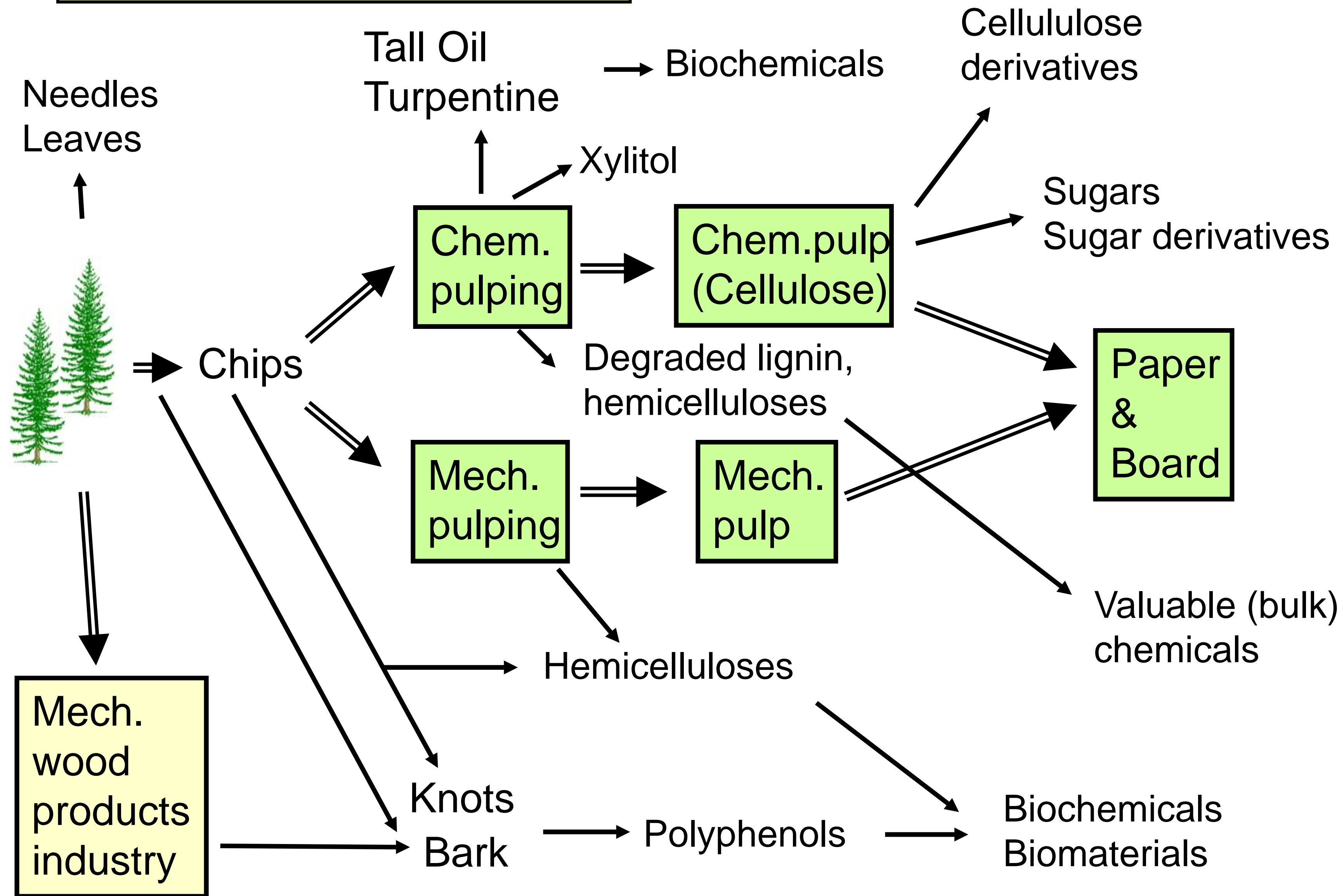
The Forest based Biorefinery: Chemical and Engineering Challenges and Opportunities

Chemical analysis in the biorefinery– Organics

Stefan Willför

Analytical methods

- To understand raw materials, processes, and products
- Already necessary for legislative reasons
  - Toxicity, environmental impact, product safety...
- Novel biorefining solutions ⇨ new analytical methods are needed


Many opportunities
Tall Oil
Turpentine
Biochemicals
Cellululose
derivatives
Needles
Leaves
Xylitol
Sugars
Sugar derivatives
Chem.
pulping
Chem.pulp
(Cellulose)
Chips
Degraded lignin,
hemicelluloses
Paper
&
Board
Mech.
pulping
Mech.
pulp
Valuable (bulk)
chemicals
Hemicelluloses
Mech.
wood
products
industry
Knots
Bark
Polyphenols
Biochemicals
Biomaterials

Which matrices / samples do we work with?

Solid plant materials: Wood, fibres, bark, needles, leaves…

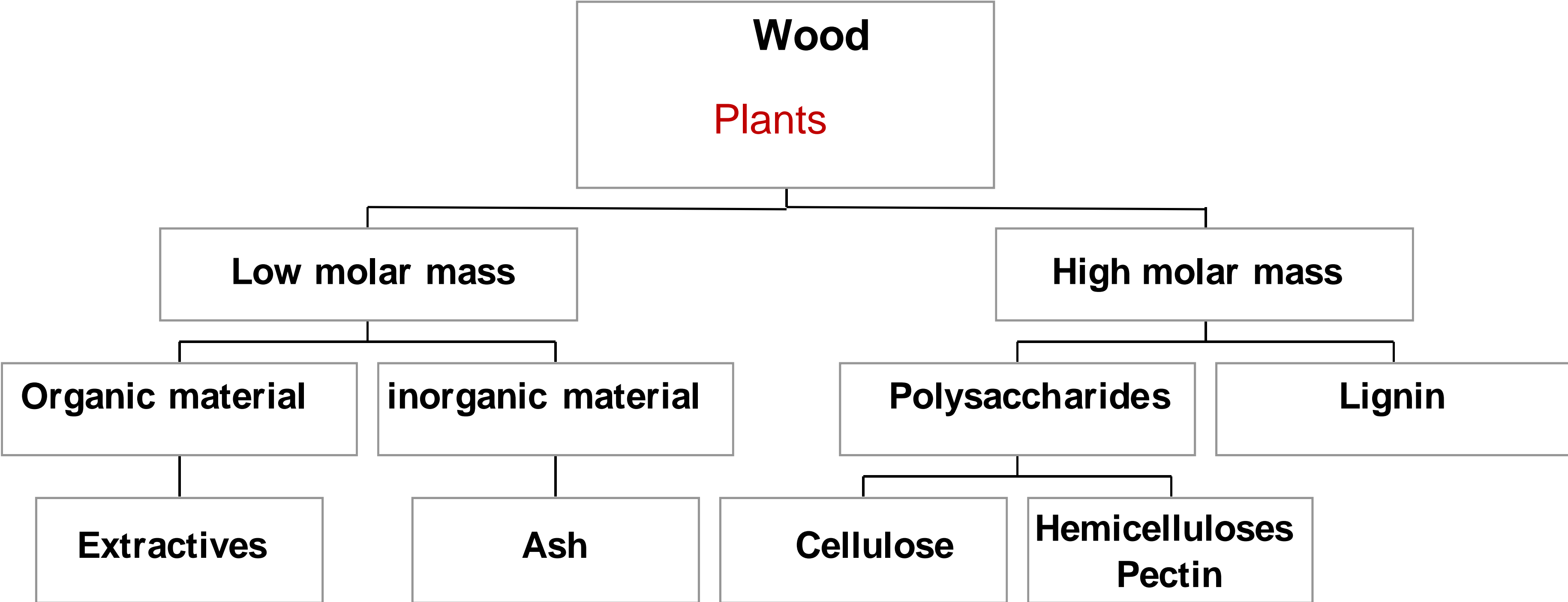
Fibre suspensions: Long fibres, fines, fillers, dissolved and colloidal material

Water effluents and process waters

Sludges

Air

Chemical compounds in wood and plants



VOCs

Proteins, tannins, cutin, starch...

Standard Methods

- Standard methods developed and critically evaluated by

  ISO, TAPPI, ASTM, CPPA, SCAN, DIN, Appita etc.

- Mostly physical tests, measurements and summative analyses

COD/BOD/TOC

- Oxygen demand (OD) is an important parameter for determining the amount of organic pollution in water
- Biochemical Oxygen Demand (BOD)
- Chemical Oxygen Demand (COD)
- Total Organic Carbon (TOC)

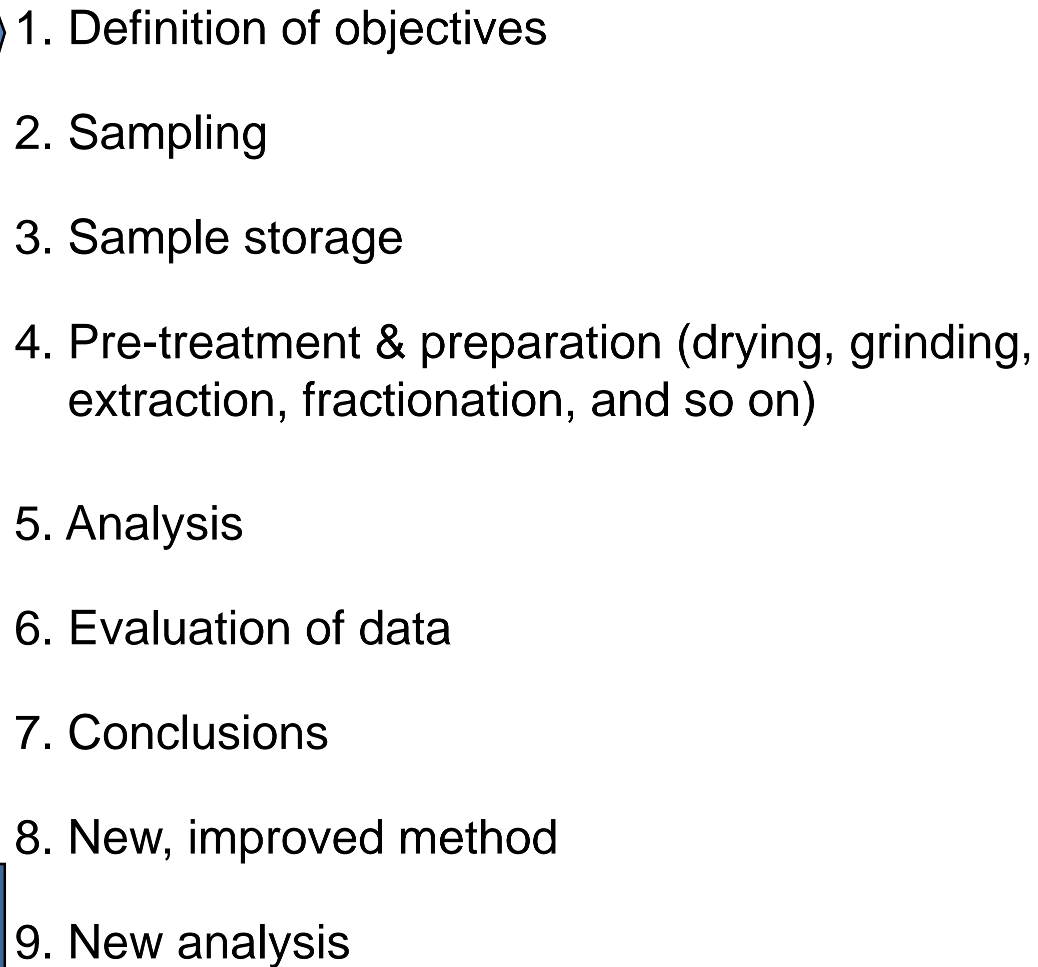
Analytical Process Studies

- Process steady-state, normal/abnormal states
- Process variations and dynamics

- Off-line analyses
- On-line measurements/analyses
- In-line measurements

- Need of automation of analyses, on all levels

Steps in an analytical determination



1. Definition of objectives
2. Sampling
3. Sample storage
4. Pre-treatment & preparation (drying, grinding, extraction, fractionation, and so on)
5. Analysis
6. Evaluation of data
7. Conclusions
8. New, improved method
9. New analysis

Definition of objectives

- What are your aims?
  - E.g. what information do we want to obtain, quantitatively or qualitatively, single compounds or compound groups…
  - Nice-to-know/part of a larger study with a specific aim/publication…?
- What are your resources?
  - Time, people, equipment, €?

Sampling

Proper sampling extremely important!

No analysis data is better than the sample it is based on !

If possible, take the samples yourself!

