ALEXYS™ Carbohydrate Analyzer

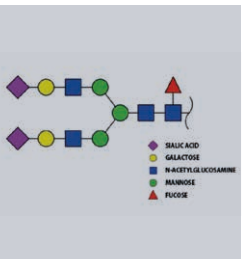
Easy, affordable, and reliable carbohydrate analysis in routine and R&D


F&B


Prebiotics


Plants

Sweeteners



Glycans

High Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD)

Antec® Scientific

ALEXYS™ the most Dedicated Analyzer for Carbohydrates

Why ALEXYS?

For over 33 years Antec Scientific has been the leader in Electrochemical detection for HPLC. Based on this experience and the market demand for more reliable and affordable carbohydrate analysis, we developed a fully dedicated High Performance Anion-Exchange Chromatography system with Pulsed Amperometric Detection (HPAEC-PAD) – called the ALEXYS Carbohydrate Analyzer.

For consistent results and highest reproducibility, day-to-day, user-to-user, and lab-to-lab, the ALEXYS Carbohydrate Analyzer is the best choice!

When choose ALEXYS?

The ALEXYS Carbohydrate Analyzer is the perfect instrument in all cases where you need:

- Highest sensitivity without derivatization
- Superieur separation - assured with SweetSep™ columns
- Access to dedicated flow cells
- Versatility – ideal for R&D and routine use

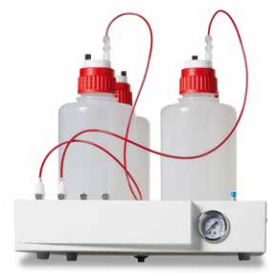
The ALEXYS Carbohydrate Analyzer is the ideal instrument for the analysis of all classes of sugars from mono- to penta-saccharides, including oligosaccharides and polysaccharides, e.g., Trans-galactooligosaccharides (TGOS), Fructooligosaccharides (FOS) according to AOAC methods and guidelines. Sugar acids, such as sialic acids, sugar alcohols e.g., erythritol, xylitol, sorbitol, mannitol, etc., sugar phosphates, and sugar nucleotides in biological systems, plant, and F&B products including N-glycans from glycoprotein can be easily analyzed.

ALEXYS™ Carbohydrate Analyzer

The ALEXYS Carbohydrate Analyzer consists of the following modules:

- Eluent bottle tray
- Quaternary HPLC pump
- Inert autosampler
- Column thermostat
- Electrochemical detector
- Flow Cell

Eluent Bottle Tray ET210



- Sparging followed by blanketing for minimal gas consumption
- Carbonate free, assuring highest reproducibility
- Works with He or cost-efficient N_2

The ET210 eluent tray with special valving enables to blanket the mobile phases with an inert gas atmosphere using N_2 or He. The inert atmosphere in the solvent bottle prevents diffusion of air into the mobile phase and will keep it free of CO_2 and O_2. Dissolved CO_2 can be problematic, especially in carbohydrate analysis using strong alkaline eluents. At pH > 12, CO_3^{2-} ions can be formed in the mobile phase, causing variations in retention times, decrease in column selectivity and loss in resolution. Keeping the mobile phase free of carbon dioxide is one of the key factors for reproducible carbohydrate analyses. Additionally, the bottles are kept closed airtight and pressurized with a small overpressure, so no gas flows out, thus saving N_2 or expensive He, compared to continuous sparging.

Quaternary HPLC Pump P6.1L

- Minimal pulsation for lowest noise
- High accuracy at µL flow rates
- Automated piston wash

The ALEXYS pump – P6.1L –consists of a dual piston design for lowest flow pulsations. This assures zero detectable noise from the piston stroke for sensitive electrochemical detection. For highest fidelity and flexibility, a quaternary gradient pump is built into the system that uses low-pressure mixing for gradient formation and solvent blending from up to four solvents. Ideal for demanding gradient elution or for additional equilibration and/or rinsing with different solvents. An automated piston wash is present to remove traces of salt and other contaminants from the backside of the pistons, increasing the lifetime of the pump seals. The pump head can deliver flow rates in the low µL/min range up to 10 mL/min with pressures up to 400 bar (5800 psi).

