HPLC WEBINAR WORKSHOP

Dr. Peirovi. A, Pharm. D.
Head of Core Facility
Pasteur Institute of Iran
Tehran, Iran
1316943551
afshin700@yahoo.com

What Is HPLC (High Performance Liquid Chromatography)? Brief History and Definition

Liquid chromatography was defined in the early 1900s by the work of the Russian botanist, Mikhail S. Tswett. His pioneering studies focused on separating compounds [leaf pigments], extracted from plants using a solvent, in a column packed with particles.

What Is High Performance Liquid Chromatography (HPLC)? The acronym *HPLC*, coined by the late Prof. Csaba Horváth for his 1970 Pittcon paper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi [35 bar]. This was called *high pressure* liquid chromatography, or HPLC.

…What Is HPLC

- The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi [400 bar] of pressure, and incorporated improved injectors, detectors, and columns. HPLC really began to take hold in the mid-to late-1970s. With continued advances in performance during this time [smaller particles, even higher pressure], the acronym HPLC remained the same, but the name was changed to *high performance* liquid chromatography.
- Martin, in collaboration with Anthony T. James, went on to develop gas chromatography (the principles of which Martin and Synge had laid out in their landmark 1941 paper) beginning in 1949. In 1952, during his lecture for the Nobel Prize in Chemistry (shared with Synge, for their earlier chromatography work) Martin announced the successful separation of a wide variety of natural compounds by gas chromatography

HPLC 2 common method

- Normal Phase.

Polar stationary phase and non-polar solvent.

- Reverse Phase.

Non-polar stationary phase and a polar solvent.

