Study on the development of a rapid analytical method for inorganic elements in animal blood

（動物血液の迅速無機元素分析法の開発に関する研究）

Abstract of the thesis

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The utilization of inductively coupled plasma mass spectrometry (ICP-MS) can be obtained information on multiple elements covering the entire mass range from lithium (Li) to uranium (U) on the condition of low cost, simple operation and short analysis time, which can also be obtained information on the definite amount of inorganic elements even if small samples (<0.1mL). Consequently, these methods have been assumed one of the useful techniques for chemometric analysis requiring a large amount of measurement data. Study on the development of a rapid analytical method for inorganic element was performed using semi-quantitative analysis which is one of the measurement systems of ICP-MS.

In this study, this method was applied to plural biological samples, and the measuring data obtained from this method was analyzed, it was employed to examine whether the type of feed and the type of disease could be classified. As a definitive purpose, we would like to lead the novel development of these techniques for applications in the fields of veterinary medicine and animal husbandry.

1. The analysis of multiple elements for biological samples using semi-quantitative analysis by ICP-MS and the investigation of the applicability for chemometrics analysis.

This chapter was placed to the preliminary experiment for this study, it was performed to examine whether the biological samples could be measured by using semi-quantitative analysis by ICP-MS. Also it was performed to investigate whether the type of the feed stuff could be distinguished by the data from multiple elements in plasmas obtained from lambs, or whether the difference of depilation disease could be distinguished by the data from multiple elements in serum obtained from dogs. Multiple elements in plasma obtained from Romney lambs (*Ovis aries*), younger than 1 year old, fed by different feed were simultaneously measured by ICP-MS. It was

employed to examine whether the type of feeds could be distinguished using the multivariate data. Twenty elements in plasma obtained from lambs were analyzed by a semi-quantitative method of ICP-MS, obtained data were then analyzed by principal component analysis (PCA). As a result, the lambs were divided into three groups on a score plots depending on the different conditions, it was suggested to be distinguished the fattening conditions. Discriminant analyses of the elements were performed using linear discriminant analysis (LDA) with forward stepwise regression, the following discriminant function was made by Br and Rb. The accuracy of classification of each group, as shown by 10-fold cross-validation, proved the effectiveness of the established discriminant function.

On the other hand, it was employed to examine whether the disease groups could be distinguished using the information of multiple elements in serum obtained from depilation disease dog and normal dog. As a result, the distribution of a score plots based on PCA was not able to classify clearly to two categories depending on disease. Although the discriminant analyses of the elements were performed using LDA, and discriminant function was made by Rb and Sr, some discrimination rate was less than 90%. Because the concentrations of macroelement such as Na, K and Ca were lower than the concentrations of these elements in blood of normal mammals, it was thought to be required for the verification of accuracy and the improvement of the low result of macroelement by using semi-quantitative ICP-MS analysis. Also the differences of classification contribution by each sample were seen, it was thought that the quality of data from multivariate analysis was needed to investigate the applicability for PCA and discriminant function. In chapter 2, we were performed the verification of accuracy of multiple elements in biological samples using semi-quantitative ICP-MS analysis.

2. The verification of the accuracy for multiple elements in biological samples

using semi-quantitative analysis by ICP-MS.

The comparison of measurement data for bovine serum by semi-quantitative analysis with full-quantitative analysis that was fully utilized as traditional analysis, and the verification of analytical results using semi-quantitative ICP-MS were performed. Both semi- and full-quantitative analysis by ICP-MS was performed for bovine serum. The ratio of concentrations (%) was calculated as a comparison of each method against the other. The ratio of the concentrations from a total of thirty-seven elements ranged from 85.0% to 118%, except for K and Ca. While the analytical results obtained for K and Ca using the semi-quantitative analysis was shown low concentrations, each ratio was 65.3% and 44.0% in comparison with the ratio of the concentrations from full-quantitative analysis. The accuracy could be improved by adding both elements to the calibration standard when performing semi-quantitative analysis for K and Ca (the ratio of concentration for full-quantitative analysis, K: 106%, Ca: 95.1%). Additional recovery tests were performed to evaluate accuracy by semi-quantitative analysis for bovine serum. The results of the recovery test for K and Ca were 103 ± 5.8% and 96.6 ± 5.8%, and the results of the recovery test for multiple elements except for K and Ca ranged from 88.6% to 118%. From additional recovery tests and the comparison with full-quantitative analysis, it was shown that the measurement data obtained from bovine serum by using semi-quantitative method were accurate.

