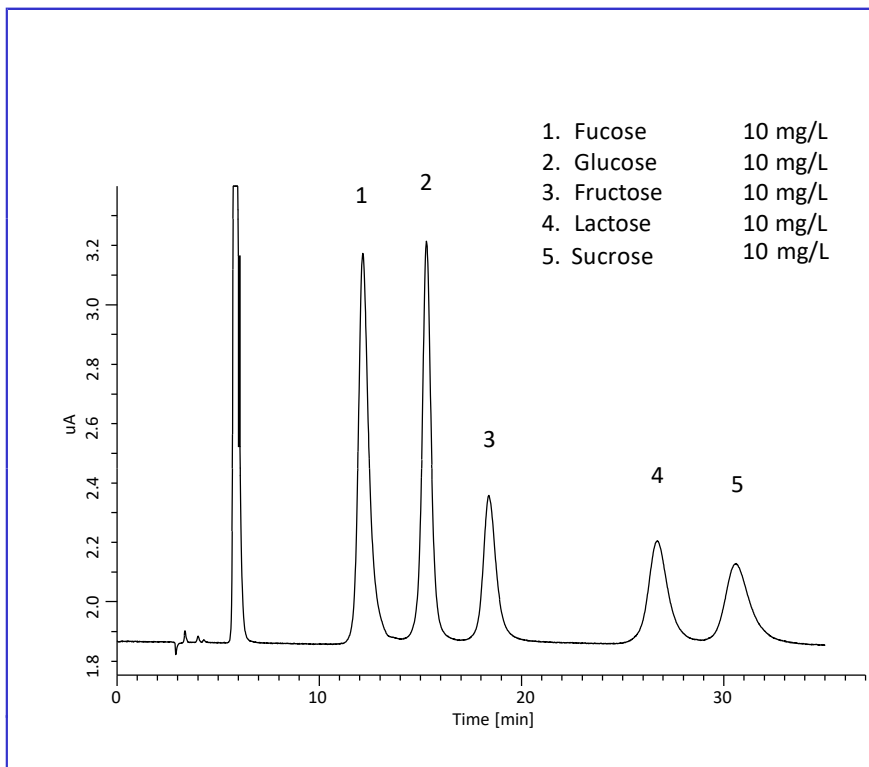


Highly Sensitive and Simple Method for Sugar Analysis Using HPLC-ECD

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

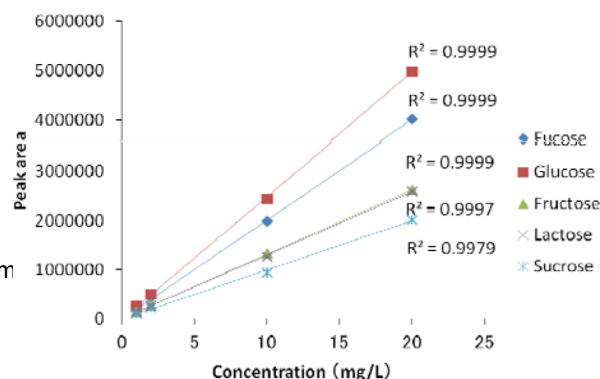


Conditions

System	: LC800 system with ECD
Column	: InertSphere Sugar-1 (5 μ m, 150 \times 4.6 mm I.D.)
Eluent Flow rate	: 100 mM NaOH*
Col.	: 0.5 mL/min
Temp.	: 25 $^{\circ}$ 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 μ L

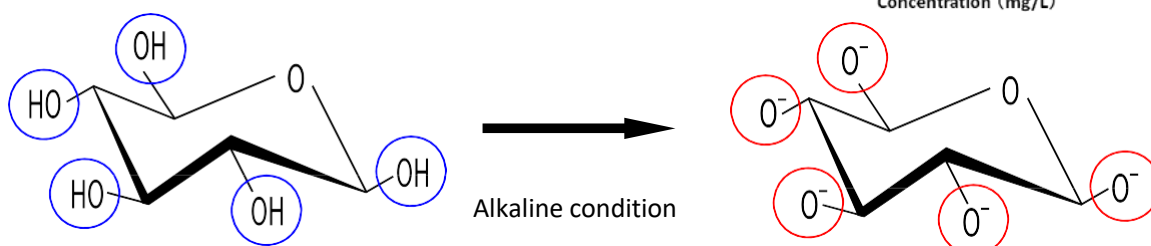
* Eluent was stored in a polypropylene bottle with CO₂ trap cartridge.

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 solution as mobile phase.



