

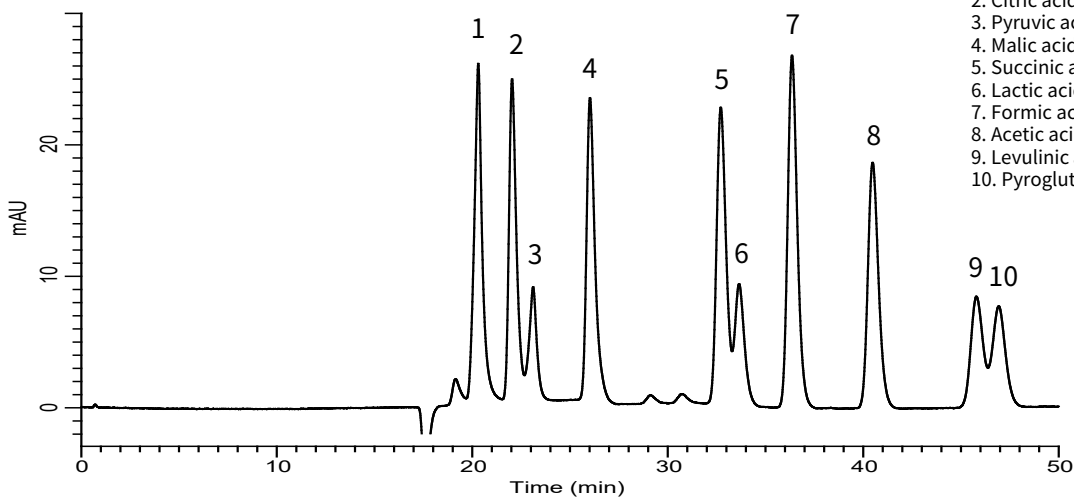
# Analysis of Organic Acid Using InertSphere FA-1 - A Postcolumn BTB Method

Organic acids are used as sour flavorings, antioxidants, and antibacterial agents in foods, and their properties are attracting significant attention. In this report, InertSphere FA-1 was used in the analysis of organic acids and separation was made using ion exclusion mode; which is the most commonly used method for organic acid analysis. The sulfonic acid group on the column repels organic acids ionically and elution is made in order from the smallest pKa.

In addition, a post-column method using BTB was used, which has high selectivity and is resistant to contaminants. A simple pretreatment method with detection using a visible absorption wavelength (440 nm) offers high-precision analysis with fewer interfering peaks due to the presence of contaminants.

(Y. Yui)

## Example: Measurement of standards

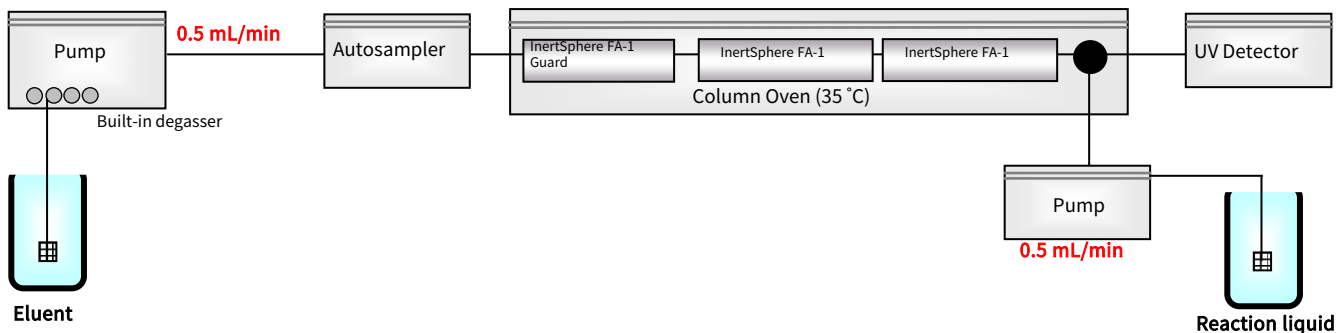


1. Phosphoric acid (phosphate)
2. Citric acid (citric acid)
3. Pyruvic acid (pyruvate)
4. Malic acid (malate)
5. Succinic acid (succinate)
6. Lactic acid (lactic acid)
7. Formic acid (formic acid)
8. Acetic acid (acetic acid)
9. Levulinic acid (levulinic acid)
10. Pyroglutamic acid (pyroglutamic acid)  
(1 mg/mL each)

