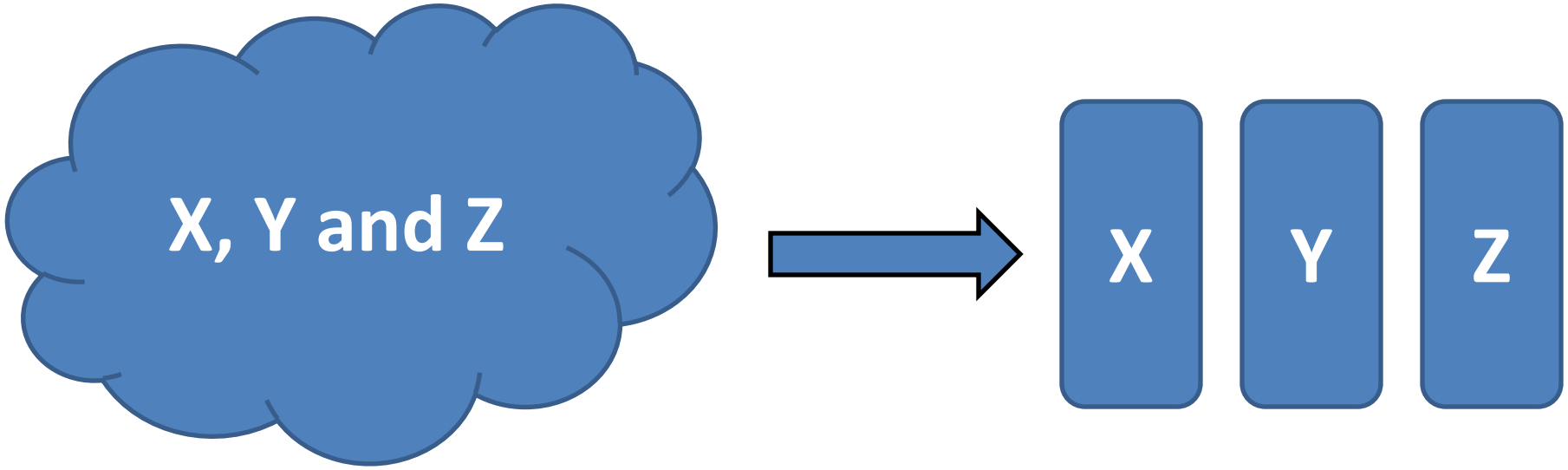
The Analytical Challenge
Chapter 1, pp. 1-22, #1,7,8,9,10

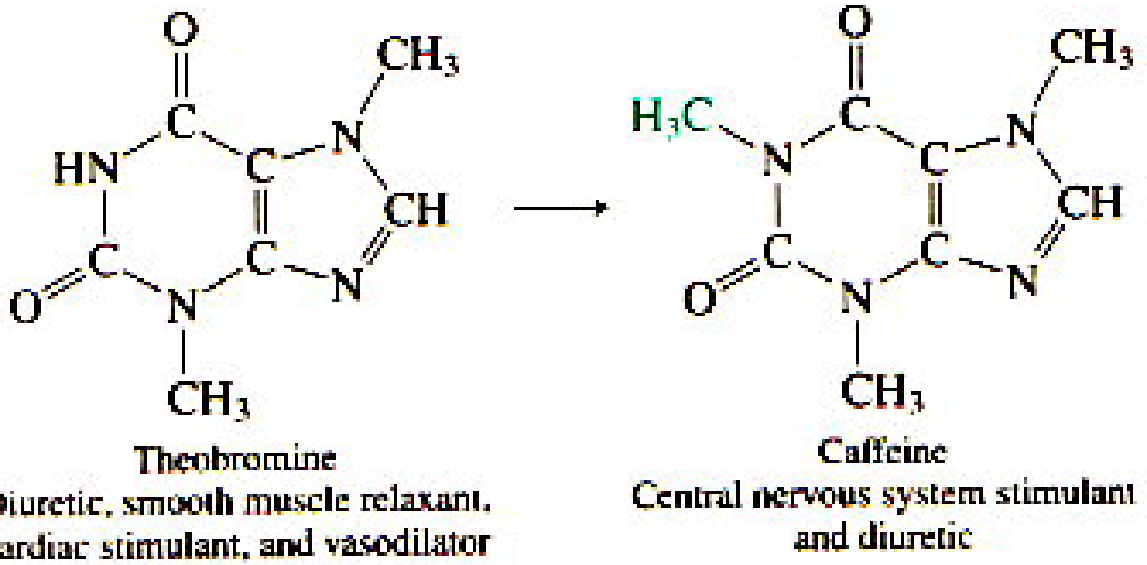


Complex Sample

Individual volume zones of each analyte

Very few analytical methods provide both qualitative and quantitative analysis of complex mixtures. Some form of SEPARATION is needed up front.

