



Principal Component and Distinction Analyses by Components in Nutritional Drink

In the analysis by fluorescence fingerprinting, the autofluorescence (fluorescence fingerprint) characteristic to a component can be obtained and the multivariate analysis of multiple characteristic fluorescence intensities can be performed. Therefore, it is expected that the analysis can be applied to the "product classification" and "classification of non-defective and defective items."

This time, PARAFAC¹⁾ analysis was used for commercially available nutritional drinks, and the contained components were presumed and the classification of the samples based on the contained ingredients was performed. The analysis examples are introduced here.

For the measurement, F-7100 installed with the microplate accessory, on which multiple samples can be loaded, and the automatic filter accessory, which allows the elimination of higher order lights from the scattered light, was used.



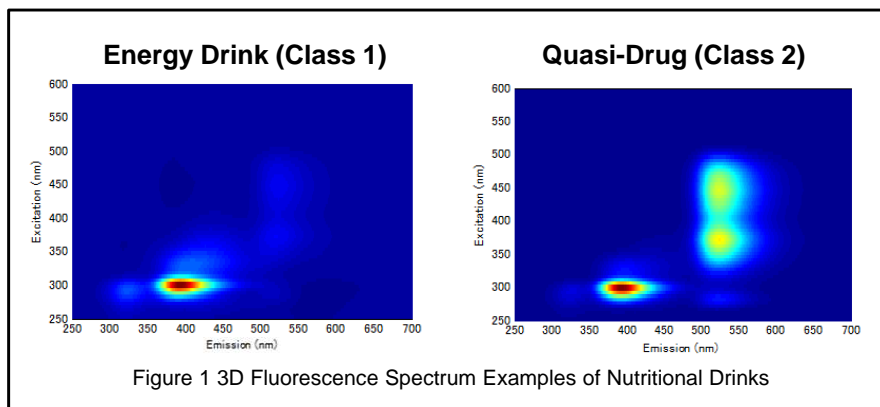
F-7100 Fluorescence Spectrophotometer

¹⁾ PARAFAC: Parallel Factor Analysis, Solo[®] 8.1.1 (Eigenvector Research, Inc., USA) was used.

Presumption of Components in Nutritional Drink by PARAFAC Analysis

- ✓ The fluorescence intensity was standardized (FL No.150002) with 100 µg/L of Quinine Sulphate (QS).
- ✓ Nutritional drinks are mainly classified into drugs, quasi-drugs, and energy drinks (soft drinks). This time, one each of quasi-drug and energy drink samples was analyzed and the 3D fluorescence spectra were obtained (Figure 1). Then, 3D peaks were separated by PARAFAC analysis and the contained components were presumed based on the past report examples.

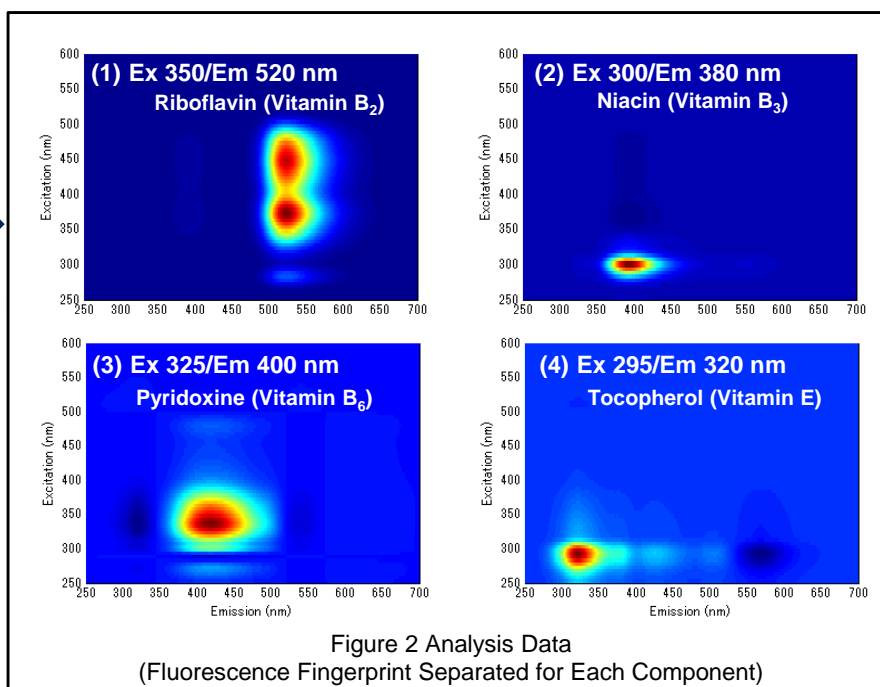
3D Fluorescence Spectra of Nutritional Drinks and PARAFAC Analysis Results



