HPLC analysis methods used for analyzing sugars and sugar derivatives in IL media obtained from lignocellulosic biomass

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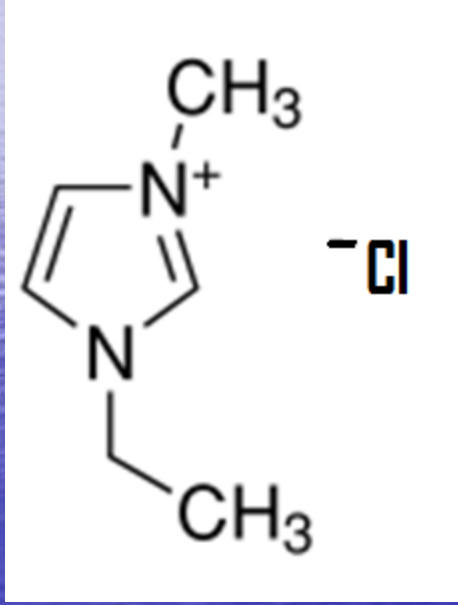
POKE Workshop, December, 11-12, 2013 Tallinn

1-Ethyl-3-methylimidazolium chloride

EmimCl or $[emim]^+ [Cl]^-$

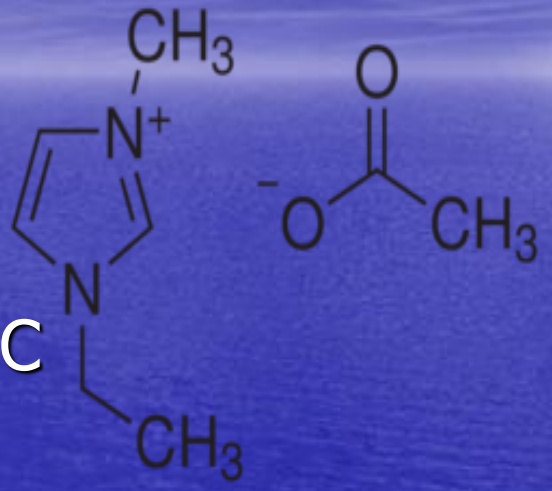


Molar mass:	146,62 g/mol
Density	1,1120 g/cm^3 (at 80 °C)
Melting point	77-80 °C ← impurities effect
Flash point:	186 °C
Viscosity	47.4 mPas (at 80 °C)
Flame point	515 °C
Solubility in water	∞

Very hygroscopic

Information concerning melting point varies in literature

1-Ethyl-3-methylimidazolium acetate
EmimOAc or $[emim]^+ [CH_3COO]^-$



- Molar mass: 170,21 g/mol
- Density 1.027 g/cm^3 at 25 °C
- Melting point > 30 °C
- Flash point: 164 °C
- Viscosity 10 mPas (at 80 °C)
- Solubility in water ∞

SUGARS in trees

- Cellulose
- Hemicelluloses
  - (Galacto)glucomannans 20-25 % of softwood's wood substance (water & alkali soluble)
  - Glucanes, galactanes & arabinogalactanes
- Pectines
  - Galacturonic acid units & some rhamnose units

    (→ Gives negative charge to fibers)

Saccharides & their derivatives

- Furfural
- 5-HMF
- Cellobiose
- Galactose
- Glucose
- Mannose
- Fructose
- Arabinose
- Xylose

Valuable platform chemicals

(galacturonic acid)

(glucuronic acid)

monosaccharides

analyzed by:

- **GC**
- **HPLC**
- **CE**

Focus/aim

Norway spruce (*Picea abies*)

Scots pine (*Pinus sylvestris*)

Silver Birch *(Betula pendula)*

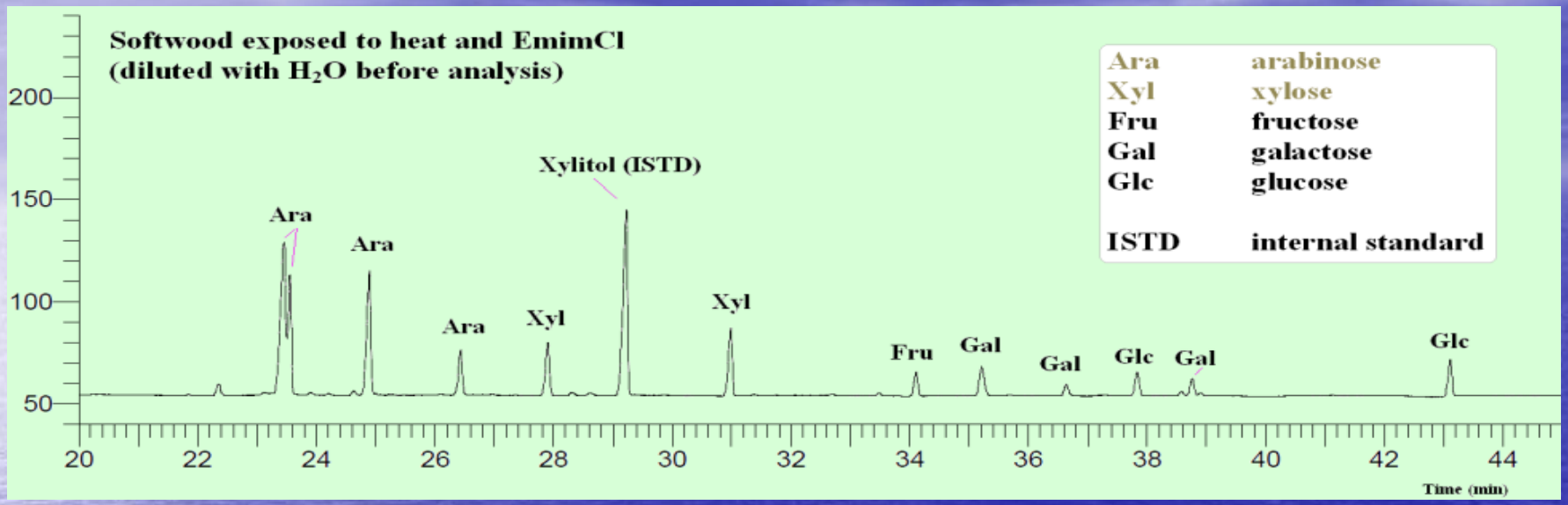
- debarking
- sawing
- chipping
- screening
- freeze-drying

IL

HEAT

SUGARS & THEIR DERIVATIVES

…GC analysis



Analysis of carbohydrates on extract

Without acid methanolysis

- calibration samples: STD sugars with xylitol in MeOH
- ISTD: xylitol instead of sorbitol

