Differential Mobility Separation Enhances the Quantification of Lysophosphatidic Acid in Plasma

Using SelexION® Technology

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Lysophosphatidic acid (LPA) is a phospholipid signaling molecule in the class of lipid mediators, functioning by signaling through G-Protein coupled receptors or nuclear receptor proteins. LPA has been associated with a wide range of biological processes.

Accurate LPA measurement in plasma has proven difficult.[1] A bottleneck for accurate quantitation of LPA in plasma using current analytical methods is the chemical interferences. The most abundant LPA fragments are typically the best fragments for quantification, however the most intense MRM transitions for LPA have strong matrix interferences when monitored in plasma. There is an MRM transition that has good specificity in plasma however it has much lower signal intensity.

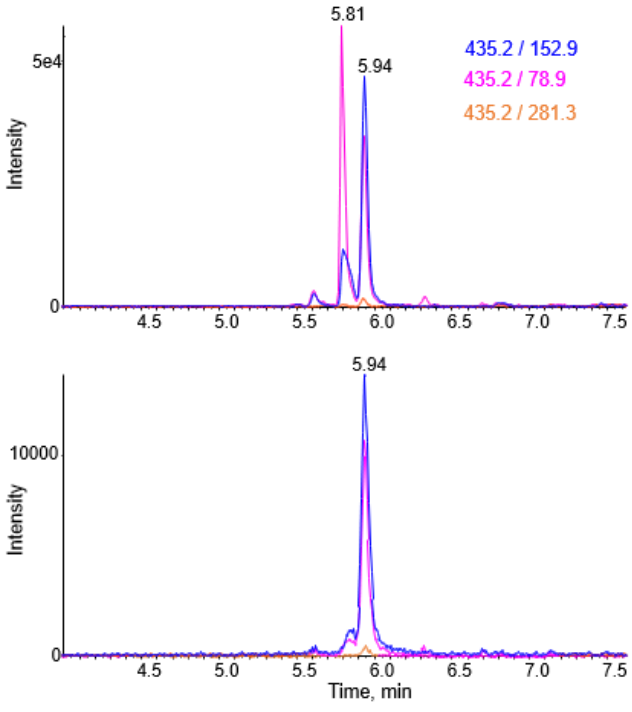

Figure 1. Comparison MRM transition without and with SelexION Technology in Plasma. (Top) The MRM transitions 153 and 79 show strong interferences as a double peak, making assignment difficult. The 3rd MRM transition does not have the same interference but has much lower intensity by comparison. (Bottom) In comparison, when using SelexION Technology, the interference is removed, allowing for a much more confident quantification.

Here, SelexION Technology is used for the quantification of LPA 18:1 in plasma which removes the matrix interferences observed when measuring plasma sample, allowing the use of the higher intensity MRM transitions for quantitation of LPA with much greater confidence.

Key Features of DMS for Lipid Separation

- SelexION Technology is a planar differential mobility device (DMS) that separates analytes based on differences in their chemical properties, prior to entering the instrument orifice, thus providing an orthogonal level of selectivity[2,3]
- DMS provides an extra level of selectivity, removing matrix interferences during measurement of target analytes when analyzing complex sample matrices like plasma sample, improving signal-to-noise
- Ability to use chemical modifiers with SelexION Technology allows lipid classes and subclasses to be resolved
- Differential charge site topography makes phospholipids ideal candidates for differential mobility separation with SelexION Technology[4]
- Minutes to install and remove the SelexION cell makes it easy to include differential mobility in quantitative studies

Methods

Sample Preparation: Lipid standards were purchased from Avanti Polar Lipids, Inc, USA. As a biological sample, human standard plasma was extracted and diluted 1:10 in Methanol for measurement.

Chromatography: Separation was performed using an ExionLC™ System using a Phenomenex Kinetex C18 100x2.1mm 2.6µm 100Å column. Chromatography details can be download from the SCIEX user community.[5]

Mass Spectrometry: Samples were analyzed using a QTRAP® 6500+ system equipped with SelexION Technology using Analyst® Software 1.7. Complete method information can be downloaded from the SCIEX user community.[5]

Data Processing: Separation results were visualized using PeakView® Software 2.2 and quantitation curves were analyzed using MultiQuant™ Software 3.0.

Optimization of Differential Mobility Separation

First, the compound and the source optimization parameters were determined for LPA 18:1 using infusion and the standard automated optimization tools of the Analyst software. Three transitions were used, two with higher intensity but less specificity, and a third with good specificity but less intensity.

