Differential Mobility Separation with SCIEX SelexION®- A Novel Technique for the Bioanalysis of Poorly Fragmenting Molecules like Valproic Acid

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Major Challenges for Valproic acid (VPA) Bioanalysis

- **Poor fragmentation -** Valproic acid (VPA) produces no stable product ions due to its simple chemical structure, this makes multiple reaction monitoring (MRM) not feasible. Scientists must rely on multiple ion monitoring (MIM) or pseudo MRM by selecting the parent ion in both Q1 and Q3 for quantitation.
- **High background noise -** Analysis of VPA in using MIM or pseudo MRM leads to high chemical noise due to a lack of selectivity
- **Isobaric interference in complex matrix –** Due to the lack of selectivity using MIM isobaric interferences from the matrix may interfere at the retention time of the analyte. Separating these interferences and requires longer chromatography gradients which limits sample throughput.
- **Limit of Quantitation (LOQ) -** Both high background and isobaric interference limits LOQ that can be achieved.

SelexION® Technology for Bioanalytical Quantitation

- SelexION technology is a planar differential mobility separation device (DMS) that separates compounds based on their respective size, shape, charge state and chemical interaction, prior to entering the mass spec and adds an orthogonal level of separation and selectivity prior to the instrument orifice
- SelexION Technology is compatible with the fast cycle times and the narrow LC peak widths required for bioanalytical quantitation with UHPLC.
- The SelexION is highly robust, reproducible, and stable for use in regulated bioanalysis.

Figure 1. The SCIEX Triple Quad™ 5500 LC-MS/MS System with SelexION® technology.

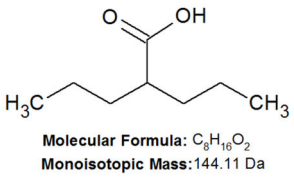

Figure 2. Structure of Valproic acid.

Key benefits of the SelexION® Technology for VPA Bioanalysis

- Elimination of high background noise in MIM or pseudo MRM mode without any change in the optimal compound dependent parameters
- Removal of isobaric interferences from biological matrix adds a new dimension of selectivity for the

method and made quantitation possible with a simplified protein precipitation extraction technique which saved time and cost per sample.

- Improvement in signal to noise ratio and LOQ of the method due to lower baseline noise and removal of interferences.
- Easy to maintain, and can be installed or removed in minutes with no need to break vacuum or use any tools.

INTRODUCTION

Valproic acid (VPA) is one of the most widely used antiepileptic drugs for grand mal epilepsy and petit mal epilepsy, often with other adjunctive therapeutic agents. VPA is an analog of the natural fatty acid valeric acid and also known as 2-propyl pentanoic acid (Figure 2). Administration of VPA in humans may results in rare but serious hepatotoxicity as metabolism by liver enzymes is the major route of elimination. Therefore developing a bioanalytical method to clearly define its pharmacokinetics is crucial.

Numerous techniques have been reported for the quantification of VPA in biological samples including: fluorescence polarization immunoassay, thin layer chromatography-derivatization, homogeneous enzyme-linked immunoassay, gas chromatography, high performance liquid chromatography and high performance liquid chromatography with tandem mass spectrometry (LC–MS/MS).

However, LC-MS/MS methods for VPA do have challenges due to some of the reasons listed above which emphasize the need for developing a novel technique for its quantitation in biological matrices. In this tech note we employ the SelexION DMS to develop a specific and sensitive method for the quantitation of VPA in human plasma over the range of 50 to 8000 ng/mL.

Experimental

Sample Preparation

In a microfuge tube 0.2 mL VPA spiked plasma was vortexed for 30 seconds with 0.6 mL methanol, then centrifuged for 10 minutes at 13000 rpm. The supernatant was transferred in an autosampler vial and 10 µL was injected into the LC-MS system.

Chromatographic conditions

Table 1. Chromatography conditions.

Column	Atlantis dC18 (100 × 2.1mm, 3.0 µ)	
Mobile Phase A	5 mm Ammonium Acetate	
Mobile Phase B	Methanol	
Flow rate	0.5 mL/min	
Column temperature	50 °C	
Gradient	Time	%B
	0.00	10
	0.50	20
	1.00	50
	1.50	80
	3.00	80
	3.50	10
	5.00	10
Auto sampler Temperature	5°C	
Injection volume	10 µL	

Mass spectrometric conditions

The SCIEX Triple Quad™ 5500 System equipped with SelexION Technology and a Turbo V™ source was used. Scan type of Q1 Multiple Ions (Q1MI) was used for monitoring VPA. DMS parameters were optimized for VPA in T infusion mode with the goal to maximize signal intensity and reduce the background interference. The mass spectrometry and DMS optimised conditions are given in Table 1.

