ENHANCING MASS SPECTROMETRY SENSITIVITY BY REDUCING CHROMATOGRAPHIC FLOW RATES WITH IONKEY/MS

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There is consistent pressure for scientists to achieve lower limits of quantitation as many are limited by smaller sample volumes available for analysis or are challenged to detect more potent analytes in biological matrices. This has led scientists to investigate microflow LC as an alternative to standard flow LC (2.1-mm column format) as this technique has shown to increase sensitivity and ionization efficiency as well as reduce ion suppression. This white paper will review the sensitivity benefits that can be expected when operating at a microscale flow rate with the Waters ionKey/MS™ System in comparison to a 2.1-mm column format, as well as explain why this signal enhancement is possible.

INTRODUCTION

The need for greater sensitivity in an LC/MS analysis has driven development of more efficient ion sources and ion optics in mass spectrometers. Even with such advances, biological assays still may demand greater sensitivity than what may be currently available with a standard LC/MS system. Additional sensitivity gains can be realized by reducing the amount of solvent flow to the ion source while reducing the column diameter from a traditional 2.1-mm diameter to a microflow format. At these reduced flow rates, sensitivity gains of 10X to 20X can readily be achieved.

At flow rates greater than 100 µL/min, a significant portion of sensitivity is lost due to poor ionization efficiency and limited sampling efficiency. An electrospray plume generated from conventional LC flow rates can be quite broad and divergent. The inlet to a mass spectrometer only has the ability to sample a portion of the electrospray plume. Most commonly, this is done by positioning the electrospray probe orthogonally to the inlet and sampling on the edges of the plume where fine droplets are present (Figure 1, top). As the solvent flow rate is reduced, the electrospray plume decreases in size and becomes more convergent (Figure 1, bottom). This allows the inlet of the mass spectrometer to become more efficient and capture a greater percentage of the plume. This results in an increase in ion signal.

Mass-sensitive detection, not concentration-sensitive

The increase in sensitivity or ion signal is roughly proportional to the decrease in column diameter and flow rate.[1] This behavior is similar to a UV detector, which responds to the concentration of the analyte in the mobile phase rather than the absolute amount or mass of the analyte. This has led electrospray to be characterized as a concentration-sensitive technique, even though it has been generally understood that electrospray is a mass-sensitive phenomena.[2] Although concentration and sensitivity appear to follow the same trend, the ion signal at low flow rates does not increase as rapidly as the concentration of an analyte within the mobile phase, showing that the two are not directly coupled.

In actuality, a mass spectrometer is a mass-flow-sensitive detector[3] where signal response is proportional to the amount of sample reaching the detector per unit time. To illustrate this, an infusion was performed with a constant concentration of analyte at increasing flow rates (Figure 2). With the solution of analyte (verapamil) maintained at the same concentration, the higher flow rate results in a larger signal as there is a greater amount or mass of the sample entering the mass spectrometer per unit time.

