ERASMUS 2014 – laboratory works
ANALYTICAL CHROMATOGRAPHY

Title: Determination of sugar profile in honey by HPAEC-PAD (high performance anion-exchange liquid chromatography with pulsed amperometry detection)

Theoretical:

Description: Carbohydrate analysis with high-performance anion-exchange chromatography (HPAEC) uses pulsed amperometric detector (PAD). It requires no pre- and postcolumn derivatization and it offers excellent resolution and sensitivity. The major advantages of HPAEC are speed of analysis (e.g. monosaccharide analysis can be done during 15 min) and high resolution (e.g. separation of anomers). What is defined as high-performance anion-exchange chromatography may also be interpreted as high pH anion-exchange chromatography because of use of highly alkaline eluents in this form of chromatography.

Retention times are closely related to pKa of the analytes. Effect of molecular weight and shape of molecules (tetraoses are retained longer than trioses). The longer the degree of polymerization, the longer the retention time.

Quantification of analytes by PAD, PAD belongs to electrochemical detectors and it has a higher sensitivity than other detectors (very low detection limitr, piko moles concetration).

Principle and terms:
Sugars (weakly acidic nature of carbohydrates) → ionization at high pH → anions in alkaline mobile phase → separation on functional groups of stacionary phase in column (resolution according to pKa, molecular weight and shape of sugars) → oxidation of individual sugar anions at the surface of a gold electrode at selected potential → measuring the electrical current generated by oxidation → detection at detection voltage (gold electrode, reference electrode).

Equipment:
Dionex system consists of a pump with degass unit, eluent containers, autosampler, valve injection, chromatography oven, gold and reference electrode (Ag/AgCl), electrochemical detector, PC, chromatography workstation.

Column guard and analytical column (stacionary phase):
Anion exchange stacionary phase, pellicular resin (MicroBead latex cross-linking, polystyren/divinylbenzene polymers fills in guard column CarboPac PA1 Guard (4 x 50 mm) and column CarboPac PA1 Analytical (4 x 250 mm) (separation of neutral and amino sugars) (monosaccharides and disaccharides), CarboPac MA-1 (separation of alditols), CarboPac PA100 (separation of oligosaccharides).

Max back pressure: 4000 psi (28 MPa)

Eluent (mobile phase) (example from our lab):
A container – deionized water (18 MΩ cm^{-1} resistance or higher).
B container - the mixture of sodium hydroxide solution (NaOH) and sodium acetate (NaOAc) solution.
C container - alkaline solution of sodium hydroxide (NaOH) (200 mmol l^{-1}).
D container - alkaline solution of sodium hydroxide (NaOH) (600 mmol l^{-1}).

Isocratic separation of sugars.
Gradient separation of sugars.

Practical:

Analyzed sample: sample of honey

The aims:
To determine sugars profile and sugars amount in the analyzed sample

Standards preparation:
Solution of D-glucose, D-fructose and sucrose at desirable concentrations (for preparation of calibration curve).

Sample (analyt) preparation:
To weigh, dissolve, fitrate (pass through a 0.45 mm filter prior to injection), put into vials.
To make sequence of analysis, start of batch, evaluate (peak integration)and calculate (quantification) of sugars amount.

Examples purification of analyzed comple samples:
Deproteinization (hydrophobic filter cartridge, precipitation, …).
Remove phenolic fraction (PVP filter cartridge).
Remove salts (cation exchanger cartridge).

Chromatography conditions for the analysis of sugars:
Columns: CarboPac PA1 Analytical
CarboPac PA1 Guard
Eluent: A - water
C - 16 mmol l^{-1} NaOH, regeneration of column by means of 200 mmol l^{-1} NaOH
Flow rate: 0.25 ml/min
Isocratic elution
Injection volume: 10 μl
Temperature: 25 °C
Detection: pulsed amperometry (PAD), gold electrode
Program: time, potential (waveform), composition of eluent.