When? Time of year?
Where? Forest/plantation/wood yard/debarking station? Height from ground?
How? Amount, storage, packaging material?
Representative samples
No chemical or physical changes

Sampling and sample characteristics (height and age of tree and/or sample, soil, place in forest, wind direction…)
(or process data during the sampling)

Quantitative analyses: Sample can be frozen
Physico-chemical analyses: Fresh samples

Sample storage

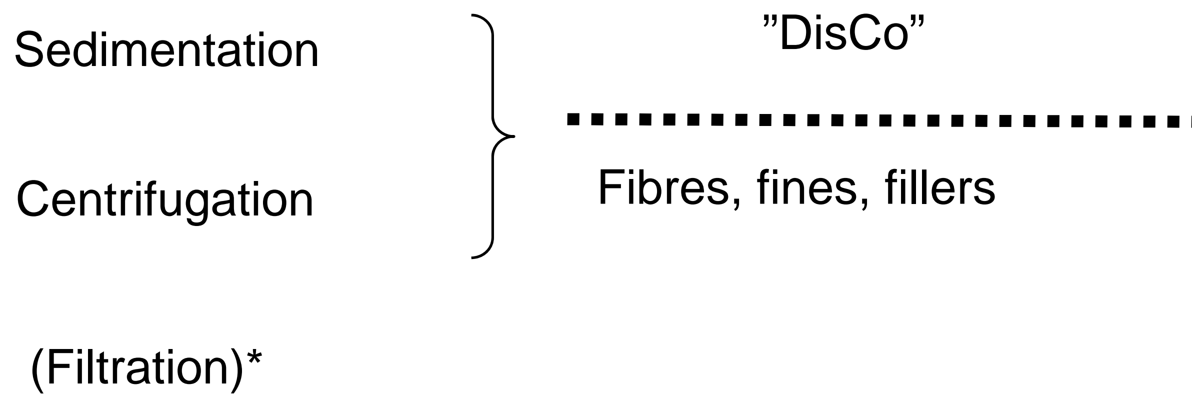
Short storage times: Cold room

Long storage times: Freezer

Very sensitive materials can even be
frozen in liquid nitrogen and deep-freezed (-80°C)

Is it reasonable to first dry (freeze-dry) the sample?

Heterogeneous mixtures, like fibre suspensions should be fractionated before freezing



*Not the best way of separation. Why?

Pretreatment and preparation

For example:

- drying
- grinding
- extraction
- fractionation

Dry matter content

Important for calculating the analysis results

Drying in oven at 105ºC, weighing before and after

Special balances with an IR lamp (requires more sample)

Air- or oven-drying

- When air-drying a sample, the risk for oxidation of sensitive compounds is enhanced
- Oven-drying at modest temperatures can in some cases be ok (fast), but avoid elevated temperatures
- Note: hydrophilic extractives and sugars can form an impenetrable layer when air-dried long times!
  - Subsequent extractions may not work

Freeze drying

Vacuum

Low temperatures

Freeze drying is used as an alternative drying method to avoid physical and chemical changes in the material that may occur during normal drying

Frozen water is removed from the material through sublimation, i.e. ice is directly transformed into gas under vacuum

Grinding

- Solid samples usually need to be finely divided to ensure complete solvent penetration and extraction
- Some risks associated with grinding:
  - The grinding equipment can get very hot
  - Loss of volatile compounds
  - Contamination
  - Loss of sample
  - Oxidation, polymerization, radicals…

Extraction

Why extract?

To extract:

To separate or obtain from a mixture by pressure, distillation, treatments with solvents, or the like.

Ideal extraction:

Rapid, simple, inexpensive, quantitative, no waste, sample ready for analysis

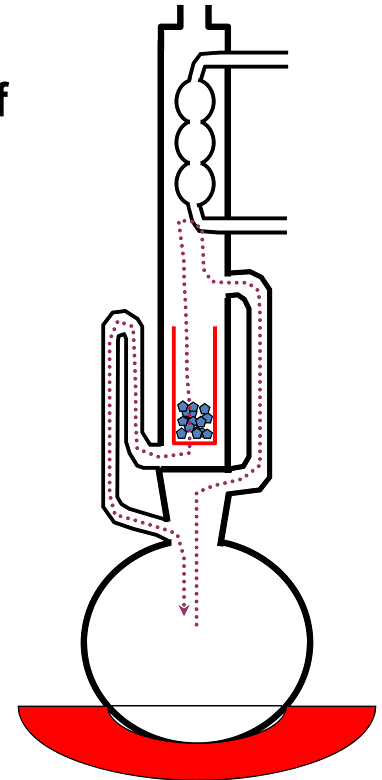
Solid samples (should be finely divided first): Soxhlet, Reflux, SFE, ASE, Ultrasonic bath/bar, PHWE…

Liquid samples: Separation funnel, SPE…

Soxhlet

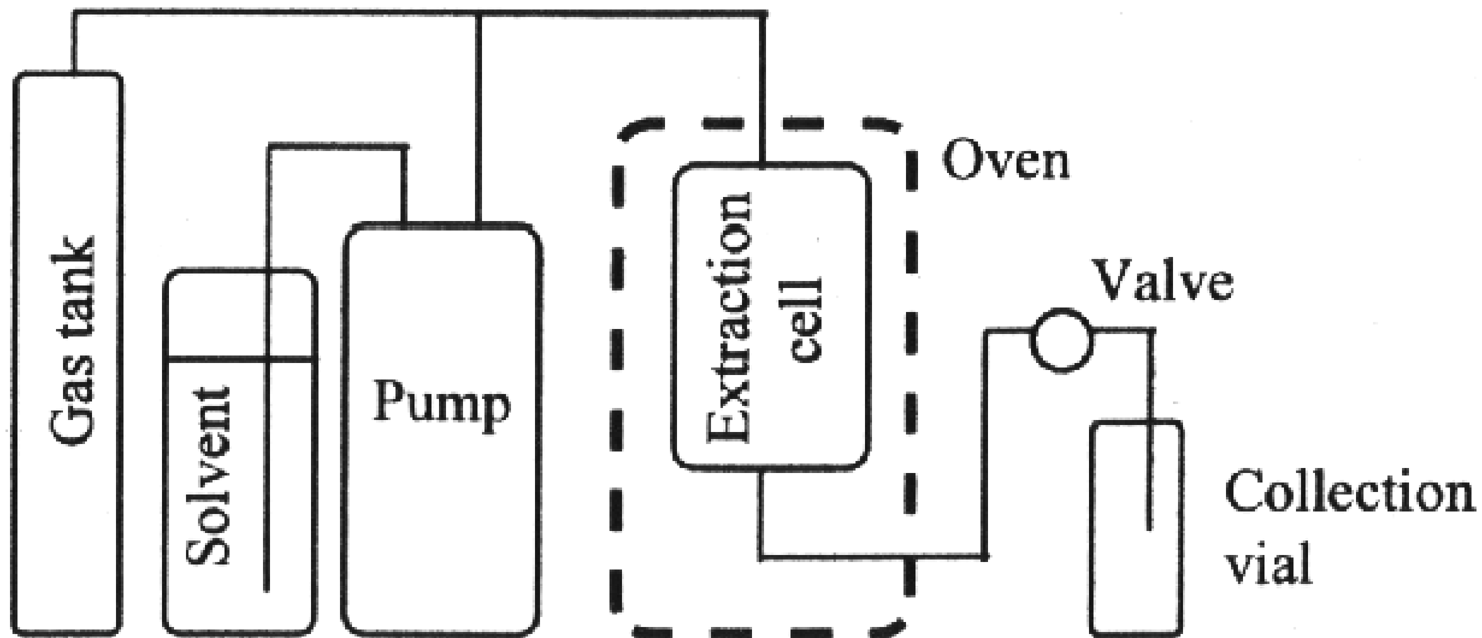
Method for continuous extraction of analytes from a solid into an organic solvent.

As the flask containing the solvent is heated, vapours rise in the larger outside tube, enter the water-cooled condenser and reliquify. When the liquid level in the extractor reaches the top of the bent tube, siphoning action returns the extract-enriched solvent to the flask. High extraction yield: >15 extraction cycles



Accelerated solvent extraction (ASE)

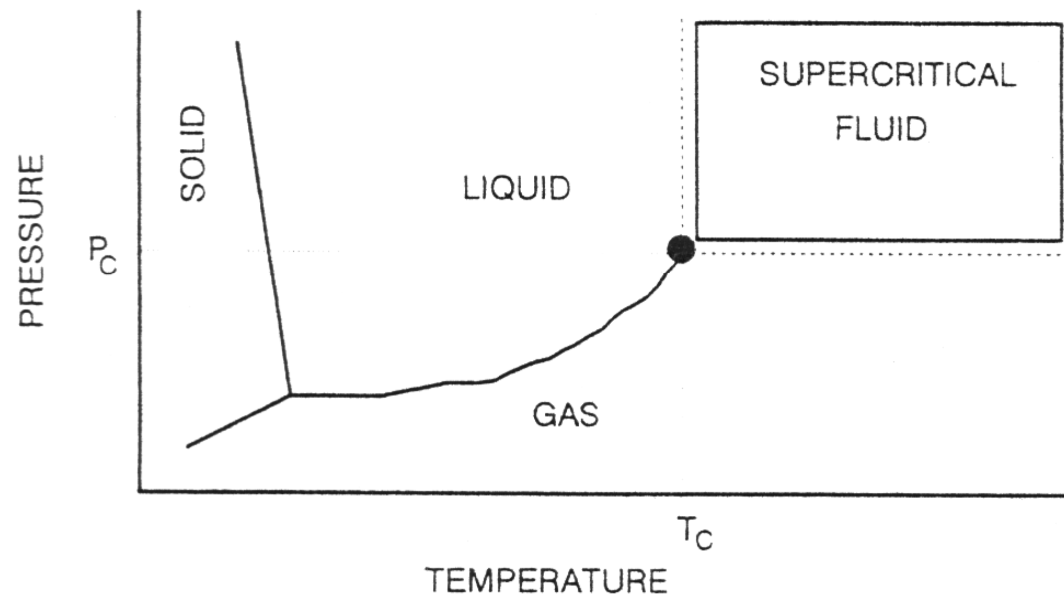
Accelerated solvent extraction (ASE) or enhanced solvent extraction (ESE) uses conventional organic solvents at high temperature under pressure. Increased temperature accelerates the extraction kinetics, while elevated pressure prevents boiling at temperatures above the normal boiling point.



Supercritical fluid extraction (SFE)

SFE: A fluid that is raised above its critical temperature (T_c) and pressure (P_c). Has the penetration and transport properties of a gas but acts like liquid when dissolving analytes from a matrix.

Critical point: The point at which a substance in one phase has the density, pressure and temperature as in another phase.



Ultrasonic Processor

Power acceleration of mass-transfer processes due to disintegration and microflow stream. Can be used for extraction purposes.

Electrical energy is converted to mechanical vibrations of ultrasonic frequencies. The vibrations cause pressure waves in the liquid which form millions of small bubbles. The expansion and implosion (cavitation) of these bubbles produce strong shear forces which intensely agitate the molecules in the solution

Common solvents

- Tetrahydro furan (THF) (boiling point 66 ºC) – lipophilics and hydrophilics, mixable with water, contains stabilizing agents!
- Acetone (56 ºC) - lipophilics and hydrophilics, also mono- and disaccharides
- Ethyl acetate (77 ºC) – for extraction of semipolar substances from water, not good for lipophilics
- Chloroform (61 ºC) – lipophilics and fatty acid calcium soaps
- Dichloro methane (DCM) (40 ºC) – lipophilics and fatty acid calcium soaps
- Diethyl ether (35 ºC) – lipophilics, volatile solvent, contains stabilizing agents!
- Methyl tert-butyl ether (55 ºC) – as DCM but also lignans (to some extent)
- Hexane (69 ºC) – selective for lipophilics, requires dry samples
- Petroleum ether (variable ºC) – as hexane

Extraction of process waters

- Liquid - liquid
- Sorption - elution on sorbents (RP, XAD)
- Freeze-drying and extraction

Extraction of dissolved and collodial components from water

- Liquid / liquid (non-miscible solvent)
- Sorption to absorbent & eluetion from it
  - RP-sorbent
  - Polymer (XAD-type: polystyrene- eller acrylate resin)
  - Silica
  - Active carbon
- Solid-Phase MicroExtraction (SPME)
  - Silica fibre coated with polymer resin
  - Dipped in the solution
  - Injection directly into GC (or HPLC)
- Drying and extraction (freeze-drying!!)

