Analysis Guidebook
Food Product Analyses
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1.1 Analysis of Fatty Acids (1) - GCMS

• Explanation
Fatty Acids exist in a great many food products. And derivatization process is used to measures them. The aims of derivatization process are as follows.

1) Weaken the polarity of compounds.
2) Lower the boiling point.
3) Increase molecular ion peak and ion intensity in high mass region.

In the case of fatty acids, derivatization process is used to achieve item 1). The methyl esterization or trimethylsilylation can be used but generally methyl esterization employing diazomethane is used for the derivatization.

Normally, the molecular ion peak that displays the molecular weight is detected for the Ei mass spectrum's saturated fatty acid methyl ester and, as determination of molecular weight is easy, a carbon count is possible. However, the molecular ion peak often does not appear when the level of unsaturation increases, which means that not only molecular weight but also the carbon count and unsaturated level cannot be determined. In such cases, the Ci mass spectrum is measured. With the Ci mass spectrum, the ion denoting the molecular weight appears as an ion (M+1) with added proton in the molecular weight for detection of molecular weight + 1 ion. Measuring the Ei and Ci mass spectra enables qualitative analysis of compounds in fatty acid methyl ester measuring. Also, the columns used in this measuring include the slightly polar column DB-1 and polar column DB-WAX. The polarity column produces peaks in the saturated and unsaturated order while the slightly polar column produces peaks in the reverse order.

• Analytical Conditions

<table>
<thead>
<tr>
<th>Instrument</th>
<th>GCMS-QP5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>DB-WAX 0.25mm×30m df=0.25µm</td>
</tr>
<tr>
<td>Col.Temp.</td>
<td>60˚C-250˚C (10˚C/min)</td>
</tr>
<tr>
<td>Inj. Temp.</td>
<td>250˚C</td>
</tr>
<tr>
<td>I/F Temp.</td>
<td>250˚C</td>
</tr>
<tr>
<td>Carrier Gas</td>
<td>He(100kPa)</td>
</tr>
<tr>
<td>Reagent Gas</td>
<td>Isobutane</td>
</tr>
</tbody>
</table>

![Fig. 1.1.1 Ei mass spectrum of C18:0](image1)

![Fig. 1.1.2 Ci mass spectrum of C18:0](image2)
1.1 Analysis of Fatty Acids (2) - GCMS

Fig. 1.1.3 Ei mass spectrum of C20:5

Fig. 1.1.4 Ci mass spectrum of C20:5

Fig. 1.1.5 Mass chromatogram of protonized molecules for fatty acid methyl ester
1.1 Analysis of Fatty Acids (3) / Derivatization
- Fat Extraction Method

• Pretreatment for Fatty Acid Analysis
Fat must be extracted from the food product and hydrolysis and methylation performed for GC and GC-MS analysis of fatty acids in food products. Here, several representative pretreatment methods will be introduced from the numerous methods available.

1. Fat Extraction
This shows an example of fat extracted from a sample.

References
1.1 Analysis of Fatty Acids (4) / Derivatization
- Preparation of Methyl Fatty Acids

2. Preparation of Methylated Fatty Acid
This shows a transmethylation method for extracting fat using an alkali catalyst that does not require fat extraction of food oils, etc. This easy method just requires hydrolysis and fatty acid extraction so labor is reduced.

Note, however, that amide-bonded fatty acid and free fatty acid do not methylate.

References
1.1 Analysis of Fatty Acids (5) / Derivatization

- Alkali Hydrolysis of Fat

3. Alkali Hydrolysis of Fat

Extracted fat is triacylglycerol which emerges as glycerol and potassium salt's fatty acid (water soluble) using alkali. Fatty acid hardly separates when acidified, which enables extraction with non-polar solvent. Here, an example of alkali hydrolysis is introduced.

**References**

4. Methyl Ester Derivative Preparation Method

High-class fatty acids are generally derived into methyl ester. The currently used methods are introduced here.

(1) Methyl Esterization using BF₃–CH₃OH

- 20mg of Fatty Acid
  - (Remove solvent if it is in solution)
  - Boil approximately 3mL of BF₃–CH₃OH over a water bath for 2 min
  - Shake 20mL of n-hexane + 20mL of distilled water
  - Water Layer
  - n-Hexane Layer
    - Add anhydrous Na₂SO₄ (suitable amount), let stand, filter
    - Filtrate
      - Remove solvent by spraying nitrogen gas over 50°C water bath
    - GC, GCMS

Fig. 1.1.9 Methyl esterization using boron trifluoride-methanol

(2) Methyl Esterization Using H₂SO₄–CH₃OH

- 20mg of Fatty Acid
  - Boil approximately 20mL of H₂SO₄–CH₃OH over a water bath for 1 hr
  - Shake 20mL of n-hexane + 20mL of distilled water
  - Water Layer
  - n-Hexane Layer
    - • Repeatedly rinse with 10mL batches of distilled water to neutralize
    - • Add anhydrous Na₂SO₄ (suitable amount), let stand, filter
    - Filtrate
      - Remove solvent
    - GC, GCMS

Fig. 1.1.10 Methyl esterization using sulphuric acid-methanol
1.1 Analysis of Fatty Acids (6) / Derivatization (2)
- Methyl Ester Derivative

(3) Methyl Esterization Using CH₂N₂
A diazomethane generator is assembled as shown in the diagram. And ethyl ether (I), 50% potassium hydroxide water solution (II), 10mg of fatty acid + 2mL of ethyl ether (III) and acetic acid are sealed in tubes.
1. A suitable amount of nitrogen gas is passed through test tube I.
2. Some 0.5 to 1mL of N-methyl-N'-nitroso-p-toluenesulfonamide with 20% ethyl ether is injected into test tube II to create diazomethane.
3. Remove test tube III from diazomethane generator once the ethyl ether liquid inside has turned yellow.
4. Leave test tube III to stand for 10 min to enrich the ethyl ether, and inject into GC or GCMS.

(4) Methyl Esterization Using Dimethylformamide Dialkylacetals (CH₃)₂NCH(OR)₂
Add 300 µL of esterification reagent to some 5 to 50mg of fatty acid. Dissolve the sample and inject the resultant reaction liquid into the GC or GCMS. (Normally it is best to heat this at 60°C for 10 to 15 min.)

(5) Methyl Esterization Using Phenyltrimethyl Ammonium Hydroxide (PTAH)
Dissolve the fatty acid in acetone, add PTAH/methanol solution (1 to 1.5M%), thoroughly stir sample and reaction reagent, leave to stand for 30 min, and induct into GC or GCMS.
This methyl esterization using on-column injection is a method where the PTAH/methanol reagent and fatty acid are mixed in advance, injected into the GC and made to react in a GC injector. Compared to other methods treatment is quick and simple and there is no volatile loss because the reaction is in a GC injector. Furthermore, harmful, dangerous reagents are not required.

Notes and cautions
- Handle diazomethane with care, as it is carcinogenic.
- For the above reason, only adjust small amounts and be sure to use a ventilating hood.
- Do not use ground glass stoppers because there is a danger of explosion.
- Small amounts of ether solution (100mL or less) can be stored in a refrigerator for several days.
- Several relatively easy-to-handle diazomethane generators are available in market.
1.2 Fatty Acids (Fish Oil) - GC

**Explanation**

Among high-class fatty acids, unsaturated fatty acids are currently in the limelight, for example, much attention is being given to the antithrombogenic effect of eicosapentaenoic acid, etc. From the outset, gas chromatographs have been used to separate and quantify high-class fatty acids. High-class fatty acids have absorptivity and high boiling points, which means that derivatization (usually methyl esterization) is performed for GC analysis. This example introduces capillary column analysis of fatty acid methyl ester in fish oil. Fig. 1.2.1 shows constant pressure analysis at 110kPa and Fig. 1.2.2 shows rising pressure analysis from 110kPa to 380kPa. Rising pressure analysis provides quicker analysis with improved sensitivity because separation hardly changes.

**References**

1) Application News No. G165
2) Gas Chromatograph Data Sheet Nos. 15, 21

**Pretreatment**

Methyl esterization of fatty acids in fish oil is performed in accordance with Fig. 1.1.11 followed by GC analysis.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>GC-17AAFw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>CBP20</td>
</tr>
<tr>
<td>Column temperature</td>
<td>210°C</td>
</tr>
<tr>
<td>Injection inlet temperature</td>
<td>230°C</td>
</tr>
<tr>
<td>Detector temperature</td>
<td>230°C (FID)</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>He 100kPa</td>
</tr>
<tr>
<td>Injection method</td>
<td>Split 1:100</td>
</tr>
</tbody>
</table>

**Recommended instrument configuration**

- **Main unit**: GC-17AAFw
- **Detector**: FID
- **Column**: DB-WAX 0.25mm×30m df=0.25µm
- **Auto injector**: AOC-20i/s
- **Data processor**: C-R7Aplus or CLASS-GC10

Fig. 1.2.1 Analysis of fatty acid methyl ester in fish oil (constant pressure)

Fig. 1.2.2 Analysis of fatty acid methyl ester in fish oil (rising pressure)
1.3 Triglycerides - GC

• Explanation
Triglycerides are compounds with a high boiling points and strong absorptivity. Separation is poor in analysis of these compounds when a short column filled with highly heat resistant packing is used with the packed-column GC.

In comparison to this kind of column a capillary column filled with fused silica offers minimal absorptivity at high separation and excellent heat resistance. However, even better heat resistance is required for high-boiling-point compounds like triglycerides.

Stainless steel capillary columns or aluminum coated ones are extremely heat resistant and, as such, are suitable for analysis of triglycerides. Also, cold on-column injector suppress discrimination of samples.

• Pretreatment
None in particular.

• Analytical Conditions

<table>
<thead>
<tr>
<th>Instrument</th>
<th>: GC-17AAFw</th>
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<tbody>
<tr>
<td>Column</td>
<td>: CBM65 0.22mm×25m df=0.10µm</td>
</tr>
<tr>
<td>Column temperature</td>
<td>: 50˚C(1min)-(20˚C/min)-240˚C-(6˚C/min)-390˚C</td>
</tr>
<tr>
<td>Detector temperature</td>
<td>: 390˚C(FID)</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>: He(1.5mL/min)</td>
</tr>
<tr>
<td>Injection method</td>
<td>: Cold on-column</td>
</tr>
</tbody>
</table>

References
1) Application News No. G130

Recommended instrument configuration

| Main unit     | : GC-17AAFw+OCI-17          |
| Detector      | : FID                      |
| Column        | : ULTRA ALLOY-65 0.53mm×30m df=0.1µm |
| Auto injector | : AOC-20i/s                |
| Data processor| : C-R7Aplus or CLASS-GC10 |
1.4 Analysis of Fatty Acids in Red Wine Using Infrared Spectrophotometry (1) - IR

•Explanation
Food products are mixtures of various compounds that require liquid chromatography (LC) or gas chromatography (GC) separation procedures for component analysis. However, injections of a large amount of samples are difficult due to column load restrictions in chromatography, so at maximum the amount of component existing in one peak of a chromatogram will be only in the µg order. Nevertheless, if a FTIR is used, infrared measuring is possible and components can be quantified.

Here, component analysis of food products using a preparative LC-FTIR method will be introduced.

•Pretreatment
Red wine that has been filtered through a membrane filter was injected into an LC. Fig. 1.4.1 shows a chromatogram detected by the UV detector. The separated substances in peaks A to C have been collected, but because there are numerous coexisting substances in the collected substances, the collected substance is re-injected into the LC using a mobile phase of water, and the chromatogram measured. The separated substances in the largest peak obtained from this operation is collected, the mobile phase vaporized from within the collected substance, this collected substance is mixed with KBr powder and measured using a diffuse reflection method.

Fig. 1.4.2 shows the infrared spectrum of peak A. Absorption of coexisting substances is overlaid but tartaric acid can be clearly confirmed.

Fig. 1.4.3 shows the infrared spectrum of peak B. The carboxylic acid peak can be confirmed in the region of 1730cm⁻¹ and, as glucose (a coexisting substance) is equal to the holding time, glucose absorption has mostly become infrared spectrum.

Fig. 1.4.4 shows the infrared spectrum of peak C. In this case there is no interference from other components and the spectrum is only for succinic acid.

•Analytical Conditions

<table>
<thead>
<tr>
<th>Instrument</th>
<th>: LC-VP Series</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>: Shim-pack SCR-102H</td>
</tr>
<tr>
<td></td>
<td>(8mmφ×300mmL)</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>: 5mM Trifluoroacetic Acid Aqueous</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>: 1mL/min</td>
</tr>
<tr>
<td>Column Temp.</td>
<td>: 50˚C</td>
</tr>
<tr>
<td>Detector</td>
<td>: UV-VIS Detector 380nm</td>
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<tr>
<td>Instrument</td>
<td>: FTIR</td>
</tr>
<tr>
<td>Resolution</td>
<td>: 4cm⁻¹</td>
</tr>
<tr>
<td>Accumulation</td>
<td>: 50</td>
</tr>
<tr>
<td>Appodization</td>
<td>: Happ-Genzel</td>
</tr>
<tr>
<td>Detector</td>
<td>: Pyroelectric Detector</td>
</tr>
</tbody>
</table>

1.4 Analysis of Fatty Acids in Red Wine Using Infrared Spectrophotometry (2) - IR

- **Fig. 1.4.1** LC chromatogram of red wine
- **Fig. 1.4.2** Infrared spectrum of peak A (T: tartaric acid peak)
- **Fig. 1.4.3** Infrared spectrum of peak B (G: glucose)
- **Fig. 1.4.4** Infrared spectrum of peak C (succinic acid)
1.5 Analysis of Decenoic Acid in Royal Jelly - LC

•Explanation
Royal jelly is widely known as a food product and herbal medicine, and its peculiar component is 10-hydroxy-δ-decenoic acid (10-HAD). The amount of this and the investigation method are vital points in composition standards for royal jelly. The following is an analysis example.

References
Study group text related to royal jelly composition standard testing method provided by Japan Royal Jelly Fair Trade Council

•Pretreatment
Distilled water is added to a specific amount of sample, dissolved through mixing, a specific amount of internal standard (benzoic acid) was added and the mixture filtered through a disposable 0.45 µm filter.

•Analytical Conditions
Instrument : HPLC
Column : STR ODS- II (4.6mm φ×150mm)
Mobile phase : 10mM sodium phosphate buffer
Liquid(pH2.6)/methanol=55/45 (v/v)
Flow rate : 1.0mL/min
Temperature : 40°C
Detection : UV-VIS Detector 210nm

Fig. 1.5.1 Analysis of decenoic acid in raw royal jelly
1.6 Analysis of Fatty Acids - LC

**Explanation**
Fatty acid can be detected using carboxyl group absorbent (210nm) in the same way as organic acid, etc. However, this kind of short wavelength is susceptible to impurities and some samples are difficult to analyze. Here, a prelabel agent is derived into a fluorescent substance and detected using a fluorescent detector. The compound labeling agent ADAM (9-Anthryldiazomethane) possessing the carboxyl group is a prelabel agent that targets the methylating agent (diazomethane) reaction. Here, direct analysis using UV absorption detection and prelabel derivatization detection using ADAM agent will be introduced.

**References**
Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)

**Pretreatment**
None.

**Analytical Conditions**
- **Instrument**: HPLC
- **Column**: Shim-pack CLC-ODS (6.0mm×150mm)
- **Mobile phase**: Acetonitrile/water = 95/5 (v/v)
- **Flow rate**: 1.0mL/min
- **Temperature**: 45˚C
- **Detection**: Fluorescence Detector (Ex365nm Em415nm)

**Fig. 1.6.1** Analysis of fatty acid using UV absorption detection

**Fig. 1.6.2** Analysis of high-class fatty acid using precolumn derivatization method with ADAM

**Fig. 1.6.3** Reaction equation for ADAM and fatty acid
1.7 Analysis of Organic Acid in Beer - LC

**Explanation**
In the case of analysis of organic acid using absorptiometry, carboxyl group absorption at 200 to 210nm is used, but some samples are difficult to analyze because of poor selectivity and impurity interference at this wavelength.
In such cases, a conductivity detector that detects ionized substances at selectively high sensitivity is used.

