

# AppliChrom® ABOA SugarSep

For HPLC analysis of

- sugars
- sugaralcohols
- alcohols
- carboxylic acids

Special chromatography material for fast and reliable analysis using HPLC-RI or HPLC-ELSD at approx. 60-80°C

## Advantages:

- no organic eluents necessary, eluent = water
- good for environment; eluent = water
- low costs for deposition of used eluents; eluent = water
- easy to handle, measurement direct possible from aqueous sample
- Low invest; measurement possible with standard-HPLC system if RI or ELSD (evaporative light scattering detector) is connected with HPLC-system.

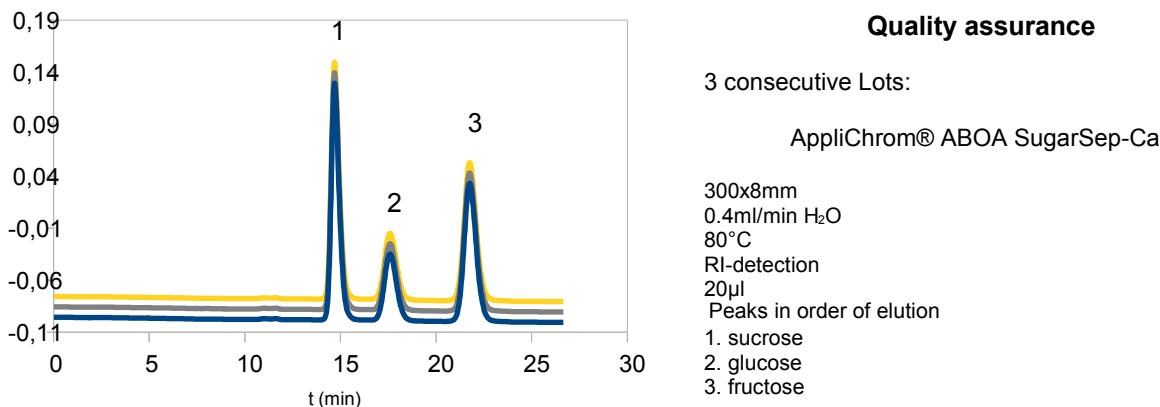
**AppliChrom ABOA SugarSep-Ca** – analysis of sugars, sugaralcohols and alcohols

**AppliChrom ABOA SugarSep-Pb** – analysis of sugars, especially from wood hydrolysate

**AppliChrom ABOA SugarSep-H** – analysis of sugars, sugaralcohols, alcohols and carboxylic acids

## Why using AppliChrom products:

AppliChrom means: high degree of reproducibility, column for column, lot for lot.



AppliChrom means: own synthesis- and developmentcenter in Oranienburg (Germany/Europe)



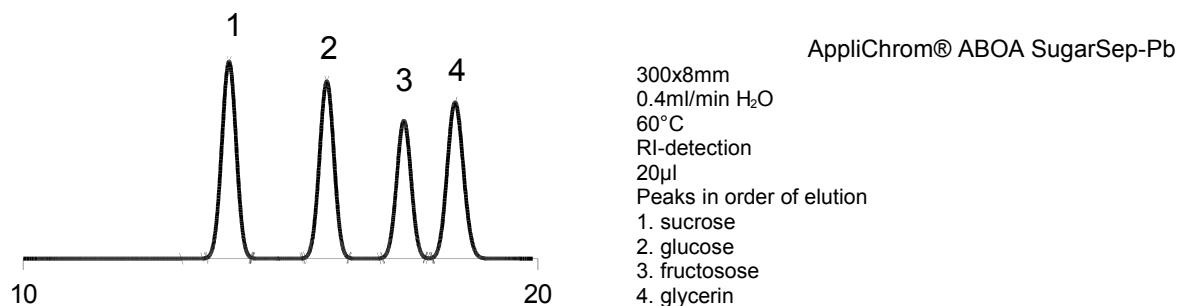
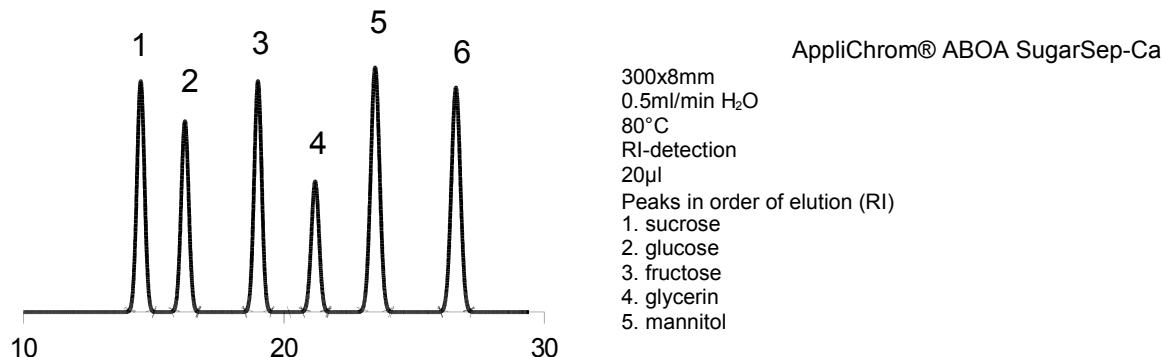
Inhouse control about the whole production process & base for furtur innovative productlines for todays and further needs of our customers.

\*) Laboratory picture taken from: Märkische Allgemeine Zeitung, 15. März 2014 (Online report: 2014-02-27): <http://www.maz-online.de/Lokales/Oberhavel/Polymere-Multitalente>