Detection of sugar in HPLC

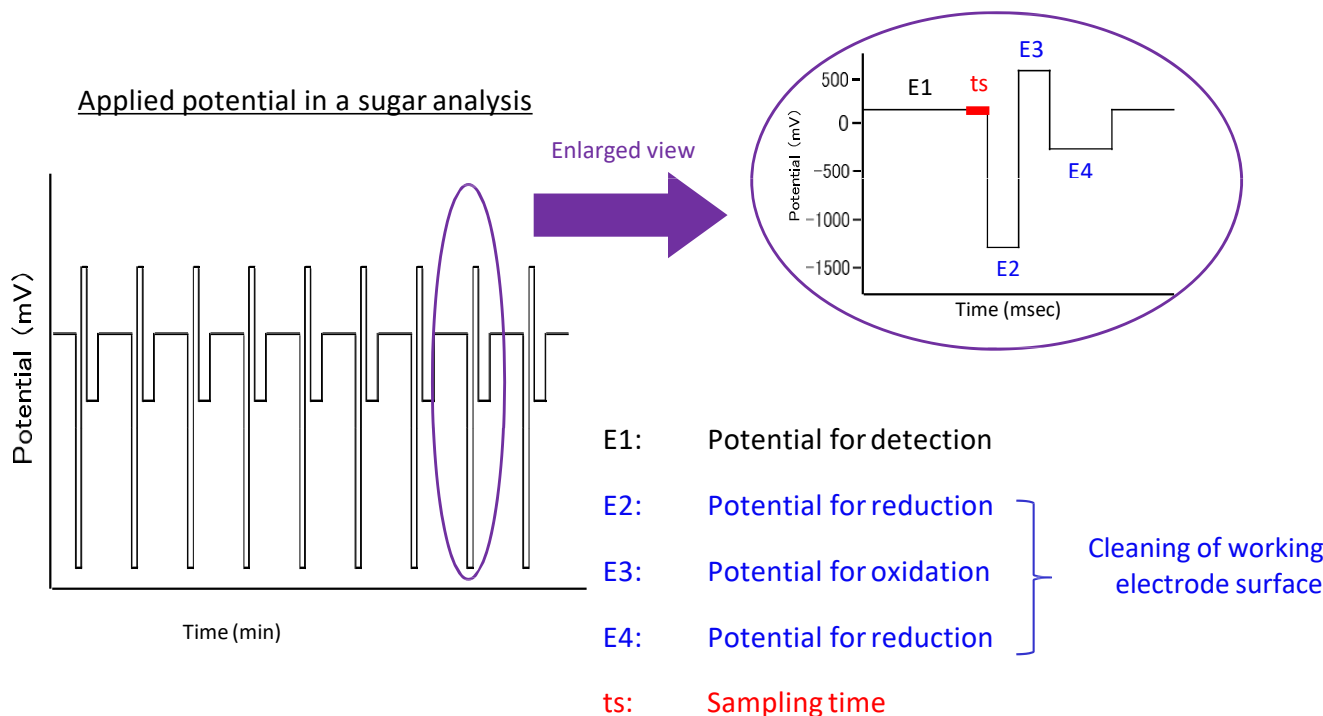
Sugars cannot be detected using UV or fluorescence detector without derivatization because sugars do not possess chromophore, such as double bond or benzene ring, in their structure. On the other hand, sugar analysis using electrochemical detector (ECD) does not require any derivatization because sugars 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.

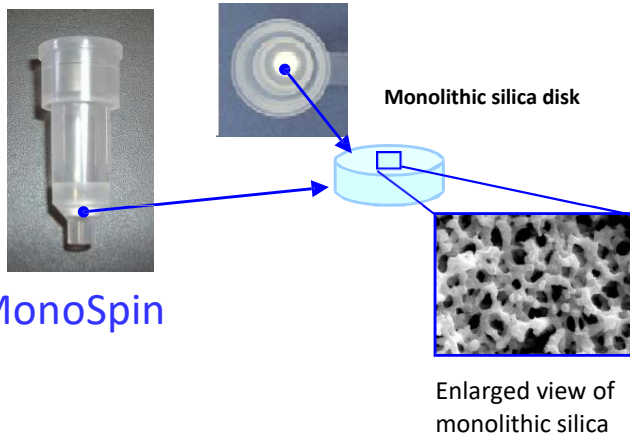
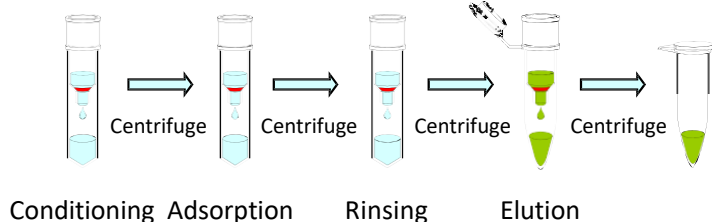


Clean-up of serum sample using MonoSpin C18

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



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

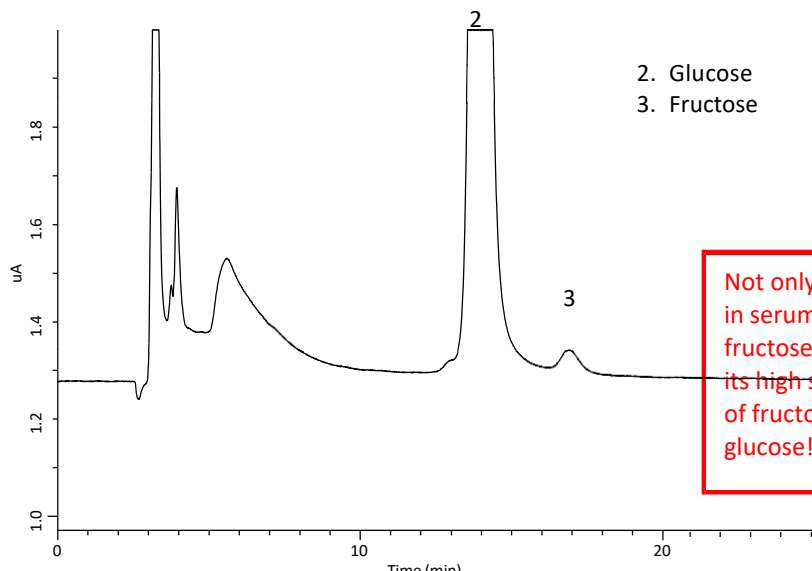
Centrifuge at 10,000 g for 1 min

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.

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