PROCESS CHEMISTRY CENTRE

ÅBO AKADEMI UNIVERSITY

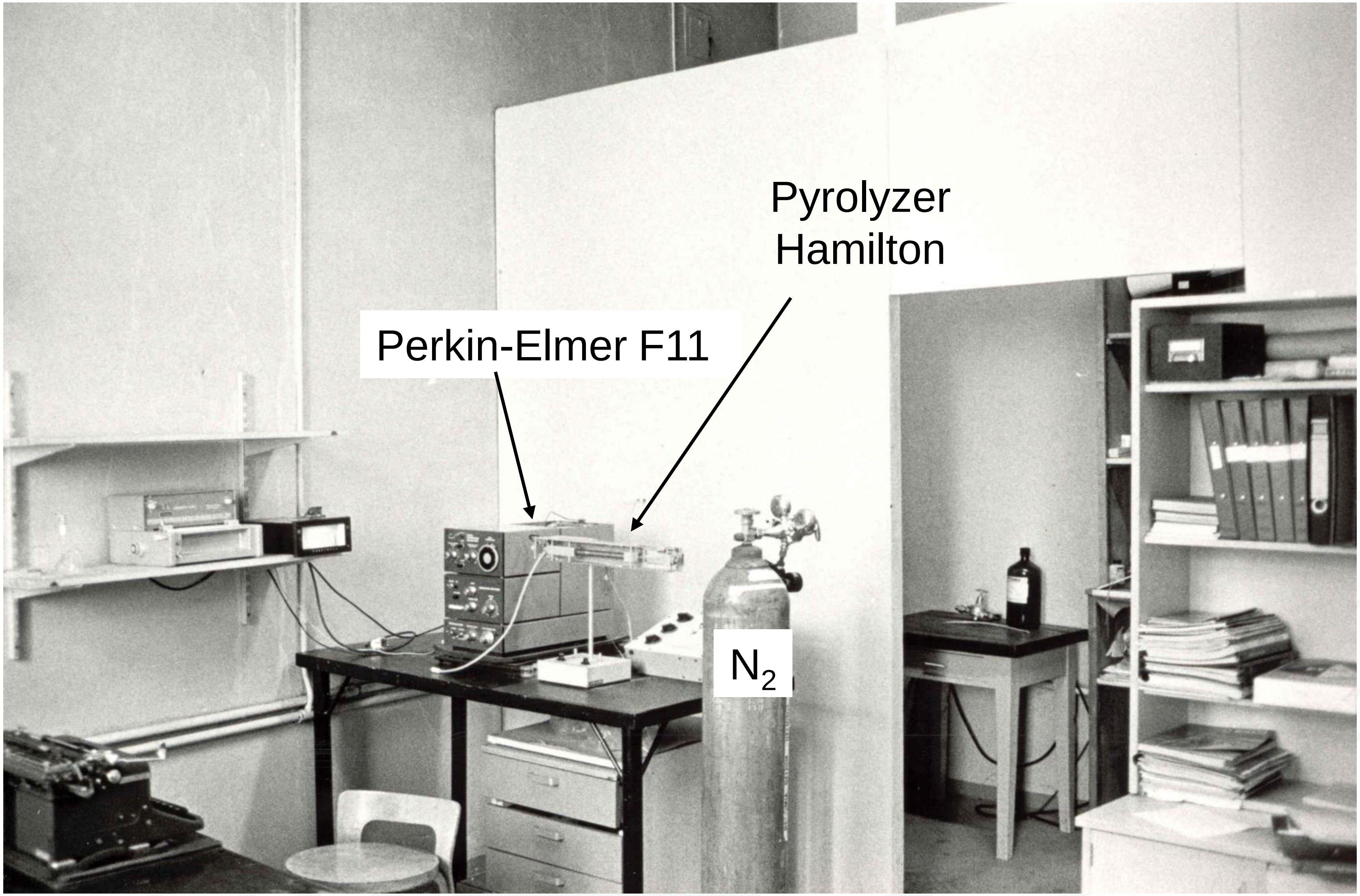
Evolution of analytical techniques for extractives

