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Technical Report

Profiling Analysis of Polysulfide Silane Coupling Agent

1. Introduction

Recently, in line with the oil-conservation movement, silica, as a non-petroleum resource, is increasingly used as a tire reinforcement filler. Fillers are typically mixed with rubber compounds used in tires to enhance reinforcement of the tire. Because silica has a hydrophilic silanol group on its surface, when added to the lipophilic elastomer rubber, it readily coheres to the rubber without actually bonding to the rubber molecule. Thus, the processibility and mechanical strength of the rubber compound is degraded, greatly affecting tire performance. For this reason, processing with a silane coupling agent is conducted to avoid this shortcoming.

Silane coupling agents contain hydrolyzable groups which react with inorganic materials, and organofunctional groups which react with organic materials. Therefore, they possess the functionality to form durable bonds between an inorganic material and an organic material. Polysulfide silane coupling agents are widely used in silica-mixed rubber compounds. The alkoxy groups at both ends of the molecule cover the surface of the silica, and the polysulfide group in the center forms a bond with the elastomer (Fig. 1). In this way, the silane coupling agent binds together the silica and elastomer to improve processibility and mechanical strength of silica-mixed rubber compounds.

Many kinds of polysulfide silane coupling agents are currently being marketed, and the silane coupling agents adopted at various tire manufacturers' development sites adhere to their own proprietary standards. Therefore, analyzing these helps to elucidate one of the characteristics associated with tire development, which is extremely important from the standpoint of tire inspection and improvement. Although it is relatively easy to analyze the differences between tires when the silane coupling agents used have completely different structures, analysis is very difficult in cases where different manufacturers are producing tires with the same chemical structure.

Assuming the analysis in the scenario above is part of a basic study, we conducted difference analysis of the principle components and sub-components in 4 different types of a commercially available polysulfide silane coupling agent, Bis (triethoxysilylpropyl) polysulfide, with the same basic skeleton from different manufacturers. The search for marker molecules that characterize the respective polysulfide silane coupling agents was conducted using Profiling

Solution and multivariate analysis software. In addition, the structure analysis of the formulas and structures of the marker molecules was conducted using a combination of LCMS-IT-TOF (high performance liquid chromatograph / ion trap time-of-flight mass spectrometer), providing MS^n analysis using accurate mass measurement, and Formula Predictor, a software program for prediction of molecular formulas.
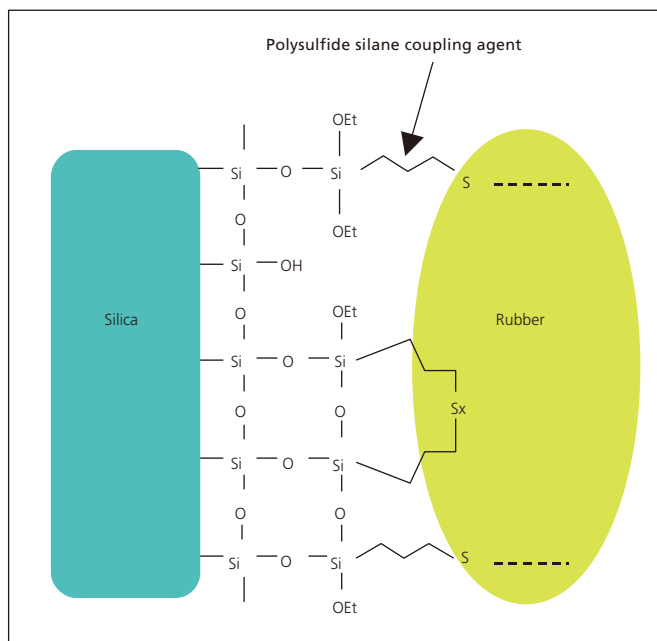

Fig. 1 Silica and Elastomer Bonds Linked by Polysulfide Silane Coupling Agent

2. Statistical Analysis Methods

2-1. Multivariate Analysis

Multivariate analysis is a statistical technique for analyzing the mutual relationship between data associated with multiple variables. Using this technique helps in the identification of a specific cause associated with a particular phenomenon, and facilitates profiling analysis to determine how to group and characterize that phenomenon.

As an application example in mass spectrometry, multivariate analysis is commonly used to compare the data acquired from multiple samples, and to identify characteristic components (marker molecules) in these samples through a "marker molecule search." It is used for analyzing proteins, lipids and other types of metabolites in the body for use in the search for specific disease markers for diagnosis, as well as the search for target molecules in drug discovery. In other fields, it is an extremely useful analytical technique for finding differences and relationships that exist among multiple samples.

2-2. Profiling Solution

Profiling Solution is used to automatically align retention time data from the high mass accuracy data acquired using Shimadzu's LCMS-IT-TOF instrument. The results are generated in the form of a tabular matrix and can be exported (Scheme 1) to third-party multivariate analysis software (e.g. SIMCA-P; Umetrics).

