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SEMIQUANTITATIVE DISCOVERY OF CERAMIDES BY ISOENERGETIC PRECURSOR ION SCAN IN THE TRIPLE QUADRUPOLE MASS SPECTROMETER: FUNDAMENTAL ISSUES AND PROOF-OF-PRINCIPLE APPLICATION

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The triple quadrupole mass spectrometer (TSQ) has boosted discovery and quantification of trace components in biological samples using class-selective scan modes. The use of suitable molecular precursors and of characteristic fragment ions allows identifying and measuring ceramides with micro-heterogeneous fatty acid or sphingosine. Collision energy in the TSQ is a key parameter to extract information from MS-MS experiments for compounds of which authentic standards are not available.

We describe the theoretical basis and proof-of-principle application of a novel scan mode of a commercial TSQ by which the molecular precursor ions of all ceramides in a sample experiment the same Center-of-Mass Collision Energy (CoM-CE), irrespective of their different m/z values over a range of homologous or chemical analogs. At this value of CoM-CE, the different precursor ions have, for the same fragmentation channel, essentially the same fragment ion cross section, thus the same response factor for quantification. This scan mode can be applied to Precursor and Neutral Loss scans, and is performed by modulating the voltage drop of a scanning mass filter according to a specific linear function of precursor ion mass. Under this instrumental condition, all precursor ions collide under the same conditions and fragment to a common charged (in Precursor Ion scan) or neutral (in Neutral Loss scan) sub-structure, and response factors are thus intrinsically homogeneous, especially for first-generation fragments. As a proof-of-principle application in the sphingolipid field, isoenergetic fast scanning during LC separation of lipid extracts highlights the presence of unanticipated ceramides that can be quantified as equivalents of known analogs without resorting to the construction of individual calibration curves. The use of a scan mode coupled to the LC separation allows improving the amount of information on the sphingolipidome that can be extracted from complex samples, and discovering components with unusual or modified fatty acids. Among displayed proof-of principle examples are the sphingolipid fractions of organs and tissues of experimental animals, and of edible seeds and nuts.