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High Throughput Microflow LC-MS: Sensitivity Gains on a Practical Timescale

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ABSTRACT

The sensitivity gains observed when using microflow LC-MS do not always translate into an everyday workflow advantage in a high throughput laboratory. The longer run times that are typical on a microflow scale deliver improved sensitivity, but decreased sample throughput when compared to UPLC.® The ability to deliver high sensitivity at run times under 3 minutes compelled the need to develop a microflow device that can function on a UPLC timescale (<3 min). The iKey™ HT is a high throughput device that demonstrates improved sensitivity over UPLC methods on a timescale that is practical for routine analysis.

INTRODUCTION

Microflow LC-MS has been shown to offer significant sensitivity gains over traditional 2.1 x 50 mm UPLC approaches.[1] The reason for the observed signal gains in microflow lies in the fact that lower flow rates improve the sampling efficiency of the electrospray plume and generate a finer and less disperse spray. Also, as the column diameter decreases, analytes elute off of the LC column with a much lower volume and result in peaks with a higher signal-to-noise (S/N) ratio as compared to its UPLC counterpart. Sensitivity gains are always welcome in an ever-changing landscape where bioanalytical demands are being escalated every day. However, one of the downsides of using a microfluidic approach is that lower flow rates can also translate into longer run times.

From a practical perspective, longer run times mean longer turnaround times for results. For time sensitive studies, delays in reporting data can impact research decisions upstream. The effect would also be felt by contract research organizations that charge per sample. Longer run times result in less profit per instrument. Run times are more than just actual hours. Sample thaw time and re-prep time also factor into analysis time. A typical pharmacokinetic study with 3 animals dosed intravenous (i.v.) and 3 animals dosed orally (p.o) will consist of approximately 66 samples. If blanks and standards are included, the number of injections increases to approximately 110 for the entire study. A typical microflow LC-MS run at 12 minutes per injection would take 1320 minutes or 22 hours to complete. The same study at 3 minutes per injection would only take 5.5 hours. The entire study could be completed in a single 8-hour work day and, additionally, three other studies could be conducted in the evening and overnight.

In this white paper, we will introduce a novel microfluidic device capable of higher pressures and flow rates which enable run times under 3 minutes while still delivering improved sensitivity over traditional UPLC systems.

METHODS

Standards were prepared in rat plasma and serially diluted to the desired concentration. In this study, six compounds, verapamil, propranolol, dextromethorphan, alprazolam, imipramine, and fluticasone were evaluated. Plasma samples were extracted by protein precipitation using a crash ratio of 1:2 with acetonitrile.

Chromatographic separation was performed on a Waters® BEH C_{18} iKey HT (300 µm x 50 mm, 1.7 µm) constructed of ZTA (zirconia toughened alumina) material that provides increased strength for withstanding higher fluidic pressures. Mobile phase A consisted of water with 0.1% formic acid while mobile phase B consisted of acetonitrile containing 0.1% formic acid. LC gradient condition and injection volume were held constant for both the microflow and the conventional UPLC experiments. A 5 µL injection volume was used for both workflows.

The only chromotagraphic variable between the microflow and conventional UPLC workflows is the flow rates in use. The flow rate of the UPLC condition was held at 600 µL/min, while the flow rate for the microflow is typically 12 µL/min. The gradient for both methods starts at 5% B and ramps to 95% B over 1 minute and this condition is held for another 0.5 minutes, and the gradient returns back to the initial condition at 1.6 minutes for re-equilibration of the system.

Mass spectrometric detection was conducted on a Waters Xevo® TQ-S mass spectrometer operating in positive ion mode. MRM transitions were determined for each compound and cone voltages and collision energy were optimized based on individual signal intensity.

