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Technical Report

# Analysis of Lipids in a NAFLD Model Mouse

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## Abstract:

Mass spectrometry is used frequently in lipid research not only for blood and urine samples, but also when using laboratory animal organs as samples. In recent years, using multivariate analysis of homogenate samples in conjunction with measuring distributions in tissue section samples is expected to accelerate research work involved in identifying disease mechanisms or discovering new drugs. Therefore, this example analyzes the variations in lipid components after administering 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) to a non-alcoholic fatty liver disease (NAFLD) model mouse.

**Keywords: MS imaging, LCMS-IT-TOF, iMScope, lipids**

## 1. Background of Research

Hepatocellular carcinoma is often caused by a hepatitis virus, but it can also be caused by alcoholic hepatitis. However, due to the increase in metabolic syndrome cases, there has also been an increase in the incidence of non-alcoholic steatohepatitis (NASH), which does not involve alcohol. Therefore, a variety of research studies are currently being conducted. Previous studies have indicated a strong relationship between the emergence of non-alcoholic fatty liver disease (NAFLD) or its progression to non-alcoholic steatohepatitis (NASH), and oxidative stress. However, the details of that mechanism and causal factors are unclear.

In recent years, animal studies have confirmed that administering 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) can suppress excessive accumulations of fat in the liver.[1)]

To identify the mechanism, the same sample can be analyzed using multiple types of mass spectrometers to take advantage of the features offered by each type. This article describes an example of analyzing an NAFLD model mouse that was administered AAPH.

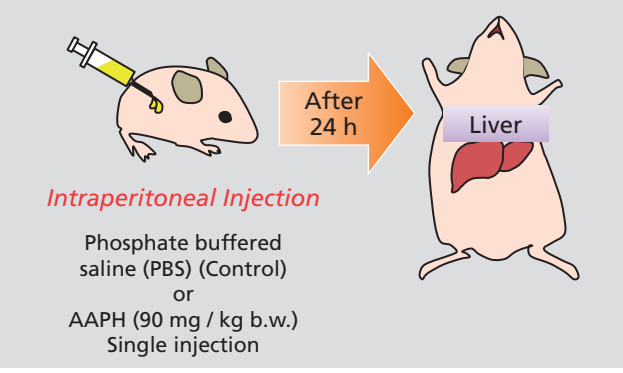


Fig. 1 Laboratory Animal Preparation

## 2. Materials and Methods

An NAFLD mouse was used as the laboratory animal model. A liver obtained 24 hours after administering a single dose of AAPH (90 mg/kg) was used for the experiment. A homogenate sample was used for LCMS-IT-TOF analysis and a thin frozen tissue section sliced 10 µm thick was used for imaging mass spectrometry. A liver from a model mouse administered phosphate buffered saline (PBS) was used as the control sample (Fig. 1).

A diagram of the principle used for imaging mass spectrometry is shown in Fig. 2. A cryostat was used to slice a 10 µm section of the mouse liver (I), which was placed on a microscope slide with conductivity provided by an ITO (indium tin oxide) coating (II). Then the sample was sprayed with a matrix to aid ionization (III) before acquiring imaging mass spectrometry data (IV).

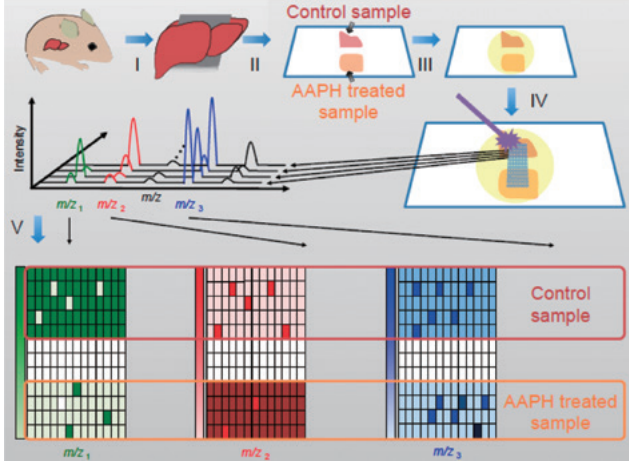


Fig. 2 Principle of Imaging Mass Spectrometry

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## 3. Variation Analysis Using LCMS-IT-TOF Data

The excised liver sample was homogenated and its lipids were measured by LCMS-IT-TOF (Fig. 3). Experimental conditions are shown in Table 1.

