Improved Peak Resolution and Repeatability Using Novel High Performance Post-Column Flow Splitters

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Abstract

Flow splitting is often used in Liquid Chromatography (LC) and Liquid Chromatography with Mass Spectroscopy (LC-MS) as a means of reducing flow to a detector or splitting a single stream between multiple detectors. Chromatographers using flow splitting in various methods have been plagued by a multitude of problems at discovery and QC levels. Inefficiently designed flow splitter technology causes significant problems that are often overlooked, underestimated, or tolerated simply due to a lack of product options. A popular method of flow splitting is "homemade" flow splitters where scientists use cut tubing to restrict flow and achieve the desired flow. In addition to being a tedious and slow task, this approach can also lead to sub-optimal analysis. Scientists are often plagued with the following issues as a result:

- Excessive peak dispersion caused by excessive post-column dwell volume
- Ineffective time usage of highly paid employees setting up equipment rather than performing analysis
- Poor split accuracy resulting in unrepeatable, inaccurate results
- DIY methods, such as cutting tube, that are archaic and difficult to reproduce

However, a new Fixed Flow Splitter from PerfectPeak® is available for use in post-column High Performance Liquid Chromatography (HPLC) and Ultra-High Performance Liquid Chromatography (UPLC) analysis. The Flow Splitter utilizes porous metal media flow restrictors as a means of creating a differential flow in the device. As a result, the product features:

- Approximately 5 µL dwell volume for industry-best peak dispersion
- Finger-tight connections for quick-installation, quick-split changes, and effective use of scientist's time
- Flow split accuracy within +/- 10%
- Lightweight, compact design to be readily installed in-line without the need for mounting apparatuses

Introduction

Recent progress in mass spectroscopy and liquid chromatography has made flow splitting a valuable tool for research involving multiple detection or multiple columns. In particular, flow splitting has proven to be very useful when performing analysis with mass spectroscopy ensuring that the flow to the mass spec does not saturate the detector and inhibit sensitive analysis.

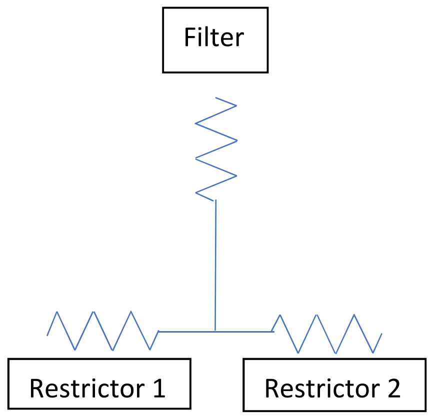


Figure 1. *The concept of a flow splitter is two (2) resistors in parallel that create a resistance.*

Creating a differential resistance is key to the functionality of the flow splitter and achieving the appropriate flow split. The PerfectPeak® Flow Splitter accomplishes this using proprietary porous metal restrictor technology where different density 316L stainless steel restrictors are employed to create this differential resistance.

(a) (b)

Figure 2. *Photograph of a Mott PerfectPeak® 2:1 Flow Splitter (a) and an image of the replacement cartridges offered with the Splitter (b).*

Experimental Procedure

Figure 3 shows a schematic diagram of a typical layout of an HPLC/UHPLC system. The PerfectPeak® Flow Splitter was evaluated using an Agilent 1260 Series binary pump system (see **Table 1** for full configuration), a Waters Symmetry C18, 5 µm MVK, and an Agilent UV detector, with the splitter placed between the column and detector.