RESULTS

A BEH C_{18} iKey HT device (300 µm x 50 mm, 1.7 µm) was developed to enable higher flow rates during high throughput analysis. The performance and reproducibility of the device were evaluated by conducting 1000 injections of a crashed plasma sample containing imipramine, fluticasone, propanolol, and tolbutamide (Figure 1). Overall, the coefficient of variation (%CV) across the study was less than 5% for all analytes. Pressure variation between the first and last injection was also evaluated and no difference was observed (Figure 2). These results demonstrated that while analyzing complex biomatrices, the system performed consistently with excellent reproducibility over time and no clogging or decreased column performance was observed.

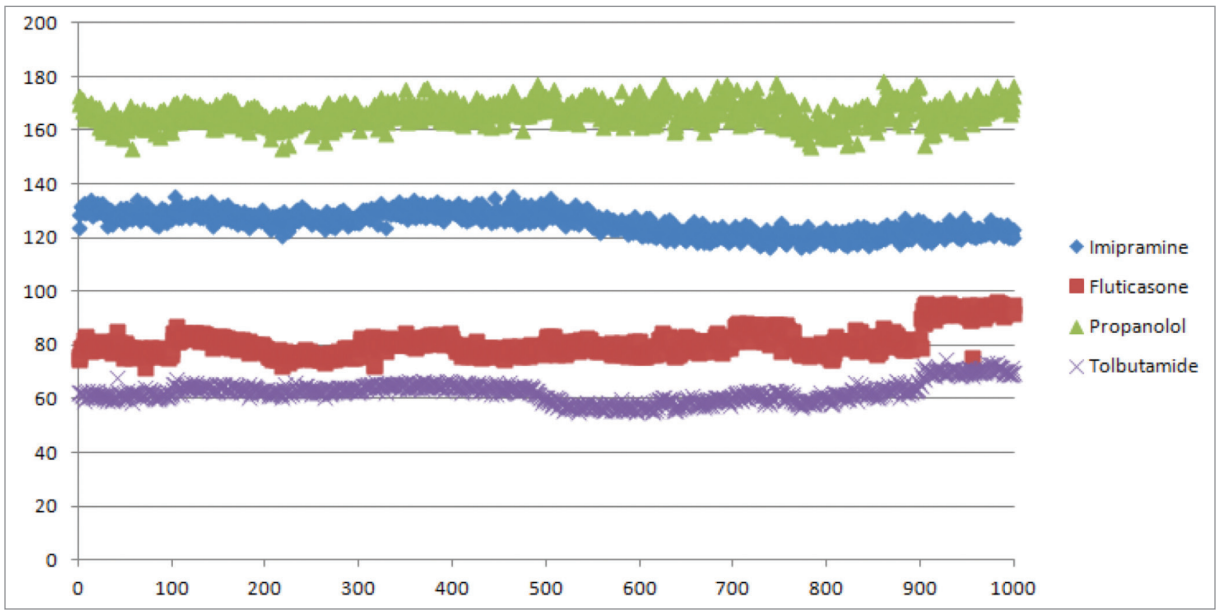

Figure 1. Reproducibility of 1000 injections of the rat plasma sample spiked with four small molecule analytes.