Liquid / liquid extraction

Solvent requirements

- Non-miscible with water (phase separation)
- Better solubility for comp. in this than in water)
- Boiling point 35 – 80°C
- Not too expensive
- Nontoxic

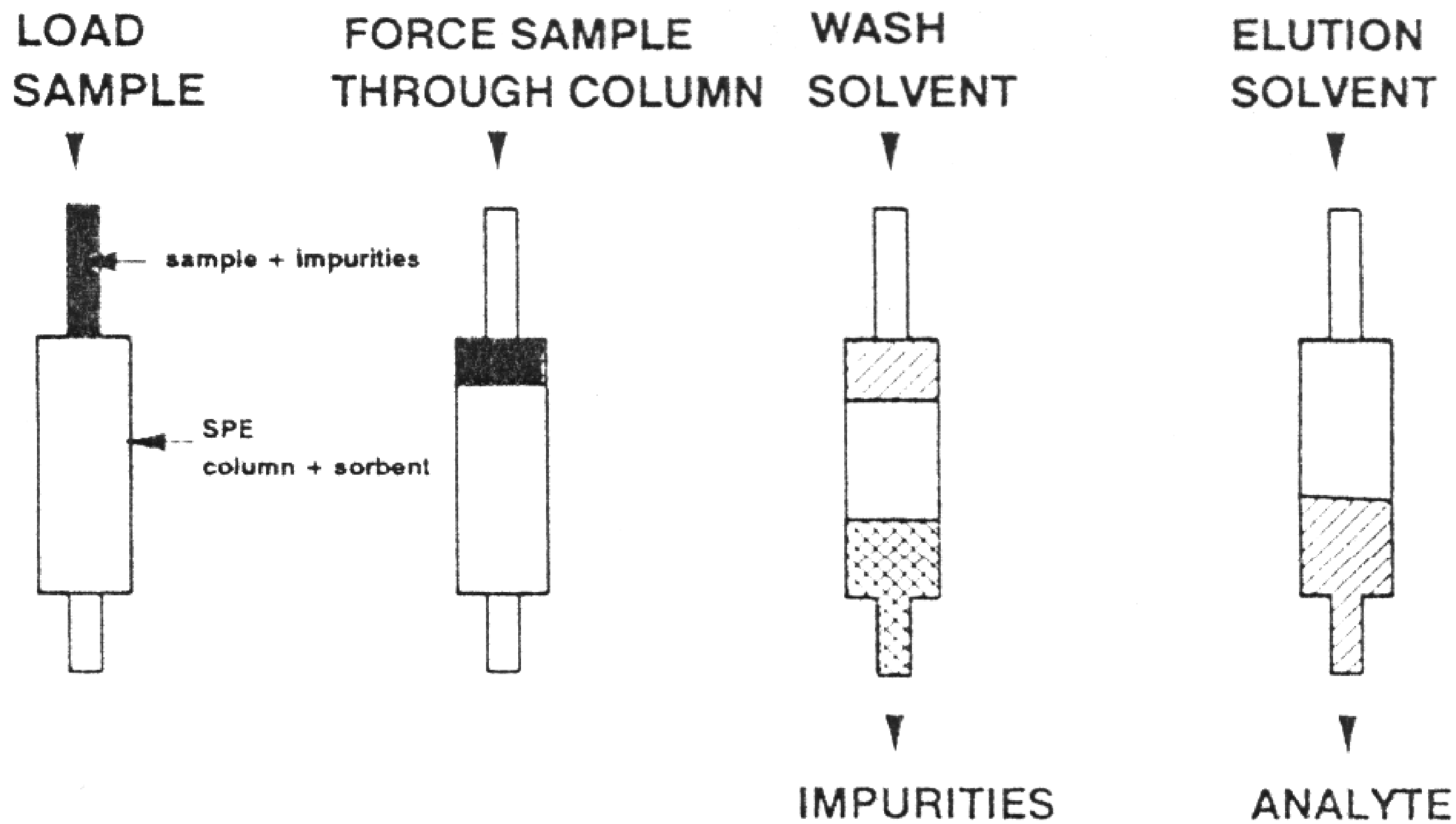
Exemples: Hexane / Petroleum ether
Diethyl ether
Dichloromethane
Methyl tert. butyl ether (MTBE)
Ethyl acetate

Liquid / liquid extraction

- Separation funnel
- Test tube (centrifugation possible)

- Adjust pH ?
- Add salt ?

Solid Phase Extraction (SPE)



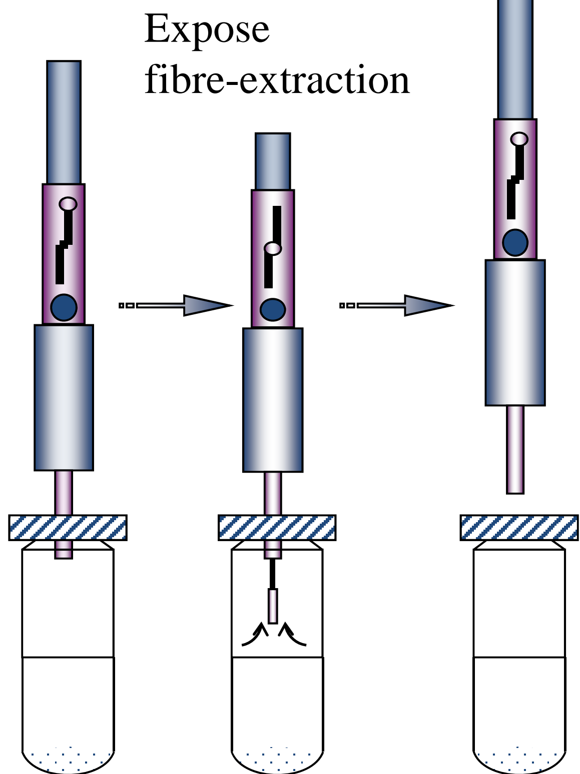
Solid-Phase Microextraction (SPME),
for volatile substances

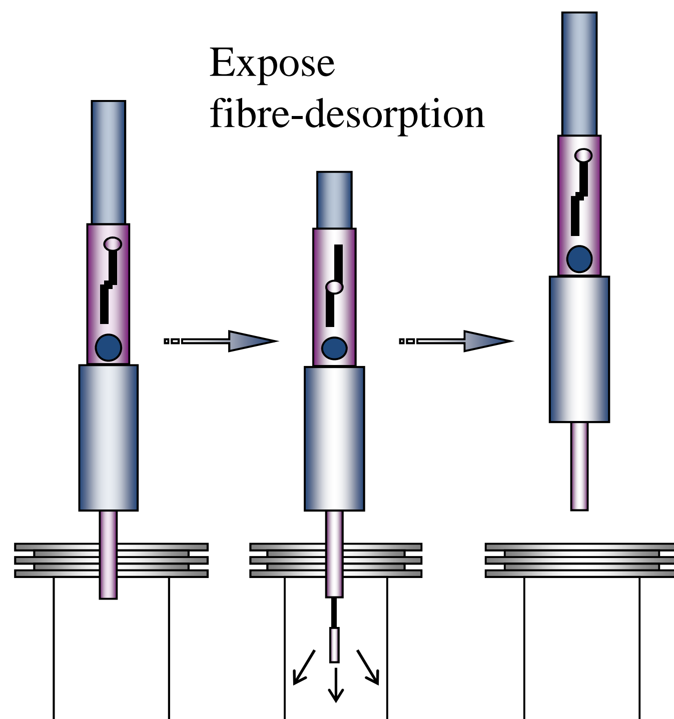
integrates

- **sampling**
- **extraction**
- **concentration**
- **and sample introduction**

into a single step

Solid-Phase Micro Extraction (SPME)





Chromatography

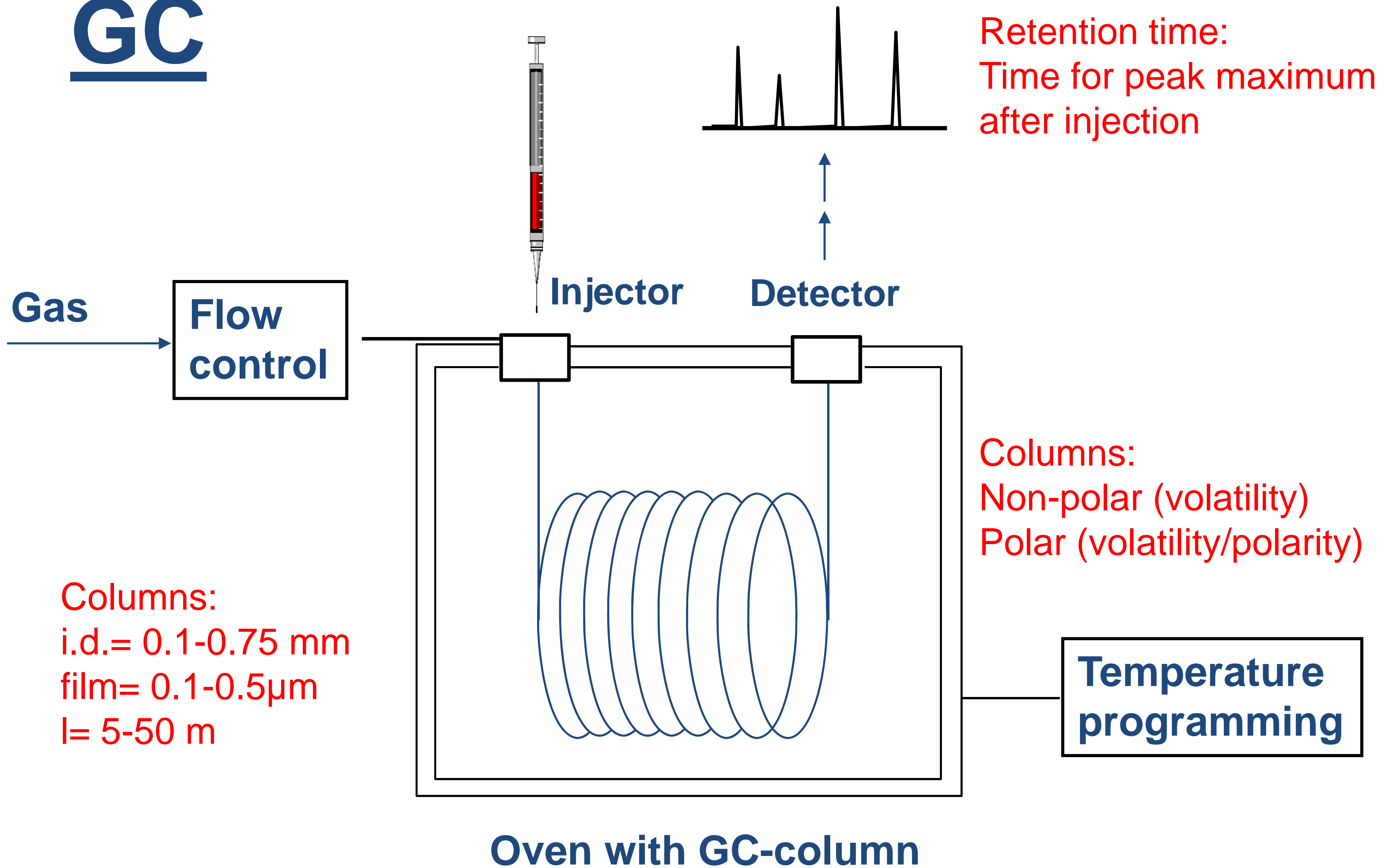
IUPAC (1993):

