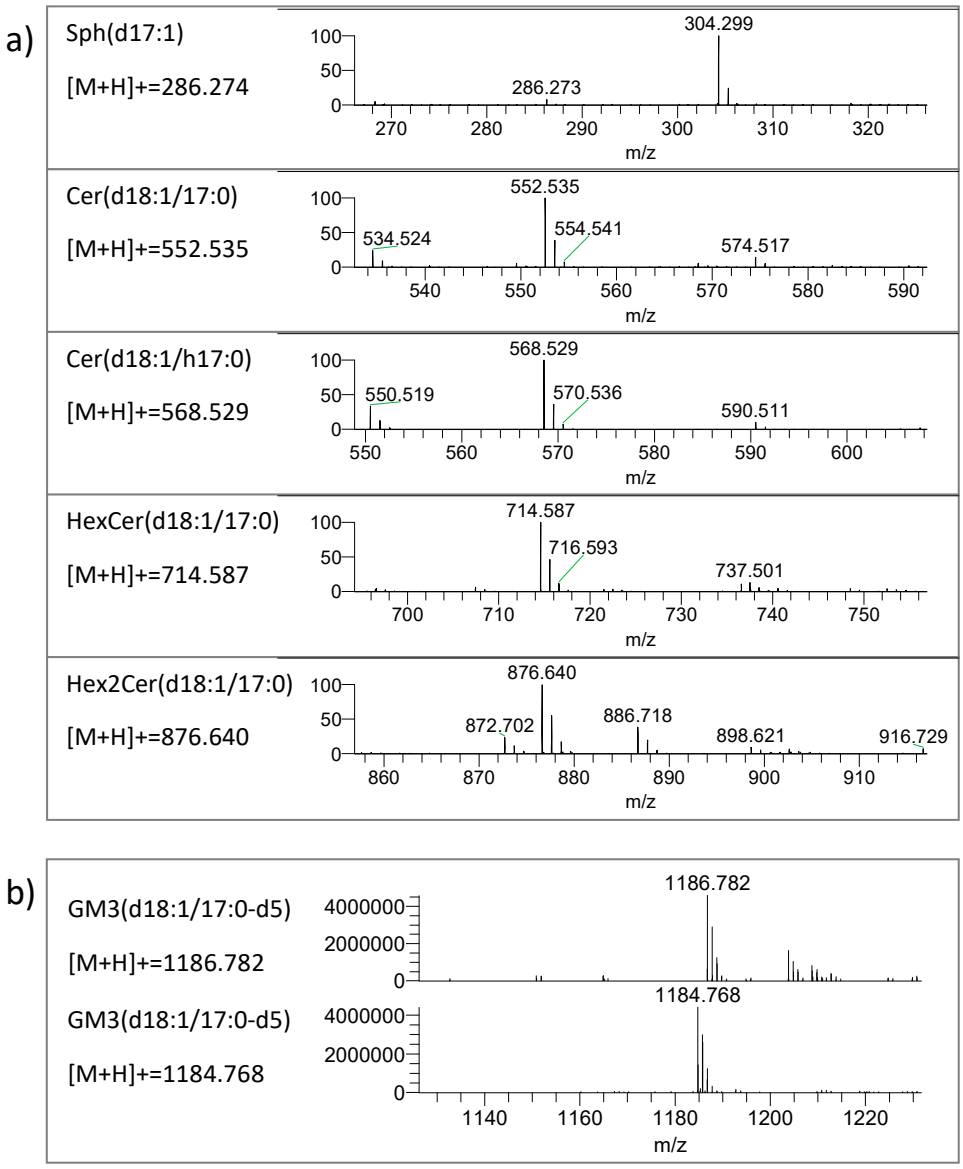
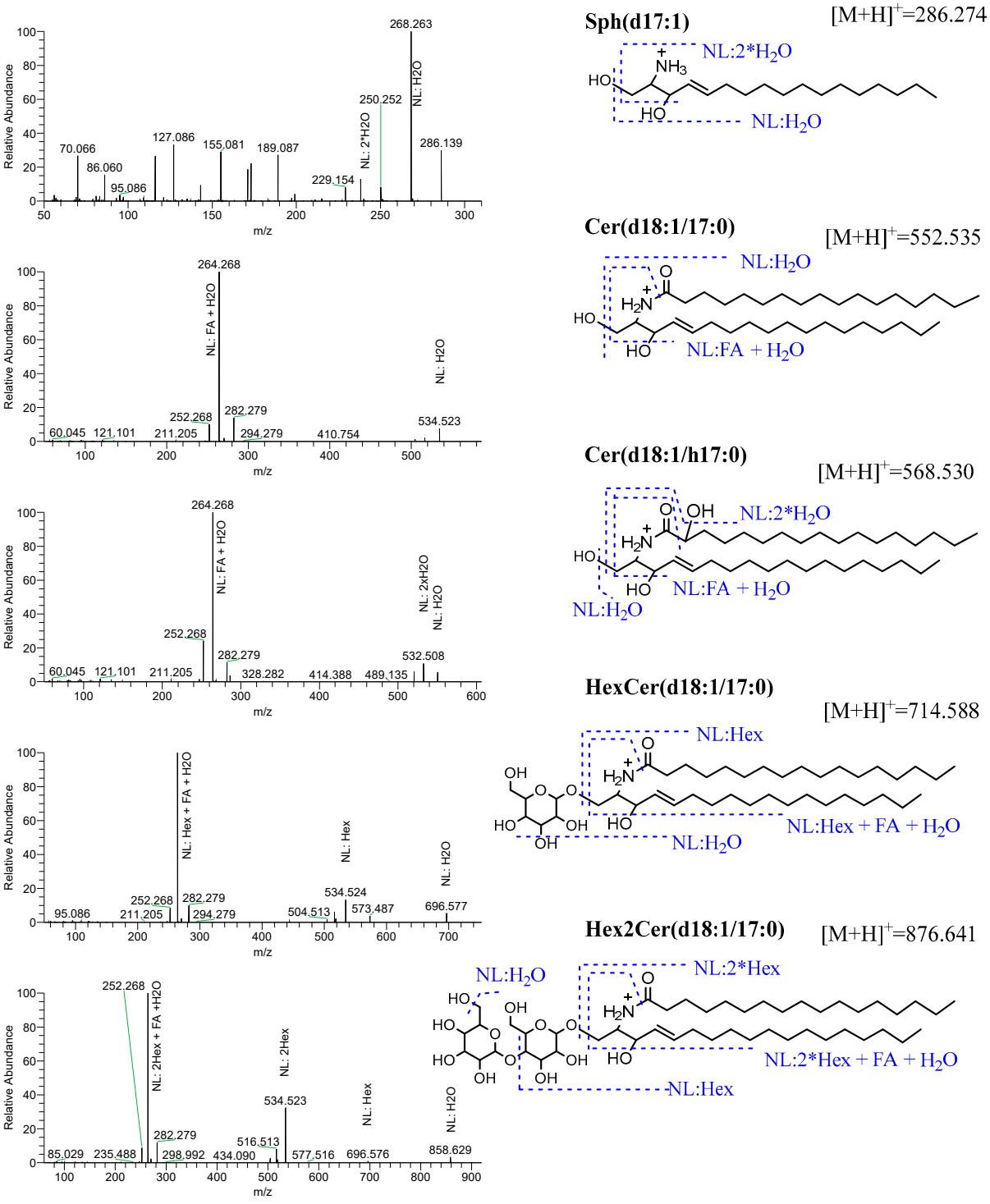
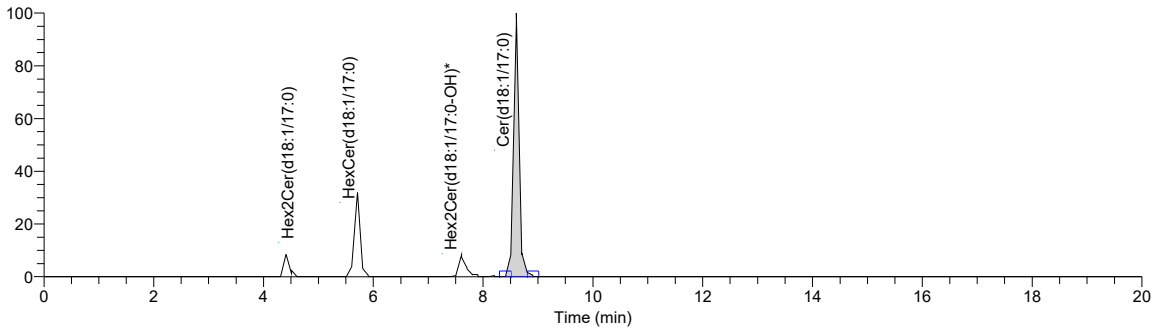
Supplementary material



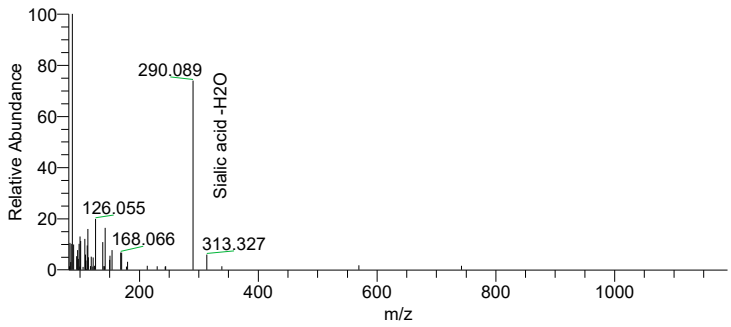
Supplementary Figure S1: a) Zoomed region of the MS spectrum of the commercial standards used for method development in this work. The [M+H]+ is osbserved as the predominant ion without significant contribution of other adducts, that should be observed along the m/z range represented. **b). Zoomed region of the MS spectrum of GM3 standard in both positive - upper panel, and negative polarity-lower panel**. Similar intensities are observed, according to the absolute signal scale used, to this end, along the y axis.



