# General theory of chromatography

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Separation of mixtures

- Chromatography is a method for separation of compounds from mixtures.

- Separation is based on different interaction strengths between compounds and stationary phase.
  - Interactions include partitioning, adsorption and electrostatic interactions.
  - The stronger is the interaction the longer is the component retained in the column.

Principle of chromatography
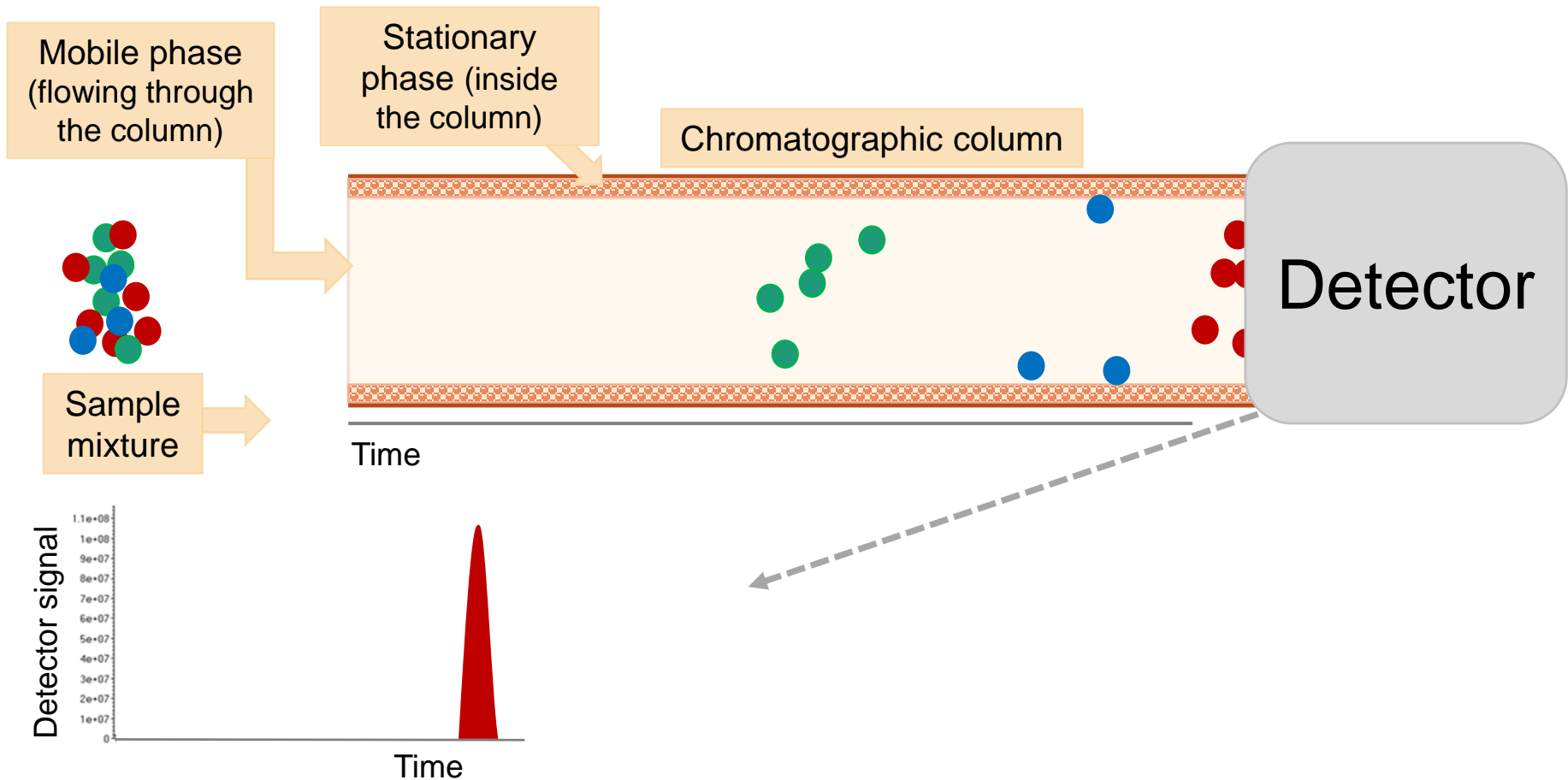
Chromatogram

- Chromatogram – plot of detector signal vs. time.