An example of a matrix generated using Profiling Solution is shown in Fig. 2. From left to right, the columns display data files, detected ion m/z values, their retention times, and signal intensities, respectively. Besides these, multiple TIC, extracted ion chromatograms and mass spectra can also be displayed. Thus, Profiling Solution can be used to easily conduct batch processing on multiple files, making it an indispensable tool for statistical analysis.
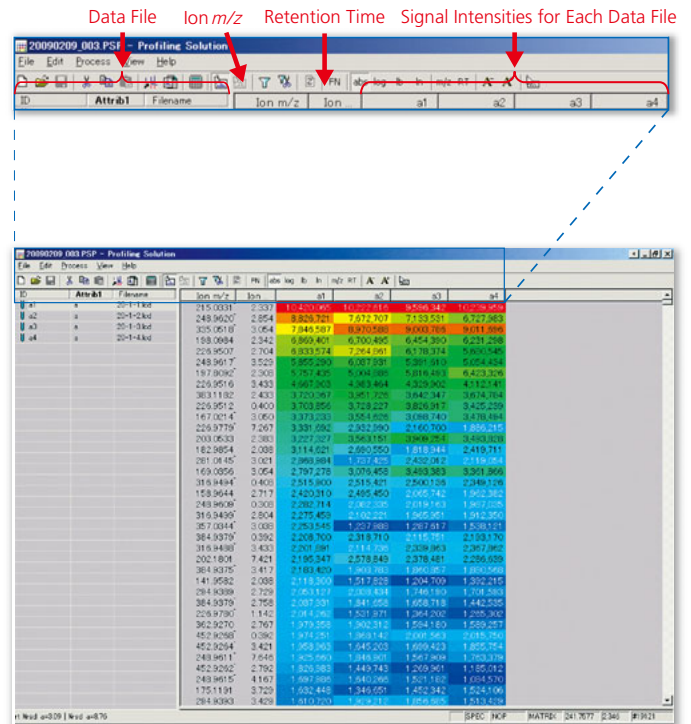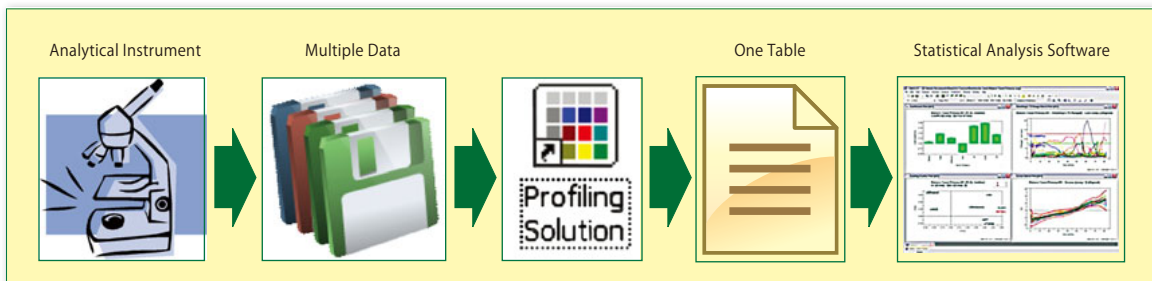

Fig. 2 Profiling Solution Window

Scheme 1 Multivariate Analysis Workflow Using Profiling Solution

3. Experimental Method

1) The analytical samples consisted of 4 types of a polysulfide silane coupling agent from different manufacturers which were diluted 1:10000 with methanol. In addition, the diluted solutions were mixed together at equal volumes to prepare a QA/QC sample to confirm system repeatability. Fig. 3 shows the structure of the principle component Bis (triethoxysilylpropyl) polysulfide (TESPP) of the polysulfide silane coupling agents.

2) LC/MS analysis was conducted using an LCMS-IT-TOF (high performance liquid chromatograph / ion trap time-of-flight mass spectrometer). A Shimadzu Shim-pack XR-ODS (75 mmL. × 2.0 mmI.D., 2.2 μm) was used. Gradient elution analysis was conducted using a 5 mmol ammonium formate aqueous solution as mobile phase A, and acetonitrile as mobile phase B. For ionization, electrospray ionization (ESI) was used, and the positive ions were measured.

3) The 4 types of polysulfide silane coupling agent samples and the QA/QC sample were each analyzed 3 times. Profiling Solution was used to conduct peak picking and retention time alignment with respect to these data, and a matrix was generated. The matrix was then exported to SIMCA-P for principle component analysis (PCA), and the differences among the various polysulfide silane coupling agent compositions were expressed visually.

4) Many of the constituents present in the polysulfide silane coupling agents that were identified in PCA were subjected to composition prediction using formula prediction software, based on information that included accurate mass, isotope pattern, and product ion information. In addition, structure prediction based on MS^n data of known substances was conducted.

