# PROFILING AND STRUCTURAL CHARACTERIZATION BY MASS SPECTROMETRY OF REGION-SPECIFIC GANGLIOSIDES IN BRAIN

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## Abstract

Gangliosides (GGs), a particular class of glycosphingolipids ubiquitously found in tissues and body fluids, exhibit the highest expression in the central nervous system, especially in brain. GGs are involved in crucial processes, such as neurogenesis, synaptogenesis, synaptic transmission, cell adhesion, growth and proliferation. For these reasons, efforts are constantly invested into development and refinement of specific methods for GG analysis. We have recently shown that ion mobility separation (IMS) mass spectrometry (MS) has the capability to provide consistent compositional and structural information on GGs at high sensitivity, resolution and mass accuracy. In the present study we have implemented IMS MS for the first time in the study of a highly complex native GG mixture extracted and purified from a normal fetal hippocampus in the $17^{th}$ gestational week (denoted FH17). The combination of electrospray ionization, ion mobility separation and high resolution mass spectrometry in the negative ionization mode enhanced ganglioside separation based not just on the *m/z* value, but also on the charge state, the carbohydrate chain length and the degree of sialylation. In the generated driftscope plot (drift time versus *m/z*), 131 distinct gangliosides characterized by high variability of the oligosaccharide core and diversity of the ceramide moiety were identified with an average mass accuracy of 12.3 ppm. As compared to previous studies where no separation techniques prior to MS were applied, IMS MS technique has not just generated valuable novel information on the GG pattern characteristic for hippocampus in early developmental stage, but also provided data related to the GG molecular involvement in the synaptic functions by the discovery of 25 novel structures modified by $CH_3COO^-$. By applying IMS in conjunction with collision induced dissociation (CID) tandem MS (MS/MS), novel GG species modified by $CH_3COO^-$ attachment, discovered here for the first time, were sequenced and structurally investigated in details. The present findings, based on IMS MS, provide a more reliable insight into the expression and role of gangliosides in human hippocampus, with a particular emphasis on their cholinergic activity at this level.

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