

Fast and Robust Microflow HPLC Method for Open-Access Product Analysis in Discovery Medicinal Chemistry

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Abstract:

The advent of high throughput synthesis and purification has created a need for more efficient ways to do reaction scouting, route development and product profiling. Microflow HPLC, generally referred to as having chromatographic columns of 0.3 to 0.5 mm i.d. and flow rates in the 2 to 60 $\mu\text{L}/\text{min}$ range, can significantly shorten the HPLC analysis time attributable to its characteristic rapid gradient mixing and fast column re-equilibration. The substantial reduction in solvent usage and waste generation offers additional benefits in cost-saving and lessening of environment impact. Using a novel microflow HPLC system, we have developed a robust short-cycle time (2.5 minutes) rapid gradient method for the analysis of synthesis products in discovery chemistry labs. The capabilities of the microflow HPLC method to deal with real world samples are investigated. The results compare favorably to those obtained using a conventional 4.6 mm column gradient HPLC system. The benefits and issues of using microflow HPLC in an open access medicinal chemistry environment are discussed.

Introduction:

Open access HPLC located in a centralized lab or distributed to bench top is used routinely by medicinal chemists for reaction scouting, route development and product profiling. The analysis is typically done with a conventional HPLC running a generic 5-min gradient method on a 50 x 4.6 mm reverse phase column at a relatively high flow rate of 3 - 4 ml per minute. An additional 2 - 3 minutes re-equilibration time is needed to re-generate the column after each run, making the injection-to-injection cycle time at about 7 - 8 minutes. The recent advance in high throughput synthesis and purification technologies has greatly elevated the productivity of discovery chemistry, thus causing a bottleneck in downstream analytical support. Microflow HPLC has been shown to significantly shorten the HPLC analysis time attributable to its characteristic rapid gradient mixing and fast column re-equilibration. The objective of this work is to develop a rapid gradient microflow HPLC method of 2 to 3 minutes cycle time which can be readily adopted for walk-up medicinal chemistry product analysis. In addition to improving analytical productivities, the substantial reduction in solvent usage and waste generation offers further benefits.

Experimental:

Microflow HPLC System:

Eksigent *express*LC-100™, a single-channel fast HPLC system with integrated CTC/PAL autosampler, Eksigent 2.08 Control Software and PeakViewer™ data analysis package was used for the development of a fast LC method for the analysis of medicinal chemistry reaction products. The microflow system is optimized for LC column internal diameters of 0.3 to 0.5 mm and flow rates in the 2 - 60 $\mu\text{L}/\text{min}$ range. The unique flow rate control mechanism allows for very accurate "time-sliced" variable volume injections from nL to μL . An in-line sample filter of 0.45 μm pore size positioned in between the injection port and the sample loop is particularly useful for preventing precipitates or particulates in the crude sample solutions contaminating the system.

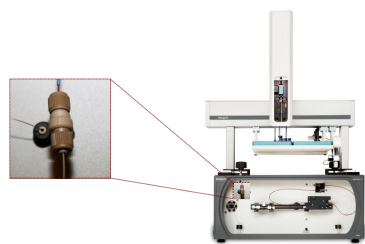


Figure 1 Eksigent *express*LC-100™ HPLC system. The in-line sample filter shown in the magnified view is particularly useful for filtering out precipitates or particulates which are often present in the crude sample solutions

Separation Conditions:

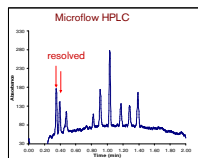
Column: Eksigent ChromXP C18EP, 3 μ , 120Å, 50 x 0.3 mm
Mobile Phase: A: water 0.1% TFA; B: acetonitrile, 0.08% TFA
Gradient: 0 min 5% B
1.5 min 95% B
2.0 min 95% B
Temperature: 45°C
Flow Rate: 12 $\mu\text{L}/\text{min}$
Detection: 4 mm path, UV@215 and 254 nm
Cycle Time: run time 2 minutes; re-equilibration time, 0.5 min
Injection Volume: 100 nL, samples diluted in methanol

Results:

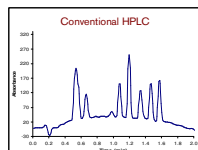
Microflow HPLC is capable of delivering precise and fast gradients that result in better chromatographic performance for complex samples under the fast ballistic gradient separation conditions. This is clearly shown below by comparing the chromatograms from the separation of a mixture of drug-like compounds on a conventional HPLC system to that on a comparably scaled microflow Eksigent system.

