TAKING ADVANTAGE OF SIGNIFICANT REDUCTIONS IN ION SUPPRESSION USING IONKEY/MS COMPARED TO STANDARD-FLOW LC/MS

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This white paper compares ion suppression levels of the Waters ionKey/MS™ System to those of a standard-flow LC/MS system. In both peptide and small molecule applications, it is shown that the ionKey/MS System delivers a significant reduction in ion suppression characteristics due to the lower flow rates utilized during its analysis.

INTRODUCTION

Ion suppression is defined as the loss of signal of an analyte of interest caused by the coelution and ionization of an interfering compound. Often in bioanalytical applications, phospholipids present in the protein-crashed plasma matrix can interfere and cause near-total elimination of the analyte of interest's signal.[1] This unwanted suppression of the signal significantly reduces the performance, precision, and accuracy of a quantitative assay and leads to inconclusive results. Accordingly, the coeluting compound must be mitigated by a gradient, stationary phase, or column length adjustment, and, in extreme cases, a sample preparation adjustment to return the assay to biological relevance.

The Waters ionKey/MS System operates in the 1-5 µL/min or microspray flow regime and as such offers considerable sensitivity improvements and ion suppression reductions compared to standard-flow LC/MS systems operating at 100s of µL/min. The sensitivity improvements are attributed to an increase in sampling efficiency by the mass spectrometer at lower flows.[2] The reduction in ion suppression is attributed to the increased surface-to-volume ratio of the smaller initial droplet size that results from the lower flows.[3] Recent scientific literature has shown that nanospray applications with flow rates of less than 100 nL/min offer significant reductions in ion suppression effects[4] and greater tolerance to salt contamination than the ESI sources utilized on standard-flow LC/MS systems.[5] Furthermore, ion suppression was shown to be completely eliminated at flow rates below 20 nL/min.[6] However, nanospray at these extremely low flow rates is not always practical in many workflows due to long delay times and a perceived lack of robustness and stability of the small diameter columns and ESI emitter tips.[7]

Microspray, exemplified by the ionKey/MS System, offers a robust,[8] easy to use, and relatively high-throughput platform alternative to nanospray while retaining a beneficial reduction in ion suppression compared to standard ESI. The reduction in ion suppression afforded by the ionKey/MS System is characterized in the following experiment.

Comparison of ion suppression characteristics

To compare the ion suppression characteristics of ionKey/MS to standard-flow LC/MS, the instrument configuration shown in Figure 1 and experimental conditions described in Table 1 were used.

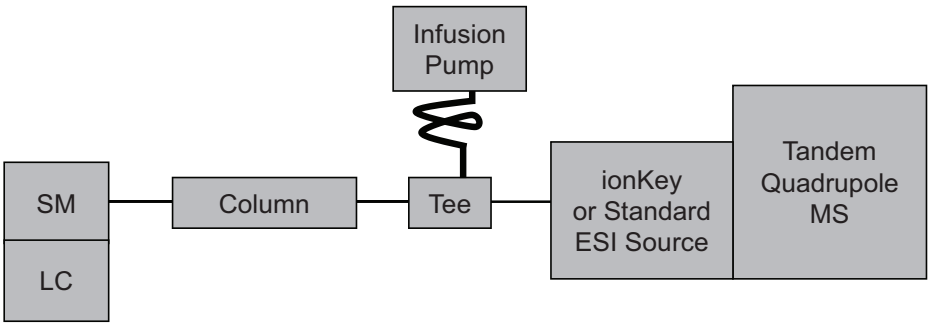

Figure 1. Instrument configuration. For standard-flow LC/MS the separation channel is a 2.1 mm x 50 mm column. The separation channel used in ionKey/MS is a 150 µm x 50 mm iKey.™

	LC system	ESI source	Total flow rate	Analytical flow rate	Post-column infusion flow rate
Standard-flow LC/MS	ACQUITY UPLC®	Standard ESI	600 µL/min	577 µL/min	23 µL/min
ionKey/MS	ACQUITY UPLC M-Class	ionKey	3 µL/min	2.89 µL/min	0.11 µL/min

Table 1. Experimental conditions. The post-column infusion flow rate is approximately 4% of the total flow rate for both flow rate regimes.