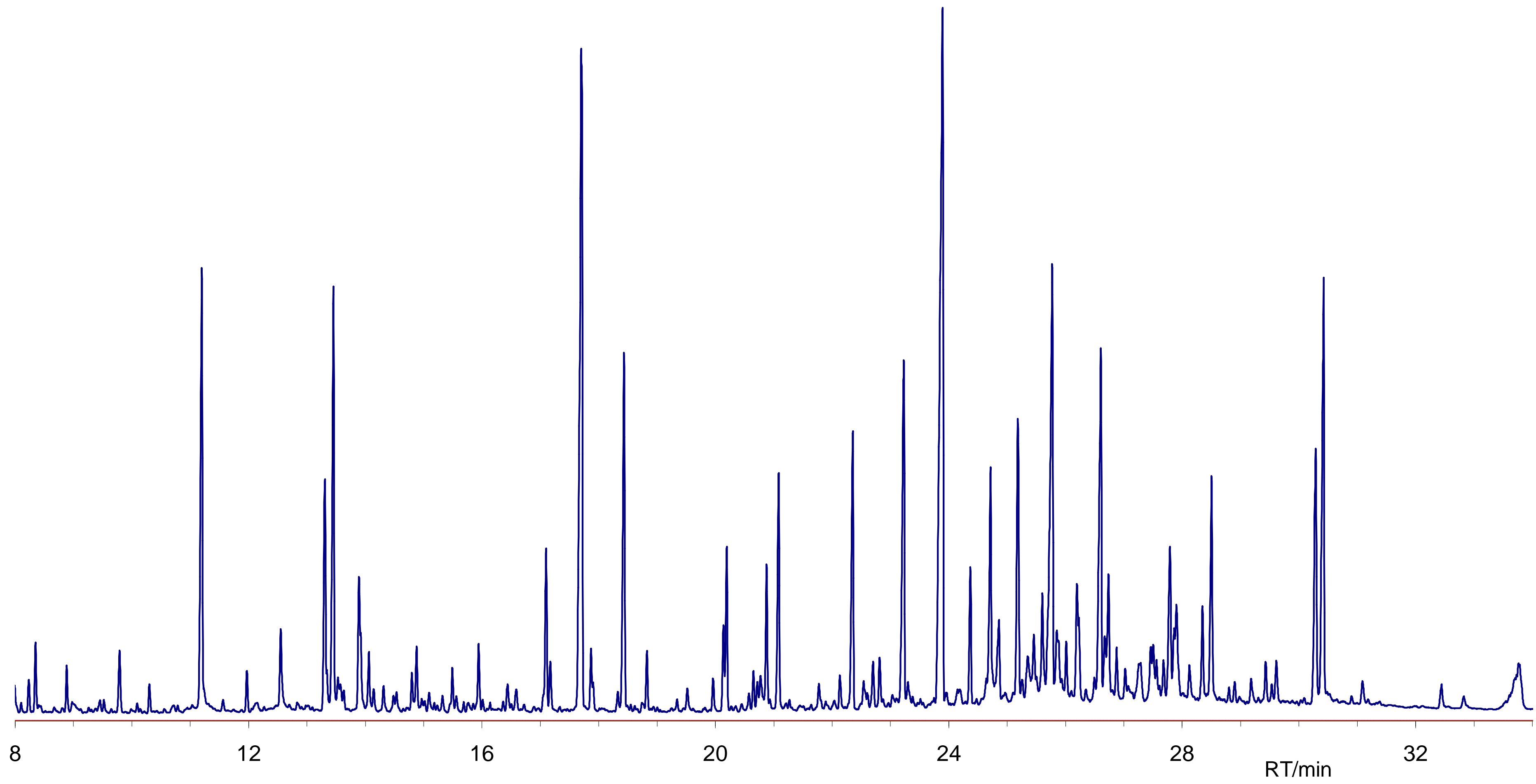
Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the other moves (mobile) in a definite direction.

Gas Chromatography (GC)

Qualitative and quantitative determination of volatile organic components, such as extractives, hemicellulose building blocks, organic acids etc.

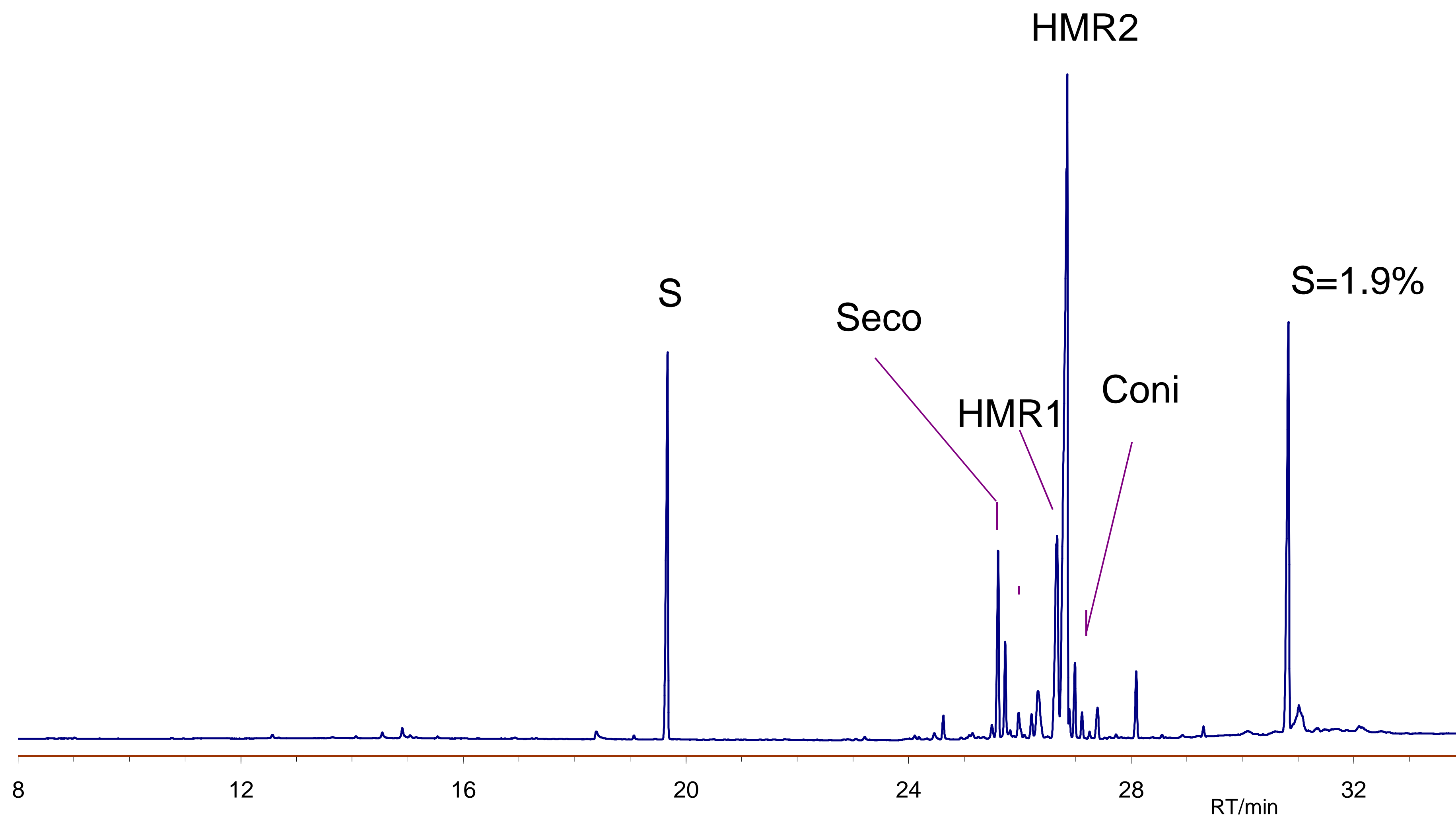
The derivatized and vaporized products are introduced to the column for separation and identified in a detector, whose response is recorded as a chromatogram.


GC
Retention time:
Time for peak maximum
after injection
Injector
Detector
Gas
Flow
control
Columns:
Non-polar (volatility)
Polar (volatility/polarity)
Columns:
i.d.= 0.1-0.75 mm
film= 0.1-0.5µm
l= 5-50 m
Temperature
programming
Oven with GC-column



Dammar resin

Picea abies



GC-detectors

- **FID (Flame Ionisation Detector) for hydrocarbons**

- **GC - MS**

- **ECD (Electron Capture Detector) for halogens**

- **N, P-FID for samples containing nitrogen and phosphor**

- **FPD (Flame Photometric Detector) for S & P**

DERIVATIZATION

GC and GC-MS analysis in the vapour phase require volatile derivatives that do not adsorb onto the column wall

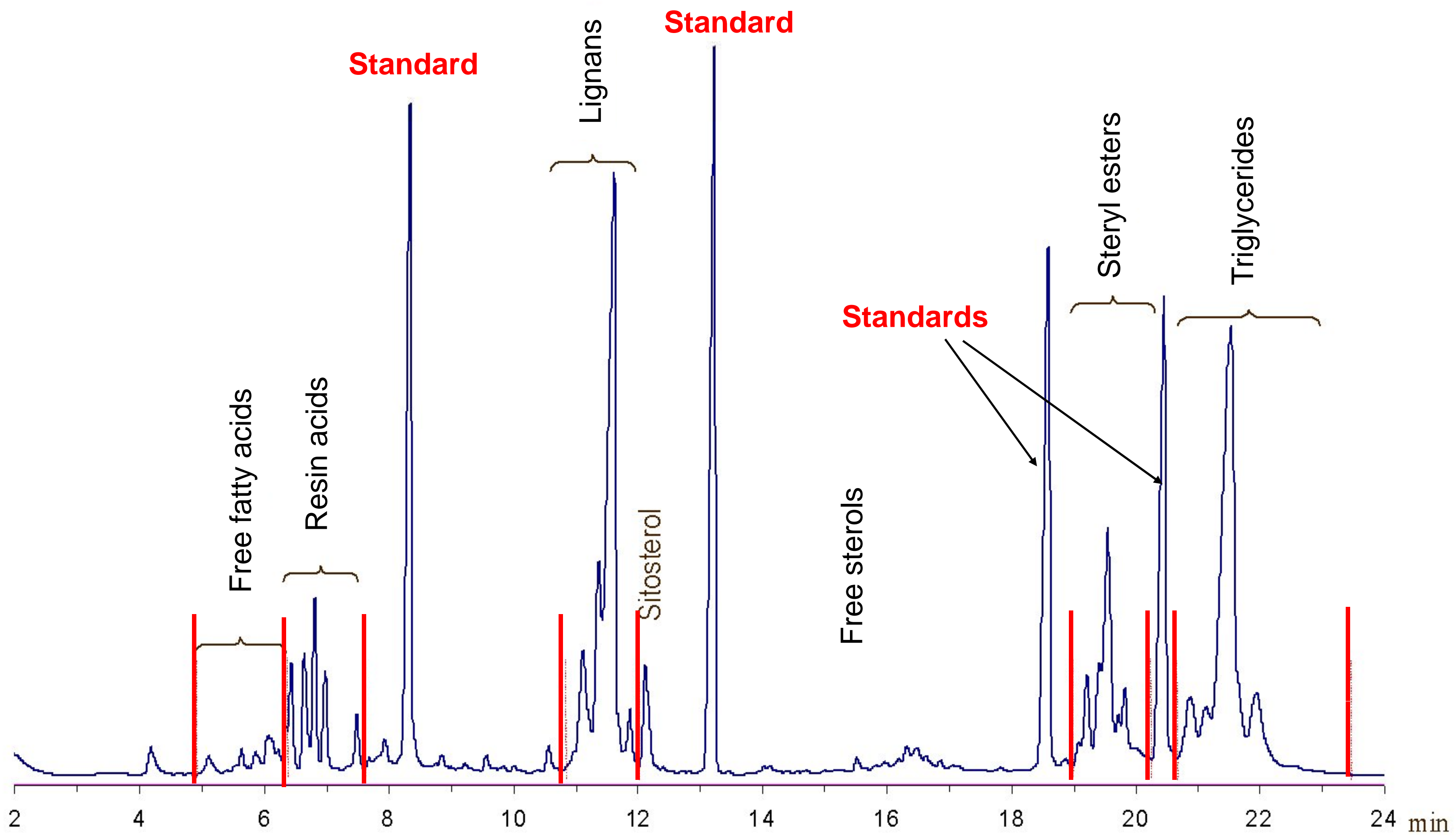
Different derivatizations for different substances, e.g. silylation or methylation for extractives, methanolysis and silylation for carbohydrates

Silyl derivatives are formed by the displacement of the active proton in -OH, -NH and -SH groups. Thus, protic sites are blocked, which decreases dipole-dipole interactions and increases volatility

Trimethylsilyl group (TMS)

Demand on standards:

- ➔ **Same compound NOT present in the sample itself**
- ➔ **Chemically similar to the sample**
- ➔ **Retention time close to the sample compounds**
- ➔ **No peak overlapping**


Standard
Lignans
Standard
Steryl esters
Triglycerides
Standards
Free fatty acids
Resin acids
Sitosterol
Free sterols
2
4
6
8
10
12
14
16
18
20
22
24
min

Benefits with GC

- Very high resolution
- Fast analyses (even less than 5 min)
- Accurate quantification, based on internal standards
- Can be combined with a mass spectrometer (MS), to get separation, quantification & identification
- Amounts down to the nanogram scale can be analysed
- Automation (injection and analytical run)
- Small sample mixtures, from, e.g. TLC & HPLC can be analysed
- Universal and also Selective detectors

Limitations with GC

- Only molecules up to about 1000 mass units
- Compounds must be stable at high temperatures
- Polar compounds must be derivatised
- Samples must be processed/pretreated before analysis
- Demands knowledge of the instrument

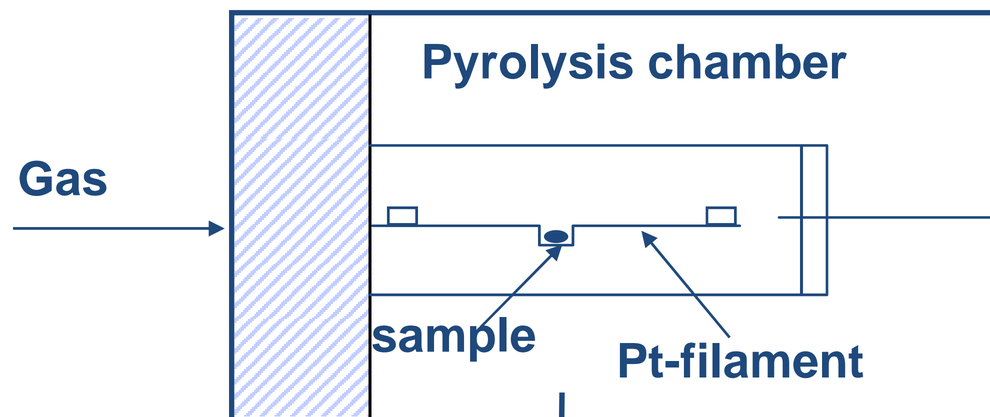
Pyrolysis-GC-MS

Qualitative and quantitative determination of:
semi-volatile and non-volatile components, such as extractives, polymers, deposits, etc.

