LT094
GL Sciences Inc.

Analysis of Preservatives in Foods --Third Report--

In May 2010, the Ministry of Health, Labour and Welfare issued Notification No. 0528-4 of the Food Safety Agency, revising the Analysis Methods for Food Additives. Potassium sorbate was included as a new additive, and an example of concurrent analysis was newly added to Appendix 2.

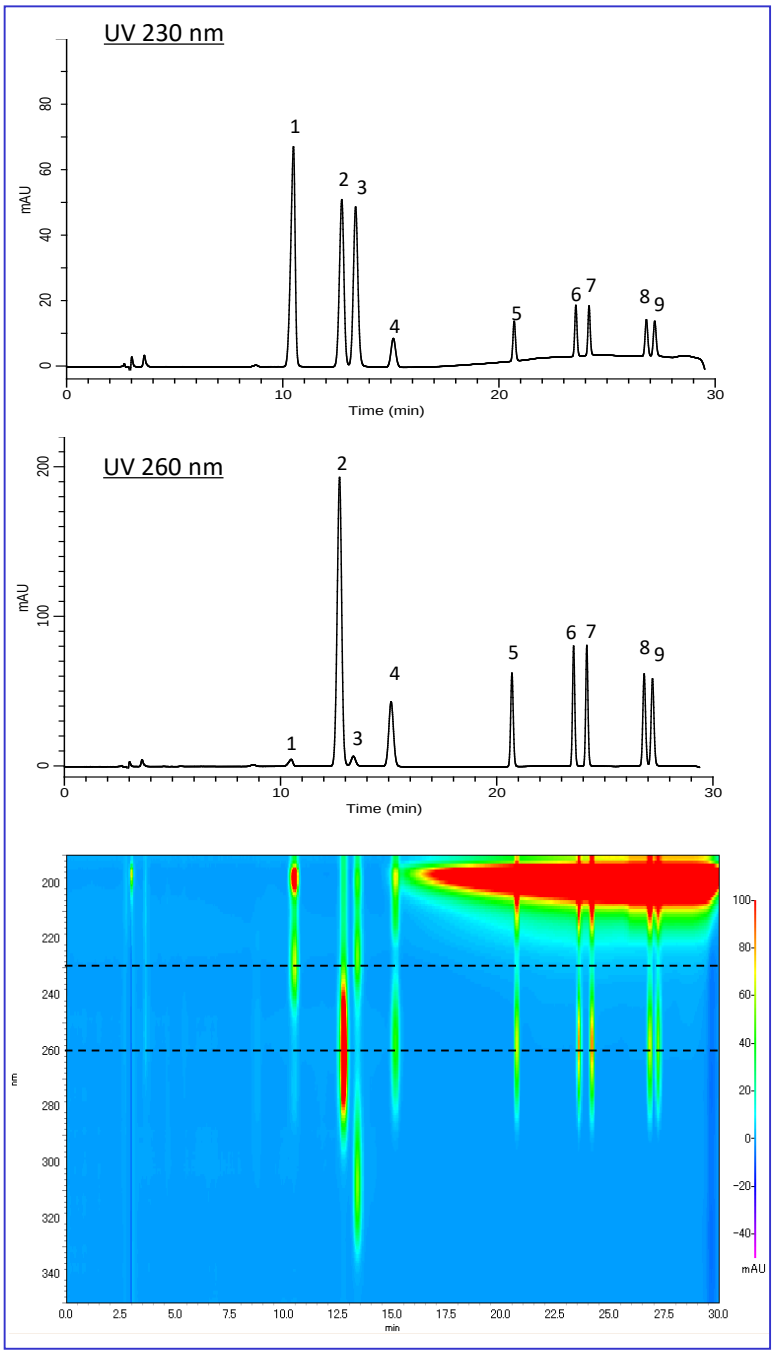
This application presents an example of the analysis using Inertsil ODS-4 in accordance with the simultaneous analysis method.

In addition, simultaneous analysis using a separation with high speed separation (8-minute cycle) and LC/MS/MS (8-minute cycle) were also found to be good, these are also presented in this report.

(Y.Tanaka)

Associated applications
No.75HPLC Analyses of Preservatives in Foods
No.76 HPLC Analysis of Preservatives in Foods -Second Report--

Example: Measurement of standard



Using an Inertsil ODS-4 column, all preservatives of interest are separated with a resolution of 1.5 or greater.

