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Application News

High Performance Liquid Chromatograph Mass Spectrometer
LCMS-2050 and LCMS-9030

Rapid Screening of Herbal Medicine Using Single Quadrupole LC-MS

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User Benefits

- Rapid screening of herbal medicine can be conducted using single quadrupole LC-MS. Direct injection analysis without separation column enables high-throughput analysis of less than one minute.
- Data analysis of the obtained mass spectrum with eMSTAT Solution™ allows easy classification and characterization of herbal medicine samples, as well as discriminant analysis to determine the group in which an unknown sample should be categorized.

■ Introduction

It is known that the medicinal properties of crude drugs, which are natural products, vary depending on their growing environment, such as the place of origin and weather, and the time of harvest. Therefore, an equivalence evaluation of a new crude drug with a present crude drug is important. Since crude drugs are composed of hundreds to thousands of components, a multifaceted component analysis is required in the chemical evaluation. In recent years, the chemical evaluation of crude drugs has also been conducted using metabolomics technology, which is a comprehensive analysis of metabolites.

In this Application News, we will present a case study of screening of Kampo medicine, so called herbal medicine, using flow injection analysis (FIA) and single quadrupole LC-MS. Although Kampo medicine is manufactured by blending multiple crude drags and has complex matrix, it can be subjected to simple screening analysis for classification and characterization by using a novel method described here.

■ Sample and pretreatment

Six commercially available Kakkonto extract granules were analyzed. 15 mL of 50% methanol aqueous solution was added to 1.5 g of Kakkonto extract granules and stirred for 15 minutes. Ultrasonic extraction was then performed for 15 minutes, and the supernatant was collected by centrifugation (12,000 rpm, 20 minutes). The supernatant was diluted 10-fold with a 50% methanol aqueous solution containing internal standards (5 ppm reserpine, 20 ppm chloramphenicol) and used as the sample for measurement.

■ Instrumentation and analytical conditions

Fig.1 shows the combination setup of Nexera™ series HPLC and LCMS-2050, a single quadrupole LC-MS. It is compact, easy to use, and provides excellent performance. FIA, in which sample is injected directly into the mass spectrometer without analytical column, generally contaminates mass spectrometer frequently, but LCMS-2050 can be applied to FIA because of its robustness and easy maintenance feature in case of contamination recovery. Table 1 shows the analytical conditions for rapid screening.

Fig.1 Nexera™/ LCMS-2050 Setup

Table 1 Analytical Conditions for Rapid Screening

[Flow Injection Conditions] (Nexera XR)		
Flow Rate	:	0.1 mL/min (0 min)→0.05 mL/min (0.1 min) →0.1 mL/min (0.65 min) →1 mL/min (1 min)
Mobile Phase	:	Water/Methanol=50/50
Injection Volume	:	1 µL
[MS Conditions] (LCMS-2050)		
Ionization	:	ESI/APCI (DUIS™), Positive and Negative Mode
Mode	:	Scan (*m/z* 50-2000)
Interface Voltage	:	+3.0 kV / -2.0 kV
Nebulizing Gas Flow	:	2.0 L/min
Drying Gas Flow	:	5.0 L/min
Heating Gas Flow	:	7.0 L/min
Desolvation Temp.	:	450˚C
DL Temp.	:	200˚C

The combination setup of Nexera series HPLC and LCMS-9030, a quadrupole time-of-flight (QTOF) LC-MS, shown in Fig. 2 was employed for detected peak identification. Table 2 shows the analytical conditions for peak identification.