**References**
Hayashi, Shimadzu Review 49 (1), 59 (1992)
Shimadzu LC Application Report No. 18
Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)

**Detection lower limit**
Approximately $3 \times 10^{-11}$ equivalent (differs depending on component)

**Pretreatment**
Beer is injected in without any pretreatment.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>2xShim-pack SCR-102H (8.0mmφ×300mm)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>5mM p-toluenesulfonic acid</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.8mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>45°C</td>
</tr>
<tr>
<td>Reaction solutions</td>
<td>5mM p-toluenesulfonic acid 20mM Bis-Tris 100µm EDTA</td>
</tr>
<tr>
<td>Cell temperature</td>
<td>48°C</td>
</tr>
<tr>
<td>Detection</td>
<td>Conductivity</td>
</tr>
</tbody>
</table>

**Peaks**
1. Phosphoric acid
2. Citric acid
3. Pyruvic acid
4. Malic acid
5. Succinic acid
6. Lactic acid
7. Formic acid
8. Acetic acid
9. Levulinic acid

Fig. 1.7.1 Analysis of beer

Fig. 1.7.2 Flowchart of organic acid analysis system
1.8 Analysis of Amino Acid in Cooking Vinegar Using Precolumn Derivatization (1) - LC

**Explanation**
A separation method (precolumn derivatization method) exists using reversed phase chromatography with a derivatization reaction performed on the sample pretreatment stage. Here, the analysis example shows an OPA (o-phthalaldehyde) precolumn derivatization method.

**References**
Application Report No. 19 (Shimadzu Corporation)

**Pretreatment**
See next page for details.

**Analytical Conditions**
- **Instrument**: HPLC
- **Column**: Shim-pack CLC-ODS (6.0mmφ×150mm) with guard column
- **Precolumn**: Shim-pack GRD-ODS (4.0mmφ×250mm)
- **Mobile phase**: (A) 10mM sodium phosphate buffer (pH 6.8)
  (B) A/acetonitrile = 2/1
  (C) 80% acetonitrile water solution
  Gradient method
- **Flow rate**: 1.0mL/min
- **Temperature**: 45°C
- **Detection**: Fluorescence Detector
  - Ex350nm Em460nm (1st class amino acid)
  - Ex485nm Em530nm (2st class amino acid)

---

Fig. 1.8.1 Analysis of cooking vinegar using prelabel amino acid analysis method
1.8 Analysis of Amino Acid in Cooking Vinegar Using Precolumn Derivatization (2) - LC

References

Pre-treatment

<table>
<thead>
<tr>
<th>Sample</th>
<th>10~20 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta)-Mercapt reagent(^1)</td>
<td>200 µL</td>
</tr>
<tr>
<td>OPA reagent(^2)</td>
<td>200 µL</td>
</tr>
<tr>
<td>NBD–Cl reagent(^3)</td>
<td>200 µL</td>
</tr>
</tbody>
</table>

Mixing and Waiting

Injection 10 µL to HPLC

1) \(\beta\)-Mercapt propionic acid | 10 µL
100 mM Borate buffer (pH 9.0) | 10 mL
2) OPA | 20 mg
Acetonitrile | 3 mL
100 mM Borate buffer (pH 9.0) | 10 mL
3) NBD–Cl | 100 mg
Acetonitrile | 10 mL

Fig. 1.8.2 Pretreatment conditions

<table>
<thead>
<tr>
<th>Time</th>
<th>Function</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>B.CONC</td>
<td>5</td>
</tr>
<tr>
<td>4.0</td>
<td>B.CONC</td>
<td>15</td>
</tr>
<tr>
<td>8.0</td>
<td>B.CONC</td>
<td>20</td>
</tr>
<tr>
<td>16.0</td>
<td>B.CONC</td>
<td>27</td>
</tr>
<tr>
<td>18.0</td>
<td>B.CONC</td>
<td>30</td>
</tr>
<tr>
<td>25.0</td>
<td>B.CONC</td>
<td>45</td>
</tr>
<tr>
<td>30.0</td>
<td>B.CONC</td>
<td>50</td>
</tr>
<tr>
<td>39.0</td>
<td>B.CONC</td>
<td>65</td>
</tr>
<tr>
<td>39.01</td>
<td>B.CONC</td>
<td>70</td>
</tr>
<tr>
<td>42.0</td>
<td>B.CONC</td>
<td>75</td>
</tr>
<tr>
<td>48.0</td>
<td>B.CONC</td>
<td>80</td>
</tr>
<tr>
<td>48.01</td>
<td>B.CONC</td>
<td>100</td>
</tr>
<tr>
<td>49.0</td>
<td>SV</td>
<td>1</td>
</tr>
<tr>
<td>53.0</td>
<td>SV</td>
<td>0</td>
</tr>
<tr>
<td>54.0</td>
<td>B.CONC</td>
<td>100</td>
</tr>
<tr>
<td>54.01</td>
<td>B.CONC</td>
<td>0</td>
</tr>
<tr>
<td>54.02</td>
<td>STOP</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1.8.3 Gradient conditions
**Analysis of Amino Acid Using Postcolumn Derivatization - LC**

**Explanation**

Fig. 1.9.1 shows a standard amino acid chromatogram created using a Shimadzu HPLC amino acid analysis system. The 17 components of a protein-configured amino acid can be automatically analyzed in a 45min cycle.

Each component is separated through gradient elution using a cation exchange column. And detection is made possible through the use of a postcolumn derivatization with fluorescence detection using OPA (o-phthalaldehyde). The OPA method is 10 times more sensitive than the ninhydrin coloring method. Also, using N-acetylcysteine on a thiol compound that exists in the reaction means that sensitive detection can be achieved for even 2nd class amines such as proline. Fig. 1.9.2 shows a chromatogram of the 17 amino acid components in Soya sauce as an application example for the food product field.

**Pretreatment**

Dilute the Soya sauce sample by 500 fold, filter through a membrane filter, and inject 10mL of filtered liquid.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Shim-pack Amino-Na</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Amino acid analysis mobile phase kit Na type</td>
</tr>
<tr>
<td>Temperature</td>
<td>60˚C</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.5mL/min</td>
</tr>
<tr>
<td>Detection</td>
<td>Fluorescence Detector (Ex348nm Em450nm)</td>
</tr>
<tr>
<td>Reaction agent</td>
<td>Amino acid reaction liquid OPA kit</td>
</tr>
<tr>
<td>A liquid</td>
<td>A liquid: Sodium hypochlorite/boric acid buffer</td>
</tr>
<tr>
<td>B liquid</td>
<td>B liquid: OPA, N- acetylcysteine/boric acid buffer</td>
</tr>
<tr>
<td>Reaction agent</td>
<td>0.2mL/min for both A and B liquids</td>
</tr>
</tbody>
</table>

**References**

Shimadzu LC Application Report No. 17
Shimadzu Application News No. L196
Yasui, Shimadzu Review, 47 (4), 365 (1990)
1.10 Simultaneous Analysis of D- and L-Amino Acids (1) - LC

• Explanation
Measurement of optical purity in the food product field is vital. In the case of amino acid, optical separation of configured amino acid is necessary because, in particular, optical purity greatly affects synthetic peptide and its physiological activity in derivatives.

Optical isomer separation methods in LC are broadly divided among the Chiral column solid phase method, Chiral mobile phase method and the Chiral derivatization method. This explanation introduces the Chiral derivatization method.

OPA/N-acetylcysteine agent was used as the derivatization agent.

References
Shimadzu Application News No. L235

• Pretreatment
None.

• Analytical Conditions

<table>
<thead>
<tr>
<th>Instrument</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Develosil ODS-UG-5 (6.0mmφ×200mm) with guard column</td>
</tr>
<tr>
<td>Precolumn</td>
<td>Shim-pack GRD-ODS (4.0mmφ×250mm)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>(A) 50mM sodium acetate, (B) methanol (A)→(B)gradient method</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.2mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>35˚C</td>
</tr>
<tr>
<td>Detection</td>
<td>Fluorescence Detector Ex350nm Em450nm</td>
</tr>
</tbody>
</table>

Fig. 1.10.1 Analysis of D-, L-amino acid standard solution
1.10 Simultaneous Analysis of D- and L-Amino Acids (2) - LC

References

Fig. 1.10.2 Chiral derivatization reaction

Fig. 1.10.3 Gradient conditions

Fig. 1.10.4 Derivatization conditions

standard solution 300µL
buffer solution1 200µL
mix
reagent A2 100µL
mix
reagent B3 100µL
mix and wait 3min
inject 20µL

*1 0.1N sodium tetraborate
*2 2% N-acetyl - L-cysteine
(0.1N sodium tetraborate solution)
*3 1.6% o-Phthalaldehyde
(methanol solution)
1.11 Nutritive Components in Processed Foods - UV

• Explanation
Japanese government national health policy since 1986 dictates that processed food must display nutritive components. Within the regulations governing this policy, energy, proteins, lipid, saccharine and table salt can be displayed. The above policy also includes directives for nutritive component analysis method standards and analyzers. Here, analysis of vitamin C using a spectrophotometer for ultraviolet and visible region will be introduced.

• Pretreatment
Reducing vitamin C is converted into oxidized vitamin C, and red osazone created through reaction of 2,4-Dinitrophenylhydrazine. This osazone is dissolved in 85% sulfuric acid and measured using a spectrophotometer. Here, vitamin C in a nutritious candy was dissolved using metaphosphoric acid solution and measured.

• Analytical Conditions
Instrument : UV Spectrophotometer
Sample : Candy
Solvent : Metaphosphoric acid solution
Cell : 10mm
Range : 0 ～ 0.5ABS
Slit : 2nm

![Fig. 1.11.1 Absorption spectrum of vitamin C](image)

![Fig. 1.11.2 Calibration curve for vitamin C](image)

![Fig. 1.11.3 Quantitative results for vitamin C](image)
1.12 Analysis of Trace Amounts of Vitamins B₁ and B₂ in Food Products Using Fluorescence Photometry (1) - RF

**Explanation**

Vitamins are one valuable form of nutrition. They help to condition physiological function in minute amounts and have been much used in physiology and pharmacology from ancient times.

Vitamin analysis differs for the characteristics (water soluble, fat soluble) and types. And the Japanese Pharmacopoeia and Standard Methods of Analysis for Hygienic Chemists state that on the whole analysis should be conducted using chromatography, absorptiometry and fluorescence photometry. The latter being often used where the vitamin is chemically processed to increase its unique fluorescence for measuring. Here, a measuring example using a fluorescence photometry will be introduced.

**Pretreatment**

Vitamin B₂ - or riboflavin as it is commonly known - is copiously contained in milk, eggs and grains and promotes growth in animals. A riboflavin deficiency leads to various inflammations such as oral ulcers and vision impairment.

Water-solution riboflavin is lime green and shows a green fluorescence. And when it is in an alkali solution, and an ultraviolet is irradiated onto that solution, it becomes a lumiflavin with strong fluorescent properties uniquely inactive.

Fig. 1.12.1 shows the creation process for lumiflavin. And measurement of lumiflavin provides a good way for quantifying vitamin B₂, which also has been adopted for the Standard Methods of Analysis for Hygienic Chemists. Here, vitamin B₂ copiously found in Soya beans was pretreated in accordance with the Standard Methods of Analysis for Hygienic Chemists and measured. Photolysis was performed in an alkali solution on the vitamin B₂ that had been hot-water extracted. And after oxidation, the liquid extracted with chloroform was measured. Vitamin B₂ itself is fluorescent and that excitation and fluorescent spectrum is shown in Fig. 1.12.2. Fig. 1.12.3 shows the spectrum after pretreatment. Fig. 1.12.4 shows the data for processed and measured Soya bean. A comparison with the standard product shows that 2 µg of vitamin B₂ exist in 1g of Soya bean.

**Analytical Conditions**

- **Instrument**: RF Spectrofluorophotometer
- **Sample**: Vitamin B₂ in Soya bean
- **Solvent**: Chloroform
- **Excitation**: 469nm
- **Slit**: Ex10nm Em10nm
1.12 Analysis of Trace Amounts of Vitamins B₁ and B₂ in Food Products Using Fluorescence Photometry (2) - RF

Fig. 1.12.3 Excitation and fluorescent spectra of lumiflavin created from photolysis

Fig. 1.12.4 Measurement of Vitamin B₂ in Soya bean

Fig. 1.12.5 Pretreatment for vitamin B₂ analysis
1.13 Analysis of Water Soluble Vitamins Using Semi-micro LC System - LC

**Explanation**
A column with an inner diameter of 4 to 6mm is usually used in HPLC analysis, but in recent years semi-micro scale columns are being employed in this area and will undoubtedly become the mainstream column for the following reasons.

1. Mass sensitivity (sensitivity based on mass) is increased.
2. The amounts of mobile phase and sample used are reduced.

Fig. 1.13.1 shows a semi-micro LC analysis example of the vitamin B group and caffeine in a vitamin drink. Some 2µL of sample was injected.

**References**
Shimadzu Application News No. L239 (C190-E065)

**Pretreatment**
A 0.45 µm membrane filter was used for filtration.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>STR ODS-II (2.0mmφ×150mm)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>10mM phosphate buffer (pH 2.6) containing 5mM hexanesulfonic acid sodium salt/acetonitrile = 9/1 (v/v)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.2mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>Detection</td>
<td>UV-VIS Detector 240nm</td>
</tr>
</tbody>
</table>

Fig. 1.13.1 Analysis of vitamin B group and caffeine in vitamin drink
1.14 Analysis of Vitamin B Group - LC

**Explanation**
Quantification methods for vitamins have shifted from biological methods to chemical methods. GC and HPLC incorporated methods are almost always used for fat-soluble Vitamins whereas GC analysis of water-soluble vitamins is complicated to the point that it is impractical thus the HPLC analysis method is the most favored. Ion conversion and normal-phase partition chromatography are used for separation but, from the point of view of column durability and analysis stability, reversed phase chromatography has become the mainstream method.

There are individual test methods for each vitamin, and chromatography simultaneous analysis capabilities for samples with comparatively few impurities and large amounts of target components are often found in medical products and drink materials. Here, the conditions for simultaneous analysis and the analysis example itself are shown for the vitamin B group.

**References**
Shimadzu HPLC Application Report No. 14

**Pretreatment**
None.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Shim-pack CLC-ODS(6.0mm×150mm)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>100mM sodium phosphate buffer (pH 2.1) containing 0.8mM octanesulfonic acid sodium salt/acetonitrile = 9:1 (v/v)</td>
</tr>
<tr>
<td>Temperature</td>
<td>40°C</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.5mL/min</td>
</tr>
<tr>
<td>Detection</td>
<td>UV-VIS Detector 210nm or 270nm</td>
</tr>
</tbody>
</table>

Fig. 1.14.1 Analysis example (210nm) of vitamin B group
Fig. 1.14.2 Analysis example (270nm) of vitamin B group
1.15 Analysis of Tocopherol in Milk - LC

**Explanation**
LC vitamin analysis is broadly separated into water-soluble vitamin analysis and fat-soluble vitamin analysis. Use of HPLC enables simultaneous analysis of the components, which has made it a popular form of analysis from the outset. Here, analysis of fat-soluble vitamin tocopherol is introduced.

**Pretreatment**
1. Add chloroform to sample for extraction.
2. After vaporizing and dry hardening the chloroform layer, the sample is dissolved in a small amount of hexane and then concentrated.
3. The dissolved liquid sample is injected.