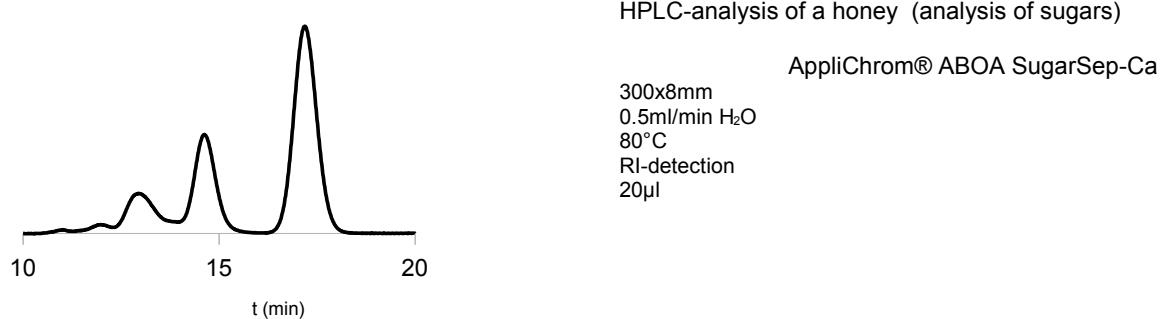
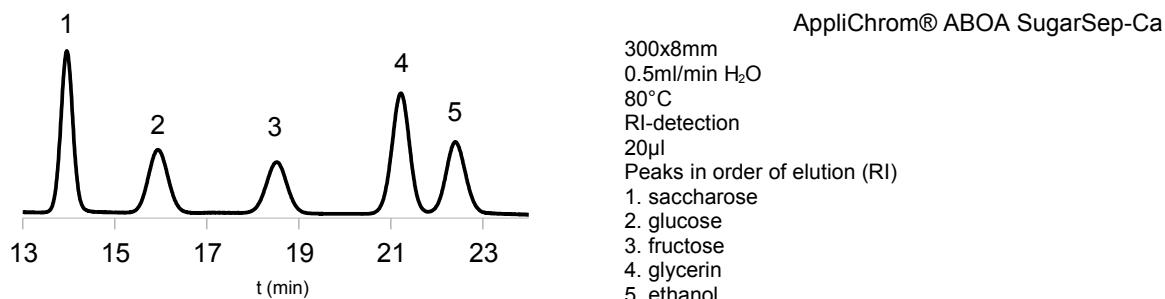
# AppliChrom® ABOA SugarSep

## Applications:

### HPLC-analysis of monosaccharides, disaccharides, sugaralcohols, glycerin



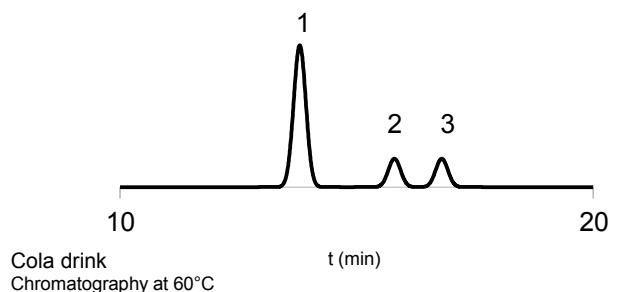
### HPLC analysis of saccharose, glucose, fructose, glycerin, ethanol & honey sample:



# AppliChrom® ABOA SugarSep

## Analysis of mono and disaccharides

HPLC-analysis of a cola-drink



### AppliChrom® ABOASugarSep-Pb for HPLC of sugars

300x8mm

0.4ml/min H<sub>2</sub>O

60°C

RI-detection

20µl

Peaks in order of elution; identification by comparison of retention times from standards

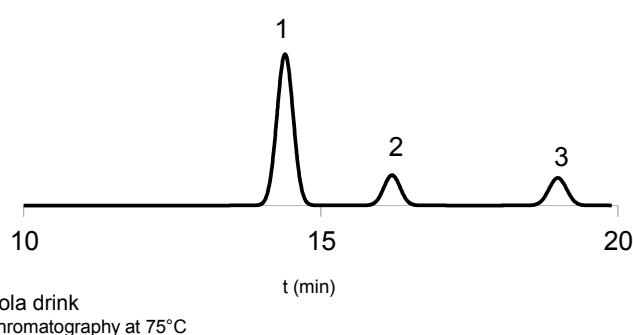
RI vs. t<sub>r</sub> [min]

1. sucrose

2. glucose

3. fructose

HPLC-analysis of a cola-drink



### AppliChrom® ABOASugarSep-Ca for HPLC of sugars

300x8mm,

0.5ml/min H<sub>2</sub>O

75°C

RI-detection

20µl

Peaks in order of elution; identification by comparison of retention times from standards

RI vs. t<sub>r</sub> [min]

1. Sucrose

2. Glucose

3. Fructose

## Fermentation control:

### AppliChrom® ABOA SugarSep-H for HPLC of sugars and acids

300x8mm,

0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,

75°C

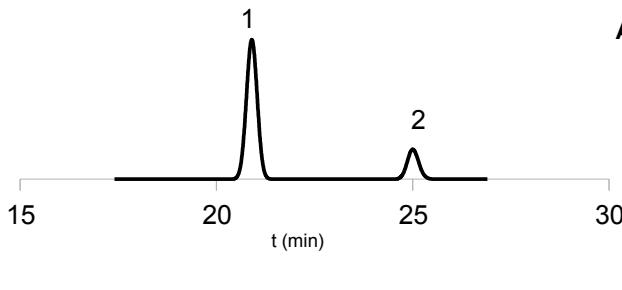
RI-detection

20µl,

Peaks in order of elution RI vs. t<sub>r</sub> [min]

1. glucose

2. DL-lactic acid (Milchsäure)



### AppliChrom® ABOA SugarSep-H for HPLC of alcohols and acids

300x8mm,

0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,

75°C

RI-detection

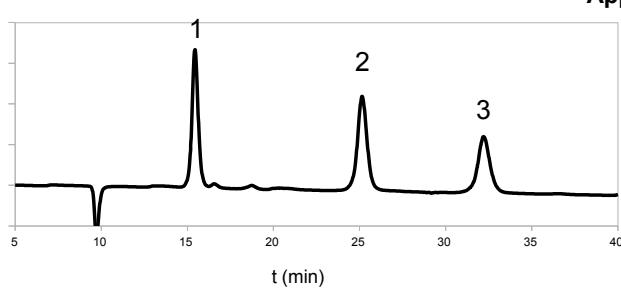
20µl,

Peaks in order of elution RI vs. t<sub>r</sub> [min]

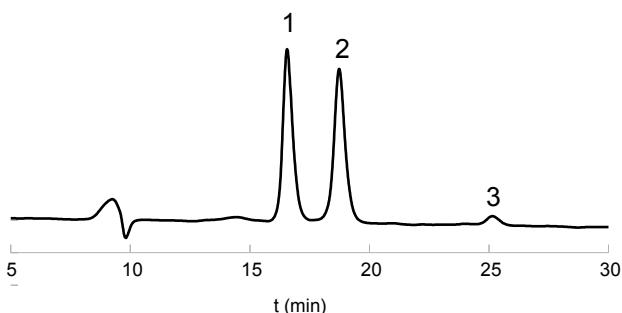
1. citric acid

2. acetic acid

3. 1,4-butandiol



### AppliChrom® ABOA SugarSep-H for HPLC of aceto balsamico – sugars and acids



300x8mm,

0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,

75°C

RI-detection

20µl,

Peaks in order of elution, RI vs. t<sub>r</sub> [min]

