

Vitamin A is a micronutrient found in foods, in addition there are other compounds that have vitamin A activity; retinols such as alcoholic retinol and esterified palmitate retinol, carotenoids such as  $\beta$ -carotene and cryptoxanthin.

See Technical Note No. 28 for An Analysis of Carotenes.

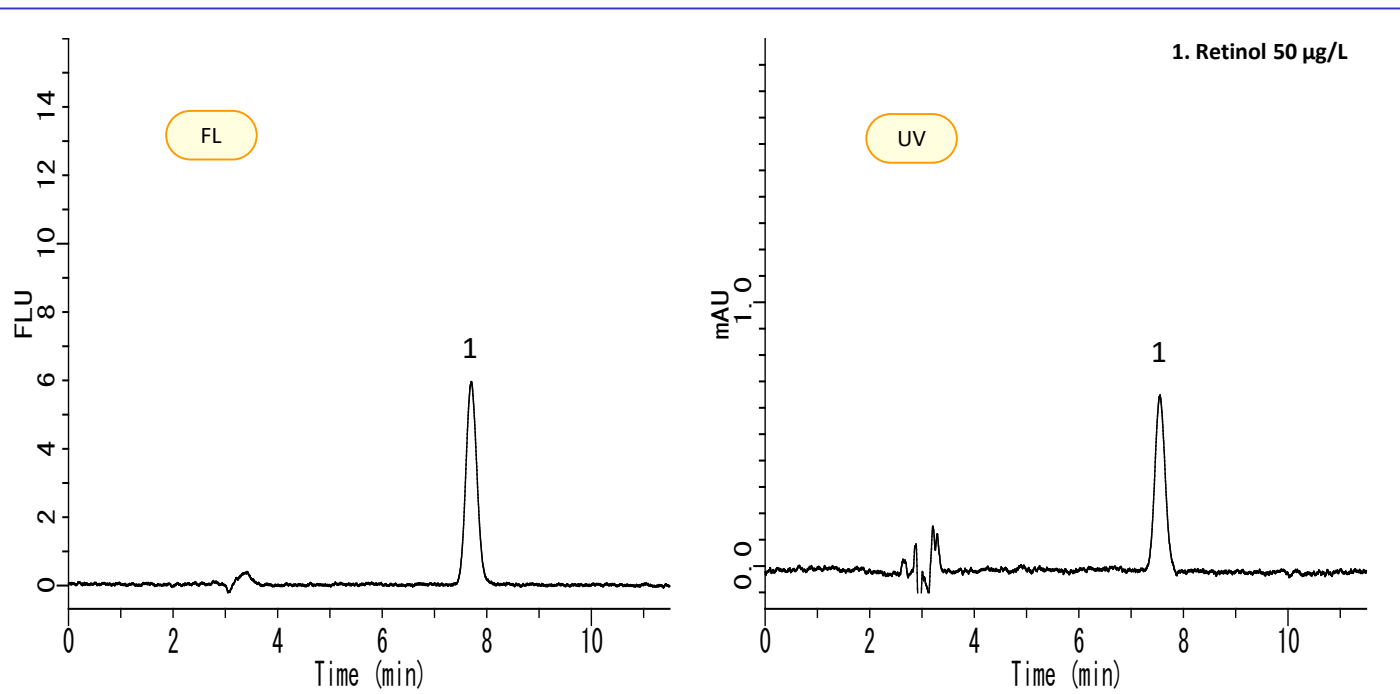
In this report, retinol was analyzed as an ingredient in accordance with the Food Sanitation Inspection Guidelines.

Retinols in samples were saponified and hydrolyzed to retinol. A liquid-liquid extraction was then performed, the concentrated sample was injected onto an HPLC with UV and fluorescence (FL) detectors.

The analysis of vitamin A in foods, as shown in the following pages, can be performed well with UV detection. However, FL detectors are more sensitive for samples with trace amounts of vitamin A.