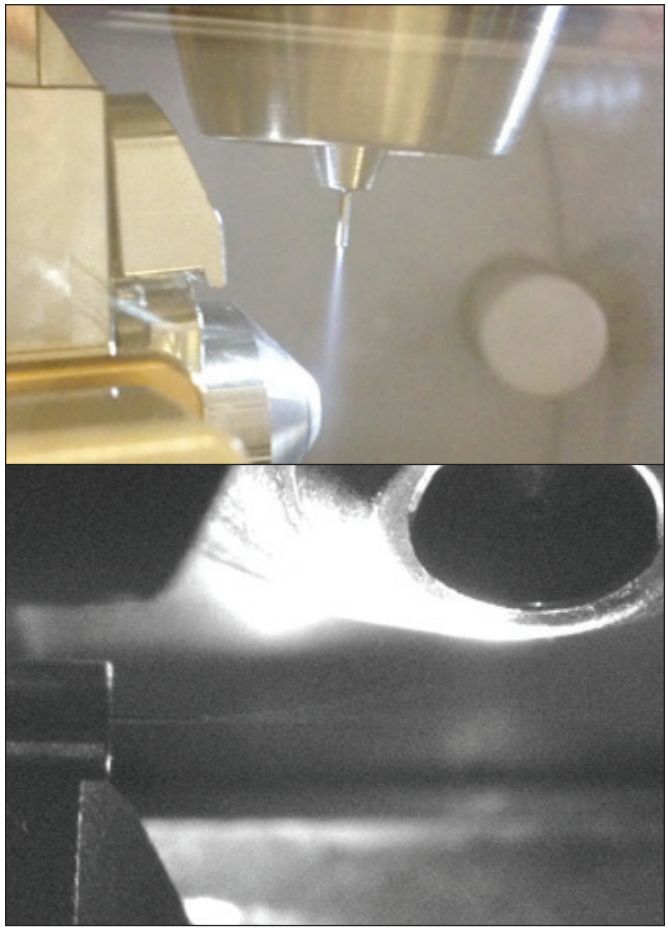

Figure 1. The size of the electrospray plume decreases as the flow rate decreases. Top: Standard ESI source operated at 600 µL/min. Bottom: ionKey/MS source operated at 3 µL/min.

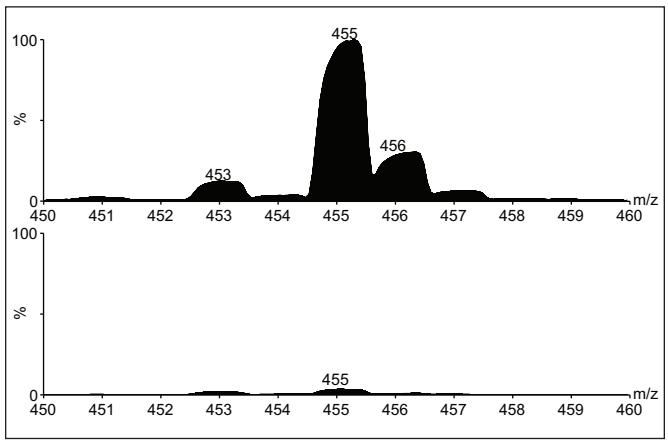

Figure 2. Infusion of an 833 pg/mL solution of verapamil. Top: Flow of 600 µL/min into a standard ESI source. Bottom: Flow of 3 µL/min with an ionKey/MS source. The higher flow exhibits a 27X increase in signal response for infusion.

Concentration-sensitive behavior is only observed when analytes are eluted as chromatographic peaks where lower flow rates result in increased signal response. Under this condition, the same amount of analyte is eluted from a column per unit time with varying amounts of solvent. The lower solvent flow generates a finer, less-disperse electrospray plume and allows for greater sampling efficiency by the mass spectrometer.

Sensitivity enhancement scaling down from 2.1-mm column format

To demonstrate the increase in sampling and ionization efficiency, numerous analytes were analyzed at flow rates between 0.45 and 600 µL/min using a combination of commercially available UPLC columns and prototype microfluidic devices (Table 1). The flow rate for each column dimension was scaled according to the square of the column's internal diameter to maintain the same linear velocity through the column. The signal response are presented in area counts to eliminate possible differences observed in peak height due to varying separation efficiencies and post-column band broadening. The gain in area counts were all compared to the equivalent separation in a 2.1-mm column.

Column ID	Column Body	Flow Rates
2.1 mm	ACQUITY UPLC® Column	200-600 µL/min
1.0 mm	ACQUITY UPLC Column	150 µL/min
300 µm	PEEK-Sil Capillary Column	12 µL/min
150 µm	iKey™	1-4 µL/min
75 µm	nanoACQUITY UPLC® Capillary Column	450 nL/min

Table 1. Screened flow rate combinations.

Sensitivity gains were achieved for a variety of analytes by comparing equal injection volumes by lowering the mobile phase flow and column diameter from a 2.1-mm I.D. column format (Figure 3). The average enhancement ranged from 2X to as much as 50X for a series of small molecule pharmaceutical analytes, depending on the flow rate (Figure 4).

The signal enhancement did not directly match the corresponding increase in peak concentration at lower flow rates for each column I.D. (Table 2).

