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Application News

CLAM™-2030 Fully Automated Sample Preparation Module for LCMS
LCMS™-8060 Liquid Chromatograph Mass Spectrometer

Measuring Primary Metabolites in Human Plasma Using an LC/MS/MS System with Fully Automated Sample Preparation Module

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User Benefits

- Capable of comprehensive quantitative analysis of primary metabolites in plasma
- Performs time-consuming sample preparation automatically.
- Eliminates the need for manual sample preparation and reduces variability in quantitative results due to manual operations.

■ Introduction

Metabolomics analysis, a technique for comprehensive analysis of large numbers of metabolites, is used in a wide range of fields and applications, including functional analysis of food, improving fermentation productivity, and elucidating physiological and pathological mechanisms. Liquid chromatograph mass spectrometers and other mass spectrometer systems are used to perform metabolomics analysis. In recent years, metabolomics analysis is increasingly being used in clinical research, such as to search for disease markers and markers that predict the efficacy and toxicity of drugs. However, sample preparation for metabolomics analysis and the operation of mass spectrometers is much more complex than for general laboratory testing. As a result, there is a risk of procedure errors and variability due to operator differences. In addition, operator workload increases as the number of samples increases, and sample preparation can become a bottleneck in the analytical workflow when analyzing a large number of samples.

A normal sample preparation protocol for primary metabolite analysis performed on an LC/MS/MS system with fully automated sample preparation module involves steps such as adding organic solvent for deproteination, removing solid components by centrifugation, and recovering supernatant. This article introduces an example of using an LC/MS/MS system with fully automated sample preparation module, comprised of a CLAM-2030 fully automated sample preparation module and LCMS-8060 liquid chromatograph mass spectrometer (Fig. 1), to perform an analysis and resolve the issue of sample preparation encountered when metabolomics analysis is applied to clinical research.

Fig. 1 LC/MS/MS System with Fully Automated Sample Preparation Module (CLAM™-2030 + LCMS™-8060)

■ Workflow for Simultaneous Analysis of Primary Metabolites on an LC/MS/MS System with Fully Automated Sample Preparation Module

Blood collection tubes need only be set in the system since the sample preparation steps are performed automatically by the CLAM-2030. After sample preparation, samples are then automatically transferred to the auto-sampler for analysis by the LC/MS/MS system. Fig. 2 shows the sample pretreatment protocol and analysis workflow, including analysis by the LC/MS/MS system. LC conditions, MS conditions, and parameter settings for MRM transitions all follow LC/MS/MS Method Package for Primary Metabolites Ver. 2 (Table 1). Analytes were set by modifying part of this Method Package.

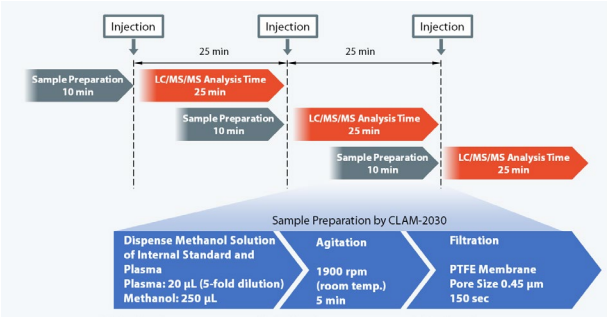

Fig. 2 Pretreatment of Plasma Samples by CLAM-2030

Table 1 LC and MS Analytical Conditions

Liquid Chromatograph	
System:	Nexera™ X2
Column:	Reversed-phase column
Mode:	Gradient elution
Injection Volume:	2 µL
Mobile Phase A:	0.1 % formic acid in Water
Mobile Phase B:	0.1 % formic acid in Acetonitrile
Flowrate:	0.25 mL/min
Mass Spectrometer	
System:	LCMS-8060
Ionization:	ESI (Positive/Negative)
Nebulizing Gas:	3 L/min
Drying Gas:	10 L/min
Heating Gas:	10 L/min
DL Temp.:	250 °C
Heat Block Temp.:	400 °C
Interface Temp.:	300 °C

■ Continuous Analysis of Clinical Samples and QC Samples Using an LC/MS/MS System with Fully Automated Sample Preparation Module

Ten clinical samples were analyzed each day for 22 days (220 samples in total) and commercial human plasma was used as a QC sample. The QC sample was analyzed first, followed by the 10 clinical samples. D3-creatine was used as an internal standard. Table 2 lists the primary metabolites detected in the QC sample using the LC/MS/MS system with fully automated sample preparation. A total of 55 components were detected, mainly amino acids, organic acids, and nucleosides included in LC/MS/MS Method Package for Primary Metabolites Ver. 2.

The peak area %RSD was calculated for each primary metabolite detected when 22 QC samples were measured. The relative peak area %RSD between each primary metabolite and D3-creatine was also calculated, and histograms were drawn showing the data range and frequency of the peak area %RSD and the relative peak area %RSD (Fig. 3). The peak area %RSD was 10 % or lower for 33 of 55 components (60 %), and 20 % or lower for 51 of 55 components (93 %). By contrast, when adjusted based on the D3-creatine internal standard, the relative peak area %RSD was 10 % or lower for 37 of 55 components (67 %), and 20 % or lower for 52 of 55 components (95 %).

