HPLC WORKSHOP

NISAR AHMED

معهد الكويت للأبحاث العلمية
KUWAIT INSTITUTE FOR SCIENTIFIC RESEARCH

HPLC WORKSHOP

Zainab-Al-Ballam

معهد الكويت للأبحاث العلمية
KUWAIT INSTITUTE FOR SCIENTIFIC RESEARCH

AN INTRODUCTION

TO

HPLC - INSTRUMENTATION

معهد الكويت للأبحاث العلمية
KUWAIT INSTITUTE FOR SCIENTIFIC RESEARCH

Instrumentation

Essential components :

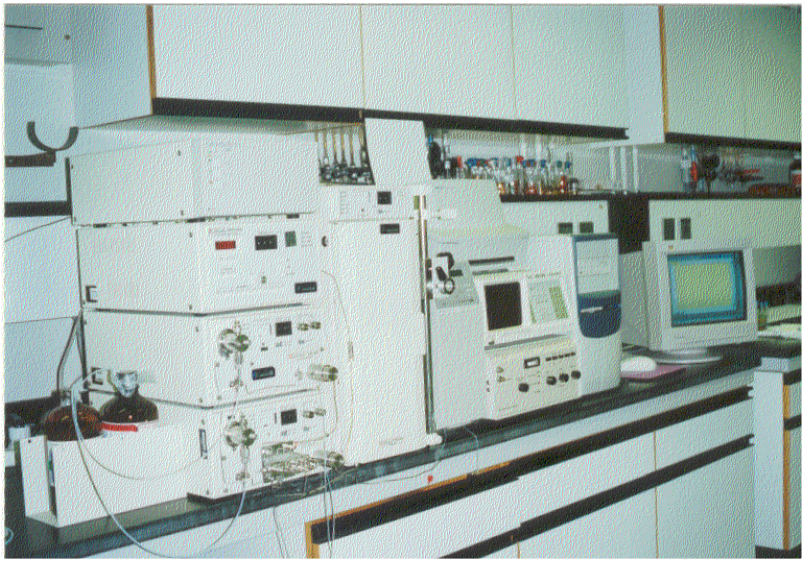
A]- Pump .

b]- injector .

C]- Column .

D]- Detector .

E]- Recorder .



Other more advanced option :

Includes Multipurpose system , Automatic injectors, Sample fraction collector .

Instrumentation

A]- **Pumps :**

Function of the pump in the HPLC is to deliver the mobile phase through the column at high pressure with a controlled flow rate .

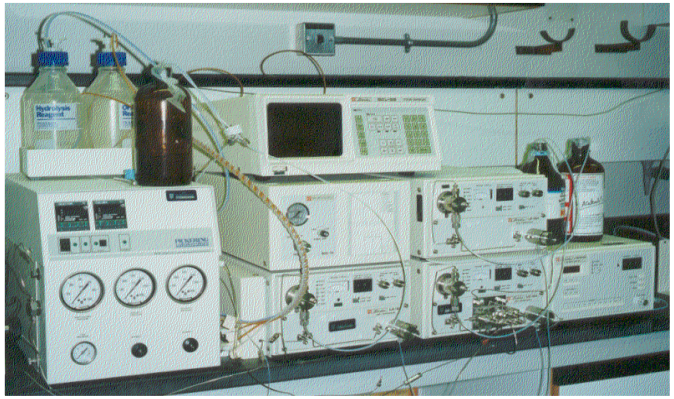
Characteristics:

1) The interior of the pump should be made of inert materials that resist corrosion by any solvent being used.

2) Pressure up to (5000-6000) psi and wide range of flow rate (0.1 > 10 ml / min.) .

3) Flow should be constant , reproducible with at least 1% .

It should be easy to set, measure, & change the flow rate.



Instrumentation

4) It should be easy to change from one mobile phase to another .

5) The internal volume of the pump and all the plumping between the pump and the injector should be as small as possible .

6) The pump should be useful for isocratic and gradient operation .

7) The pump should be adaptable to the use of small volume of mobile phase reservoir , or a heater reservoir .

8) The pump should easy to maintain and repair.

Seals, Rings, and gaskets will require occasional replacement , and will help if these are easy to access.

Problems

1- Pumps set up by a manufacturers' service technician .

2- Obtain all available operation manuals .

3- Stock an adequate supply of parts .

4- Maintain a log notebook .

5- Do not store corrosive solvents or buffers .

6- Lubricate pumps motors .

7- Solvents must be miscible .

Problems

8- Degas solvents to avoid bubbles in pumps head .

9- Confirm the pressure limits (min.=0) , (max=500)

10- Inspect pumps head & fittings for leaks .