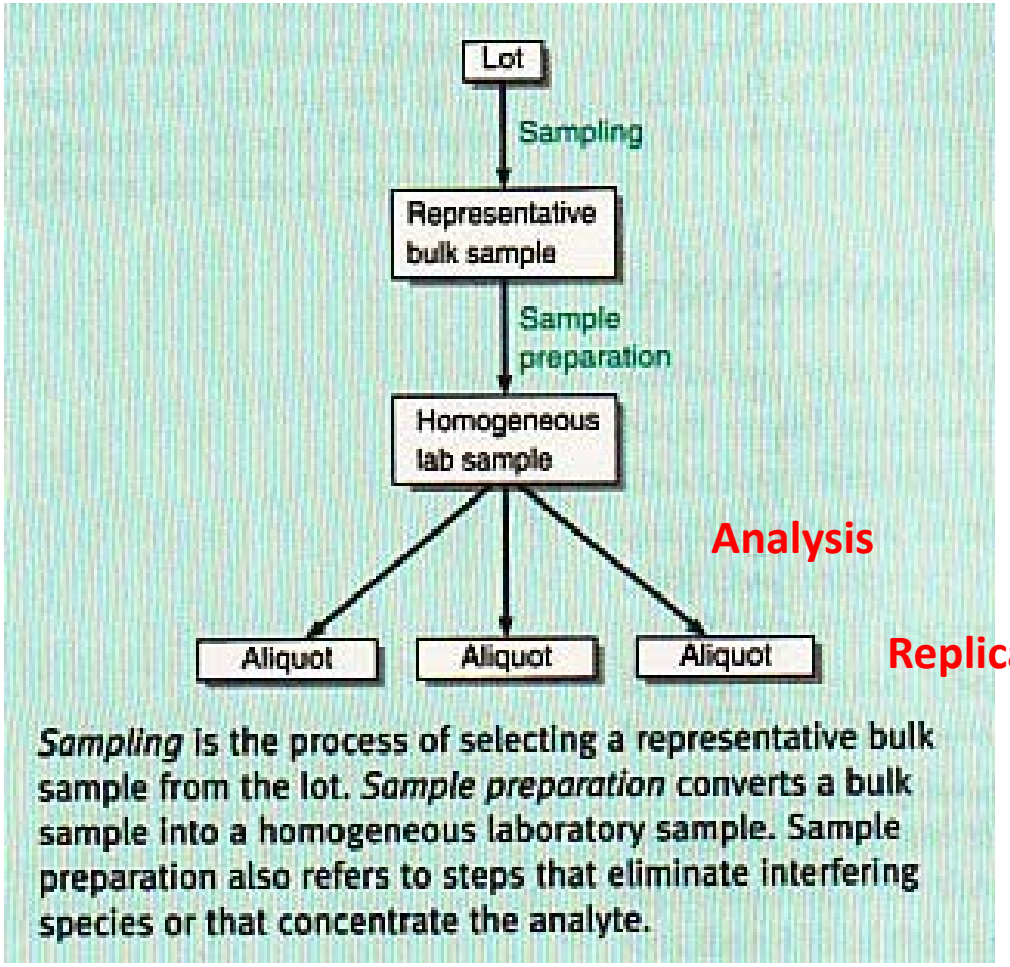
Analysis of Chocolate



How would one analyze different chocolate bars for their caffeine and theobromine content? These are the *analytes*.

*****There are many parts to this answer*****

The Analytical Process



Sample preparation is often the key step in the analytical process!

Sample Types

- **Homogeneous** sample (same composition throughout)

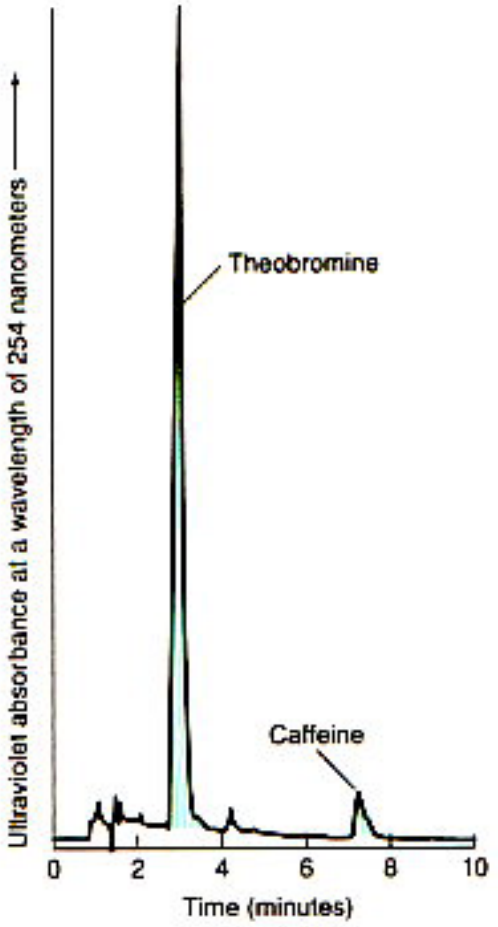
- **Heterogeneous** sample (different composition from region to region)….Random and segregated materials

