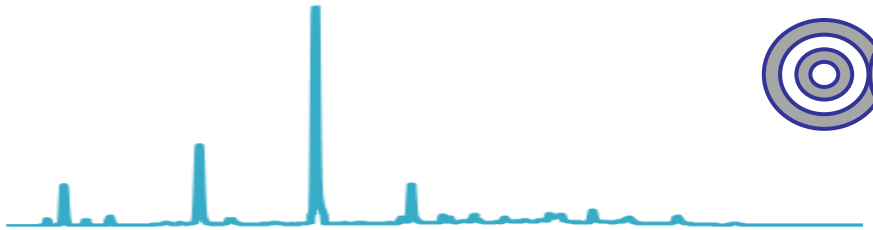
بسم الله الرحمن الرحيم

Chem 651

Advanced Studies in Instrumental Analysis

Miniaturization of Liquid Chromatography

Towards sensitive, green, and economic chromatographic techniques



Ahmad Aqel Ifseisi
Professor of Analytical Chemistry
College of Science, Department of Chemistry
King Saud University
P.O. Box 2455 Riyadh 11451 Saudi Arabia
Building: 05, Office: 2A149, Lab: 2A127
Tel. 014674198, Fax: 014675992
Web site: https://faculty.ksu.edu.sa/aifseisi
E-mail: aifseisi@ksu.edu.sa
ahmad3qel@gmail.com

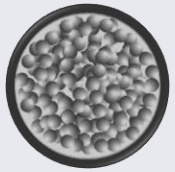
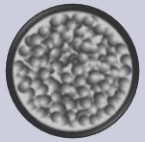
كرسي أبحاث المواد المتقدمة
Advanced Materials Research Chair
AMRC

جامعة الملك سعود
King Saud University

Liquid Chromatography Scales

The column is considered the heart of the **HPLC** & **GC** techniques. The separation of the sample components is achieved when those components pass through the column.

The move to smaller i.d. columns

	HPLC columns were originally 4.6 mm i.d. operated at 1.0–2.0 mL/min
	3.0 mm i.d. columns introduced as a means to save solvents 50% solvent savings going from a 4.6x100 mm at 1.5 mL/min to a 3.0x100 mm at 0.75 mL/min
	Short columns with 2.1 mm i.d. and sub-2 µm stationary phases particle size introduced for use with UHPLC
	In 1988, K. Karlsson & M. Novotny published a paper “separation efficiency of slurry packed liquid chromatography microcolumns with very small inner diameters”, they reported high efficiencies with micro-LC columns that had the i.d. of 44 µm

Since that time great efforts have been made to miniaturize **LC** instrumentation by carrying out theoretical, technological, and methodological studies.

-Mejía-Carmona K., TrAC 122 (2020) 115735. -Rieux L., et al, LCGC NA 29 (2011) 926. -Swart R., LC packings / Dionex, Chromedia 2008.

Definitions for the miniaturized chromatographic system

LC technique	Column i.d.	Typical flow rate	Injection volume	Relative sensitivity
Preparative	> 25 mm	> 30 mL/min	--	--
Semi-preparative	5–10 mm	5–20 mL/min	--	--
Conventional	3.2–4.6 mm	0.5–2.0 mL/min	100 µL	1
Narrowbore	1.5–3.2 mm	100–500 µL/min	19 µL	5
Microbore	0.5–1.5 mm	10–100 µL/min	4.7 µL	21
Micro LC capillary	150–500 µm	1–10 µL/min	490 nL	235
Nano LC capillary	10–150 µm	10–1000 nL/min	12 nL	3800

