LT001
GL Sciences Inc.

# HPLC Analysis of Organic Acids in Food (Ion Exchange Mode)

The organic acids found in foods are an important constituent for taste, such as sourness and umami. Recently, organic acids have become of interest in areas such as promoting digestion and antibacterial efficacy. Organic acids are also added to some foodstuffs. For these reasons, the analysis of organic acids in foodstuffs is useful for quality control, research and development and quantifying the amount of added organic acids.

## Advantages of the post-column method

Analyses of organic acids can be made using one of three methods: direct measurement with absorbance detection at UV 210 nm, conductivity, and pH-indicator. Direct measurement using UV 210 nm is not recommended because the target organic acids may be obscured by contaminants, nor is it selective.

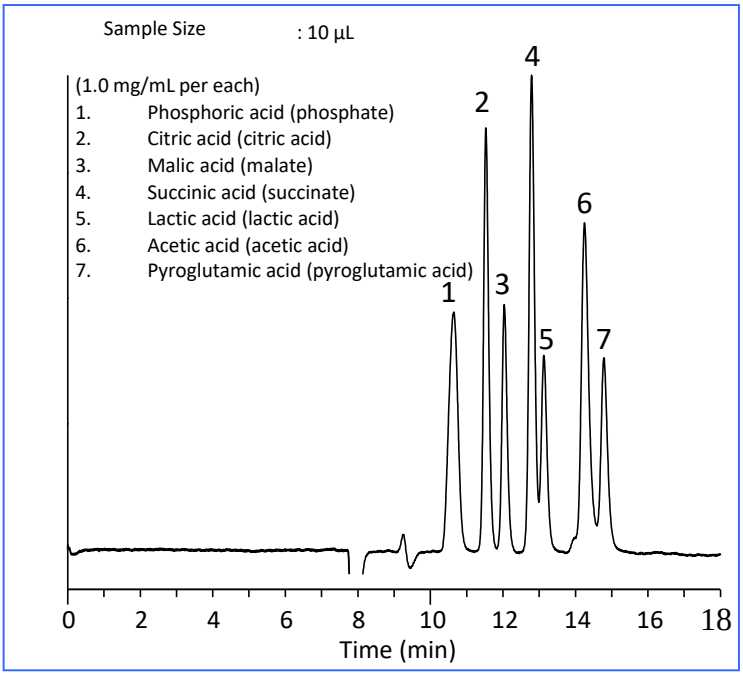
The pH indicator method using BTB (bromothymol blue) is preferable as it allows selective analysis. This is because the post-column treatment method detects the absorption of compounds at visible wavelengths.

The system described in this application note enables high-precision quantitative analysis with low interference from peaks due to contaminants with simple pretreatment.

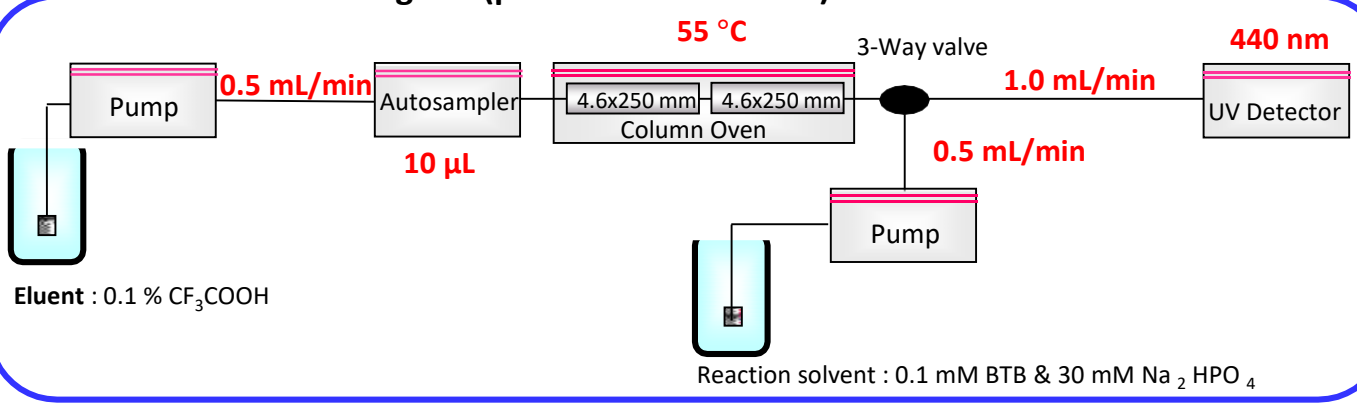
### Measurement conditions

**Conditions**

| | |
|---|---|
| **Column** | : Inetsil CX (5 µm 250 x 4.6 mm I.D.) x 2 |
| **Eluent** | : 0.1% $CF_3COOH$ |
| **Reaction solution** | : 0.1 mM BTB + 30 mM $Na_2HPO_4$ |
| **Flow rate** | : 1.0 mL/min |
| **Column Temp.** | : 55 ˚C |
| **Detector** | : VIS 440 nm |




### BTB method flow diagram (post-column method)

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# Advantages for the analysis of organic acids

## ▪Low cost

Typically, columns for organic acid analysis are expensive with typical sizes of 8.0 x 300 mm.
In this assay, two smaller columns - Inertsil CX 4. 6 x 250 mm are used.
Using these columns samples may be analyzed at low cost following the procedure described in this application note..