Heterogeneous samples (materials) need a different strategy for sampling than do homogeneous samples.

Types of Analytical Analyses

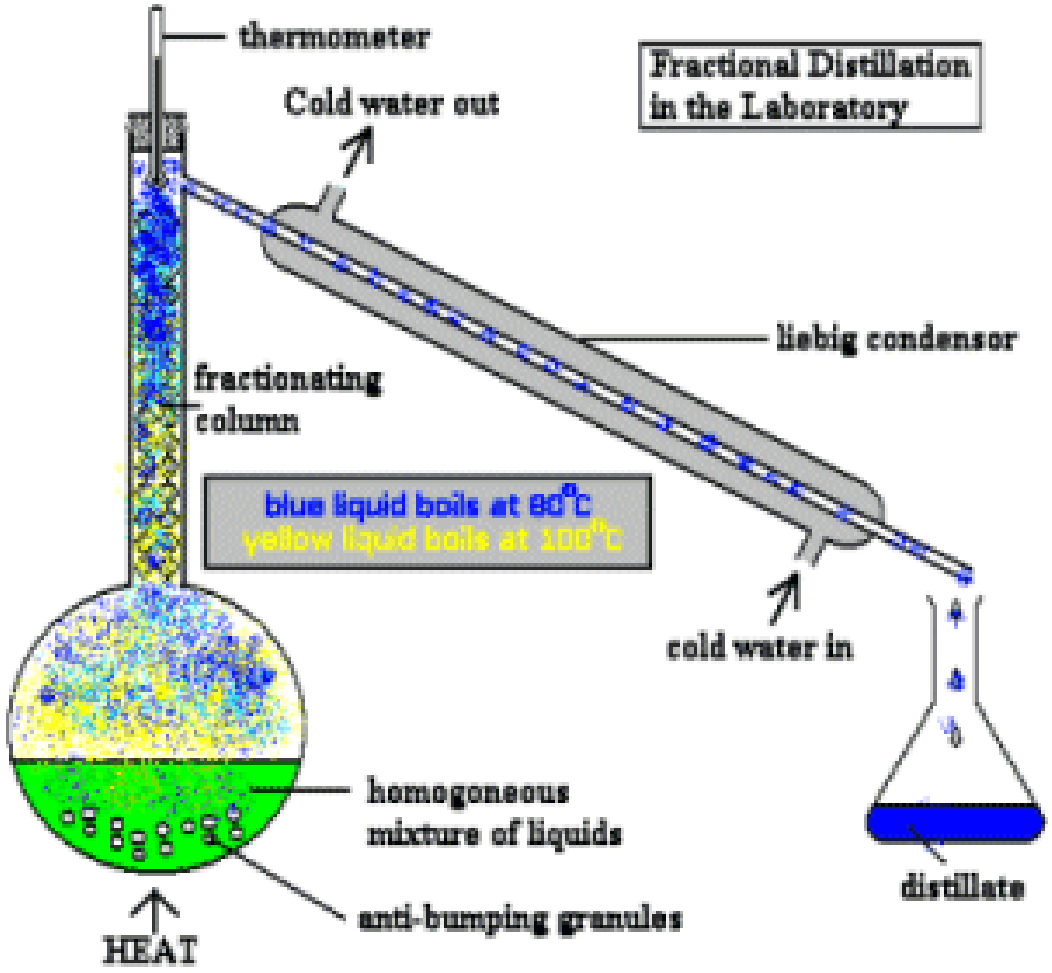
- **Qualitative analysis** (identifying *what* is in an unknown sample)

- **Quantitative analysis** (identifying *how much* analyte is present in an unknown sample)