A personal review

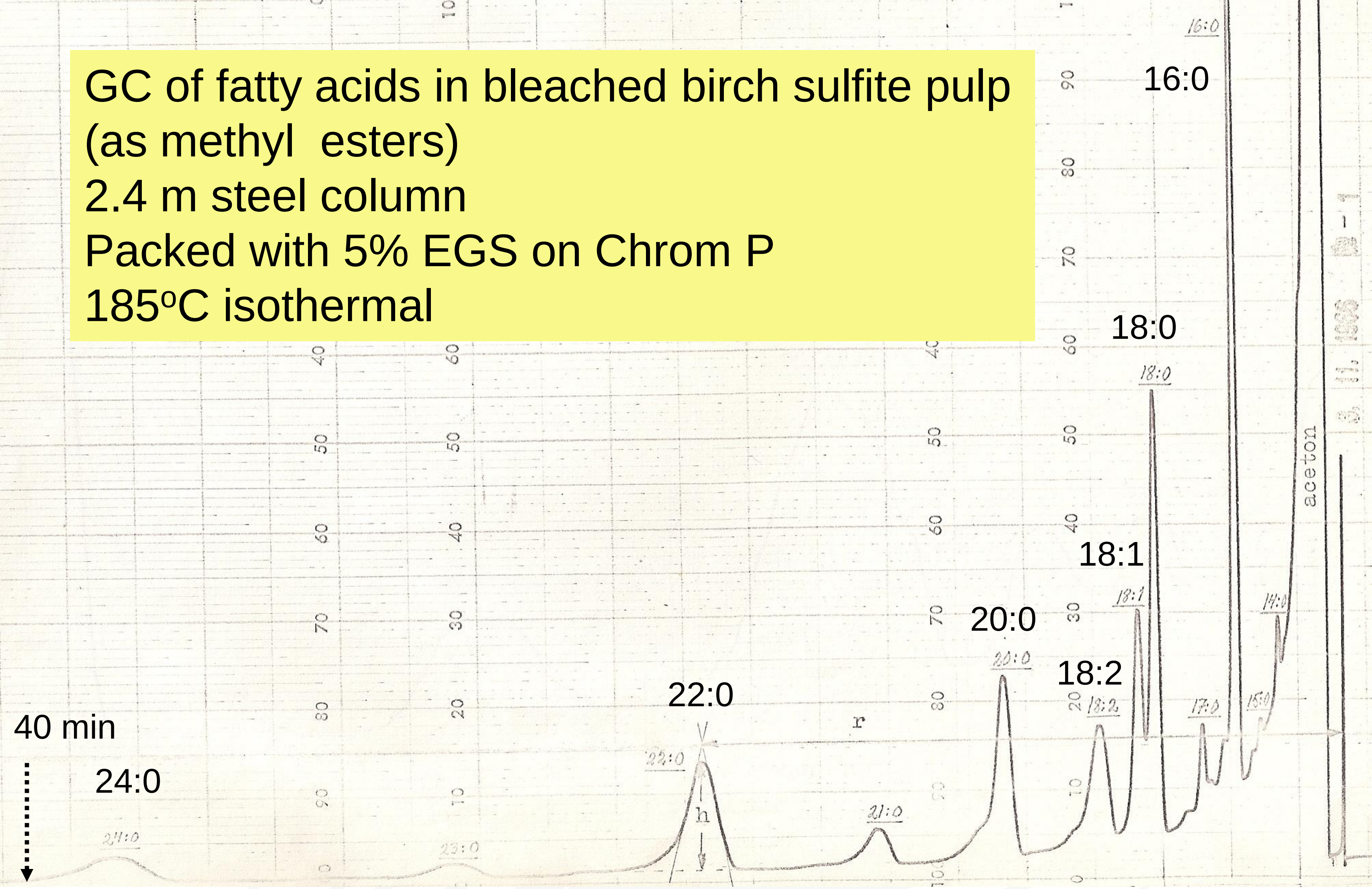
Bjarne Holmbom

Final Seminar on COST Action FP 0901, Turku/Åbo, September 17, 2013

State-of-the-art in 1966

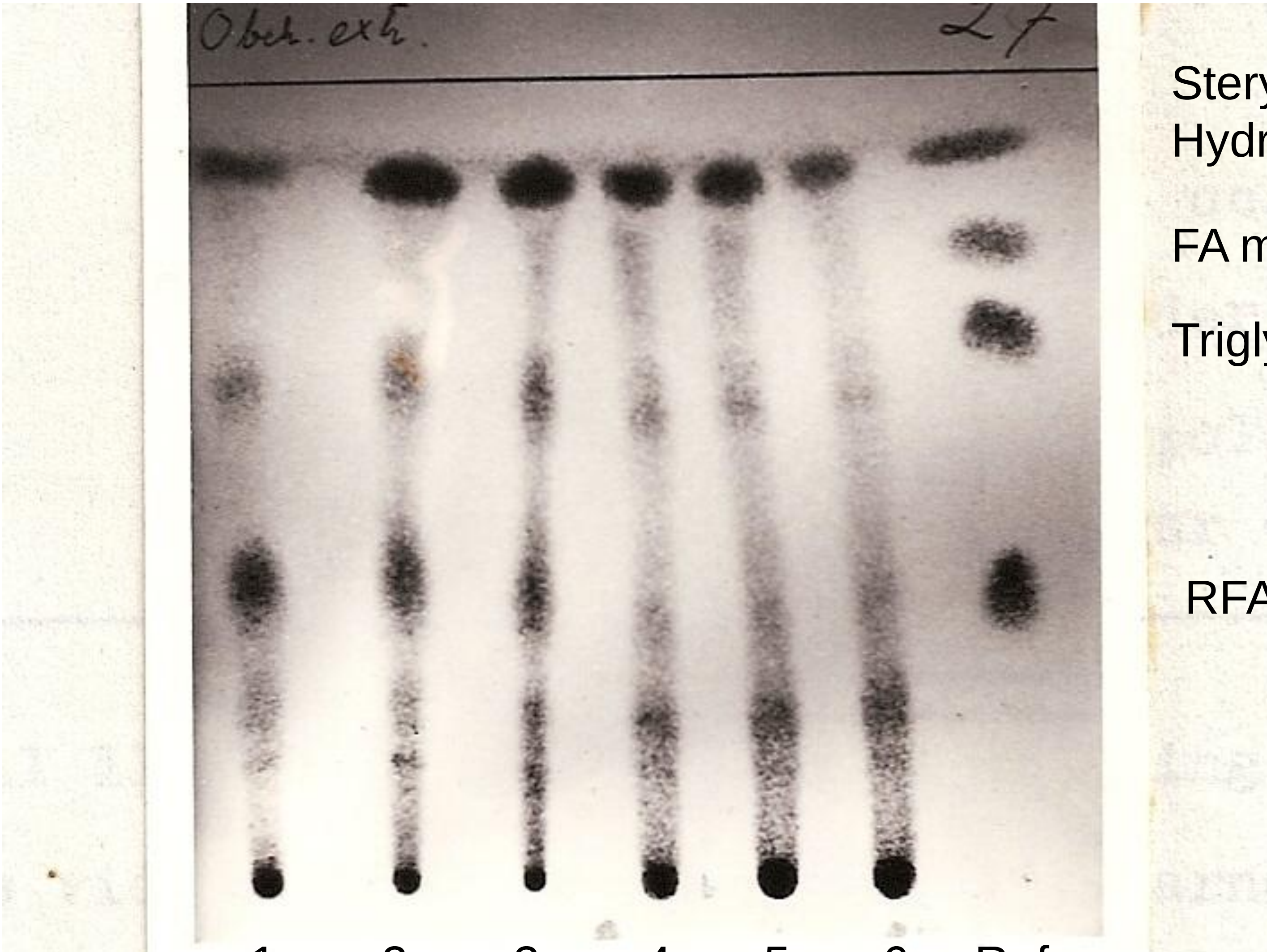


GC of fatty acids in bleached birch sulfite pulp
(as methyl esters)
2.4 m steel column
Packed with 5% EGS on Chrom P
185°C isothermal



Diploma work 1966-67

TLC



Steryl esters
Hydrocarbons

FA me ester

Triglycerides

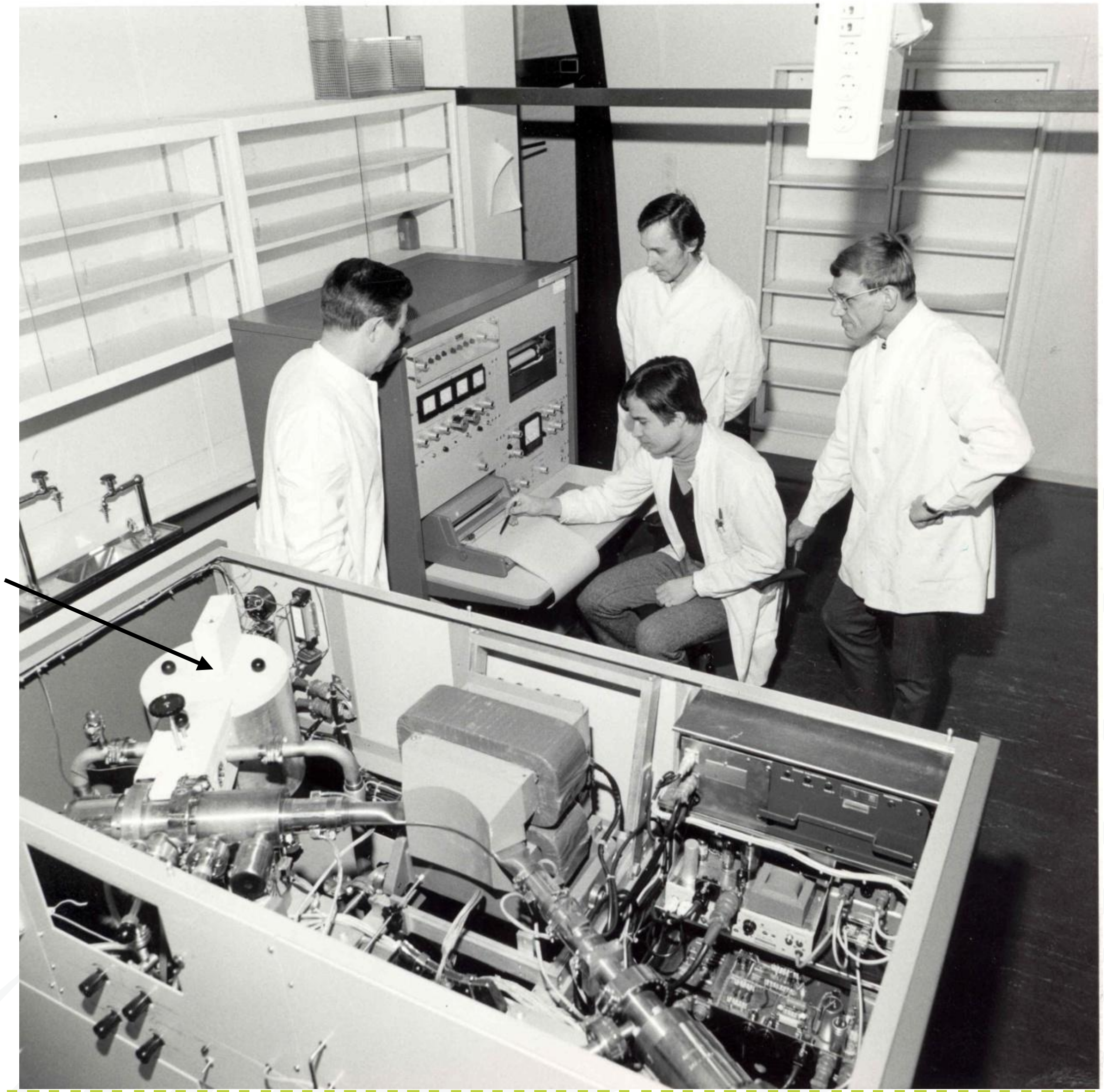
RFA´s

1	2	3	4	5	6	Ref.
Chips	Cook	D	E	H	Pulp	

Diploma work 1966-67

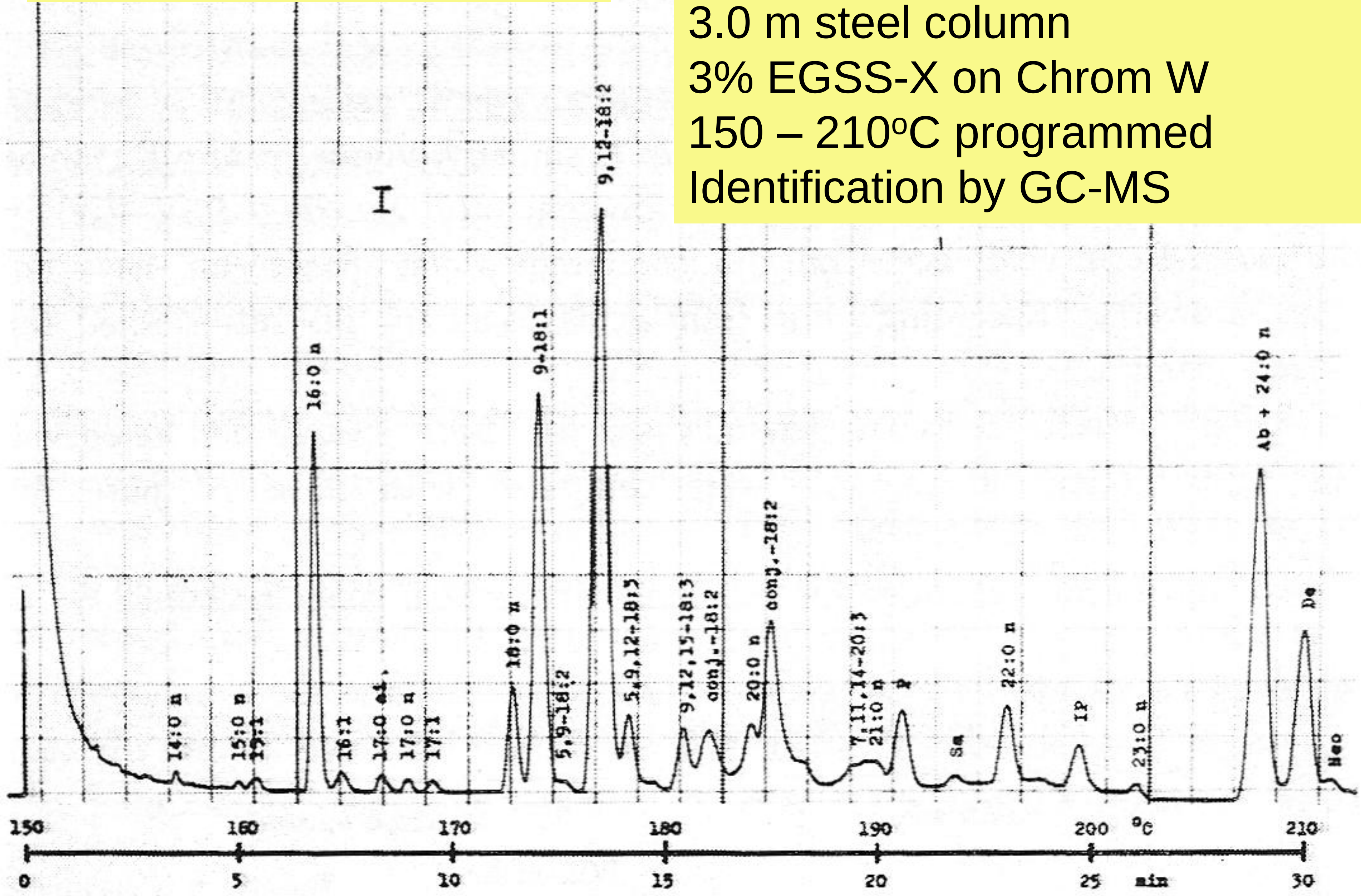
LKB 9000 GC-MS at ÅA in 1969

GC oven



B. Holmbom & E. Avela:
Acta Acad. Abo. B 1:13 **(1971)** 1-14

GC of fatty & resin acids in tall oil
1970
3.0 m steel column
3% EGSS-X on Chrom W
150 – 210°C programmed
Identification by GC-MS



