Differential Mobility Separation with SCIEX SelexION®- A Novel Technique for the Bioanalysis of poor fragmenting molecules like Valproic Acid

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INTRODUCTION

Valproic acid (VPA) is one of the most widely used antiepileptic drugs for grand mal 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-3).
Numerous techniques have been reported for its quantitation in biological samples, mainly including Gas chromatography, High performance liquid chromatography and High performance liquid chromatography with tandem mass spectrometry(LC–MS/MS). However, VPA have challenges for the determination with tandem mass spectrometry due to some of the reasons listed in “Major challenges for VPA bioanalysis” section below which emphasize the need for developing a novel technique for its quantitation in biological matrices.

Major Challenges for VPA Bioanalysis

- **Poor fragmentation** – VPA produces poor fragments with no stable product ions due to its simple chemical structure
- **MRM not feasible** - Q1 and Q3 in MRM should have same quasi molecular (Pseudo MRM) ion due to its fragmentation limitations
- **High background noise-** Analysis of VPA in Q1MI or Pseudo MRM will lead to high chemical noise
- **Isobaric interference in complex plasma matrix** - Isobaric interferences from the matrix may affect the chromatography which needs to be separated and it is time consuming
- **Limit of quantitation (LOQ)** - High background can compromise the LOQ of the method because of its interference in the blank sample.

Key benefits of SelexION® Technology for VPA Bioanalysis

- Elimination of high background noise in Q1MI or Pseudo MRM mode without any change in the optimal compound dependent parameters
- Removal of isobaric interferences from biological matrix added a new dimension of selectivity for the method
- Quantitation made possible with protein precipitation extraction technique simplified the method development time and cost per sample..

SelexION® Technology for Bioanalytical Quantitation

- SelexION® Technology is a planar differential mobility separation device (DMS) that separates compounds based on difference in their chemical and structural properties.
- Planar geometry results in high speed and minimal diffusion losses for maximum sensitivity and UHPLC compatibility.
- SelexION® Technology adds an orthogonal level of separation and selectivity prior to the instrument orifice (Figure 2).
- SelexION® Technology is compatible with fast cycle times required for monitoring multiple MRM transitions combined with narrow HPLC peaks.
- 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.

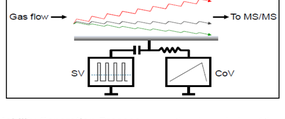


Figure 1. Differential Mobility Separation Process. Innovative planar design of the DMS cell uses an asymmetric RF waveform (SV) to separate ions based on differential mobility between the high and low fields. The compensation voltage (CoV) is used to correct the trajectory of the ion of interest which traverses the cell and into the orifice while interferences are deflected into the cell walls.

MATERIALS AND METHODS

Sample Preparation:

In an Eppendorf tube 0.2 mL VPA spiked plasma was shaken 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 were injected into the LC-MS system.

Chromatographic conditions:

LC System : LC 800 - GL sciences
Column : Atlantis dC18 (100 × 2.1mm, 3.0µ)
Column Temp : 50 °C
Flow Rate: 0.500 ml/min
Flow type : Gradient
Mobile phase : 5mm Ammonium acetate buffer and Methanol

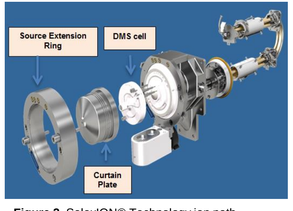


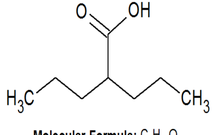
Figure 2. SelexION® Technology ion path components

Mass spectrometric conditions:

The SCIEX 5500 series 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 for the interest to maximize signal intensity and reduce the background interference. The mass spectrometry and DMS optimised conditions are given in Table 1.