Inert Autosampler AS6.1L

- Fast, accurate & reproducible
- Sample pick-up down to 100 nL
- Cooled sample tray

The autosampler AS6.1L is a new generation autosampler. It is fast, robust and easy to use, without compromising accuracy and reproducibility. The autosampler includes indispensable features for the highest injection performance. It has a dual needle with positive headspace pressure, extensive wash routines for minimal carry over and 3 injection modes including µL pick-up mode for zero sample loss. The AS6.1L can handle sample volumes down to 100 nL accurately and reproducibly. The sample tray offers temperature control from 4°C, to preserve labile samples, up to 40°C for samples which require heating.
The fast needle movement results in unsurpassed speeds of less than 30s for a full cycle. These features make the autosampler a perfect fit for high throughput routine carbohydrate analysis. The optimized fluidic path of the autosampler is metal-free and consist of inert PEEK, ETFE, Kel-F, glass, PTFE and silica-coated stainless steel to eliminate the possibility of metal contamination and improve robustness. Sample capacity: up to 108 standard 1.5 mL sample vials or up to 768 samples using two microtiter plates.

Column Thermostat CT2.1

- Temperature stability ± 0.1 °C
- Peltier element for heating and cooling
- Leak detection with auto shut off

The ALEXYS Carbohydrate Analyzer comes with a column oven / thermostat for highest reproducibility, optimal selectivity, and separation efficiency. Accurate sub ambient temperature control is essential for the reproducible separation of complex mixtures of carbohydrates. With its wide temperature control in the range of 5 °C to 85 °C any type of carbohydrate analysis can be performed.
The CT2.1 operates with a microprocessor-controlled Peltier element for precise temperature control. In combination with its high temperature stability, this allows programming of linear as well as non-linear temperature gradients. It can accommodate up to 4 anion-exchange columns of 32 cm in length.

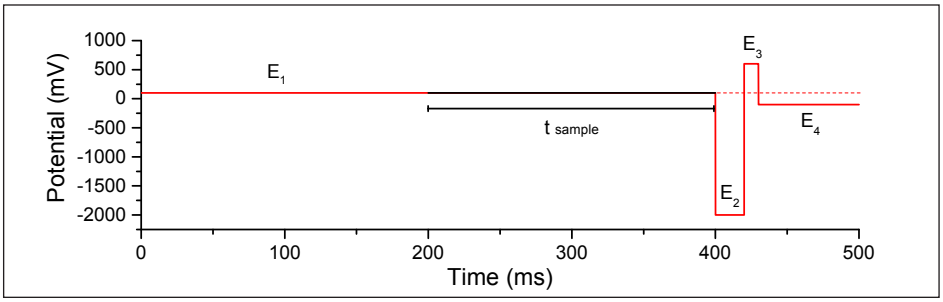
Electrochemical Detector DECADE™ Elite

- Highly sensitive electrochemical detector (ECD)
- Temperature-stabilized and Faraday-shielded flow cell compartment
- ADF (Advanced Digital Filter) for enhanced Signal-to-Noise Ratio (S/N)

To achieve unparalleled detection sensitivity, the DECADE Elite incorporates premium electronics and circuit boards throughout the entire apparatus. The electronic noise produced by the detector is less than the chemical noise, measuring values ≤ 2 pA with a dummy cell (load of 300 MΩ/0.5 µF). Additionally, the flow cell is housed within a Faraday shielded oven compartment with temperature stability of 0.1 °C, ensuring optimal baseline stability and the elimination of electrical interferences.
The Elite is fitted with an ADF low-pass digital filter specially designed by Antec Scientific. The ADF algorithm is tailored to suppress noise originating from the pump and the electrochemical flow cell. By selecting the appropriate filter settings, the signal-to-noise (S/N) ratio can be significantly improved, up to 100 times.

Why Pulsed Amperometric Detection (PAD)?

