

# The Forest based Biorefinery: Chemical and Engineering Challenges and Opportunities

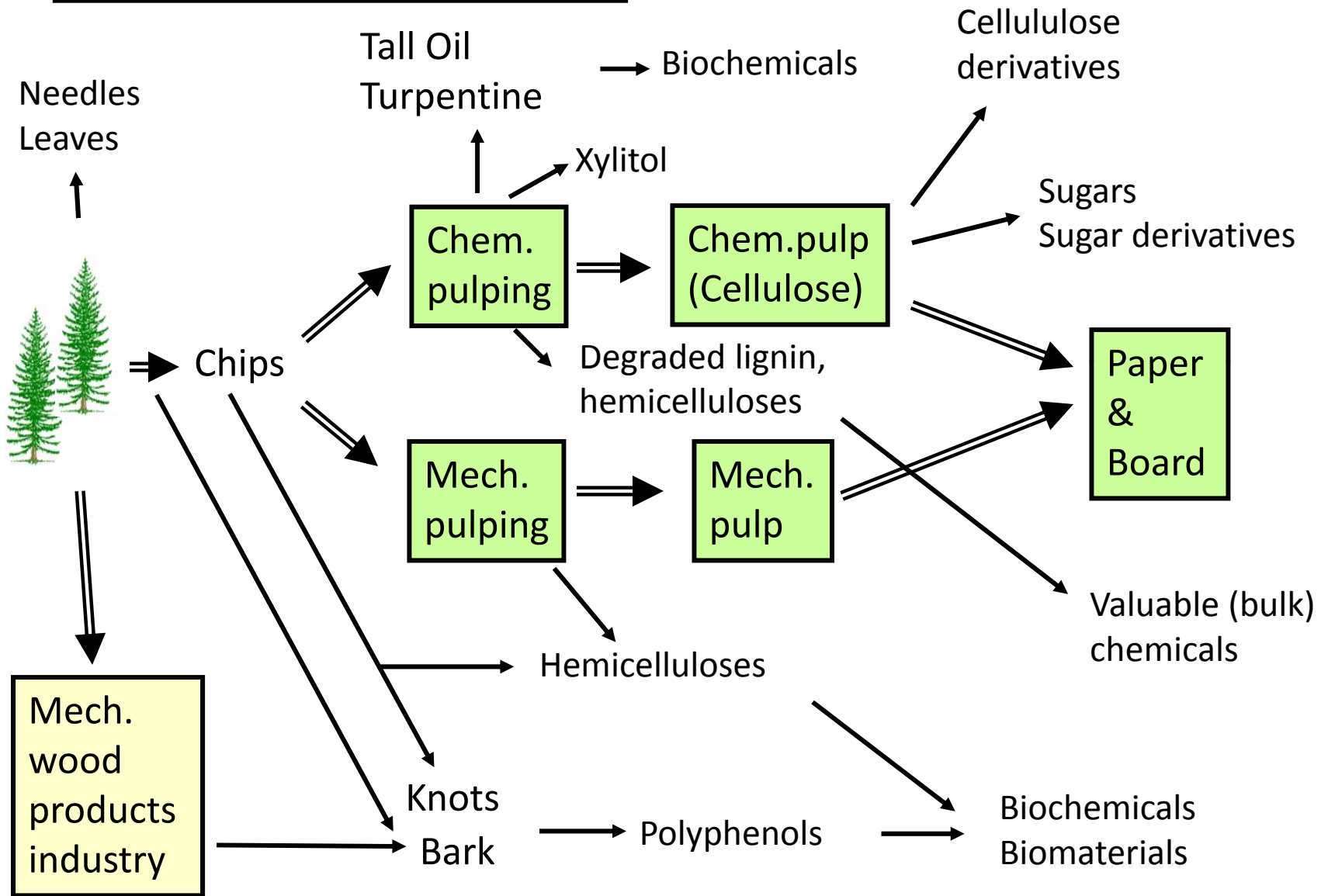
Chemical analysis in the biorefinery–  
Organics

Stefan Willför

# Analytical methods

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

# Many opportunities



## Which matrices / samples do we work with?

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

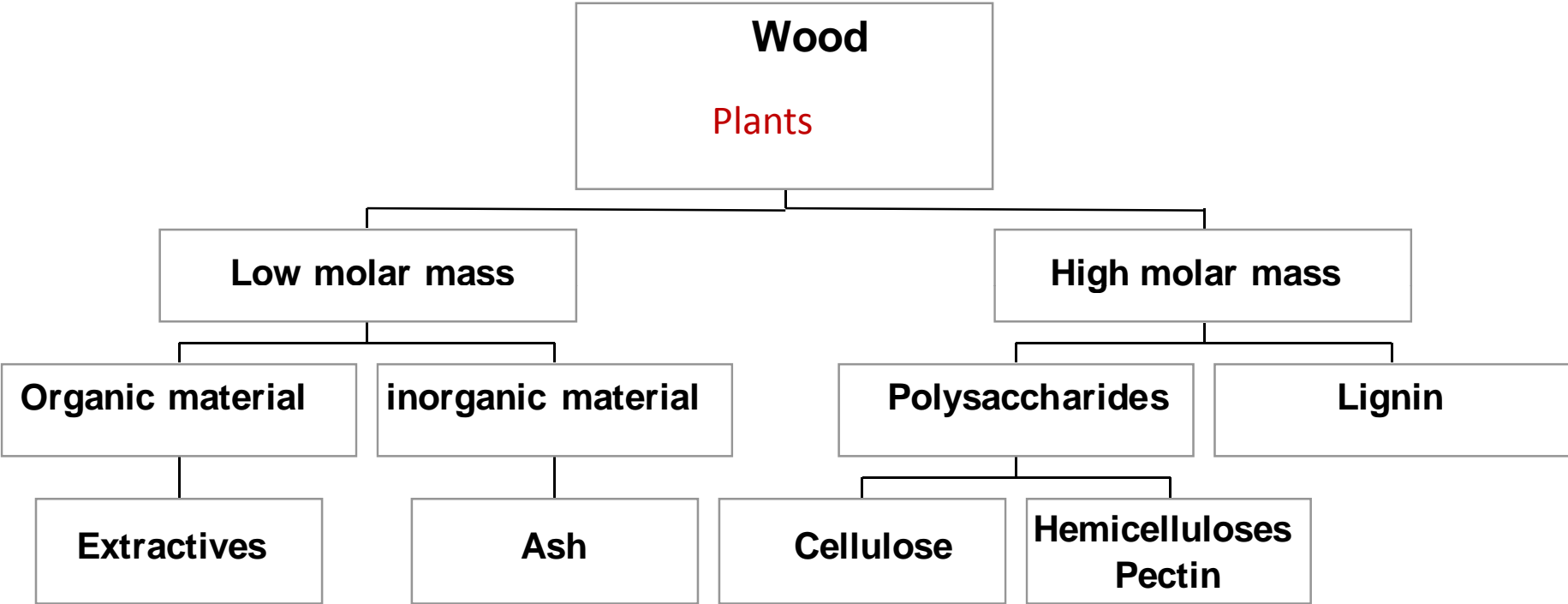
Fibre suspensions: Long fibres, fines, fillers,  
dissolved and colloidal material

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

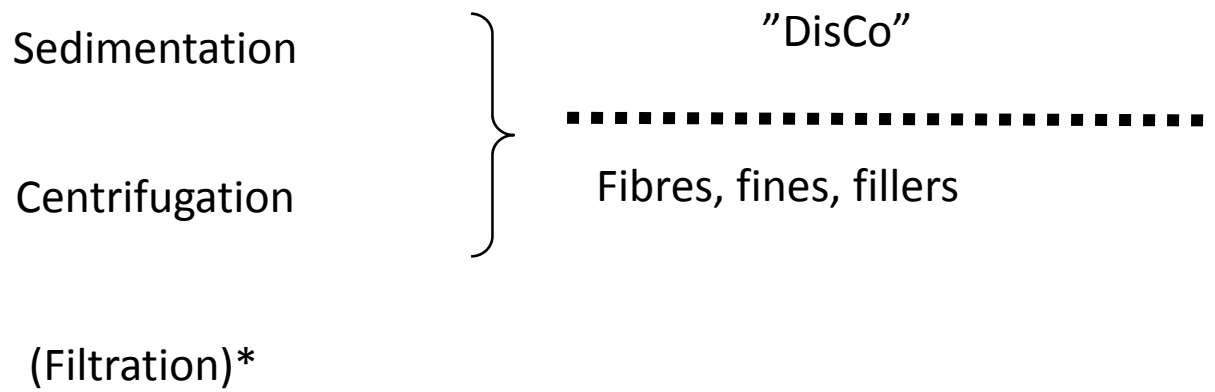
Short storage times: **Cold room**

Long storage times: **Freezer**

Very sensitive materials can even be frozen in liquid nitrogen and deep-frozen ( $-80^{\circ}\text{C}$ )

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 105°C, 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

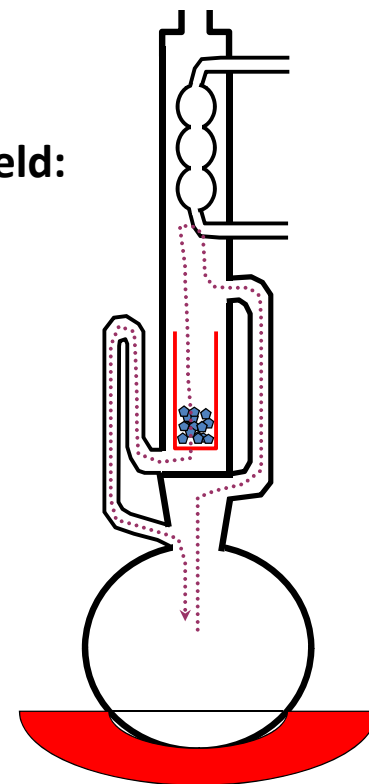
Solid samples (should be finely divided first): Soxhlet, Reflux, SFE, ASE, Ultrasonic bath/bar, PHWE...

Liquid samples: Separation funnel, SPE...

