Application Note: 440

FAIMS Removes Interference in a Validated Small Molecule Bioanalytical Assay

Michael Blackburn, M.Phil., Jennifer Mackie, Sanofi-Aventis, Alnwick, UK

Key Words

- Concomitant Medication
- FAIMS
- Interference Removal
- Quantitation
- TurboFlow™ Technology

Goal

To remove an interfering peak due to a concomitantly administered medication using FAIMS.

Introduction

The long and complex process of drug development can take up to 14 years. One important aspect of that process is pre-clinical development where absorption, distribution, metabolism and excretion (ADME) studies of drug candidates take place in animal plasma. During this phase of bioanalysis, accurate determination of pharmacokinetic parameters is critical to the evaluation of the safety and efficacy of the drug compound under development.

Bioanalytical methods are developed to determine drug concentrations and must be validated using the same matrix as the study subjects. The ideal control matrix contains no interferences, but this must be tested during the validation according to FDA guidelines. Occasionally, individual defined sources, or lots, of matrix show interferences. These matrices contain numerous components which may interfere with MS analysis. Compounds that may have MS transitions similar to the analytes and internal standards (IS) include but are not limited to: salts, proteins, peptides, lipids, (phospholipids, fatty acids, bile acids and others) and sugars.

Other factors related to compound administration may also contain potential interferences for the bioanalytical assay. Sources of interference include the dosing vehicles, phthalates (and other plasticizers), drug metabolites and concomitantly administered medications (co-med). A selective method will prevent these other compounds from adding bias or imprecision to the assay.

During method validation for a drug candidate undergoing preclinical trials, an interference appeared in the internal standard (IS) transition of the control matrix. By utilizing FAIMS and full scan MS, the interference was identified as the co-med. Even with online sample cleanup and the high selectivity of LC-MS/MS, the interference was still present and the selectivity was less than optimal.

To improve the selectivity of the assay, high-Field Asymmetric waveform Ion Mobility Spectrometry (FAIMS) was used. FAIMS increases the selectivity of analysis by selecting which ions are allowed into the MS. This technique filters out interferences and leaves the signal cleaner than LC-MS/MS alone.

Methods

Control blank and subject plasma samples were obtained from rat subjects. Samples were prepared by adding 50 µL of IS in water (50 ng/mL) to 100 µL aliquots of plasma. The samples were mixed and injected onto the Thermo Scientific Aria TLX-1 system powered by TurboFlow technology. The online sample preparation system included a 1 mm Cyclone™ extraction column (Thermo Fisher Scientific, Franklin, MA) using pH9.5 loading buffer at 5 mL/min. The analytical separation included a gradient elution profile from 5% to 50% methanol in 20 mM ammonium formate buffer (pH3). The mobile phase flow rate of 1 mL/min was used to elute the analyte and IS from a C18 analytical column (2.1 x 50 mm).

The optimal FAIMS conditions were dispersion voltage (DV) -5000V, 50% He in nitrogen at 3.5 L/min, inner and outer electrode temperatures were 65 and 95 °C, respectively. The compensation voltage (CV) was determined by infusing the analyte and ramping the CV. The ideal CV for the component of interest was -13.9 V. At this CV, no signal for the interference was observed, thus the interference was eliminated.

MS conditions included the use of the heated electrospray probe (H-ESI) and highly selective reaction monitoring MS/MS (H-SRM 0.2 Da FWHM). The vaporizer temperature was 400 °C, the sheath and auxiliary gases were 60 and 40 units, respectively. The analyte was monitored at *m/z* 217 → *m/z* 160 and the IS was monitored at *m/z* 220 → *m/z* 163.

Data were acquired and chromatographic peak areas were integrated in Thermo Scientific LCQUAN 2.5.5 software. Area ratios (analyte divided by the IS) for the standard calibration samples were plotted vs. nominal concentration. Linear regression with $1/[\text{concentration}]^2$ weighting was performed. Back-calculated concentrations for the standards and QC samples were reported.

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Results and Discussion

Prior to the inclusion of FAIMS, the drug and IS co-eluted at 1.78 min (Figure 1a and b, respectively). An interference was detected at 2.01 min in the IS transition (Figure 1b) in which there was significant overlap with the IS peak. By performing a full scan single MS experiment during analysis by TurboFlow technology, the interference appeared to be ketamine (*m/z* 238, data not shown). Ketamine might potentially lose water in the ion source to form a species at *m/z* 220, which is isobaric with the IS. Subsequent fragmentation in the collision cell produced a fragment in common with the IS (*m/z* 163).