PAD is the preferred technique for detecting all types of carbohydrates, including polyalcohols, glycoproteins, antibiotics, and more, as mandated by various USP and EP methods. These compounds tend to contaminate the working electrode surface, making conventional constant-potential amperometric detection (DC mode) challenging. The simplest PAD mode involves three steps and is commonly used for the routine analysis of carbohydrates with sufficiently high concentrations. One drawback of this 3-step PAD is the relatively long oxidative and reductive cleaning step, leading to the consumption of the electrode surface.
On the other hand, it is advisable to employ a 4-step PAD in instances where a 3-step is not necessary. This approach guarantees minimal electrode degradation, sustained detection reliability over time, and the achievement of highest sensitivity.
The Decade Elite enables the programming of various waveform types with up to 30 potential/time coordinates, using 10 mV and 10 ms increments.



Example of a 4-step PAD potential waveform for the detection of carbohydrates. In this waveform, the electrode cleaning after the measurement period (t sample at potential E1) is done by applying a short 20 ms negative potential of -2.0 V (E2), followed by a short 10 ms activation potential (E3) and a mild oxide reduction potential (E4) to create catalytic sites on the electrode surface.

Dedicated Flow Cells for Maximum Performance

Two types of electrochemical cell designs are available: a thin-layer flow cell, FlexCell™ and a wall-jet design, SenCell™. Both are optimized in terms of electrode area and spacer thickness, but each cell has its unique characteristics that suits its application.

FlexCell

- Easily removable working electrode
- Solid gold electrode for periodic polishing
- Low cost in routine use

The FlexCell is a thin-layer flow cell - it can be easily opened, and the gold electrode removed or exchanged. A side effect of the applied pulse mode is that the gold electrode is slowly consumed. After long-term use (several months) some gold on the working electrode surface can disappear. If needed, the solid gold electrode of the FlexCell allows for periodic polishing of the gold surface with the provided metal polishing kit. The traditional 3-step detection pulse has more wear than the newer 4-step pulse. The FlexCell is the recommended flow cell for routine carbohydrate analysis.

SenCell

- Adjustable Spacer Technology (AST) for highest sensitivity
- Fast equilibration
- No spacers or gaskets

The SenCell has been specially designed to be compatible with a large flow rate range to cover Capillary-, Microbore- and standard bore HPLC and at the same time to achieve the highest sensitivity by incorporating a variable detection volume.
The cell is small, robust, and easy to handle. The SenCell has unique, stepless variable spacing[1]. This Adjustable Spacing Technology (AST) is a built-in micro-positioner for the working electrode relative to the inlet. To optimize the volume of the flow cell, simply turn the micro-positioner. Optimizing the electrode distance (spacing), allows the user to obtain the highest signal to noise ratio. For many applications this improves the detection sensitivity considerably. The SenCell should be used only with a 4-step pulse to minimize the wear of the Au electrode. It is the advised flow cell for sensitive carbohydrate analysis particularly in R&D.

[1] US Patent 2014/0102916 A1

System Performance

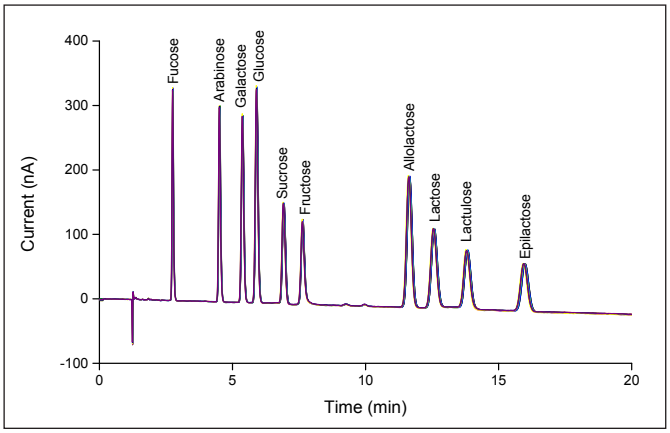
The pump flow accuracy of the ALEXYS™ Carbohydrate Analyzer was measured using a digital liquid flowmeter on one of the P6.1L pumps. The average values of the measurements are shown in Table 1. The accuracy is given as the relative error in % and % RSD.

