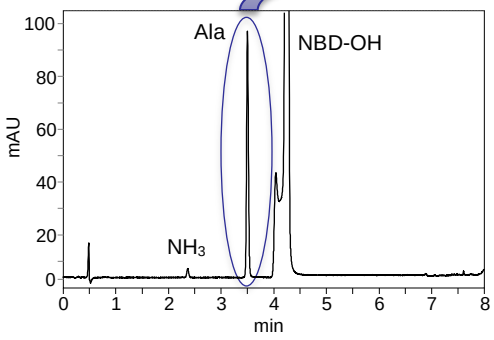
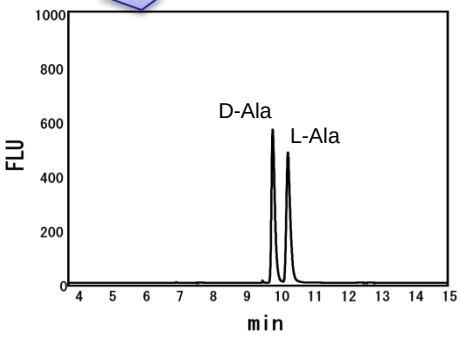
■ Chiral Amino Acid Analysis by Column Switching

In recent years, D-amino acid found in mammals and food is drawing attention and the studies regarding its relationship with diseases and food functionalities are being conducted. There are multiple techniques for the chiral analysis to separate the D-amino acid and L-amino acid, and the known methods include the HPLC analysis method after chiral derivatization, direct separation method with a chiral column, capillary electrophoresis method, and enzymatic method.
This time, the example of the chiral amino acid analysis by a chiral column with the application of the column switching is introduced here. In this method, amino acid is derivatized with NBD-F reagent first and then, high-speed analysis with an UHPLC and an UHPLC column (1.8 µm) is performed. After confirming the elution of the target amino acid, the valve is switched at the time of its introduction to the loop. The D-amino acid and L-amino acid are separated by the chiral column and the fluorescence detection is performed. In this analysis, DL-alanine (DL-Ala) approved as a food additive was chosen as the target amino acid. The analysis examples for various food products are also introduced.

■ Analysis Example of DL-Alanine Standard Sample (Primary and Secondary Chiral Analysis)



[Primary Analysis:
Chromatogram by UV Detector]