Table 2 Primary Metabolites Detected in QC Sample

Compound Name	Compound Type	Compound Name	Compound Type
2-Aminobutyric acid	Amino acid	Valine	Amino acid
4-Aminobutyric acid	Amino acid	Carnitine	Amino acid derivative
4-Hydroxyproline	Amino acid	Creatinine	Amino acid derivative
Alanine	Amino acid	Kynurenine	Amino acid derivative
Arginine	Amino acid	S-Adenosylhomocysteine	Amino acid derivative
Asparagine	Amino acid	Adenine	Base
Aspartic acid	Amino acid	Choline	Choline
Asymmetric dimethylarginine	Amino acid	Acetylcarnitine	Lipid
Citrulline	Amino acid	Inosine	Nucleoside
Cystathionine	Amino acid	Uridine	Nucleoside
Cysteine	Amino acid	Adenosine 3', 5'-cyclic monophosphate	Nucleoside
Glutamic acid	Amino acid	Adenosine monophosphate	Nucleoside
Glutamine	Amino acid	Niacinamide	Vitamin
Glycine	Amino acid	Allantoin	Purine
Histidine	Amino acid	Hypoxanthine	Purine
Isoleucine	Amino acid	Carnosine	Peptide
Leucine	Amino acid	2-Ketoglutaric acid	Organic acid
Lysine	Amino acid	Argininosuccinic acid	Organic acid
Methionine	Amino acid	Cholic acid	Organic acid
Methionine sulfoxide	Amino acid	Citric acid	Organic acid
Ornithine	Amino acid	Creatine	Organic acid
Phenylalanine	Amino acid	Guanidoacetic acid	Organic acid
Proline	Amino acid	Isocitric acid	Organic acid
Serine	Amino acid	Lactic acid	Organic acid
Symmetric dimethylarginine	Amino acid	Pantothenic acid	Organic acid
Threonine	Amino acid	Succinic acid	Organic acid
Tryptophan	Amino acid	Uric acid	Organic acid
Tyrosine	Amino acid		

Note: Partial change from compounds in Primary Metabolite LC/MS/MS Method Package Ver. 2.

These results show the LC/MS/MS system with a fully automated sample preparation module not only reduces sample preparation workload but also reduces measurement variability. Adding an internal standard also enables a more stable continuous analysis to be performed.

The peak area of D3-creatine measured while analyzing 10 clinical samples each day over 22 days (220 samples in total) is shown in Fig. 4. No instrument tuning was performed, nor was the analytical column changed during these 22 days. A peak area %RSD of 5.9 % was obtained for D3-creatine during continuous analysis. These results show that stable continuous analysis was achieved not just for QC samples but also for clinical samples.

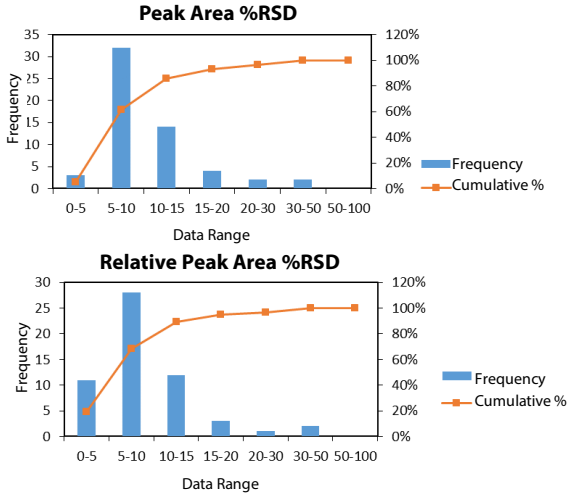

Fig. 3 Histograms Showing Data Range, Peak Area %RSD and Relative Peak Area %RSD for D3-Creatine from 22 Analyses of QC Sample

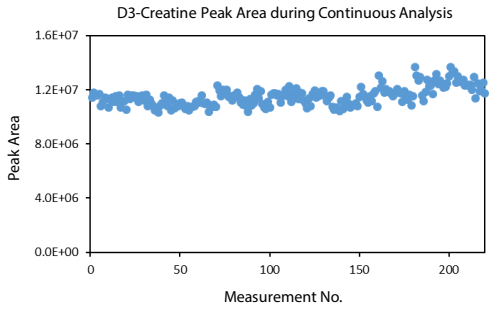

Fig. 4 Peak Area of D3-Creatine during Repeated Analysis of Clinical Samples Over 22 Days (220 Samples in Total)

■ Conclusion

Simultaneous analysis of primary metabolites was performed using an LC/MS/MS system with a fully automated sample preparation module comprised of the CLAM-2030 Fully Automated Sample Preparation Module and LCMS-8060 Liquid Chromatograph Mass Spectrometer. Stable continuous analysis was achieved for both clinical samples and QC samples. This system is expected to be developed in the future for use in the search and verification of marker candidates by metabolomics analysis.

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