The analytes of interest, a mixture of peptides and small molecules, were injected using the LC system and separated chromatographically using the same gradient conditions. Analyte detection was performed using multiple reaction monitoring (MRM) on the Xevo® TQ-S System. To generate the control data point, the analytes of interest were exposed to a post- column infusion of 66:33 acetonitrile/water (v/v) supplied by the infusion pump at the flow rates seen in Table 1. The suppressed data point was generated by exposing the analytes of interest to a post-column infusion of a 3:1 protein precipitation of rat plasma using the same infusion solvent and flow rates as the control. The diluted rat plasma was introduced into the infusion flow stream by filling an appropriately sized sample loop offline. Accordingly, the analytes are exposed to all potential suppressors present in the diluted rat plasma, including phospholipids, across the entire gradient thereby avoiding any retention time differences between the scales that could alter suppression characteristics. This instrument configuration does not provide any information on the analytes of interest and their specific coeluting suppressors present in the diluted rat plasma, but instead affords a controlled comparison of the ion suppression compared to the control for each flow regime.

Microspray using ionKey/MS offers significant reductions in ion suppression compared to the standard-flow LC/MS system for all analytes tested. The signal in height of the model peptide, LVNELTEFAK, seen in Figure 2 is suppressed approximately 6.8 times on the ionKey/MS when exposed to all potential suppressors in the diluted rat plasma compared to the control. In comparison, the signal of the standard-flow LC/MS is suppressed approximately 218 times compared to the control. Therefore ionKey/MS shows a decrease in suppression on the order of 32 times when compared to the standard-flow platform for this model peptide.

For the model small molecule shown in Figure 3, dextromethorphan, ionKey/MS shows suppression on the order of approximately 1.9 times compared to the control, while the standard-flow LC/MS shows suppression on the order of 24.5 times. Accordingly, ionKey/MS also shows a reduction in suppression for this small molecule of about 13 times when compared to its standard-flow counterpart, reinforcing the conclusion that ionKey/MS offers significant reductions in ion suppression.

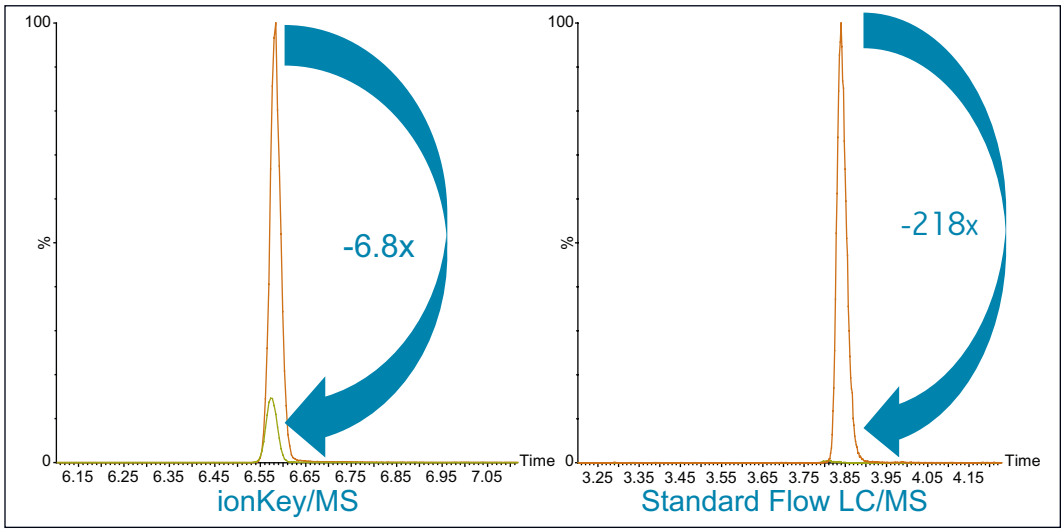

Figure 2. LVNELTEFAK suppression comparison. The red trace is performed with a 4% post column infusion of the infusion solvent (control). The green trace is performed with a 4% post column infusion of diluted rat plasma (suppressed). Signal loss = signal of neat / signal of suppressed.