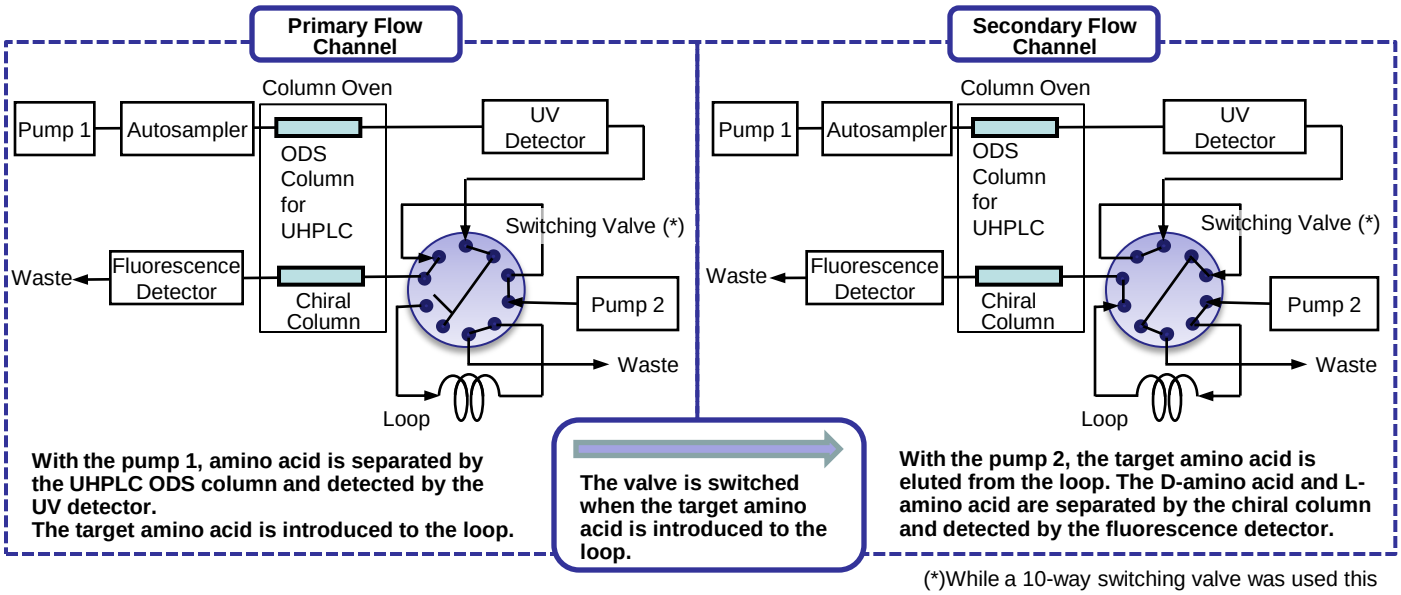


[Secondary Analysis:
Chromatogram by Fluorescence Detector]

<Derivatization Procedure>
Amino acid standard sample (20 µL)
← Buffer (*) (160 µL)
← NBD-F reagent (*) (20 µL)
(Dissolve with eluent (*))
Derivatization 60 °C , 1 min
← Neutralizing solution (*) (800 µL)
Analytical sample

(*)For ultra high-speed amino acid analysis

■ Flow Channels and Analytical Conditions



**With the pump 1, amino acid is separated by the UHPLC ODS column and detected by the UV detector.
The target amino acid is introduced to the loop.**

The valve is switched when the target amino acid is introduced to the loop.

With the pump 2, the target amino acid is eluted from the loop. The D-amino acid and L-amino acid are separated by the chiral column and detected by the fluorescence detector.

(*)While a 10-way switching valve was used this time, a 6-way switching valve can also be used.