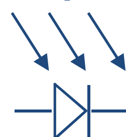
The sample is thermally degraded in an inert atmosphere. The degradation products are introduced to to GC or GC-MS for separation and identification.

Py-GC

1. Pyrolysator



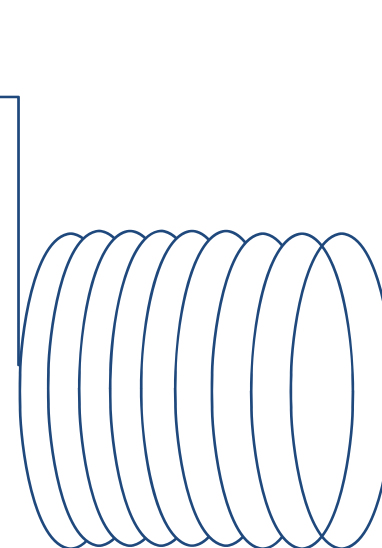
Optical cable



Photodiode

3. Detection

(FID, MS, etc.)



2. GC-column

Pyrolysis – GC - MS

- No sample treatment – fast method
- Small amounts: about 0.1 mg)
- Good separation of fragments
- All fragmentation patterns not understood

Liquid Chromatography (LC)

- Normal phase
  - Mobile phase an organic solvent
  - Stationary phase: silica, Al-oxide

Mainly for lipophilic (low-polar) compounds
Most common form of TLC

- Reversed phase
  - Mobile phase: water and/or solvents miscible with water (MeOH-water, or MeCN-water)
  - Stationary phase: Liquid film, chemically bound to silica

Non-ionized compounds,soluble in polar solvents

Liquid Chromatography (LC)

- Ion-exchange chromatography (IEC)
  - Mobile phase: water, buffered to control pH
  - Stationary phase: ionic resins

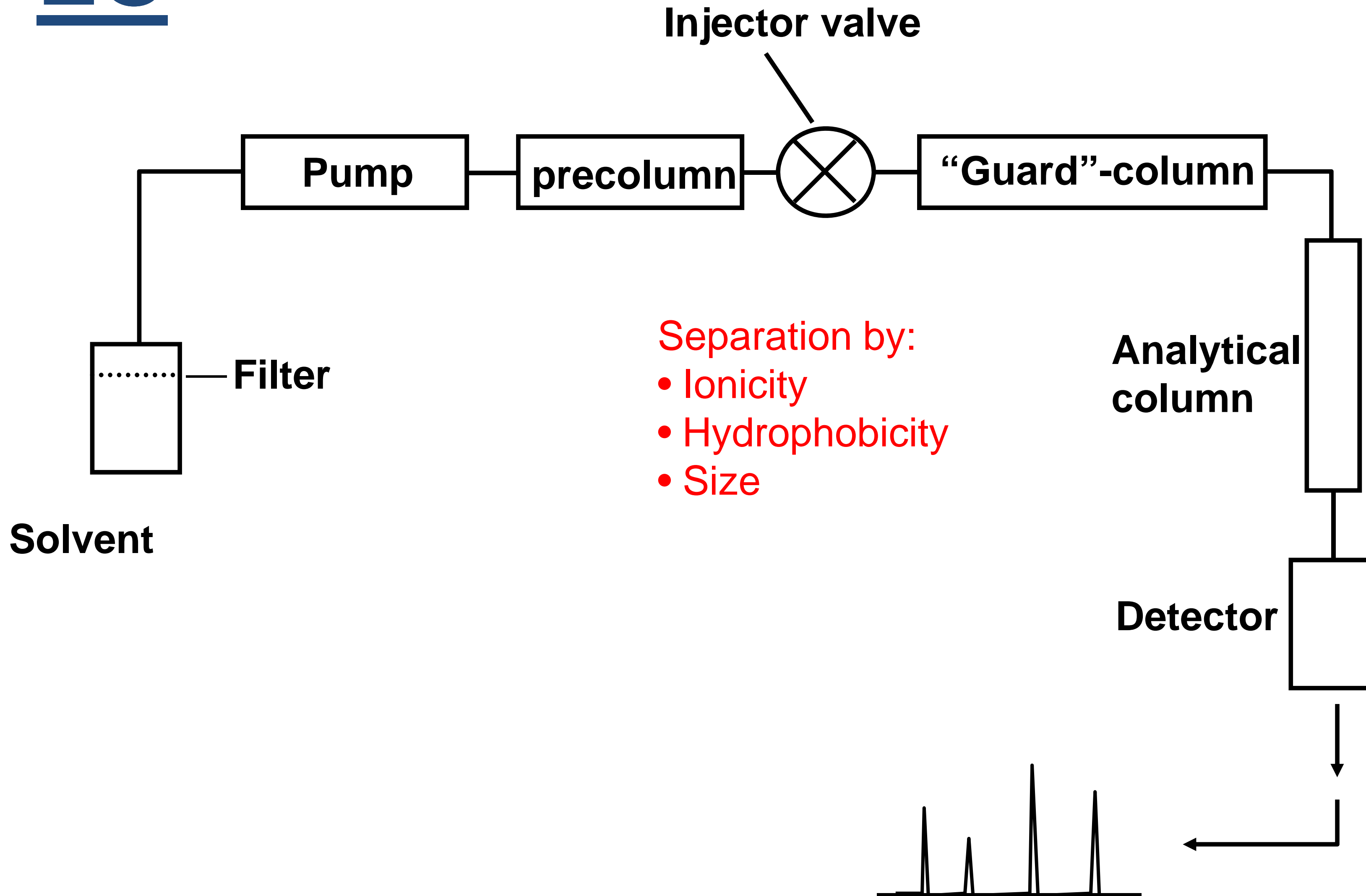
For example: Proteins, polymers, inorganic ions (ion chromatography (IC), water-soluble ionisable compounds

- SEC (Size-exclusion chromatograpy) (earlier GPC)
  - Mobile phase: Water or THF
  - Stationary phase: Porous resins

  Separation according to hydrodymamic size

  Polymers, oligomers

LC



LC-detectors

- UV-Vis & Diode array for conjugated & aromatic compounds, e.g. phenols

- Refractive index (RI) for, e.g. carbohydrates

- Fluorescence

- Mass detectors. Good & sensitive

- Multi Angle Laser Light Scattering (MALLS)

- NMR

Different MS techniques

- Single quadrupole (this is also the most common GC-MS detector)
- Triple quadrupole = tandem MS, MS/MS - for quantitative analysis, filters ions
- Ion trap – mainly for qualitative analysis, i.e., structural determination
- Time-of-flight (TOF) – for structural determination, gives exact mass (precision four decimals)

LC-MS *vs* GC-MS, advantages

- No derivatisation of non-volatile compounds is needed
- Polar and thermally unstable compounds can be analysed as such
- GC-MS MW max. approx. 750, triple-quadrupole and ion trap-LC-MS normally up to *m/z* 3000, TOF instruments up to *m/z* 16.000
- Better sensitivity and selectivity, LC-MS is therefore excellent for the quantification of selected substances in complex mixtures (LC-MS is widely used in drug analysis)

LC-MS *vs* GC-MS, some disadvantages

- LC-MS is not very suitable for rapid and reliable identification of unknown substances
- Identification of unknown compounds is difficult as the fragmentation is sparse because of the mild conditions during ionisation
- No spectra libraries are available that would enable identification

LC-MS *vs* GC-MS, disadvantages

- Some compounds are not ionised or fragmented at all
- Sometimes a time-consuming work is required, including testing of the most suitable eluent, column, and ionisation parameters
- LC-MS instruments are expensive compared to GC-MS instruments (200-400 k€ *vs* 40-50 k€)

Potential use of LC-MS

- Oligosaccharides
- Lignans, flavonoids, stilbenes
- Oligolignans
- Lignin (fragments)
- Tannins

FLASH CHROMATOGRAPHY

A fast, safe and easy way to separate individual organic compounds in gram-scale from complex mixtures of *e.g.* wood extractives and synthesis products.

The separation is achieved by forcing the sample through a packed column by an eluent (solvent mixture) under pressure.

Thin Layer Chromatography (TLC)

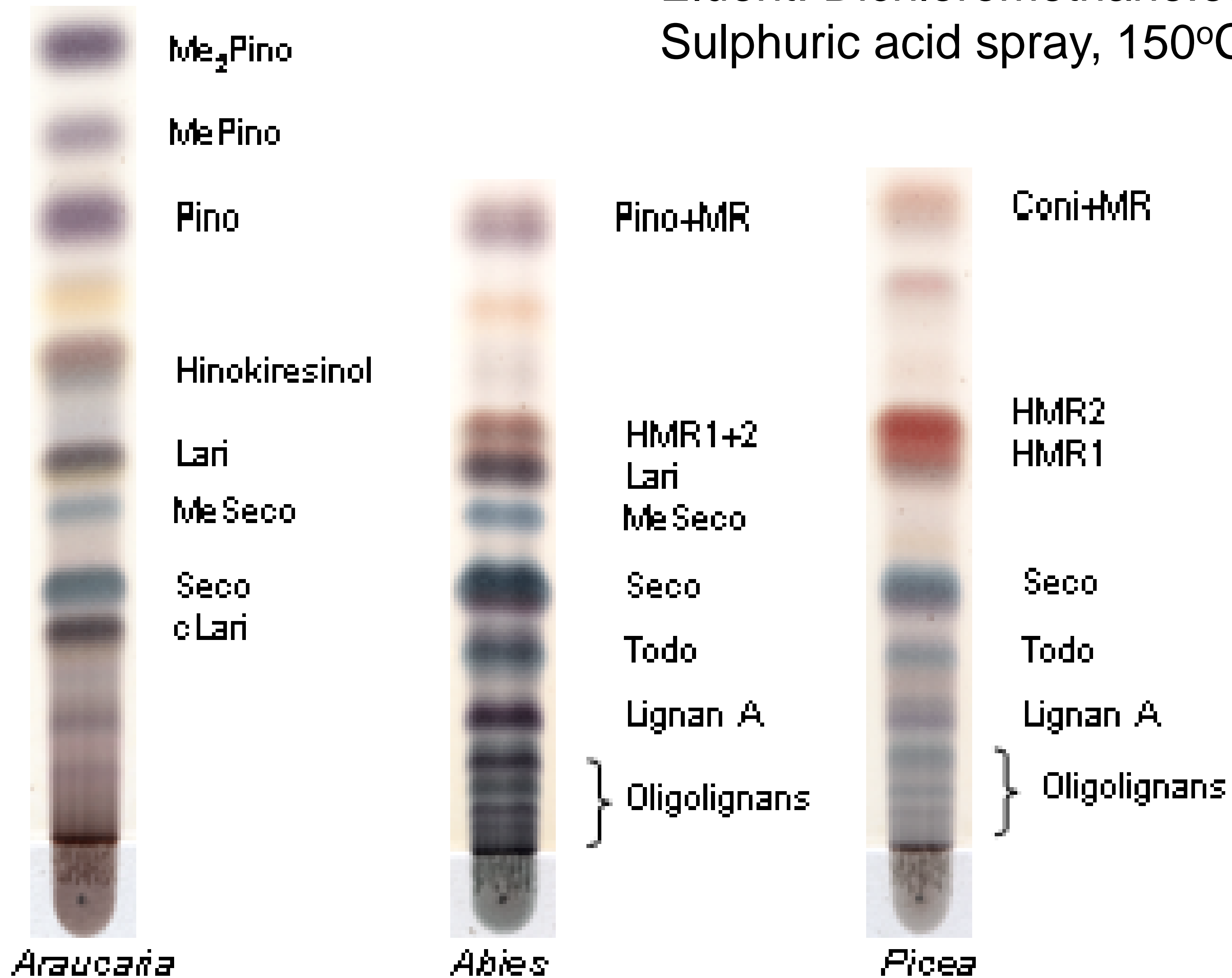
Fast, simple and cheap qualitative and semi-quantitative determination of semi-volatile and non-volatile compounds. Preparative separation for further analysis.

