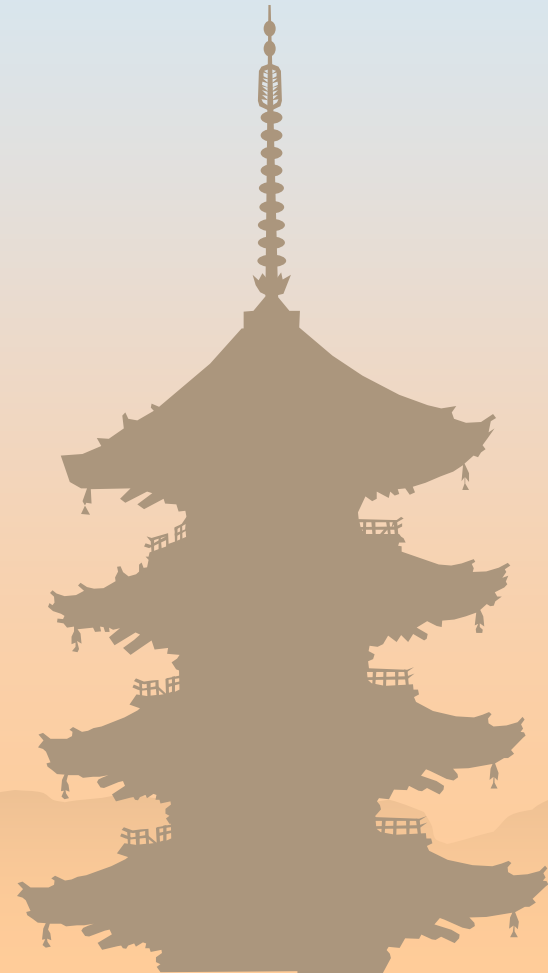


# Transportation Research and Information in Japan

Institute of Transportation Economics

**Fumio KUROSAKI**, Ph.D.





一般財団法人 運輸調査局

Institute of Transportation Economics

- 1) Institute of Transportation Economics (ITE) is a Research Institute, which specializes in the economics and management of transportation.
- 2) ITE collects literature and information about transportation.
- 3) ITE publishes the monthly Journal "Transportation and Economics" (Japanese).
- 4) ITE is a secretariat of the Japan Society of Transportation Economics.



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Institute of Transportation Economics

Institute of Transportation Economics cooperates with HERMES Project as an observer status, like TRB.

The HERMES Project\*:

Establishing a CompreHEnsive transport Research Information Management and Exchange System

\* The Project is funded under the EU Framework

# Transportation Modes in Japan



## 1) Railways

*Japanese National Railways*  
were reformed in 1987.

→ 6 Passenger Railways + 1 Freight Company

*J a p a n* has many private railways and Metros as well

## 2) Road

## 3) Air

*JAL, ANA, etc.*

## 4) Sea

# Who performs Research about Transportation ?

1) *Universities*

2) *Private Companies*

*Railway Companies, Auto Manufactures*

3) *Industrial organizations*

4) *Research Institute*

*Railway Technical Research Institute, ITE, etc*

5) *Others*

→ *Various organizations perform the research.*

# Field of the Research about Transportation

## *Ex) Railway Sector*

- 1) Civil Engineering*
- 2) Mechanical Engineering*
- 3) Electricity Engineering*
- 4) Transportation Economics*
- 5) Other Fields*

→ *Transportation covers various fields.*

# Who has Results of the Research?

*Usually researchers submit their papers to Engineering/Academic Societies.*

## *<Engineering / Academic Societies in Japan>*

- 1) Japan Societies of Civil Engineers*
- 2) The Institute of Electrical Engineers in Japan*
- 3) The Japan Society of Mechanical Engineers*
- 4) The Japan Society of Transportation Economics*
- 5) Other engineering/academic societies*

