Chapter 28: High-Performance Liquid Chromatography (HPLC)

- Scope
- Instrumentation – eluants, injectors, columns
- Modes of HPLC
  - Partition chromatography
  - Adsorption chromatography
  - Ion chromatography
  - Size exclusion chromatography

HPLC

- Most widely used separation technique
- Broad applicability – organic & inorganic
- Can be very sensitive, accurate & precise
- Suitable for separation of nonvolatile species
- Has found numerous uses in industry, clinical settings, environmental areas, pharmaceuticals, etc.

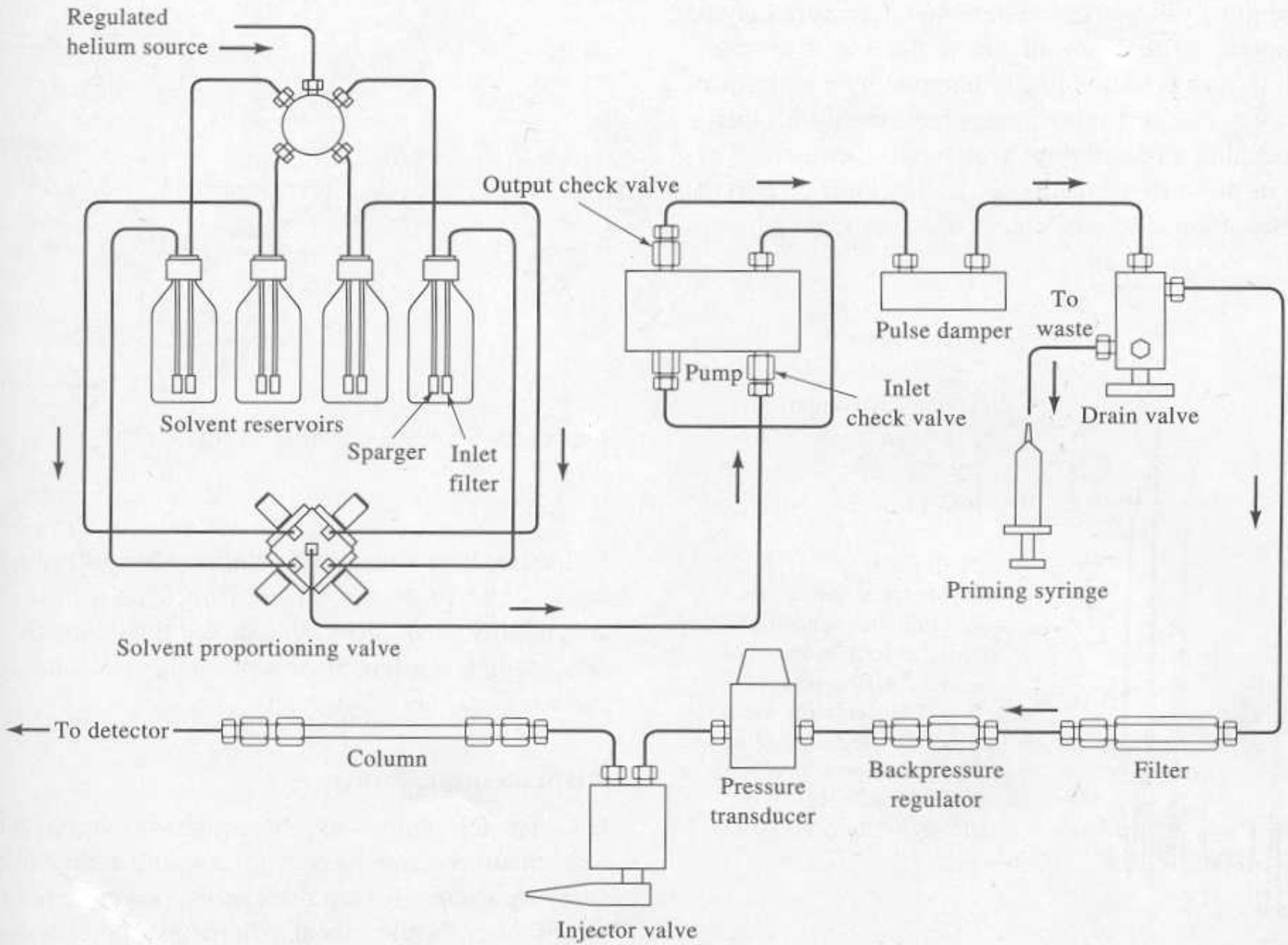


Figure 28-4 Schematic of an apparatus for HPLC. *(Courtesy of Perkin-Elmer Corporation, Norwalk, CT.)*

Solvents (mobile phase) – are stored in special reservoirs connected to the pumping system – must be free of particles that can clog components & free of bubble forming gases that get trapped in column or detector

Three basic ways to degas solvents

1) vacuum or suction filter (0.4 or 0.2 µm)
2) ultrasonicate (with vacuum)
3) He purge (sparge units often built in)

