Separation of Bile Acid Isomers Based on Differential Mobility Spectrometry

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ABSTRACT

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, differential ion mobility spectrometry (DMS, SelexION® Technology) was used as a methodology for the separation of bile-acid isomers. SelexION® device is an ion mobility technology which separates molecules based on their dipole moment instead of m/z. This separation was used in conjunction with liquid 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® technology is sufficient for a clear separation of isomers, enhancing the selectivity of the measurement, and also allowing for infusion-based fast quantification without the need for LC separation.

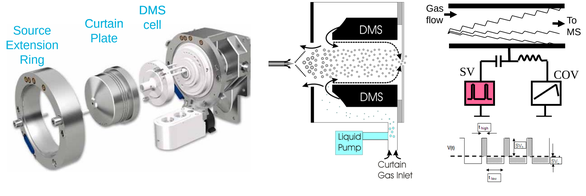

Figure 1. High Selectivity Quantification using SelexION® Technology on the QTRAP® 6500 LC-MS/MS System. The SelexION® Technology is an easy to install differential mobility separation (DMS) device that can be installed on a QTRAP® 5500 or 6500/6500+ LC-MS/MS system, attaching in front of the curtain plate (Left). Gas draws the ions towards the orifice (Middle) while a Separation Voltage (SV) in the form of an asymmetric waveform is applied to the flat plates, which alternates between high field, K(E) and low field, K(0). This moves the charged ion back and forth between plates, an ion will have net drift base on its high and low field mobility. A separation voltage (SV) is applied as the filtering voltage and the compensation voltage (CoV, a small DC offset between the plates) is applied as the restoring voltage, which can be tuned for the compound of interest. Other co-eluting nearly isobaric species that tune with different compensation voltages will be filtered away (right).

Methods

Sample Preparation: Commercially available bile acid standards were prepared in 1 mM in 10% DMSO as stock solution. Two groups of bile acid standards were used: taurodeoxycholic acid (TDCA), taurochenodeoxycholic acid (TCDCA), tauroursodeoxycholic acid (TUDCA) with monoisotopic mass of 499.3 Da and formula $C_{26}H_{45}O_6NS$ and glycodeoxycholic acid (GDCA), glycochenodeoxycholic acid (GCDCA) and glycoursodeoxycholic acid (GUDCA) with monoisotopic mass of 449.3 Da and formula $C_{26}H_{43}O_5N$. For direct infusion, compounds were prepared in 50% MeOH at 0.1 µM concentration.

Liquid Chromatography: For LC separation compounds were prepared in 50% MEOH at 10 nM concentration was done on a SCIEX ExionLC™ AD HPLC system. . The samples were analyzed on a QTRAP® 6500+ LC-MS/MS system equipped with a SelexION® device.

Results

Using the SelexION® device, the bile acid standards were infused individually and as mixtures to determine the CoV values of the different isomers. As shown in Figure 3, the species of the two groups were infused and the compensation voltage ramped over a range of -40V to 30V. The total ionogram shows the presence of a number of separate peaks.

The LC-DMS-MS analysis were also performed. The addition of DMS to LC separation has several advantages:

- removal of chemical background for better quantification
- less requirement effort for development of chromatographic separation
- there is no requirement for retention time assignment
- less reliance on compound specific fragments which may be low abundant.

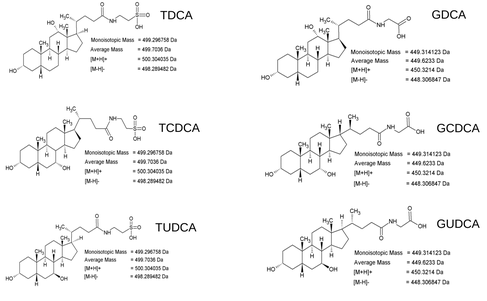

Figure 2. Bile acids are derivatives of cholesterol, which carry a short carboxylic acid chain on the C17 atom of the cholesterol back bone. A range of modifications can occur on the carboxylic function, which define the class of the bile acid. Two groups of isobaric bile acids were analyzed by SelexION® Technology: 1) Taurocholic acid (left), a conjugate of bile acid and taurine and 2) Glycocholic acid, which is a conjugate of bile acid with glycine. The isomers within each of the two classes differ only with respect to the location or configuration of a hydroxyl group. In the DCA-Variant, the hydroxyl group is located on the C-12 of the cholesterol skeleton, while in the CDCA and the UDCA variants, the hydroxyl group is located on the C-7 atom in alpha- or in beta-configuration, respectively.