The wavelengths of absorption for sorbic acid and benzoic acid esters are in the region UV 260 nm, and those for dehydroacetic acid are in the region of 230 to 310 nm.

If the separation is difficult , contamination is present or to adjust the sensitivity, adjust the wavelength as appropriate.

HPLC conditions

Guard column : Cartridge guard column E Inertsil ODS-4 (5 µm, 10 x 4.0 mm I.D.)

Column : Inertsil ODS-4 (5 µm, 250 x 4.6 mm I.D.)

Eluent : A) CH_3OH/H_2O/phosphate buffer* (pH 4. 0). = 2/17/1, v/v/v
B) CH_3OH/H_2O/phosphate buffer (pH 4. 0) = 14/5/1, v/v/v
A/B = 50/50 - 10 min - 50/50 -10 min - 0/100 - 5 min - 0/100 - 0.1 min - 50/50 - 10 min - 50/50 ,v/v

Flow rate :1.0 mL/min

Column temperature :40 ˚C

Detected : UV 230 nm, 260 nm (GL-7452A PDA Detector)

Injection volume :20 µL

* 0.2 mol/L Phosphate Buffer: Dissolve 27.0 g of KH_2PO_4 in ultrapure water. Adjust to pH 4.0 with phosphoric acid and make up to a total volume of 1000 mL

1. Benzoic acid (10 mg/L)
2. Sorbic acid (10 mg/L)
3. Dehydroacetic acid (10 mg/L)
4. Methyl Parahydroxybenzoate (10 mg/L)
5. Ethyl parahydroxybenzoate (10 mg/L)
6. Isopropyl parahydroxybenzoate (10 mg/L)
7. Propyl Parahydroxybenzoate (10 mg/L)
8. Isobutyl p-hydroxybenzoate (10 mg/L)
9. Butyl *p*-hydroxybenzoate (10 mg/L)

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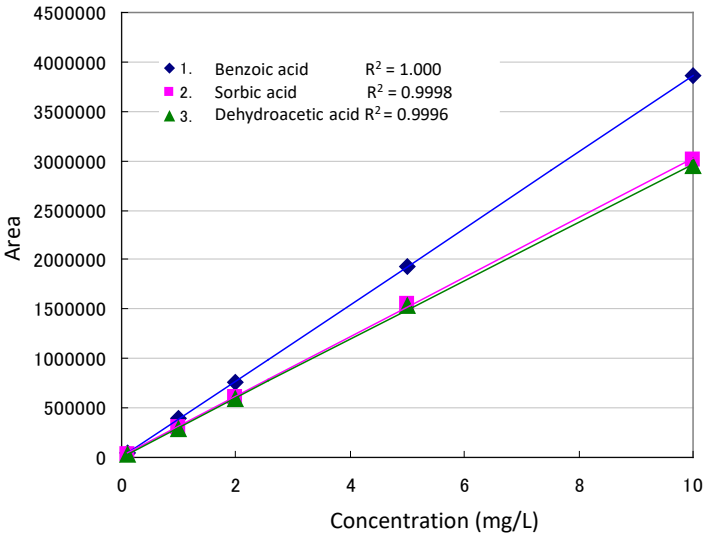
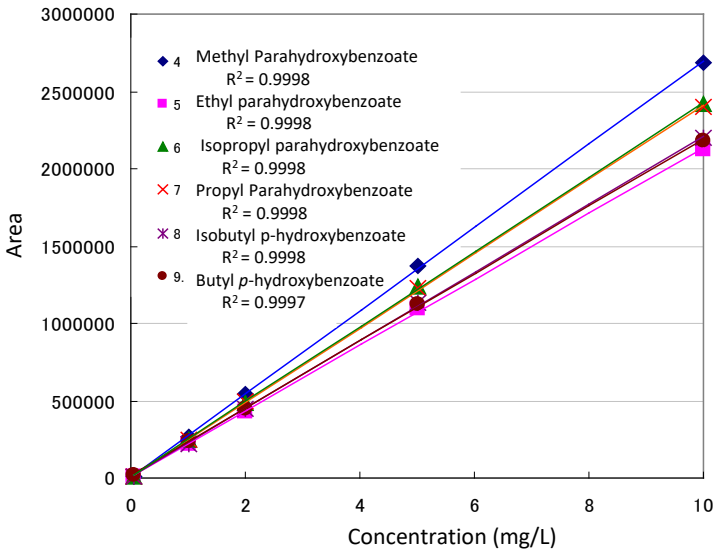
Calibration curve