Can purchase HPLC solvents & water - still

HPLC pumping systems typically employ two reciprocating or piston pumps

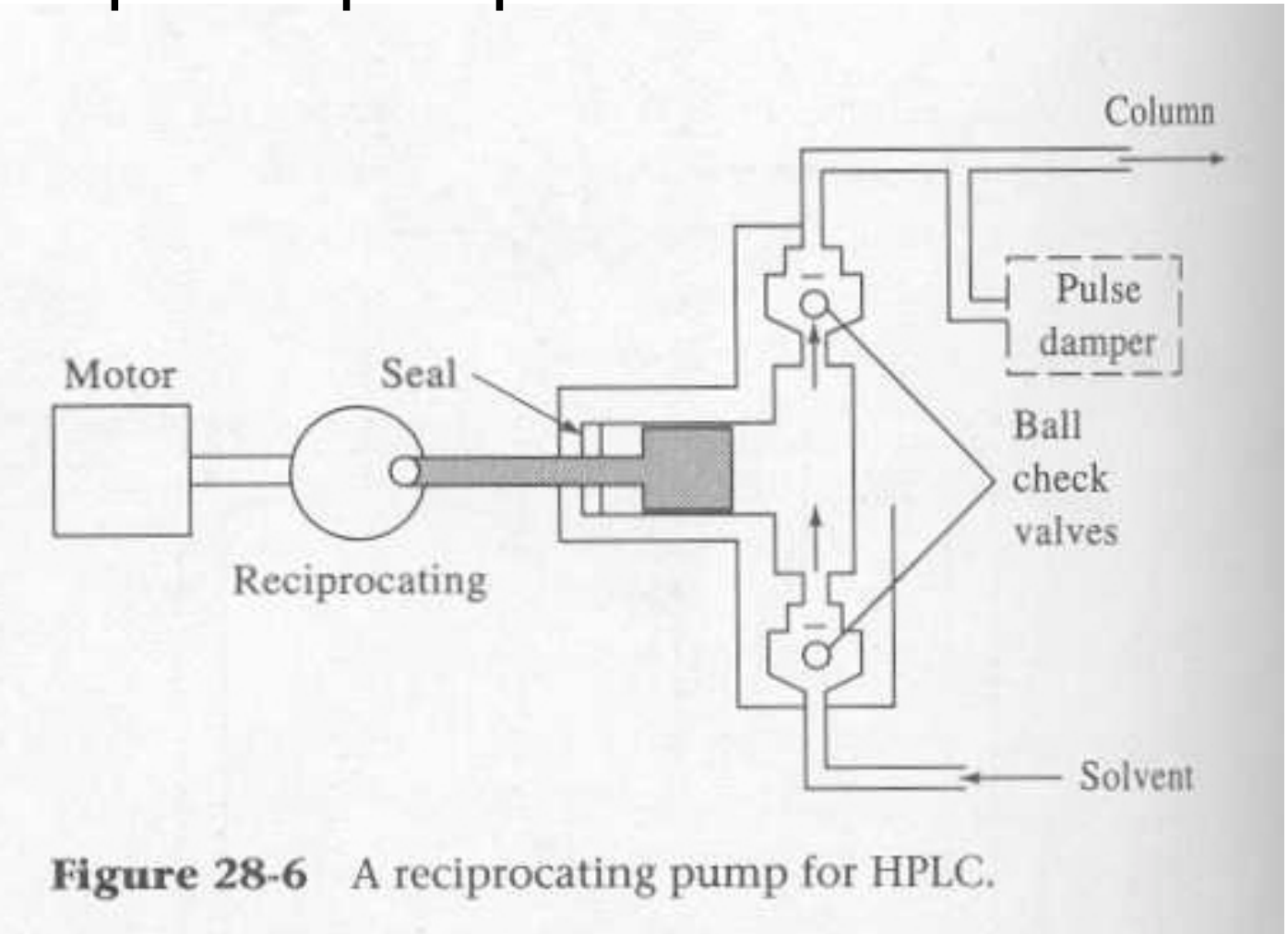


Figure 28-6 A reciprocating pump for HPLC.

Check valves & pump seals need to be replaced

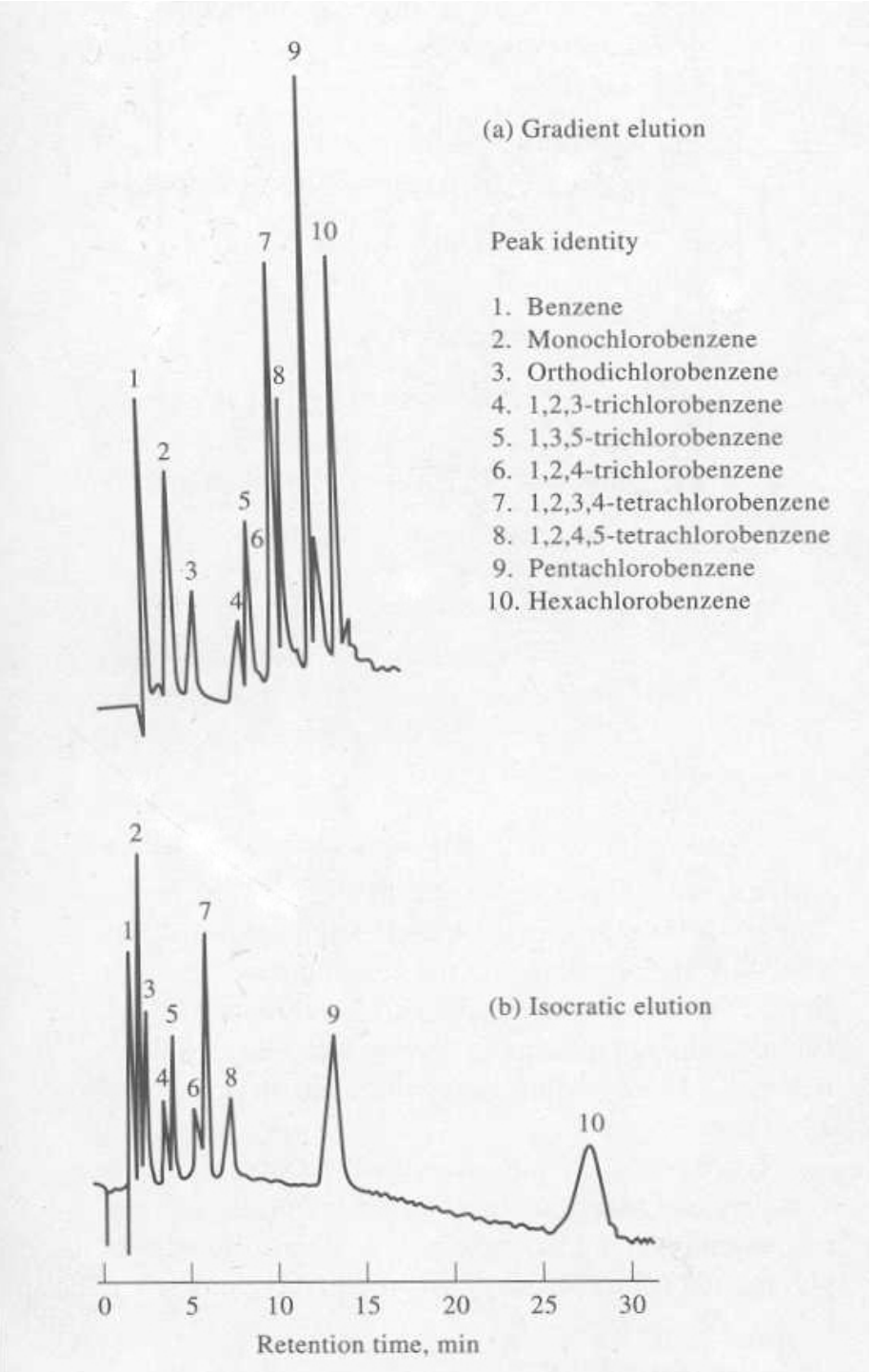
Pulse-free flow is never really achieved

