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C180-E060A

Analysis Guidebook

Food Product Analyses

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4. Aromas and Odors

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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

Instrument	: GCMS-QP5000
Column	: DB-WAX 0.25mm×30m df=0.25μm
Col.Temp.	: 60℃-250℃ (10℃/min)
Inj. Temp.	: 250℃
I/F Temp.	: 250℃
Carrier Gas	: He(100kPa)
Reagent Gas	: Isobutane

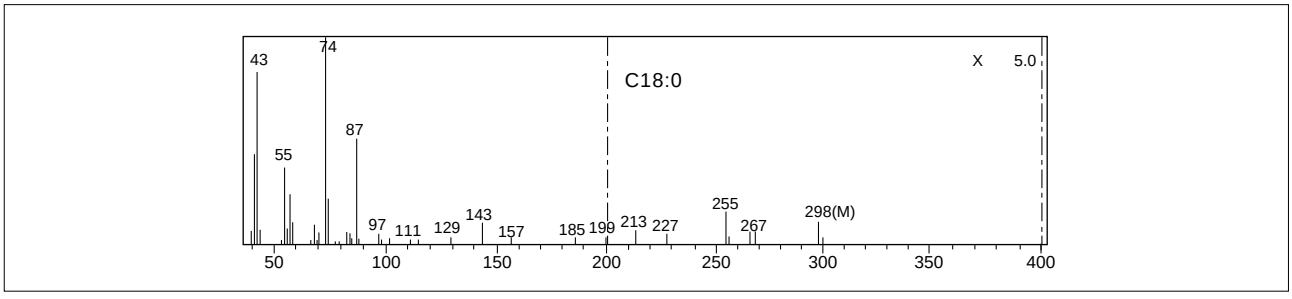


Fig. 1.1.1 Ei mass spectrum of C18:0

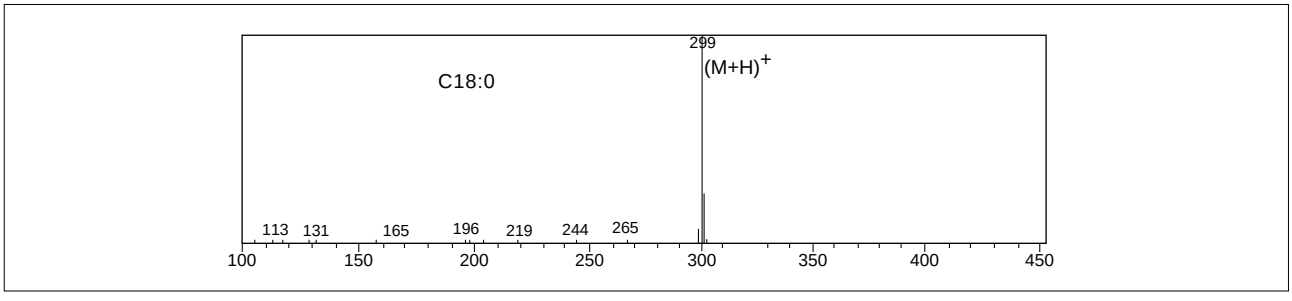


Fig. 1.1.2 Ci mass spectrum of C18:0

1.1 Analysis of Fatty Acids (2) - GCMS

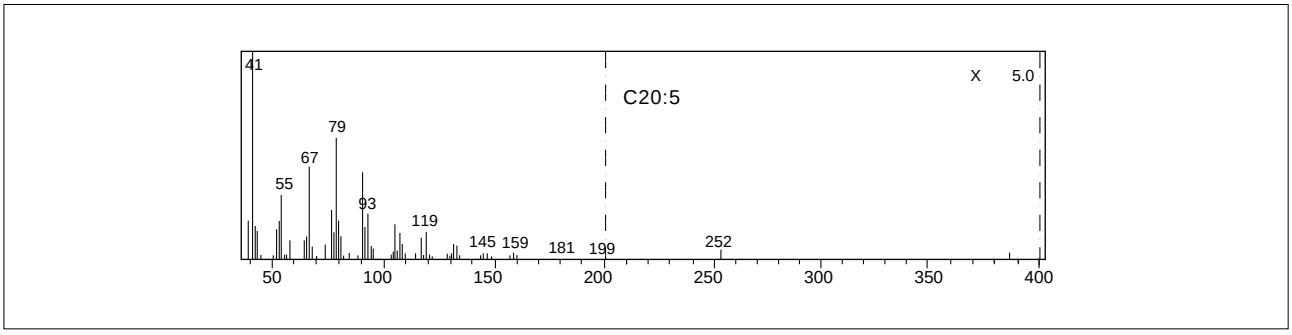


Fig. 1.1.3 Ei mass spectrum of C20:5

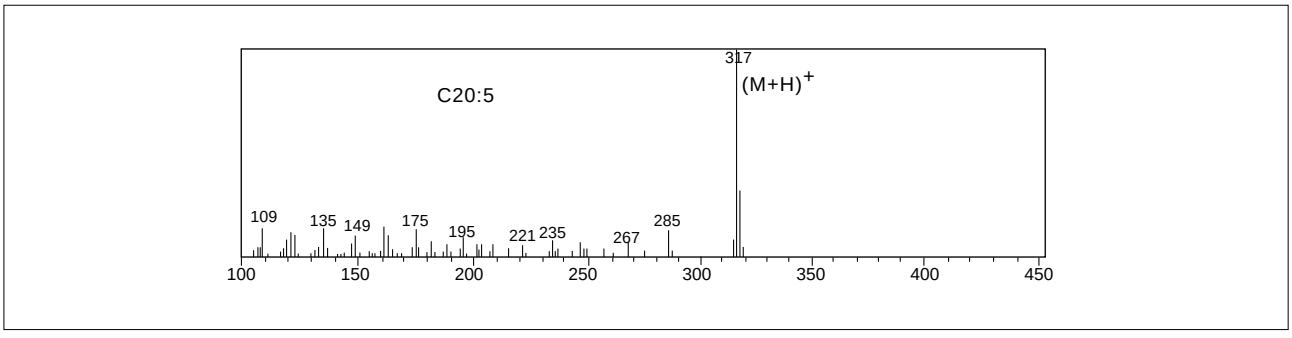


Fig. 1.1.4 Ci mass spectrum of C20:5

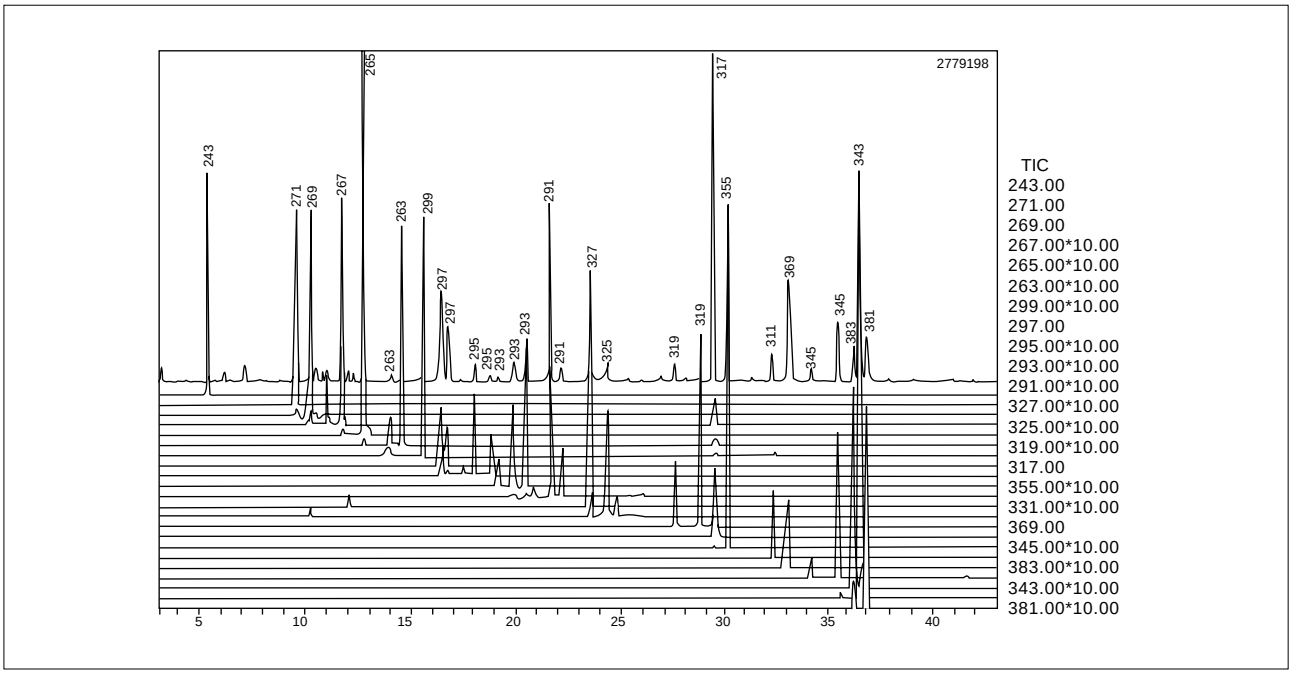
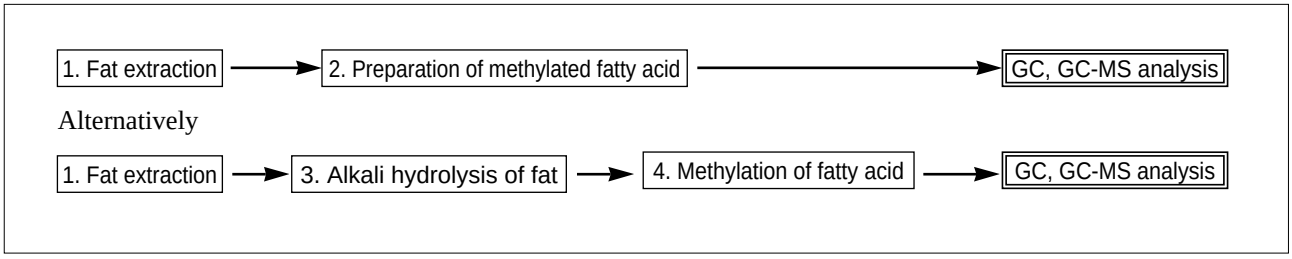


Fig. 1.1.5 Mass chromatogram of protonized molecules for fatty acid methyl ester

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

Standard Methods of Analysis for Hygienic Chemists and Notes 1990 Appended supplement (1995)
Pharmaceutical Society of Japan Edition, published by Kanehara & Co., Ltd (1995)

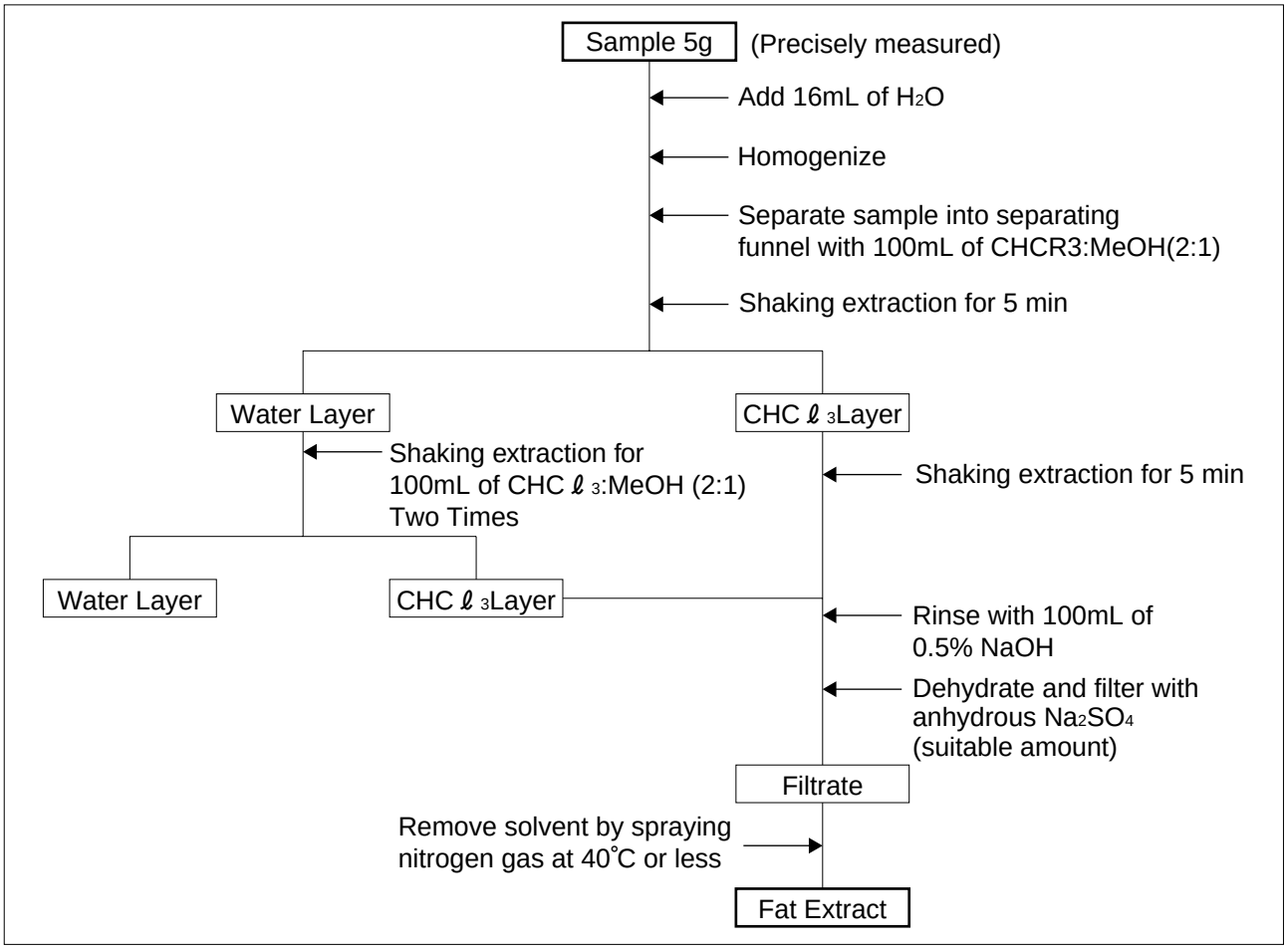


Fig. 1.1.6 Fat extraction method

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

Standard Methods of Analysis for Hygiene Chemists and Notes 1990 Appended supplement (1995)
Pharmaceutical Society of Japan Edition, published by Kanehara & Co., Ltd (1995)

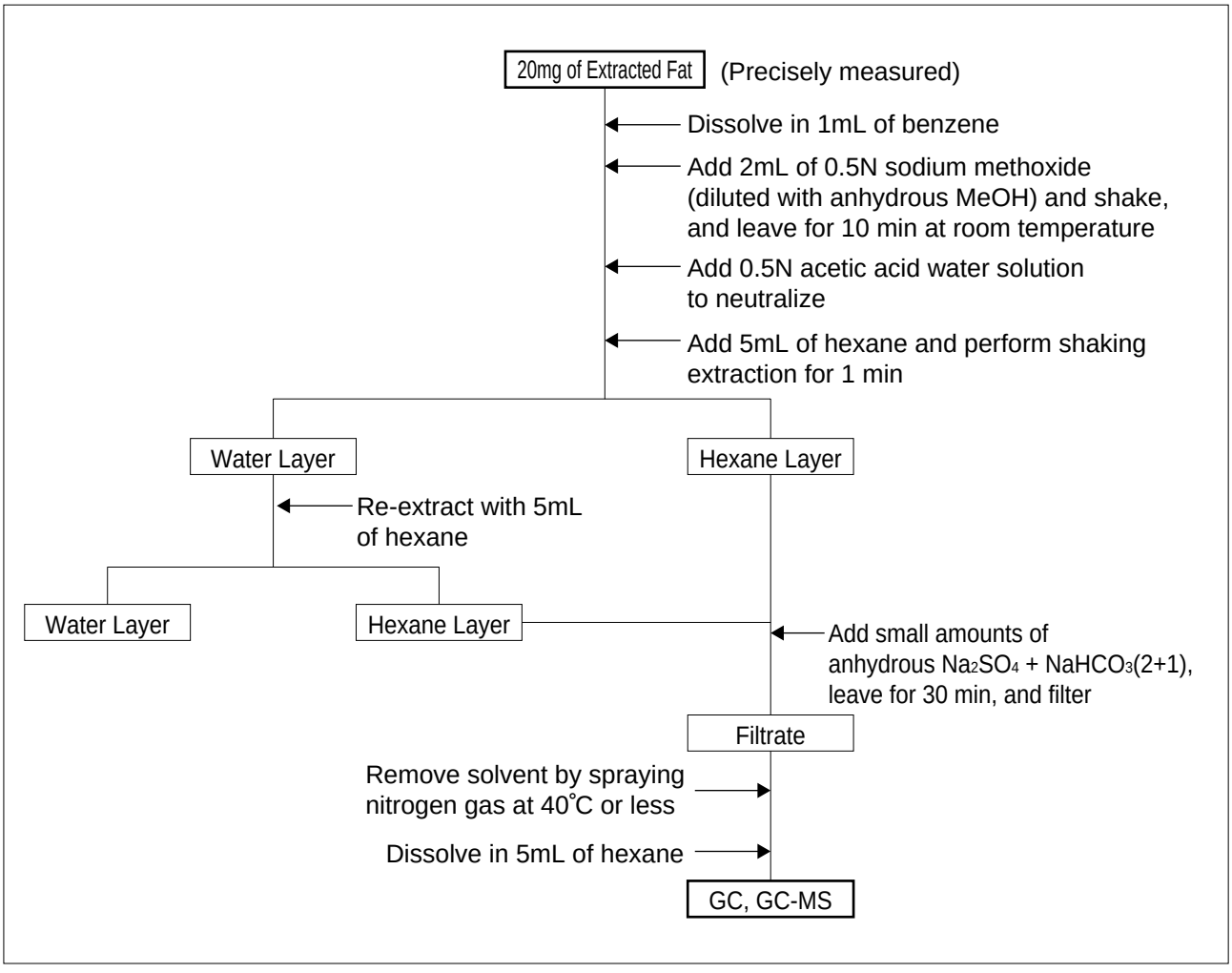


Fig. 1.1.7 Preparation of methylated fatty acid

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

Organic Chemistry Testing Guidebook No. 5, Handling Biological materials, Toshio Goto, Tetsuo Shiba, Teruo Matsuura ed, Kagaku-Dojin Publishing Company, INC (1991)

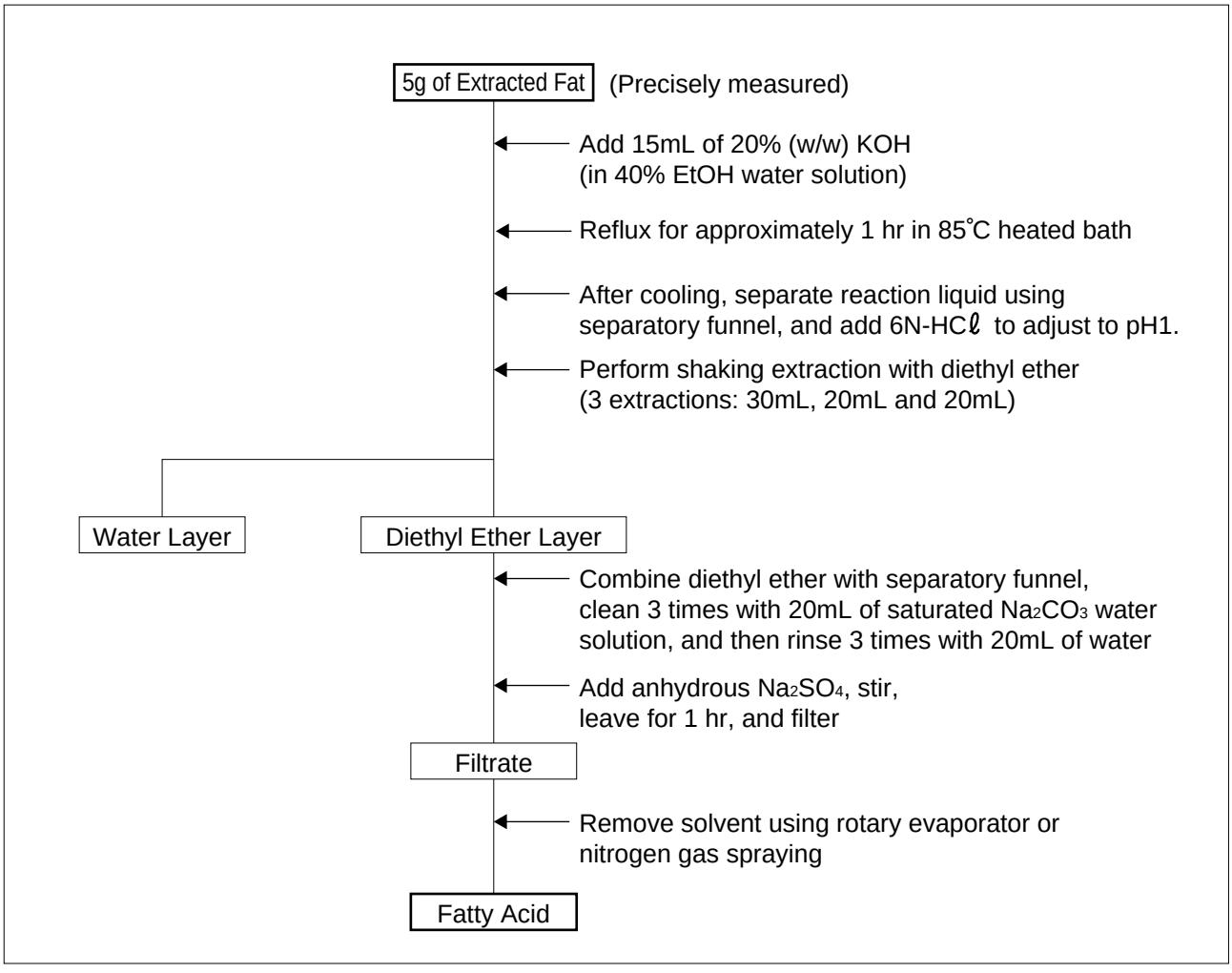


Fig. 1.1.8 Alkali hydrolysis of fat

1.1 Analysis of Fatty Acids (6) / Derivatization (1)
- Preparation of Methyl Ester Derivative

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 BF_3-CH_3OH

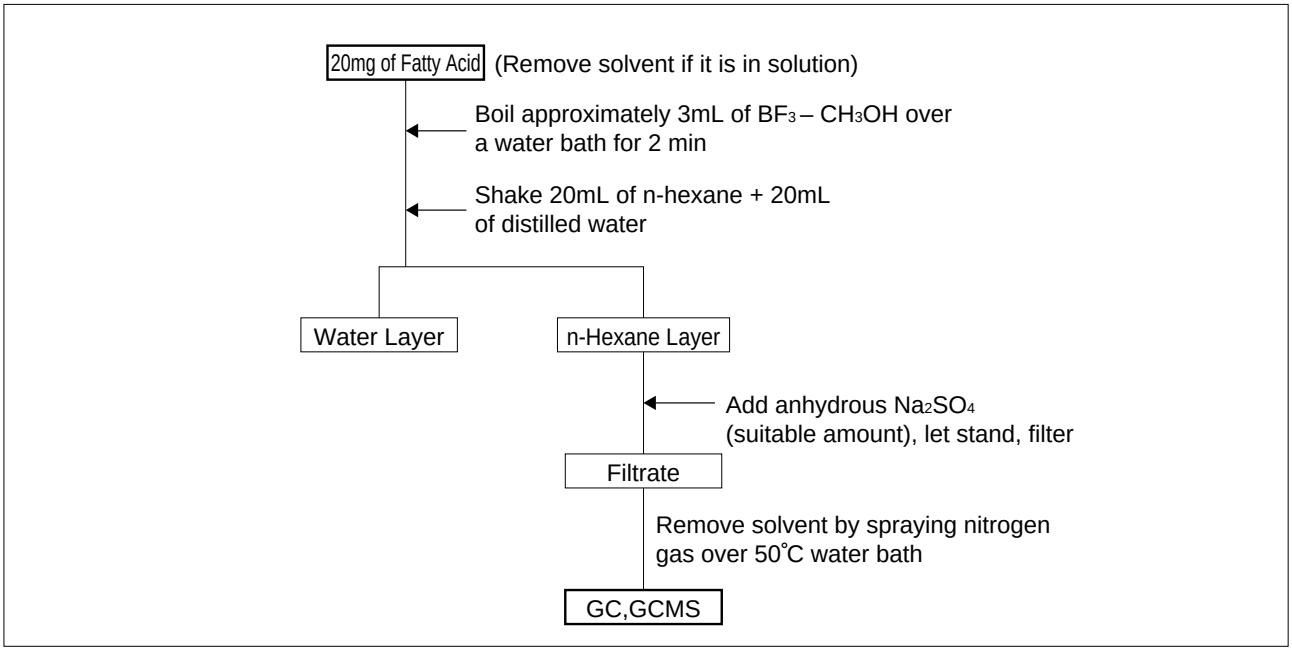


Fig. 1.1.9 Methyl esterization using boron trifluoride-methanol

(2) Methyl Esterization Using H_2SO_4-CH_3OH

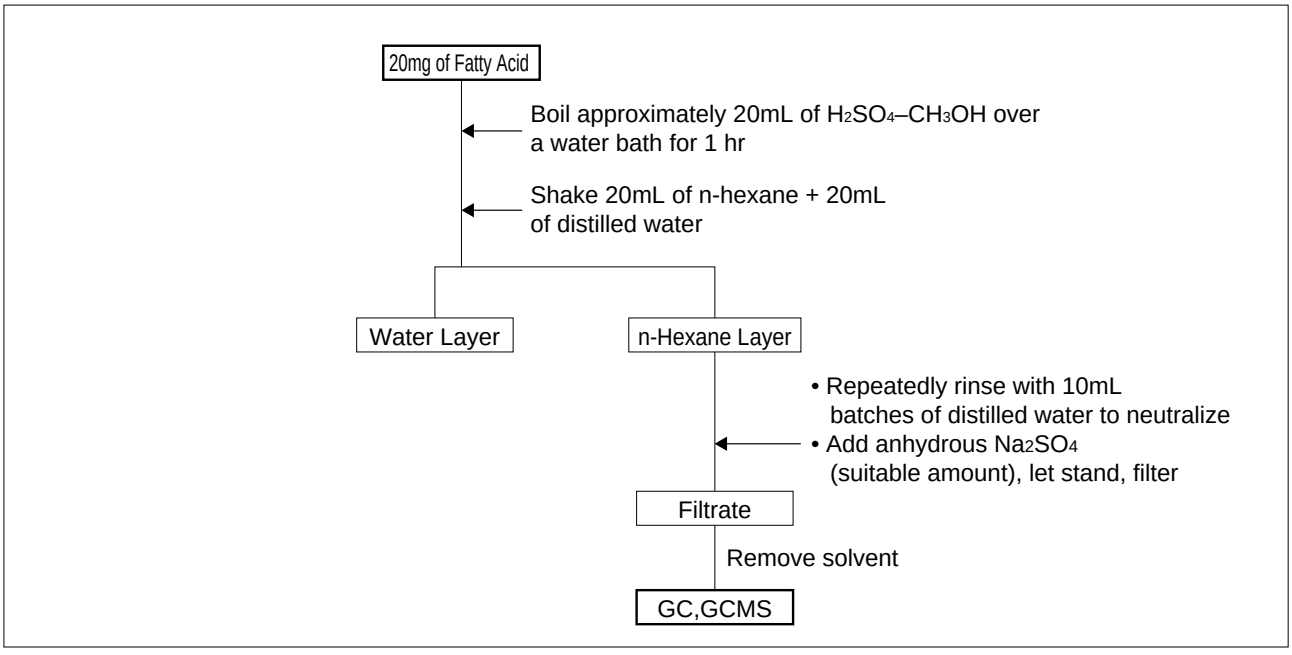


Fig. 1.1.10 Methyl esterization using sulphuric acid-methanol

1.1 Analysis of Fatty Acids (6) / Derivatization (2)
- Methyl Ester Derivative

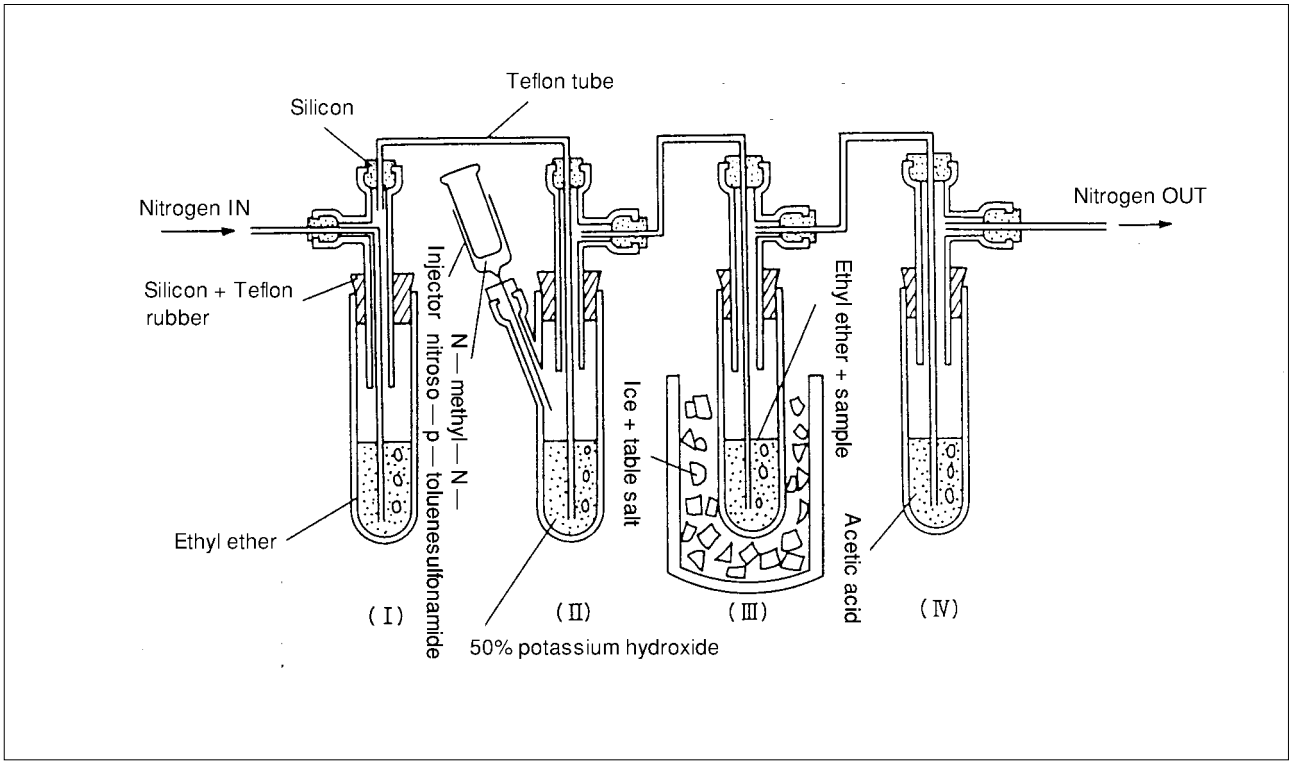


Fig. 1.1.11 Methyl esterization using diazomethane

(3) Methyl Esterization Using CH_2N_2

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.

♦ Notes and coutions
- 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.

(4) Methyl Esterization Using Dimethylformamide Dialkylacetals $(CH_3)_2NCH(OR)_2$

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℃ 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 injectionn 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.

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 absorptivety 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.

•Pretreatment

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

•Analytical Conditions

Instrument	: GC-17AAFw
Column	: CBP20 0.22mm×25m df=0.25µm
Column temperature	: 210˚C
Injection inlet temperature	: 230˚C
Detector temperature	: 230˚C(FID)
Carrier gas	: He 100kPa (0.52mL/min at 210˚C)
Injection method	: Split 1:100

References

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

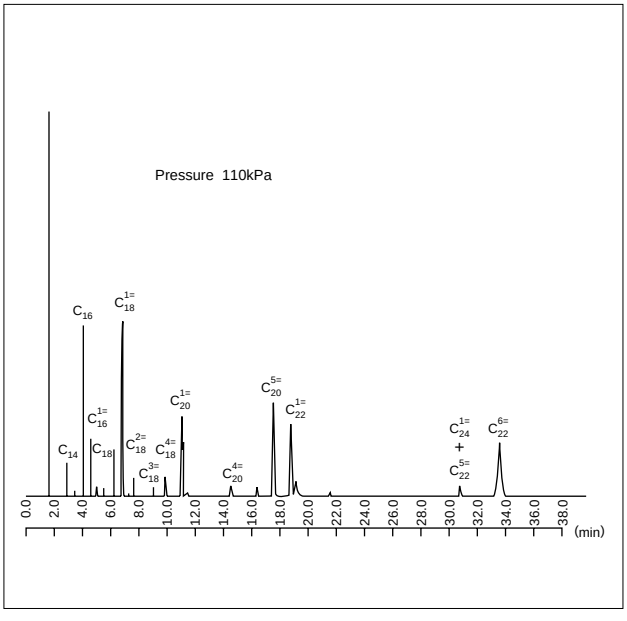


Fig. 1.2.1 Analysis of fatty acid methyl ester in fish oil (constant pressure)

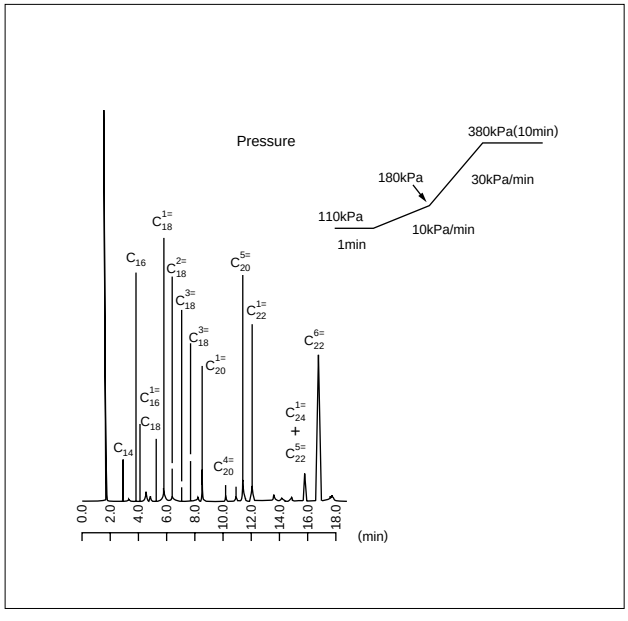


Fig. 1.2.2 Analysis of fatty acid methyl ester in fish oil (rising pressure)

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

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

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

References

1) Application News No. G130

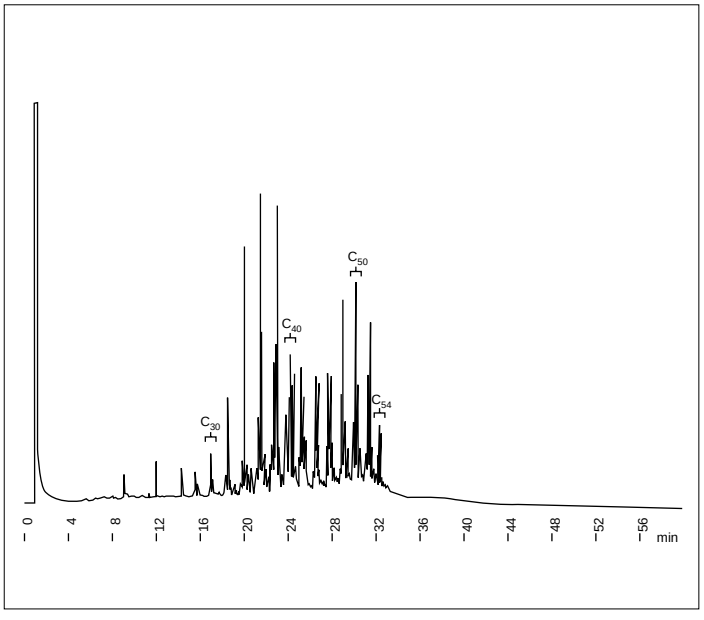


Fig. 1.3.1 Analysis of triglyceride in butter

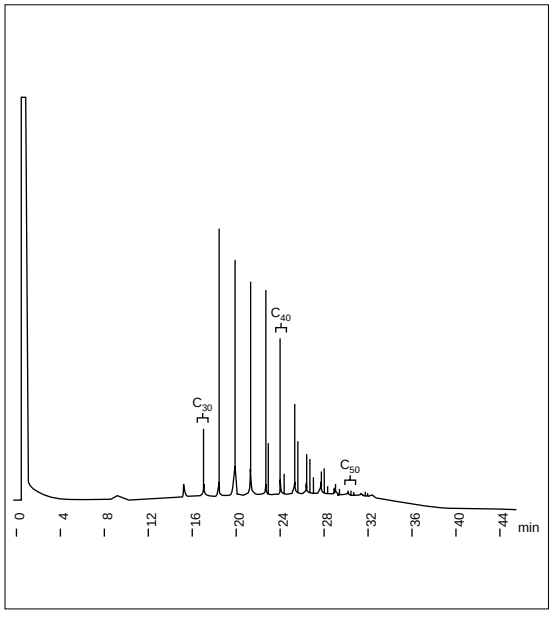


Fig. 1.3.2 Analysis of triglyceride in palm oil

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

Instrument	: LC-VP Series
Column	: Shim-pack SCR-102H (8mmϕ×300mmL)
Mobile Phase	: 5mM Trifluoroacetic Acid Aqueous
Flow Rate	: 1mL/min
Column Temp.	: 50˚C
Detector	: UV-VIS Detector 380nm

Instrument	: FTIR
Resolution	: 4cm^{-1}
Accumulation	: 50
Appodization	: Happ-Genzel
Detector	: Pyroelectric Detector

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

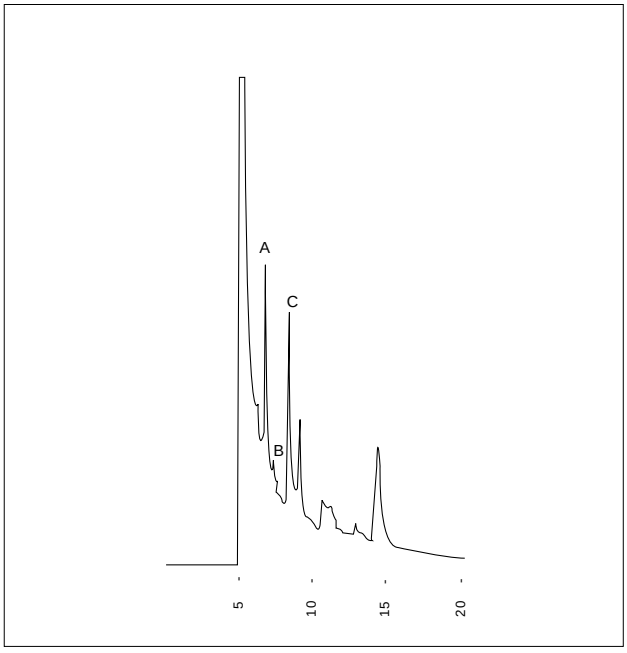


Fig. 1.4.1 LC chromatogram of red wine

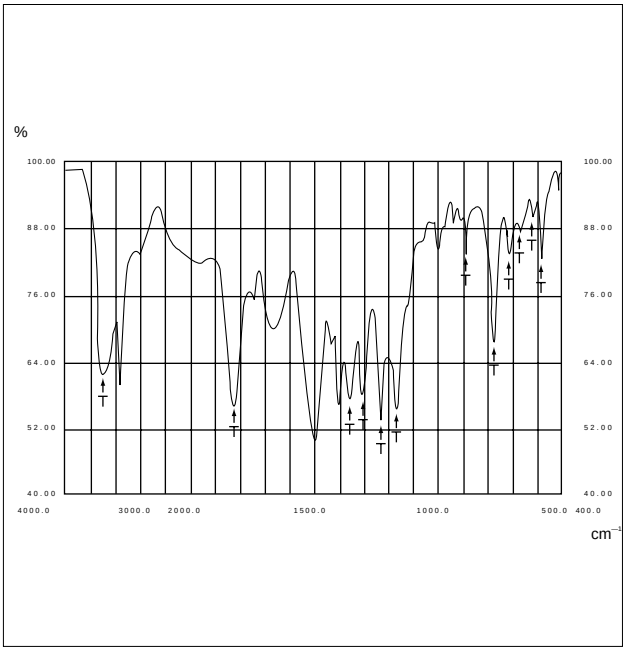


Fig. 1.4.2 Infrared spectrum of peak A (T: tartaric acid peak)

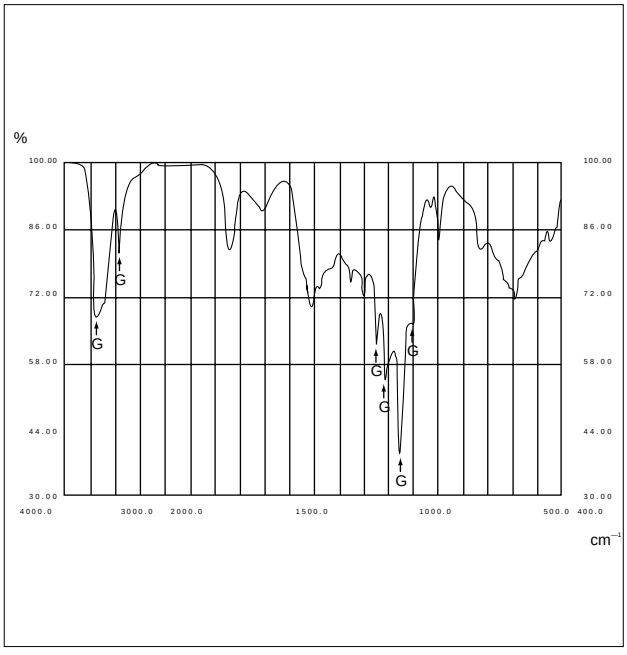


Fig. 1.4.3 Infrared spectrum of peak B (G: glucose)

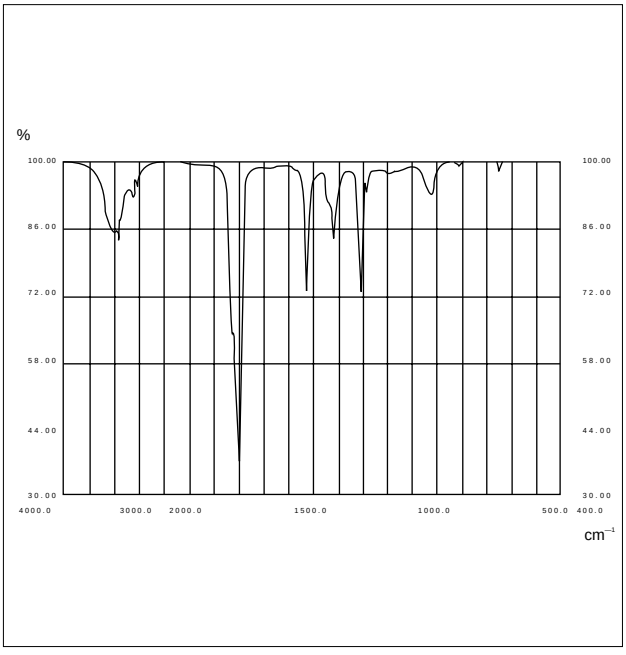


Fig. 1.4.4 Infrared spectrum of peak C (succinic acid)

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 (4.6mmϕ×150mm)
Mobile phase	: 10mM sodium phosphate buffer liquid(pH2.6)/methanol=55/45 (v/v)
Flow rate	: 1.0mL/min
Temperature	: 40℃
Detection	: UV-VIS Detector 210nm

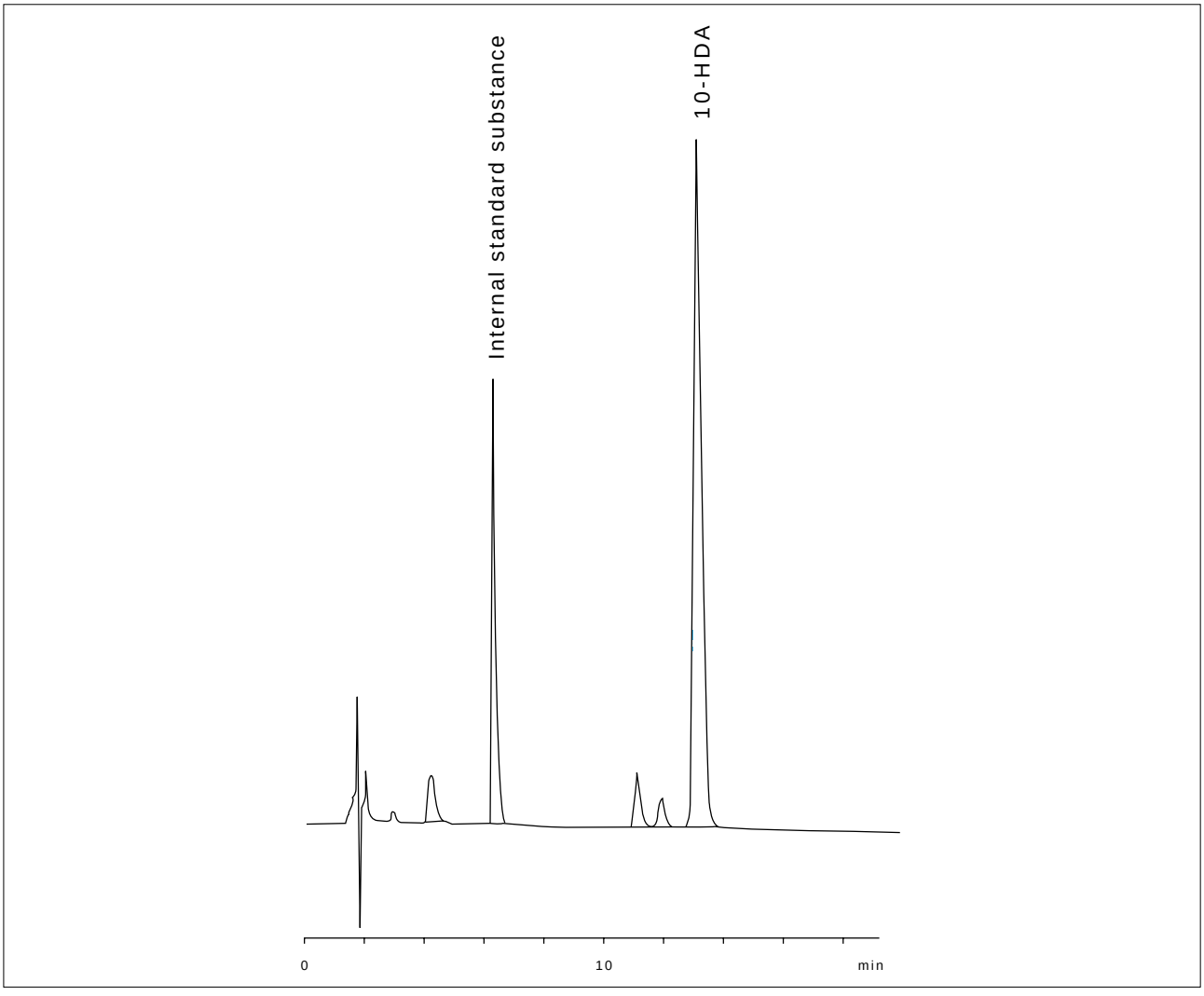


Fig. 1.5.1 Analysis of decenoic acid in raw royal jelly

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-Anthryl-diazomethane) 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.