4. Analytical Conditions

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Column:	Shim-pack XR-ODS (75 mmL. × 2.0 mmI.D., 2.2 μm)
Mobile phase A:	water containing 5 mmol ammonium formate
Mobile phase B:	acetonitrile
Time program:	70%B (0–0.11 min)–100%B (6.67–12 min) –70%B (12.01–15 min)
Mobile phase flow rate:	0.45 mL/min
Column temperature:	40°C
Sample injection Volume:	1 μL
Ionization mode:	ESI$^+$
Nebulizer gas flow rate:	1.5 L/min
Probe voltage:	+4.5
CDL temperature:	200°C
BH temperature:	200°C
Drying gas pressure:	0.1 MPa
Scan range:	m/z 100–1000

$C_{18}H_{42}O_6Si_2S_x$ (x=2–10)
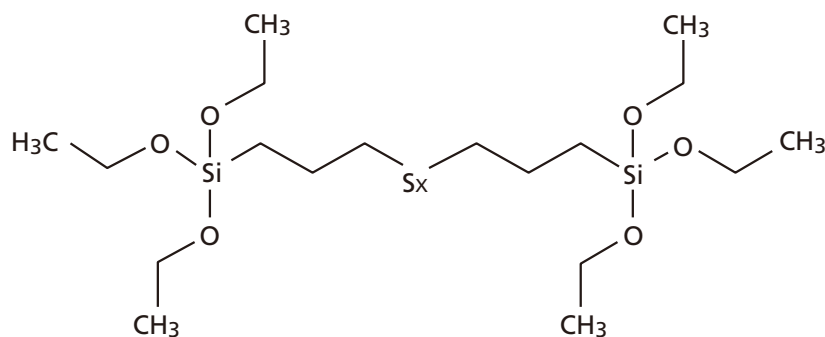
Fig. 3 Structure of TESPP

5. Results and Discussion

5-1. Results of LC/MS Analysis of Polysulfide Silane Coupling Agents

Fig. 4 shows the total ion chromatograms (TICs) of the 4 types of polysulfide silane coupling agents (Sample A, B, C and D). A complete picture of the components detected using these conditions can be seen in the TIC. In addition, comparing the TICs reveals some of the differences between the samples. Multiple peaks associated with the principle components are detected, and the distribution based on the number of S was suggested. Due to the similarity of the TICs of Sample A and Sample B among the 4 sample types, the principle components in these samples were presumed to be similar overall.

Fig. 5 shows the mass spectra of the peaks having a retention time of 6.4 min. The ion of m/z 524 was assigned to the ammonium adduct molecular ion $C_{18}H_{42}O_6Si_2S_3$, based on the accurate mass. This was presumed to be Bis (triethoxysilylpropyl) trisulfide (TESPT), one of the principle components of the polysulfide silane coupling agents, and the presumption was confirmed based on the observed product ion spectra obtained from the MS^2 and MS^3 analysis.
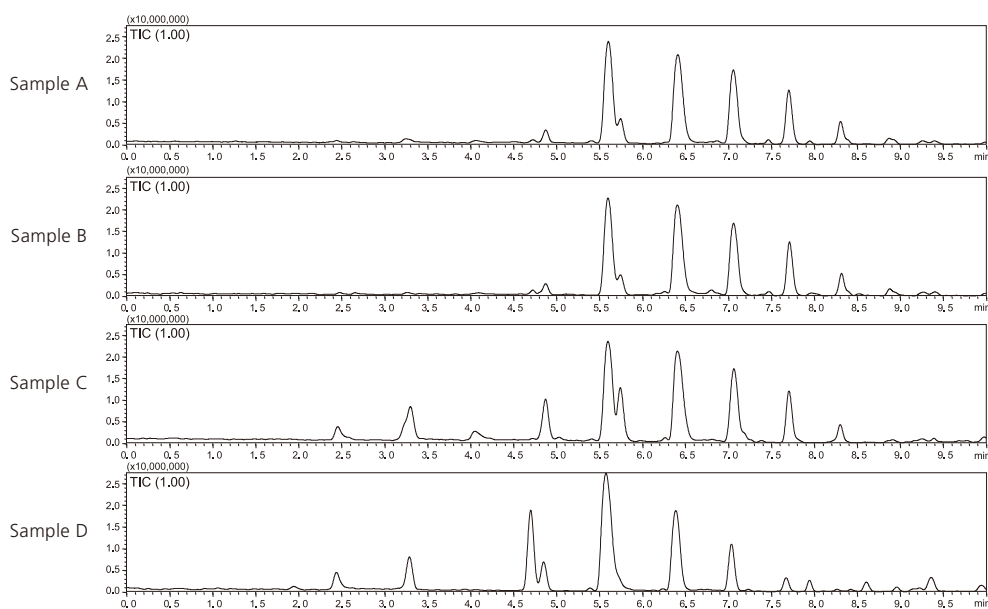