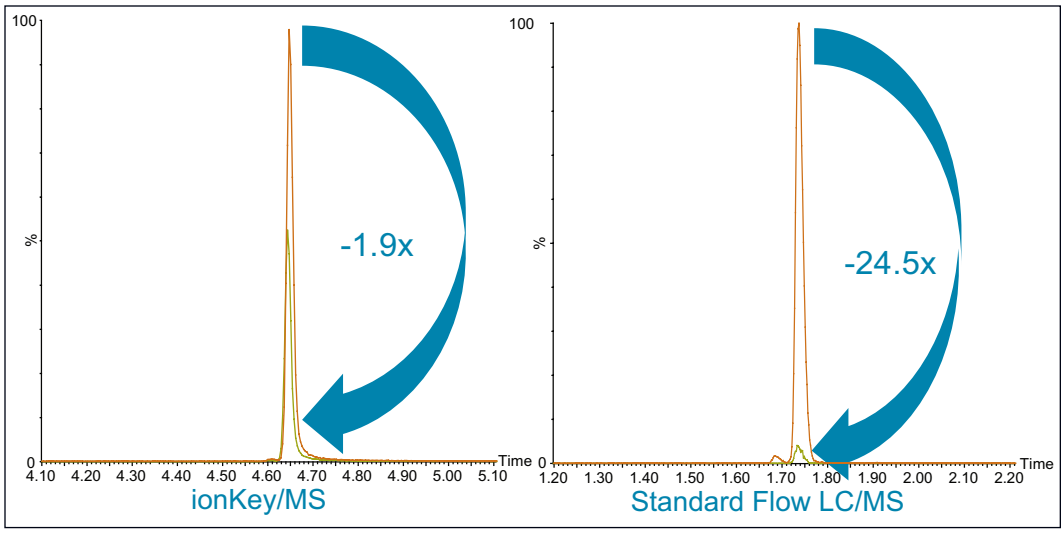

Figure 3. Dextromethorphan suppression comparison.

Effect on quantification

The quantitative extent of area suppression is analyte-dependent and the reduction in suppression between the ionKey/MS and the standard-flow LC/MS is not the same for each analyte and class of analyte as seen in Figure 4 and 5. This can be attributed to the fact that the surface activity and timeframe for the analyte to reach the surface of the droplet, as well as the size of the droplet, differ drastically between the two flow regimes, effectively altering ionization efficiency. Moreover, it is interesting to note that although ionKey/MS does suffer ion suppression effects for all analytes, it is far less and not nearly as pervasive as that which is encountered when using standard-flow LC/MS. In fact, the analyte-of-interest's signal on the standard-flow LC/MS is nearly totally eliminated, making quantitative area measurements exceedingly difficult.

