

Carbohydrate analysis with the 817 Bioscan

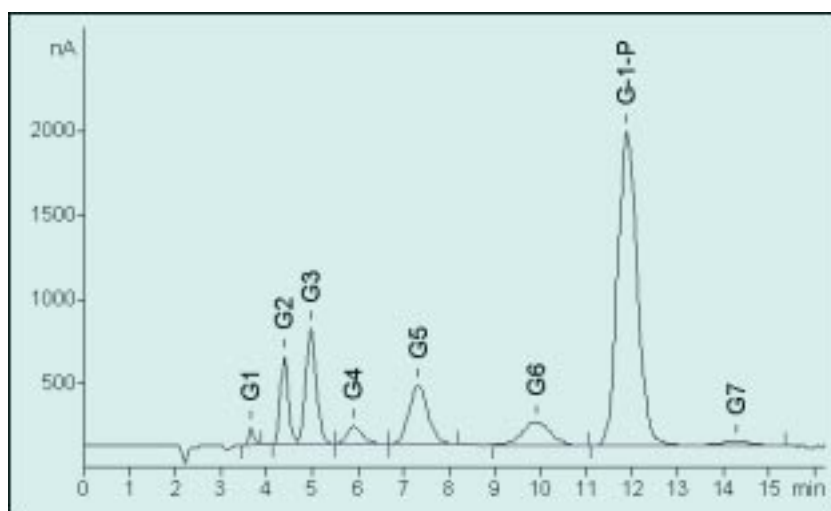
The 817 Bioscan is an electrochemical detector that can be operated in three different modes depending on the analytical requirements. In the DC mode a constant potential is applied to the working electrode. The analytes are then oxidized or reduced and the resulting current is measured. The scan mode allows current/potential curves to be recorded. The operator can use the recorded voltammograms to determine the optimal working potential for the pulse mode. This operating mode utilizes three different potentials that are applied in a cyclic manner. A suitable working potential (e.g. E1 = +50 mV for 400 ms) is followed by two cleaning steps (e.g. E2 = +750 mV for 200 ms; E3 = -50 mV for 400 ms), which ensure that after each cycle an active, newly conditioned electrode surface is available. Current measurement takes place during the last 100 ms of the working potential period. Pulsed amperometric detection is particularly suitable for the determination of carbohydrates.

A wide range of applications

On the market for just about one year, the 817 Bioscan is used today for a variety of applications all over the world. One example is the determination of the artificial sweetener sucralose in cola drinks. Another customer analyzes enzymatic reaction products with the help of the Bioscan. Special enzymes convert glucose and maltose into trehalose or gentiobiose. The enzymatic degradation of starch or cellulose to oligomers with up to seven glucose units can also be monitored (see Figure below).



The 817 Bioscan opens up numerous applications – be it as a stand-alone sugar analyzer or as a component of a modular IC system.

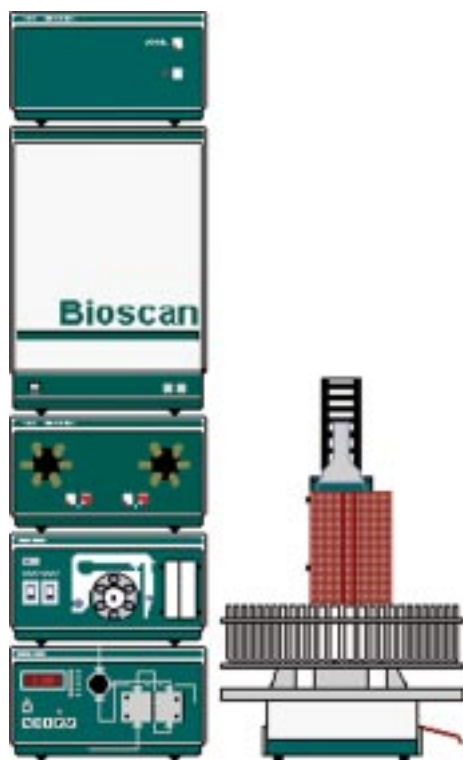


Glucose-1-phosphate G-1-P (2 mol/L) in a mixture of G1 to G7 oligomers.