Results of measurements at UV 230 nm



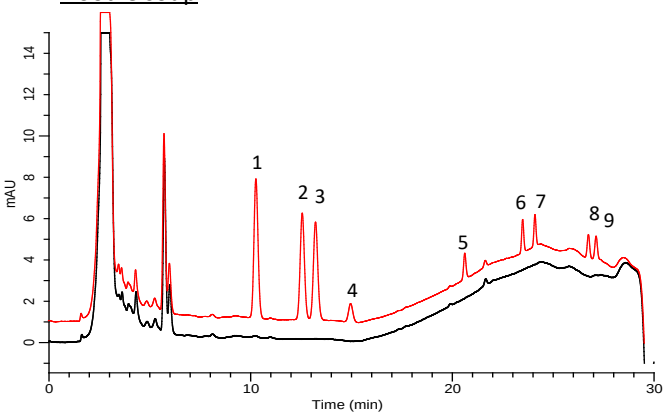
Results of measurements at UV 260 nm

Example of food analysis

Example of pretreatment

For pretreatment, steam distillation is described in the test method. In this test, samples were diluted and filtered through a 0.45 µm filter.

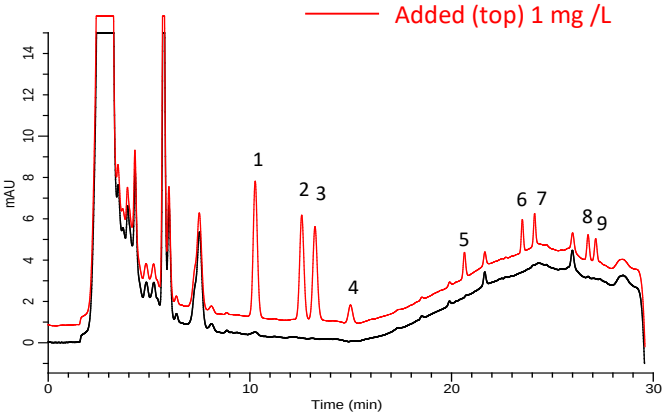
Noodle soup



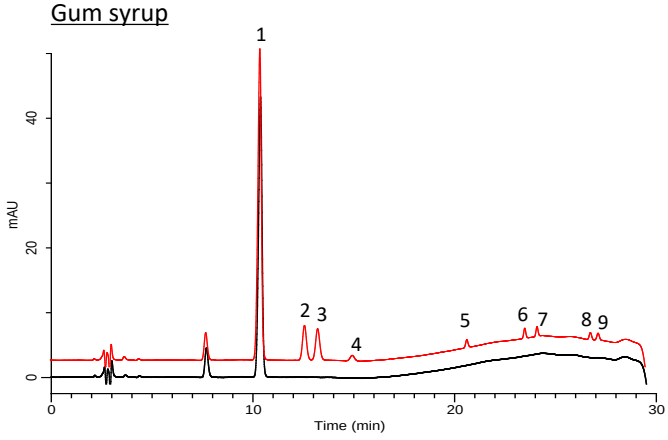
Soy sauce

Not added (bottom)