In GC the analyte affinity for the column is influenced by temp

In HPLC the solvent strength affects an analytes retention on column

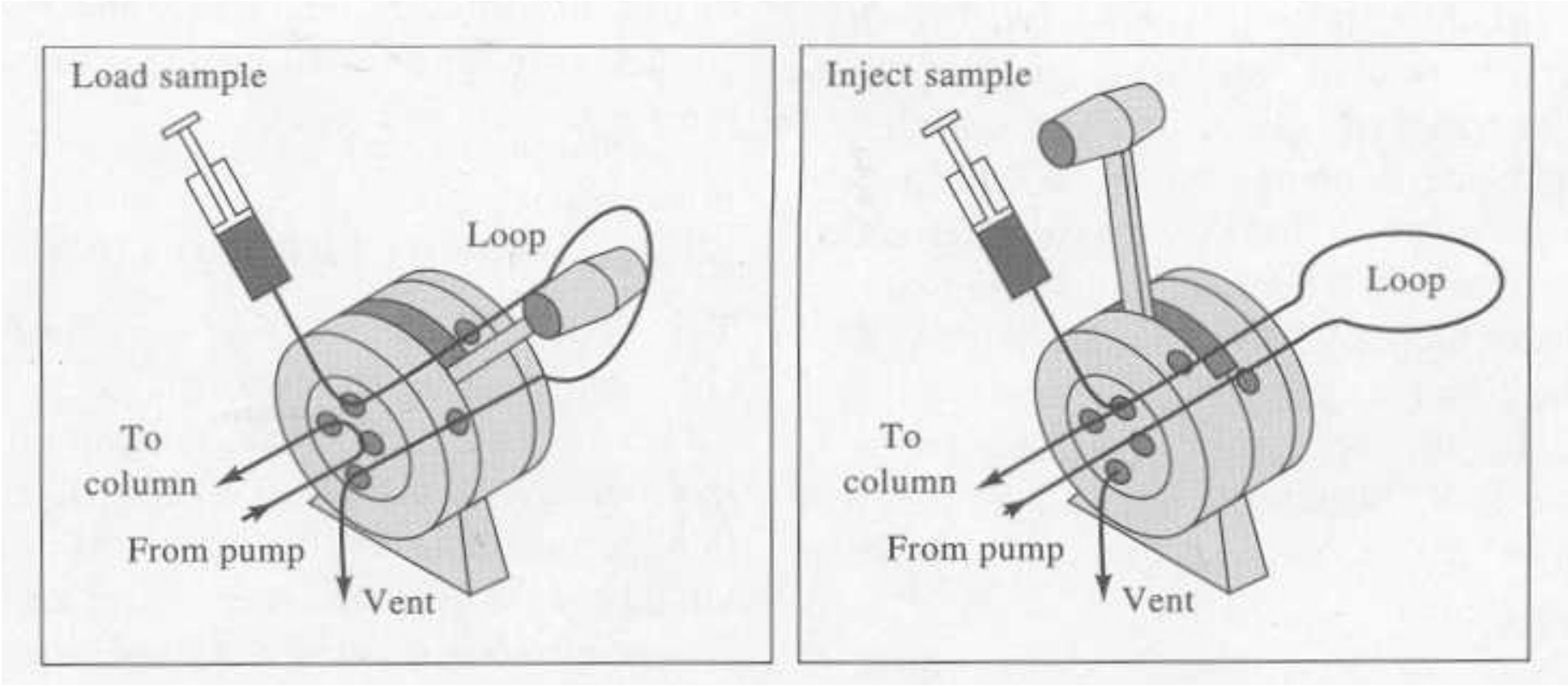
Therefore, analogous to temp programming in GC, do solvent programming in HPLC

This is also referred to as gradient elution



Gradient elution dramatically improves the efficiency of separation

HPLC sample injectors are
exclusively 6 port valves that
are overfilled by syringe giving
extreme accuracy & precision – typical
volumes are 10 to 50 µL but can be larger