An eluent and the analytes rise in the stationary phase due to capillary forces. The analytes are separated according to their affinity to the stationary phase (most commonly: silica)

Aluminium or glass plates coated with a silica layer (different thickness for different purposes).

TLC of ethanol extracts of knots

Silica plates
Eluent: Dichloromethane:ethanol 93:7
Sulphuric acid spray, 150°C, 3 min



UV-VIS spectrophotometer

Simple, sensitive and non-destructive qualitative and quantitative determination of chromophore-containing components in gas, liquid and solid phases.

Photo absorption measurement by comparison of the intensity of narrow beams of light coming through two channels, one with and one without sample. Sample scanning with different wave lengths of light gives a UV/VIS-absorption spectrum.

Some other analytical methods

- Capillary electrophoresis (CE)
- Supercritical fluid chromatography (SFC)
- Infra Red spectrometry (IR, FTIR)
- Nuclear Magnetic Resonance (NMR)
- Flow Cytometry
- Near Infra Red Spectrometry (NIR)
- Elemental composition
- X-Ray based methods

Carbohydrate analysis of plant cell wall material

Stefan Willför, T. Tamminen, J. Puls, C. Laine,
A. Suurnäkki, B. Saake, K. Uotila, H. Simolin,
J. Hemming, A. Pranovich, B. Holmbom

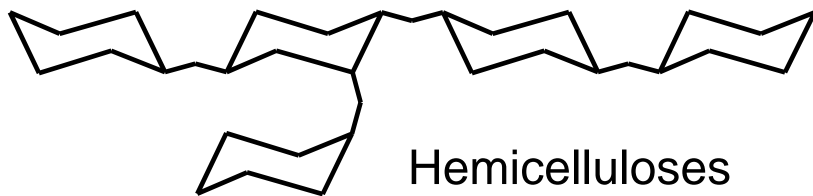
Materials

- Oat spelt
- Wheat straw
- Spruce TMP
- Aspen stemwood
- Bleached birch kraft pulp

Methods

- In-house methods

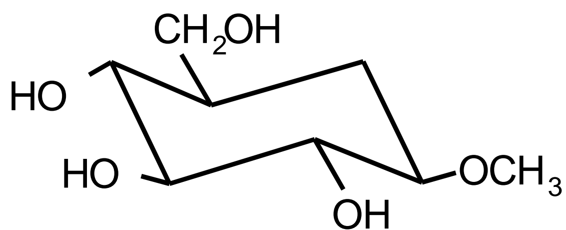
- Acid hydrolysis
- Acid methanolysis
- Enzymatic hydrolysis
  - Works only on delignified samples

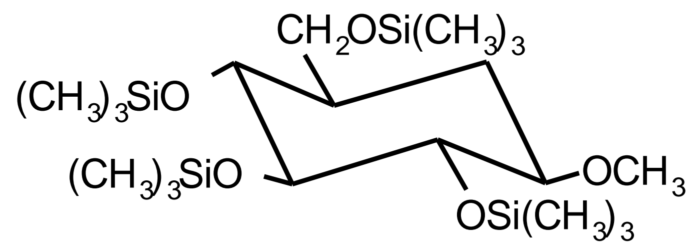


Hemicelluloses

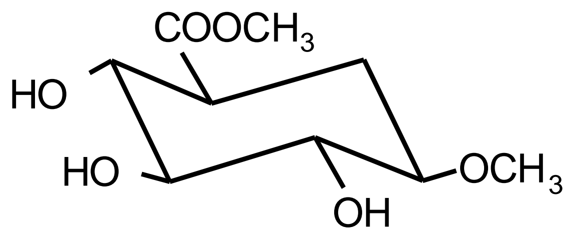
GAS CHROMATOGRAPHY

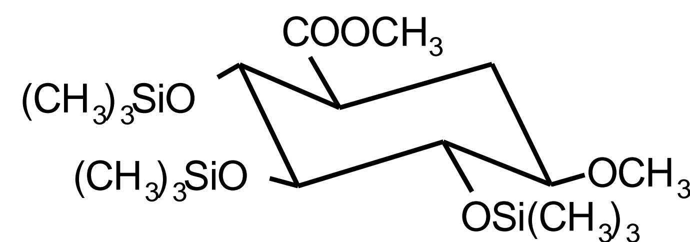
METHANOLYSIS





SILYLATION

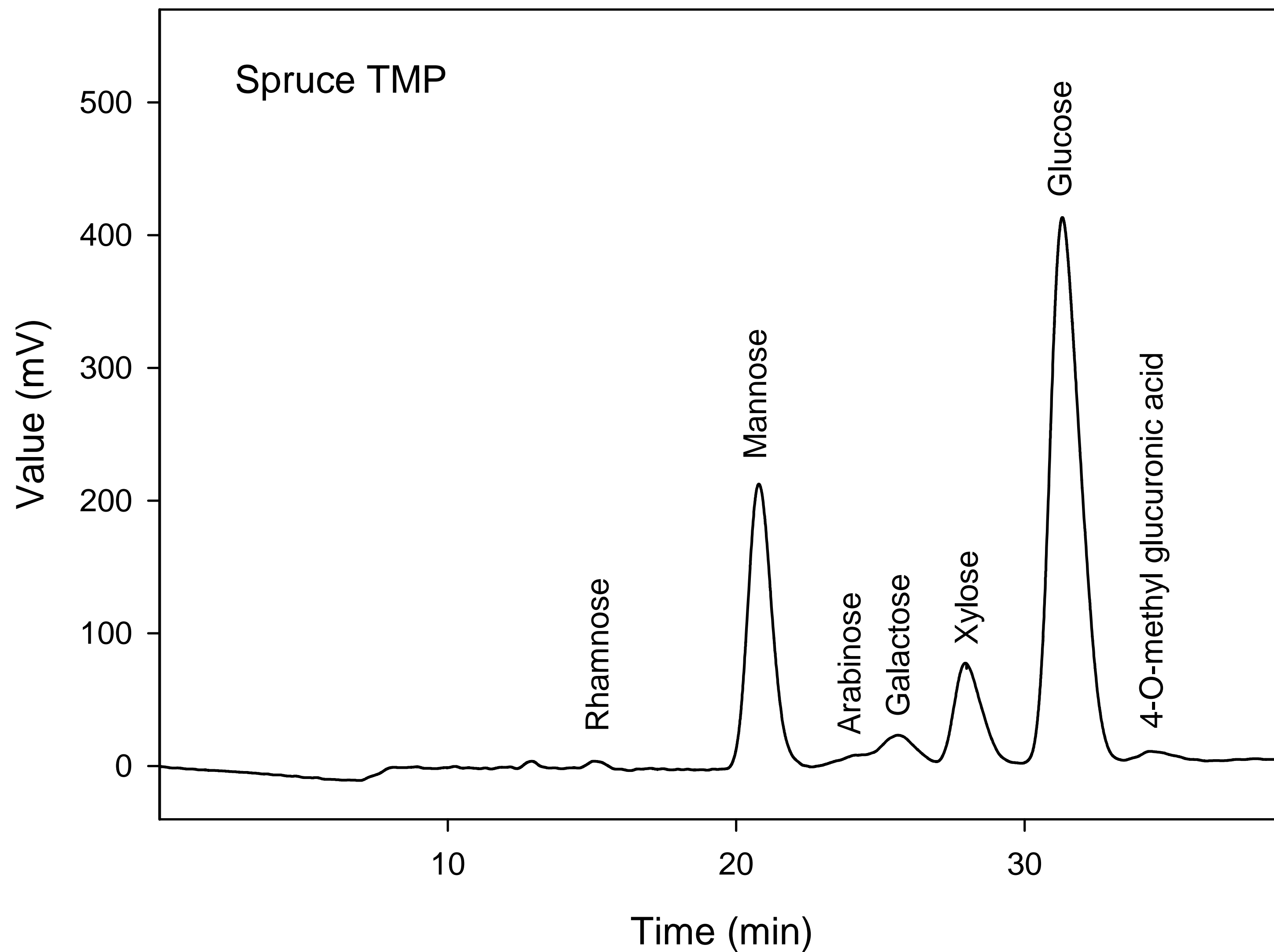




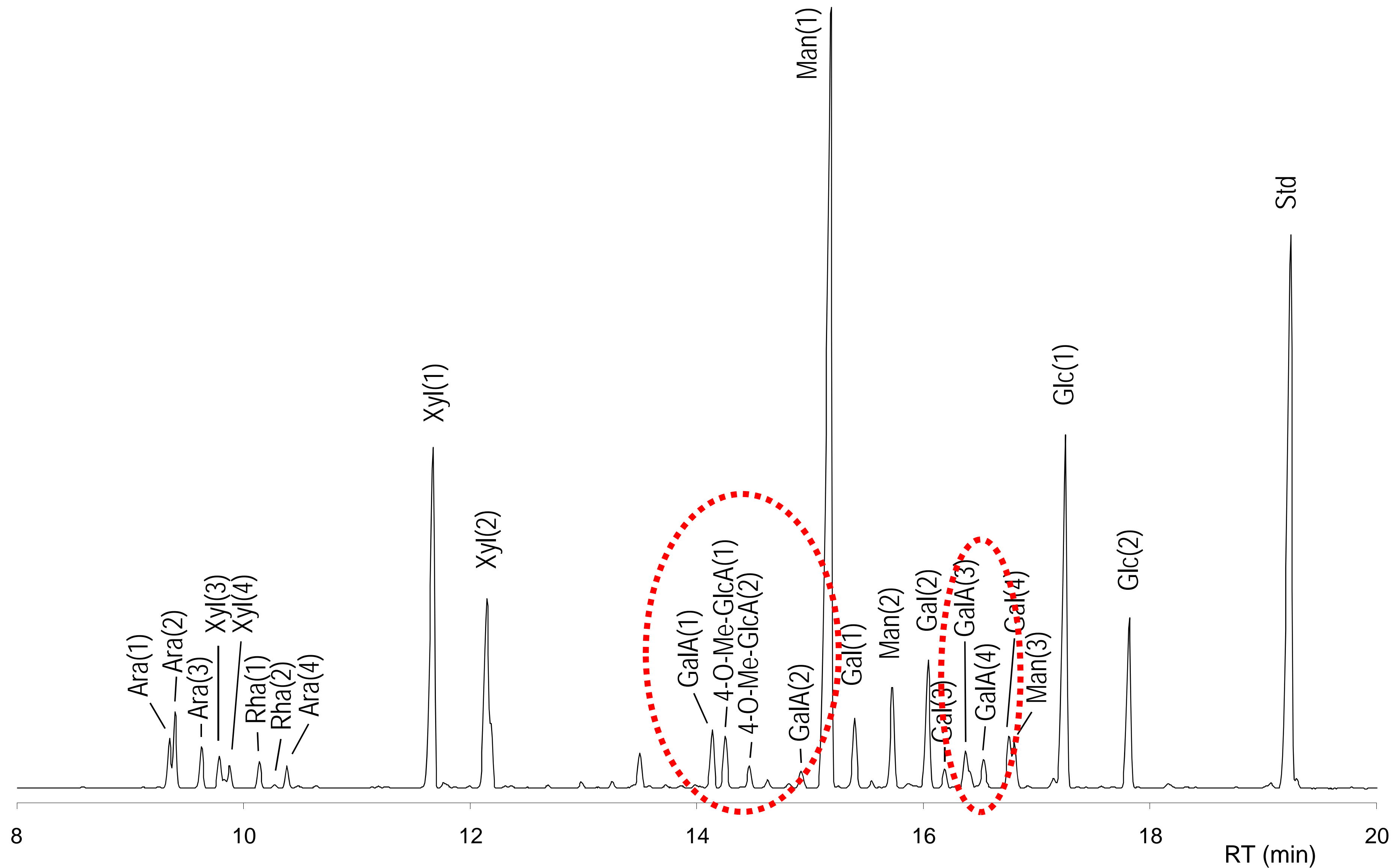
	Acid hydrolysis	Acid methanolysis	Enzymatic hydrolysis
Sample amount	100 mg	10 mg	500 mg
Depolymerization	Two-step H_2SO_4	One-step 2 M HCL in water-free MeOH	Enzyme mixture
Internal standard	Yes, after depolymerization		
Conditions	30°C, 1 h + 120°C, 50 min	100°C, 5 h	40°C, 48 h
Calibration	No	Yes	No

- GC-FID (HP-1, HP-5)
- GC-MS (HP-5)
- HPAEC-PAD (i.e. Dionex system)
- HPAEC-Borate