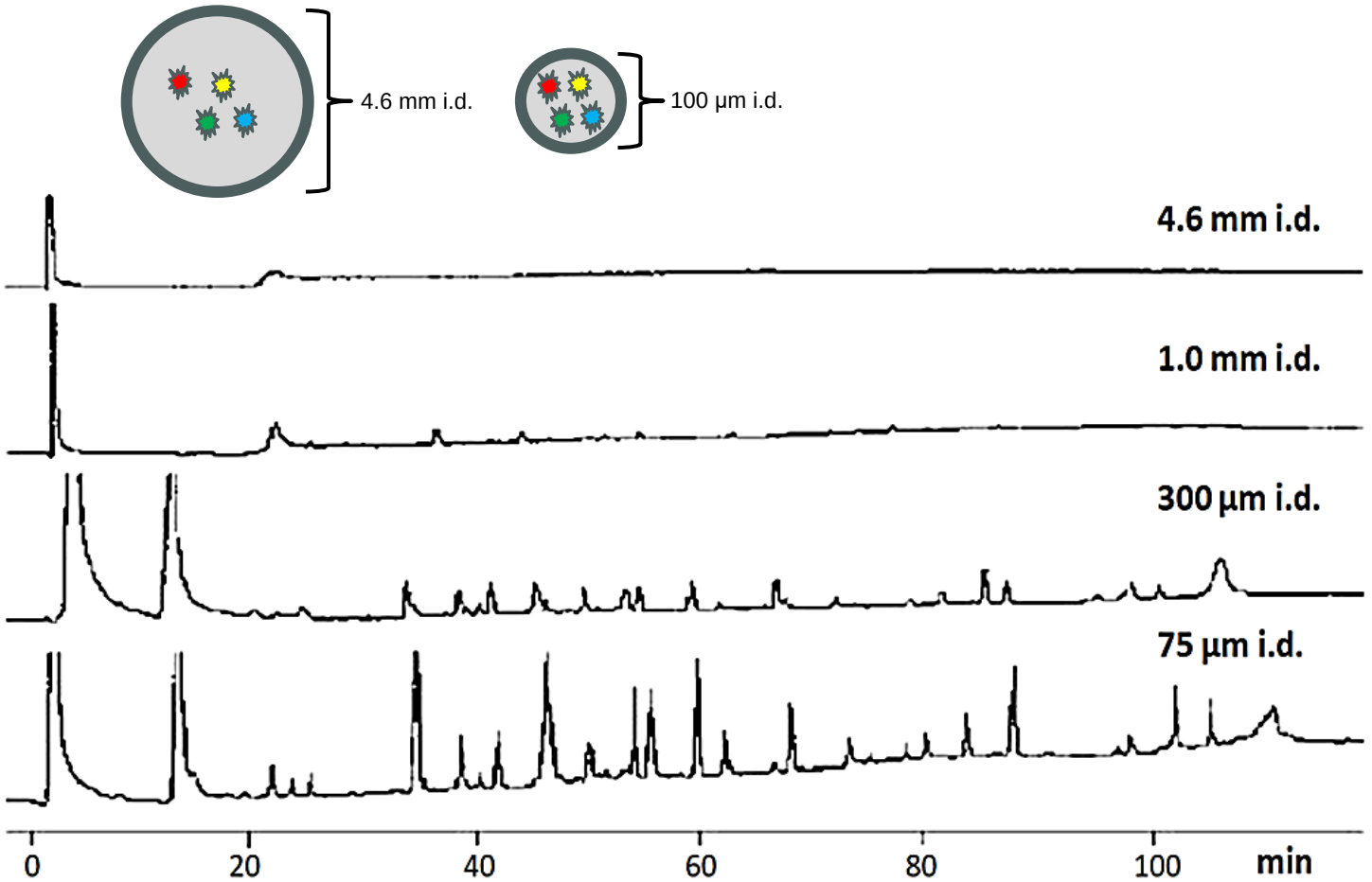
LC technique	Column i.d.	Typical flow rate	Injection volume
Preparative	> 5 mm	> 5 mL/min	---
Conventional	2.1–4.6 mm	0.5–2.5 mL/min	5–25 µL
Micro LC	0.5–2.1 mm	10–500 µL/min	0.5–5 µL
Capillary LC	100–500 µm	0.5–10 µL/min	10–500 nL
Nano LC	10–100 µm	10–500 nL/min	< 10 nL

-Mejía-Carmona K., TrAC 122 (2020) 115735. -Rieux L., et al, LCGC NA 29 (2011) 926. -Swart R., LC packings / Dionex, Chromedia 2008.

Motivation Behind Downscales LC

Advantages of using down scales LC	
Improving detection sensitivity	Easier coupling to MS interfaces
Lower solvent & sample consumption	Reduce analytical costs and wastes
Less subject to the effects of viscous heating	

Improving detection sensitivity



A sensitivity comparison of different column i.d. for digested myoglobine.

↓ Column i.d. → ↓ Sample dilution & diffusion → ↑ Sensitivity

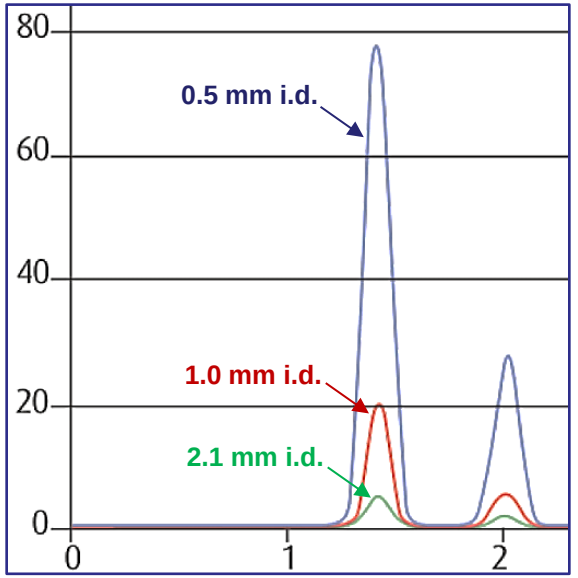
↑ Sensitivity → ↓ Detection limits

Down scale factor:

$$f = \frac{(i.d._{thicker})^2}{(i.d._{thinner})^2}$$

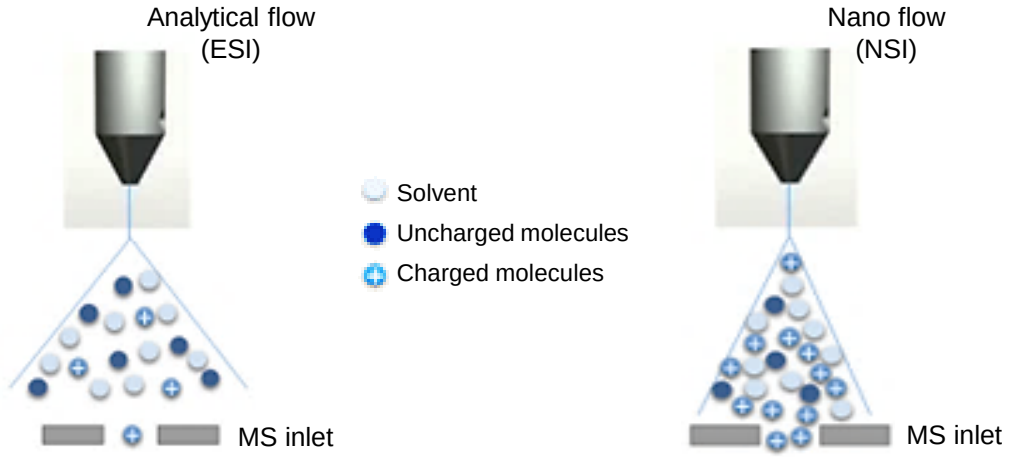
Example: (4.6 mm *vs.* 100 µm)

f **= 4600^2 / 100^2 = 2100**



Easier coupling to mass spectrometer interfaces

Better flow rate compatibility with MS detectors.



analytical *vs.* low flow ionization

The smaller volumetric flow rate results in smaller droplet sizes, that are easily evaporated, which increase the ionization efficiency and improve detection sensitivity.

Less subject to the effects of viscous heating

Column i.d.	Flow rate	Power
4.6 mm	2.0 mL/min	24 W
2.0 mm	0.38 mL/min	4.5 W
1.0 mm	95 µL/min	1.1 W
500 µm	24 µL/min	280 mW
250 µm	5.9 µL/min	71 mW
100 µm	940 nL/min	11 mW
50 µm	240 nL/min	2.9 mW

Considerations for reducing viscous friction.

$$Power = F\,\Delta P$$

1 W	= 14.33 calories/min
	= 7.17 calories/mL

Power represents the enough power to boil mobile phase before it exits the column.

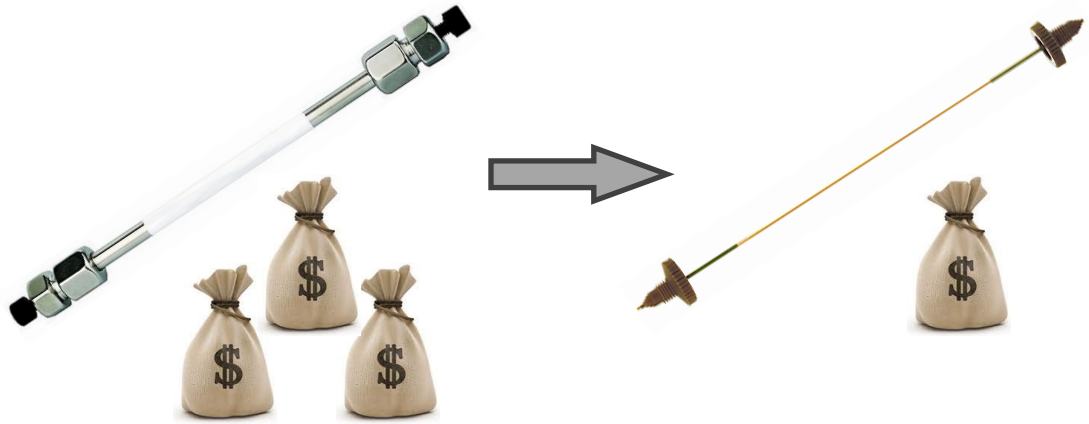
Lower solvent and sample consumption

Lower mobile phase solvents, samples, and stationary phase materials consumption.