Rotary Injection Valve

Common for HPLC, rare in GC

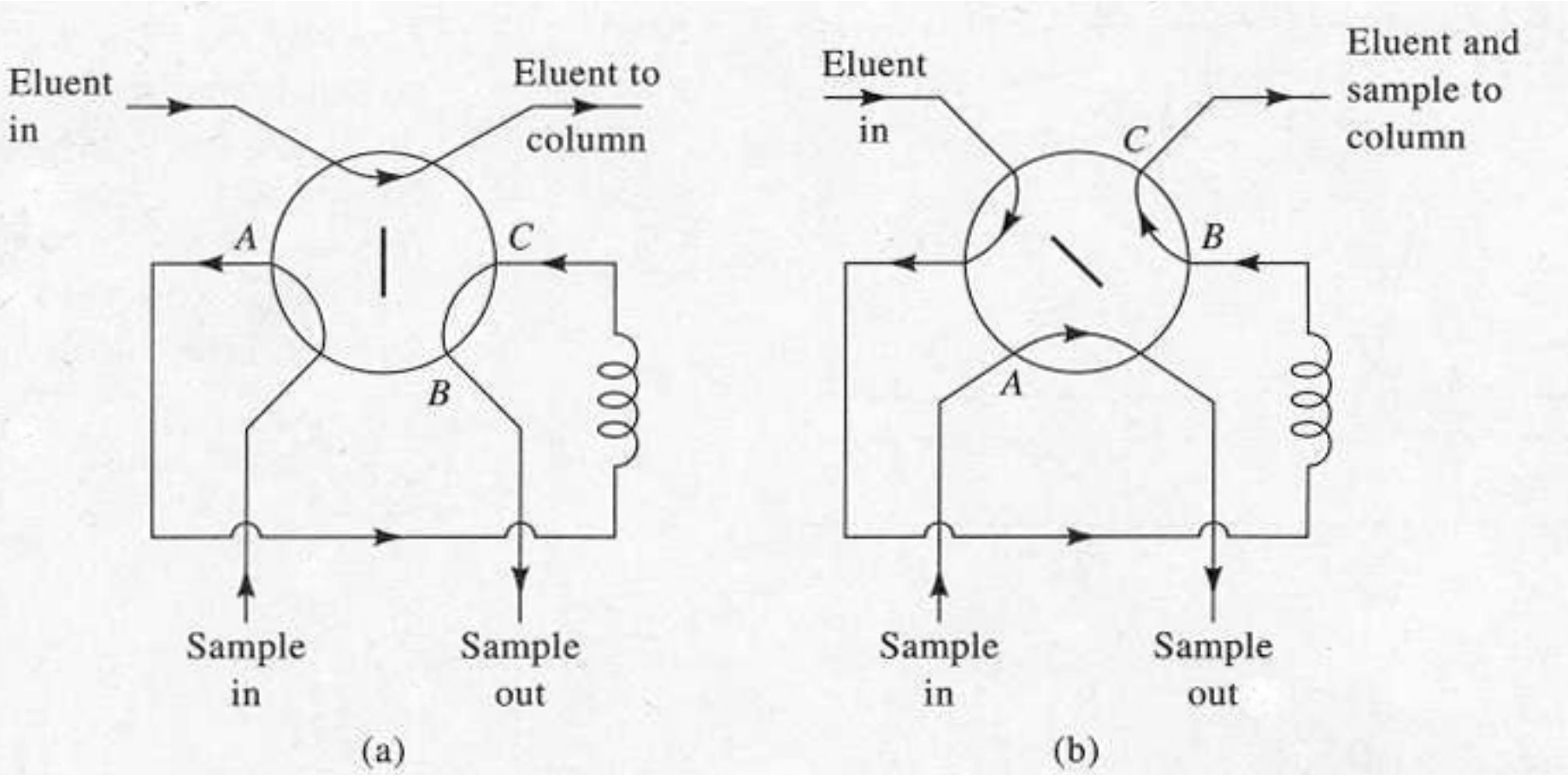
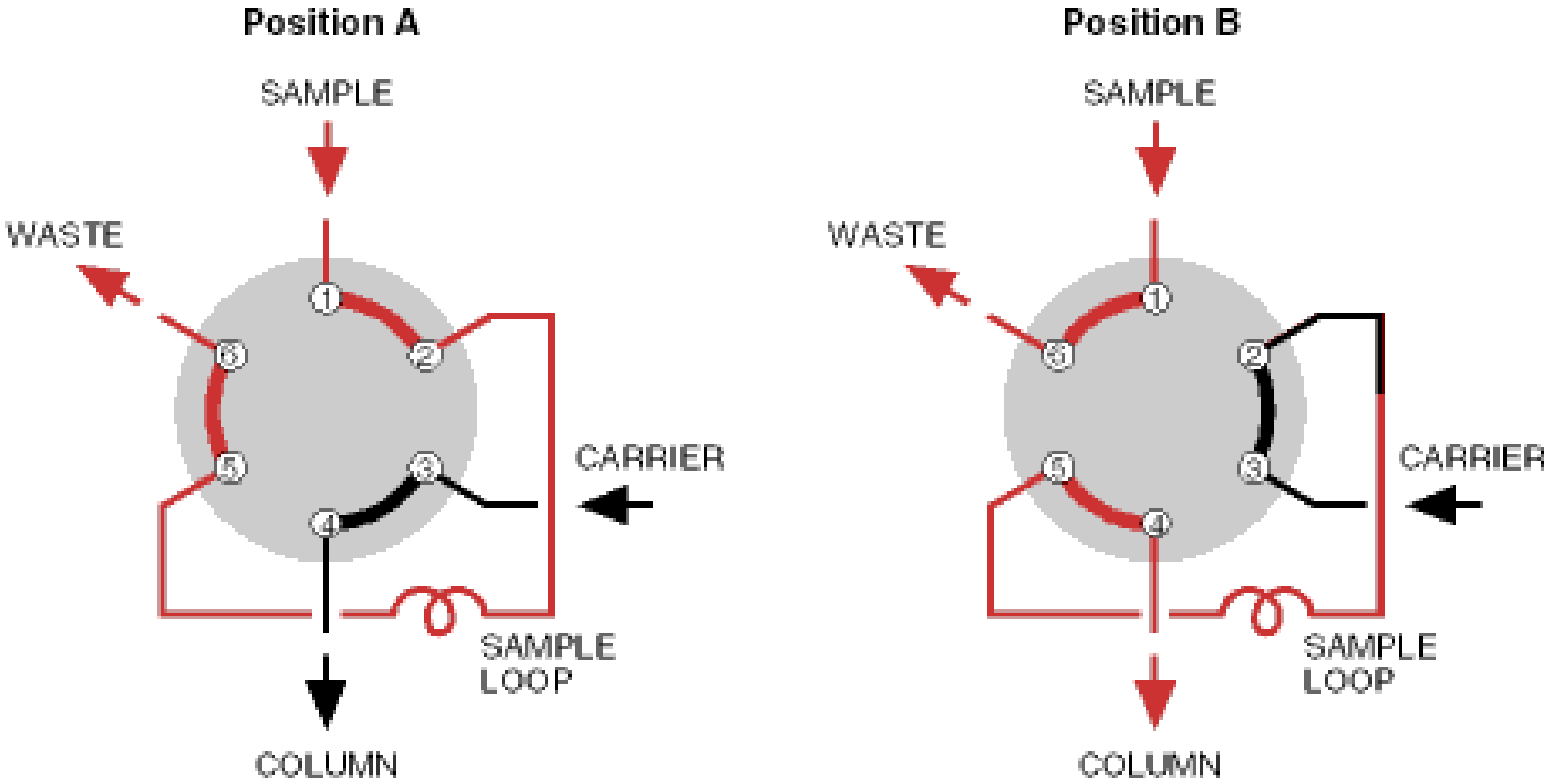