# How to get research results in Japan

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engineering society.*

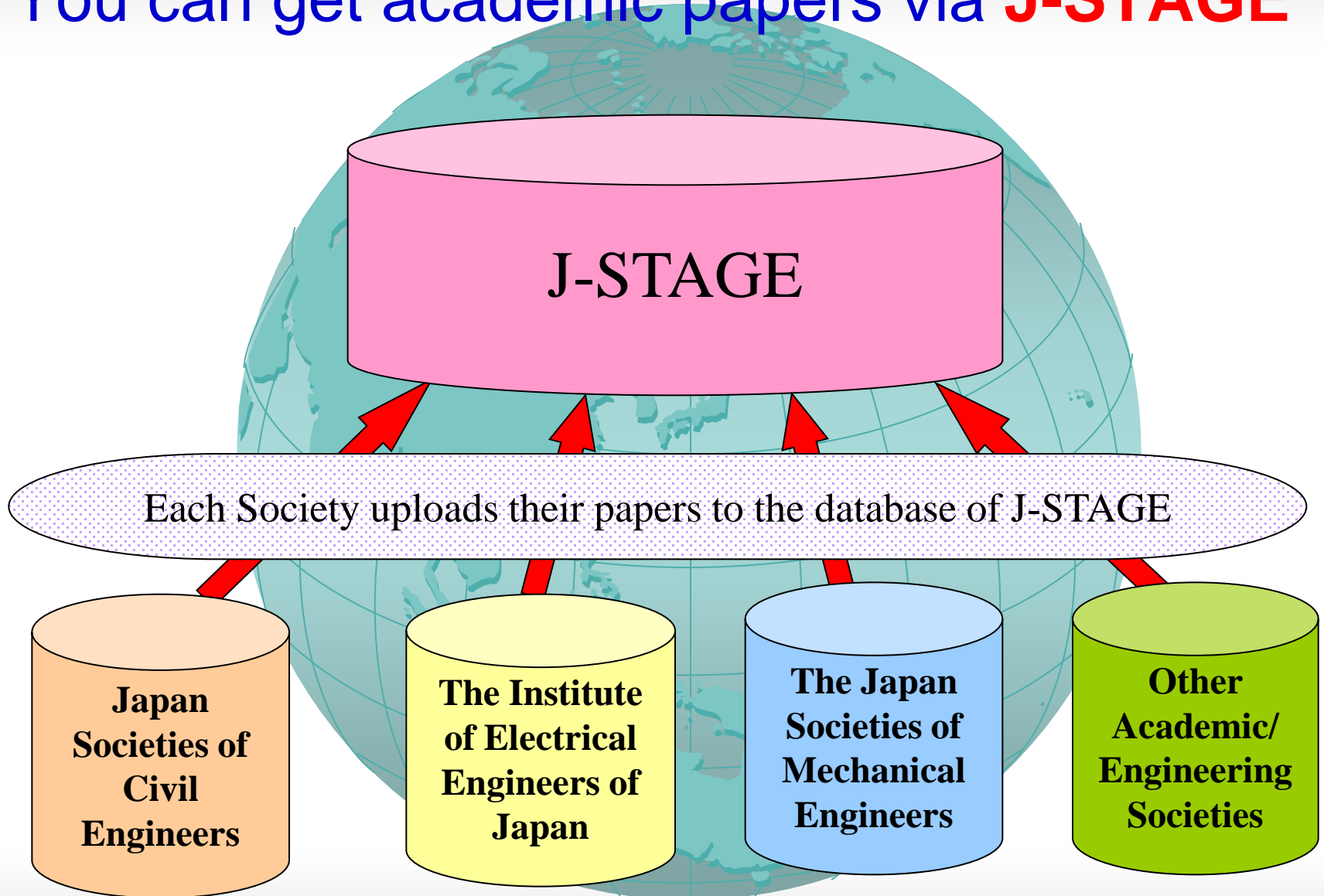
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All | New title(0) | New issue(15) | Release announcement(0)

**May 02, 2012 [New issue]**  
The new issue of [Biological and Pharmaceutical Bulletin](#) is now available.  
Vol. 35 No. 5

**May 02, 2012 [New issue]**  
The new issue of [Chemical and Pharmaceutical Bulletin](#) is now available.  
Vol. 60 No. 5

**May 02, 2012 [New issue]**  
The new issue of [IEEJ Transactions on Electronics, Information and Systems](#) is now available.  
Vol. 132 No. 5

**May 02, 2012 [New issue]**  
The new issue of [IEEJ Transactions on Fundamentals and Materials](#) is now available.  
Vol. 132 No. 5