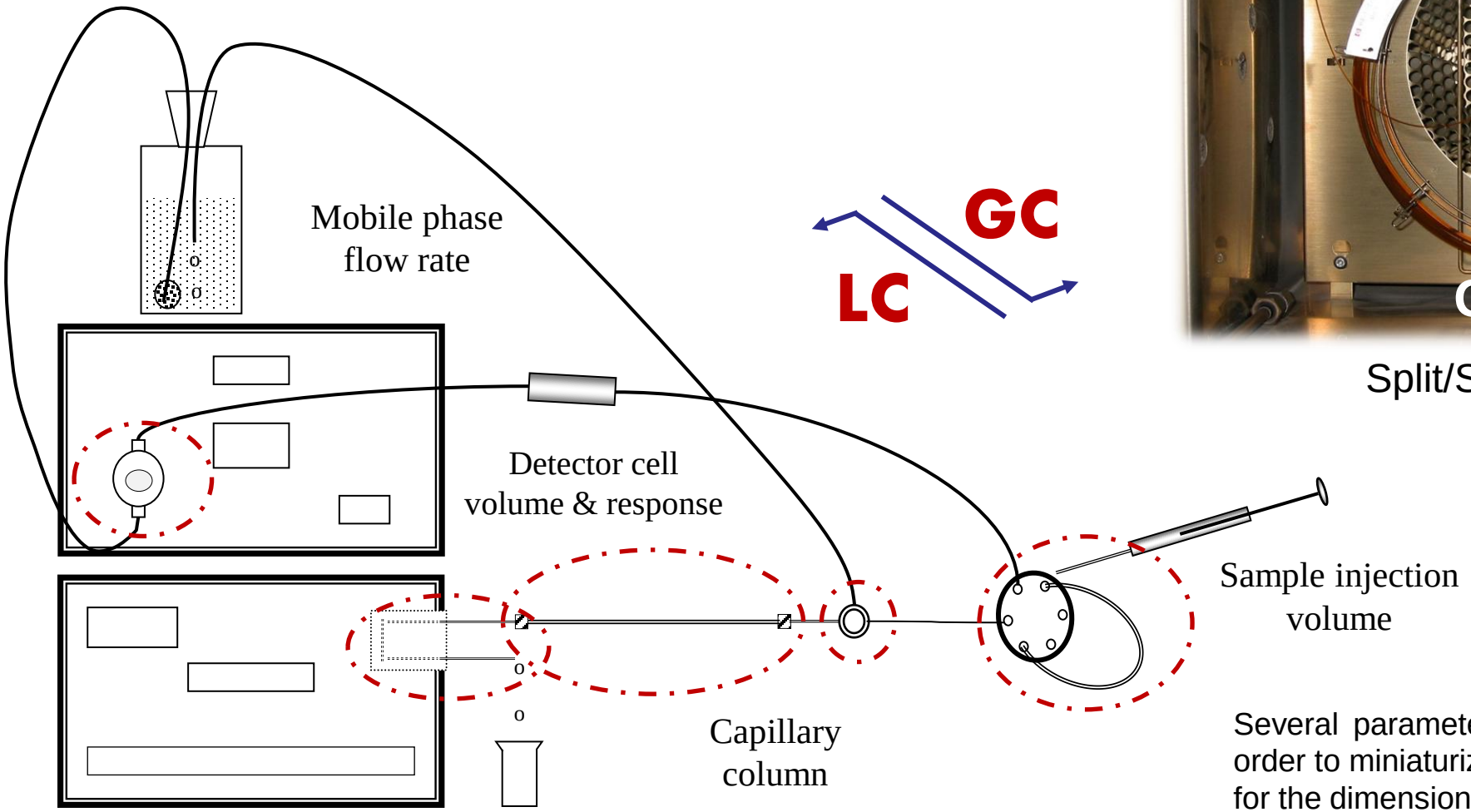
4.6 mm column i.d.	15 cm column length	100 µm column i.d.
1.0 mL/min 60 mL/h	**Solvents (mobile phase)** Flow rate	1.0 µL/min 60 µL/h (~1000 times lower)
10 µL	**Samples (injection volume)**	4.0 nL (~2500 times lower)
2.5 mL	**Materials (stationary phase)** Empty column volume	1.18 µL (~2100 times lower)