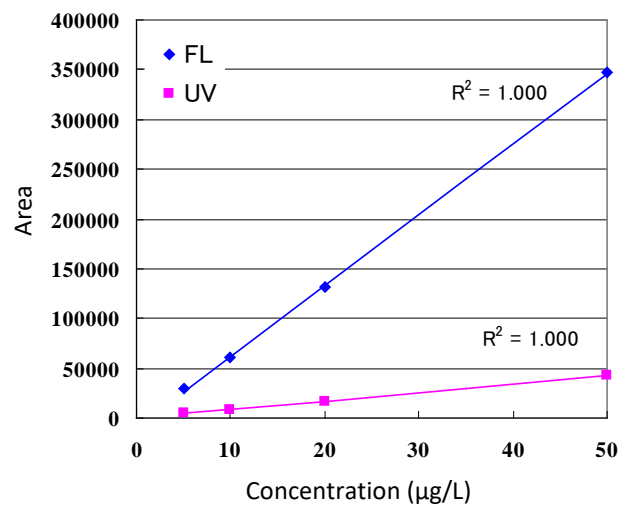
(K.Suzuki)

### Example: Measurement of Standard



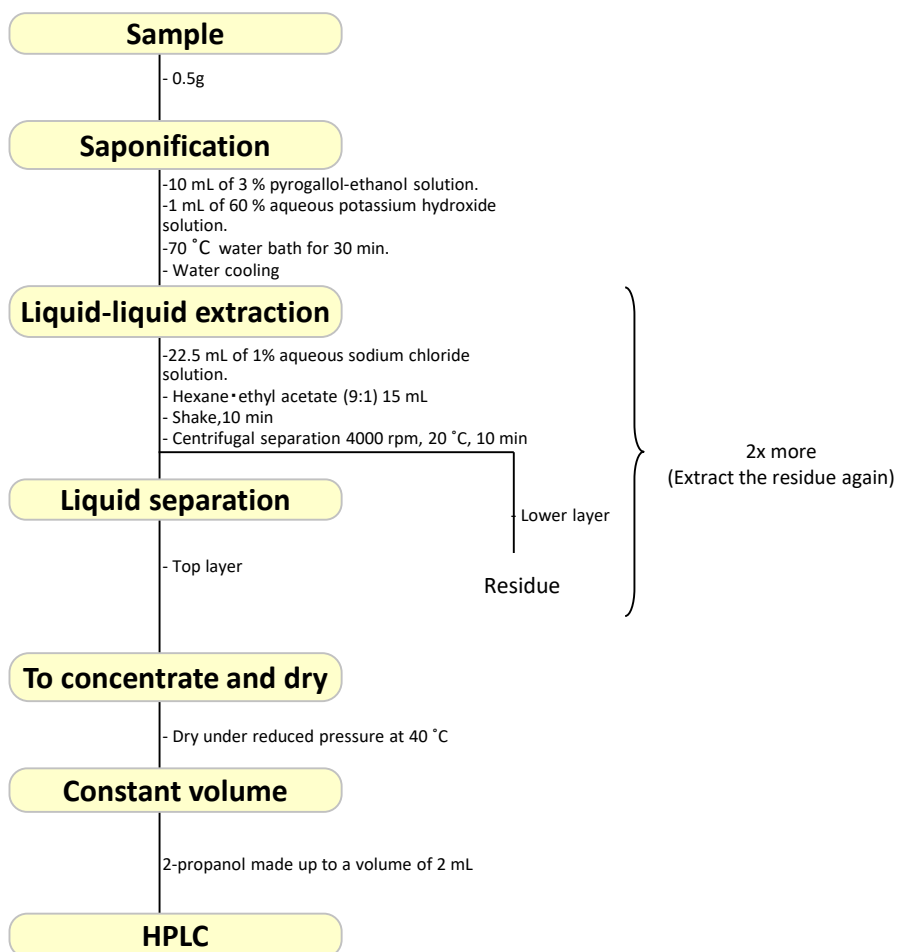
#### HPLC conditions

|                           |   |
|---------------------------|---|
| <b>Column</b>             | : Inertsil ODS-3<br>(5 $\mu\text{m}$ , 250 x 4.6 mm I.D.)                 |
| <b>Eluent</b>             | : A) $\text{CH}_3\text{OH}$<br>B) $\text{H}_2\text{O}$<br>A/B = 95/5, v/v |
| <b>Flow rate</b>          | : 1.0 mL/min  |
| <b>Column temperature</b> | : 40 $^\circ\text{C}$   |
| <b>Detected</b>           | : UV 325 nm<br>FL Ex 340 nm Em 460 nm                                     |
| <b>Injection volume</b>   | : 20 $\mu\text{L}$  |