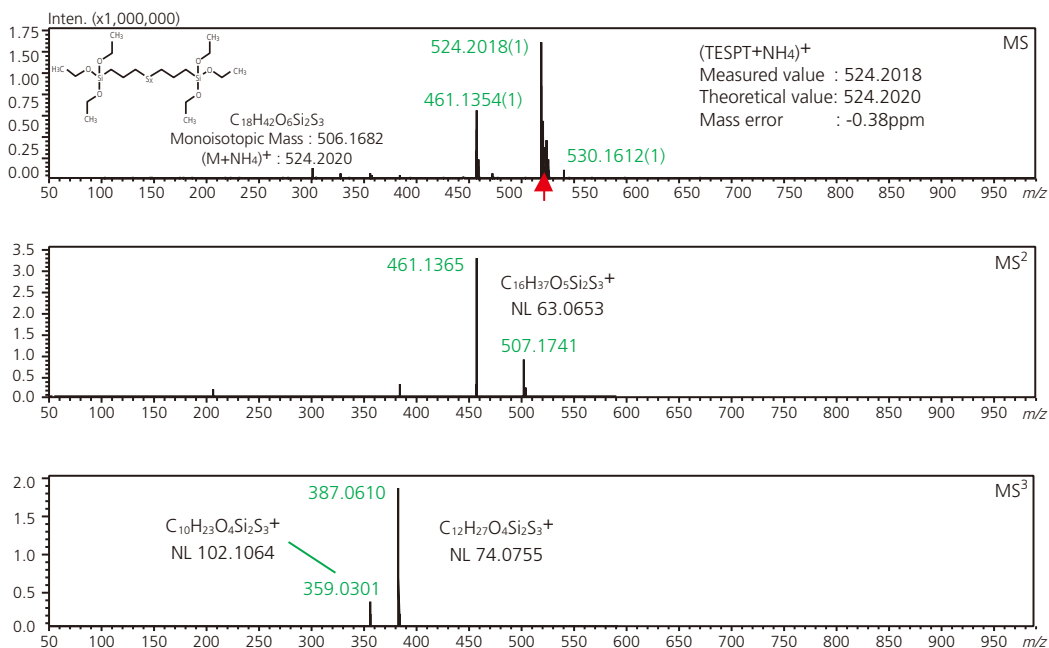Fig. 4 TICs of 4 Types of Polysulfide Silane Coupling Agents

Fig. 5 Mass Spectra of TESTP

Fig. 6 shows the structure of TESPT, in addition to the predicted cleavage positions of the product ions obtained from MS² and MS³. Prediction of the ion cleavage positions in the known substance based on its structure provides useful information for predicting the structures of unknown related substances.

Fig. 7 shows the extracted ion chromatograms of the ammonium adduct molecular ion of TESPP (x = 1 to 8). Comparing the chromatograms makes it possible to compare the x distribution in TESPP. Similar distributions are found in Samples A, B and C,

confirming that x = 2 to 8. On the other hand, the distribution in Sample D compared to that in the other 3 samples indicates a shift of x to a smaller value, indicating that x = 1. This confirmed the presence of Bis (triethoxysilylpropyl) monosulfide (TESPM) only in Sample D.