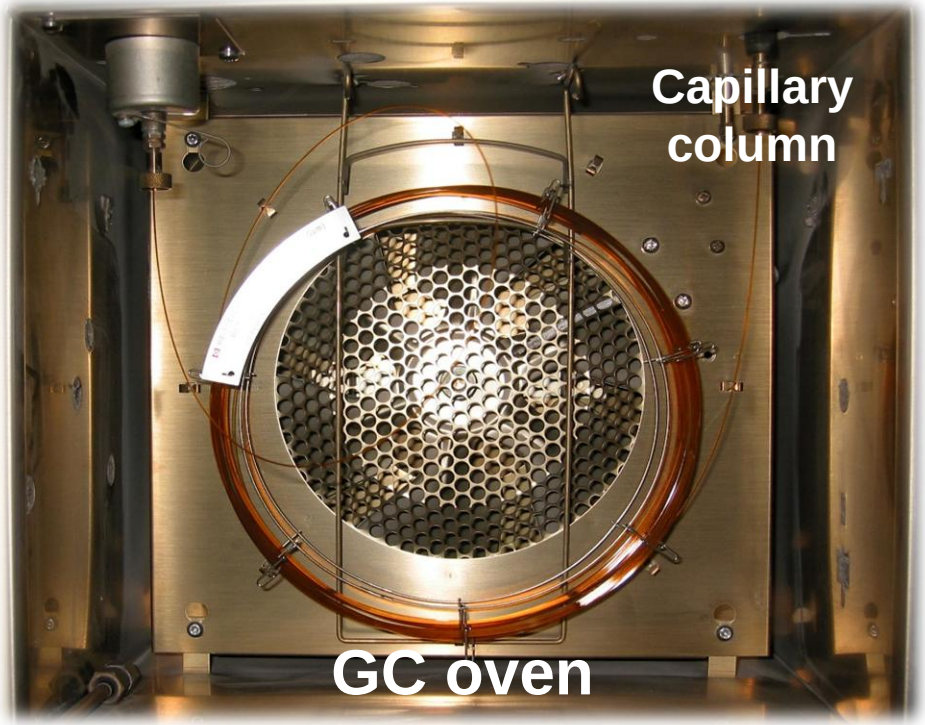
Good for the expensive samples and deuterated solvent in the coupling of **LC** to **NMR**.

Reduce analytical costs and wastes



Miniaturized LC System Challenges



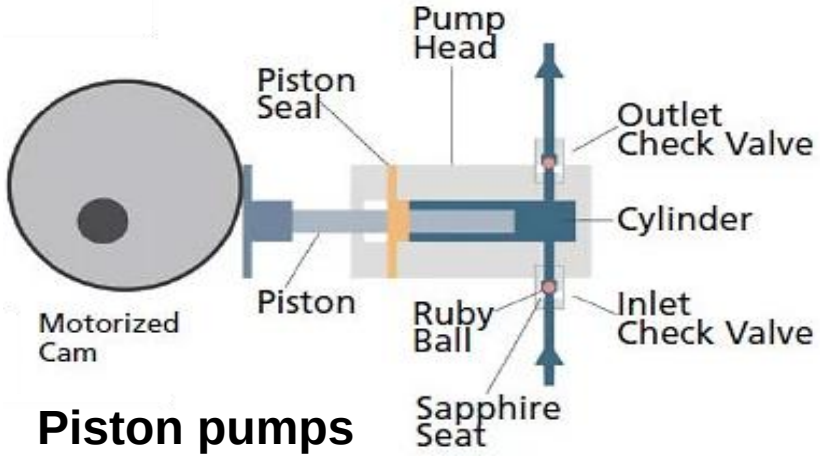


Split/Splitless injection

Several parameters are needed to be adjusted in order to miniaturize **HPLC** systems & to be suitable for the dimensions of capillary columns

Mobile phase flow rates

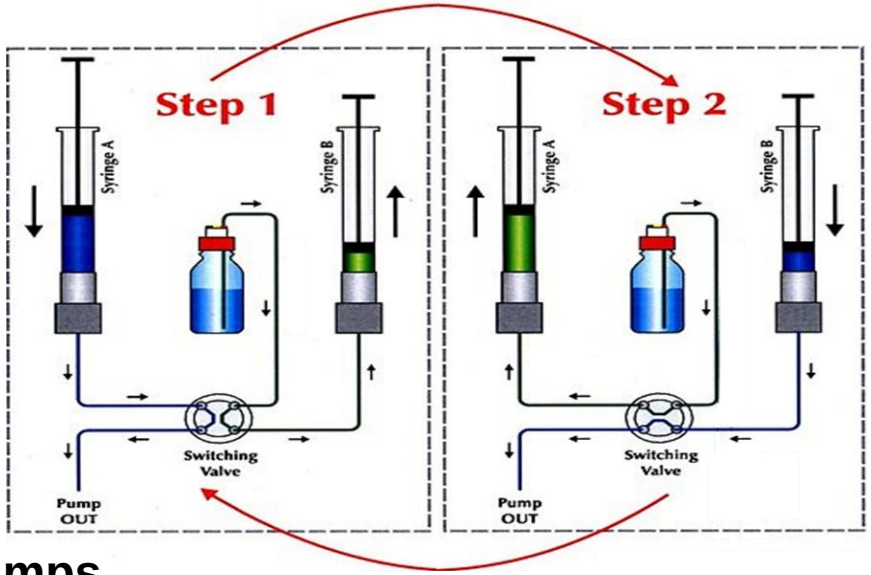
Low mobile phase flow rates (µL/min, nL/min) scale.