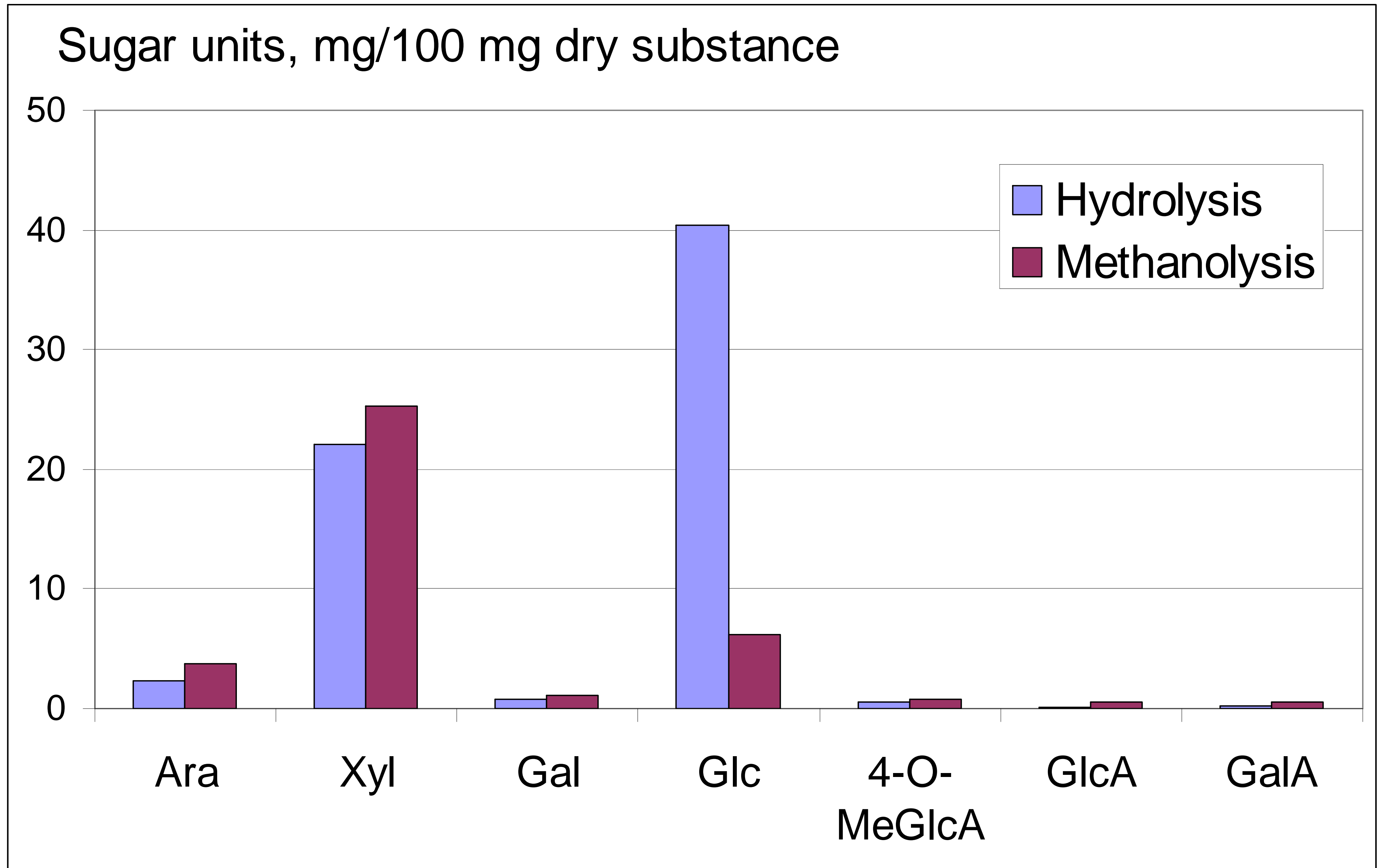
HPAEC-Borate, spruce TMP



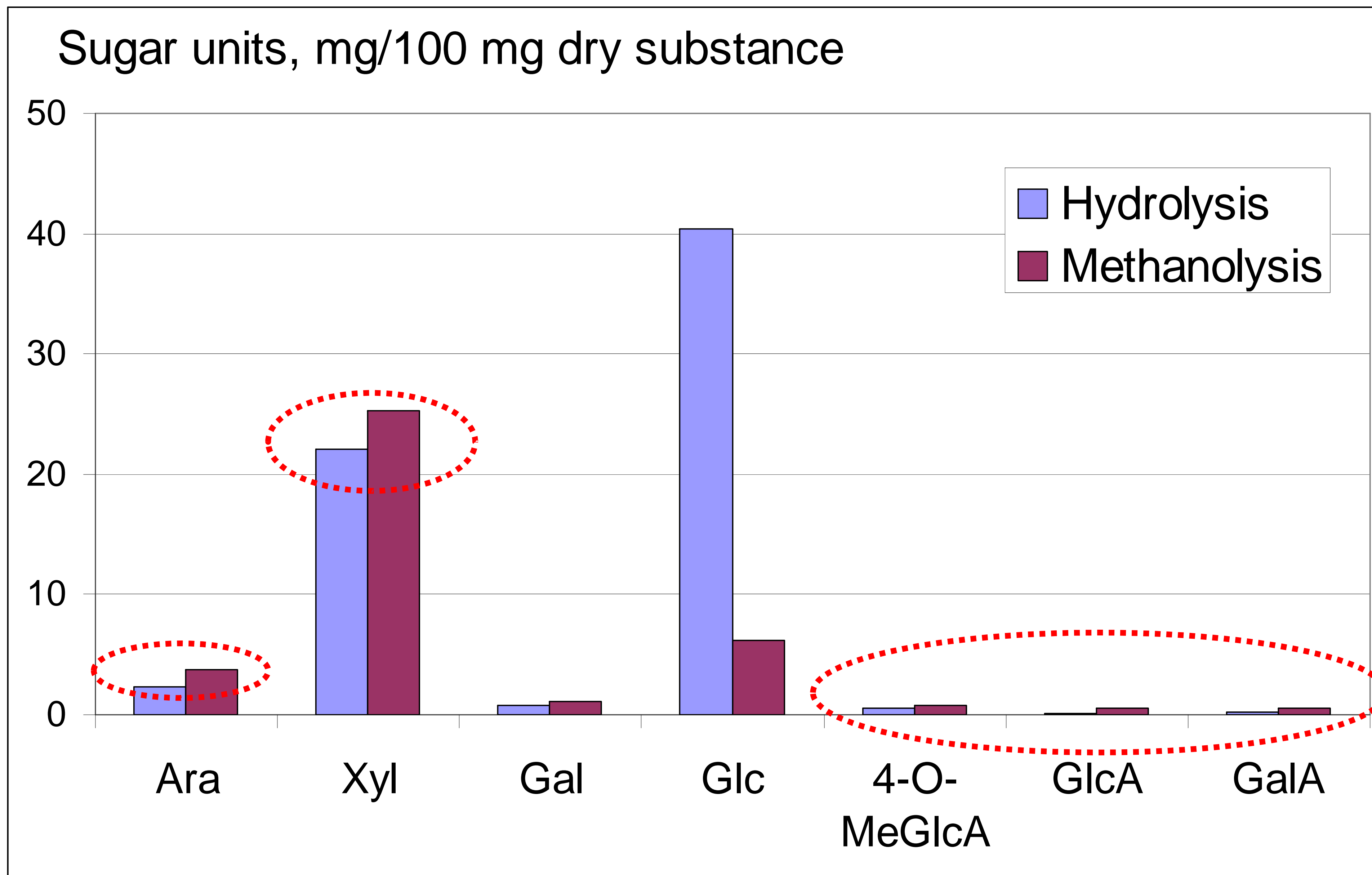
GC-FID, HP-1, spruce TMP



Example, wheat straw



Example, wheat straw



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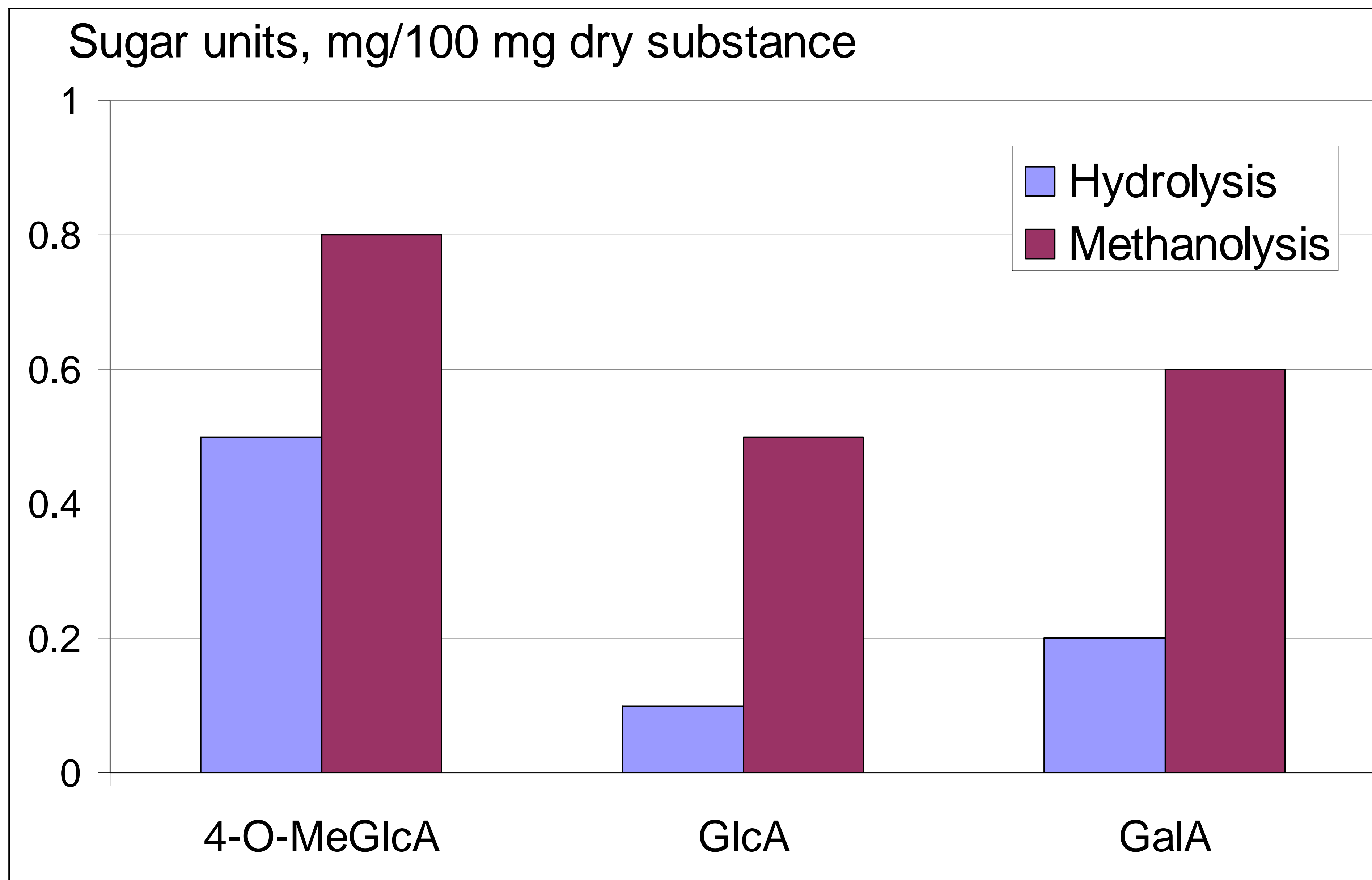
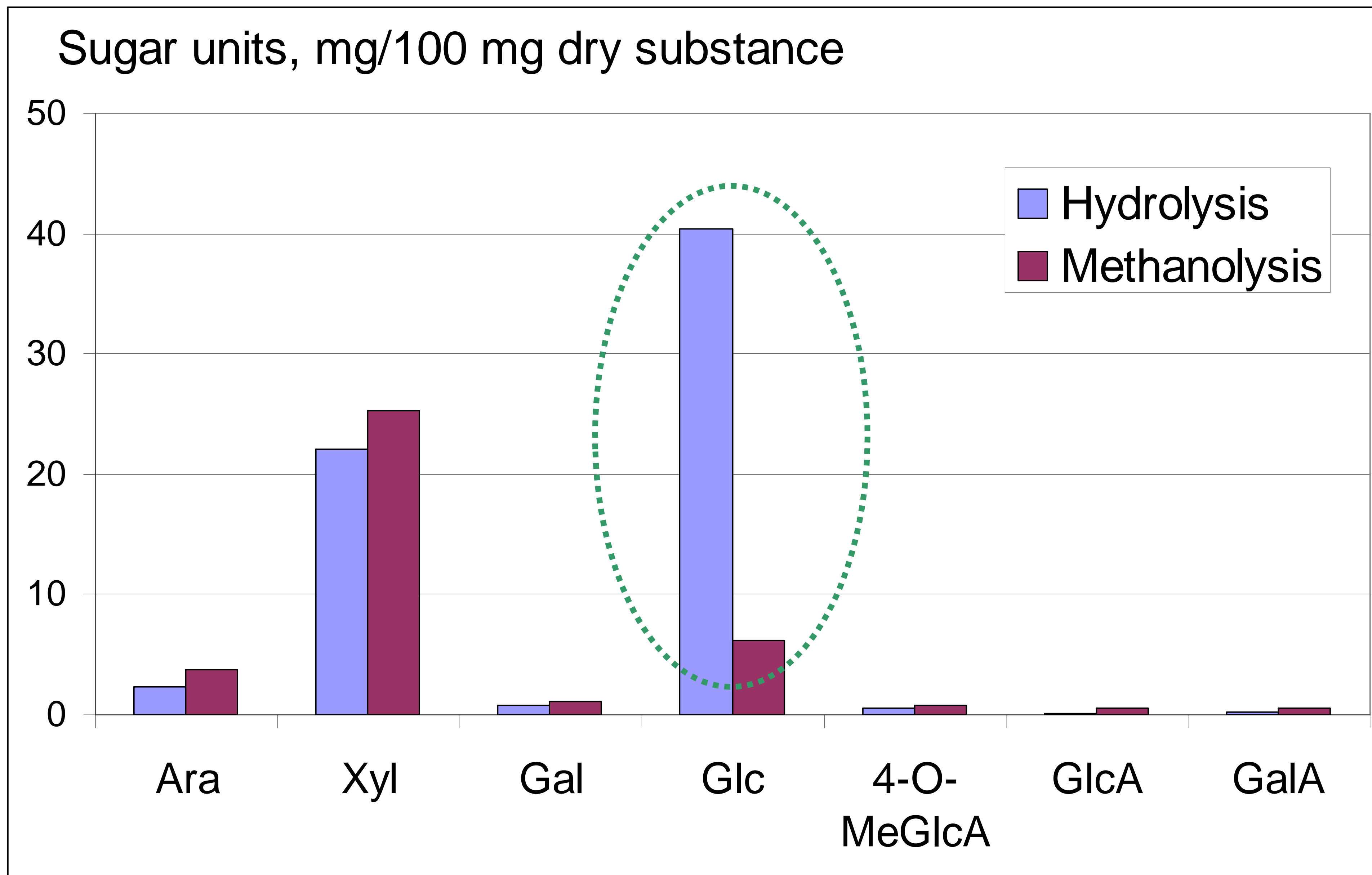