The LC/MS analysis results confirmed the similarity of component compositions of Sample A and Sample B, and that the distribution of the principle component of Sample D is different from that in the other 3 samples.
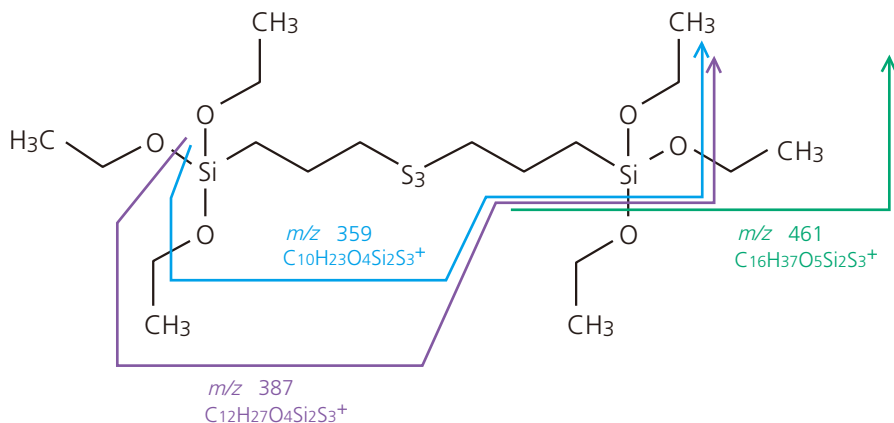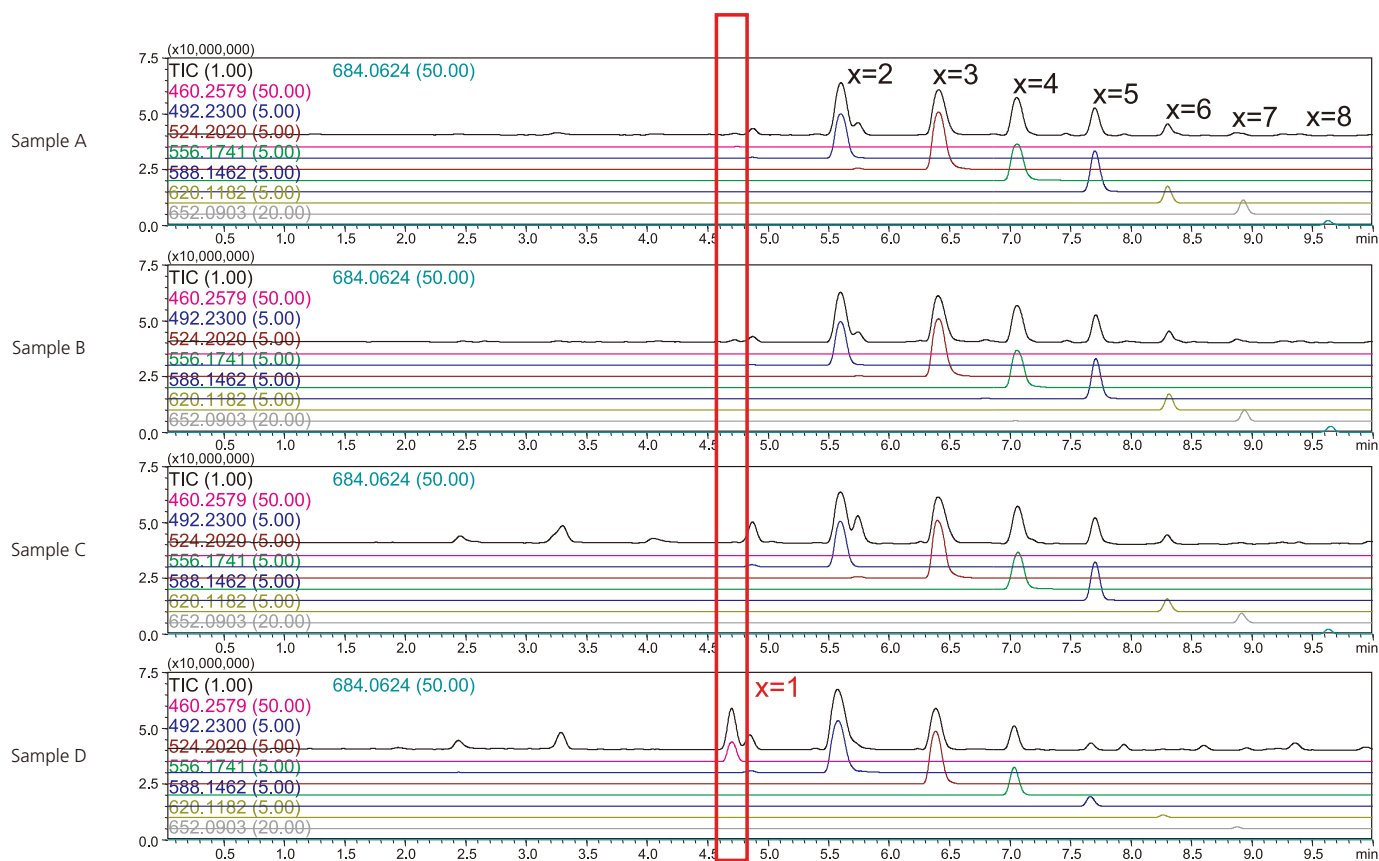

Fig. 6 Predicted Cleavage Positions of TESPT

Fig. 7 Extracted Ion Chromatograms of 4 Types of Polysulfide Silane Coupling Agents

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5-2. Principle Component Analysis (PCA)

The data obtained above were used to create a matrix after picking peaks and aligning retention times using Profiling Solution (Fig. 8). In the matrix, in addition to the retention time and *m/z* values, the signal intensities are displayed using different colors. Also, the extracted ion chromatograms of components of interest can be displayed to check the peak shape.

The matrix generated in Profiling Solution was exported to SIMCA-P, and principle component analysis (PCA) was conducted. Fig. 9 shows the PCA results. Each point in the score plot displays 1 point of analysis data, and the closer the point positions are to one another, the higher the degree of data similarity. The high similarity of Sample A and Sample B as judged from the TIC is in agreement with the high degree of similarity indicated by their close positional

distribution in the score plot (Fig. 9).

Each point in the loadings plot represents a peak in the extracted ion chromatograms, and the points that are distributed in the same direction as each of the points in the score plot are judged to have a large degree of influence on that data (indicating characteristic similarity).