1971

Electronic integrator

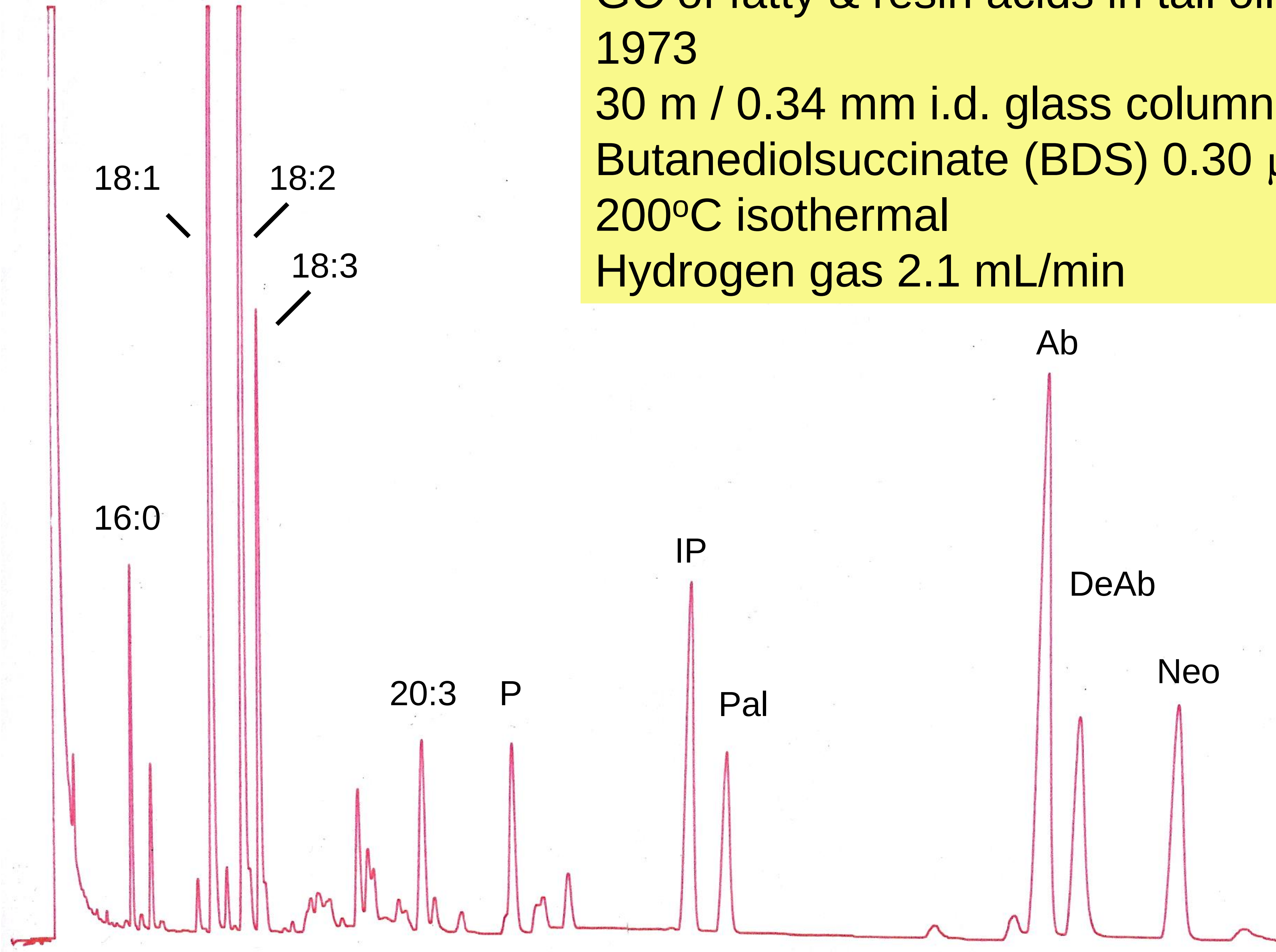
Glass capillary columns

Home-made

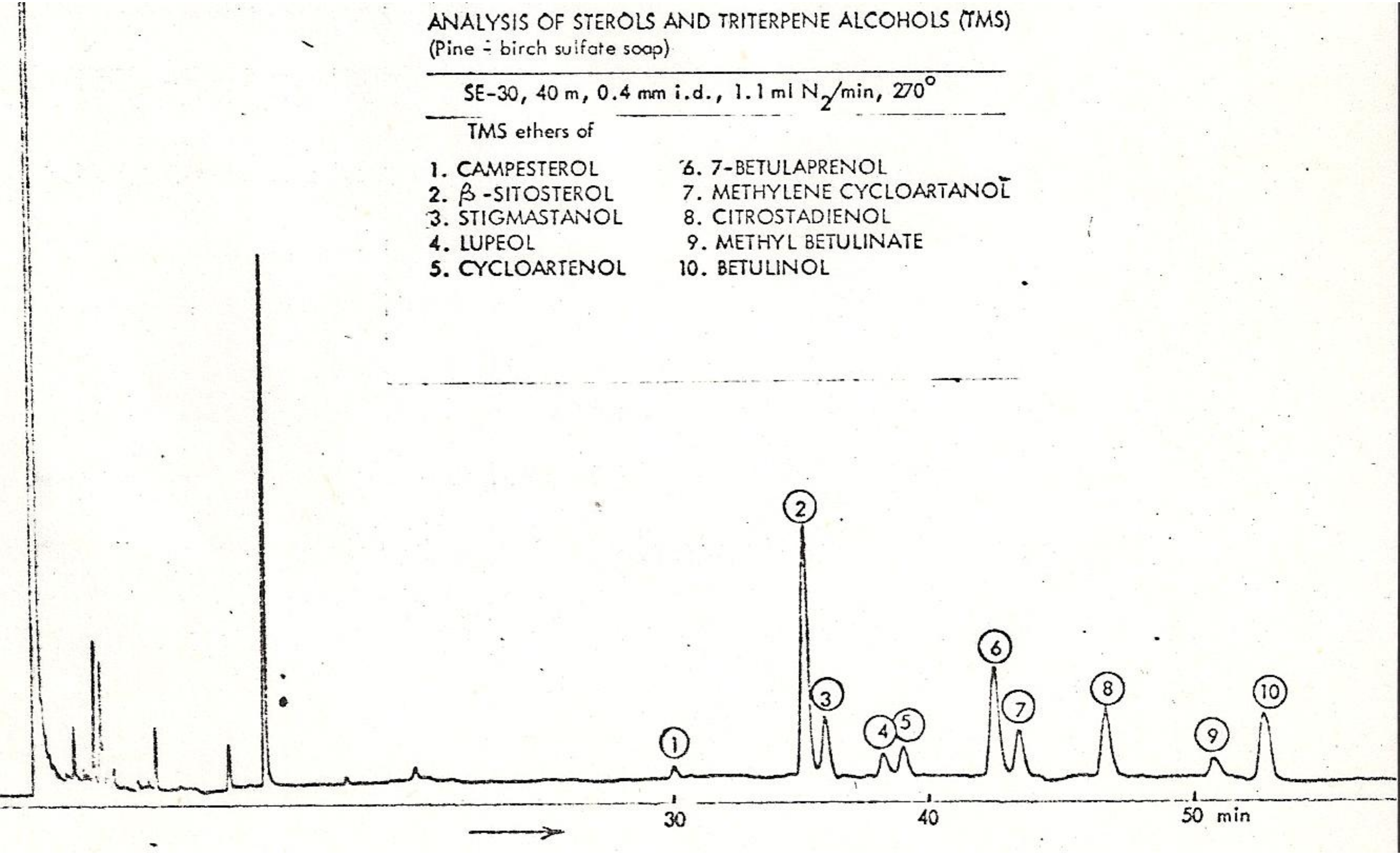
Also in GC-MS

First experiments in 1970

GC of fatty & resin acids in tall oil
1973
30 m / 0.34 mm i.d. glass column
Butanediolsuccinate (BDS) 0.30 μm
200ºC isothermal
Hydrogen gas 2.1 mL/min