Analytical to prep scale pumps
> 0.1 mL/min

Piston pumps

Pulsation-free for flow rates < 100 µL/min

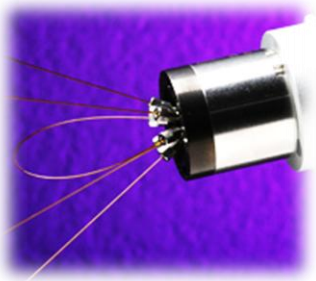


Syringe pumps

Sample injection volume

Injection volume accuracy and reproducibility.

Analytical injector
10–100 µL
sample loop



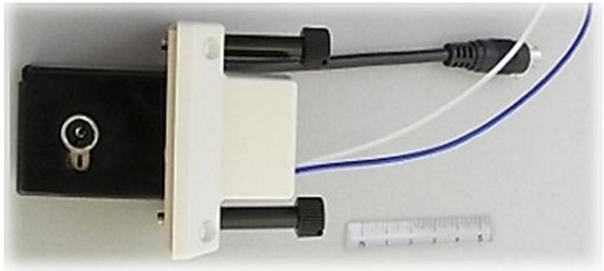
Nanovolume injector
& switching valve

4–20
nanoliters

Internal sample
injectors

External injectors (VICI external injector – manual or automatic injector).

Detector cell volume



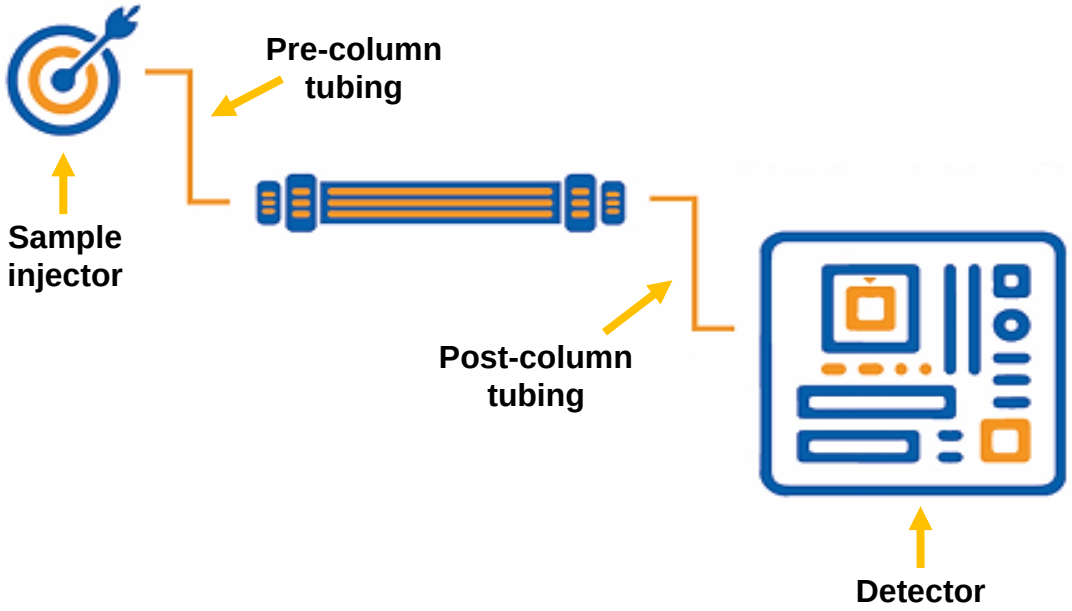
Standard analytical flow cell
5–10 mm path length,
2.5–18 µL cell volume

Nano flow cell
3–20 mm path length,
< 20 nL cell volume

Detector cell volume in the **UV/Vis** or spectroscopic photo detectors.

Extra column volume

Pre-column and post-column tubings and connections are very influential in these scales.

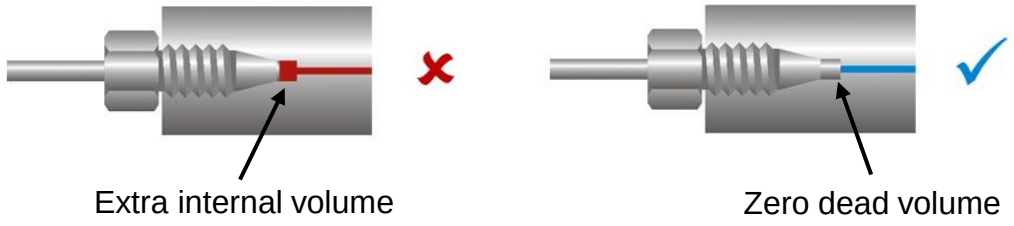


Extra-column volume must be minimized.