Figure 27-4 A rotary sample valve: valve position (a) for filling sample loop *ACB* and (b) for introduction of sample into column.

Injector for HPLC

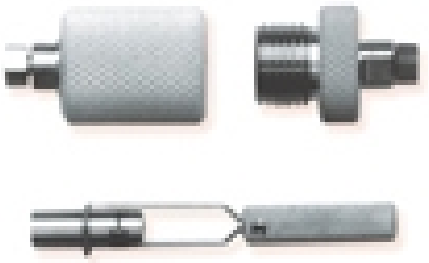
6 port rotary valve



Columns

- usually stainless steel
- can be PEEK (poly ether ether ketone)
- may cost $200-$1000 packed
- Length 10-30 cm, ID 4-10 mm
- Packings are 3, 5, or 10 µm particle size
- Most common 25 cm, 5 µ, 4.6 mm ID
- N = 40,000 to 60,000
- Normally packed under 6000 psi pressure at factory as a slurry

Guard columns are normally used before the analytical column to protect & increase lifetime of column – operator usually slurry or dry packs short guard column regularly with same or similar packing used in analytical column (old column material) – can purchase guard systems, cartridges, etc.



Detectors for HPLC

- Ideal characteristics same as GC
- Exception is temp range
- Low dead volume 1 to 10 µL

Most common detector is UV-vis absorbance detector

Three types

1) Filter instrument – optical filters, Hg lamp
2) Variable wavelength – monochromator
3) Diode array detector- provide spectra

Many HPLC detectors available

For universal & selective detection

TABLE 28-1 Performances of LC Detectors

LC Detector	Commercially Available	Mass LOD (commercial detectors)[a]	Mass LOD (state of the art)[b]
Absorbance	Yes[c]	100 pg–1 ng	1 pg
Fluorescence	Yes[c]	1–10 pg	10 fg
Electrochemical	Yes[c]	10 pg–1 ng	100 fg
Refractive index	Yes	100 ng–1 μg	10 ng
Conductivity	Yes	500 pg–1 ng	500 pg
Mass spectrometry	Yes[d]	100 pg–1 ng	1 pg
FT–IR	Yes[d]	1 μg	100 ng
Light scattering[e]	Yes	10 μg	500 ng
Optical activity	No	—	1 ng
Element selective	No	—	10 ng
Photoionization	No	—	1 pg–1 ng

1) Filter based UV-vis detector – Typically set at 254 nm using the most prominent band in Hg spectrum – can also use 313, 365, 334 nm and other lines as well
2) Variable wavelength detectors – use continuum source like (D_2 or H_2) & a monochromator, select any λ, less sensitive
3) PDA - D_2 or H_2 source, disperse & focus on diode array, get complete spectrum every 1 sec, powerful, expensive, less sensitive, lots of data generated