•Pretreatment

None.

•Analytical Conditions

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

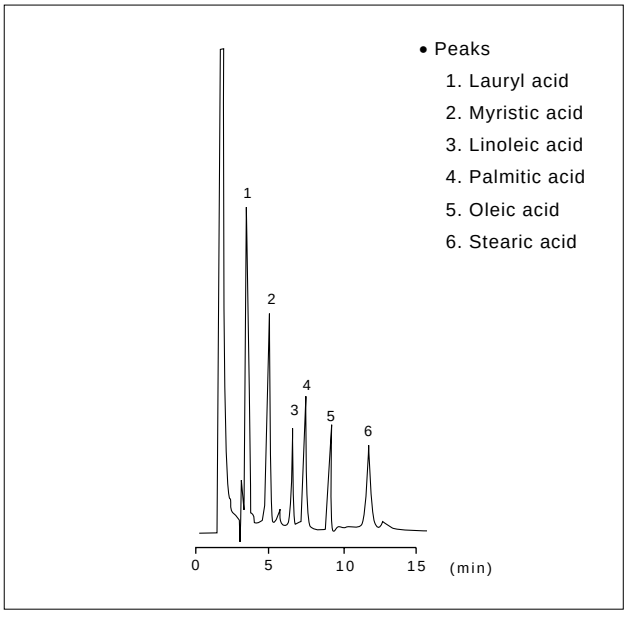


Fig. 1.6.1 Analysis of fatty acid using UV absorption detection

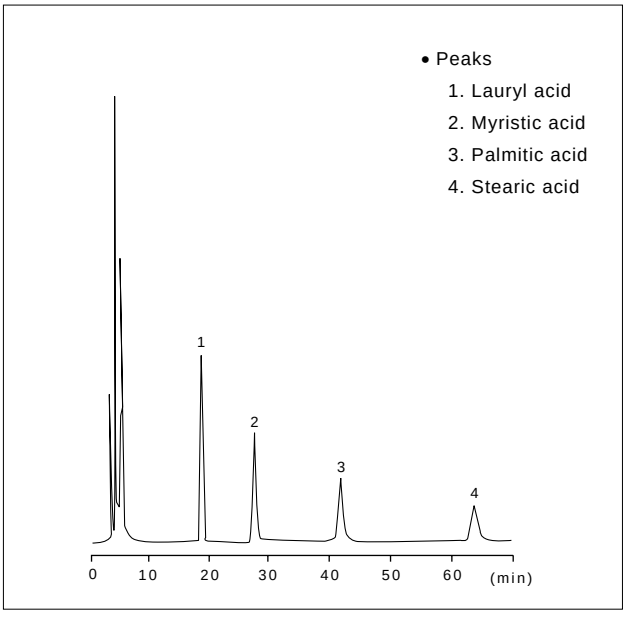


Fig. 1.6.2 Analysis of high-class fatty acid using precolumn derivatization method with ADAM

References

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

References

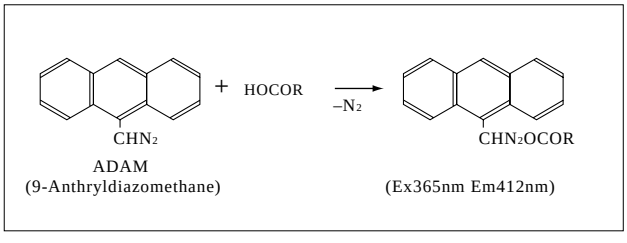


Fig. 1.6.3 Reaction equation for ADAM and fatty acid

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 3×10^{-11} equivalent (differs depending on component)

•Pretreatment

Beer is injected in without any pretreatment.

•Analytical Conditions

Instrument	: HPLC
Column	: 2×Shim-pack SCR-102H (8.0mmϕ×300mm)
Mobile phase	: 5mM p-toluenesulfonic acid
Flow rate	: 0.8mL/min
Temperature	: 45℃
Reaction solutions	: 5mM p-toluenesulfonic acid 20mM Bis-Tris 100μm EDTA
Reaction liquid flow rate	: 0.8mL/min
Cell temperature	: 48℃
Detection	: Conductivity

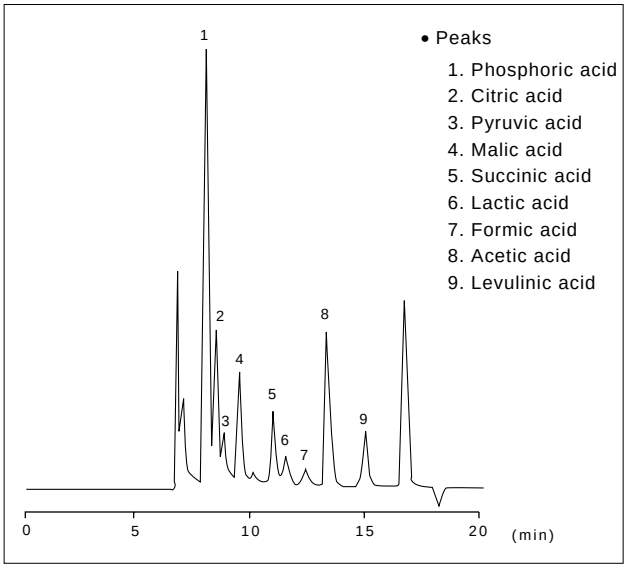


Fig. 1.7.1 Analysis of beer

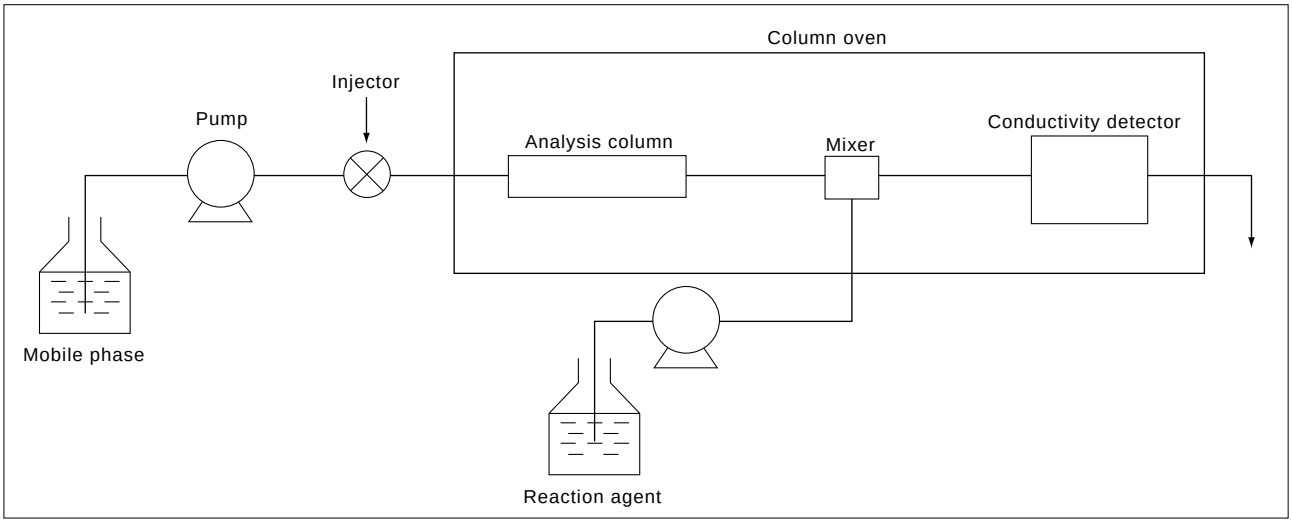


Fig. 1.7.2 Flowchart of organic acid analysis system

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℃
Detection : Fluorescence Detector
Ex350nm Em460nm (1st class amino acid)
Ex485nm Em530nm (2st class amino acid)

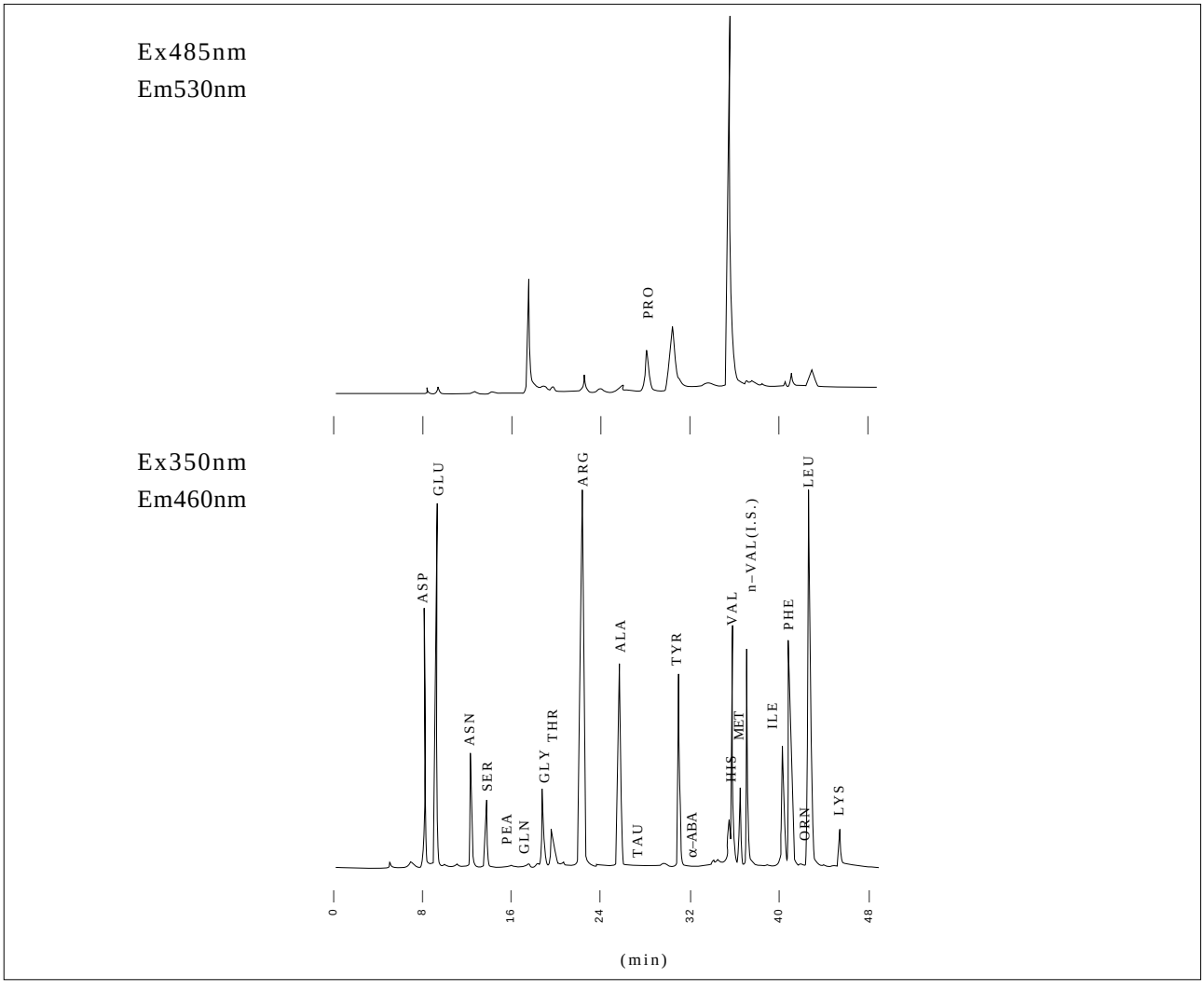
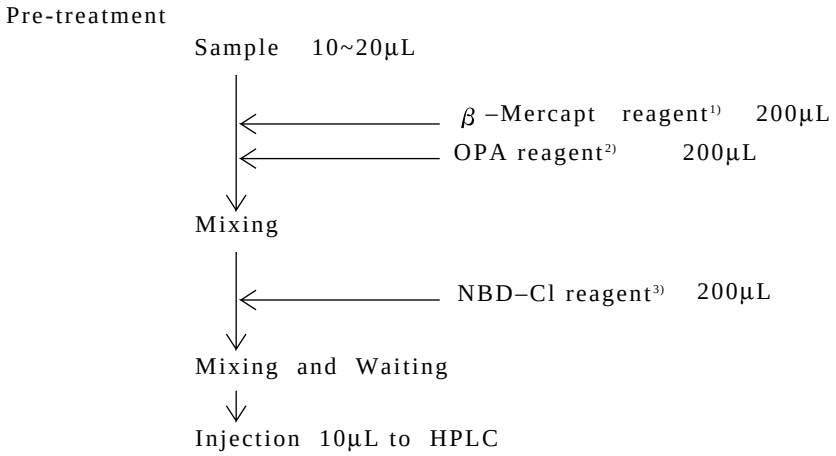


Fig. 1.8.1 Analysis of cooking vinegar using prelabel amino acid analysis method

1.8 Analysis of Amino Acid in Cooking Vinegar Using Precolumn Derivatization (2) - LC

References



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

Fig. 1.8.2 Pretreatment conditions

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

Fig. 1.8.3 Gradient conditions

1.9 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.

References

Shimadzu LC Application Report No. 17
Shimadzu Application News No. L196
Yasui, Shimadzu Review, 47 (4), 365 (1990)

•Pretreatment

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

•Analytical Conditions

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

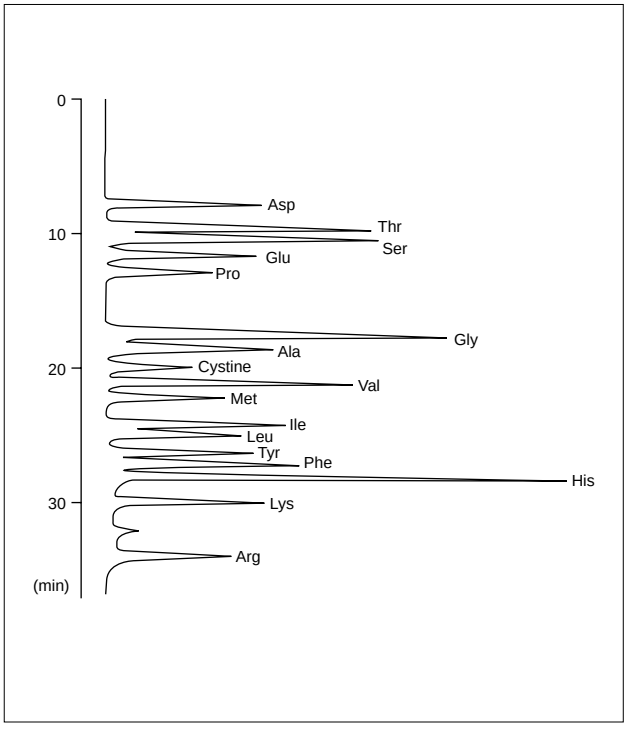


Fig. 1.9.1 Analysis of standard solution of 17 components in protein-configured amino acid

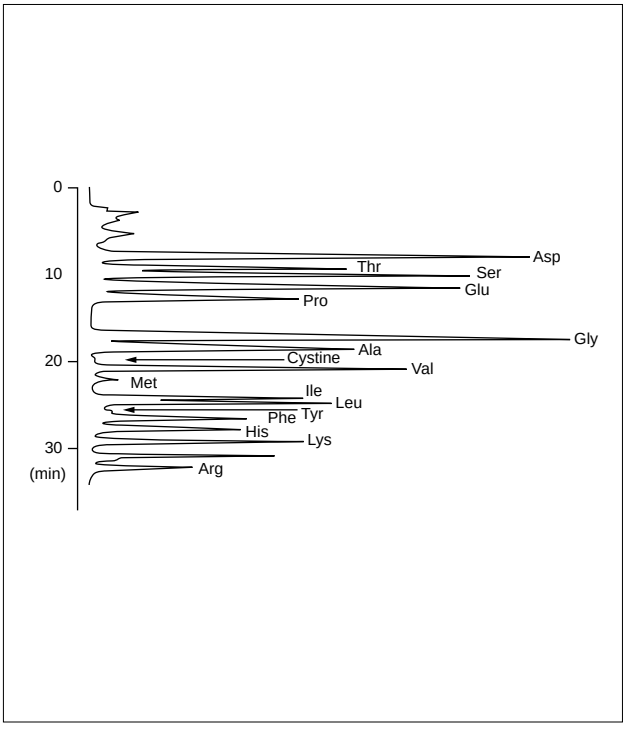


Fig. 1.9.2 Analysis of Soya sauce

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.

•Pretreatment

None.

•Analytical Conditions

Instrument	: HPLC
Column	: Develosil ODS-UG-5 (6.0mmϕ×200mm) with guard column
Precolumn	: Shim-pack GRD-ODS (4.0mmϕ×250mm)
Mobile phase	: (A) 50mM sodium acetate (B) methanol (A)→(B)gradient method
Flow rate	: 1.2mL/min
Temperature	: 35℃
Detection	: Fluorescence Detector Ex350nm Em450nm

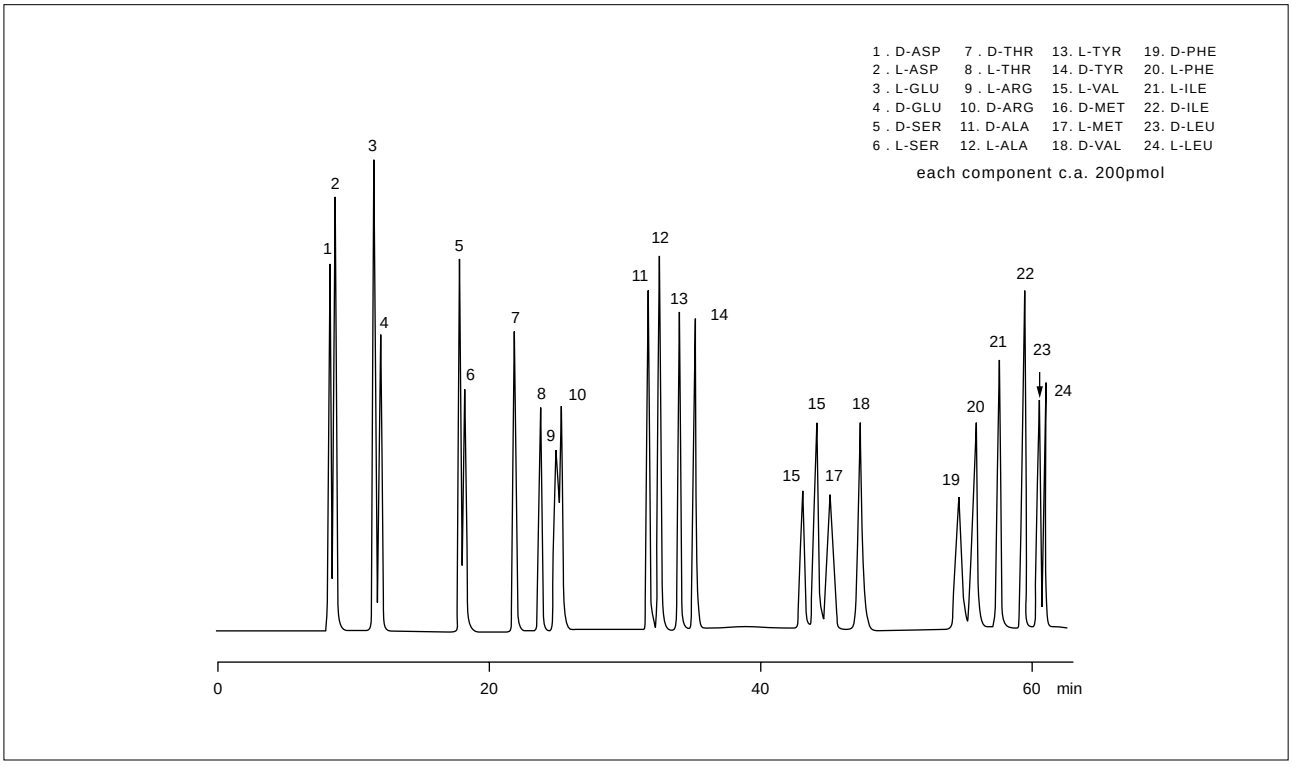


Fig. 1.10.1 Analysis of D-, L-amino acid standard solution

References

N. Nimura and T. Kinoshita, J. Chromatogr., 352, 169 (1986)

Murakita, et al Clinical Chemistry, Supplement No. 2, pp71b, 21 (1992)

Murakita, et al Summary of Symposium on Separation Science and Related Techniques, pp101 (1993)

Shimadzu Application News No. L235

1.10 Simultaneous Analysis of D- and L-Amino Acids (2) - LC

References

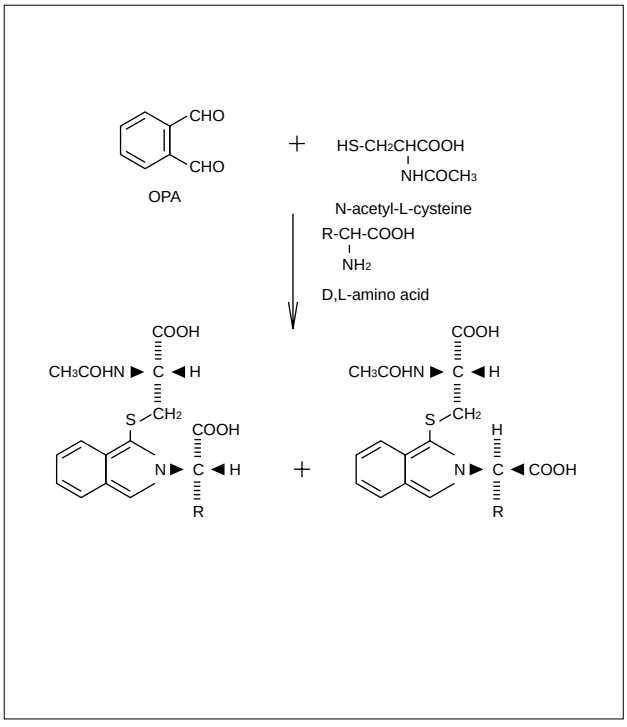


Fig. 1.10.2 Chiral derivatization reaction

TIME	FUNCTION	VALUE
16	BCONC	24
24	BCONC	24
29	BCONC	40
50	BCONC	40
59	BCONC	67
59.01	BCONC	80
64	BCONC	80
64.01	BCONC	0
65	STOP	

Fig. 1.10.3 Gradient conditions

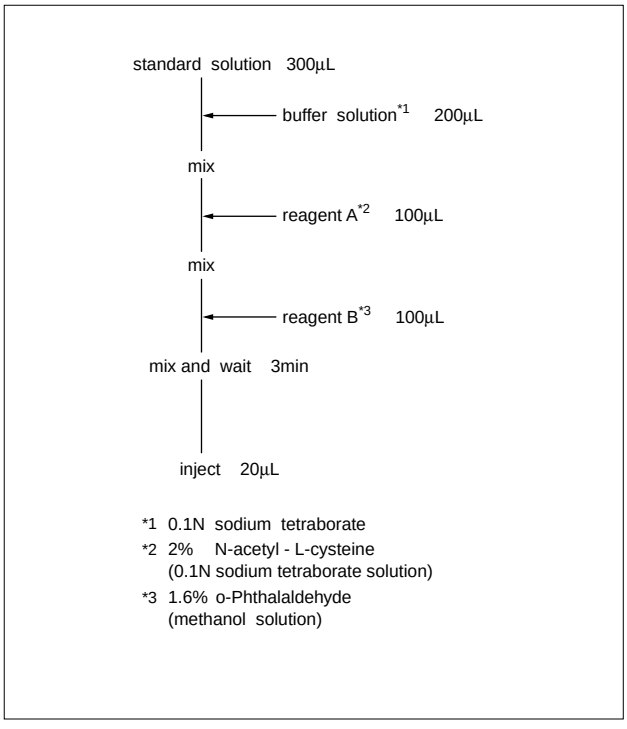


Fig. 1.10.4 Derivatization conditions

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

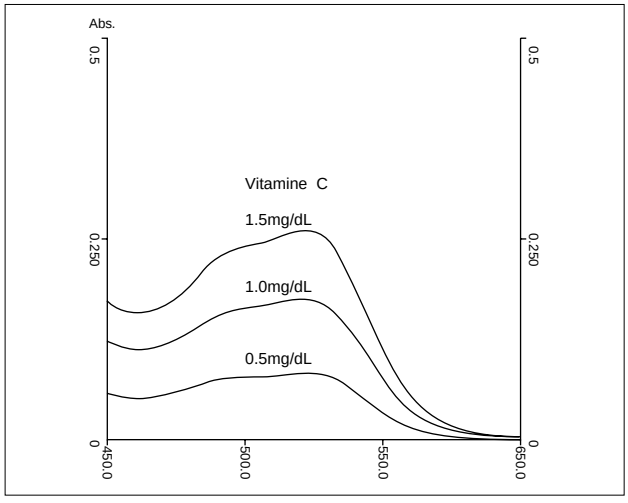


Fig. 1.11.1 Absorption spectrum of vitamin C

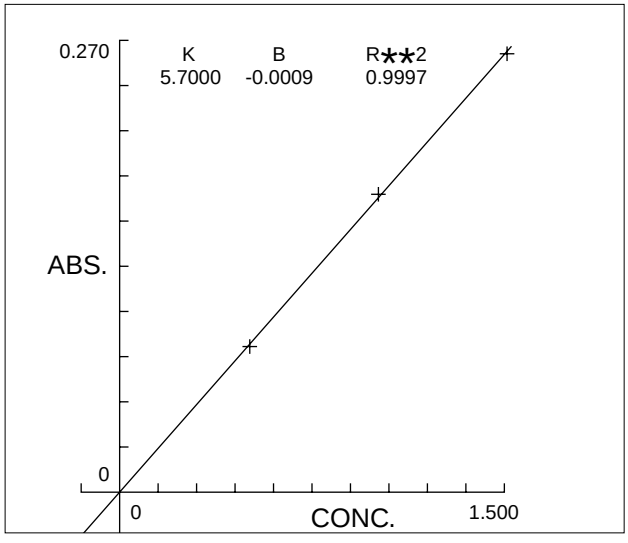


Fig. 1.11.2 Calibration curve for vitamin C

STD. NO. CONC. ABS.

01	0	0
02	0.5000	0.0870
03	1.0000	0.1780
04	1.5000	0.2620

Standard mg/dL

CONC.=K★ABS.+B

K	B	R★★2
5.7000	-0.0009	0.9997

NO.	ABS.		CONC.	
01	0.1940	C=	1.1048	Balance Food
02	0.1640	C=	0.9338	Candy

Fig. 1.11.3 Quantitative results for vitamin C

1.12 Analysis of Trace Amounts of Vitamins B_1 and B_2 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 B_2 - 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 B_2, which also has been adopted for the Standard Methods of Analysis for Hygienic Chemists. Here, vitamin B_2 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 B_2 that had been hot-water extracted. And after oxidation, the liquid extracted with chloroform was measured. Vitamin B_2 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 B_2 exist in 1g of Soya bean.

•Analytical Conditions

Instrument	: RF Spectrofluorophotometer
Sample	: Vitamin B_2 in Soya bean
Solvent	: Chloroform
Excitation	: 469nm
Slit	: Ex10nm Em10nm

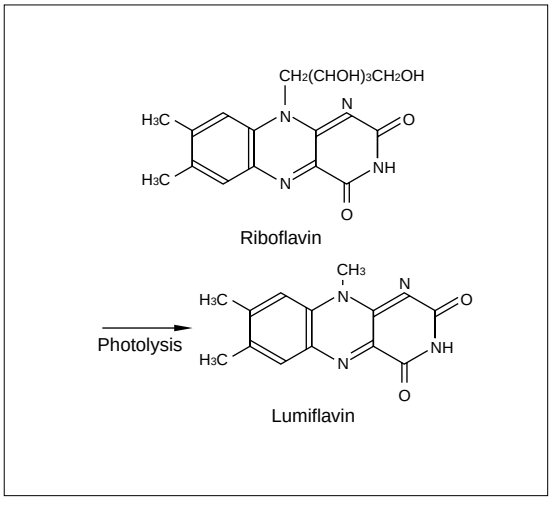


Fig. 1.12.1 Creation process of lumiflavin

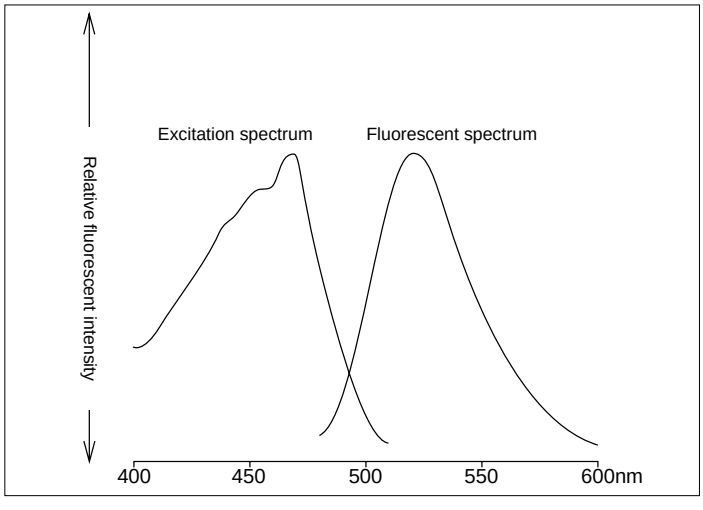


Fig. 1.12.2 Excitation and fluorescent spectrum of vitamin B2 (riboflavin)

1.12 Analysis of Trace Amounts of Vitamins B_1 and B_2 in Food Products Using Fluorescence Photometry (2) - RF

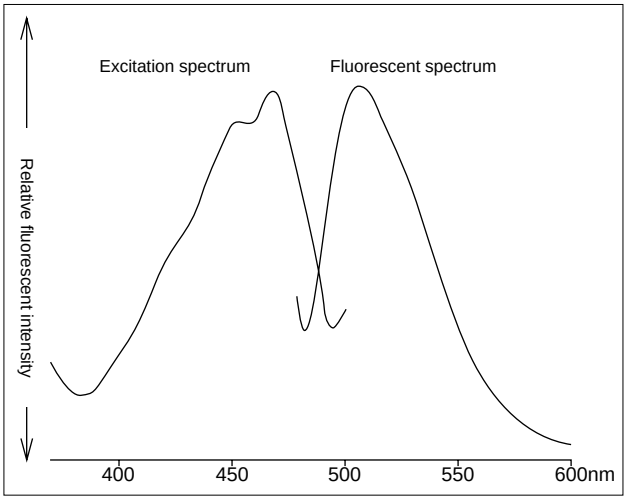


Fig. 1.12.3 Excitation and fluorescent spectra of lumiflavin created from photolysis

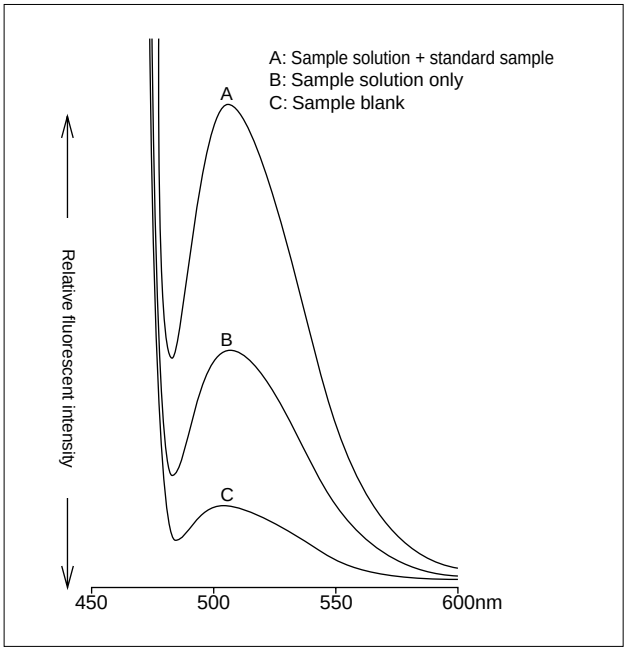


Fig. 1.12.4 Measurement of Vitamin B2 in Soya bean

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

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

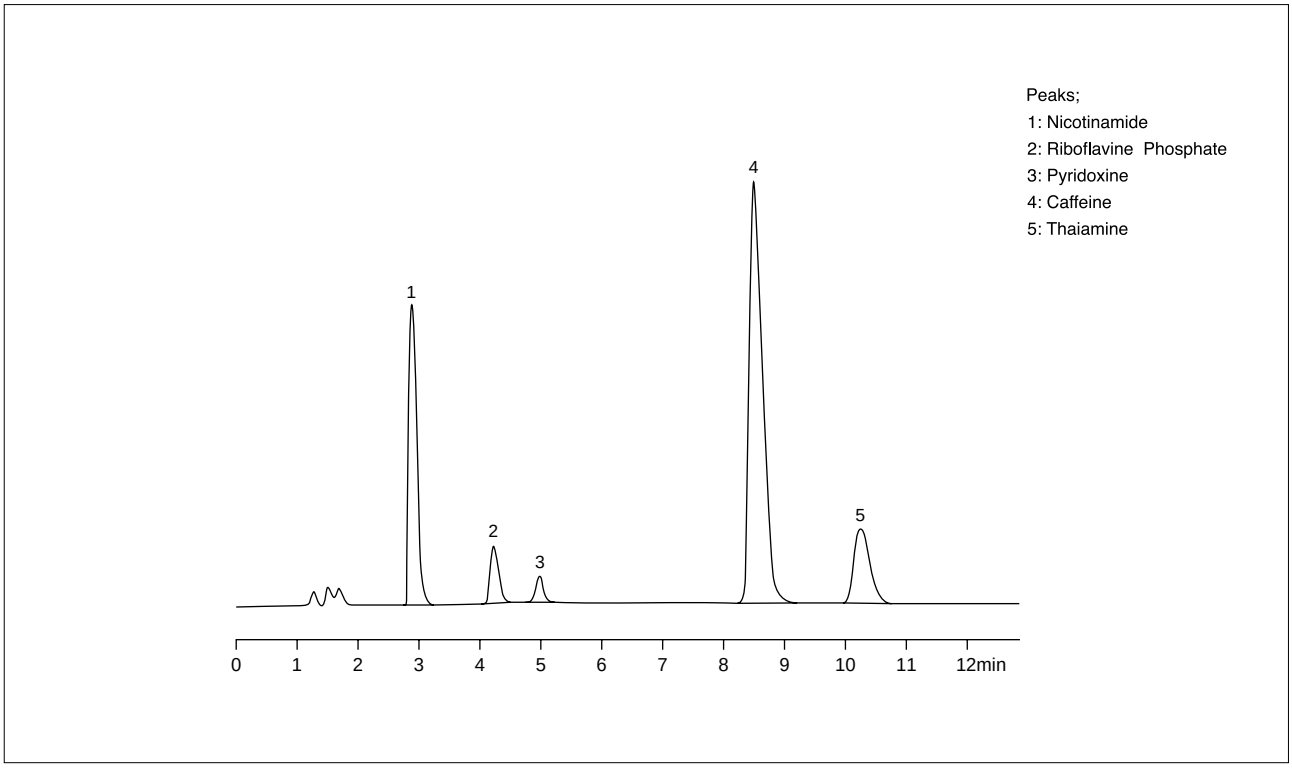


Fig. 1.13.1 Analysis of vitamin B group and caffeine in vitamin drink

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

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

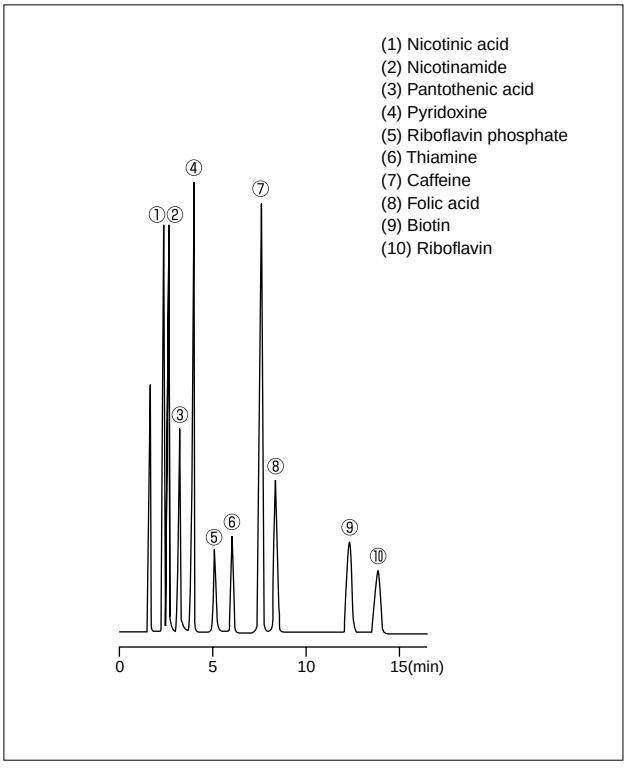


Fig. 1.14.1 Analysis example (210nm) of vitamin B group

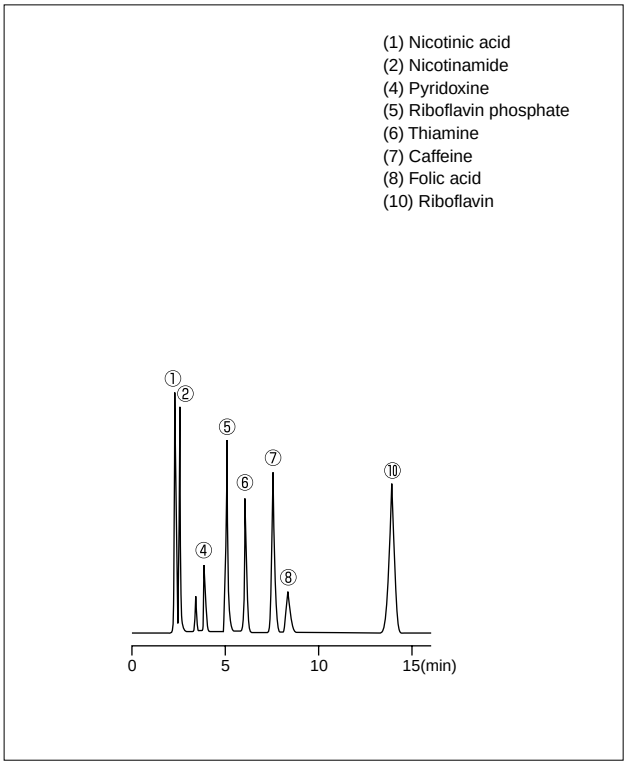


Fig. 1.14.2 Analysis example (270nm) of vitamin B group

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.