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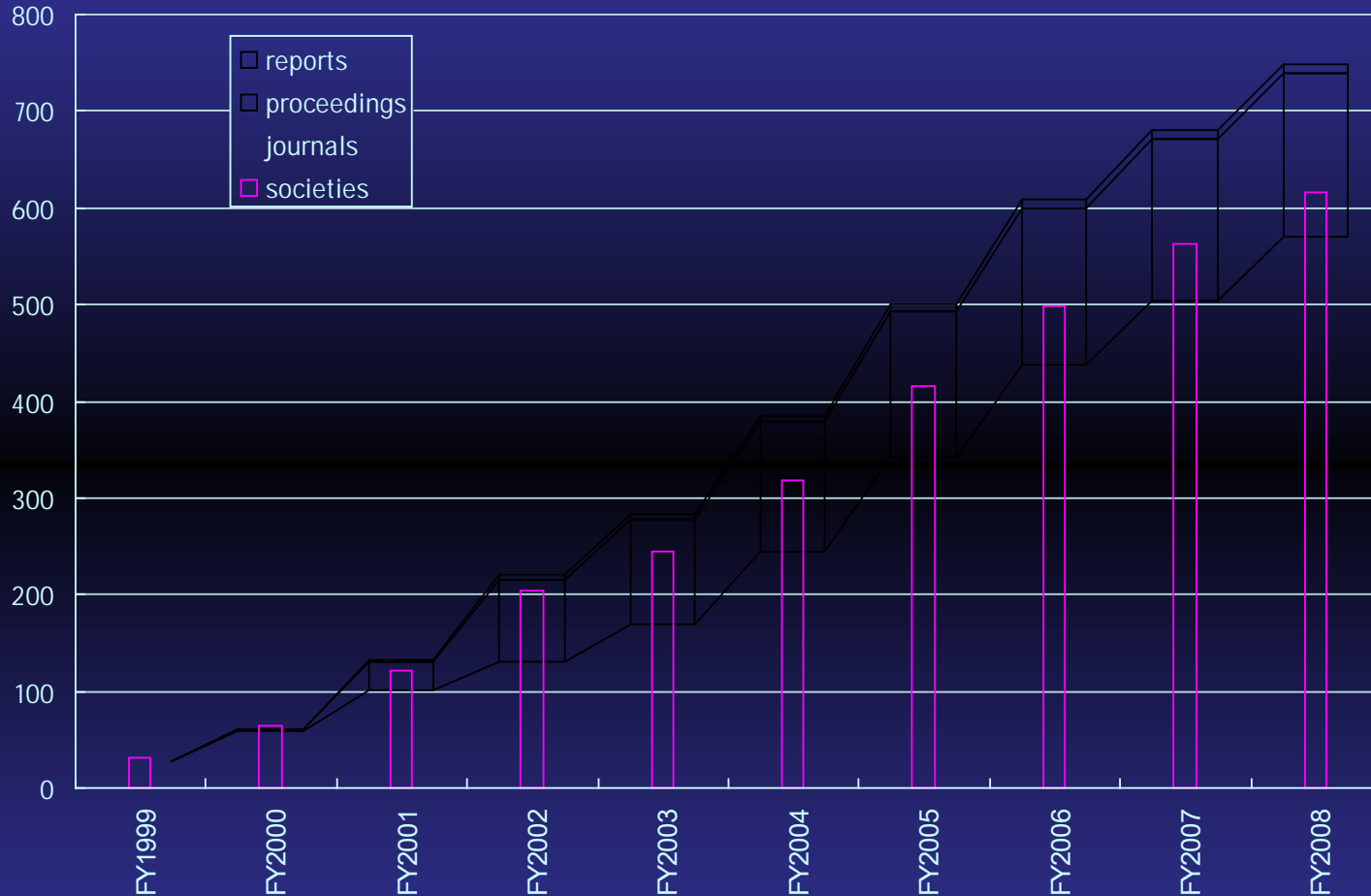
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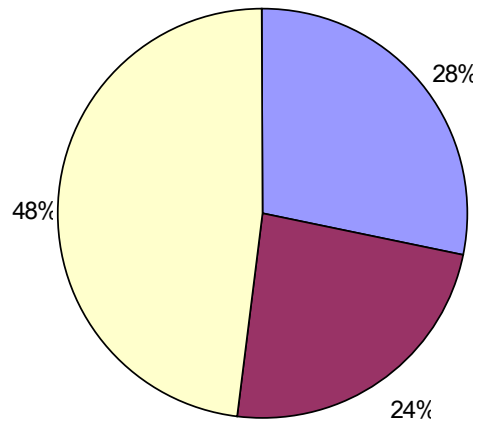
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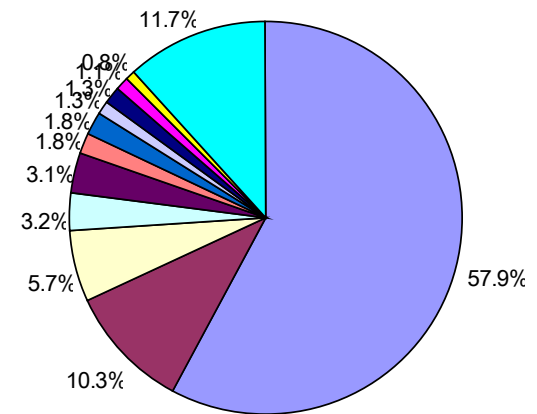
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## Determination of Hydroxylated Polycyclic Aromatic Hydrocarbons in Airborne Particulates by High-Performance Liquid Chromatography with Fluorescence Detection

