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Application News

High Performance Liquid Chromatography

Dual stream HPAEC-PAD system for fast analysis of carbohydrates

No. SCA_190_037

Analysis of complex mixtures of carbohydrates is a challenging subject in analytical chemistry. Separating them normally requires multiple specific ligand exchange columns, and the lack of UV-absorbance limits the detection choice to refractive index detection (RID). Common methods for carbohydrate analysis, therefore, suffer from poor chromatographic resolution and detection limits.

High performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) offers increased resolving power and sensitivity for the analysis of complex mixtures of carbohydrates. However, due to the required regeneration and equilibration, analysis times are relatively long (app. 1hr) [1].

This Application Note proposes a novel dual stream HPAEC-PAD system, which effectively doubles the throughput of HPAEC-PAD analysis with minimal additional cost.

Need for regeneration

Atmospheric carbon dioxide is a big interference factor in HPAEC. It reacts with sodium hydroxide in the eluent and alters retention times of the analytes, as HPAEC is highly sensitive to changes in anion concentration. The formed carbonate ions tend to adhere and accumulate on the column, decreasing retention and in turn decreasing resolving power and reproducibility over time.

The solution is to regenerate the column after each analysis with a high sodium hydroxide concentration.

Proposed system configuration

The configuration of the system is based on the Shimadzu Prominence system, consisting of two LC-20AD pumps, a SIL-20AC autosampler and an Antec Decade Elite electro chemical detector.

The pumps were equipped with built in solenoid valves, one is equipped with a solvent selection valve and is dedicated to regeneration and column equilibration. The other pump utilizes a low pressure gradient elution (LPGE) kit and is used for analysis. Even though monosaccharide analysis is performed in isocratic mode, the LPGE valve is very useful for method development, optimization and the possibility to use acetate gradients for oligosaccharide mapping.

The pumps are interfaced using a 10-port 2-position valve, which alternates the two columns between either the “Run” or the “Regeneration” flowline, as displayed in figure 1.

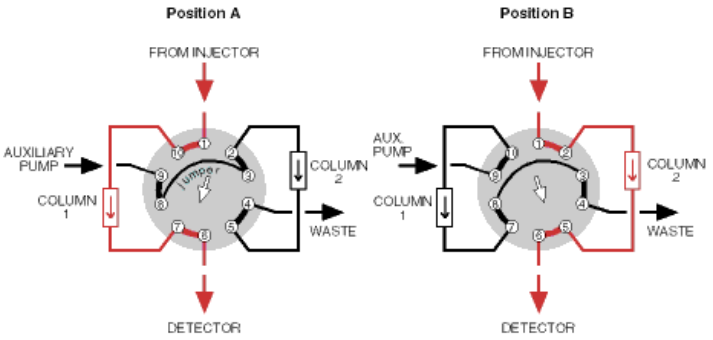

Figure 1: Proposed rotary valve configuration [2]

Hydroxide eluents need to be kept in poly propylene bottles due to borate formation when kept in glass. These borates form complexes with carbohydrates [3], altering their chromatographic behavior and reducing the sensitivity of PAD detection by lowering the oxidation efficiency [4].

Because carbon dioxide gas may diffuse through polypropylene bottles, the hydroxide eluents are continuously sparged with helium that is additionally filtered with a soda-lime scrubber. The helium flow is delivered using a DGU-10B helium degasser.

To save helium gas, the helium flow is passed through all solvent bottles containing sodium hydroxide in series.

The water is separately sparged for half an hour at first and then blanketed with helium as it is kept in a glass bottle.

Method

The method for the proposed system consists of consecutive analysis and regeneration cycles between which the columns are alternated (figure 2).

While in the regeneration flowline the column is regenerated using a high concentration of NaOH (200 mM) for 15 minutes, after which a step gradient is applied to the NaOH concentration that is used for the analysis. The column is re-equilibrated at this concentration to ensure reproducible chromatography and to decrease the need for detector stabilization time when the columns are switched.

A small amount of time must be reserved after the column switch to establish total stabilization, as the PAD detector is highly sensitive to the minor changes in eluent composition and back-pressure when the columns are switched. Five minutes was found to be sufficient.

