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Application Note

The iKey Separation Device as a More Efficient, Yet Simple-To-Use, Alternative to Conventional Capillary Columns

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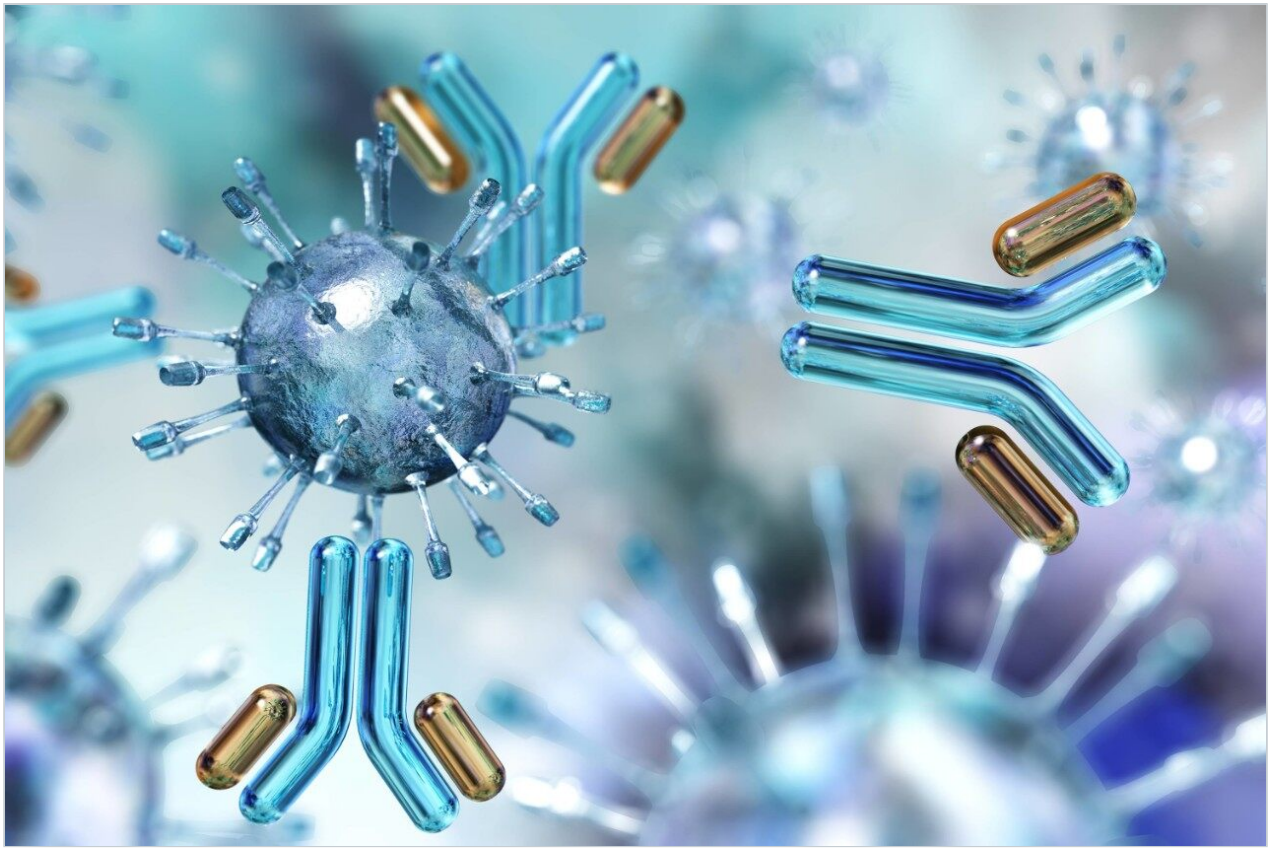

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief evaluates the chromatographic performance of iKey Separation Devices in comparison to equivalent ACQUITY UPLC M-Class Columns

Benefits

iKey Separation Devices outperform conventional 150 µm I.D. microflow columns by providing improved chromatographic performance, spray stability, and usability through the easy-to-use, plug-and-play design.

Introduction

Microflow chromatography, coupled with a high-sensitivity mass spectrometer, offers many benefits over standard-flow chromatography. The reduced flow rate, typically at less than 5 µL/min, increases ionization efficiency and analysis sensitivity while requiring less sampleand consuming less mobile phase. These advantages make microflow chromatography the tool of choice in analyzing complex samples with limited availability, such as in biomarker discovery, proteomics, and several other types of -omics research. Taking full advantage of a traditional microflow LC-MS system requires expert attention as, for example, fluidic connections need to be handled with great care. Small leaks or improperly set fittings make the system susceptible to extra column dispersion, acorresponding loss in efficiency and sensitivity, and potentially even carryover issues.