Source Parameters		Compound Parameters		DMS Parameters	
Curtain Gas	30	DP	-55	DMS Temp	Medium
CAD	NAP	EP	-10	Modifier	None
Ion Spray Voltage	4500 (-Ve)	CE	NAP	Separation Voltage (SV)	3000
Temp	650	CXP	NAP	CoV	-2.2
GS1	55	Q1MI	143.1	DMO offset (DMO)	3
GS2	50	Dwell Time	200	DMS Resolution (DR)	Low

Table 1: Mass Spectrometry & DMS Conditions



Molecular Formula: $C_8H_{16}O_2$
Monoisotopic Mass: 144.11 Da

Figure 3. Structure of Valproic acid

RESULTS AND DISCUSSIONS

VPA have carboxyl side chain in its molecular structure. So tuning VPA produced high intense peak of 143.1 in negative ion mode. Though the abundance of deprotonated ion [M-H]⁻ was maximum, poor fragmenting pattern of VPA leading to low sensitivity is a challenge for its quantitation.

Most of the published literatures suggested that the use of Pseudo-MRM is an effective way to overcome, 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 would be very high. Moreover it can affect the sensitivity of the assay too.

By considering these facts, 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. The optimized Mass and DMS parameters are given in the Table-1.

A simple protein precipitation technique using methanol for the extraction of VPA from Human plasma was chosen. Significant matrix interference observed from these samples, however SelexION® removed all these which was evidenced by the calibration curve plotted from the same samples but with and without SelexION® (Figure 6).

It was also observed that extensive chromatographic condition optimization was not required when SelexION® was used which itself add additional orthogonal selectivity for the VPA ions. Refer Fig (4&5) which shows, the chromatogram with and without SelexION®. An interference peak just next to the retention time of VPA was completely eliminated by SelexION®.

In this method, Linearity was established in the range of 50 to 8000 ng/ml in Human plasma with correlation coefficient r = 0.99, demonstrated excellent relationship between analyte area response and concentration.

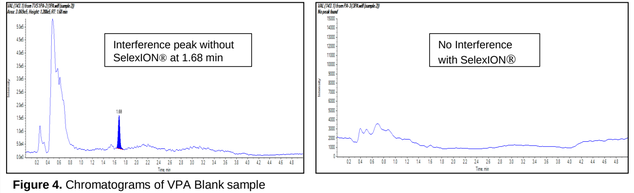


Figure 4. Chromatograms of VPA Blank sample

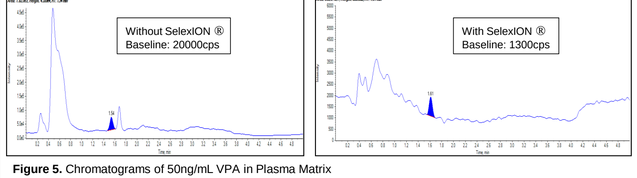


Figure 5. Chromatograms of 50ng/mL VPA in Plasma Matrix

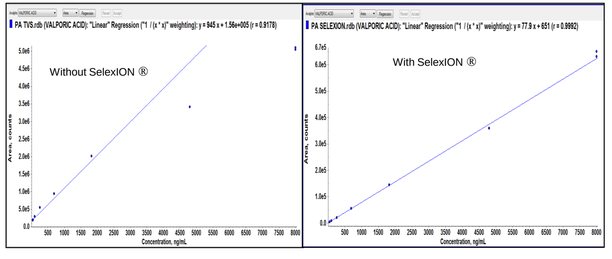


Figure 6: Calibration curve of VPA (50 to 8000 ng/ml) in Plasma Matrix

CONCLUSIONS

- A novel technique for the Bioanalysis of poor fragmenting molecules like Valproic acid was established using SCIEX SelexION® in 5500 series system
- Differential Mobility Separation (DMS) using SelexION® Technology provides an orthogonal level of selectivity by separating components and interfering ions based on their chemical properties and ion mobility
- Matrix interference and background noise was significantly reduced to improve selectivity and thus improved the signal to noise ratio
- Linearity was established in the range of 50 to 8000 ng/ml in Human plasma with correlation coefficient r = 0.99

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2. Planar differential mobility spectrometer as a pre-filter for atmospheric pressure ionisation mass spectrometry. (2010).International Journal of Mass Spectrometry. 298(1-3): 45-54.
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TRADEMARKS/LICENSING