References

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

•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-NH_2 (6.0mmϕ×150mm)
Mobile phase	: n-hexane/isopropyl alcohol = 100/4 (v/v)
Temperature	: 40℃
Flow rate	: 1.5mL/min
Detection	: UV-VIS Detector 297nm

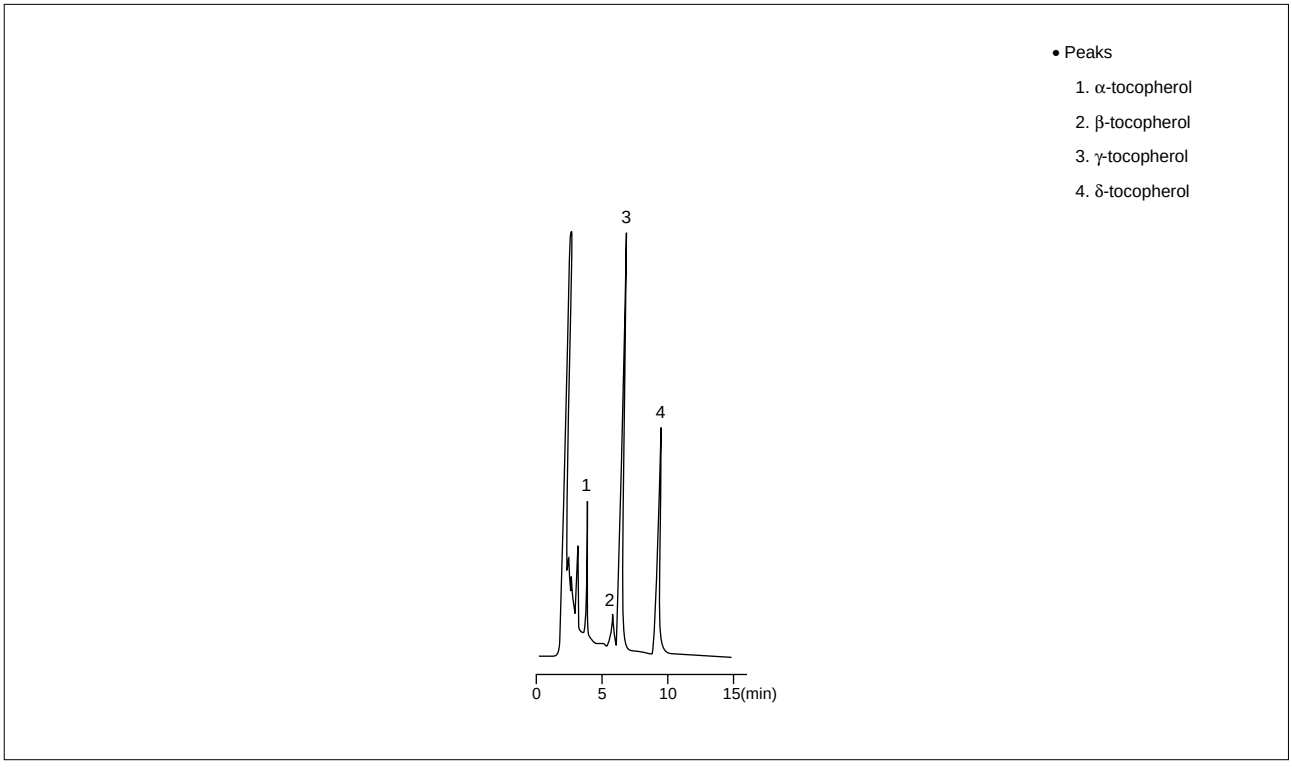


Fig. 1.15.1 Analysis of tocopherol types in milk

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-Ⅱ (4.6mmϕ×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℃
Flow rate	: 1.0mL/min
Detection	: UV-VIS Detector 260nm

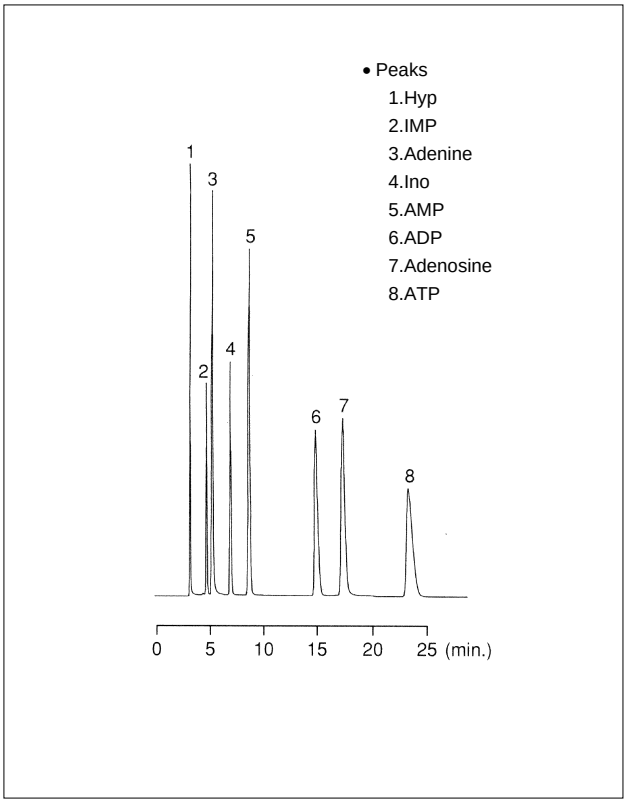


Fig. 1.16.1 Analysis of adenine derivative components

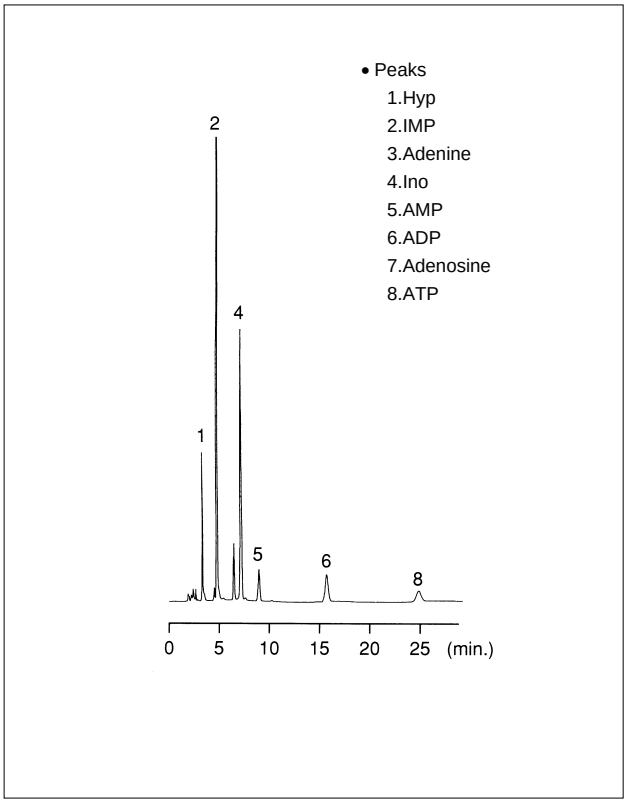


Fig. 1.16.2 Analysis of tuna meat

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 elation 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)
Mikami, Egi, Shimadzu Review, Nos. 44 (3), 47 (1987)
Shimadzu HPLC Application Report No. 11 (C196-E036)

•Pretreatment

Beer was injected without any pretreatment.

•Analytical Conditions

Instrument	: HPLC
Column	: Shim-pack CLC-NH_2 (6.0mmϕ×15cm)
Mobile phase	: Acetonitrile/water = 60/40 (v/v)
Temperature	: 25℃
Flow rate	: 1.0mL/min
Detection	: Refractive index detector

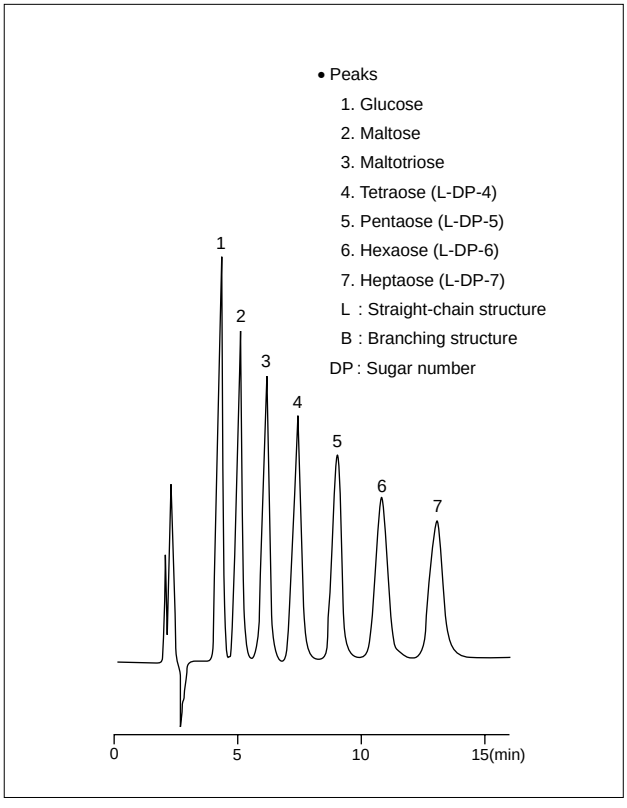


Fig. 1.17.1 Analysis of sugar and oligosaccharide standard samples

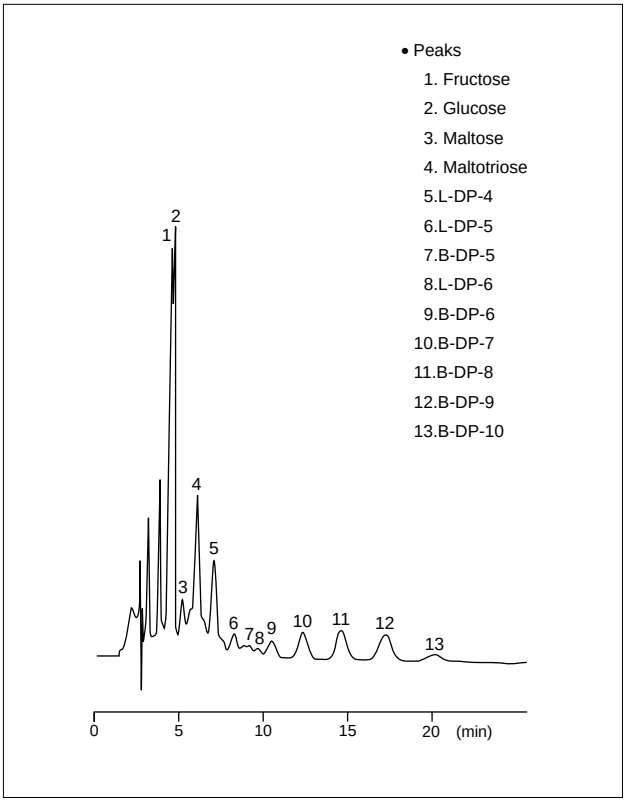


Fig. 1.17.2 Analysis of oligosaccharides in beer

1.18 Analysis of Nonreducing Sugar Using Postcolumn Derivatization with Fluorescence Detection - LC

•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

T. Kinoshita, et al, J. Liquid Chromatogr., No. 14 (10), 1929 (1991)
Shimadzu Application News No. L 226

•Pretreatment

None.

•Analytical Conditions

Instrument	: HPLC
Column	: Asahipak NH2P-50 (4.6mmϕ×250mm)
Mobile phase	: Acetonitrile/water = 65/35 (v/v)
Temperature	: 40℃
Flow rate	: 1.0mL/mi
Reaction liquid	: 20mM taurocyamine, 0.1M potassium tertraborate water solution containing 1mM sodium periodate (using 10NKOH solution to adjust to pH 12.5)
Reaction liquid rate	: 1.0mL/min
Reaction temperature	: 150℃
Detection	: Fluorescence Detector (Ex320mm Em450nm)

References

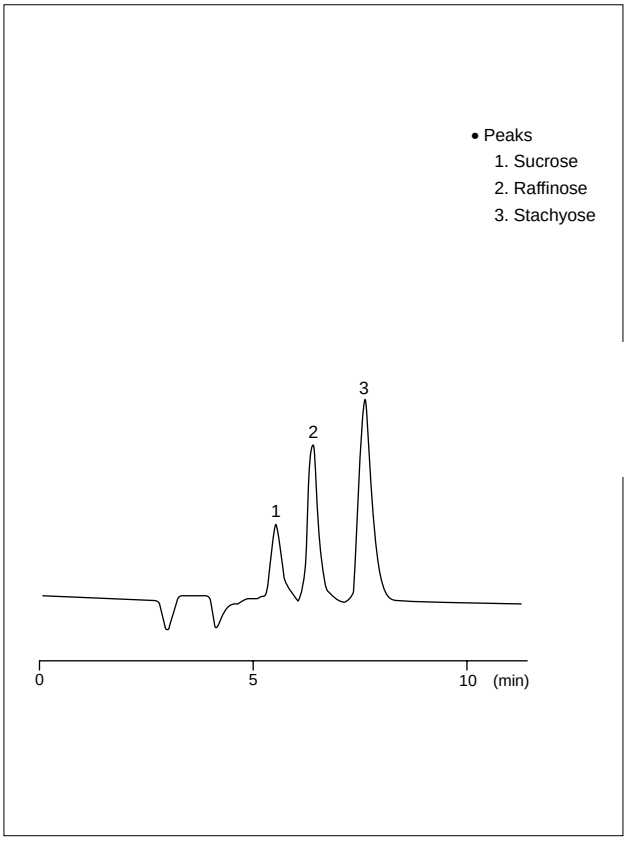


Fig. 1.18.1 Analysis of nonreducing oligosaccharide

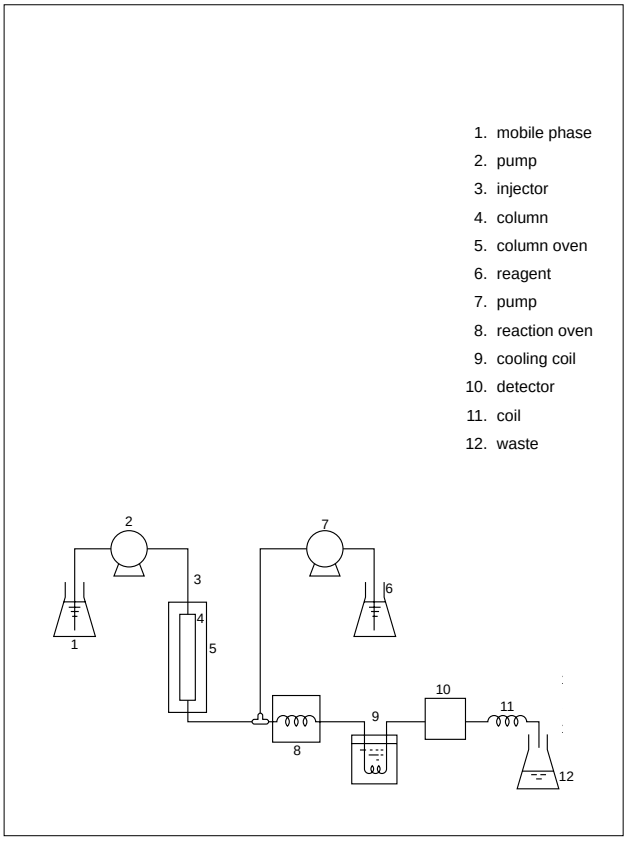


Fig. 1.18.2 Flowchart diagram of nonreducing sugar analysis system

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

[Pickle liquid]

Instrument	: HPLC
Column	: Shim-pack SCR-101C (7.9mmϕ×300mm)
Mobile phase	: Water
Temperature	: 80℃
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℃
Flow rate	: 1.0mL/min
Detection	: Refractive index detector

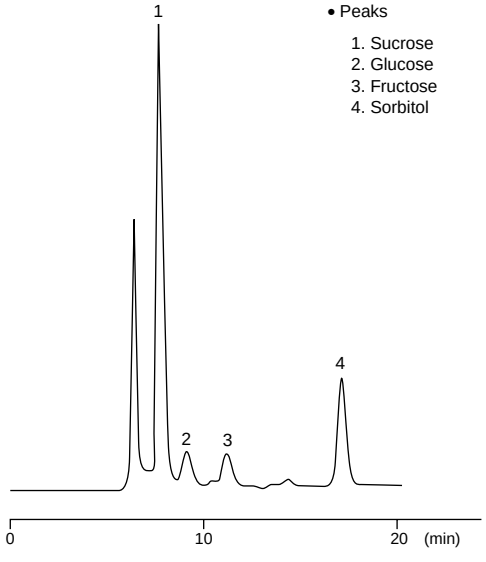


Fig. 1.19.1 Analysis of pickle liquid

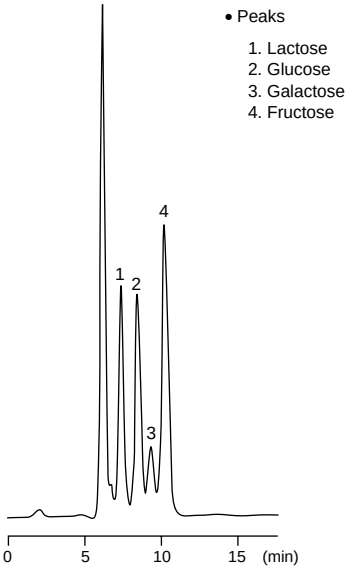


Fig. 1.19.2 Analysis of yogurt

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 Linear gradient
Temperature	: 65℃
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℃
Detection	: Fluorescence Detector (Ex320nm Em430nm)

References

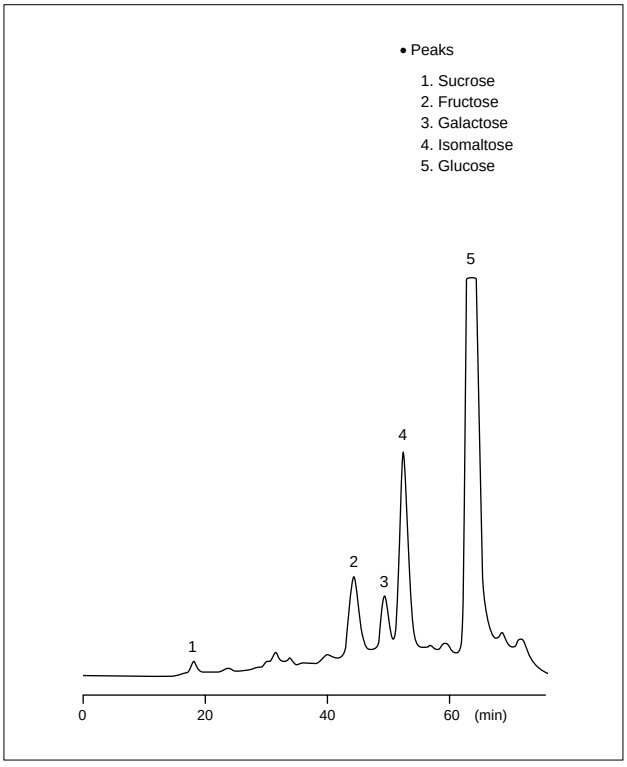


Fig. 1.20.1 Analysis of miso using reducing sugar analysis system

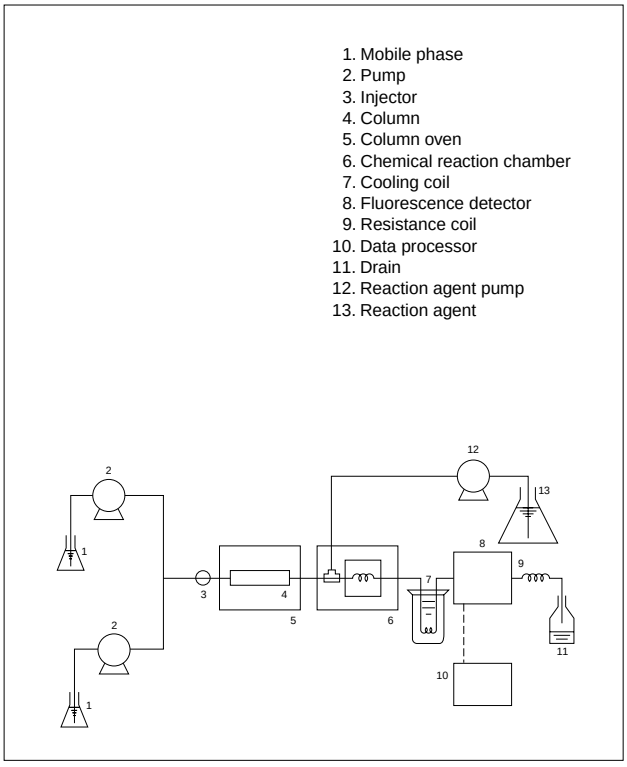


Fig. 1.20.2 Flow line diagram of reducing sugar analysis system

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

Instrument	: GC-14BPF
Column	: 10%PEG6000 3mm×1m on shimalite TPA (glass)
Col. Temp.	: 150˚C
Inj. Temp.	: 230˚C
Det. Temp.	: 200˚C(FID)
Carrier gas	: N_2

References

1) Standard Methods of Analysis for Hygienic Chemists (annotation) 455 (1990), edited by the Pharmaceutical Society of Japan
2) Ministry of Health and Welfare (currently Ministry of Health, Labour and Welfare), Environmental Health Bureau, Food Sanitation Testing Policy, 33-35 (1989)

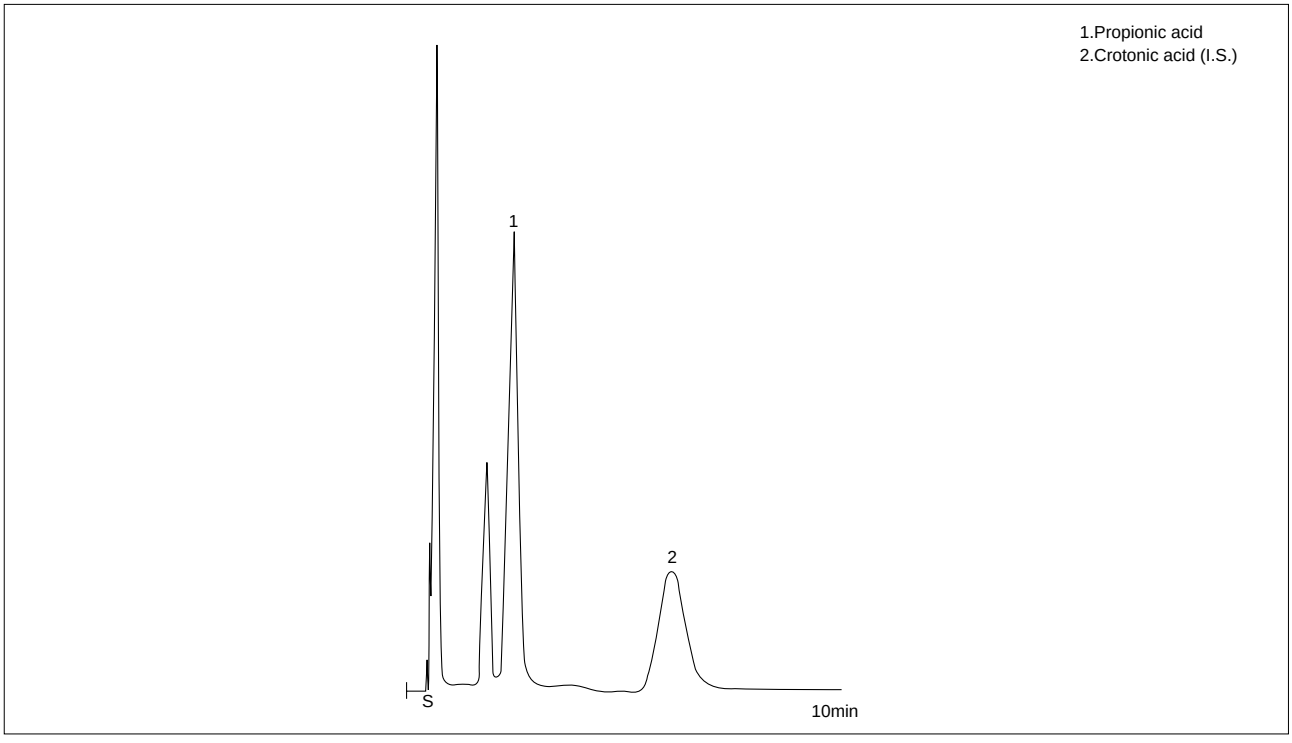


Fig. 2.1.1 Analysis of propionic acid

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	: N_2

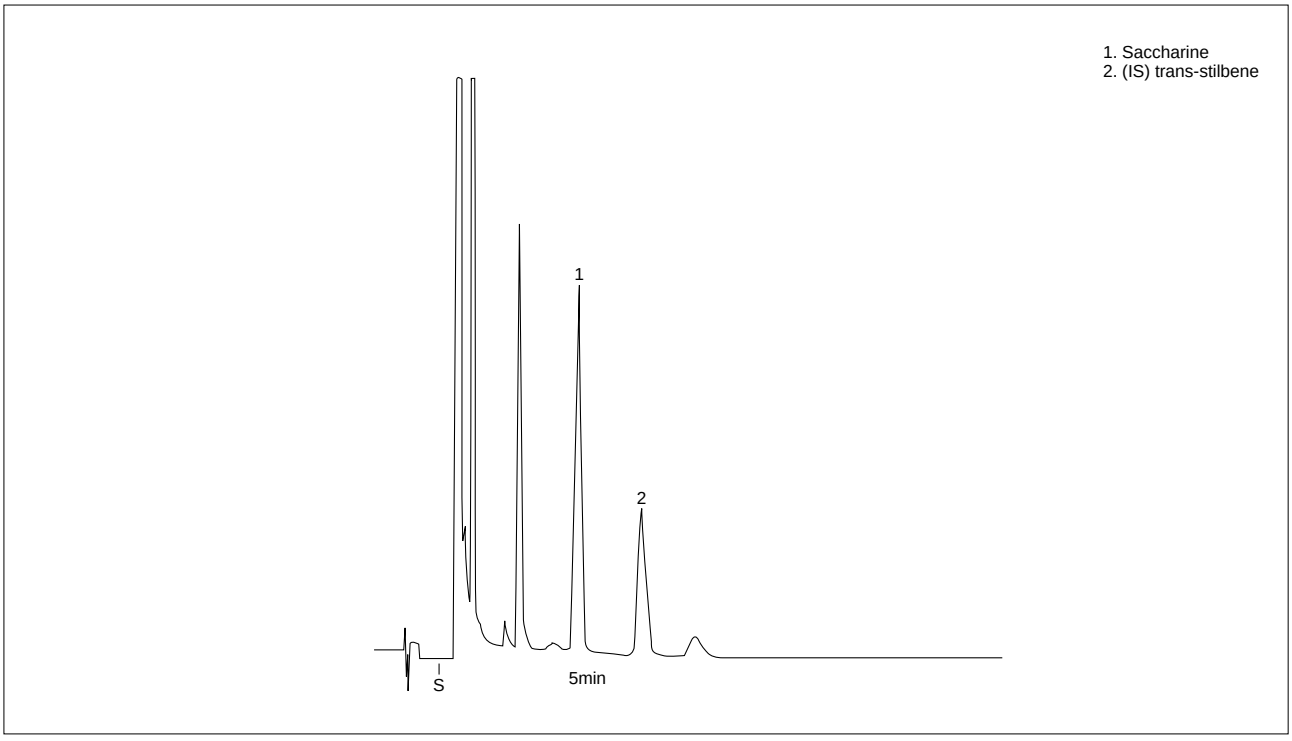


Fig. 2.2.1 Analysis of saccharine

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

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

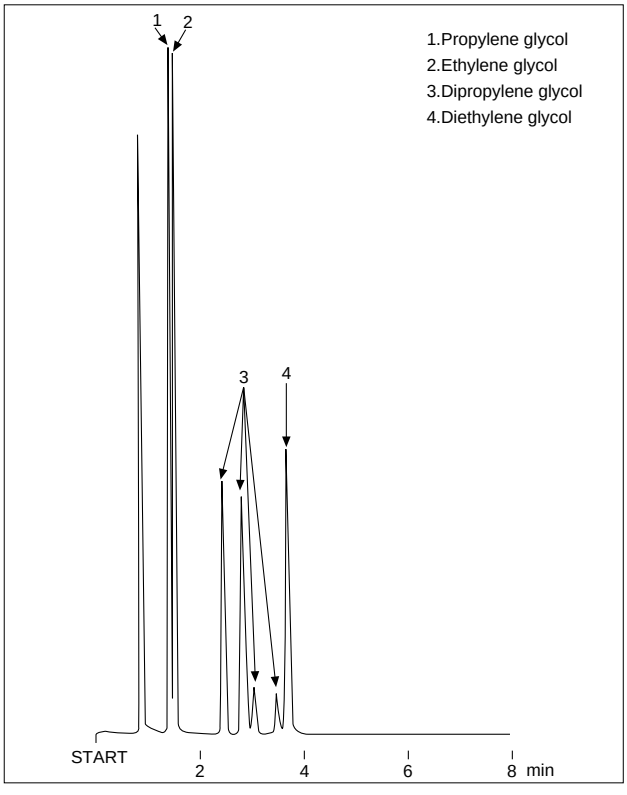


Fig. 2.3.1 Analysis of glycols (standard products)

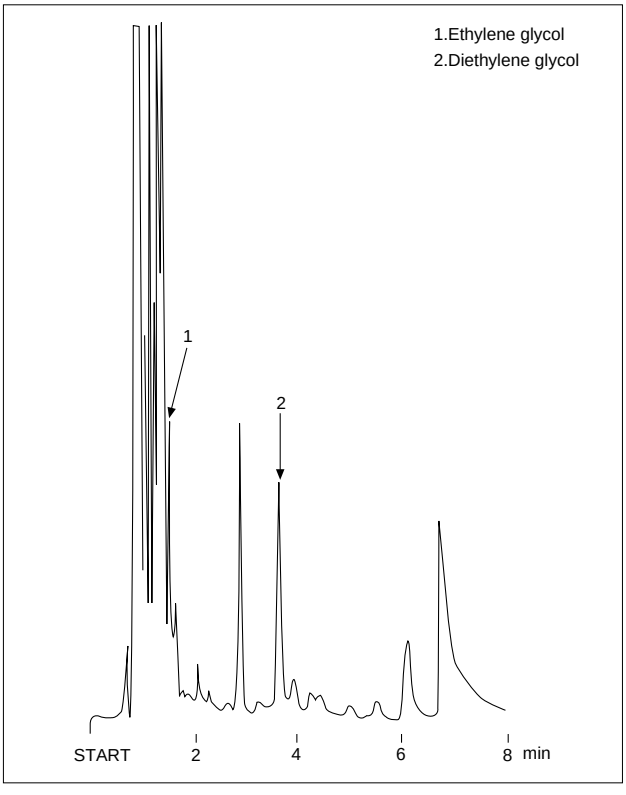


Fig. 2.3.2 Analysis of shop-sold wine with glycols added

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

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%H_3PO_4 3mm × 2m on chromosorb W(glass)
Col. Temp. : 185˚C
Inj. Temp. : 230˚C
Det. Temp. : 250˚C(FID)
Carrier gas : N_2

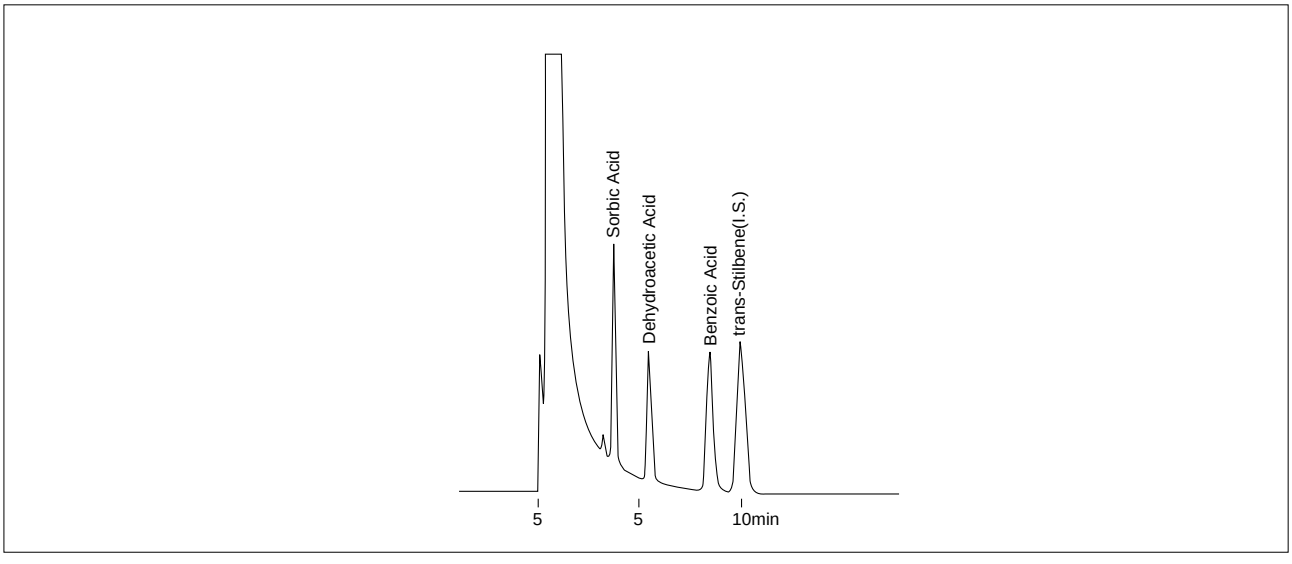


Fig. 2.4.1 Analysis of Preservatives

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

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 naphthylethylenediamine for measurement.
- Benzoic acid in a food product
  The benzoic acid preservative was separated and extracted from soy sauce using steam distillation in readiness for UV absorption measurement.
- Sorbic acid in a food product
  The sorbic acid preservative was separated and extracted from boiled fish paste using steam distillation in readiness for UV absorption measurement.
- 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	: H_2O
Cell	: 10mm
Range	: 0 ~ 2Abs

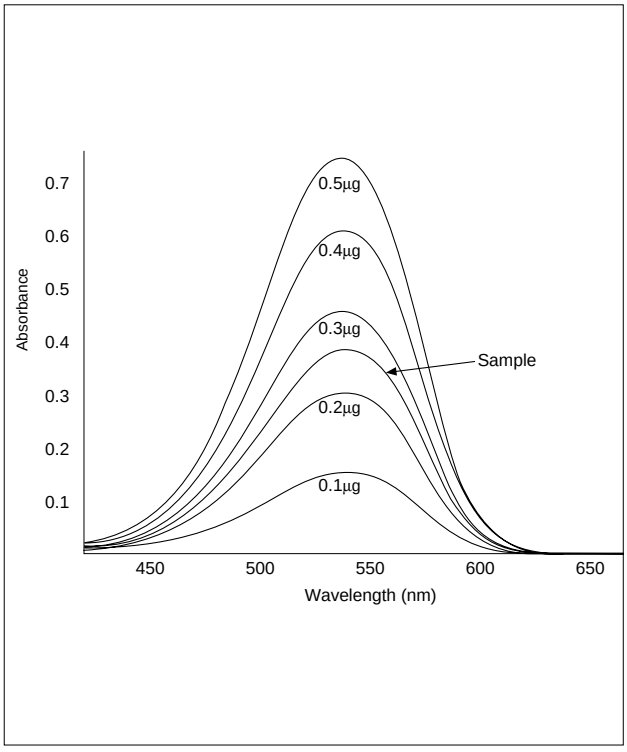


Fig. 2.5.1 Absorption spectrum for sodium nitrite

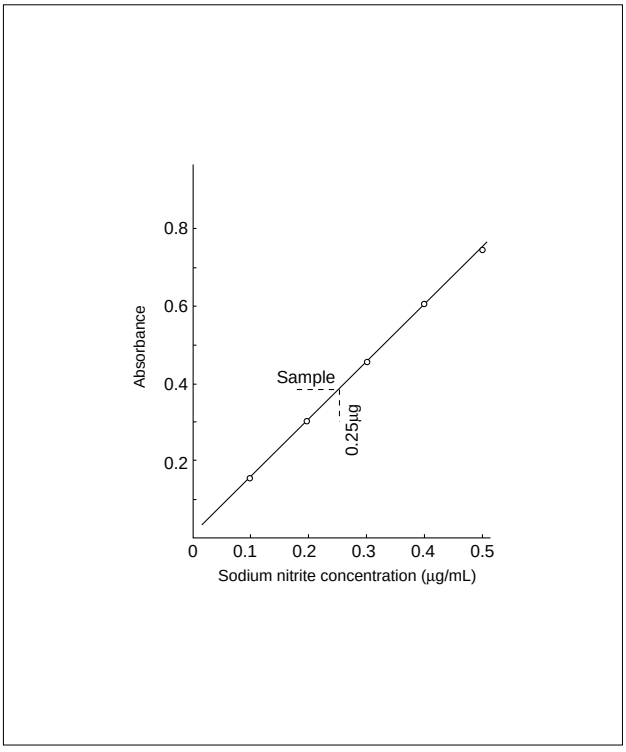


Fig. 2.5.2 Calibration curve for sodium nitrite

2.5 Analysis of Preservatives in Food Products with Absorption Photometry (2) - UV

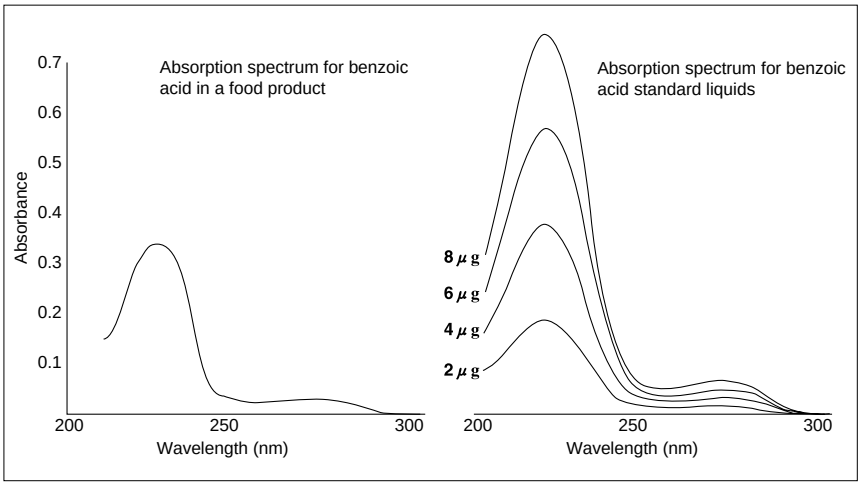


Fig. 2.5.3 Absorption spectrum for benzoic acid

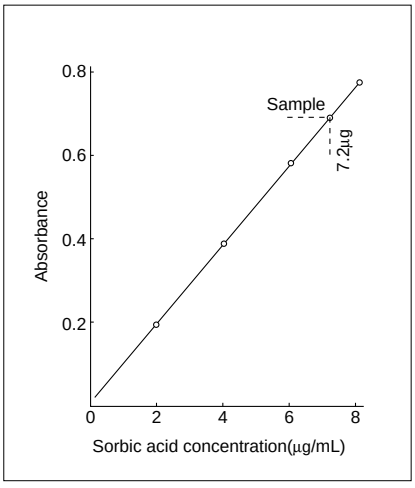
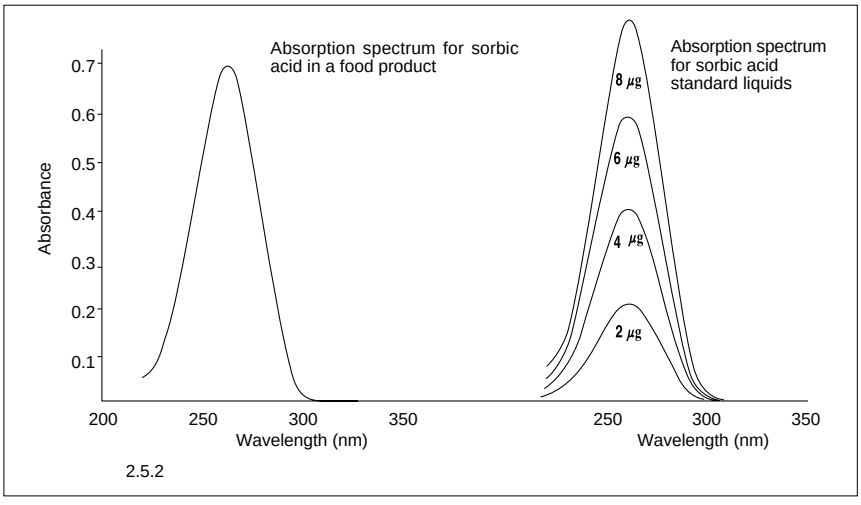


Fig. 2.5.4 Calibration curve for sorbic acid



2.5.2

Fig. 2.5.5 Absorption spectrum for sorbic acid

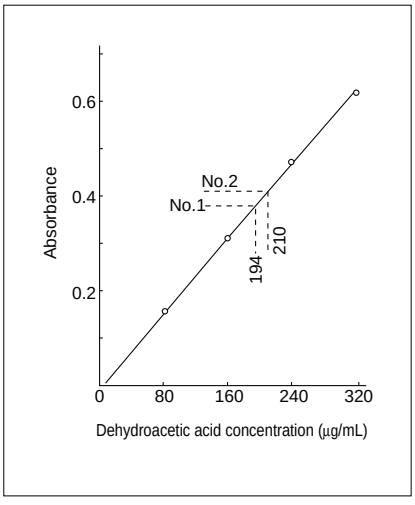
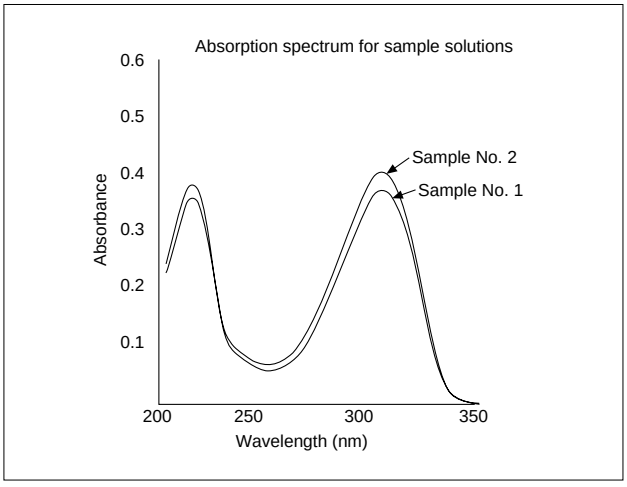