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Analytical Sciences, Vol. 20 (2004) No. 1 pp.129-132

[Abstract](#)

## Determination of Phenytoin and Its Major Metabolites in Human Serum by High-Performance Liquid Chromatography with Fluorescence Detection

Shuuji HARA, Jun HAGIWARA, Misa FUKUZAWA, Nobufumi ONO and Takeshi KURODA

Analytical Sciences, Vol. 15 (1999) No. 4 pp.371-375

[Abstract](#)

## Highly Sensitive Determination of Histamine by Narrow-Bore High-Performance Liquid Chromatography Using Postcolumn Fluorescence Detection

Kazuo KURUMA and Toshiyuki SAKANO

Analytical Sciences, Vol. 15 (1999) No. 5 pp.489-492

[Abstract](#)

## Simultaneous Determination of Phenolic Xenoestrogens by Solid-Phase Extraction and High-Performance Liquid Chromatography with Fluorescence Detection

Yen SUN, Mitsuhiro WADA, Naotaka KURODA, Kuni HIRAYAMA, Hiroyuki NAKAZAWA and Kenichiro NAKASHIMA

Analytical Sciences, Vol. 17 (2001) No. 6 pp.697-702

[Abstract](#)

## Determination of Selenomethionine by High-Performance Liquid Chromatography-Fluorescence Detection Coupled with On-line Oxidation

Chiaki AOYAMA, Makoto TSUNODA and Takashi FUNATSU

Analytical Sciences, Vol. 25 (2009) No. 1 pp.63-65

[Abstract](#)

## Direct Determination of Platelet-Produced Thromboxane B<sub>2</sub> in Human Serum by Column-

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## Determination of Selenomethionine by High-Performance Liquid Chromatography-Fluorescence Detection Coupled with On-line Oxidation

Chihai KISHIMOTO, Makoto TATEYAMA,<sup>1</sup> and Takashi FURUKAWA

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 7-3-1 Hongo, Bunkyo, Tokyo 113-0032, Japan

A simple and sensitive determination method for selenomethionine (Se-Met) using an HPLC-fluorescence detection system coupled with an online chemical reactor has been developed. NBD-F (6-fluoro-1,2,3,4-tetrahydro-8-quinolinesulfonamide) was used as the fluorescence reagent for Se-Met. NBD-Se-Met was separated from NBD-Met (methionine) at 20 min after with within 30 min. Applying an optimal oxidation potential, selenomethionine fluorescence intensity of NBD-Se-Met (Se-Met) was linear in the range of 0.010–0.10 μmol with a correlation coefficient of 0.997. Detection limit (S/N = 3) was calculated as 0.01 μmol, which is comparable to that of indirectly coupled plasma mass spectrometry. This simple and sensitive method should be useful for the determination of Se-Met in physiological samples, such as serum or urine.

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### Introduction

Selenomethionine (Se-Met) is an essential trace element for human beings.<sup>1,2</sup> Data from toxicology studies and epidemiological studies have shown that Se intake is inversely related to cancer risk.<sup>3</sup> Moreover, many studies suggest that selenium supplementation reduces the incidence of prostate cancer.<sup>4,5</sup> In recent years, free selenium compounds contribute to the anticancer effect of selenium intake.

