Increasing Mass Spec Sensitivity with the Novel ionKey/MS System, Making Microscale LC-MS Routine, Robust, and Accessible to Scientists at any Skill Level

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Achieving ultra low limits of detection and quantitation for mass spectrometry is a consistent challenge for analytical scientists. One commonly overlooked technique to increase mass spectrometry sensitivity is to decrease the chromatographic flow rate. This enables scientists to reach lower limits of detection and quantitation compared to 2.1 mm ID analytical scale chromatography. Operating at these low flow rates can be challenging due to the difficulty of making quality connections and troubleshooting. However with the introduction of the ionKey/MS system, any scientist, regardless of skill level can now perform microscale LC-MS in routine laboratories with UHPLC chromatographic performance, robustness and reliability. In addition to the increase in sensitivity, operating at these low flow rates can enable scientists to perform more analyses with their precious samples, implement micro-sampling techniques, dramatically reduce solvent consumption and increase uptime by reducing the amount of solvent entering the mass spectrometer.

Enhancing mass spectrometry sensitivity is an ongoing analytical quest. Scientists' desire to achieve lower limits of quantitation is driven by several factors: restricted sample volumes; the need to detect more potent therapeutics dosed at lower levels; desire to find biomarkers at ultralow concentrations; and many other application requirements [1,2]. While the need for higher sensitivity LC/MS analyses has led to the development of more efficient ion sources and ion optics, scientists continue to seek even lower detection limits.

It has been demonstrated that reducing chromatographic flow rates combined with a smaller column diameter can achieve higher sensitivity results [3,4,5,6]. Microscale LC/MS provides additional benefits beyond sensitivity gain. In addition to reduced injection volumes, microscale LC/MS greatly decreases solvent consumption which means reduced solvent storage and disposal costs and less environmental impact.

Despite these benefits, microscale LC/MS presents a number of challenges to analysts as the methodology is extremely difficult to perform in a production environment. It is complicated to make efficient fluidic connections and minimise dispersion and troubleshooting can be very challenging as leaks are often difficult to detect. Plugging of the small scale chromatographic components by the relative dirtiness of biological samples can be a concern as well at this scale of chromatography. Finally, the inherent complexity of microscale LC/MS limits the number of experienced scientists capable of setting up a reproducible system and achieving the desired results.

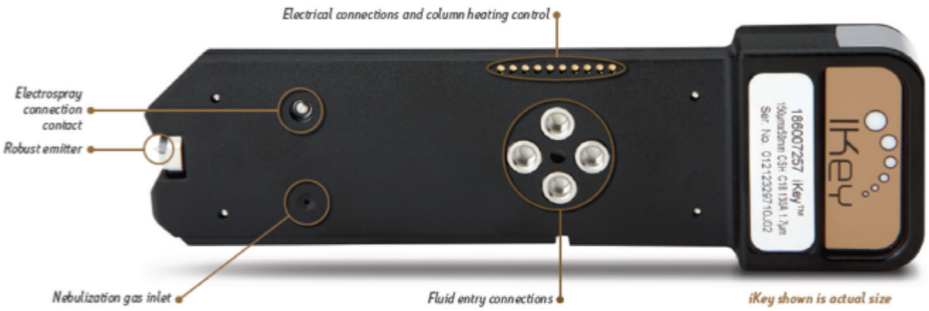

Figure 1. iKey Separation Device

ionKey/MS as an Emerging Technology

The commercial introduction of the ionKey/MS system by Waters in 2014 addresses many of the challenges of microscale LC/MS. The system was developed to provide reproducible and robust UHPLC separations, provide up to 40x sensitivity improvement over 2.1 m I.D. columns, and provide plug and play ease of use making it accessible to lab personnel at all skill levels.

The most unique aspect of ionKey/MS is the iKey separation device (**Figure 1**), which replaces difficult to use, delicate, fused-silica tubing, fragile electrospray emitters, and simplifies the user experience. The iKey is about the size of a smart phone and incorporates a rigid monolithic substrate made of ceramic, chosen for its strength and inertness. The ceramic substrate is inscribed with a 150 µm channel packed with 1.7 µm UHPLC chromatographic particles. The ceramic substrate is then encased in an injection moulded housing containing a column heater, electronic and fluidic connections and an electrospray emitter. When the iKey device is locked into position in the source, all of the electronic and fluidic connections are made automatically, thus eliminating any potential variability.