1. glucose

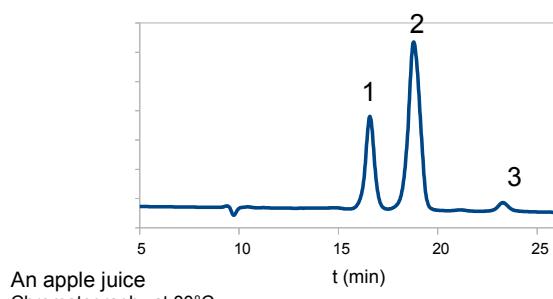
2. fructose

3. acetic acid

# AppliChrom® ABOA SugarSep

## Influence of Temperature on HPLC:

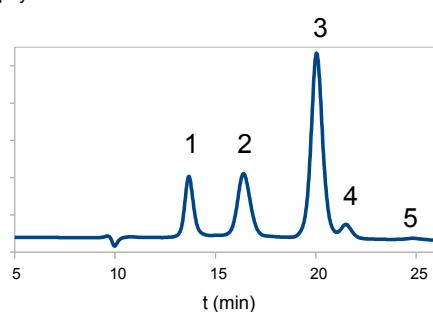
Sample: an apple juice



### AppliChrom® ABOA SugarSep-H for apple juice HPLC at high temperature

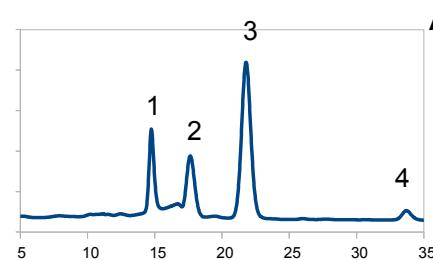
300x8mm,  
0.4ml/min 0.1%  $\text{H}_2\text{SO}_4$ ,  
80°C  
RI-detection  
20 $\mu\text{l}$ ,  
Peaks in order of elution, RI vs.  $t_r$  [min]  
1. glucose  
2. fructose/malic acid  
3. sorbit

(Sucrose hydrolyses; malic acid and fructose signals overlap)



### AppliChrom® ABOA SugarSep-H for apple juice HPLC at low temperature

300x8mm,  
0.4ml/min 0.1%  $\text{H}_2\text{SO}_4$ ,  
30°C  
RI-detection  
20 $\mu\text{l}$ ,  
Peaks in order of elution, RI vs.  $t_r$  [min]  
1. sucrose  
2. glucose  
3. fructose  
4. malic acid  
5. sorbitol



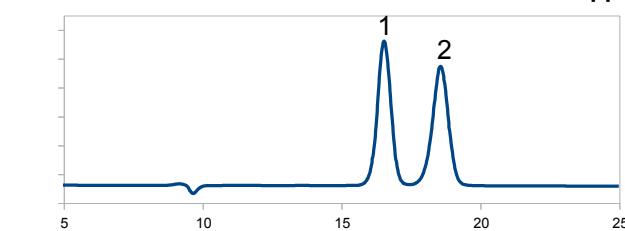
### AppliChrom® ABOA SugarSep-Ca for apple juice HPLC at high temperature

300x8mm,  
0.4ml/min 0.1%  $\text{H}_2\text{SO}_4$ ,  
80°C  
RI-detection  
20 $\mu\text{l}$ ,  
Peaks in order of elution, RI vs.  $t_r$  [min]  
1. sucrose  
2. glucose  
3. fructose  
4. sorbitol

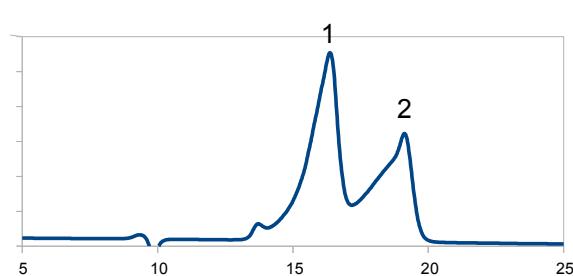
## HPLC of A Cola-drink:

Suppression of sucrose hydrolysis by lowering temperature; selection of eluent and column

### AppliChrom® ABOA SugarSep-H in cola-drink HPLC at high temperature



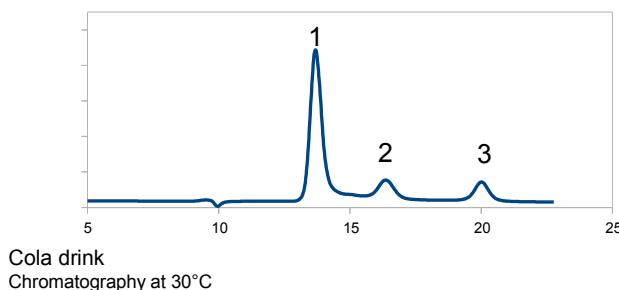
300x8mm,  
0.4ml/min 0.1%  $\text{H}_2\text{SO}_4$ ,  
80°C  
RI-detection  
20 $\mu\text{l}$ ,  
Peaks in order of elution, RI vs.  $t_r$  [min]  
1. glucose  
2. fructose



**AppliChrom® ABOA SugarSep-H**  
300x8mm,  
0.4ml/min 0.1%  $\text{H}_2\text{SO}_4$ ,  
55°C  
RI-detection  
20 $\mu\text{l}$ ,  
Peaks in order of elution, RI vs.  $t_r$  [min]  
1. glucose  
2. fructose

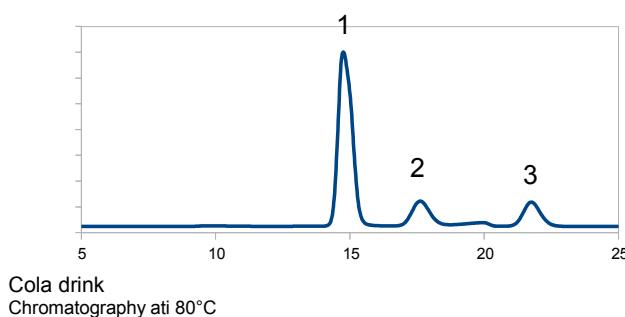
(hydrolysis during chromatography is observed at 55°C =>  
lower temperature e.g. 30°C is recommended)

# AppliChrom® ABOA SugarSep



## AppliChrom® ABOA SugarSep-H

300x8mm,  
0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,  
30°C  
RI-detection  
20µl,  
Peaks in order of elution, RI vs. t<sub>r</sub> [min]  
1. sucrose  
2. glucose  
3. fructose