Among several chemical forms, selenomethionine (Se-Met) has been reported to be responsible for the chemoprotective property of Se.<sup>6,7</sup> Se-Met, which is biologically indistinguishable from methionine, is routinely incorporated into methionine-containing proteins in the place of methionine. Se-Met is a major natural food-form of selenium and has been reported for human dietary supplementation of Se.<sup>8</sup> Accurate determination of Se-Met is necessary for a better understanding of mechanisms governing the anticancer effect produced by selenium as well as for the monitoring of selenium intake in humans.

The determination methods for Se-Met are mainly based on HPLC coupled with indirectly coupled plasma mass spectrometry (ICP-MS) detection.<sup>9–14</sup> Although ICP-MS enables accurate determination of Se, several problems are mentioned by Francoulet et al.<sup>9</sup> First, interference by polyatomic species (e.g., <sup>76</sup>Ge<sup>+</sup>, <sup>76</sup>As<sup>+</sup>, <sup>76</sup>Se<sup>+</sup>, <sup>76</sup>Br<sup>+</sup>, <sup>76</sup>Kr<sup>+</sup>) and <sup>76</sup>Se<sup>+</sup> from the major selenium species (Se<sup>0</sup>, Se<sup>2+</sup>, Se<sup>4+</sup>, Se<sup>6+</sup>) are problematic. The second point is inappropriate use of addition experiments. For example, when either the standard or the sample component is a seleno-*l*-homocysteine, the difference chromatography method is complicated because gain is difficult. The third point is

assignment of the peaks at or near the void volume. Therefore, applications performed with only one HPLC-ICP-MS system are thought to be insufficient to confirm the identity of a component.

Consequently, it is significant to develop a determination method for Se-Met using HPLC with fluorescence detection because it provides high sensitivity and shows high selectivity in quantification. However, on-line fluorescence determination method for Se-Met and selenomethionine using a photodiode array (PDA) as a fluorescence detection system. The standard deviation limit obtained from online detection is 10 pmol for Se-Met. To improve the sensitivity, the photo-polymerized free detection by online detection system, which provides 100-fold higher sensitivity than on-line fluorescence detection.

In this paper, using 4-(2-mercaptoethyl)-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) as a fluorescence detection reagent, a sensitive determination method for Se-Met is described. Thus the detection of NBD-F from larger wavelength fluorescence (NBD-F) provides higher sensitivity than PDA in most cases.<sup>15,16</sup> Furthermore, we found out that the fluorescence intensity of NBD-Se-Met was enhanced by oxidative reaction. Utilizing an on-line chemical reactor, our method enabled highly sensitive determination of Se-Met.

### Experimental

#### Materials

Se-Met was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Egg yolk lecithin and standard solutions were obtained from Wako Pure Chemical Co. (Tokyo, Japan). Selenogen was from Sigma Chemical Co. (St. Louis, MO). Chloroform, acetic acid, and acetonitrile were from Kanto Reagent Co. (Tokyo). NBD-F

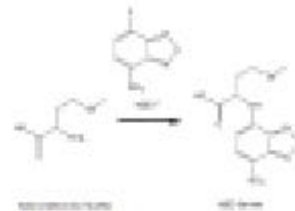


Fig. 1. Chemical reaction of selenomethionine by NBD-F.

Acetonitrile and 1-propanol of HPLC grade were from Kanto Chemical Co., Inc. Water was purified using a Milli-Q system (Millipore, Bedford, MA). All other chemicals were of reagent grade.

#### Fluorescence detection procedure

Procedure of fluorescence detection was based on our previous method.<sup>17</sup> The reaction scheme for the determination is shown in Fig. 1. To 20 μL of Se-Met solution, 20 μL of 200 mM borate buffer (pH 8.0) and 30 μL of 10 mM NBD-F in acetonitrile were added. After 3 min at 40°C, 100 μL of 0.1 M 1-propanol buffer (pH 8.0) was added to stop the reaction. Twenty microliters of the reaction solution were subjected to HPLC analysis.