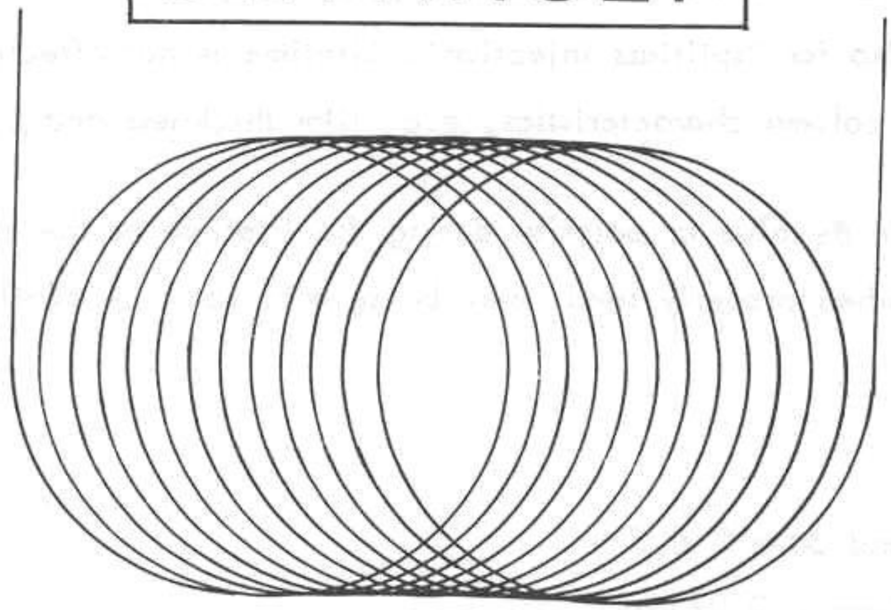
TMS – Sterols 1973



Tall oil refining plant in Lappeenranta 1977 - 2005

Oy Separation Research Ab brochure 1975

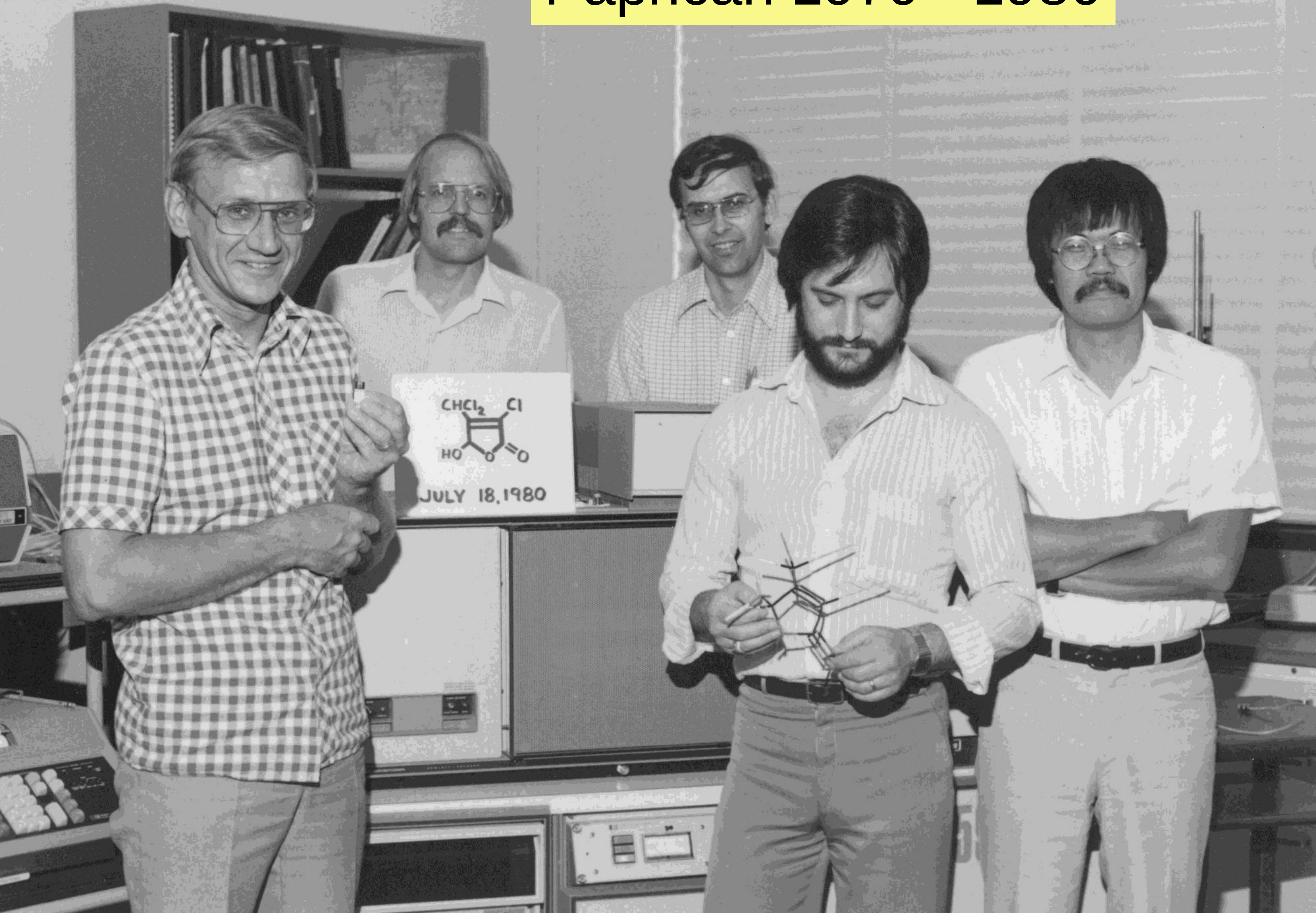
ULTRASEP®



High-Efficiency Glass Capillary Columns

With ULTRASEP Glass Capillary Columns as "the heart" of your gas chromatographic or GC-MS system you obtain separations close to what is theoretically possible.