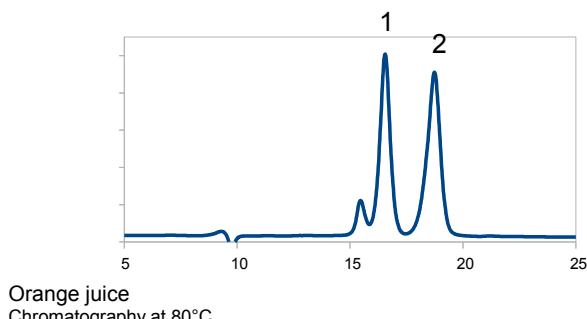


## AppliChrom® ABOA SugarSep-Ca

300x8mm,  
0.4ml/min 0.1% H<sub>2</sub>O,  
80°C  
RI-detection  
20µl,  
Peaks in order of elution, RI vs. t<sub>r</sub> [min]  
1. sucrose  
2. glucose  
3. fructose

## An Orange juice:

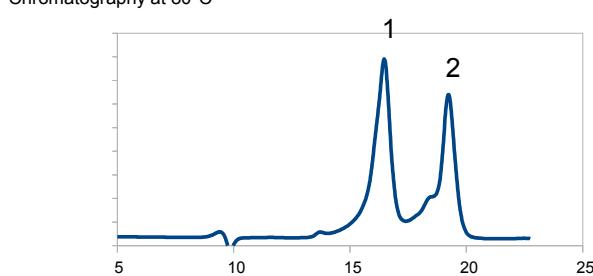
**Suppression of hydrolysis by temperature lowering gives an increase of informations of sample.**  
**Comparison AppliChrom ABOASugarSep-H and SugarSep-Ca.**



## AppliChrom® ABOA SugarSep-H

300x8mm,  
0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,  
80°C  
RI-detection  
20µl,  
Peaks in order of elution, RI vs. t<sub>r</sub> [min]  
1. glucose  
2. fructose

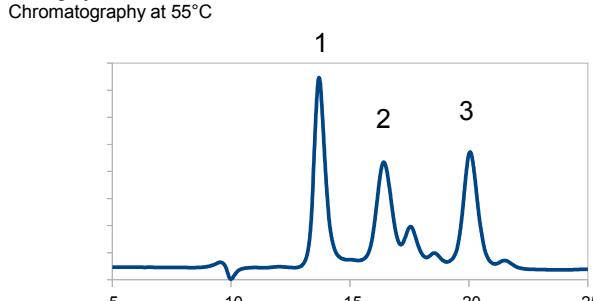
(Hydrolysis of sucrose)



## AppliChrom® ABOA SugarSep-H

300x8mm,  
0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,  
55°C  
RI-detection  
20µl,  
Peaks in order of elution, RI vs. t<sub>r</sub> [min]  
1. glucose  
2. fructose

(Hydrolysis of sucrose)

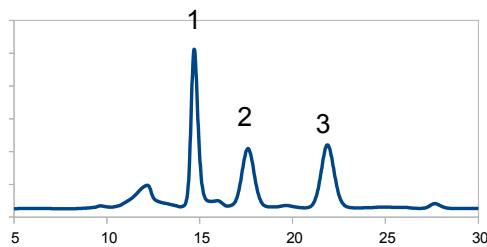


## AppliChrom® ABOA SugarSep-H

300x8mm,  
0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,  
30°C  
RI-detection  
20µl,  
Peaks in order of elution, RI vs. t<sub>r</sub> [min]  
1. sucrose  
2. glucose  
3. fructose

(further peaks unassigned)

# AppliChrom® ABOA SugarSep



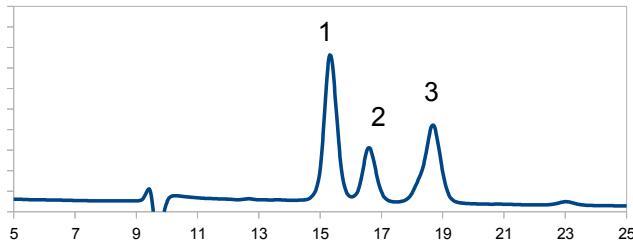
Orange juice  
Chromatography at 80°C

## AppliChrom® ABOA SugarSep-Ca

300x8mm,  
0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,  
80°C  
RI-detection  
20µl,  
Mainpeaks in order of elution RI vs. t<sub>r</sub> [min]  
1. sucrose  
2. glucose  
3. fructose

(further peaks unassigned)

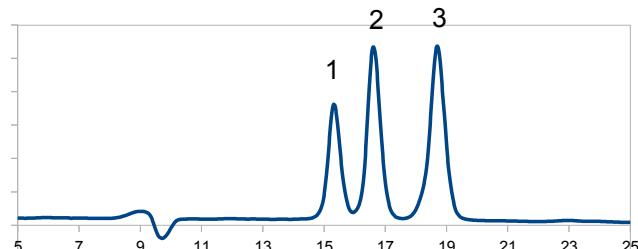
## Analysis of maturationprocess of red currants:



Red currants (hitherto green),  
Chromatography at 80°C

## AppliChrom® ABOA SugarSep-H

300x8mm,  
0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,  
80°C  
RI-detection  
20µl,  
Peaks in order of elution, RI vs. t<sub>r</sub> [min]  
1. citric acid  
2. glucose  
3. fructose



Red currants,  
mellow (red)  
Chromatography at 80°C

## AppliChrom® ABOA SugarSep-H

300x8mm,  
0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,  
80°C  
RI-detection  
20µl,  
Peaks in order of elution, RI vs. t<sub>r</sub> [min]  
1. citric acid  
2. glucose  
3. fructose

(further peaks not assigned)

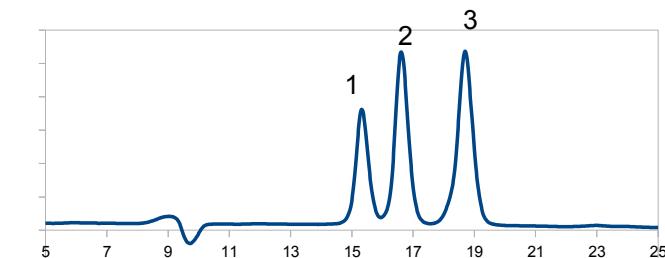
## Observation:

Mellow red currants: decrease of tartratic acid concentration, increase of sugarconcentraiton.

