

Transferring microLC methods to conventional HPLC systems

Studies show that isocratic and gradient methods that are developed on the ExpressLC-800® system from Eksigent can be easily converted to a method appropriate for a conventional HPLC system.

Introduction

Liquid chromatographic (LC) method development is a time-consuming process even for the simplest of samples, since the variables that affect separation include mobile phase, stationary phase chemistry and physical characteristics, temperature, injection volume, dissolution solvent, and others. Often the process is reduced to simply choosing a common mobile phase and a favorite C18 column. While it is clear that the wider the range and number of separation variables investigated, the greater the assurance that the resulting method will be optimized both for the current and future applications, such studies are usually truncated since they require both a great deal of method development time and detailed analysis.

An attractive alternate approach for LC method development involves microLC which uses 0.3 mm ID columns. MicroLC method development has the advantage of short gradient delays, re-equilibration times in the range of seconds, small sample volumes and a total mobile phase consumption less than 1% that required for conventional analysis.

In addition, there is a commercially available parallel HPLC system containing eight, fully-independent chromatography channels based on a microLC platform—the Eksigent ExpressLC-800 system. This system combines the previously mentioned benefits of microLC with the high throughput of parallel analyses.

The ExpressLC-800 system enables eight different mobile phase/column combinations to be simultaneously evaluated, reducing overall method development time by as much as 90%. The developed method can then be run on a single channel microLC (0.3 mm ID column) system or a conventional LC (4.6 mm ID column) system.

However, a primary question when considering microLC method development can be simply stated: *if a method is developed in the microLC format, how easily can it be converted to conventional LC?*

To at least partially answer this question, two studies were performed comparing the results of running methods developed on a microLC system translated to a conventional HPLC system. One study involved a method developed for an isocratic separation while the other was for a gradient separation. Both microLC and conventional columns were packed with the same stationary phases.



Isocratic separation

A test sample composed of four compounds was created: uracil 0.1 mg/mL, diethylphthalate 2.0 mg/mL, naphthalene 0.5 mg/mL, and acenaphthene 1.5 mg/mL. The separation conditions used on both systems were as follows:

Column stationary phase	ProntoSil C18CL, 3 μ , 120Å
Column length	50 mm
Column temperature	Ambient
Mobile phase	30% H ₂ O / 70% acetonitrile
Run time	4 minutes
Detection wavelength	260 nm

To adapt the microLC method developed on the ExpressLC-800 HPLC system, only the flow rate and injection volume were scaled-up according to the ratio of the cross-sectional areas of the two columns. For this study we went from a 0.3 mm ID microLC column to a 4.6 mm conventional column and the appropriate scale-specific separation conditions were:

	Conventional LC	MicroLC
Column ID	4.6 mm	0.3 mm
Flow rate	1.0 mL/min	4.3 μ L/min
Injection volume	5 μ L	40 nL

In scaling up separation methods, the basic rule of thumb is to increase the flow rate by the ratio of the cross-sectional areas of the columns. Here we modified an isocratic method developed on a 0.3 mm ID column to one suitable for a 4.6 mm ID column. The conversion factor used was therefore 235. Of course, an instrument designed to operate with the column ID chosen is required. The microLC system used was an Eksigent ExpressLC-800 with UV-absorbance detection. The resulting separation is shown in figure 1. Figure 2 shows the same separation on a conventional HPLC, an Agilent 1100 Series LC.

From examining the two chromatograms, it can be seen that by increasing the flow rate from 4.3 μ L/min to 1.0 mL/min, the scale-up of the method is effective. Qualitatively,

the separations appear to be nearly the same. A more quantitative comparison of four separation attributes including retention time (RT), peak width at half-height (PW 0.5) and plate count (Plates) is given in Table 1.