Calibration curve for retinol

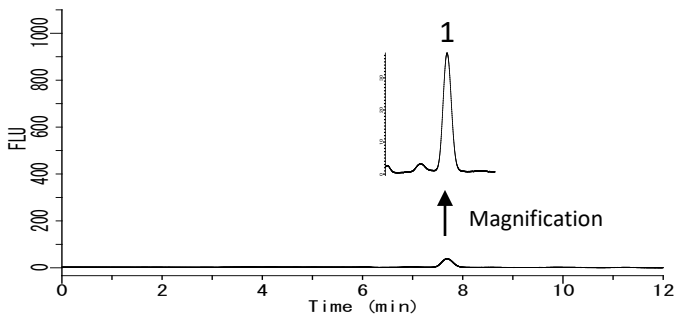
## Example of pretreatment



## Example: Sample Measurement

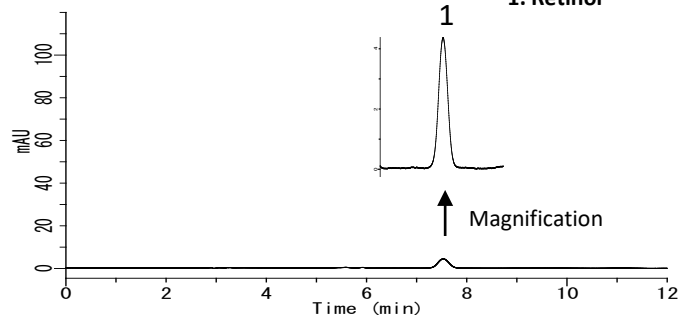
FL

### Powdered milk

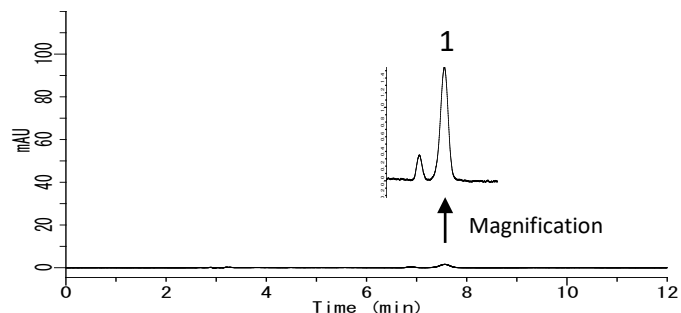
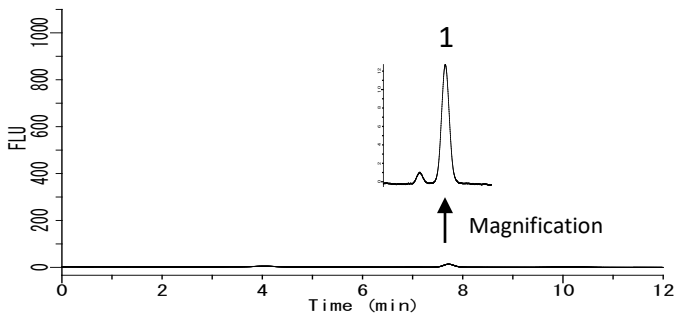