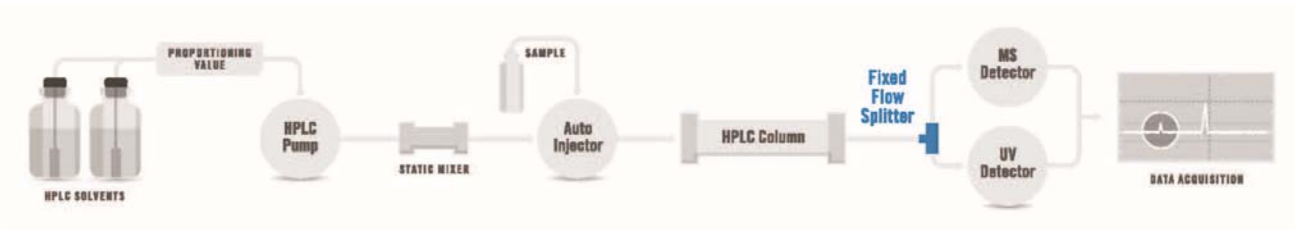


Figure 3. *Schematic diagram of the low-pressure gradient experimental test system.*

The HPLC was controlled using the Agilent Chemstation Software.

Table 1 Configuration of Agilent 1260 Infinity HPLC	
Pump	G1312B
Auto Injector	G1329B
Column Compartment	G1316A
Detector, VWD	G1314B

Repeat Injection Test

The Mott PerfectPeak® Flow Splitter was tested for injection-to-injection reproducibility with a small molecule analysis method. This method is described in **Table 2**. The method used was modified from an Agilent application note on the USP method for the evaluation of caffeine and acetaminophen (https://www.agilent.com/cs/library/applications/5991-5920EN.pdf). One mg/mL of caffeine and acetaminophen was prepared in deionized water. The method was run under the following conditions:

Table 2 Method used for the Analysis of Caffeine and Acetaminophen	
Column	Zrobax Eclipse Plus C18 4.6x100mm, 3.5µm
Mobile Phase	Water: Methanol: Acetic Acid (69:28:3)
Temperature	45 °C
Flow Rate	1.0 mL/min
Injection Volume	5 µL
Detection	UV, 275 nm

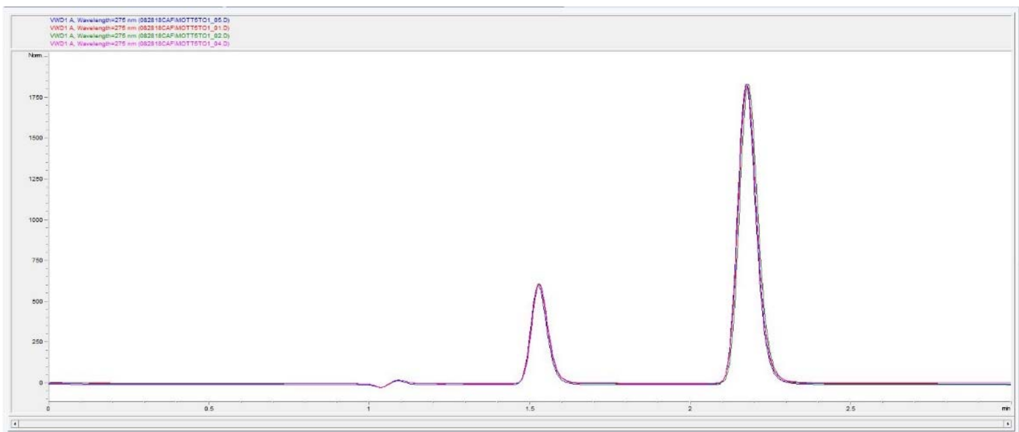


Figure 4. *This figure is the data overlay of the caffeine acetaminophen analysis from the UV detector at 275 nm.*

Peak retention was monitored upon successive injections for flow stability. It was found that upon four (4) consecutive injections, the caffeine and acetaminophen peaks had a 0.11% RSD and 0.11% RSD respectively. The USP requirements of RSD for replicated injections is no more than 2.0% variation. The results of the testing can be found in **Figure 4**, where peak resolution was 10.3 on average.