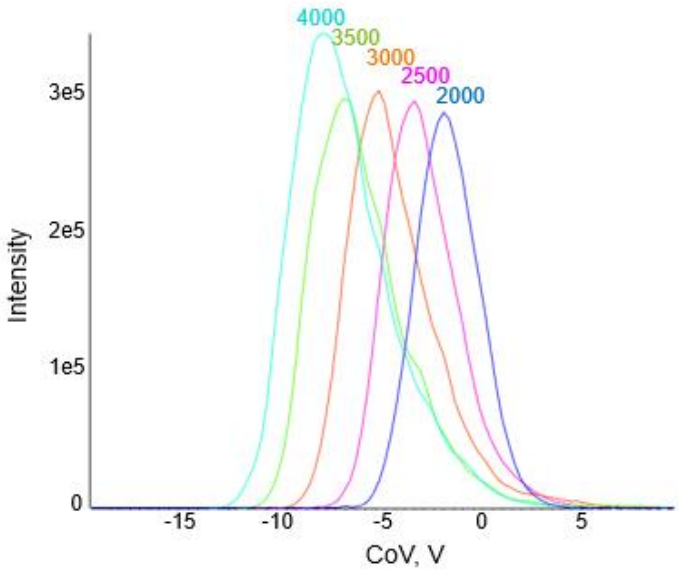

Figure 2. Separation Voltage Optimization. Using syringe infusion to tee LPA 18:1 into the LC stream, compensation voltages were ramped at a series of fixed separation voltages. The optimum of SV = 4000 was selected to use because of best intensity.

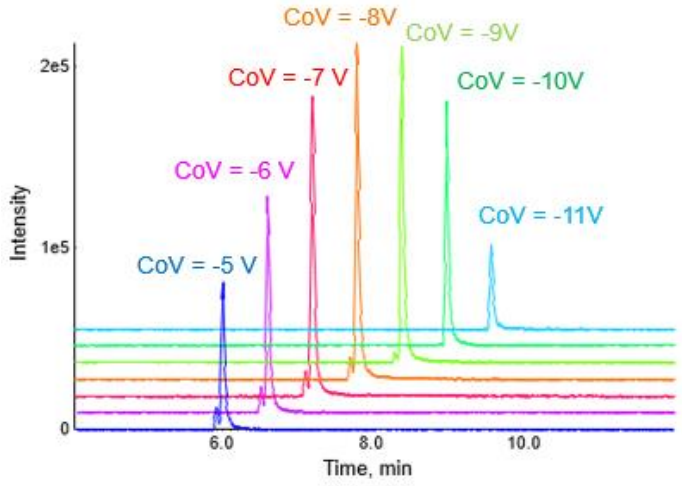

Figure 3. LC Chromatograms with On-Column CoV Optimization. In the on-column experiment, the separation voltage is set to 4000V based on the infusion optimization experiment. Then seven experiments are defined with each CoV set at discrete values from -5 to -11V monitoring a LPA MRM transition. Maximum intensity is found at CoV value of -8V, in correspondence to the optimized CoV found by T-infusion.

Using the optimized compound conditions, the DMS parameters were tuned next. Because the SelexION Device is sensitive to source temperature and mobile phase composition, it is important to tune the separation parameters under similar flow conditions as will be used during the LC-MS analysis. However, because a large CoV range must be tested, it is more practical to do this by direct infusion where the analyte is present over a long time. As such, the compound was introduced via T-infusion with a flow rate of 10 µL/min and simultaneous flowrate of mobile phase A and B at a ratio of 50:50 with a flow rate of 400 µL/min. The compensation voltage (CoV) was ramped from -20V to 10V at different separation voltages (SV) in the presence of 2-propanol as a modifier and a CoV of –7.9V was determined as CoV at SV = 4000V (Figure 2).

Next, the DMS parameters were confirmed using an on-column test. Using the same modifier as infusion test, the separation voltage of 4000V was selected. Then the CoV was stepped across the range of -5 to -11V using a set of 7 experiments (Figure 3). This on-column confirmation experiment is critical to fine tune the CoV settings under the exact chromatographic conditions used in the study. While the CoV value can be quickly determined by direct infusion or T-infusion, it may slightly shift when applied in the final chromatographic method due to solvent composition differences.

Comparison of Quantitation with and without Differential Mobility Separation

The utility of the SelexION device for the quantification of the LPA 18:1 in plasma was examined. Measuring the LPA without separation shows strong matrix interferences, especially for the highly abundant MRM transitions 435->153 and 435-> 79 (Figure 1, top). This hampers the proper quantification of this important signaling lipid in plasma due to the risk of chromatographic changes and the possible co-elution of the LPA with the matrix interferences. In contrast, interferences are strongly reduced by SelexION Technology, as shown in Figure 1 (bottom). Although the total signal intensity is decreased during differential mobility separation, the selectivity is enhanced, allowing to detect the endogenous LPA 18:1 at very good S/N ratio.

Using DMS allows the much higher intensity MRM transitions to be used for the quantitation experiments as the DMS provides the additional selectivity.

