AB SCIEX SelexION™ Technology: A New Solution to Selectivity Challenges in Quantitative Bioanalysis

Differential Mobility Separations Enhanced with Chemical Modifiers: A New Dimension in Selectivity

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High selectivity is a key component of successful quantitative bioanalysis. The ever increasing sensitivity and throughput requirements of bioanalytical assays often pose method development challenges. One of the most common issues encountered is the presence of a matrix interference which must be eliminated by adjusting HPLC conditions, or modifying sample preparation.

Interferences can be present as an un-resolved chromatographic peak, or as a high baseline. In some cases, separation of isomers is required. If a high baseline problem cannot be solved, LOQ's and dynamic range are adversely impacted. Resolving a difficult chromatographic interference can require slower chromatography or more complicated and labor intensive sample clean-up. It also slows down data review if peak integration has to be manually adjusted on a sample by sample basis.

Significant advances have been made in increasing MS/MS selectivity beyond the gold standard MRM. For example, MRM^3 on the QTRAP® 5500 system adds additional selectivity by increasing the number of fragmentation steps. Ion mobility presents another attractive option by introducing additional selectivity during sample introduction, following atmospheric pressure ionization. Although ion mobility techniques have been used extensively for qualitative applications, they have traditionally lacked the required ruggedness and speed required for quantitative bioanalysis.

The AB SCIEX SelexION™ Technology with the QTRAP® 5500 system brings the power of differential ion mobility separation to complex bioanalysis, enabled by multiple new innovations in ion mobility.

Key SelexION™ Technology Innovations

- The introduction of chemical modifier adds a new dimension to selectivity and dramatically increases separation capacity.
- Planar geometry results in high speed and minimal diffusion losses for maximum sensitivity and UHPLC compatibility.
- Highly robust, reproducible, and stable for use in regulated bioanalysis.
- Easy to maintain, and can be Installed or removed in minutes with no need to break vacuum or use any tools.

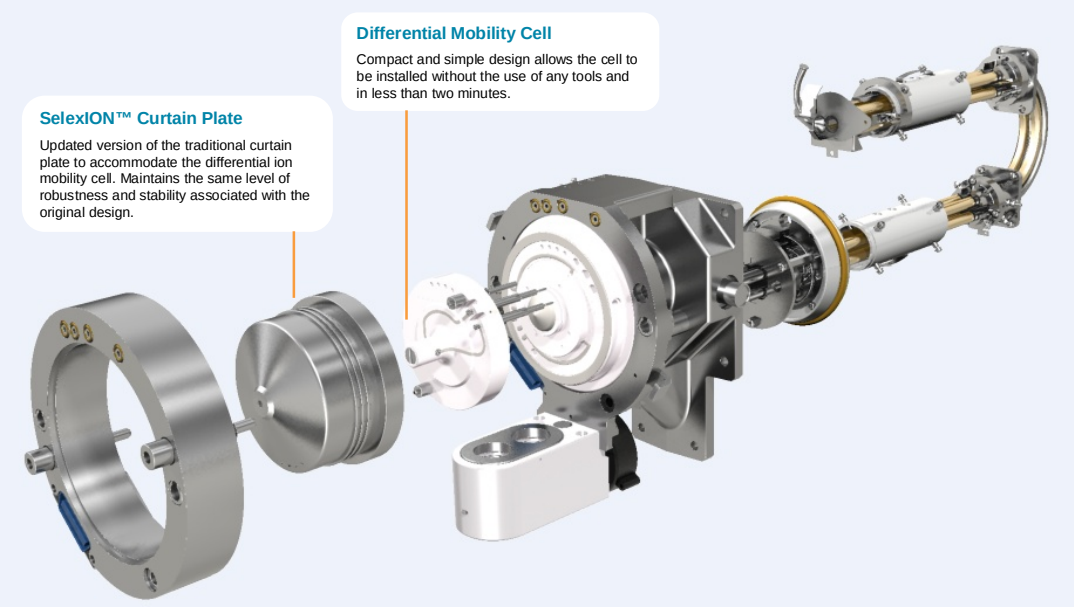

Figure 1. SelexION™ Technology. The DMS interface is directly coupled to the orifice plate. A modified curtain plate accommodates the DMS cell which can be easily installed and removed without the use of any tools and without venting the system. The source extension ring enables use of the standard AB SCIEX Triple Quad™ and QTRAP® system sources.