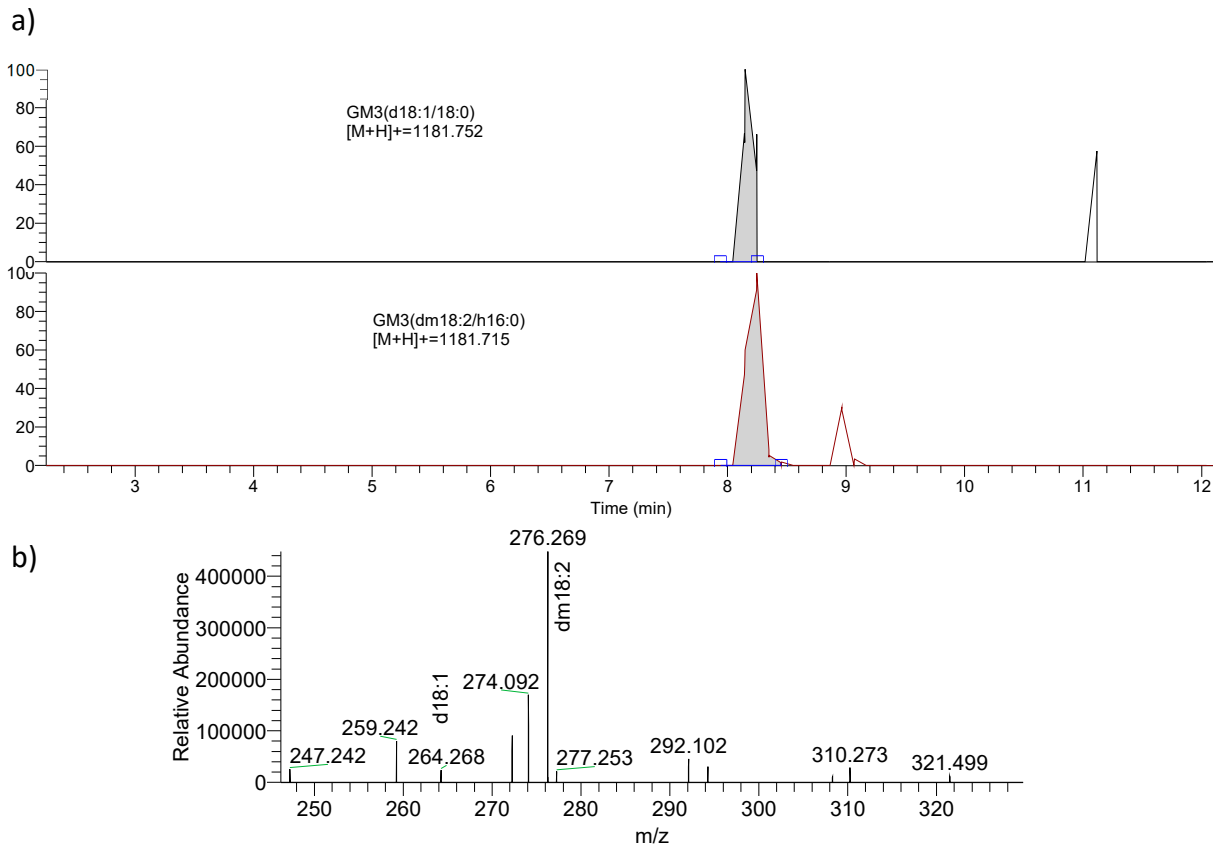
Supplementary Figure S2: MS/MS spectrum in positive polarity of the five commercial standards used in method development for ceramides and glycoceramides. Schematic molecular structures, with fragmentation patterns, are depicted on the right and correlated with the masses annotated in the spectrum., matching theoretical values within a 10 ppm tolerance threshold.



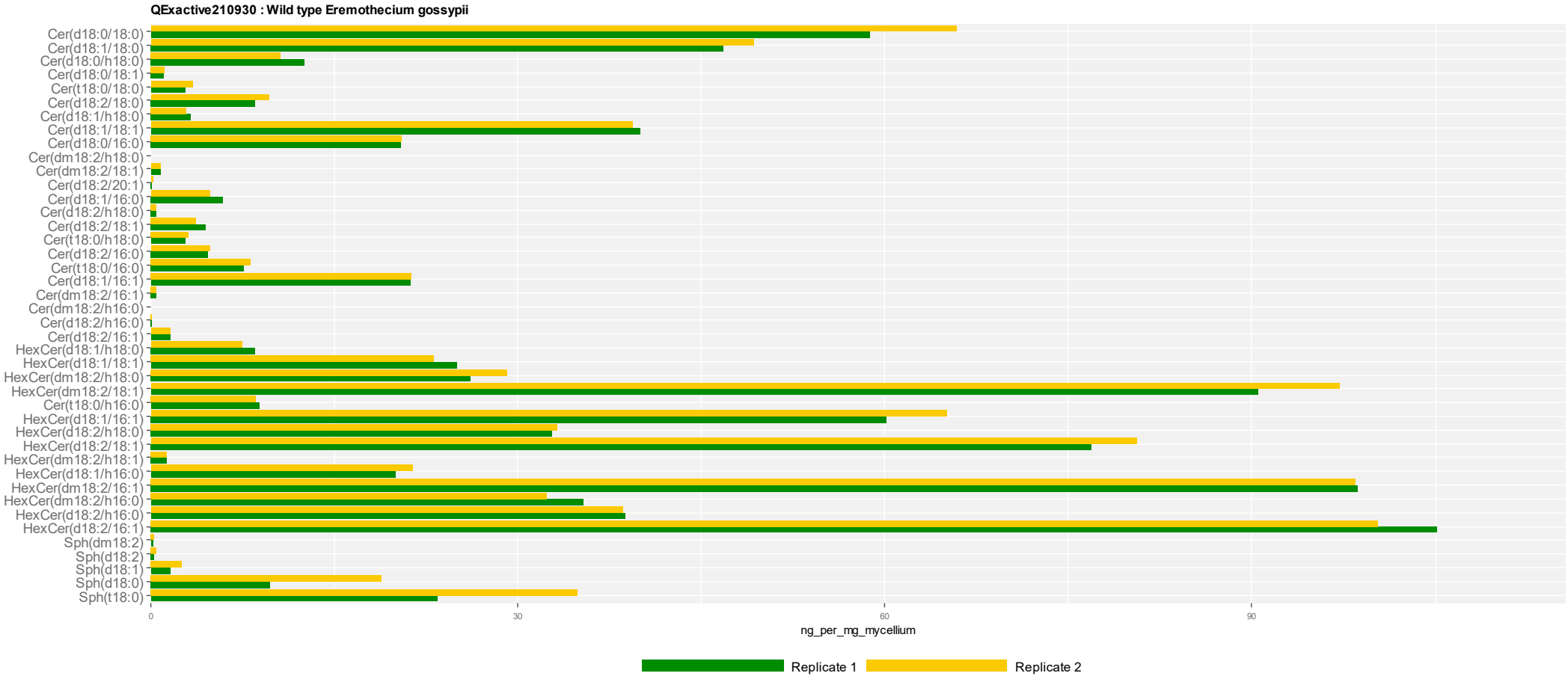
Supplementary Figure S3: LC-MS/MS chromatogram for the commercial standard Cer(d18:1/17:0). Correct signal appears at 8.8 min (shaded). The other ghost peaks belong