Fig. 2.5.6 Calibration curve for dehydroacetic acid



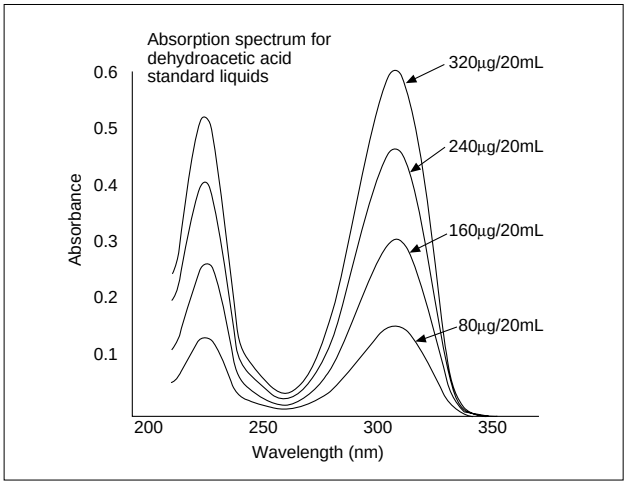


Fig. 2.5.7 Absorption spectrum for dehydroacetic acid

2.6 Color Control of Food Products (1) - UV

•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%

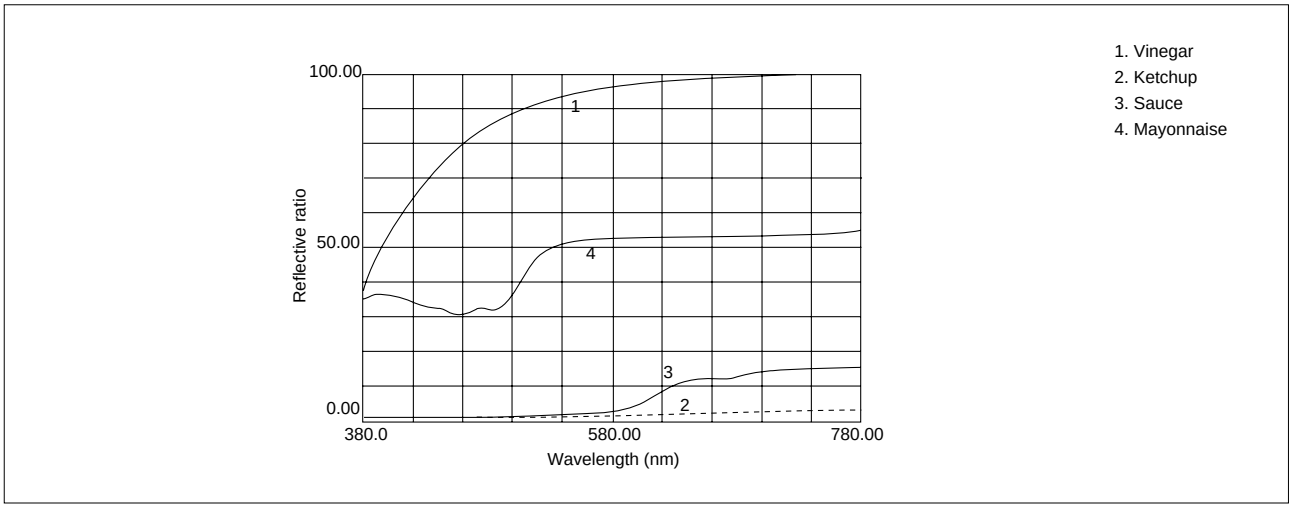


Fig. 2.6.1 Transmittance and reflectance spectra

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

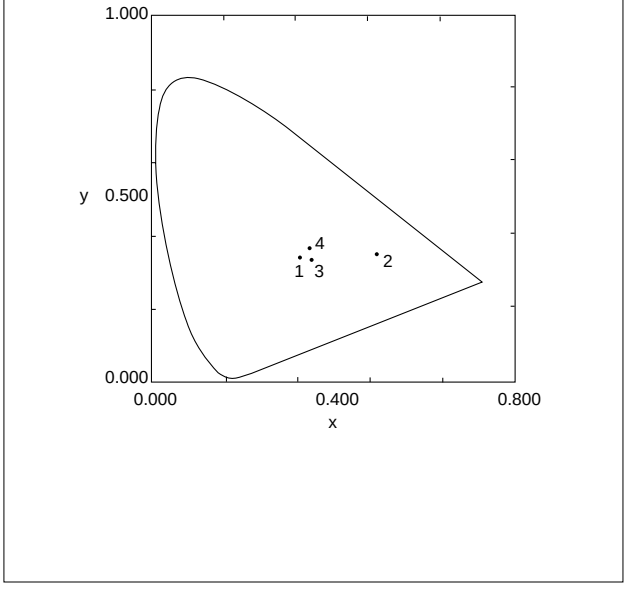


Fig. 2.6.3 CIE (x,y) chromaticity diagram

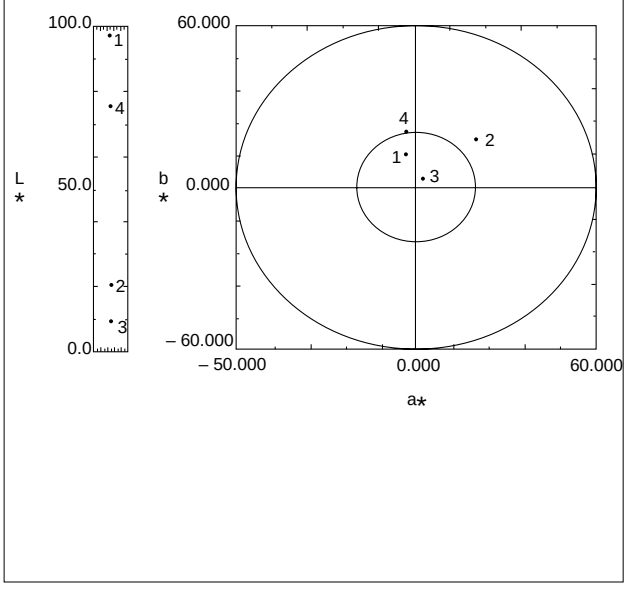


Fig. 2.6.4 UCS (Lab) chromaticity diagram

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(4.6mmϕ × 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

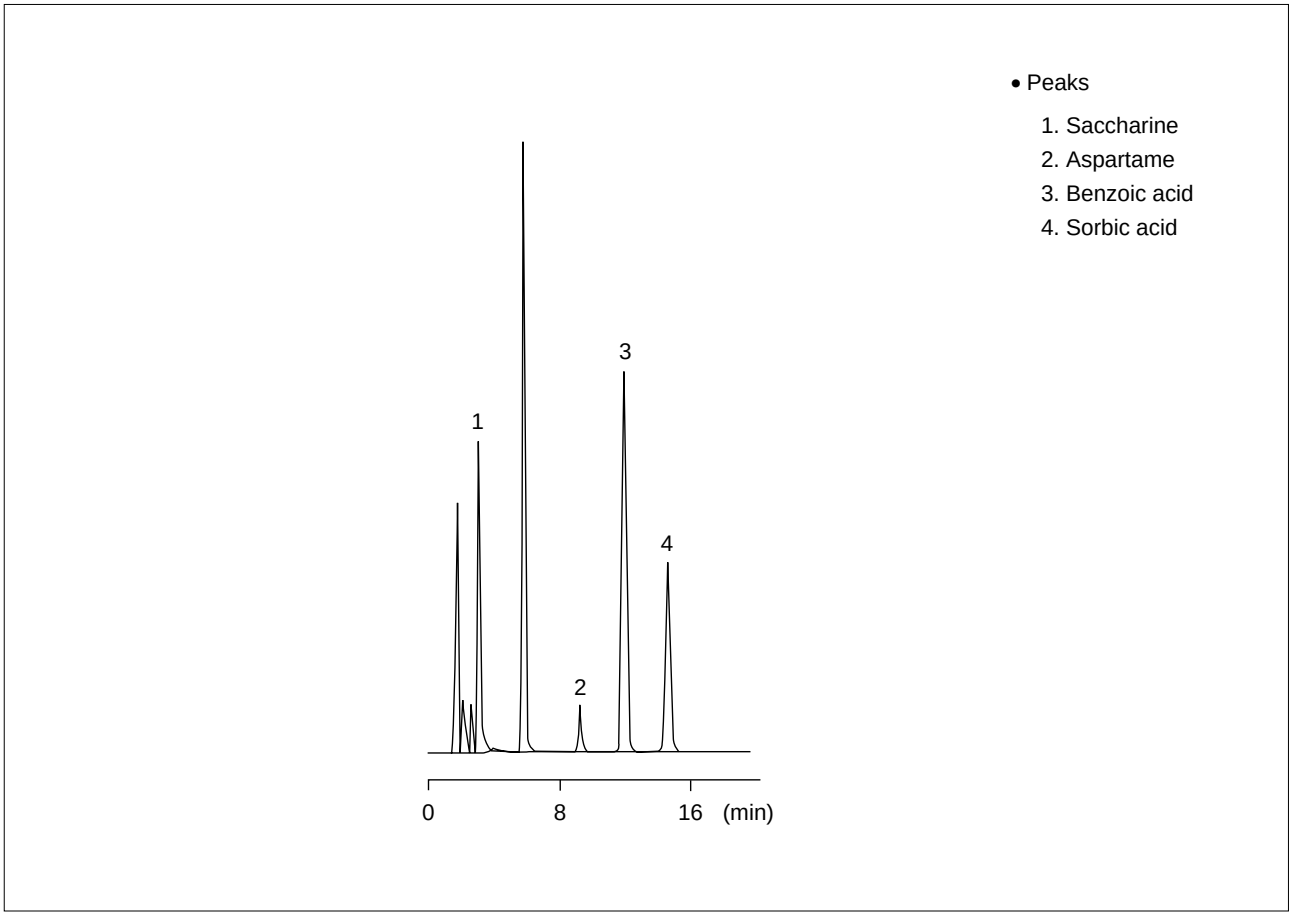


Fig. 2.7.1 Analysis of sweetener in soft drink

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)

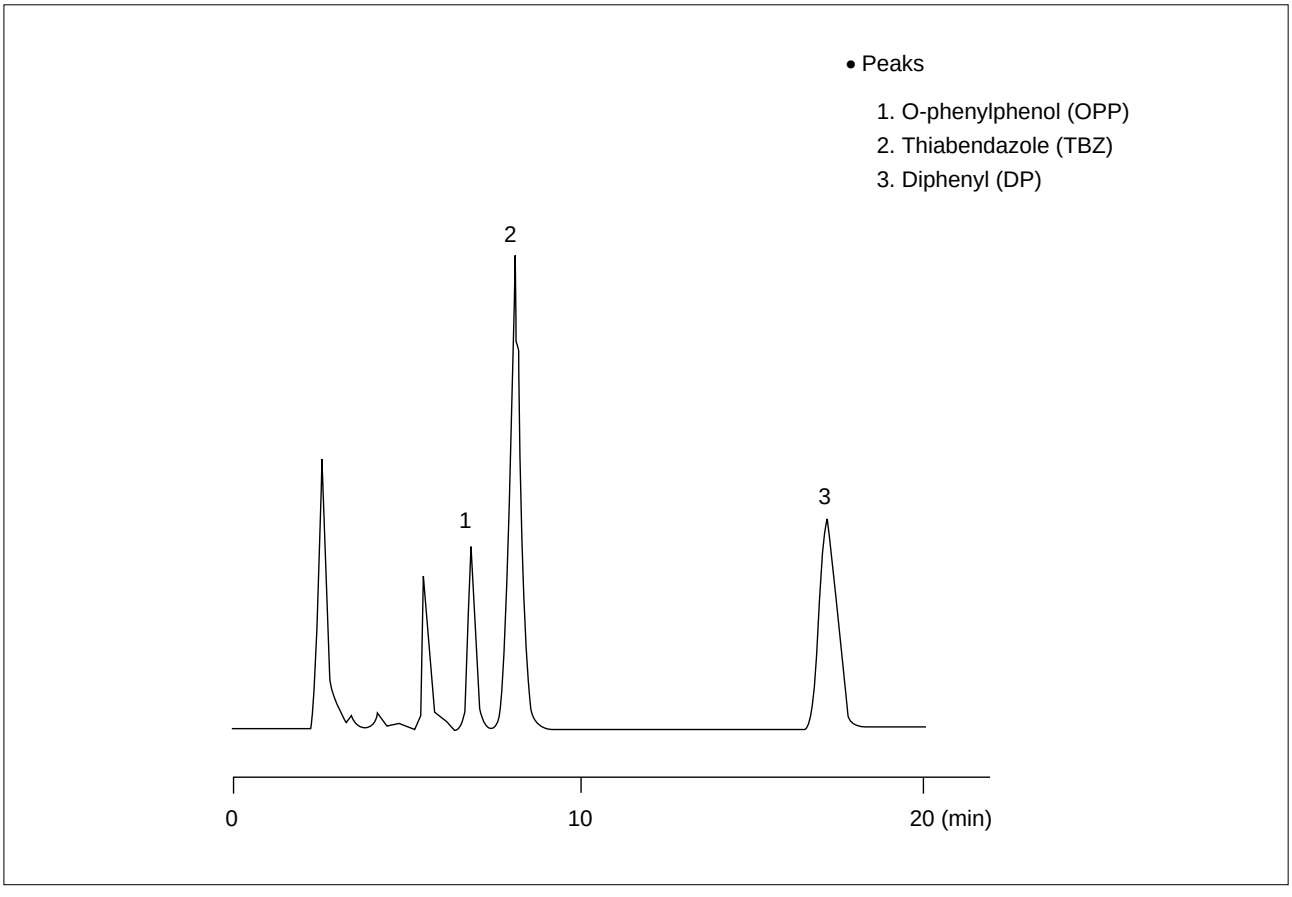


Fig. 2.8.1 Analysis of fungicide in orange

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

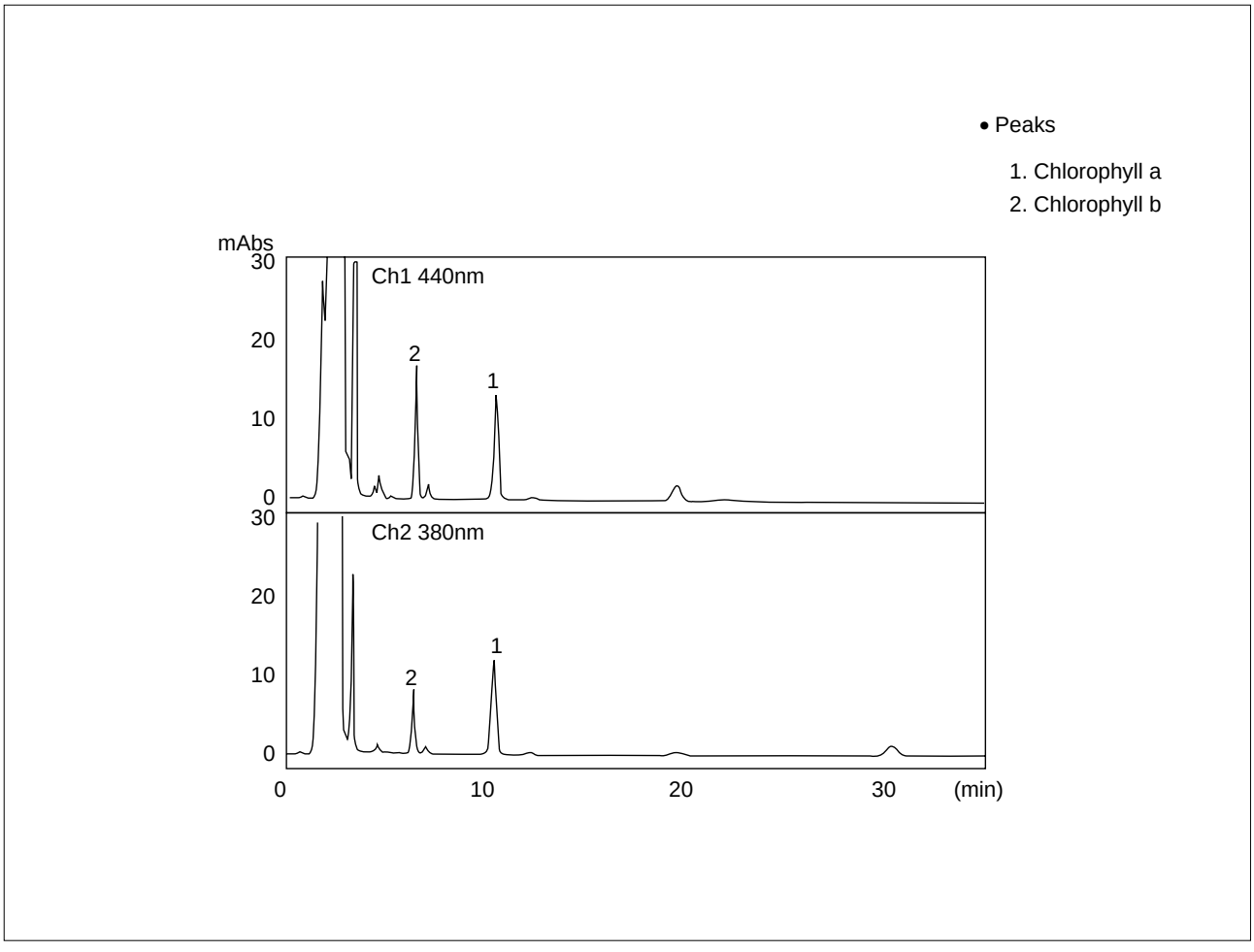


Fig. 2.9.1 Analysis of chlorophyll in spinach

2.9 Analysis of Chlorophyll in Spinach (2) - LC

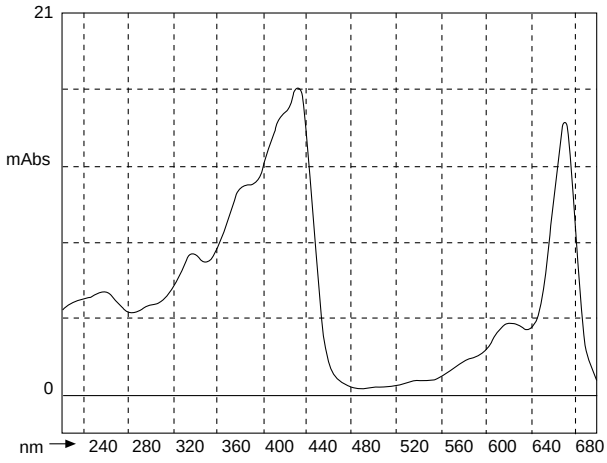


Fig. 2.9.2 Spectrum of chlorophyll a

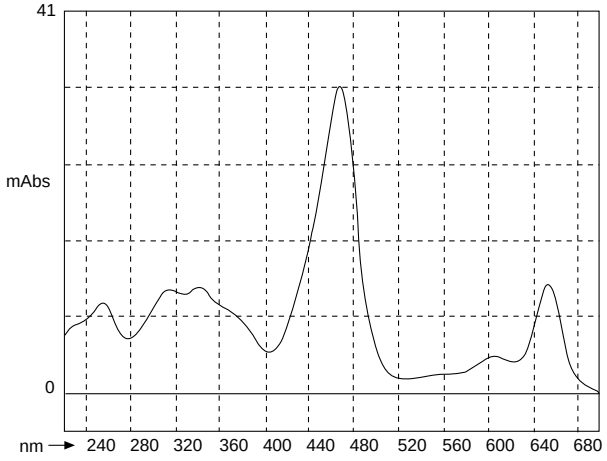


Fig. 2.9.3 Spectrum of chlorophyll b

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.

References

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

Shimadzu Application News No. L214 (C190-E050)

•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 (6.0mmϕ × 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

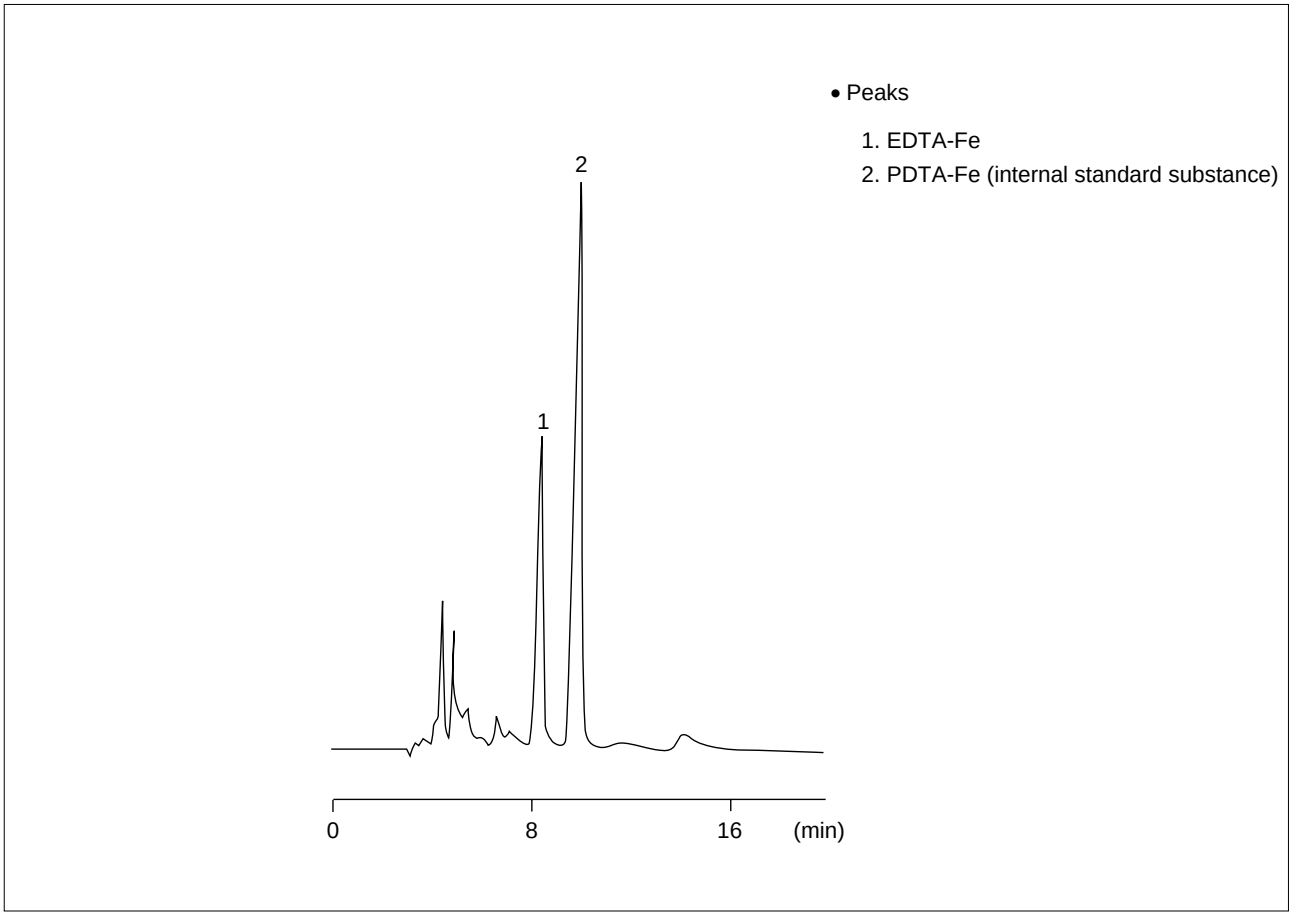


Fig. 2.10.1 Analysis of EDTA in mayonnaise

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

Instrument : HPLC
Column : STR ODS-Ⅱ (4.6mmϕ × 150mm)
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

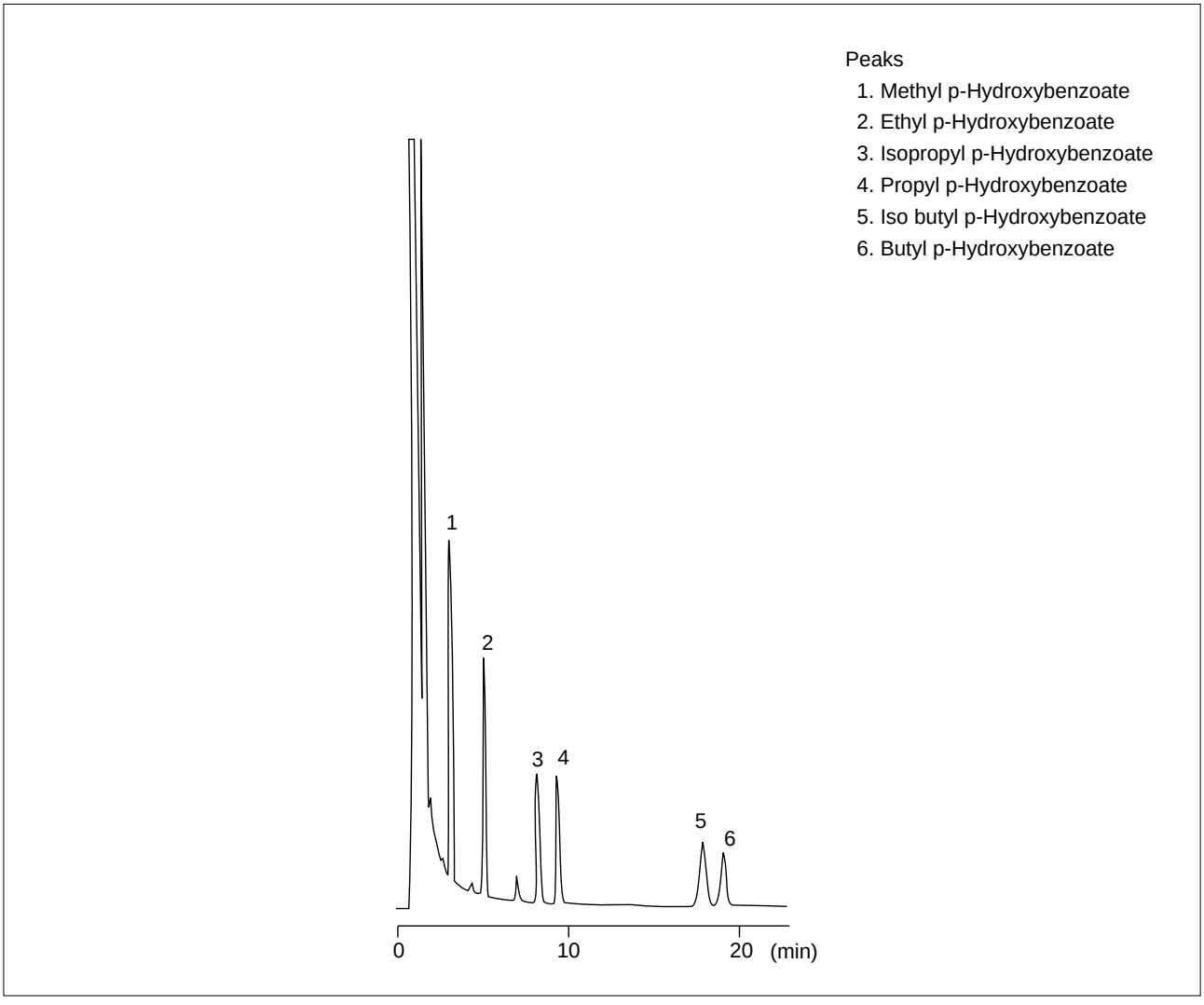


Fig. 2.11.1 Analysis of p-hydroxybenzoates in soy sauce

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

Time	B concentration
0.00 min (initial condition)	0%
15:00 min	20%
45.00 min	40%
55.00 min	70%
55.01 min	0%
65.00 min	0%

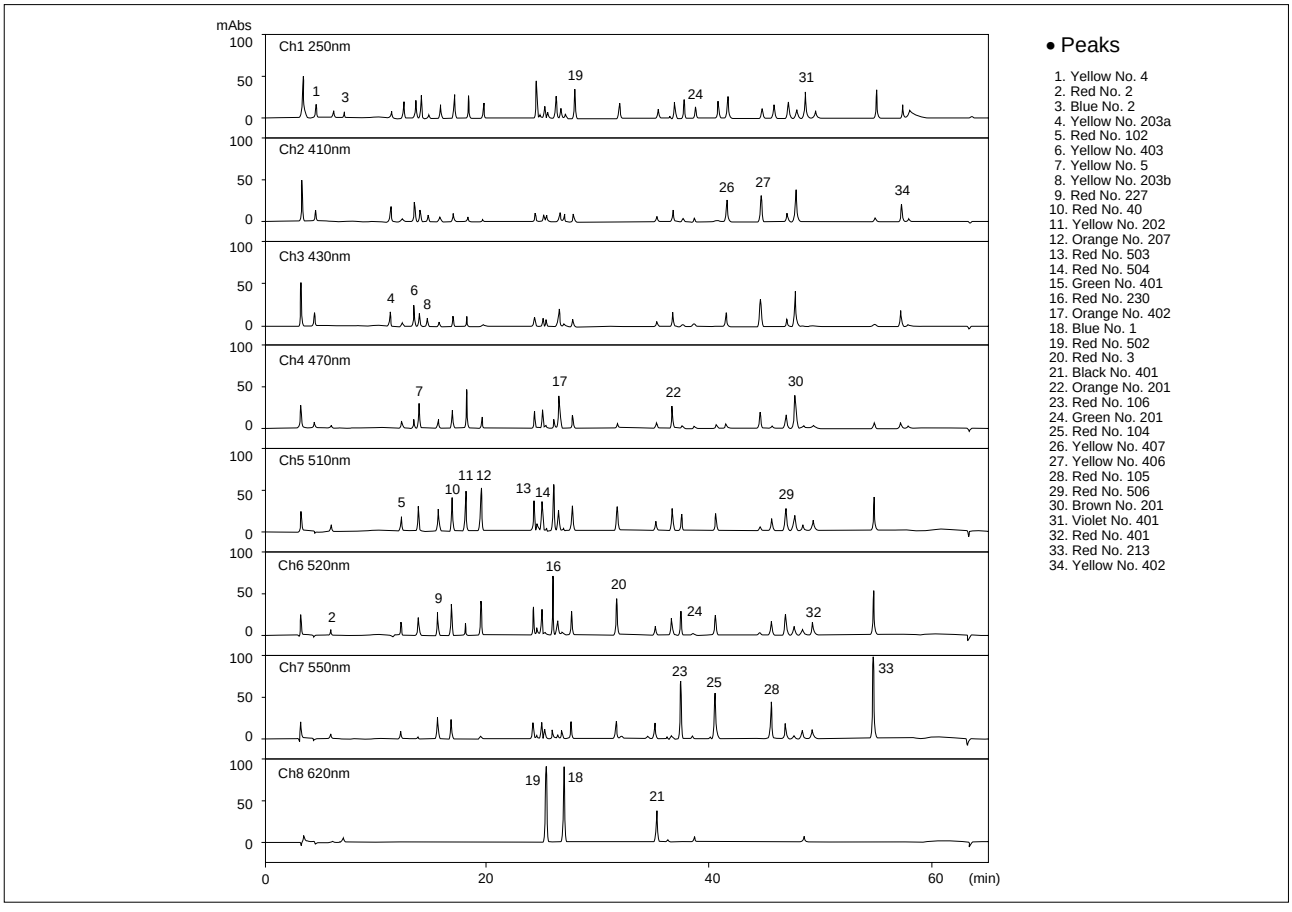


Fig. 2.12.1 Simultaneous analysis of water-soluble tar pigments

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-14BPFpsc
Column : DB-1 0.25mm × 60m df = 0.25µm
Col. Temp. : 60˚C(2min)-180 (20˚C/min)-265˚C (10˚C/min)
Inj. Temp. : 240˚C
Det. Temp. : 270˚C(FPD)
Carrier gas : He 1.2mL/min
Injection : Splitless (1min)

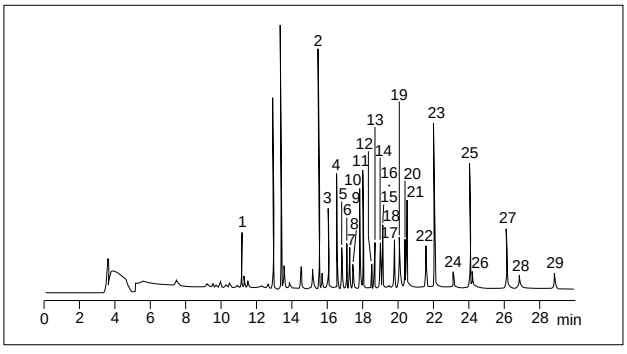


Fig. 3.1.1 Analysis example for onions using FPD

Organophosphorous Pesticides

1. Dichlorvos (DDVP)
2. Salithion
3. Dimethoate
4. Cyanophos (CYAP)
5. Diazinon
6. Disulfoton
7. Formothion
8. Iprobenfos (IBP)
9. Dichlofenthion (ECP)
10. Methylparathion
11. Chlorpyrifosmethyl
12. Fenitrothion (MEP)
13. Malathion
14. Fenthion
15. Chlorpyrifos
16. Parathion
17. α-CVP
18. β-CVP
19. Phenthoate
20. Propaphos
21. Methidathion (DMTP)
22. Prothiophos
23. Isoxathione
24. Ethion
25. Cyanofenphos (CYP)
26. Edifenphos (EDDP)
27. Pyridaphenthion
28. EPN
29. Phosalone

Chart 3.1.2 List of the 29 organophosphorus Pesticides components

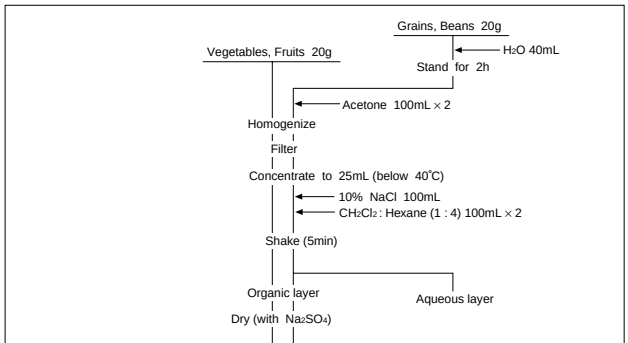


Fig. 3.1.3 Pretreatment flowchart

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

3.2 Analysis of Pesticides Using NCI (1) - GCMS

•Explanation

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)
	-300˚C(3˚C/min)(7min)
Inj.Temp.	: 280˚C
I/F Temp.	: 280˚C
Carrier Gas	: 120kPa(2min)-250kPa(2kPa/min)

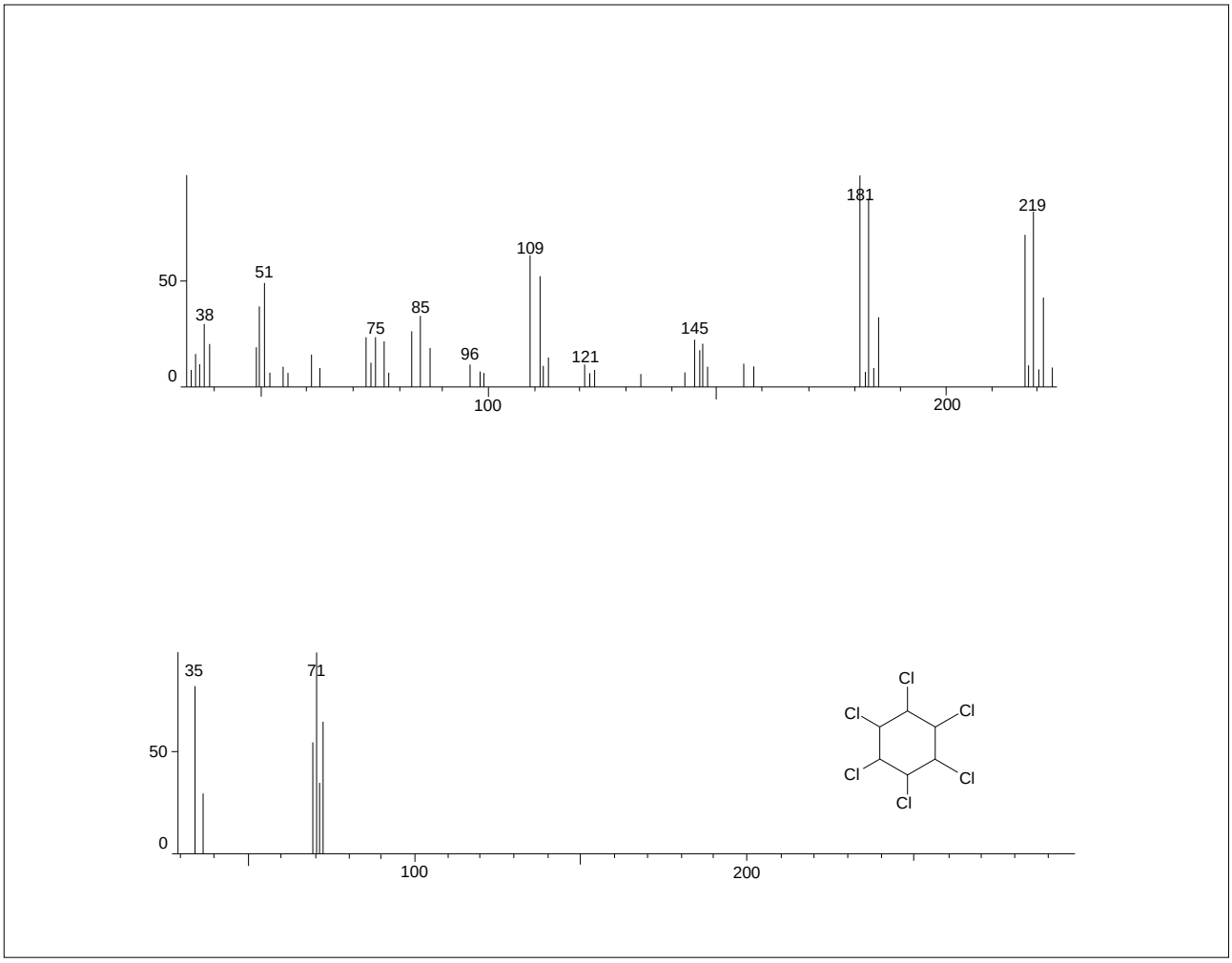


Fig. 3.2.1 α-BHC mass spectrum (upper: EI, lower: NCI)

3.2 Analysis of Pesticides Using NCI (2) - GCMS

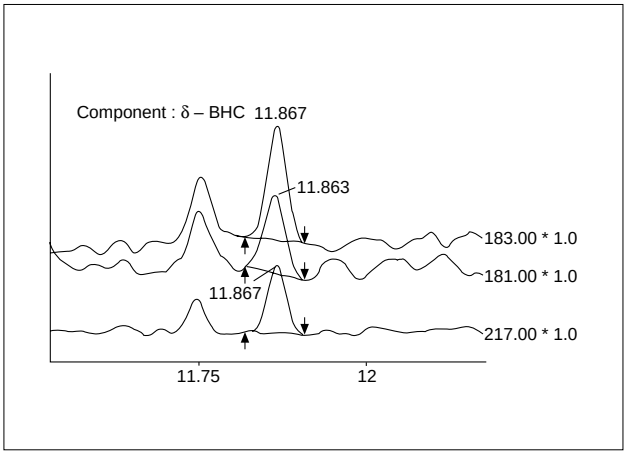


Fig. 3.2.2 SIM chromatogram using EI

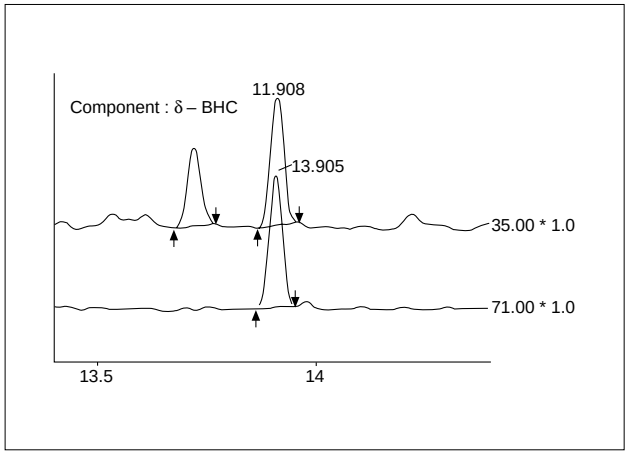


Fig. 3.2.3 SIM chromatogram using NCI

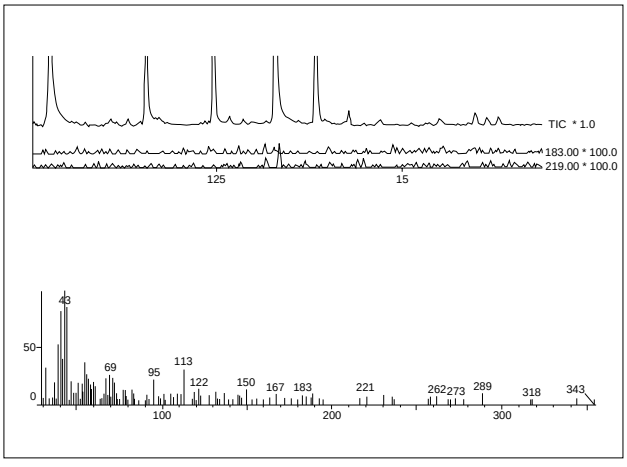


Fig. 3.2.4 MC and mass spectrum using EI

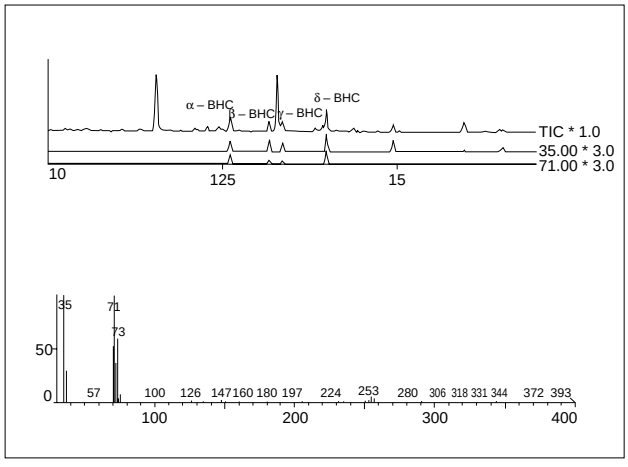


Fig. 3.2.5 MC and mass spectrum using NCI

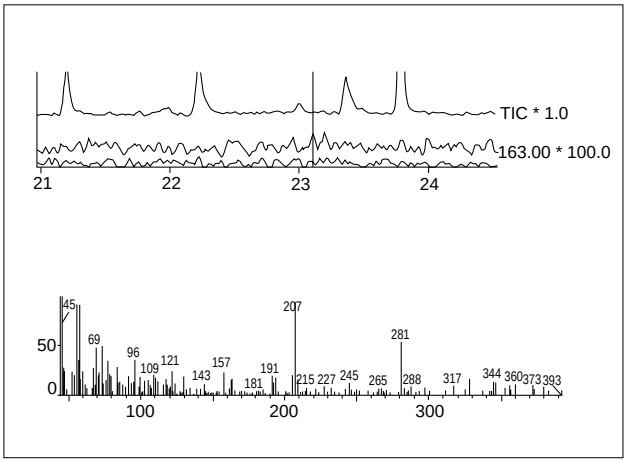


Fig. 3.2.6 MC and mass spectrum using EI

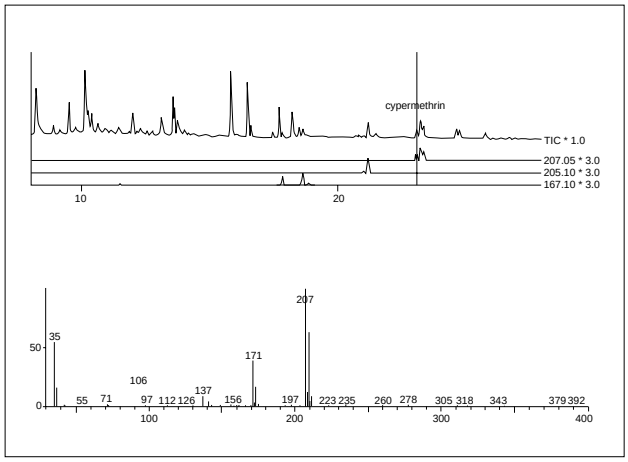


Fig. 3.2.7 MC and mass spectrum using NCI

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.