# Soxhlet

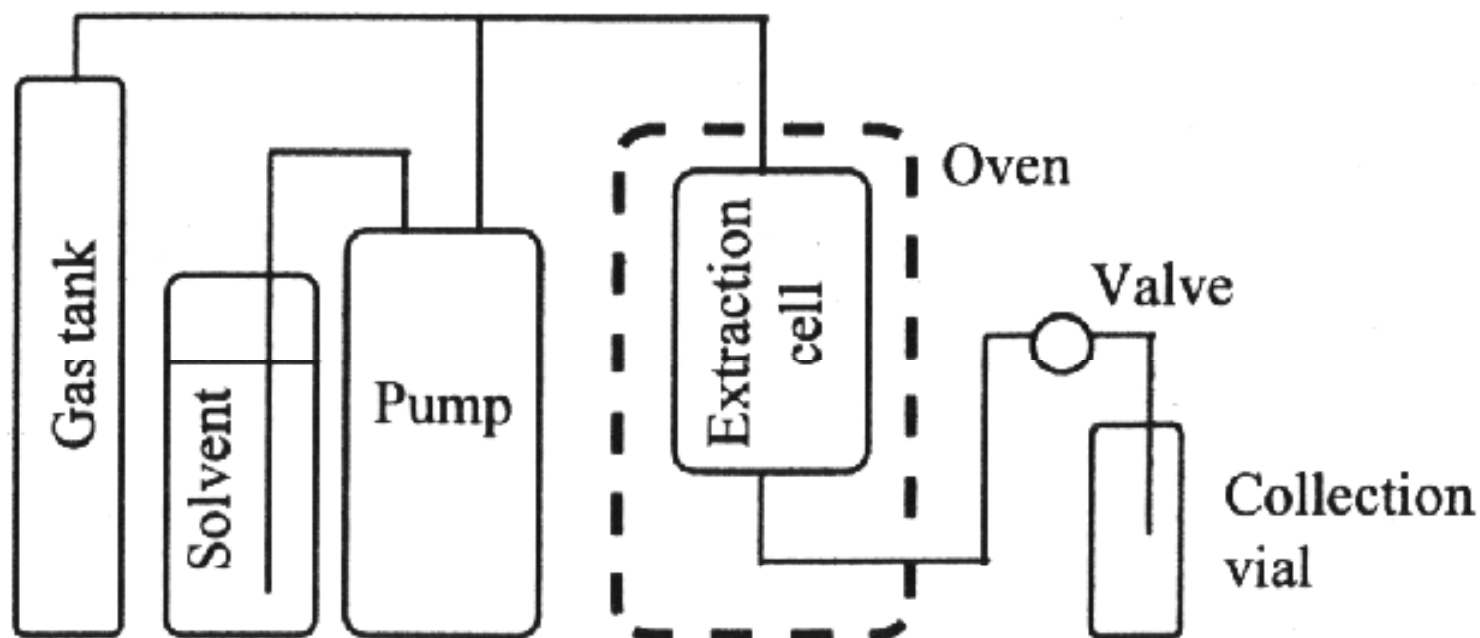
Method for continuous extraction of analytes from a solid into an organic solvent.

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)

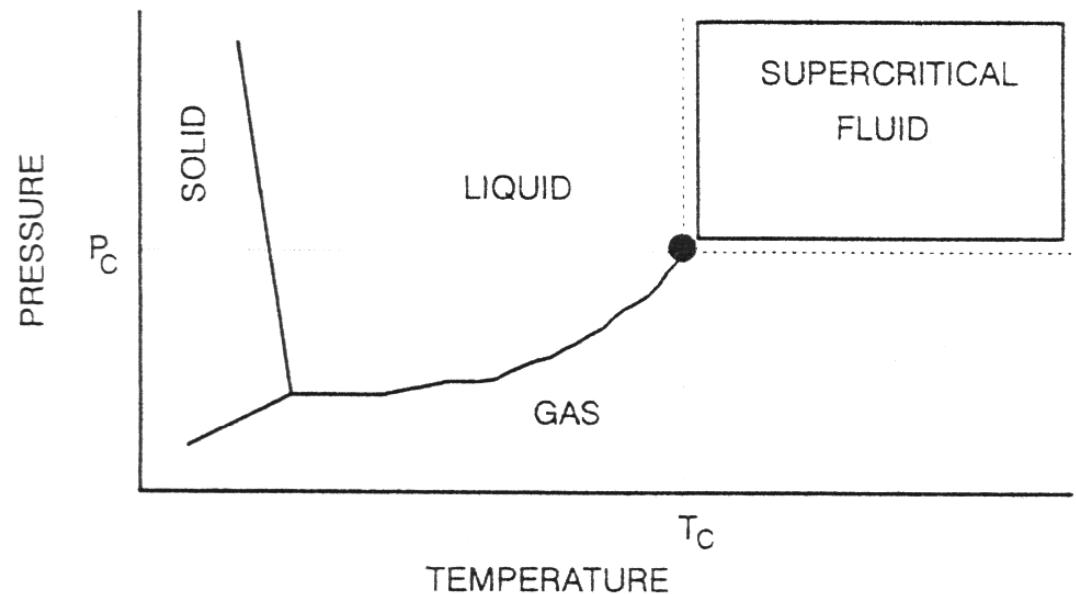
Accelerated solvent extraction (ASE) or enhanced solvent extraction (ESE) uses conventional organic solvents at high temperature under pressure. Increased temperature accelerates the extraction kinetics, while elevated pressure prevents boiling at temperatures above the normal boiling point.



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



# Extraction of process waters

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

# Extraction of dissolved and colloidal components from water

- Liquid / liquid (non-miscible solvent)
- Sorption to absorbent & elution 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)

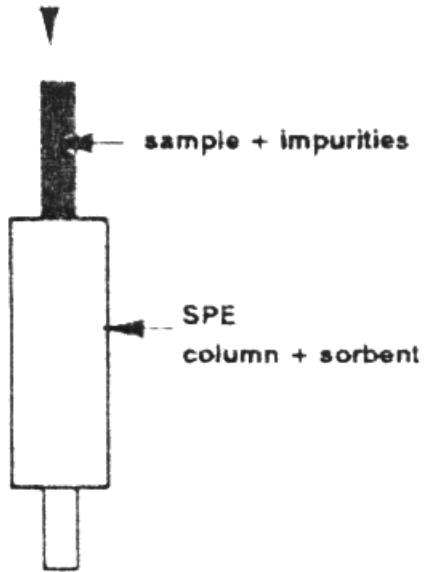
Ethyl acetate

# Liquid / liquid extraction

- Separation funnel
- Test tube (centrifugation possible)
  
- Adjust pH ?
- Add salt ?

