

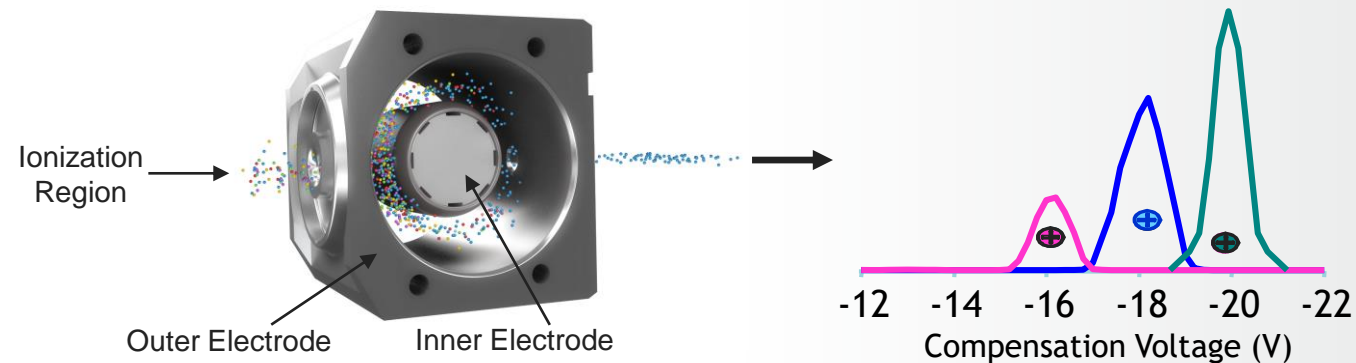
Compensation Voltage Determination on a LC Time Scale

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The FAIMS device separates gas phase ions flowing in the gap between two concentric cylindrical electrodes by applying an RF dispersion voltage (DV) to displace the ions within the gap based on their differential mobility. Ions are selectively transmitted into the inlet of a mass spectrometer by a DC potential called compensation voltage (CV). As the CV is changed, a new population of ions is introduced for mass analysis. This is known as *CV Fractionation*. Determining the optimal FAIMS conditions for a specific analyte, known as *Targeted CV Optimization*, requires scanning the CV while infusing a reference standard to determine the optimal CV. Here, a method for optimizing the CV for each analyte during a LC run is described.



Sample Information:

Compound	Precursor (m/z)
Albuterol	240.16
Alprazolam	309.09
Atenolol	267.17
Atrazine	216.10
Caffeine	195.19
Closantel	662.86
Cyclosporin	1202.85
Cyclosporin-2	601.93
Ethyl Sulfate (EtS)	125.13
Indoxacarb	528.08
Ketoconazole	531.16
Lidocaine	235.34
Myclobutanil	289.11
Tolbutamide	271.11
Warfarin	309.11

Instrument Information:

Liquid Chromatography System

Thermo Scientific™ Vanquish™ Flex
Accucore™ Vanquish™ C18+ column

FAIMS

Thermo Scientific™ FAIMS Pro Duo

Mass Spectrometer

Thermo Scientific™ TSQ Altis™
Thermo Scientific™ TSQ Altis™ Plus

Data Analysis Software

Thermo Scientific™ Freestyle™ 1.8

- FAIMS has been shown to improve proteomic analysis by removing the chemical background that obfuscates low abundance precursor ions in biological samples^{1,2,3}
- CV Fractionation occurs when FAIMS separation at select CVs transmits specific populations of ions to the MS, thus increasing number of precursors available for MS/MS

1. Hebert, et al., *Anal. Chem.*, **2018**, *90*, 9529–9537.

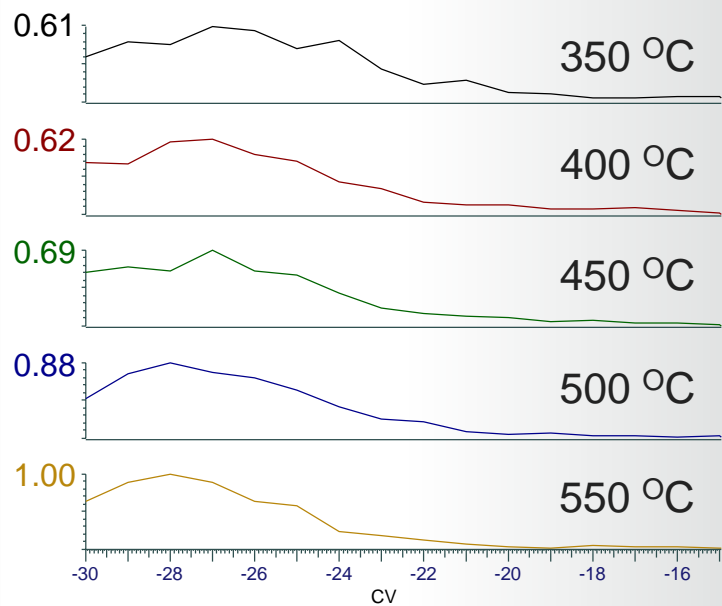
2. Schweppe, et al., *Anal. Chem.*, **2019**, *91*, 4010-4016.