•Analytical Conditions

Instrument : GCMS-QP5000
Column : DB-1 0.32mm × 30m df = 0.25μm
Column temperature : 50˚C(2min)-140˚C(20˚C/min) -220˚C(7˚C/min)-310˚C (15˚C/min)(6/min)
Injection inlet temperature : 280˚C
Interface temperature : 300˚C
Carrier gas : He(40kPa)
Injection method : Spitless(2min)

Component	Selected ions (m/z)
d27-TBT	295, 293, 316
TBT	277, 275, 291
Tetra-BT	291, 289
TPeT	303. 305
d15-TPT	366, 364
TPT	351, 349

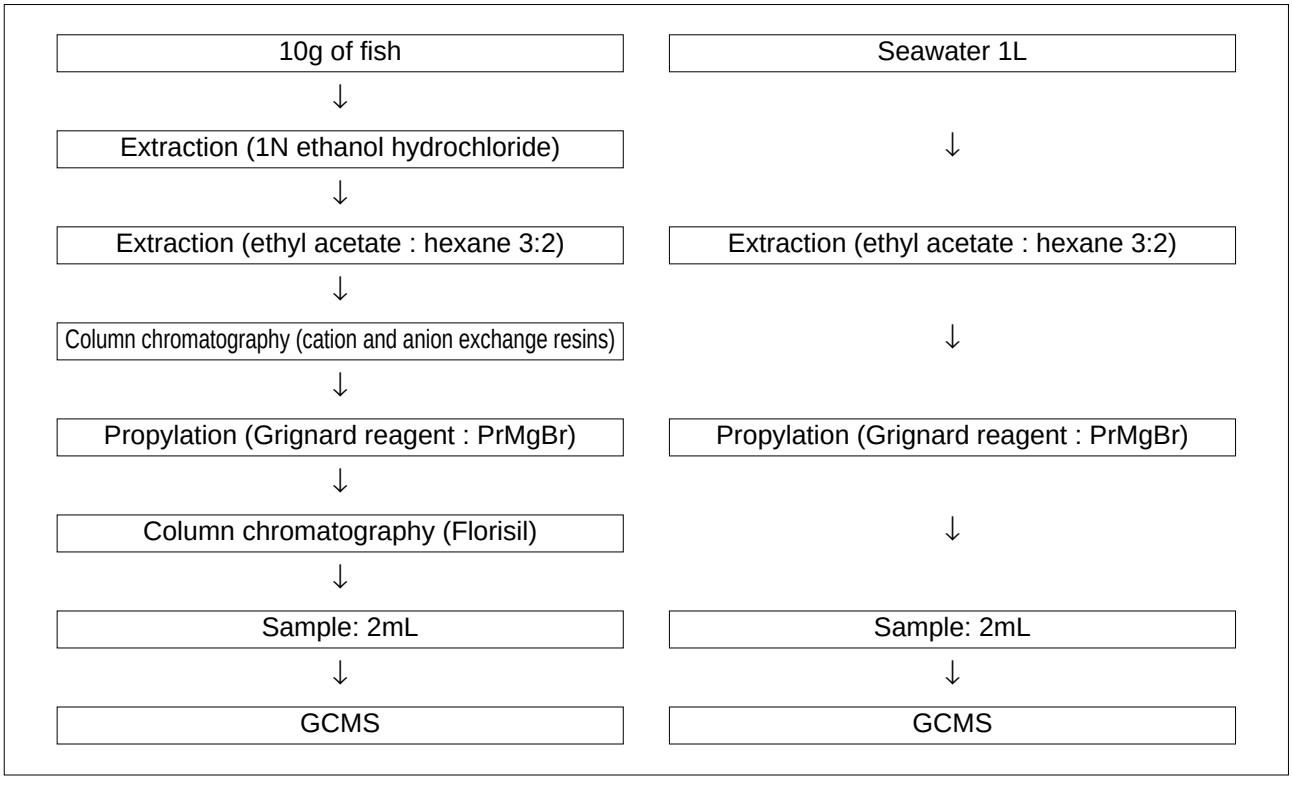


Fig. 3.3.1 Extraction methods for organotin in fish and seawater

3.3 Analysis of Organotin in Fish (2) - GCMS

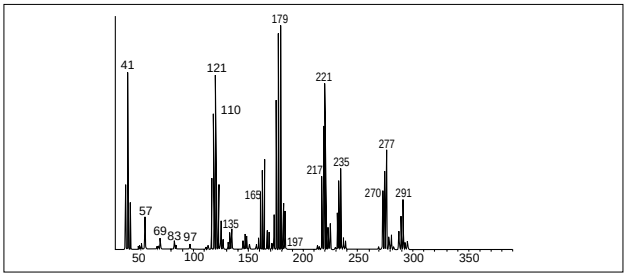


Fig. 3.3.2 TBT mass spectrum

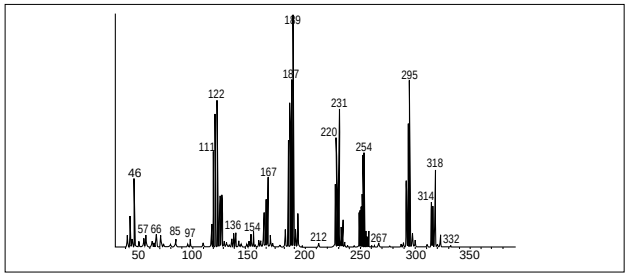


Fig. 3.3.3 d27-TBT mass spectrum

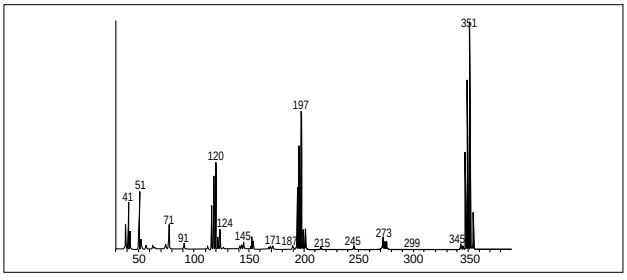


Fig. 3.3.4 TPT mass spectrum

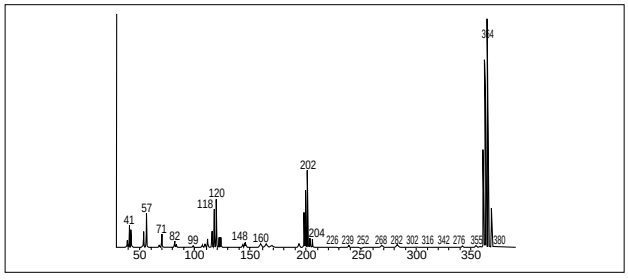


Fig. 3.3.5 d15-TPT mass spectrum

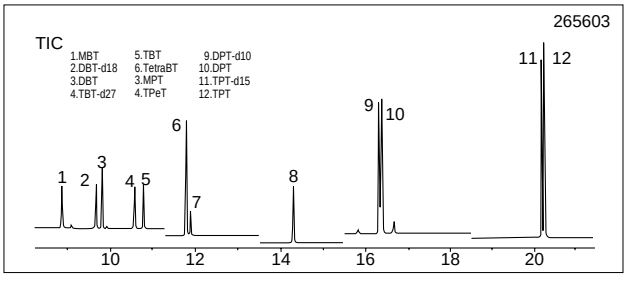


Fig. 3.3.6 SIM chromatogram of standard sample

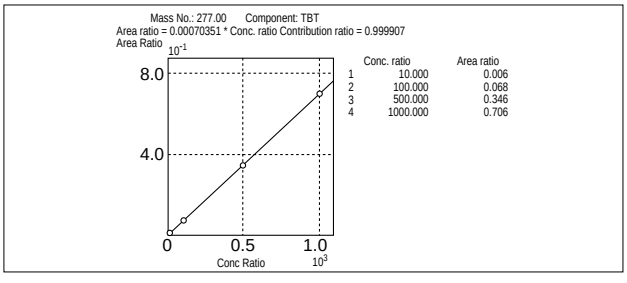


Fig. 3.3.7 TBT calibration curve (10 to 1000ppb)

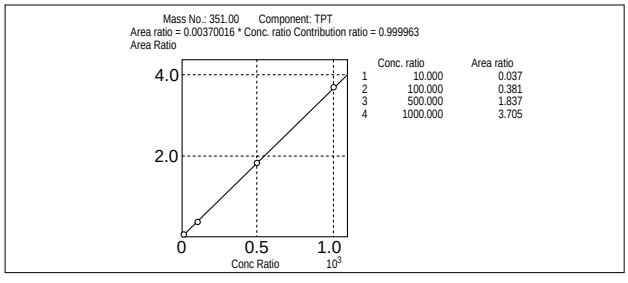


Fig. 3.3.8 TPT calibration curve (10 to 1000ppb)

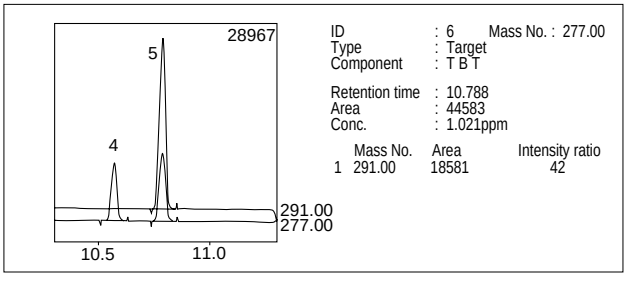


Fig. 3.3.9 SIM chromatogram of TBT in fish (sea bass)

Component	Sea bream (μg/g)	Sea bass (μg/g)	K port (μg/L)	W port (μg/L)
TBT	0.436	0.782	0.173	0.068
TPT	0.014	0.010	0.019	0.078

Chart 3.3.10 Quantitative results for tin in fish and seawater

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

Instrument : GCMS-QP5000
Column : DB-1 0.25mm × 30m df=0.25μm
Col.Temp. : 50˚C(2min)-130˚C(20˚C/min)
-300˚C(3˚C/min)(7min)
Inj.Temp. : 280˚C
I/F Temp. : 280˚C
Carrier Gas : 120kPa(2min)-250kPa(2kPa/min)

	Component	Molecular weight
1	Metamidophos	141
2	Dichlorvos	220
3	Propamocarb	188
4	Acephate	183
5	Isoprocarb	193
6	Fenobucarb	207
7	Ethoprophos	242
8	Chlorproham	213
9	Bendaiocarb	223
10	Dimethipin	210
11	α-BHC	288
12	Dimethoate	229
13	Thiometon	246
14	β-BHC	288
15	γ-BHC	288
16	σ-BHC	288
17	Terbufos	288
18	Diazinon	304
19	Ethiofencarb	225
20	Etrimfos	292
21	Pirimicarb	238
22	Metribuzin	214
23	Bentazone	254
24	Methylparathion	263
25	Carbaryl	201
26	Heptachlor	370
27	Fenitorthion	277
28	Methiocarb	225
29	Dichlofluanid	332
30	Esprocarb	265
31	Pirimiphos-methyl	305
32	Thiobencarb	257
33	Malathion	330
34	Aldrin	362
35	Fenthion	278
36	Parathion	291
37	Chlorpyrifos	349
38	Diethofencarb	267
39	Captan	299
40	Heptachlor epoxide	386
41	Pendimethalin	281
42	α-Chlorfenvinphos	358
43	Pyrifenox	294

	Component	Molecular weight
44	Chinomethionat	234
45	β-Chlorfenvinphos	358
46	Quinalphos	298
47	Phenthoate	320
48	Triadimenol	295
49	Vamidothion	287
50	Trichlamide	339
51	Methoprene	310
52	Flutolanil	323
53	Dieldrin	378
54	Prothiophos	344
55	Myclobutanil	288
56	p,p'-DDE	316
57	Pretilachlor	311
58	Endrin	378
59	Fensulfothion	308
60	Chorobenzilate	324
61	p,p'-DDD	318
62	o,p'-DDT	352
63	Mepronile	269
64	Lenacil	234
65	Edifenphos	310
66	Captafol	347
67	p,p'-DDT	352
68	Propiconazol	341
69	EPN	323
70	Dicofol	370
71	Phosalone	367
72	Mefenacet	298
73	Amitraz	293
74	Cyhalothrin	449
75	Bitertanol	337
76	Pyridaben	364
77	Inabenfide	338
78	Permethrin	390
79	Cyfluthrin	363
80	Cypermethrin	415
81	Flucythrinate	451
82	Fenvalerate	419
83	Fluvalinate	502
84	Pyrazoxyfen	437
85	Deltamethlrin	503
86	Tralomethrin	661

Chart 3.4.1 List of pesticides and molecular weights

3.4 Simultaneous Analysis of Pesticides (2) - GCMS

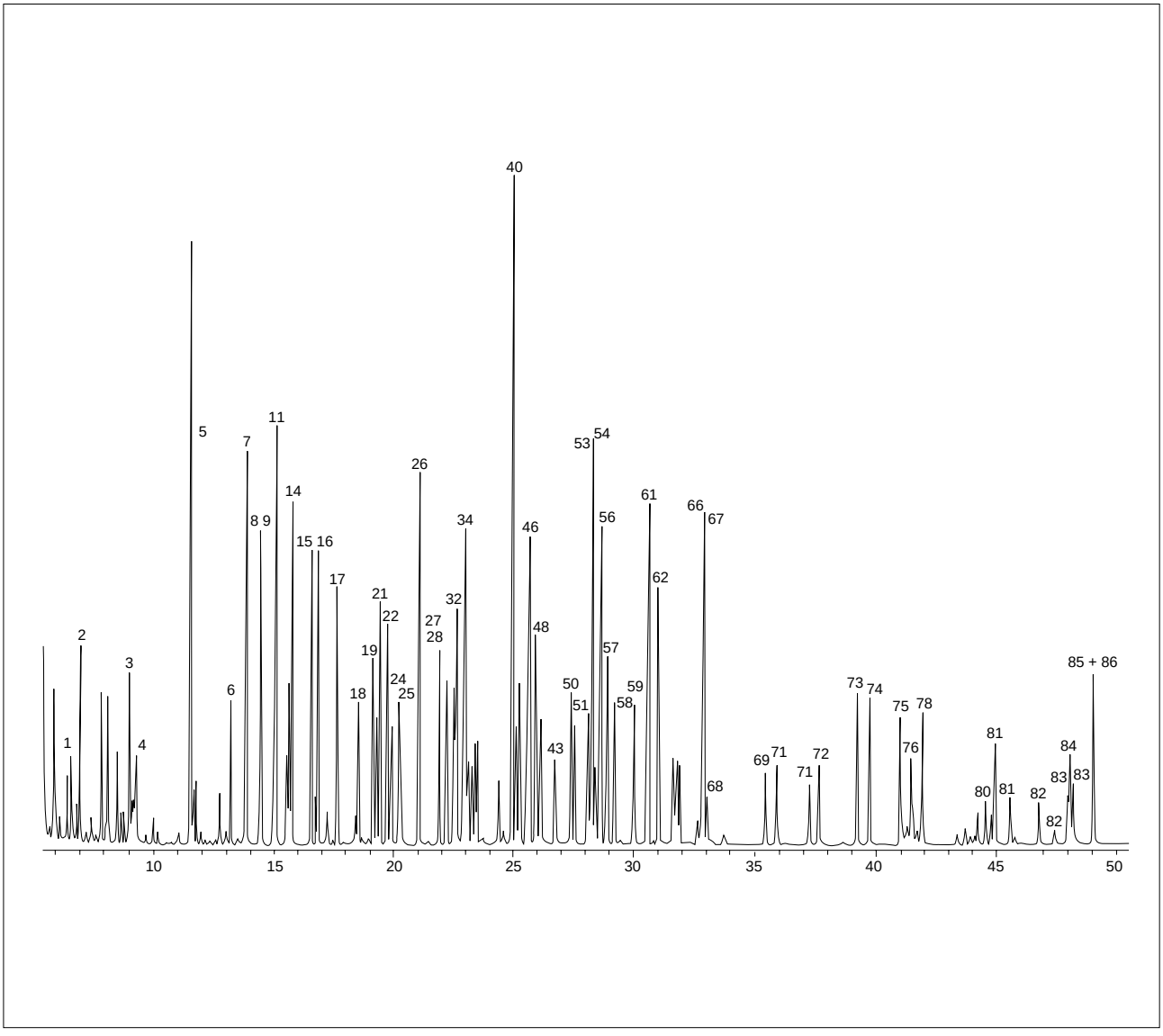


Fig. 3.4.2 Analysis of 86 pesticides using DB-1

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.

References

Shimadzu Application News No. L246 (C190-E068)

•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
λ = 210nm to 300nm

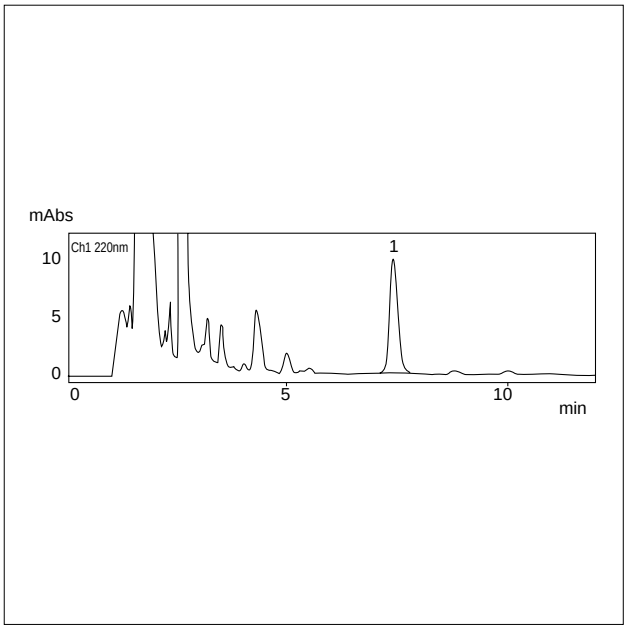


Fig. 3.5.1 Chromatogram of imazalil in imported orange sample (220nm)

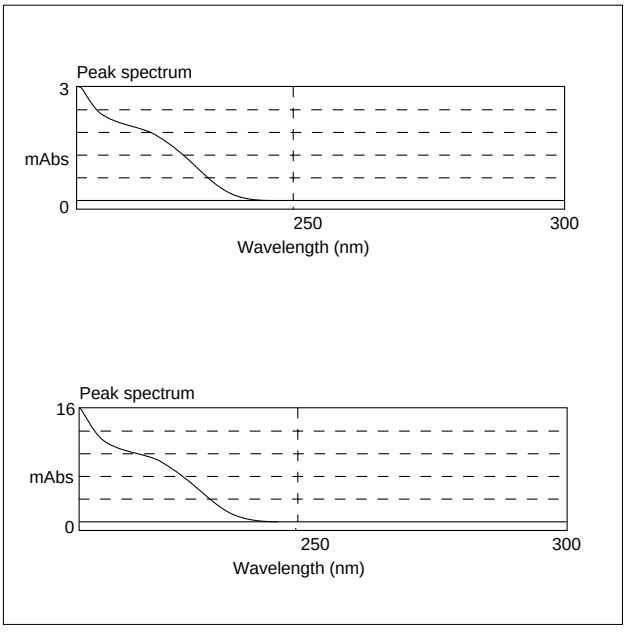


Fig. 3.5.2 Spectra of imazalil (upper: standard sample, lower: sample)

3.6 Analysis of N-Methylcarbamate Pesticides in Lemons (1) - LC

•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. Shinbujirou 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

•Pretreatment

See flowchart in Fig.3.6.3.

•Analytical Conditions

- Separation Conditions

Instrument	: HPLC
Column	: STR ODS-Ⅱ (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)

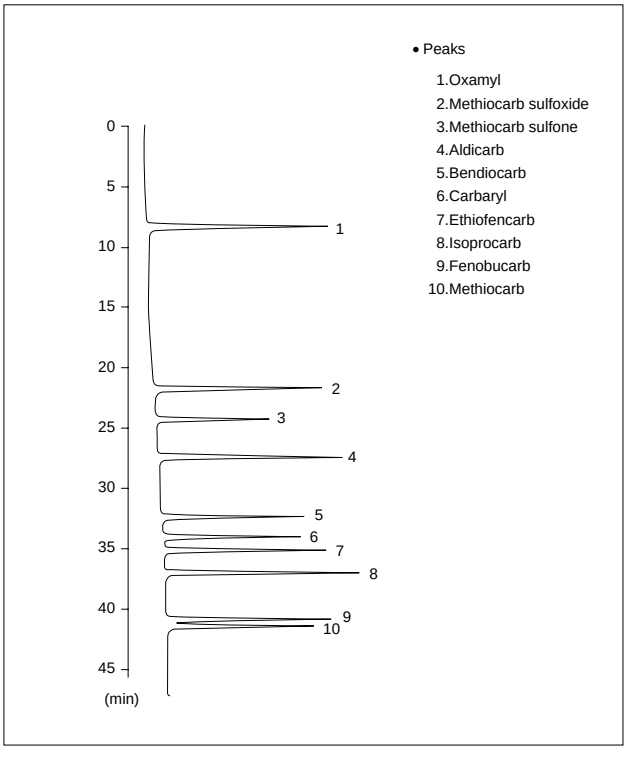


Fig. 3.6.1 Analysis of N-Methylcarbamate pesticides

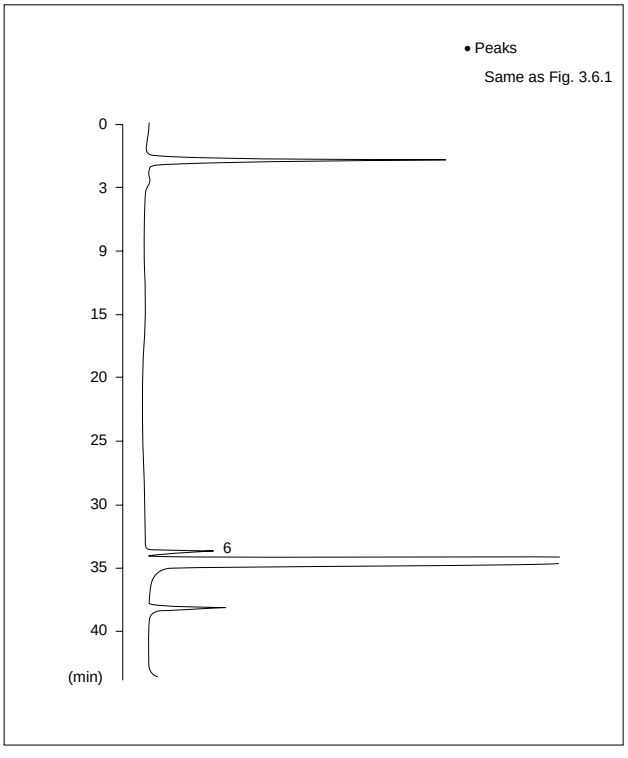


Fig. 3.6.2 Analysis of lemon

3.6 Analysis of N-Methylcarbamate Pesticides in Lemons (2) - LC

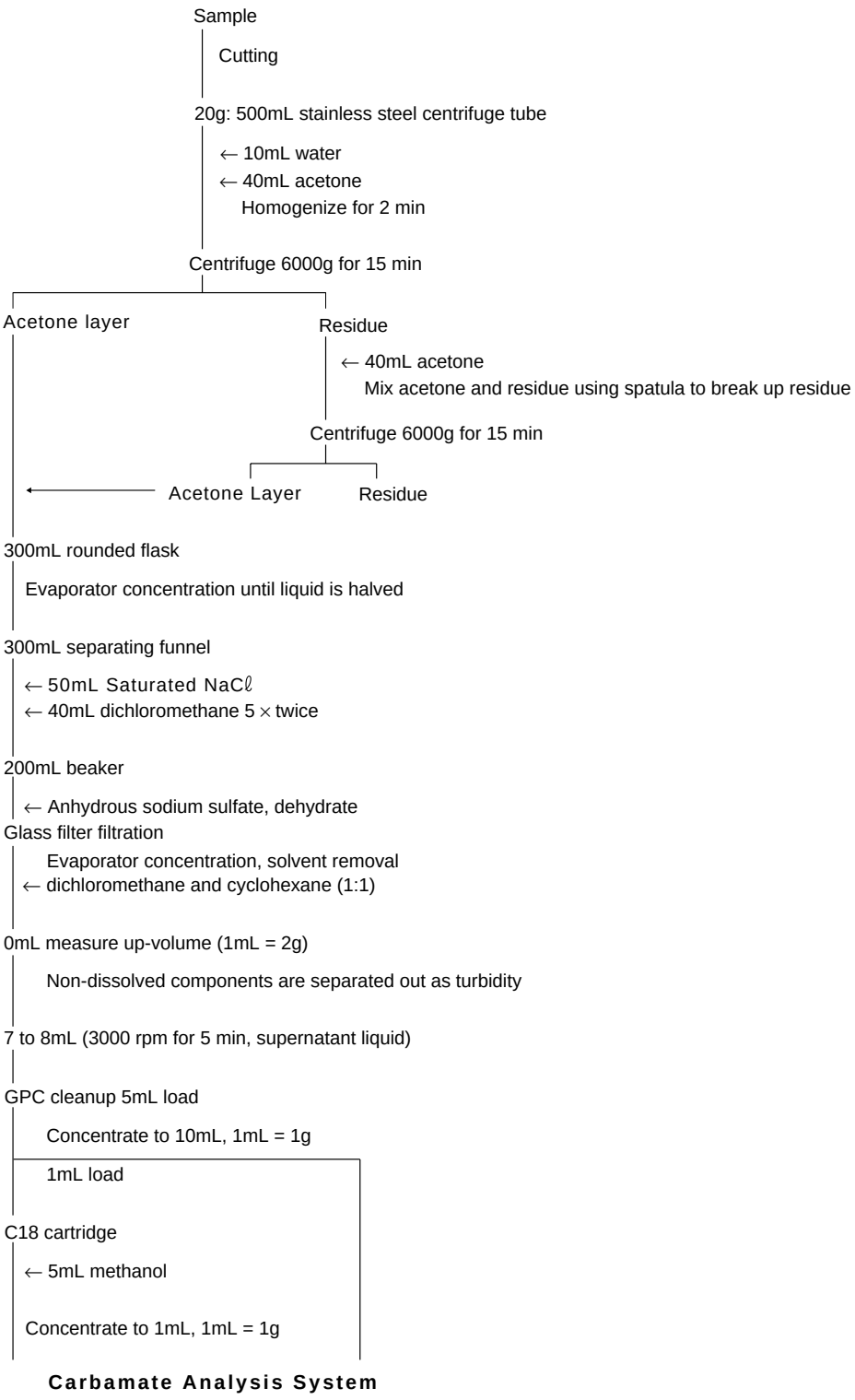


Fig. 3.6.3 Pretreatment of extract for carbamate analysis
(Provided by Dr. Shinbujirou Hori and Dr. Hirotaka Obana, Food Chemistry Dept. of the Osaka Prefectural Institute of Public Health)

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)

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

Chart 3.7.1 Analytical conditions

Column	: Shim-pack VP-ODS (4.6mmI.D.×150mmL)
Mobile phase	: Water/Acetonitrile=7/3(v/v)
Flow Rate	: 1.0mL/min
Temperature	: 40˚C
Detection	: Fluorescence (Ex279nm Em307nm)

Chart 3.7.3 Analytical conditions

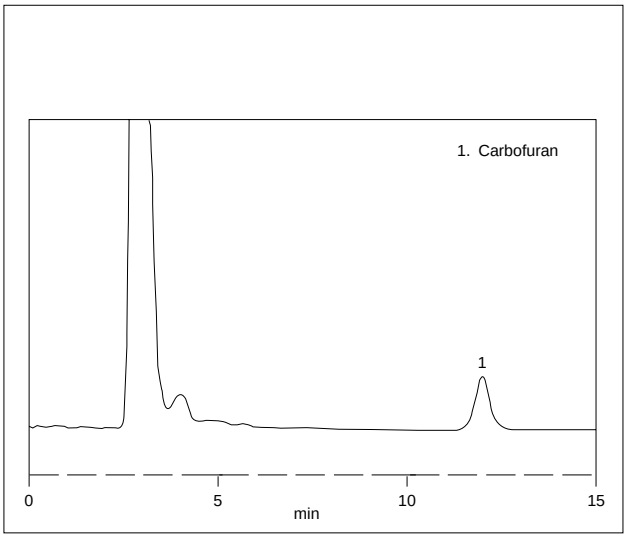


Fig. 3.7.2 Analysis of carbofuran standard product using post-column derivatization method

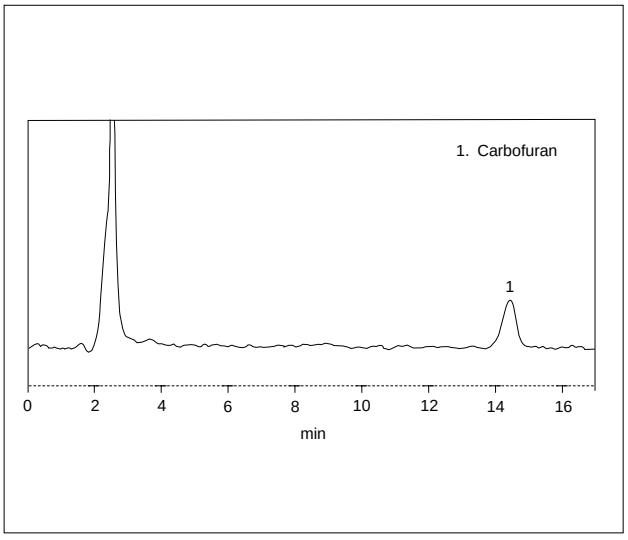


Fig. 3.7.4 Analysis of carbofuran standard product using direct fluorescent detection method

4.1 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℃ for 60 min.

•Analytical Conditions

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

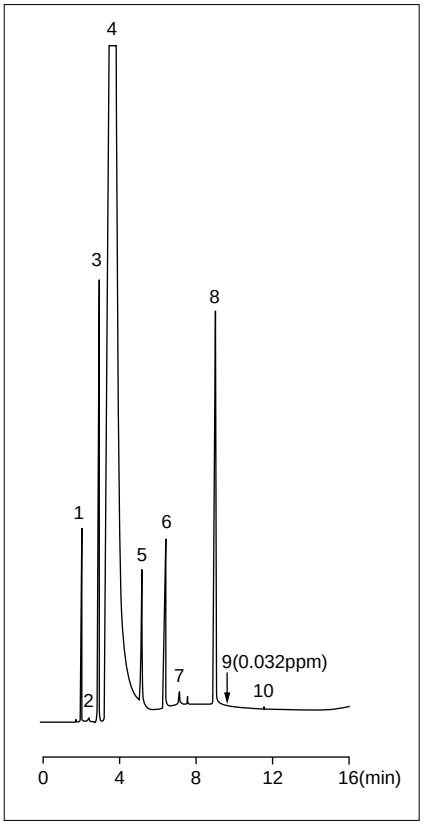


Fig. 4.1.1 Analysis of brewage

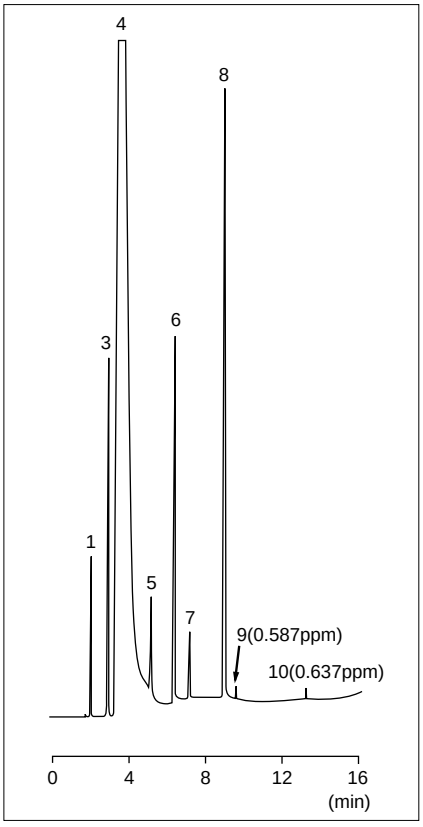


Fig. 4.1.2 Analysis of shochu

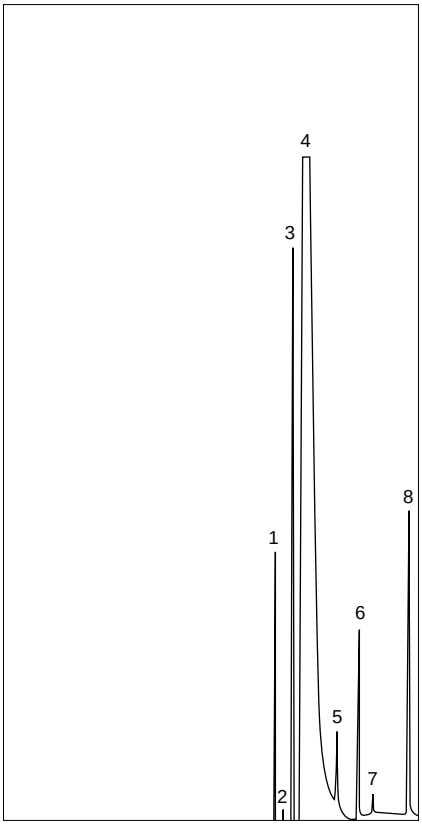


Fig. 4.1.3 Analysis of whisky

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

•Explanation

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℃ for 60 min.

•Analytical Conditions

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

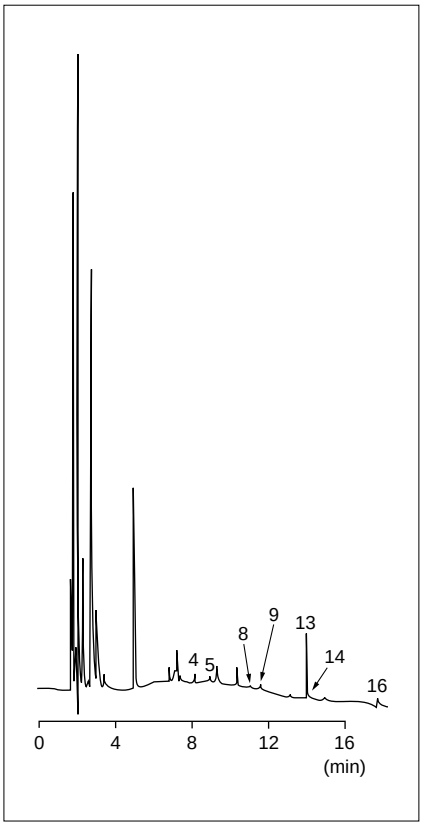


Fig. 4.2.1 Analysis of refined green tea

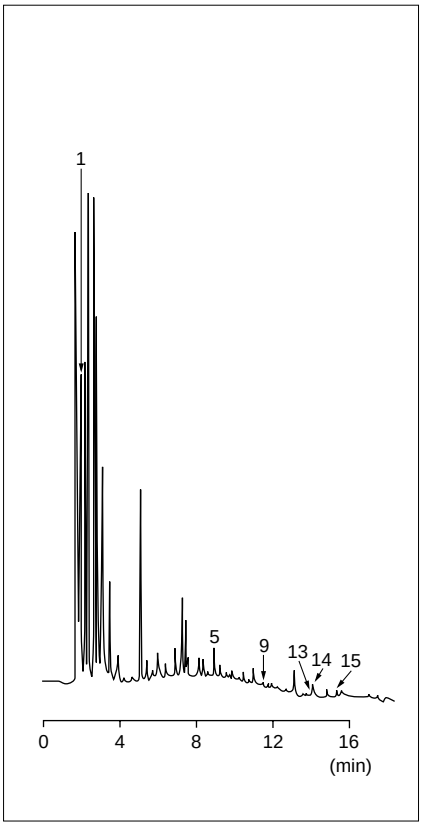


Fig. 4.2.2 Analysis of coarse green tea

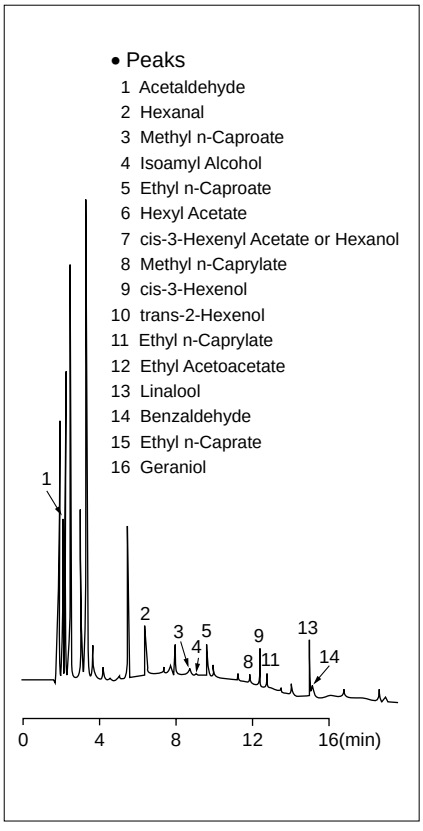


Fig. 4.2.3 Analysis of black tea

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℃ for 30 min.

•Analytical Conditions

Instrument	: GC-14BPF+HSS-2B
Column	: CBP1 0.53mm × 25m df = 3.0µm
Col. Temp.	: 50℃(15min)-5℃/min-200℃
Inj. Temp.	: 230℃
Det. Temp.	: 230℃(FID)
Carrier gas	: He(10.5mL/min)
Injection method	: Direct Injection
Injection volume	: 0.8mL

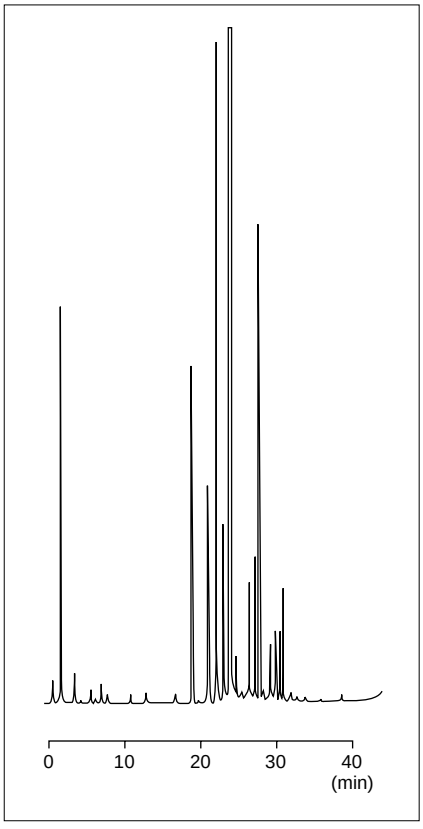


Fig. 4.3.1 Analysis of orange oil

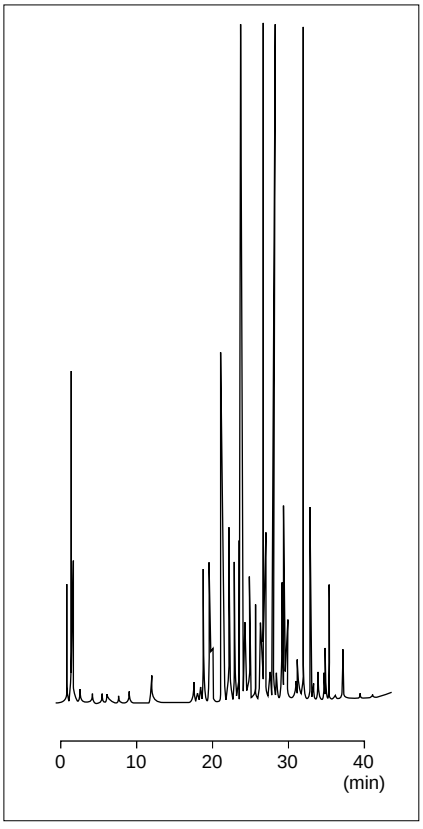


Fig. 4.3.2 Analysis of lavender oil

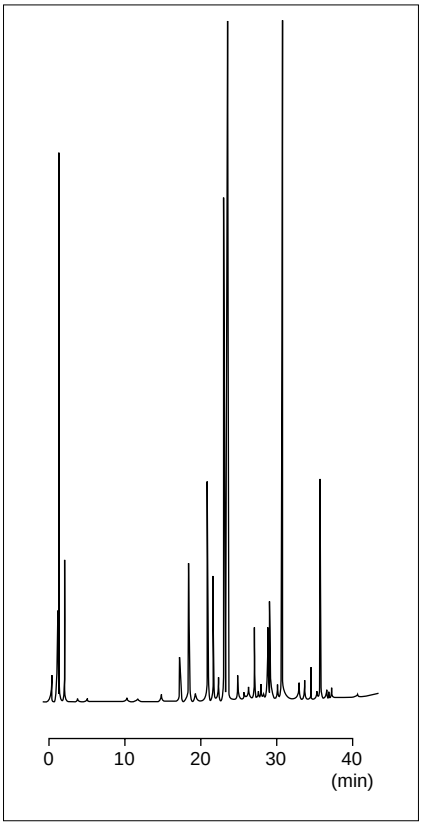


Fig. 4.3.3 Analysis of spearmint oil

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

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

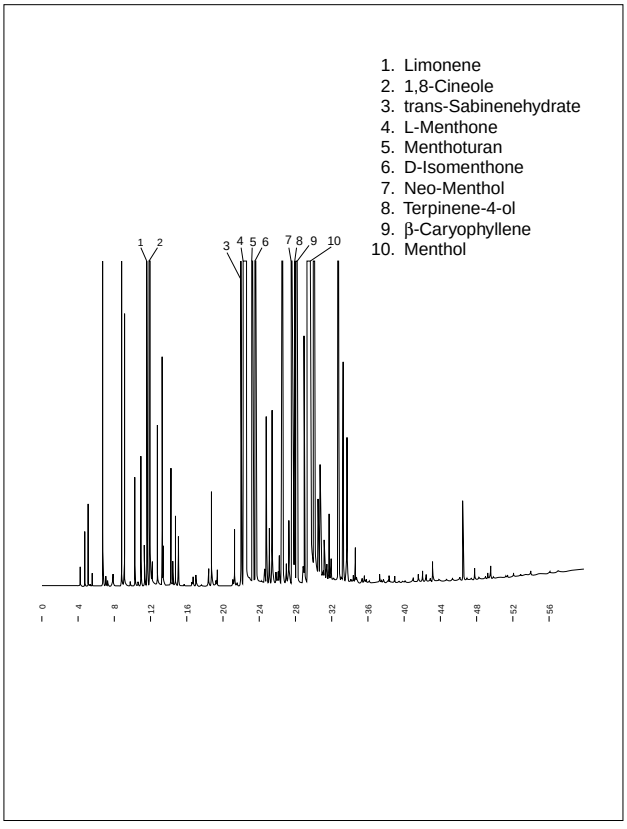


Fig. 4.4.1 Analysis of peppermint oil

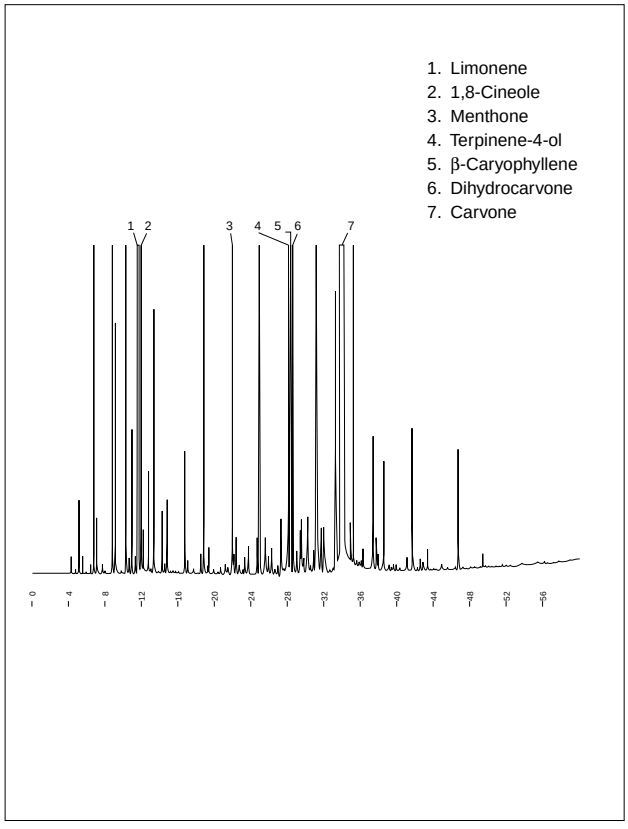


Fig. 4.4.2 Analysis of spearmint oil

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

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℃ for 40 min.