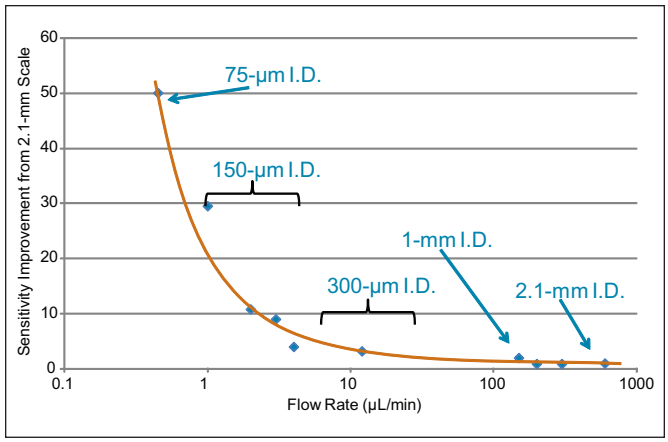

Figure 3. The average signal enhancement with reducing column diameters and flow rates in comparison to a 2.1-mm format for a series of small molecules (lidocaine, propanolol, dextromethorphan, fluconazole, alprazolam, and verapamil). All injections were made with the same concentration solution and a volume of 1 µL.

Column Diameter	Average Sensitivity Enhancement, Small Molecule/Peptides	Eluting Peak Concentration
2.1 mm	1X	1X
1.0 mm	2X / 3X	4.4X
300 µm	3.2X / 6X	49X
150 µm	9X / 16X	196X
75 µm	50X / >100X	784X

Table 2. Average sensitivity enhancement.

The amount of signal enhancement varied depending on the chemical properties of each analyte. The sensitivity enhancement observed for a separation on a 150-µm I.D. Waters iKey separation device, for instance, varied from 9X for verapamil, to 83X for alprazolam at 1 µL/min (Figure 4).

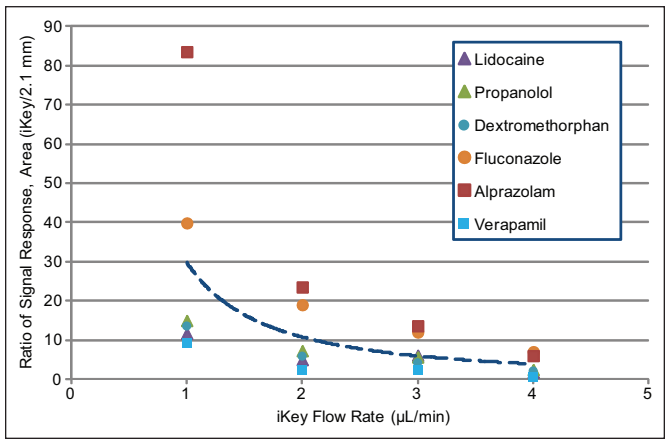

Figure 4. The signal enhancement with the Waters iKey device in comparison to a 2.1-mm format for a series of small molecules at flow rates from 1 to 4 µL/min (lidocaine, propanolol, dextromethorphan, fluconazole, alprazolam and verapamil).

A typical chromatogram comparing the Waters iKey with a 2.1-mm column is shown in Figure 5.

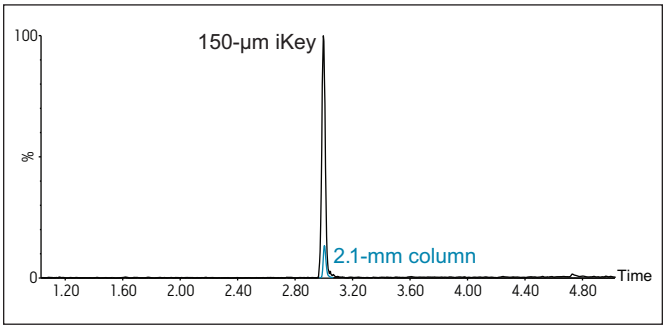

Figure 5. Chromatographic response of a 62.5-pg/mL injection of verapamil on a 150-µm iKey separation device and a 2.1-mm column. The retention times have been adjusted to better show the comparison.

It has been suggested that analytes with hydrophobic functional groups of large molecular volume have increased droplet surface affinity.[4] This may account for the improvement in ESI response. Peptides generally exhibited a better response as compared to small molecules (Figure 6).