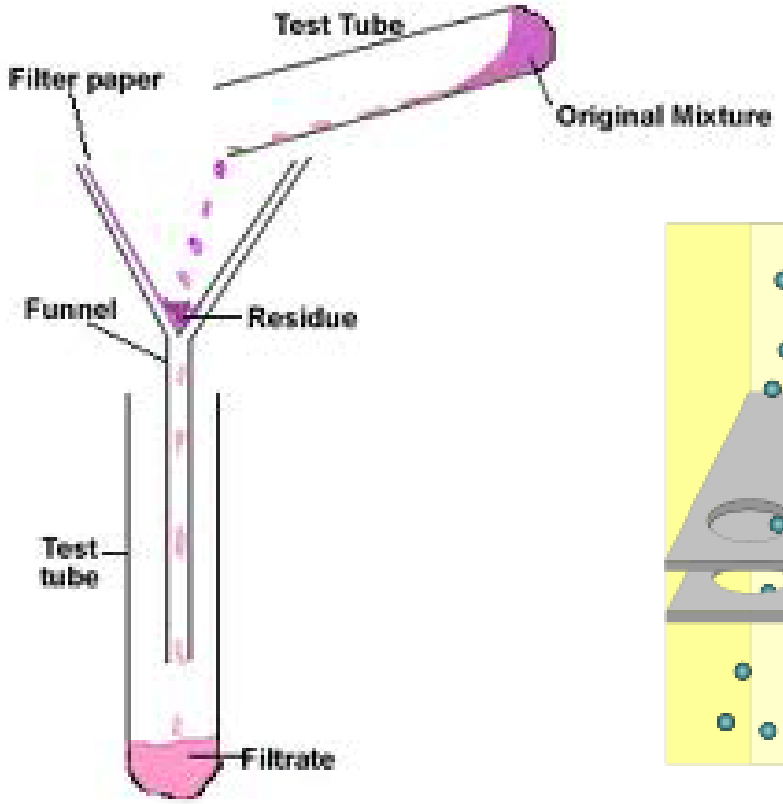
Distillation

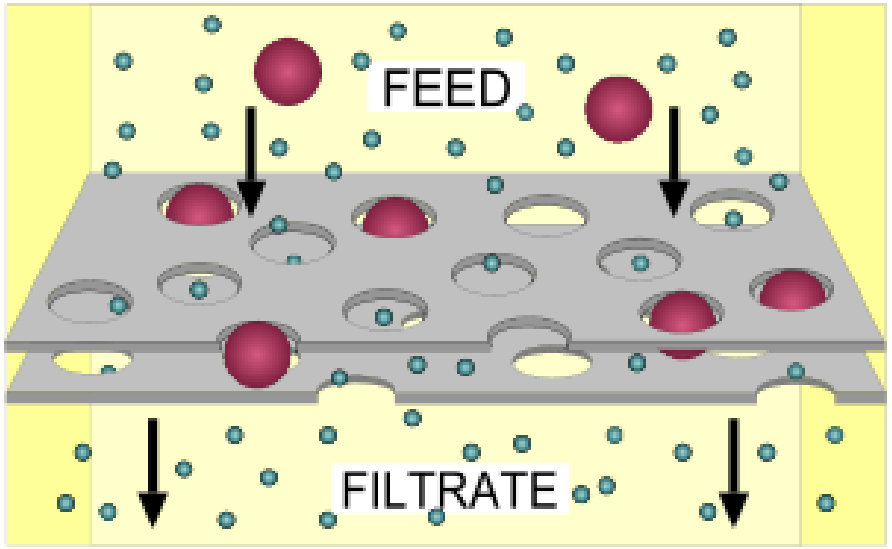
Process by which mixtures are separated based on differences in component volatilities.