Analytical Conditions

Instrument:
 F-7100 Fluorescence Spectrophotometer
 Slit on excitation side: 5 nm
 Slit on fluorescence side: 5 nm
 Scan speed: 30,000 nm/min
 Response: Automatic
 Photomultiplier vol.: 500V
 Spectral correction: ON

PARAFAC Analysis



PARAFAC analysis indicated that at least four components are contained. Based on the examples of the excitation and fluorescence wavelengths of each component reported in the past, the components were estimated as below.

- (1) Riboflavin (Vitamin B₂)
- (2) Niacin (Vitamin B₃)
- (3) Pyridoxine (Vitamin B₆)
- (4) Tocopherol (Vitamin E)



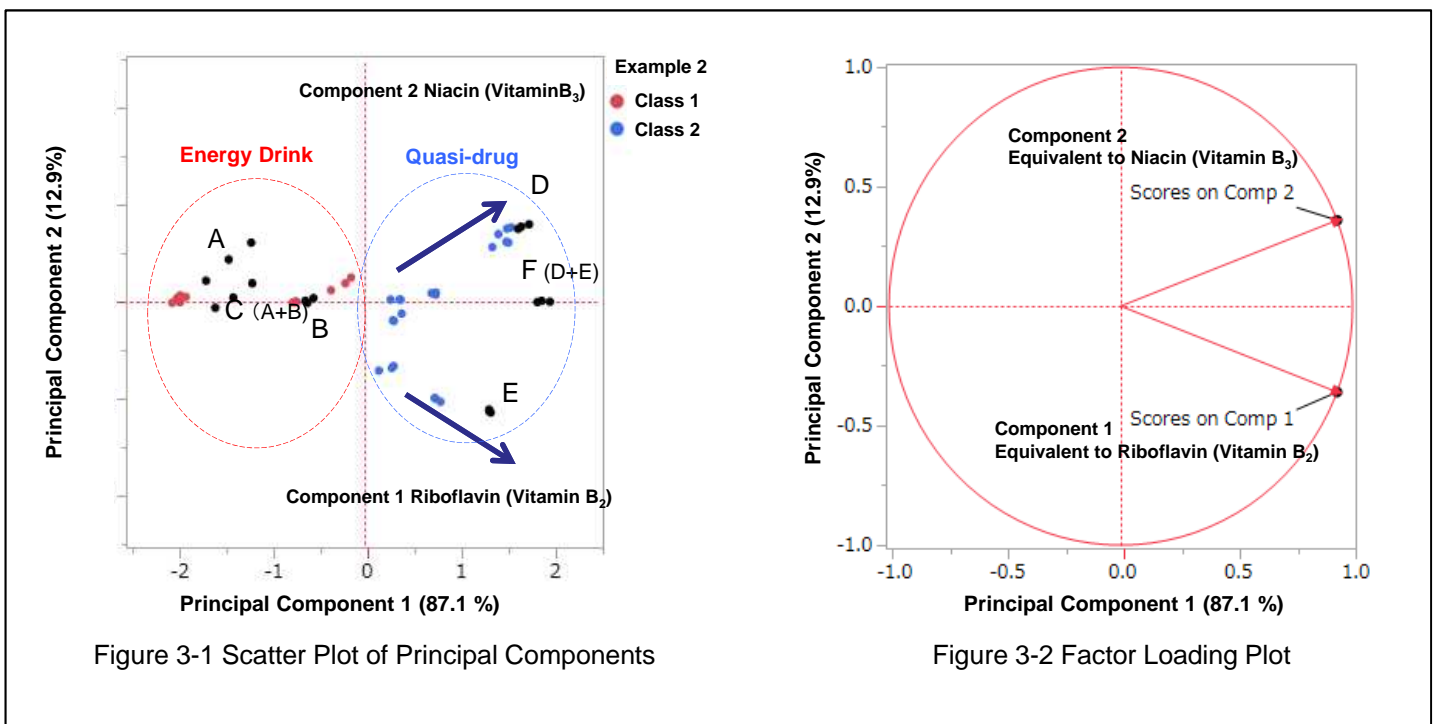
Principal Component Analysis for Components in Nutritional Drink