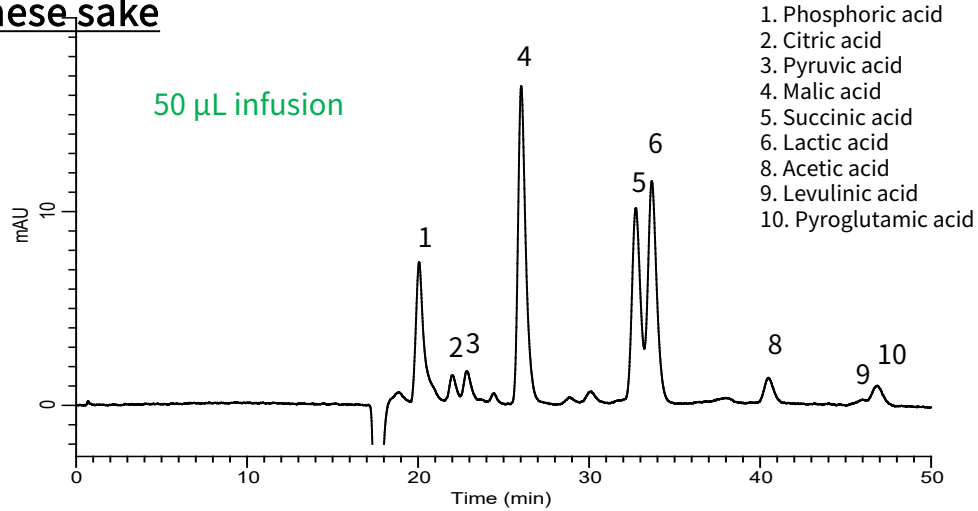
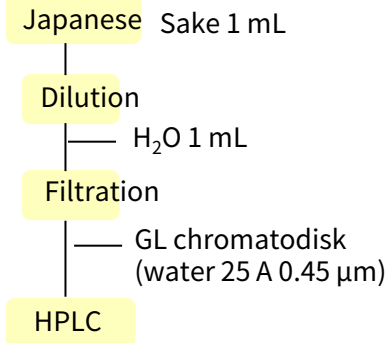
### HPLC conditions

Mobile phase	: 3 mM HClO <sub>4</sub>
Reaction liquid	: 0.1 mM BTB + 30 mM Na <sub>2</sub> HPO <sub>4</sub>
Guard column	: InertSphere FA-1 Guard (9 μm, 5.0 mm I. D. × 300 mm)
Column	: InertSphere FA-1 (9 μm, 300 mm I. D. × 7.8 mm I. D.) <b>two-stranded ligation</b>
Flow rate of mobile phase	: 0.5 mL/min
Flow rate of reagent	: 0.5 mL/min
Column temperature	: 35 °C
Detected	: VIS 440 nm
Injection volume	: 10 μL

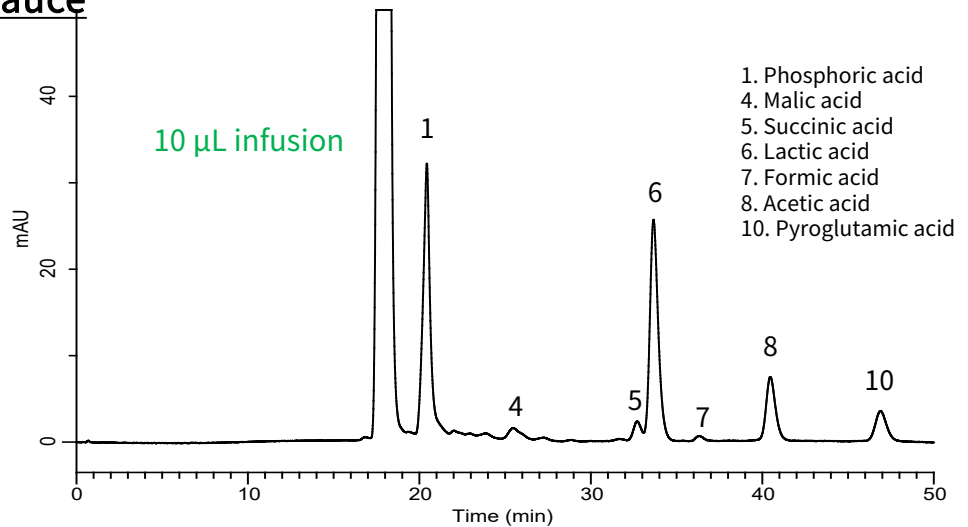
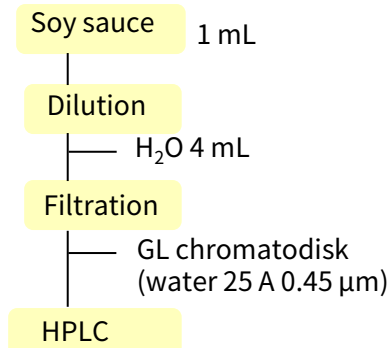
## Flow diagram