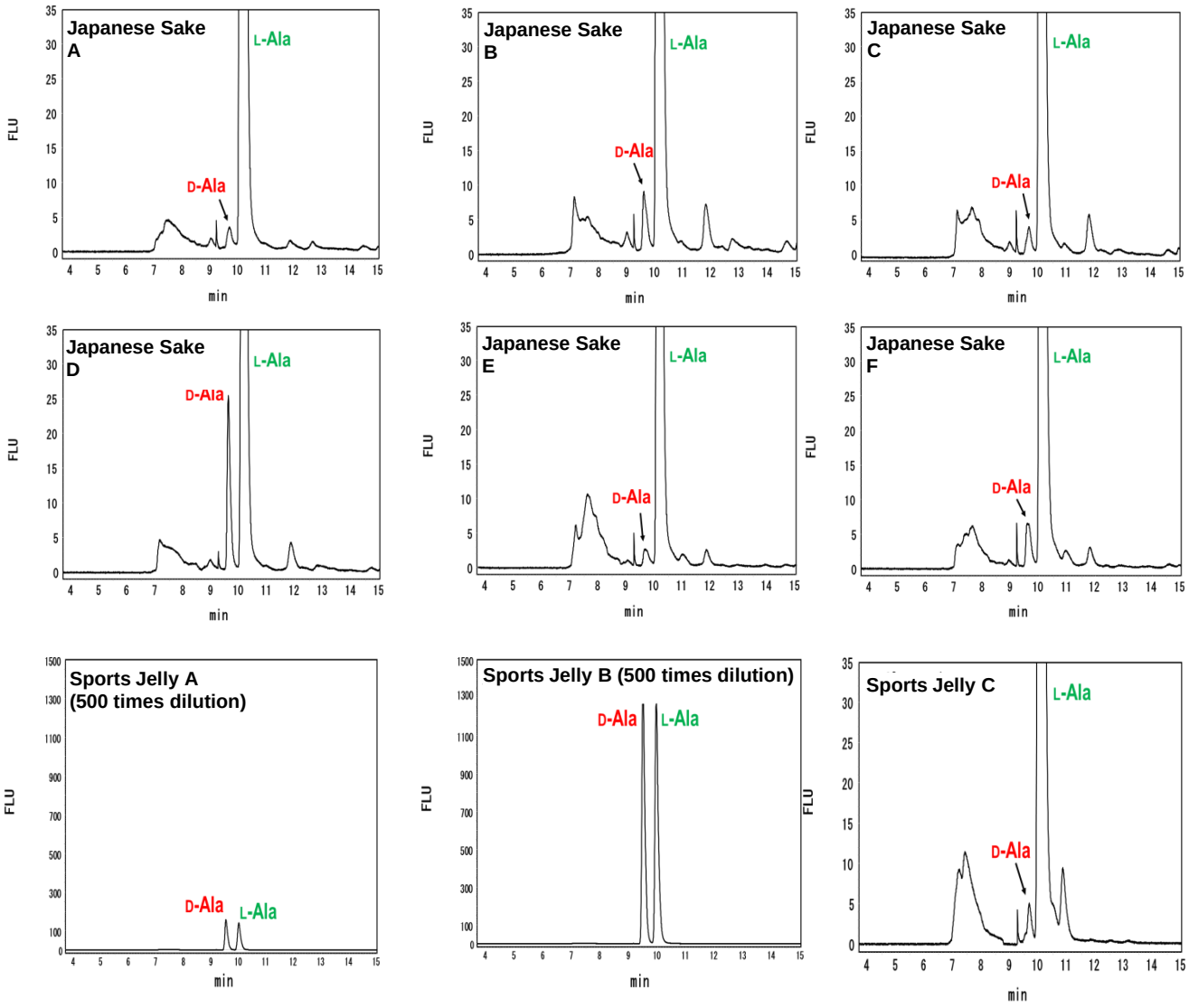
<Primary Analytical Conditions>
Column : Analytical column for ultra high-speed amino acid analysis (1.8 µm)
3.0 mm I.D.×50 mm
Eluents : Eluents A, B for ultra high-speed amino acid analysis
Gradient elution
Flow rate : 0.55 mL/min
Column temperature : 37 °C
Detection wavelength : VIS470 nm
Injection vol. : 10 µL

<Secondary Analytical Conditions>
Column : CHIRALPAK ZWIX (+) (3 µm)
4.0 mm I.D. × 250 mm (Daicel Corporation)
Eluents : Contains 50 mM formic acid and 25 mM diethylamine
Acetonitrile/Methanol/Water = 49/49/2
Flow rate : 0.8 mL/min
Column temperature : 37 °C
Detection wavelength: FL Ex 470 nm Em 540 nm

Primarily, an UHPLC and an UHPLC column (1.8 µm) were used to elute alanine at about 3.5 minutes and the analysis time was 8 minutes. At the time of the introduction of the eluted alanine to the loop, the valve was switched. Alanine was then introduced to the chiral column and the separation of the D-alanine and L-alanine was achieved.

■Chiral Amino Acid Analysis by Column Switching

■Analysis Examples of D-Alanine and L-Alanine in Various Food Products (Secondary Chiral Analysis)



<Sample Preparation>

Sample
↓ ← Dilute to 10 times with 0.02N hydrochloric acid (*)
Centrifuge 10000 rpm, 15 min
↓
Supernatant
↓
Derivatization (Refer to the procedure in P1)
↓
Analytical sample (10 µL injection)

(*) When the dilution factor is different, it is described separately.

D-Ala and L-Ala were detected from sake, sports jelly, and nutritional products. By employing the column switching, the online process from the high-speed amino acid analysis to the chiral separation was possible.

* This analysis was conducted as a part of a collaboratively with Keio University Faculty of Pharmacy, Division of Physical Pharmaceutical Chemistry

Main system configuration: LaChromUltra (L-2160U Pump, L-2200U Autosample, L-2300 Column Oven, L-2400U UV Detector, L-2485U Fluorescence Detector)
LaChrom Elite (L-2130 Pump), Ultra High-speed Amino Acid Analysis Kit, Chiral Column, Switching Valve, Tube, etc.

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.