In this example, the direction of distribution of Var_355, Var_395 and Var_272 in the loadings plot is the direction of distribution of Sample C in the score plot, leading to the conclusion that these peaks show the characteristics of Sample C. Similarly, it was concluded that Var_142, Var_235, Var_113 and Var_284 show the characteristics of Sample D. Fig. 9 shows the list of peaks character-istic of Sample C or Sample D from the loadings plot.
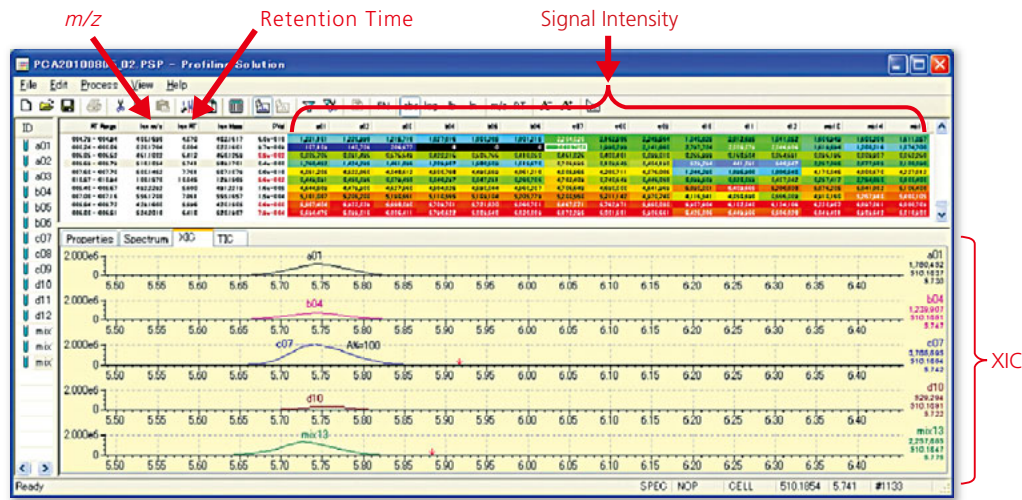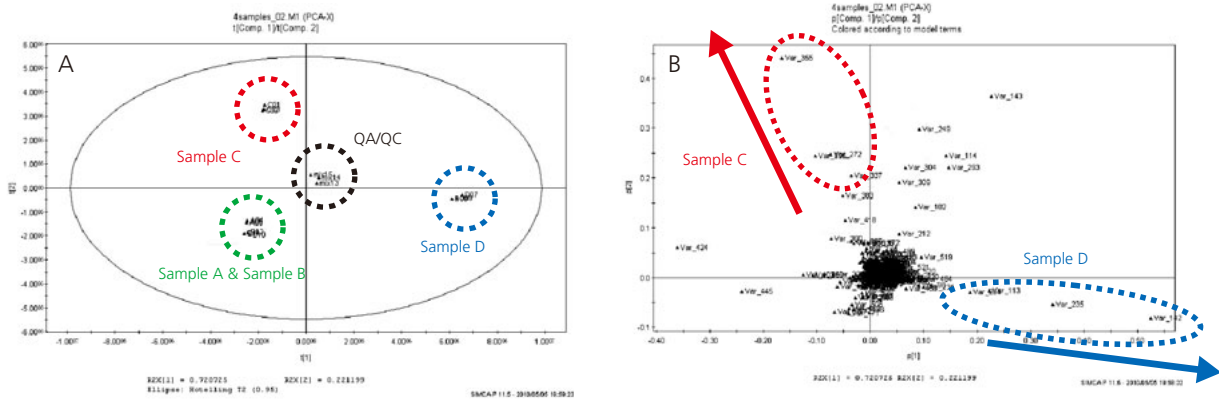

Fig. 8 Matrix Generated Using Profiling Solution

Number	*m/z*	Retention Time (min)	Sample
Var_235	397.190	4.716	Sample D
Var_113	295.084	4.717	
Var_284	465.214	4.719	
Var_142	323.115	4.720	
Var_355	510.186	4.742	Sample C
Var_272	447.118	4.745	
Var_395	542.158	4.776	

Fig. 9 Principle Component Analysis (PCA) Results for 4 Polysulfide Silane Coupling Agent Samples and List of Peaks Characteristic of the Samples
(A: Score Plot, B: Loadings Plot, C: List of Peaks Indicating Characteristic Association)

5-3. Formula and Structure Prediction

Fig. 10 shows the extracted ion chromatograms of peaks indicating characteristic associated with Sample C or Sample D. We confirmed that Component 1, derived from m/z 295, 323, 397, and 465, exists in large quantity in Sample D. On the other hand, Component 2, derived from m/z 510 and 447, and Component 3 derived from m/z 542, were confirmed to be present in large quantity in Sample C.