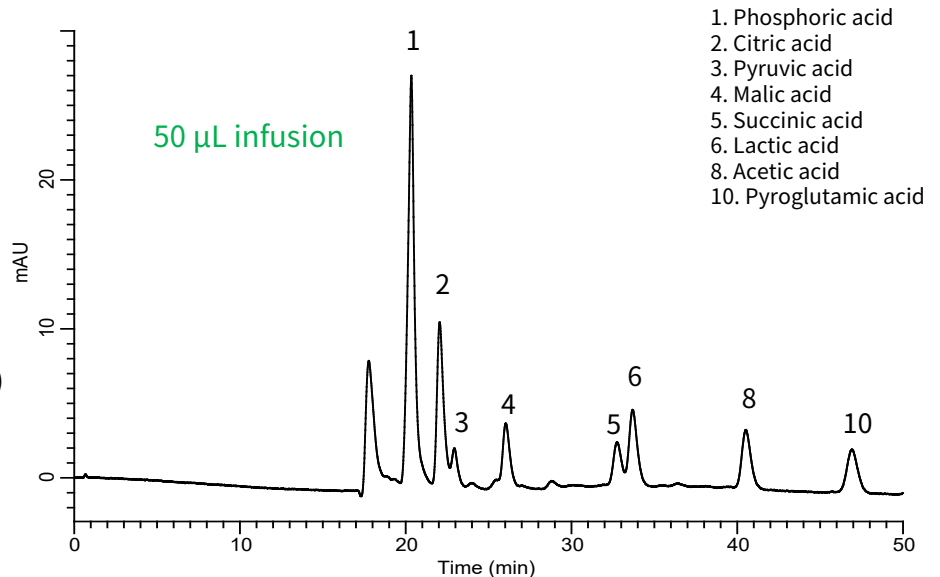
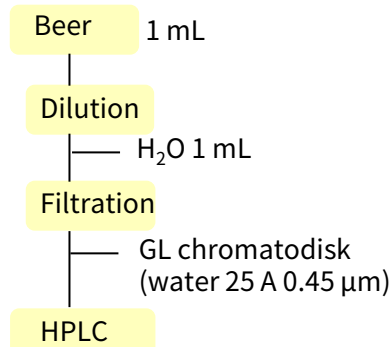
## Examples of pretreatment and analysis of organic acids in Japanese sake



## Examples of Pretreatment and Analysis of Organic Acids in Soy Sauce



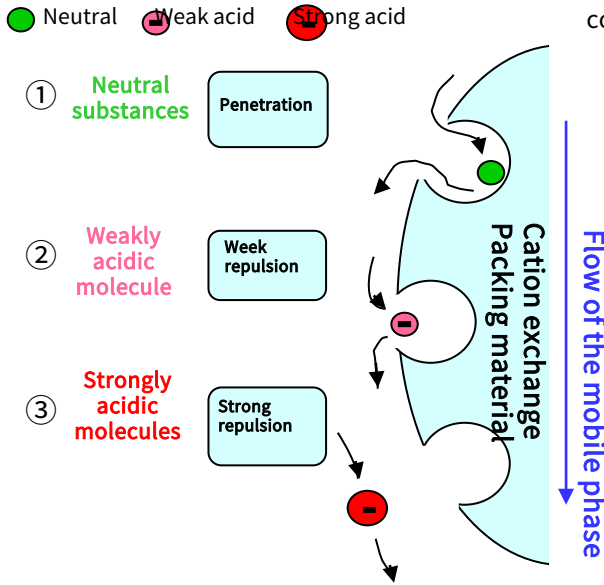
## Examples of pretreatment and analysis of organic acids in beer



\*The assay conditions for each application are the same as the analysis of the standard example on the previous page, only the injection volume is changed.