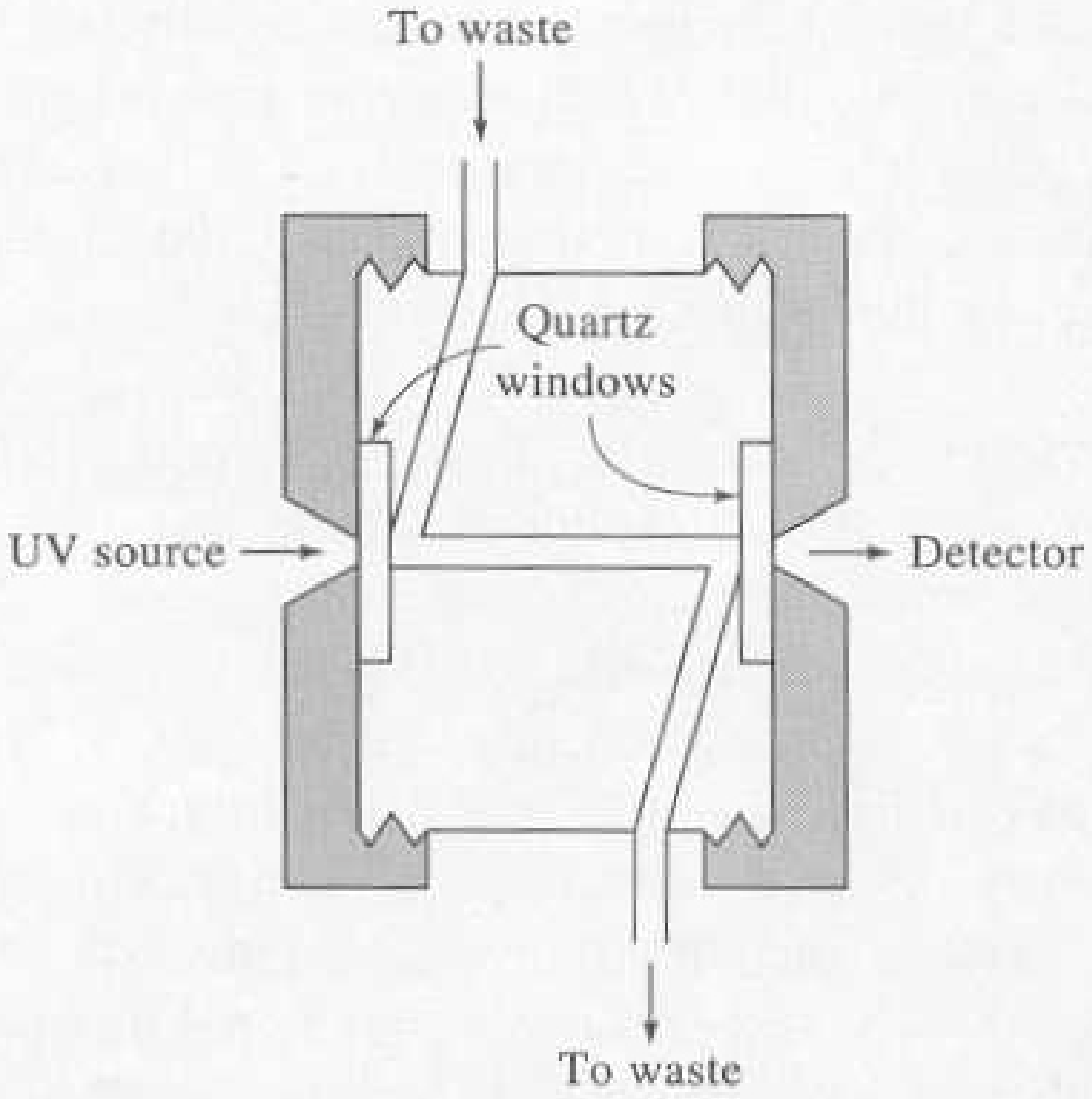


Figure 28-9 Ultraviolet detector cell for HPLC.

