Analysis of Ceramides using GC/Q-TOF with a Novel Soft Ionization EI Source

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Introduction

Ceramides are part of the class of sphingolipids that have multiple functions, from being components of cell membranes to acting as secondary messengers in signaling events for different cell functions. Ceramides are commercially available in personal care products to replenish natural ceramides produced by the body to retain the skin's moisture.[1] Internally, ceramides are being investigated as potential biomarkers for neurodegenerative diseases.

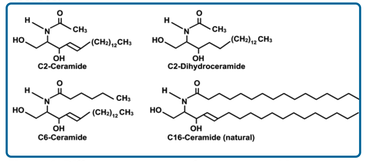

Figure 1: Different structures of select ceramides

Ceramide lipids consist of an amide (i.e., sphingosine, phytosphingosine or 6-hydroxyl sphingosine) linked with a fatty acid (i.e., non-H fatty acid, alpha-OH fatty acid, or omega-OH fatty acid). Their heterogeneity increases with the length and the degree of saturation of the fatty acid. This study addresses the determination of ceramides and sphinganines with up to C26 amide chain length by high resolution accurate mass GC/Q-TOF using a prototype soft ionization electron ionization (EI) source. Ceramides identified in a porcine brain extract and a chicken egg extract known to contain ceramides are discussed here.

Experimental

GC Method

Ceramide standards with acyl amide chains varying from C2:0 to C24:0 were obtained from Avanti Polar Lipids, Inc (Alabaster, AL). The extract of porcine brain (860052P) and chicken egg (860051P) were obtained also from Avanti Polar Lipids, Inc. Individual stock solutions of the test compounds and brain and chicken egg extracts were derivatized with 100 µL BSTFA-TMCS (99:1) and 100 µL of pyridine for 30 min at 70°C. The derivatized solutions were evaporated to dryness using a gentle stream of purified nitrogen and were redissolved in 1 mL spectroscopic grade isooctane. The GC separation was done on a 15 m x 0.25 mm id x 0.1 µm film thickness DB1-HT capillary column using He as carrier gas at 1.8 mL/min. The oven temperature was programmed from 90°C to 250°C at 4°C/min and from 250°C to 325°C (hold 3 min) at 10°C/min. The split/splitless injector was operated at 305°C.

[1]*Draelos, Z.D., Skin Moisturization, Cosmetic Science and Technology Series, Vol. 25*

Experimental

Name	Molecular Formula	MI^{+}-with TMS Derivitization
C2:0-Ceramide	$C_{20}H_{39}NO_3$	448.3715
C6:0-Ceramide	$C_{24}H_{47}NO_3$	541.4341
C8:0-Ceramide	$C_{26}H_{51}NO_3$	569.4654
C10:0-Ceramide	$C_{28}H_{55}NO_3$	597.4967
C12:0-Ceramide	$C_{30}H_{59}NO_3$	625.528
C14:0-Ceramide	$C_{32}H_{63}NO_3$	652.5515
C16:0-Ceramide	$C_{34}H_{67}NO_3$	681.5906
C16:0-D31-Ceramide	$C_{34}H_{36}D_{31}NO_3$	712.7852
C18:1-Ceramide	$C_{36}H_{69}NO_3$	707.6062
C18:0-Ceramide	$C_{36}H_{71}NO_3$	709.6219
C20:0-Ceramide	$C_{38}H_{75}NO_3$	737.6532
C22:0-Ceramide	$C_{40}H_{79}NO_3$	765.6845
C24:1-Ceramide	$C_{42}H_{81}NO_3$	791.7002
C24:0-Ceramide	$C_{42}H_{83}NO_3$	793.7158

Table 1: Ceramide Standards Analyzed by GC/Q-TOF

The accurate mass measurements were done with an Agilent 7200 Series GC/Q-TOF high resolution mass spectrometer (Figure 2) equipped with a prototype soft ionization electron ionization (EI) source (Figure 3) heated to 200°C. The spectral data were acquired at 5 Hz with a mass range of 50-900 m/z. Data analysis was performed using the Agilent MassHunter Qualitative Analysis software.

Figure 2: Agilent 7200 Series GC/Q-TOF mass spectrometer with (inset) Prototype Soft Ionization EI source

The prototype EI source can be tuned and operated at lower ionization energies (i.e., 15, 12, and 10 eV) to enhance the relative intensity of the molecular ion (MI), and yield fragment ions at high m/z values that can be used to identify compounds.

Fragmentation of Ceramides

Fragmentation of Ceramides

The TMS derivatives of ceramides yield pseudo-molecular ions by loss of a methyl group from one of the silylated OH groups on the sphingoid backbone (M^{+}-15). Predominant ions that are characteristic to all ceramides include *m/z* 426.3344, resulting from the neutral loss of the amide ligand and retention of TMS groups on the oxygen atoms, *m/z* 311.2765, cleavage of the C2-C3 of the fatty acid sphingoid backbone, and *m/z* 243.1231, cleavage of the backbone at the double bond on C5 (Figure 4a-c).

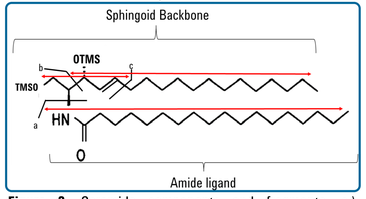

Figure 3: Ceramide components and fragments. a.) Cleavage of the amide ligand b.) C2-C3 cleavage of TMS-derivatized backbone and c.) cleavage of the C5 at the double bond of the backbone.