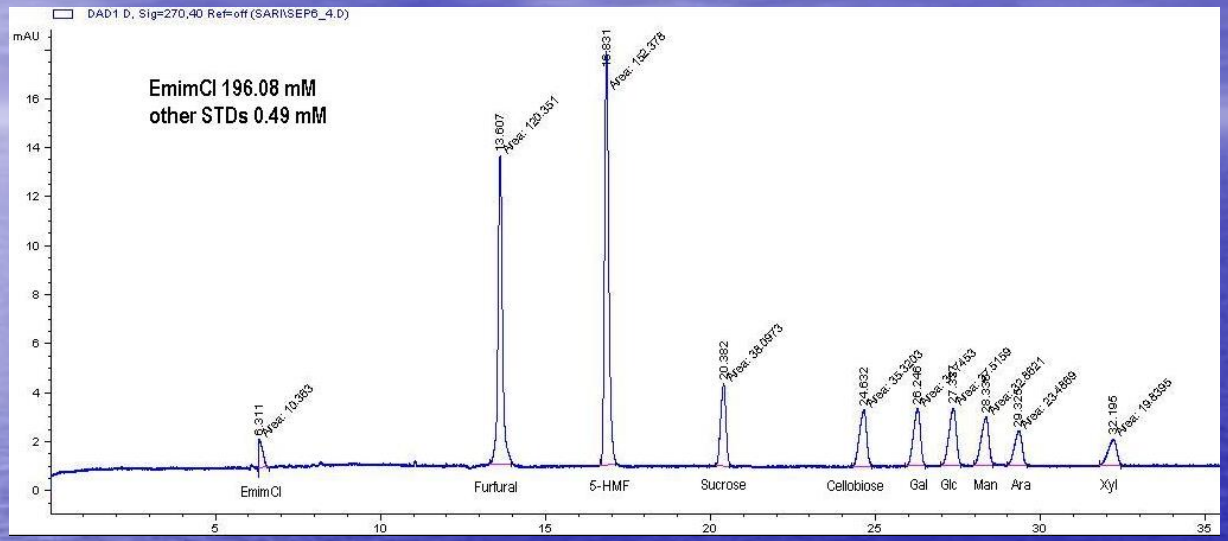
→Evaporation (N_2) + vacuum oven →

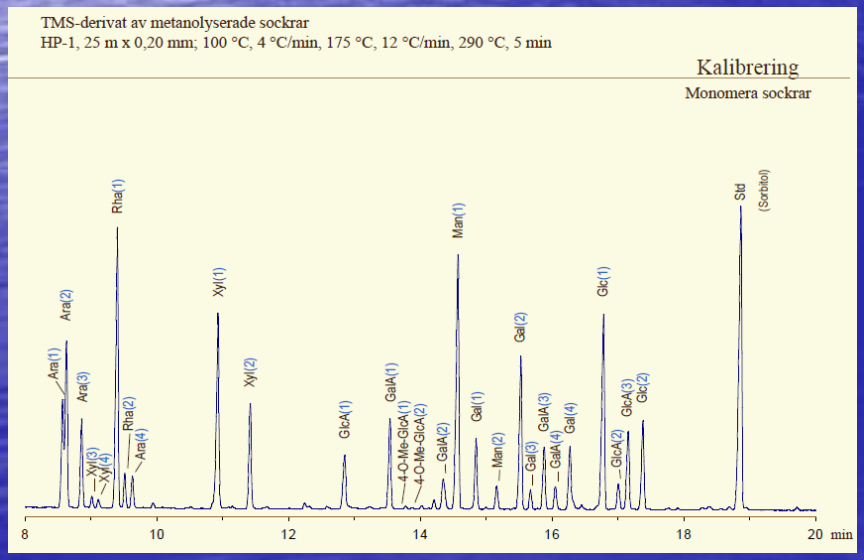
- silylation:

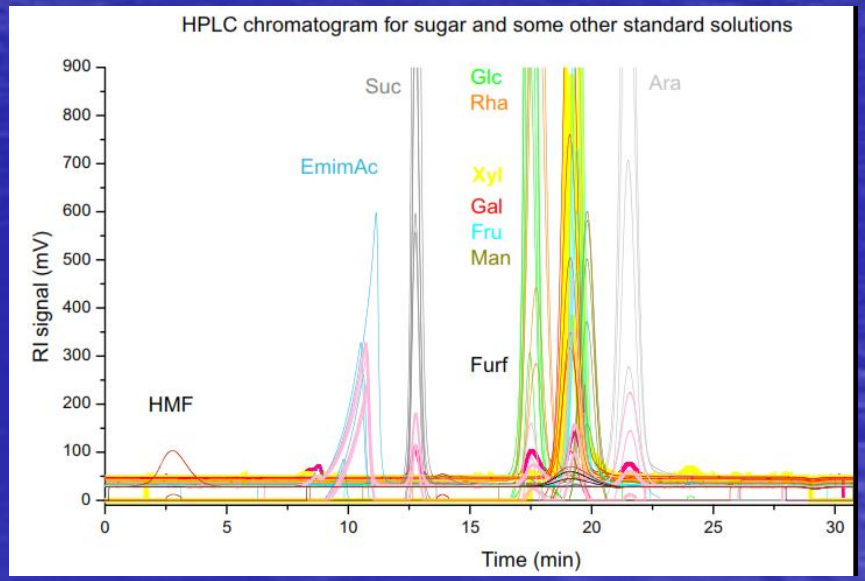
  pyridine, HMDS and TMCS

→ shaking (and ultrasonic bath)