Added (top) 1 mg /L

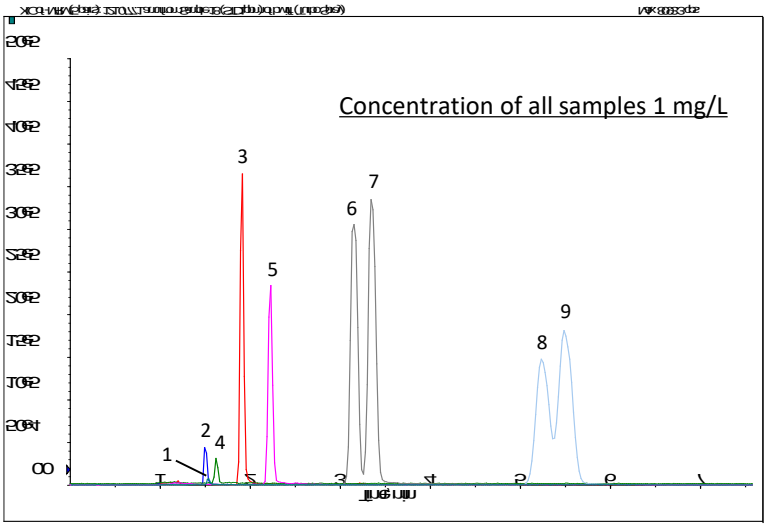


Gum syrup



Example of high-speed analysis using LC/MS/MS

LC/MS, LC/MS/MS or GC are used as confirmatory tests.
This section provides an example of fast analysis using LC/MS/MS with an 8-minute cycle.
MS allows selective identification of the preservative for this purpose.



1. Benzoic acid (Q1:121, Q3:77, Nega)
2. Sorbic acid (Q1:113, Q3:67, Posi)
3. Dehydroacetic acid (Q1:169, Q3:85, Posi)
4. Methyl Parahydroxybenzoate (Q1:153, Q3:109, Posi)
5. Ethyl parahydroxybenzoate (Q1:167, Q3:139, Posi)
6. Isopropyl parahydroxybenzoate (Q1:181, Q3:139, Posi)
7. Propyl Parahydroxybenzoate (Q1:181, Q3:139, Posi)
8. Isobutyl p-hydroxybenzoate (Q1:195, Q3:139, Posi)
9. Butyl p-hydroxybenzoate (Q1:195, Q3:139, Posi)

HPLC conditions

System : LC800 HPLC system
4000 Q TRAP

Column :Inertsil ODS-4 (2 µm, 75 x 2.1 mm I.D.)

Elution : A) 0.05% HCOOH in (H_2O/CH_3CN = 5/95).
B) 0.05 % HCOOH in (H_2O/CH_3CN = 95/5)
A/B = 95/5 - 0.2 min - 63/37 - 5.3 min - 63/37 - 0.01 min - 95/5 - 2.5 min - 95/5, v/v

Flow rate : 0.5 mL/min

Column temperature :40 ˚C

Detection : LC/MS/MS (4000 Q TRAP: ESI, Positive, MRM)

CUR	CAD	IS	TEM	GS1	GS2
50	3	5500	700	60	50

: LC/MS/MS (4000 Q TRAP : ESI, Negative, MRM)

CUR	CAD	IS	TEM	GS1	GS2
40	2	-4500	700	80	60

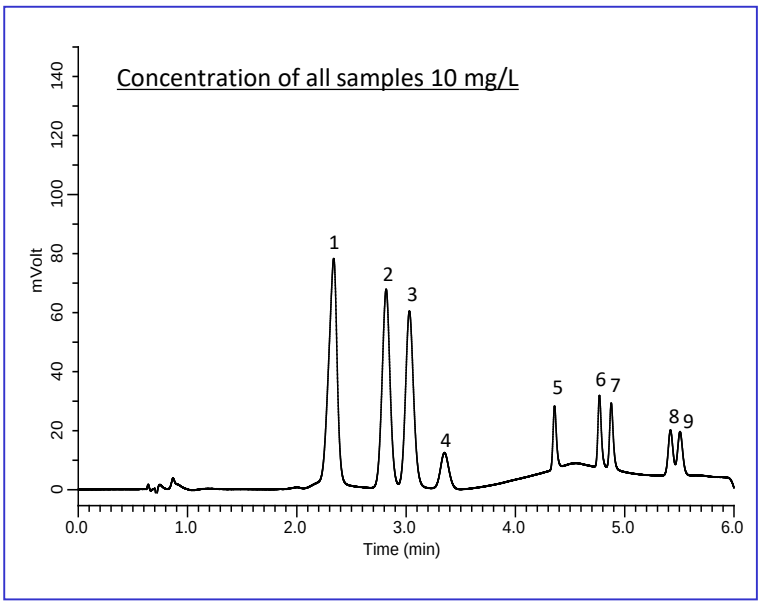
Injection volume : 2 µL

Analytical column : Inertsil ODS-4 2 µm, 75 x 2.1 mm I.D.
Cat.No. 5020-81203

* Reduce the organic solvent concentrations for complete separation of peaks 8 and 9

Example of high-speed analysis

Using Inertsil ODS-4, Benzoates can be well separated even with fast separation (in less than 8 minutes). Benzoates have wavelengths with maximum absorption near 260 nm. If detection is difficult at UV 230 nm, set the wavelength to UV 260 nm.