**Analytical Conditions**
- **Instrument**: HPLC
- **Column**: Shim-pack CLC-NH₂ (6.0mmφ×150mm)
- **Mobile phase**: n-hexane/isopropyl alcohol = 100/4 (v/v)
- **Temperature**: 40°C
- **Flow rate**: 1.5mL/min
- **Detection**: UV-VIS Detector 297nm

**Peaks**
1. α-tocopherol
2. β-tocopherol
3. γ-tocopherol
4. δ-tocopherol

References
Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)
Shimadzu HPLC Application Data book (C190-E001)

Fig. 1.15.1 Analysis of tocopherol types in milk
1.16 Analysis (Measurement of K Value) of Nucleotide in Tuna Meat - LC

• Explanation
Nucleic acid base and nucleotide are usually analyzed using reversed phase chromatography as they can be simultaneously analyzed. Here, a separation example using reversed phase chromatography for 8 adenine derivative components is shown. This form of analysis is applied to measuring of fish freshness indicated by the K value (freshness constant) because the 4 kinds of nucleotides, hypoxanthine and inosine can be individually quantified.

\[
K = \frac{Hyp+Ino}{Hyp+Ino+IMP+AMP+ADP+ATP}
\]

References
Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)

• Pretreatment
1. Add 25mL of 1M perchloric acid to 10g of tuna meat and homogenize.
2. Centrifugally separated (3000 rpm for 5 min).
3. Skim off top layer, and add 1M potassium bicarbonate solution to adjust sample to pH 6.5.
4. Remove the created potassium perchlorate sediment, and filter top layer through membrane filter.
5. Inject 5μL of filtered solution.

• Analytical Conditions
Instrument: HPLC
Column: STR ODS-2 (4.6mm φ×150mm)
Mobile phase: A liquid/B liquid = 100/1 (v/v)
  A liquid: 100mM Phosphoric acid (triethylammonium)buffer (pH 6.8)
  B liquid: acetonitrile
Temperature: 40°C
Flow rate: 1.0mL/min
Detection: UV-VIS Detector 260nm

Fig. 1.16.1 Analysis of adenine derivative components
Fig. 1.16.2 Analysis of tuna meat
1.17 Analysis of Oligosaccharide in Beer - LC

**Explanation**
In the case of analysis of sugars using the partition method, the mobile phase is a mixture of water and acetonitrile used with an aminopropyl column. The elution position can be adjusted by changing the water to acetonitrile ratio.

Fig. 1.17.1 shows an analysis example of monosaccharide and oligosaccharide standard solutions and Fig. 1.17.2 shows an analysis example of oligosaccharide in beer.

**References**
Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)
Shimadzu HPLC Application Report No. 11 (C196-E036)

**Pretreatment**
Beer was injected without any pretreatment.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Shim-pack CLC-NH₂ (6.0 mm × 15 cm)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile/water = 60/40 (v/v)</td>
</tr>
<tr>
<td>Temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>Detection</td>
<td>Refractive index detector</td>
</tr>
</tbody>
</table>

Fig. 1.17.1 Analysis of sugar and oligosaccharide standard samples

Fig. 1.17.2 Analysis of oligosaccharides in beer
**Explanation**
Nonreducing sugars such as sucrose, raffinose and stachyose can be analyzed at high sensitivity and high selectivity by adding taurocyamine (as a fluorescent reaction agent for postcolumn fluorescence detection) to reducing sugar.

Fig. 1.18.1 shows an analysis example for mixed standard solutions of sucrose, raffinose and stachyose. Some 500pmol of each component was injected.

**References**
Shimadzu Application News No. L 226

**Pretreatment**
None.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Asahipak NH2P-50 (4.6mmφ×250mm)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile/water = 65/35 (v/v)</td>
</tr>
<tr>
<td>Temperature</td>
<td>40°C</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0mL/mi</td>
</tr>
<tr>
<td>Reaction liquid</td>
<td>20mM taurocyamine, 0.1M potassium tetraurate water solution containing 1mM sodium periodate (using 10NKOH solution to adjust to pH 12.5)</td>
</tr>
<tr>
<td>Reaction liquid rate</td>
<td>1.0mL/min</td>
</tr>
<tr>
<td>Reaction temperature</td>
<td>150°C</td>
</tr>
<tr>
<td>Detection</td>
<td>Fluorescence Detector (Ex320nm Em450nm)</td>
</tr>
</tbody>
</table>

**References**
1.19 Analysis of Sugar in Yogurt - LC

**Explanation**
The ligand conversion chromatography column SCR-101 series consists of the 101N, 101C and 101P types with ends made respectively of Na, Ca and Pb. And the retaining behavior of sugars differs with each one. In particular, in the case of sugar alcohol analysis, 101C or 101P is recommended. Also, glucose and galactose separation is possible with the 101C type. Fig. 1.19.1 shows an analysis example of a Japanese pickle liquid and Fig. 1.19.2 shows an analysis example of sugar in yogurt.

**References**
Shimadzu LC Application Report No. 11 (C196-E036)
Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)

**Pretreatment**
[Analysis of Japanese pickle liquid]
1. Filter the pickle liquid through a membrane filter.
2. Inject 10 µL of filtered liquid.

[Analysis of yogurt]
1. Add perchloric acid to the yogurt, and mix to deproteinize.
2. Centrifugally separate, and filter upper layer through a membrane filter.
3. Inject 10 µL of filtered liquid.

**Analytical Conditions**

[Pickel liquid]
- Instrument: HPLC
- Column: Shim-pack SCR-101C (7.9mmφ×300mm)
- Mobile phase: Water
- Temperature: 80°C
- Flow rate: 0.8mL/min
- Detection: Refractive index detector

[Yogurt]
- Instrument: HPLC
- Column: Shim-pack SCR-101C (7.9mmφ×300mm)
- Mobile phase: Water
- Temperature: 85°C
- Flow rate: 1.0mL/min
- Detection: Refractive index detector

**Fig. 1.19.1 Analysis of pickle liquid**

**Fig. 1.19.2 Analysis of yogurt**
1.20 Analysis of Fermented soybean paste (Miso) Using Reducing Sugar Analysis System - LC

**Explanation**
Anion exchange chromatography using a boric acid buffer as the mobile phase is capable of analyzing disaccharide and monosaccharide simultaneously. Generally differential refraction calculation is not a suitable form of detection in this type of analysis because the concentration and pH of the boric acid buffer have to be changed (gradient method). The optimum detection method _ postcolumn fluorescence detection method _ will be introduced here.

HPLC is regarded as suitable for the analysis of sugars. But sometimes the sample contains a lot of impurities or concentration is extremely low, so postcolumn fluorescence detection employing L-arginine (a base amino acid) as the detection agent is used to improve selectivity and sensitivity.

**References**
Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)
Shimadzu LC Application Report No. 4
Mikami, Ishida, Shimadzu Review, Nos. 40 (4), 63 (1983)

**Pretreatment**
After extraction using distilled water, filter the sample through a membrane filter.

**Analytical Conditions**

| Instrument | : HPLC |
| Column     | Shim-pack ISA-07/S2504 |
| Mobile phase | A: 0.1M potassium borate buffer (pH 8.0) |
|            | A: 0.4M potassium borate buffer (pH 9.0) |
| A/B        | 100/0 → 0/100 |
| Temperature | 65˚C |
| Flow rate  | 0.6mL/min |
| Reaction liquid | 3% boric acid water solution containing 1% L-arginine |
| Reaction liquid rate | 0.5mL/min |
| Reaction temperature | 150˚C |
| Detection  | Fluorescence Detector (Ex320nm Em430nm) |

**Peaks**
1. Sucrose
2. Fructose
3. Galactose
4. Isomaltose
5. Glucose

![Fig. 1.20.1 Analysis of miso using reducing sugar analysis system](image1)

![Fig. 1.20.2 Flow line diagram of reducing sugar analysis system](image2)

References
2.1 Propionic Acid in Cookies and Bread - GC

• Explanation
Propionic acid is one of the components that form flavor and fragrance, included in fermented products such as miso, soy sauce and cheese as a microbial metabolite. It is also used as a preservative in cookies and bread because of its low toxicity and minimal effect on bread yeast.

When propionic acid is analyzed using GC with FID, the total calculation of the natural propionic acid, which is inherently included in the food, and the added propionic acid is obtained as the quantitative value.

• Pretreatment
Propionic acid was extracted using steam distillation method.

• Analytical Conditions

<table>
<thead>
<tr>
<th>Instrument</th>
<th>GC-14BPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>10%PEG6000 3mm×1m on shimalite TPA (glass)</td>
</tr>
<tr>
<td>Col. Temp.</td>
<td>150°C</td>
</tr>
<tr>
<td>Inj. Temp.</td>
<td>230°C</td>
</tr>
<tr>
<td>Det. Temp.</td>
<td>200°C(FID)</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>N₂</td>
</tr>
</tbody>
</table>

Fig. 2.1.1 Analysis of propionic acid

Recommended Instrument Configuration

Main unit : GC-17AAFw
Detector : FID
Column : DB-WAX 0.32mm × 30m df=0.5μm
Auto injector : AOC-20i/s
Data processor : CLASS-GC10
2.2 Saccharine and Sodium Saccharine - GC

•Explanation
Saccharine and sodium saccharine are used as artificial sweeteners. Saccharine is only used in chewing gum because it does not dissolve easily in water whereas sodium saccharine does and is widely used in pickles and jams.
Saccharine and sodium saccharine are extracted from food products and refined, and after being methylated, they are analyzed by GC with FID or FPD. Here, a GC with FID analysis example will be introduced.

References
Standard Methods of Analysis for Hygienic Chemists (annotation) 493 to 495 (1990), edited by the Pharmaceutical Society of Japan.

•Pretreatment
1. Extract and refine sample by dialysis extraction or direct extraction.
2. Produce a derivative (methylate) of saccharine using diazomethane, etc.
3. Dissolve in ethyl acetate, etc. and use this liquid as the sample.

•Analytical Conditions
Instrument : GC-14BPF
Column : 5% SE-30 3mm×1.5m on chromosorb W (glass)
Col. Temp. : 190˚C
Inj. Temp. : 250˚C
Det. Temp. : 230˚C(FID)
Carrier gas : N₂

Recommended Instrument Configuration
Main unit : GC-17AAFw
Detector : FID
Column : DB-1 0.25mm × 30m df=0.25μm
Auto injector : AOC-20i/s
Data processor : CLASS-GC10

Fig. 2.2.1 Analysis of saccharine
2.3 Ethylene Glycols in Wine - GC

• Explanation
Normally wine does not contain ethylene glycol but there have been reports of temporary errors where diethylene glycol was mixed into wine. Here, ethylene glycol and diethylene glycol have been added to wine and directly analyzed by GC. Analysis was possible without any interference from impurities in the wine.

References
Shimadzu Application News No. G110

• Pretreatment
Ethylene glycol and diethylene glycol were added to a shop-sold wine for direct analysis.

• Analytical Conditions
Instrument : GC-14APF
Column : ULBON HR-20M 0.25mm×25m df=0.25µm
Col. Temp. : 150°C
Inj. Temp. : 200°C
Det. Temp. : 200°C(FID)
Carrier gas : He 2mL/min
Injection : Split 1:30

Recommended Instrument Configuration
Main unit : GC-17AAFW
Detector : FID
Column : DB-WAX 0.25mm × 30m df=0.25µm
Auto injector : AOC-20i/s
Data processor : CLASS-GC10

Fig. 2.3.1 Analysis of glycols (standard products)

Fig. 2.3.2 Analysis of shop-sold wine with glycols added
2.4 Sorbic Acid, Dehydroacetic Acid and Benzoic Acid - GC

**Explanation**
The preservatives sorbic acid, dehydroacetic acid and benzoic acid are analyzed by UV absorption spectrum method or GC method. The UV method is fast and efficient but can be affected by coexisting substances such as fragrances, whereas GC has the advantage of being able to easily separate out such substances. Here, these preservatives were extracted from a food product by direct extraction or steam distillation and refined to be analyzed by GC with FID.

**References**
Standard Methods of Analysis for Hygienic Chemists (annotation) 445 to 451 (1990), edited by the Pharmaceutical Society of Japan

**Pretreatment**
1. Direct extraction
   Add saturated saline solution and sulfuric acid, homogenize with strong acidity and extract with ethyl ether. Reversely extract the ether layer using sodium hydrogen carbonate solution, re-extract using ethyl ether, and concentrate. GC analyze the final liquid as an acetone.

2. Steam distillation
   Pulverize the sample, add water, and neutralize pH. Add tartaric acid solution and salt and perform steam distillation. Extract residue using ethyl ether as previously described.

**Analytical Conditions**

**Instrument**
: GC-14BPF

**Column**
: 5% DEGS + 1%H₃PO₄ 3mm × 2m on chromosorb W(glass)

**Col. Temp.**
: 185°C

**Inj. Temp.**
: 230°C

**Det. Temp.**
: 250°C(FID)

**Carrier gas**
: N₂

---

**Recommended Instrument Configuration**

<table>
<thead>
<tr>
<th>Component</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main unit</td>
<td>GC-17AAFw</td>
</tr>
<tr>
<td>Detector</td>
<td>FID</td>
</tr>
<tr>
<td>Column</td>
<td>DB-WAX 0.25mm × 30m df=0.25μm</td>
</tr>
<tr>
<td>Auto injector</td>
<td>AOC-20i/s</td>
</tr>
<tr>
<td>Data processor</td>
<td>CLASS-GC10</td>
</tr>
</tbody>
</table>

---

**Fig. 2.4.1 Analysis of Preservatives**
2.5 Analysis of Preservatives in Food Products with Absorption Photometry (1) - UV

**Explanation**
Various preservatives are added to preservative and processed foods to prevent putrefaction and to keep freshness. The use of these food additives is strictly governed by the Food Sanitation Law to ensure that concentrations do not exceed the permitted safe concentrations for human consumption. Here, preservatives in food products regulated by the Food Sanitation Law were analyzed with a Shimadzu double-beam spectrophotometer after pretreatment in accordance with the law.

**Pretreatment**
- Sodium nitrite in a food product
  The sodium nitrite preservative in meat was separated by distillation, and sulfamic acid was diazotized using nitrite acid under acidity of hydrochloric acid, and colored with naphthylethlenediamine for measurement.
- Benzoic acid in a food product
- Sorbic acid in a food product
- Dehydroacetic acid in a food product
  The dehydroacetic acid preservative was separated and extracted from bean jam using steam distillation in readiness for UV absorption measurement.

**Analytical Conditions**
- **Instrument**: UV Spectrophotometer
- **Reference**: blank
- **Solvent**: H2O
- **Cell**: 10mm
- **Range**: 0 – 2Abs

![Fig. 2.5.1 Absorption spectrum for sodium nitrite](image1)
![Fig. 2.5.2 Calibration curve for sodium nitrite](image2)
2.5 Analysis of Preservatives in Food Products with Absorption Photometry (2) - UV

Fig. 2.5.3 Absorption spectrum for benzoic acid

Fig. 2.5.4 Calibration curve for sorbic acid

Fig. 2.5.5 Absorption spectrum for sorbic acid

Fig. 2.5.6 Calibration curve for dehydroacetic acid

Fig. 2.5.7 Absorption spectrum for dehydroacetic acid
•Explanation
Color control is an important factor in quality control, as colors have large psychological effect and consumer image of products largely depends on the color of their coating resin or paint. Thus, colorimeters, which determine color of objects, are widely used in various fields.
Colorimetry methods are largely divided into two: one is spectral colorimetry in which a spectrophotometer is used to measure reflectance or transmittance spectrum, and the tristimulus values X, Y and Z are determined by calculation; the other is the direct reading of the tristimulus values where a photoelectric photometer is used to directly measure the tristimulus values.
Here, a measurement example using the color measurement software with the spectrophotometer UV-3100PC will be introduced.

Color Measurement of Processed Food Products available in consumer market
The colors of processed foods can greatly enhance their appearance for marketing purposes, which makes color control an important facet of the food industry. Here, color measurement was performed on shop-sold flavoring products.
1. Vinegar
2. Ketchup
3. Sauce
4. Mayonnaise

Vinegar was analyzed using transmittance measurement and the other products by reflective measurement. Fig. 2.6.1 shows the spectra for the products. Next, based on these spectrum data, the x, y, Z stimulus values and L*, a*, b* values were calculated under the conditions of C illuminant and 2* field of view. Fig. 2.6.2 shows the printout of the results. Also, under the same conditions, CIE (xy) and UCS chromaticity diagrams were drawn up (see Fig. 2.6.3 and Fig. 2.6.4). The xy chromaticity diagram shows chromaticity (hue and saturation) using the x and y chromaticity coordinates. The closer to the center, the lower the saturation. Color differences can be discerned at a glance. In the Lab chromaticity diagram, the left-side L* displays brightness between zero and 100 while a* and b* on the right denote chromaticity. Plus a* is the red direction, minus a* the green direction, plus b* the yellow direction and minus b* the blue direction. The closer to the center, the lower the saturation and the closer to the edge, the higher the saturation. This is the chromaticity diagram most widely used.