Table 1 Analytical Method

| | |
|---|---|
| Mass range | *m/z* 500–1500 |
| Polarity | Positive / negative |
| Column | L-column2 ODS size 2.1 × 150 mm, 3 µm (Chemicals Evaluation and Research Institute) |
| Buffer | <A> 0.2 % formic acid in water : acetonitrile : methanol = 6 : 17 : 17 (v/v)<br><B> 0.2 % formic acid in methanol |

Fig. 3 LCMS-IT-TOF

Results from statistical analysis of the experiment data are shown in Fig. 4. A comparison of results from the AAPH administered group and control group indicate variations in multiple lipid components.

Of the varied lipid components those that indicated characteristic variations are listed in Table 2.

Table 2 Lipid Component Variation Due to Administration of AAPH

| | *m/z* | | *m/z* |
|---|---|---|---|
| A | 786.568 (+) | E | 832.5429 (+) |
| B | 810.5616 (+) | F | 822.5151 (+) |
| C | 806.5357 (+) | G | 780.4237 (+) |
| D | 785.5436 (+) | H | 801.4930 (+) |

As shown in this data, the components that vary in response to the administered drug can be identified by using a homogenate liver sample, comparing the control and administered groups, using statistical analysis tools.

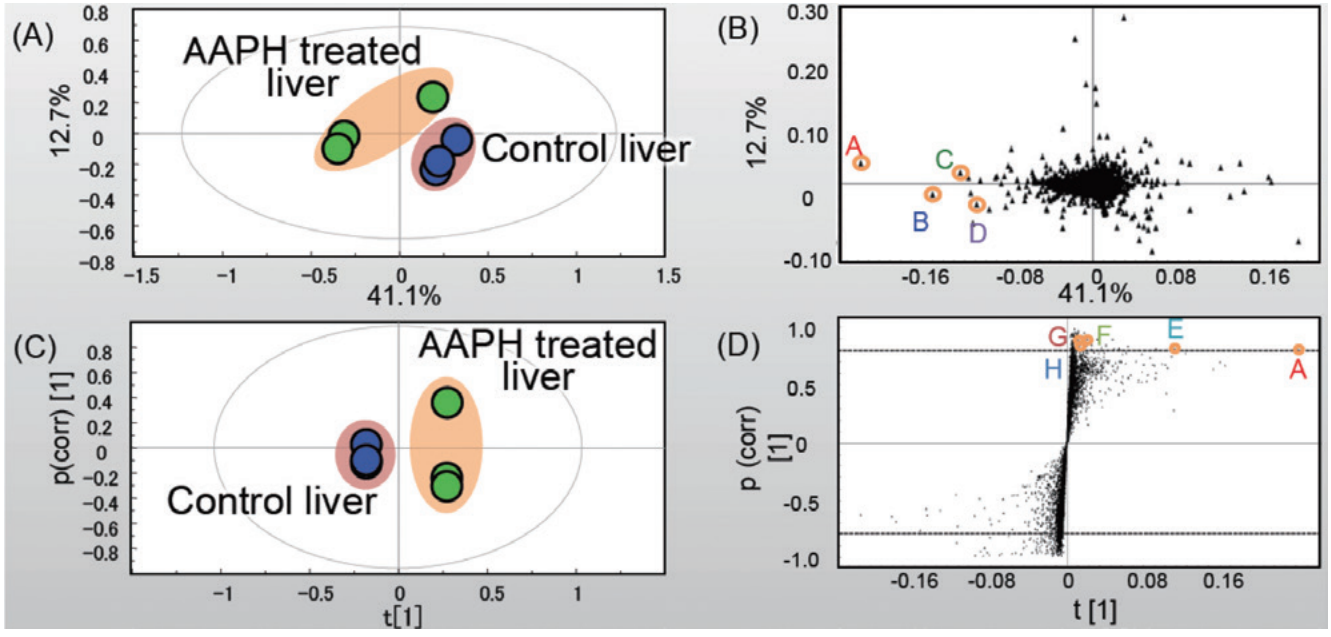


Fig. 4 Lipid Variation Analysis Results After Administering AAPH
(A) PCA score plot, (B) PCA loading plot, (C) OPLD-DA score plot, (D) OPLS-DA S-plot

## 4. Visualization of Lipids by Imaging Mass Spectrometry

In section 3, Variation of lipids in the homogenate sample was analyzed by LC-MS.