# AppliChrom® ABOA SugarSep

## Analysis of mellow red currants –

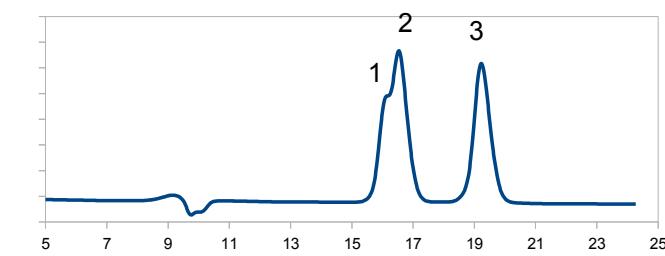
### Influence of temperature, peakpositions, chromatograms



Red currants,  
**mellow (red)**  
Chromatography at **80°C**

#### AppliChrom® ABOA SugarSep-H

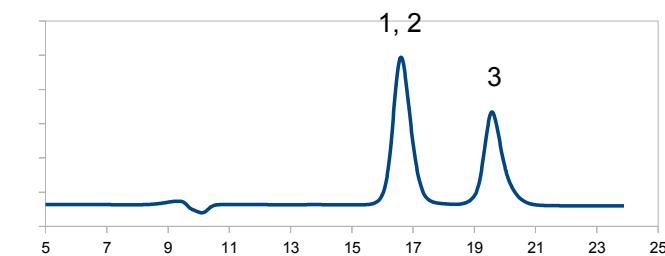
300x8mm,  
0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,  
80°C  
RI-detection  
20µl,  
Peaks in order of elution RI vs. t<sub>r</sub> [min]  
1. citric acid  
2. glucose  
3. fructose



Red currants,  
**mellow (red)**  
Chromatography at **55°C**

#### AppliChrom® ABOA SugarSep-H

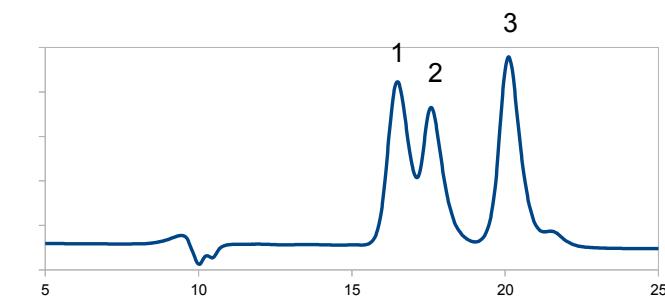
300x8mm,  
0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,  
**55°C**  
RI-detection  
20µl,  
Peaks in order of elution, RI vs. t<sub>r</sub> [min]  
1. citric acid  
2. glucose  
3. fructose



Red currants,  
**mellow (red)**  
Chromatography at **45°C**

#### AppliChrom® ABOA SugarSep-H

300x8mm,  
0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,  
**45°C**  
RI-detection  
20µl,  
Peaks in order of elution, RI vs. t<sub>r</sub> [min]  
1. citric acid  
2. glucose  
3. fructose



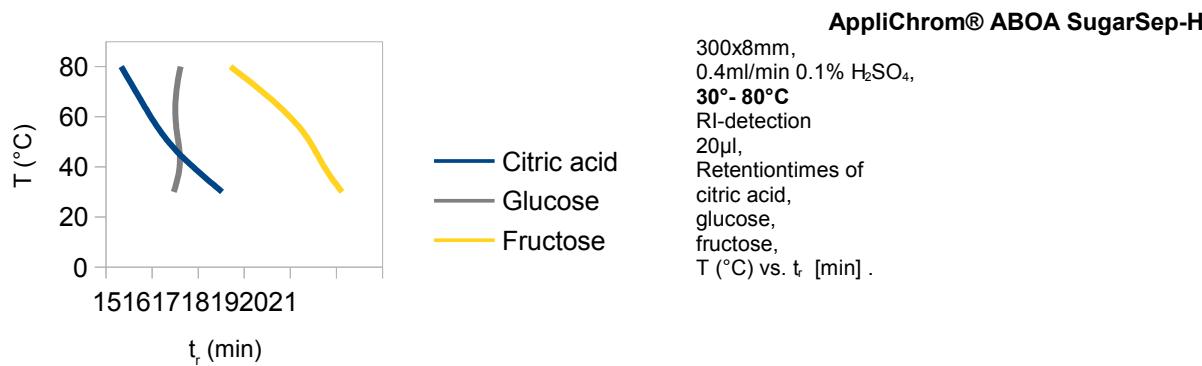
Red currants,  
**mellow (red)**  
Chromatography at **30°C**

#### AppliChrom® ABOA SugarSep-H

300x8mm,  
0.4ml/min 0.1% H<sub>2</sub>SO<sub>4</sub>,  
**30°C**  
RI-detection  
20µl,  
Peaks in order of elution, RI vs. t<sub>r</sub> [min]  
1. glucose  
2. citric acid  
3. fructose

# AppliChrom® ABOA SugarSep

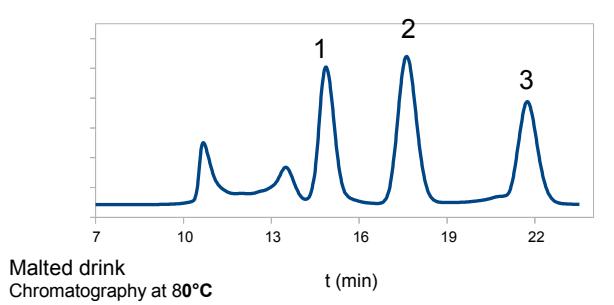
Influence of temperature on peak positions:



Peakpositions of analytes depend on temperature. Changes of temperature can change order of peak elution, can produce peak overlapping and can change selectivity.