Filtration

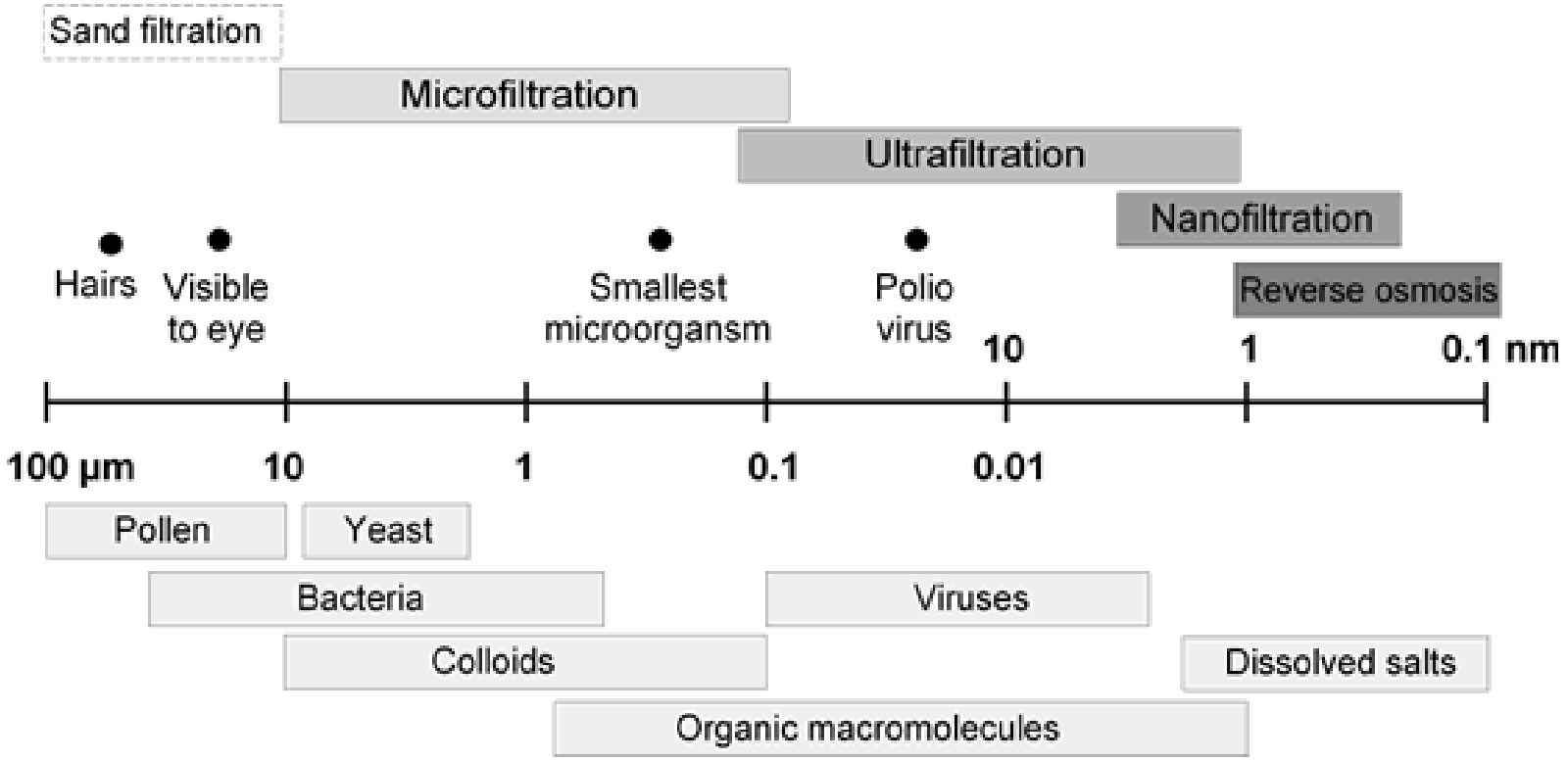
Process by which solids are separated from fluids (liquids or gases) by interposing a medium through which only the fluid can pass.





Pressure difference between feed and permeate

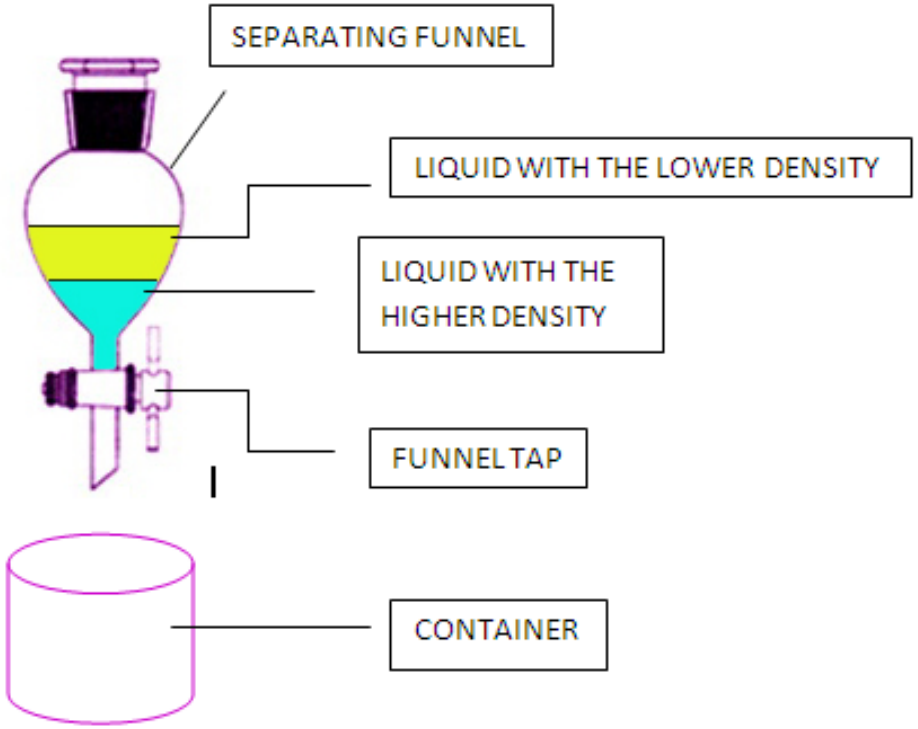
Membrane Filtration



Powerful methods for analyte separation/isolation.