HPLC conditions

System : LC800 HPLC system

Column : Inertsil ODS-4 HP (3 µm, 150 x 2.1 mm I.D.)

Eluent : A) CH_3OH/H_2O/phosphate buffer* (pH 4. 0).
= 2/17/1, v/v/v
B)3 CHOH/H_2O/phosphate buffer (pH 4. 0)
= 14/5/1, v/v/v
A/B = 50/50 - 2.3 min - 50/50 - 1.2 min - 0/100 - 1.5 min - 0/100 - 0.1 min - 50/50 - 3 min - 50/50 , v/v

Flow rate : 0.5 mL/min

Column temperature : 40 ˚C

Detected : UV 230 nm (10 mm UV flow cell)

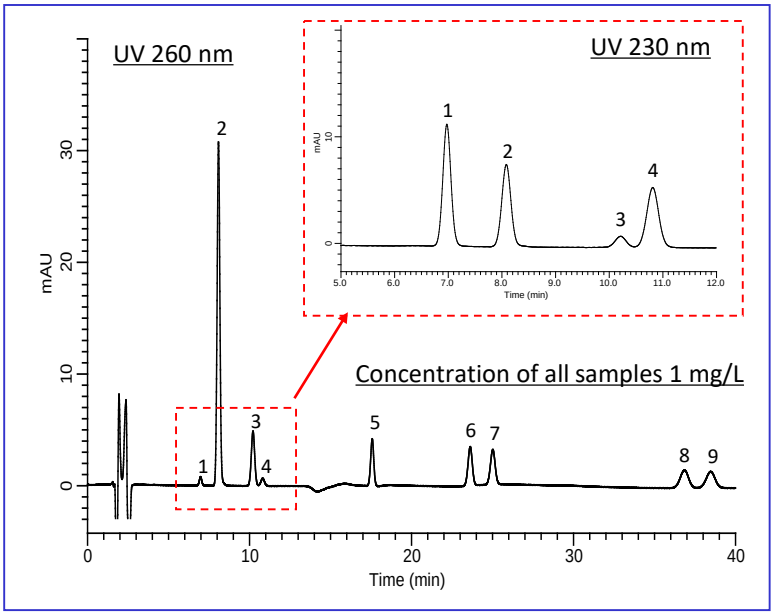
Injection volume : 5 µL

* 0.2 mol/L Phosphate Buffer: 27.0 g of KH_2PO_4 was added to ultrapure water.
Add phosphoric acid to pH 4.0. and make up to a total volume of 1000 mL

Analytical column : Inertsil ODS-4 HP 3 µm, 150 x 2.1 mm I.D.
Cat.No. 5020-14002

Example of analysis with citrate buffer

An example using citrate buffer is also shown in the concurrent analysis example. The separation time is relatively long, but it can be analyzed under these conditions.



HPLC conditions

System : GL-7400 HPLC system

Guard column : Cartridge guard column E Inertsil ODS-4 (5 µm, 10 x 4.0 mm I.D.)

Column : Inertsil ODS-4 (5 µm, 150 x 4.6 mm I.D.)

Eluent : A) CH_3OH/CH_3CN/5 mM citrate buffer* = 1/2/7, v/v/v

B)CH_3OH/CH_3CN/5 mM citrate buffer = 5/4/11, v/v/v

A/B = 100/0 -10 min- 100/0 -5 min- 0/100 -22 min- 0/100, v/v

Flow rate : 1.0 mL/min

Column temperature : 40 ˚C

Detected : UV 230 nm, 260 nm

Injection volume :20 µL

* 5 mM Citrate Acid Buffer: Add 7.0 g of citric acid monohydrate and 6.0 g of trisodium citrate dihydrate to ultrapure water and make up to a total volume of 1 L,
Dilute 10-fold before use.

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