3. Pfammatter, et al., *Molecular & Cellular Proteomics*, **2018**, *17*, 2051-2067.

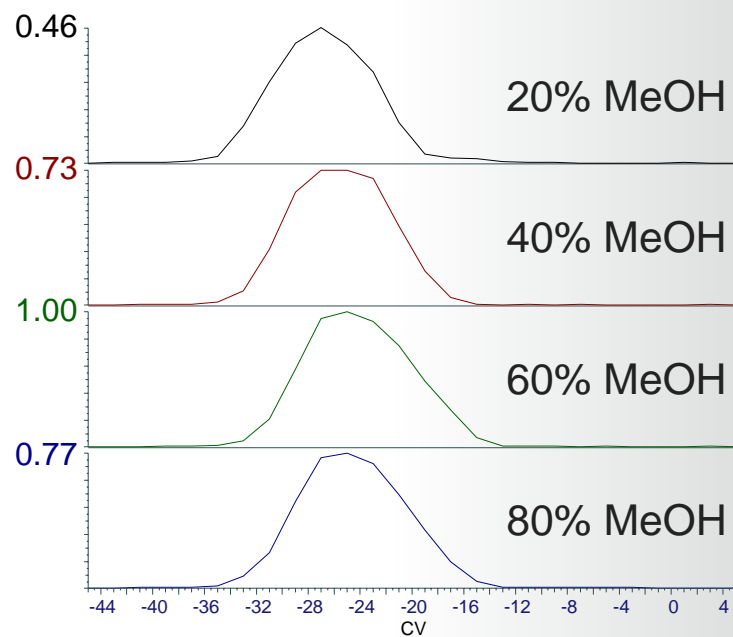
- Targeted CV analysis occurs when the CV is empirically determined and applied during LC-MS methods for improved signal-to-noise of analytes of interest
- Multiple conditions within the ionization source affect the solvation of the ion plume, therefore affecting the CV peak shape and optima (including mobile phase composition, ion source temperature, and gas dynamics)
- These dependencies make it imperative that CV optimization occurs under identical conditions as subsequent analysis

Results – Source Conditions Affect CV Peak

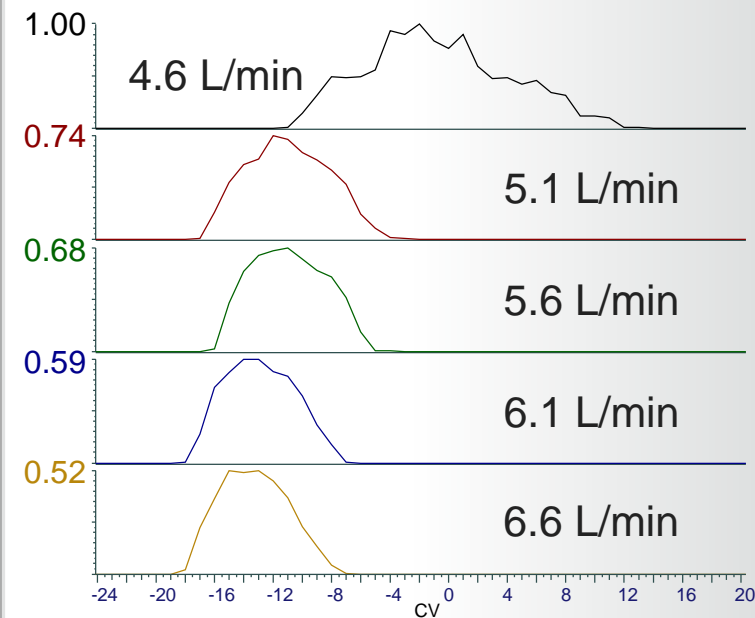
HESI temperature directly affects the ion plume's level of solvation. As this temperature is increased, the CV peak for albuterol narrows and is shifted to more negative values. In general, ion intensity increases with HESI temperature.