=> correct and accurate column temperature is an important parameter for adjusting the correct selectivity

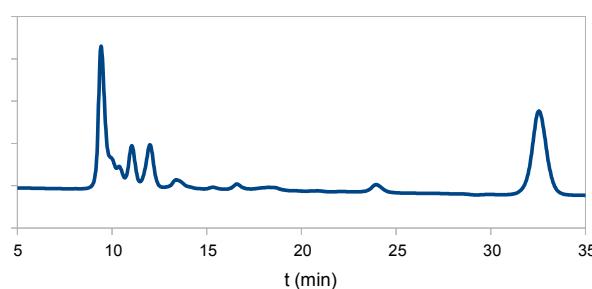
## HPLC of a malted drink and of a pilsener beer: AppliChrom ABOA SugarSep-Ca



A malted drink

### AppliChrom® ABOA SugarSep-Ca

300x8mm,  
0.4ml/min,  
 $H_2O$   
**80°C**  
RI-detection  
20 $\mu$ l,  
Peaks in order of elution, RI vs.  $t_r$  [min]  
1. maltose (14,8min)  
2. glucose (17,5min)  
3. fructose (21,8min)



A pilsener beer

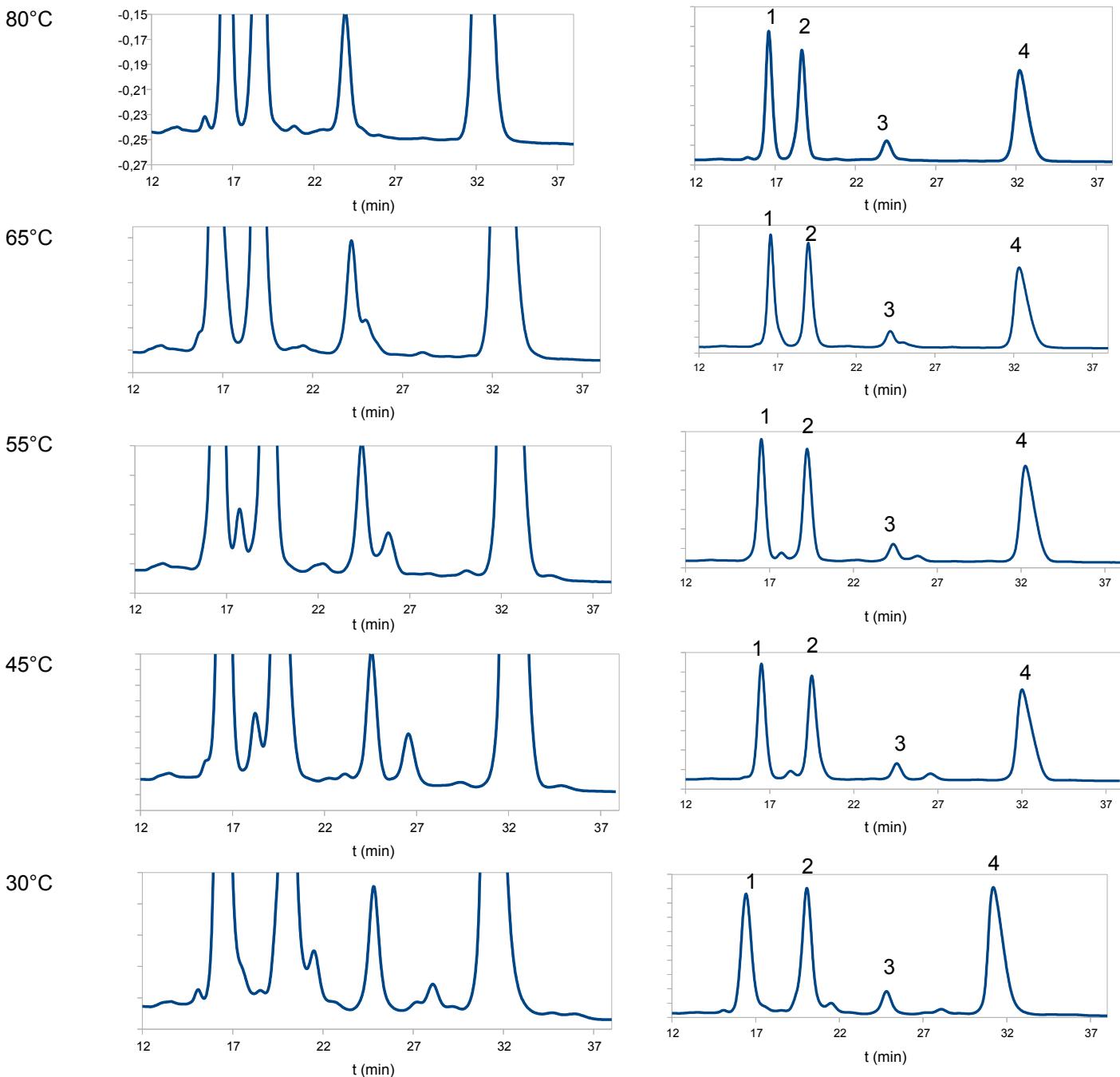
### AppliChrom® ABOA SugarSep-Ca

300x8mm,  
0.4ml/min,  
 $H_2O$   
**80°C**  
RI-detection  
20 $\mu$ l

# AppliChrom® ABOA SugarSep

HPLC analysis: standard solution for wine analysis  
(obtained from: Deutsche Weinanalytiker e.V.), lot 1050807 (blue label).

HPLC-column-  
temperature



AppliChrom® ABOA SugarSep-H

300x8mm,  
0.4ml/min,  
0.1%  $\text{H}_2\text{SO}_4$ ,  
**30°C- 80°C**  
RI-detection

20 $\mu$ l, standard solution for wine analysis of: Deutsche Weinanalytiker e.V.,  
lot 1050807 (blue label).

Mainpeaks in order of elution RI vs.  $t_r$  [min]

- 1. glucose
- 2. fructose
- 3. glycerin
- 4. ethanol

Plus further wine typical fruit acids

=> HPLC-selectivity is adjusted by column temperature.

**Result:** HPLC-selectivity of AppliChrom ABOA SugarSep column can be adjusted by variation of column temperature to actual analytical sample.

# AppliChrom® ABOA SugarSep

**Further analytes that are analysed:**  
Urea, allantoin, acrylamid, methacrylamid,...

## Effects:

=> Retentiontimes of some analytes depend on temperature.

=> Hydrolysis of some analytes occur at elevated temperatures if eluent  $H_2SO_4$  is used

(e.g. sucrose => glucose + fructose). If sugars are analysed that can hydrolyse it can be good to test additional AppliChrom ABOA SugarSep-Ca in pure water to see if the results are equal.

=> Not only sugars and alcohols can be HPLC analysed with AppliChrom ABOA SugarSep-H, also additional carboxylic acids and fruit acids can be analysed.

## Fields of application for HPLC-column AppliChrom ABOA SugarSep-H:

=> analysis of monosaccharides, alcohols, fruit acids, lactic acid,... at lower temperature also of disaccharides.

