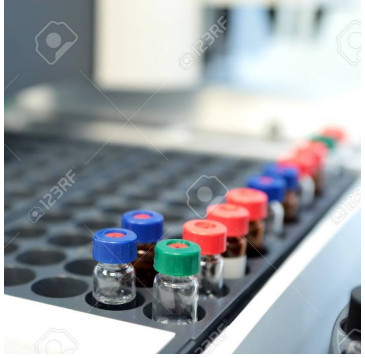
Technical Note

ALEXYS High-throughput Carbohydrate Analyzer



The finest HPAEC-PAD applications for carbohydrate analysis

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Carbohydrates in Dutch candy
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Fructans in infant formula
TGOS in food products
Profiling of FOS

Lactose Free Products
Lactose in dairy & meat
Lactose in lactose-free products

Artificial Sweeteners
Sugar alcohols
Sucralose

Glycoproteins
N-glycans
Monosaccharides
Sialic acids

Automated column switching

- **ALEXYS™ High-throughput Carbohydrate Analyzer**
- **Column switching using integrated valve control**
- **Robust and cost effective solution for routine analysis**
- **Adaptable for different applications**

Summary

This technical note describes a high-throughput HPAEC-PAD method that uses automated column switching to speed up cycle time and increase sample throughput. The ALEXYS™ High-Throughput Carbohydrate Analyzer uses two columns running in alternating mode: one column is being used for the analysis, while the other column is washed and re-equilibrating, reducing idle time between injections. An example application using this method for analyzing lactose and its isomers is described, highlighting the ability of the method to deliver twice the sample-throughput with excellent analytical performance.

Automated column switching

Introduction

In a number of HPLC and HPAEC methods, a post-run wash step is required to regenerate and re-equilibrate the column before the next injection. This additional step extends the total run time and limits the effective measurement throughput. Developing a method that eliminates or shortens the waiting period between runs would significantly improve efficiency and reduce costs, particularly in high-throughput workflows or when operational expensive instruments such as mass spectrometers are used.

This technical note presents a dual-column setup designed to enhance productivity by alternating between two analytical columns using a switching valve. As an example, a lactose measurement of 10 minutes followed by a washing step of 15 minutes was demonstrated. The ALEXYS™ High-throughput Carbohydrate Analyzer (Figure 1) uses an additional column, a 10-port column switching valve and a second HPLC (washing) pump. The eluent bottles are equipped with dual suction lines to deliver the same eluent to both pumps. No other instrumental modifications were needed.

The method is based on switching columns. While one column is actively used for sample analysis (‘online’), the second column is simultaneously regenerated and equilibrated (‘offline’) [2 – 4]. This process runs continuously and automatically within the method and enables a smooth transition between injections. Depending on the separation, regeneration, and equilibration time, this setup can double the sample throughput compared to the single-column approach. This approach significantly increases instrument utilization and sample capacity without compromising analytical performance, making it suitable for routine batch analysis where large sample quantities are processed.

Method

Instrumentation

The ALEXYS™ High-throughput Carbohydrate Analyzer (Figure 1) is configured for dual column operation and consisting of the ET 210 eluent tray (for storage and N_2 blanketing of mobile phases during the analysis), two P 6.1L quaternary LPG pumps, AS 6.1L autosampler, an external, electrically actuated 10-port, 2-position PEEK switching valve, an optional CT 2.1 column thermostat, and the DECADE Elite electrochemical detector with single cell control (SCC). In this configuration, one quaternary LPG pump is used for the actual analysis, while the other pump is used for regenerating the column.

Figure 1. ALEXYS High-throughput Carbohydrate Analyzer, SCC. The column thermostat CT2.1 is an optional part.

The two LPG pumps made it possible to run an isocratic or a gradient method for the analysis, and enabled different wash gradients if necessary. The SenCell™ with Au working electrode and HyREF (Pd) reference electrode was selected for the detection of the carbohydrates [5]. A single autosampler, detector, and flow cell ensure consistent detection response across analysis on two columns.

