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## Quantification of oxysterols by liquid chromatography high-resolution tandem mass spectrometry

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The aim of the current study was to develop a reliable, robust and fast method for the quantification of oxysterols in biological fluids by liquid chromatography-high-resolution mass spectrometry method (LC-MS/HR-MS). Here, a short, sensitive and specific LC-MS/HR-MS method has been developed for the quantification of 7 $\alpha$ -, 7 $\beta$ -, 4 $\beta$ -, 24-, 25-, 27-hydroxycholesterol, 7-ketocholesterol and cholestan-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol. Sample preparation included a saponification step, liquid/liquid extraction, and derivatization with N,N-dimethylglycine (DMG). Baseline chromatographic separation of the isobaric compounds was achieved. The mass analyzer was a quadrupole-Orbitrap hybrid instrument (QExactive, Thermo Fisher Scientific). We applied full scan, targeted selected ion monitoring (SIM) and parallel reaction monitoring (PRM). Neither full scan nor SIM gave convincing signals due to base line noise and unspecific signals. Only the exploitation of a collision induced dissociation in the HCD cell in combination with mass resolution setting of 35,000 resulted in high abundant signals for low concentrations and linear calibration lines by multiplexing with the corresponding deuterated internal standard. The run time is 7.8 min. This run-time meets the expectations of routine analysis and to our knowledge this is one of the shortest methods for the analysis of oxysterols. LOD and LOQ were determined by functional testing with serial dilutions of control and calibrator samples. LOQ for all oxysterols were found below 1 ng/ml. The method has been successfully applied to determine the concentrations of oxysterols in human plasma from patients with cardiovascular disease and to compare these results with control samples.

### Biography

Zoltan Pataj has received his PhD in Analytical Chemistry in 2011 from University of Szeged, Hungary. He was working as Post Doc researcher in Olomouc, Czech Republic and Tübingen, Germany. He is a Humboldt fellow at the University Hospital in Regensburg.

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