Innovative Planar Design

SelexION™ Technology brings differential mobility separation (DMS) to the QTRAP® 5500 system in a compact, easy to use device. It is integrated in the ion source region directly in front of the orifice and behind the curtain plate (Figure 1). The DMS cell consists of two parallel flat plates (10 x 30 mm, 1 mm gap) with an RF voltage (the Separation Voltage, SV) applied across the plates. Unlike traditional ion mobility, ions are not separated in time as they traverse the cell. They are separated in trajectory based on difference in their mobility between the high field and low field portions of the applied RF (Figure 2). As the ions migrate towards the walls of the DMS cell at different rates, they will be separated. By applying a second voltage offset (the Compensation Voltage, CoV) the trajectory of the desired ions can be corrected along the axis of the cell and towards the orifice. Other species will migrate away from the straight line due to the difference in mobility compared to the analyte of interest.

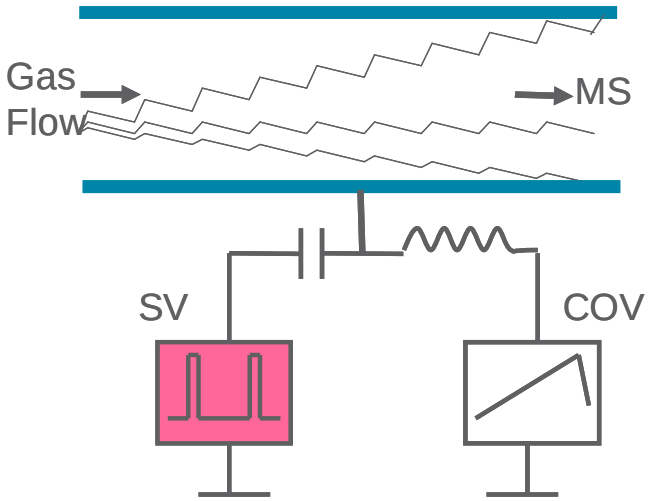

Figure 2. Differential Mobility Separation Process. Innovative planar design of the DMS cell uses an asymmetric RF waveform (SV) to separate ions based on differential mobility between the high and low fields. The compensation voltage (CoV) is used to correct the trajectory of the ion of interest which traverses the cell and into the orifice while interferences are deflected into the cell walls.

This planar design yields a stable, easy to tune system with high resolving power over a short distance. This gives high speeds and short residence times, resulting in minimal diffusion losses and enabling the use of short MRM cycle times. By simply turning off the separation voltage, the cell becomes transparent with ions moving normally along the centre line of the device. This means that it is possible to transmit ions through the mobility cell when not using the DMS mode. A significant signal loss occurs in transparent mode, so we recommend dismounting the device for maximum sensitivity in non-DMS mode.

Chemical Modifiers – The Second Dimension

Volatile reagents can be introduced into the gas flow which chemically modifies how the ions interact with curtain gas during the DMS separation. Different species will have different affinities to form clusters with this chemical modifier. As the clustered ions move between the high field and low field portions of the applied RF, they will have different rates of clustering and de-clustering.

In the high field portion, ions will de-cluster due to the higher energy available. But in the low field, the cluster will form again. This interaction dramatically increases the separation capacity of the DMS cell, taking advantage of chemical properties such as proton affinity to add another dimension in the differential mobility effect.

In this application note, we will demonstrate how the SelexION™ Technology can be used to solve selectivity challenges while maintaining rugged bioanalytical performance.