System setup and operation

The dual-column workflow is based on the programmable column switching valve. At any given time, one column is ‘online’ with the detector for analysis while the other column is ‘offline’ and connected with the wash pump for regeneration

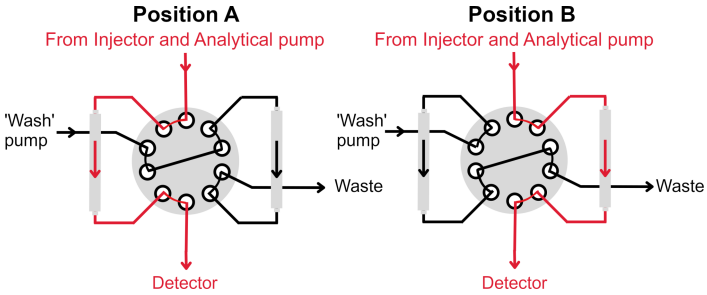
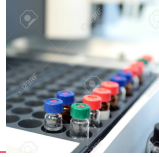


Figure 2. Flow path configuration of the 10-port 2-position switching valve for high-throughput analysis. One pump is dedicated as the analytical pump and the other pump is dedicated as the regeneration (‘Wash’) pump.



and equilibration. The flow path for this operation is illustrated in Figure 2. Before operation, the valve and the actuator must be initialized and configured according to the manual 250_7001 [6]. The valve actuator is connected to a control module. The control module is then connected to the DECADE Elite electrochemical detector, allowing for direct control of the valve position in the Chromatography Data System (CDS) software.

Requirements

Apart from instrumentation, several additional factors should be considered. The dual-column approach is most beneficial when the column washing step is significantly longer than the analytical run time. The column performance should be comparable for both, since the same application is run on both columns. It is important that the run times are the same, therefore column selectivity and efficiency should be (nearly) the same. Users may choose to have column-specific calibration or use a shared or averaged calibration file. An example will be discussed below, which may be helpful for the users to decide which calibration approach best fits their workflow.

Under ideal conditions, this setup can reduce the total cycle time to approximately half that of a single-column method. When a large number of samples are analyzed, the time savings quickly offset the additional calibration runs, making this high-throughput system highly cost-effective.

In this example, a fully optimized HPAEC-PAD method for separation and detection was first developed using a single column. Once established, two high-throughput methods—one for each column—were created. These methods are identical in every respect except for the programmable valve-switching event. One method includes a command to switch the valve from position ‘A’ to ‘B,’ while the other switches from ‘B’ to ‘A.’ The valve-switching event was scheduled at the end of the cycle time to maintain consistent system performance.

The timing of the valve and column switch depends on whichever step —elution or washing— takes longer. Columns can only be switched once both the analytical run and the wash step have been completed. In case the wash step is significantly longer, it may be possible to start the wash already while waiting for the other column to finish the wash step. But such alternative approach is beyond the scope of the current investigation.

For example, if the elution time is 10 minutes and the regeneration + equilibration time is 15 minutes, the cycle time of the high-throughput method should be set to 15 minutes (the elution time can be extended accordingly). Conversely, if the elution takes longer than the regeneration step, the equilibration time can be adjusted accordingly.

It is essential that the regeneration/wash pump gradient program always begins at the same composition as the final equilibration step. This ensures a stable baseline at the start of each analysis and consistent reproducibility.

Practical implementation

The analysis workflow proceeds as follows:

Step 1, System initialization: Begin by switching on the pump flow. After 10 minutes, switch on the electrochemical cell manually or load one of the two high-throughput methods onto the instrument. Allow approximately 30 minutes for the baseline to stabilize before injecting any samples. Additionally, perform a thorough wash of the sample needle and loop, and verify that the syringe and buffer tubing is free of air bubbles.

Step 2, Method and valve position: Verify that the selected (start) method matches the current valve position. For instance, if column 1 is assigned to valve position ‘A’, ensure that the method includes a command to switch the valve to position ‘B’ at the end of the run (see Figure 3). The valve position can be checked and changed manually using the remote control supplied with the valve actuator and control module (Figure 4).

DECADE Elite Detector Method

Main | Output | Time Table

	Time [min]	Parameter	Value
1	0.10	Sensor autozero	Active
2	14.98	Valve position	Load
3			

Figure 3. Example of valve position settings in the acquisition method. Valve position is switched at the end of the run (t = 14.98 minute, cycle time 15 minutes).

Figure 4. Manual remote control for valve actuator showing the actual valve position.