11- Dirt, Sticking, or malfunctioning check valves can cause inaccurate flow .

12- Life time of pump seals .

13- Verify Flow rate with graduated cylinder and stop watch .

14- Used pump-priming procedures .

B]- **Injection System**

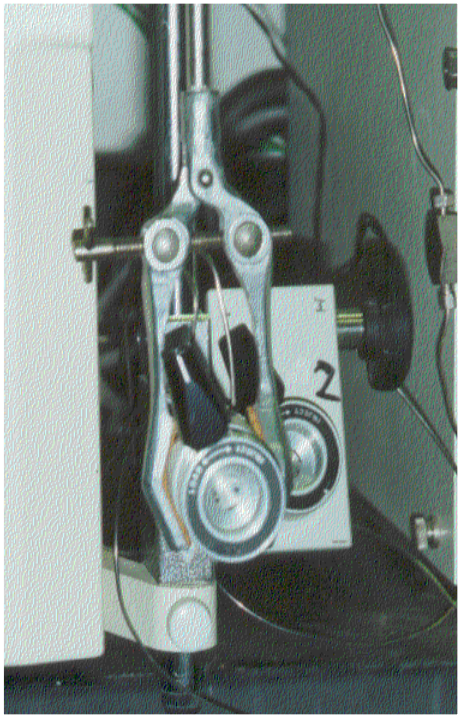
Sample is introduced into the column with an injection valve such as the six port valve .

-The valve has two positions : LOAD & INJECT.

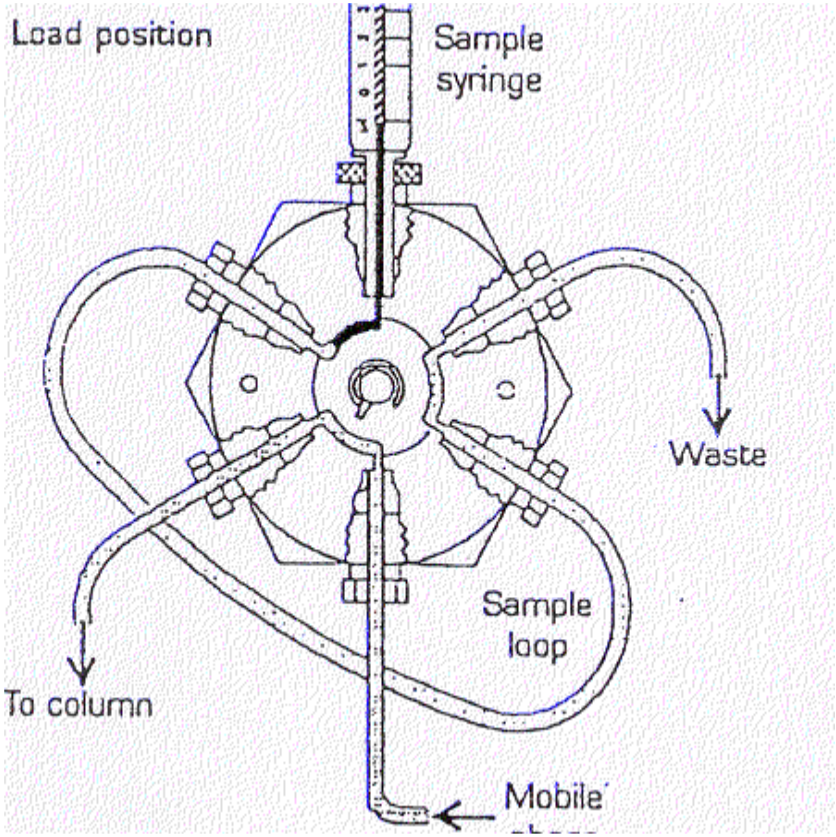
When the valve has rotated to the load position, the sample load can be fitted by a syringe while the eluent flow directly to the column.

When the valve is rotated to the inject position, eluent sweeps the sample in the loop into the column.