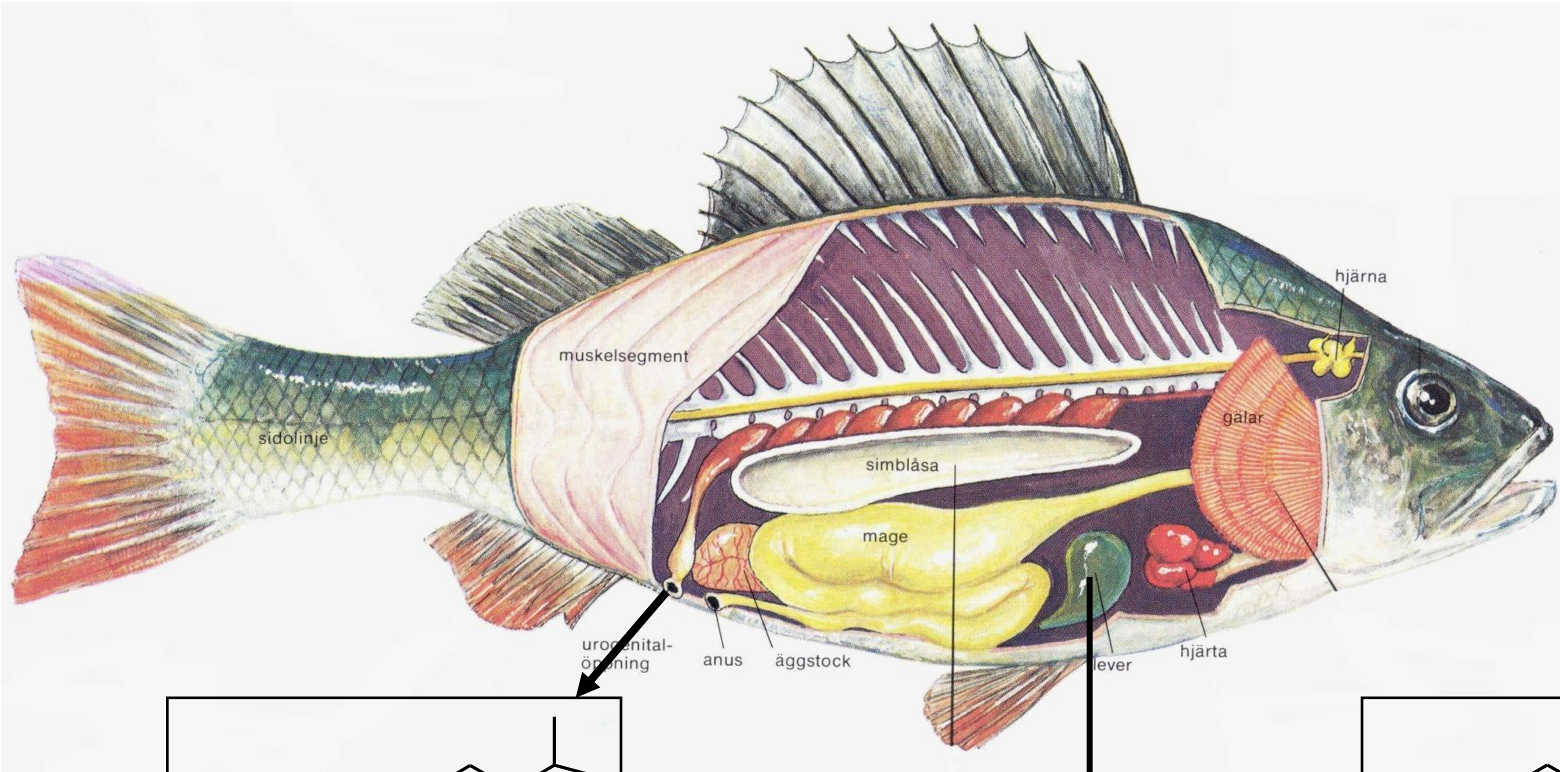
Paprican 1979 - 1980

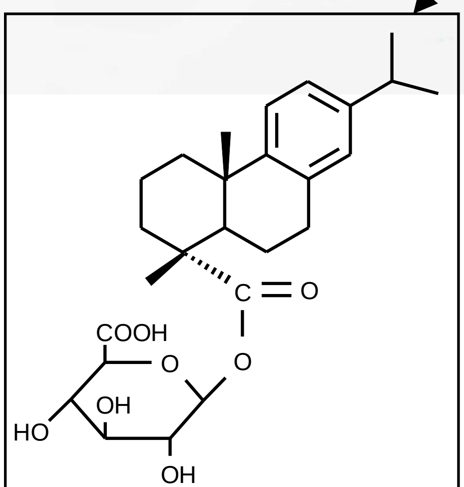


Forest Products Chemistry team in 1981

George Kruzynski

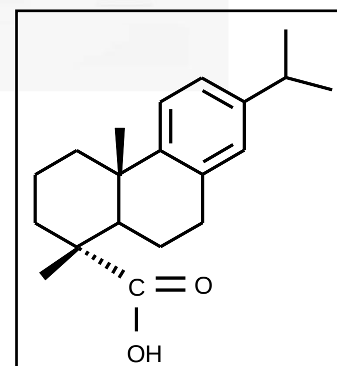
Resin acid uptake and metabolism





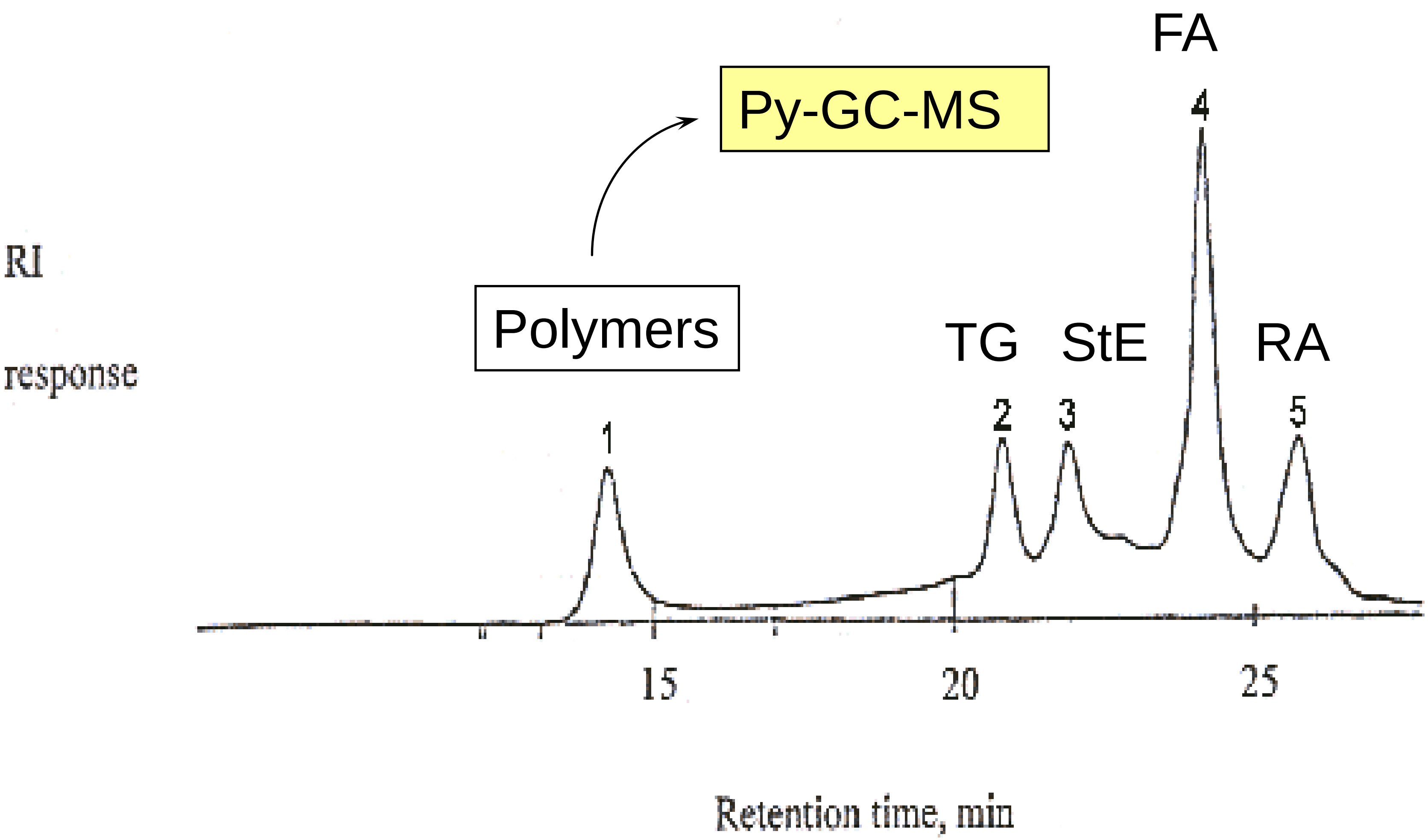
Liver

Bile



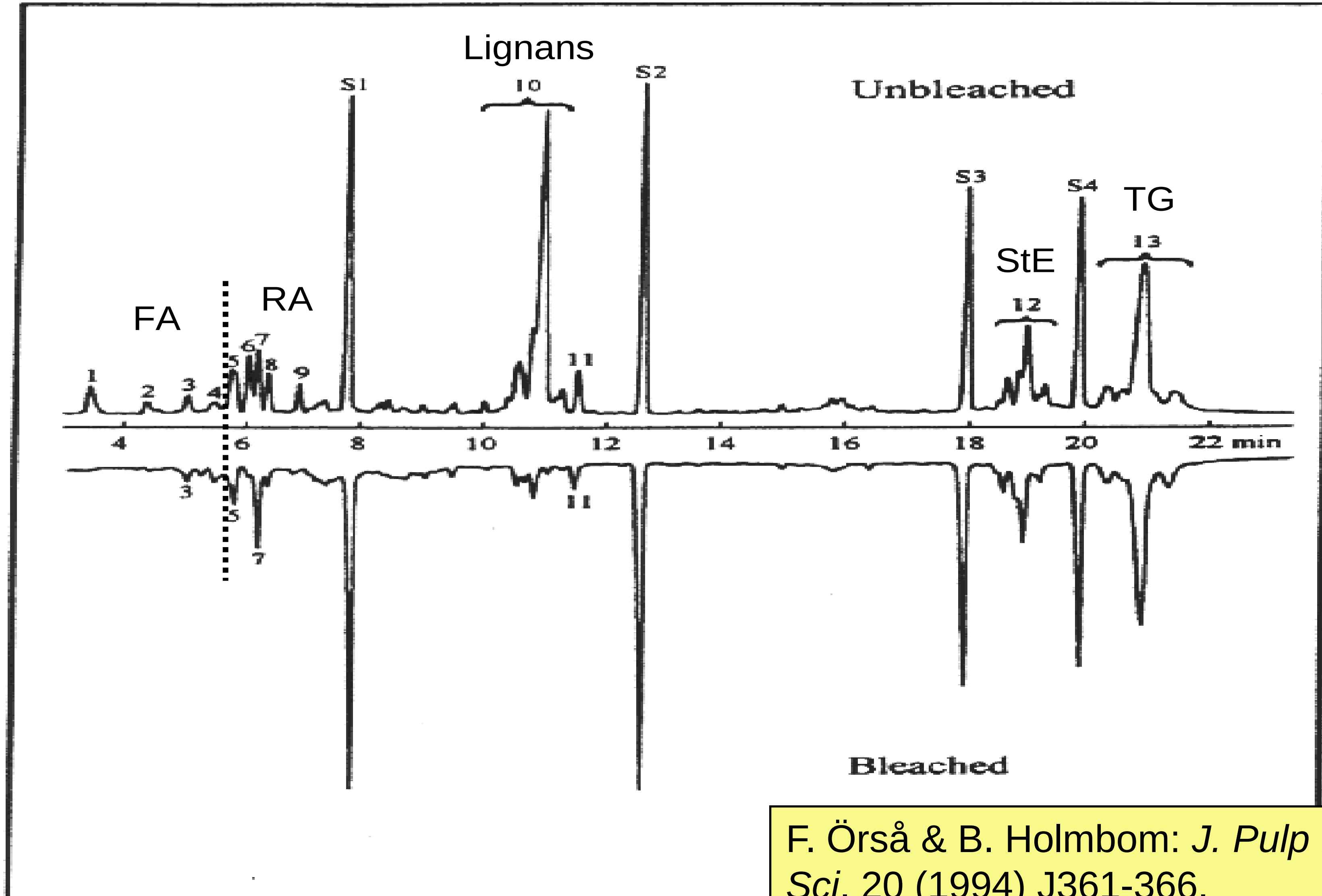
A. Oikari, E. Ånäs, G. Kruzynski, B. Holmbom
Bull. Environ. Contam. Toxicol. 33 (1984) 233-240.

HPLC - SEC of THF extract of DIP



J. Sjöström & B. Holmbom: *J. Chromatogr*. 411 (1987) 363-370.

Group analysis by short-column GC
Wood pitch in spruce TMP



F. Örså & B. Holmbom: *J. Pulp Pap. Sci*. 20 (1994) J361-366.

Short-column GC essentials

- Temperature-programmed injector
- On-column injection
- Columns
  - Short (5-7 m)
  - Thin-film (0.15 μm)
  - Wide-bore (0.53 mm)
- 4 internal standards
- Accurate integration

Paper mill process waters

Centrifugation

Supernatant
Dis. + Coll.

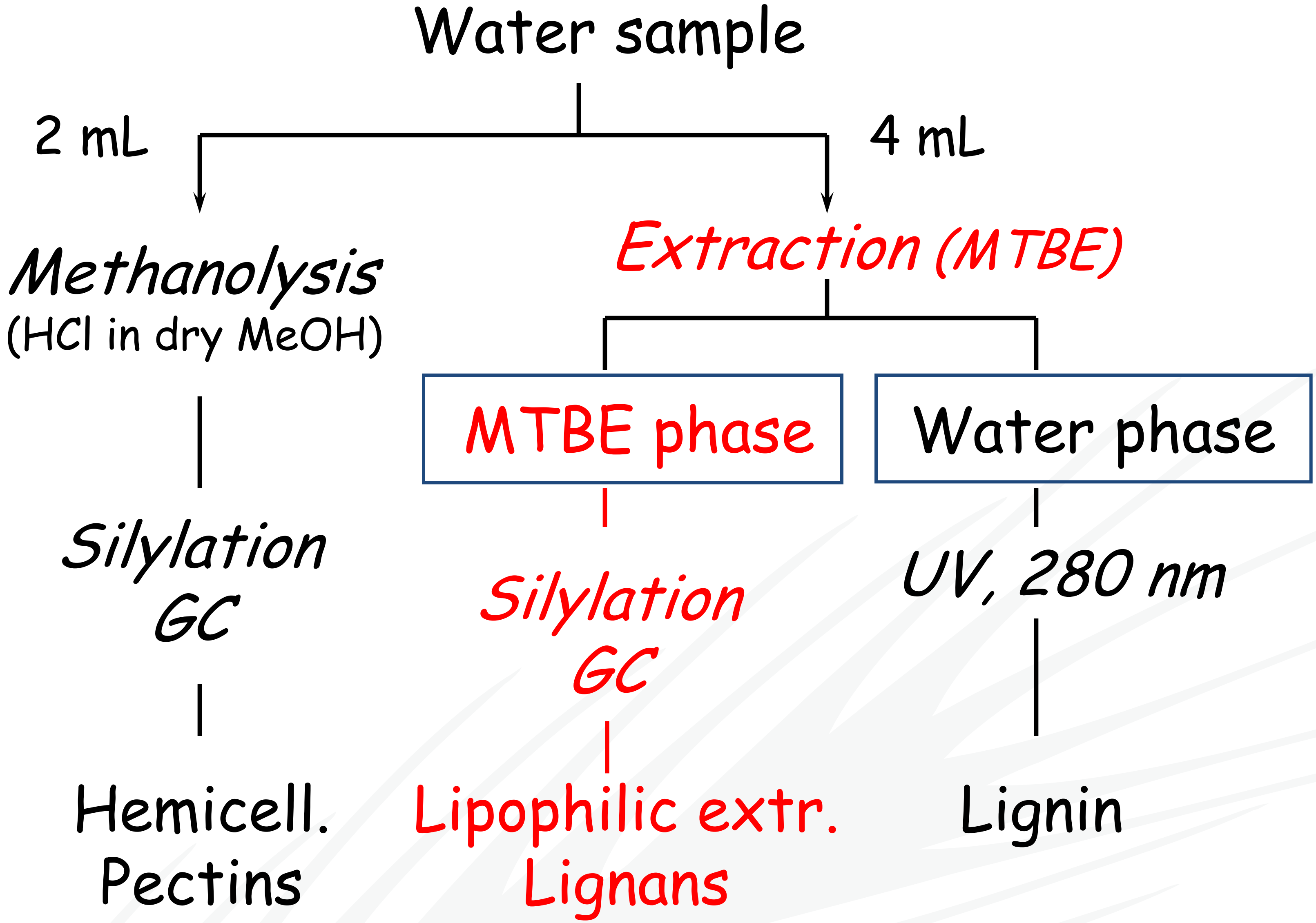
Microfiltration
(0.1-0.2 µm)