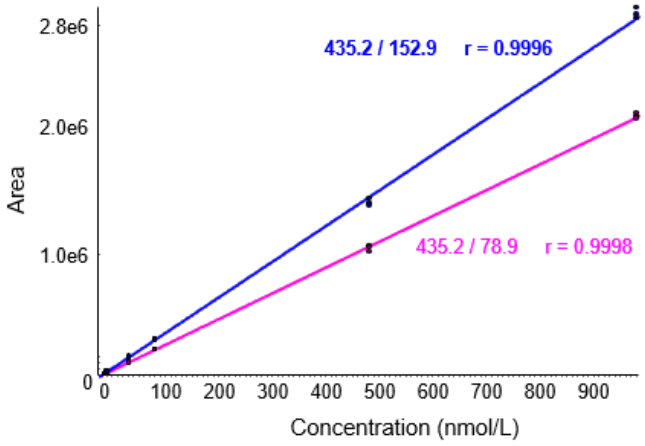

Figure 4: Calibration Curves for LPA 18:1. The two high intensity MRM transitions 435-> 79 and 435->153 are shown for the concentration range of 5 and 1000 nM.

Quantification of LPA 18:1 in plasma

The total LPA concentration in human plasma was reported with a large variation between 80 nM to 1.2 µM, depending on the method used. [1] calibration curve was measured between 5 nM and 1000 nM, covering two orders of magnitude, and adequate for measuring the endogenously present concentrations of LPA in plasma. Using differential mobility separation, the calibration curve was found to be linear for both MRM transitions (Figure 4). The statistics for both curves are shown in Table 1, with a relative CV of >5%.

Using this calibration curve, the endogenous LPA 18:1 concentration in plasma was determined with a S/N of >140 without signal smoothing (Table 2).

Table 1. Statistics of Calibration Curves for LPA 18:1. The relative standard deviation is well below 5%. For curves shown in Figure 4.

Concentration [nM]	# Values	Mean	Std Dev	%CV
435.2 / 152.9				
5	*3 of 3*	*1.48 e4*	*2.42 e2*	*1.6*
10	*3 of 3*	*3.24 e4*	*1.39 e3*	*4.3*
50	*3 of 3*	*1.44 e5*	*4.00 e3*	*2.8*
100	*3 of 3*	*2.83 e5*	*4.94 e3*	*1.7*
500	*3 of 3*	*1.36 e6*	*2.91 e4*	*2.1*
1000	*3 of 3*	*2.85 e6*	*4.34 e4*	*1.5*
435.2 / 78.9				
5	*3 of 3*	*1.10 e4*	*3.60 e2*	*3.3*
10	*3 of 3*	*2.35 e4*	*2.15 e2*	*0.9*
50	*3 of 3*	*1.03 e5*	*1.91 e3*	*1.9*
100	*3 of 3*	*2.06 e5*	*2.53 e3*	*1.2*
500	*3 of 3*	*1.00 e6*	*2.49 e4*	*2.5*
1000	*3 of 3*	*2.04 e6*	*2.18 e4*	*1.1*

Table 2. Statistics of Measuring the Endogenous Single LPA 18:1 Species in 1:10 Diluted Plasma, using SelexION Technology. The S/N ratio of the endogenous species is >140, highlighting the confidence detection. The calculated concentration was corrected for the sample dilution.

Plasma	MRM	Area	Retention Time	S/N	Calc Concentration [nM]
1	*435.2 / 152.9*	*1.91 e4*	*5.94*	*199.5*	*60.11*
1	*435.2 / 78.9*	*1.37 e4*	*5.94*	*141.6*	*50.94*
2	*435.2 / 152.9*	*1.90 e4*	*5.95*	*212.2*	*60.08*
2	*435.2 / 78.9*	*1.43 e4*	*5.94*	*185.2*	*60.24*
3	*435.2 / 152.9*	*1.97 e4*	*5.94*	*208.8*	*60.34*
3	*435.2 / 78.9*	*1.45 e4*	*5.93*	*150.5*	*60.33*

Conclusions

A method has been developed here for the quantitation of Lysophosphatidic acid (LPA) in plasma using SelexION Technology for reducing matrix interferences and increasing method robustness. The method was applied to measure endogenous LPA in a series of plasma samples. With this method, a concentration of ca 60 mM in human plasma samples was determined, which is in line with the reported values in the literature.[1]

References

1. Onorato, Joelle M., Petia Shipkova, Anne Minnich, Anne-Françoise Aubry, John Easter, and Adrienne Tymiak. 2014. “Challenges in Accurate Quantitation of Lysophosphatidic Acids in Human Biofluids.” Journal of Lipid Research 55 (8): 1784–96. https://doi.org/10.1194/jlr.D050070.
2. Schneider et al, (2010) Planar Differential Mobility Spectrometer as a Pre-Filter for Atmospheric Pressure Ionization Mass Spectrometry, Int. J. Mass Spectrom., 298 (1-3), 45-54.
3. SelexION® Technology: A New Solution to Selectivity Challenges in Quantitative Analysis - Differential Mobility Separations Enhanced with Chemical Modifiers: A New Dimension in Selectivity. SCIEX Technical Note RUO-MKT-02-3251-B.
4. Differential Mobility Separation for Improving Lipidomic Analysis by Mass Spectrometry - SelexION® Technology on QTRAP® Systems. SCIEX Technical note RUO-MKT-02-4802-A.
5. Download complete method information from SCIEX User Community.

Document number: RUO-MKT-02-9274-A

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