HPLC & GC chromatograms vs. CE electropherogram

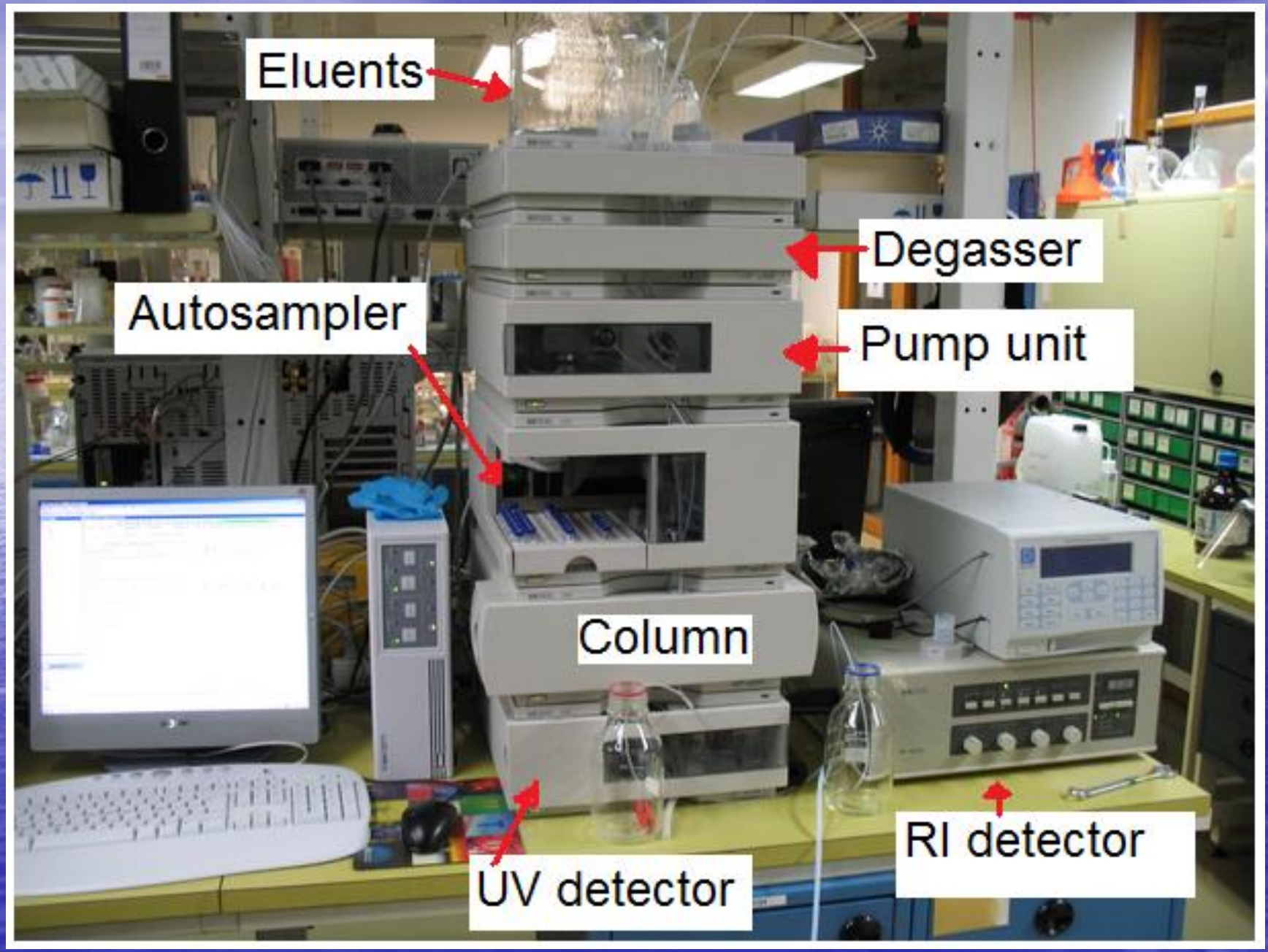






HPLC

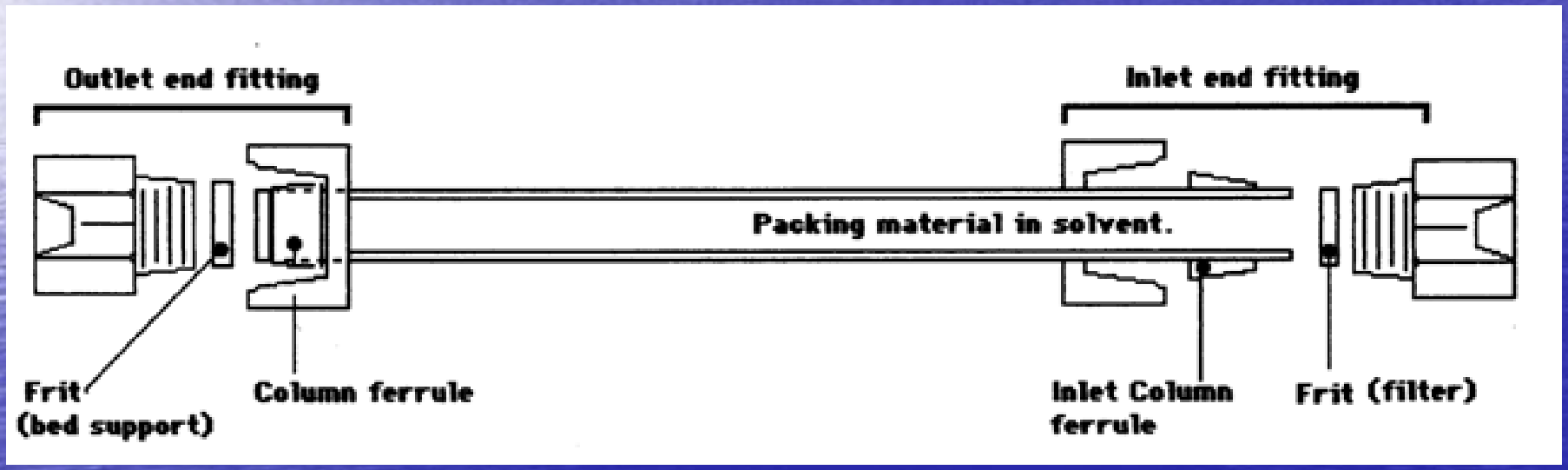
High Pressure/Performance Liquid Chromatography


Eluents
Degasser
Autosampler
Pump unit
Column
UV detector
RI detector

HPLC column

- heavy-walled stainless steel
- equipped with end-fitting containing porous frits

→ Frits filter incoming sample and support the compressed bed of HPLC packing wetted with the mobile phase.



(**Guard column** = protective column
placed in-line between injector and main column)

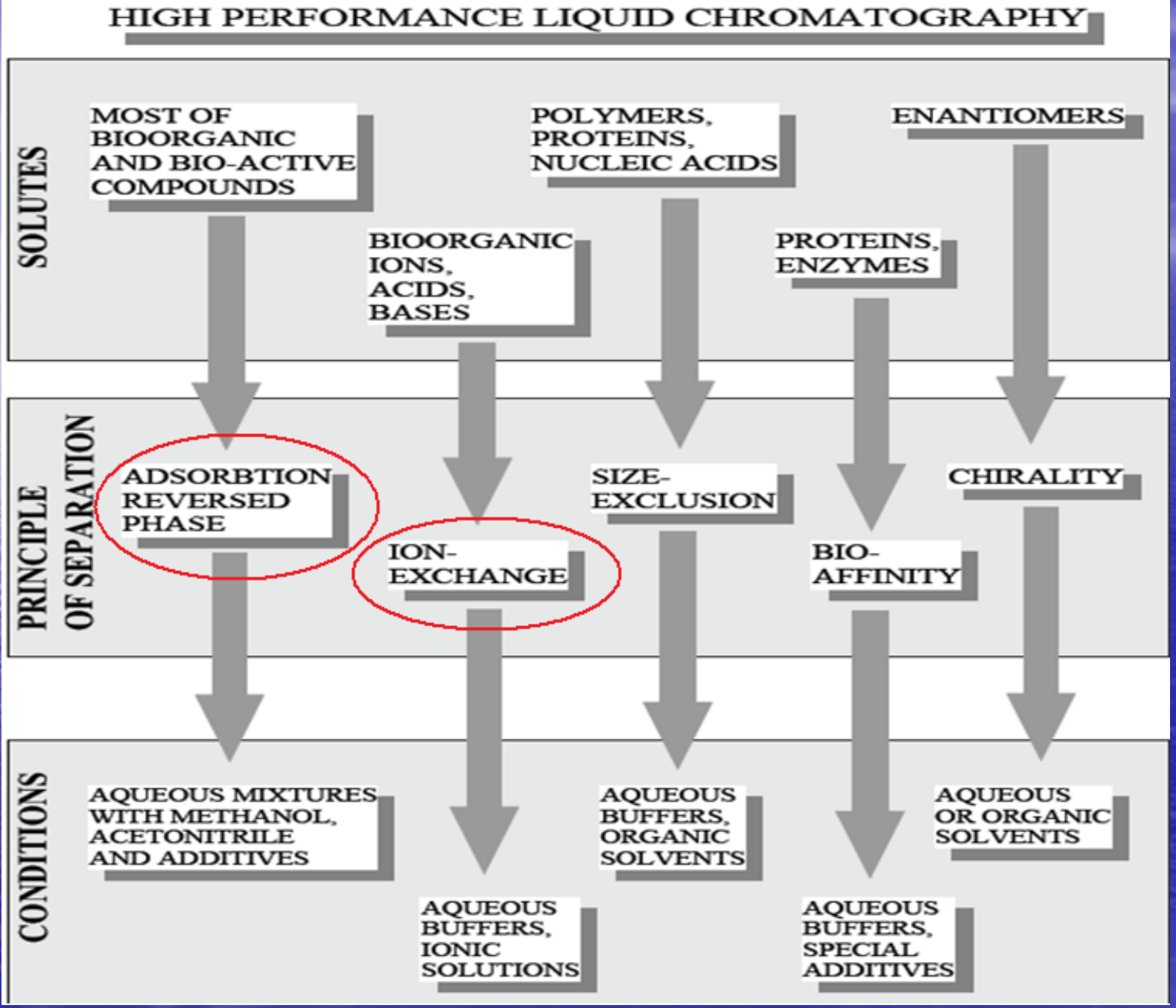
(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

HPLC Column Packings

- Silica and bonded-phase silica
  - Classic, Type II, and hybrid silica
- Polymer reverse phase
- Zirconium and MS bonded-phase
- Ion exchange: polymer, Zr, & Si
- Size separation: polymer and silica

(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

Modes of HPLC.



http://www.tut.fi/units/ymp/kem/opintojaksot/KEM-4350/vanhat/L6.pdf
Lecture 6.HPLC. Phases and columns.