Filtrate
Dissolved

Filtration in
DDJ (76 µm)

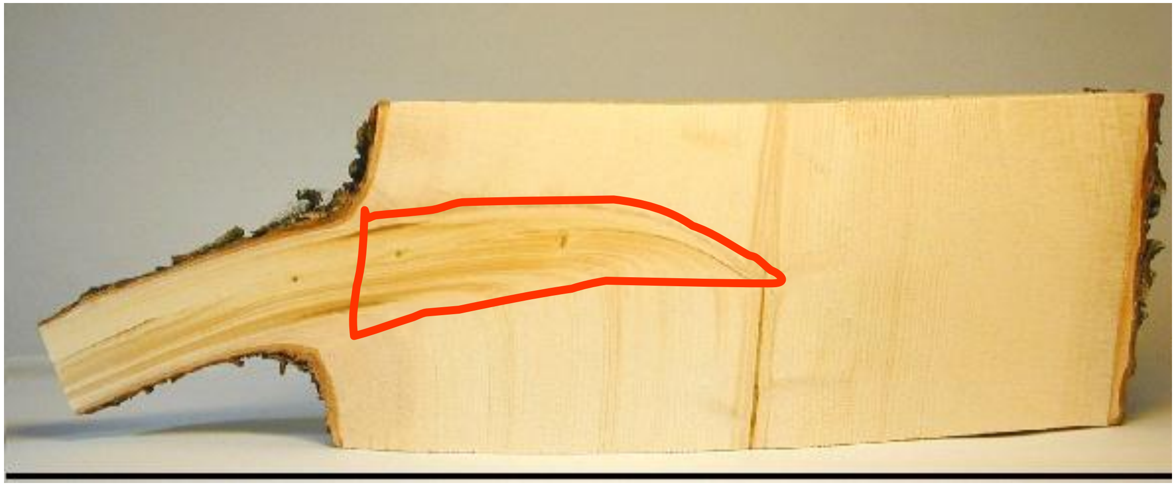
Filtrate
Diss. + Coll.
Fines & Particles

B. Holmbom: In: Analytical Methods in Wood Chemistry, Pulping and Papermaking. (Eds: E. Sjöström and R. Alén), Springer Verlag, Berlin 1998, pp. 269-285.



Holmbom 1998

In 1998, as it happened



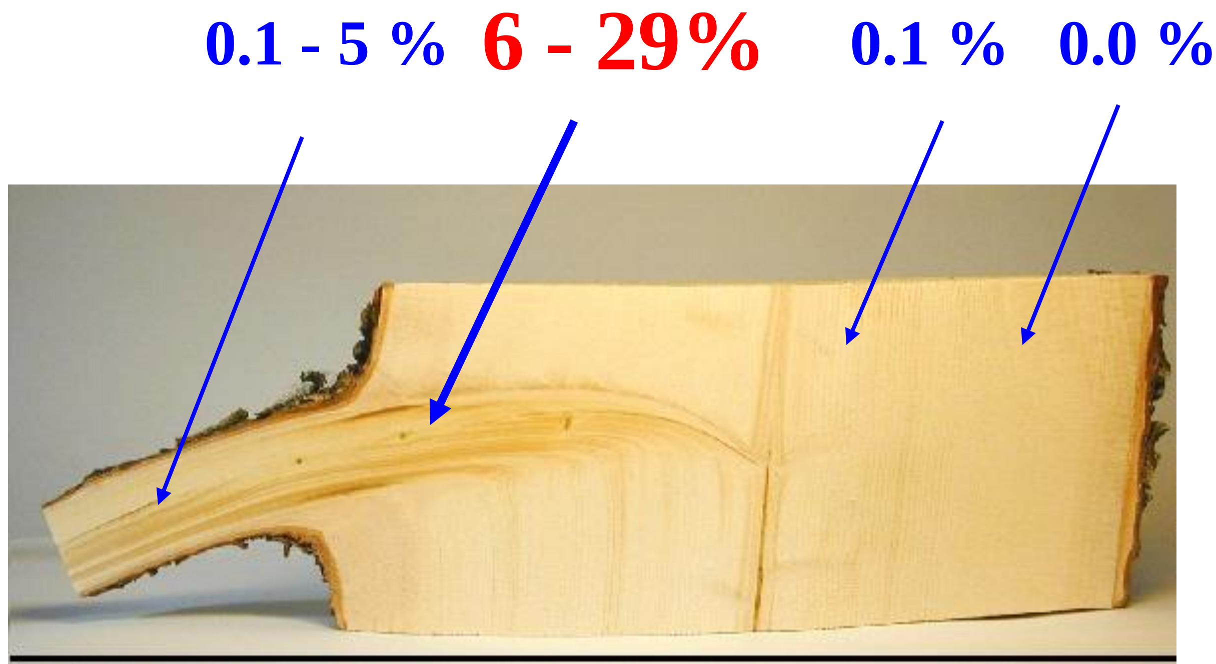
We sampled a spruce knot

Analysis: it contained 10 wt.% of lignans !

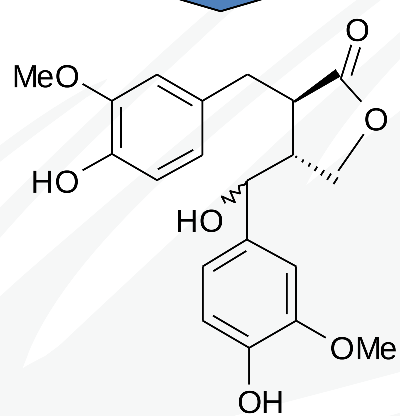
The start for extensive research on knots

Lignans in *Picea abies*

PhD work
Stefan Willför



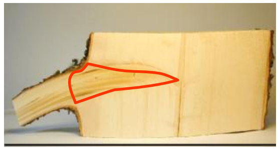
70-85% of the lignans



Hydroxymatairesinol (HMR)

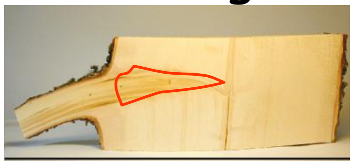
The knot story

Analysis
10% lignans !



1998

More spruce knots → 6-29% lignans



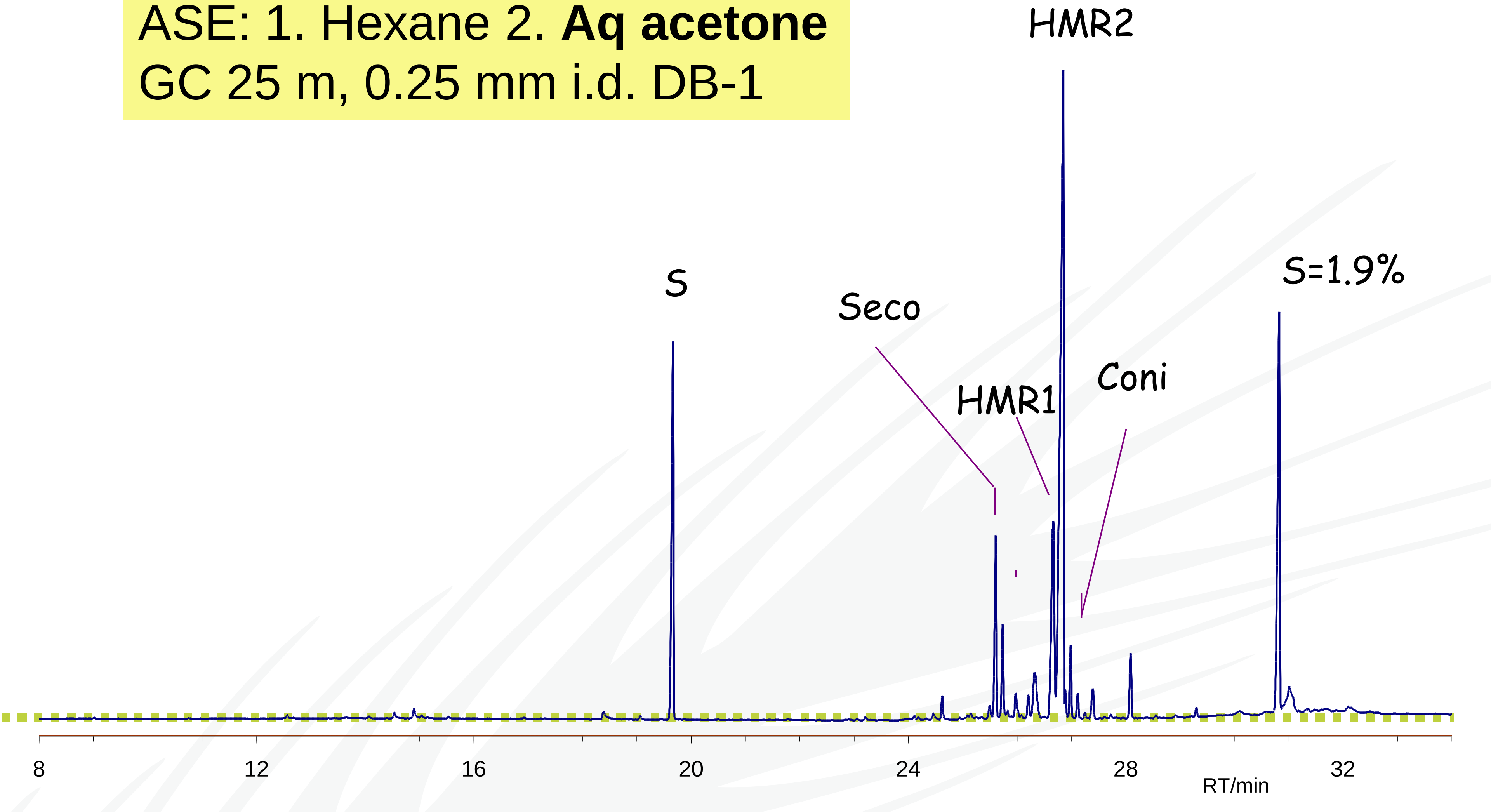
Knots from other tree species → 60+ species
5-15% polyphenols