## Order Informations:

SASH102508	HPLC-Column AppliChrom® ABOA SugarSep-H, 10 $\mu$ 250mm x 8mm	Price available upon request
SASH103008	HPLC- Column AppliChrom® ABOA SugarSep-H, 10 $\mu$ 300mm x 8mm	Price available upon request
SASPB102508	HPLC-Column AppliChrom® ABOA SugarSep-Pb, 10 $\mu$ 250mm x 8mm	Price available upon request
SASPB103008	HPLC-Column AppliChrom® ABOA SugarSep-Pb, 10 $\mu$ 300mm x 8mm	Price available upon request
SASCA102508	HPLC-Column AppliChrom® ABOA SugarSep-Ca, 10 $\mu$ 250mm x 8mm	Price available upon request
SASCA103008	HPLC-Column AppliChrom® ABOA SugarSep-Ca, 10 $\mu$ 300mm x 8mm	Price available upon request

Precolumns, other column dimensions, other counterions (AppliChrom ABOA SugarSep-Na,...), OEM columns or OEM bulkmedia on request, .

How can we serve you?

## AppliChrom products for sugar & polysaccharid characterisation available (HPLC, HILIC, GPC/SEC)

- Monomers, sugars, sugaralcohols
- Dimeric sugars and oligomeric sugars
- Oligomeric and polymeric sugars, polysaccharidderivatives

## HPLC & HILIC methods for saccharides

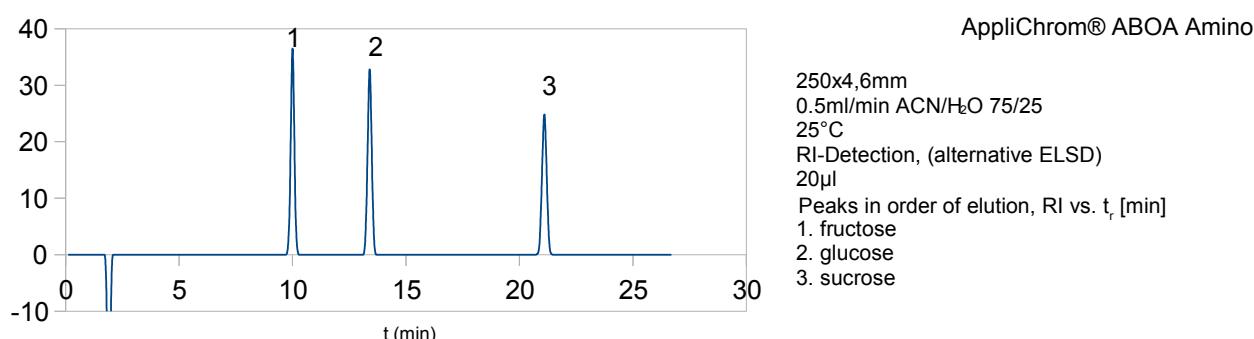
### AppliChrom OTU Amino columns for sugar-HPLC:

HPLC analysis of mono- and disaccharides, of oligosaccharides in ACN/H<sub>2</sub>O (70/30) – alternative selectivity to AppliChrom ABOA SugarSep series.

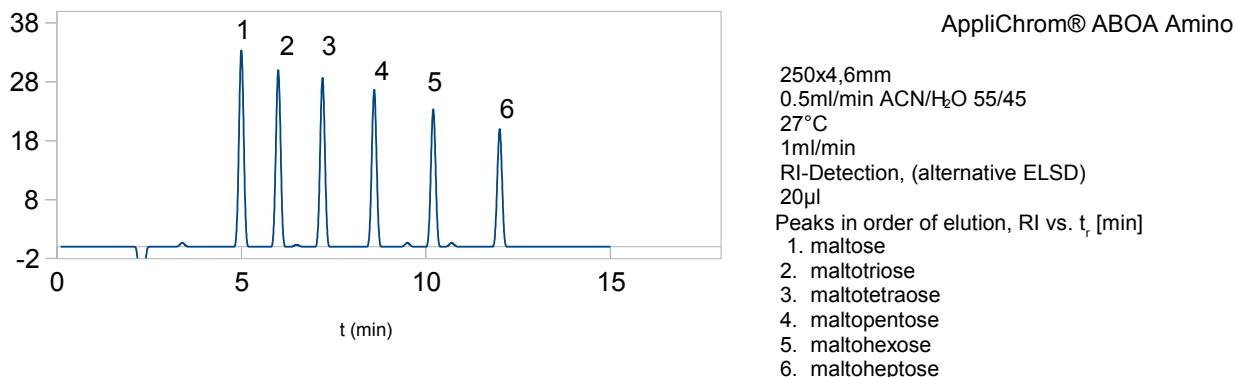
### AppliChrom OTU DioHILIC Columns for sugar-HPLC:

HPLC analysis of mono- and disaccharides, of oligosaccharides in ACN/H<sub>2</sub>O – alternative selectivity to AppliChrom OTU Amino columns.

## HPLC Analysis of low molecular weight sugars & oligosaccharides:



# AppliChrom® ABOA SugarSep



## GPC/SEC-methods for saccharides

### AppliChrom GPC columns for water soluble samples:

**AppliChrom ABOA SuperOH-P-series for GPC:** polymeric polar aqueous GPC/SEC columns for oligomeric/polymeric sugars resp. Polysaccharides, 100 - > 20Mio dalton, fields of application: molecular weight distribution, oligomeric and polymeric saccharides, Starchderivatives, starchdegradationproducts, pectins, pullulan, alginat, dextran, dextransulfat, hyaluronic acid, heparin,... Measurements in water.

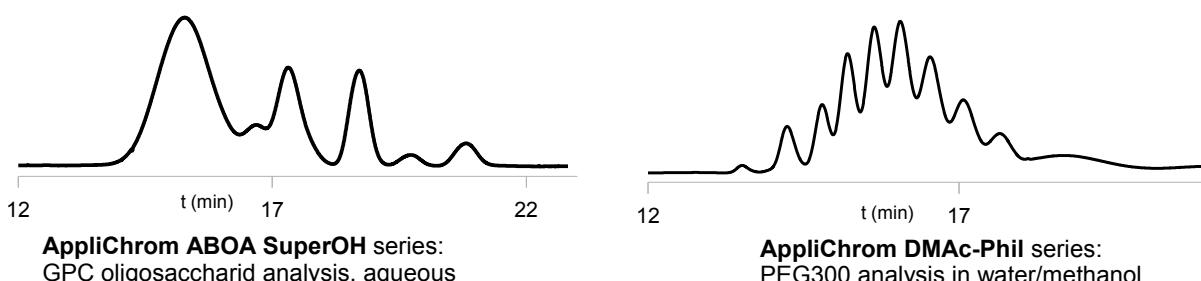
**AppliChrom ABOA SuperOH-P-serie for GPC:** polymeric aqueous GPC/SEC columns for the molecular weight range of 100 - > 10Mio dalton, including polyamines, polycations and polyelectrolytes like Polyethylenimin, chitosan, p-DADMAC, measurements in water – no ion-exclusion mechanism, pure SEC/GPC!