Table 2. MS and DMS parameters.

Source		Compound		DMS	
Curtain Gas	30	DP	-55	DMS Temp	Medium
CAD	-	EP	-10	Modifier	None
Ion Spray Voltage	4500	CE	NAP	Separation Voltage (SV)	3000
		CXP	NAP	CoV	-2.2
Temp	650	Q1MI	143.1	DMO offset (DMO)	3

Mass spectrometric conditions

Analyst® version 1.6.3 was used for mass spectrometer data acquisition and processing. A $1/x^2$ weighted linear regression was used to calculate concentrations.

RESULTS AND DISCUSSIONS

VPA has a carboxyl side chain in its molecular structure and produces an intense peak of 143.1 in negative ion mode. Though the abundance of the deprotonated ion $[M-H]^-$ was high, the product ion spectrum produces no intense daughter ions. Most published methods for VPA suggest the use of Pseudo-MRM as an effective way to overcome this with the same Q1 and Q3 ions and low collision energy. But this technique cannot effectively filter all the background interferences and as a result, the baseline noise is high and this affects the sensitivity of the assay too.

To overcome these limitations acts, we used the Differential Mobility separation technique with SCIEX SelexION® a novel method for the quantitation of VPA in biological matrix in Q1 Multiple Ion scan mode. Addition of the SelexION DMS device had a dramatic impact on reducing the baseline noise in the chromatogram as can be seen in Figure 3, the baseline noise was reduced from 7.5e5 cps to 2000 cps.

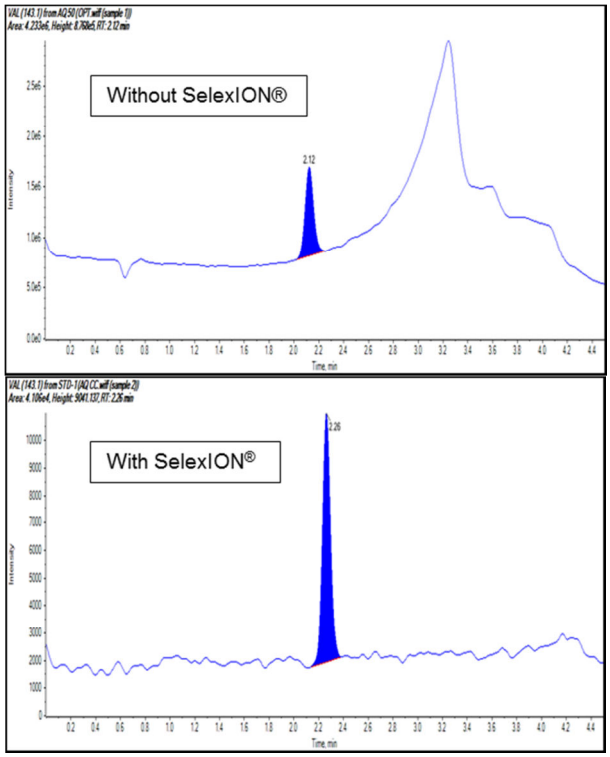

Figure 3. Chromatograms of a 50ng/mL VPA neat standard without the SelexION (top) and with SelexION (below). The baseline is greatly reduced from 7.5e5 cps to 2000 cps. The retention time of VPA is 2.26 minutes.

We adopted a simple protein precipitation technique using methanol for the extraction of VPA from human plasma. In the matrix extracted samples chromatographic conditions and mobile phases were optimized in order to obtain symmetric LC peak shape of VPA. In blank human plasma an interfering peak (1.68 mins) was observed that extensive chromatographic development could not separate. However the orthogonal selectivity of the SelexION® completely eliminated the interfering peak which can be seen in (Figure 4.).