3. The classification of the fattening condition and the fattening period using the bovine serum

It was performed to investigate whether the fattening region and the feed stuff (the following; the fattening condition) could be distinguished by the data from multiple elements in serum obtained from cattle (Holsteins, 16.3-21.3 months of age) using ICP-MS method evaluated accuracy with chapter 2. Twenty-four elements in serum

obtained from cattle were analyzed by a semi-quantitative method of ICP-MS. The data were then analyzed by PCA and partial least squares discriminant analysis (PLS-DA). As a result, the cattle were divided into two groups on a score plots depending on the different conditions. Discriminant analyses of the elements were performed using LDA with forward stepwise regression, the following discriminant function was made by Br, Mo and I. This discriminant function classified the samples from each group for the fattening condition and for the fattening period by nearly 100% probability.

As second approach, to investigate whether the types of the fattening period for cattle could be distinguished using the data of elements in the serum, the samples and analytical results using in first approach were reclassified as fattening middle group (N=38) and fattening final group (N=78). PCA and PLS-DA were carried out for data obtained from cattle, the cattle were divided into two groups on a score plots depending on the fattening period though the overlap on figure was seen. Discriminant analyses of the elements were performed using LDA, the following discriminant function was made by P, Ca, Ti and Se. The discriminant function classified the bovine serum from each group by 100% probability.

In this study, same multivariate data was used for the classification of the fattening condition and the fattening period. If the condition which divide each group differed, the behavior of elemental conditions also differed among each group, it was suggested that the utilization of classification responded to each purpose was effective.

4. Selection examination of the variables (elements) in the classification for the fattening condition using a linear discriminant function

Because the development of the selection of the most suitable variables and the combination of variables were taken a large burden whenever a sample changes, it was

performed to investigate whether the combination of fixed flexible variables would be possible. When the serum of cattle groups (a total of 27 samples) was newly measured, and the measuring data to a total of three groups of a cattle group and two cattle above-mentioned groups was classified, the discriminat function using Br, Mo, Rb, Sr, I and Ba had the highest distinction accuracy, and the distinction rate was 99.3%. By the classification of biological samples such as bovine serum, it was suggested that the selection of halogen such as Br and I was a very important.

It was performed to investigate whether discriminant function using 6 elements could be used as flexible technique for all animal species, multiple elements in serum from horses (90 samples) were measured. As a result, creating the discriminant function to a total of four groups of a horse group and three cattle above-mentioned groups were classified the bovine serum from each group by 98.3% probability. Discriminat function using Br, Mo, Rb, Sr, I and Ba could be classified according to the high accuracy of cattle three groups and a horse group, it was shown that this technique was possible to flexibility use even if different animals species are classified.

In chapter 2 of this study, for semi-quantitative method that had few performed example and full-quantitative that had lots of performed example, the usefulness of measurement data obtained from semi-quantitative method can be made clear according to evaluate the accuracy in bovine serum. The research of classification and discrimination was performed easily and quickly by using this method, which is thought to be able to promote the establishment of more classification systems. Because the composition of the inorganic elements was different for different types of samples, the elements and the concentrations in the optimal calibration standard needed to change with the types of samples. Preparation of the calibration standards

that were able to ensure the accuracy of measuring elements in the different sample types was necessary.

Since macroelement in plasma and serum of animals are high concentration in vivo, and those concentrations should stay constant in metabolism in vivo, it was suggested that macroelement were difficult to become the index of discrimination. On the other hand, it is expected that the variation of trace elements takes place other factor (the fattening condition and the environment condition) rather than metabolism in vivo, trace elements was suggested to be important variable quantities for the classification of plural groups. As the reason the characteristics to be inspected of elements for animals are restricted macroelement, it was thought that the elemental concentration except for macroelement in blood is low, and the measurement sensitivity with generic method is low. The utilization of ICP-MS was taught us the fluctuation of trace elements that did not become measuring objects, and which might lead to the development of robust discriminant technique and the discovery of novel function for trace elements.

In chapter 3, two kinds of group divisions such as the fattening conditions and the fattening period were performed using multivariate data set from same serum samples. There were significant differences ($p<0.05$) in all trace elements on the fattening condition. But on the fattening period, there were no significant differences ($p<0.05$) in trace elements such as Mo and I which were selected as variables of discriminant function in fattening condition. The behavior of multiple elements in serum for the fattening condition and for the fattening period was suggested to differ completely, it was shown that the utilization of classification system responded to each purpose was possible.

In chapter 4, the discriminant function in the variables that consists of elements contained in given quantity in soil could be made clear to be utilizable also for

distinction of the fattening conditions of different animal species. The utilization of flexible valiables could be reduced the burden of the development every samples or every animal species, and it was suggested to be rapidly enabled the introduction of inspection technique.

A rapid analytical method for inorganic element using semi-quantitative analysis data can be used in studies of pet animals and domestic animals, and a novel disease diagnosis and a method for distinguishing domestic animals fed special feeds can be developed. As a definitive purpose, we would like to lead the novel development of these techniques for applications in the fields of veterinary medicine and animal husbandry.