Green HPLC Technologies for Solvent and Waste Reduction

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Abstract

HPLC is a nearly universal analytical technique applied on a routine basis around the world for separation, quantitation and identification of chemical compounds by industry and academia. Approximately 130,000 analytical HPLC instruments are in use today, with another 16,000 instruments sold yearly. The majority of these instruments use a typical column diameter of 4.6 mm internal diameter (I.D.) with most analytical methods using a flow rate of 1 ml/min for this sized column. If the typical HPLC is used 20 hours a week, a single conventional HPLC would require 60 L of solvent (typically acetonitrile, methanol, isopropanol, hexane, and/or aqueous buffers) to operate and generate 60 L of mixed organic/aqueous waste per year. This translates into 8 million liters of solvent produced each year to supply mobile phase for analytical HPLC while producing the equivalent of nearly 40,000 55-gallon drums of waste per year.

In this poster, we will analyze the potential impact of various HPLC technologies for corporate green initiatives. Conventional HPLC, ultra high pressure LC, SFC, and micro-flow HPLC all provide similar information but have different solvent requirements. Besides the analytical performance characteristics of each LC technology we will compare their relative environmental impacts.

HPLC Generates Millions of Liters of Waste per Year

A conventional HPLC is defined as operating with flow rates from 1 to 10 mL/min. As of 2006 there are currently 131,000 conventional HPLCs in operation around the world, which accounts for 71% of all installed liquid chromatography instruments. By scaling down the instrument components, including the chromatography column, less solvent is needed to generate the same analytical information. Mobile phase and column chemistries remain unchanged. When flow rates are scaled down by the ratio of the column volumes, the linear velocity, and hence, the retention time of the analytes remains the same. All existing HPLC methods can be easily scaled down to MicroLC instruments, yielding the same information plus a 200-fold savings in mobile phase waste generation.

These mobile phases consist of acetonitrile, methanol, hexane, isopropanol, and/or water all mixed together with low concentrations of analyte, acid or base. Many of these components are toxic to humans and harmful to the environment. Disposal requires special handling and solvent waste is usually incinerated. Incineration is energy intensive, especially for mixed aqueous solvents, which make up the majority of LC solvent waste, and this incineration contributes to greenhouse gas emissions.

ExpressLC-100: 4.3 ul/min x **640 days** = 4L waste

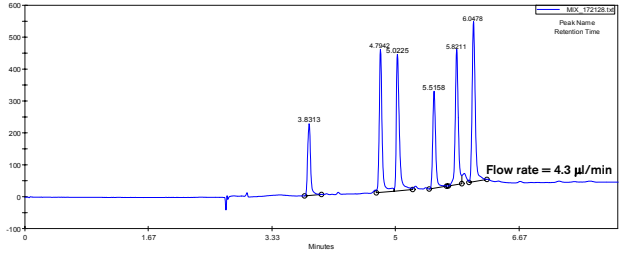
Conventional HPLC: 1 ml/min x **2.8 days** = 4L waste

Conventional HPLC Methods Scale Down to MicroLC without Re-optimization

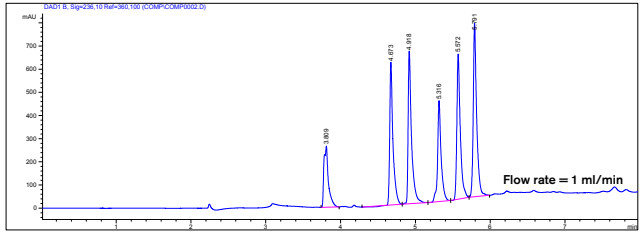
Eksigent has developed a new pump technology called Microfluidic Flow Control (MFC). MFC works by using flow rate measurements of each mobile phase to control a rapid, electronically adjusted pressure source. Micro-scale mixing occurs in a volume of 300 nL, resulting in low delay volumes. This rapid mixing virtually eliminates the mixing delays required in conventional systems at the start of the method and at re-equilibrations. To scale down a conventional 10 uL injection, requires only 40 nL. This system utilizes an electronically controlled injection valve coupled with our MFC flow delivery system. After filling the sample loop with a conventional autosampler, the valve is then toggled from load to inject and back to the load position, based on integrating the flow rate over time to reach the desired injection volume. Eksigent utilized its expertise in microfluidic chip development to develop a highly sensitive, non-dispersive UV absorbance flow cell. The detection system offers dynamic range, linearity, and sensitivity comparable to conventional detection systems.