•Analytical Conditions

Instrument : UV-3101PC with color measurement software
Sample : Vinegar, ketchup, sauce, and mayonnaise
Reference : MgO
Range : 0 to 100%

Fig. 2.6.1 Transmittance and reflectance spectra
2.6 Color Control of Food Products (1) - UV

Measurement results of x, y, Z and L*, a*, b* values

Title : COLOR MEASUREMENT
Comment : UV-3100PC + ISR-3100
Illuminant : C  Field of view (degree) : 2
Reference value : 0.00 0.00 0.00 0.00 0.0000 0.0000
Sample ID  L*  a*  b*  Y  x  y
1  97.44  -2.67  12.39  93.51  0.3286  0.3406 Vinegar
2  20.59  21.05  17.83  3.14  0.4985  0.3491 Ketchup
3  9.46  2.88  3.37  1.06  0.3546  0.3345 Sauce
4  75.63  -2.77  20.77  49.29  0.3511  0.3657 Mayonnaise

Fig. 2.6.2 Measurement results

Fig. 2.6.3 CIE (x,y) chromaticity diagram

Fig. 2.6.4 UCS (Lab) chromaticity diagram
2.7 Analysis of Sweetener in Soft Drink - LC

**Explanation**
This is an example of simultaneous analysis of the sweeteners aspartame, saccharine, benzoic acid, sorbic acid and glycyrrhizic acid.

**References**
Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)

**Pretreatment**
A soft drink was directly injected without pretreatment.

**Analytical Conditions**
- **Instrument**: HPLC
- **Column**: STR ODS-M (4.6mm φ × 150mm)
- **Mobile phase**: 40mM sodium acetate buffer (pH 4.0)/methanol = 3/1 (v/v)
- **Temperature**: 40°C
- **Flow rate**: 1.0mL/min
- **Detection**: UV-VIS Detector 250nm

**Peaks**
1. Saccharine
2. Aspartame
3. Benzoic acid
4. Sorbic acid

Fig. 2.7.1 Analysis of sweetener in soft drink
2.8 Analysis of Fungicide in Oranges - LC

**Explanation**
In Japan the use of o-phenylphenol (OPP), thiabendazole (TBZ) and diphenyl is permitted for preventing mold in citrus. Here, the simultaneous analysis of these components using fluorescent detection will be introduced.

**References**
Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)

**Pretreatment**
1. Add 0.5g of anhydrous sodium acetate, 15g of anhydrous sodium sulfate and 40mL of ethyl acetate to 10g of orange, and homogenize twice.
2. Filter using glass filter.
3. Add 2.5mL of butanol to the acquired ethyl acetate layer.
4. Concentrate at 40˚C until 2.5mL is obtained.
5. Add methanol to dilute to 10mL and filter through membrane filter.
6. Inject 5µL of filtrate.

**Analytical Conditions**
- **Instrument**: HPLC
- **Column**: Shim-pack CLC-ODS (6.0mmφ × 150mm)
- **Mobile phase**: Acetonitrile/methanol/water = 30/35/35 (v/v/v)
  - Prepare it to pH 2.4 with phosphoric acid containing 10mM sodium dodecyl acetate.
- **Temperature**: 40˚C
- **Flow rate**: 1.0mL/min
- **Detection**: Fluorescence Detector (Ex285nm Em325nm)

**Peaks**
1. O-phenylphenol (OPP)
2. Thiabendazole (TBZ)
3. Diphenyl (DP)

Fig. 2.8.1 Analysis of fungicide in orange
2.9 Analysis of Chlorophyll in Spinach (1) - LC

**Explanation**
Chlorophyll is the green pigment required for photosynthesis in seed plants and seaweed. Chlorophyll is divided into types a to e, being dependant on its structure. Here, an analysis example for chlorophyll a and b in spinach will be introduced. Generally, chlorophyll shows a spectrum with characteristic absorption maximum at the 430 to 450 and 650nm regions. The detection and spectrum is provided by a photodiode array UV-Vis detector.

**References**
Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)

**Pretreatment**
1. Add 10mL of acetone to 10g of spinach, and mix.
2. Centrifugal separation (3000 rpm for 10 min).
3. Inject 10µL of the supernatant liquid.

**Analytical Conditions**
- **Instrument**: HPLC
- **Column**: STR ODS- II (4.6mmφ × 150mmL)
- **Mobile phase**: Methanol
- **Temperature**: 40˚C
- **Flow rate**: 1mL/min
- **Detection**: Photodiode array detection from 220nm to 700nm

**Peaks**
1. Chlorophyll a
2. Chlorophyll b

Fig. 2.9.1 Analysis of chlorophyll in spinach
2.9 Analysis of Chlorophyll in Spinach (2) - LC

Fig. 2.9.2 Spectrum of chlorophyll a

Fig. 2.9.3 Spectrum of chlorophyll b
2.10 Analysis of EDTA in Mayonnaise - LC

**Explanation**
EDTA in mayonnaise was analyzed after chelation of Fe ion. Reversed-phase ion pair chromatography with tetrabutylammonium ions was used for separation. In this analysis, a polymer column (ODP), instead of a silica column (ODS), was used because of the high pH of the mobile phase and the basicity of the tetrabutylammonium. The following chromatogram shows the measurement of marketed mayonnaise with PDTA (the internal standard substance) and EDTA added.

**Pretreatment**
1. Add chloroform to sample, mix together, and centrifugally separate (12000 r.p.m for 2 min, twice).
2. Add 0.01M FeCl3 solution to water layer and mix together.
3. Inject 20µL of sample.

**Analytical Conditions**
- **Instrument**: HPLC
- **Column**: Asahipak ODP-50 (6.0mm φ × 150mm)
- **Mobile phase**: 20mM sodium phosphate buffer (pH 6.9) containing 10mM tetrabutylammonium hydrogensulfate (adjust to pH 7.5 with 4M of NaOH)
- **Temperature**: 40°C
- **Flow rate**: 0.8mL/min
- **Detection**: UV-VIS Detector 255nm

**Peaks**
1. EDTA-Fe
2. PDTA-Fe (internal standard substance)

References
Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)
Shimadzu Application News No. L214 (C190-E050)

Fig. 2.10.1 Analysis of EDTA in mayonnaise
2.11 Analysis of p-Hydroxybenzoates in Soy Sauce - LC

**Explanation**
LC is a great force in the analysis of preservatives used in food products. In particular, LC is useful for simultaneous analysis of such components. Here, an analysis example for p-hydroxybenzoates added to soy sauce will be introduced.

**References**
Shimadzu Application News No. L222 (C190-E032)

**Pretreatment**
1. Add pure water to soy sauce until diluted by 10 fold.
2. Filter through membrane filter.
3. Inject 10µL of filtrate.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>HPLC</th>
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<tbody>
<tr>
<td>Column</td>
<td>STR ODS- II (4.6mmφ × 150mm)</td>
</tr>
</tbody>
</table>
| Mobile phase | 10mM sodium phosphate buffer  
(pH 2.6)/methanol = 1/1 (v/v) |
| Temperature | 40˚C |
| Flow rate   | 1.5mL/min |
| Detection   | UV-VIS Detector 270nm |

Peaks
1. Methyl p-Hydroxybenzoate
2. Ethyl p-Hydroxybenzoate
3. Isopropyl p-Hydroxybenzoate
4. Propyl p-Hydroxybenzoate
5. Iso butyl p-Hydroxybenzoate
6. Butyl p-Hydroxybenzoate

Fig. 2.11.1 Analysis of p-hydroxybenzoates in soy sauce
2.12 Simultaneous Analysis of Water-soluble Tar Pigments - LC

**Explanation**
Synthetic and natural compounds are used as food pigments, and HPLC is a powerful tool for analyzing such compounds. The photodiode array analysis, which allows simultaneous analysis at multiple wavelengths and spectrum display, further facilitates the analysis and identification of unknown components. Here, a simultaneous analysis example for water-soluble tar pigments will be introduced showing multi-chromatograms for each absorption wavelength using a photodiode array detector.

**References**
Masaaki Ishikawa et al; Summary of the 31st Annual Conference of the Japan Hygienic Chemistry Council (1994)

**Pretreatment**
None.

**Analytical Conditions**
- **Instrument**: HPLC
- **Column**: STR ODS-II (4.6mmφ × 150mm)
- **Mobile phase**: A: 20mM ammonium phosphate buffer (pH 6.8)/isopropanol = 25/1 (v/v)
  - B: Acetonitrile
- **Gradient elution of 2 liquids**
  - **Temperature**: 40°C
  - **Flow rate**: 1.0mL/min
  - **Detection**: Photodiode Array detection from 220nm to 700nm

**Gradient Conditions**

<table>
<thead>
<tr>
<th>Time</th>
<th>B concentration</th>
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<td>0.00 min</td>
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</tr>
<tr>
<td>15.00 min</td>
<td>20%</td>
</tr>
<tr>
<td>45.00 min</td>
<td>40%</td>
</tr>
<tr>
<td>55.00 min</td>
<td>70%</td>
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<tr>
<td>55.01 min</td>
<td>0%</td>
</tr>
<tr>
<td>65.00 min</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Peaks**
1. Yellow No. 4
2. Red No. 2
3. Blue No. 2
4. Yellow No. 203a
5. Red No. 102
6. Yellow No. 403
7. Yellow No. 5
8. Yellow No. 203b
9. Red No. 227
10. Red No. 40
11. Yellow No. 202
12. Orange No. 207
13. Red No. 503
14. Red No. 504
15. Green No. 401
16. Red No. 230
17. Orange No. 402
18. Bluish No. 1
19. Red No. 502
20. Red No. 3
21. Black No. 401
22. Orange No. 201
23. Red No. 106
24. Green No. 201
25. Red No. 104
26. Yellow No. 407
27. Yellow No. 406
28. Red No. 105
29. Red No. 506
30. Brown No. 201
31. Violet No. 401
32. Red No. 401
33. Red No. 213
34. Yellow No. 402

![Fig. 2.12.1 Simultaneous analysis of water-soluble tar pigments](image-url)
**3. Residual Pesticides**

### 3.1 Organophosphorus Pesticides in Farm Products (Onions) - GC

**Explanation**
To achieve good separation, using a capillary column is effective in simultaneous analysis of organophosphorus pesticides. An FPD or FTD detector can be used, but if analysis target is limited to organophosphorus pesticides, FPD is the best selection because of its high selectivity. Here, an analysis example where organophosphorus pesticides (29 components with absolute weight of 0.1 to 1.0ng) is added to shop-sold onions, pretreatment performed, and analysis performed using a GC with FPD will be introduced.

Selective and highly sensitive quantitative analysis of phosphorus compounds is possible if an FPD with P filter is used. This analysis method also eliminate the interference from sulfur compounds in onions and garlic.

**References**
Yasuhiko Sotoumi, Yumiko Nakamura, Yukari Hasegawa, Mamoru Fujimori, Yoshio Ito; Hygienic Chemistry, 36, 249 to 357 (1990)

---

**Pretreatment**
This pretreatment (Fig. 3.1.2) is an extraction method for screening to assist fast processing (*see reference material). There are two methods, one for soft fatless vegetables and fruits and the other for hard fat-loaded grain and beans. The latter includes the process of pulverization and defatting. In this example the former method was used on onions.

**Analytical Conditions**
- **Instrument** : GC-14BPPpsc
- **Column** : DB-1 0.25mm × 60m df = 0.25µm
- **Col. Temp.** : 60°C(2min)-180 (20°C/min)-265°C (10°C/minute)
- **Inj. Temp.** : 240°C
- **Det. Temp.** : 270°C (FPD)
- **Carrier gas** : He 1.2mL/min
- **Injection** : Splitless (1min)

**Recommended Instrument Configuration**
- **Main unit** : GC-17AAFw
- **Detector** : FPD-17c(P-filter)
- **Column** : DB-1 0.25mm × 60m df=0.25µm or DB-1 0.25mm × 30m df=0.25µm
- **Auto injector** : AOC-20i/s
- **Data processor** : C-R7Aplus or CLASS-GC10

---

**Fig. 3.1.1 Analysis example for onions using FPD**

**Fig. 3.1.3 Pretreatment flowchart**

3.2 Analysis of Pesticides Using NCI (1) - GCMS

**Explaination**
Trace analysis is required for the measurement of residual pesticides in vegetables and fruits, but it is difficult to extract only pesticides, even after a cleanup pretreatment. NCI is an effective method for this analysis. Generally, positive ions are detected in mass spectrometry, but negative-ion analysis may be used depending on the compound. The negative ions of such compounds allow microanalysis with minimal interference from the matrix. Trace amount of pesticides that cannot be detected using the conventional EI method can be detected by this method.

**Analytical Conditions**
- **Instrument**: GCMS-QP5050A
- **Column**: DB-1 0.25mm × 30m df=0.25µm
- **Col.Temp.**: 50°C(2min)-130°C(20°C/min)
- **I/F Temp.**: -300°C(3°C/min)(7min)
- **Inj.Temp.**: 280°C
- **I/F Temp.**: 280°C
- **Carrier Gas**: 120kPa(2min)-250kPa(2kPa/min)

---

Fig. 3.2.1 α-BHC mass spectrum (upper: EI, lower: NCI)
3.2 Analysis of Pesticides Using NCI (2) - GC/MS

Fig. 3.2.2 SIM chromatogram using EI

Fig. 3.2.3 SIM chromatogram using NCI

Fig. 3.2.4 MC and mass spectrum using EI

Fig. 3.2.5 MC and mass spectrum using NCI

Fig. 3.2.6 MC and mass spectrum using EI

Fig. 3.2.7 MC and mass spectrum using NCI
3.3 Analysis of Organotin in Fish (1) - GCMS

**Explanation**
Organotins such as tributyltin (TBT) and triphenyltin (TPT) are widely used as antifouling paints for ships and fishing nets, which has led to seawater and marine life pollution problems. Conventionally, such compounds are analyzed using GC-FPD, but here an analysis example for GCMS with superb qualitative accuracy will be introduced. Though tripentyltin (TPeT) is often used as the internal standard substance, this is not the best selection because TBT, TPT and TPeT have different recovery rates. In this example, a deuterium label compound that makes full use of GCMS features was used as the internal standard substance.

The advantage of the deuterium label compound as standard substance is that it is materially identical to the target compound but does not exist in the sample.

**Pretreatment**
Fig. 3.3.1 shows the methods of extraction from fish and seawater.

<table>
<thead>
<tr>
<th>Component</th>
<th>Selected ions (m/z)</th>
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<tbody>
<tr>
<td>d27-TBT</td>
<td>295, 293, 316</td>
</tr>
<tr>
<td>TBT</td>
<td>277, 275, 291</td>
</tr>
<tr>
<td>Tetra-BT</td>
<td>291, 289</td>
</tr>
<tr>
<td>TPeT</td>
<td>303, 305</td>
</tr>
<tr>
<td>d15-TPT</td>
<td>366, 364</td>
</tr>
<tr>
<td>TPT</td>
<td>351, 349</td>
</tr>
</tbody>
</table>

**Analytical Conditions**
- **Instrument**: GCMS-QP5000
- **Column**: DB-1 0.32mm × 30m df = 0.25µm
- **Column temperature**: 50°C(2min)-140°C(20°C/min)
- **Injection inlet temperature**: -220°C(7°C/min)-310°C(15°C/min)(6/min)
- **Interface temperature**: 300°C
- **Carrier gas**: He(40kPa)
- **Injection method**: Spitless(2min)

**Fig. 3.3.1 Extraction methods for organotin in fish and seawater**
3.3 Analysis of Organotin in Fish (2) - GCMS

Fig. 3.3.2 TBT mass spectrum

Fig. 3.3.3 d27-TBT mass spectrum

Fig. 3.3.4 TPT mass spectrum

Fig. 3.3.5 d15-TPT mass spectrum

Fig. 3.3.6 SIM chromatogram of standard sample

Fig. 3.3.7 TBT calibration curve (10 to 1000ppb)

Fig. 3.3.8 TPT calibration curve (10 to 1000ppb)

Fig. 3.3.9 SIM chromatogram of TBT in fish (sea bass)

Chart 3.3.10 Quantitative results for tin in fish and seawater
Residual Pesticides

3.4 Simultaneous Analysis of Pesticides (1) - GCMS

**Explanation**
Residual Pesticides on vegetables and fruits are a matter of concern. There are various kinds of pesticides used, among which approximately 200 are subjected to regulations. A good way of analyzing these pesticides is simultaneous GCMS measurement.