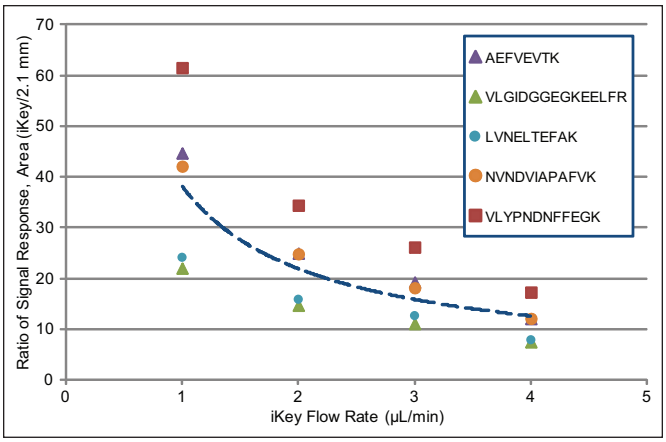

Figure 6. The signal enhancement with the Waters iKey device in comparison to a 2.1-mm format for a series of tryptic peptides from an Enolase digest at a flow rates from 1 to 4 µL/min.

The 150-µm iKey separation channel dimension appears to be an inflection point where the observed sensitivity gain begins to increase non-linearly with reduced flow rate (Figure 4). At this scale, the flow rate can be altered from 1 to 4 µL/min with minimal impact on chromatographic performance (Figure 7). At nanoflow rates (< 1 µL/min), sensitivity gains may be observed at the expense of instrument throughput. The LC system volume (including sample loop) has a greater impact on nanoflow separations (75-µm I.D.) and it creates a gradient delay and long column equilibration time. The 150-µm iKey separation channel dimension provides enhanced sensitivity while maintaining rapid throughput. Additionally, improved chromatography and narrower peaks are observed at 150-µm I.D. as compared to a 75-µm I.D. column format. The sharper peaks provide better resolution and sensitivity realizing the true benefit benefits of a UPLC® separation.

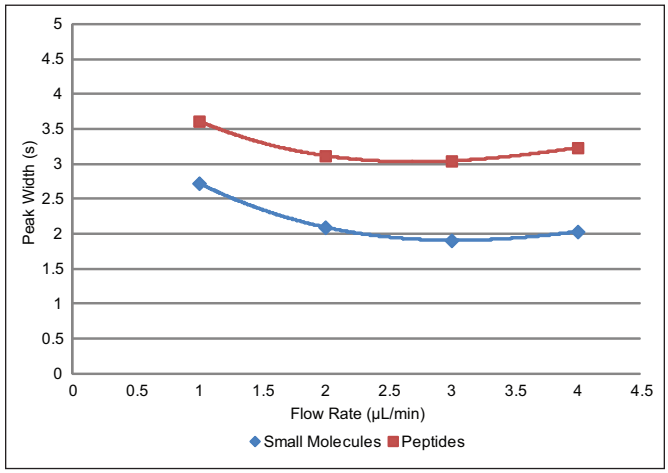

Figure 7. Average peak width for a small molecule separation (5-min gradient, 5 to 95 %B) and a peptide separation (10-min gradient, 5 to 45 %B).

SUMMARY

- Sensitivity gains were observed for both small molecules and peptides by comparing equal injection volumes with reduced mobile phase flow and column diameter, from 2.1-mm to 150-µm I.D.
- The signal enhancement realized at microliter/minute flow rates in this study ranged from 2X to 83X and is molecule-dependent.
- The 150-µm iKey separation channel dimension offers a unique balance between enhanced sensitivity and optimal throughput.

References

1. G. Hopfgartner, K. Bean, J. Henion, and R Henry, J Chromatogr A, 647 (1993) 51.
2. T. R. Covey ***et al***, R. Cole (Eds) Electrospray and MALDI Mass Spectrometry: Fundamentals Practicalities and Biological Applications, Second Edition, Wiley and Sons Inc., 2010, 443.
3. B.L. Karger and P. Vouros, J Chromatogr, 323 (1985) 13.
4. C, G. Enke ***et al***, Anal Chem, 72 (2000) 11.

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