

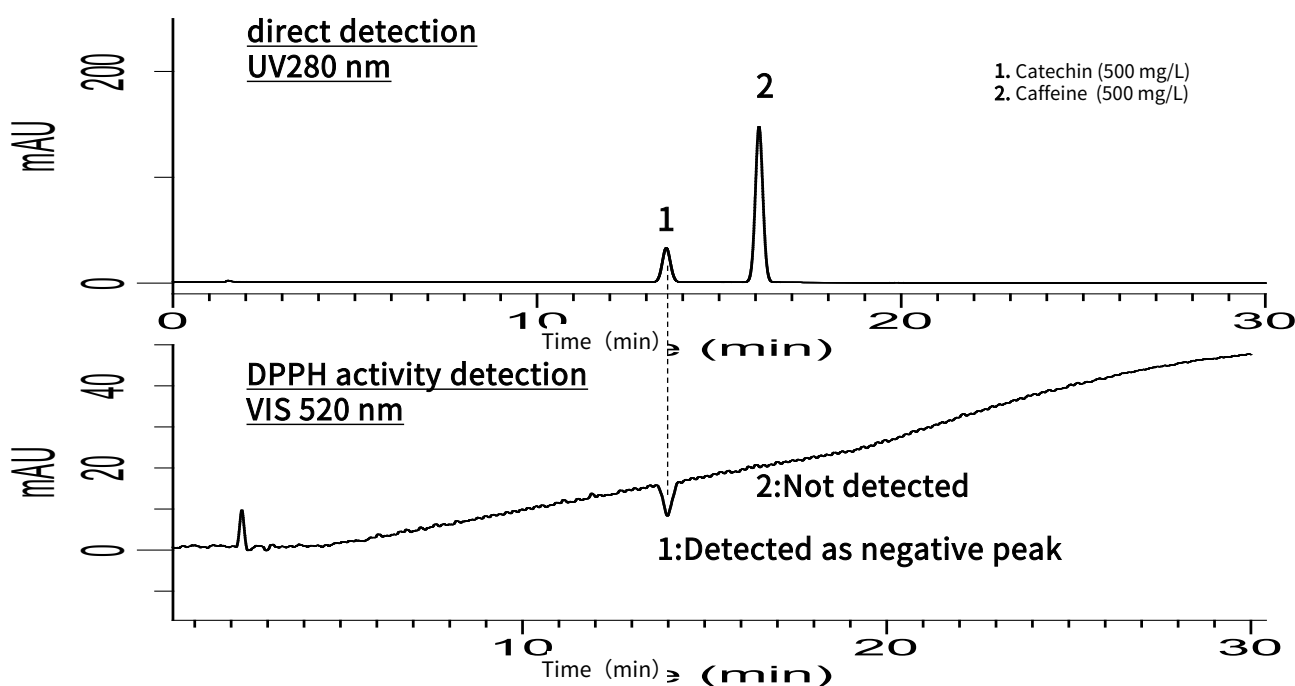
# Analysis of Antioxidant capacity by post-column HPLC using DPPH radical

We will introduce an example of the antioxidant capacity measurement of food using post-column HPLC using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH), which is known as a stable radical. DPPH is known as a purple stable radical and becomes colorless when reduced by antioxidants. DPPH radical scavenging activity is known as an index of antioxidant substances that utilize this reaction. By measuring this, it is possible to relatively evaluate the antioxidant capacity. This time, we performed measurements together with UV direct detection, so we will introduce them. There is a known method of calculating by converting to the amount of Trolox, but we will omit it here.

(K. Suzuki)

## Example of standard solution analysis

It can be confirmed that catechin has DPPH activity. On the other hand, we can see that the caffeine peak has very low DPPH activity despite having a higher UV absorption.



### HPLC Condition

**Column** : InertSustain C18  
(5 μm, 150 × 4.6 mm I.D.)

**Cat. No.** : 5020-07345

**Eluent** : A) 0.1% H<sub>3</sub>PO<sub>4</sub> in H<sub>2</sub>O\*  
B) CH<sub>3</sub>OH/CH<sub>3</sub>CN = 9/1, v/v

Time(min)	A (vol%)	B (vol%)
0.0	90	10
15.0	80	20
30.0	60	40
30.1	90	10
40.0	90	10

\*1 mL of special grade phosphoric acid (85%) added to 1 L of radical ultrapure water