•Analytical Conditions

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

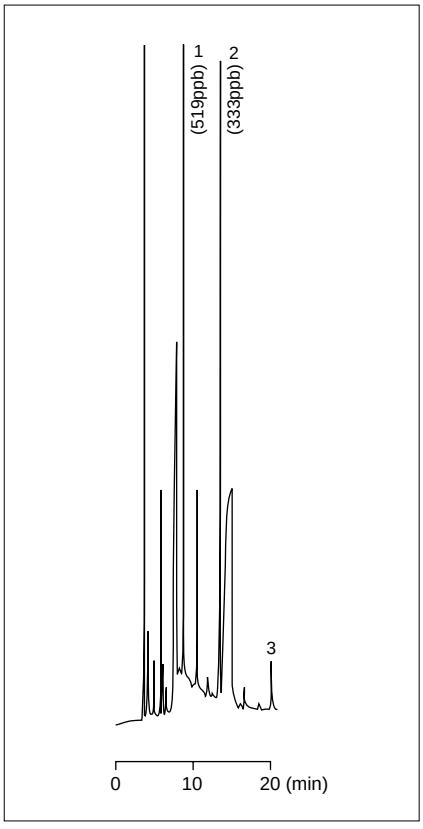


Fig. 4.5.1 Analysis of strong soy sauce

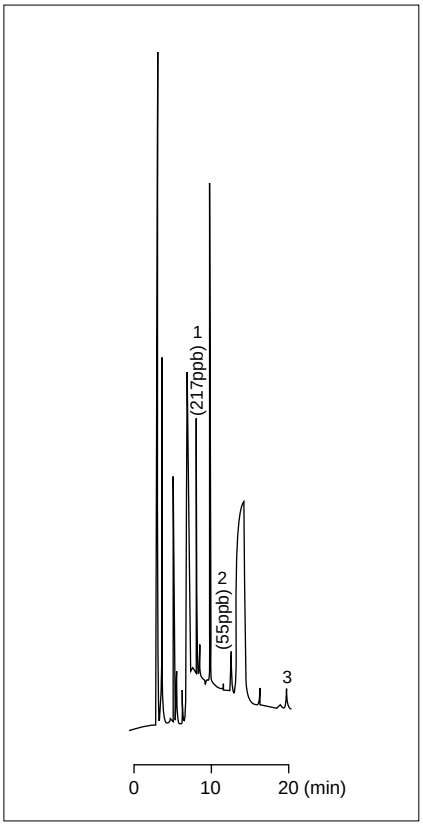


Fig. 4.5.2 Analysis of Japanese sake

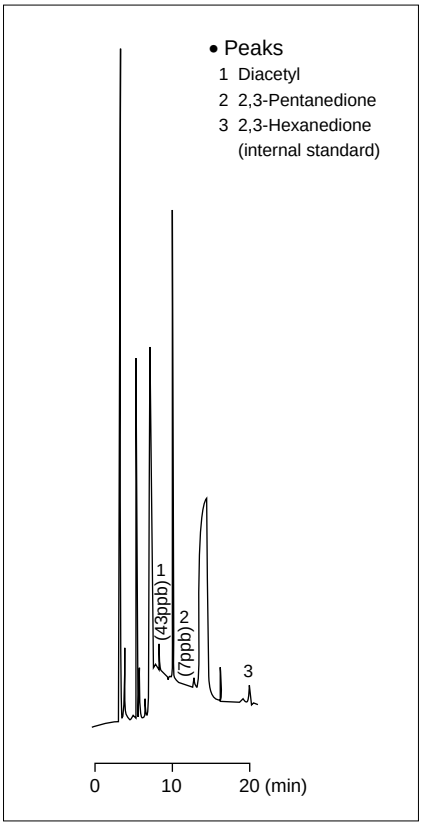


Fig. 4.5.3 Analysis of shochu

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 fruits 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℃ for 30 min.

•Analytical Conditions

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

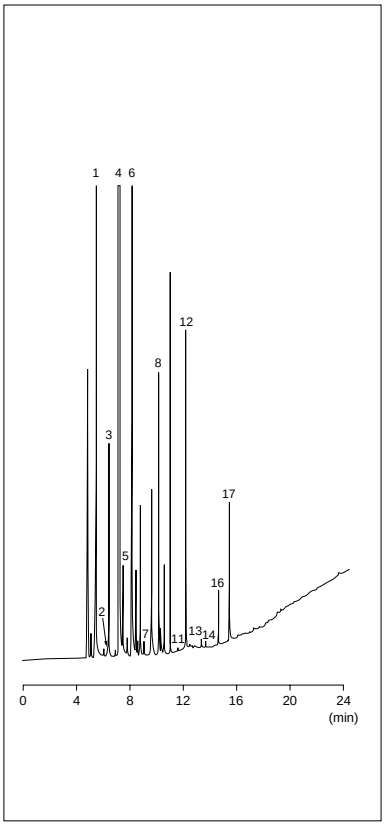


Fig. 4.6.1 Analysis of melon

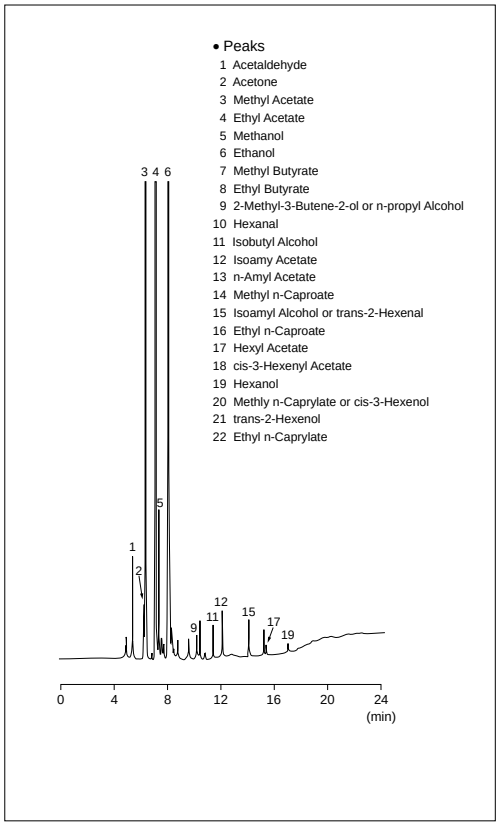


Fig. 4.6.2 Analysis of strawberry

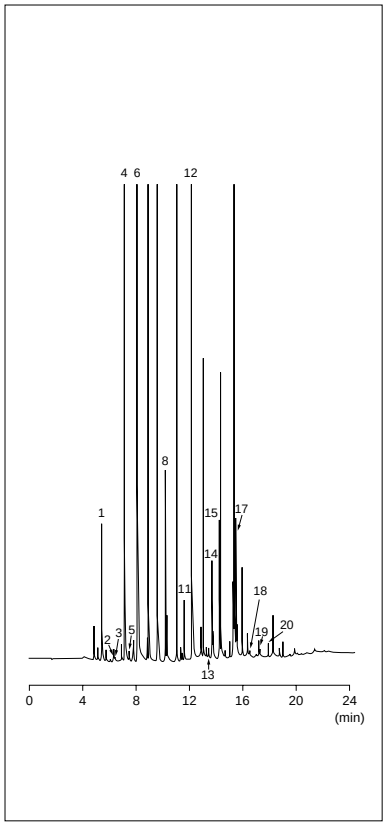


Fig. 4.6.3 Analysis of banana

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℃ for 30 min.

•Analytical Conditions

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

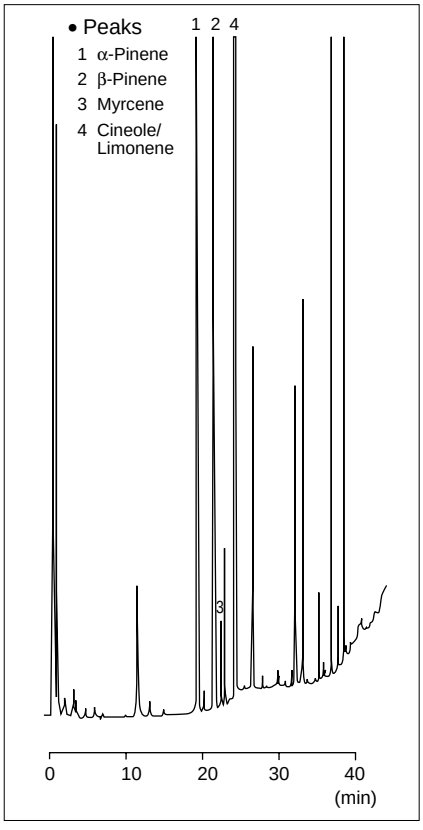


Fig. 4.7.1 Analysis of perilla (1.5g)

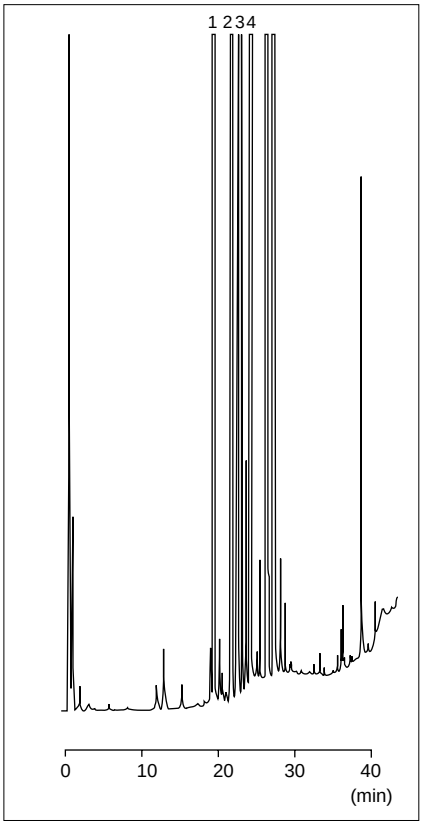


Fig. 4.7.2 Analysis of parsley (2g)

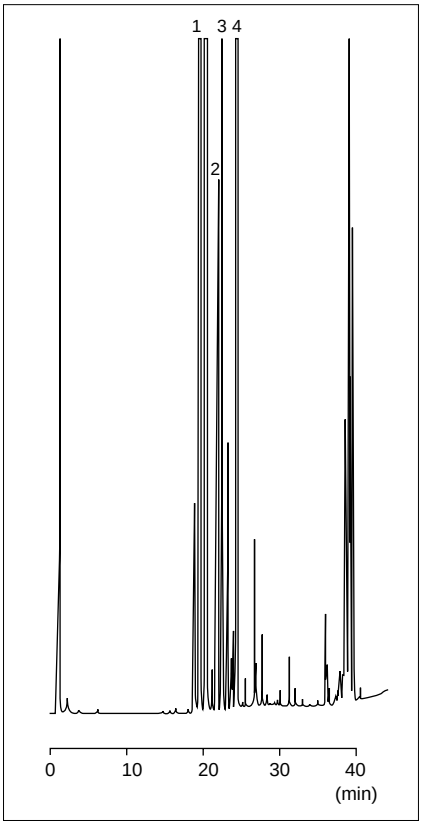


Fig. 4.7.3 Analysis of ginger (10g)

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℃ for 40 min.

•Analytical Conditions

Instrument	: GC-14BPF+HSS-2B
Column	: CBP1 0.53mm × 25m df = 3.0μm
Col. Temp.	: 90℃-6℃/min-230℃
Inj. Temp.	: 300℃
Det. Temp.	: 300℃(FID)
Carrier gas	: He(4.3mL/min)
Injection method	: Direct
Injection volume	: 0.8mL

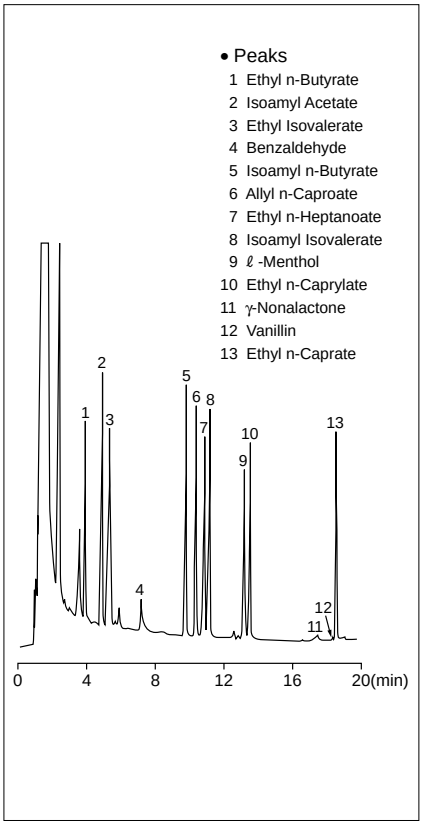


Fig. 4.8.1 Analysis of standard flavoring agent

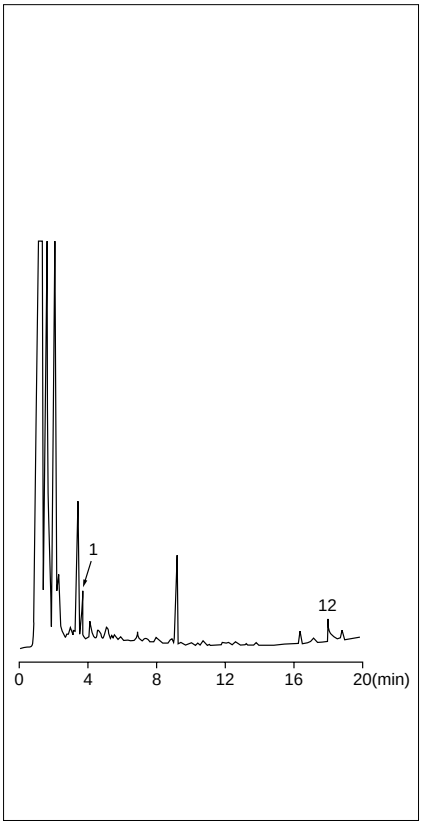


Fig. 4.8.2 Analysis of cookie (6.4g)

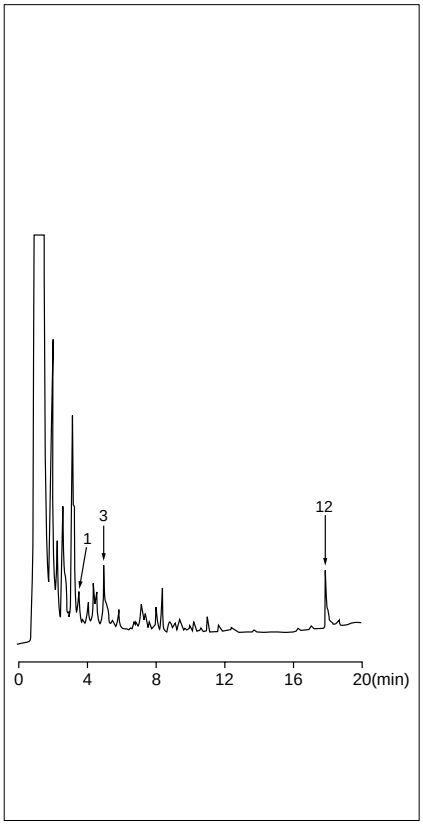


Fig. 4.8.3 Analysis of chocolate (with nut cream) (3.9g)

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

Instrument	: GCMS-QP5000
Column	: DB-1701 0.32mm × 30m df = 1.0μm
Col.Temp.	: 40℃(8min)-200℃(20℃/min)(5min)
Int.Temp.	: 230℃
I/F Temp.	: 230℃
Carrier Gas	: He(20kPa)
– P&T –	
Instrument	: Tekmar 3000J
Sample Size	: 5mL(35℃)
Trap Tube	: Tenax GR
Purge	: 11min
Dry Purge	: 3min
Desorb	: 225℃, 8min

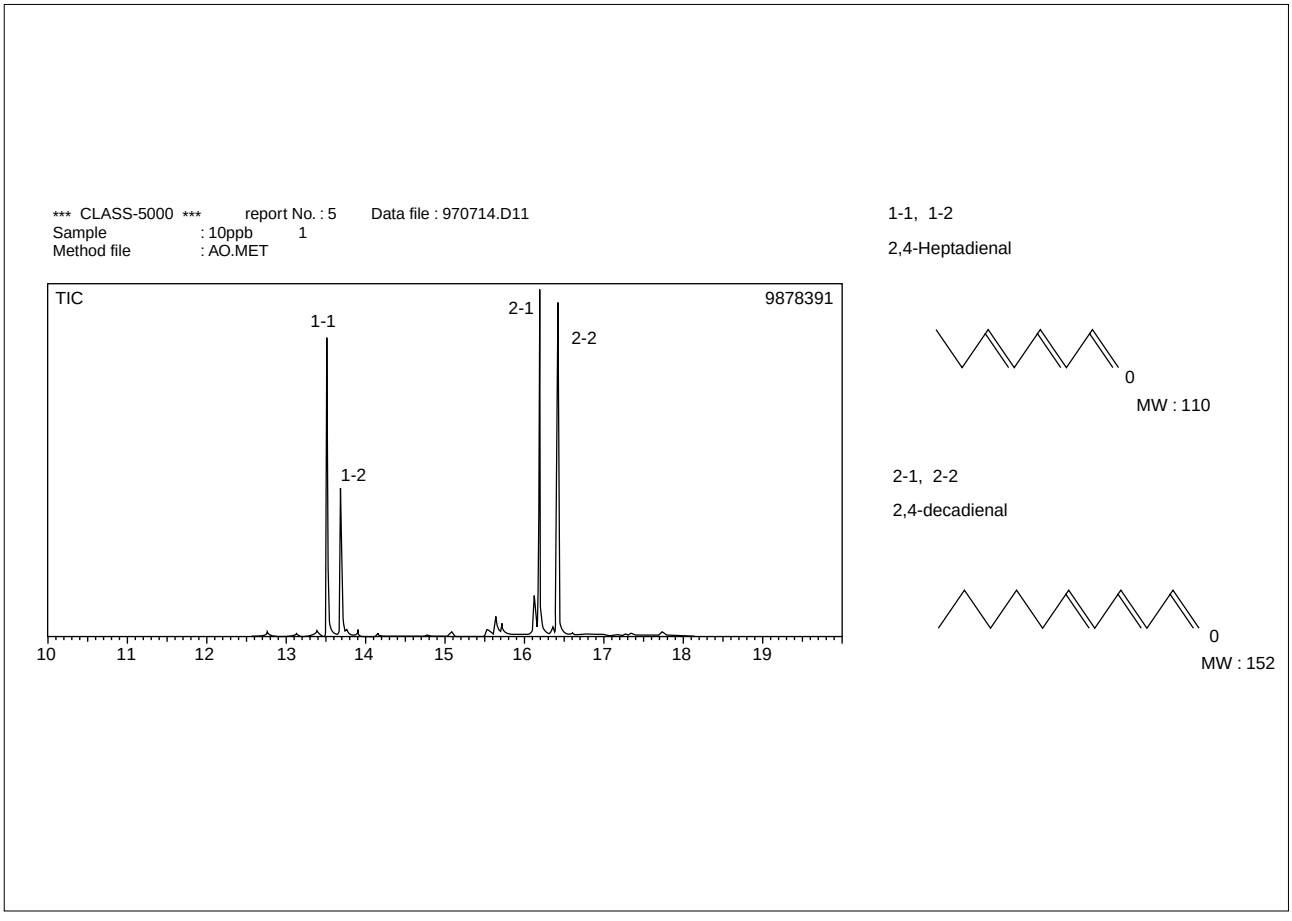


Fig. 4.9.1 TIC chromatogram of fishy smell components

4.9 Analysis of Fishy Smell in Water (2) - GCMS

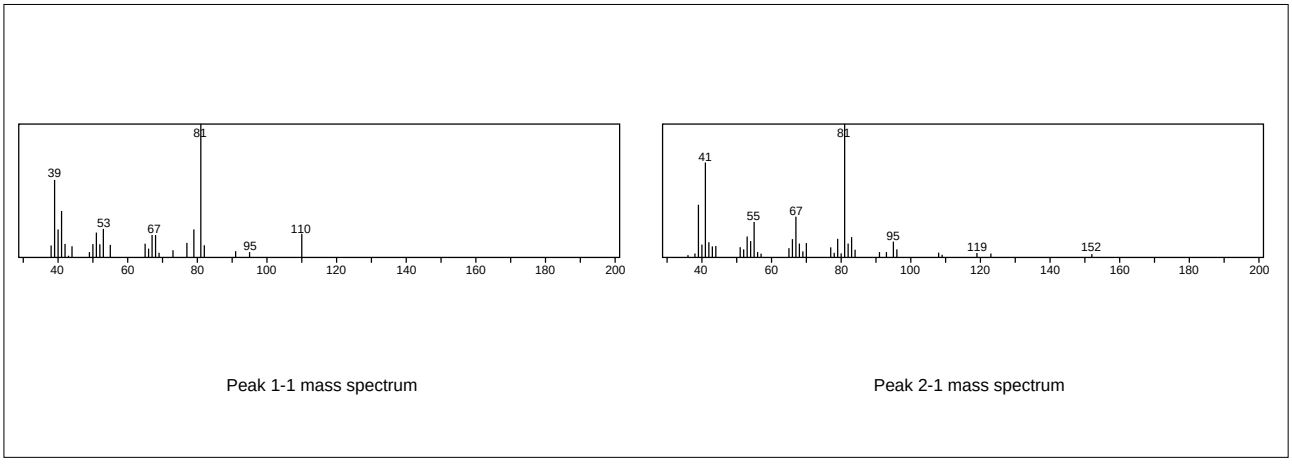


Fig. 4.9.2 Mass spectrum

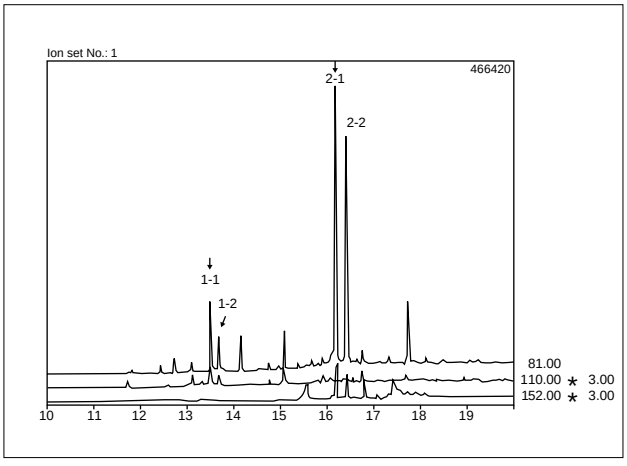


Fig. 4.9.3 SIM chromatogram of 100ppt

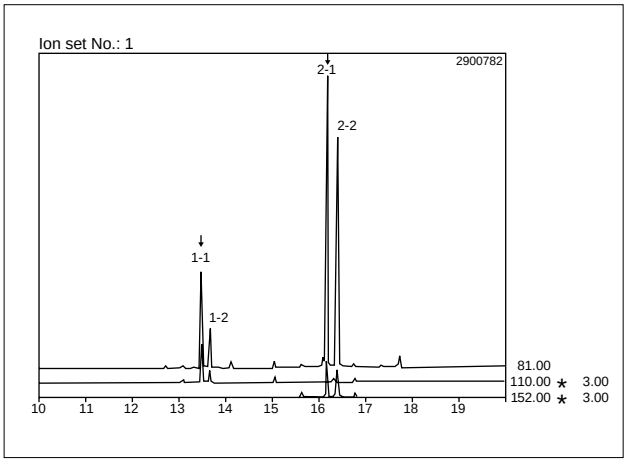


Fig. 4.9.4 SIM chromatogram of 1ppb

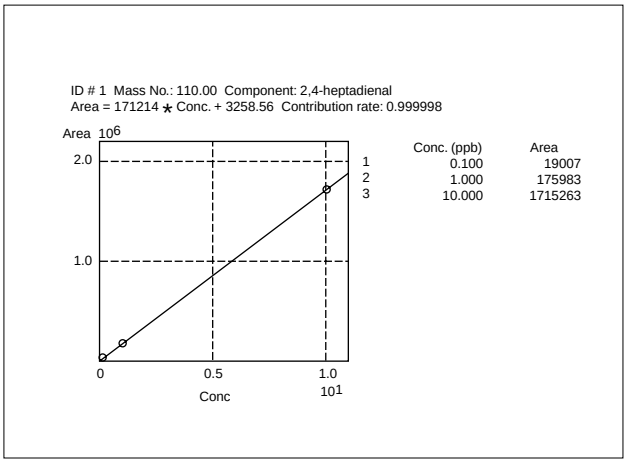


Fig. 4.9.5 Calibration curve for 2,4-heptadienal

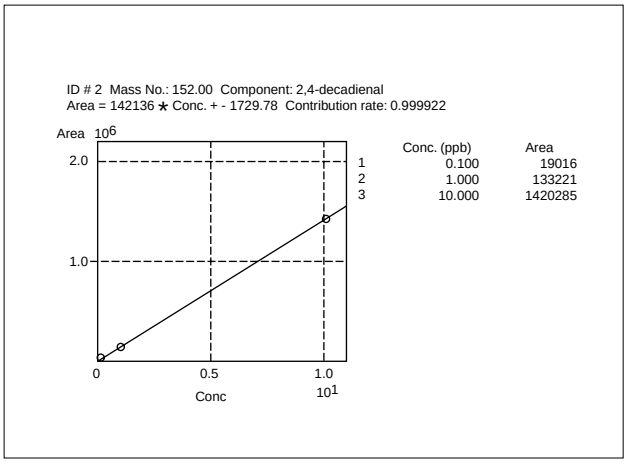


Fig. 4.9.6 Calibration curve for 2,4-decadienal

4.10 Analysis of Alcohols (1) - GCMS

•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℃(5min)-250℃(5℃/min)(5min)
Int.Temp. : 250℃
Carrier Gas : He(35kPa)
: HS –
Instrument : HSS-4A
Sample Size : 10mL
Sample Temp. : 60℃
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℃(3min)
Thermal : 250℃(5min)

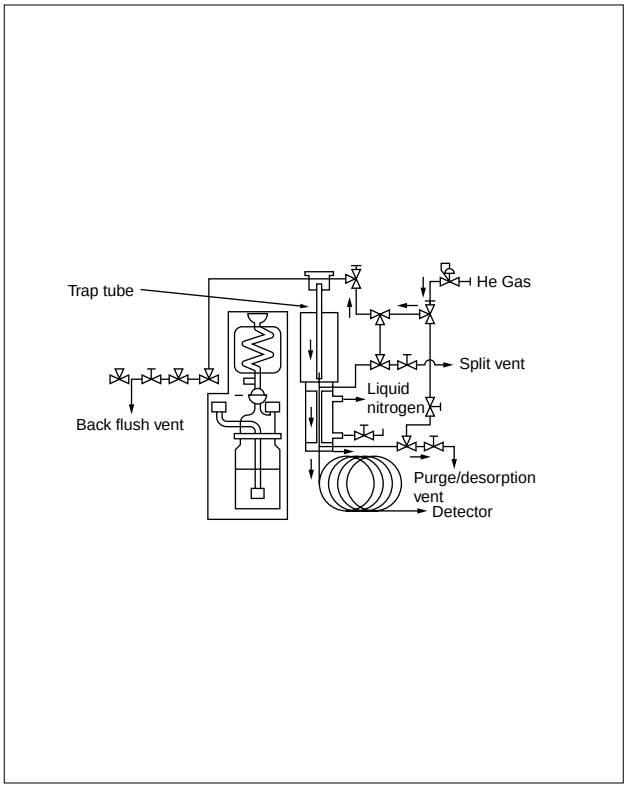


Fig. 4.10.1 CP4010 flow line diagram

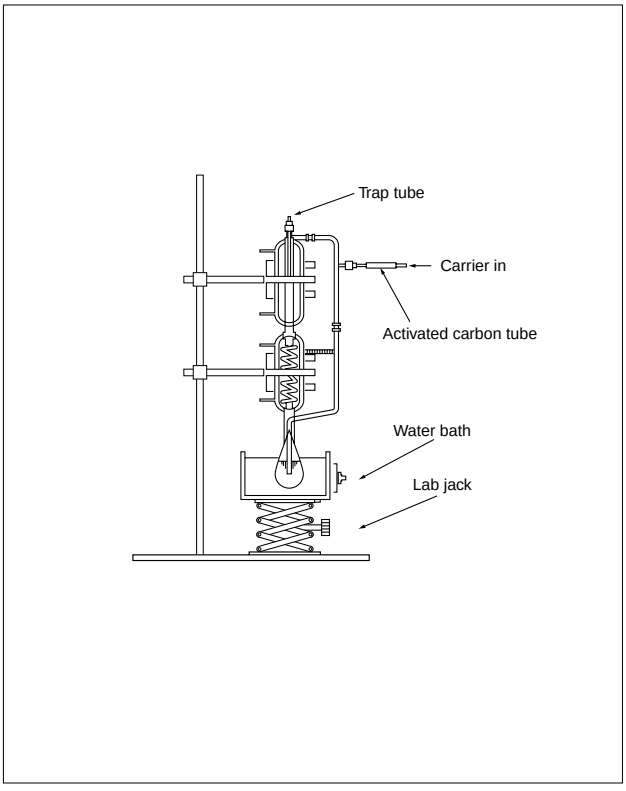


Fig. 4.10.2 Schematic diagram of Tenax Trapping Set

4.10 Analysis of Alcohols (2) - GCMS

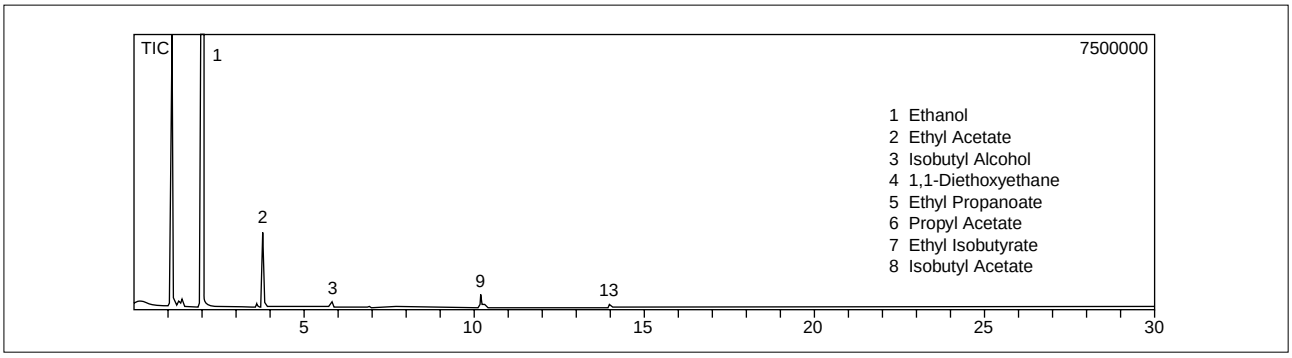


Fig. 4.10.3 TIC chromatogram of Japanese sake (HS method)

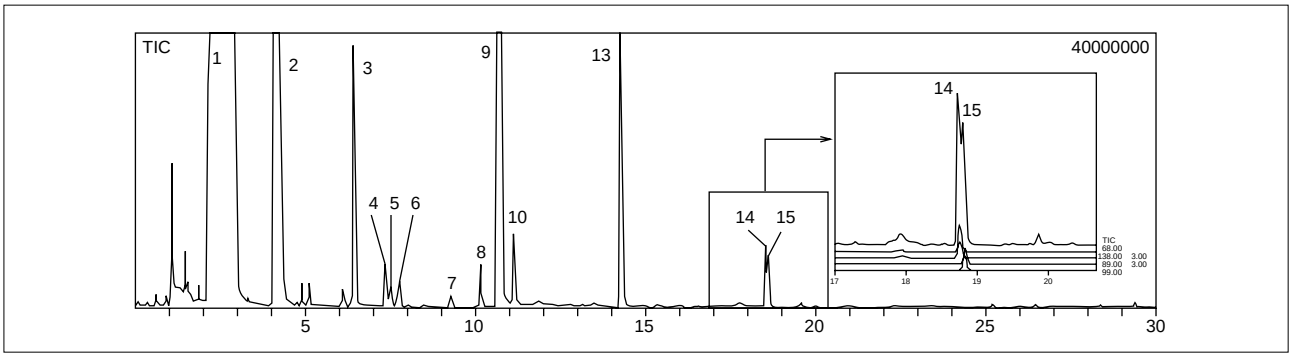


Fig. 4.10.4 TIC chromatogram of Japanese sake (TCT method)

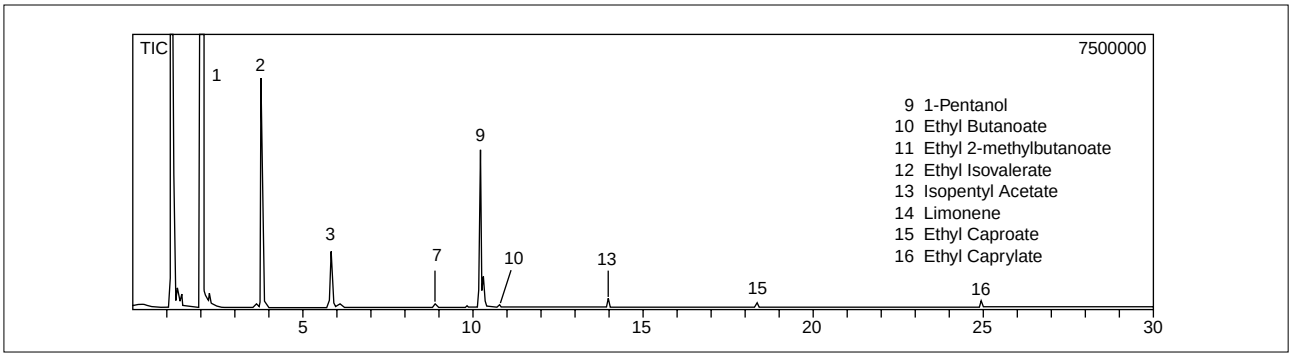


Fig. 4.10.5 TIC chromatogram of wine (HS method)

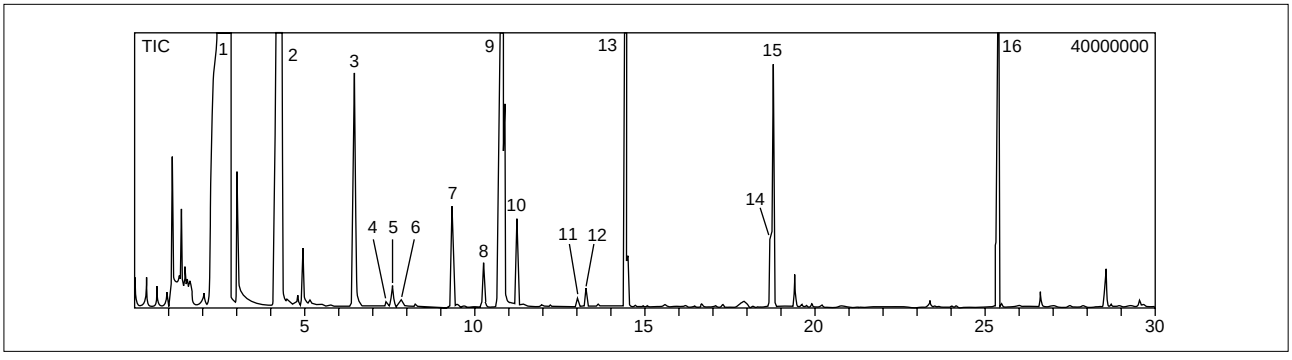


Fig. 4.10.6 TIC chromatogram of wine (TCT method)

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

Instrument	: GCMS-QP5000
Column	: DB-624 0.25mm × 60m df = 1.4µm
Col. Temp.	: 40℃(5min) – 230℃(5℃/min)(5min)
I/F Temp.	: 230℃
Carrier gas	: He(100kPa)
– TCT –	
Instrument	: CP4010(TCT mode)
Sample amount	: 10g (room temperature)
Trap tube	: Tenax TA
Pre-cool	: –150℃, 5min
Pre-flush	: 50℃, 1min
Thermal desorption	: 250℃, 10min, 20mL/min

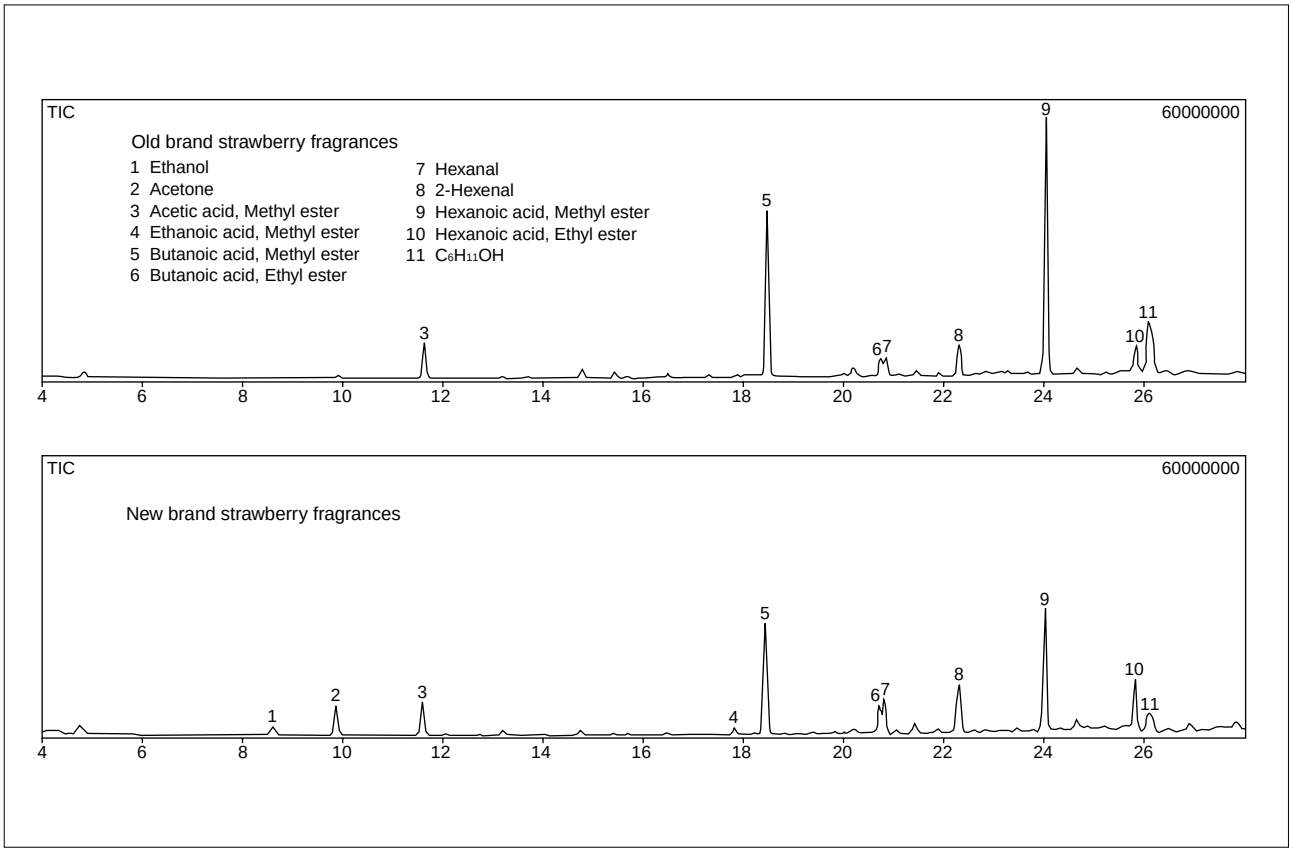


Fig. 4.11.1 TIC chromatogram of strawberry fragrance components (upper: old brand, lower: new brand)

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. Conventionally, 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 desorped 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℃(2min)-140℃(30℃/min)
	–220℃(10℃/min)
I/F Temp.	: 250℃
Carrier Gas	: He(50kPa)
– TCT –	
Instrument	: GP4010(TCT mode)
Sample Size	: 25mL(50℃)
Trap Tube	: Tenax GR
Purge	: 50℃, 15min, 100mL/min
Desoption	: 250℃, 5min