Silica columns

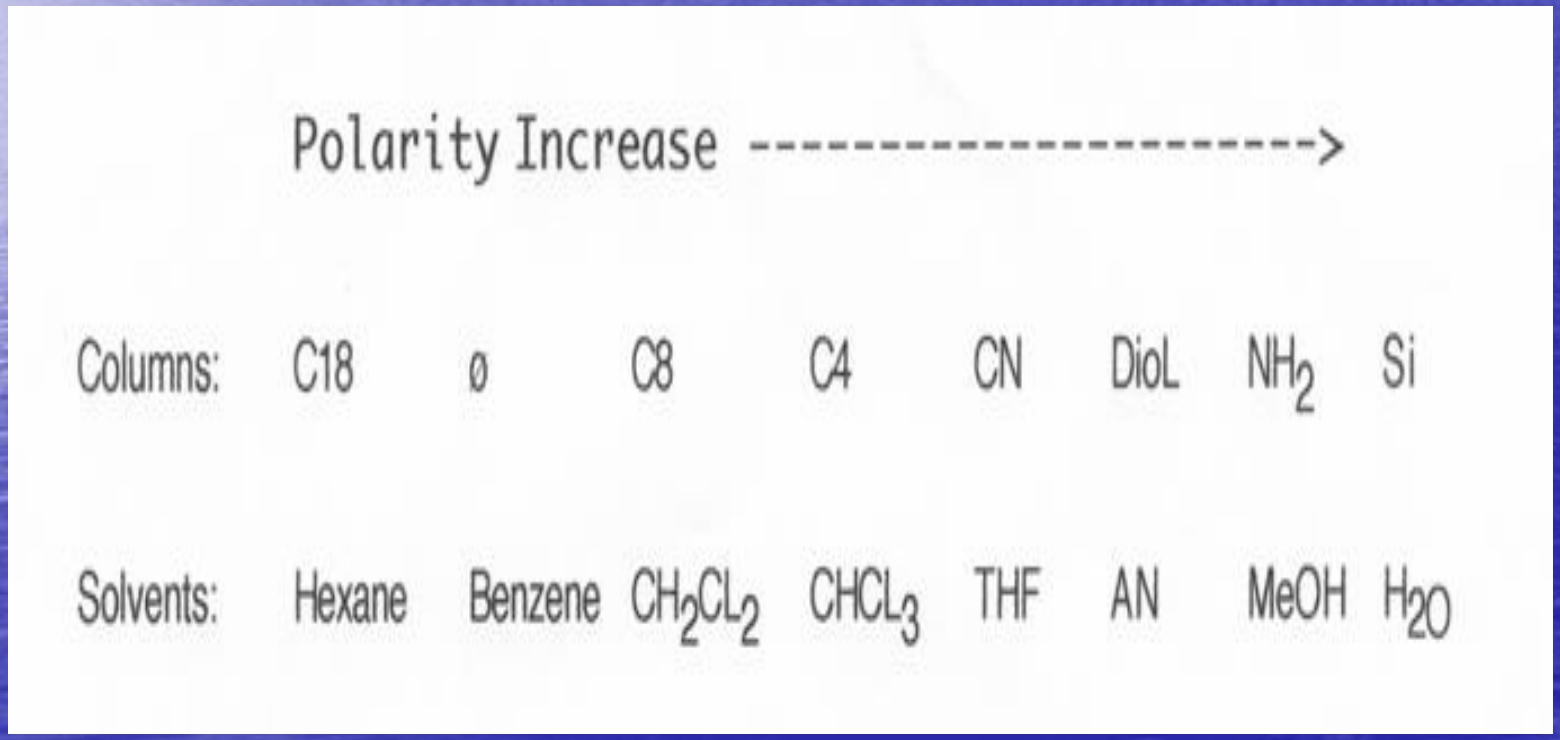
POLARITY

Column	Phase	Solvents	Application
C18	Octyldecyl	AN, MeOH, H_2O	General nonpolars
C8	Octyl	AN, MeOH, H_2O	General nonpolars
Phenyl	Styryl	AN, MeOH, H_2O	Fatty acids, double bonds
Cyano	Cyanopropyl	AN, MeOH, THF, H_2O	Ketone, aldehydes
Amino	Aminopropyl	H_2O, AN, MeOH, THF, $CHCl_3$, CH_2Cl_2	Sugars, anions
Diol	Dihydroxyhexyl	AN, MeOH, THF, H_2O	Proteins
SAX	Aromatic Quaternary amine	Salt buffers AN, MeOH, H_2O	Anions
SCX	Aromatic Sulfonic acid	Salt buffers AN, MeOH, H_2O	Cations
DEAE	Alkyl ether Ethyl 2° amine	Salt buffers AN, MeOH, H_2O	Proteins, anions
CM	Alkyl ether Acetic acid	Salt buffers AN, MeOH, H_2O	Proteins, cations
SI	(none)	Hexane, methylene	Polar organics, positional isomers
	Silanols	Chloride, chloroform	

(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

How to select solvents/eluents

- Select solvents with polarity opposite the column polarity.



(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

…HPLC solvents some criteria for selection

- UV cutoffs
  → for wavelength selection
- Molar mass
  → for MS contaminations
- Boiling point
  → for high temperature work

