High-throughput Simultaneous Analysis of Oxysterols, Plant Sterols and Cholesterol Precursors using Gas-chromatography Coupled to Tandem Mass Spectrometry

M. Peer, S. Matysik, G. Schmitz

University Hospital Regensburg, F.-J.-Strauss-Allee 11, 93053 Regensburg, Germany

The importance of analyzing a high number of species simultaneously with mass spectrometric methods to uncover biosynthetic and metabolic pathways has increased tremendously in recent years. In particular, various disorders of cholesterol synthesis and metabolism can be evaluated by the determination of distinct biomarkers. The simultaneous quantitative determination of cholesterol precursors, plant sterols and oxysterols in plasma can be used for an early evaluation and diagnosis of inherited disorders or metabolic diseases. Moreover, the intermediate molecules formed from cholesterol towards steroid hormones, transcriptional regulators and bile acid synthesis or detoxification are becoming increasingly important.

We developed a reliable and rapid GC method coupled to triple quad MS detection allowing quantitative analysis of cholesterol precursors (e.g. desmosterol, lanosterol), plant sterols (e.g. sitosterol, campesterol) and oxysterols (24-OH-, 25-, 27-OH-cholesterol, 7-ketocholesterol) in plasma. Deuterium labeled compounds were used as internal standards. A simple derivatization step to form trimethylsilyl ethers was included in the sample preparation. Positive chemical ionization with ammonia as reagent gas was applied to generate high abundant precursor ions. The definition of highly sensitive precursor/product ion transitions, especially for coeluting substances allowed fast GC run times under 8.5 minutes. Using multiple reaction monitoring (MRM) mode, detection limits in the pg/ml range could be achieved for most of the compounds. The whole protocol was validated concerning accuracy, precision and recovery. The presented method can be applied for high-throughput sterol profiling in various clinical and epidemiological studies and is also suitable for tissue and cell-culture analysis