Cell for UV-vis detector for HPLC

- Low vol

Diode Array Detector

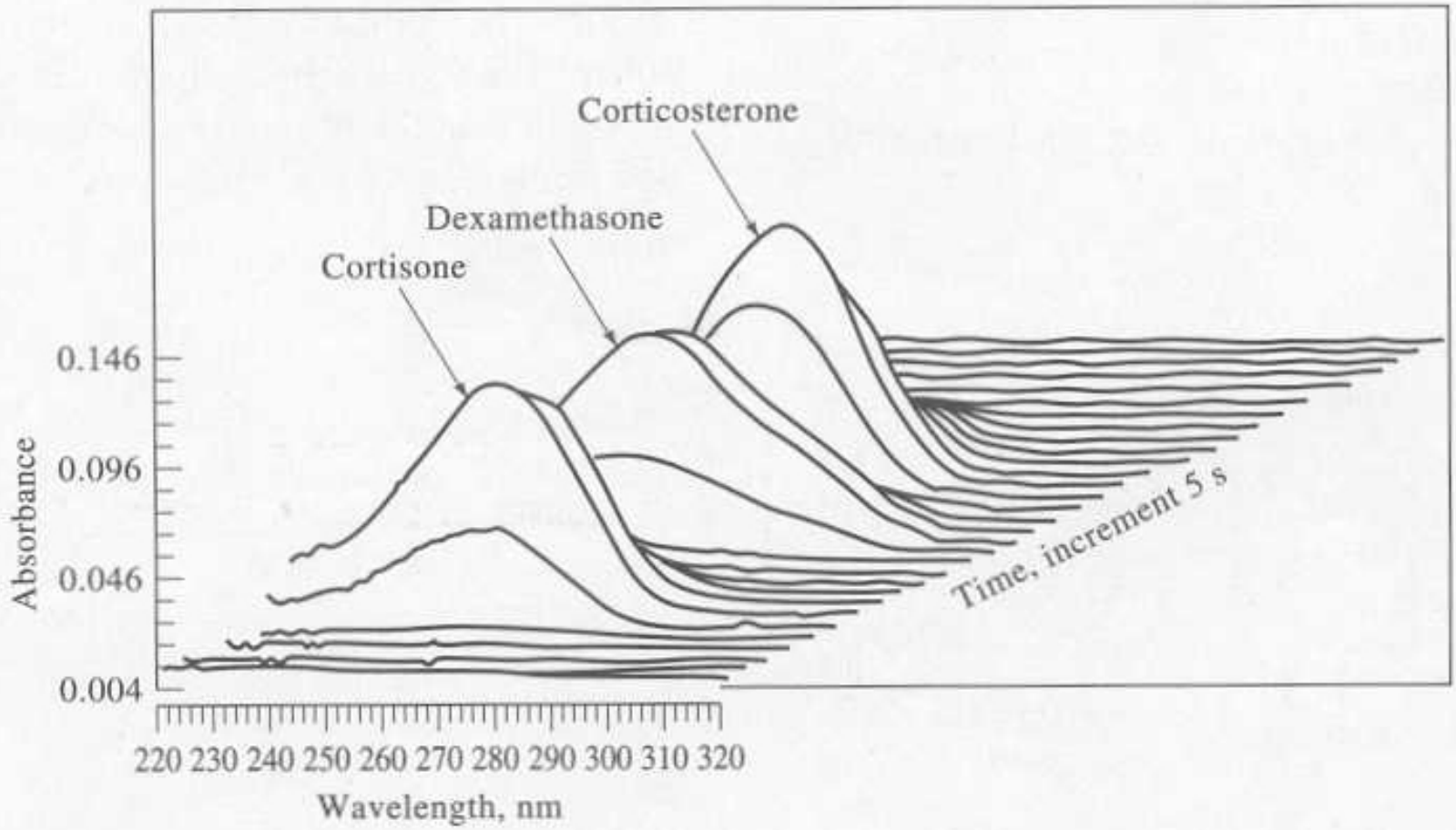


Figure 28-10 Absorption spectra of the eluent from a mixture of three steroids taken at 5-second intervals. *(Courtesy of Hewlett-Packard Company, Palo Alto, CA.)*

Fluorescence detector – normally
fixed wavelength filter fluorometer
excitation filter & emission filter
can be changed for particular λ of interest
gives selectivity based on:

- ability to exhibit fluorescence
- excitation wavelength
- emission wavelength

Variable λ monochromator based
fluorescence detectors also available

Filter based detectors usually more sensitive

Refractive index detector (RI) -

responds to nearly all solutes

but has poor sensitivity – detects changes in refractive index as sample passes through as long as solute has different RI than solvent – analogous to TCD in GC

Electrochemical Detection

- Amperometric – fix potential & measure current (i)
- Conductometric – measure conductivity
- Coulometric – fix potential & integrate i
- Voltammetric – vary potential & measure i
- Potentiometric – measure potential

Can use 2 or 3 electrode design with Pt or carbon electrodes (glassy C or C paste)