- ✓ In addition to the above PARAFAC analysis, the 3D fluorescence spectrum was measured three times for each of commercially available nutritional drinks (11 kinds) so as to presume the contained ingredients. The principal component analysis, which is the main multivariate analysis method²⁾, and distinction analysis³⁾ were performed for the presumed ingredients, (1) Riboflavin (vitamin B₂) and (2) Niacin (Vitamin B₃). JMP®, multivariate analysis software, was used for the analysis.
- ✓ Nutritional drinks (A, B) classified as energy drinks (Class 1) and the sample (C) prepared by mixing A and B at 1:1 were analyzed. Nutritional drinks (D, E) classified as quasi-drugs (Class 2) and the sample (F) prepared by mixing D and E at 1:1 were also analyzed.

2) JMP®12.2(SAS Institute Inc., Cary, NC, USA)

3) Principal component analysis: The method to contract the multivariate data into low-dimensional data.
Distinction analysis: The method to analyze unknown samples by using the classification of known components.

Principal Component Analysis



- ✓ Based on the principal component analysis, it was confirmed that each sample is classified as an energy drink or a quasi-drug. By reviewing the factor loading plot (Figure 3-2) showing the ratio of the effects by the Components 1 and 2 as vectors, it is observed that the concentrations of those components increased in the direction of the vector in Figure 3-1.
- ✓ A, B, and C, analyzed as the samples were classified into Class 1 while D, E, and F were classified into Class 2. The results indicated that the products were appropriately classified as energy drinks or quasi-drugs.

[Accessory Used for Analysis]

Microplate Accessory
(5J0-0139)



Automatic Filter
Accessory (5J0-0158)