Experimental

Sample Preparation: A standard curve of Clenbuterol was prepared in pooled urine in the concentration range of 63 pg/mL to 125 ng/mL. Quality control samples were also prepared at 0.625, 6.25, and 62.5 ng/mL, respectively. Clenbuterol-d9 was used as internal standard

Chromatography: A Shimadzu Prominence HPLC system equipped with a Hypersil Gold C18 column (2x50mm, 3µm) was used for this analysis. The run consisted of a 3 minute water / acetonitrile gradient (0% to 95% ACN) at a flow rate of 450 µL/min. The column was heated to 40 °C.

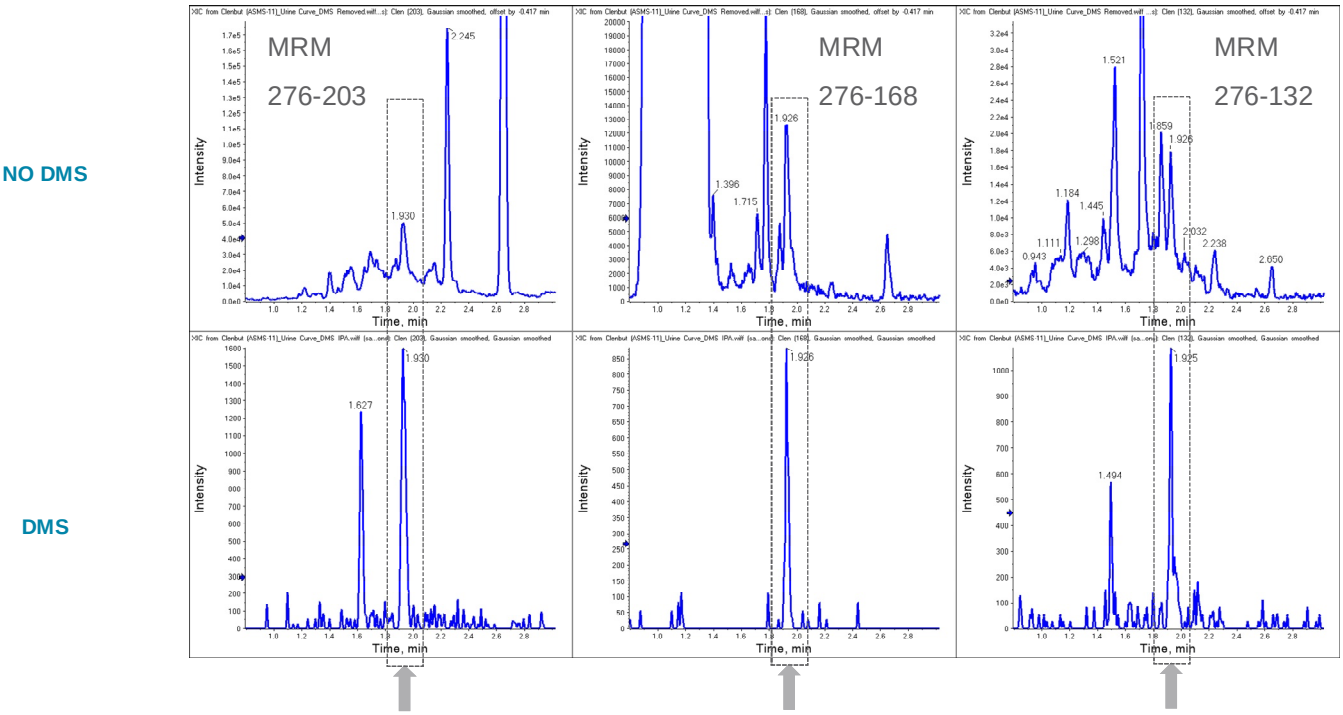

Figure 3. Analysis of Clenbuterol in urine. This assay suffers from matrix interferences in all three MRM channels (top). Using SelexION™ Technology with isopropanol as a chemical modifier resulted in the reduction or elimination of interferences in all three MRM channels (bottom).

Differential Mobility Separation: A QTRAP® 5500 system equipped with the SelexION™ Technology interface was used for all experiments. Isopropanol was used as a chemical modifier and was introduced into the curtain gas using an integrated pump. Modifier flow rate was calculated to give a final gas phase composition of 1.5% for the modifier. The DMS cell was operated at a separation voltage (SV) of 2800V. A Turbo V™ source was used in ESI mode. The MRM transitions of m/z 276 **à** 203, 276 **à** 168, and 276 **à** 132 were monitored for the analyte as discussed below.