The sample is then introduced to and separated in the iKey and transported directly to the integrated emitter, which converts the eluent into an aerosol. The plume of fine droplets in the aerosol are ionised giving them a positive charge at which point they enter the vacuum of the MS where they are further separated.

Sensitivity Enhancements

A mass spectrometer is a mass-flow-sensitive detector where signal response is proportional to the amount of sample reaching the detector per unit time. At flow rates greater than 100 µL/min, a significant portion of sensitivity is lost due to poor ionisation 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. As the solvent flow rate is reduced, the electrospray plume decreases in size and becomes more convergent. 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.

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.

Concentration-sensitive behaviour 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 smaller droplets. Smaller droplets go through less Coulomb Fission events and have a greater surface to volume ratio compared to larger droplets allowing for a higher percent of analytes eluting to become ionised. The less-disperse electrospray plume allows for greater sampling efficiency by the mass spectrometer.

Figure 2. Increased Signal Response

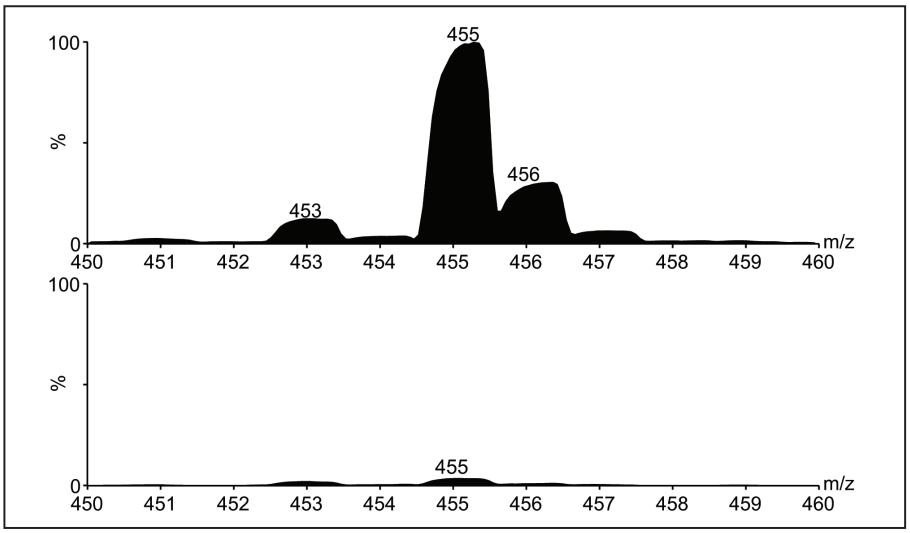

Figure 2. Infusion of an 833 pg/mL solution of verapamil. Top: Flow of 600 µL/min into a standard ESI source. Bottom: Flow of 3 µL/min with an ionKey/MS source. The higher flow exhibits a 27X increase in signal response for infusion.

To demonstrate the increase in sampling and ionisation efficiency, numerous analytes were analysed at flow rates between 0.45 and 600 µL/min using a combination of commercially available UHPLC columns and iKey separation devices. 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 is 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 was compared to the equivalent separation in a 2.1-mm column.

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)**.