# Solid Phase Extraction (SPE)

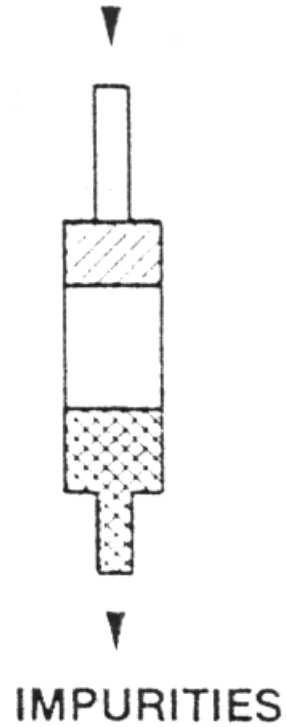
LOAD  
SAMPLE



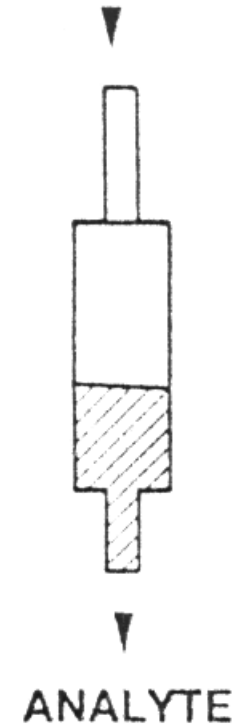
FORCE SAMPLE  
THROUGH COLUMN



WASH  
SOLVENT



ELUTION  
SOLVENT



# Solid-Phase Microextraction (SPME), for volatile substances

integrates

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

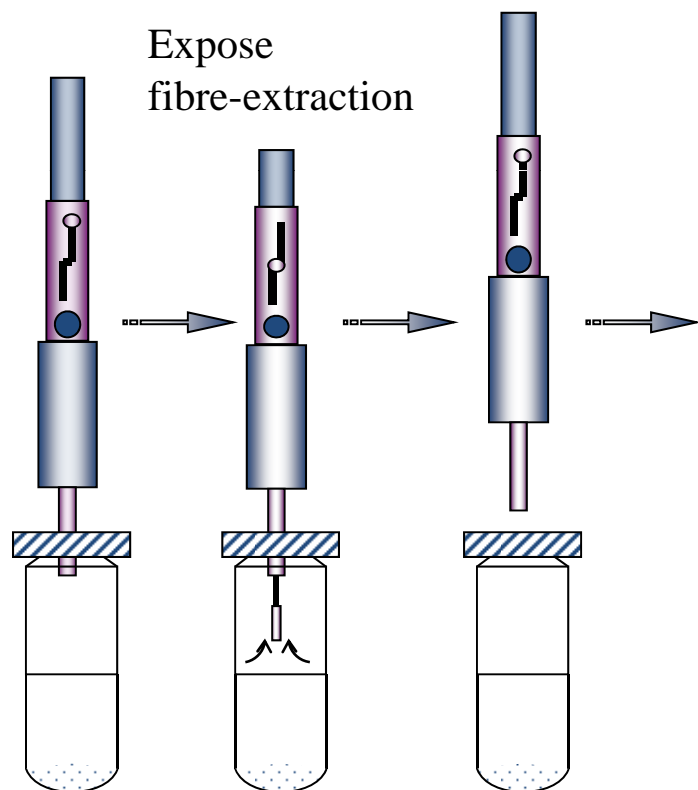
into a single step



# Solid-Phase Micro Extraction (SPME)

## Extraction

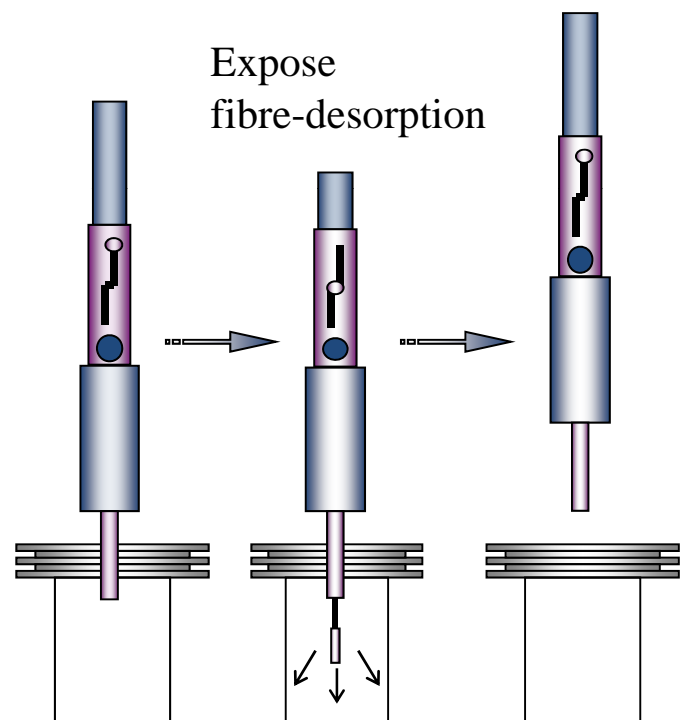
Pierce sample septum



A

## Desorption

Pierce GC inlet septum



B