## ▪Rapid analysis

The reduction in column size shortens the assay time by approx.16 minutes, allowing a large number of samples to be processed per day.

## ▪High resolution

Inertsil CX is a cation-exchange column in which benzene sulfone groups are chemically bonded to maternal silica gel. Therefore, compared to polymer-based columns, a higher number of theoretical plates can be achieved. Organic acids can be quantified with concentrations up to 0.1 µg/µL.

## ▪Low pressure

When a 4.6 mm internal diameter column is used with a total of 500 mm length, the column is durable because of the low operating pressure (approx. 6 MPa ( 60 bar)), even at a flow rates of 0.5 mL/min.

**Analytical Column**

**Inertsil CX**

| | |
|---|---|
| Base Material | : High purity spherical silica gel |
| Particle Size | : 5 µm |
| Surface Area | : 450 $m^2/g$ |
| Purity | : 99.999 % |
| Pore Size | : 100 Å |
| Pore Volume | : 1.05 mL/g |
| Functional Group | : Benzene sulfone |
| End-capping | : None |
| Carbon Loading | : 14 % (ion-exchange capacity: 0.5 meqv $g^{-1}$) |

Functional Group


| Internal diameter (mm) | Length (mm) | Cat.No. |
|---|---|---|
| 4.6 | 250 | 5020-07146 |

# Organic Acids in Food Dressings

**・Food Dressing pretreatment**

Dressings typically contain xanthan gum as a thickener. This xanthan gum affects the peak shape and must be removed using chloroform, if the peaks continue to tail, further treatment with chloroform will be required.
Because oils also affect the assay, the oil in any oil-type dressings must be removed by emulsification with a surfactant (Tween 20). Non-oil dressings can be analyzed with chloroform treatment alone.

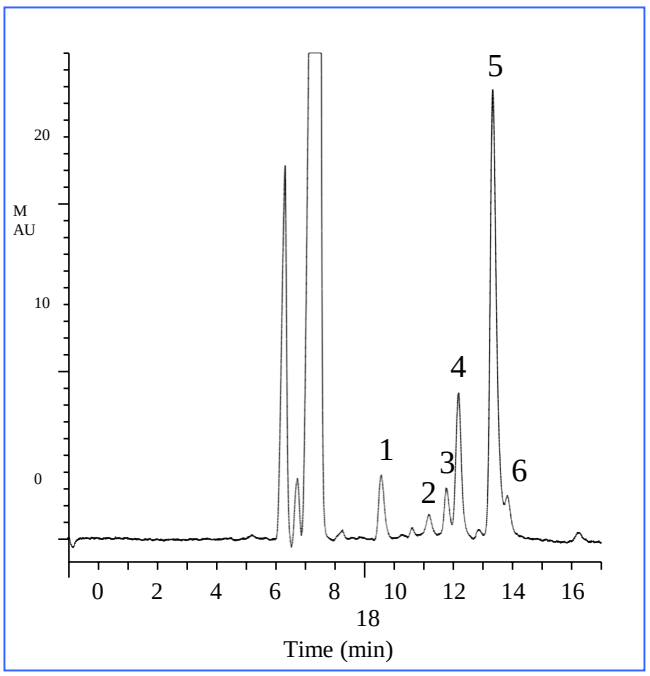
## Food Dressing Pretreatment Method

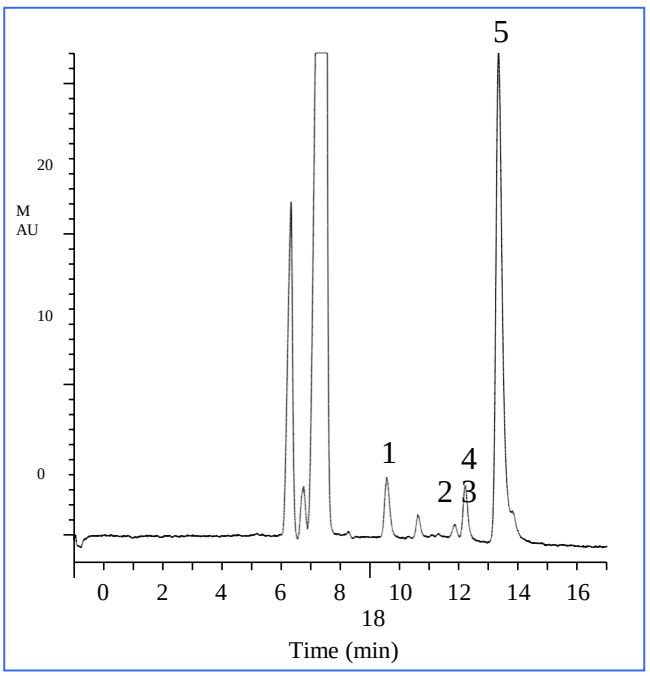
- 1 mL of dressing
- Dilution
  - $H_2O$ 9 mL
- Emulsification
  - Tween20 6.5 mL
- Cleaning
  - Chloroform 50 mL
- Centrifugal separation
  - (3,000 rpm, 10 min)
- Filtration
  - 0.45 µm filter
- HPLC