- The separated compounds appear as maxima, called peaks, in the chromatogram.

- Retention time – time it takes for a component to pass through the system under set conditions.

Chromatogram of methylated fatty acids of linseed oil

Peak

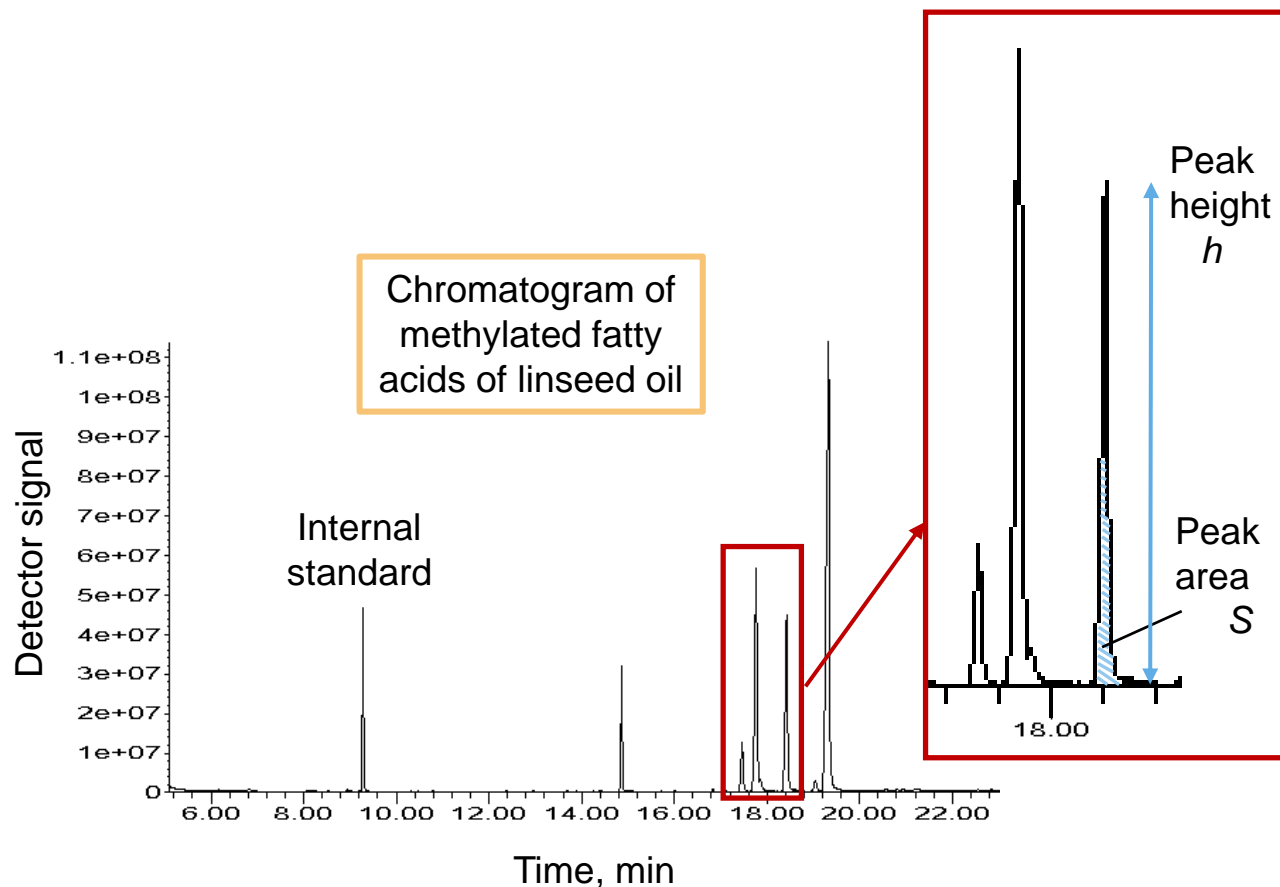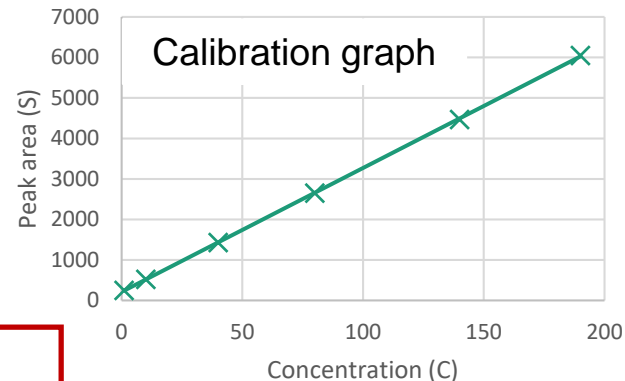Retention time of the component
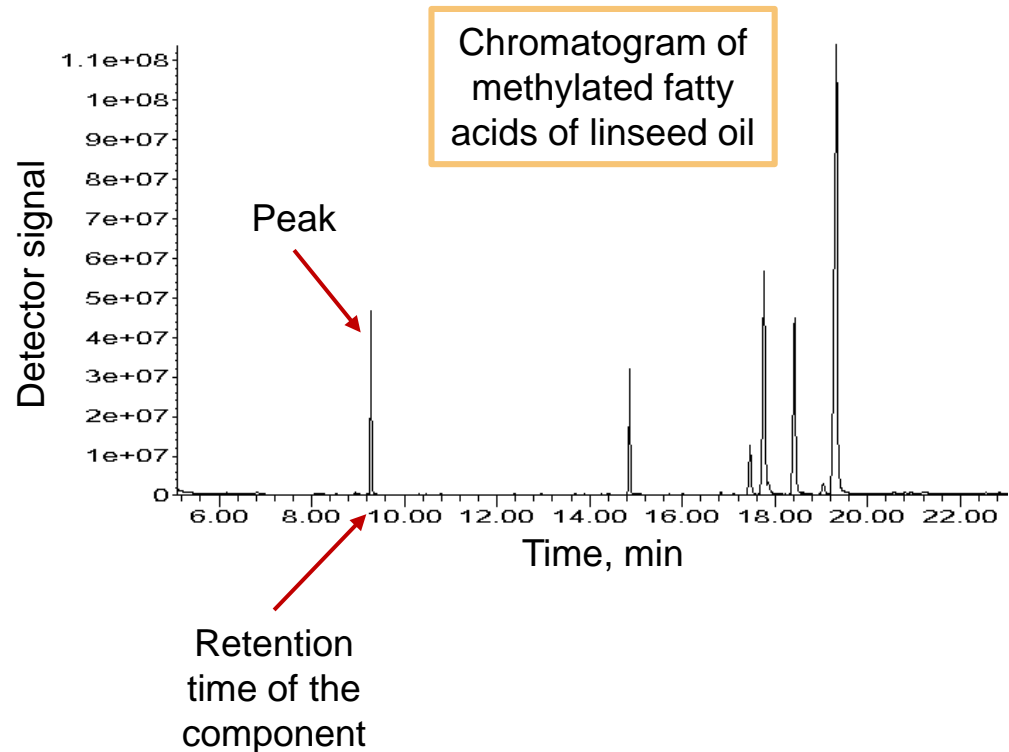
Identification of compounds