# 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.

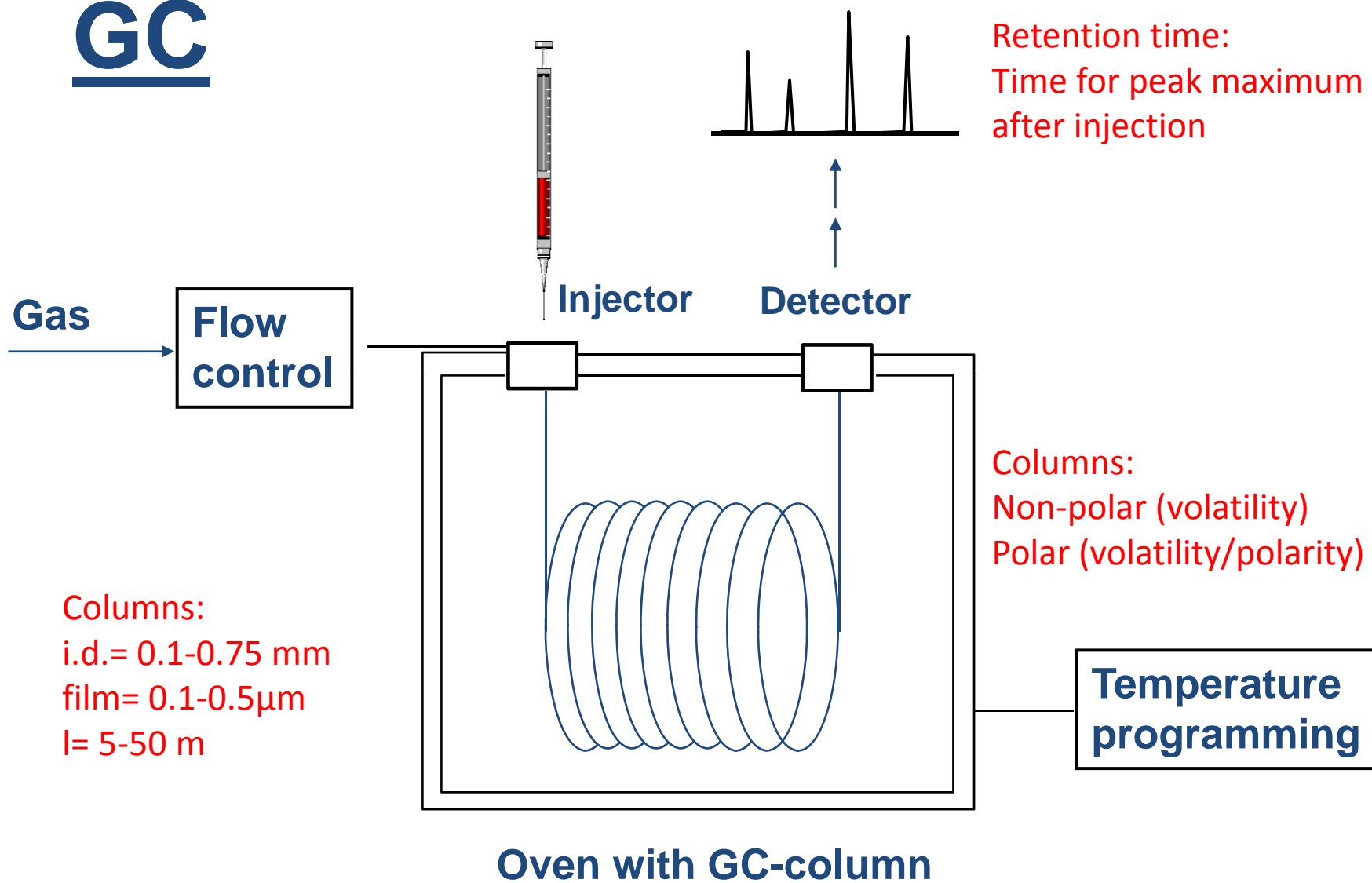


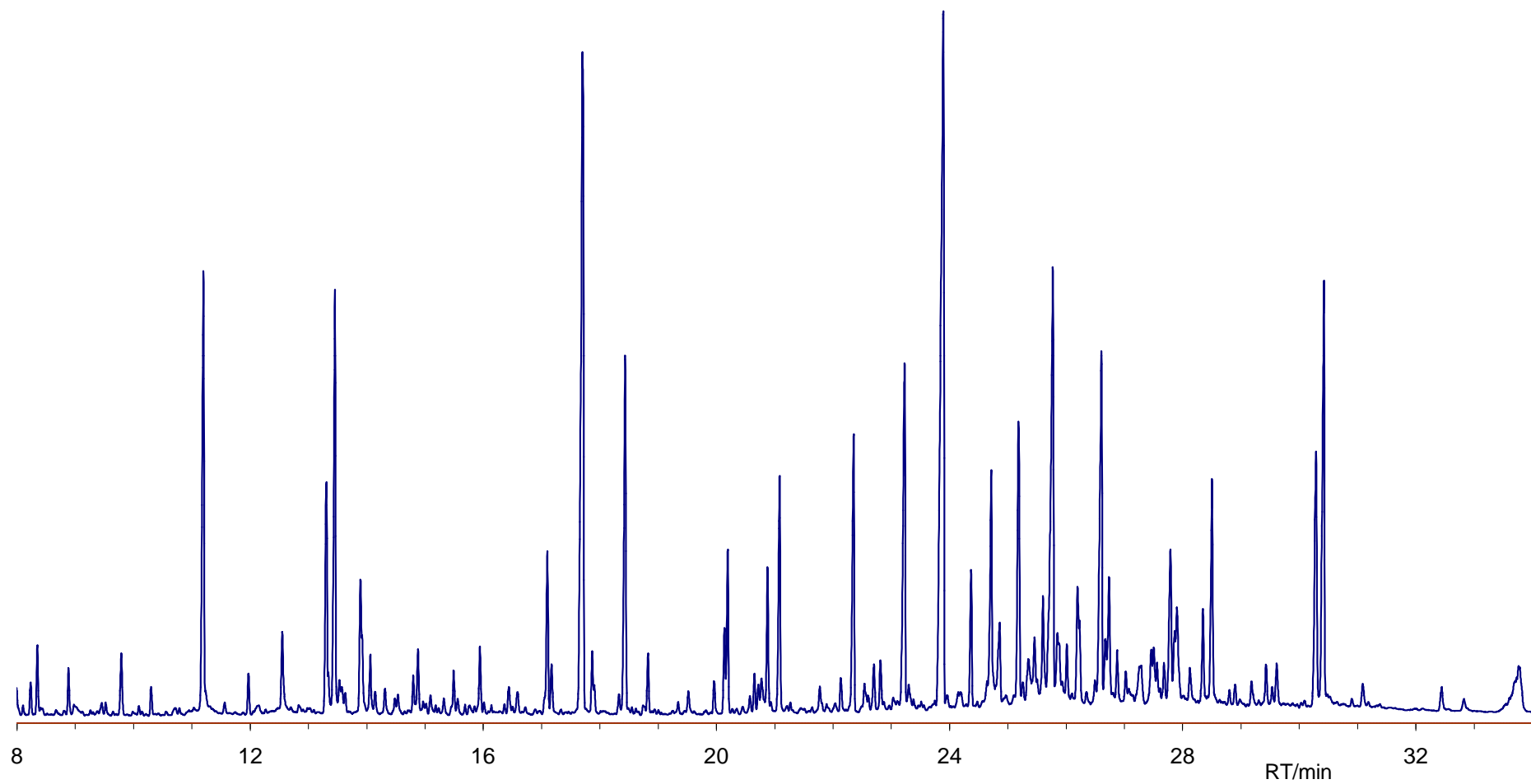
# **Gas Chromatography (GC)**

**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.**

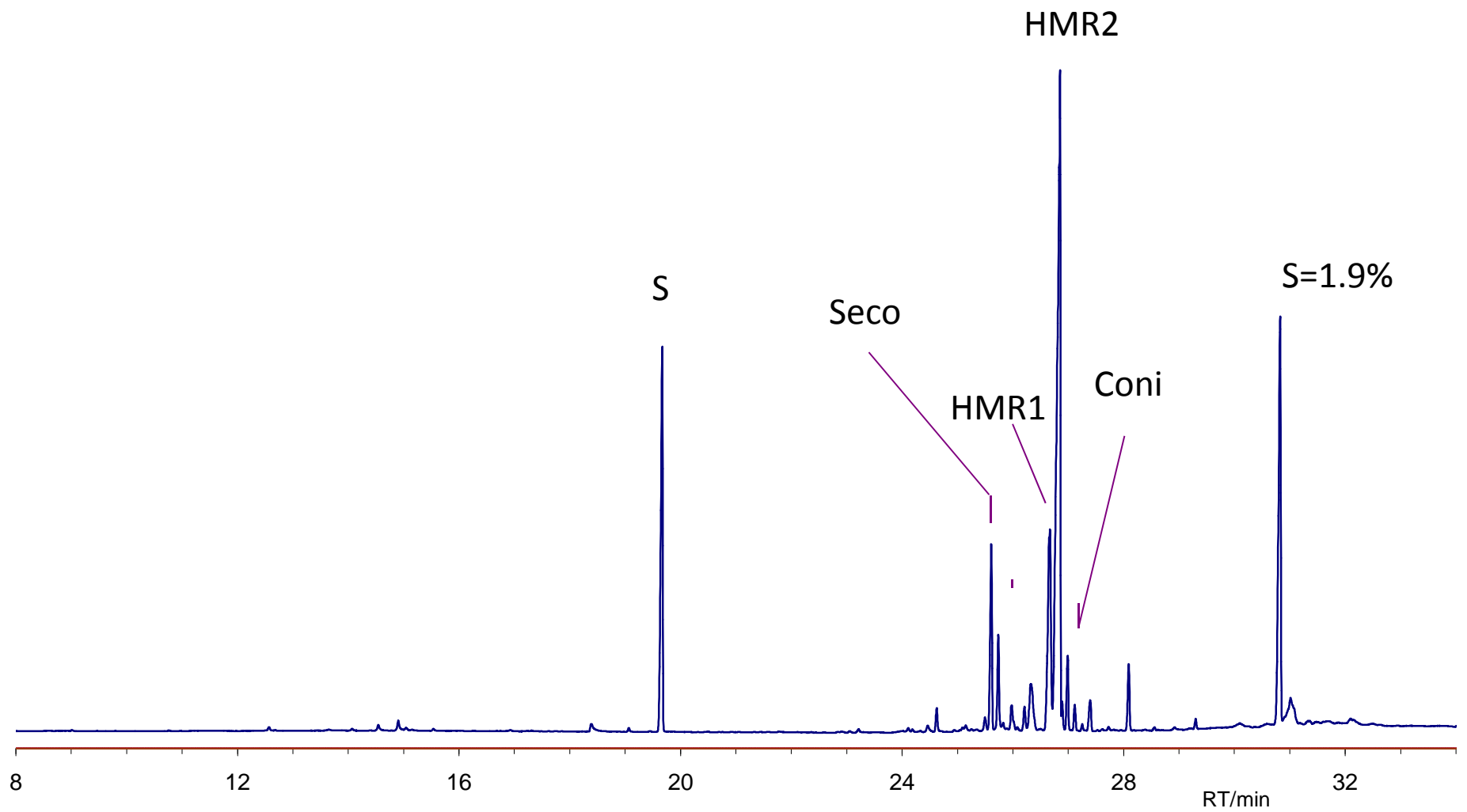
# GC





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

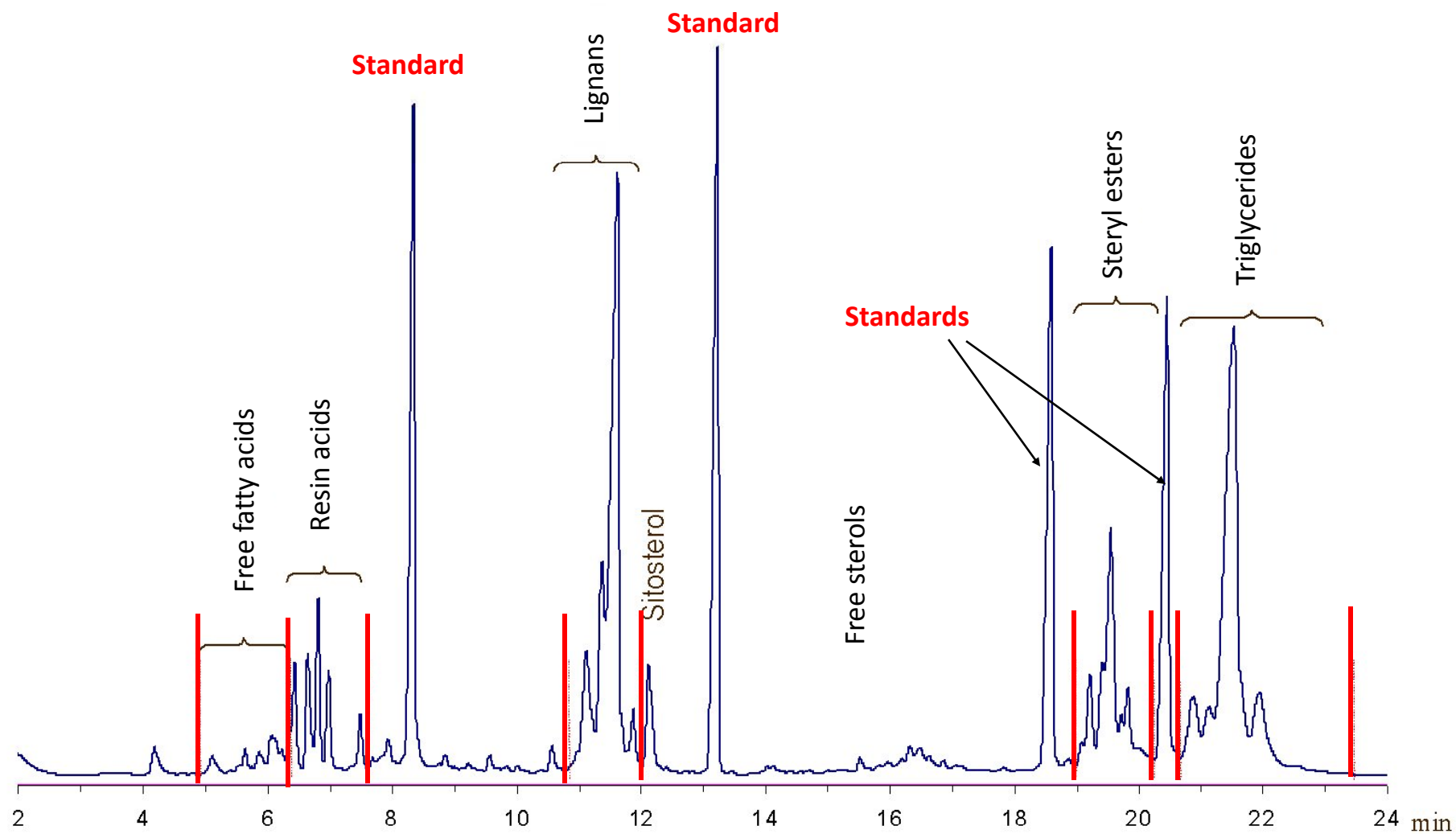
Silyl derivatives are formed by the displacement of the active proton in -OH, -NH and -SH groups. Thus, protic sites are blocked, which decreases dipole-dipole interactions and increases volatility