The composition of the LC modifier at an analyte's elution and ionization point also affects CV position. Here, the CV optima for lidocaine are shifted as methanol content is changed.

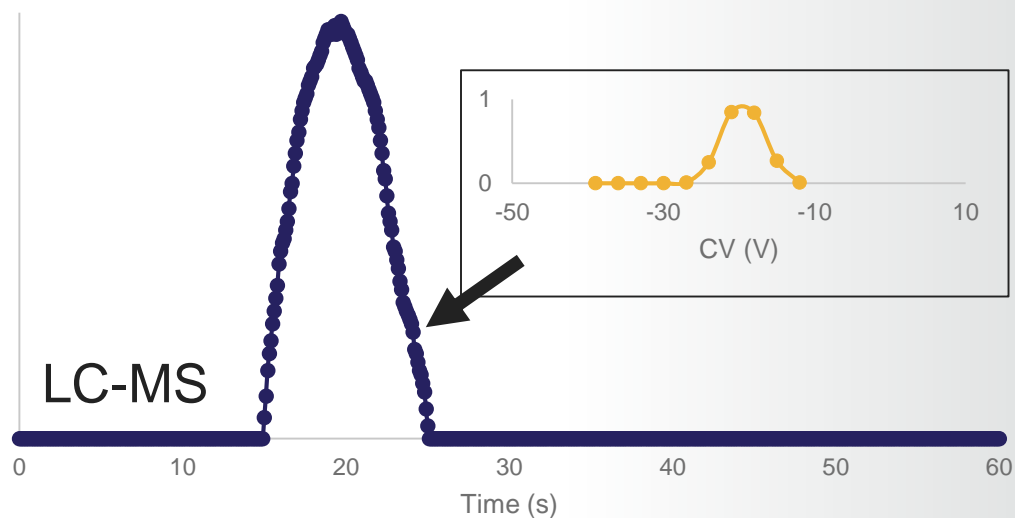


FAIMS gas flow rate has the largest impact on CV and ion intensity. When increased, the CV peak for caffeine is shifted and is more symmetrical due to reduced ion solvation. Intensity decreases with excess FAIMS gas flow.

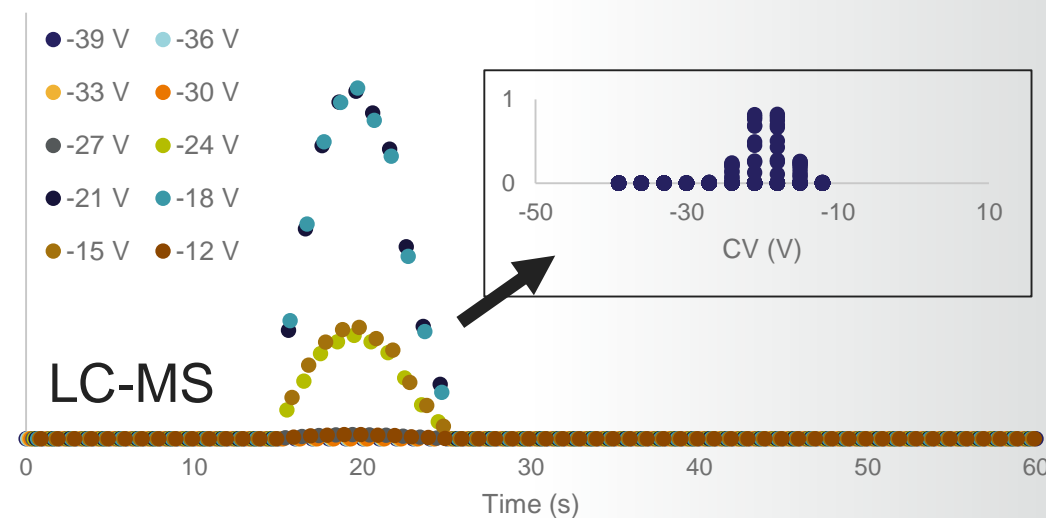


Results – CV Determination From LC Peaks

Historically, CV optima are determined by first scanning the CV range while infusing the analyte. For reasons described previously, this often requires teeing standards into the LC flow at similar source settings and mobile phase composition as subsequent LC-MS analyses (inset). This is done manually and requires sufficient sample for optimization.