Liquid-Liquid (Solvent) Extraction

Process by which components in a liquid mixture are separated based on their relative solubilities (partitioning) in two immiscible liquids (organic – water).



$$K = C_{phase\ 1} / C_{phase2}$$

Partition coefficient

Sample Preparation

Weighing the chocolate and extracting the fat.

Maximize extraction with small particles

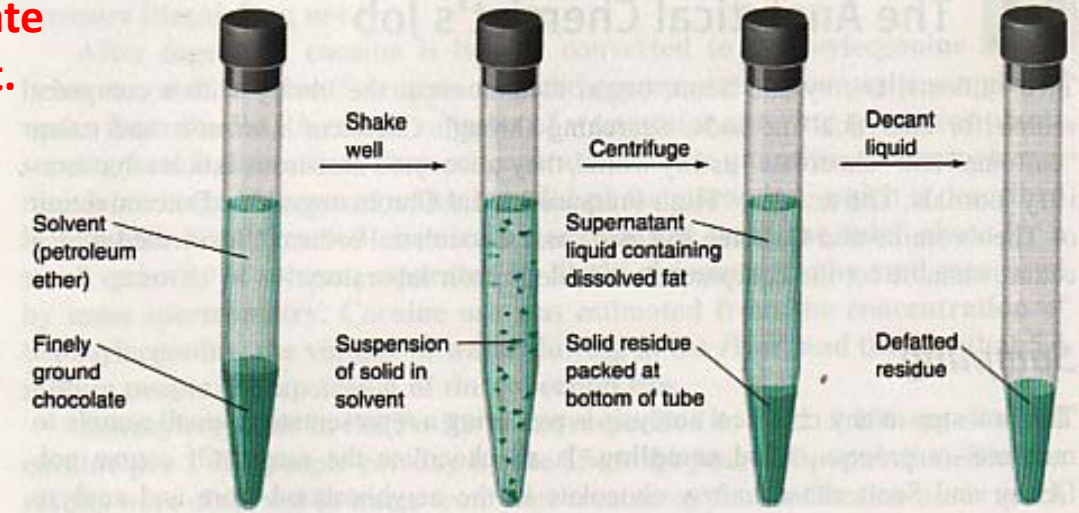


Figure 0-2 Extracting fat from chocolate to leave defatted solid residue for analysis.

Liquid extraction used to remove fat from the solid chocolate.

Caffeine and theobromine are insoluble in the ether.

