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MEASUREMENT OF CERAMIDE SPECIES IN DIFFERENT BIOLOGICAL MODELS BY LC-MS/MS

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Introduction

Ceramide (Cer) is one of the most important sphingolipids subclasses actively involved in apoptosis and cell death. Recently, it has been proposed as pharmaceutical target in pathologies related to its over productions and, in this optic, its specific involvement within the more complex sphingolipid pathways is needed. Liquid Chromatography coupled to mass spectrometry (LC-MS/MS) is the most important technique to study the Cer metabolism, and the unique able to provide an in-depth profile of all sphingolipid species.

Here we present the optimized LC-MS/MS procedure to quantify Cer species in different biological matrices and in different biological models related to deregulation of Cer metabolism.

Materials and methods

Sphingolipids from different tissues and cells were fortified with internal standard N-lauroyl-D-erythro-sphingosine (0.2 nmol), and extracted with a customized procedure modified from the one described by Merrill et al. (1).

The LC-MS/MS (Dionex Ultimate 3000 tandem AB Sciex 3200 Q Trap), operated in the positive electrospray ionization (ESI) mode. Identification and quantification of all targeted Cer was accomplished by multiple reactions monitoring (MRM) by following the transition from the $[MH^+]$ species to the common ion fragment 264.4 m/z. The Cer content was expressed as pmol normalized to total protein content (mg). The analysis run to run lasted 22 min at flow rate 0.3 mL/min.

Results and Conclusions

As low as 50 fmoles can be detected on-column and quantified with ±15% precision and within ±20% of target level; the method was linear up to 400 pmoles in a typical 150 microL extract. Examined biological samples (>50), include mouse retinas and hearts, human Chordoma tumor biopsies, C38 and meningioma cell cultures. The method here presented resulted suitable for all the tested samples provided by *in vivo* and *in vitro* studies. In particular, the method was useful as a direct read-out for pharmacological treatments aimed to lower the Cer content in different pathological models related to the Cer overproduction.