Ketamine was in the commercially supplied control plasma. The problem with this interference is that it changes the integrated area of the IS for the Standard and QC samples but not for the unknown samples. Though the method may appear to pass the acceptance criteria, the determined concentrations of the drug in the study samples may be biased by the presence of the interference.

FAIMS successfully removed the interference. Figure 2 shows the compensation voltage ramp during infusion of IS (*m/z* 220) and ketamine (*m/z* 238). The IS emerges from FAIMS at CV = -13.9 V (blue trace, right-most peak) and is transmitted into the MS. In contrast, ketamine emerges at CV = -17.2 V (green trace). The interference due to ketamine (blue trace, left-most peak) is separated by FAIMS and is not transmitted into the MS when the CV is set to transmit the IS (CV = -13.9 V). Thus, when FAIMS is set to transmit the analyte and IS (CV = -13.9), the interference is filtered out. Because the stable-labeled IS shows FAIMS characteristics identical to the analyte, LC-FAIMS-MS/MS analysis requires only one CV value.

A representative chromatogram for the TurboFlow LC-FAIMS-MS/MS analysis of the analyte at the LLOQ in plasma is shown in Figure 3. Using a 1:1 split after the column but before the source increased the signal and signal-to-noise ratio for the analyte by 4.5-fold and 2000-fold, respectively. The upper trace is for the analyte and the lower trace is for the IS. When compared to previous results, implementing FAIMS with a split yielded a 350% transmission increase relative to the 'without FAIMS' experiment. Note that the interference is not present in Figure 3.

Figure 4 shows results from the standard and QC samples as determined by linear regression in Thermo Scientific Watson LIMS software. All calibration and QC samples were within the FDA guidance criteria.