Here, an example of a simultaneous analysis of 86 pesticides using GCMS is shown.

### Analytical Conditions

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</tr>
<tr>
<td>2 Dichlorvos</td>
<td>220</td>
</tr>
<tr>
<td>3 Propanocarb</td>
<td>188</td>
</tr>
<tr>
<td>4 Acephate</td>
<td>183</td>
</tr>
<tr>
<td>5 Isoprocarb</td>
<td>193</td>
</tr>
<tr>
<td>6 Fenobucarb</td>
<td>207</td>
</tr>
<tr>
<td>7 Ethophos</td>
<td>242</td>
</tr>
<tr>
<td>8 Chlorproham</td>
<td>213</td>
</tr>
<tr>
<td>9 Benfocarb</td>
<td>223</td>
</tr>
<tr>
<td>10 Dimethipin</td>
<td>210</td>
</tr>
<tr>
<td>11 α-BHC</td>
<td>288</td>
</tr>
<tr>
<td>12 Dimethoate</td>
<td>229</td>
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<tr>
<td>13 Thiometon</td>
<td>246</td>
</tr>
<tr>
<td>14 β-BHC</td>
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<td>15 γ-BHC</td>
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<td>22 Mefentrazone</td>
<td>214</td>
</tr>
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</tr>
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</tr>
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<td>27 Fenitrothion</td>
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<td>28 Methiocarb</td>
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<td>265</td>
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<td>33 Malathion</td>
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<td>34 Aldrin</td>
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<td>36 Parathion</td>
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<tr>
<td>37 Chlorpyrifos</td>
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</tr>
<tr>
<td>38 Diethofencarb</td>
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<td>39 Captan</td>
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<tr>
<td>40 Heptachlor epoxide</td>
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<tr>
<td>41 Pendimethalin</td>
<td>281</td>
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<tr>
<td>42 α-Chlorpenevinphos</td>
<td>358</td>
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<td>43 Pyrifluous</td>
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<table>
<thead>
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<td>45 β-Chlorfenvinphos</td>
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<tr>
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<td>50 Trichlormethane</td>
<td>339</td>
</tr>
<tr>
<td>51 Methoprene</td>
<td>310</td>
</tr>
<tr>
<td>52 Flutolanil</td>
<td>323</td>
</tr>
<tr>
<td>53 Dieldrin</td>
<td>378</td>
</tr>
<tr>
<td>54 Prothionphos</td>
<td>344</td>
</tr>
<tr>
<td>55 Myclobutanil</td>
<td>288</td>
</tr>
<tr>
<td>56 p,p'-DDT</td>
<td>316</td>
</tr>
<tr>
<td>57 Ethion</td>
<td>311</td>
</tr>
<tr>
<td>58 Endrin</td>
<td>378</td>
</tr>
<tr>
<td>59 Fenvaltrion</td>
<td>308</td>
</tr>
<tr>
<td>60 Chlorbenzilate</td>
<td>324</td>
</tr>
<tr>
<td>61 p,p'-DDD</td>
<td>318</td>
</tr>
<tr>
<td>62 α,p'-DDT</td>
<td>352</td>
</tr>
<tr>
<td>63 Meproxylin</td>
<td>269</td>
</tr>
<tr>
<td>64 Lenacil</td>
<td>234</td>
</tr>
<tr>
<td>65 Edifenphos</td>
<td>310</td>
</tr>
<tr>
<td>66 Captan</td>
<td>347</td>
</tr>
<tr>
<td>67 p,p'-DDT</td>
<td>352</td>
</tr>
<tr>
<td>68 Propiconazol</td>
<td>341</td>
</tr>
<tr>
<td>69 EPN</td>
<td>323</td>
</tr>
<tr>
<td>70 Dicofol</td>
<td>370</td>
</tr>
<tr>
<td>71 Phosalone</td>
<td>367</td>
</tr>
<tr>
<td>72 Mefenacet</td>
<td>298</td>
</tr>
<tr>
<td>73 Amitraz</td>
<td>293</td>
</tr>
<tr>
<td>74 Cyhalothrin</td>
<td>449</td>
</tr>
<tr>
<td>75 Bietanel</td>
<td>337</td>
</tr>
<tr>
<td>76 Pyriaben</td>
<td>364</td>
</tr>
<tr>
<td>77 Inabentide</td>
<td>338</td>
</tr>
<tr>
<td>78 Permethrin</td>
<td>390</td>
</tr>
<tr>
<td>79 Cyfluthrin</td>
<td>363</td>
</tr>
<tr>
<td>80 Cypermethrin</td>
<td>415</td>
</tr>
<tr>
<td>81 Flucythrinate</td>
<td>451</td>
</tr>
<tr>
<td>82 Fenvalerate</td>
<td>419</td>
</tr>
<tr>
<td>83 Fluvinate</td>
<td>502</td>
</tr>
<tr>
<td>84 Pyrazoxylene</td>
<td>437</td>
</tr>
<tr>
<td>85 Deltamethrin</td>
<td>503</td>
</tr>
<tr>
<td>86 Trolomevine</td>
<td>661</td>
</tr>
</tbody>
</table>

Chart 3.4.1 List of pesticides and molecular weights
3.4 Simultaneous Analysis of Pesticides (2) - GCMS

Fig. 3.4.2 Analysis of 86 pesticides using DB-1
3.5 Analysis of Imazalil in Oranges - LC

**Explanation**
Fungicide imazalil is mostly contained in imported oranges and bananas imported to Japan. Here, analysis of imported oranges will be introduced. The target component was confirmed by comparison with UV spectrum of standard Sample using a photodiode array UV detector.

**Pretreatment**
Performed in accordance with Standard Methods of Analysis for Hygienic Chemists, annotation (supplement 1995).

**Analytical Conditions**
- **Instrument**: HPLC
- **Column**: STR ODS- II (4.6mmφ × 150mm)
- **Mobile phase**: 5mM (sodium) phosphate buffer (pH = 6.9/acetonitrile = 45/55 (v/v))
- **Flow rate**: 1.0mL/min
- **Temperature**: 40˚C
- **Detection**: Photodiode array detection
  \[ \lambda = 210\text{nm} \text{ to } 300\text{nm} \]

**References**
Shimadzu Application News No. L246 (C190-E068)

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*Fig. 3.5.1 Chromatogram of imazalil in imported orange sample (220nm)*

*Fig. 3.5.2 Spectra of imazalil (upper: standard sample, lower: sample)*
### Explanation

HPLC is introduced in the Official Gazette as an effective method for analyzing residual N-Methylcarbamate widely used as pesticides and herbicides. Fig. 3.6.1 shows the chromatogram of a standard pesticide sample containing 8 components specified in the Official Gazette, using the "Shimadzu Carbamate Analysis System" complying to the Official Gazette. Fig. 3.6.2 shows a chromatogram of a lemon to provide a practical application example of this system. The lemon sample was provided by Dr. Shimbujirou Hori and Dr. Hirotaka Obana at the Food Chemistry Dept. of the Osaka Prefectural Institute of Public Health after the pretreatment shown in Fig. 3.6.3.

### References

Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)
Shimadzu Application News No. L231 (C190-E061)
Yasui, Hayashi, Mikami; Shimadzu Review, 57 (2), 113 (1995)
Shimadzu LC Application Report No. 20

### Analytical Conditions

- **Separation Conditions**
  - **Instrument**: HPLC
  - **Column**: STR ODS-2 (4.6mmφ × 150mm)
  - **Mobile phase**
    - A: 10% methanol/water solution
    - B: 90% methanol/water solution
    - Binary gradient
  - **Temperature**: 50°C
  - **Flow rate**: 0.8mL/min

- **Reaction Conditions**
  - **Reaction liquid 1**: 50mM sodium hydroxide
  - **Temperature**: 100°C
  - **Reaction liquid 2**: o-phthalaldehyde/boric acid buffer
  - **Temperature**: 50°C
  - **Flow rate**: 0.4mL/min

- **Detection**: Fluorescence Detector
  - *(Ex340nm Em445nm)*

### Peaks

1. Oxamyl
2. Methiocarb sulfoxide
3. Methiocarb sulfone
4. Aldicarb
5. Bendiocarb
6. Carbaryl
7. Ethiofencarb
8. Isoprocarb
9. Fenobucarb
10. Methiocarb

---

**Fig. 3.6.1 Analysis of N-Methylcarbamate pesticides**

**Fig. 3.6.2 Analysis of lemon**
3.6 Analysis of N-Methylcarbamate Pesticides in Lemons (2) - LC

Residual Pesticides

Fig. 3.6.3 Pretreatment of extract for carbamate analysis
(Provided by Dr. Shinbujirou Hori and Dr. Hirotaka Obana, Food Chemistry Dept. of the Osaka Prefectural Institute of Public Health)
**3.7 Analysis of Carbofuran in Water - LC**

**Explanation**

In 1999, the then Ministry of Health and Welfare added the pesticides carbofuran to the water quality standard items to be monitored using LC. Carbofuran is one form of N-methylcarbamate pesticides widely used as a pesticide or herbicide. Here, the post-column derivatization method and direct measurement of the sample using natural fluorescence are used to analyze carbofuran.

**References**

Shimadzu Application News No. L260, L231 (C190-E061)

---

**Chart 3.7.1 Analytical conditions**

<table>
<thead>
<tr>
<th>Column</th>
<th>Shim-pack VP-ODS (4.6mmI.D.×150mmL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>Water/Methanol=55/45(v/v)</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.8mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>50°C</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>0.05M NaOH</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.4mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>100°C</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>120mM Borate/10mM NaOH containing 0.25mM OPA and 0.25mM β-Mercaptopropionic acid</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.4mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>50°C</td>
</tr>
<tr>
<td>Detection</td>
<td>Fluorescence (Ex340nm Em445nm)</td>
</tr>
</tbody>
</table>

**Chart 3.7.3 Analytical conditions**

<table>
<thead>
<tr>
<th>Column</th>
<th>Shim-pack VP-ODS (4.6mmI.D.×150mmL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>Water/Acetonitrile=7/3(v/v)</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1.0mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>40°C</td>
</tr>
<tr>
<td>Detection</td>
<td>Fluorescence (Ex279nm Em307nm)</td>
</tr>
</tbody>
</table>

---

**Fig. 3.7.2 Analysis of carbofuran standard product using post-column derivatization method**

**Fig. 3.7.4 Analysis of carbofuran standard product using direct fluorescent detection method**
**4. Aromas and Odors**

### 4.1 Aromatic Components of Alcohols - GC

**Explanation**
The headspace method enables analysis of volatile components in solids and liquids without complicated pretreatment. The following are the advantages of the headspace GC.

1) Components with low boiling points can be analyzed at high sensitivity.
2) Induction of components with high boiling points into GC can be prevented, reducing the analysis time.
3) Contamination of GC injection port and column is minimized because non-volatile components are not inducted into the GC.

Here, several analysis examples for volatile components in sake and whisky will be introduced.

**Pretreatment**
Shop-sold sake and whisky were sealed in 5mL vials and kept at 100˚C for 60 min.

**Analytical Conditions**

| Instrument | GC-14BPFsc + HSS-2B |
| Column | CBP20 0.32mm × 25m df = 0.5μm |
| Col. Temp. | 50˚C(5min)-10˚C/min-200˚C |
| Inj. Temp. | 230˚C |
| Det. Temp. | 230˚C(FID) |
| Carrier gas | He(1.35mL/min) |
| Injection method | Split(1:16) |
| Injection volume | 0.4mL |

**Recommended Instrument Configuration**

| Main unit | GC-14BPFsc |
| Detector | FID |
| Column | DB-WAX 0.32mm × 30m df=0.5μm |
| Headspace sampler | HSS-2B |
| Data processor | C-R7Aplus |
4.2 Aromatic Components of Tea - GC

**Explaination**
Volatile components in solid samples like tea can be easily analyzed using the headspace method. With this method, sample extraction by steam distillation is not required, as the sample is simply sealed to be analyzed.

**Pretreatment**
3g of tea leaf in 10mL of distilled water was kept at 100°C for 60 min.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>GC-14BPFsc+HSS-2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>CBP20 0.32mm × 25m df = 0.5µm</td>
</tr>
<tr>
<td>Col. Temp.</td>
<td>50°C(5min)-10°C/min-200°C</td>
</tr>
<tr>
<td>Inj. Temp.</td>
<td>230°C</td>
</tr>
<tr>
<td>Det. Temp.</td>
<td>230°C(FID)</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>He(1.4mL/min)</td>
</tr>
<tr>
<td>Injection method</td>
<td>Split(1:15)</td>
</tr>
<tr>
<td>Injection volume</td>
<td>0.4mL</td>
</tr>
</tbody>
</table>

**Peaks**
1. Acetaldehyde
2. Hexanal
3. Methyl n-Caproate
4. Isopropanol Alcohol
5. Ethyl n-Caproate
6. Hexyl Acetate
7. cis-3-Hexenyl Acetate or Hexanol
8. Methyl n-Caprylate
9. cis-3-Hexanol
10. cis-3-Hexenol
11. Ethyl n-Caprylate
12. Ethyl Acetoacetate
13. Linalool
14. Benzaldehyde
15. Ethyl n-Caprate
16. Geraniol

**Recommended Instrument Configuration**

| Main unit     | GC-14BPFsc |
| Detector      | FID |
| Column        | DB-WAX 0.32mm × 30m df=0.5µm |
| Headspace sampler | HSS-2B |
| Data processor | C-R7Aplus |
4.3 Essential Oil (Headspace Analysis) - GC

**Explanation**
This is an analysis example for essential oil used as flavors for food products.

**Pretreatment**
Essential oils were sealed in 5µL vials and kept at 40°C for 30 min.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>GC-14BPF+HSS-2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>CBP1 0.53mm × 25m df = 3.0µm</td>
</tr>
<tr>
<td>Col. Temp.</td>
<td>50°C(15min)-5°C/min-200°C</td>
</tr>
<tr>
<td>Inj. Temp.</td>
<td>230°C</td>
</tr>
<tr>
<td>Det. Temp.</td>
<td>230°C(FID)</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>He(10.5mL/min)</td>
</tr>
<tr>
<td>Injection method</td>
<td>Direct Injection</td>
</tr>
<tr>
<td>Injection volume</td>
<td>0.8mL</td>
</tr>
</tbody>
</table>

![Fig. 4.3.1 Analysis of orange oil](image1)
![Fig. 4.3.2 Analysis of lavender oil](image2)
![Fig. 4.3.3 Analysis of spearmint oil](image3)

**Recommended Instrument Configuration**

- **Main unit**: GC-14BPF + WBC attachment or GC-17AAFw + WBI-17
- **Detector**: FID
- **Column**: DB-1 0.53mm × 30m df=5.0µm
- **Headspace sampler**: HSS-2B or HSS-4A
- **Data processor**: C-R7Aplus
4.4 Essential Oil (Direct Analysis) - GC

**Explanation**
Here, direct GC analysis examples of peppermint oil and spearmint oil used as flavorings are introduced.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>GC-14BPFsc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>ULBON HR-20M 0.25m × 50m df = 2.5μm</td>
</tr>
<tr>
<td>Col. Temp.</td>
<td>60°C-3°C/min-220°C</td>
</tr>
<tr>
<td>Inj. Temp.</td>
<td>250°C</td>
</tr>
<tr>
<td>Det. Temp.</td>
<td>250°C(FID)</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>He(1.4mL/min)</td>
</tr>
<tr>
<td>Injection method</td>
<td>Split(1:15)</td>
</tr>
<tr>
<td>Injection volume</td>
<td>0.2μL</td>
</tr>
</tbody>
</table>

**Recommended Instrument Configuration**

- **Main unit**: GC-17AAFW
- **Detector**: FID
- **Column**: ULBON HR-20M 0.25mm × 50m df=0.25μm
- **Headspace sampler**: AOC-20i/s
- **Data processor**: C-R7Aplus or CLASS-GC10

---

Fig. 4.4.1 Analysis of peppermint oil

Fig. 4.4.2 Analysis of spearmint oil

1. Limonene
2. 1,8-Cineole
3. trans-Sabinenehydrate
4. L-Menthone
5. Mentholuran
6. D-Isomenthone
7. Neo-Menthol
8. Terpinene-4-ol
9. β-Caryophyllene
10. Menthol

1. Limonene
2. 1,8-Cineole
3. Menthone
4. Terpinene-4-ol
5. β-Caryophyllene
6. Dihydrocarvone
7. Carvone
4.5 Diketones - GC

**Explanation**
This introduces analysis examples using a headspace system with ECD for diketones contained in brewed products such as sake.