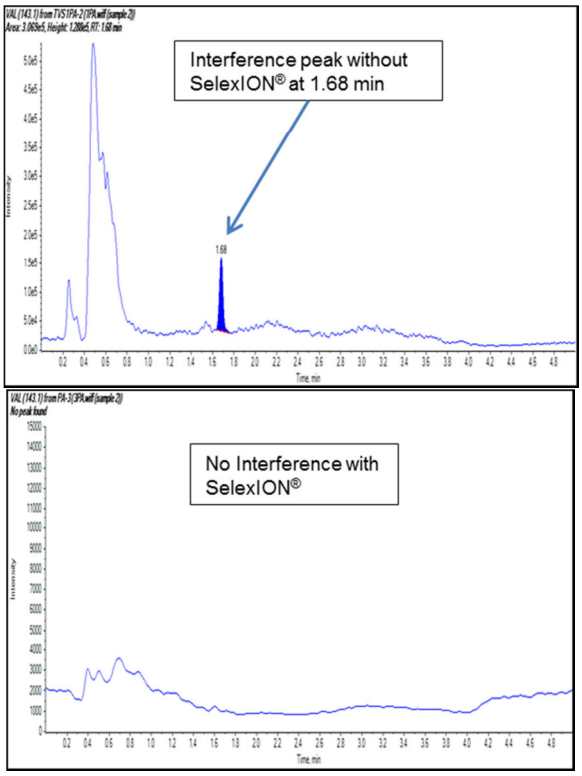

Figure 4. Chromatograms of blank extracted human plasma showing elimination of an interfering peak by using the SelexION device.

With the baseline noise lowered and the interfering peak removed with SelexION® the signal to noise at the LLOQ was improved 4-fold from 52.5 to 12.1 (Figure 5).

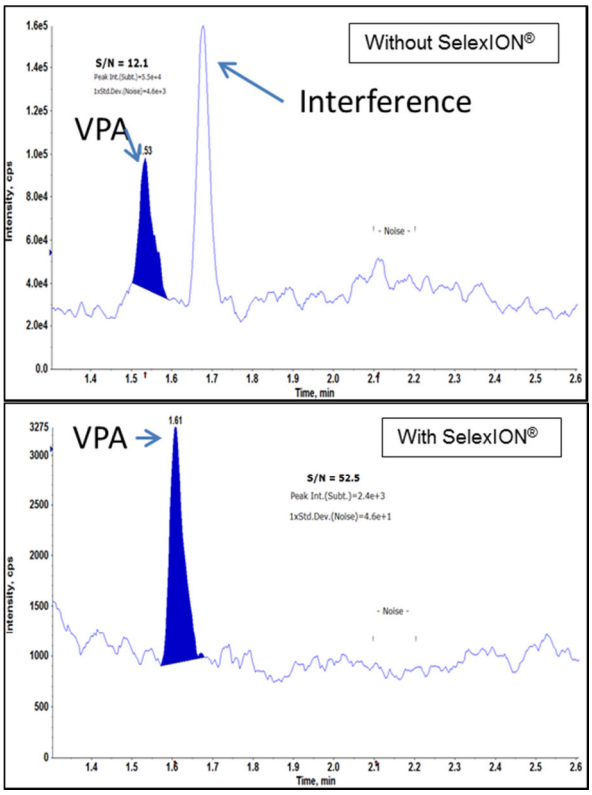

Figure 5. The SelexION device reduced baseline noise and resulted in a 4-fold increase in signal to noise at the LLOQ. The retention time of the interference peak

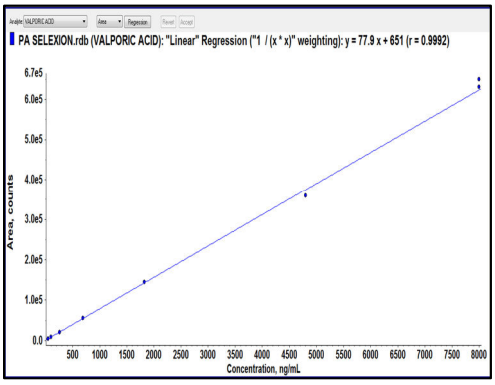

Figure 6. Calibration curve of VPA (50 to 8000 ng/ml) in Plasma.

There was excellent relationship between analyte area response and concentration and linearity was established in the range of 50 to 8000 ng/ml in human plasma with correlation coefficient r = 0.99 (Figure 6). The accuracy and precision of the calibration standards is shown in Table 3.

Table 3. Accuracy and precision statistics

Standards	Average Accuracy (%), N=3
STD A	97.3
STD B	106.2
STD C	98.9
STD D	100.8
STD E	102.1
STD F	92.5
STD G	102.7

CONCLUSIONS

A novel technique for the bioanalysis of a poor fragmenting analyte like valproic acid was established using SCIEX SelexION® on a SCIEX Triple Quad™ 5500 System. The Differential Mobility Separation (DMS) using SelexION Technology provides an orthogonal level of selectivity by separating components and interfering ions based on size, shape, charge state and chemical interaction. The SelexION was able to reduce the baseline noise in the Q1 MI scan and remove an interfering peak in the chromatogram and improved the signal to noise ratio at the LLOQ.

REFERENCES

1. Liquid chromatography–tandem mass spectrometry method for simultaneous determination of Valproic acid and its ene-metabolites in epilepsy patient plasma. Journal of Pharmaceutical Analysis 6 (2016)112–116.

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