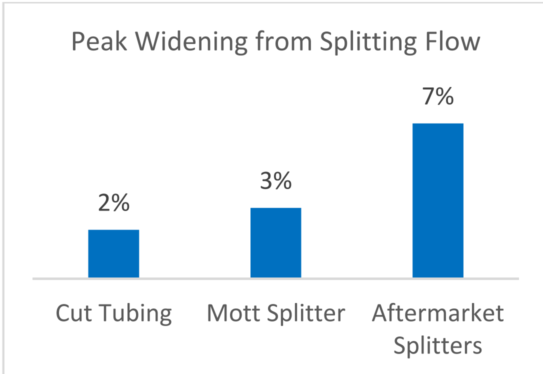
Dispersion Analysis

The PerfectPeak® Flow Splitter was also tested for dispersion or peak spreading and compared to the system where no splitter was installed. The intention of this test is to showcase the quick peak response exhibited because of the minimal volume of the device. Testing was performed on the Agilent 1260 instrument under the method conditions described in **Table 3**. The splitter was compared to a cut tubing/DIY splitter, and a competitive device. A sample of acetone was prepared at 6µL/mL in deionized water.

Table 3 Peak Dispersion Test Method	
Column	Waters Symmetry C18 5µm MVK 3.9x150 mm
Mobile Phase	Acetonitrile: Water (60:40)
Temperature	25 °C
Flow Rate	1.0 mL/min
Injection Volume	1 µL
Detection	UV, 254 nm

This test was performed with three nominal 10:1 splitters and results were averaged over repeat injections. The results of this analysis found in the table below show that the PerfectPeak® Flow Splitter has negligible impact on peak shape and resolution even when compared with the conventional DIY tubing setup.

Table 4 Calculated Values if Internal Bandwidth Spreading and Theoretical Plate Count		
	Average IBW	Average N (plate count)
Column	0.0462	12213.93
Cut Tubing	0.0472	11878.05
PerfectPeak® Splitter	0.0476	11966.46
Aftermarket Splitter Components	0.0493	11221.58



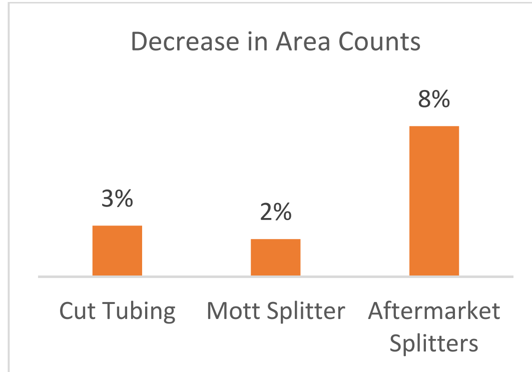


Figure 5a-b. *This figure shows the percentage effect of peak widening (a) and percentage effect of area counts (b) when using a flow splitter relative to no splitter installed and typically used aftermarket splitters / components.*

From the data in **Table 4**, the greatest theoretical plate count can be found when using the system with no splitter components installed. As components are added to the fluid stream, volume is added thus increasing the dispersion of the peaks and decreasing the plate count. This can negatively impact resolution when peaks are found close to each other. The PerfectPeak® Flow Splitter resulted in the least amount of reduction in peak shape, even when compared to a cut tubing splitter.

Additional tests were performed at a leading contract lab, Mercachem, by analytical group leader Colinda van Tilburg. The tests compared a generic "T" with cut tubing to the PerfectPeak® to analyze the effects in a UPLC-MS-ELSD setup. The results dramatically improved peak shape and reduced peak broadening.