Next, LC-DMS-MS analysis was performed. The addition of DMS to the LC separation has several advantages: including the removal of chemical background for better quantification, less requirement for development of chromatographic separation, no requirement for retention time assignment and less reliance on compound specific fragments which may be low abundant.

As show in Figure 4, the same transition was used, but with the addition of DMS, specific isomers can be determined by use of the respective specific compensation voltages.

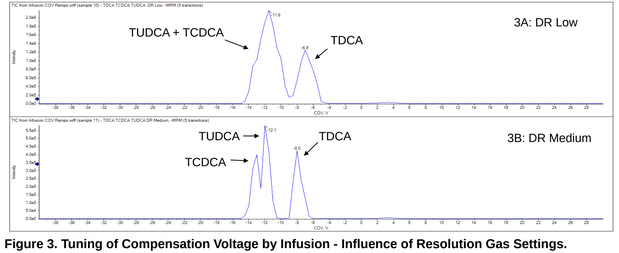

Figure 3. Tuning of Compensation Voltage by Infusion - Influence of Resolution Gas Settings. Applying the resolution gas parameter (DR) to the SelexION® separation leads to an increased residence time of the analytes in the DMS cell, which enhances the resolution of the analyte peaks. In this experiment, the bile acids were directly infused and the Compensation Voltage (CoV, abscissa) was ramped from -40V to +30V. In Fig. 3A, low resolution gas was applied, and the two isomers TUDCA and TCDCA could not be separated. A higher resolution gas setting is required (Fig 3B, DR Medium) to separated these isomers.

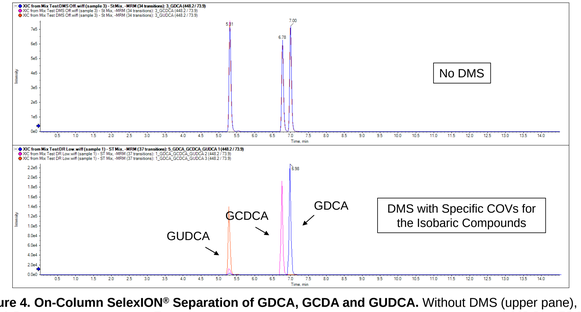

Figure 4. On-Column SelexION® Separation of GDCA, GCDA and GUDCA. Without DMS (upper pane), only LC separation is possible. For all three isomeric compounds, the same transition is used (448.2 -> 73.9) and each of the three entries in the SRM table detect all three isomers. Adding the compound-specific Compensation Voltage (lower pane) to the SRM-table of transitions allows only the isomer with the corresponding CoV to enter the MS for detection.

Finally, to show the utility of SelexION® technology in conjunction with chromatographic separation, a short separation method was performed using isocratic LC conditions.

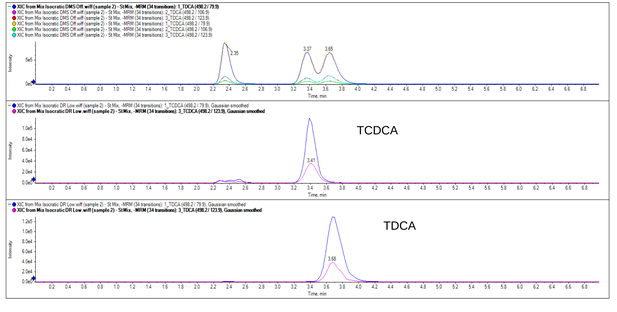

Figure 5: An Isocratic LC Method was Tested for Very Fast Separation of Bile Acids. Without SelexION® technology the two isobaric bile acid TDCA and TCDCA cannot be completely separated from each other. A confident identification and accurate quantitation would be difficult due to the overlap of the two LC peaks. A separation of these isobaric bile acids is possible by SelexION® Technology using the specific compensation voltages for these compounds.

CONCLUSIONS

- SelexION® technology can be used in clinical research studies to separate bile acid isomers by direct infusion, opening up the possibility for a very fast flow injection based quantitative bile acid assay.
- Furthermore, SelexION® technology, as an orthogonal separation dimension to LC-MS analysis, enhances the selectivity and therefore improves the confidence of identification as well as reliable quantitation of the isomers.

TRADEMARKS/LICENSING

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