## Non-oil Food Dressing Pretreatment Method

- 1 mL of dressing
- Dilution
  - $H_2O$ 9 mL
- Cleaning
  - Chloroform 50 mL
- Centrifugal separation
  - (3,000 rpm, 10 min)
- Filtration
  - 0.45 µm filter
- HPLC

1. Phosphoric acid (phosphate)
2. Malic acid (malate)
3. Succinic acid (succinate)
4. Lactic acid (lactic acid)
5. Acetic acid (acetic acid)
6. Pyroglutamic acid (pyroglutamic acid)



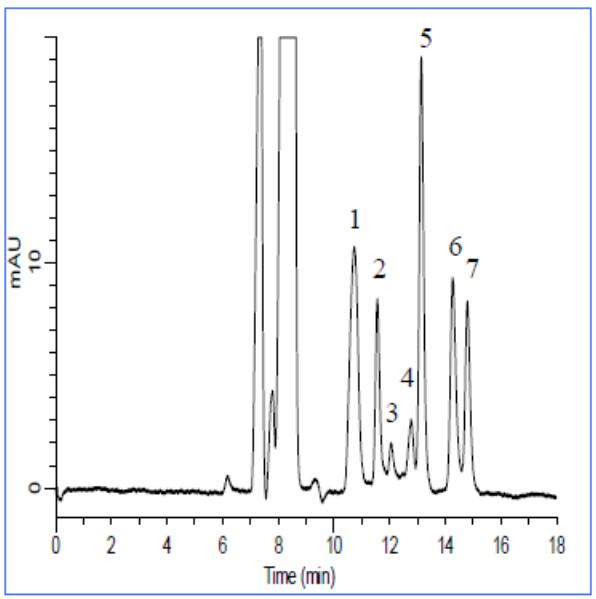



# Organic acids in soy sauce

## Pretreatment method

1 mL of Soy sauce
↓
Dilution
— $H_2O$ 9 mL
↓
Filtration
— 0.45 µm filter
↓
HPLC

1. Phosphoric acid (phosphate)
2. Citric acid (citric acid)
3. Malic acid (malate)
4. Succinic acid (succinate)
5. Lactic acid (lactic acid)
6. Acetic acid (acetic acid)
7. Pyroglutamic acid (pyroglutamic acid)

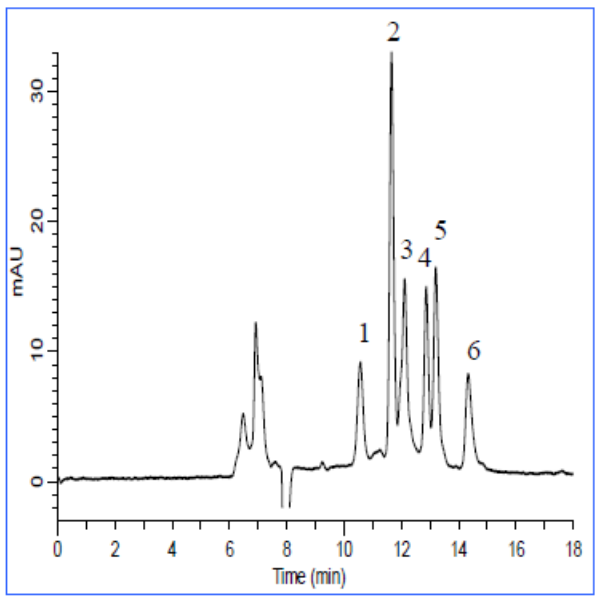



# Organic acids in red wine

## Pretreatment method

1 mL of Red Wine
↓
Dilution
— $H_2O$ 1 mL
↓
Filtration
— 0.45 µm filter
↓
HPLC

1. Phosphoric acid (phosphate)
2. Citric acid (citric acid)
3. Malic acid (malate)
4. Succinic acid (succinate)
5. Lactic acid (lactic acid)
6. Acetic acid (acetic acid)



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