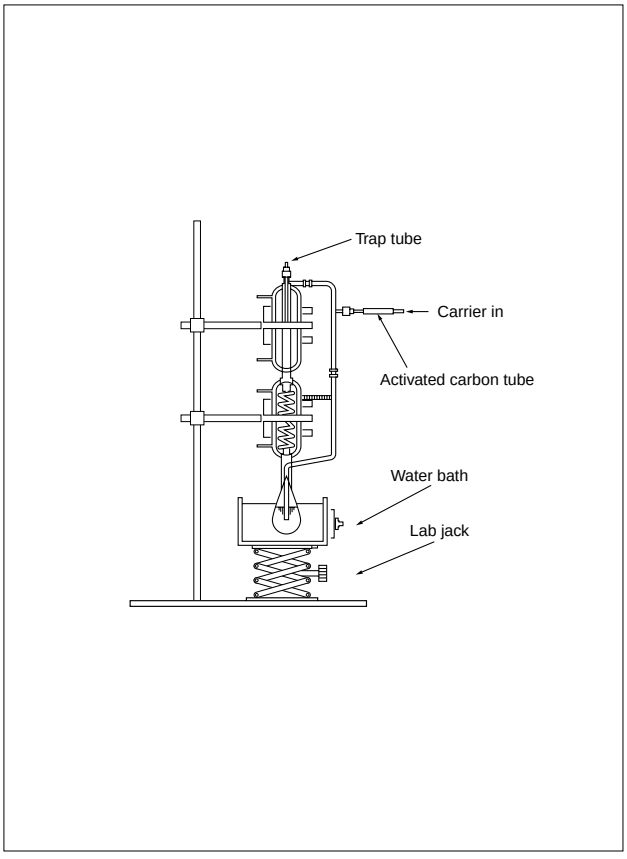


Fig. 4.12.1 Schematic diagram of Tenax Trapping Set

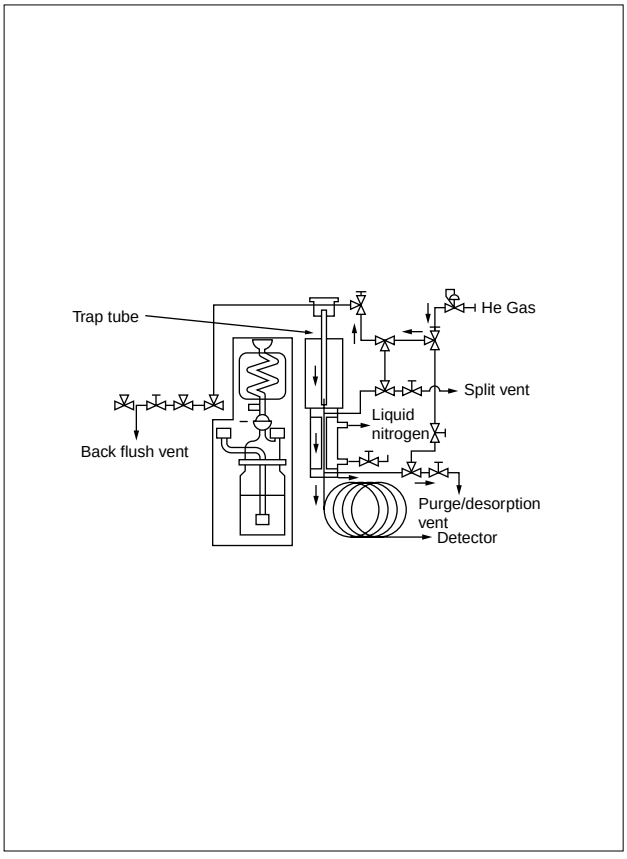


Fig. 4.12.2 TCT main unit flow line diagram

4.12 Analysis of Beverage Odors (2) - GCMS

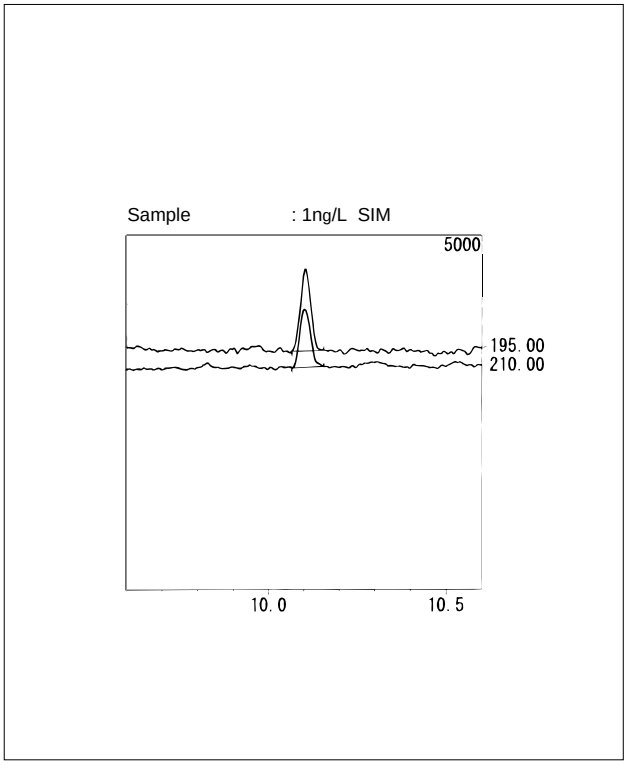


Fig. 4.12.3 SIM chromatogram (1ng/L)

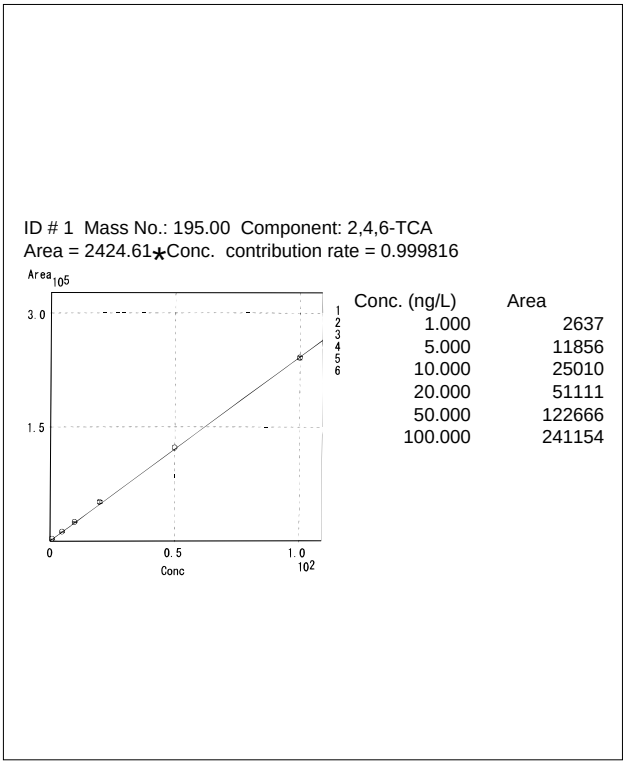


Fig. 4.12.4 TCA calibration curve (1 to 100ng/L)

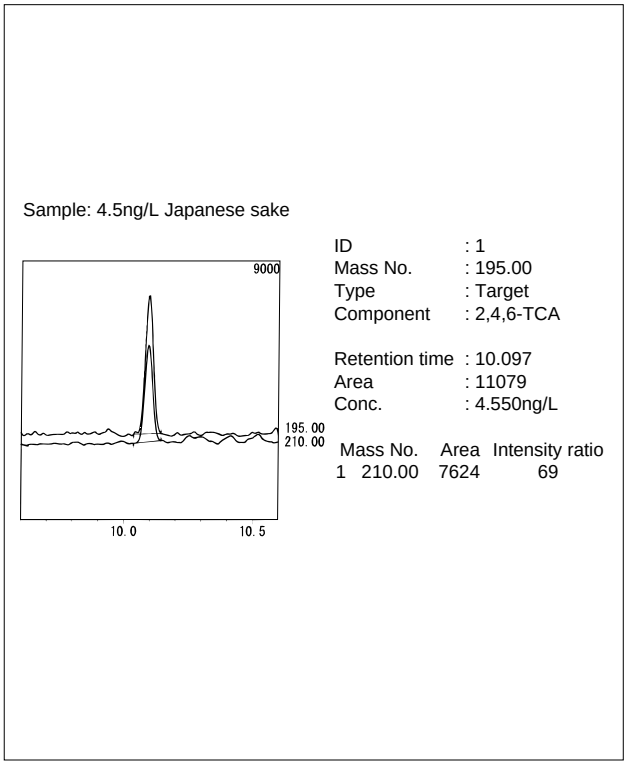


Fig. 4.12.5 Analysis of Japanese sake (4.5ng/L added)

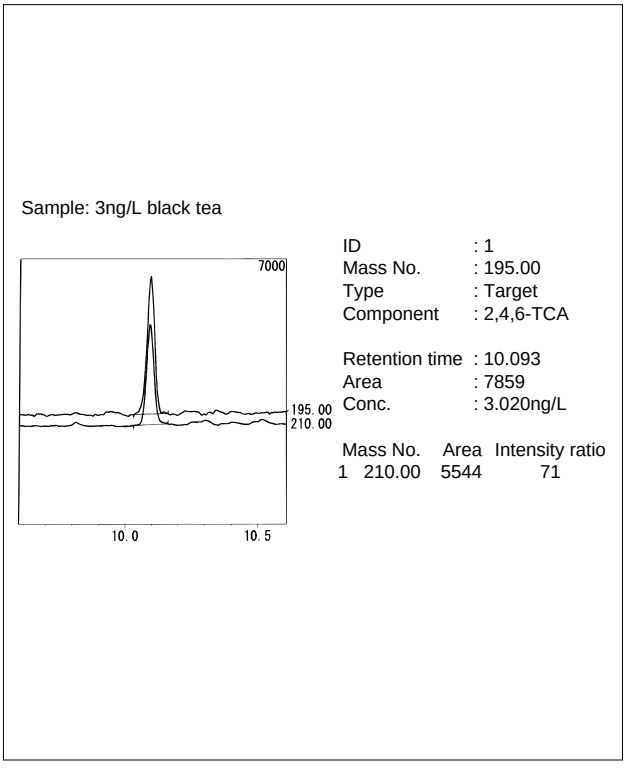


Fig. 4.12.6 Analysis of black tea (3ng/L added)

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℃(5min)-210℃(3/min)(30/min)
Inj.Temp.	: 250℃
Int.Temp.	: 230℃
Carrier Gas	: He(180kPa)
Injection	: Split(100:1)

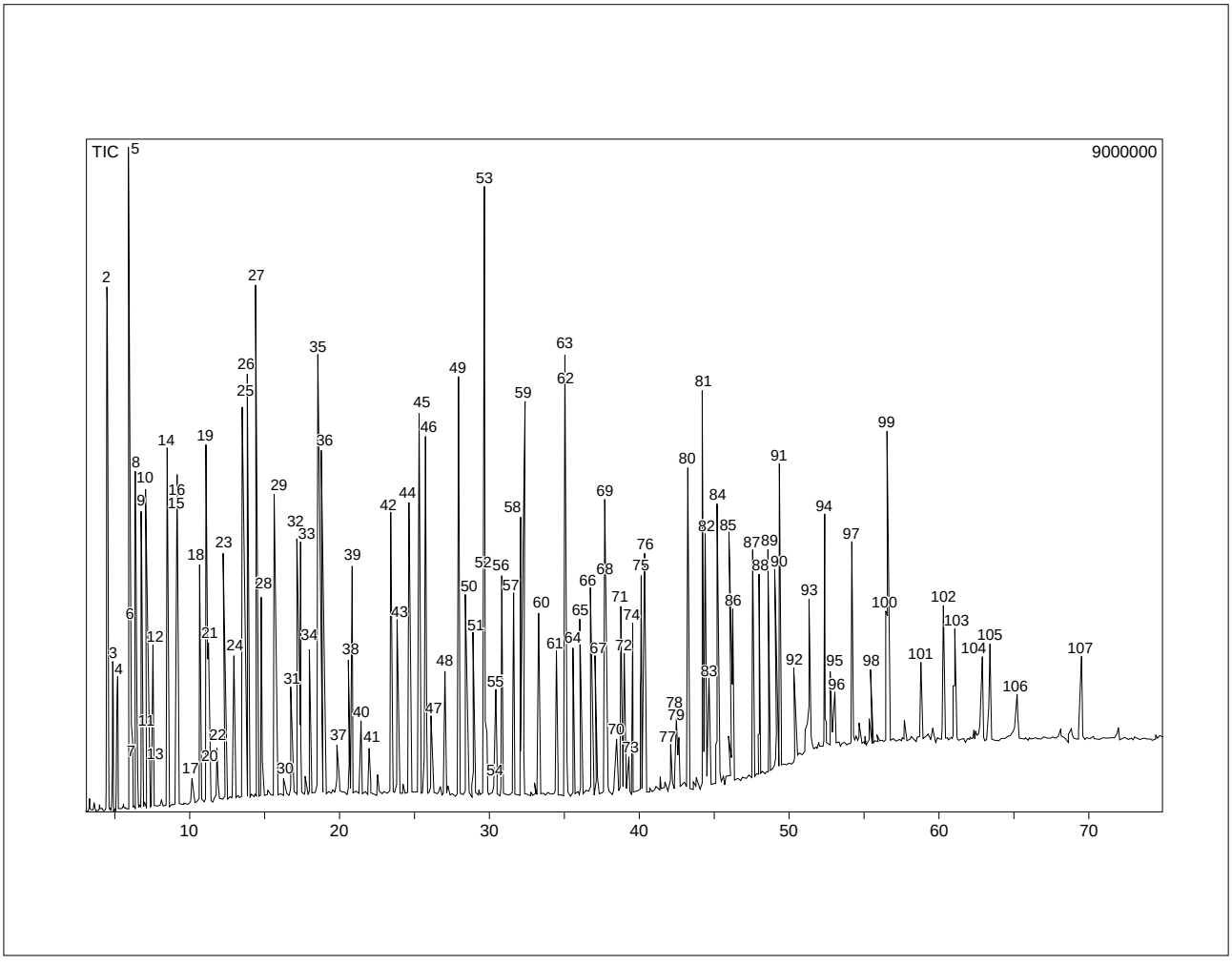


Fig. 4.13.1 TIC chromatogram of fragrant components

4.13 Analysis of Fragrant Material (2) - GCMS

	Compound	Alcohol	Ester	Aldehyde	Ketone	Terepene	Others
1	Ethyl acetate		O				
2	Diethyl acetal			O			
3	Ethyl alcohol	O					
4	Ethyl propionate		O				
5	i – Butyl acetate		O				
6	Chloroform						O
7							
8	Ethyl n-butyrate		O				
9	Ethyl 2-methyl butyrate		O				
10	Ethyl i-valerate		O				
11	n-Butyl acetate		O				
12	n-Hexanal			O			
13	i-Butyl alcohol	O					
14	n-Amyl acetate		O				
15	n-Butyl alcohol	O					
16	Methyl i-amyl ketone				O		
17							
18	n-Amyl propionate		O				
19	Limonene					O	
20	2-Methyl butyl alcohol	O					
21	n-Amyl furmate		O				
22	c-2-Hexenal			O			
23	Ethyl caproate		O				
24	n-Amyl alcohol	O					
25	i-Amyl n-butyrate		O				
26	n-Hexyl acetate		O				
27	Methyl n-hexyl ketone				O		
28	i-Amyl i-valerate		O				
29							
30							
31	Ethyl lactate		O				
32	n-Hexanol	O					
33	Ethyl n-hexyl ketone				O		
34	Allyl caproate		O				
35							
36	Methyl n-heptyl ketone				O		
37	t-3-Hexenol	O					
38							
39	Ethyl caprylate		O				
40	Acetic acid						O
41	Furfural			O			
42	Methyl n-octyl ketone				O		
43	Tetrahydro furfuryl alcohol	O					
44	Benzaldehyde			O			
45	Ethyl nonanoate		O				
46	Linalool					O	
47							
48	Diethyl malonate		O				
49	Methyl n-nonyl ketone				O		
50	Ethytl levulinate		O				
51	Methyl benzoate		O				
52	Ehtyl caprate		O				
53	l-Menthol					O	
54							

Chart 4.13.2 Component names 1

4.13 Analysis of Fragrant Material (3) - GCMS

	Compound	Alcohol	Ester	Aldehyde	Ketone	Terepene	Others
55	Furfuryl alcohol	O					
56	Ethyl benzoate		O				
57	Phenyl diethyl acetate		O				
58							
59	Methyl n-decyl ketone				O		
60	Benzyl acetate		O				
61	Methyl phenyl acetate		O				
62	Dimethyl benzyl carbinyl acetate		O				
63	Allyl caprate		O				
64	Ethyl phenyl acetate		O				
65	Allyl β-cyclohexyl propionate		O				
66	Phenethyl acetate		O				
67	Anethol					O	
68	Caproic acid						O
69	Ethyl laurate		O				
70	t-2-Decenal			O			
71	Benzyl n-butyrate		O				
72	Benzyl alcohol	O					
73	Phenetyl propionate		O				
74	i-Butyl phenyl acetate		O				
75	Dimethyl benzyl carbinylbutyrate		O				
76	Phenyl ethyl alcohol	O					
77							
78							
79	Phenyl ethyl propionate		O				
80	Phenethyl i-valerate		O				
81	Methyl n-tridecyl ketone				O		
82	Anisaldehyde			O			
83	γ-Nonalactone						O
84	Ethyl myristate		O				
85	Triacetine						O
86	Methyl cinnamate		O				
87	Benzylidene acetone				O		
88	Ethyl cinnamate		O				
89	γ-Decalactone						O
90	Eugenol					O	
91	Phenethyl caproate		O				
92	δ-Decalactone						O
93	Heliotropine						O
94	γ-Undecalactone						O
95	Anisalcohol	O					
96	Cinnamy alcohol	O					
97	Diethyl sebacate		O				
98							
99	γ-Dodecalactone						
100	Phenethyl octanoate		O				
101	δ-Dodecalactone						O
102	TEC						O
103	Benzophenone				O		
104	Ethyl vanillin						O
105							
106	vanilline						O
107	Benzyl benzoate		O				

Chart 4.13.2 Component names 2

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.

•Pretreatment

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

•Analytical Conditions

Instrument	: ICPS-7500
	: HVG-1 (hydride generator)
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	: 0.6mL/min (Hydride generating method: 2.5mL/min)
Observation method	: Horizontal/axial
Sample injection system	: Coaxial nebulizer/cyclone chamber, hydride generator

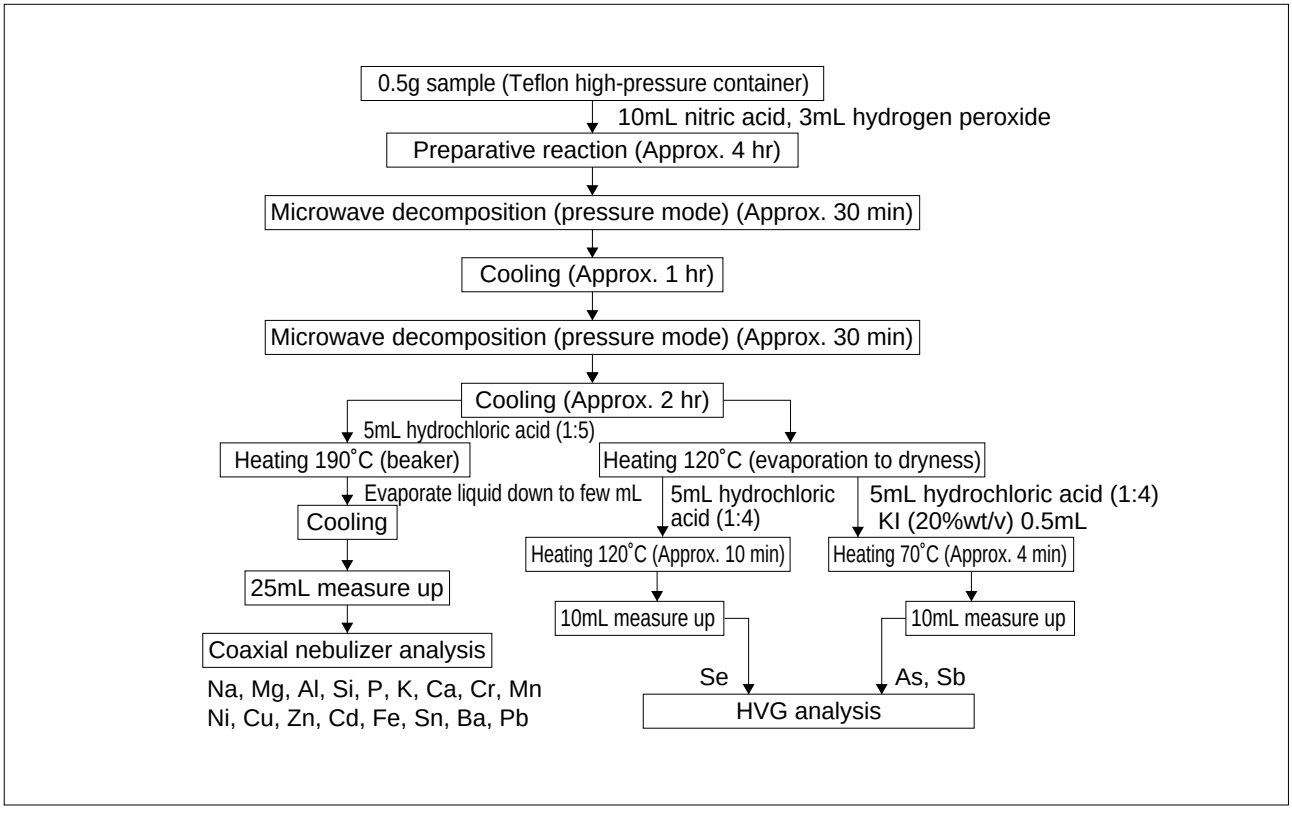


Fig. 5.1.1 Microwave decomposition flowchart

5.1 Analysis of Inorganic Components in Powdered Milk (2) - ICP-AES

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

* HVG hydride generator used

Chart 5.1.2 Powdered milk analysis results (μg/g)

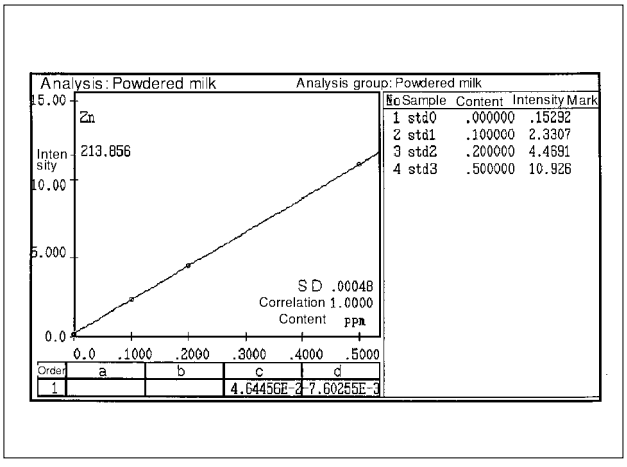


Fig. 5.1.3 Zn calibration curve

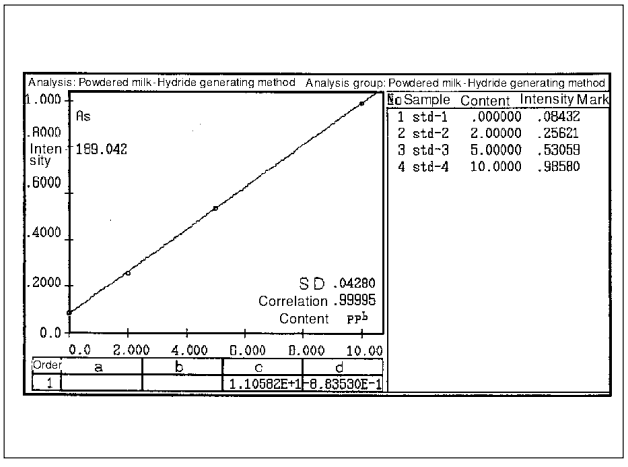


Fig. 5.1.4 As calibration curve

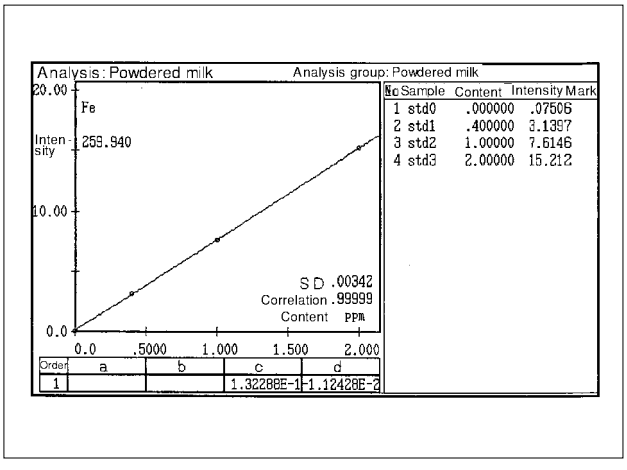


Fig. 5.1.5 Fe calibration curve

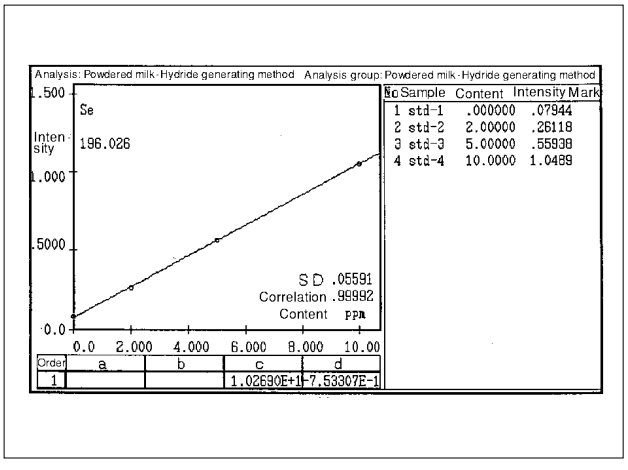


Fig. 5.1.6 Se calibration curve

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 l.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

Element	NIST-SRM 1549		BCR-CRM 063	
	Quantitative value	Guaranteed value	Quantitative value	Guaranteed value
Na	0.51	0.47±0.03	0.46	0.457±0.016
K	1.68	1.69±0.03	1.76	1.78±0.07
Ca	1.31	1.3 ±0.03	1.29	1.26±0.03
Mg	0.123	0.120±0.003	0.118	0.112±0.003
P	1.07	1.06±0.2	1.02	1.04±0.03
Fe*	2.3	1.78±0.10	2.6	2.06±0.25
Zn*	47.4	46.1±2.2	43	(42)
Mn*	0.27	0.26±0.06	0.25	(0.226)

* : 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-D_2

Air not added

Stage	Temperature (ßC)	Time (sec)	Heat mode	Gas	Inner gas flow rate
1	70	3	lamp	Ar	0.20
2	120	30	lamp	Ar	0.50
3	400	20	lamp	Ar	0.50
4	500	10	lamp	Ar	1.00
5	700	10	step	Ar	1.00
6	700	3	step	Ar	0.0H
7	2400	3	step	Ar	0.0H
8	2600	2	step	Ar	1.00

Chart 5.2.1 Heat program

	Measurement results	Added amount
Air added	10.5ppb	10.0ppb
Air not added	10.4ppb	10.0ppb

Chart 5.2.2 Measurement results for Pb in milk

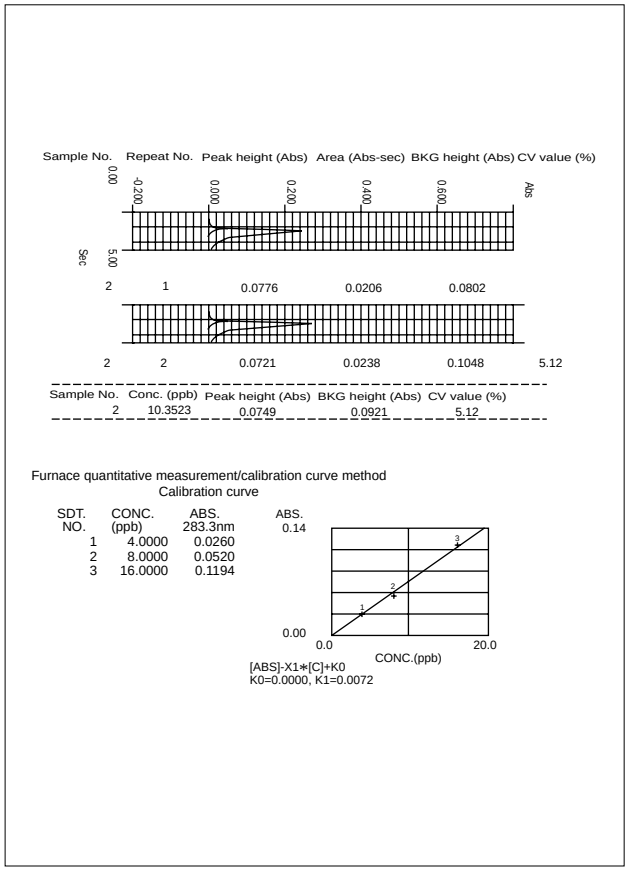


Fig. 5.2.3 Peak profile and calibration curve of Pb in milk

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(4.6mmϕ × 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(4.6mmϕ × 100mm)
Mobile phase : 3.0mM oxalic acid
Temperature : 40˚C
Flow rate : 1.2mL/min
Detection : Conductivity

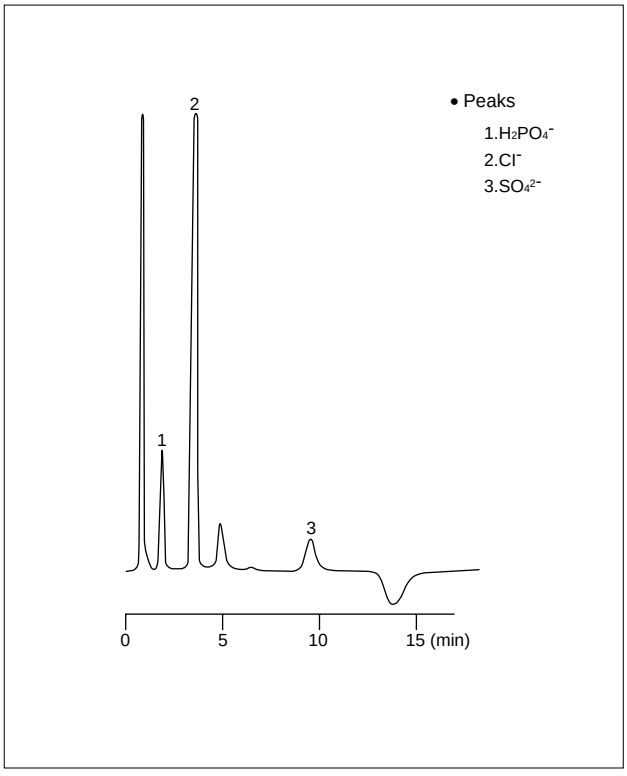


Fig. 5.3.1 Analysis of inorganic anions

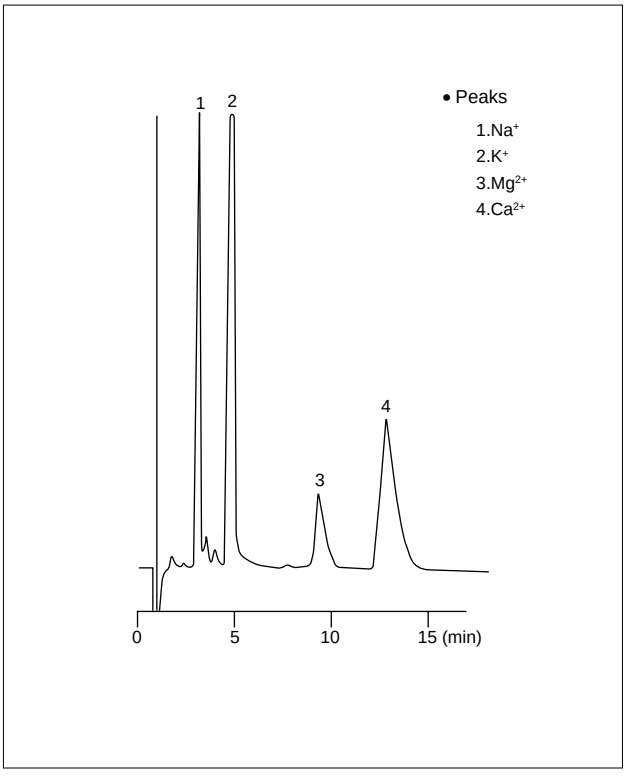


Fig. 5.3.2 Analysis of inorganic cations

5.3 Analysis of Inorganic Ions in Milk (2) - LC

References

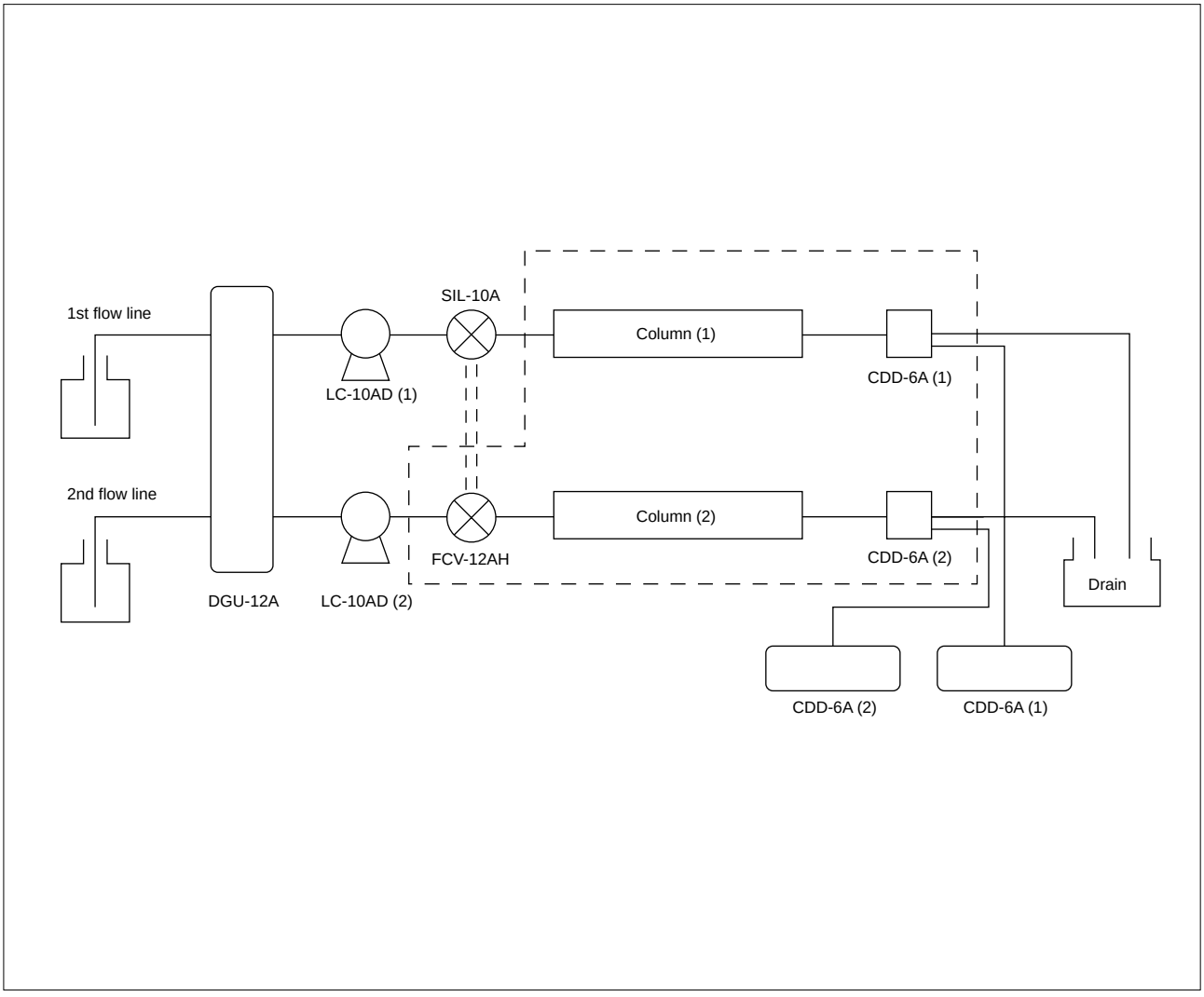


Fig. 5.3.3 Diagram of dual flow line system

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-D_2

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 150ßC
↓
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

Lighting Conditions

Element	: Pb
Turret No.	: 1
Lamp current Low (mA)	: 10
Lamp current High (mA)	: 0
Wavelength (nm)	: 283.3
Slit width (nm)	: 0.5
Lighting mode	: BGC-D2

Temperature Program

Final stage No. of concentration in oven : 5
Concentration frequency : 1

	Temperature (ßC)	Time (sec)	Heat mode	Sensitivity	Gas	Inner gas flow rate	Sampling	Previous stage (sec)
1	110	30	Ramp	Regular	Gas #1	0.20	Off	0
2	250	10	Ramp	Regular	Gas #1	0.20	Off	0
3	600	20	Ramp	Regular	Gas #1	1.00	Off	0
4	600	20	Step	Regular	Gas #1	1.00	Off	0
5	600	5	Step	High	Gas #1	0.00	Off	0
6	2100	3	Step	High	Gas #1	0.00	On	2
7	2600	2	Step	Regular	Gas #1	1.00	Off	0

Autosampler Mixing Conditions

Adding Conc. (ppb)	Sample amount	R2 (Pb: 10ppb standard solution)	R1 (pure water)	Total
Blank	0 L	0 L	200 L	200 L
0	100 L	0 L	100 L	200 L
1	100 L	20 L	80 L	200 L
2	100 L	40 L	60 L	200 L
3	100 L	60 L	40 L	200 L

Pb: 10ppb standard solution and pure water containing approximately 1.1mol/L of nitric acid
Inj. Vol. : 20 L

Chart 5.4.2 Measurement parameters

5.4 Analysis of Pb in White Sugar Using Atomic Absorption Spectrophotometry (2) - AA

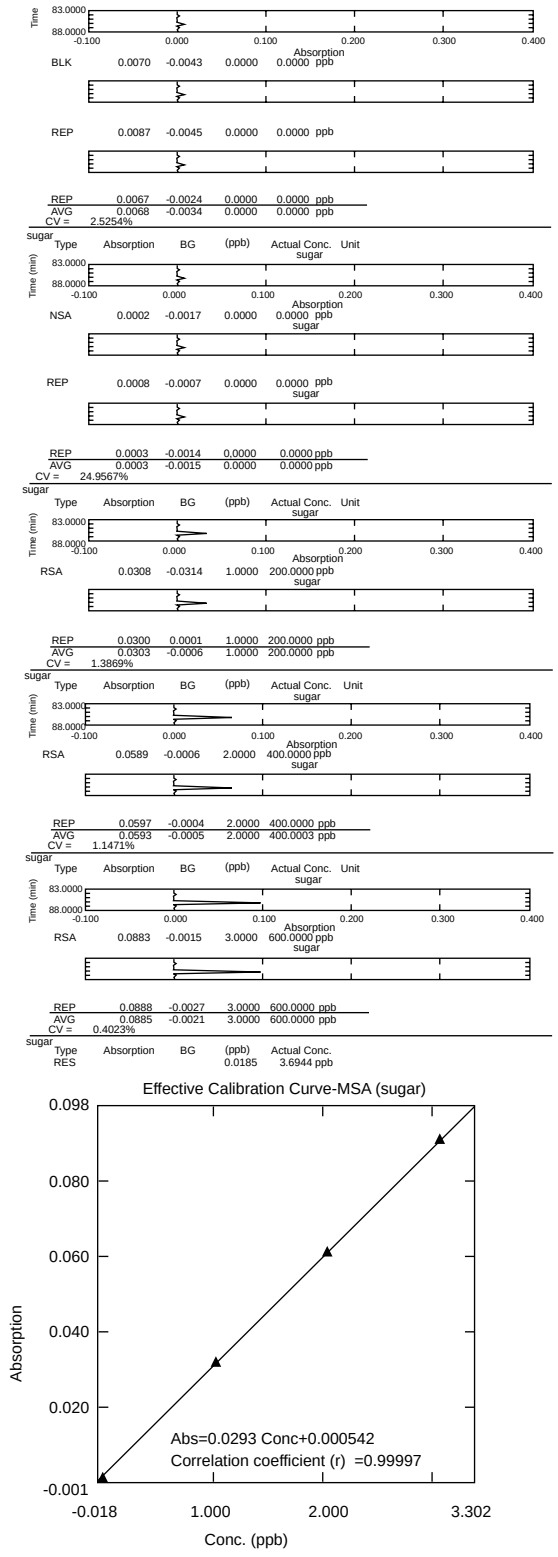


Fig. 5.4.3 Measurement results for Pb in refined white sugar

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

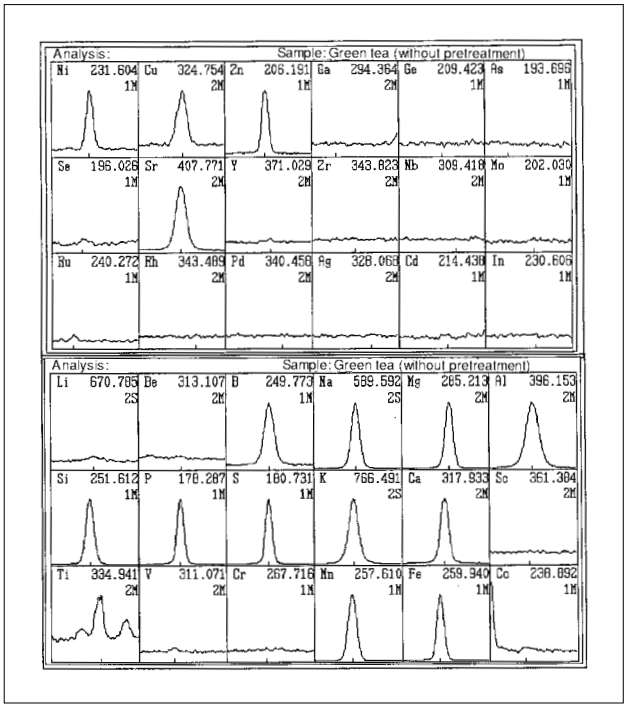


Fig. 5.5.1 Profile example for qualitative analysis

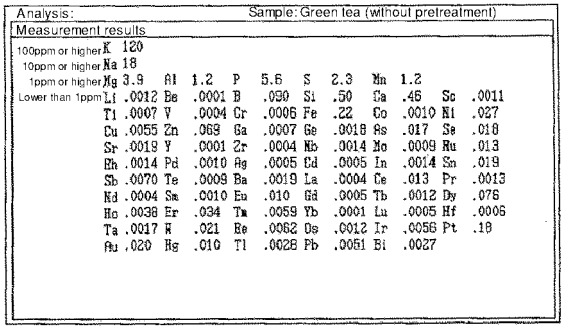


Fig. 5.5.2 Semi-quantitative value for qualitative analysis

5.5 Analysis of Inorganic Components in Canned Drink (Green Tea) (2) - ICP-AES

Element	Direct introduction	Wet decomposition
Fe	0.249	0.260
Ni	0.029	0.029
Al	1.27	1.30
Pb	<0.001	<0.001
Sn	<0.001	0.002
Cu	0.0057	0.0053
Zn	0.083	0.089
Cr	0.0008	0.0007

Chart 5.5.3 Green tea analysis results (μg/mL)

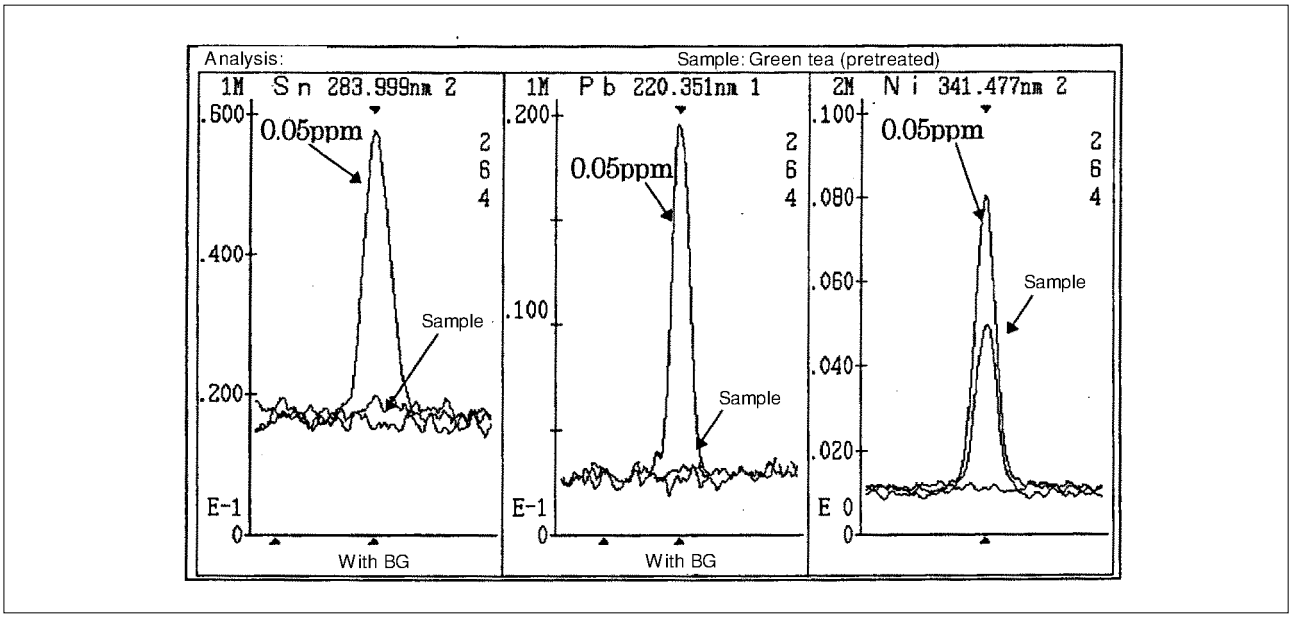


Fig. 5.5.4 Spectrum line profile

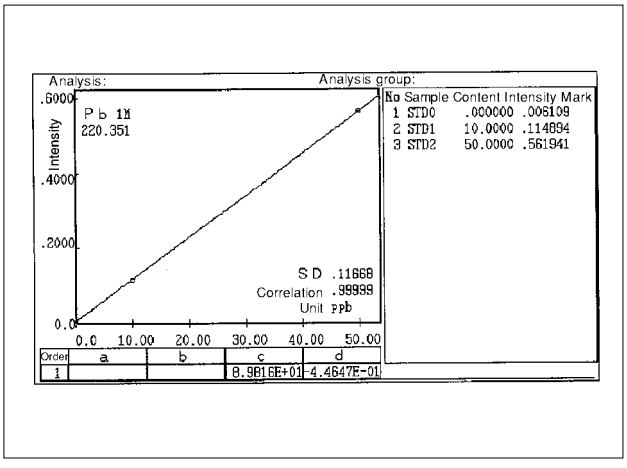


Fig. 5.5.5 Pb calibration curve

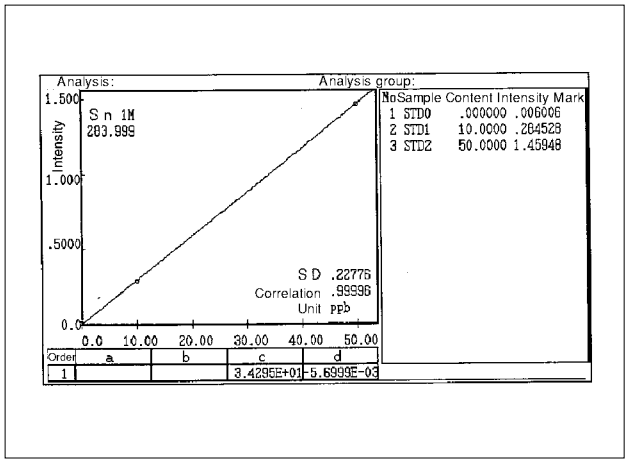


Fig. 5.5.6 Sn calibration curve

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.