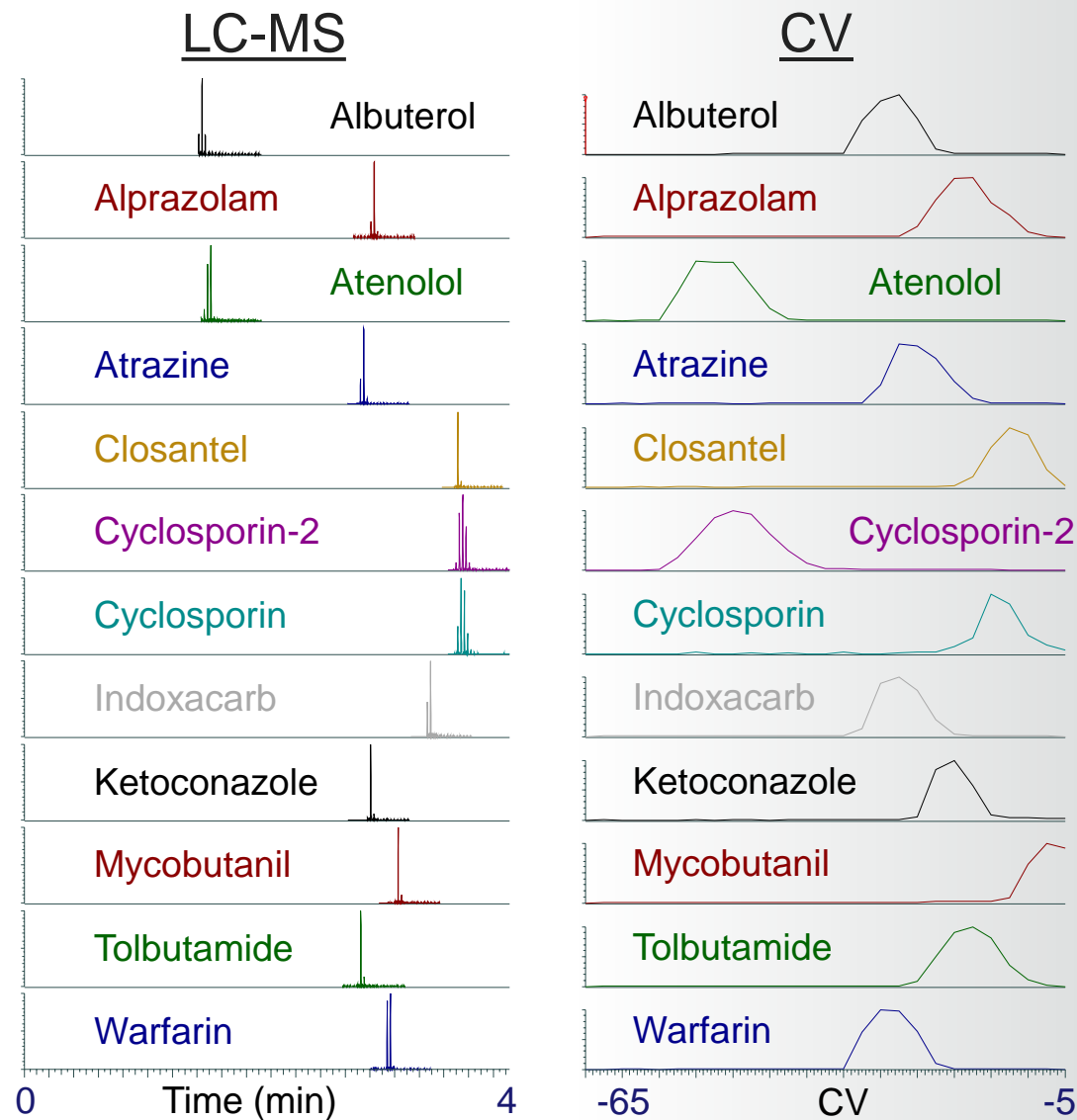
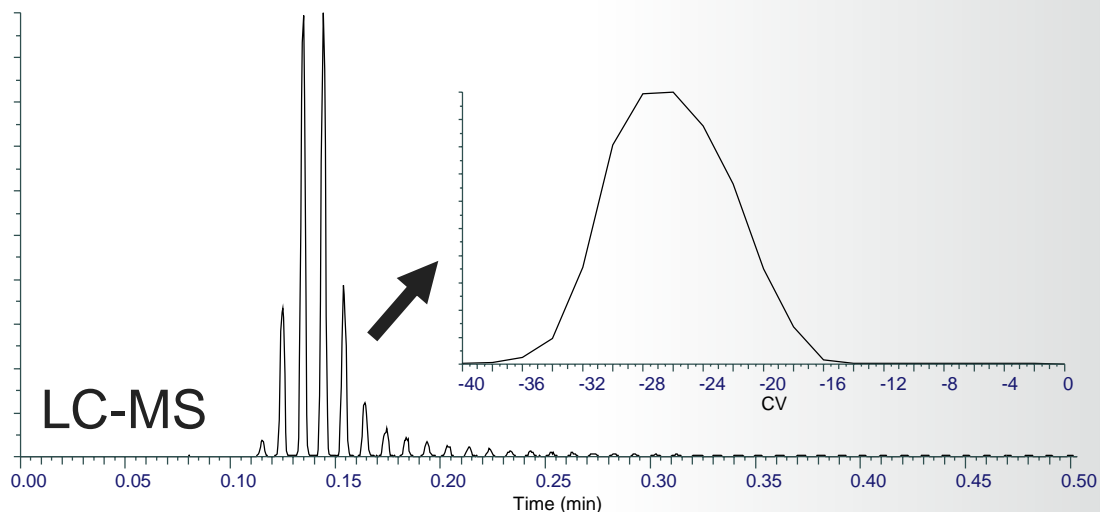


