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Improved Quantitative Approach for Monitorization of Gangliosides Structural Diversity in Fungal Cell Factories by LC-MS/MS

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Current advancements in MS metabolomics gravitates towards enhanced high-throughput analysis with unequivocal identification of hundreds of biomolecules in a run. These new methods aim at monitoring - in a quantitative manner, all species concerning a biological problem. Thereby, for discrimination of a set of structure-related and/or building blocks-sharing metabolites, fine chemical elucidation is essential. Such is the case of this work, dealing with a wide plethora of glycolipids – recombinant gangliosides, that comprises chimeric structures arising from metabolic engineering.

Gangliosides naturally occur in higher animals with important (patho)-physiological properties and are composed of a sphingoid core bound to a glycan moiety including several units of sialic acid. The combinatorial diversity grows exponentially in synthetic biology approaches, e.g., use of microbial cell factories, with an expanded building blocks assortment. A dedicated platform accounting for this complexity, meeting the high-throughput and unequivocal identification requirements, is herein presented. The targeted LC-MS/MS methodology developed includes internal standard analogues-based absolute quantification, adapted to gangliosides bioavailability in fungal – recombinant - organisms and includes hitherto uncharacterized structures, with unusual sphingoid bases and both simple and hydroxylated fatty acids. The addition of glycans to the polar head was also successfully monitored for up to 4 monomers including hexoses and acidic residues. Specific endeavour was required to avoid interferences between more than 100 species, often isobaric species, by separation in the chromatographic stage and discrimination via MS and MS^n *info* in a high-end device (Vanquish/Q-Exactive, Thermo™). This platform represents an improved methodology to study the biochemical diversity associated to gangliosides for natural and metabolically engineered biosynthetic pathways.