Sample Preparation

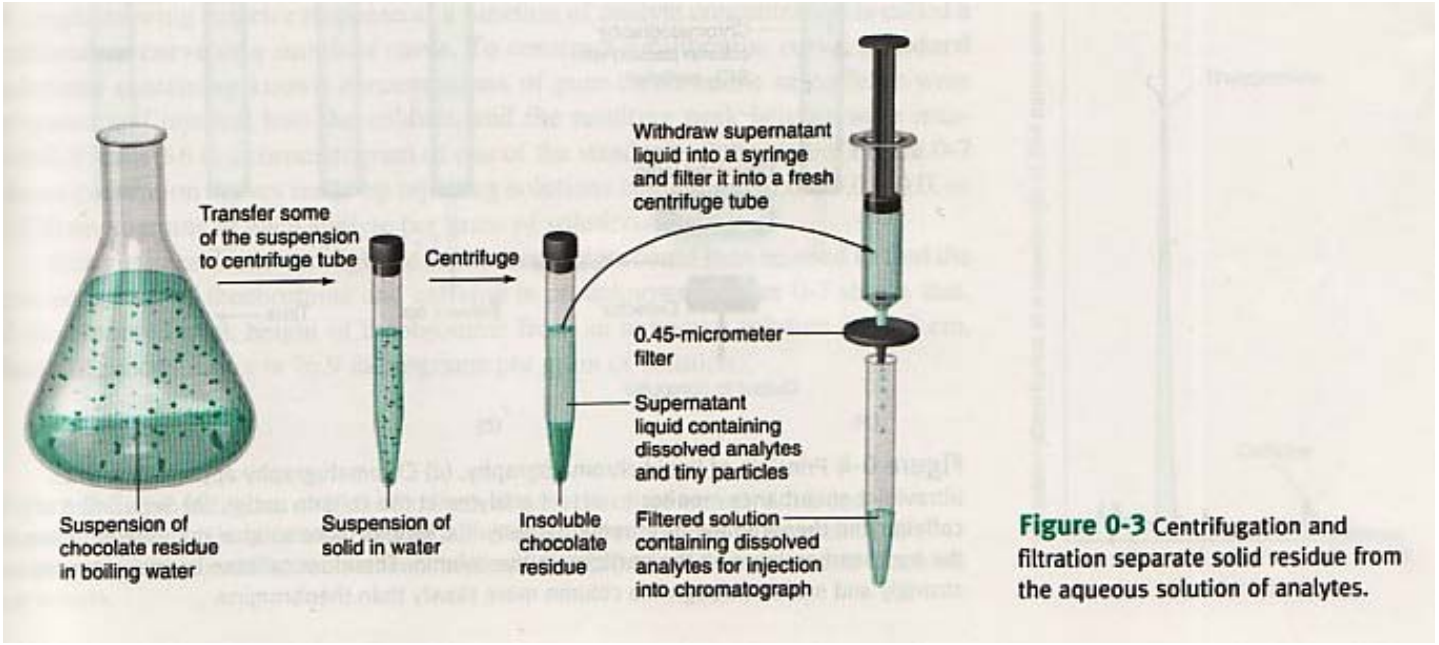


Figure 0-3 Centrifugation and filtration separate solid residue from the aqueous solution of analytes.

Caffeine and theobromine dissolved into the water. Sample is then filtered and made ready for the real analysis.

Analysis – Separation and Detection

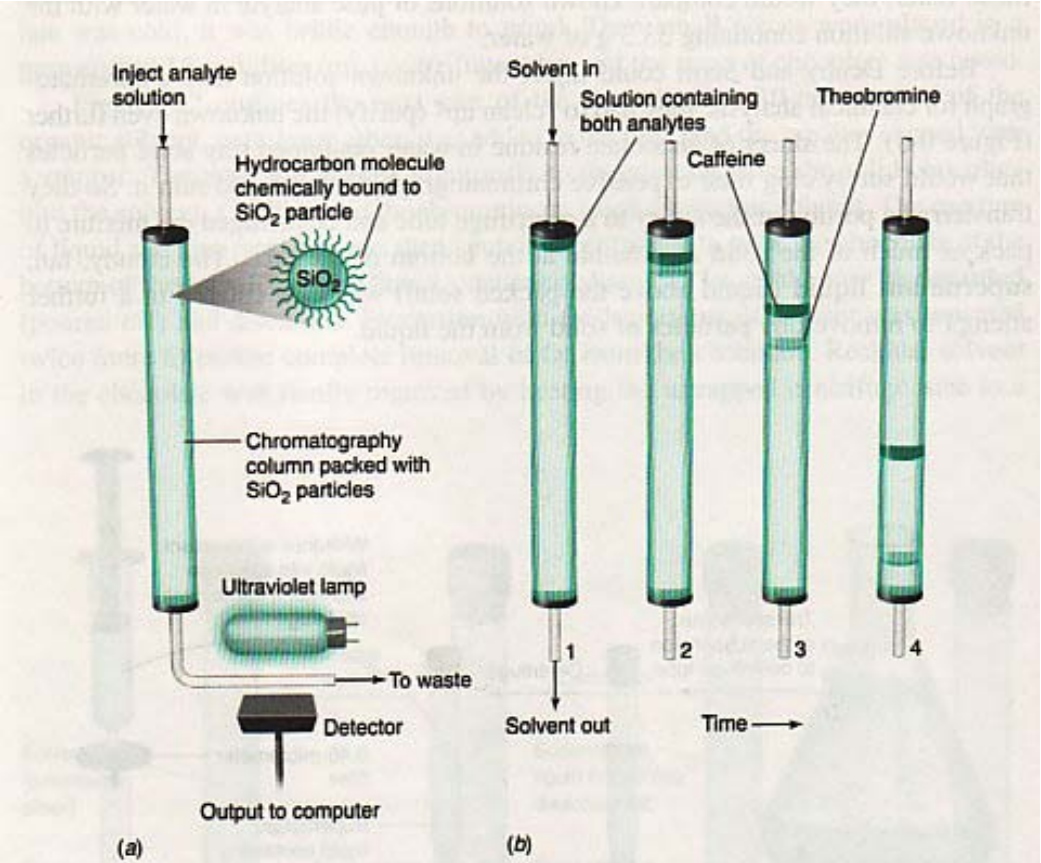


Figure 0-4 Principle of liquid chromatography. (*a*) Chromatography apparatus with an ultraviolet absorbance monitor to detect analytes at the column outlet. (*b*) Separation of caffeine and theobromine by chromatography. Caffeine is more soluble than theobromine in the hydrocarbon layer on the particles in the column. Therefore caffeine is retained more strongly and moves through the column more slowly than theobromine.