An alternative to this manual method utilizes a single LC-MS injection to optimize CV at the exact LC and source conditions. As the analyte elutes, the CV is looped through a predetermined range with each MS scan. As the range repeats across the LC peak, the CV peak can be determined by plotting the ion current at each CV setting (inset).



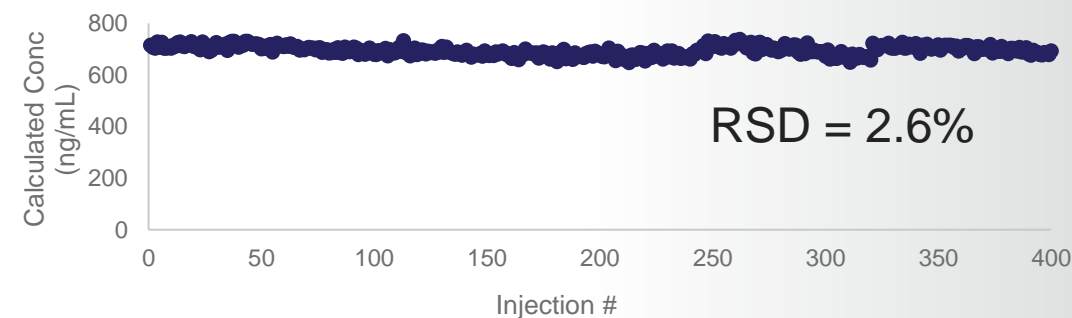
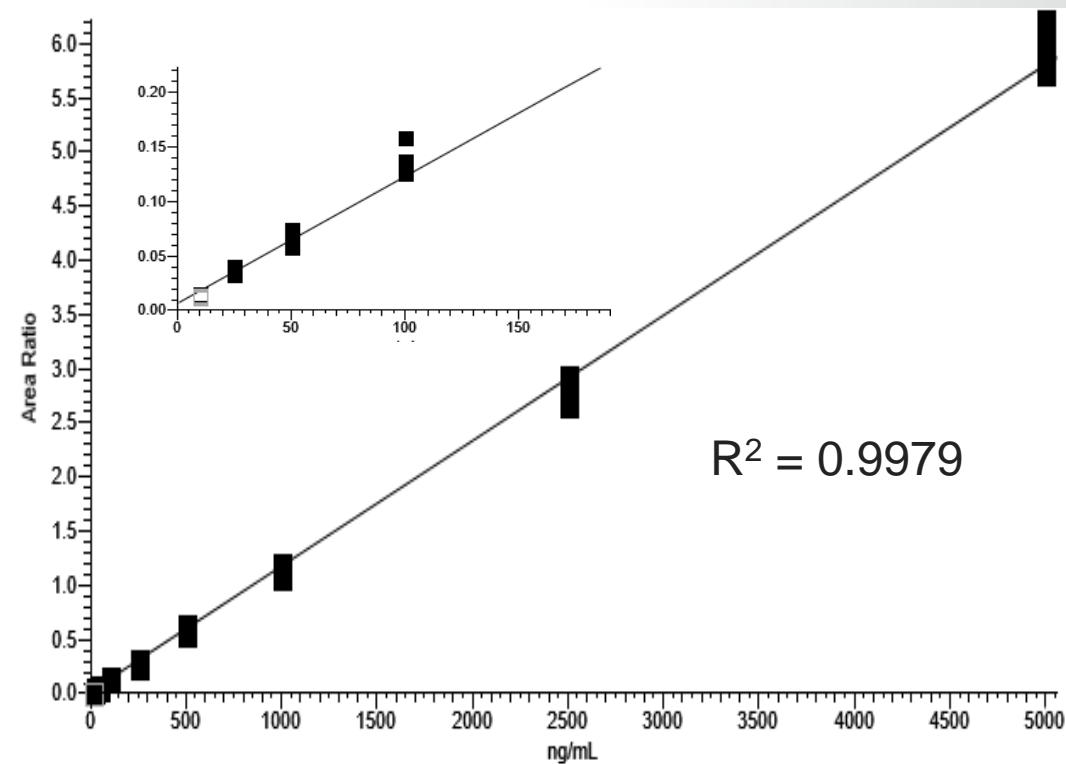
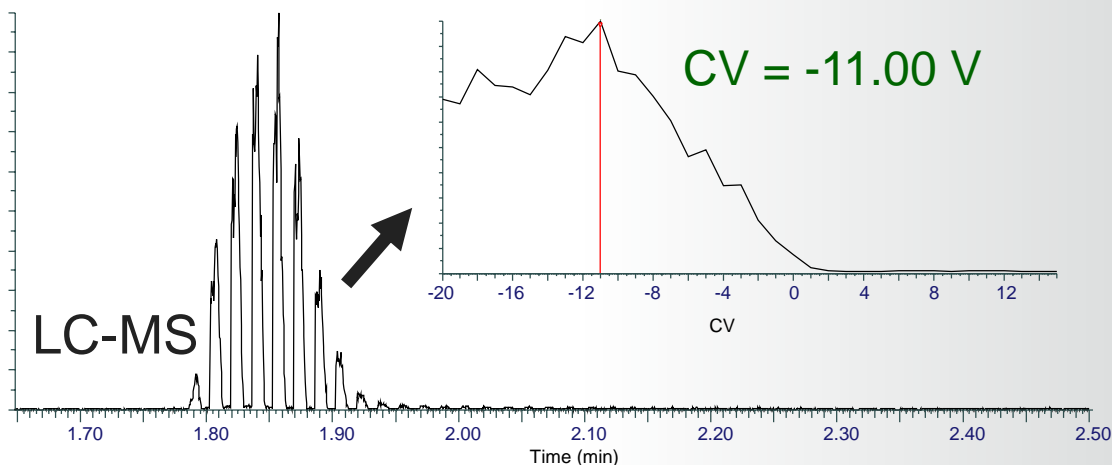
Results – CV Determination From LC Peaks