Fig. 2 Nexera/ LCMS-9030 Setup

Table 2 Analytical Conditions for Peak Identification

[Flow Injection Conditions] (Nexera XR)		
Flow Rate	:	0.1 mL/min
Mobile Phase	:	Water/Methanol=50/50
Injection Volume	:	1 µL
[MS Conditions] (LCMS-9030)		
Ionization	:	ESI, Positive or Negative Mode
Mode	:	MS and MS/MS
Nebulizing Gas Flow	:	3.0 L/min
Drying Gas Flow	:	10.0 L/min
Heating Gas Flow	:	10.0 L/min
Interface Temp.	:	300˚C
DL Temp.	:	250˚C
HB Temp.	:	400˚C

■ Data analysis

The obtained mass spectral data were converted to JCAMP format using LabSolutions™ LCMS and analyzed by eMSTAT Solution, which is equipped with a statistical analysis mode and a discriminant analysis mode, allowing even those that are not so familiar with statistical procedures to easily perform everything from statistical analysis to discriminant analysis (Fig. 3). For each sample, four consecutive analyses were performed to create a data set for multivariate analysis. Data obtained in positive mode were corrected by reserpine, and data obtained in negative mode were corrected by chloramphenicol.

■ Rapid Screening

Scan analysis of the five Kakkonto extracts A-E revealed 91 peaks in the positive mode and 199 peaks in the negative mode. The results of principal component analysis (PCA) of the data obtained in positive mode are shown in Fig. 4. Kakkonto B and E were plotted in proximity, indicating similar characteristics. Kakkonto A was found to have significantly different characteristics compared to the other Kakkonto samples. Fig. 5 shows the results of PCA of the negative mode data. Kakkonto A and C were plotted in proximity, indicating that they had similar characteristics.

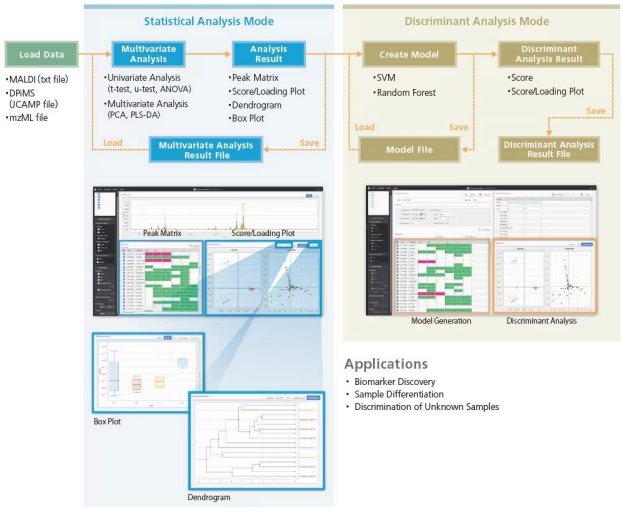


Fig. 3 Workflow on eMSTAT Solution™

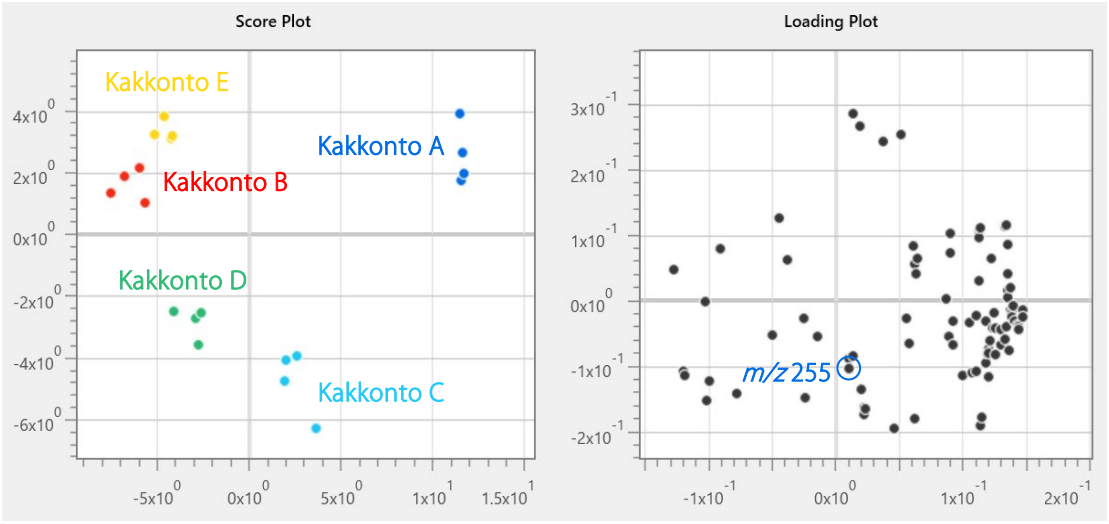


Fig. 4 Results of PCA (Positive Mode)

