LC Technical Note
LT101
GL Sciences Inc.

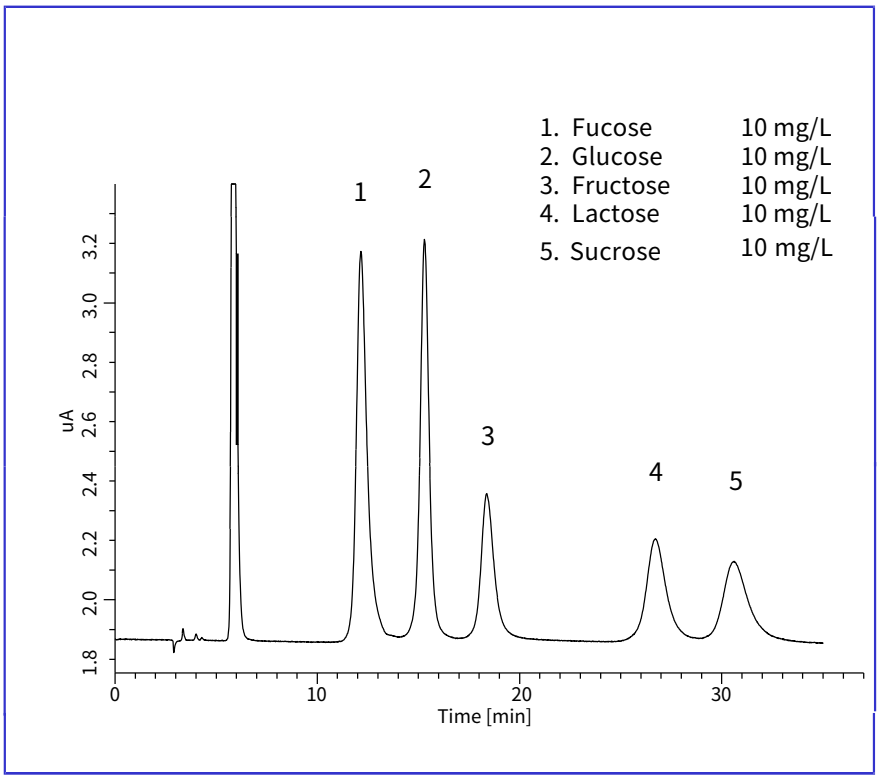
Highly Sensitive and Simple Method for Sugar Analysis Using HPLC-ECD

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



Conditions

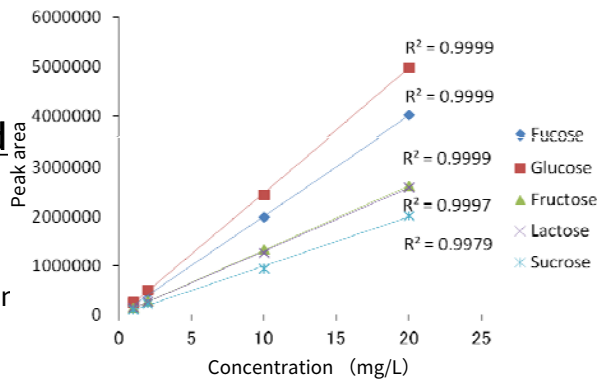
System : LC800 system with ECD
Column : InertSphere Sugar-1 (5 µm, 150×4.6 mm I.D.)
Eluent : 100 mM NaOH*
Flow rate : 0.5 mL/min
Col. Temp. : 25 °C
Detection : ECD Pulse Mode (ED723, Gold)

E1: 150 mV	t1: 600 ms
E2: -1500 mV	t2: 50 ms
E3: 600 mV	t3: 50 ms
E4: -200 mV	t4: 100 ms
	ts: 50 ms