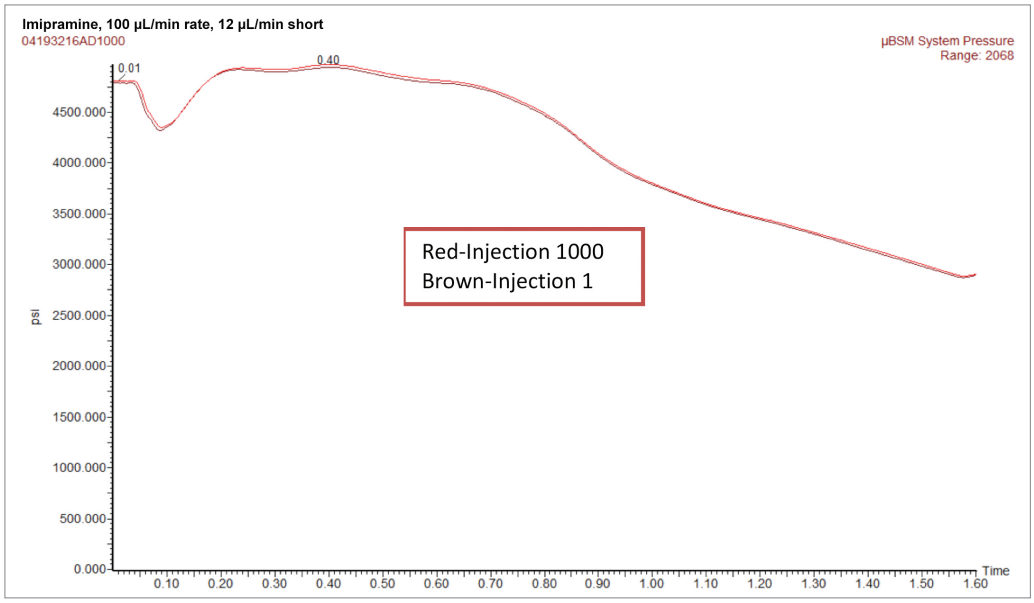

Figure 2. Overlay of pressure traces of injection 1 and injection 1000 for Impramine.

SENSITIVITY GAIN

To demonstrate the increase in sensitivity when using the iKey HT, several analytes were compared under identical conditions to UPLC data. Sensitivity gains for each compound in plasma are shown in the corresponding overlays where the red trace represents the iKey HT data and the green trace represents the conventional UPLC data. For all compounds, sensitivity gains were observed. The level of sensitivity gain is compound dependent, and ranges from 1.6x for verapamil to 6.1x for fluticasone (Figure 3).