Figure 3. Sensitivity Gains

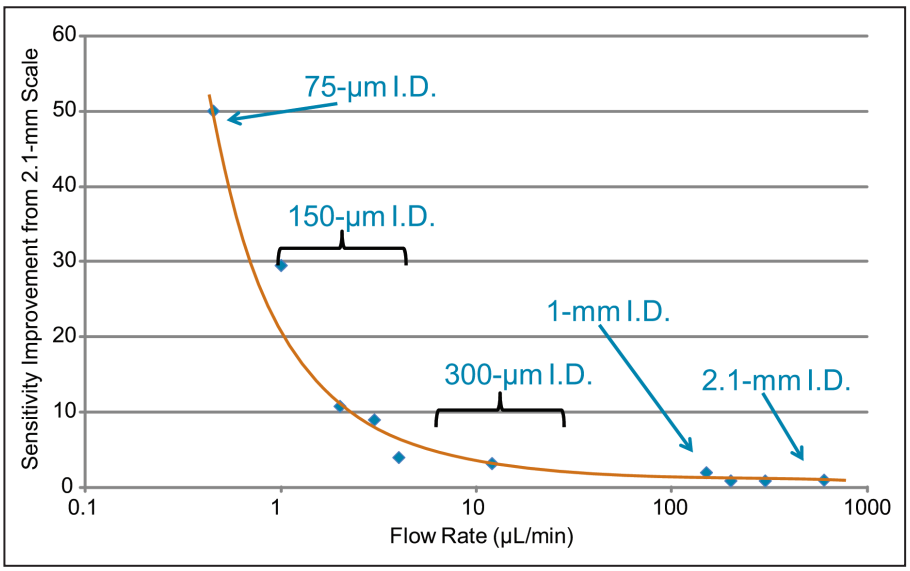

Figure 3. The average signal enhancement with reducing column diameters and flow rates in comparison to a 2.1-mm format for a series of small molecules (lidocaine, propanolol, dextromethorphan, fluconazole, alprazolam, and verapamil). All injections were made with the same concentration solution and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

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).

Figure 4. Signal Enhancement

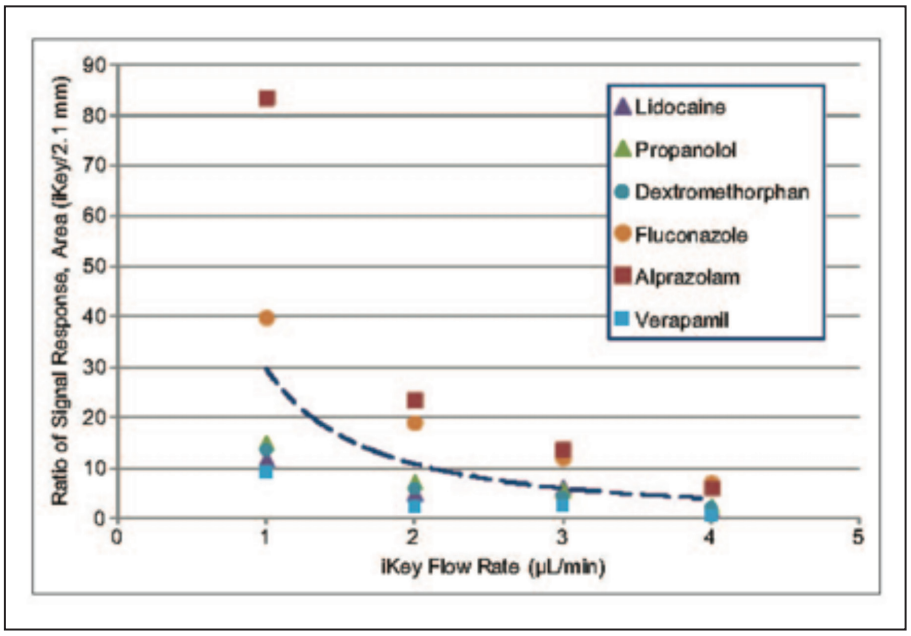

Figure 4. The signal enhancement with the Waters iKey device in comparison to a 2.1 mm format for a series of small molecules at flow rates from 1 to 4 µL/min (lidocaine, propanolol, dextromethorphan, fluconazole, alprazolam, and verapamil).

The amount of signal enhancement varies depending on the chemical properties of each analyte. (Table I).

Table 1. Average Sensitivity Enhancement

Column Diameter	Flow Rate	Average Sensitivity Enhancement, Small Molecule Peptides	Eluting Peak Concentration
2.1 mm	200 – 600 µL/min	1X	1X
1.0 mm	150 µL/min	2X / 3X	4.4X
300 µm	12 µL/min	3.2X / 6X	49X
150 µm	150 µL/min	9X / 16X	196X
75 µm	450 nL/min	50X / >100X	784X

A typical chromatogram comparing the ionKey/MS system with a 2.1-mm column is shown in Figure 5. The 150-µm iKey separation channel dimension provides enhanced sensitivity while maintaining rapid throughput.

