

## Introduction

Carbohydrates are one of the most important components in many food products and play an important role in many physiological processes. The determination of carbohydrates is of interest to the food industry and many fields in life sciences. Analytes of interest include simple mono- or disaccharides (such as glucose and sucrose), polysaccharides (starch, cellulose) and glycoproteins.

Here we present a simple and sensitive analysis method for the determination of Carbohydrates using Isocratic Anion Exchange Chromatography (IC) and Pulsed Amperometric Detection (PAD) on a gold working electrode.

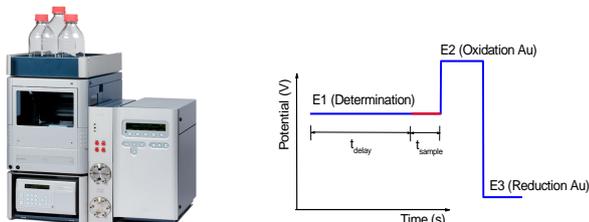


Fig. 1. ALEXYS Analyzer, right: schematic representation of PAD pulse form.

## Method

HPLC	ALEXYS Carbohydrates Analyzer
Columns	ALC-5, 250 x 4.6 mm (ID), particle size 7 $\mu$ m ALC-5 guard column
Flow cell	Flexcell (or VT), with Au working electrode and HyREF
Degassing	Continuous sparging of solvent with Helium 5.0
Sample	2 $\mu$ mol/L Glucose, Fructose, Lactose and Sucrose standard
Mobile phase	30 mM NaOH, 1 mM Ba(OH) <sub>2</sub> and 1 mM NaOAc
Flow rate	2 mL/min
Temperature	30 °C column and flow cell
E-cell	E1, E2, E3: 0.05, 0.75, -0.80 V ts, t1, t2, t3: 0.06, 0.55, 0.13, 0.12 s
I-cell	300 - 500 nA

## Analysis of mono- and disaccharides

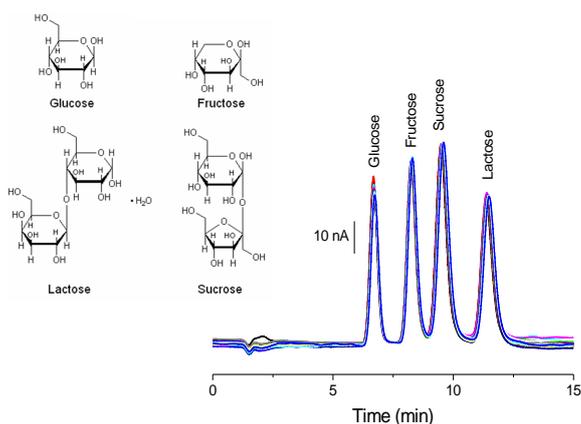


Fig. 2. Overlay of 23 chromatograms of a standard mixture of 2  $\mu$ M Glucose, 2  $\mu$ M Lactose, 4  $\mu$ M Fructose and 4  $\mu$ M Sucrose in water (20  $\mu$ l injected), ADF 0.01 Hz. Plate numbers are 14900, 10900, 12000 and 17300 plates/meter, respectively.

## References

- [1] D.C. Johnson et al., J. Chromatogr., 640, 79 (1993)
- [2] W.R. LaCourse, Pulsed Electrochemical detection in HPLC, Wiley-Interscience (1997)

## Reproducibility, Linearity and LOD

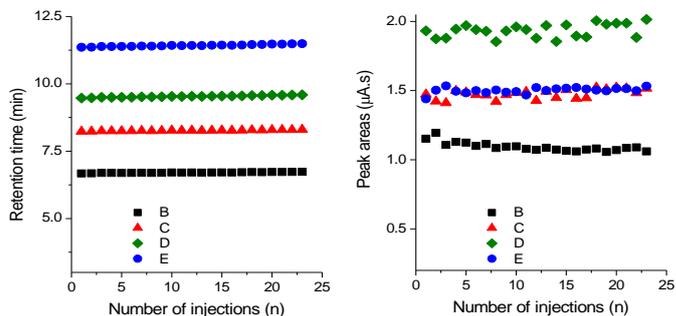


Fig. 3. Reproducibility ( $n=23$ ) of 20  $\mu$ L injections of a standard mixture of 2  $\mu$ M Glucose, 2  $\mu$ M Lactose, 4  $\mu$ M Fructose and 4  $\mu$ M Sucrose in water.

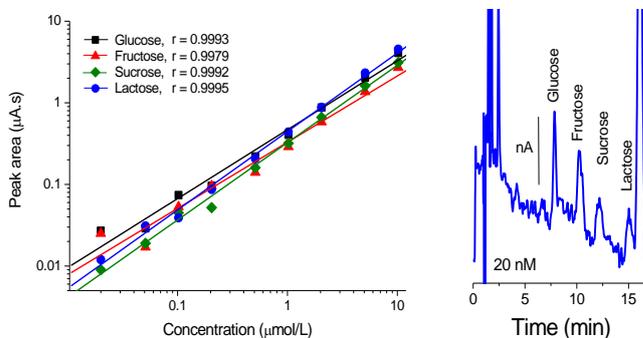


Fig. 4. Calibration plots in the range of 20 nmol/L - 10  $\mu$ mol/L (20  $\mu$ L). Right: calculated LOD values ( $3 \times$  peak-to-peak noise): Glucose, lactose: 10 nM; sucrose, fructose: 15 nM (VT03 flow cell). Chromatogram: 20 nM standard mixture.

## Analysis of carbohydrates in malt beer & apple juice

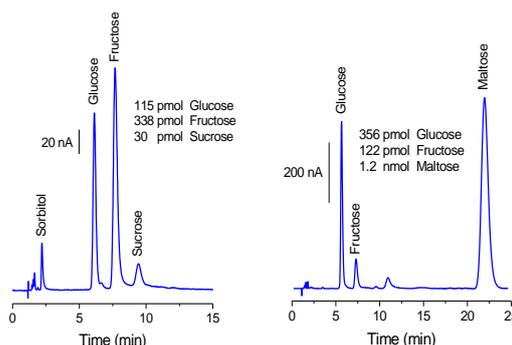


Fig. 5. Left: Apple juice sample ( $E1=+50$  mV, 10  $\mu$ L, diluted 10000 x). Right: Malt-beer sample ( $E1=+50$  mV, 20  $\mu$ L, sample degassed in ultrasonic bath for 10 minutes and diluted 1000 x).

## Conclusion

The ALEXYS Carbohydrates Analyzer provides a reliable solution for the routine analysis of carbohydrates. The Flexcell with exchangeable working electrode and pH resistant, maintenance free HyREF has been designed for extreme measurement conditions. Complex samples can be analysed using a binary gradient system. Excellent reproducibility and detection sensitivity have been demonstrated.