	Status	Run	Method Name	SV	Sample ID	Sample	Inj.Vol. [µL]	Sample Type	Lvl	File Name
1		☑	250811_Column1_Analysis_ValvePosA	1	ID103	10 uM standard_column 1_1	3.000	Standard	1	\%q\%y%m%oCM25011-%H%M-%q %Q_v%2v
2		☑	250811_Column2_Analysis_ValvePosB	1	ID104	10 uM standard_column 2_1	3.000	Standard	1	\ Data\ID103\250811CM25011-1124-ID103 10 uM standard_column 1_1_v01.prm
3		☑	250811_Column1_Analysis_ValvePosA	1	ID103	10 uM standard_column 1_2	3.000	Standard	1	\%q\%y%m%oCM25011-%H%M-%q %Q_v%2v
4		☑	250811_Column2_Analysis_ValvePosB	1	ID104	10 uM standard_column 2_2	3.000	Standard	1	\%q\%y%m%oCM25011-%H%M-%q %Q_v%2v
5		☑	250811_Column1_Analysis_ValvePosA	1	ID103	10 uM standard_column 1_3	3.000	Standard	1	\%q\%y%m%oCM25011-%H%M-%q %Q_v%2v
6		☑	250811_Column2_Analysis_ValvePosB	1	ID104	10 uM standard_column 2_3	3.000	Standard	1	\%q\%y%m%oCM25011-%H%M-%q %Q_v%2v
7		☑	250811_Column1_Analysis_ValvePosA	1	ID103	10 uM standard_column 1_4	3.000	Standard	1	\%q\%y%m%oCM25011-%H%M-%q %Q_v%2v
8		☑	250811_Column2_Analysis_ValvePosB	1	ID104	10 uM standard_column 2_4	3.000	Standard	1	\%q\%y%m%oCM25011-%H%M-%q %Q_v%2v
9		☑	250811_Column1_Analysis_ValvePosA	1	ID103	10 uM standard_column 1_5	3.000	Standard	1	\%q\%y%m%oCM25011-%H%M-%q %Q_v%2v
10		☑	250811_Column2_Analysis_ValvePosB	1	ID104	10 uM standard_column 2_5	3.000	Standard	1	\%q\%y%m%oCM25011-%H%M-%q %Q_v%2v
11		☑	250811_standby	10	standby	standby	3.000	Bypass	–	\%q\%y%m%oCM25011-%H%M-%q %Q_v%2v

Figure 5. Example of a sequence file in DataApex Clarity CDS containing alternating methods, 5 consecutive injections of the same sample from the same vial on both columns. Sample ID column is used for column identifier. File name column is programmed to automatically separate the generated data files into its respective folder based on the column identifier (folder 'ID103' or 'ID104'). A standby method is placed at the end of the sequence.

Step 3, Sequence setup: The sample sequence must always start with the method corresponding to the currently active column, followed by the method for the second column, continuing in an alternating pattern throughout the sequence. To avoid confusion, ensure to include column identifier in the data file name. Alternatively, a separate data output folder for each column may be used. In Clarity CDS, this action can be performed automatically using the file name parameter. An example of the sequence file is shown in Figure 5.

Step 4, Data processing: The generated data can be processed using either a shared calibration file or two separate calibration files (one for each column). Although ideally two identical columns are used, slight differences in retention time may occur due to batch variability. For optimal results, two separate calibration files during data processing were used (requiring additional calibration runs).

Step 5, Post analysis: When the analysis is complete, apply a standby method to turn off the cell and reduce the flow rate during idle periods (Figure 5, last line in the sequence file).

Example application

In Application Note 220_041 [5], the dual-column setup was used to analyze lactose and its isomers in lactose-free labeled products. This application note demonstrates the performance of the ALEXYS High-Throughput Carbohydrate Analyzer in combination with 2.1 mm ID microbore SweetSep AEX200 columns. All target compounds eluted within 10 minutes (Figure 6), followed by a 15-minute wash and equilibration step. Compared to the single-column approach described in Application Note 220_009 [8], the total cycle time was reduced from 30 minutes to 15 minutes—effectively doubling sample throughput. In this study, a total of 60 standards (10 replicates per column at three different concentrations) and 30 calibration runs (15 concentrations ranging from 0.1 to 80 µM on each column) were analyzed. This example also demonstrates the performance of the overall dual-column system with SweetSep AEX200 columns as follows:

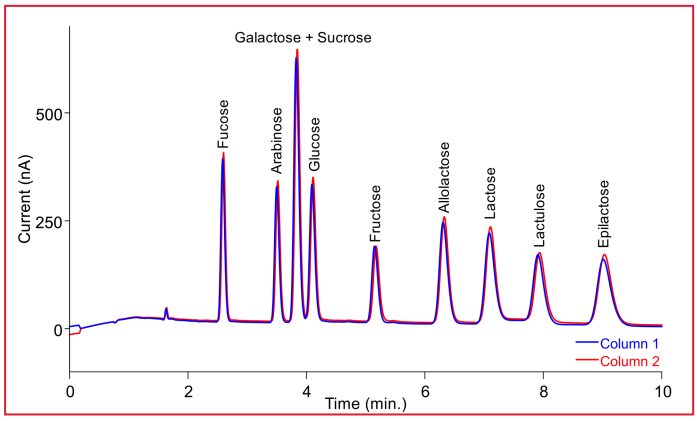
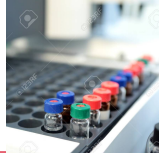


Figure 6. Overlay of the chromatograms obtained from a 3 µL injection of a 10 µM standard mix in DI water on column 1 (blue line) and on column 2 (red line).

- Retention times for all analytes were highly comparable between the two columns, demonstrating excellent batch-to-batch reproducibility of the SweetSep AEX200 columns.
- Retention time RSD values were ≤ 0.25% for all compounds in the standard mix at all tested concentrations (0.1 µM, 1 µM, and 10 µM, n = 10 for each concentration for each column). For comparison, the single-column approach described in Application Note 220_009 (n = 10, same concentrations) shows comparable retention time RSD values (≤ 0.30%) [8].
- Peak area RSD values at 0.1 µM (n = 10) on both columns were < 1.2%. Peak area RSD were improved with increasing concentration, i.e. at 10 µM RSD (n = 10) were < 0.5%. A single-column approach shows RSD of <2.4% at 0.1 µM (n = 10) and <1.31% at 10 µM (n = 10) [8].
- Linearity for all sugars were evaluated from 0.1–80 µM concentration range. Calibration curves were built using quadratic fitting, ignoring the origin, and weighted with 1/amount^2. The relative standard errors between 1.3–2.7% were observed. Calibration curves accuracy were within ±4% for all calibration curves on both columns, with coefficient of determination (r^2) > 0.999.



Calibration curves of the single-column approach using the same fitting as above shows slightly higher values for relative standard errors from 1.0—4.2% and comparable r^2 values (> 0.999).

- The calculated detection limits for all sugars on both columns range between 0.9 to 4.7 ng/mL, and the calculated limit of quantification (LOQ) range between 3.0 to 18.2 ng/mL, demonstrating excellent detection sensitivity on both columns. Although the calculated lactose LOQs (11.2 and 11.5 ng/mL) were higher compared to the single column approach (5 ng/mL), they still remain far below regulatory threshold (85-fold below the upper limit) and sufficient for reliable quantifications of lactose in lactose-free products.
- Quantification of lactose in three samples was performed using different calibration approaches, i.e. dual calibration (one calibration per column), single calibration averaging the responses of two columns, and a shared calibration for both columns (column 2 uses calibration of column 1 or vice versa). The amounts of lactose found in all samples using either single calibration approaches only differs by 0.1 mg/L compared to the dual calibration. These differences contributed to a recovery values ±3% compared to the dual calibration. Dual calibration generally gave recovery values closest to 100% (see the example in Table 1 below), while shared or averaged calibrations provided nearly equivalent results with the advantage of simpler data processing.

Discussion

The dual-column configuration presented here significantly increases sample throughput. However, a few operational considerations must be taken into account to ensure optimal performance and maximize efficiency.
First, when both columns are used for the same applications, the dual-column configuration relies on consistent performance from each column. Minor differences in separation performance can often be corrected by re-adjusting the method. Using the same method parameters (mobile phase composition) for both columns may result in a slightly different retention time and may be used as long as all peaks are well separated. If one column deteriorates more quickly than the other due to contamination, wear, or extended use, run-to-run reproducibility may be affected. It is therefore important to establish a 'good' column performance for each column used, and to monitor these parameters. When issues such as inconsistent peak shapes, slight retention time shifts, or compromised resolution on one channel appear, corrective actions such as column regeneration or replacement should be taken. A practical advantage of this setup is its ability to operate in single-column mode. This feature can serve as a temporary solution while awaiting a replacement column, thereby minimizing system downtime.

Table 1. Amount recovery in Lactose-free Kwark sample on both column using different calibration approaches. Dual calibration (column 1 uses Cal 1, column 2 uses Cal 2) shows the recovery closer to 100% compared to the other calibration approaches.