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





# 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

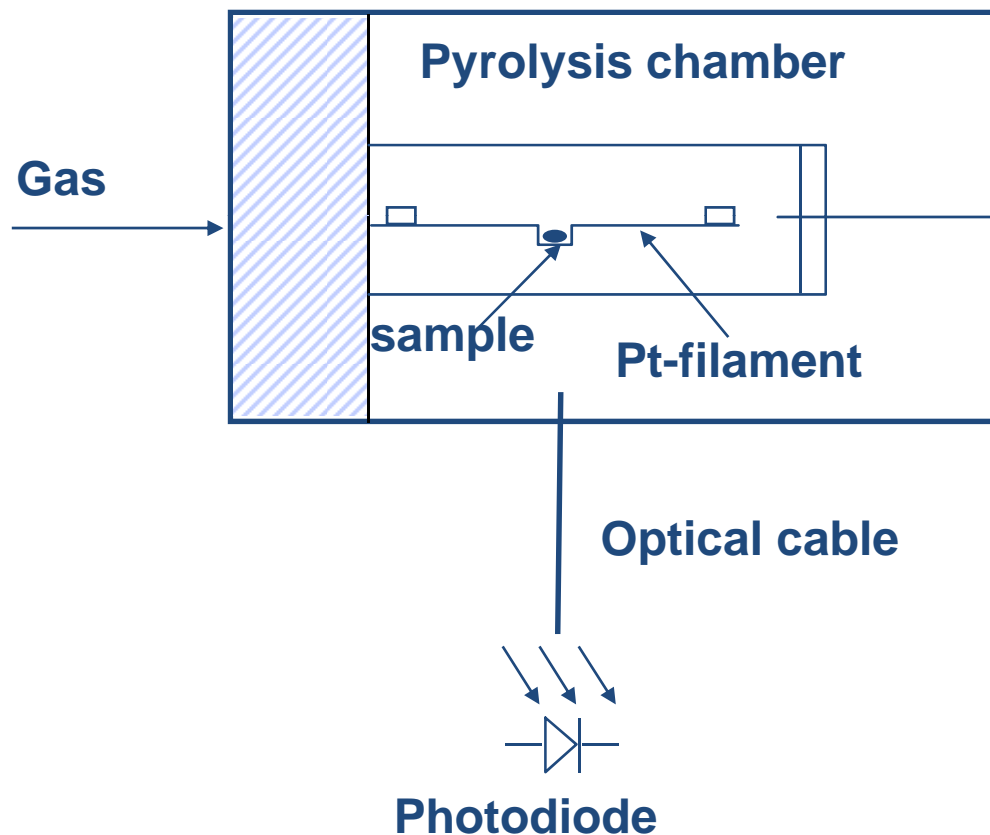
# Pyrolysis-GC-MS

**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 GC or GC-MS for separation and identification.**

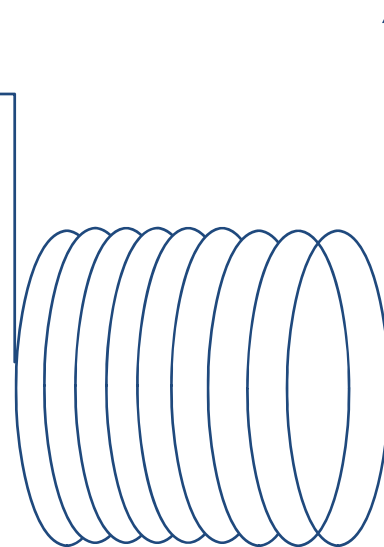
# Py-GC

## 1. Pyrolysator



## 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

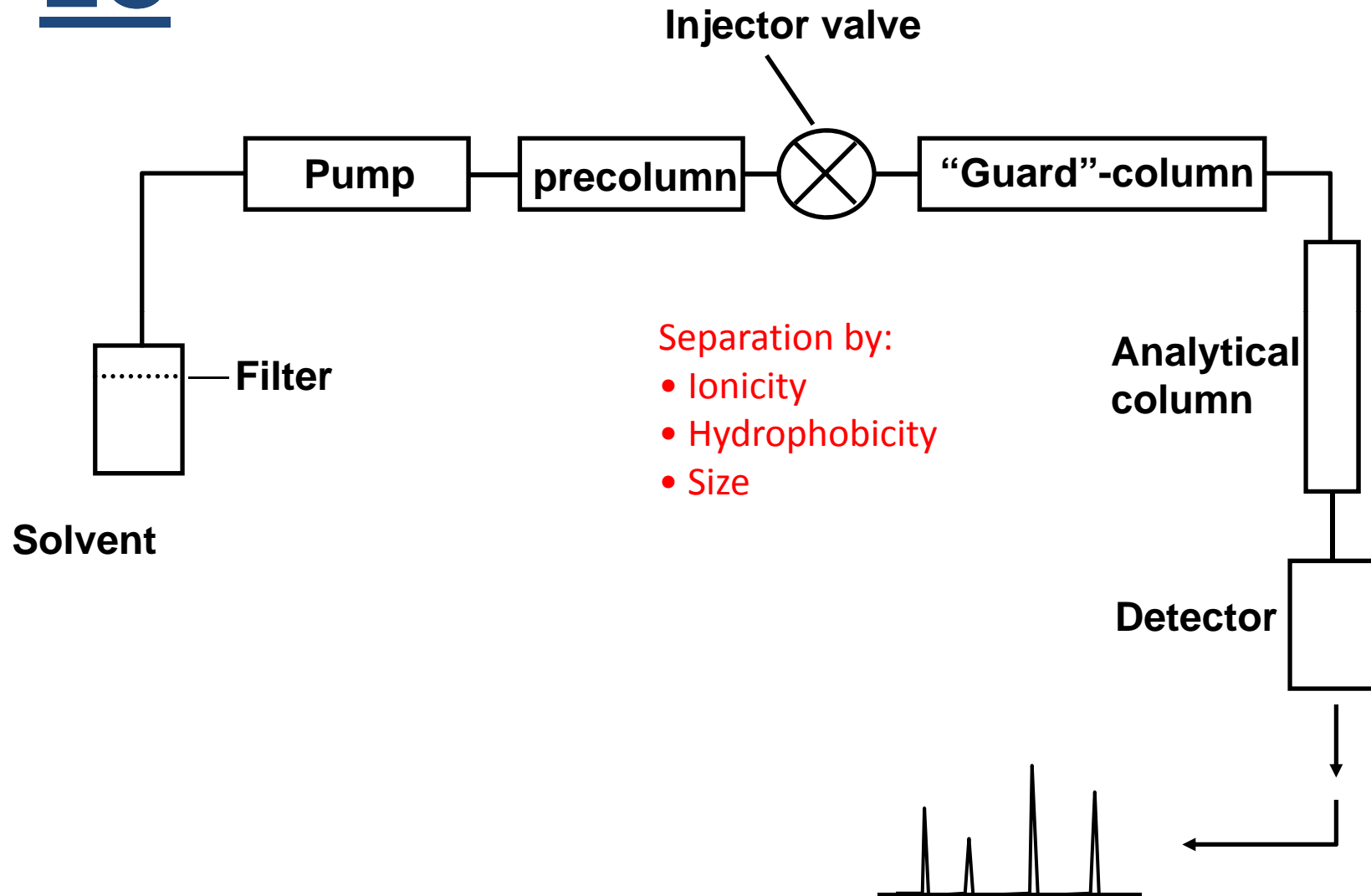
For example: **Proteins, polymers, inorganic ions (ion chromatography (IC), water-soluble ionisable compounds**

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

Separation according to hydrodynamic 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).**

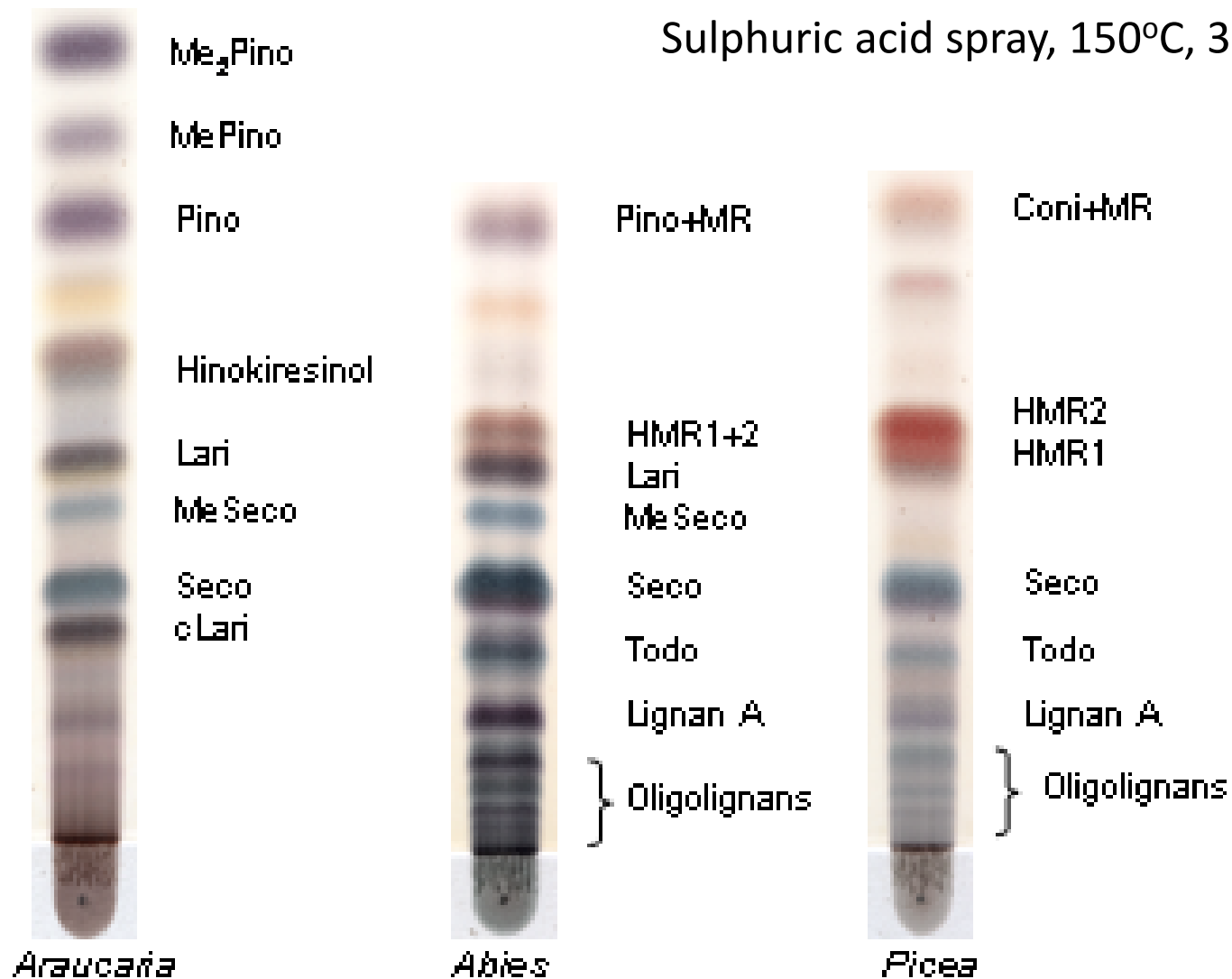


TLC of ethanol extracts of knots

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



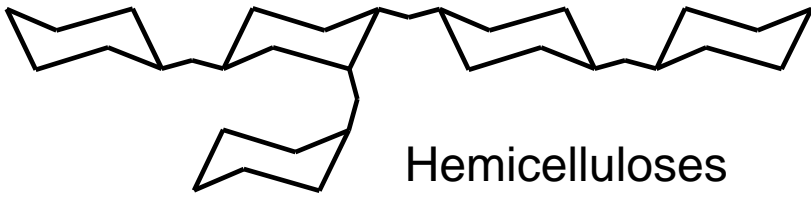
European Polysaccharide  
Network Of Excellence

# Materials

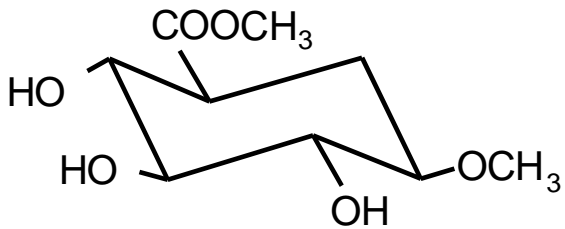
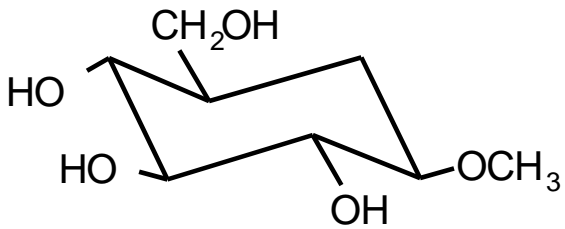
- Oat spelt
- Wheat straw
- Spruce TMP
- Aspen stemwood
- Bleached birch kraft pulp

# Methods

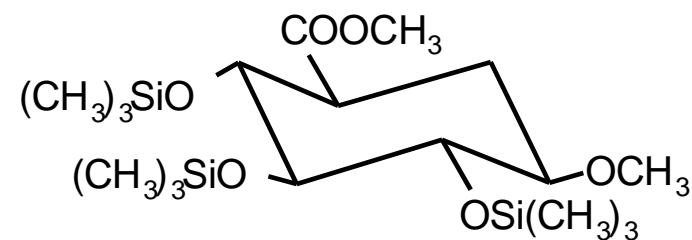
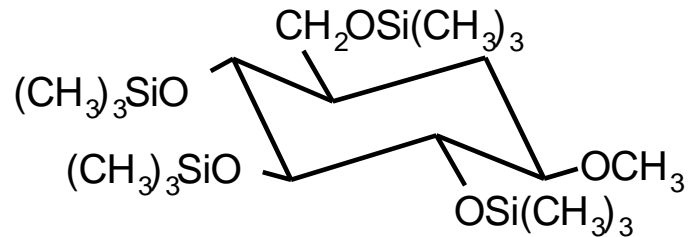
- In-house methods
- Acid hydrolysis
- Acid methanolysis
- Enzymatic hydrolysis
  - Works only on delignified samples