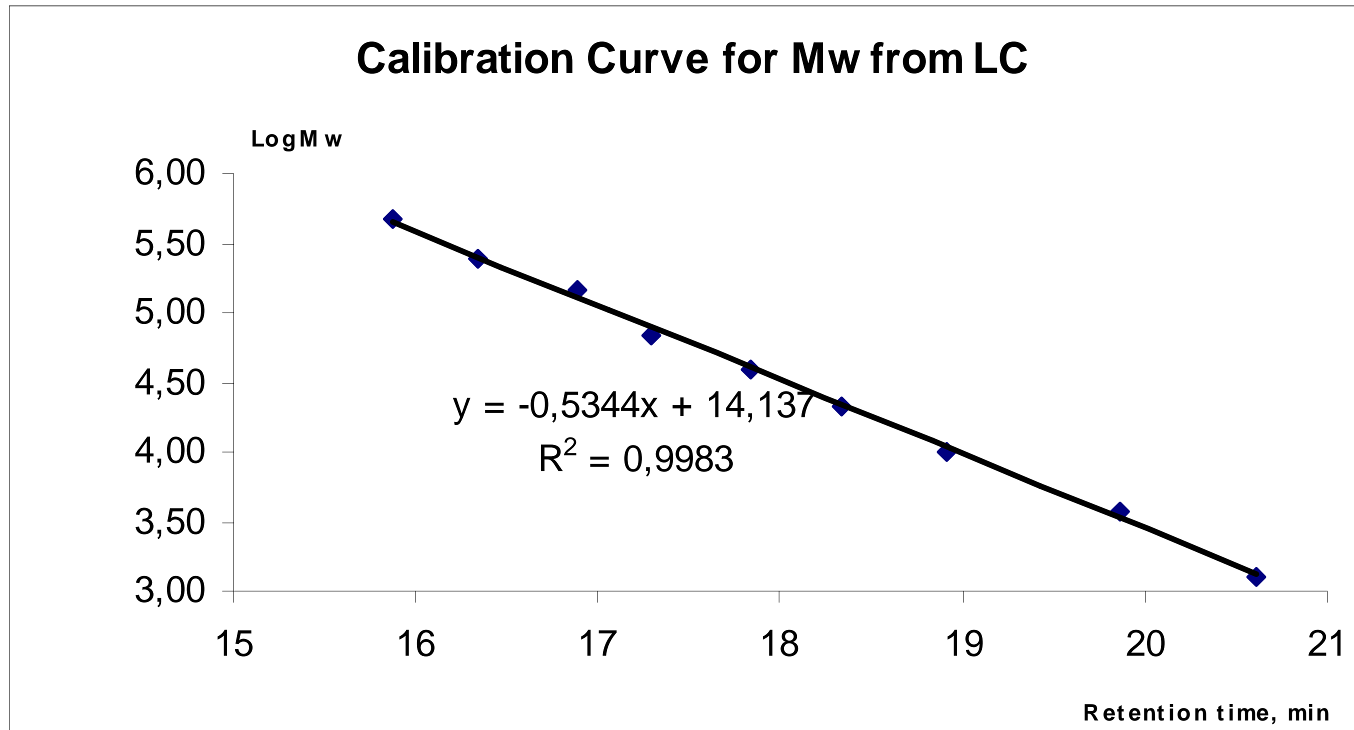
Example, wheat straw



Conclusions

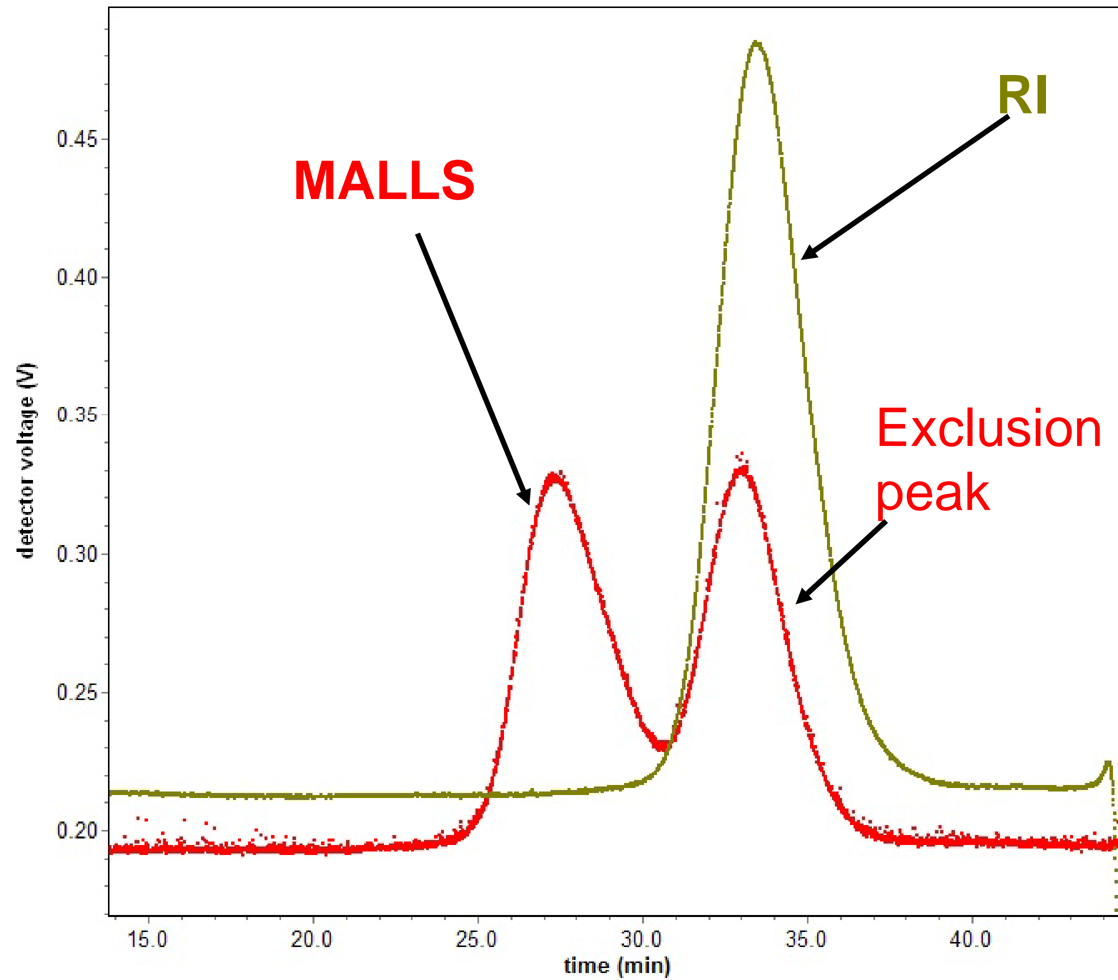
- For the total carbohydrate amount, a combination of methanolysis and hydrolysis should be used
- Calibrate the system of choice for each set of new plant materials to analyze
  - Time, strength of acid, temperature important
- Details in Willför et al., Industrial Crops and Products, 2009

Mw using refractive index



- Factors:
  - Calibration: Dextran standards (right ones???)
  - New calibration needed frequently
  - Only Mw (hydrodynamic volume)

MALLS



- Polydispersity:
  Mw/Mn, Mz/Mn
- Molar mass moments (g/mol):
  Mn, Mw, Mz
- rms radius moments (nm): Rn, Rw, Rz

Conclusions

- MALLS sensitive to larger molecules – RI proportional to concentration
- Dc/dn values…
- Report on the method you have used
  - MALLS gives the true molar mass
  - RI "equals" the hydrodynamic volume, dependent on suitable calibration polymers
  - Other methods – e.g. MALDI…
- Both values can be useful!

Wood
(extr.-free)

Acid treatment
72% and dilute H_2SO_4

Lignin residue
“Klason lignin”

Sugar monomers

Total amount of lignin

Klason lignin in wood and pulp

Extraction with organic solvent (acetone/water 9:1)

Treatment with 72% sulphuric acid at room temperature

Treatment with 3% sulphuric acid at 100°C

Gravimetric determination of insoluble lignin

The soluble lignin is determined spectrophotometrically using UV light at 280 nm. Adding these two together gives the total amount

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Acetyl bromide method

- Wood or pulp sample, about 1-10 mg
- 5 mL 20% AcBr in pure acetic acid
- 0.1 mL perchloric acid (70%)
- 50°C, 3 h
- Neutralisation with NaOH
- Dilution
- UV-Vis at 280 nm
- Calibration with pure lignin preparations

Lignin in process waters

- Easily by UV-spectrometry at 280 nm
- Calibration with pure lignin preparations
- Extractives, such as lignans, give a contribution
- Should be extracted before the determination

STRUCTURAL ANALYSIS
of
isolated lignin preparations
or by
direct analysis

Analysis of lignin preparations

- No ideal methods to isolate lignin without changing its structure

  - Milled Wood Lignin (Björkman-lignin)
    - Grinding in ball mill
    - Extraction with dioxane-water (96:4)
    - Purification by precipitation

  - Enzymatic hydrolysis ofpolysaccharides
    - Rest: lignin
    - Tedious (much work, much time!)

Analysis av lignin preparations

- Elementary analysis (C, H, O)
- Methoxyl groups separately
- Molar mass determinations
- NMR
  - Proton and C-13
  - Phosphorus derivatives too
- UV-Vis
- FTIR
- Py-GC-MS
- Chemical degradation and analysis of the pieces

Direct analysis of lignin in wood and pulp samples

- Degradation + GC
  - Acidolysis
  - Thioacidolysis
  - Permanganate oxidation
  - Pyrolysis

Lignin analysis; summary

Because of the heterogeneity of lignin there is
no universal degradation method giving all needed
Information about the structure

Most methods are time-consuming, demands
experience and are not quantitative

Specific structural information can be obtained

By combination of several methods the structure
of lignin can be described fairly well