Figure 3: Picture of concept system

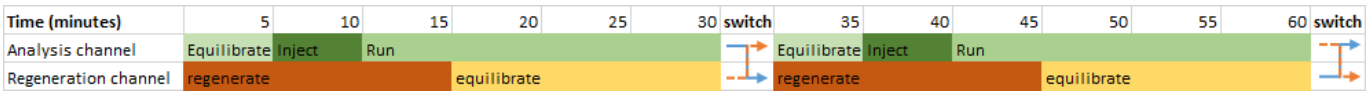

Figure 2: Visual representation of the analysis cycles. Blue and orange arrows represent different columns. Transition from the “regenerate” to the “equilibrate” phase consists of a step gradient from a high to a low NaOH concentration.

Prove of concept – Results

Carbopac PA20 columns are recommended for analysis of mono- and di-saccharides although for this proof of concept a PA20 and a PA100 were used.

A length of about 10 cm of .003” ID PEEK tubing was used as a restriction to compensate for the pressure difference between the columns at the used flowrate (app. 80 bar).

The method was split up into two separate method files, one for each column, so the data was easily recognized and grouped for post-run analysis. The used methods are displayed in table 1 and 2.

A mix-standard consisting of 10-3 M of each arabinose, galactose, glucose and xylose in deionized water was used for the analyses.

A series of 26 runs, 13 for each column, was performed to assess the repeatability for the “off-line” regeneration and equilibration.

Table 1: HPLC method used

System:		Concept dual flowline HPAEC-PAD system
Column:	Ch 1:	Carbopac PA100
Column:	Ch 2:	Carbopac PA20
Mobile phase:	Ch 1:	A) Deionized water B) 40 mM NaOH
Mobile phase:	Ch 2:	A) 200 mM NaOH B) 10 mM NaOH
Gradient:	Ch 1:	0 – 30 min 20 %B
Gradient:	Ch 2:	0 – 17.5 min 100 %A
Flow Rate:		0.5 mL/min
Column Temp.:		30 °C
Injection Vol.:		20 µL

Example chromatograms for both columns are displayed in figure 4 and 5. Statistical results of the thirteen runs of column 2 (PA20) are displayed in table 3. Statistical results were calculated using the LabSolutions browser.

Table 2: Detector Settings (PAD)

Range:	20 µA
E1:	0.10 V
t1:	0.4 s
ts:	0.1 s
E2:	- 2.00 V
t2:	0.02 s
E3:	0.60 V
t3:	0.01 s
E4:	- 0.10 V
t4:	0.07 s

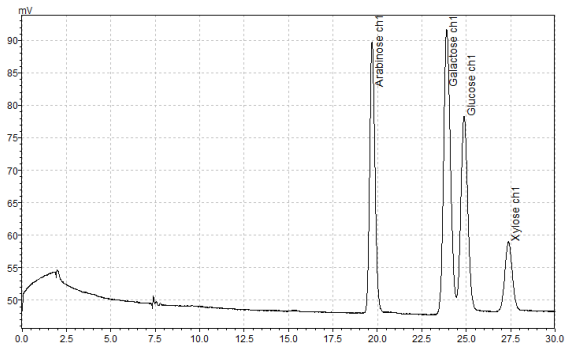

Figure 4: Chromatogram of column 1 run (PA100)

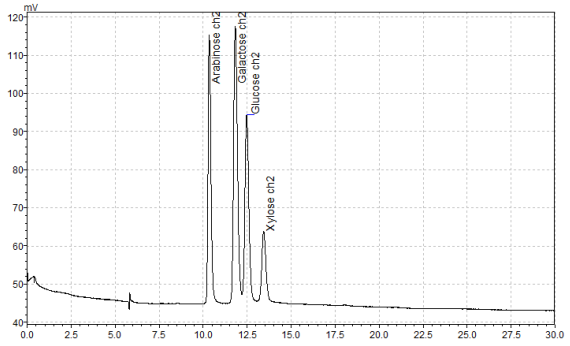

Figure 5: Chromatogram of column 2 run (PA20)

The repeatability of the retention times was found to be good with very low relative standard deviations (RSD) of < 0.06 %.