	MicroLC	Conventional
Stationary phase	ProntoSil C18CL, 3µ, 120Å	
Column length	150 mm	
Column I.D.	**0.3 mm**	**4.6 mm**
Injection Volume	**0.04 ul**	**10 ul**
Flow Rate	**0.0043 ml/min**	**1 ml/min**
Mobile phase A	H_20 w/ 0.1% TFA	
Mobile phase B	ACN w/ 0.1% TFA	
Gradient	0-->4 minutes 2 --> 95% B	
UV detection	236 nm	

MicroLC column: 0.3 mm I.D x 150 mm



Conventional column: 4.6 mm I.D x 150 mm



Resolution and Selectivity Comparable when using MicroLC with a Solvent Savings of >99%.

Peaks: 1- Caffeine
2- Indomethacin
3- Methyl paraben
4- Ketoprofen
5- Butyl Paraben
6- Amitriptyline

	MicroLC	Conventional
Solvent Used/Waste Generated (15 min. run + 5 min. equilibration)	**0.086 mL**	**20 mL**

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

Other LC Techniques

Supercritical fluid chromatography (SFC) uses approximately 10% of the organic solvent of a conventional LC system providing a savings in organic/mixed waste generation. However, the remaining 90% is comprised of supercritical CO_2, hence the mobile phase chemistry is very different than conventional analytical LC and existing HPLC methods would need to be re-optimized for SFC, requiring time and effort without a guarantee of success. SFC provides a substantial green benefit when compared to conventional preparative chromatography.

Ultra-high pressure LC promises faster separations by using smaller particle sizes and high flow rates. Although a decrease in run time is realized, approximately the same solvent is used, just more of it in a shorter period of time. Manufacturers of high-pressure instruments also provide method optimization tools in order to use less solvent. In this scenario, your method and column dimensions are actually re-optimized, providing solvent savings of 2 to 5 fold.

	Conventional	High-Pressure LC	Method Reoptimization	SFC	Micro LC
Mobile Phase Chemistry	aqueous/organic	aqueous/organic	aqueous/organic	carbon dioxide/organic modifiers	aqueous/organic
Method Optimization (vs. conventional)	no change	increase flow rate/reduce retention time	use algorithms to estimate new parameters	all new method parameters	reduce flow rate/ same retention time
Column Dimensions (vs. conventional)	no change	smaller particles (1.9 u)	shorter column, smaller I.D. (2.1 to 1 mm), smaller particles (1.9 u)	same as conventional	smaller I.D. (0.3 mm)
Solvent Savings (vs. conventional)	0	0	2-5 fold	10 fold	**200 fold**

Conclusion

MicroLC, that is, liquid chromatography performed with small I.D. chromatography columns, typically 0.3 mm, and low flow rates (4 ul/min), provides a significant savings in solvent usage and mixed waste generation over conventional HPLC. Wide-scale use of micro-flow HPLC would reduce toxic waste generated by chemical researchers in academia and industry by millions of liters per year. Any HPLC method that has been validated and developed over the past decades can be directly transferred to MicroLC by scaling only column I.D., flow rate, and sample volume, yielding the same separation, sensitivity and retention times, but with a significant savings in solvent usage and mixed waste generation. MicroLC has the most potential of existing techniques to significantly reduce analytical HPLC waste.

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