Waters introduced the ionKey/MS System and iKey Separation Devices in 2014. The system integrates the microflow UPLC separation directly into the MS source through the plug-and-play design of the iKey. This addressed the problems associated with conventional microflow chromatography. The fitting-less device connects the separation iKey Separation Devices outperform conventional 150 µm I.D. microflow columns by providing improved chromatographic performance, spray stability, and usability through the easy-to-use, plug-and-play design. The iKey Separation Device as a More Efficient, Yet Simple-To-Use, Alternative to Conventional Capillary Columns channel inside the iKey to the system with minimal dispersion and uservariability. This enables the laboratory to utilize highly sensitive microflow LC-MS without worrying about the integrity of fluidic connections and extra-column dispersion. The iKey Separation Devices and the ionKey/MS System transformed microflow chromatography from what was once a hard-to-use system to a simple plug-and-play experience.

Results and Discussion

The chromatographic performance of iKey Peptide CSH C18 Separation Device, 1.7 µm, 150 µm x 100 mm (p/n 186007259) and ACQUITY UPLC M-Class Peptide CSH C18 Column, 130Å, 1.7 µm, 150 µm x 100 mm (p/n 186007480) was investigated using microflow chromatographic separations of proteolytic peptide standards. The MassPREP Enolase Digest with Phosphopeptides Mix (p/n 186003286) was diluted to 10 fmol/µL and directly introduced to an ACQUITY UPLC M-Class System via 5 µL full loop direct injection. The mixture was separated with a 2 µL/min water/acetonitrile gradient, each with 0.1% formic acid, running from 3 to 40% acetonitrile over 15 minutes. Five peptide SRMs (Table 1) were monitored on a Xevo TQ-S MS equipped with corresponding ion sources: an ionKey Source for iKeys and a NanoFlow ESI Source for ACQUITY UPLC M-Class Columns.

Peptide	Sequence	Parent (m/z)	Daughter (m/z)	Cone voltage (V)	Collision energy (eV)
T6	SIVPSGASTGVHEALEMR	614.31	201.12 771.37	30	24
T11	NVNDVIAPAFVK	643.86	745.46 1073.60	30	25
T43	VNQIGTLSESIK	644.85	834.45 947.54	30	25
T43p	VNQIGpTLSESIK	684.90	816.35 929.44	30	27
T38	TAGIQIVADDLTVTNPK	878.48	1172.61 1413.76	30	33

Table 1. Monitored proteolytic peptides and their SRM conditions.

Chromatographic performance was assessed using the results of five replicate chromatographic runs on four iKeys and four ACQUITY UPLC M-Class Columns. Figure 1 and Figure 2 show SRM chromatograms

representative of each chromatographic device. Table 2 presents the chromatographic data obtained with each set of devices. Notably, retention times on the iKeys are almost identical (within 2%) to those on the ACQUITY UPLC M-Class Columns of same dimensions, thus existing methods for 150 µm I.D. columns may be used on iKeys without the need for redeveloping the method from scratch. The retention time variation within four ACQUITY UPLC M-Class Columns was less than 2% RSD and less than 1% RSD for four iKeys over a wide range of analyte retention times. The retention time variation within five replicate injections on a single iKey/column was excellent, at less than 0.1% RSD. The average peak width measured at 13.4% peak height (4σ peak width) from iKeys was 25% less than that from equivalent ACQUITY UPLC M-Class Columns, which demonstrates that superior chromatographic efficiency can be achieved with iKey Separation Devices (Figure 2, Table 2). The high efficiency of iKeys can also be confirmed from the observed peak capacity values. The iKeys were found to produce a half-height peak capacity of 294, while the ACQUITY UPLC M-Class Column's peak capacity was 206. Accordingly, users can resolve 40% more peaks when using the same gradient run on iKeys compared to the equivalent ACQUITY UPLC M-Class Columns (Figure 1). In addition, the sharper peaks produced by iKeys result in increased peak heights and improvements in assay sensitivity.

