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Progress in isotopic measurements by LC-based methods for nutritional investigation

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On one hand, the use of labeled internal standards is a common practice in mass spectrometry for accurate quantitative analysis of biomolecules in complex mixtures such as in biological fluids to mimic the sample analyte being quantitated. On the other hand, advances in mass spectrometry to quantitate the isotopic enrichment in molecules and/or the mass isotopomer distribution have over the recent years led to extensive use of stable isotope labeled substrates in clinical metabolic studies. Indeed, determining fluxes through metabolic pathways is an important step for depth understanding of metabolic regulation, or for evaluate the functional effects of nutrients. Pratically, the isotopic measurements have analytical limitations due to the study design (e.g. dose of tracer introduced into the biological systems). Consequently, two different mass spectrometers are normally used in tracer study such as organic mass spectrometer (MS) and isotope ratio mass spectrometer (IRMS) according to the level of isotopic enrichment in the analyte of interest. IRMS coupled to diverse peripherals can provide high-precision isotopic ratio measurements for light stable isotopes. In the area of 13C analysis, the last breakthrough was the commercialization of the first interface used for the coupling between the liquid chromatography (LC) and IRMS in 2004. So far, in the last 6 years, a multitude of methods have been published using this device (e.g. for carbohydrates, amino acids and glutathion) despite few analytical constraints (e.g. an aqueous LC mobile phase is mandatory for the LC-IRMS coupling). The talk will be focused on the instrumental strategy and on the analytical performance using LC-MS and LC-IRMS to measure 13C isotopic ratio. Examples obtained from in vivo metabolism studies in the area of nutrition & medicine will be reported.