(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

…HPLC solvents

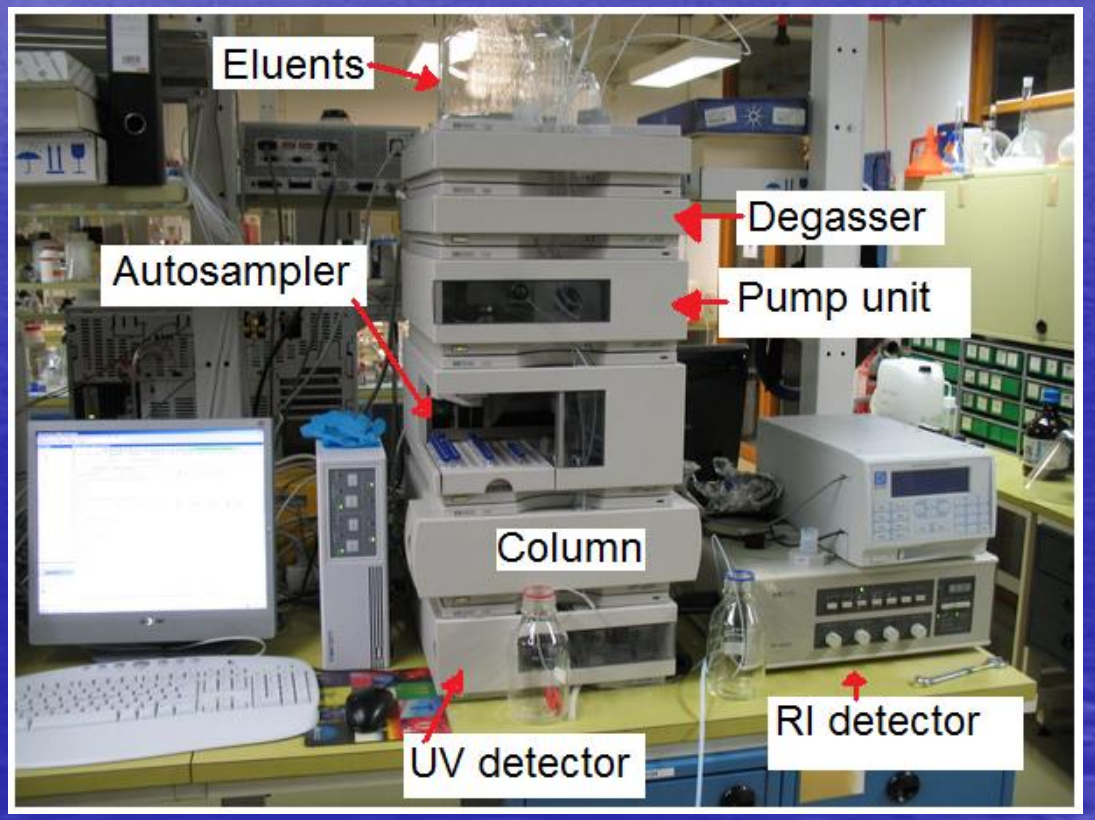
Solvent	Formula	MW (Da)	Boiling Point (°C)	UV Cutoff (nm)
Acetonitrile	CH_3CN	41.05	81.6	190
Chloroform	$CHCl_3$	119.38	61.7	245
Dichloromethane	CH_2Cl_2	84.93	40.0	235
Ethanol	CH_3CH_2OH	46.08	78.5	210
Ethyl acetate	$CH_3CO_2CH_2CH_3$	88.12	77.1	260
Diethyl ether	$(CH_3CH_2)_2O$	74.12	34.5	220
Heptane	$CH_3(CH_2)_5CH_3$	100.21	98.4	200
Hexane	$CH_3(CH_2)_4CH_3$	86.18	69	200
Isopropanol	$CH_3CH(OH)CH_3$	60.11	82.4	210
Methanol	CH_3OH	32.04	65	205
n-Propanol	$CH_3CH_2CH_2OH$	60.11	97.4	210
Tetrahydrofuran	C_4H_8O	72.12	66	215
Toluene	$C_6H_5(CH_3)$	92.15	110.6	285
Water	H_2O	18.02	100	none

(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

HPLC, High Performance Liquid Chromatography

Hewlett Packard 1100 series HPLC

- refractive index detector
- diode array UV detector
- HPX-87K carbohydrate column

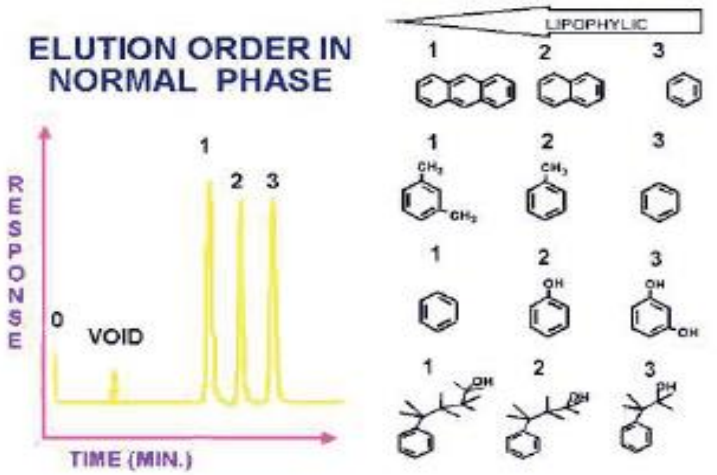


Analysis conditions for RP-C8 HPLC column

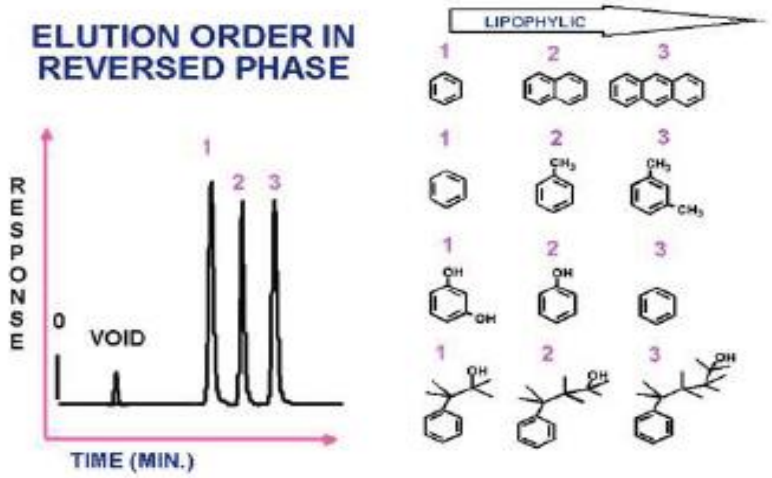
	Also Tried:
• **Flow**: **0.8** and **0.4 ml/min**	(0.1-1)
• **Temperature**: **25 °C**	(25–60 °C)
• **Injection volume**: **1.00 µl**	(1-5)

- **Detectors**: **RI** and UV
- **Eluents**: **water** (H_2O/ACN, H_2O/MeOH)

Reversed phase & normal phase → Difference



For **normal phase** elution order is that the polar solutes elute later than non-polar lypophilic ones.



For reversed phase elution order is that the polar solutes elute before non-polar ones.

http://www.tut.fi/units/ymp/kem/opintojaksot/KEM-4350/vanhat/L6.pdf
Lecture 6.HPLC. Phases and columns.

Reverse-Phase Chromatography

- Polar (hydrophilic) compounds elute before nonpolar (lipophilic) compounds
- Ionization of compounds disturbs analysis

→often prevented by adding buffer

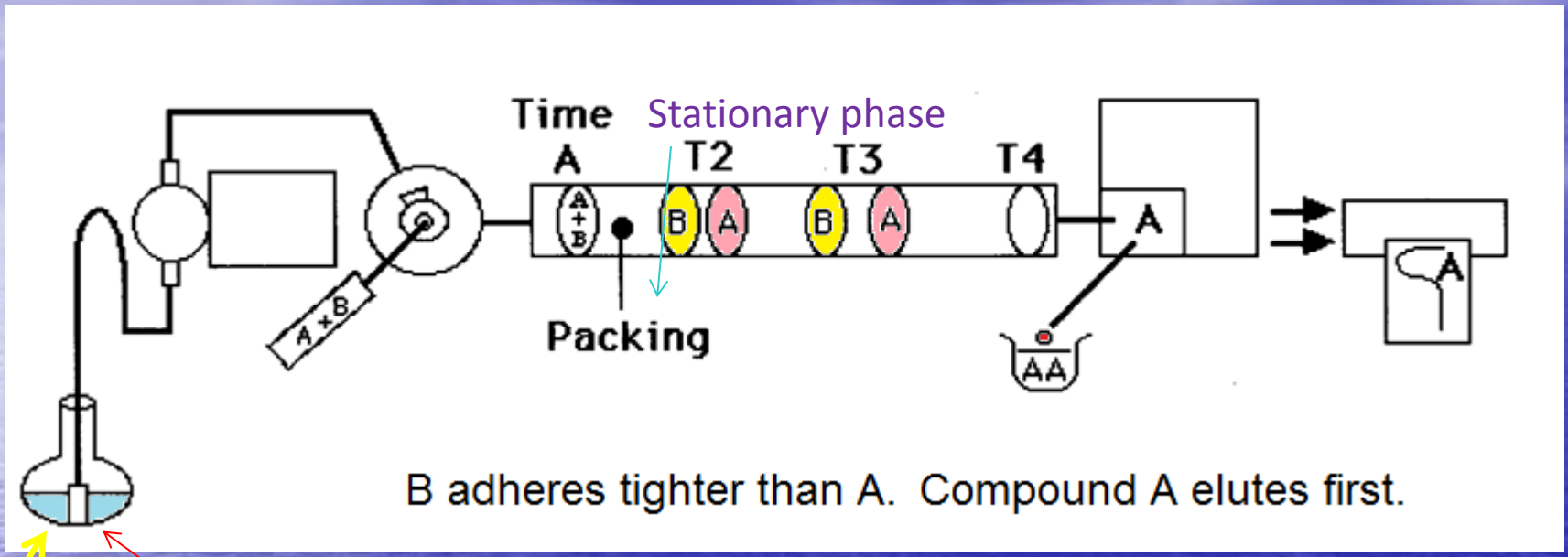
e.g. phosphate, acetate or carbonate

- Nonpolar columns require polar solvents
- Water/Acetonitrile or water/methanol

(when neutral compounds are analyzed)