**Pretreatment**
5mL of solution samples or 3g of solid samples were sealed in vials and kept at 60˚C for 40 min.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>GC-14APE+HSS-2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>DB-WAX 0.25mm × 60m</td>
</tr>
<tr>
<td>df</td>
<td>0.25µm</td>
</tr>
<tr>
<td>Col. Temp.</td>
<td>40˚C</td>
</tr>
<tr>
<td>Inj. Temp.</td>
<td>200˚C</td>
</tr>
<tr>
<td>Det. Temp.</td>
<td>200˚C (ECD, Current 0.5nA)</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>He (1.7mL/min)</td>
</tr>
<tr>
<td>Injection method</td>
<td>Split (1:15)</td>
</tr>
<tr>
<td>Injection volume</td>
<td>0.4mL</td>
</tr>
</tbody>
</table>

Fig. 4.5.1 Analysis of strong soy sauce

Fig. 4.5.2 Analysis of Japanese sake

Fig. 4.5.3 Analysis of shochu

*Peaks*
1. Diacetyl
2. 2,3-Pentanedione
3. 2,3-Hexanedione
   (internal standard)

**Recommended Instrument Configuration**

- Main unit: GC-14BPEsc
- Detector: ECD
- Column: DB-WAX 0.25mm × 60m df=0.25µm
- Headspace sampler: HSS-2B
- Data processor: C-R7Aplus
**4.6 Fruit Fragrances - GC**

**Explanation**
This introduces several analysis examples using a headspace system for various fruit fragrances. The results show how lower alcohol and esters form distinctive fruit fragrances.

**Pretreatment**
10g of fruit samples were sealed in vials and kept at 60°C for 30 min.

**Analytical Conditions**
- **Instrument**: GC-14BPFsc+HSS-2B
- **Column**: DB-WAX 0.25mm × 60m df = 0.25µm
- **Col. Temp.**: 50°C(5min)-10°C/min-200°C
- **Inj. Temp.**: 230°C
- **Det. Temp.**: 230°C(FID)
- **Carrier gas**: He(1.1mL/min)
- **Injection method**: Split(1:18)
- **Injection volume**: 0.8mL

---

**Fig. 4.6.1 Analysis of melon**

**Fig. 4.6.2 Analysis of strawberry**

**Fig. 4.6.3 Analysis of banana**

**Recommended Instrument Configuration**
- **Main unit**: GC-14BPFsc
- **Detector**: FID
- **Column**: DB-WAX 0.25mm × 60m df=0.25µm
- **Headspace sampler**: HSS-2B
- **Data processor**: C-R7Aplus
4.7 Vegetable Fragrances - GC

•Explanation
This introduces several analysis examples using a headspace system for many vegetable fragrances. The results show how terpene compounds are a main component in providing vegetables with earthy, fresh fragrances.

•Pretreatment
Suitable amount of vegetable samples were sealed in vials and kept at 40°C for 30 min.

•Analytical Conditions

Instrument : GC-14BPF+HSS-2B
Column : CBP1 0.53mm × 25m df = 3.00μm
Col. Temp. : 50°C(15min)-5°C/min-200°C
Inj. Temp. : 230°C
Det. Temp. : 230°C(FID)
Carrier gas : He(10.5mL/min)
Injection method : Direct
Injection volume : 0.8mL

Peaks
1 α-Pinene
2 β-Pinene
3 Myrcene
4 Cineole/ Limonene

Fig. 4.7.1 Analysis of perilla (1.5g)
Fig. 4.7.2 Analysis of parsley (2g)
Fig. 4.7.3 Analysis of ginger (10g)

Recommended Instrument Configuration
Main unit : GC-14BPF + WBC attachment or GC-17AAFw + WBI-17
Detector : FID
Column : DB1 0.53mm × 30m df = 5.0μm
Headspace sampler : HSS-2B or HSS-4A
Data processor : C-R7Aplus
4.8 Flavoring Agent for Food Product - GC

**Explanation**
This introduces several analysis examples using a headspace system for flavoring agents that give sweet fragrances to cookies, etc. Examples of sweets are also given.

**Pretreatment**
Suitable amount of standard flavoring agent and sweets were sealed in vials and kept at 130°C for 40 min.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>GC-14BPF+HSS-2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>CBP1 0.53mm × 25m df = 3.0µm</td>
</tr>
<tr>
<td>Col. Temp.</td>
<td>90°C-6°C/min-230°C</td>
</tr>
<tr>
<td>Inj. Temp.</td>
<td>300°C</td>
</tr>
<tr>
<td>Det. Temp.</td>
<td>300°C(FID)</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>He(4.3mL/min)</td>
</tr>
<tr>
<td>Injection method</td>
<td>Direct</td>
</tr>
<tr>
<td>Injection volume</td>
<td>0.8mL</td>
</tr>
</tbody>
</table>

**Peaks**

1. Ethyl n-Butyrate
2. Isoamyl Acetate
3. Ethyl Isovalerate
4. Benzoic Acid
5. Isoamyl n-Butyrate
6. Allyl n-Caprate
7. Ethyl n-Heptanoate
8. Isoamyl Isovalerate
9. 2-Menthol
10. Ethyl n-Caprylate
11. γ-Nonalactone
12. Vanillin
13. Ethyl n-Caprate

**Recommended Instrument Configuration**

- **Main unit**: GC-14BPF-WBC attachment or GC-17AAFw + WBI-17
- **Detector**: FID
- **Column**: DB1 0.53mm × 30m df = 5.0µm
- **Headspace sampler**: HSS-2B or HSS-4A
- **Data processor**: C-R7Aplus
4.9 Analysis of Fishy Smell in Water (1) - GCMS

**Explanation**
Fishy smells are attributed to unsaturated aldehyde in uroglene Americane and has become a problem in drinking water supplies along with musty smell ever since vast outbreaks of it occurred in Lake Biwa in 1995. The 4 compounds of unsaturated aldehyde with carbon number 7 or 10 trans, cis-2,4-heptadienal and trans, cis-2,4-decadienal are the cause of this fishy smell. The purge & trap method is more effective than the headspace method to analyze these substances because of the low vapor pressure. The threshold values of these substances as odors are several 100ppb, and the lower detection limit of this method is several ppb.

**References**
Shimadzu Application News No. M181

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>GCMS-QP5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>DB-1701 0.32mm × 30m df = 1.0µm</td>
</tr>
<tr>
<td>Col.Temp.</td>
<td>40°C(8min)-200°C(20°C/min)(5min)</td>
</tr>
<tr>
<td>Int.Temp.</td>
<td>230°C</td>
</tr>
<tr>
<td>I/F Temp.</td>
<td>230°C</td>
</tr>
<tr>
<td>Carrier Gas</td>
<td>He(20kPa)</td>
</tr>
<tr>
<td>Instrument</td>
<td>Tekmar 3000J</td>
</tr>
<tr>
<td>Sample Size</td>
<td>5mL(35°C)</td>
</tr>
<tr>
<td>Trap Tube</td>
<td>Tenax GR</td>
</tr>
<tr>
<td>Purge</td>
<td>11min</td>
</tr>
<tr>
<td>Dry Purge</td>
<td>3min</td>
</tr>
<tr>
<td>Desorb</td>
<td>225°C, 8min</td>
</tr>
</tbody>
</table>

---

**Fig. 4.9.1 TIC chromatogram of fishy smell components**

---

**TIC**

- 1-1, 1-2: 2,4-Heptadienal
- 2-1, 2-2: 2,4-Decadienal
4.9 Analysis of Fishy Smell in Water (2) - GCMS

Fig. 4.9.2 Mass spectrum

Fig. 4.9.3 SIM chromatogram of 100ppt

Fig. 4.9.4 SIM chromatogram of 1ppb

Fig. 4.9.5 Calibration curve for 2,4-heptadienal

Fig. 4.9.6 Calibration curve for 2,4-decadienal


**Explanation**

There are two headspace methods: static headspace method and dynamic headspace method. Generally, the term headspace method refers to the static headspace method.

The dynamic headspace method refers to a method where purge gas is continuously fed into the sample to purge out volatile elements, and then the volatile elements are concentrated onto the trapping agent. After concentration, target components are desorped and analyzed by GCMS. This method enables microanalysis because it involves the concentration of the sample.

Here, the difference between the static and dynamic headspace methods will be shown using Japanese sake and wine.

A Chrompack CP4010 and a Tenax trapping set were used in the dynamic headspace analysis.

Sensitivity was clearly higher in dynamic headspace analysis.

**Analytical Conditions**

- **Instrument**: GCMS-QP5000
- **Column**: DB-1701 0.32mm × 30m df = 1.0µm
- **Col.Temp.**: 40°C(5min)-250°C(5°C/min)(5min)
- **Int.Temp.**: 250°C
- **Carrier Gas**: He(35kPa)
- **HS – Instrument**: HSS-4A
- **Sample Size**: 10mL
- **Sample Temp.**: 60°C
- **Thermostat**: 30min
- **Injection**: 0.8mL
- **– TCT – Instrument**: CP4010+Tenax Trap Set
- **Sample Size**: 20mL(room Temp.)
- **Purge**: 20mL/min(5min)
- **Trap Tube**: TenaxGR(0.1g)
- **Precool**: –150°C(3min)
- **Thermal**: 250°C(5min)

---

**Fig. 4.10.1 CP4010 flow line diagram**

**Fig. 4.10.2 Schematic diagram of Tenax Trapping Set**
4.10 Analysis of Alcohols (2) - GCMS

Fig. 4.10.3 TIC chromatogram of Japanese sake (HS method)

Fig. 4.10.4 TIC chromatogram of Japanese sake (TCT method)

Fig. 4.10.5 TIC chromatogram of wine (HS method)

Fig. 4.10.6 TIC chromatogram of wine (TCT method)
Aromas and Odors

4.11 Analysis of Strawberry Fragrances - GCMS

• Explanation
Strawberry fragrance components consist of fatty acid methyl esters from C2 to C6. Old and new varieties of marketed strawberries were compared and the correlation between type and fragrance studied. Normally, steam distillation or the headspace method is used for pretreatment of fragrance components; however, sometimes problems occur with the heating process. In the case of strawberries, heat destroys cells and release large amounts of special esters that are sometimes mistaken for fragrance components. Here, the Chrompack CP4010 + GCMS system (TCT + GCMS) was used to dry-air purge the sample without heating to enable optimum measurement of strawberry fragrances.

• Analytical Conditions

<table>
<thead>
<tr>
<th>Instrument</th>
<th>GCMS-QP5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>DB-624 0.25mm × 60m df = 1.4µm</td>
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<td>Col. Temp.</td>
<td>40°C (5min) – 230°C (5°C/min)(5min)</td>
</tr>
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<td>I/F Temp.</td>
<td>230°C</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>He(100kPa)</td>
</tr>
<tr>
<td>Instrument</td>
<td>CP4010 (TCT mode)</td>
</tr>
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<td>Sample amount</td>
<td>10g (room temperature)</td>
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<tr>
<td>Trap tube</td>
<td>Tenax TA</td>
</tr>
<tr>
<td>Pre-cool</td>
<td>−150°C, 5min</td>
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<tr>
<td>Pre-flush</td>
<td>50°C, 1min</td>
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<td>Thermal desorption</td>
<td>250°C, 10min, 20mL/min</td>
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Fig. 4.11.1 TIC chromatogram of strawberry fragrance components (upper: old brand, lower: new brand)
**4.12 Analysis of Beverage Odors (1) - GCMS**

**Explanation**
2,4,6-trichloroanisole (2,4,6-TCA), a cause of musty odor, is contained in wood and paper-manufactured packing materials, and its transfer to food products and drinking water may cause problem. The perceptual threshold value of TCA in water is extremely low at the ppt level. Conventional, 2,4,6-TCA was analyzed by solvent extraction or steam distillation method, but these methods require a lot of time and are extremely complicated; moreover, the poor collection rate would make ppt-level measurement difficult.

Here, measurement was conducted using a combination of the Chrompack CP4010 and Tenax trapping set. In this method, the sample is purged to collect the target components in the trap tube. The trap tube is heated by the TCT mode of the CP4010, and the desorbed components are analyzed by GCMS.

This system setup is an offline one, so the Tenax unit is easy to clean and there is no sample memory.

**Analytical Conditions**
- **Instrument**: GCMS-QP5000
- **Column**: DB-1701 0.32mm × 30m df = 1.0µm
- **Col.Temp.**: 50°C(2min)-140°C(30°C/min)
  -220°C(10°C/min)
- **I/F Temp.**: 250°C
- **Carrier Gas**: He(50kPa)
- **Sample Size**: 25mL(50°C)
- **Trap Tube**: Tenax GR
- **Purge**: 50°C, 15min, 100mL/min
- **Desoption**: 250°C, 5min

Fig. 4.12.1 Schematic diagram of Tenax Trapping Set

Fig. 4.12.2 TCT main unit flow line diagram
4.12 Analysis of Beverage Odors (2) - GCMS

Sample: 4.5ng/L Japanese sake

Fig. 4.12.5 Analysis of Japanese sake (4.5ng/L added)

Sample: 3ng/L black tea

Fig. 4.12.6 Analysis of black tea (3ng/L added)
4.13 Analysis of Fragrant Material (1) - GCMS

**Explanation**
Many fragrant components are contained in food products. These components are compounds of alcohols, esters, aldehydes, ketones, terpenes and others. The amount and mixture ratio of these components determine the aroma, and any aroma can be artificially synthesized by mixing these components. Here, some 100-aroma components were mixed together and analyzed by GCMS.

**Analytical Conditions**
- **Instrument**: GCMS-QP5000
- **Column**: DB-WAX 0.25mm × 60m df = 0.25µm
- **Col.Temp.**: 70˚C(5min)-210˚C(3/min)(30/min)
- **Inj.Temp.**: 250˚C
- **Int.Temp.**: 230˚C
- **Carrier Gas**: He(180kPa)
- **Injection**: Split(100:1)

![Fig. 4.13.1 TIC chromatogram of fragrant components](image_url)
### 4.13 Analysis of Fragrant Material (2) - GCMS

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<th>Terepene</th>
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Chart 4.13.2 Component names 1
## 4.13 Analysis of Fragrant Material (3) - GCMS

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<th>Compound</th>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl vanillin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzyl benzoate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5. Inorganic Metals

5.1 Analysis of Inorganic Components in Powdered Milk (1) - ICP-AES

• Explanation
The microwave sample decomposition method is quicker than the conventional wet decomposition method and takes place in a sealed system to prevent external contamination and volatilization loss of components such as As and Se. It is an extremely useful method to decompose the sample when the sample amount is small, or when a micro-amount element is to be analyzed. Here, powdered milk was liquidized using a microwave decomposition unit and analyzed using ICP-AES. The ICP-AES, which causes little self-absorption and has a wide dynamic range, enables analysis of major components like sodium and calcium, as well as minor components such as cadmium and lead, in the same solution. Arsenic, selenium and antimony can be analyzed at higher sensitivity by using a hydride generator.