Fig. 11 shows the mass spectrum of Component 1. The formula prediction results indicated the assignment of m/z 465 as the sodium adduct molecular ion of Bis (triethoxysilylpropyl) monosulfide (TESPM, composition formula: $C_{18}H_{42}O_6Si_2S$), and the assignment of m/z 323.115, 397.190, and 295.084 as fragmentation ions. Fig. 12 shows the predicted cleavage positions of TESPM. This suggested that cleavage occurred in the same way as in TESPT, shown in Fig. 6.
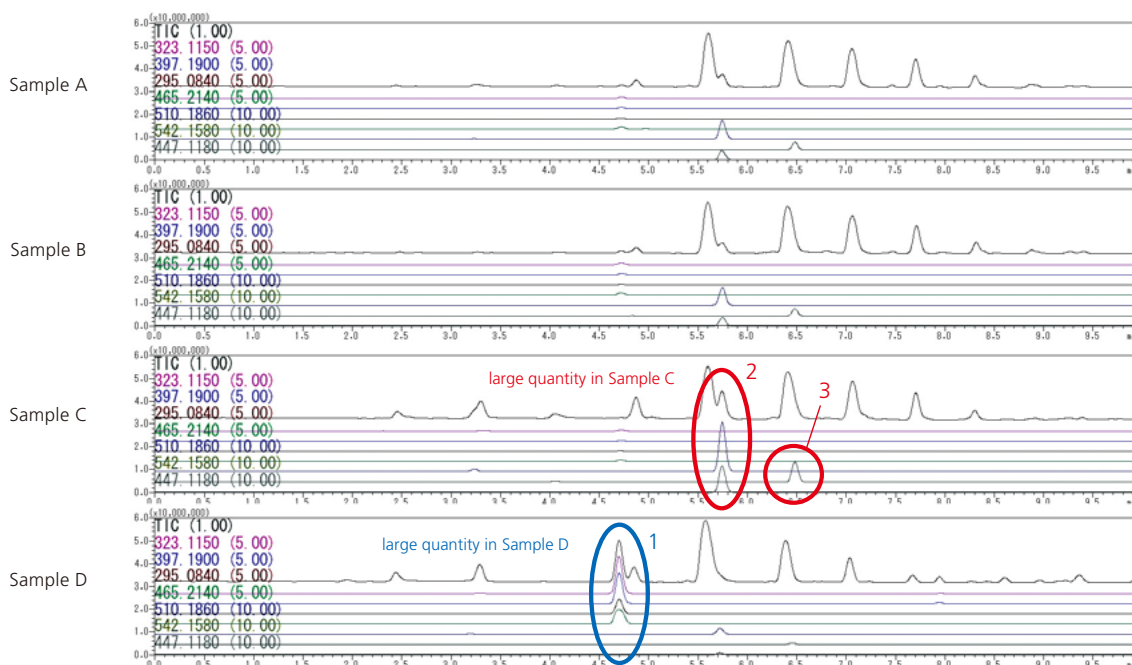

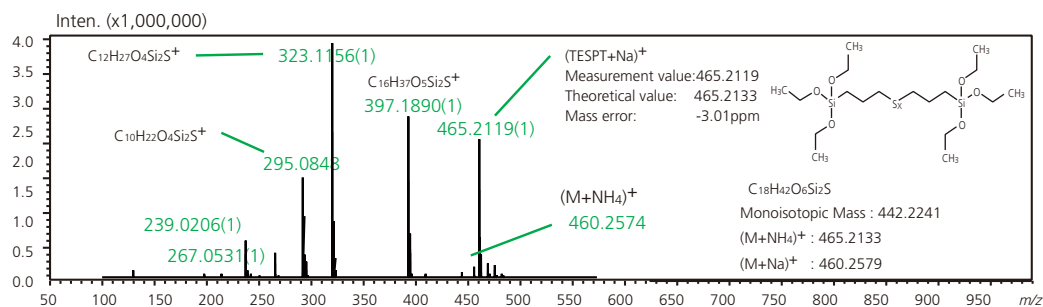**Fig. 10 Extracted Ion Chromatograms of Components Present in Large Quantity in Sample C or Sample D**
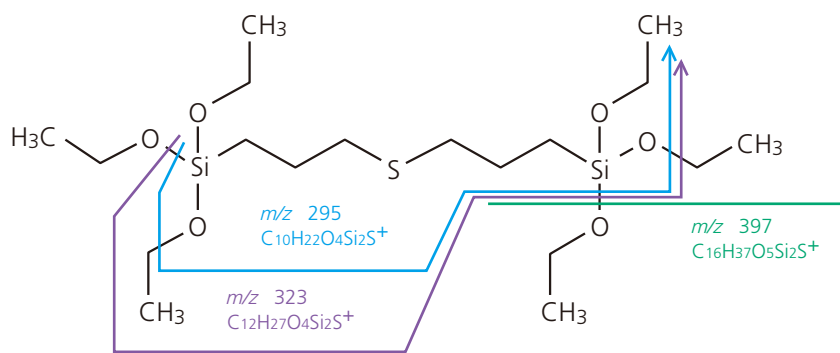