Technical sep. of knots → Production of knots

Biotesting Applications → HMR as dietary supplement

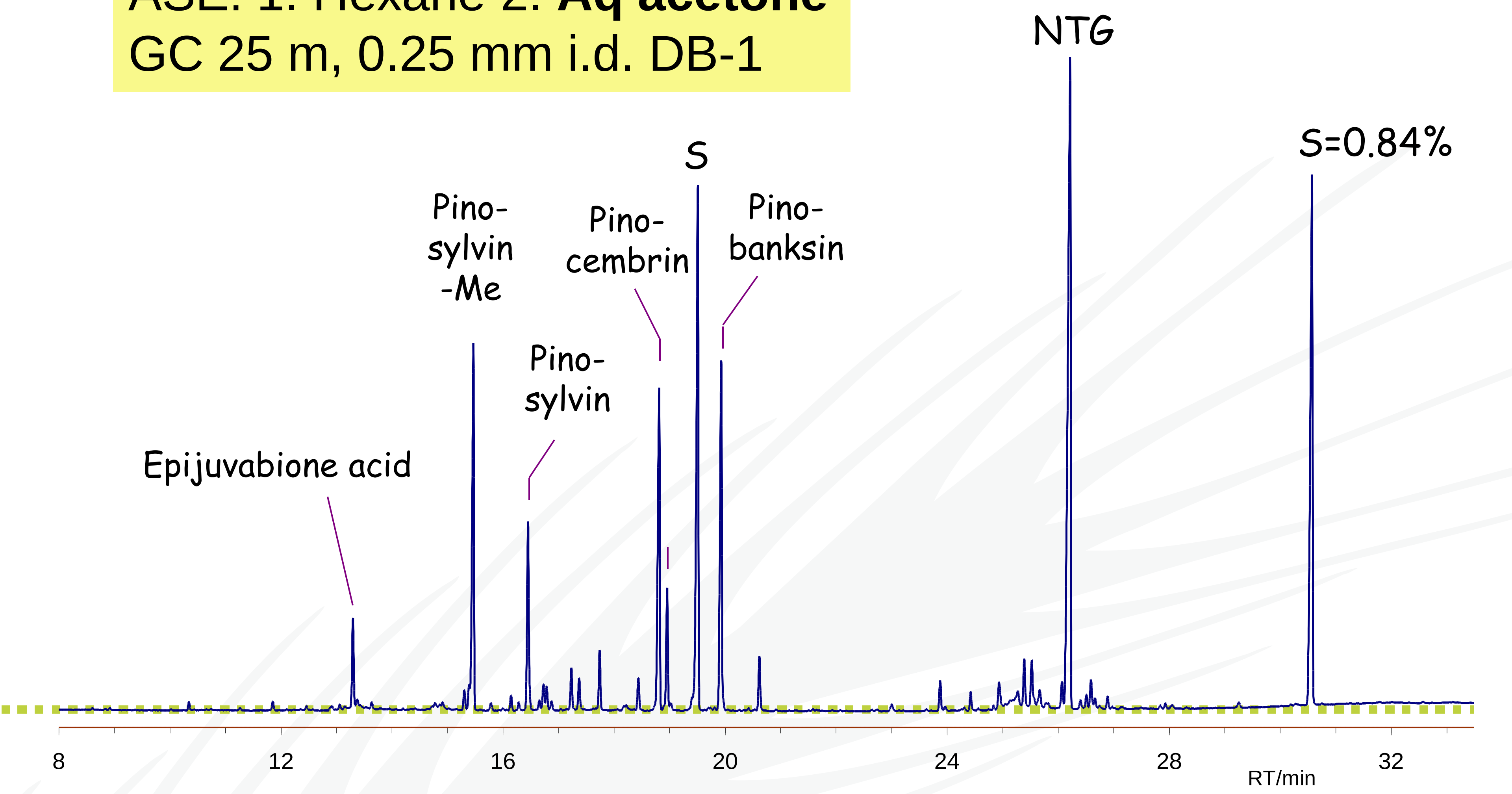
Picea abies

Knots
ASE: 1. Hexane 2. **Aq acetone**
GC 25 m, 0.25 mm i.d. DB-1

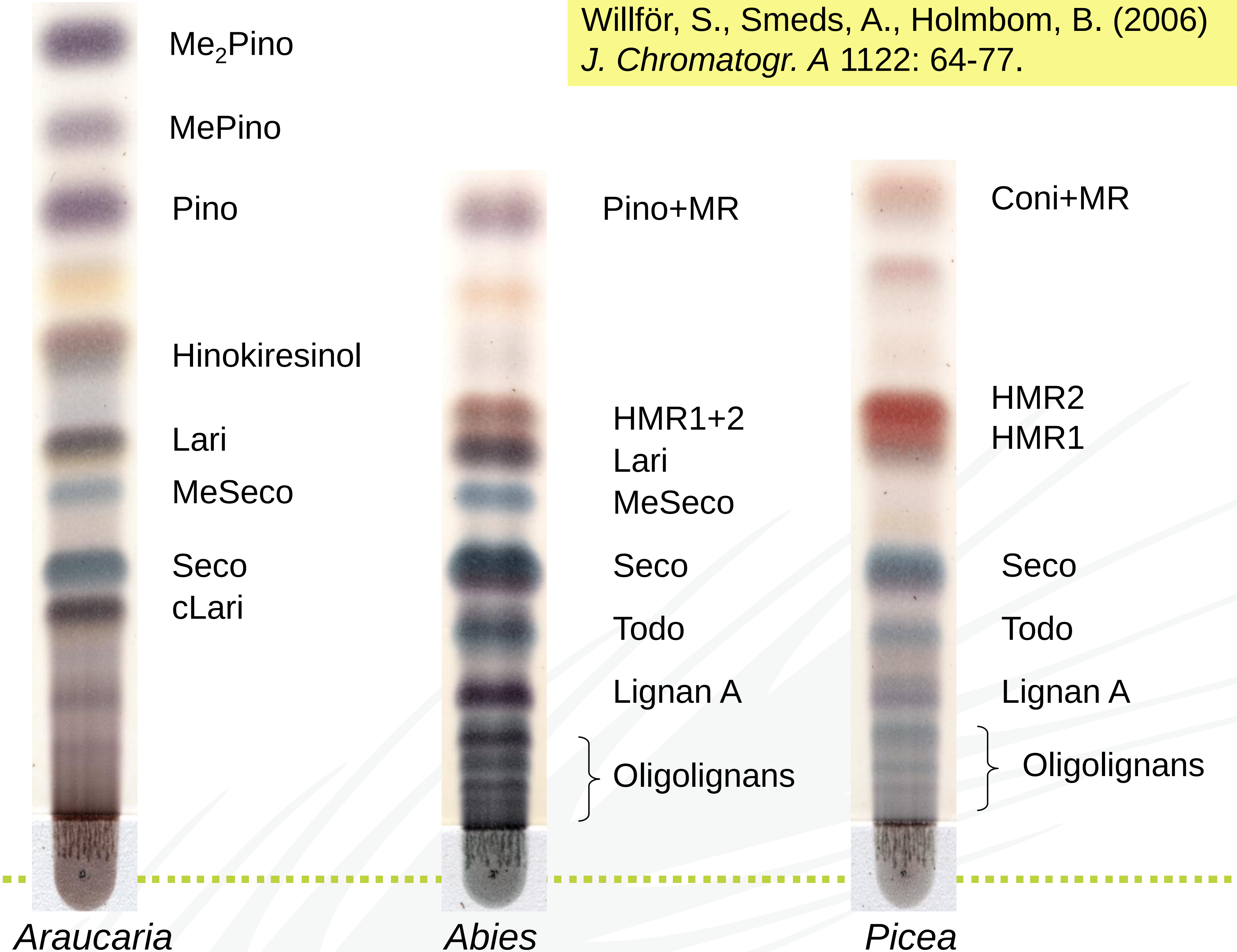


Pinus banksiana

Knots
ASE: 1. Hexane 2. **Aq acetone**
GC 25 m, 0.25 mm i.d. DB-1



Willför, S., Smeds, A., Holmbom, B. (2006) *J. Chromatogr. A* 1122: 64-77.