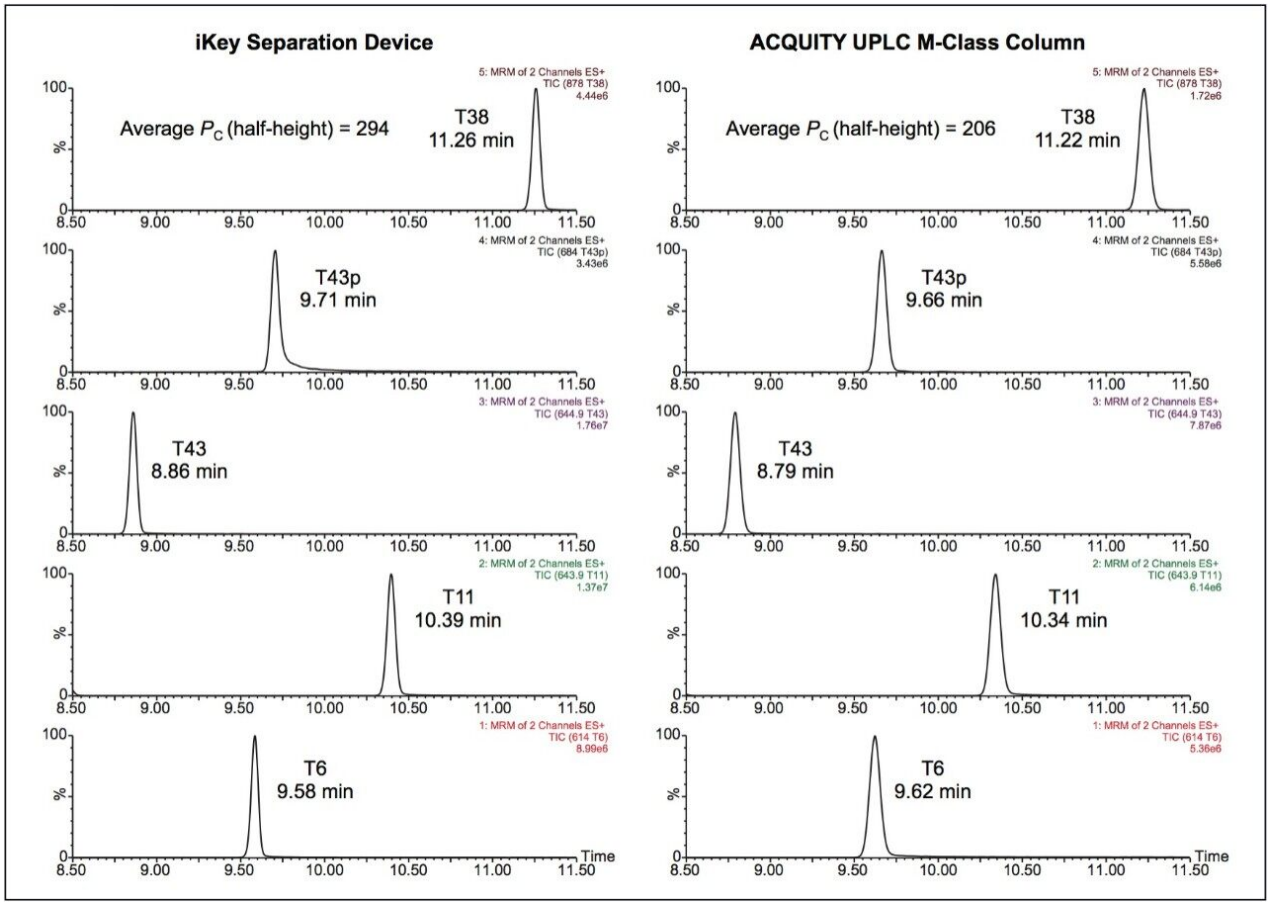

Figure 1. Typical chromatograms using an iKey Separation Device (left), and an ACQUITY UPLC M-Class Column (right). The reported peak capacity values are averages of half-height peak capacity values for all five peaks.