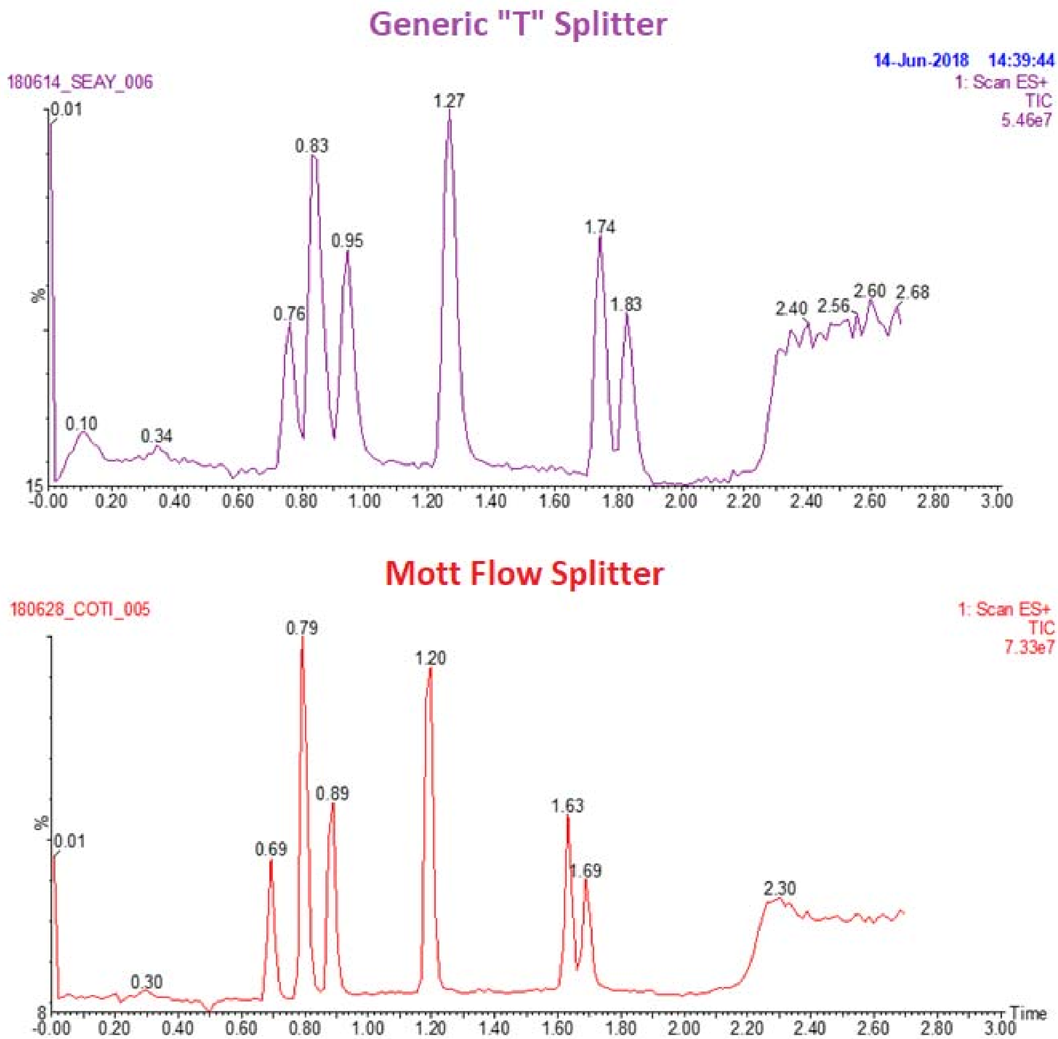


Figure 6. *Reduction in peak broadening are observed by converting a method setup from a generic "T" with cut tubing to the Mott PerfectPeak® Flow Splitter.*

Test Method:
UPLC: Waters I-Class
Acq. Method: UPLC_AN_ACID
Column: XSelect CSH C18 (50x2.1mm 2.5µm)
Flow: 0.6 ml/min; Column temp: 40°C
Eluent A: 0.1% formic acid in acetonitrile
Eluent B: 0.1% formic acid in water
Gradient: t=0 min 5% A, t= 2.0 min 98% A, t=2.7 min 98%
Post-time: 0.3 min
Detection PDA: 210-320nm
Detection ELSD: gas pressure 40 psi, drift tube temp: 50°C
Detection MSD: ESI (pos/neg) mass range 100-800