(Robert Franzén, KEM-4350 Kromatografia ja Massaspektroskopia, Kromatografia – peruskäsitteet, menetelmät & laitteet, lecture material, TTY/Kemia, 2005.)
(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

Separation model



Solvent inlet filter

Mobile phase/solvent mixture/ eluent

Isocratic elution

– Constant mobile phase composition

Gradient elution

– mobile phase composition is changed stepwise or continuously (reproducible)

(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

Retention times are different for different chemical compounds but they also depend on the following method parameters:

- eluents/solvent mixture (+ gradient or isocratic)
- flow in the column
- temperature
- injection volume

→ new calibration curves* must be done when any method parameter is changed

*) In this research needed for ILs and sugars

Resolution

$$R = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\sqrt{N}\,\frac{K'}{1 + K'}$$

K' = Retention factor = $(V_A - V_0) / V_0$

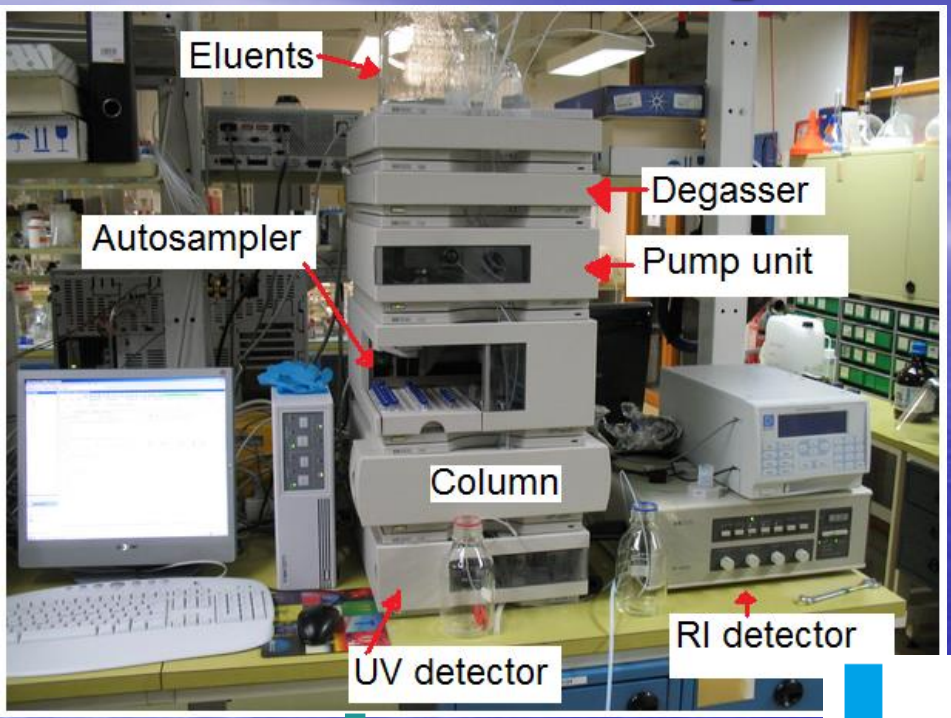
Alpha = Sep. factor = $(V_A - V_0) / V_B - V_0)$

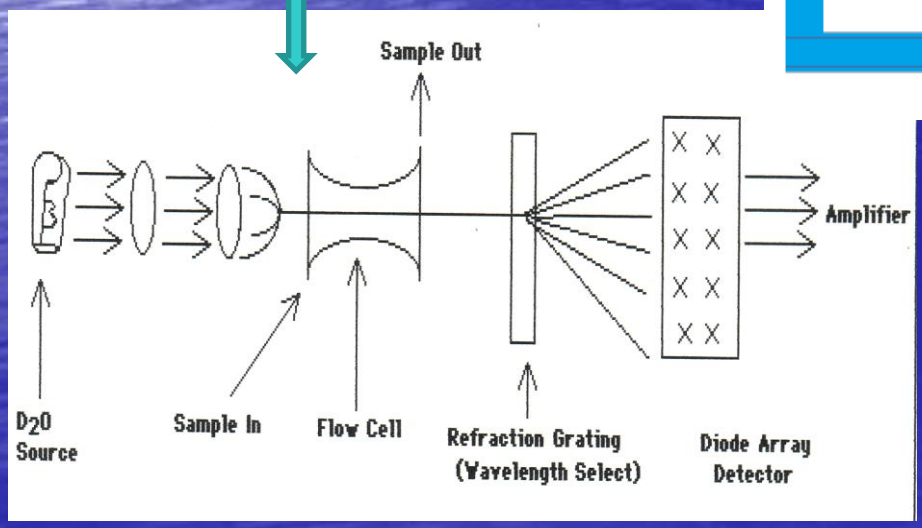
N = Retention factor = $16\,(V_A / V_B)$

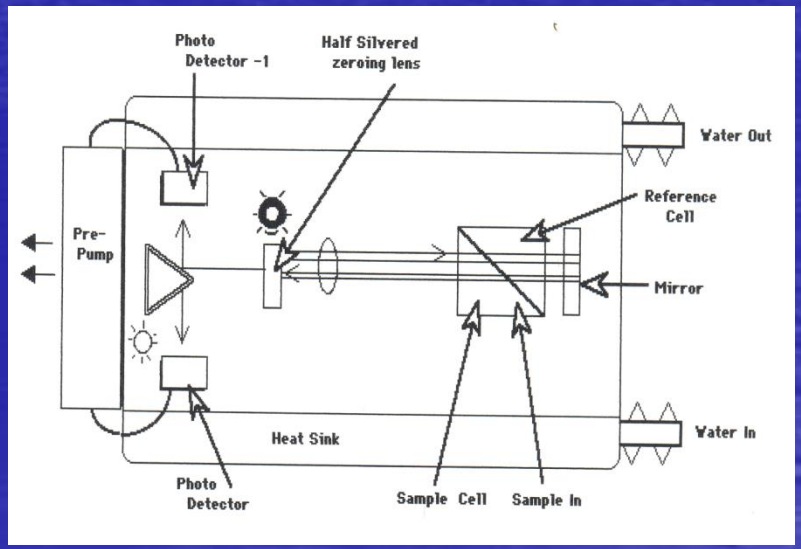
Resolution (*R*)–A measure of the completeness of a separation. Influenced by *K'* (solvent polarity), *N* (column efficiency, and *a* (system chemistry

(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

HPLC analysis







Challenges in monosaccharide analysis (HPLC)

- anomer mutarotation → wide & split peaks
- loss of reducing sugars (T)
- formation of Schiff bases ($R^1R^2C=NR^3$)
- shortened column lifetime
- long analysis time
- salt interferences

http://www.sepscience.com/Sectors/Enviro/Articles/521-/Overcoming-Challenges-in-Carbohydrate-Separations

Problems with HPLC

(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

- 70 % are column problems.
- 80 % are due to bad water.