Fig. 11 Mass Spectrum of Component 1

Fig. 12 Predicted Cleavage Positions of TESPM

Fig. 13 shows the MS^n spectra of Component 2. The formula prediction results indicated the assignment of m/z 510 as the $C_{17}H_{40}O_6Si_2S_3$ ammonium adduct molecular ion, suggesting that it had a structure with 1 CH_2 fewer than TESPT. This presents candidates having 2 types of structures, that is, one with the structure where the TESPT -OCH_2CH_3 replaces -CH_3, and the other where the methylene chain $(CH_2)_3$ replaces $(CH_2)_2$. Next, shifting attention to neutral loss (NL) in MS^2, where only a neutral loss of 63 was confirmed in TESPT (Fig. 5), in Component 2, a neutral loss of 49 was observed. This corresponds to a decrease of 1 CH_2 in neutral loss as compared to TESPT. Similarly, looking at NL in MS^3, where only a loss of 74 was confirmed in TESPT (Fig. 5), in Component 2, the neutral loss of 60 was again 1 CH_2 shorter. Thus, the difference is believed to be due to the neutral loss of CH_2 in the structure. As shown in Fig. 6, by comparing the neutral losses it is proposed that the structure of Component 2 is similar to TESPT but in which -CH_2CH_3 is replaced with -OCH_3, as shown in Fig. 15.

Formula prediction was also conducted for Component 3, in which it was determined that the formula was $C_{17}H_{40}O_6Si_2S_4$. Here we presumed that the structure had 1 S more than Component 2, and even in the sub-components, it was suggested that the distributions are based on the number of S.

From the above, Components 2 and 3 are believed to be byproducts or contaminants, confirming that the degree of mixing in such components differed according to the manufacturer.
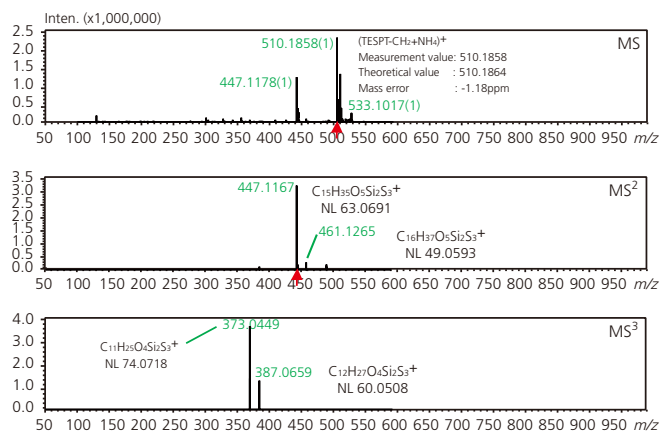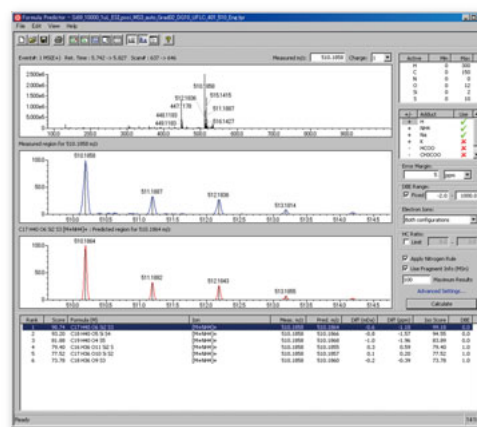

Fig. 13 MS^n Spectra of Component 2
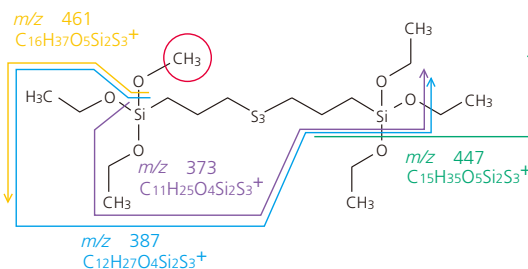

Fig. 14 Formula Prediction Results for Component 2

Fig. 15 Predicted Structure and Cleavage Positions of Component 2

6. Conclusions

1) From the TIC, it was observed that the compositions of Sample A and Sample B are similar, and the results supporting this conclusion were obtained through statistical analysis as well.

2) It was confirmed from the extracted ion chromatograms that only Sample D was present in TMSPM, and the results supporting this conclusion were obtained through statistical analysis as well.

3) Statistical analysis was used to confirm the sub-components that influence Sample C, enabling prediction of their formulas and structures, respectively. This result suggested the possibility that sub-components with distributions based on the number of S, depending on the manufacturer, were mixed in with the products, or the presence of contaminants in the products.

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