Separation column: Metrosep Carb 1 – 250 (6.1013.000)
 Eluent: 100 mmol/L sodium hydroxide, 170 mmol/L sodium acetate
 Flow rate: 1 mL/min
 Temperature: 35 °C

817 Bioscan in the sugar industry

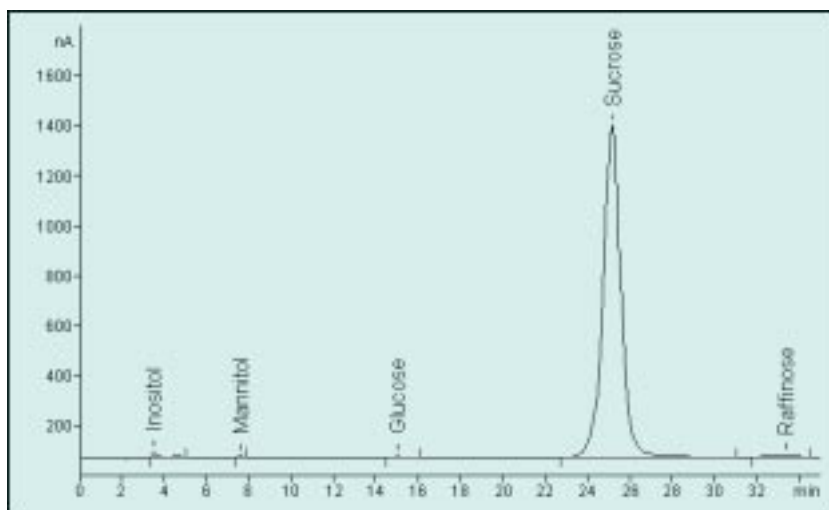
Sugar refineries and sugar mills are one of the main application areas of the 817 Bioscan. Ion chromatography with pulsed amperometric detection is ideal for monitoring the individual steps involved in processing sugar beet or sugar cane. For example, the degree of inversion of the raw sugar can be checked using the Bioscan. Apart from the main component, sucrose, the glucose and fructose contents are also determined. The following figure shows the chromatogram of a raw sugar sample taken from the crystallization stage of a sugar beet refinery. Its sucrose content is 93.6% referred to the dry substance.



Modular IC system consisting of 762 IC Interface, 817 Bioscan, 812 Valve Unit with two injectors, 754 Dialysis Unit, metal-free 709 IC Pump and 766 IC Sample Processor.

Determination of galactose and lactose in a soluble, spray-dried whey protein concentrate [2g dry substance (DS) dissolved in 1 L ultrapure water and then diluted 1 : 10].

Separation column: Metrosep Carb 1 – 250 (6.1013.000)
 Eluent: 100 mmol/L sodium hydroxide
 Flow rate: 1 mL/min
 Temperature: 32 °C
 Results: 30.1 mg galactose / 100 g DS;
 3815.6 mg lactose / 100 g DS

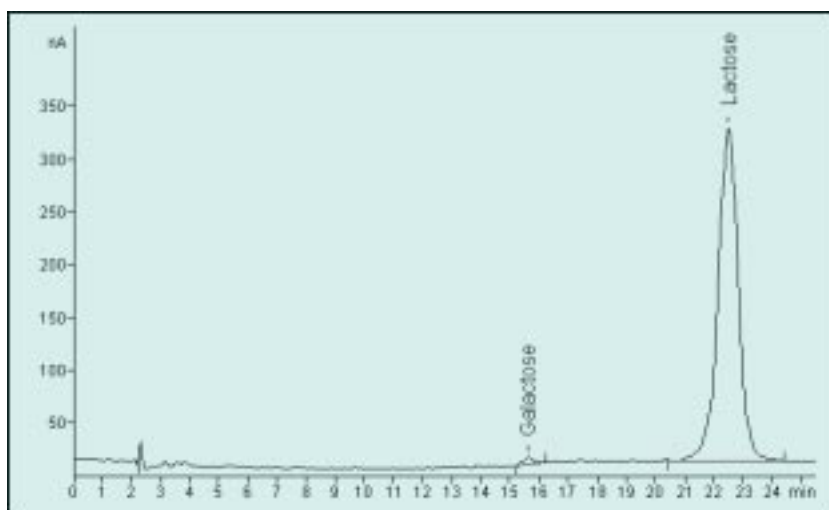


Analysis of a raw sugar melt (diluted 1 : 1000 with ultrapure water).

Separation column: Metrosep Carb 1 – 250 (6.1013.000)
 Eluent: 100 mmol/L sodium hydroxide
 Flow rate: 1 mL/min
 Temperature: 32 °C
 Results: 550 ppm inositol; 380 ppm mannitol; 60 ppm glucose; 64.58% sucrose;
 0.81% raffinose

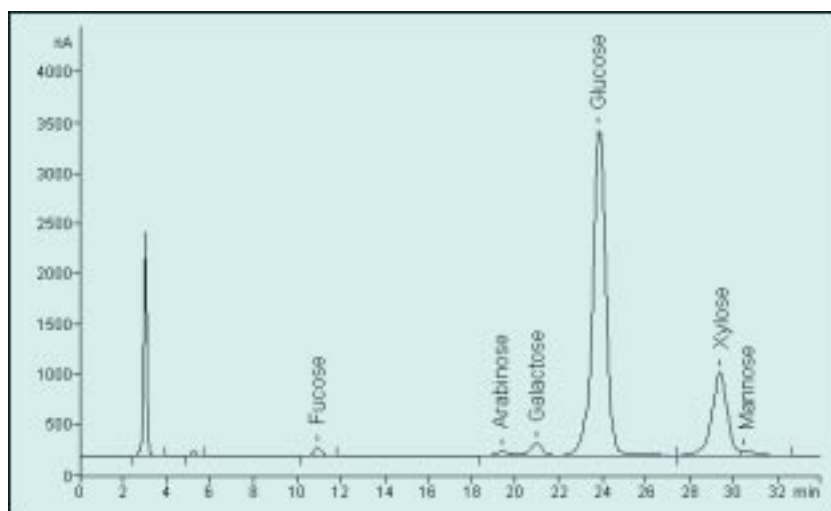
Interesting combinations with Metrohm IC modules

