Separation and analysis of co-eluting isobaric metabolites using differential ion mobility

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Abstract:

The aim of this project was to evaluate an LCMS system equipped with the SelexION differential ion mobility for the analysis of co-eluting isobaric glucuronides of Losartan. In this study, two tetrazole conjugated glucuronides of Losartan were generated from *in vitro* incubations in monkey hepatocytes. Both glucuronides were co-eluted using a fast LC method (typical bioanalytical method). The differential ion mobility technique separated both glucuronides with direct infusion. A 3D chromatogram was generated consisted of x and y axis which represent conventional LC-MS chromatogram whereas the z dimension was the compensation voltage (CoV) window of interest. CoV values for each isomers were optimized and then used to generate specific MS/MS spectra even for the co-eluted molecules.

Introduction:

LC-RAD-MS (online radioactive detector coupled with LCMS system) is a standard technique used for qualitative and quantitative metabolic profiling. Typically, a poor separation may reduce the accuracy of the quantification or characterization particularly for co-eluting peaks. Differential ion mobility (SelexION) is based on relative ion mobility of two charged species in an electric field in a presence of an organic modifier enriched countercurrent gas [1]. This technique has shown to be able to separate isobaric ions such as isomers and interferences [2]. Separations in ion mobility mode often do not correlate well with chromatographic based separation techniques. Here, we demonstrated the separation of two co-eluting glucuronides of Losartan using a direct infusion on a QTRAP 5500 fitted with a SelexION device. These results were compared to those obtained using a fast LC and a long LC gradient.

Methods:

Table 1: Experimental conditions*

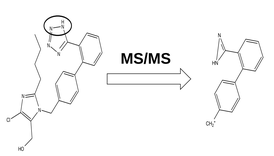
	"BA" Fast Method	Optimized method
Sample	Losartan incubated in Monkey hepatocytes (6h)	
Mass Spectrometer	AB SCIEX QTRAP 5500 equipped with SelexION	
(U)HPLC	Agilent 1290	
SRM transition	*m/z* 599.1 → 207.0	
DMS conditions	Separation Voltage 2500V, modifier 3% Isopropyl Alcohol	
Compensation Voltage (CoV)	Ramp (-5V to +5V) or Fixed (-1.5 or 0.8V)	
Column type	Zorbax XDB-C8 (2.1 x 30 mm, 3.5 µm)	
Buffers	(A) 10 mM ammonium acetate + 0.1 %FA (B) ACN + 0.1 %FA	
Flow rate / Col. Temp.	0.5 mL/min / 40°C	
Gradient	50 to 95 %B in 5 min	5 to 95 %B in 15 min

* Infusion had same MS parameters

Figure 1: Major sites of Glucuronidation of Losartan and major MS/MS fragment

Glucuronide of Losartan
$[M+H]^+$ = 599 Da

Losartan
$[M+H]^+$ = 423 Da



MS/MS

Major Fragment [2]
$[M+H]^+$ = 207 Da

Figure 2: HPLC separation (15 min run)

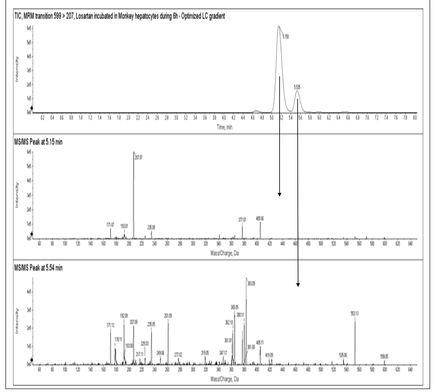


Figure 3: Ion Mobility separation (Infusion or 5 min run)

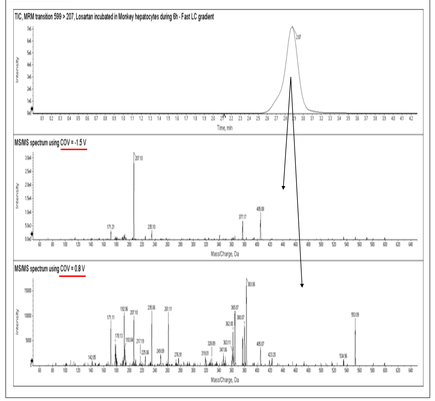
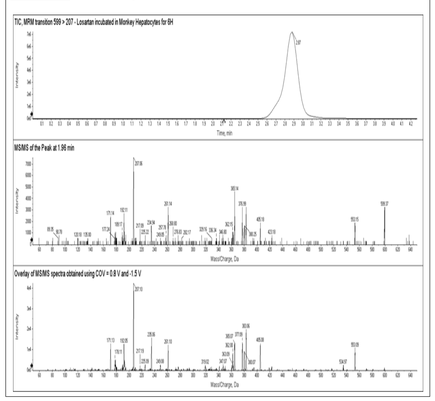


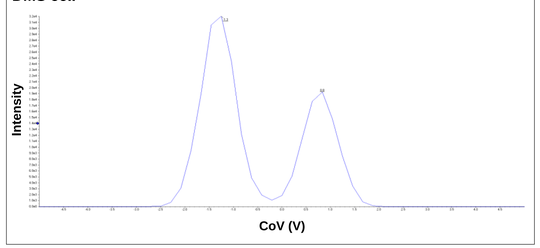
Figure 4: None optimal LC method without the SelexION



Isobaric compounds selectivity:

- A good LC method was able to separate isomers and to obtain selective MS/MS spectra of each glucuronides (Fig. 2).
- In fast analysis condition, both isomers are co-eluted. The resulting MS^2 spectrum was the overlay of the MS^2 spectra of each glucuronides (Fig. 4).
- Optimum CoV values for each glucuronides were used to run MS/MS experiment and obtain selective MS/MS spectra (Fig. 3) [3].
- The ion mobility separation can then be used to confirm the number of entities and obtain selective structural information.