**METHANOLYSIS**



**SILYLATION**



**GAS CHROMATOGRAPHY**

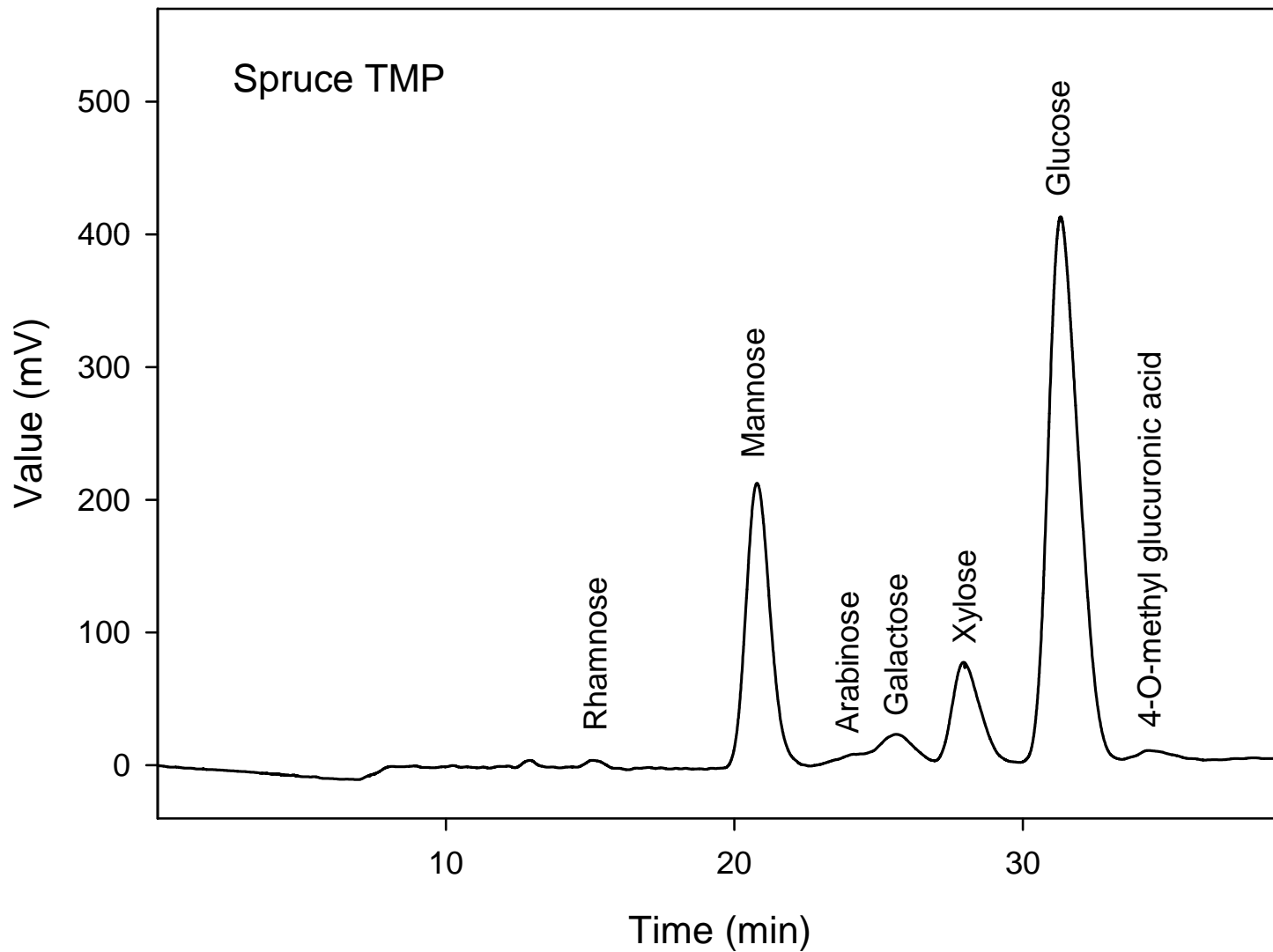


	Acid hydrolysis	Acid methanolysis	Enzymatic hydrolysis
Sample amount	100 mg	10 mg	500 mg
Depolymerization	Two-step $H_2SO_4$	One-step 2 M HCL in water-free MeOH	Enzyme mixture
Internal standard	Yes, after depolymerization		
Conditions	30°C, 1 h + 120°C, 50 min	100°C, 5 h	40°C, 48 h
Calibration	No	Yes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

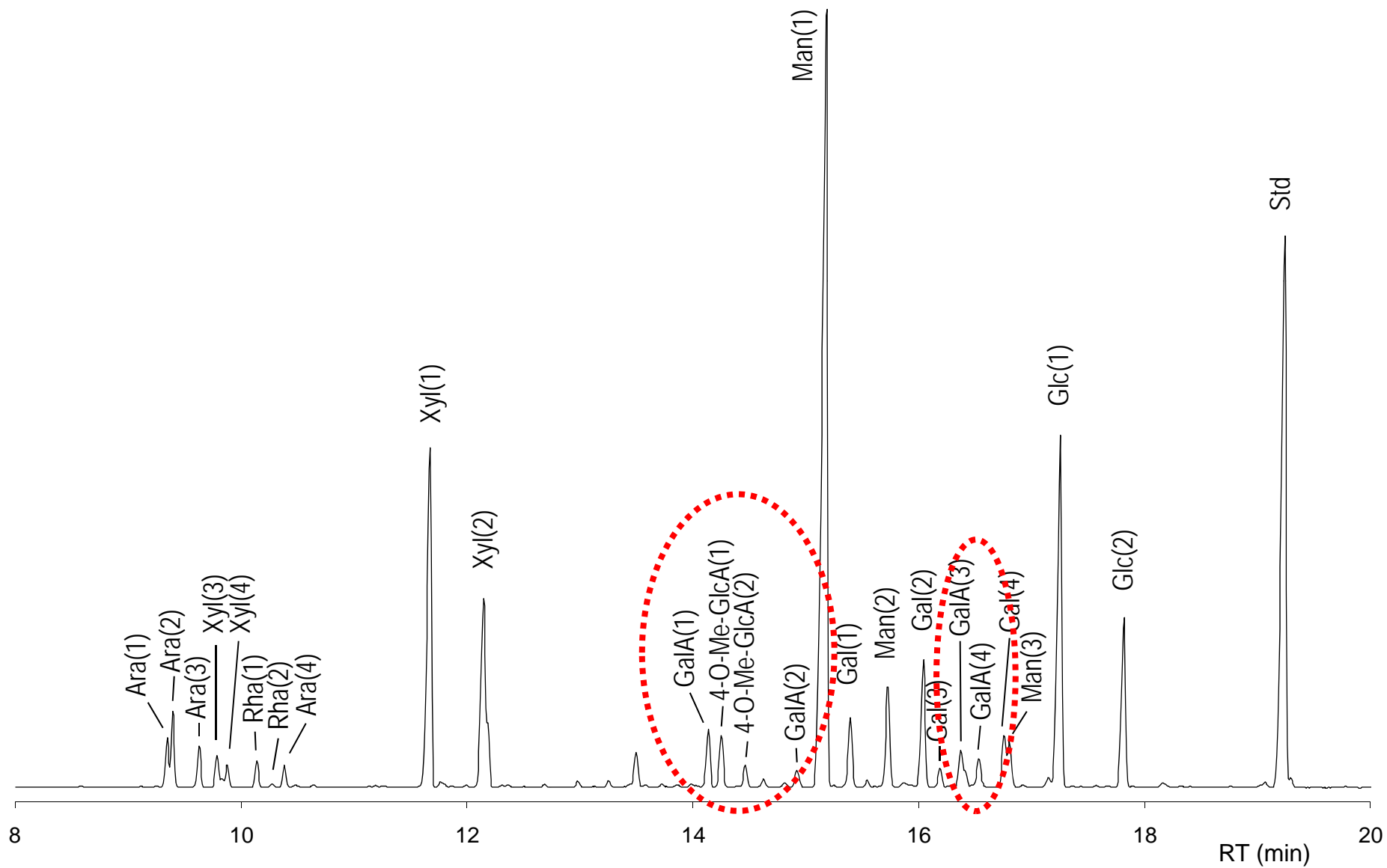


- GC-FID (HP-1, HP-5)
- GC-MS (HP-5)
- HPAEC-PAD (i.e. Dionex system)
- HPAEC-Borate