Trouble-shooting

- Column QA with standards, (4-std test mixture)
- Treating Column killers.
- Reverse order system diagnosis.
- System Pacification

HPLC Column Killers

- Bonded Phase Loss - low pH, temp.
- End Voids - packing dissolved
- Adhering organic compounds
- Pressure increases
- Center voids - channeling

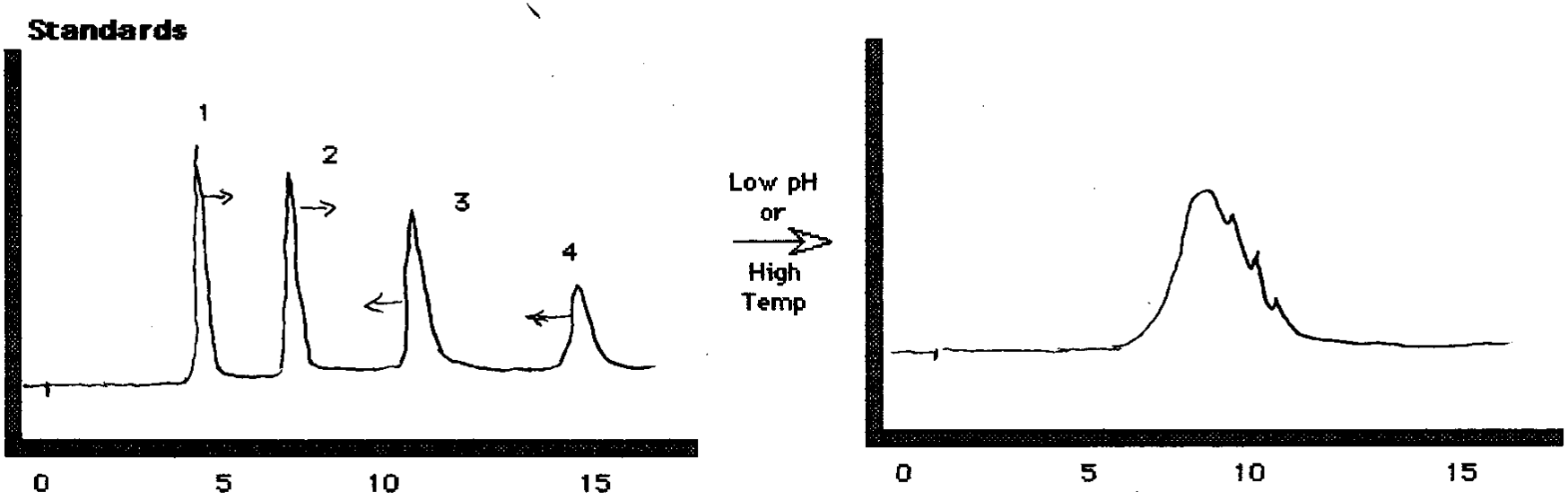
(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

Loss of Bonded-phase

(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

Effect of Bonded Phase Loss

Caused by hydrolysis of Si-O-Si bonds → Avoid by keeping pH 2.5-7.5!

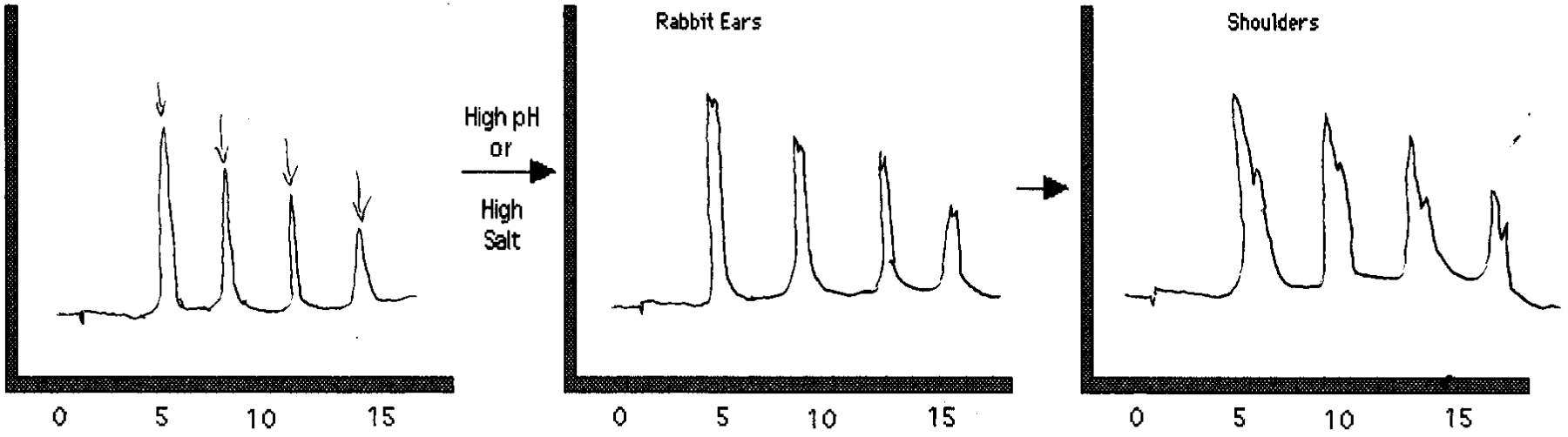


Dissolving Packing

(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

Effect of Dissolved Packing - End Void

Packing dissolves at pH > 8 or at high salt conc. → open & repack column head!

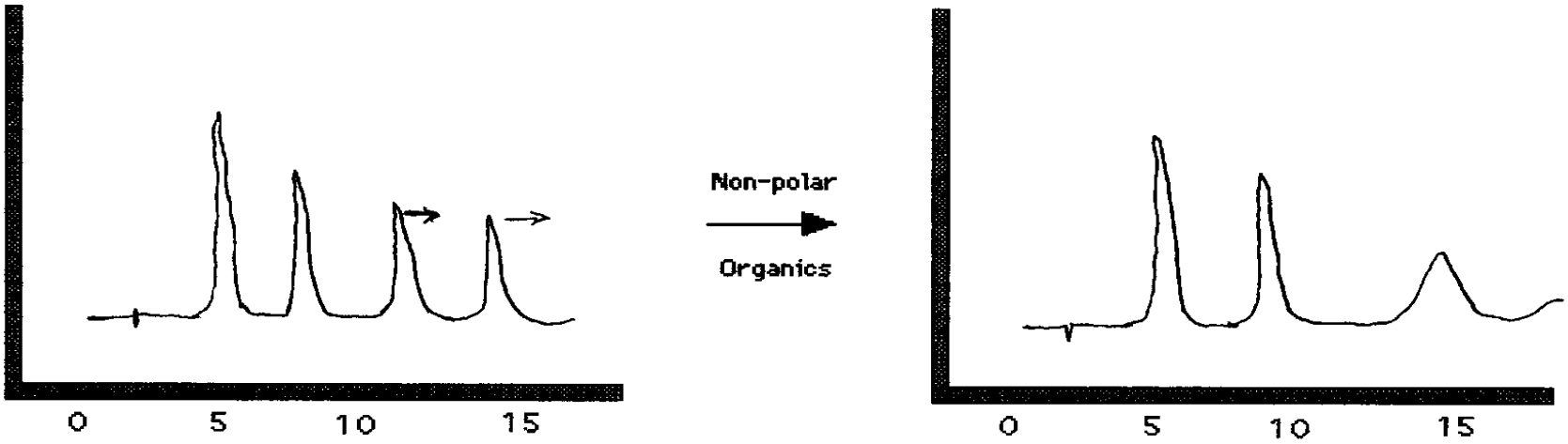


Bound Non-polar Material

(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

Effect of Bound Non-Polar Material

Late peak disappear.→ Treatment: wash with strong solvent, use clean water.