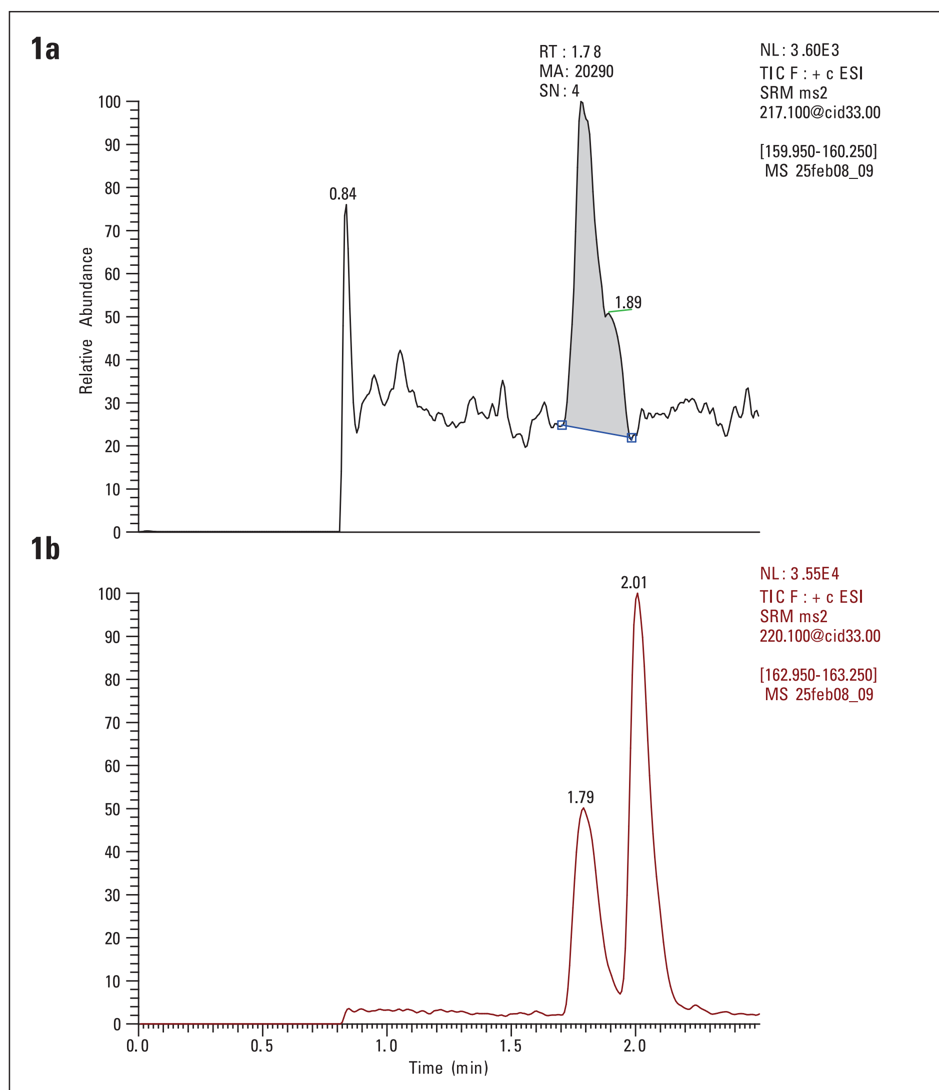

Figure 1: Representative LC-MS/MS chromatograms for the analyte at the LLOQ. Figure 1a, the upper trace (analyte) shows a peak (retention time 1.78 min) with signal-to-noise ratio of 4 and significant chemical background. Figure 1b, the lower trace (IS) shows a peak for the IS at the same retention time as the analyte and a second peak at 2.01 min for the interference. Integration of the IS was adversely affected by interference.

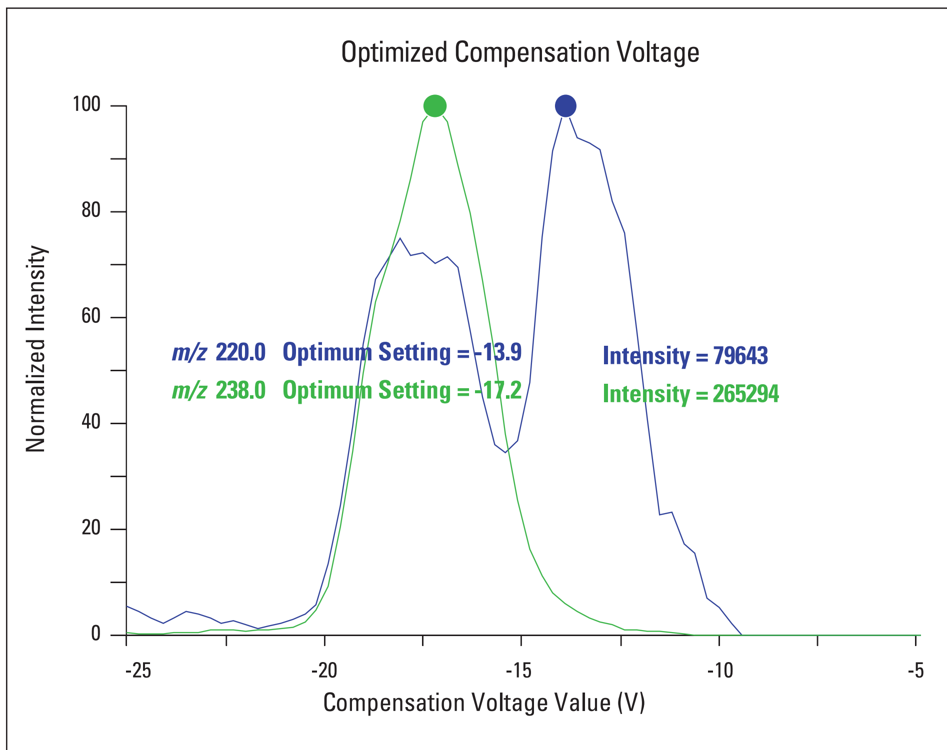

Figure 2: CV scan. A reference solution of IS and ketamine was infused and the CV was ramped from -25 to -5 V. The blue trace (*m/z* 220) shows two peaks, one due to the IS (CV = -13.9 V) and the other due to ketamine (CV = -17.2 V). Ketamine forms *m/z* 220 via in-source fragmentation prior to FAIMS separation. The CV for analysis of the analyte is identical to the stable-labeled IS at -13.9 V. Ketamine is excluded from the MS because it does not emerge when FAIMS is set to -13.9 V.