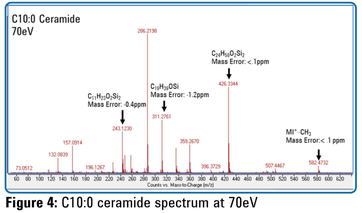

Figure 4: C10:0 ceramide spectrum at 70eV

Amide Ligand Saturation and Unsaturation Probed with Soft Ionization

Comparing amide side chains of the same length that are fully saturated to mono-unsaturated, it is evident the double bond located on the amide chain has some inherent stability, resulting in increased MI, as seen in Figure 5 with C18:1 ceramide in comparison to its fully saturated counterpart C18:0.

The overall stability of ceramides containing the unsaturated amide chain results in larger molecular ion as the ionization energy decreases, such as can be seen in Figure 6 for C18:1 ceramide.

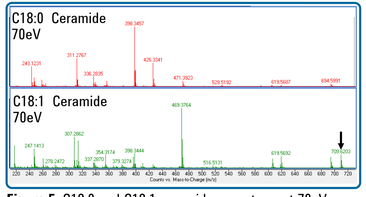

Figure 5: C18:0 and C18:1 ceramides spectrum at 70eV

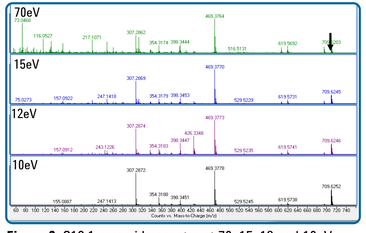

Figure 6: C18:1 ceramide spectra at 70, 15, 12 and 10eV

Soft ionization also provided some insight on how the molecule fragments. The transfer of a TMS group to the N atom and cleavage at the C2-C3 (*m/z* 311.2765) is a favored pathway over the neutral elimination of the amide (*m/z* 426.3344). At lower ionization energies, the latter pathway is the preferred mechanism of fragmentation.

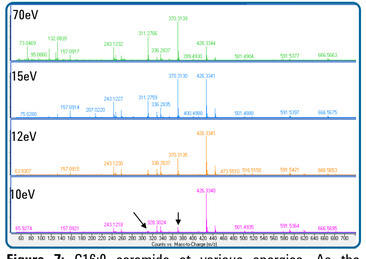

Figure 7: C16:0 ceramide at various energies. As the electron energy decreases, fragments *m/z* 370.3104 and *m/z* 311.2766 (<1 ppm error) resulting from TMS transfer from O to N, decrease.

Ceramide Extracts

Porcine Brain Extract

The ceramide extract from porcine brain was analyzed with the same methodology used on the ceramides standards. In addition to the reported ceramides (C16:0, predominant species C18:0, C22:0, C24:0, and C24:1), the following ceramides were found: C20:0, C22:1, C22:0, C23:1, C23:0, C25:0 and C26:0.

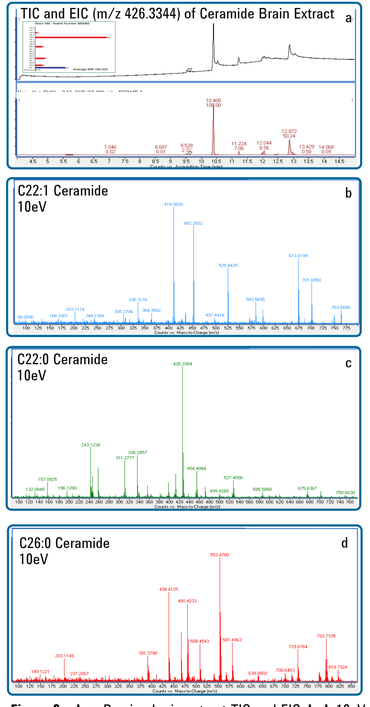

Figure 9a-d: a. Porcine brain extract TIC and EIC. **b-d.** 10eV spectra from select ceramides found in the porcine brain extract.

Chicken Egg Extract

The ceramide chicken egg extract has a different profile from the porcine brain extract, displaying the following: predominant species C16:0, C18:0, C22:0, and C24:1, Additionally, C14:0, C20:0, C22:1, and C24:0 ceramides were found.

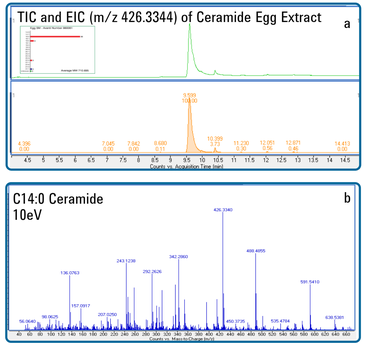

Figure 10a-c: a. Egg extract TIC and EIC. **b-c.** 10eV spectra from select ceramides found in the egg extract.

Conclusions

- It has been demonstrated that **GC/Q-TOF** is an excellent tool for the analysis of ceramide compounds.
- **Soft Ionization** reduces or eliminates the low m/z ions that do not contain useful structural information and also provides some insight to preferred fragmentation patterns.
- The signature fragment ions with **accurate mass** can be utilized to identify ceramides.

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