Ceramides are part of the class of sphingolipids that have multiple functions, from being components of cell membranes to acting as intracellular messengers regulating cell growth and differentiation. They are also commercially available in personal care products to reproduce natural ceramide profiles in the skin's epidermis.1 Internally, ceramides are being investigated as potential biomarkers for neurodegenerative diseases.

### Experimental

**GC Method**

Ceramide standards with amino acid chains ranging from C12 to C44 were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). The extract of porcine brain (M3052P) and chicken egg obtained from Avanti Polar Lipids. Inc. Individual stock solutions of the test compounds and brain and chicken egg extracts were derivatized with pyridine (2.0% v/v) and triethylamine (5.0% v/v) in acetonitrile. The supernatants were then incubated at 70 °C for 30 min. The derivatized solutions were then analyzed using a gas chromatograph equipped with a capillary column (DB-5, 60 m × 0.25 mm i.d., 0.25 µm film thickness). The GC separation was done on a HP series 1120 (Agilent Technologies, CA) with temperatures programmed to increase from 40 °C to 150 °C at a rate of 1 °C/min followed by 1 °C/min increments. The final stage was held at 150 °C for 8 min. The split/splitless injector was operated at 250 °C.

**TIC and EIC**

The TIC and EIC spectrum were obtained using GC/Q-TOF with a soft ionization source. The mass spectrometer was run in electron ionization (EI) mode at 70 eV. The source temperature was 230 °C, and the capillary voltage was 3000 V. The source pressure was 20 mTorr. The ion source was operated in the positive ion mode. The mass spectrometer was calibrated using National Institute of Standards and Technology (NIST) reference standards.

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### Conclusions

- It has been demonstrated that GC/Q-TOF is an excellent tool for identifying ceramide derivatives.
- Soft ionization reduces or eliminates the low m/z ions that do not contain useful structural information and provides some insight to preferred fragmentation patterns.
- The fragmentation pattern with highest m/z can be utilized to identify ceramides.

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