Figure 5: Separation of two isobaric glucuronides of Losartan in the DMS cell



Testing of the MS Response Linearity:

- The MS response linearity was tested using Losartan, presumably the glucuronides have the similar response linearity as Losartan.
- The drug was quantified using Glyburide as Internal Standard (IS) and the SelexION as the separation technique (Fig. 6).
- SelexION showed similar linearity as conventional chromatography based technique (Table 2).
- The sensitivity in the ion mobility using direct infusion was limited by the ion suppression.

Figure 6: (a) Losartan and IS are separated and (b) quantified in direct infusion

(a) (b)

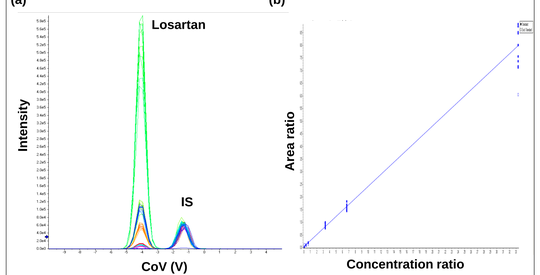
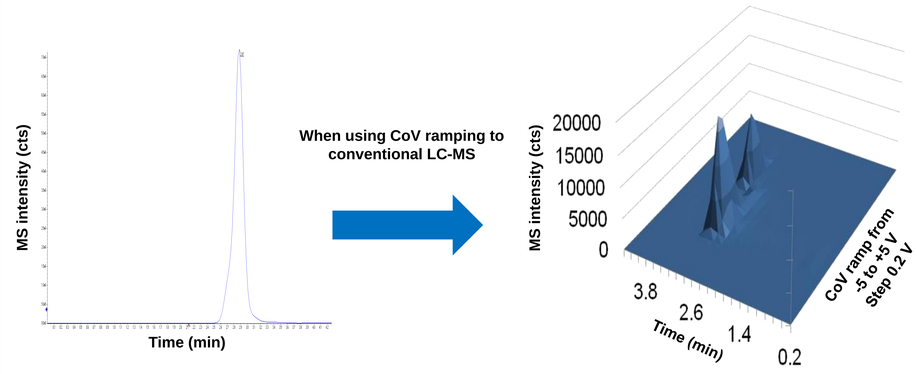


Table 2: Linearity comparison in SelexION and LC based technique

	SelexION	(U)HPLC
R^2 value	0.99535	0.99807
% CV (Ā)	7.12	1.78
Slope	y = 0.23846 x + 0.04161	y = 0.53117 x -0.08580
QC accuracy (Ā)	85%	78%
Run time (min)	12-40	120-400

3-Dimensional Separation:

Figure 7: Glucuronides of Losartan were separated using a the third dimension (CoV ramping) on a conventional LC-MS analysis with a fast gradient. On both pictures, *x* and *y* axes represent conventional LC-MS trace (SRM). The *z* axis is a CoV ramp from -5 to +5 V with a step of 0.2 V.



Co-elution of isobaric compounds requires additional method development or complementary techniques. The number of isobaric co-eluting species cannot be determined using solely LC-MS technology. In this experiment, the ion mobility device was able to separate isomers for ions possessing different CoV values. This added dimension of separation is captured in the 3-D-chromatogram (above). The method consisted of ca. 50 successive CoV ramps of a discreet window of interest (- 5 V to + 5 V, steps 0.2 V). The run time for a complete ramp depended on the ramping window, ramping step and number of MRM transitions monitored. The scan speed limitation is mitigated by measuring a broader peak width. Therefore, to be able generate a 3D chromatogram on a sharp peak (e.g. UPLC peak), the scan speed has to be increased.

Results and Conclusions:

- ✓ SelexION technique adds an additional level of selectivity for separating isobaric species.
- ✓ Two glucuronide isomers of Losartan were successfully separated and analyzed using the differential ion mobility.
- ✓ Developing a separation with the SelexION was similar to developing an LC method requiring a compromise between resolution and sensitivity.
- ✓ This technology can be used to remove endogenous compounds from complex matrices [4].
- ✓ A preliminary CoV ramping allowed to identify selective CoV optimum for both glucuronides.
- ✓ Using selective CoV optimum, selective MS/MS spectra were generated and were identical to those obtained with an HPLC peak separation.
- ✓ Co-elution of isobaric entities can now be identified using the SelexION compensation voltage ramping.
- ✓ The SelexION mobility module can be used for quantification yielding results similar to conventional LC separations. Quantification using the ion mobility was 10 times faster and didn't require a LC method development (no LC column used). The only limiting factor observed during quantification using infusion was the potential for ion suppression relative to the matrix of interest.
- ✓ The differential ion mobility system delivers a new dimension of selectivity for applications requiring the separation of isobaric metabolites. A 3D-chromatogram can be processed using multiple CoV ramps combined to a LC column separation.
- ✓ The scan speed for ramping the compensation voltage is a technical challenge and may not be applicable to short UPLC peaks. In addition, the data processing requires a lot of manual steps. An automated data processing solution is currently being developed in-house.

References:

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Acknowledgement:

- Helen Gu and Dr. WenKui Li for their strong contribution
- Dr. Tsu-Han Lin and Dr. Handan He for their continuous support