Table 1: Pump flow accuracy (n=10)

Parameter	Results			
Flow rate	0.05 mL/min	0.2 mL/min	1.0 mL/min	5.0 mL/min
Avg. Flow rate (mL/min)	0.0509	0.2010	1.0047	4.9691
Accuracy (%)	1.8	0.5	0.5	0.6
RSD (%)	0.17	0.16	0.19	0.19

With typical % RSD values of ≤ 0.19, excellent flow accuracy is achieved assuring consistent and reproducible results.

To illustrate the repeatability of the analysis, a mixture consisting of 10 carbohydrates was separated on the ALEXYS Carbohydrate Analyzer. Below the overlay of the chromatograms of 10 consecutive injections is depicted.



Repeatability measurement: Overlay chromatogram of 10 consecutive injections using the ALEXYS Analyser equipped with a SweetSep™ AEX200 column, 4.0 mm ID × 200 mm

In Table 2 the data from the repeatability measurements are listed.

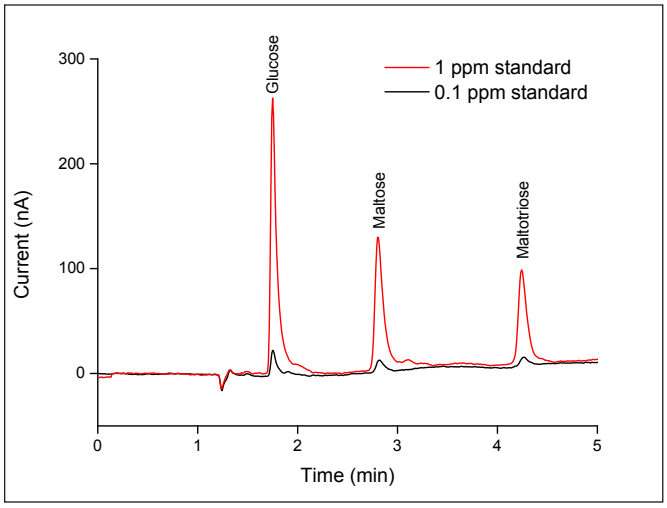
Table 2: Repeatebility (n=10)

No.	Compound	Retention Time		Peak Area	
		Average (min)	RSD (%)	Average (nA.s)	RSD (%)
1	Fucose	2.76	0.16	1271.51	0.24
2	Arabinose	4.52	0.12	1570.68	0.28
3	Galactose	5.38	0.14	1887.01	0.23
4	Glucose	5.91	0.12	2345.41	0.20
5	Sucrose	6.93	0.21	1311.08	0.36
6	Fructose	7.64	0.11	1180.47	0.59
7	Allolactose	11.65	0.15	2707.21	0.39
8	Lactose	12.58	0.13	1904.92	0.52
9	Lactulose	13.83	0.12	1476.78	0.50
10	Epilactose	15.98	0.11	1356.35	0.53

Outstanding repeatability in carbohydrate analysis is demonstrated by typical % RSD values ranging between 0.11 and 0.21 for retention times and between 0.20 and 0.53 for peak areas.

System Performance (cont.)

To highlight the exceptional sensitivity of the ALEXYS Carbohydrate Analyzer, mono-, di-, and trisaccharides have been separated, close to their limit of detection (LOD). The chromatogram below displays a standard mix comprising Glucose, Maltose, and Maltotriose.