Fittings & connections

Fittings and connections at the downscale **LC** are not straightforward. The impact of column connection on band broadening.

How to achieve a perfect connection?



Conventional analytical scale (outer diameter) almost standard in all companies (1/16 inch).

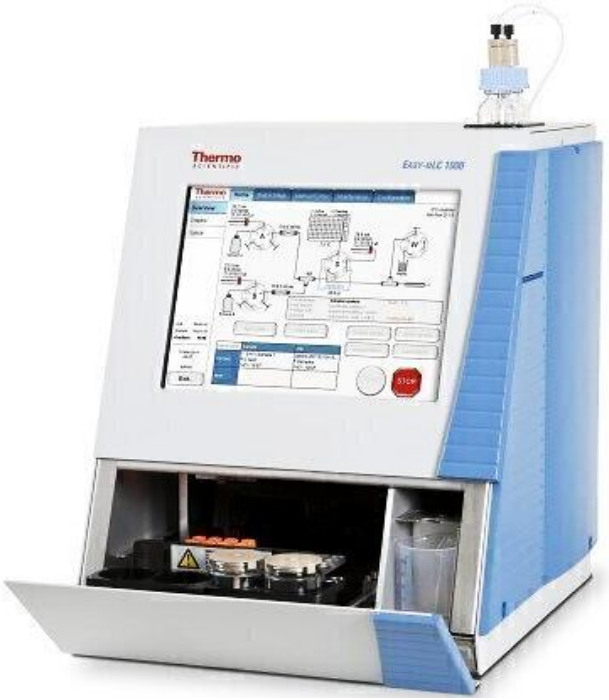
Solvents leakage

Leaks can occur anywhere within the **HPLC**

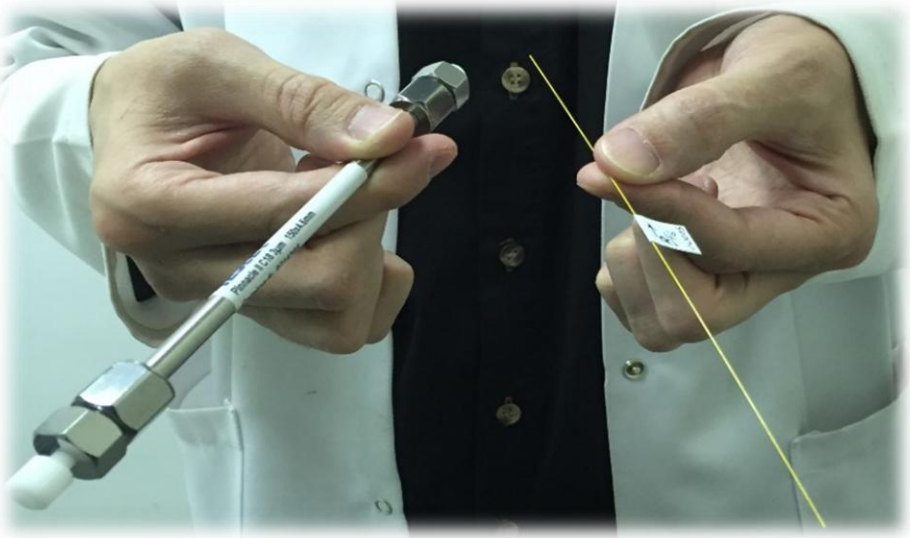
Leaks at the fittings	Leaks at the pump
Leaks at the injector	Leaks at the column
Leaks in the detector	

Difficult to detect mobile phases leakage.