UV

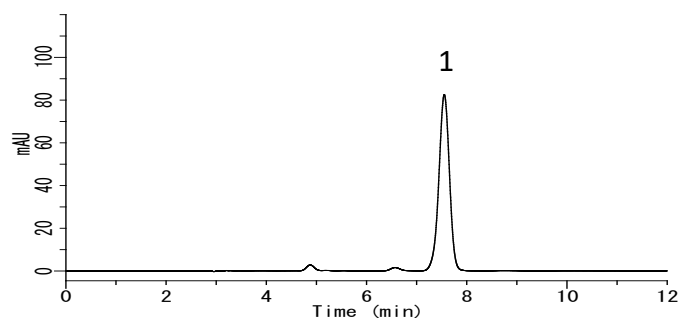
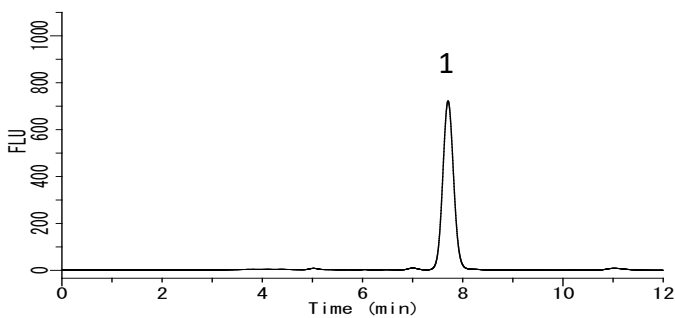
### 1. Retinol



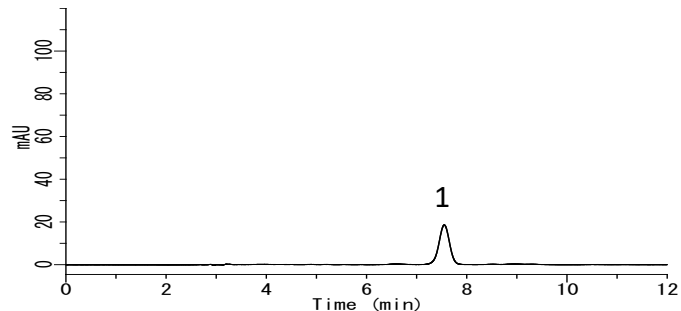
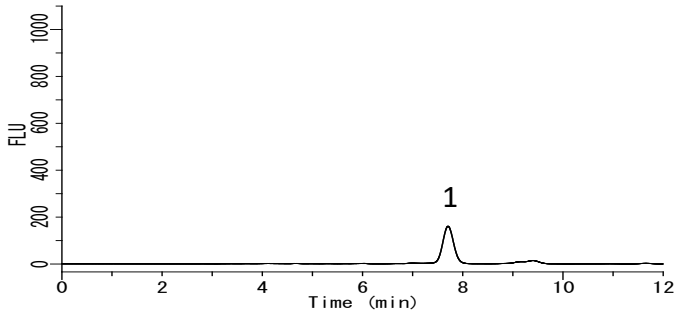
### Chicken



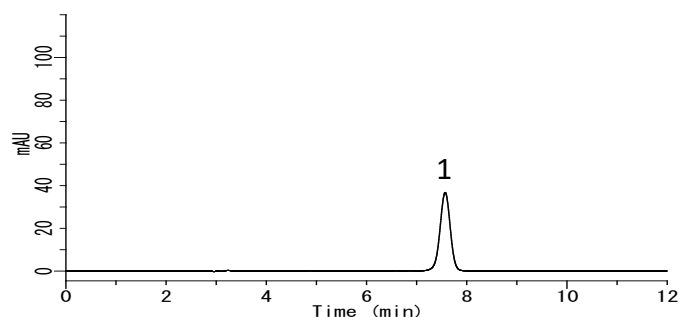
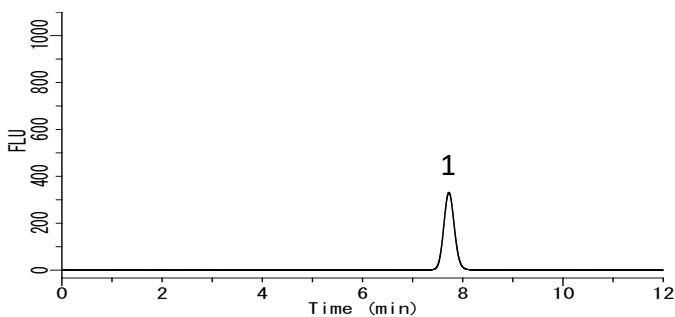
### Broiled eel