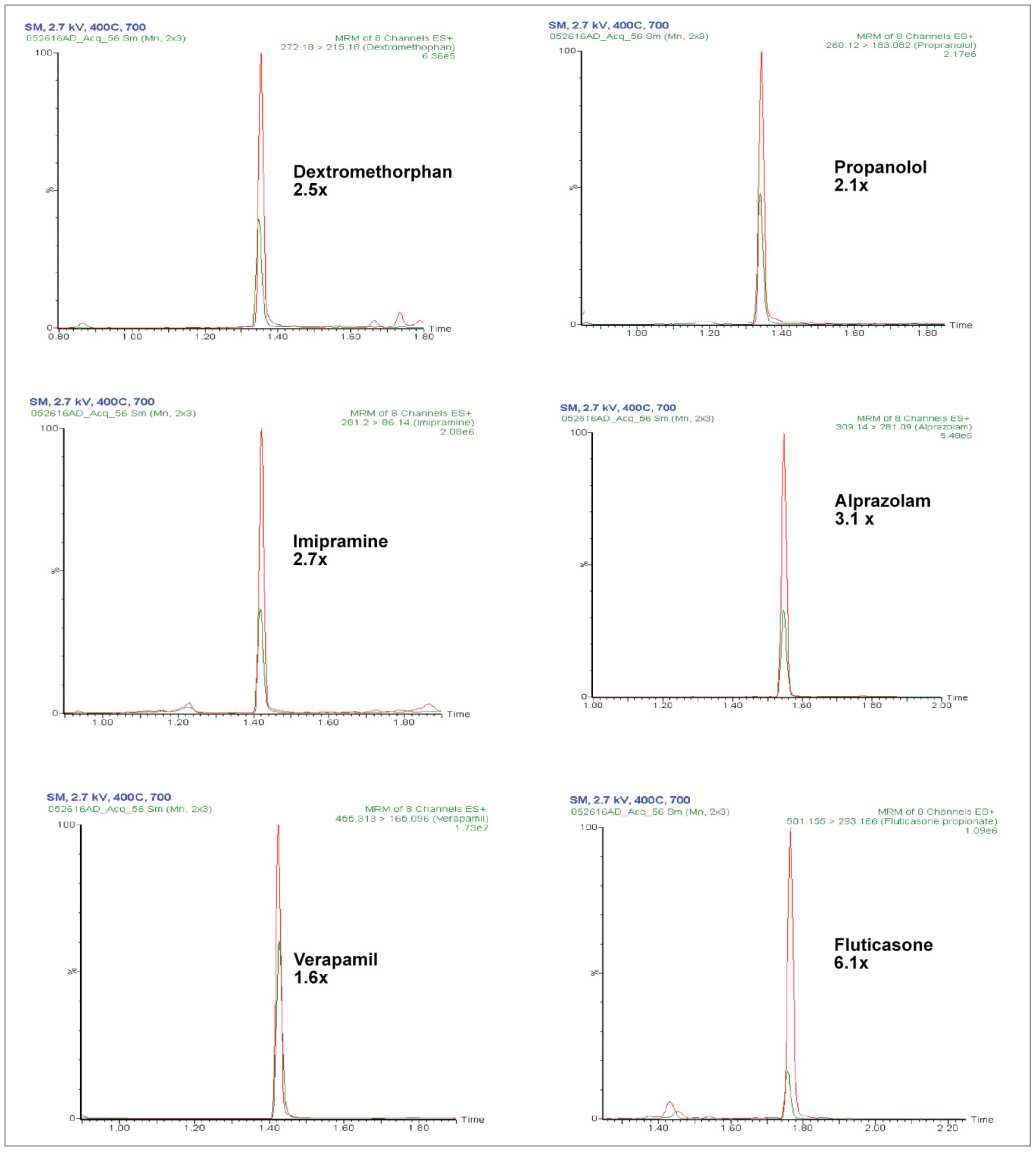

Figure 3. Sensitivity gains using (300 µm x 50 mm) BEH C_{18} iKey HT (red) compared to (2.1 mm x 50 mm) BEH C_{18} UPLC (green) under identical injection volume and gradient conditions.

FLOW RATE DEPENDENCE

To investigate the effect of flow rate on sensitivity, we varied the flow rate from 8 µL/min to 18 µL/min. As expected, the retention time changed accordingly at higher flow rates. However, no appreciable difference in signal intensity was observed except for fluticasone which performed better at higher flows (Figure 4). This uniform response with changes in flow rate differs from what is observed using the 150 µm iKey where response tends to increase at lower flow rates.[2] For high throughput applications this allows us to perform faster analysis without loss in sensitivity.