• Analytical Conditions

Instrument: ICPS-7500
- HVG-1 (hydride generator)
- 27.12MHz
- 1.2kW
- Ar 14.0L/min
- Ar 1.2L/min
- Ar 0.7L/min
- Ar 3.5L/min
- 0.6mL/min
  (Hydride generating method: 2.5mL/min)

Observation method: Horizontal/axial
Sample injection system: Coaxial nebulizer/cyclone chamber, hydride generator

• Pretreatment
See Fig. 5.1.1 for details of the operation flow for microwave decomposition.

Fig. 5.1.1 Microwave decomposition flowchart
# 5.1 Analysis of Inorganic Components in Powdered Milk (2) - ICP-AES

## Chart 5.1.2 Powdered milk analysis results (µg/g)

<table>
<thead>
<tr>
<th>Element</th>
<th>Measured value</th>
<th>Element</th>
<th>Measured value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>1259</td>
<td>Si</td>
<td>23</td>
</tr>
<tr>
<td>Mg</td>
<td>376</td>
<td>Ba</td>
<td>1.4</td>
</tr>
<tr>
<td>P</td>
<td>2238</td>
<td>Ni</td>
<td>0.21</td>
</tr>
<tr>
<td>K</td>
<td>4644</td>
<td>Sn</td>
<td>0.2</td>
</tr>
<tr>
<td>Ca</td>
<td>3960</td>
<td>Cr</td>
<td>0.04</td>
</tr>
<tr>
<td>Mn</td>
<td>0.34</td>
<td>Cd</td>
<td>0.022</td>
</tr>
<tr>
<td>Fe</td>
<td>75</td>
<td>Pb</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Cu</td>
<td>2.8</td>
<td>As</td>
<td>0.007*</td>
</tr>
<tr>
<td>Zn</td>
<td>23</td>
<td>Sb</td>
<td>0.002*</td>
</tr>
<tr>
<td>Al</td>
<td>3.0</td>
<td>Se</td>
<td>0.03 *</td>
</tr>
</tbody>
</table>

* HVG hydride generator used

## Figures

- **Fig. 5.1.3 Zn calibration curve**
- **Fig. 5.1.4 As calibration curve**
- **Fig. 5.1.5 Fe calibration curve**
- **Fig. 5.1.6 Se calibration curve**
5.1 Analysis of Inorganic Components in Powdered Milk (3) - ICP-AES

**Explanation**
Here, a standard powdered milk was analyzed after incineration. The results show that nearly all the inorganic components conformed to the guaranteed values.

**Sample**
Non-fat Milk Powder (SRM 1549:NIST)
Skim Milk Powder (CRM 063:BCR)

**References**
- Standard Methods of Analysis for Hygienic Chemists (Annotation), edited by the Pharmaceutical Society of Japan, published by Kanehara & Co., Ltd
- Analysis Manual for the Standard Tables of Food Composition in Japan 5th rev, edited by the Resources Council of the former Science and Technology Agency, published by the Japan Resources Association

**Pretreatment**
1g of sample was placed on a platinum dish and incinerated to ash over 12 hours at 550˚C using an autoclave. 1mL of nitric acid was added to the incinerated sample to dissolve it. Finally, ultra pure water was added to make 100mL of the sample solution.

**Analytical Conditions**
Instrument: ICPS-8000
High frequency: 27.12MHz
High frequency output: 1.2kW
Cooling gas: Ar 14.0L/min
Plasma gas: Ar 1.2L/min
Carrier gas: Ar 0.7L/min
Purge gas: Ar 3.5L/min
Sample suction rate: 1.0mL/min
Observation method: Horizontal
Sample induction: Coaxial nebulizer

---

<table>
<thead>
<tr>
<th>Element</th>
<th>NIST-SRM 1549</th>
<th>BCR-CRM 063</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quantitative value</td>
<td>Guaranteed value</td>
</tr>
<tr>
<td>Na</td>
<td>0.51</td>
<td>0.47±0.03</td>
</tr>
<tr>
<td>K</td>
<td>1.68</td>
<td>1.69±0.03</td>
</tr>
<tr>
<td>Ca</td>
<td>1.31</td>
<td>1.3 ±0.03</td>
</tr>
<tr>
<td>Mg</td>
<td>0.123</td>
<td>0.120±0.003</td>
</tr>
<tr>
<td>P</td>
<td>1.07</td>
<td>1.06±0.2</td>
</tr>
<tr>
<td>Fe*</td>
<td>2.3</td>
<td>1.78±0.10</td>
</tr>
<tr>
<td>Zn*</td>
<td>47.4</td>
<td>46.1±2.2</td>
</tr>
<tr>
<td>Mn*</td>
<td>0.27</td>
<td>0.26±0.06</td>
</tr>
</tbody>
</table>

*: µg/g ( ): Reference value

Chart 5.1.7 Analysis results for standard powdered milk (wt-%)
5.2 Analysis of Pb in Milk Using Atomic Absorption Spectrophotometry - AA

**Explanation**

Lead is harmful to human body and stricter regulations are being applied to lead in food and pharmaceutical products. Lead can be effectively detected by electrothermal atomization with atomic absorption. Analysis of Pb in milk generally involves the flame method or electrothermal atomization where an acid is added and the sample is thermally decomposed. However, these methods require time-consuming pretreatment.

With direct analysis using electrothermal atomization, oxygen is often added during incineration to enhance the decomposition of organic matter in milk. However, the oxygen causes the deterioration of the graphite tube. Here, the use of a platform tube, instead of the graphite tube, allowed accurate measurement without the addition of oxygen or air.

**Analytical Conditions**

- **Instrument**: AA
- **Wavelength**: Pb 283.3nm
- **Lamp current Low (mA)**: 10
- **Lamp current High (mA)**: 0
- **Slit width (nm)**: 0.5
- **Background correction**: BGC-D₂

### Chart 5.2.1 Heat program

<table>
<thead>
<tr>
<th>Stage</th>
<th>Temperature (°C)</th>
<th>Time (sec)</th>
<th>Heat mode</th>
<th>Gas</th>
<th>Inner gas flow rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>3</td>
<td>lamp</td>
<td>Ar</td>
<td>0.20</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>30</td>
<td>lamp</td>
<td>Ar</td>
<td>0.50</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>20</td>
<td>lamp</td>
<td>Ar</td>
<td>1.00</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>10</td>
<td>lamp</td>
<td>Ar</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>700</td>
<td>10</td>
<td>step</td>
<td>Ar</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>700</td>
<td>3</td>
<td>step</td>
<td>Ar</td>
<td>0.0H</td>
</tr>
<tr>
<td>7</td>
<td>2400</td>
<td>3</td>
<td>step</td>
<td>Ar</td>
<td>0.0H</td>
</tr>
<tr>
<td>8</td>
<td>2600</td>
<td>2</td>
<td>step</td>
<td>Ar</td>
<td>1.00</td>
</tr>
</tbody>
</table>

### Chart 5.2.2 Measurement results for Pb in milk

<table>
<thead>
<tr>
<th>Measurement results</th>
<th>Added amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air added</td>
<td>10.5ppb</td>
</tr>
<tr>
<td>Air not added</td>
<td>10.4ppb</td>
</tr>
</tbody>
</table>

### Chart 5.2.3 Peak profile and calibration curve of Pb in milk

Fig. 5.2.3 Peak profile and calibration curve of Pb in milk

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5.3 Analysis of Inorganic Ions in Milk (1) - LC

• Explanation
Ion chromatography is the best method for analyzing inorganic ions in food products. In particular, use of a dual flow line system allows simultaneous analysis of anions and cations, which is useful in ion balance measurement. Here, an application example for analysis of inorganic ions in milk will be introduced.

References
Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)
Yagi, Funato, Ito; Analytical Chemistry, 38 (11), 655 (1989)

Quantitative lower limit:
Approximately 0.1 to 1ppm with standard product (differs depending on component)

• Pretreatment
The sample was injected into column after deproteinization with ultrafiltration membrane.

• Analytical Conditions

  • Anions
  Column: Shim-pack IC-A3(4.6mm φ × 150mm)
  Mobile phase: 8.0mM p-hydroxy benzoic acid
                3.2mM tris hydroxy aminomethane
  Temperature: 40°C
  Flow rate : 1.5mL/min
  Detection : Conductivity

  • Cations
  Column: Shim-pack IC-C3(4.6mm φ × 100mm)
  Mobile phase: 3.0mM oxalic acid
  Temperature: 40°C
  Flow rate : 1.2mL/min
  Detection : Conductivity

Fig. 5.3.1 Analysis of inorganic anions
Fig. 5.3.2 Analysis of inorganic cations
5.3 Analysis of Inorganic Ions in Milk (2) - LC

References

Fig. 5.3.3 Diagram of dual flow line system
### 5.4 Analysis of Pb in White Sugar Using Atomic Absorption Spectrophotometry (1) - AA

**Explanation**

Lead is harmful to human body and stricter regulations are being applied to lead in food and pharmaceutical products. Lead can be effectively detected by the electrothermal atomization with atomic absorption. The 13th revision of the Japanese Pharmacopoeia requires the measurement of lead, instead of heavy metal, using the electrothermal atomization method in purity tests for refined white sugar.

Here, analysis was performed in accordance with the Pharmacopoeia, with pretreatment (see Chart 5.4.1) and sample preparation using an autosampler for the standard addition method.

Chart 5.4.2 shows the measurement parameters and Fig. 5.4.3 shows the measurement results. Lead was not detected in the analyzed white sugar, but 1ppb of lead was clearly detected in the calibration curve. It can be said that this analysis method is effective for the detection of 0.5 ppm lead in white sugar (5ppb or less in processed solution), which is specified in the standard.

**Analytical Conditions**

| Instrument | AA |
| Wavelength | Pb 283.3nm |
| Lamp current Low (mA) | 10 |
| Lamp current High (mA) | 0 |
| Slit width (nm) | 0.5 |
| Background correction | BGC-D2 |

Accurately load 0.050g of sample into polytetrafluoroethylene container
↓
Add 0.5mL of nitric acid to dissolve sample
↓
Seal container and heat for 5 hr at 150°C
↓
Cool. add water to accurately make 5mL of sample solution
↓
Analyze using standard addition method for AA (electrothermal)

**Chart 5.4.1 Pretreatment for Pb in refined white sugar**

<table>
<thead>
<tr>
<th>Temperature Program</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final stage No. of concentration in oven : 5</td>
</tr>
<tr>
<td>Concentration frequency : 1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (sec)</th>
<th>Heat mode</th>
<th>Sensitivity</th>
<th>Gas #1</th>
<th>Gas Inner flow rate</th>
<th>Gas #2</th>
<th>Sampling</th>
<th>Previous stage (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>110</td>
<td>Ramp</td>
<td>Regular</td>
<td>Gas #1</td>
<td>0.20</td>
<td>Off</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>Ramp</td>
<td>Regular</td>
<td>Gas #1</td>
<td>0.20</td>
<td>Off</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>600</td>
<td>Ramp</td>
<td>Regular</td>
<td>Gas #1</td>
<td>1.00</td>
<td>Off</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>600</td>
<td>Step</td>
<td>Regular</td>
<td>Gas #1</td>
<td>1.00</td>
<td>Off</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>600</td>
<td>Step</td>
<td>High</td>
<td>Gas #1</td>
<td>0.00</td>
<td>Off</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>2100</td>
<td>Step</td>
<td>High</td>
<td>Gas #1</td>
<td>0.00</td>
<td>On</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>2600</td>
<td>Step</td>
<td>Regular</td>
<td>Gas #1</td>
<td>1.00</td>
<td>Off</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Autosampler Mixing Conditions**

<table>
<thead>
<tr>
<th>Adding Conc. (ppb)</th>
<th>Sample amount</th>
<th>R2 (Pb 10ppb standard solution)</th>
<th>R1 (pure water)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0 L</td>
<td>0 L</td>
<td>200 L</td>
<td>200 L</td>
</tr>
<tr>
<td>0</td>
<td>100 L</td>
<td>0 L</td>
<td>100 L</td>
<td>200 L</td>
</tr>
<tr>
<td>1</td>
<td>100 L</td>
<td>20 L</td>
<td>80 L</td>
<td>200 L</td>
</tr>
<tr>
<td>2</td>
<td>100 L</td>
<td>40 L</td>
<td>60 L</td>
<td>200 L</td>
</tr>
<tr>
<td>3</td>
<td>100 L</td>
<td>60 L</td>
<td>40 L</td>
<td>200 L</td>
</tr>
</tbody>
</table>

Pb: 10ppb standard solution and pure water containing approximately 1.1mol/L of nitric acid
Inj. Vol.: 20 L
5.4 Analysis of Pb in White Sugar Using Atomic Absorption Spectrophotometry (2) - AA

**Effective Calibration Curve-MSA (sugar)**

- Abs = 0.0293
- Correlation coefficient (r) = 0.99997

**Measurement results for Pb in refined white sugar**

![Graph showing effective calibration curve for Pb in white sugar](image)
5.5 Analysis of Inorganic Components in Canned Drink (Green Tea) (1) - ICP-AES

**Explanation**
Samples like green tea can be directly inducted for ICP and AA analysis without pretreatment as long as there is no sediment. Here, inorganic components in shop-sold canned drink (green tea) were qualitatively and quantitatively analyzed using an ultrasound nebulizer with the ICP-AES. Here, semi-quantitative values and spectrum line profiles were obtained for approximately 72 elements with qualitative analysis. Almost identical quantitative results were obtained for the directly inducted sample and the sample treated by conventional wet decomposition.

**References**
- Standard Methods of Analysis for Hygienic Chemists (Annotation), edited by the Pharmaceutical Society of Japan, published by Kanehara & Co., Ltd
- Analysis Manual for the Standard Tables of Food Composition in Japan 5th rev, edited by the Resources Council of the former Science and Technology Agency, published by the Japan Resources Association

**Pretreatment**
1. Direct introduction sample
   After opening seal, place 50mL of sample in plastic container, add 1mL of nitric acid and an internal standard element Y to 100ppb, and agitate the mixture.
2. Wet decomposition sample
   After opening seal, place 50mL of sample in a 100mL beaker and boil on a hotplate (approximately 190˚C). When the whole sample has been reduced to 10mL, add 5mL of nitric acid and 1mL of hydrochloric acid and thermally decompose it for approximately 2 hours. After cooling, add ultra pure water to make it exactly 50mL and agitate it.

**Analytical Conditions**
- Instrument: ICPS-8000
- Ultrasound nebulizer UAG-1
- High frequency: 27.12MHz
- High frequency output: 0.8kW
- Cooling gas: Ar 14.0L/min
- Plasma gas: Ar 1.2L/min
- Carrier gas: Ar 0.7L/min
- Purge gas: Ar 3.5L/min
- Sample suction rate: 1.5mL/min
- Observation method: Horizontal

Fig. 5.5.1 Profile example for qualitative analysis

Fig. 5.5.2 Semi-quantitative value for qualitative analysis
5.5 Analysis of Inorganic Components in Canned Drink (Green Tea) (2) - ICP-AES

Chart 5.5.3 Green tea analysis results (µg/mL)

<table>
<thead>
<tr>
<th>Element</th>
<th>Direct introduction</th>
<th>Wet decomposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>0.249</td>
<td>0.260</td>
</tr>
<tr>
<td>Ni</td>
<td>0.029</td>
<td>0.029</td>
</tr>
<tr>
<td>Al</td>
<td>1.27</td>
<td>1.30</td>
</tr>
<tr>
<td>Pb</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sn</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Cu</td>
<td>0.0057</td>
<td>0.0053</td>
</tr>
<tr>
<td>Zn</td>
<td>0.083</td>
<td>0.089</td>
</tr>
<tr>
<td>Cr</td>
<td>0.0008</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

Fig. 5.5.4 Spectrum line profile

Fig. 5.5.5 Pb calibration curve

Fig. 5.5.6 Sn calibration curve
5.6 Analysis of Inorganic Components in Brown Rice and Leaves (1) - ICP-MS

**Explanation**
Plant standard substances were analyzed using ICP-MS. Simultaneous analysis of lead and cadmium, as well as micro amounts of inorganic components such as arsenic and selenium, is possible in the same solution because the ICP-MS has a wide dynamic range and extremely high sensitivity.