HPAEC-PAD for the sensitivity assessment of mono-, di- and trisaccharides

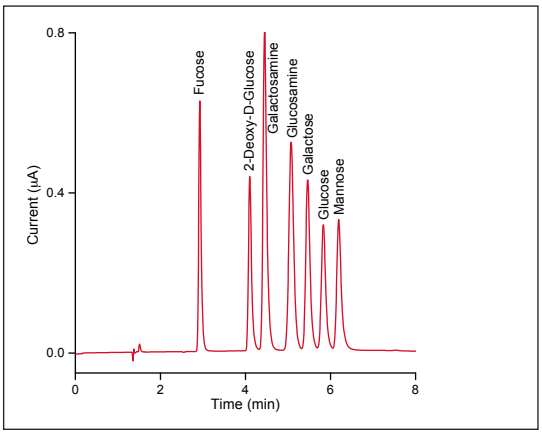
The ALEXYS Carbohydrate Analyzer showcases remarkable sensitivity, revealing typical limits of detection (LODs) ≤ 0.055 ppm and limits of quantitation (LOQs) ≤ 0.185 ppm for Maltotriose. For Glucose and Maltose even lower levels are measurable. This underscores its ability for direct carbohydrate detection without the need for derivatization.

Table 3: LODs and LOQs for mono-, di-, and trisaccharide

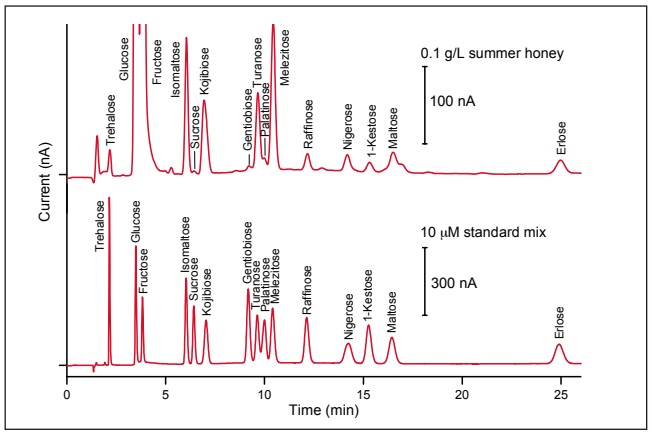
No.	Name	S/N	LOD (ppm)	LOD (µM)	LOQ (ppm)	LOQ (µM)
1	Glucose	17.088	0.018	0.097	0.059	0.325
2	Maltose	7.563	0.040	0.116	0.132	0.386
3	Maltotriose	5.420	0.055	0.110	0.185	0.366

Examples

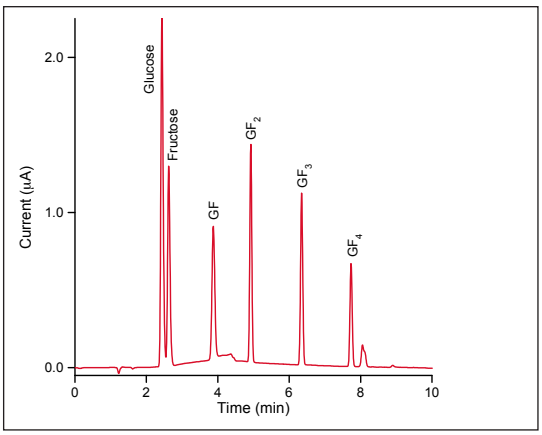
Mono- up to Pentasaccharides



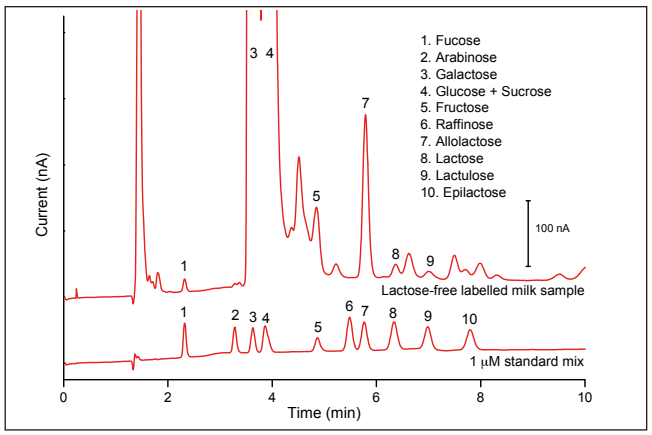
Isocratic separation of monosaccharides on a SweetSep™ AEX20 column, 4.0 mm ID × 200 mm. 10 µL inj. of a 10 µM of monosaccharides standards in water (HPAEC-PAD).