The peak areas were not found to be sufficiently stable with RSD values of about 3 % on average. It was further noted that the peak areas showed a linear decrease as a function of time, as shown in figure 6 for arabinose, while the S/N ratios remained the same.

As such it was opted to apply an internal standard method to correct for this decrease in detector response. The internal standard (IS) method was applied by dividing the peak areas of galactose, glucose and xylose by the peak area of arabinose. The correction lead to a more than 3-fold decrease in %RSD for all components (see table 3).

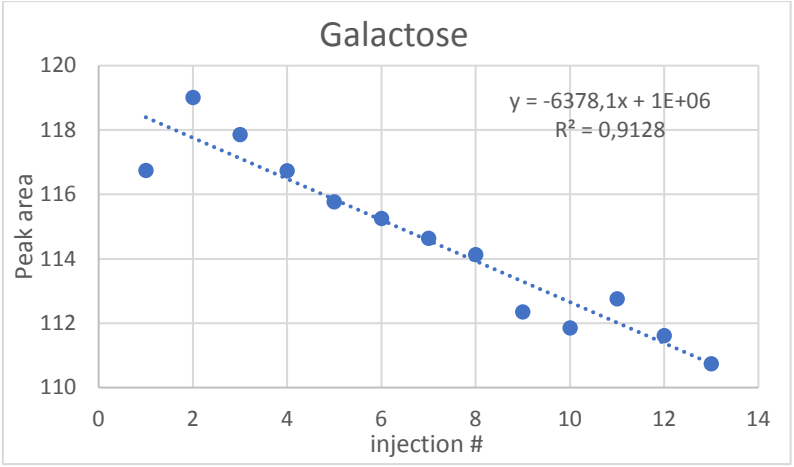

Figure 6: Graph of linear response decrease for galactose

Table 3: Statistical results for repeat injections on the concept system

Compound	Variable	Average	%RSD n = 13	RSD % n = 13 IS-Method
Arabinose 15.013 mg/L	Ret. time	10.358	0.040	-
	Area	762,594	2.602	IS
	S/N ratio	263.44	4.399	-
Galactose 18.016 mg/L	Ret. time	11.843	0.052	-
	Area	1,049,458	2.965	0.401
	S/N ratio	270.22	4.465	-
Glucose 18.016 mg/L	Ret. time	12.491	0.058	-
	Area	736,991	2.993	0.871
	S/N ratio	185.41	4.529	-
Xylose 15.013 mg/L	Ret. time	13.459	0.058	-
	Area	284,424	4.464	1.256
	S/N ratio	69.16	4.457	-

Discussion

The decrease in detector response is probably caused by either fouling of the electrode surface by oxidation products or by the oxidation of the electrode surface itself.

Normally this is circumvented by regenerating the detector with a high concentration of NaOH together with the column. However, in the proposed system set-up, regenerating the detector significantly increases equilibration times as the analysis flowline is then saturated with high NaOH eluent. This would need to be flushed before analysis adding about 10 minutes to the total analysis time. This time consists of 1 minute of column dead time (for PA20), 2 x 1.5 minutes of system dead time, an estimated 2 minutes of actual regeneration time and another 5 minutes for stabilization.

It is hard to set this method up representatively without two identical columns, as the PA100 shows much more retention, resulting in much longer regeneration times when these have to follow after analysis.

If no gradient has to be applied, a simplified system could be used without a mixer, which would decrease this flushing time.

Options to circumvent this problem should be further considered and tested using a system with two PA20 columns.

Conclusions

A novel dual-stream system for the fast analysis of carbohydrates has been proposed. Although the system shows high relative standard deviations (RSD) in its current form, these problems may be circumvented by applying an internal standard method or by adjusting the system and method to regenerate the detector cell together with the columns, at the cost of slightly elongated analysis.

References

1. Application News SCA_190_029 Shimadzu Europa GmbH
2. VICI Corporation, accessed 30-5-2017 URL: https://www.vici.com/support/app/2p_japp
3. Earl W. Malcolm and John W. Green, A Study Of The Borate-Carbohydrate Complex, Journal Of The Chemical Society, 1964
4. M. Levy and E. Doisy, The Reaction Of Borate And Sugars, St. Louis University School of Medicine, 1929