SHIMADZU
Excellence in Science

Application News

No. B62

Imaging Mass Microscope

MS Imaging of Low Molecular Weight Metabolites Using a Sublimation/Recrystallization Method with 9-Aminoacridine (9AA)

MS imaging technology initially appeared in the first half of the 1980s and has continued to undergo technological innovation to the present day. It is used in a wide variety of fields including drug discovery and metabolomics research. Technological improvements are still being pursued to this day to enhance capabilities including sensitivity, spatial resolution, and repeatability.

Although various matrices that employ ionization have been developed over the years, it is important to select the matrix appropriate for the targeted compound.

In addition to matrix selection, the coating method also has a significant effect on analysis results. Consequently, a number of coating methods appropriate for the targeted compound have been examined. These are broadly divided into the spray method and the sublimation method, and since each method has its advantages and disadvantages, currently both are widely used. Shimadzu has developed the iMLayer matrix vapor deposition system employing the sublimation method (Fig. 1), which can control the matrix coating thickness. Matrix coating methods have been further examined using this instrument.

This article introduces a sublimation/recrystallization method we developed for 9AA, with which recrystallization was previously said to be difficult. Examples of MS imaging using this method are also introduced for low molecular weight metabolites (LMWMs) in a mouse liver.

R. Yamaguchi, E. Matsuo, T. Yamamoto

Effects of Differences in Matrix Coating Methods on MS Imaging Analysis

Table 1 summarizes the effect that each matrix coating method has on matrix crystal formation and MS imaging analysis.

Compared to the sublimation method, the spray method results in coarse matrix crystals and there is a possibility of a reduction in spatial resolution due to bleeding of components contained in samples. Furthermore, uniformity is poor and repeatability is also unfavorable due to the dependence on surrounding environmental factors including temperature and humidity when the matrix solution is dried for crystallization. However, the extraction efficacy of compounds contained in samples is high, which leads to increased sensitivity.

In contrast, the sublimation method excels in terms of crystal fineness, insusceptibility to bleeding, uniformity, and repeatability, and is known to be indispensable for high spatial resolution imaging. On the other hand, this method has no extraction efficacy of components in samples and thus may prove disadvantageous in terms of sensitivity.

Measurement sensitivity in practice depends on the structure of the compound for detection. For example, the sublimation method provides sufficient sensitivity in the analysis of phospholipids and allows MS imaging to be performed at sufficient sensitivity for drugs such as amiodarone (refer to Application News B61).

However, in the detection of LMWMs, such as ADP and ATP that are contained in mouse liver, sufficient sensitivity cannot be obtained since matrix coating using the sublimation method has no extraction efficacy. This means that while most examples involve coating 9AA use the spray method when performing MS imaging, the spatial resolution is relatively low. For this reason, we examined the conditions for applying the sublimation/recrystallization method, which is used for DHB and CHCA, to 9AA.

Fig. 1 iMLayer Matrix Vapor Deposition System

Table 1 Effects of Matrix Coating Methods on Crystal Formation and MS Imaging Analysis

	Spray Method	Sublimation Method
Crystal fineness	limited	good
Insusceptibility to bleeding	poor	good
Uniformity	limited	good
Repeatability	limited	good
Extraction efficacy	good	poor

Matrix Sublimation/Recrystallization Method

Sublimation/recrystallization using 9AA as a matrix was performed. As shown in Fig. 2, filter paper saturated with 5 % methanol is placed in the same container as a sublimated sample where they are kept at a constant temperature of 37 °C for five minutes. During this time, the 5 % methanol in the filter paper evaporates, soaks into the sample, and when compounds in the sample are extracted, a tiny amount of 9AA crystals dissolve at the same time. Next, the sample is completely dried out for 10 minutes in a vacuum desiccator and the dissolved 9AA recrystallizes.

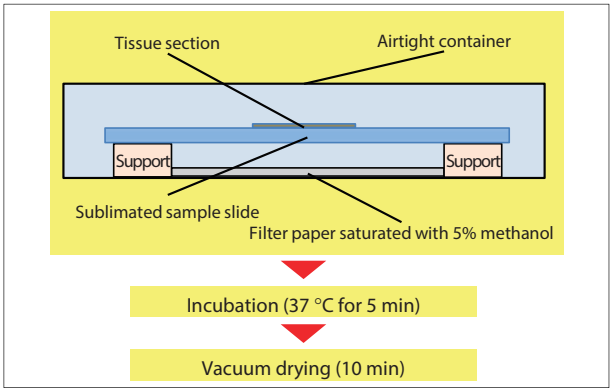

Fig. 2 9AA Sublimation/Recrystallization Method

Fig. 3 iMScope *TRIO* Imaging Mass Microscope

Table 2 iMScope *TRIO* Measurement Parameters

Parameter	Setting
Measurement area	62500 (250 × 250) points
Analysis mode	Negative
Molecular mass range	*m/z* 300 - 650
Laser frequency	1 kHz
Laser irradiation count	50 shots
Laser irradiation diameter	Approx. 5 µm
Laser irradiation interval (spatial resolution)	5 µm

Improving Detection Sensitivity in MS Imaging that Uses the Sublimation/Recrystallization Method

MS imaging analysis was performed on the mouse liver sample that underwent 9AA sublimation and recrystallization, using the iMScope *TRIO* imaging mass microscope (Fig. 3) according to the measurement parameters listed in Table 2.