- Qualitative analysis is based on …
  - retention time.
  - additional information may be provided by the detector.

Chromatogram of methylated fatty acids of linseed oil

Internal standard

Methyl palmitate
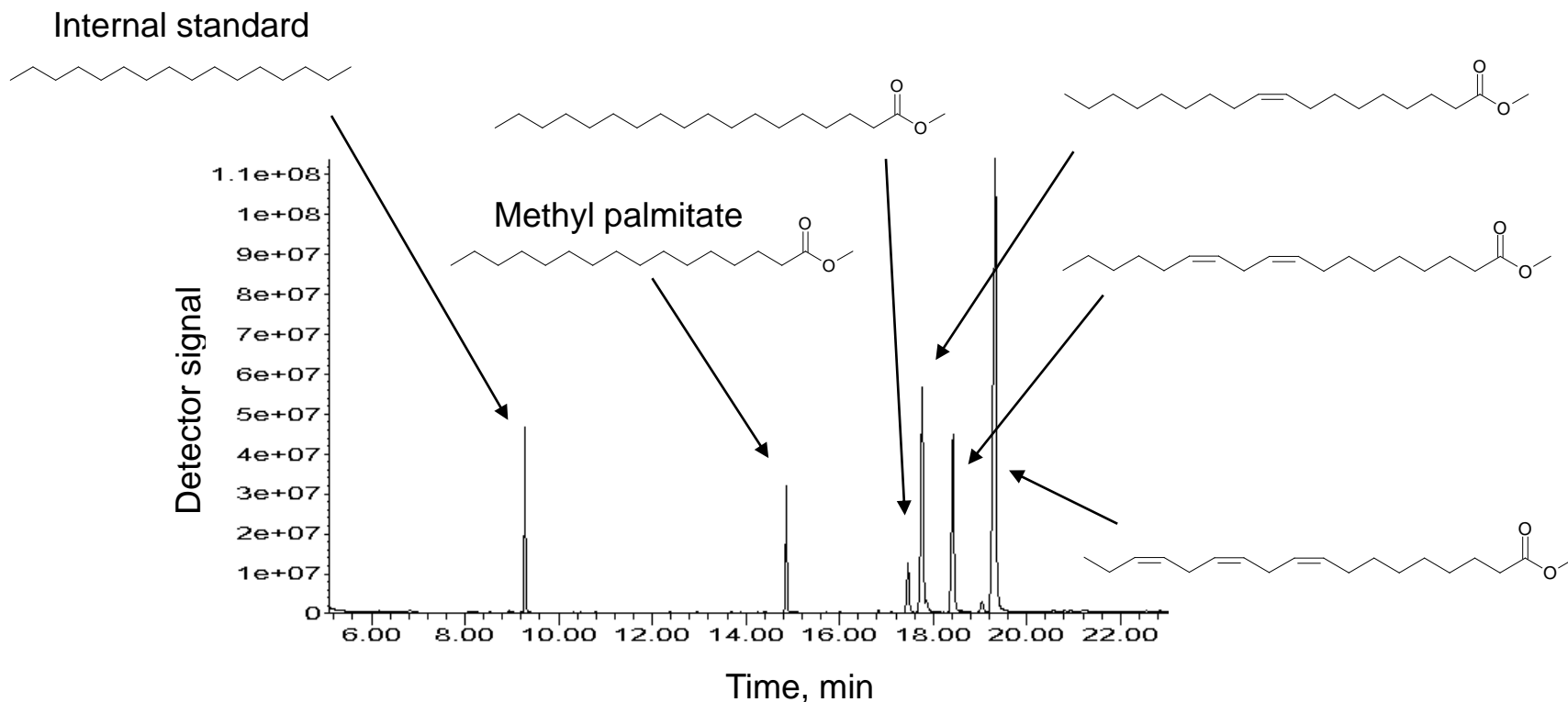
Quantification of compounds

- Quantitative analysis ...
  - is based on peak areas or heights.
  - requires calibration with standards.

Calibration graph

Chromatogram of methylated fatty acids of linseed oil

Internal standard

Detector signal

Time, min

Peak height h

Peak area S

18.00

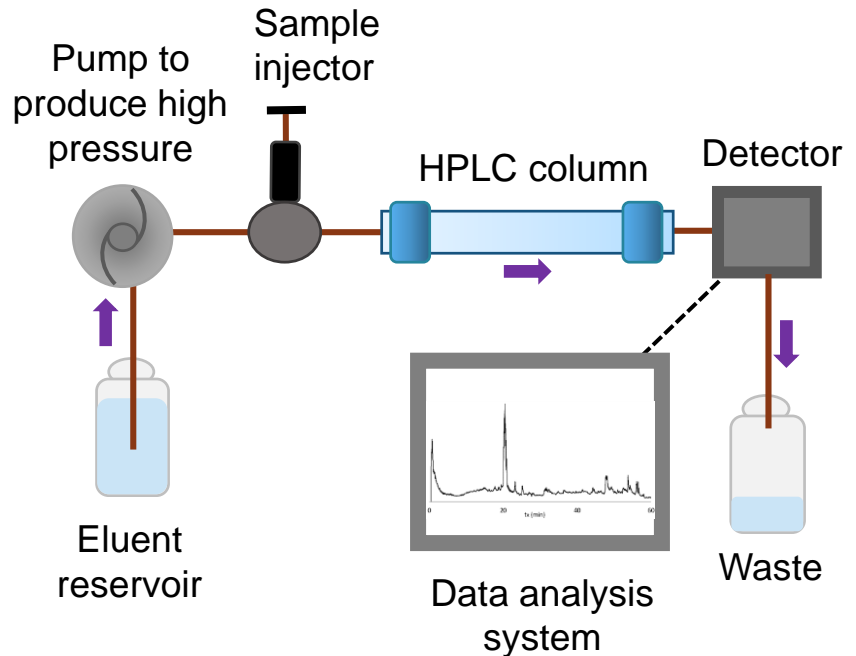| Component	Methyl linoleate
Retention time	18.427 min
Peak height	43927793
Peak area	1655315163
Content in linseed oil sample[1]	12.9 g per 100 g of sample