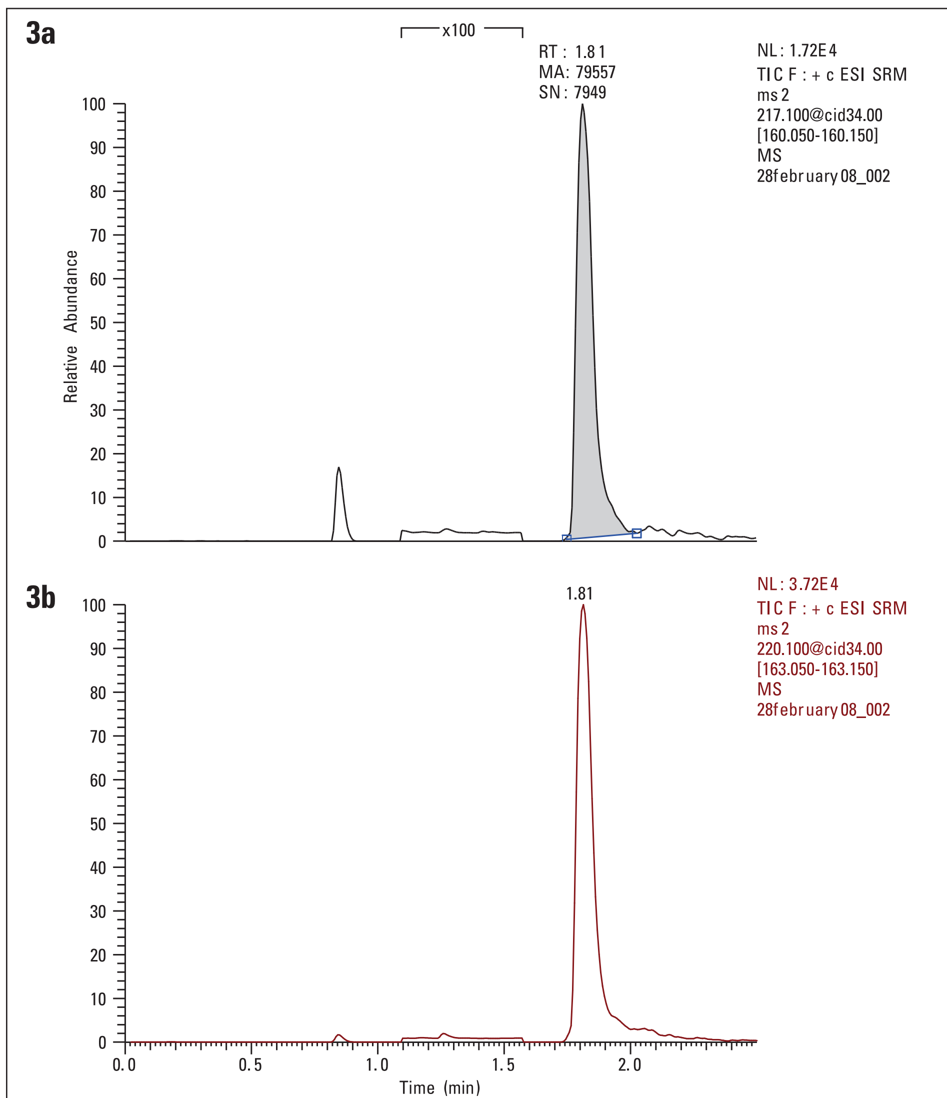

Figure 3: Representative LC-FAIMS-MS/MS chromatograms for the analysis of the analyte at the LLOQ in plasma at CV = -13.9V. The LC flow was split post-column to 0.5 mL/min. The signal-to-noise ratio is 7949 which is 2000-fold better than the experiment performed without FAIMS. The % transmission for FAIMS was 4.5 times greater relative to the standard LC-MS experiment.

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Standard/QC Info | Graph | All Samples | Intra-Assay Precision | Parameter Estimates | Titer-Plates