Figure 5. Improved Sensitivity

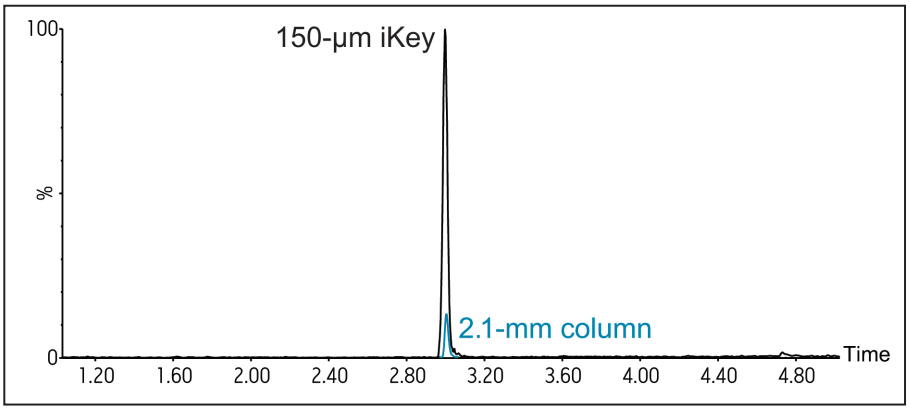

Figure 5. Chromatographic response of a 62.5-pg/mL injection of verapamil on a 150-µm iKey separation device and a 2.1-mm column. The retention times have been adjusted to better show the comparison.

Proof of Concept

Sensitivity Enhancement for the Quantification of Glucagon in Human Plasma

Glucagon for injection (rDNA origin) is a polypeptide hormone identical to human glucagon and is used to treat severe hypoglycemia (low blood sugar). As a research tool, accurate quantification of glucagon from biological matrices is used to better understand diabetes as a function of disease progression and/or drug treatment. Many assays, using different methodologies, exist for glucagon analysis in biological samples. With recent advances in MS and chromatography there has been a trend toward the analysis of large molecules by LC-MS/MS given the technique has the advantage of shorter development times, greater accuracy and precision, the ability to multiplex, and readily distinguish between closely related analogues, metabolites or endogenous interferences.

In this example [7], a combination of selective µElution mixed-mode SPE sample preparation, optimal MS precursor and fragment choice, and the ionKey/MS System were used for the highly selective and sensitive quantification of glucagon in human plasma. Compared to analytical scale (2.1 mm I.D.), the ionKey/MS system offered increased sensitivity and facilitated the use of smaller sample volumes. For a 250 pg/mL plasma extracted sample, the same injection volume (5 µL) on ionKey/MS yielded 4X greater signal to noise and a 10X improvement in sensitivity versus 2.1 mm scale **(Figure 6)**.

Figure 6. Sensitivity Enhancement over 2.1 mm I.D.

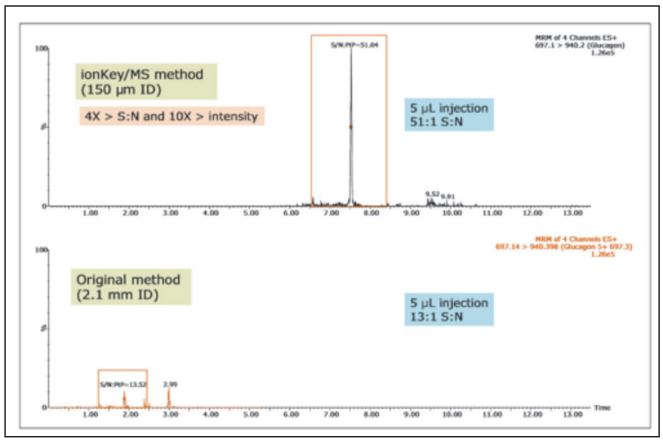

Figure 6. Comparison of 250 pg/mL glucagon extracted from human plasma (200 µl): iKey (150 µm I.D.) vs. traditional analytical flow (2.1 mm I.D.).

