There are several methods to analyze sugar by HPLC. Our previous notes showed an HPLC method using fluorescence detector coupled with post-column derivatization (No. 6) and that using RI detector (No. 91). In this note, sugar was detected with electrochemical detector (ECD). Sensitivity of this method was comparable to that of fluorescence detection although derivatization is not necessary. (C. Aoyama)

A chromatogram obtained from standard solution

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fucose 10 mg/L</td>
</tr>
<tr>
<td>2</td>
<td>Glucose 10 mg/L</td>
</tr>
<tr>
<td>3</td>
<td>Fructose 10 mg/L</td>
</tr>
<tr>
<td>4</td>
<td>Lactose 10 mg/L</td>
</tr>
<tr>
<td>5</td>
<td>Sucrose 10 mg/L</td>
</tr>
</tbody>
</table>

Conditions:

- **System**: LC800 system with ECD
- **Column**: InertSphere Sugar-1 (5 μm, 150 × 4.6 mm I.D.)
- **Eluent**: 100 mM NaOH
- **Flow rate**: 0.5 mL/min
- **Col. Temp.**: 25 °C
- **Detection**: ECD Pulse Mode (ED723, Gold)
  - E1: 150 mV t1: 600 ms
  - E2: -1500 mV t2: 50 ms
  - E3: 600 mV t3: 50 ms
  - E4: -200 mV t4: 100 ms
- **Inj. Volume**: 10 μL

* Eluent was stored in a polypropylene bottle with CO2 trap cartridge.

How are sugars separated in this method?

Sugars are ionized under strong alkaline condition. Therefore, sugars can be retained and separated on anion-exchange column using alkaline aqueous solution as mobile phase.

Calibration curves

More technical information is here...  
http://www.glsciences.com/applications.html
Detection of sugar in HPLC

Sugars cannot be detected using UV or fluorescence detector without derivatization because sugars do not possess chromophore, such as double bond or benzene ring, in their structure. On the other hand, sugar analysis using electrochemical detector (ECD) does not require any derivatization because sugars are detected by oxidation of carbohydrates on a working electrode surface and monitoring the resulting current. The sensitivity is approximately 1000-fold higher than that of refractive index (RI) detector, which can also detect sugars without derivatization. ECD has major advantages in sugar analysis.

<table>
<thead>
<tr>
<th>Detector</th>
<th>Sensitivity (approx.)</th>
<th>Cautions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrochemical detector (ECD)</td>
<td>10 ng</td>
<td>Standard HPLC pump and autosampler can be used if materials for their wetted parts are alkali-resistant.</td>
</tr>
<tr>
<td>Fluorescence detector (FL, coupled with post-column derivatization)</td>
<td>10 ng</td>
<td>Additional pump and reaction unit are required for derivatization.</td>
</tr>
<tr>
<td>Refractive index detector (RI)</td>
<td>10 μg</td>
<td>Gradient elution cannot be carried out.</td>
</tr>
<tr>
<td>Evaporative light scattering detector (ELSD)</td>
<td>1 μg</td>
<td>Non-volatile agents cannot be performed, and calibration curve is not linear.</td>
</tr>
</tbody>
</table>

Cleaning of electrode surface and pulsed-amperometric detection mode

In sugar analysis using ECD, oxidized analyte is adsorbed to the working electrode surface. It is necessary to remove analyte bound to the surface because it may diminish the sensitivity.

ED723 can be used with not only dynamic current mode (DC), in which constant potential is applied throughout an analysis, but also pulsed-amperometric detection mode (PAD). For example, potential program shown below is applied periodically. Analytes bound to working electrode surface is removed by applying strong reduction and oxidation potential after each time current is measured. Surface of working electrode can be maintained in good condition by applying PAD mode.