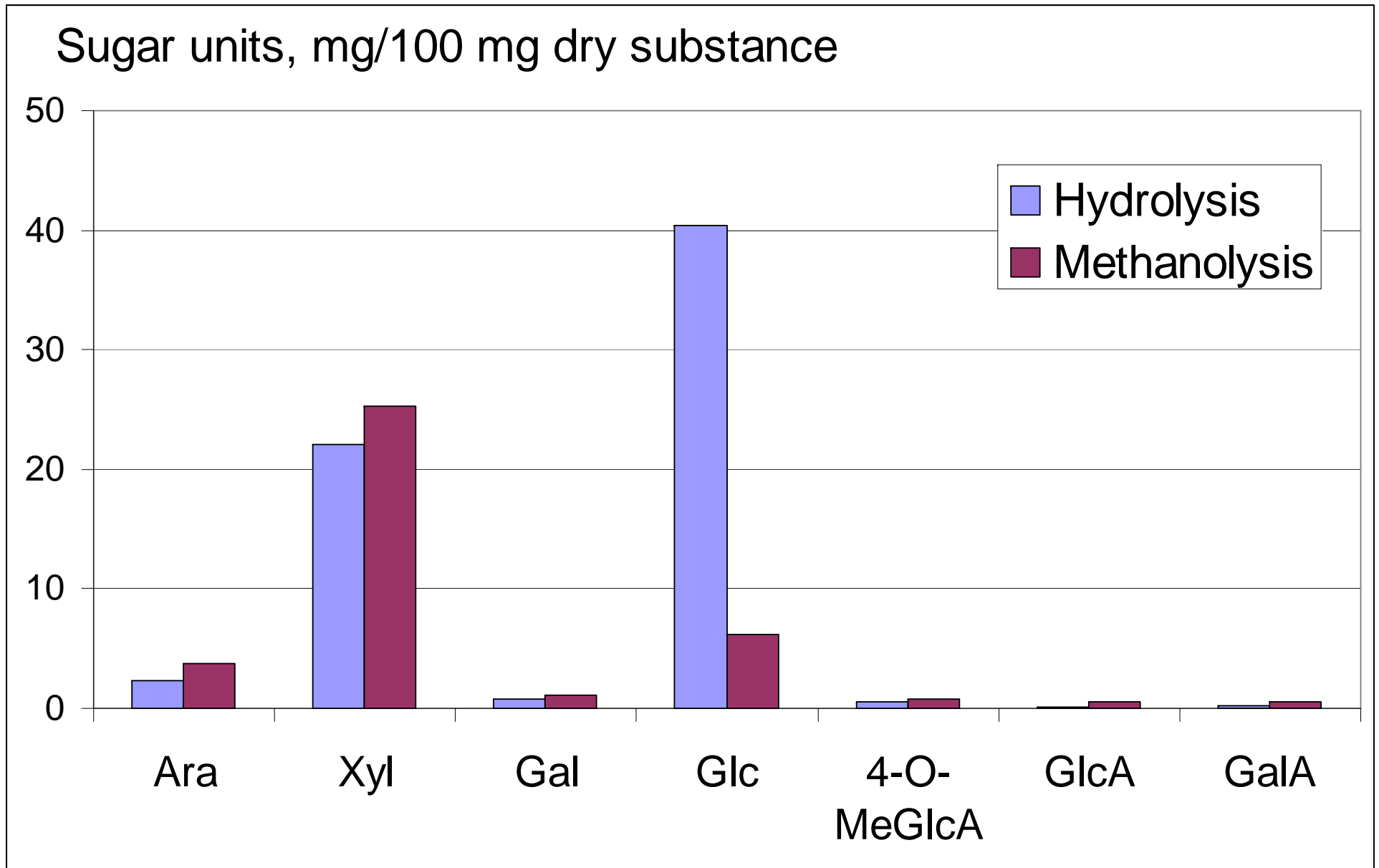
# HPAEC-Borate, spruce TMP



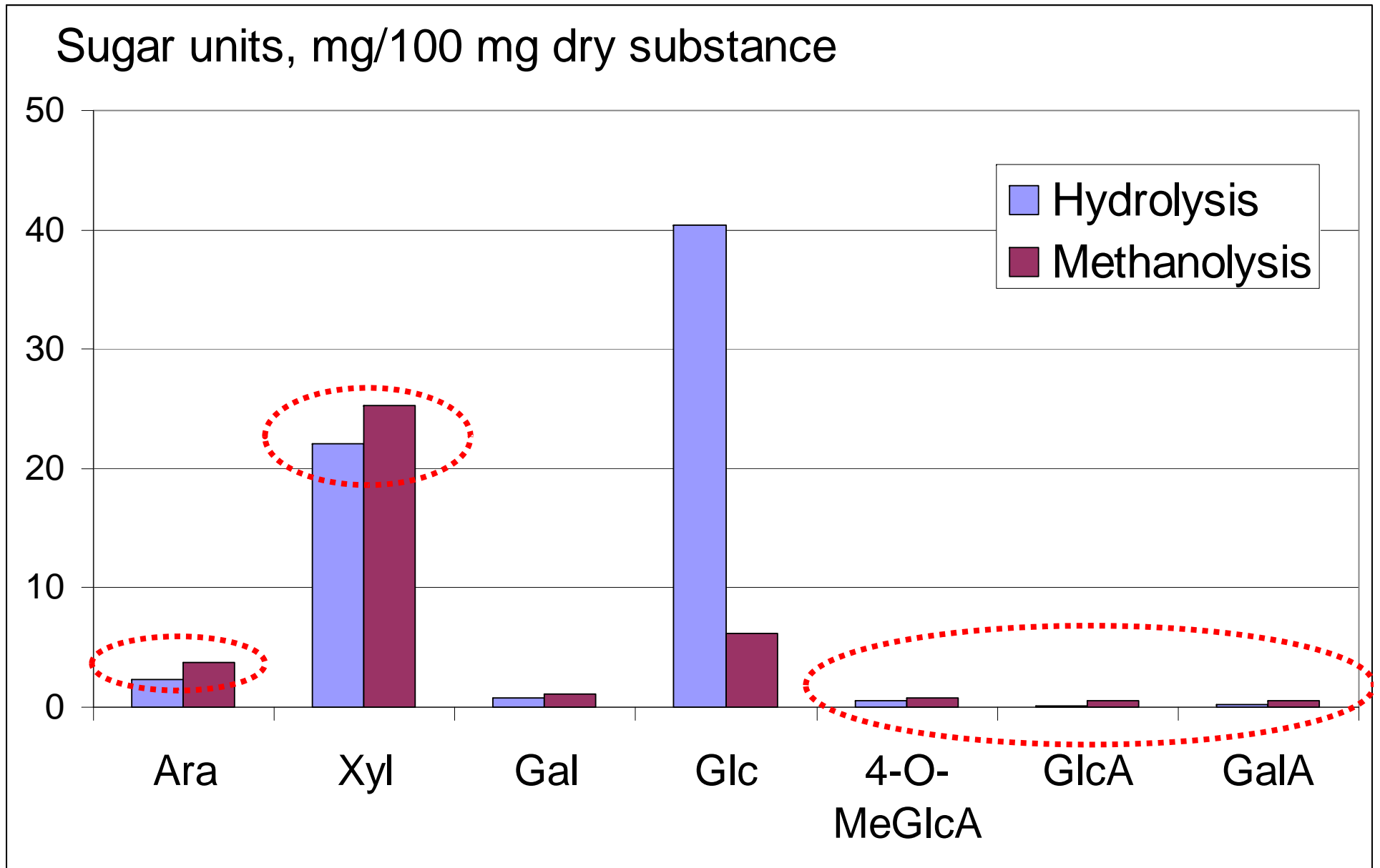
# GC-FID, HP-1, spruce TMP



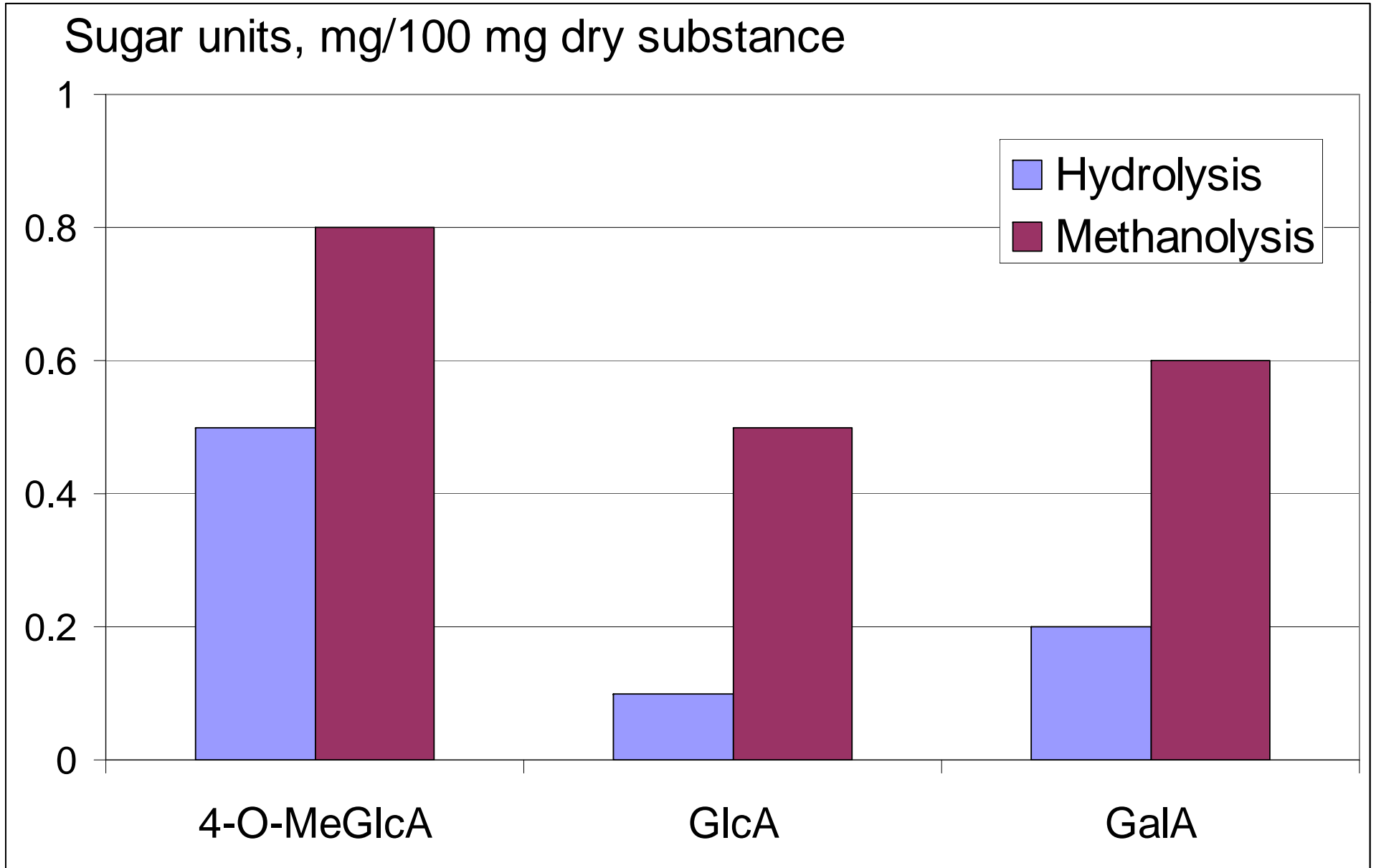
# Example, wheat straw



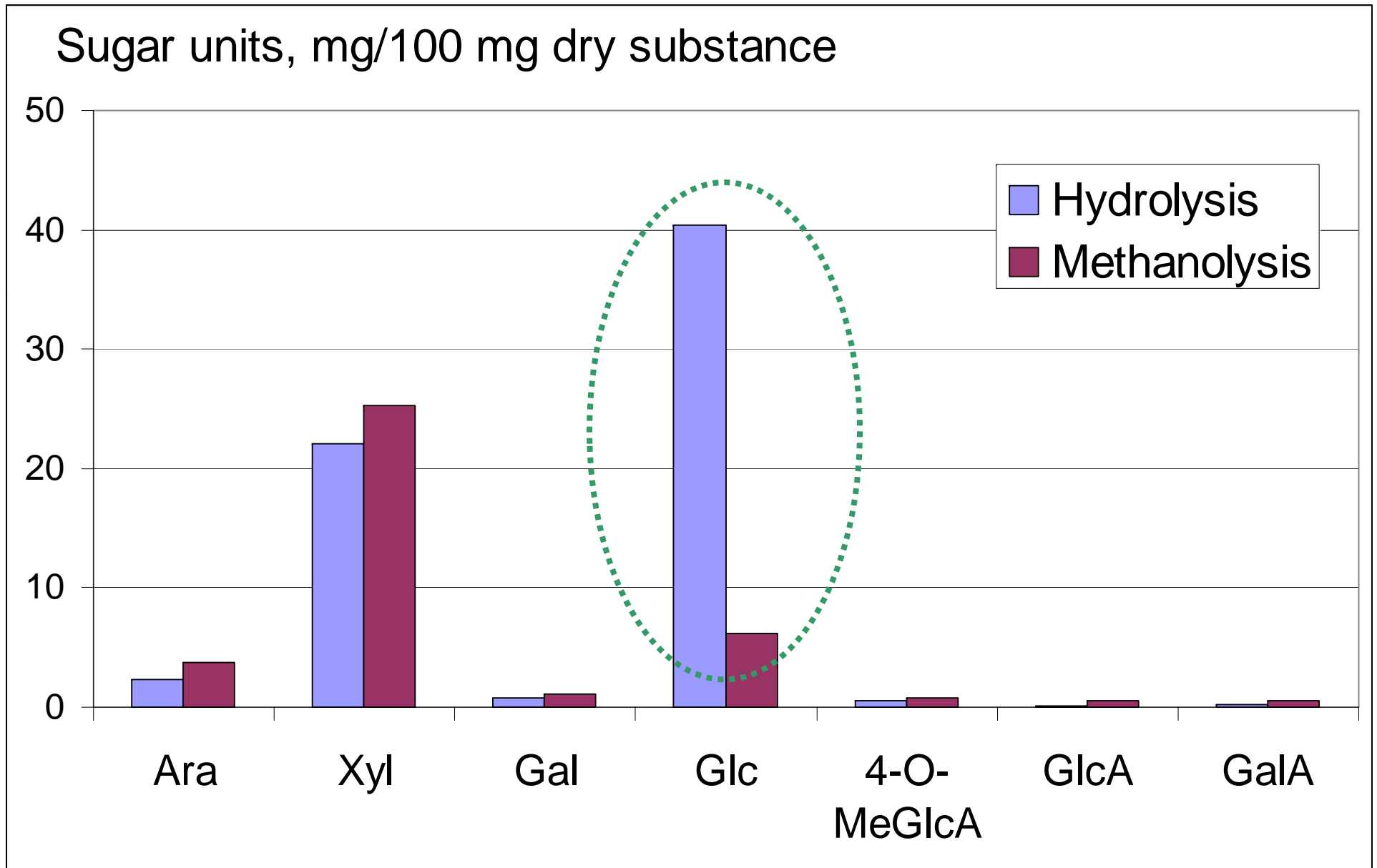
# Example, wheat straw



# Example, wheat straw



# Example, wheat straw

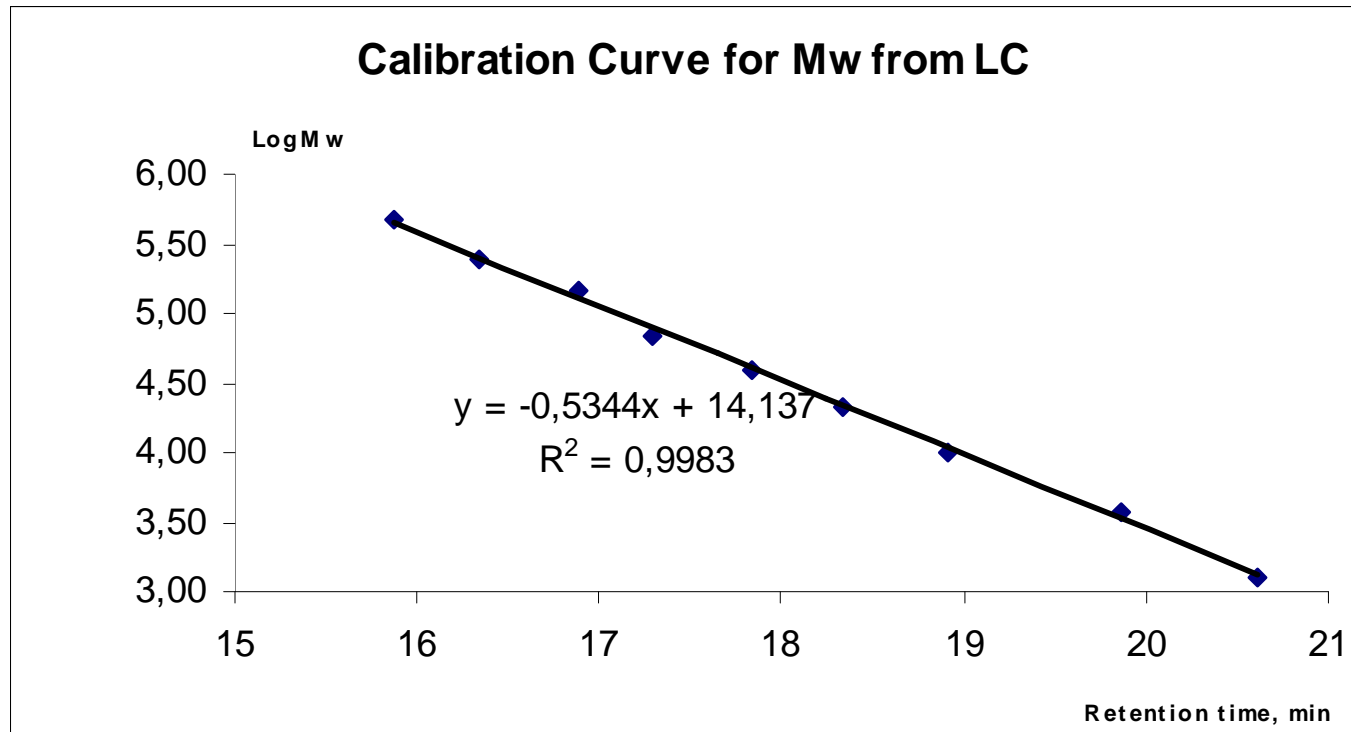


# Conclusions

- 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

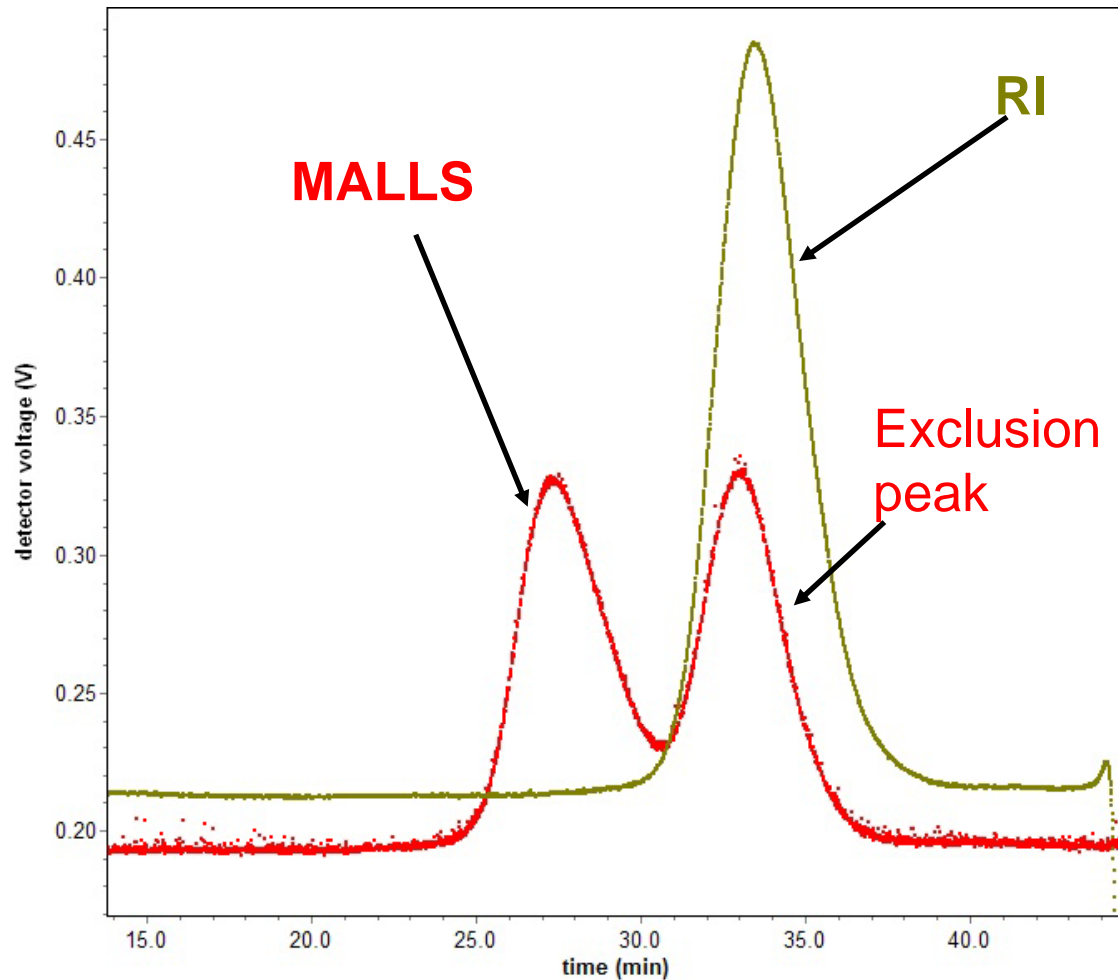


# Mw using refractive index



- 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, R<sub>w</sub>, R<sub>z</sub>

# 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<sub>2</sub>SO<sub>4</sub>

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

P. Klason, Arkiv Kemi 3(5):17 (1906)

# 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 of polysaccharides
    - Rest: lignin
    - Tedious (much work, much time!)

# Analysis of 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