When considering the effects of drug administration or genetic modifications in model mice, it is important to visualize not only the variations in substances for the overall tissue, but also the distribution of such substances. Therefore, imaging mass spectrometry has attracted interest as a means of using tissue sections to visualize such distributions. Imaging mass spectrometry involves spraying tissue section samples with matrix and then directly measuring the masses.

The technique of visualizing the distribution of biologically derived substances based on the peak intensity at each *m/z* value in MS spectra acquired at each measurement position is often used for pharmacokinetic analysis or to search for biomarkers. Herein the technique was used to visualize the distribution of lipid components that varied in response to administering AAPH.

## 4-1. Using the iMScope to Visualize the Distribution of Lipid Components

Experimental conditions for the iMScope imaging mass microscope are shown in Table 3. Experimental results are shown in Fig. 6. Results from HE staining a series of sections adjacent to the section analyzed above are shown in Fig. 5.

By analyzing the sections using the positive mode, we successfully visualized the distribution of the 8 lipids listed in Table 2, which varied in the LCMS-IT-TOF analysis (shown in Fig. 6 bordered with a dashed line). In addition, we were able to extract other lipids with characteristic distributions to such lipids within the specified mass range.

One of the major benefits of imaging mass spectrometry is that it simultaneously provides an analysis of the distribution of substances at target *m/z* values, and information about the distribution of various other substances detected using the same conditions. This feature may be as beneficial as the label-free measurement capability. In this case, some of the lipids were identified based on reference literature for measurement results obtained using the iMScope.[2, 3)]

Table 3 Analytical Method

| | |
|---|---|
| Mass range | *m/z* 600–1000 |
| Polarity | Positive / negative |
| Spatial resolution | 50 µm |
| Measurement points | 250 × 250 |
| Matrix | 1,5-diaminonaphthalene (1,5-DAN) |