Electrochem. detector nearly universal

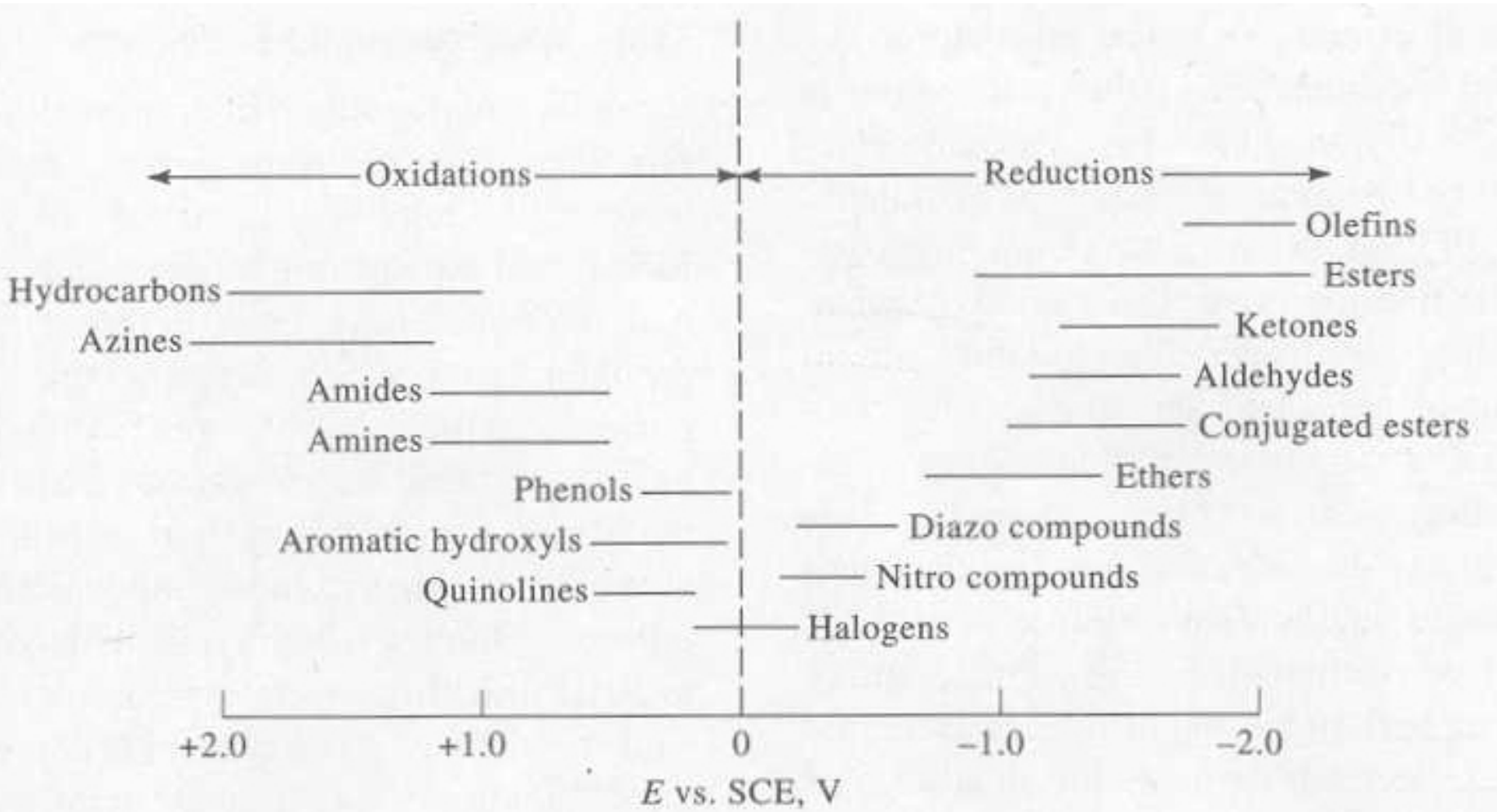


Figure 28-12 Potentially detectable organic functional groups by amperometric measurements. The horizontal lines show the range of oxidation or reduction potentials wherein compounds containing the indicated functional groups are electroactive.

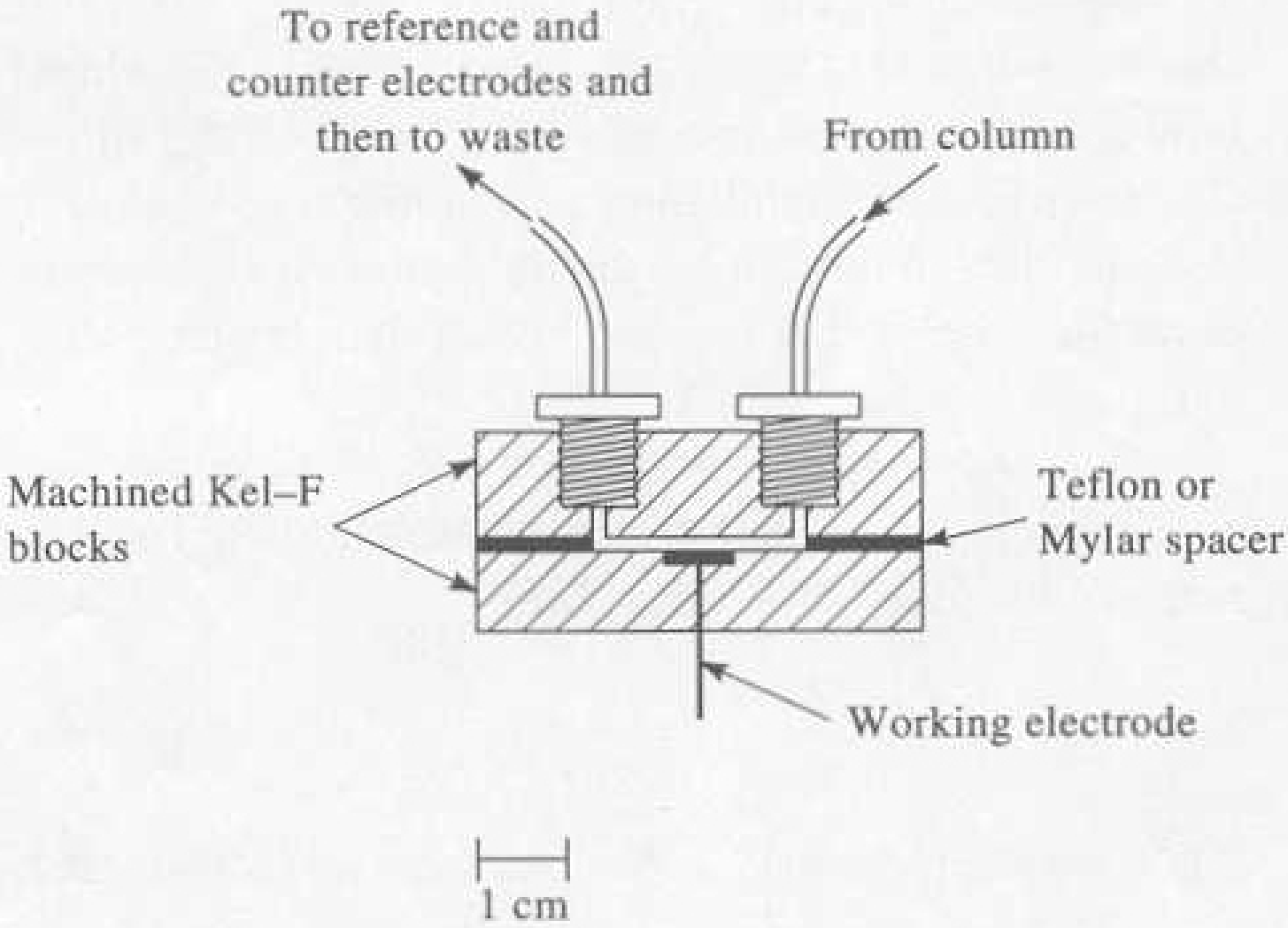


Figure 28-13 Amperometric thin-layer detector cell for HPLC.

Other HPLC detectors

- LC-MS using thermospray – new popularity (pharmaceuticals)
- Evaporative light scattering - polymers
- LC-FTIR
- LC-plasma emission or ICP-MS