

Profiling of Acyl-CoAs in Biological Tissues using Online SPE-LC-FTMS (Orbitrap)

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Introduction: We present a robust online-SPE-LC/FTMS-based method for the quantification of acyl CoAs in a wide range of biological tissues. The method requires minimal sample preparation and enables structural analysis to be performed by accurate mass determination in MS and MSⁿ. Quantification is performed using high resolution full MS scans and automated data processing using the open source XCMS software package.

Methods: All experiments were carried out on an Ultimate 3000 (Dionex) comprising an autosampler with cooled tray and a column oven with switching unit coupled to an Orbitrap (ThermoScientific). The sample extraction procedure comprises three steps: (1) addition of buffer and internal standards, (2) homogenization and (3) centrifugation. The supernatant is injected directly into the SPE-LC-FTMS system. Data acquisition was performed in full scan mode using a resolution of 60.000 FWHM.

Results and Discussion: A combination of online SPE/LC and high resolution mass spectrometry (LTQ Orbitrap XL) was used to identify and to determine the relative levels of multiple CoA-activated compounds in samples of mouse liver and muscle without prior sample extraction and solvent evaporation steps. The selectivity and sensitivity provided by the Orbitrap system in full MS-scan mode was moreover comparable to that obtained using triple quadrupole SRM-Scans and enabled the generation of profiles of CoA-activated compounds from less than 10mg of tissue. The use of non-targeted, full MS data acquisition and automated peak detection, integration, alignment and grouping using the open source software XCMS provided data about every detectable compound eluted from the LC-system: hundreds of features in the LC-MS data including the CoA-activated compounds were detected in this manner. Identifications were confirmed by accurate mass data, data dependent MS/MS scans and retention times.