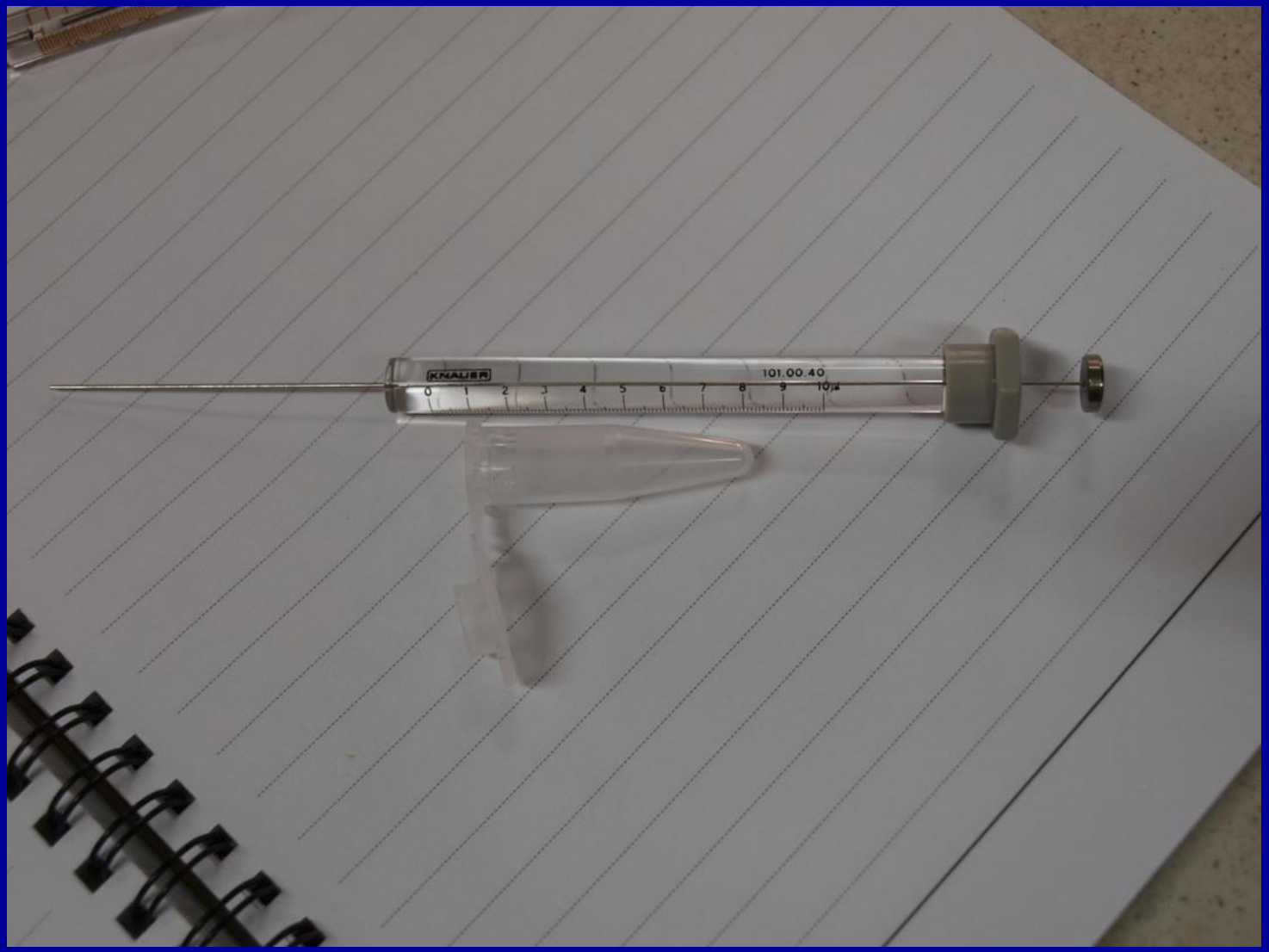
HPLC instrument

Pump, Column oven, Detector

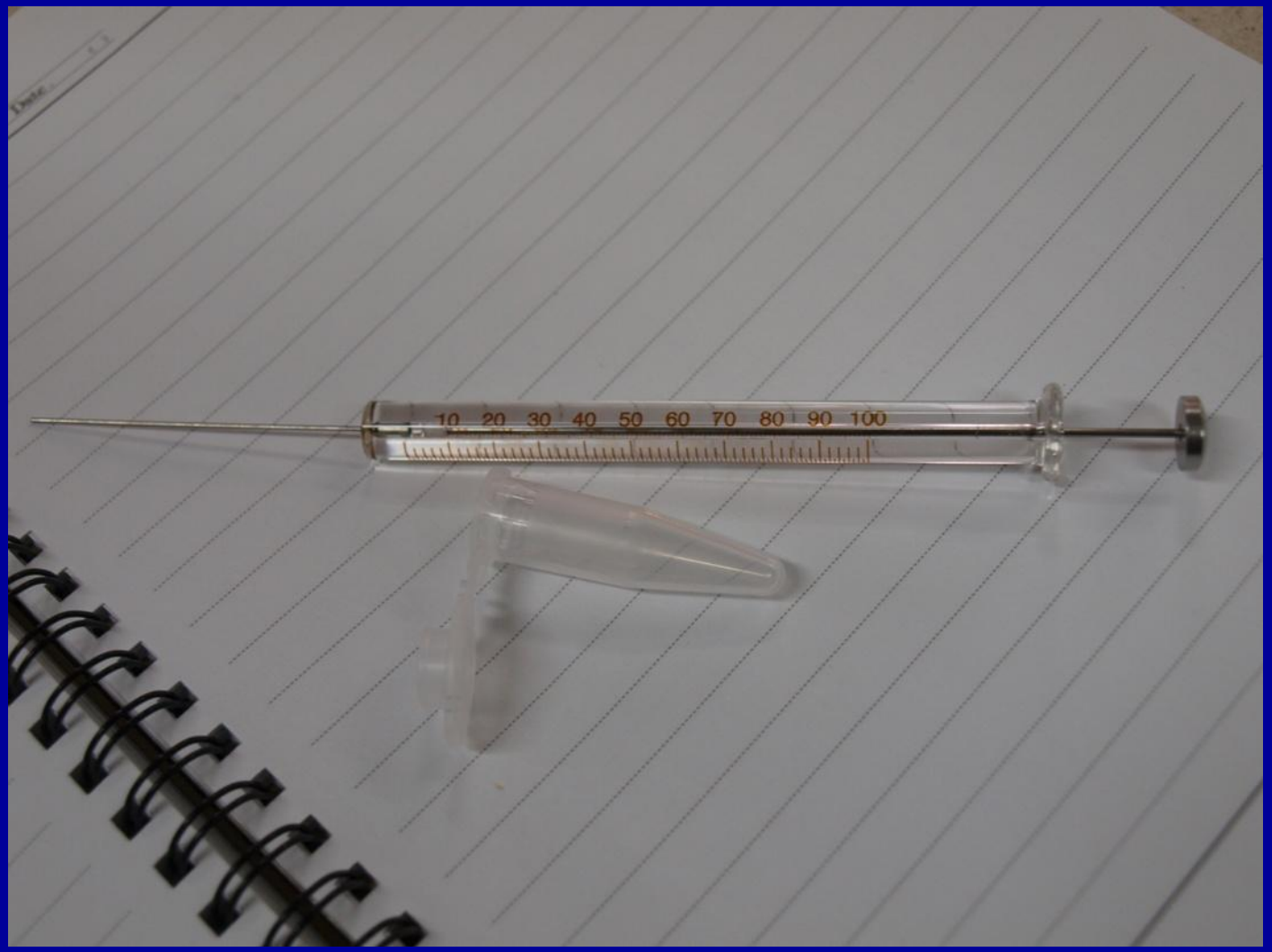
Reservors

Injector

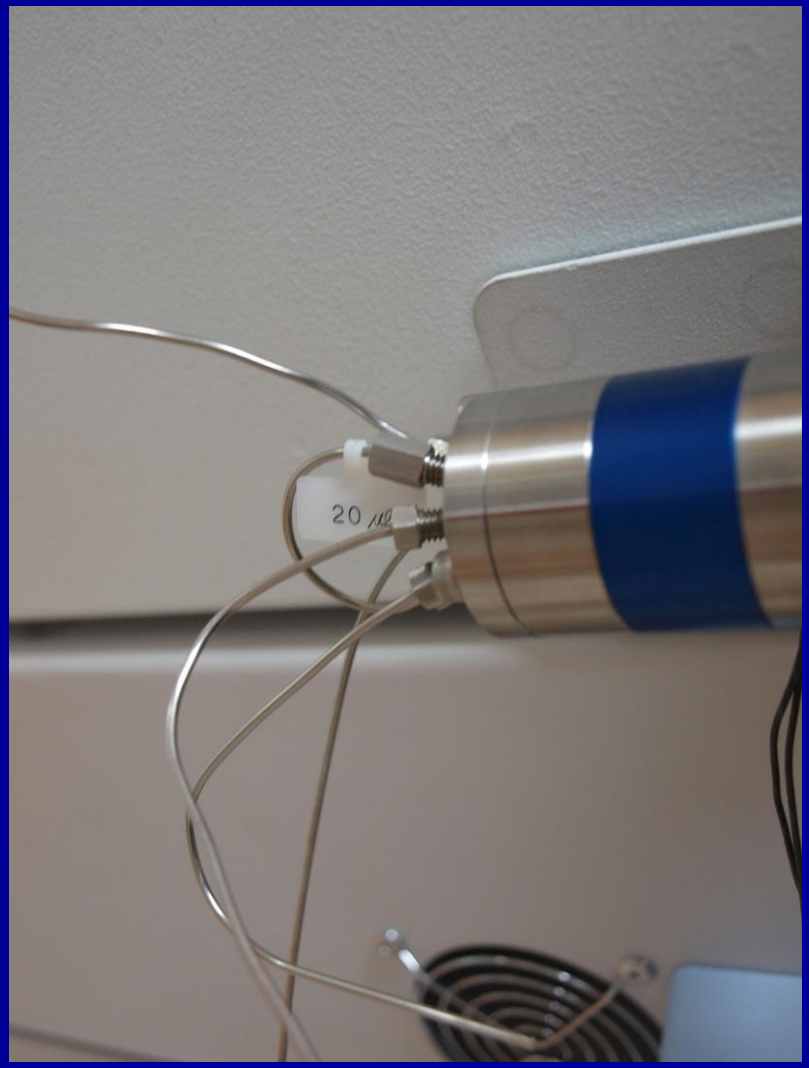
Micro syringe 10



Micro syringe 100



Loop



Column, Column oven

Waste

Bonded group in reversed phase

- C-2 Ethyl Silyl $-Si-CH_2-CH_3$
- C-8 Octyl Silyl $-Si-(CH_2)_7-CH_3$
- **C-18 Octadecyl Silyl** $-Si-(CH_2)_{17}-CH_3$
- CN Cyanopropyl Silyl $-Si-(CH_2)_3-CN$