Analysis – Separation and Detection

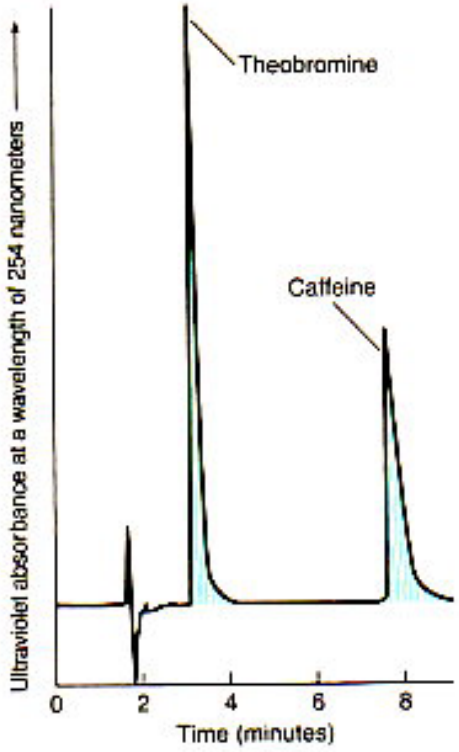


Figure 0-6 Chromatogram of 20.0 microliters of a standard solution containing 50.0 micrograms of theobromine and 50.0 micrograms of caffeine per gram of solution.

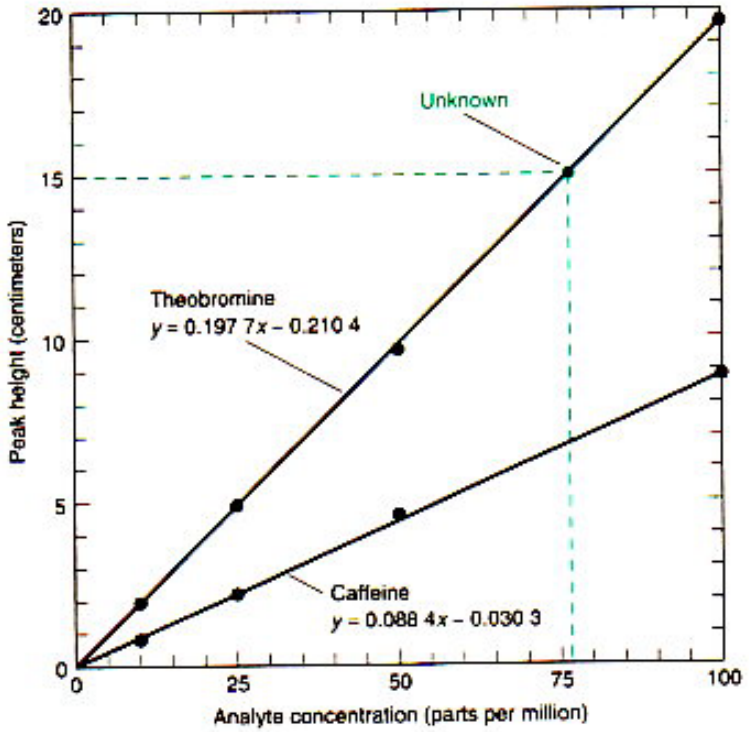


Figure 0-7 Calibration curves, showing observed peak heights for known concentrations of pure compounds. One part per million is 1 microgram of analyte per gram of solution. Equations of the straight lines drawn through the experimental data points were determined by the *method of least squares;* described in Chapter 4.

Calibration curve = Standard curve = Response curve

The Data

Table 0-1 Analyses of dark and white chocolate

	Grams of analyte per 100 grams of chocolate	
Analyte	Dark chocolate	White chocolate
Theobromine	0.392 ± 0.002	0.010 ± 0.007
Caffeine	0.050 ± 0.003	0.000 9 ± 0.001 4

Uncertainties are the *standard deviation* of three replicate injections of each extract.

Average ± std. dev.

Values for theobromine are reproducible (<1%)
Values for caffeine are less reproducible (6%)

General Steps in Chemical Analysis

- Formulating the question (*Is the water safe to drink*?)
- Selecting the analytical method(s) (instrument) to be used.
- Sampling (properly selected sample - *garbage in, garbage out*)
- Sample preparation (convert sample into form suitable for analysis)
- Analysis (reproducibility, sensitivity, stability)
- Reporting and Interpretation (verbal and written communication)
- Drawing conclusions (*What do the results mean*?)