### AppliChrom ABOA DMSO-Phil-P-series for GPC:

Applications: high molecular weight starch, lignins, humic substances, UF-resin, MUF-resin, urea resin, melamin resin.

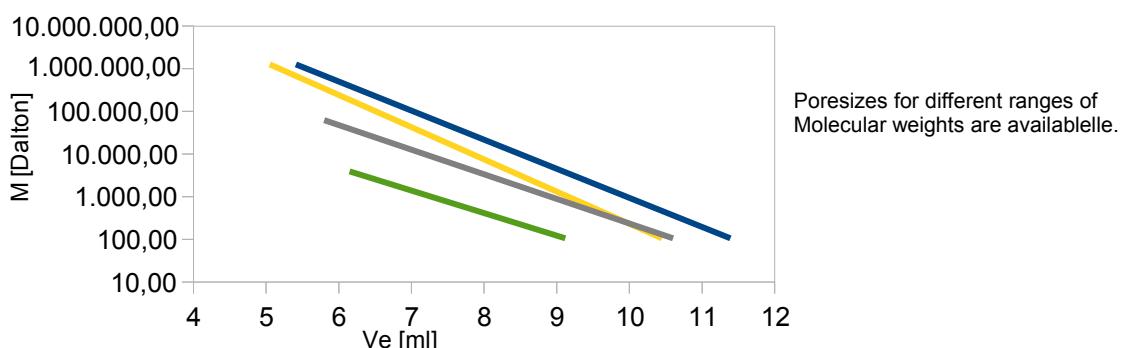
## GPC/SEC-methods for saccharides:

### GPC-analysis of polysaccharid degradation product & HPLC-analysis with GPC column



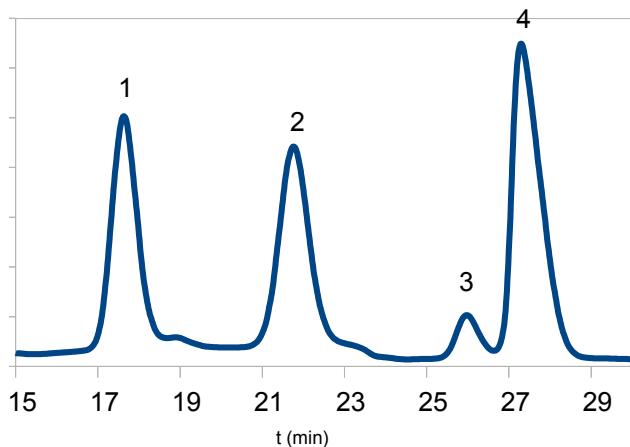
### Poresizes:

Ranges of **AppliChrom ABOA SuperOH** GPC/SEC-columns, 300x8mm measured in water using PEG/PEO-standards: porosities 100, 200, 300, 400



# AppliChrom® ABOA SugarSep

HPLC-analysis of a standardsolution for wine analysis using  
AppliChrom ABOA SugarSep-Ca column



## AppliChrom® ABOA SugarSep-Ca

300x8mm,

0.4ml/min,

H<sub>2</sub>O

80°C

RI-detection

20µl, Standardlösung zur Weinanalytik der Deutschen Weinanalytiker e.V., lot 1050807 (blue label).

Peaks in order of elution, RI vs. t<sub>r</sub> [min]

1. glucose

2. fructose

3. glycerin

4. ethanol

**Analytes that are analysed using HPLC-column AppliChrom ABOA SugarSep-Ca or AppliChrom ABOA SugarSep-Pb:**

### Sugars:

In order of elution for AppliChrom ABOA SugarSep-Ca:

Stachyose, melezitose, raffinose, gentiobiose, cellobiose, trehalose, isomaltose, sucrose, maltose, melibiose, mactose, maltulose, lactulose, glucose, xylose, galactose, mannose, rhamnose, fructose, inositol, arabinose, digitoxose, adonitol, erythritol, mannitol, salicin, dulcitol, xylitol, sorbitol, ribose

### Alcohols:

Methanol, ethanol, iso-propanol, 1,2-butandiol, 1,3-butandiol, 1,4-butandiol, ethylenglycol, diethylenglycol, triethylenglycol, 1,2,3-propantriol, glycerin, 1-propanol, 1,2-propandiol, 1,3-propandiol, tert-butanol, 2-butanol, iso-butanol, 1-butanol

**Analyte that are analysed using HPLC column AppliChrom ABOA SugarSep-H:**

### Acids:

L-Malic acid, D-lactic acid, L-lactic acid, citric acid, ascorbic acid, vitamin C, fumaric acid, shikimi acid, succinic acid, acetic acid, oxalic acid, acrylic acid, adipic acid, furan-2-carboxylic acid, galacturonic acid, gluconic acid, glutaric acid, glycolic acid, pyruvic acid, maleic acid, malonic acid, hydroxy butyric acid, propionic acid, formic acid, beta-keto glutaric acid, hippuric acid, iso-valerianic acid, n-butyric acid, n-valerianic acid, pyroglutaminic acid, 2-oxoisovalerianic acid, N-acetylneurameric acid, iso-butyric acid, 3-phenyl propionic acid,

### Alkohole:

Methanol, ethanol, iso-propanol, 1,2-butandiol, 1,3-butandiol, 1,4-butandiol, ethylenglycol, diethylenglycol, triethylenglycol, 1,2,3-propantriol, glycerin, 1-propanol, 1,2-propandiol, 1,3-propandiol,

### Saccharides:

Arabinose, arabitol, cellobiose, digitoxose, fucose, fructose, galactose, glucose, isomaltose, lactose, lactulose, maltose, maltotriose, manntioli, mannose, melezitose, melibiose, meso-erythritol, N-acetylglucosamin, N-acetylgalactosamin, raffinose, rhamnose, ribose, ribitol, sorbitol, sorbose, stachyose, sucrose/saccharose, trehalose, xylitol, xylose.

\*) Di- and oligosaccharides can be analysed in many cases using AppliChrom ABOA SugarSep-H with the eluent 0.1% H<sub>2</sub>SO<sub>4</sub> - but in this case the Column temperature should be (instead of 60-90°C) lowered to approx. 30°C to avoid decomposition of oligosaccharides. It has to be tested.