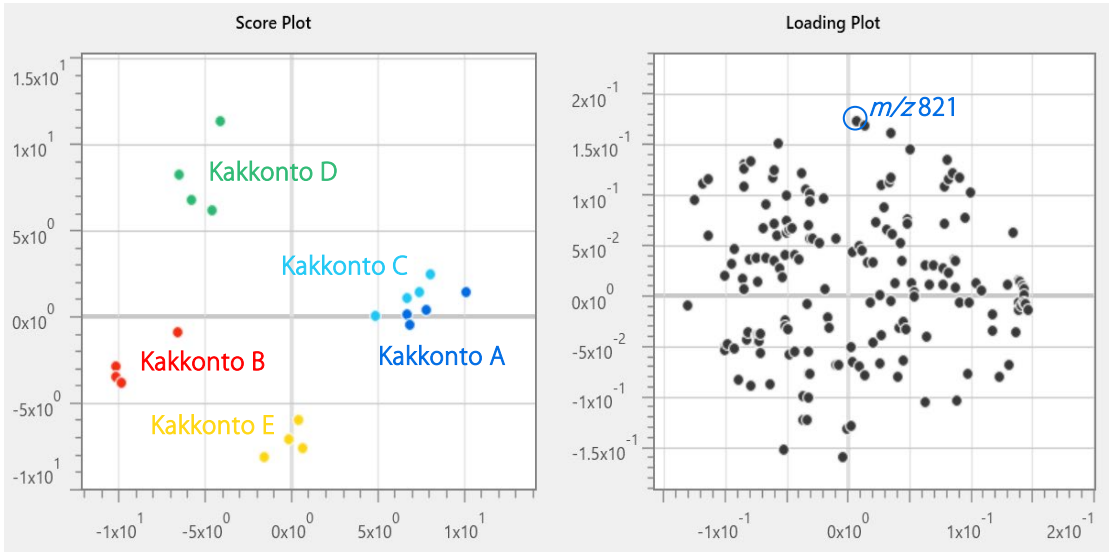


Fig. 5 Results of PCA (Negative Mode)

Kakkonto B and E, which showed similar characteristics in the positive mode PCA, were grouped into one and subjected to PCA again. Based on the results, a discriminant model (algorithm: RandomForest) was created to determine which present Kakkonto-type the unknown Kakkonto belongs to. As a result of discriminant analysis of the separately analyzed Kakkonto F using this discriminant model (Fig. 6), Kakkonto F was plotted in proximity to Kakkonto B and E and was determined to be the type similar to Kakkonto B and E (average score: 88.5).