to in-source fragmentation of other structures. Hex2Cer(d18:1/17:0) interferes in the chromatogram through in-source loss of the 2 hexoses of the glycan moiety (4.7 min). HexCer(d18:1/17:0) generates a ghost peak by release of its hexose. (5.9 min). Cer(d18:1/h17:0) easily decomposes by loss of a water molecule from the hydroxylated fatty acid, in such a way that the 2nd isotopic peak is present in the chromatogram of the non-hydroxylated counterpart.



Supplementary Figure S4: MS/MS spectrum in negative polarity of the GM3(d18:1/18:0-d5) standard used in method development for ganglioside analysis. Mass annotated in the spectrum matches theoretical values, within 10 ppm tolerance, of the fragment ion consisting of the deprotonated sialic acid unit.



Supplementary Figure S5: a) LC-MS/MS chromatogram in positive polarity, based on the sphingoid base fragment ion, for the isobaric species GM3(d18:1/18:0) and GM3(d18:2/h16:0), that elute at similar retention times. **b) Chimeric MS/MS spectrum with information for both species**, given that precursor ions have similar masses and retention times. Quantification is only possible when using MS/MS fragments that allow unequivocal identification, such as the annotated ions corresponding to the sphingoid base.



Supplementary Figure S6: Quantitative LC-MS/MS analysis of ceramides (Cer) and glucosyl ceramides (HexCer) in a biological matrix extracted from the wild type fungus *Eremothecium gossypii.* Quantitative values were extrapolated using internal standards for each group of molecules and calculated versus the initial amount of the mycelia of fungus obtained. A total of 42 species in the wild type organism were detected, including the five sphingoid types

and occasionally alpha-hydroxylation in the fatty acyl chain. Concentrations observed ranged between values close to the observed limit of detection, around one nanogram of the species per milligram of mycelium, and a few hundreds of ng per mg of mycelium.