The combination with other modules from the Metrohm IC range opens up numerous additional applications for the 817 Bioscan. One interesting example is the use of the 754 Dialysis Unit for inline sample preparation. With the modular IC system shown at left, dairy products such as whole milk, yogurt, powdered milk, protein concentrates or baby food can be analyzed fully automatically. In this way galactose and lactose were determined in the intermediate products of a milk processing plant. As an example, the figure below shows the chromatogram of a soluble, spray-dried whey protein concentrate that has been prepared from casein whey. The sample contains approx. 80% protein, 4.8% fat and 4.1% water. Care must be taken that the system is working optimally during inline dialysis using the 754 Dialysis Unit and the 812 Valve Unit as injection and control valve. In particular, the counterpressure must be equal on both sides of the semipermeable dialysis membrane in order to avoid any filtering effects and to ensure that the separation of the analytes from the matrix only takes place by diffusion owing to the difference in concentration. In addition, both the dialysis time and the transfer time must be optimized. The chromatogram shown here was recorded after a dialysis time of 14 min; the transfer time was 0.55 min.



Demanding applications in the paper industry

The separation of arabinose, galactose, glucose, mannose and xylose plays a decisive role in many of the applications using the 817 Bioscan. In samples from the paper industry, large differences in the concentrations of these analytes are to be expected. In the sulfuric acid digestion of wood chips the glucose content may be between 70 and 99%. In contrast, the mass fractions of arabinose, galactose and mannose are very low and lie between 0 and 5%. If the standard eluent 100 mmol/L sodium hydroxide were used at a flow rate of 1 mL/min then all five monosaccharides would be eluted between 13 and 16 min on the Metrosep Carb 1 – 250. In order to achieve a clear separation of the analytes, a mixture of sodium hydroxide and sodium acetate at a much lower concentration is therefore used as the eluent (see Figure below). Helium must be passed through this solution to prevent the uptake of carbon dioxide from the surrounding atmosphere. As a result of the weak eluent the background current in the flow-through measuring cell of the Bioscan drops below 20 nA, whereas the optimal working range lies between 150 and 1000 nA. In comparison with the standard eluent the detection sensitivity is also considerably lower and diminishes even further during the analysis. It is therefore necessary to work with an internal standard. In principle the problem can be solved by the post-column addition of sodium hydroxide. For this the 752 IC Pump Unit is used, its pulsation being suppressed by a combination of various capillaries. The eluent with the separated analytes and the post-column reagent (300 mmol/L sodium hydroxide) are combined directly after the separation column in a T piece and mixed in a special capillary; the liquid flow then reaches the amperometric detector cell (order number for T piece + mixing capillary: 6.2758.000). The chromatogram shown below was obtained with the system described above. The background current in the flow-through measuring cell of the Bioscan was now 192 nA. Fucose was used as internal standard for the analysis and showed an excellent recovery of 101.7%.



Determination of monosaccharides in a digestion solution of wood chips (diluted 1 : 10 with ultrapure water).

Separation column: Metrosep Carb 1 – 250 (6.1013.000)
 Eluent: 2.5 mmol/L sodium hydroxide, 0.5 mmol/L sodium acetate
 Flow rate: 1.25 mL/min
 Post-column reagent: 300 mmol/L sodium hydroxide
 Flow rate: 0.43 mL/min
 Temperature: 32 °C
 Results: 10.0 ppm fucose;
 7.6 ppm arabinose;
 20.8 ppm galactose;
 653.5 ppm glucose;
 197.7 ppm xylose;
 11.9 ppm mannose

Metrohm IC separation columns for carbohydrates

All the applications described here were carried out on the Metrosep Carb 1 – 250 column (250 mm x 4.6 mm; order no. 6.1013.000). This IC separation column is outstandingly suitable for the determination of carbohydrates using alkaline eluents and pulsed amperometric detection. A shorter version of the sugar column is also available – the Metrosep Carb 1 – 150 (150 mm x 4.0 mm; order no. 6.1013.010). Its ion exchange capacity is about half that of its «tall sister»; the retention times are correspondingly shorter. The short Metrosep Carb 1 thus guarantees rapid ion chromatographic determinations and is particularly suitable for the separation of larger sugar molecules.