HPAEC-PAD of honey on a SweetSep™ AEX200 column, 4.0 mm ID × 200 mm. Top: 10 µL inj. of a 0.1 g/L Swiss summer honey sample. Bottom: 10 µL inj. of a 10 µM standard of 15 sugars present in honey.

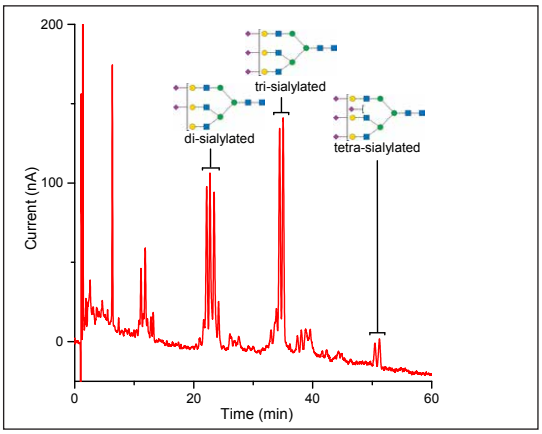


Separation of short-chain fructooligosaccharides up to DP5 by HPAEC-PAD. SweetSep™ AEX200 column, 4.0 mm ID × 200 mm. 10 µL inj. of 10 ppm mixtures GFs.

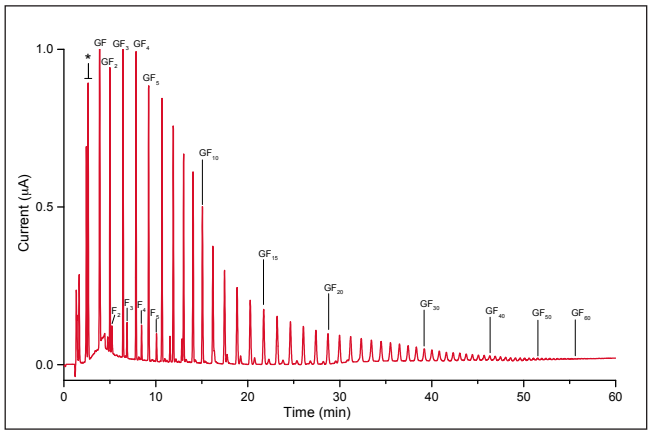


Lactose intolerance: Analysis of milk on a SweetSep™ AEX200 column, 4.0 mm ID × 200 mm. Top: 10 µL inj. of a 10 g/L lactose-free labelled milk. Bottom: 10 µL inj. of < 10 µM standard of 11 sugars commonly found in milk.

Oligosaccharides & N-Glycans



Separation of N-glycans standard containing di-, tri-, and tetra sialylated oligosaccharides by HPAEC-PAD on a SweetSep™ AEX200 column, 4.0 mm ID × 200 mm.



Gradient separation of inulin from chicory. 10 µL inj, 200 ppm. SweetSep™ AEX200 column, 4.0 mm ID × 200 mm using HPAEC-PAD.

Applications Overview

Food and beverages

220_002 - Carbohydrates in food products
220_003 - Mono- and disaccharides
220_006 - Carbohydrates in instant coffee
220_016 - Carbohydrates in food according to AOAC
220_019 - Sugars in meat & fish
220_025 - Carbohydrates in honey

Prebiotics food additives

220_020 - TGOS in food products
220_021 - Profiling of fructooligosaccharides
220_022 - Fructans in infant formula

Lactose-free products

220_009 - Lactose-free products
220_018 - Lactose in dairy & meat products

Carbohydrates in plants

220_004 - Carbohydrates in plants

Artificial sweeteners

220_013 - Sugar alcohols

Compositional analysis of glycans

220_003 - Mono- and disaccharides

Link to application notes. Scroll down to access.