Inj. Volume : 10 µL

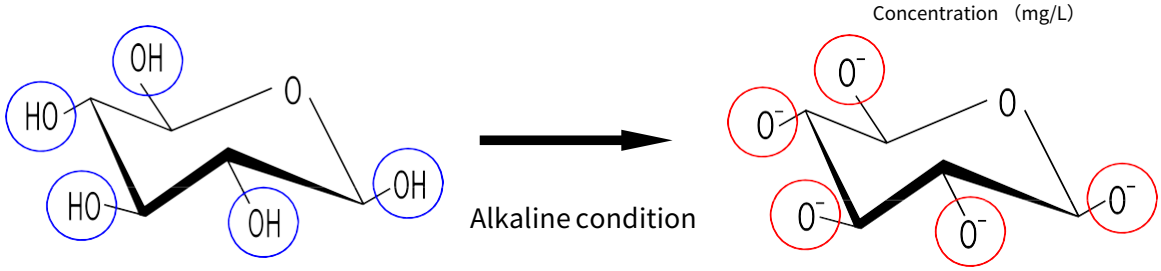
* Eluent was stored in a polypropylene bottle with CO_2 trap cartridge.

Calibration Curves



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 solutior as mobile phase.



GL Sciences

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.

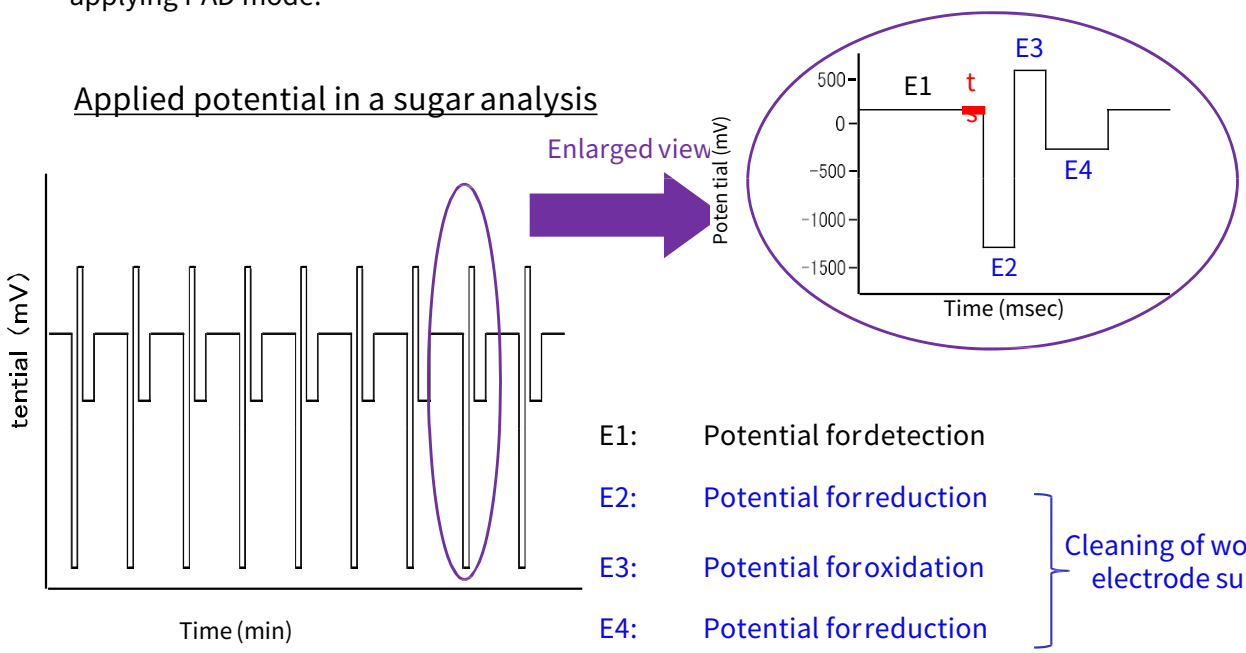
Detector	Sensitivity (approx.)	Cautions
Electrochemical detector(ECD)	10 ng	Standard HPLC pump and autosampler can be used if materials for their wetted parts are alkali-resistant.
Fluorescence detector (FL, coupled with post-column derivatization)	10 ng	Additional pump and reaction unit are required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