## What is ion exclusion mode?

Ion exclusion mode is a method to perform separation by ionic exchange group of the packing materials and strength of repulsive force between ion of target component.



### Determinants of separation time

1. [Penetration into the pores]  
The penetration power into the pore is determined by the size of the charge (pKa value)

Higher pKa = greater penetration

2. [Electrostatic exclusion due to the negative charge of the packing material]  
Molecules with a large negative charge (small pKa) and are subject to large electrostatic exclusion and elute more quickly.

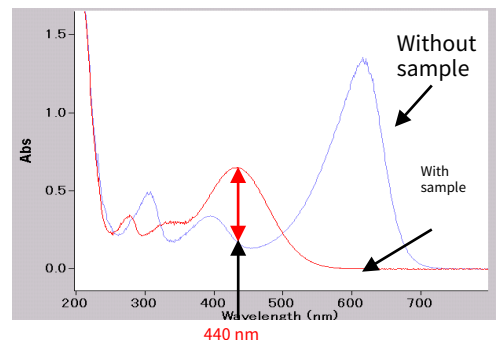
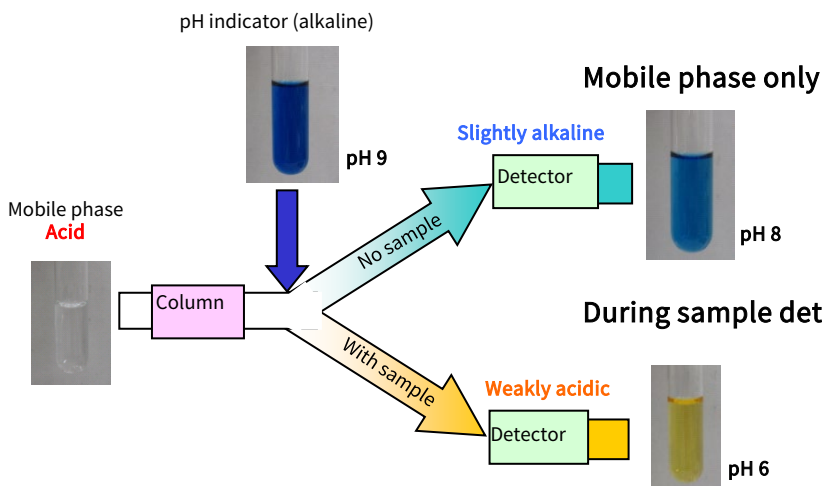
Small pKa = fast elution

Because molecules are separated by ionic strength, the stronger the anionic strength, the faster the elution.

Elution order

## With the BTB (bromothymol blue) method

Because detection using the BTB method is made using a visible wavelength at 440 nm, even samples such as crops and dressings that contain many contaminants can be analyzed. A highly selective assay is available. (see Technical note No. 1)



Absorption spectrum

The use of BTB reagent results in low absorption at 440 nm for weakly alkaline mobile phase only and a high absorption for weakly acidic samples. This difference is used to detect the peaks of interest.

## Column

Analytical column: InertSphere FA-1 9  $\mu\text{m}$ , 300 x 7.8 mm I.D.  
Cat.No. 5020-11003

Guard column: InertSphere FA-1 Guard 9  $\mu\text{m}$ , 50 x 6.0 mm I. D.  
Cat.No. 5020-10998

- Base Material : Styrene-divinylbenzene-based polymer
- Particle size : 9  $\mu\text{m}$
- Functional Group : Sulfonic acid group
- Counter-ion : H<sup>+</sup>
- Degree of linking : 8 %
- USP code : L17



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