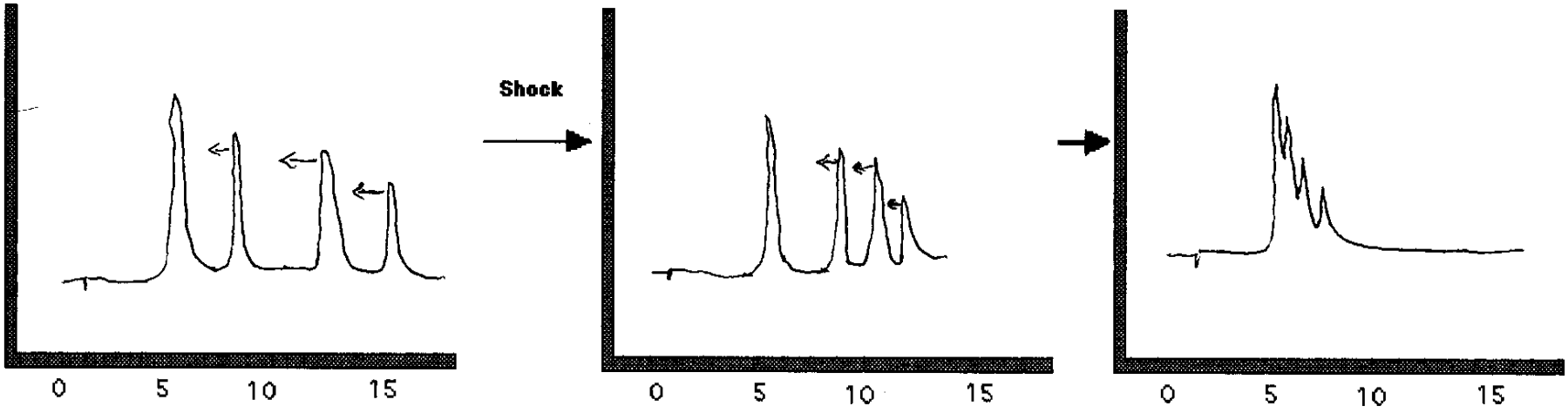


Center Void Formation

(Marvin C. McMaster, HPLC, a practical user's guide, 2nd edition, Wiley 2007.)

Effect of Channeling - Center Void

Avoid shocking or reversing the column. → Save shock & column reversal for treatment, run reversed.



Sequence: VIC-RIC_2 2009-03-20 14-35-43

Use method from data file | Ready/Reprocess Data Mode

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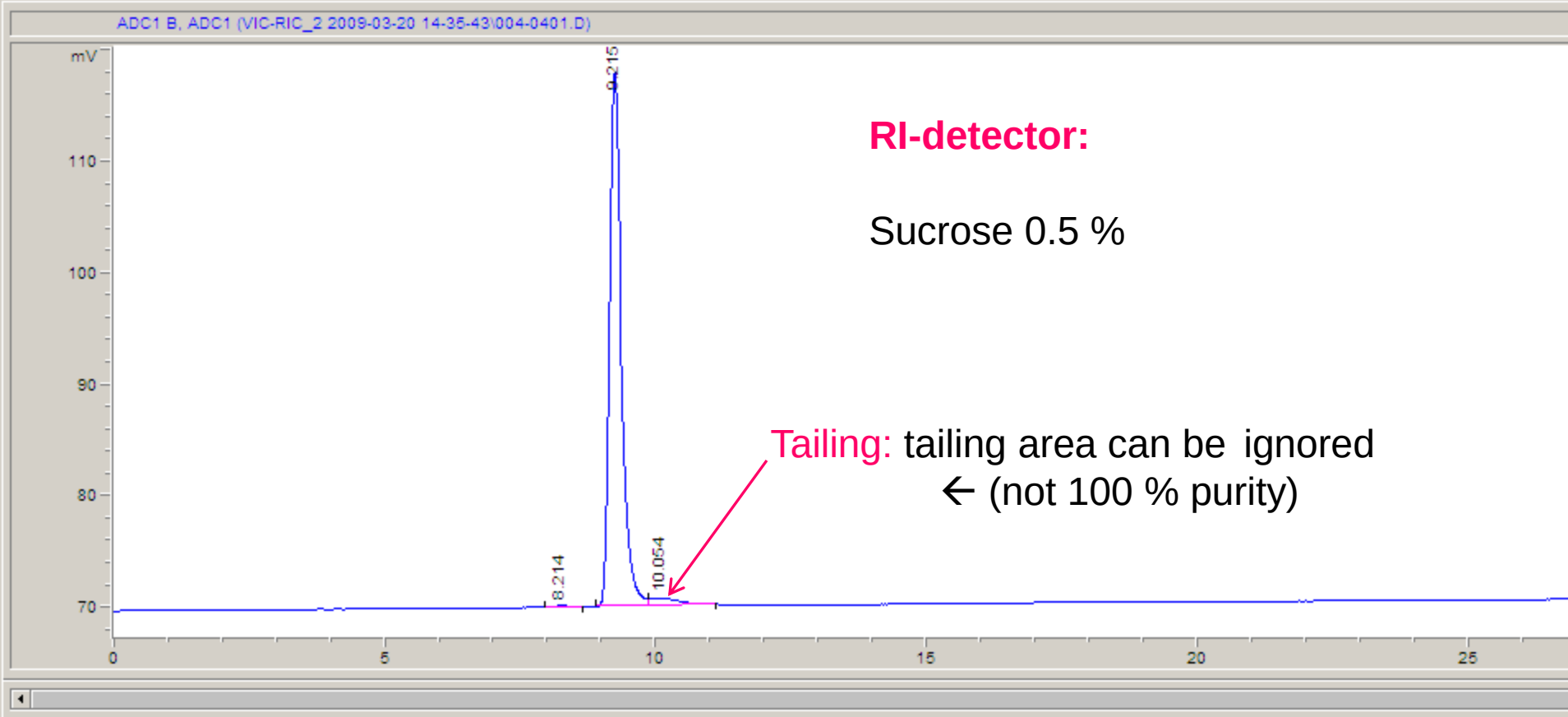
3	1	Vial 3	Xylitol 3%	RIC_AND_VICTORIA.M	Sample			0	0	1	1	00
4	1	Vial 4	Sucrose 0,5%	RIC_AND_VICTORIA.M	Sample			0	0	1	1	00
5	1	Vial 5	Sucrose 1%	RIC_AND_VICTORIA.M	Sample			0	0	1	1	00

Integration | Calibration | Signal | Purify

Report: Short | 1) ADC1 B, ADC1...43\004-0401.D)



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Date	20-Mar-09, 16:13:23
Sample	Sucrose 0.5%

#	Time	Area	Height	Width	Area%	Symme
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2	9.215	772.1	47.8	0.2443	66.022	0.638
3	10.054	27.4	7.1E-1	0.5046	2.345	0.387
4	29.741	367.2	10.8	0.5485	31.399	1.205

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Thank You!

DoRa

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cost

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