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

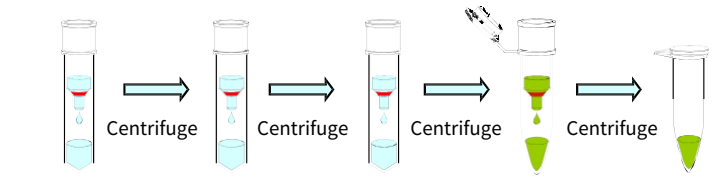
Cleaning of working electrode surface

ts: Sampling time

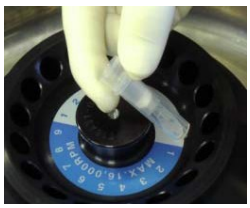
Clean-up of serum sample using MonoSpin C18

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.

Example of procedures

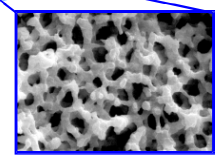


Conditioning Adsorption Rinsing Elution



MonoSpin

Monolithic silica disk



Enlarged view of monolithic silica

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.

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

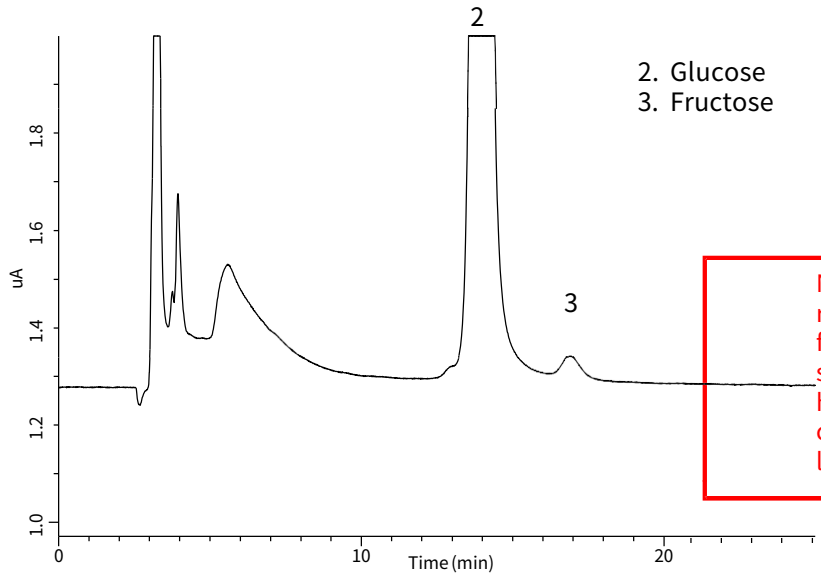
Put the spin column into collection tube

↓ + 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

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!

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.

GL Sciences Inc. Japan
22-1 Nishishinjuku 6-chome
Shinjuku-ku, Tokyo
163-1130, Japan

Phone: +81-3-5323-6620
Fax: +81-3-5323-6621
Email: world@gls.co.jp
Web: www.glsciences.com

GL Sciences Inc. USA
4733 Torrance Blvd. Suite 255
Torrance, CA 90503
USA

Phone: +1-310-265-4424
Fax: +1-310-265-4425
Email: info@glsciencesinc.com
Web: www.glsciencesinc.com

GL Sciences B.V.
Dillenburgstraat 7C
5652AM, Eindhoven
The Netherlands

Phone: +31-40-254-9531
Email: info@glsciences.eu
Web: www.glsciences.eu

GL Sciences (Shanghai) Limited
Tower B, Room 2003
Far East International Plaza
No.317 Xianxia Road, Changning District
Shanghai, China 200051

Phone: +86-21-62782272
Email: contact@glsciences.com.cn
Web: www.glsciences.com.cn

GL Sciences

International Distributors

Visit our Website at www.glsciences.com/distributors