### Butter

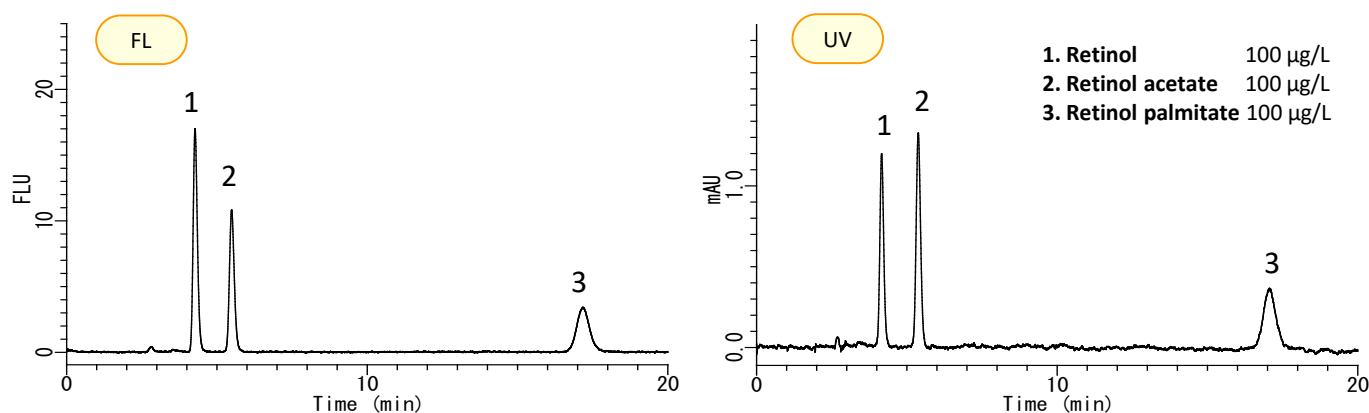


### Pork (liver)



## Example: Measurement of Standard

It has been reported that the retinol can be separated and detected by extracting and analyzing samples without saponification.



### HPLC conditions

**Columns** : Inertsil ODS-3  
(5 µm, 250 x 4.6 mm I.D.)

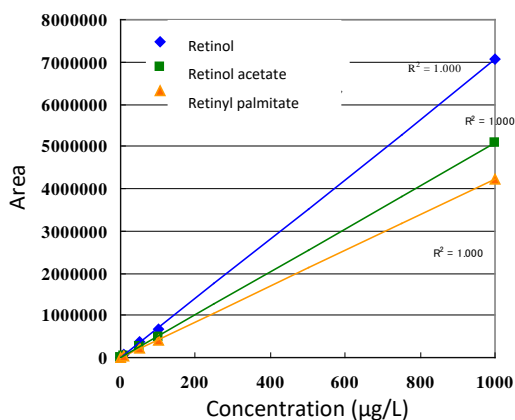
**Eluent** : A) C<sub>2</sub>H<sub>5</sub>OH  
B) H<sub>2</sub>O  
A/B = 95/5, v/v

**Flow rate** : 1.0 mL/min.

**Column temperature** : 40 °C

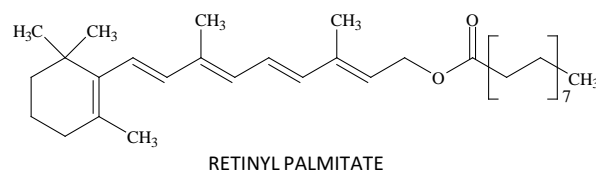
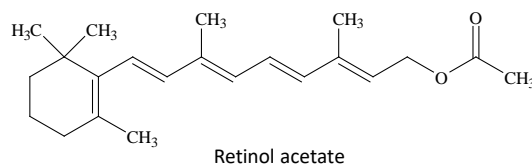
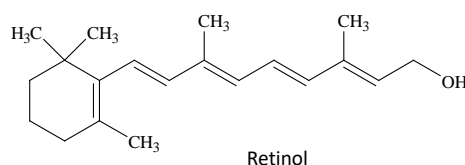
**Detected** : UV 325 nm  
FL Ex 340 nm Em 460 nm

**Injection volume** : 20 µL



Example of a calibration curve using a fluorescence detector.

### Structural formula



Structures are created using Chemistry 4-D Draw provided by ChemInnovation Software, Inc.

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