**Flow rate** : 1.0 mL/min

**Col.Temp.** : 40 °C

**Detection** : UV 280 nm (UV)  
VIS 520 nm (DAD)

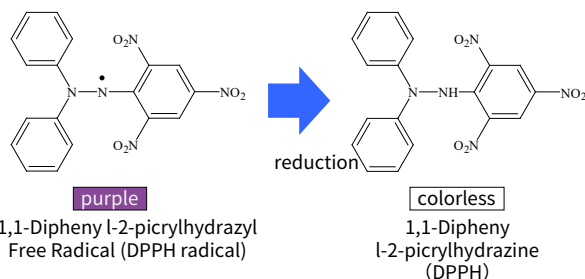
**Inj.Vol.** : 10 μL

**Reaction solution** : 50 mg/L DPPH radical in MeOH

**Reaction flow rate** : 0.5 mL/min

**Reaction temperature** : 40 °C

### Structure

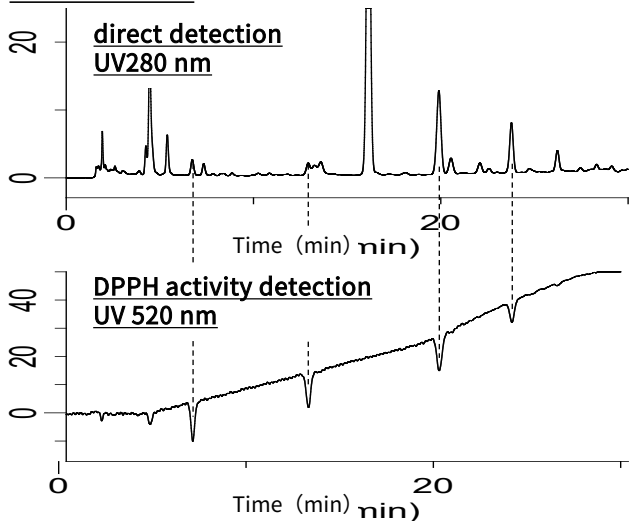


Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

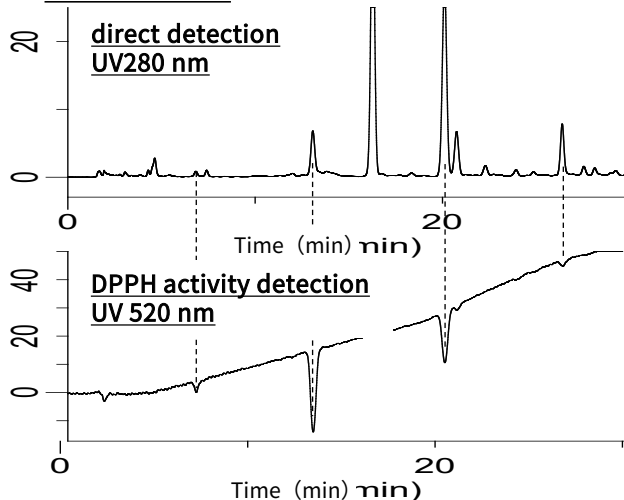
## Analysis example

Using LC Technical Note No. 145 as a reference, two types of tea were passed through a 0.45  $\mu\text{m}$  GL chromatodisk and measured.

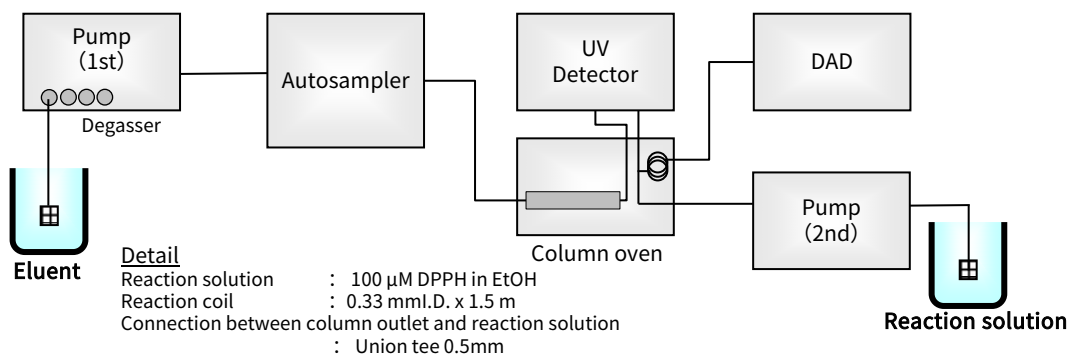
### Green Tea A



### Green Tea B



### Flow diagram



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