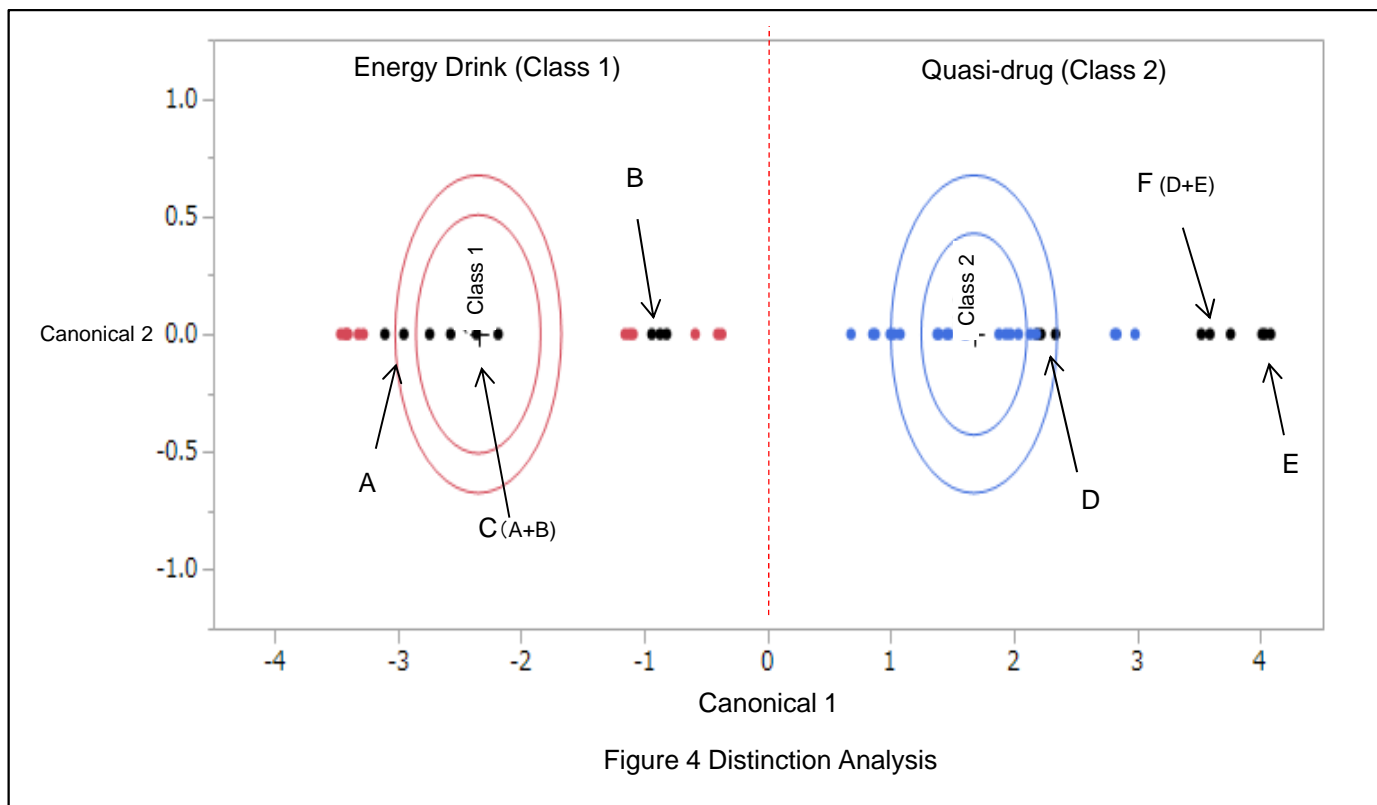




Classification by Distinction Analysis of Components in Nutritional Drink

- ✓ After the principal component analysis, a distinction analysis was performed. JMP3®, the same multivariate analysis software used for the principal component analysis, was used for the analysis.
- ✓ The samples were, as they were for the principal component analysis, nutritional drinks (A, B) classified as energy drinks (Class 1) and the 1:1 mixture of A and B (C), and the nutritional drinks (D, E) classified as quasi-drugs (Class 2) and the 1:1 mixture of D and E (F).

Distinction Analysis



- ✓ The results of the distinction analysis indicated that the commercially available nutritional drinks, based on the canonical 1 of the horizontal axis, were distinguished as energy drinks (Class 1) or quasi-drugs (Class 2). The nutritional drinks A, B, and C were classified as energy drinks (Class 1) while D, E, and F, were classified as quasi-drugs (Class 2). It was confirmed that these samples were classified as energy drinks or quasi-drugs as presumed.

Note 1: These data are an example of measurement; the individual values cannot be guaranteed.

Note 2: Nutritional drinks were classified as either OTC drugs or quasi-drugs, depending on the contained components, by the Pharmaceuticals and Medical Devices Act. This is the evaluation method for the purposes of research and development and process control, therefore, the classification is different from what is described in the Pharmaceuticals and Medical Devices Act.

[KEY WORDS]

Bio/Medical Science/Food/Pharmaceutical, Food, Fluorescence Spectrophotometer, F-7000, F-7100, F-2700, Quinine Sulfate, FL Intensity Standardization, PARAFAC, Multivariate Analysis, Fluorescence Fingerprint, Quinine Sulfate Dihydrate