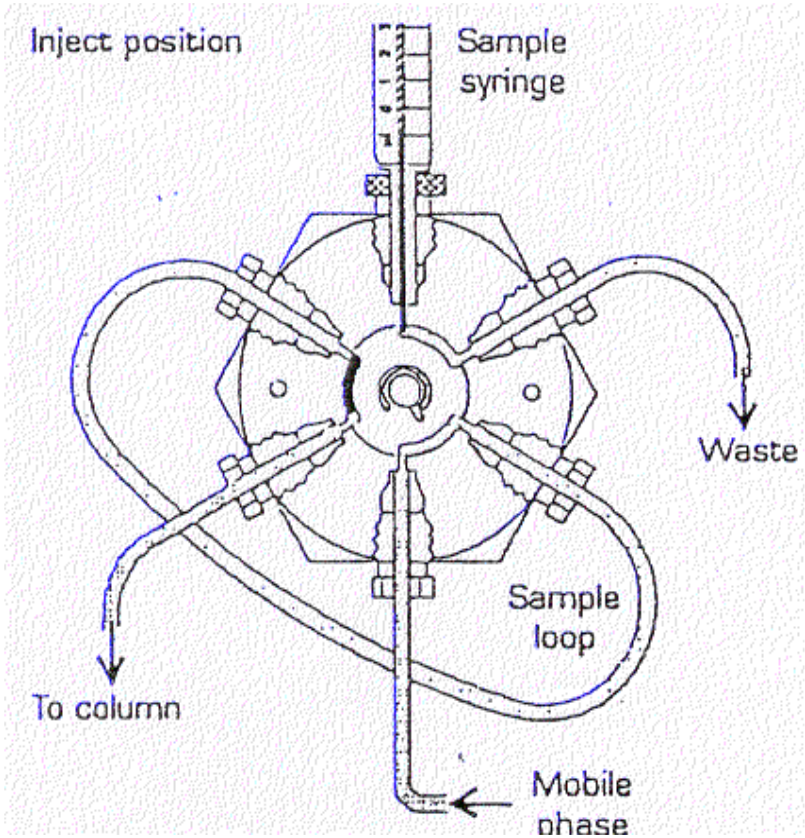
-Loops are available in size 5 µl to 2 ml (2000µl) .



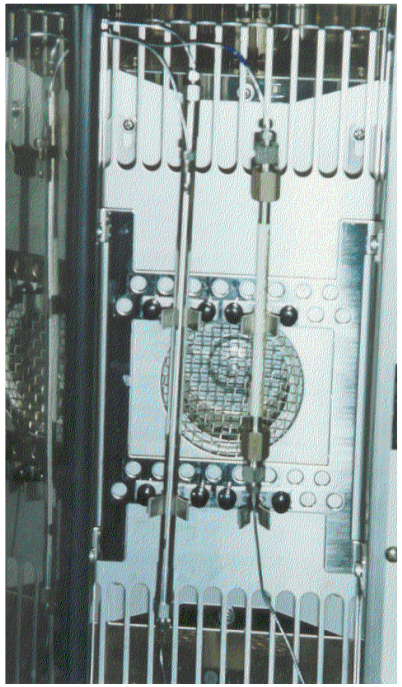
Load position



Inject position



C]- **Column**



1- Columns are open tubular structures, usually made of stainless steel .

2- Columns contains the stationary phase .

3- Factors important in producing efficient columns include;

- narrow particle size

Distribution in the packing & minimal dead volume in the tubing , fittings , cells, and other components of HPLC instruments .

C]- **Column**

4- Column selection .

5- Column packing, (bonded phases, ion exchange , ion pair, size exclusion).

6- Column evaluation .

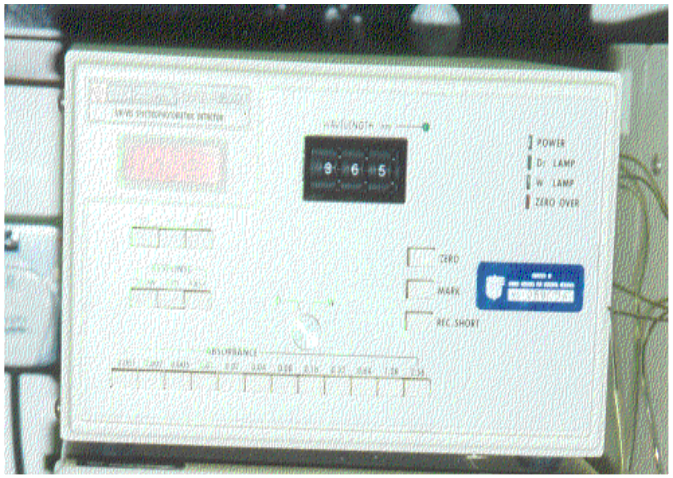
7- Column specification .

8- Filters , Pre-column , guard column , column storage , Column regeneration .

D]- **Detectors**

The function of the HPLC detector is to continuously and instantaneously monitor the mobile phase emerging from the column.

Common HPLC detectors :



1- UV Visible detector .

2- RI detector .

3- Fluorescence detector .

4- Electrochemical detector .

5- Conductivity detector .

6- Photoconductivity detector .

Electronic Data Processing in HPLC

The main goal in using Electronic data system is to measure analysis accuracy and precession ,while reducing operator attention.

- There are several types of data systems , each differing in terms of available feature .

- Use the modern data acquisition techniques can aid in the signal analysis .

Thank you