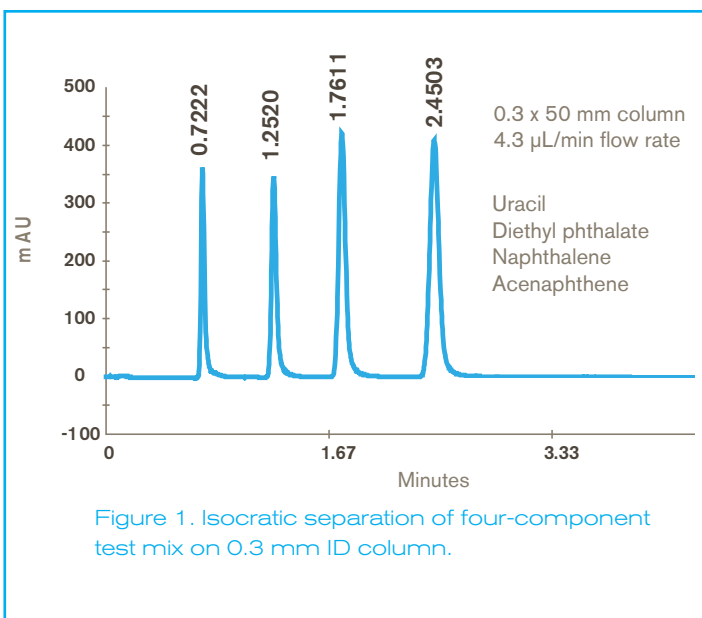


Figure 1. Isocratic separation of four-component test mix on 0.3 mm ID column.

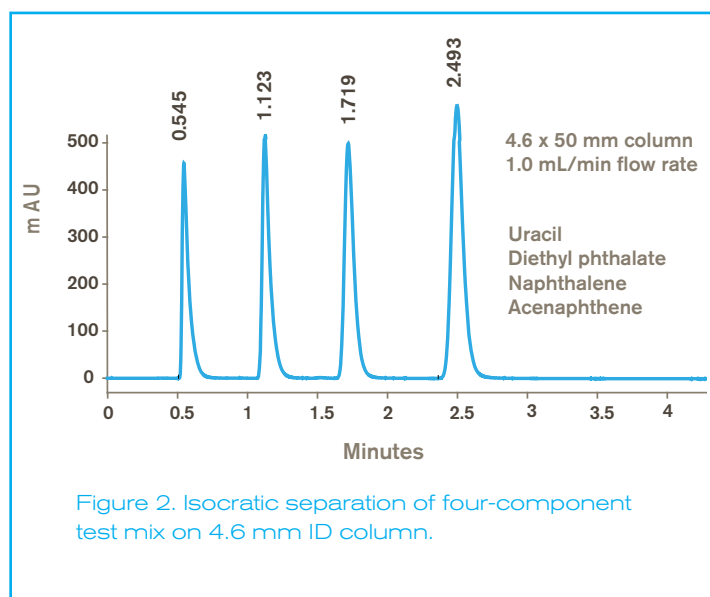


Figure 2. Isocratic separation of four-component test mix on 4.6 mm ID column.

		Uracil	Diethyl phthalate	Naphthalene	Acenaphthene
RT (min)	MicroLC	0.72	1.25	1.76	2.45
	Conventional	0.54	1.1	1.7	2.5
PW 0.5 (sec)	MicroLC	1.6	2.3	3.3	4.4
	Conventional	2.7	3.3	4.2	5.3
Plates (N)	MicroLC	N/A	5575	5735	6034
	Conventional	N/A	2380	3424	4497

Table 1. Quantitative comparison of four-component test mix

The results for all three parameters are quite similar. This implies that the isocratic conditions effectively transfer between the two systems with a simple change in flow rate. To verify that there is not a substantial difference in resolution, we compared the resolution between adjacent peaks. The results given in Table 2 indicate that there is no reason for concern in this area. The bottom line appears to be that the method developed on the microLC system was successfully scaled-up to the conventional system by increasing the flow rate by the ratio of the column cross-sectional areas.

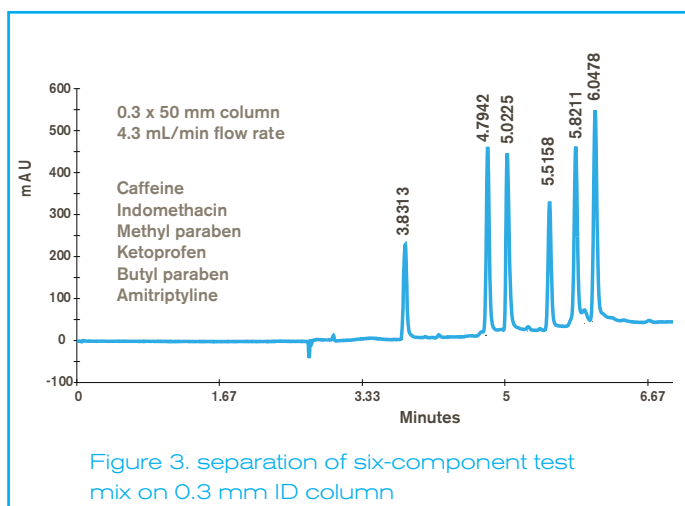
Resolution	Peak 1 - 2	Peak 2 - 3	Peak 3 - 4
MicroLC	9.2	6.4	6.3
Conventional	6.9	5.7	5.8

Table 2. Resolution between adjacent peaks for isocratic separation.