Effect of Tubing Connected Downstream of Flow Splitter on Split Ratio

The PerfectPeak® Flow Splitter was tested with different lengths of tubing connected downstream of the splitter to investigate the impact on split ratio. The intent of this section is to show the user what they would expect their flow split to be with tubing connected downstream of the PerfectPeak® Flow Splitter device. The testing was conducted at 1 mL/min with isopropyl alcohol as the test liquid. The PerfectPeak® Flow Splitter might have a higher than expected pressure drop. The idea behind this is the benefit of greater resistance in the device which will allow the splitter to maintain accuracy with greater lengths and various configurations of tubing downstream. Nominal 10:1, 5:1, 3:1, 2:1 and 1:1 splitters were tested with up to 3 feet of tubing connected downstream of the device. Initial testing was performed with a 0.002” ID (Black) PEEK tubing and 0.030” ID (Green) PEEK tubing. **Figure 7a,** below on the left shows the measured flow split with different lengths of tubing connected downstream of the splitter. The black tubing downstream of the splitter is likely the worst case. **Figure 7b,** on the right shows similar data with the green PEEK tubing connected downstream of the splitter device. We recommend the shortest and most open tubing possible when setting up your device.

Nominal 10 to 1		
Tubing	Flow Split	% Change
0"	9.89	
6"	8.38	15.3%
1'	7.09	28.3%
3'	5.07	48.7%
Nominal 5 to 1		
Tubing	Flow Split	
0"	4.74	
6"	4.09	13.7%
1'	3.56	24.9%
3'	2.81	40.7%
Nominal 3 to 1		
Tubing	Flow Split	
0"	3.07	
6"	2.78	9.4%
1'	2.51	18.2%
3'	2.04	33.6%
Nominal 2 to 1		
Tubing	Flow Split	
0"	1.93	
6"	1.81	6.2%
1'	1.75	9.3%
3'	1.53	20.7%

(a)

Nominal 10 to 1		
Tubing	Flow Split	% Change
0"	10.16	
6"	10.16	0.0%
1'	10.16	0.0%
3'	10.15	0.1%
Nominal 5 to 1		
Tubing	Flow Split	
0"	4.8	
6"	4.8	0.0%
1'	4.8	0.0%
3'	4.8	0.0%
Nominal 3 to 1		
Tubing	Flow Split	
0"	2.9	
6"	2.9	0.0%
1'	2.9	0.0%
3'	2.9	0.0%
Nominal 2 to 1		
Tubing	Flow Split	
0"	1.93	
6"	1.93	0.0%
1'	1.93	0.0%
3'	1.93	0.0%

(b)

Figure 7a-b. *The measured flow split of a PerfectPeak® Flow Splitter with tubing connected downstream of the splitter. Table (a) shows a likely worst case with 0.002” ID tubing.* ***.*** *Table (b) shows a likely best case with 0.030” tubing.*

Change in Flow Split during Gradient Analysis

The Mott PerfectPeak® Flow Splitter was tested for flow split with different viscosity fluids that would be representative of a gradient analysis. The testing consisted of preparing an isocratic solution to flow through an HPLC pump connected to the flow splitter. The flow was measured by collecting the effluent from the outlet of the splitter and measuring the mass of liquid accumulated over a set period of time. Multiple 5:1 PerfectPeak® Flow Splitters were chosen for the analysis. The low viscosity solution was 100% acetonitrile (0.3 cP). The mid viscosity fluid was a combination of 50%/50% acetonitrile and isopropyl alcohol (1.3 cP). And the high viscosity solution was 100% isopropyl alcohol (2.2 cP). The results of this test can be found in **Figure 8**.