SweetSep™ Columns

The use of Antec Scientific SweetSep™ HPAEC columns is highly recommended for robust and reliable separation of all classes of carbohydrates.

Link to SweetSep columns

Specifications

General Specifications ALEXYS Carbohydrate Analyzer

Power	100-240V, 50/60 Hz, 660 W
Dimension (h x w x d)	105 x 81 x 70 cm (41.3 x 31.9 x 27.6 inch)
Weight	71 kg
Recommended operating temperature	+22 °C (± 3 °C)
PC control	Ethernet (LAN)
Software capable of controlling ALEXYS	DataApex Clarity™ (version 8.3 and up) Thermo Scientific™ Chromeleon™ CDS (version 7.2 SR5 and up)
Recommended operation flow rate	0.2 - 1.5 mL/min
Maximum system pressure	345 bar (5000 psi)
Eluent bottle Tray ET210	
Format	Eluent tray with 4 integrated gas line connections and manual flow regulator valve
Suitable gas source	Inert gas (helium or nitrogen)
Operating pressure range	0.2 - 0.4 bar (3 - 6 psi); overpressure relieve active at 0.7 bar
Flow Path	
Bottles for mobile phase	2 L PPCO with gastight caps for blanketing purpose
Fluidic connections, low pressure inlet	1/8" o.d. FEP tubing
Fluidic connections, high pressure	1/16" o.d. PEEK tubing with PEEK connectors for the high-pressure parts
Quaternary HPLC Pump P6.1L	
Gradient formations	Quaternary pump with degasser and mixer for isocratic and gradient use
Pump head material	Ceramic
Degasser	Gas permeation using Teflon® AF amorphous fluoropolymer membrane, 4 channels
Inert Autosampler AS6.1L	
Sample tray cooling/heating	4 - 40 °C
Sample plate	up to 108 standard 1.5 mL sample vials or up to 768 samples using two microtiter plates.
Injection volume range	5 - 100 µL based on standard sample loop (100 µL)
Recommended injection modes	Full loop fill or partial loop fill
Needle wash	Standard simple wash, and option to program 2 different wash solutions
Column Thermostat CT2.1	
Heating and cooling system	microprocessor controlled Peltier element and fan supported 2-way air circulation
Temperature range	5 - 85 °C
Electrochemical Detector DECADE Elite	
Flow cell compartment	Faraday shielded compartment with integrated oven +7°C above ambient to 60°C; accommodates flow cells and optionally the column
PAD mode	Preprogrammed specific 4-step pulse (no Au wear) for carbohydrate analysis. Up to 30 potential/time coordinates waveforms programmable with 10 mV and 10 ms increments.
Filter (ADF)	0.5 - 0.001 Hz; OFF: for unprocessed data
ECD flow cell	SenCell or FlexCell with gold working electrode and HyREF™ (palladium) reference
Cell working volume	0 – 0.7 µL
Max temperature limit for flow cells	50°C

Specifications

Ordering information

Detector only	
176.0035B	DECADE Elite SCC electrochemical detector
116.4321	SenCell 2 mm Au HyREF
Recommended ALEXYS analyzer	
180.0057W	ALEXYS Carbohydrate Analyzer—gradient (quaternary LPG)
116.4321	SenCell 2 mm Au HyREF
186.ATC00	CT2.1 Column Thermostat
Column	
260.0010	SweetSep™ AEX200, 4 × 200 mm column, 5 µm
260.0030	SweetSep™ BIT, 4 × 50 mm borate ion trap
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.

*) The ALEXYS Carbohydrate Analyzer can also be controlled under Thermo Scientific Chromeleon™ CDS. For the DECADE Elite electrochemical detector only, control drivers are also available for Waters Empower™, Agilent OpenLab CDS, and Agilent OpenLab CDS Chemstation Edition. Please Contact Antec for more details.

For research purpose only. 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 and may be adjusted accordingly. Specifications mentioned in this application note are subject to change without further notice.

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