In order to compare the analysis results of a sample that was matrix coated by the sublimation method and a sample that underwent sublimation/recrystallization, the average spectra of the analysis area were compared (Fig. 4). For the sublimation method, the peak (*m/z* 385.14) originating from the 9AA matrix was strongly detected (▼ in Fig. 4) and most peaks originating from LMWMs could not be detected. However, by performing sublimation/recrystallization, the intensity of peaks originating from LMWMs increased (▼ in Fig. 4), demonstrating the increased detection sensitivity.

In addition, we found that sublimation/recrystallization yields much clearer MS images for several LMWMs used in the comparative experiments. (Fig. 5).

We developed a sublimation/recrystallization method for 9AA, with which recrystallization was previously said to be difficult, and succeeded in achieving highly sensitive MS imaging without losing the advantages of the sublimation method.

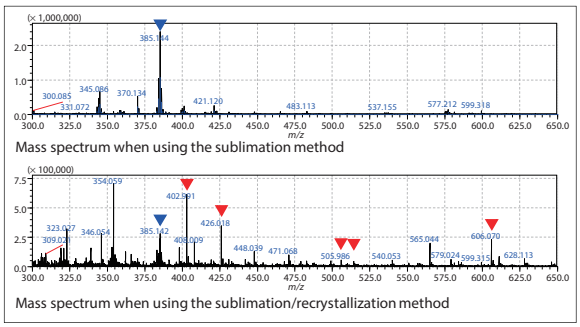

Fig. 4 Mass Spectra (Comparison of Sublimation Method and Sublimation/Recrystallization Method)

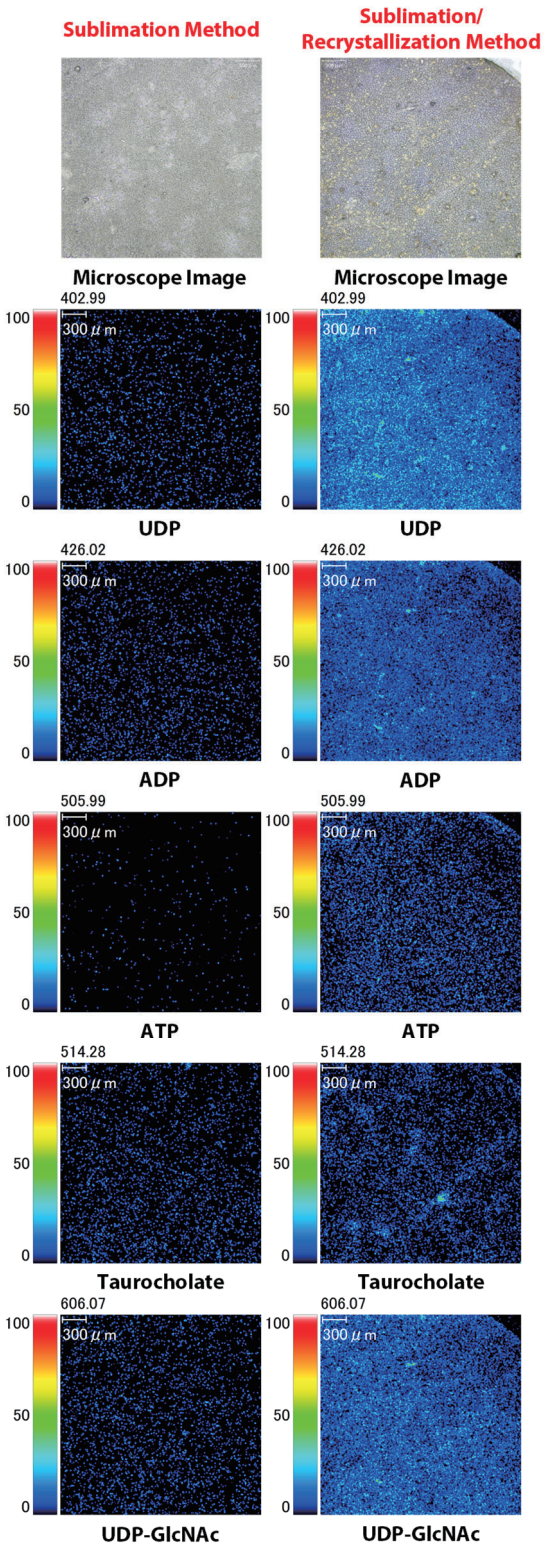

Fig. 5 MS Images (Comparison of Sublimation Method and Sublimation/Recrystallization Method)

First Edition: Dec. 2017

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/