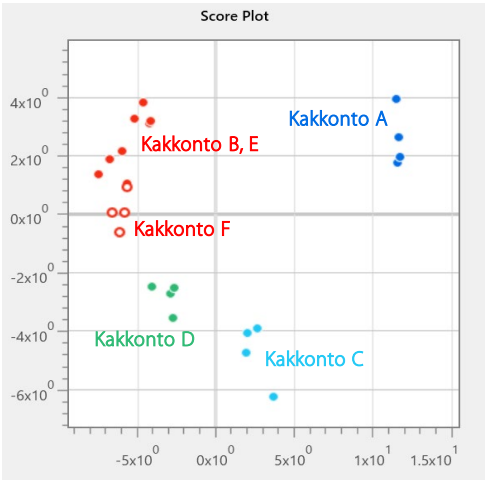


Fig. 6 Results of Discriminant Analysis

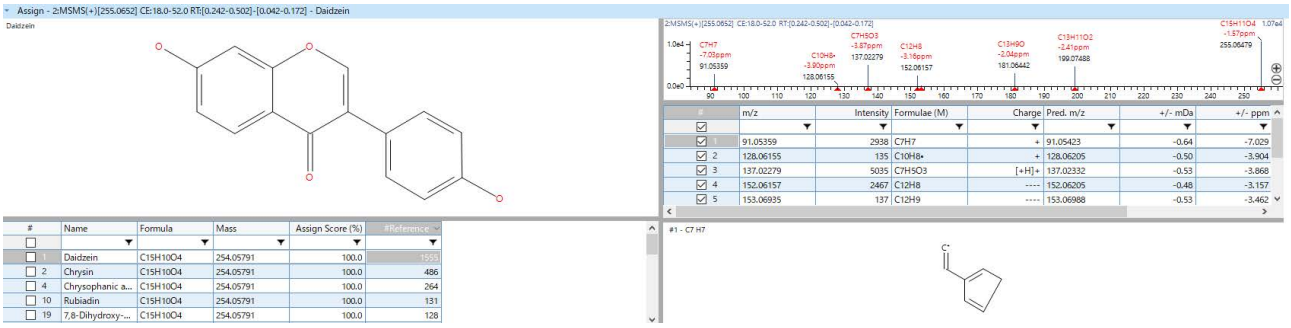
■ Peak Identification by QTOF LC-MS

Among the peaks detected by single quadrupole LC-MS, some peaks were thought to be active componets in Kakkonto. To identify these peaks with high accuracy, MS and MS/MS analyses were performed using QTOF LC-MS. The obtained data were analyzed with the analysis software LabSolutions Insight Explore™, and MS/MS spectral information was searched online based on the ChemSpider database. As a result, as shown in Fig. 7, the peaks at *m/z* 255 and *m/z* 821 detected in positive and negative mode respectively were estimated to be daidzein and glycyrrhizin, for example. The difference between the theoretical *m/z* and measured *m/z* was less than 1 mDa and was able to be estimated with high accuracy.

■ Conclusion

Using Kakkonto extract granules as samples, we introduced a simple screening analysis of Chinese herbal medicine (Kampo medicine). Using FIA and single quadrupole LC-MS, 290 peaks including active medicinal components were detected in the samples, which were able to be classified and characterized by PCA. The results were also used for discriminant analysis to determine which present Kakkonto-type the unknown Kakkonto was in proximity to. This method provides improved efficiency to screening analyses for a large number of Kampo medicines and crude drugs owning to the high throughput performance of one minute analysis time.

・Identification Result of Peak at *m/z* 255 (+)



・Identification Result of Peak at *m/z* 821 (-)

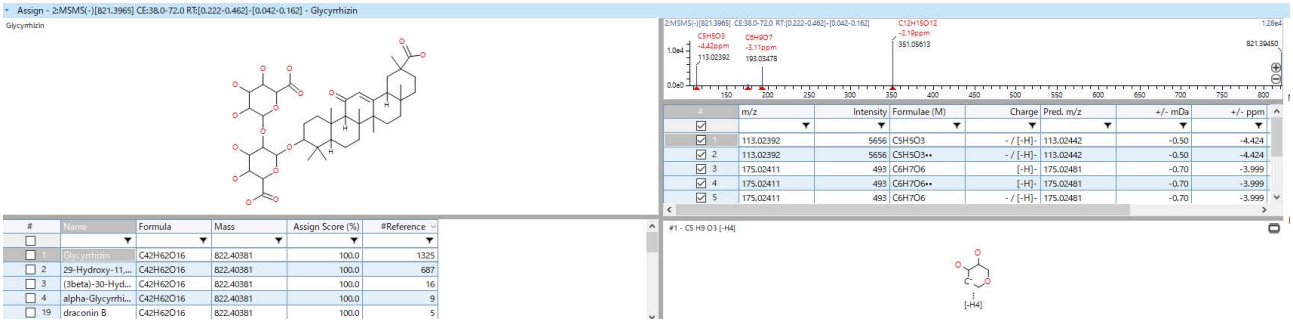


Fig. 7 Identification Results using QTOF LC-MS

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