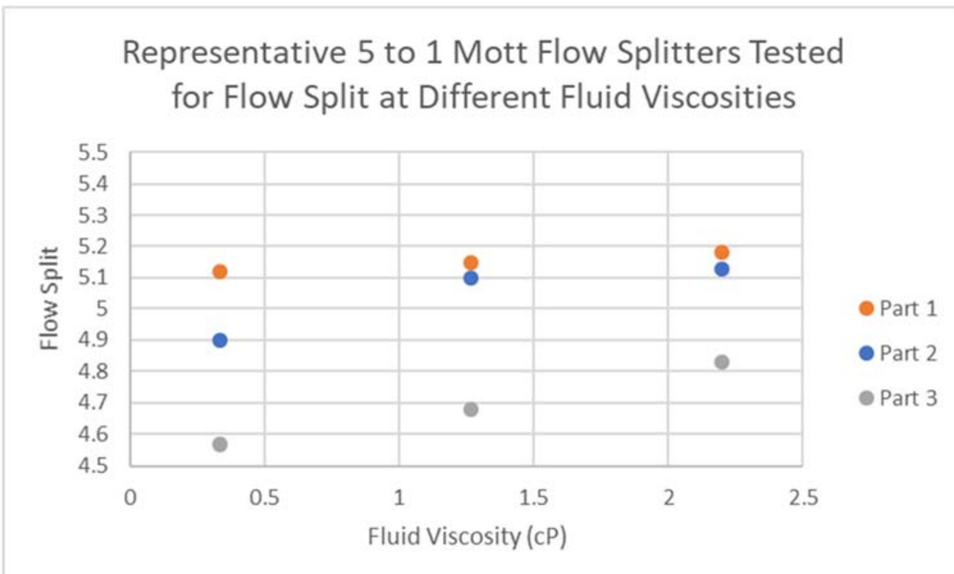


Figure 8. *This figure represents the flow split of a Mott PerfectPeak® Flow splitter when different fluid viscosities are used to mimic a gradient analysis.*

The results found in **Figure 8** indicate that the flow is likely to change 5% over the course of this extreme gradient. The effect of the drift in flow split when running a gradient should be minimal, however the user should expect a slight difference.

Summary

The PerfectPeak® Flow Splitter is excellent for use in post column flow splitting for HPLC, UPLC and LC-MS analysis. This splitter was evaluated for repeatability upon consecutive injections, peak dispersion, the effect of downstream tubing connected to the splitter on flow split and the effect of gradient on flow split. The data shows the PerfectPeak® Flow Splitter improved performance across the board for all these tests. The low dwell volume, finger-tight design, and high accuracy design alleviates repeatability issues and wasted time for scientists requiring flow splitting in their methods.

More Info on PerfectPeak® Flow Splitters…

Mott's new line of PerfectPeak® Flow Splitters are available in 1:1, 2:1, 3:1, 5:1 and 10:1 split ratios with the use of interchangeable cartridges and finger-tight fittings. These interchangeable cartridges allow a quick replacement at the convenience of the user. Split ratios can be changed in seconds without the trouble of cutting tubing and measuring flows to make sure the user gets the flow just right. PerfectPeak® Flow Splitters are also engineered with an internal volume of only ~5 µL, the lowest dwell volume available on the market today for industry best peak dispersion. Additionally, PerfectPeak® Flow Splitters have a convenient free spin threaded cap that will not tangle tubing when changing cartridges but becomes locked into place when a cartridge is installed into the device. The design also features superior filtration and reliability by employing a Media Grade 0.1 filter that is one of the thinnest filters on the market, leading to minimal pressure drop over time while protecting the resistor components of the splitter. Finally, this is all packaged in a small, lightweight tee body that will not kink instrument lines when connected. The footprint of the device is under 2 inches in size in any dimension and rated for up to 15,000 PSIG. For more information and case studies, please visit our website at www.mottcorp.com/splitter.

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