**Samples**
Brown rice (Rice flour: NIES No. 10)
Tomato leaves (NIST SRM1573)
Citrus leaves (NIST SRM1572)

**Pretreatment**
Place 0.1g of sample in a Teflon pressure decomposition container, add 1mL of nitric acid, seal the container and heat for 2 hours at 170°C. After cooling, add 10ppb of internal standard elements (Ho, Rh) and measure up to 50mL using ultra pure water. Use this solution as the sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rice flour NIES No. 10</th>
<th>Tomato Leaves NIST SRM1573</th>
<th>Citrus Leaves NIST SRM1572</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td>M/Z</td>
<td>Guaranteed value</td>
<td>Quantitative value</td>
</tr>
<tr>
<td>V</td>
<td>51</td>
<td>–</td>
<td>0.049</td>
</tr>
<tr>
<td>Cr</td>
<td>52</td>
<td>0.22*</td>
<td>0.31</td>
</tr>
<tr>
<td>Co</td>
<td>59</td>
<td>0.02*</td>
<td>0.05</td>
</tr>
<tr>
<td>Ni</td>
<td>60</td>
<td>0.39±0.04</td>
<td>0.41</td>
</tr>
<tr>
<td>Cu</td>
<td>63</td>
<td>3.3±0.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Zn</td>
<td>66</td>
<td>22.3±0.9</td>
<td>23.3</td>
</tr>
<tr>
<td>As</td>
<td>75</td>
<td>0.11*</td>
<td>0.12</td>
</tr>
<tr>
<td>Se</td>
<td>82</td>
<td>0.02*</td>
<td>–</td>
</tr>
<tr>
<td>Mo</td>
<td>98</td>
<td>0.42±0.05</td>
<td>0.45</td>
</tr>
<tr>
<td>Cd</td>
<td>111</td>
<td>0.32±0.02</td>
<td>0.29</td>
</tr>
<tr>
<td>Pb</td>
<td>208</td>
<td>–</td>
<td>1.6</td>
</tr>
</tbody>
</table>

*: Reference value

Chart 5.6.1 Analysis results for plants (µg/g)
5.6 Analysis of Inorganic Components in Brown Rice and Leaves (2) - ICP-MS

Fig. 5.6.2 Mass spectrum of Pb208  Fig. 5.6.3 Mass spectrum of Cd111  Fig. 5.6.4 Mass spectrum of As75

Fig. 5.6.5 As calibration curve  Fig. 5.6.6 Pb calibration curve  Fig. 5.6.7 Cd calibration curve
5.7 Analysis of Inorganic Components in Processed Food Products - ICP-AES

**Explanation**
This is an analysis example for processed food. Various elements included in food products are divided into essential ones and harmful ones. The ICP emission spectrometry, which allows simultaneous analysis of these elements, is quite useful for comprehending the mutual relationships between elements.

**Samples**
Tuna, bean curd dressed with liquid starch, vegetables boiled in miso, rice and vegetable porridge, rice gruel

**References**
- Standard Methods of Analysis for Hygienic Chemists (Annotation), edited by the Pharmaceutical Society of Japan, published by Kanehara & Co., Ltd
- Analysis Manual for the Standard Tables of Food Composition in Japan 5th rev, edited by the Resources Council of the former Science and Technology Agency, published by the Japan Resources Association

**Pretreatment**
Homogenize each sample in a homogenizer, take 10g for each, add 10mL of nitric acid and 2mL of sulfuric acid and thermally decompose them until white smoke of sulfuric acid appears. After cooling, measure up to 100mL. Use these as samples.

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Element</th>
<th>Tuna</th>
<th>Bean curd dressed with liquid starch</th>
<th>Vegetables boiled in miso</th>
<th>Rice and vegetable porridge</th>
<th>Rice gruel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>560</td>
<td>1150</td>
<td>1610</td>
<td>972</td>
<td>10.3</td>
</tr>
<tr>
<td>Mg</td>
<td>77</td>
<td>874</td>
<td>103</td>
<td>18.9</td>
<td>10.9</td>
</tr>
<tr>
<td>P</td>
<td>460</td>
<td>303</td>
<td>351</td>
<td>83</td>
<td>47.8</td>
</tr>
<tr>
<td>K</td>
<td>747</td>
<td>603</td>
<td>628</td>
<td>82</td>
<td>38.9</td>
</tr>
<tr>
<td>Ca</td>
<td>23.3</td>
<td>145</td>
<td>219</td>
<td>22.3</td>
<td>10.5</td>
</tr>
<tr>
<td>Mn</td>
<td>0.13</td>
<td>1.01</td>
<td>1.20</td>
<td>0.48</td>
<td>0.43</td>
</tr>
<tr>
<td>Fe</td>
<td>2.10</td>
<td>3.25</td>
<td>3.40</td>
<td>0.36</td>
<td>0.21</td>
</tr>
<tr>
<td>Zn</td>
<td>1.16</td>
<td>2.39</td>
<td>2.35</td>
<td>1.21</td>
<td>1.21</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Pb</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
</tr>
</tbody>
</table>

Chart 5.7.1 Analysis results (µg/g: wet weight)
5.8 Analysis of Na in Food Products Using Atomic Absorption Spectrophotometry - AA

• Explanation
The Nutrition Improvement Law requires the content of sodium, as well as calories and protein content, to be shown in the nutrient labels on public-consumed processed food products. The flame atomic absorption method is generally used to analyze sodium in food products. Here, an analysis example for sodium in shop-sold bread will be introduced.

• Pretreatment
There are several possible pretreatment methods, including dilution extraction, dry incineration and wet decomposition, among which diluted hydrochloric acid extraction method is the most suitable for measurement of sodium and potassium. This method allows relatively quick analysis of these substances. Commercially available bread was pulverized in a pulverizer and pretreated as shown in Fig. 5.8.1. The Analysis Method for Standard Tables of Food Composition in Japan 5th revision recommends centrifugal separation and using the supernatant liquid, instead of filtration.

Sample 5 g

↓ Add 200 mL 1% hydrochloric acid

Shake 30 min

↓

Filter 5B

↓

Take filtrate 4 mL

↓

Measure up 100 mL

Fig. 5.8.1 Pretreatment flowchart

Analytical Conditions

<table>
<thead>
<tr>
<th>Instrument</th>
<th>AA-6200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>Na 589.5 nm</td>
</tr>
<tr>
<td>Lamp current (mA)</td>
<td>12</td>
</tr>
<tr>
<td>Slit width (nm)</td>
<td>0.2</td>
</tr>
<tr>
<td>Background correction</td>
<td>None</td>
</tr>
<tr>
<td>Flow type</td>
<td>Air-acetylene</td>
</tr>
<tr>
<td>Fuel gas (C₂H₂)</td>
<td>1.8L/min</td>
</tr>
<tr>
<td>Support gas (air)</td>
<td>8L/min</td>
</tr>
<tr>
<td>Burner angle</td>
<td>45°</td>
</tr>
</tbody>
</table>

Chart 5.8.3 Analysis results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measurement result</th>
<th>Value shown in label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td>499 mg</td>
<td>502 mg</td>
</tr>
</tbody>
</table>

Fig. 5.8.2 Sodium calibration curve
6. Others

6.1 Analysis of Shellfish Toxins (1) - LC

**Explanation**
In recent years shellfish poisoned with paralytic shellfish toxins are found in various regions, causing major damage to the marine product industry and serious problems in food hygiene.

Paralytic shellfish toxin is a neurotoxin produced by a phytoplankton dinoflagellate, and is known by the component names such as saxitoxin or gonyautoxin. Post column derivatization fluorescent detection LC analysis method was used by Nagashima and Oshima to analyze this shellfish toxin.

Here, this method is used in an analysis example for gonyautoxin (GTX) 1-4 standard sample.

**Analytical Conditions**

**- Separation conditions**

**Instrument** : HPLC

**Column** : STR ODS-II (4.0mmI.D. × 150mmL)

**Mobile phase** : 10mM (sodium) phosphate buffer

(pH 7.0) containing 4mM (sodium) heptanesulfonate

**Temperature** : 40°C

**Flow rate** : 0.8mL/min

**- Reaction Conditions**

**Primary reaction** : 50mM (sodium) borate buffer

liquid (pH 9.5) containing 5mM periodic acid

**Flow rate** : 0.4mL/min

**Temperature** : 60°C

**Secondary reaction liquid** : 110mM of phosphoric acid buffer (pH 2.1)

**Flow rate** : 0.4mL/min

**Temperature** : 40°C

**- Detection** : Fluorescence detector

(Ex330nm  Em390nm)

**References**

LC talk, No. 36 from Shimadzu Corporation (1995)


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Fig. 6.1.1 Analysis example for gonyautoxin (GTX) 1-4 standard sample
6.1 Analysis of Shellfish Toxins (2) - LC

References

Fig. 6.1.2 Flow line

1. 9. Degassing units
2. Low-pressure gradient unit
3. 10. Solvent delivery units
4. Mixer
5. Auto injector
6. Column oven
7. Analysis column
8. Chemical reaction tank
9. Blender
10. Fluorescent detector
11. Reaction coils
12. M1, M2 Mobile phases
13. R1, R2 Reaction liquids

Fig. 6.1.3 Structural formula of shell toxin components

R1 R2 R3
H H H STX
OH H H neoSTX
OH H OSO₃⁻ GTX1
H H OSO₃⁻ GTX2
H OSO₃⁻ H GTX3
OH OSO₃⁻ H GTX4
6.2 Analysis of Oxytetracycline - LC

• **Explanation**
  Shop-sold pig liver was extracted using the official gazette method and oxytetracycline was added to make a solution of 0.5ppm for analysis.

**References**
Official Gazette extra No. 245 (December 26, 1995)

• **Pretreatment**
  The sample was pre-treated as shown in Chart 6.2.2 in accordance with the official gazette.

**References**

---

**Analytical Conditions**

- **Instrument**: HPLC
- **Column**: STR ODS-II (4.6mmφ × 150mm)
- **Mobile phase**: 1M imidazole buffer/methanol = 77/23 (v/v)
- **Temperature**: 40°C
- **Flow rate**: 1.0mL/min
- **Detection**: Fluorescence detector Ex380nm Em520nm

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**Chart 6.2.2 Pretreatment flowchart for oxytetracycline**

**Fig. 6.2.1 Analysis example of oxytetracycline**

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**Fig. 6.2.1 Analysis example of oxytetracycline**
6.3 Analysis of Closantel - LC

**Explanation**
Shop-sold pig liver was extracted using the official gazette method and closantel was added to make a solution of 1ppm for analysis.

**References**
Official Gazette extra No. 245 (December 26, 1995)

**Pretreatment**
The sample was pre-treated as shown in Chart 6.3.2 in accordance with the official gazette.

**Analytical Conditions**
- **Instrument**: HPLC
- **Column**: STR-ODS- II (4.6mm × 150mm)
- **Mobile phase**: Methanol/20mM sodium dihydrogenphosphate (pH 3.3) = 7/2 (v/v)
- **Temperature**: 40°C
- **Flow rate**: 1.0mL/min
- **Detection**: UV-VIS Detector 369nm

References
6.4 Analysis of Fumonisin in Sweet Corn (1) - LC

**Explanation**
The mycotoxin family member fumonisin is related to fusarium branch and is known to be the cause of equine leukoencephalomalacia and lung edema in pigs. Recent research also points to its involvement in human esophageal cancer. Here, this component was analyzed using pre-label fluorescent derivatization and detection incorporating OPA agent.

**References**
G.S.Shepland, et al., J. Liquid Chromatogr., 13, 2077 (1990)

**Pretreatment**
200µL of thiol agent and 200µL of OPA agent was added to 100µL of sample solution. After mixed and left to stand for 3 min, 10µL of the mixture was inducted into HPLC.

Thiol agent: 0.1M (sodium) borate buffer (pH 9.2) containing 50mM 3-mercaptopropionic acid OPA agent: A/B = 1/4 mixture
A: 0.25M o-Phthalaldehyde methanol solution
B: 0.1M (sodium) borate buffer (pH 9.2)

**Analytical Conditions**
Instrument: HPLC
Column: STR ODS- II (4.6mmφ ×150mm)
Mobile phase: Methanol/50mM citric acid buffer (pH 4.3) (7/3, v/v)
Temperature: 40°C
Flow rate: 1.0mL/min
Detection: Fluorescence Detector
Ex335nm Em440nm

![Fig 6.4.1 Analysis example of fumonisn in sweet corn](image-url)
6.4 Analysis of Fumonisin in Sweet Corn (2) - LC

References

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Fig. 6.4.2 Pretreatment flowchart for sweet corn
6.5 Simultaneous Analysis of Synthetic Antibacterial Agent (1) - LC

**Explanation**
HPLC is recognized as the best method for analysis of food-residual (especially fish and meat) antibacterial agents and antibiotics.

**References**
Hamada, Murakita; Shimadzu Review, 52 (2), 107 (1995)
Murayama, Uchiyama, Saito, Food Hygiene Journal, 32, 155 (1991)
Milk Hygiene Volume 79, April 1993 (from the former Ministry of Health and Welfare)

**Pretreatment**
Fig. 6.5.2 shows the method recommended by the former Ministry of Health and Welfare (currently the Ministry of Health, Labour and Welfare).

**Analytical Conditions**
- **Instrument**: HPLC
- **Column**: STR ODS- II (4.6mmφ × 150mm)
- **Mobile phase**:
  - (A) water/acetic acid = 100/0.3 (v/v) (NaClO4 included)
  - (B) acetonitrile/water/acetic acid = 90/10/0.3 (v/v/v) (NaClO4 included)
  - Gradient elution of 2 liquids
- **Temperature**: 40°C
- **Flow rate**: 2.0mL/min
- **Detection**: Photodiode array detector at 195nm to 600nm

Fig. 6.5.1 Simultaneous analysis example for 19 synthetic antibacterial agent components
6.5 Simultaneous Analysis of Synthetic Antibacterial Agent (2) - LC

References

Fig. 6.5.2 Pretreatment flowchart for simultaneous analysis of 19 synthetic antibacterial agent components
**Explanation**

In the application fields for ion chromatography, microanalysis technology has become a vital facet, requiring analyzers with even greater sensitivity. Based on a wealth of acquired non-suppressor type ion chromatograph technology, Shimadzu has come up with an auto suppressor that reduces the background level of the mobile phase to improve the S/N for even higher sensitivity where Cl ion detection limit is 1ppb (S/N = 3). Here, analysis examples for inorganic ions in drinking water using this suppressor type ion chromatograph HIC-SP will be introduced.

<table>
<thead>
<tr>
<th>Column</th>
<th>Shim-pack IC-SA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase</td>
<td>14mM Sodium Hydrogen Carbonate solution</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1.0mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>Detection</td>
<td>HIC-10ASP Suppressor system</td>
</tr>
<tr>
<td>Inj. Vol.</td>
<td>50µL</td>
</tr>
</tbody>
</table>

**Chart 6.6.1 Analytical conditions for anions**

![Chart 6.6.1 Analytical conditions for anions](image)

<table>
<thead>
<tr>
<th>Column</th>
<th>Shim-pack IC-SC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase</td>
<td>6.5mM Methane Sulfonic acid solution</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1.0mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>Detection</td>
<td>HIC-10ASP Suppressor system</td>
</tr>
<tr>
<td>Inj. Vol.</td>
<td>50µL</td>
</tr>
</tbody>
</table>

**Chart 6.6.3 Analytical conditions for cations**

![Chart 6.6.3 Analytical conditions for cations](image)

**References**

Shimadzu Application News No. H37