Confirmatory and quantitative analysis of fatty acid esters of hydroxy fatty acids in serum by solid phase extraction coupled to liquid chromatography tandem mass spectrometry

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A novel class of endogenous mammalian lipids endowed with antidiabetic and anti-inflammatory properties has been recently discovered. These are fatty acid esters of hydroxy fatty acids (FAHFAs) formed by condensation between a hydroxy fatty acid and a fatty acid. FAHFAs are present in human serum and tissues at low nanomolar concentrations. Therefore, high sensitivity and selectivity profiling analysis of these compounds in clinical samples is demanded. An automated qualitative and quantitative method based on on-line coupling between solid phase extraction and liquid chromatography–tandem mass spectrometry has been here developed for determination of FAHFAs in serum with the required sensitivity and selectivity. Matrix effects were evaluated by preparation of calibration models in serum and methanol. Recovery factors ranged between 73.8 and 100% in serum. The within-day variability ranged from 7.1 to 13.8 %, and the between-days variability varied from 9.3 to 21.6 %, which are quite acceptable values taking into account the low concentration levels at which the target analytes are found. The method has been applied to a cohort of human serum samples to estimate the concentrations profiles as a function of the glycaemic state and obesity. Statistical analysis revealed three FAHFAs with levels significantly different depending on the glycaemic state or the body mass index. This automated method could be implemented in high-throughput analysis with minimum user assistance.