Determining CV optima by injection generates a chromatogram as shown below for lidocaine (500 μ L/min at 500 $^{\circ}$ C). Each peak within the LC-MS peak corresponds to a full cycle of CVs from the looping method. The CV plot (inset) is produced by processing the .raw file in Freestyle. On the right, LC and CV data are shown for a mixture of 12 drug compounds submitted as a single four minute method.



Results – CV Stability During Analysis

Once the CV is optimized, it is stable for subsequent injections under these same experimental conditions. To demonstrate, an ethanol metabolite, EtS, was analysed in urine by first determining CV data (below) at 500 μ L/min on a TSQ Altis Plus mass spectrometer. Subsequent injections using a CV of -11V was then used to produce calibration curves (upper right) and 400 duplicate injections over five days (lower right).



Conclusions

- CV Fractionation occurs when FAIMS separation at select CVs transmits specific populations of ions to the MS, thus increasing number of precursors available for MS/MS (generally $<10\mu\text{L}/\text{min}$)
- Targeted CV analysis occurs when the CV is empirically determined and applied during LC-MS methods for improved signal-to-noise of analytes of interest (generally $>10\mu\text{L}/\text{min}$)
- Multiple conditions within the ionization source affect the solvation of the ion plume, therefore affecting the CV peak shape and optima (including mobile phase composition, ion source temperature, and gas dynamics)
- These dependencies make it imperative that CV optimization occurs under identical conditions as subsequent analysis
- The CV Scan Method utilizes a single LC-MS injection to optimize CV at the exact LC and source conditions for multiple compounds
- Once optimized, the CV value is stable, allowing for routine analysis