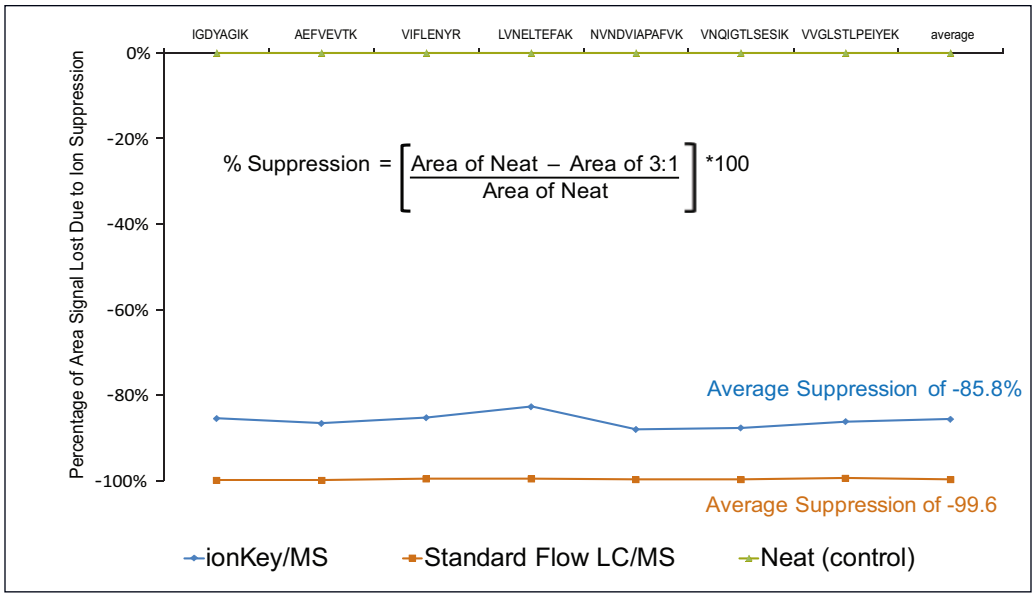

Figure 4. Percent area suppression of peptides on ionKey/MS vs. standard-flow LC/MS.

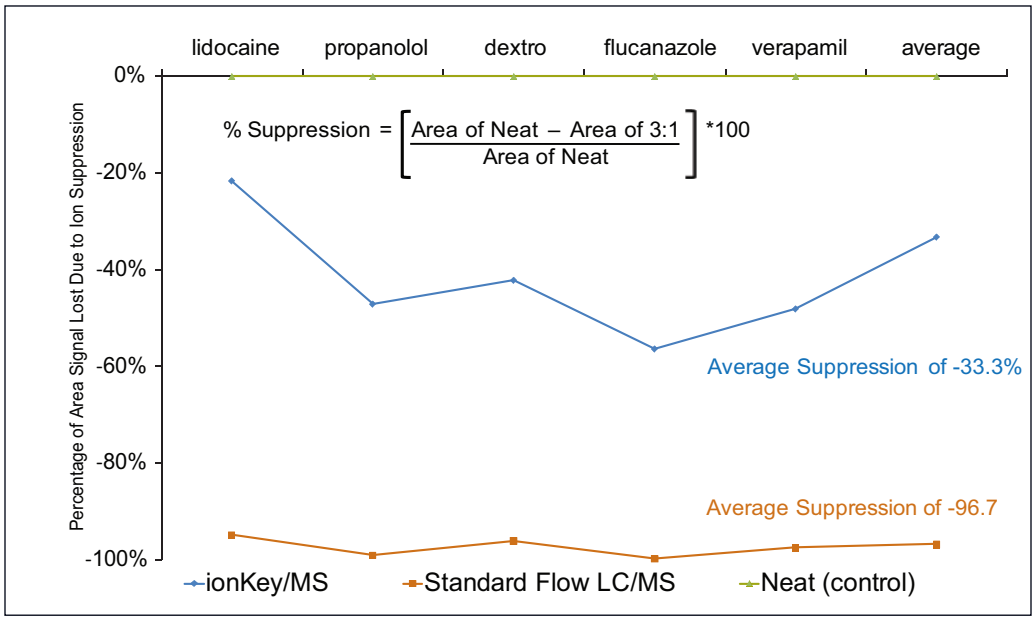

Figure 5. Percent area suppression of small molecules on the on ionKey/MS vs standard-flow LC/MS.

CONCLUSIONS

The beneficial implications of the substantial reduction of ion suppression resulting from matrix interferences when using ionKey/MS in a typical workflow will be numerous. In the familiar case of a coeluting compound causing suppression of your analyte of interest in a standard-flow LC/MS separation, it is highly probable that the reduction in signal suppression afforded by ionKey/MS will allow you to avoid significant extra effort in sample preparation and chromatographic troubleshooting. Furthermore, the LLOQ of a user's assay will be substantially reduced, allowing detection of trace quantities of the analyte of interest. The reduction in LLOQ is a result of the sensitivity gain realized when moving to lower flow rates[9] being a summation of the increased sampling efficiency and the

decrease in ion suppression. ionKey/MS is beneficial and more sensitive than standard-flow LC/MS as it is fundamentally less prone to ion suppression due to the lower flow rates utilized, making it an attractive platform for a variety of applications.

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