Robustness of the ionKey/MS System in the Analysis of Pharmaceutical Compounds in Biological Fluids

The robustness and reliability of pharmacokinetic (PK) data is an essential part of bioanalysis. In this study [8], the robustness of the ionKey/MS System was tested with a common sample preparation scheme of affinity isolation followed by the digestion and subsequent analysis of peptides generated from the sample preparation. **Figure 7** illustrates the peak shape at injection 1 and injection 1000 for two of the signature peptides monitored during the study. The study demonstrated the system to be robust over 1,000 injections using injection volumes of 1 µl. This volume is roughly equivalent to a 200 µl injection on a standard 2.1 mm I.D. column. The system was demonstrated to be capable of maintaining excellent peak symmetry and resolution over a continuous testing period of 5 days.

Figure 7. Robust Chromatographic Performance

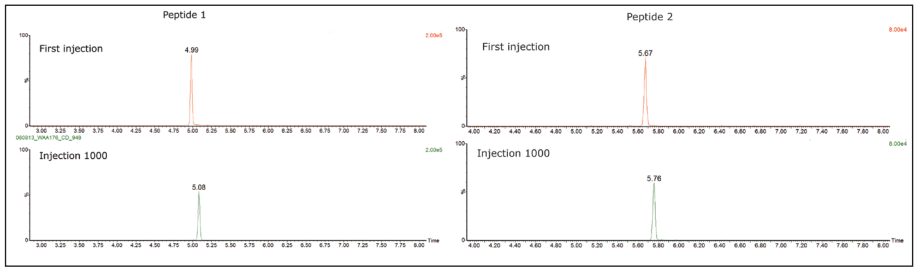

Figure 7. MRM chromatograms of two signature peptides from a therapeutic mAb digested with trypsin following immunoaffinity isolation from mouse serum.

To demonstrate the robustness of the fluidics, pressure traces were recorded throughout the entire study and the first and the last pressure traces are displayed in Figure 8. There was no discernible increase in the system pressure, indicating that none of the frits, tubing or connective fittings had been blocked over the course of the study.

Figure 8. No Pressure Increase Over 1,000 Injections

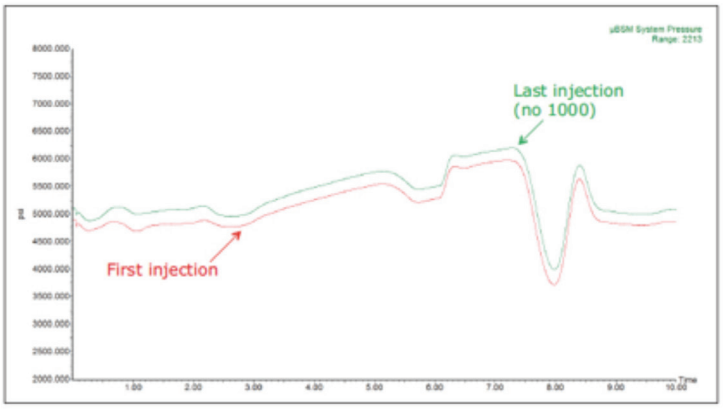

Figure 8. ionKey/MS System pressure traces recorded for the first and the last (1000th) injection of a mAb isolated by immunoaffinity from mouse serum and digested with trypsin.

Conclusion

The ionKey/MS system provides a dramatic increase in sensitivity over 2.1 mm microscale platforms. The system has been demonstrated to be robust and reproducible even while injecting dirty biological matrices at sample loads many times that of the column volume which would not be attempted at 2.1 mm ID analytical scale chromatography. The novel plug and play iKey separation device provides ease of use and enables scientists at all levels to achieve the analytical benefits of microscale LC/MS, of not only sensitivity but a reduction in solvent usage, storage and disposal cost, less environmental impact, and the ability to obtain more information about your samples.

References

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