Applied potential in a sugar analysis

- E1: Potential for detection
- E2: Potential for reduction
- E3: Potential for oxidation
- E4: Potential for reduction
- ts: Sampling time

Cleaning of working electrode surface

Enlarged view

<table>
<thead>
<tr>
<th>Potential (mV)</th>
<th>Time (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>0</td>
</tr>
<tr>
<td>E2</td>
<td>-500</td>
</tr>
<tr>
<td>E3</td>
<td>1000</td>
</tr>
<tr>
<td>E4</td>
<td>-1500</td>
</tr>
</tbody>
</table>

Potential

- E1: Potential for detection
- E2: Potential for reduction
- E3: Potential for oxidation
- E4: Potential for reduction
- ts: Sampling time
MonoSpin is a series of spin columns for solid phase extraction (SPE). Owing to the high permeability of monolithic silica disk packed into the spin column, the procedures, such as conditioning, sample loading, washing, and elution can be carried out only by centrifuging the column. It is also the advantage that the elution volume is only 200 μL.

MonoSpin C18, which is used in this note, has octadecyl group on the surface of silica as a functional group. Sugar is not retained on MonoSpin C18 at all, whereas hydrophobic compounds are retained. Therefore, hydrophobic interfering substances can be easily removed only by passing sample solution through MonoSpin C18.

**An example of procedures**

1. **Conditioning**
2. **Adsorption**
3. **Rinsing**
4. **Elution**

**Procedures for clean-up**

- Attach the spin column to tube for waste fluid
- ↓ + Methanol 200 μL
- Centrifuge at 10,000 g for 1 min
- ↓ + Water 200 μL
- Centrifuge at 10,000 g for 1 min
- Put the spin column into collection tube
- ↓ + Serum sample 200 μL
- Centrifuge at 10,000 g for 1 min
- Collected sample was injected into HPLC system after 5-fold dilution with water

**Preparation of sample**

Serum sample: Three times volume of acetonitrile was added to the sample, and the solution was centrifuged. Obtained supernatant was loaded onto MonoSpin C18.

**A chromatogram obtained from serum sample after clean-up using MonoSpin C18**

Not only glucose, concentration of which is relatively high in serum (70-110 mg/dL during fasting state), but also fructose were successfully detected with ECD because of its high sensitivity although it is known that concentration of fructose in serum is 100-fold lower than that of glucose!
Electrochemical detector, ED723
Cat.No. 6001-72305

- Following three modes can be applied: DC mode, PAD mode, and DC scan mode.
- Oven (20～45℃) is equipped as standard feature.

HPLC column for sugar analysis, InertSphere Sugar-1
Cat.No. 5020-11001

- InertSphere Sugar-1 is an anion-exchange column packed with polymer particles. Quaternary ammonium group is chemically bonded.
- Oligosaccharides and sugar phosphates also can be retained and separated with use of gradient elution.
- InertSphere Sugar-1 can be washed with methanol.
- Cartridge guard column is also available.

Smart HPLC system, LC800
Cat.No. 6001-88010

- Binary high-pressure gradient pump, autosampler, column oven, and electrochemical detector can be contained in a single unit if ECD option is added.
- Sample cooling system of autosampler and automatic plunger rinsing mechanism of pump are equipped as standard feature.

Cat. No. 6010-92050 Polypropylene bottle (1 L) with CO₂ trap cartridge
Cat. No. 6010-92060 Polypropylene bottle (2 L) with CO₂ trap cartridge

Contact us

GL Sciences, Inc. Japan
22-1 Nishishinjuku 6-chome, Shinjuku-ku, Tokyo, 163-1130 Japan
TEL: +81-3 (5323)6620  FAX: +81-3 (5323)6621

GL Sciences, Inc. USA
4733 Torrance Blvd. Ste 255, Torrance, CA 90503
Tel: (310)265-4424  FAX (310)265-4425

Distributors Outside of Japan and USA
GL Sciences uses distributors in many countries.
You can find a local distributor in your country in the following url.
http://www.glsciences.com/distributors/
E-MAIL: world@gls.co.jp