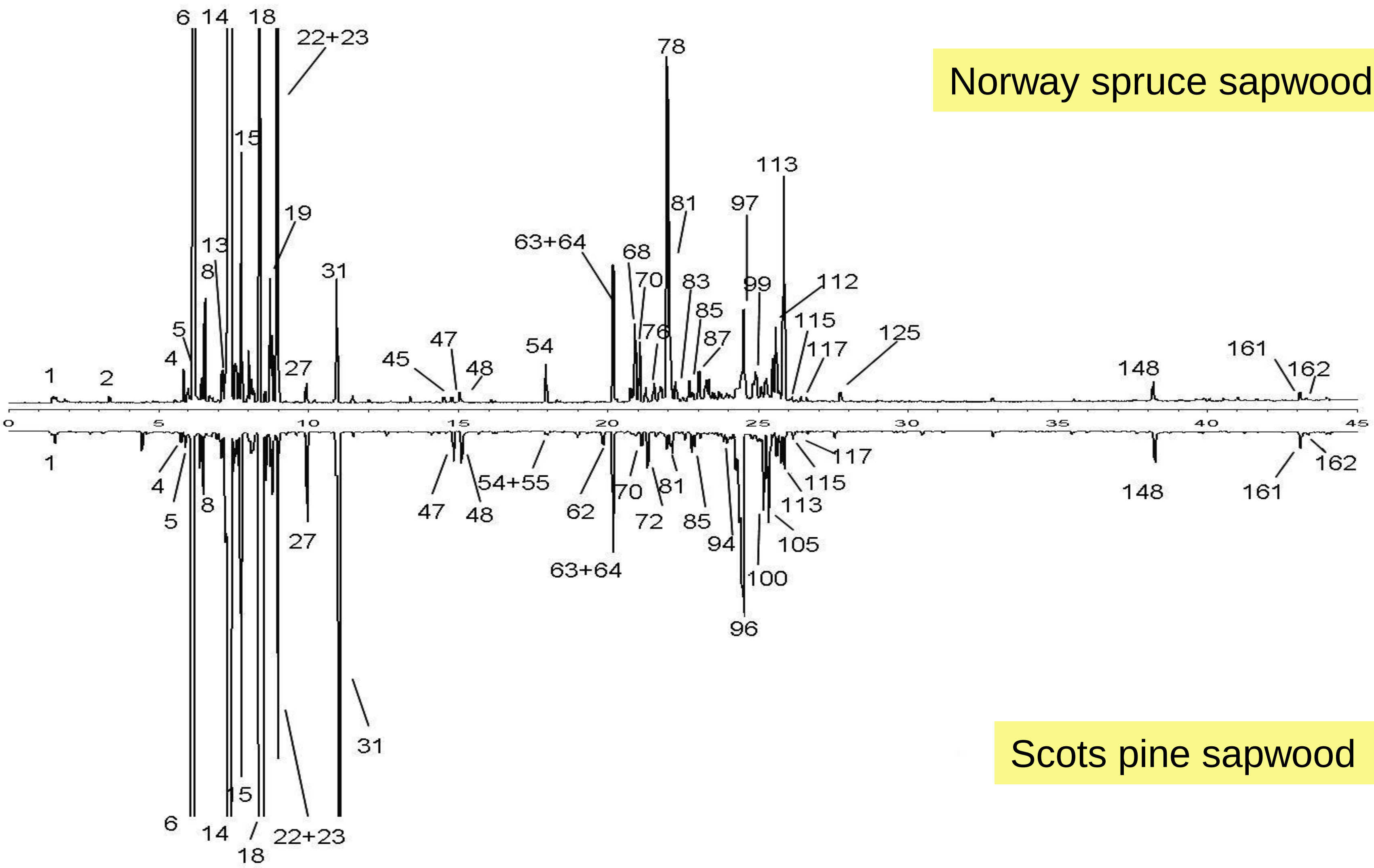
New techniques developed in the 1990´s - applied on extractives in the 2000´s

- ASE
- SPME
- LC-MS/MS
- ToF-SIMS, XPS
- Flow cytometry (FCM)

Accelerated Solvent Extraction (ASE)

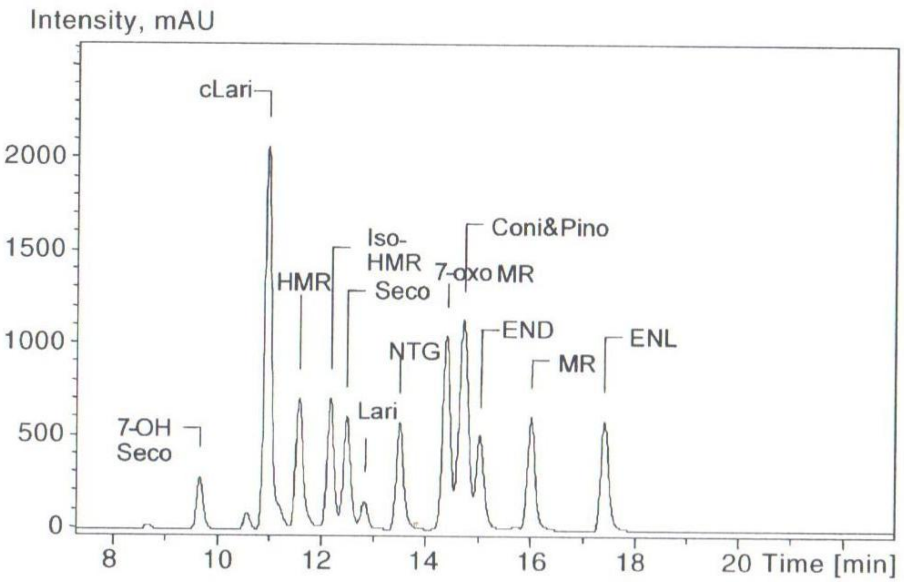
- Controlled automated, fast extraction with small amounts of solvents
- Pressurised, up to 200°C
- Requires optimisation (temp., cycles, flush between cycles)
- Sequential extraction
  - 1. Lipophilic components (hexane)
  - 2. Acetone/EtOH + 5-10% water

Solid-Phase Microextraction (SPME)



Wajs, A., Pranovich, A., Reunanen, M., Willför, S., Holmbom, B. (2007) *J. Essential Oil Res.* 19, 125-133.

LC and LC – MS/MS



13 lignan ref. mixture
RP8 HPLC (UV 280 nm)

HPLC most used for polyphenols
Especially in biological matrixes

LC-MS/MS gives extreme
sensitivity
selectivity

HPLC also coupled to H-NMR

Unique for biological fluids

Willför, S., Smeds, A., Holmbom, B. (2006) *J. Chromatogr. A* 1122: 64-77.

Surface-specific analysis

- XPS (ESCA)
  - Coverage of extractives in per cent
- ToF-SIMS
  - A few molecular layers
  - Mapping and identification of extractives
  - Lateral resolution like optical microscope

Flow Cytometers

Laser light scattering

500,000 €

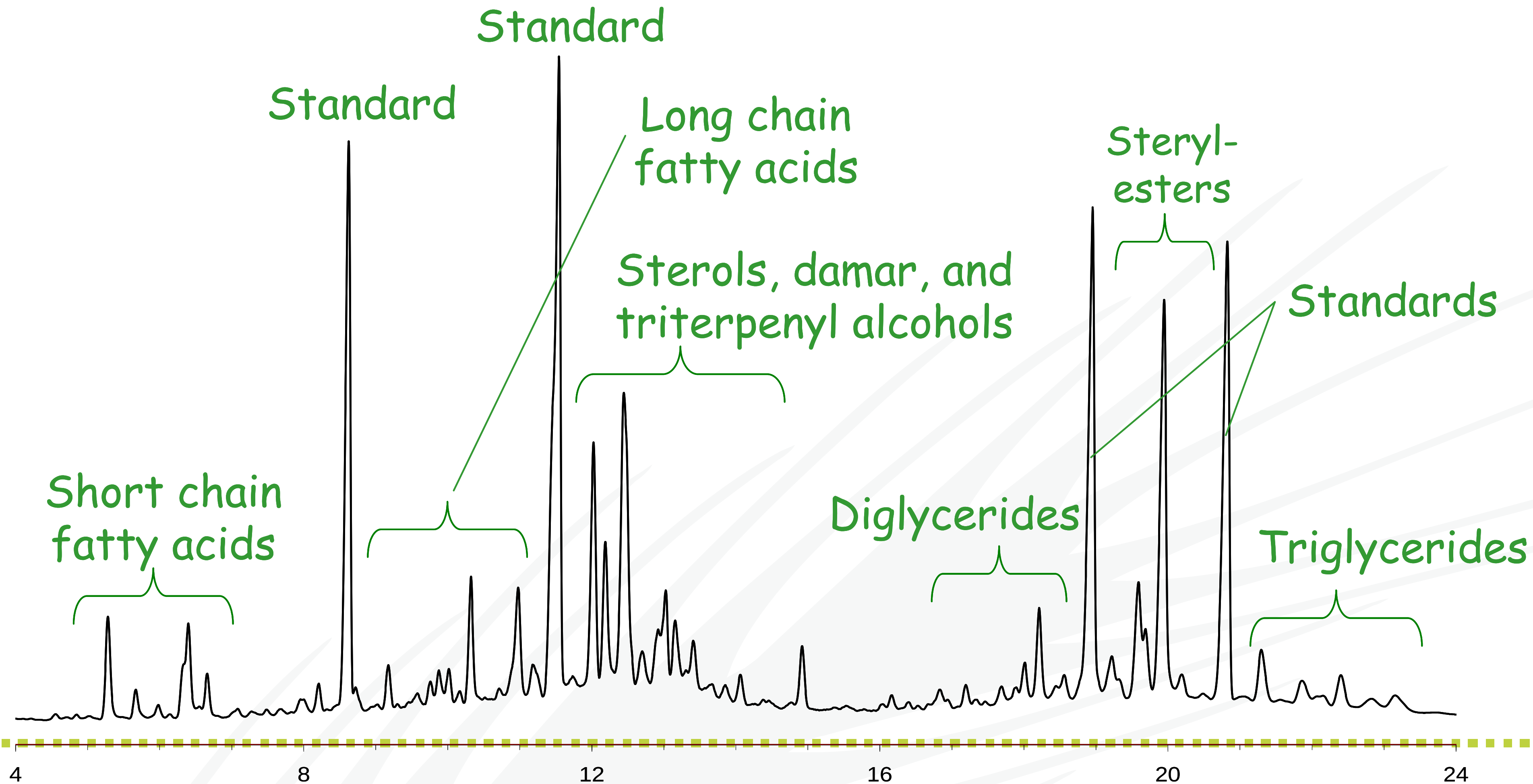
50,000 €
Portable

FCM can count particles 0.2 µm – 100 µm

- Fines
- Wood pitch (colloidal / aggregated)
- Bacteria / Fungi (Live or dead)
- DIP-soap particles
- White pitch particles

Lari Vähäsalo, the pioneer

Acetone extracts of mixed tropical hardwoods



Conclusions

- GC, GC-MS and TLC still key techniques, especially for lipophilic extractives
- GC more convenient and accurate now thanks to computers and automation
- HP-SEC can "see all extractives"
- HPLC for complicated polar extractives
- LC-MS/MS for biological fluids
- ToF-SIMS and XPS for surface mapping / analysis

Challenges Needs

- Accurate, reliable quantification
  - Appropriate calibration
  - Reproducibility between laboratories
  - Certified calibration mixtures
- Less laborious, automated analytical procedures

Extraction problems

- Air drying can "fix" the extractives
- Due to re-distribution by sublimation ("self-sizing") ?
- Also oxidation-polymerisation possible
- Freeze-drying recommended

For GC: to remember

- Never integrate a tailing peak
- Use several internal standards - for internal control of derivatisation and GC conditions
- Change in temperature – change in polarity – change in elution profile

Final words

- It has been exciting and fun (most of the time)
- Extractives are fascinating
  - in their diversity
  - in their biological / biomedical functions
- Biosciences need chemistry
- Still many opportunities and adventures