Compounds	Amount recovery on column 1 (%)			Amount recovery on column 2 (%)		
	Cal1	Cal2	Avg. cal	Cal1	Cal2	Avg. cal
Allolactose	101%	102%	102%	98%	100%	99%
Lactose	103%	106%	105%	98%	100%	100%

Second, using a dual calibration provides more reliable peak identification and the highest accuracy for quantification. In contrast, a shared or averaged calibration reduces data processing time and simplifies the workflow, making it a suitable choice when both columns exhibit closely matched separation performance—albeit with a slight compromise in accuracy compared to dual calibration. When a single calibration is applied, it is recommended to use a wider peak identification window to ensure correct peak assignment.

Third, a mismatch between the method and the valve position in the beginning of a run will result in one repeated injection on the same column. This situation is resolved in the next injection where the correct method is used to switch the valve. Alternatively, to circumvent this issue, an additional valve switching event can be programmed to ensure the correct valve position in the beginning of the run. It is important that this event is executed immediately after injection. A time delay between the injection and valve switching at the beginning of the run may result in the sample injected into the wrong column.

Lastly, this configuration can theoretically be adapted for two different columns or even two different applications. It is important to re-optimize each method and valve switching timing, especially if different gradients, run times, or mobile phase compositions were used. This is particularly relevant when adapting the system for target analytes beyond the example shown here (lactose and its isomers), as elution gradient and equilibration times may differ.

Overall, the automated dual-column switching setup is highly versatile. It can be tailored for different gradients, run times, or even switched to single-column operation if needed, offering flexibility for a wide range of applications and ultimately providing robust high-throughput carbohydrate analysis.

References

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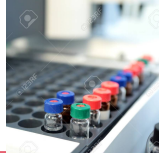
Conclusion

The dual-column setup with an integrated, automated switching procedure provides a practical and efficient way to increase sample throughput with excellent analytical performance. Alternating injections between the two columns minimizes idle time between injections, making it suitable for routine analysis. This approach is extremely versatile and can be used for different applications. A single column measurement mode can be used, ensuring continued productivity even when one of the columns fails. Overall, this method offers a higher sample throughput in routine quality control and compliance testing laboratories with large sample quantities.



Ordering information

ALEXYS analyzer	
180.0059WA	ALEXYS High-throughput Carbohydrate Analyzer, SCC
116.4321	SenCell 2 mm Au HyREF
186.ATC00	CT2.1 Column Thermostat
Columns	
260.0011	SweetSep™ AEX200, 2.1 x 200 mm column, 5 µm
260.0031	Borate ion trap, 2.1 x 50 mm column, 10 µm
260.0100*	Pre-column filter PEEK, 0.5 µm
Software#	
195.0035	Clarity CDS single instr. incl LC, AS module

*) In case samples might contain particulate matter it is advised to use a pre-column filter.

#) Antec ECD drivers are available for Chromeleon CDS, OpenLAB CDS and Empower CDS. The ALEXYS Carbohydrates Analyzer (full system) can also be controlled under Thermo Fisher Scientific Chromeleon™ CDS. Please contact Antec for more details.

Reagents, standards and sample prep accessories

NaOH 50%, carbonate –free	Fisher Scientific, pn SS254-500
Sodium acetate (NaOAc), 100%	Sigma Aldrich, pn 79714
DI water 18.2 MΩ.cm, TOC < 5 ppb	YoungIn Chromass Aquapuri Essence+ 393
Fucose	Sigma Aldrich, pn F2252-5G
Arabinose	Sigma Aldrich, pn A3131
Galactose	Sigma Aldrich, pn G0750
Sucrose	Sigma Aldrich, pn S9378
Glucose	Sigma Aldrich, pn G8270
Fructose	Sigma Aldrich, pn F0127
Allolactose	Carbosynth, pn OG09259
Lactose	Carbosynth, pn OL04771
Lactulose	Sigma Aldrich, pn 61360-5G
Epilactose	Carbosynth, pn OG04727
Potassium hexacyanoferrate(II) trihydrate	Fluka, pn 60280
Zinc sulfate heptahydrate	Sigma Aldrich, pn 31665-500g-M

For research purpose only not for use in diagnostic procedures. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system and DECADE Elite detector. The actual performance may be affected by factors beyond Antec's control. Specifications mentioned in this application note are subject to change without further notice.

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