•Analytical Conditions

Instrument : ICPM-8500
High frequency : 27.12MHz
High frequency output : 1.2kW
Cooling gas : Ar 7.0L/min
Plasma gas : Ar 1.5L/min
Carrier gas : Ar 0.62L/min
Sample induction unit : Coaxial nebulizer/water cooling chamber (5˚C)
Sample suction rate : 0.4mL/min
Ion induction system : Pt sampling cone Pt skimmer cone

Sample		Rice flour NIES No.10		Tomato Leaves NIST SRM1573		Citrus Leaves NIST SRM1572	
Element	M/Z	Guaranteed value	Quantitative value	Guaranteed value	Quantitative value	Guaranteed value	Quantitative value
V	51	–	0.049	–	0.89	–	0.19
Cr	52	0.22*	0.31	4.5±0.5	3.2	0.8 ±0.2	0.81
Co	59	0.02*	0.05	0.6*	0.42	0.02*	0.06
Ni	60	0.39±0.04	0.41	–	1.2	0.6 ±0.3	0.77
Cu	63	3.3±0.2	3.0	11 ±1	9.4	16.5 ±1.0	14
Zn	66	22.3±0.9	23.3	62 ±6	56	29 ±2	29
As	75	0.11*	0.12	0.27±0.05	0.28	3.1 ±0.3	3.1
Se	82	0.02*		–	0.09	–	0.04
Mo	98	0.42±0.05	0.45	–	0.49	0.17±0.09	0.12
Cd	111	0.32±0.02	0.29	3*	2.6	0.03±0.01	0.11
Pb	208	–	1.6	6.3 ±0.3	5.8	13.3 ±2.4	12.0

*: 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

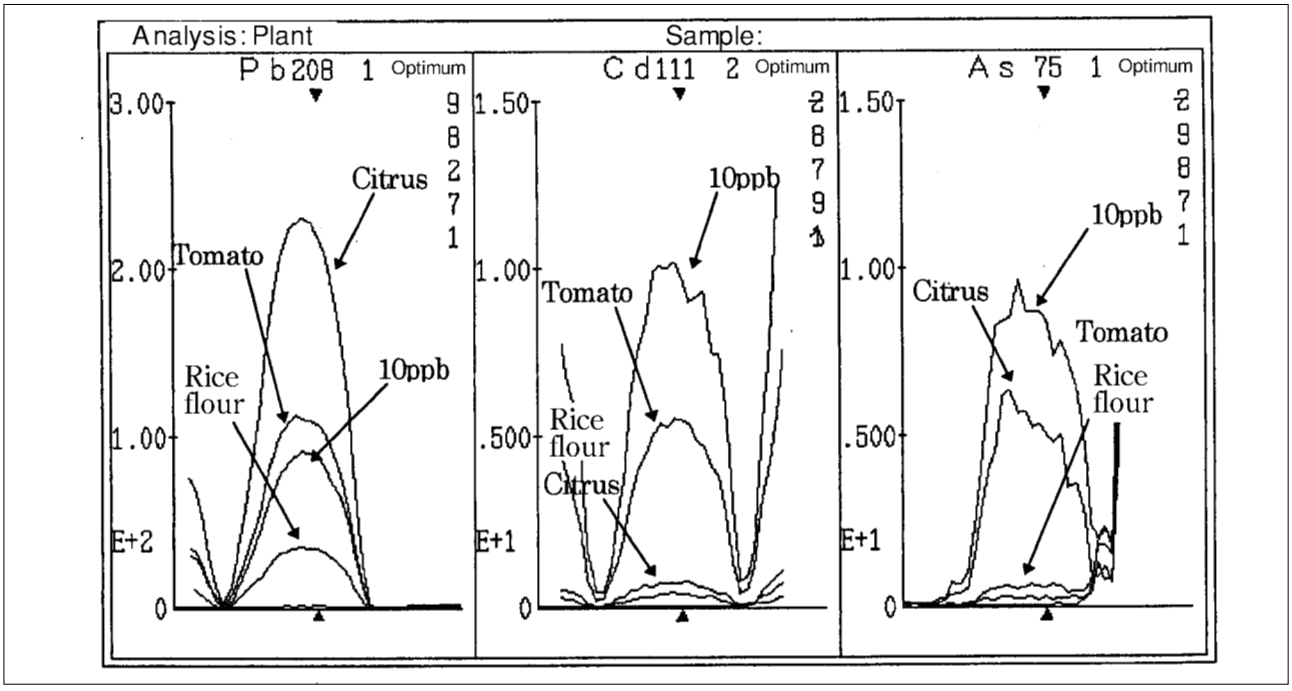


Fig. 5.6.2 Mass spectrum of Pb208 Fig. 5.6.3 Mass spectrum of Cd111 Fig. 5.6.4 Mass spectrum of As75

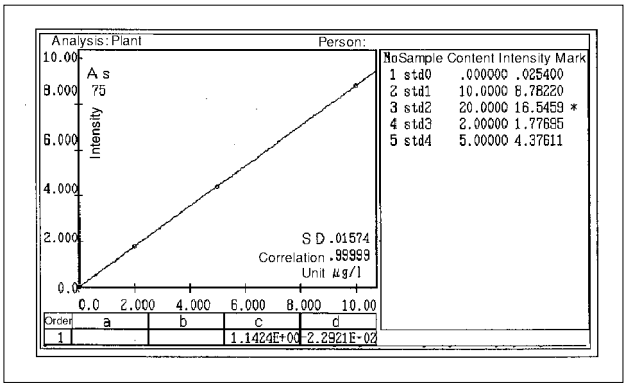


Fig. 5.6.5 As calibration curve

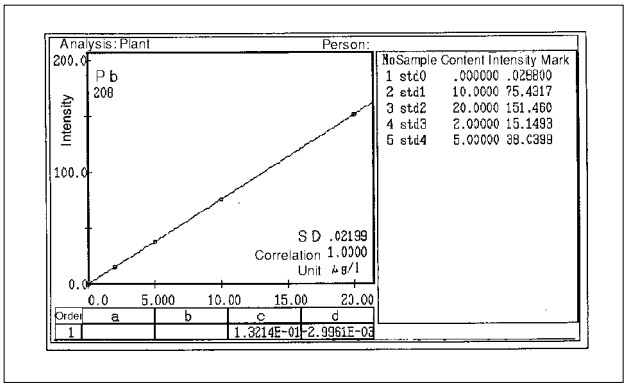


Fig. 5.6.6 Pb calibration curve

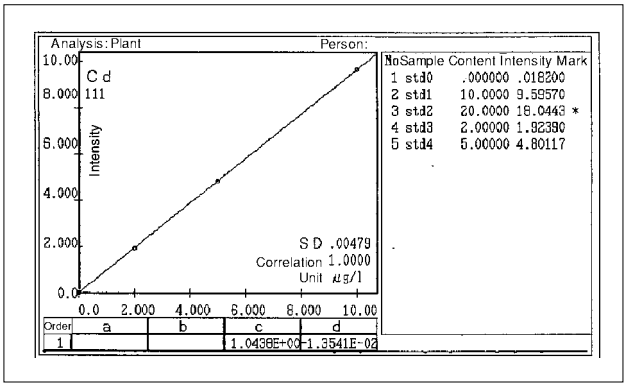


Fig. 5.6.7 Cd calibration curve

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

Instrument	: ICPS-8000
High frequency	: 27.12MHz
High frequency output	: 1.2kW
Cooling gas	: Ar 14.0L/min
Plasma gas	: Ar 1.2L/mln
Carrier gas	: Ar 0.7L/min
Purge gas	: Ar 3.5L/min
Sample suction rate	: 0.6mL/min
Observation method	: Horizontal
Sample induction	: Coaxial nebulizer/ double tube chamber

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

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.

•Analytical Conditions

Instrument	: AA-6200
Wavelength	: Na 589.5nm
Lamp current (mA)	: 12
Slit width (nm)	: 0.2
Background correction	: None
Flow type	: Air-acetylene
Fuel gas (C_2H_2)	: 1.8L/min
Support gas (air)	: 8L/min
Burner angle	: 45˚

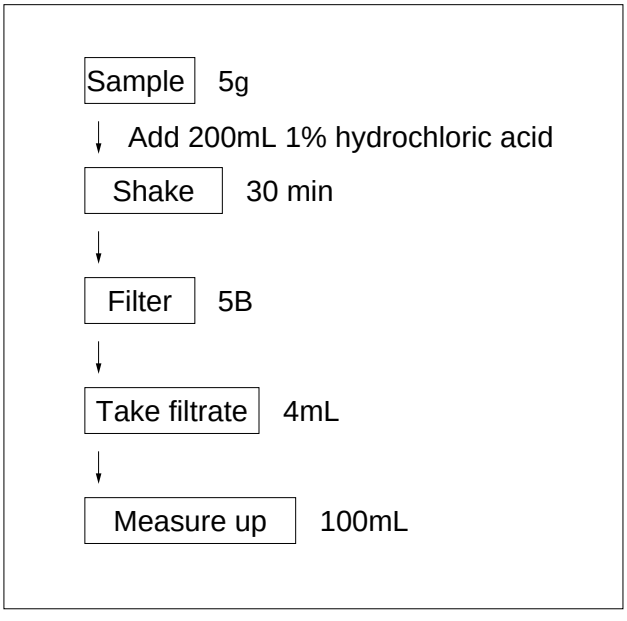


Fig. 5.8.1 Pretreatment flowchart

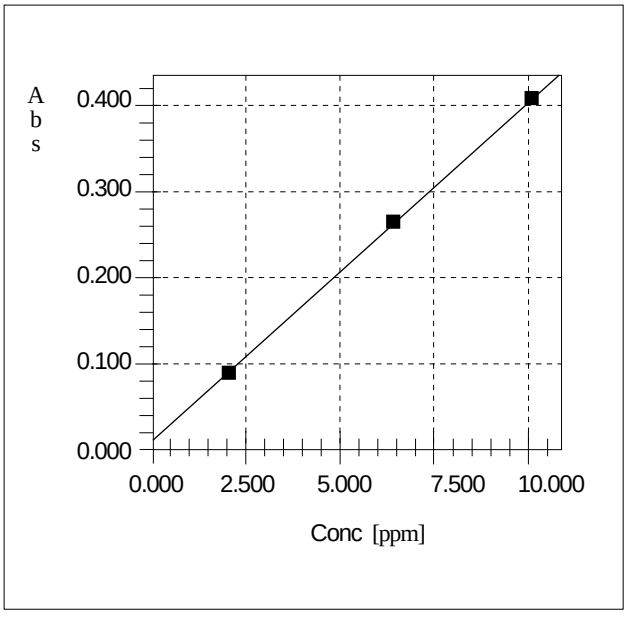


Fig. 5.8.2 Sodium calibration curve

Sample	Measurement result	Value shown in label
Bread	499mg	502mg

Chart 5.8.3 Analysis results

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.

References

LC talk, No. 36 from Shimadzu Corporation (1995)
Y.Nagashima, et.al.,
Nippon Suisan Gakkai, 53 (5), 819 (1978).
Y.Oshima, et.al., "Mycotoxins and Phycotoxins '88",
Elsevier Science Publishers, New York, 1989, 319.

•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 liquid : 50mM (sodium) borate buffer (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)

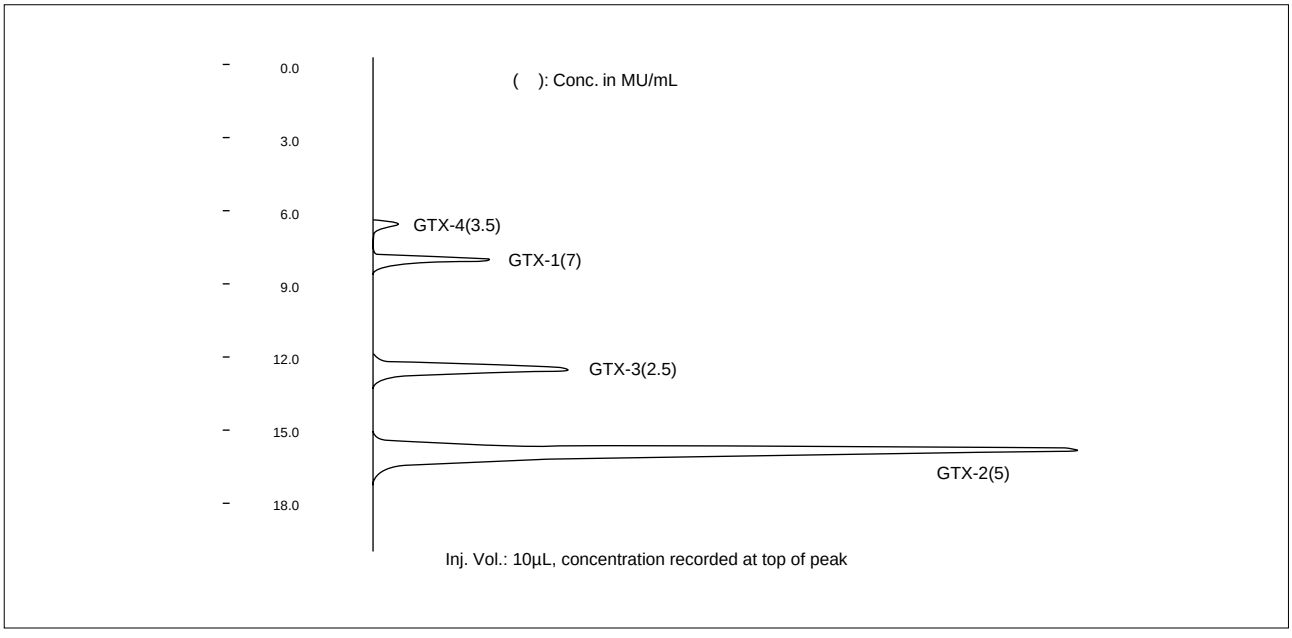
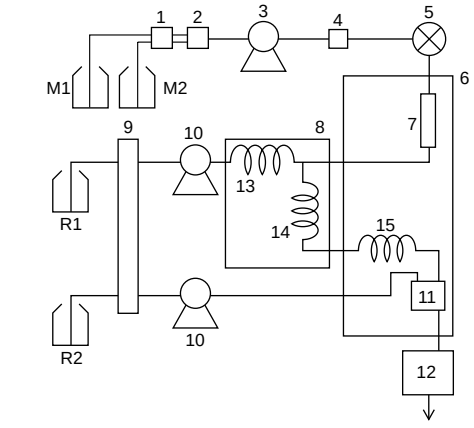


Fig. 6.1.1 Analysis example for gonyautoxin (GTX) 1-4 standard sample

6.1 Analysis of Shellfish Toxins (2) - LC

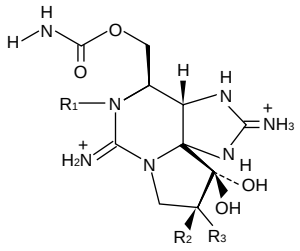
References



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
11. Blender
12. Fluorescent detector
13. 14. 15. Reaction coils
M1, M2 Mobile phases
R1, R2 Reaction liquids

Fig. 6.1.2 Flow line



R1	R2	R3	
H	H	H	STX
OH	H	H	neoSTX
OH	H	OSO_3^-	GTX1
H	H	OSO_3^-	GTX2
H	OSO_3^-	H	GTX3
OH	OSO_3^-	H	GTX4

Fig. 6.1.3 Structural formula of shell toxin components

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.

•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

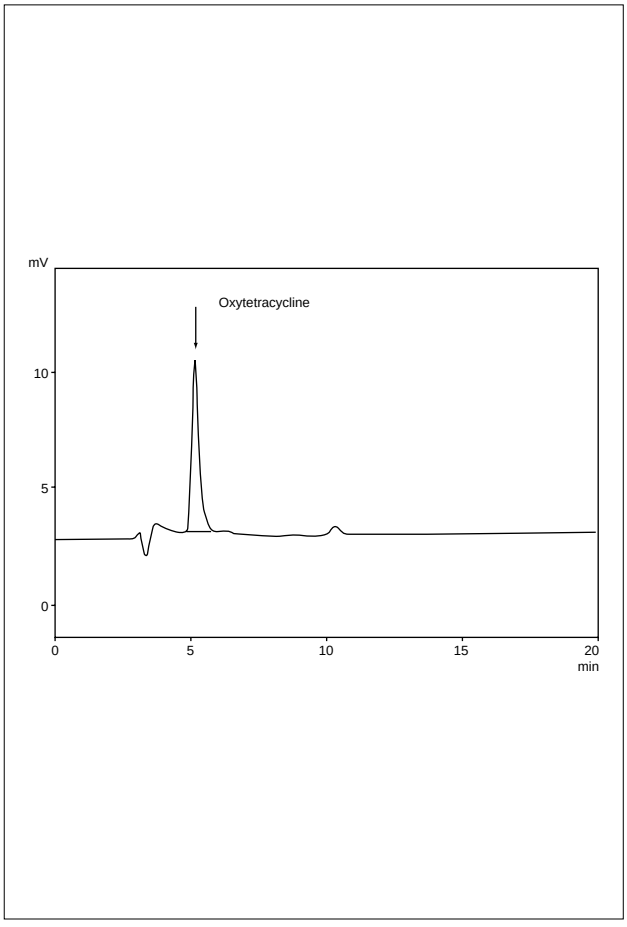


Fig. 6.2.1 Analysis example of oxytetracycline

References

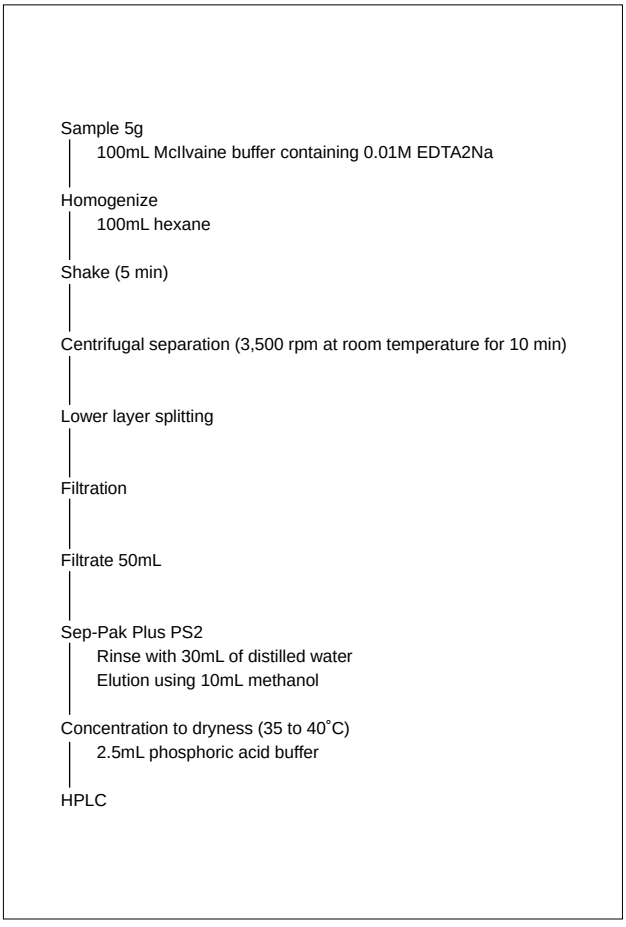


Chart 6.2.2 Pretreatment flowchart for oxytetracycline

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 (4.6mmϕ × 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

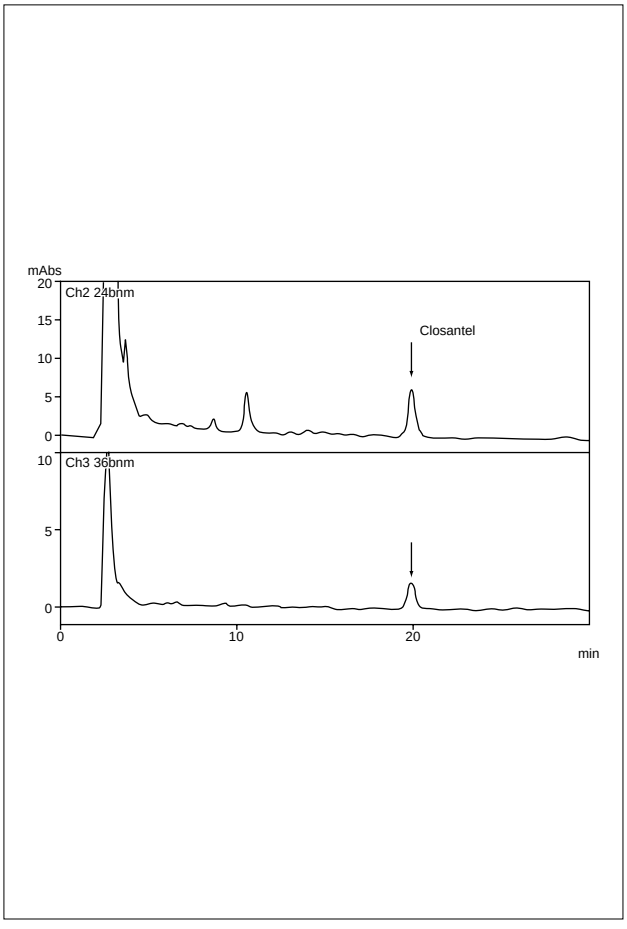


Fig. 6.3.1 Analysis example of closantel

References

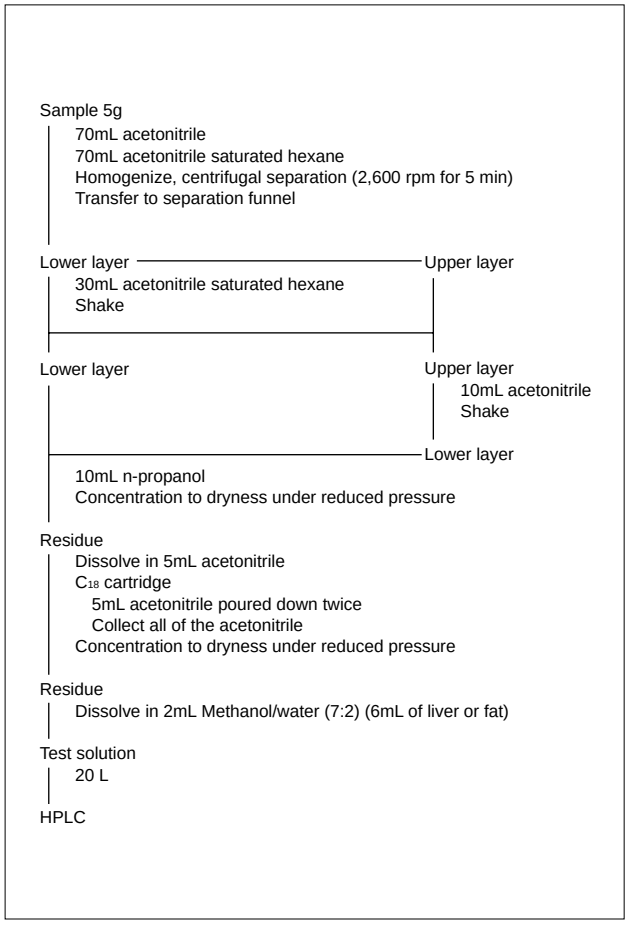


Chart 6.3.2 Pretreatment flowchart for closantel

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

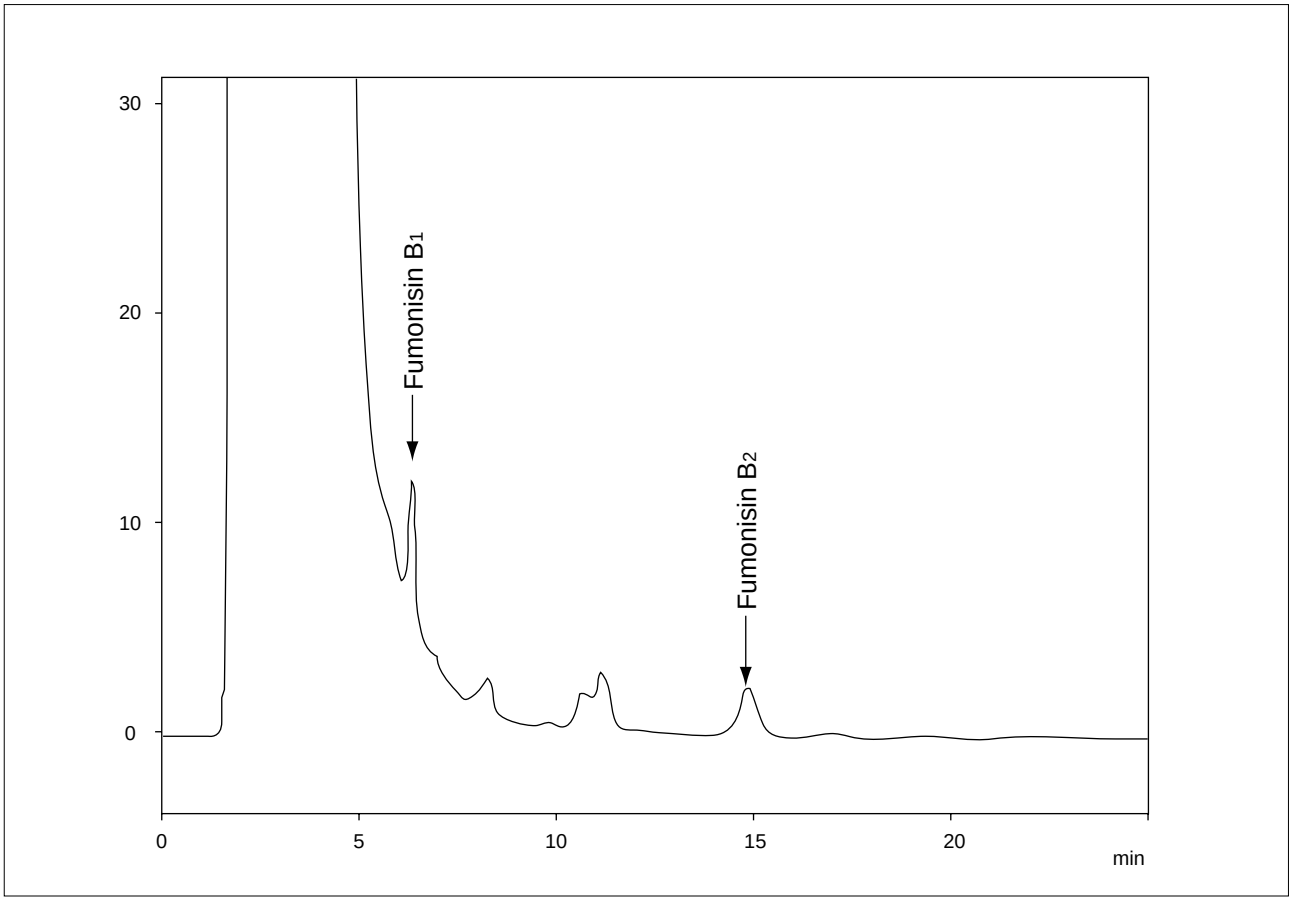


Fig 6.4.1 Analysis example of fumonisin in sweet corn

6.4 Analysis of Fumonisin in Sweet Corn (2) - LC

References

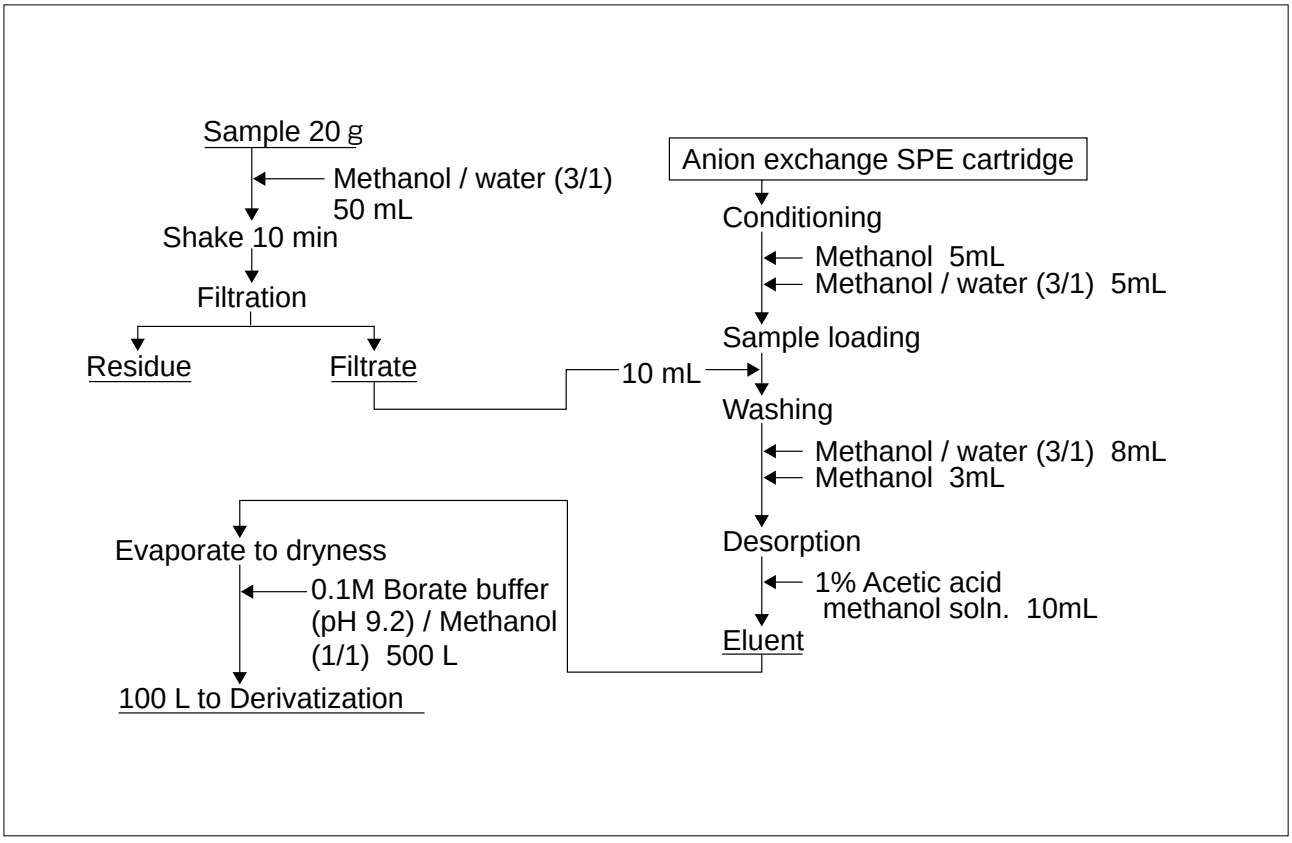


Fig. 6.4.2 Pretreatment flowchart for sweet corn

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

nstrument : 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

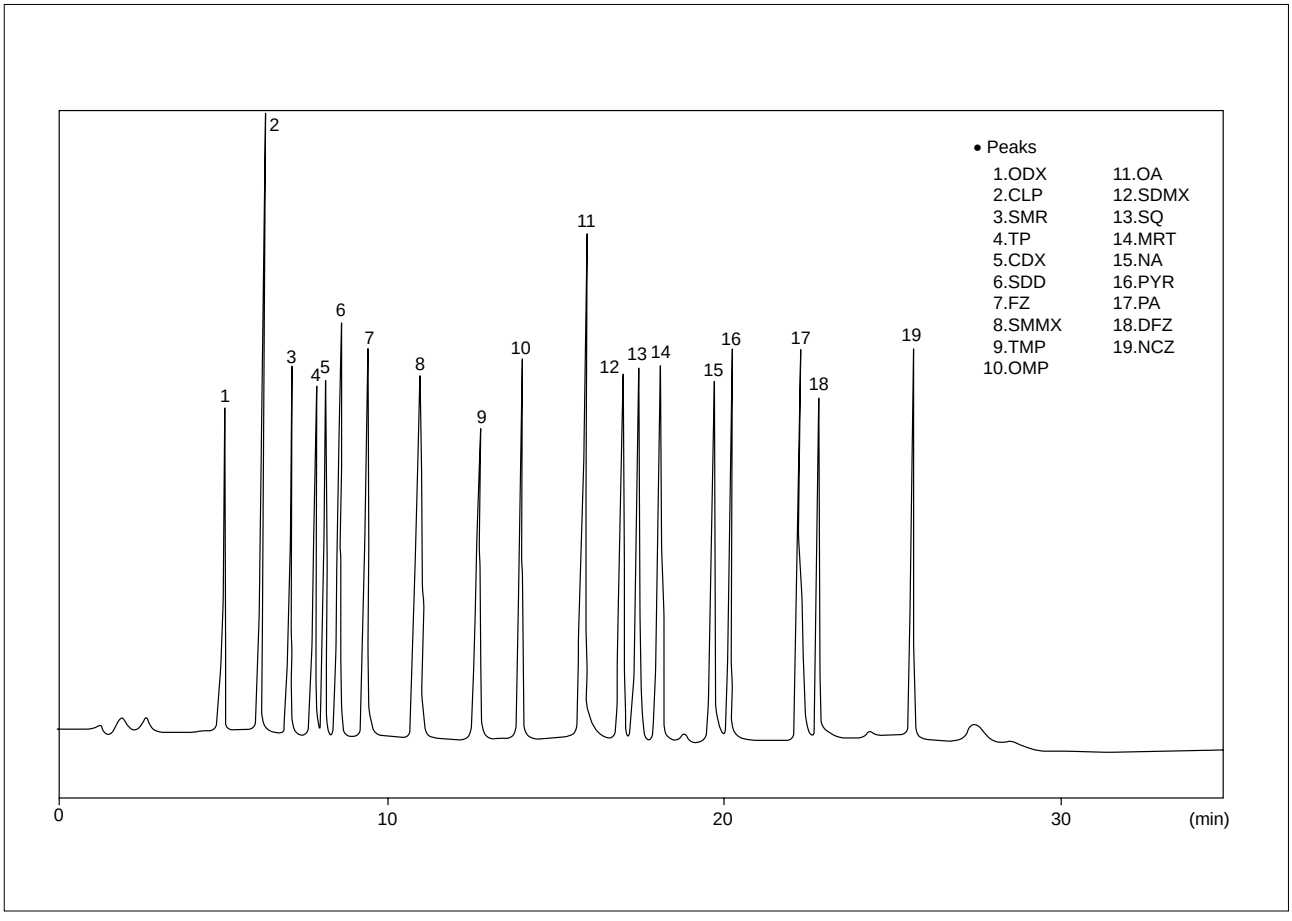


Fig. 6.5.1 Simultaneous analysis example for 19 synthetic antibacterial agent components

6.5 Simultaneous Analysis of Synthetic Antibacterial Agent (2) - LC

References

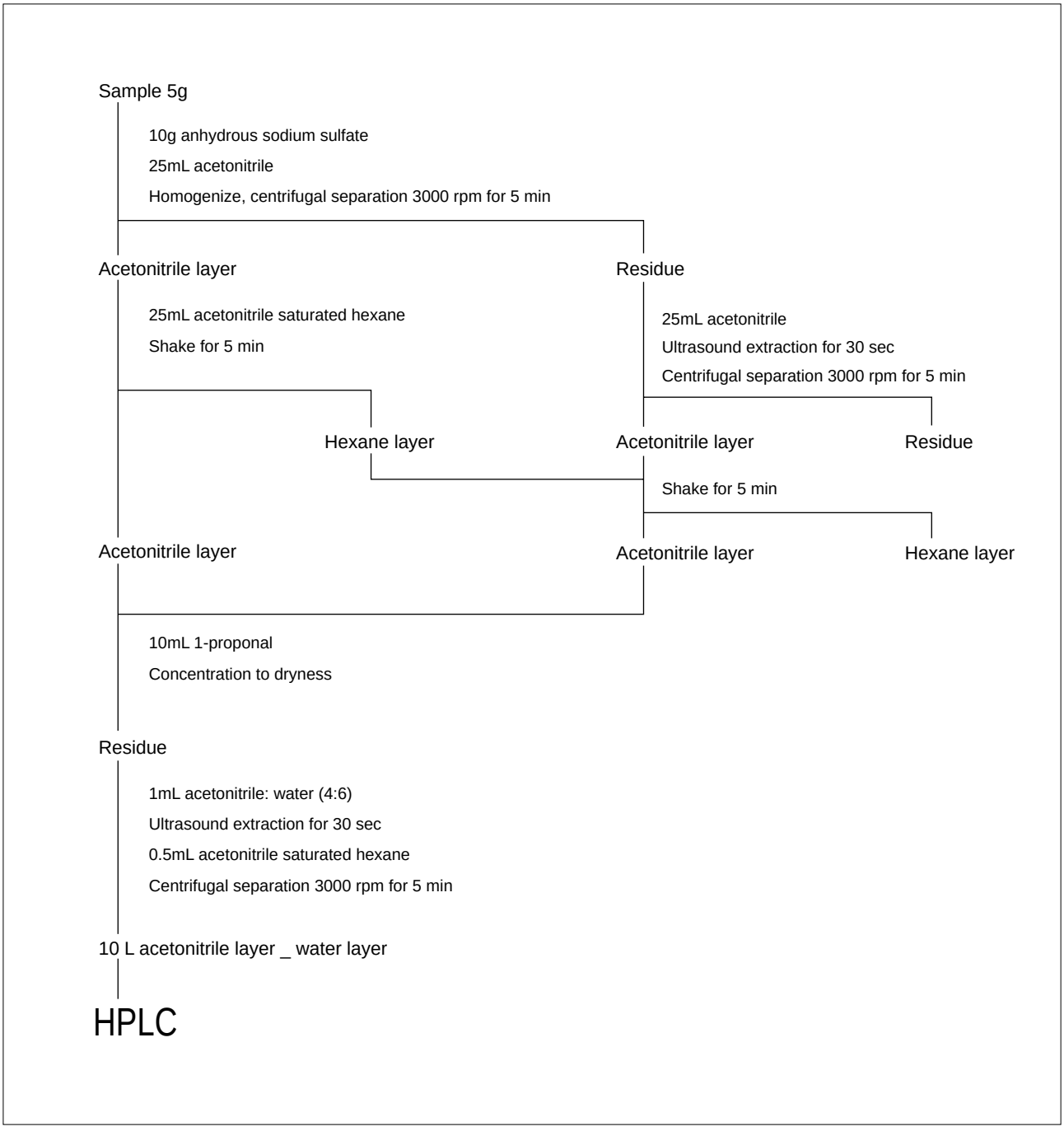


Fig. 6.5.2 Pretreatment flowchart for simultaneous analysis of 19 synthetic antibacterial agent components

6.6 Analysis of Inorganic Ions in Drinking Water - LC

•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.

References

Shimadzu Application News No. H37

Column	:	Shim-pack IC-SA1
Mobile Phase	:	14mM Sodium Hydrogen Carbonate solution
Flow Rate	:	1.0mL/min
Temperature	:	30˚C
Detection	:	HIC-10ASP Suppressor system
Inj.Vol.	:	50µL

Chart 6.6.1 Analytical conditions for anions

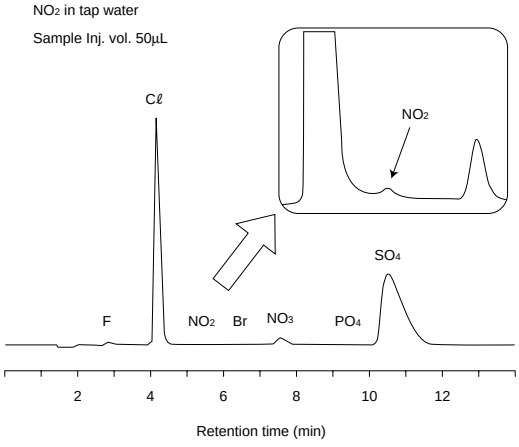


Chart 6.6.2 Analysis example of anions in drinking water

Column	:	Shim-pack IC-SC1
Mobile Phase	:	6.5mM Methane Sulfonic acid solution
Flow Rate	:	1.0mL/min
Temperature	:	30˚C
Detection	:	HIC-10ASP Suppressor system
Inj.Vol.	:	50µL

Chart 6.6.3 Analytical conditions for cations

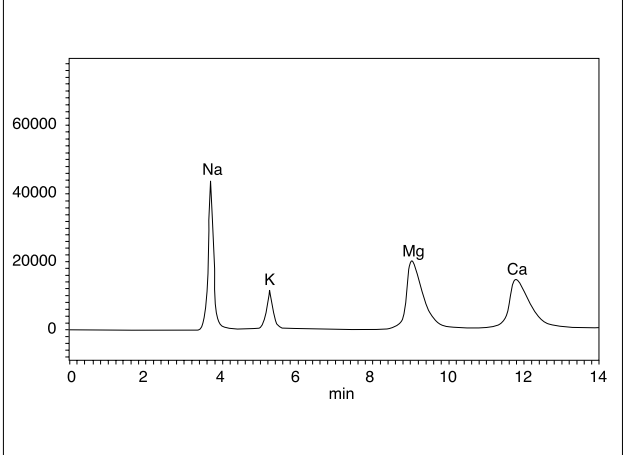


Chart 6.6.4 Analysis example of cations in drinking water

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