[1]Obtained from calibration graph.

Gas Chromatography

- Mobile phase is a gas (carrier gas)

- Probably the most efficient method for the separation of volatile compounds.
  - Boiling point < 500°C
  - Thermally stable

- Separation is mostly based on the difference in boiling points of the components.
  - Sensitive, rapid and reliable.

- Common detectors are mass spectrometric (MS) and flame ionization (FID) detectors
  - MS detector enables the identification of compounds

Flow/
pressure
control

Sample
injector

Detector

Data analysis
system

Column

Oven

Carrier gas
(He, N_2, H_2)
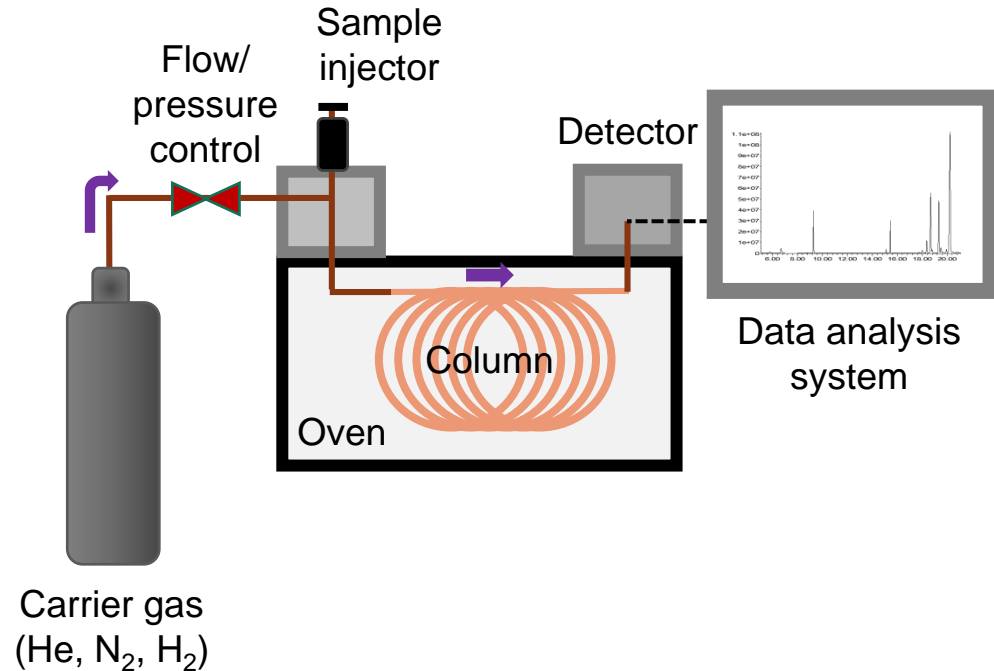
Liquid Chromatography

- Usually High Performance Liquid Chromatography (HPLC) is used

- Mobile phase is a liquid (eluent).
  - Analyte must be soluble.

- Column is densely filled with stationary phase, so high pressure is required to pump the eluent.

- Separation is based on the difference in polarity of the components.

- Common detectors are UV-Vis absorbance and mass spectrometric (MS) detectors
  - MS detector enables the identification of compounds

Pump to produce high pressure

Sample injector

HPLC column

Detector

Eluent reservoir

Data analysis system

Waste

Summary

Chromatography is a powerful method for **separating mixtures** into components.

The separated components are detected by a detector, which enables recording a chromatogram.

Both **qualitative** and **quantitative analysis** is possible.

The main methods for the analysis of cultural heritage materials are **Gas Chromatography (GC)** and **Liquid Chromatography (LC)**.