Figure 2

Eksigent *express*LC-100
0.3 x 50 mm, C18 EP, 120Å
25 $\mu\text{L}/\text{min}$, 44° C
Injection V = 100 nL
A/B water/ACN, with 0.1% TFA
5% B to 95% B in 2 minutes
30 sec re-equilibration
UV @ 215 nm



Agilent 1100
4.6 x 50 mm, C18 EP, 120Å
5 mL/min, 50°C
Injection V = 12.5 μL
A/B water/ACN, with 0.1% TFA
5% B to 95% B in 2 minutes
30 sec re-equilibration
UV @ 215 nm



The microflow HPLC method offers speed and resolution for the analysis of a broad range of commonly encountered medicinal chemistry compounds, starting materials and products, as shown below for the analysis of random samples selected from an industrial discovery medicinal chemistry lab

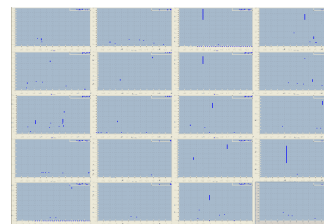


Figure 3 Typical HPLC results from open-access medicinal chemistry samples. The separation condition is described in the Experimental section.

Despite the fact that the samples from med chem labs are notoriously "dirty" the Eksigent microflow system has proven to be very reliable and robust. This in part is attributable to the in-line filter which prevents unwanted particulates or precipitates from entering the system. More than 1000 injections can be routinely carried out with no deterioration of the chromatographic performances. As a general practice, the microfilter capsule is replaced daily.

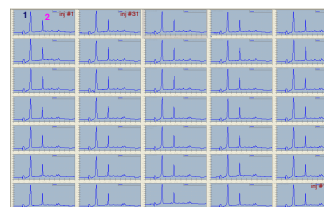


Figure 4 Repeated injections of 30 different samples. Shown here are injections #1, #31, #61 --- etc.

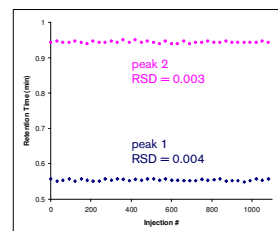


Figure 5 The relative standard deviations for the sub-1 min retention times of peak 1 and peak 2 in Figure 4 are 0.4 % and 0.3 %, respectively.

Discussion:

It is well recognized that Microflow HPLC is particularly suited for fast gradient HPLC applications attributable to its efficient mass transfer in small spaces that results in rapid gradient mixing and re-equilibration. As illustrated in the table below, the Eksigent Microflow LC, for example, has a 10X to 50X advantage in terms of system delay over other commercial systems designed for larger i.d. columns and higher flow rates. The advantages are corroborated in this study.

HPLC	Column (mm)	Delay V (uL)	Void V (uL)	V_{delay} / V_{column}
Eksigent Micro-flow LC	0.3 x 50	0,35	3	0.1
Conventional System A	4.6 x 50	750	600	1.3
Conventional System B	2.1 x 50	150	120	1.3
Conventional System C	1.0 x 50	150	30	5

The practicality of using microflow HPLC for routine, high-volume, fast cycle time analysis has been demonstrated. The underlying concern that capillary LC columns might not be sufficiently robust for rather "dirty" medicinal chemistry samples was unfounded. First of all, the amounts injected onto the column are proportionally scaled down. Secondly, undesirable particulates that may be present in the crude solution are effectively removed by the in-line filter.

Reduction in solvent usage and waste generation not only makes economic sense, but also contributes to a cleaner environment. A quick calculation would show that 4 liters and 1000 liters of organic solvent would be consumed per year for a microflow HPLC at 12 $\mu\text{L}/\text{min}$ and a conventional HPLC at 3 mL/min, respectively (assuming 24 hrs x 250 working days). The contrast is fairly dramatic when considering the depictions below.



Conclusions:

We demonstrate here the utility and benefits of a rapid gradient microflow HPLC method for the analysis of medicinal chemistry samples that is well-suited for routine operations in an open-access discovery chemistry lab environment. Compared with methods using conventional 4.6 mm columns, the method developed in this study shortens the injection-to-injection cycle time by a factor of 3. The method is very robust as demonstrated in the analysis of real-world "dirty" samples. The substantial reductions in solvent usage and waste generation would result in additional benefits of operational cost-saving and lessening of environmental impacts.