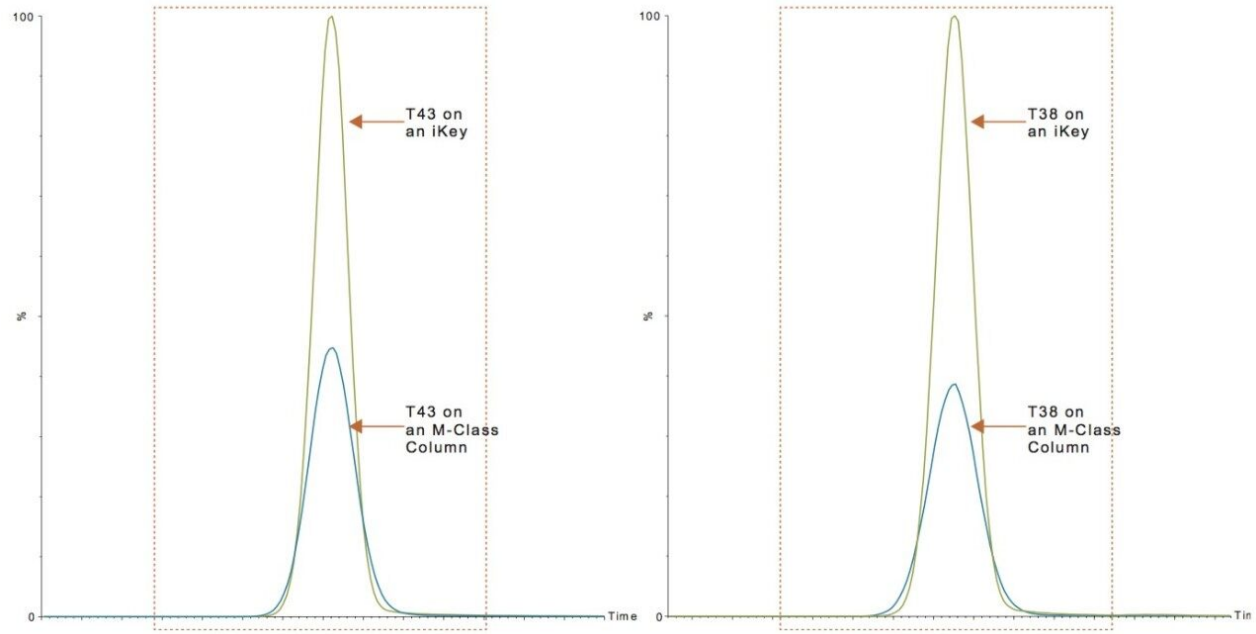

Figure 2. Comparison of chromatographic peaks from the 150 µm ACQUITY UPLC M-Class Column (blue traces) and the iKey Separation Device (green traces). Retention times were offset for easy comparison. T43 (left) is an early eluter, and T38 (right) is a late eluter.

iKey Separation Device		ACQUITY UPLC M-Class Column, 150 µm
	Retention time (minute)	
8.759 (0.77% RSD)	**Early eluter**	8.956 (1.63% RSD)
11.169 (0.56% RSD)	**Late eluter**	11.369 (1.12% RSD)
	Peak width, 4σ (second)	
5.54 (3.76% RSD)	**Early eluter**	7.50 (4.36% RSD)
5.90 (3.35% RSD)	**Late eluter**	7.98 (2.40% RSD)
294	**Average peak capacity at half-height**	206

Table 2. Chromatographic Performance of 150 µm ACQUITY UPLC M-Class Columns and iKey Separation Devices, averaged over five replicate injections on four columns/iKeys. Values in parentheses are % relative standard deviations (% RSD) within the four columns or the four iKeys.

The detector sensitivity is another factor that influences the peak height. On an electrospray MS interface, a stable spray increases MS sensitivity by providing a stable ion signal with a low background noise. It can often be difficult to produce a stable spray when using a typical microflow fused silica capillary emitter. Whether it is a pulled or an etched tip, a fused silica emitter tip is mechanically fragile and easily damaged upon exposure to high voltage. Users must replace emitters to maintain system performance. The stainless steel emitter on iKey Separation Devices, on the other hand, provides stable and consistent spray throughout the lifetime of the device. With the metal emitter being an integral part of each iKey, users do not need to make a post-column fluidic connection to a separate emitter. This simplifies the lab workflow by saving setup time. It also prevents users from compromising analysis sensitivity and the system reproducibility by accidentally damaging the capillary end or creating a void.The detector sensitivity is another factor that influences the peak height. On an electrospray MS interface, a stable spray increases MS sensitivity by providing a stable ion signal with a low background noise. It can often be difficult to produce a stable spray when using a typical microflow fused silica capillary emitter. Whether it is a pulled or an etched tip, a fused silica emitter tip is mechanically fragile and easily damaged upon exposure to high voltage. Users must replace emitters to maintain system performance. The stainless steel emitter on iKey Separation Devices, on the other hand, provides stable and consistent spray throughout the lifetime of the device. With the metal emitter being an integral part of each iKey, users do not need to make a post-column fluidic connection to a separate emitter. This simplifies the lab workflow by saving setup time. It also prevents users from compromising analysis sensitivity and the system reproducibility by accidentally damaging the capillary end or creating a void.

Conclusion

iKey Separation Devices outperform equivalent ACQUITY UPLC M-Class Columns by providing more stable spray, 40% greater peak capacity, and corresponding increases in assay sensitivity. The device-to-device retention time reproducibility is excellent for both iKeys and ACQUITY UPLC M-Class Columns at less than 2% RSD. The retention times on the iKey Separation Devices are within 2% of those observed on equivalent ACQUITY UPLC M-Class Columns running the same gradient method. iKey Separation Devices offer the unique advantage of easy-to-use, highly sensitive microflow chromatography through an innovative plug-and-play design. That existing ACQUITY UPLC M-Class Column methods can be applied to iKey Separation Devices underscores the accessibility of this new format of microflow chromatography.

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