Eliminating Interferences and Maintaining Ruggedness

LC-MS/MS analysis of clenbuterol in urine samples is known to exhibit a high degree of interferences in all major MRM transitions that can be monitored. These interferences also vary greatly in terms of complexity and intensity levels between different samples, posing a significant bioanalytical challenge.

Using DMS with isopropanol as a modifier, interferences are reduced or completely eliminated in all three MRM transitions in the urine matrix (Figure 3). Comparing urine from multiple different subjects, major interferences are present across different samples with significant variation in the profile. Using the selectivity of DMS, the chromatogram is dramatically cleaner and the data is much easier to process.

High inter-day and intra-day reproducibility is critical for success. The AB SCIEX SelexION Technology demonstrates excellent stability and reproducibility. A batch consisting of a standard curve in duplicate (concentration range: 63 pg/mL – 125 ng/mL in urine) and three QC levels (n=5), was run four times on four consecutive days. Each batch contained a total of 60 samples. Inter-day and intra-day precision and accuracy were shown to be well within bioanalytical validation guidelines (Table 1).

QC Level	Day	N	Mean	STD DEV	Percent CV	Accuracy
0.625 ng/mL	1	5 of 5	0.588	0.070	12.0	93.3
	2	5 of 5	0.624	0.063	10.1	99.0
	3	5 of 5	0.632	0.062	9.8	101.1
	4	5 of 5	0.694	0.140	20.2	110.1
	Average				**13.0**	**100.9**
6.25 ng/mL	1	5 of 5	6.49	0.59	9.1	103.8
	2	5 of 5	6.27	0.49	7.8	100.3
	3	5 of 5	6.32	0.61	9.6	101.2
	4	5 of 5	6.30	0.67	10.6	100.9
	Average				**9.3**	**101.5**
62.5 ng/mL	1	5 of 5	56.2	5.5	9.8	90.0
	2	5 of 5	62.1	8.9	14.3	99.4
	3	5 of 5	55.1	3.4	6.2	88.2
	4	5 of 5	59.5	4.4	7.5	95.1
	Average				**9.4**	**93.2**

Table 1. Clenbuterol QC Results. Inter-day and intra-day precision and accuracy over four days for clenbuterol in urine. SelexION Technology demonstrated excellent stability for satisfying bioanalytical acceptance criteria.

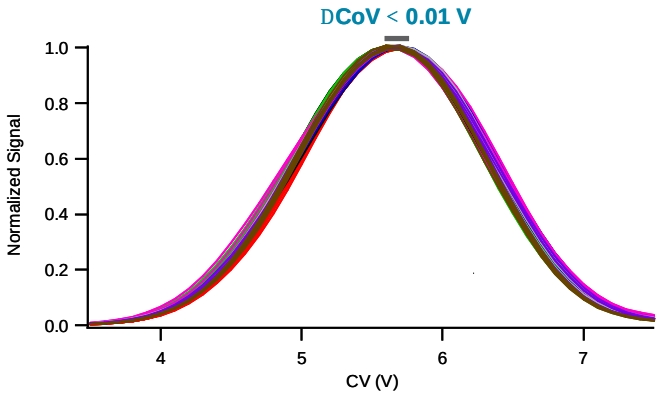

Figure 4. High Stability of Compensation Voltage. CoV stability is a key requirement for reproducible bioanalytical quantification with DMS. CoV remained within 0.01V when measured repeatedly over 24 hours demonstrating excellent stability.

A key component in SelexION™ Technology is the excellent stability of compensation voltage over time (Figure 4). This is critical for quantitative precision and accuracy.

Overcoming High Baseline

Quantification of Pentoxifylline in protein precipitated plasma is limited by a very high baseline in the MRM transition of m/z 279.2 **à** 99.2. Due to the relatively low masses used in this transition, chemical noise limits both the LOQ and assay linear range. By using DMS with methanol introduced as a modifier, the baseline is lowered dramatically (Figure 5). At 10 ng/mL in plasma, signal to noise was improved by a factor of 20 times on the QTRAP® 5500 system compared to the same conditions without DMS.

