DIFFERENTIAL MOBILITY SEPARATION (DMS)-BASED SEPARATION OF BILE ACID ISOMERS

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Introduction:
Bile acids are involved in a wide range of biological functions including lipid resorption, immunological functions and metabolic regulation. Through metabolic transformations, isomeric and isobaric variants are generated, which makes the unequivocal identification and quantification of individual chemical species difficult. Here we introduce Differential Mobility Separation (DMS) as a methodology for the separation of bile-acid isomers. DMS is an ion mobility technology which separates molecules based on their dipolar moment. We show DMS separation in conjunction with chromatographic separation (LC-DMS-MS) as well as with direct infusion (DMS-MS). While the combination with chromatographic separation may improve selectivity, the separation power of SelexIon is sufficient for a clear separation of isomers, allowing for infusion-based fast quantification without the need for LC development.

Methods:
Bile acid standards were prepared in 10% DMSO at a stock solution of 1 mM. Two groups were used: taurodeoxycholic acid (TDCA), taurochenodeoxycholic acid (TCDCA), tauroursodeoxycholic acid (TUDCA) with monoisotopic mass of (499.2967) and formula (C26H45NO6S) and glycodeoxycholic acid (GDCA), glycochenodeoxycholic acid (GCDCA) and glycoursodeoxycholic acid (GUDCA) with monoisotopic mass of (449.3141) and formula (C26H43NO5). For direct infusion, compounds were prepared in water/methanol 50/50, 0.1% FA at a concentration of 1 µM. For LC-separation preparation was done in the same solvent at a concentration of 0.1 nmol/ml with 1 µl injection volume. The samples were measured on a QTRAP® 6500 LC-MSMS system equipped with a SelexION® (DMS) device and coupled to an ExionLC™ system for the LC experiments.

Results
Using the DMStechnology, we directly infused the bile acid standards individually and as mixtures to determine the CoV values of the different isomers. To this end, the compensation voltagewas ramped over a range between -30V to 0V. The results showed almost baseline separation of the different isomers. The next aim was to combine DMS with chromatographic separation. The combination of DMS with chromatography has several advantages: Firstly, DMS showed a markedreduction of chemical background for better quantification, resulting in improvement of the signal to noise ratio (give a value NNN). A further advantage is less requirement for development of chromatographic separation, which was shown by the elimination of isomers from extracted ion chromatograms. This also eliminated the requirement for retention time assignment and less reliance on compound specific fragments which may be low abundant. For example, DMS allows to use a highly abundant fragment without the need for compound specificity.