#### HPLC condition for the determination of Se-Met

The separation of an L-7500 pump (Shimadzu, Tokyo) a 7.0 μm mixed sample injector (Shimadzu, Kyoto, Japan), C18 with a 30 μm sample loop, an ODS-100 μm (5 μm) column (1.5 × 10<sup>3</sup> μm, TOYO SO, Tokyo) as a separation column, a Chloasorb III ODS (Shimadzu, Chloasorb, MA) as an alternative column as F-2000 fluorescence detector (Shimadzu) and a 1.500 μm (Shimadzu). The mobile phase consisted of water-acetonitrile-1-propanol (70:30:0.02, v/v/v). Flow rate was set at 1 mL/min. Fluorescence detection was performed at 340 nm with excitation at 470 nm. A 10 pmol standard NBD-Se-Met was injected to optimize the reaction period.

### Results and Discussion

First, to find out proper conditions for the chromatographic separation of NBD-Se-Met from 20 other amino acids, we tried to separate with a mobile phase that consisted of water-acetonitrile and TEA. However, sufficient separation was not obtained in any compositions of the three solvents. Hence, we added 1-propanol in the mobile phase because we have already succeeded in simultaneous determination of 21 amino acids with 1-propanol.<sup>18</sup> As a result, the addition of 0.1 mL of 1-propanol improved the separation. Finally, a mobile phase that consisted of water-acetonitrile-1-propanol (70:30:0.02, v/v/v) was chosen with optimum sensitivity (Fig. 2).

However, the peak area of NBD-Se-Met was about 30–44% smaller when compared to other amino acids. It was likely due

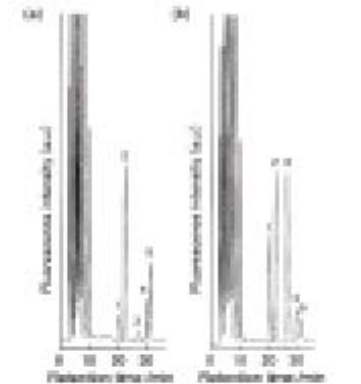


Fig. 2. Chromatograms obtained from standard solution (a) without and (b) with oxidation reagent (NBD-F) at optimized reaction period. Injected amount of NBD-Se-Met was 10 pmol for Se-Met, 100 μM for other 20 amino acids. Peaks in the chromatogram (a) NBD-Se-Met (1.500 μm) 1.500 μm, 1.500 μm, 1.500 μm, 1.500 μm.

oxidation through which the fluorescence of a selenomethionine is quenched by electron transfer.<sup>19</sup> The electron transfer from C(S<sub>2</sub>)<sub>2</sub> group of Se-Met to the excited state of fluorescently labeled amino acids causes the quenching of fluorescence. The peak area of NBD-selenomethionine, in which C(S<sub>2</sub>)<sub>2</sub> group is expected to react with other than C(S<sub>2</sub>)<sub>2</sub> group, was between the area of NBD-Se-Met and that of NBD-cysteine, whose side chain does not react with C(S<sub>2</sub>)<sub>2</sub> group.

Based on our idea mentioned above, we expected that the fluorescence intensity of NBD-Se-Met could be enhanced by chemical modification of C(S<sub>2</sub>)<sub>2</sub> group. In a previous report, adding an on-line chemical reactor resulted sensitive determination of NBD-leucine, which does not react with NBD-F by HPLC-fluorescence detection system.<sup>17</sup> The chemical modification of the methyl group of arginine, which often quenches fluorescence of this, seemed to narrow the fluorescence. Therefore, highly sensitive fluorescence detection of NBD-Se-Met should be achieved by adding a similar system.

We examined the effect of oxidative chemical modification on the fluorescence intensity of NBD-Se-Met as online oxidation experiments. As shown in Fig. 1, the maximum response of the fluorescence was obtained at 470 nm (in 10 solvent). The fluorescence intensity was 10-fold higher than without the reaction. Based on the result, we selected 470 nm as the optimum position. From the measured selenomethionine fluorescence intensity, it was determined that the content of NBD-Se-Met in one amino acid solution at 470 nm. Mixtures of 10 pmol of the chemical structures of various amino acids were shown to be oxidized by Se-Met.<sup>20</sup> In the mobile phase, NBD-Se-Met should be converted to a selenomethionine sulfone having a stable free-

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