Miniaturized LC system



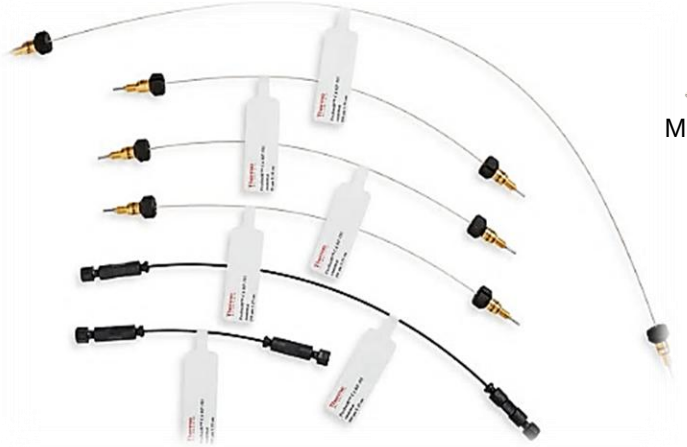
Commercial **nanoLC** systems



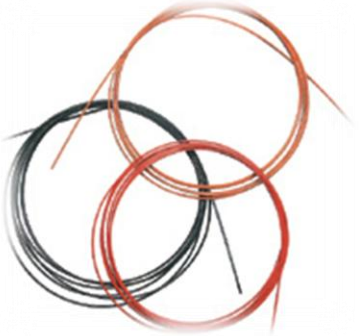
Conventional **LC** modification

-Mejía-Carmona K., et al., Trends in Analytical Chemistry 122 (2020) 115735.
-Rieux L., et al., LCGC North America 29 (2011) 926.
-Swart R., LC packings / Dionex, Netherlands, Chromedia 2008.

Capillary HPLC Columns



Micro-loop



Micro-tubing

Nano-UV cell

Micro-adapter

Fittings

Tubing sleeves

Micro-tee

Micro-union

Micro-cross

Miniaturized liquid chromatographs commercially available

Manufacturer	Brand name	Flow (µL/min)	Pressure (bar)
Waters	Acquity UPLC M-Class nanoAcquity UPLC	0.2–100 0.2–100	1034 690
Agilent	1260 Infinity nanoflow LC 1200 Series capillary	0.1–1.0 1–100	400 400
Shimadzu	Prominence LC-20AD nano	0.001–5.0	400
Thermo	EASY-nano LC 1200 Dionex™ UltiMate 3000 series	0.002–2.0 0.001–50	1200 800
AB Sciex	Ekspert™ nanoLC 400	0.1–50	690

Instrument
Columns
Accessories
Maintenance

Miniaturized LC Applications

Natural
products & plants

Industrial

Clinical &
toxicology

Food

Petrochemical

Environmental

Pharmaceutical

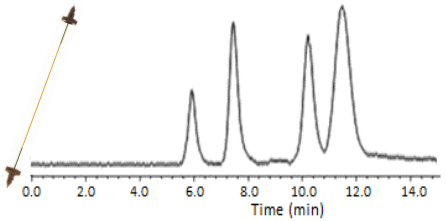
Forensic &
narcotics

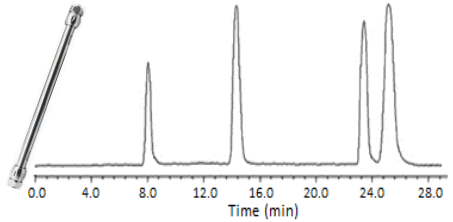
C_{18} conventional column

Vs.

Capillary monolithic column

Determination of **BTEX** in water samples



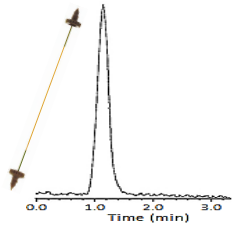


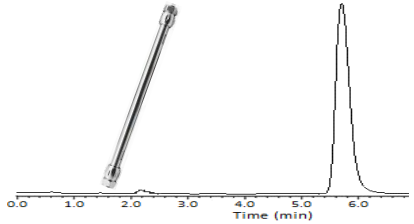
BTEX sources

	C_{18} commercial column	Monolith capillary column
Column dimension	100 x 4.0 mm, 3 µm	200 x 0.10 mm
Flow rate	500 µL/min	0.40 µL/min
Injection volume	10 µL	4.0 nL
Detector cell volume	10 µL	3.0 nL
Analysis time	27 min	12 min
N/m (tol)	48,900	33,400
R_s (o-xyl – m/p-xyl)	1.78	1.33
LOD (tol)	0.05 µg/mL	0.01 µg/mL
LOL (tol)	200 µg/mL	150 µg/mL
Sampling rate	≈ 2 sample/h	≈ 5 sample/h
Solvents consumption	30.0 mL/h	0.024 mL/h

-Aqel et al, Curr Anal Chem 16 (2020) 223

Determination of **caffeine** in food samples





	C_{18} commercial column	Monolith capillary column
Column dimension	150 x 4.6 mm, 3 µm	150 x 0.53 mm
Flow rate	500 µL/min	41 µL/min
Retention time	5.78 min	1.16 min
Detector cell volume	10 µL	1.0 µL
Injection volume	10 µL	1.0 µL
Plates number	28,400	12,700
LOD	0.20 µg/mL	0.05 µg/mL
LOQ	0.70 µg/mL	0.16 µg/mL
LOL	150 µg/mL	250 µg/mL
Sampling rate	≈ 10 h^{-1}	≈ 40 h^{-1}
Solvents consumption	30.0 mL/h	2.46 mL/h

-ALOthman et al, Food Chem 132 (2012) 2217


Thank You!