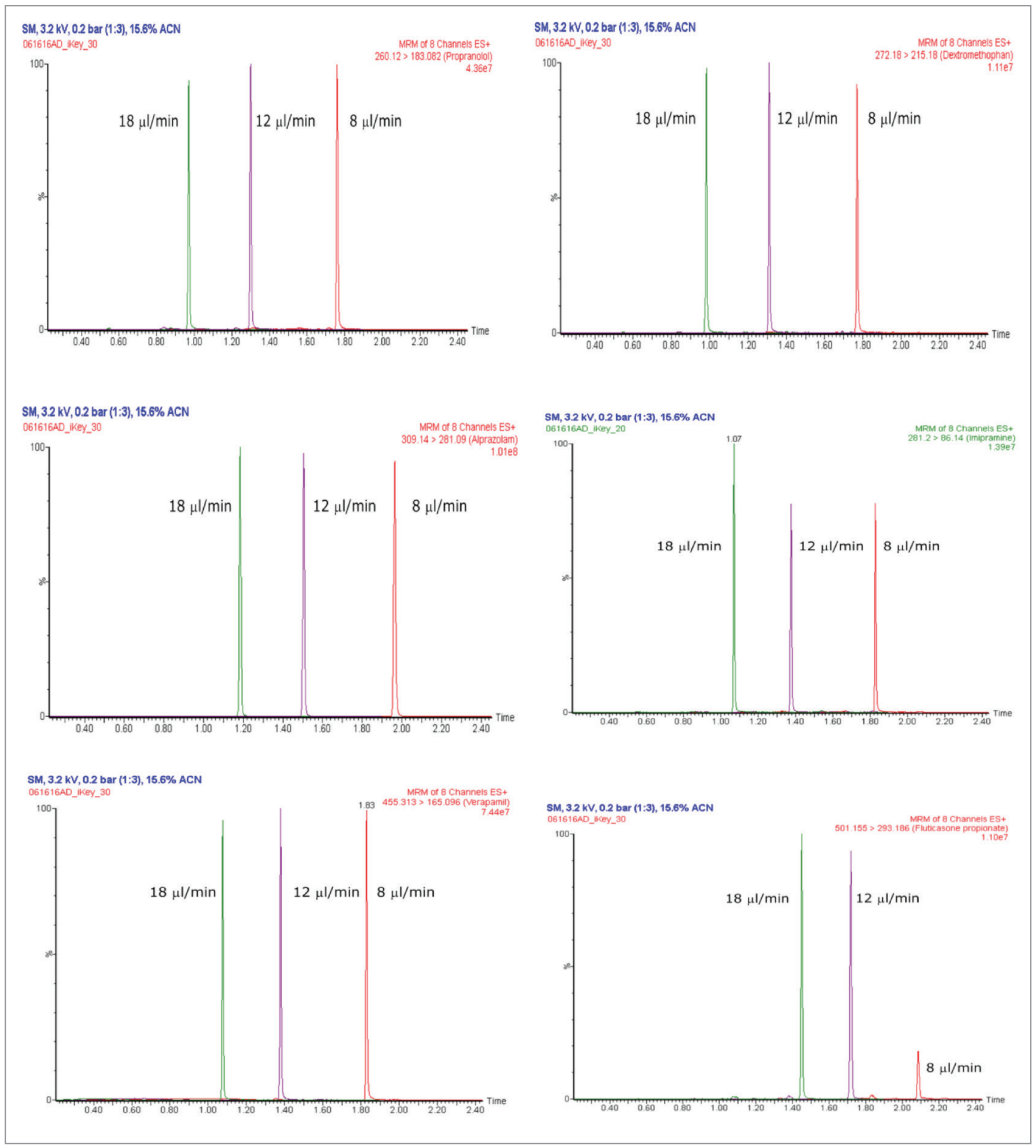

Figure 4. Sensitivity comparison when using different flow rates. Flow rates varied from 8 µL/min (red) to 12 µL/min (purple) to 18 µL/min (green).

CONCLUSIONS

- Microflow LC/MS using a 300 µm iKey HT resulted in sensitivity gains of at least 2x in all of the compounds tested when compared to conventional UPLC. The sensitivity gains were manifested on a UPLC timescale (<3 min) which demonstrated the feasibility of incorporating microflow into an everyday workflow. With the 300 µm ikey HT, the sensitivity varies insignificantly as the flow rate was changed from 8 µL/min to 18 µL/min.
- The ability to maintain cycle times under 3 minutes is a key requirement in a high throughput laboratory. It enables the user to conduct a study in a 96-well plate format and report the data within the same day. The 300 µm iKey HT allows for greater flexibility to support multiple workstreams (small and large molecule research) because the users can choose to operate in the high sensitivity mode with a 150 µm iKey or opt for the high throughput mode using a 300 µm iKey HT. Both options provide enhanced sensitivity and improved performance over the conventional UPLC alternative.

References

1. P.D. Rainville, J. Langridge, M. Wrona, I. Wilson, and R. Plumb, *Bioanalysis*, 7(11), 1397–1411 (2015).
2. Y.W. Alelyunas, G. Roman, J. Johnson, C. Doneanu, and M. Wrona, *J. Appl. Bioanal.* 1(4), 128–135 (2015).

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