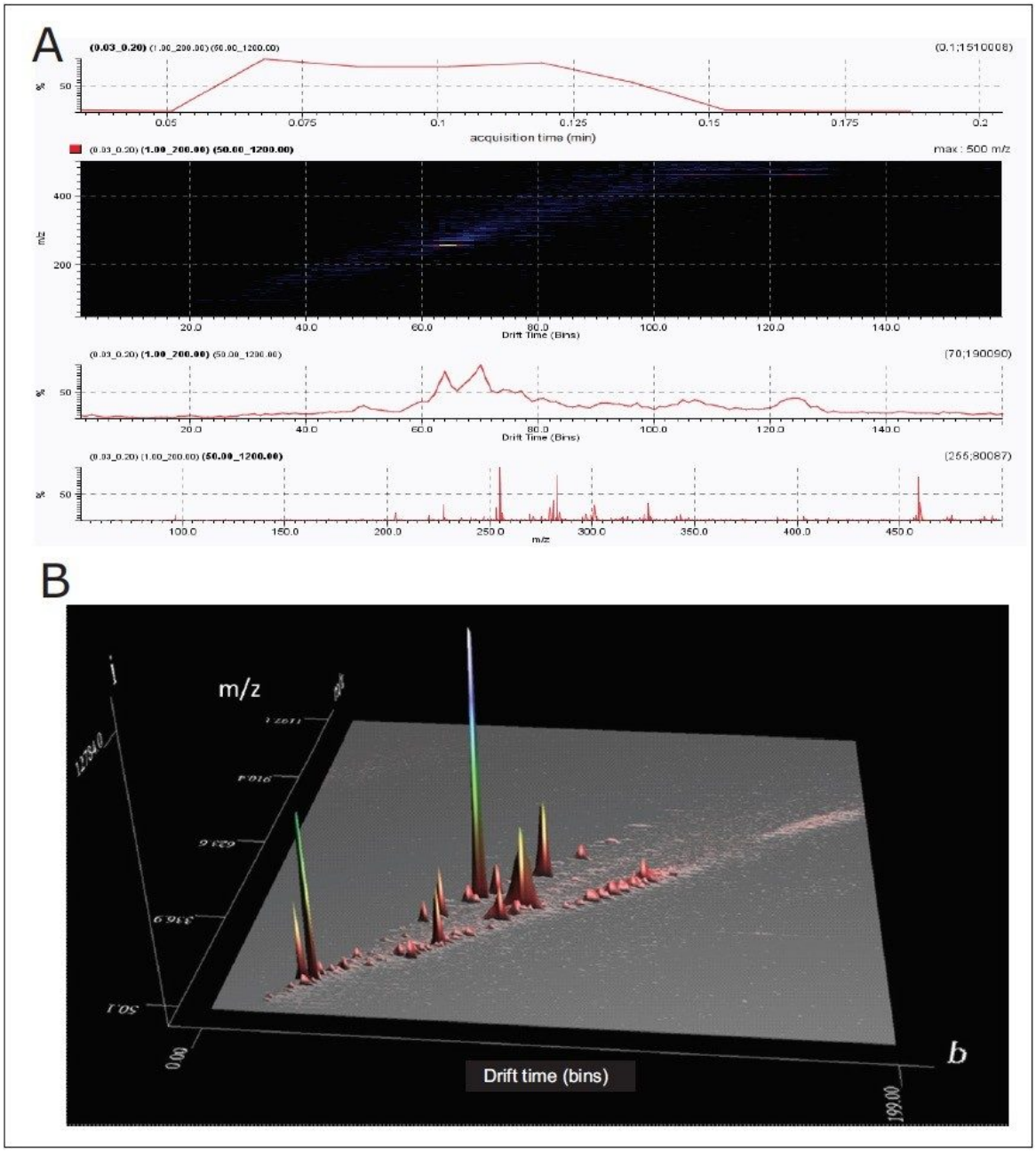

Figure 3. The entire DART-IMS-TOF analysis requires just a few seconds (0.1 min). Lipids are separated by ion mobility on the millisecond time-scale (panel A). Software processing of the data allows the generation of 3D molecular maps based on drift time, exact mass, and intensity of the signal relative to the various analytes present in the oils. Isobaric species are separated by ion mobility.