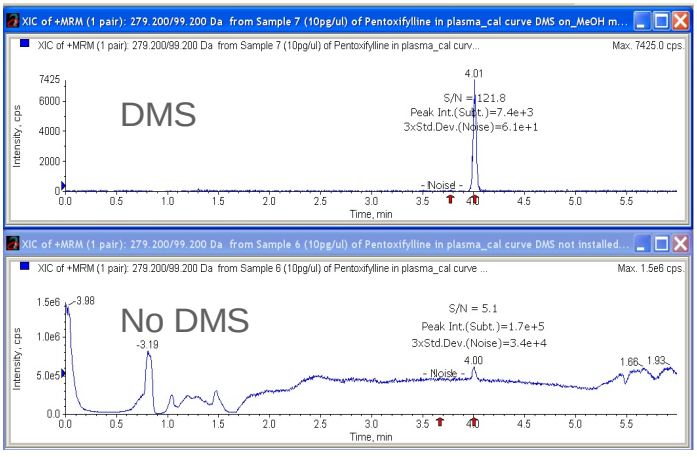

Figure 5. Pentoxifylline in Protein Precipitated Plasma. Very high baseline is successfully removed with DMS, resulting in a 20-fold improvement in signal to noise at 10 ng/mL.

Separating Isomers

AB SCIEX SelexION™ Technology enables separation of isobaric analytes. This can enable shorter run times if the analytes do not need to be resolved chromatographically. Ephedrine and pseudoephedrine are isobaric diastereomers. When using acetone as a chemical modifier in the DMS cell, the two analytes exhibit different mobility coefficients and are completely resolved by using different compensation voltages (CoV) as shown in Figure 6. Since the analytes are not resolved chromatographically in this case, and their MRM transitions are identical, their separation using DMS is essential to their quantitation. The optimum CoV values can be easily determined from the plot.

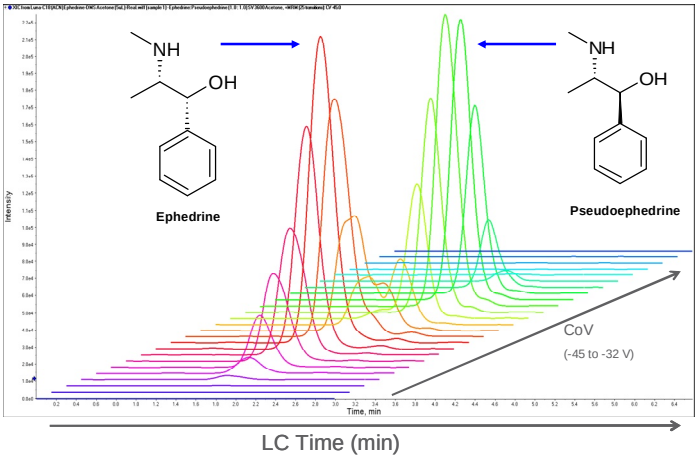

Figure 6. Separation of Ephedrine and Pseudoephedrine with SelexION Technology. Analytes are completely resolved in CoV space even though they are not resolved in chromatographic space.

Conclusions

- AB SCIEX SelexION™ Technology with the QTRAP® 5500 System is a powerful tool enabling a new dimension in selectivity while maintaining ruggedness.
- Using chemical modifiers in differential mobility separations dramatically increases resolving power.
- Innovative planar geometry of SelexION Technology yields a compact and rugged device and allows the DMS cell to remain in place even when ion mobility is not in use.
- Excellent inter-day and intra-day precision and accuracy can be achieved with SelexION Technology, to satisfy bioanalytical validation requirements.
- Whether facing the challenge of resolving a chromatographic interference, eliminating a high baseline, or separating isomers, AB SCIEX SelexION Technology with the QTRAP 5500 System offers a powerful new tool to help the bioanalytical scientist solve tough selectivity challenges.

References

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