Standard Samples

Nominal Conc.	Rep.	Instrument Response	ID	Conc. Found	Status	%Found - Added/Added	Response Per ng/mL	Dilution Factor	Position	User ID	Deactive Decisio
1	1	0.257934	Cal H	1.08	Active	8.00	0.257934	1	2		
1	2	0.207155	Cal H	0.838	Active	-16.20	0.207155	1	3		
1	3	0.255909	Cal H	1.07	Active	7.00	0.255909	1	12		
2.5	1	0.542389	Cal G	2.46	Active	-1.60	0.216956	1	4		
5	1	1.120506	Cal F	5.24	Active	4.80	0.224101	1	5		
10	1	2.156805	Cal E	10.2	Active	2.00	0.215681	1	6		
50	1	10.343201	Cal D	49.7	Active	-0.60	0.206864	1	7		
100	1	20.238921	Cal C	97.5	Active	-2.50	0.202389	1	8		
150	1	30.005935	Cal B	145	Active	-3.33	0.200040	1	9		
200	1	41.862083	Cal A	202	Active	1.00	0.209310	1	1		
200	2	40.444462	Cal A	195	Active	-2.50	0.202222	1	10		
200	3	42.822744	Cal A	206	Active	3.00	0.214114	1	11		

Slope	0.20729476266276
Intercept	3.33551460809111E
R-Squared	0.99406198835092
LLOQ	1
ULOQ	200
Mean Std %Diff	4.38
S.D. Std %Diff	4.36
Mean Std Response	0.217723
S.D. Std Response	0.019591
Regression Type	LINEAR
WeightingFactor	1/X**2

QC Samples

Nominal Conc.	Rep.	Instrument Response	ID	Conc. Found	Status	%Found - Added/Added	Response Per ng/mL	Dilution Factor	Position	User ID
1	1	0.228495	QCLLOQ	0.941	Active	-5.90	0.228495	1	25	
1	2	0.208105	QCLLOQ	0.843	Active	-15.70	0.208105	1	37	
1	3	0.187562	QCLLOQ	0.744	>20% Theoretical	-25.60	0.187562	1	49	
1	4	0.223729	QCLLOQ	0.918	Active	-8.20	0.223729	1	61	
1	5	0.232754	QCLLOQ	0.962	Active	-3.80	0.232754	1	73	
1	6	0.249332	QCLLOQ	1.04	Active	4.00	0.249332	1	85	
2.5	1	0.460712	QCL	2.06	>15% Theoretical	-17.60	0.184285	1	26	
2.5	2	0.546329	QCL	2.47	Active	-1.20	0.218532	1	38	
2.5	3	0.583995	QCL	2.66	Active	6.40	0.233598	1	50	
2.5	4	0.531624	QCL	2.40	Active	-4.00	0.212650	1	62	
2.5	5	0.578101	QCL	2.63	Active	5.20	0.231240	1	74	
2.5	6	0.541833	QCL	2.45	Active	-2.00	0.216733	1	86	
50	1	11.422383	QCM	54.9	Active	9.80	0.228448	1	27	
50	2	11.646823	QCM	56.0	Active	12.00	0.232936	1	39	
50	3	11.002980	QCM	52.9	Active	5.80	0.220060	1	51	
50	4	11.363088	QCM	54.7	Active	9.40	0.227262	1	63	
50	5	10.265602	QCM	49.4	Active	-1.20	0.205312	1	75	
50	6	11.122781	QCM	53.5	Active	7.00	0.222456	1	87	
200	1	42.253663	QCH	204	Active	2.00	0.211268	1	28	
200	2	45.567244	QCH	220	Active	10.00	0.227836	1	40	
200	3	45.554431	QCH	220	Active	10.00	0.227772	1	52	
200	4	46.400875	QCH	224	Active	12.00	0.232004	1	64	
200	5	49.163991	QCH	237	>15% Theoretical	18.50	0.245820	1	76	
200	6	42.335957	QCH	204	Active	2.00	0.211680	1	88	

Instrument Response (0–45) vs. Nominal Conc.

Figure 4: Watson LIMS results from a validation analysis of the analyte in rat plasma. For the regression analysis, the standard calibration samples were between 1 and 200 ng/mL. Each standard calibration sample was analyzed in one replicate except for the LLOQ and ULOQ samples (three replicates each). All standard results were within the FDA guidelines for accuracy and precision. The QC samples at the LLOQ, < 3x LLOQ, mid-range, and ULOQ concentrations were analyzed in six replicates each and were determined to be within the FDA criteria for accuracy and precision.

Conclusions

FAIMS successfully prevented the ketamine co-med interference from adversely affecting the accuracy and precision of the bioanalytical method. This technology is simple to optimize and the analysis yielded increased signal by 4.5 times and the signal to noise ratio increased by 2000 fold relative to data acquired without FAIMS. Additionally, FAIMS reduced chemical background. This is a robust technique requiring little intervention as demonstrated in this GLP validated assay.

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