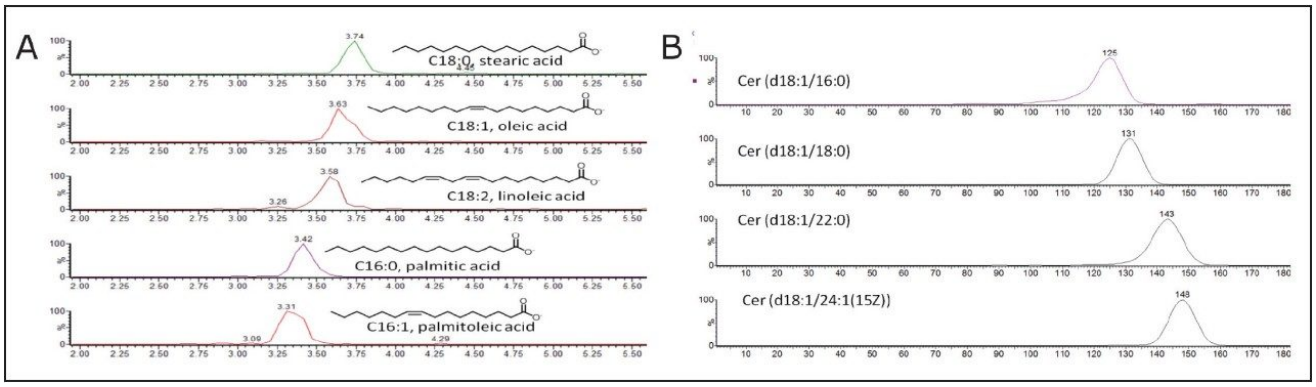

Figure 4. Ion mobility separation of fatty acids from olive oil (panel A), and ceramides from human sebum (panel B) after rapid DART ionization in negative and positive ion mode, respectively. Differences in the acyl chain length or number of double bonds affect the shape and size of lipid molecules, resulting in characteristic drift times.

A comparison of lipid profiles of human sebum, shown in Figure 5, and edible oils shown in Figure 6 was done based on the separation capabilities of IMS-TOF/MS. HDMS Compare Software was used for a rapid binary comparison of different driftograms (masses versus drift time matrices). The drift time and spectral information associated with the components responsible for the differentiation can be extracted from the dataset and analyzed to better understand the underlying reasons for the observed differences.

Figure 5. Comparison of sebum skin oils from two human subjects. Overlaying individual molecular maps clearly show areas where the samples are significantly different. Ion mobility data analysis and processing was done using HDMS Compare Software. Key areas of significant differences between two samples were clearly visualized and identified with two different colors. Regions of interest were easily selected and expanded in Zoomed Map view for further interrogation of important sample differences.

HDMS Compare Software also allows importing a list of target ions (mass and drift time) and reporting changes in the levels of these targets, as shown in Figure 6.

Figure 6. Comparison of edible oils. HDMS Compare Software was used to determine molecular difference in fish oil versus olive oil. The software automatically identified significant differences between the two oils in the levels of a selected list of ions, including m/z 301 (eicosapentanoic acid; EPA), 303 (arachidonic acid), and 327(docosahexaenoic acid; DHA).

Conclusion

- The combination of a desorption ionization technique such as DART with ion mobility-TOF offers a convenient solution for lipidomic profiling.
- Post-ionization separation by ion mobility allows resolution of complex mixtures of lipids.
- Software solutions provide overlay driftograms (plots of masses versus drift time) to compare different samples.
- More generally, these results suggest that the combination of desorption ionization techniques and the ion mobility approach is suitable for the rapid screening of bioactive lipids, including fatty acids and ceramides.
- Potential applications include phenotypic fingerprinting and comparative lipidomics in the areas of

personalized medicine, disease diagnostics, food analysis, and traditional medicines.

References

1. Yew JY, Cody RB, Kravitz EA. Cuticular hydrocarbon analysis of an awake behaving fly using direct analysis in real-time time-of-flight mass spectrometry. Proc Natl Acad Sci U S A. 2008 May 20;105(20):7135-40. Epub 2008 May 12.
2. Dear GJ, Munoz-Muriedas J, Beaumont C, Roberts A, Kirk J, Williams JP, Campuzano I. Sites of metabolic substitution: investigating metabolite structures utilising ion mobility and molecular modelling. Rapid Commun Mass Spectrom. 2010 Nov 15;24(21):3157-62.

Featured Products

Travelling wave (T-Wave) <https://www.waters.com/134663694>

720004611, February 2013