Gradient separation

For the evaluation of the gradient separation method, a test sample composed of six compounds (caffeine, indomethacin, methyl paraben, ketoprofen, butyl paraben and amitriptyline) was created with a final concentration of approximately 0.1 mg/mL each. The separation conditions used on both systems were as follows:

Column stationary phase	ProntoSil C18CL, 3 μ , 120Å
Column length	150 mm
Column temperature	Ambient
Mobile phase A	0.1% TFA
Mobile phase B	acetonitrile w/ 0.1% TFA
Gradient	2 – 95% B, linear, 4 minutes
Detection wavelength	236 nm



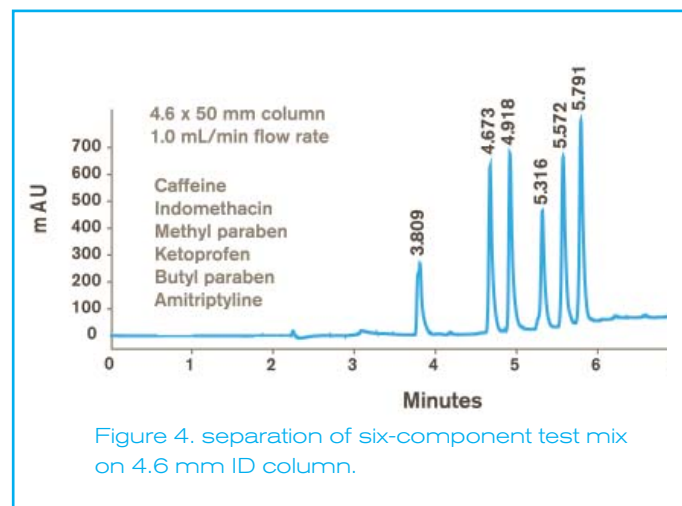
To adapt the microLC method developed on the ExpressLC-800 HPLC system, only the flow rate and injection volume were scaled-up. The gradient profile remained the same for both systems. The appropriate scale-specific separation conditions were:

	Conventional LC	MicroLC
Column ID	4.6 mm	0.3 mm
Flow rate	1.0 mL/min	4.3 μ L/min
Injection volume	10 μ L	40 nL

The results of the gradient separation of the six-component test mixture on the microLC are shown in Figure 3 and those for the conventional HPLC are shown in Figure 4.

From examining the two chromatograms, it can be seen that the scale-up of the method by increasing the flow rate from 4.3 μ L/min to 1.0 mL/min is also effective for gradient separations. Once again, the separations appear to be qualitatively similar. To quantitatively compare the two, we compared peak retention time (RT) and peak width at half-height (PW 0.5).

In Table 3, both parameters are very close. This implies that the gradient conditions accurately transfer between the two systems. To verify that there is not a substantial difference in resolution, we compared the resolution between adjacent peaks. The results given in Table 4 indicate that there is also no reason for concern in this area. As with the isocratic separation method, it appears that the gradient method developed on the microLC system, once scaled-up, is also effective with conventional system.



		Caffeine	Indomethacin	Methyl paraben	Ketoprofen	Butyl paraben	Amitriptyline
RT (min)	MicroLC	3.83	4.79	5.02	5.52	5.82	6.05
	Conventional	3.81	4.67	4.92	5.32	5.57	5.79
PW 0.5 (sec)	MicroLC	2.5	2.3	2.5	2.4	2.4	2.6
	Conventional	3.3	2.6	2.8	2.8	2.8	2.8

Table 3. Six-component test mix

Resolution	Peak 1 - 2	Peak 2 - 3	Peak 3 - 4	Peak 4 - 5	Peak 5 - 6
MicroLC	12.0	2.9	6.0	3.8	2.7
Conventional	10.2	3.6	5.7	3.5	2.9

Table 4. Resolution between adjacent peaks

Conclusion

Studies have clearly demonstrated that isocratic and gradient methods that are developed on Eksigent's microLC system can be easily converted to a method appropriate for a conventional HPLC system. These results should help reduce chromatographers' apprehensions about microLC, allowing them to more widely adopt microLC and realize its benefits.

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