Common Reverse Phase Solvents

Methanol

- Acetonitrile
- Tetrahydrofuran
- Water

HPLC solvents mobile phase and samples

- In HPLC method we have to use very pure mobile phase, there are several commercial brand of HPLC solvents(for preparing mobile phase) furthermore both sample and mobile phase should be filtered through 0.22 micron filter and is better that we degas both before starting our process , if we have some particle more than 0.22 micron or a turbid mobile phase or sample , it is not possible to apply them on instrument, air bubble could be detect and we can't have a good result with air bubble, we should remove air bubble before starting HPLC

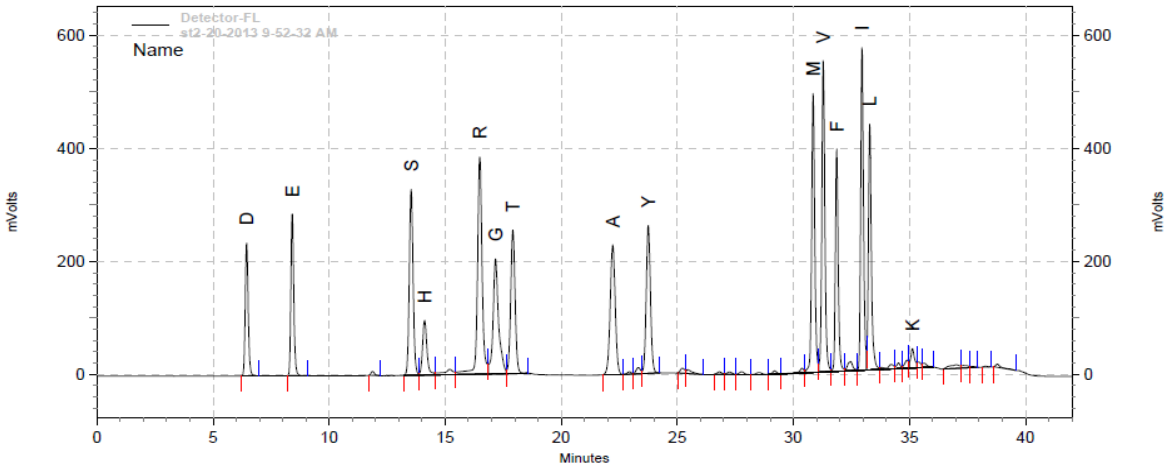
UPLC

- What Is Ultra Performance Liquid Chromatography (UPLC Technology)?

  In 2004, further advances in instrumentation and column technology were made to achieve very significant increases in resolution, speed, and sensitivity in liquid chromatography. Columns with smaller particles [1.7 micron] and instrumentation with specialized capabilities designed to deliver mobile phase at 15,000 psi [1,000 bar] were needed to achieve a new level of performance.
- Basic research is being conducted today by scientists working with columns containing even smaller 1-micron-diameter particles and instrumentation capable of performing at 100,000 psi [6,800 bar]. This provides a glimpse of what we may expect in the future

STD graph of amino acids

Method: C:\ChromGate\Projects\AAA\Method\OPA\opa tsk 1.met
Injection: 10 ul



Detector-FL
Results

Name	Retention Time	Area	Area %
D	6.450	104226724	3.93
E	8.417	123173745	4.65
S	13.533	190409714	7.18
H	14.117	63425898	2.39
R	16.483	266195822	10.04
G	17.167	169339381	6.39
T	17.917	166737880	6.29
A	22.217	172656616	6.51
Y	23.750	166444584	6.28
M	30.850	229511680	8.66
V	31.283	249149723	9.40
F	31.867	167753514	6.33
I	32.950	238404018	8.99
L	33.283	193968909	7.32
K	35.133	19811931	0.75
Totals		2521210139	95.12

Thank You