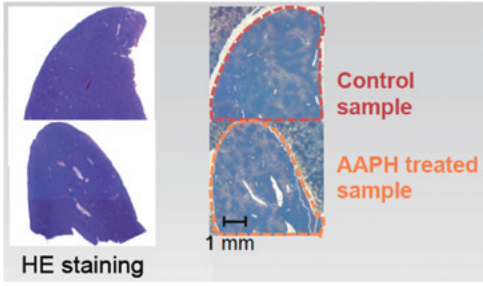


Fig. 5 Image of HE Stained Consecutive Sections

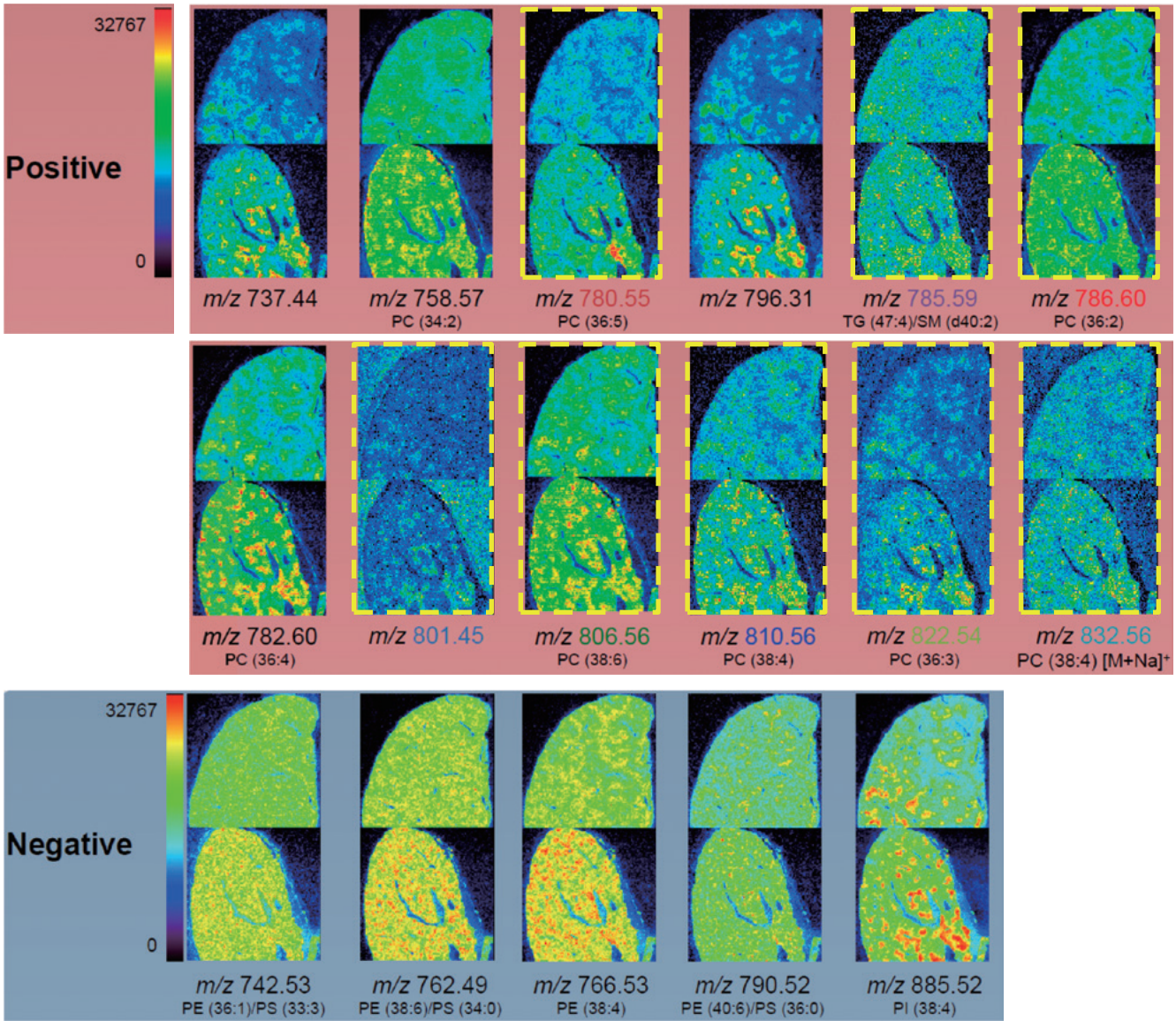


Fig. 6 Visualization of Localized Lipid Components Using the iMScope

## 4-2. Using the AXIMA Confidence to Visualize the Distribution of Lipid Components

Experimental conditions for the AXIMA Confidence are shown in Table 4. Experimental results are shown in Fig. 7.

Because the AXIMA Confidence ionizes components in a vacuum, whereas the iMScope ionizes them at atmospheric pressure, it sometimes provides different detection results. Furthermore, in this case ionization was performed using two types of mass spectrometers with different laser diameters. Therefore, results cannot be simply compared as though the tests were similar. Nevertheless, even with the AXIMA Confidence, a general-purpose mass spectrometer, we were able to visualize the distribution of lipids that showed variation when being analyzed by other techniques, as well as other lipids being observed within the specified mass range, and we were also able to clarify the differences between those two groups. We believe that these results demonstrate the benefits of imaging mass spectrometry.

Table 4 Analytical Method

| | |
|---|---|
| Mass range | *m/z* 500–1000 |
| Polarity | Positive |
| Spatial resolution | 20 µm |
| Measurement point | About 15,000 (control + AAPH treated) |
| Matrix | 1,5-diaminonaphthalene (1,5-DAN) |

## 5. Summary

This article describes visualizing the distribution of variations in lipid components that occurred due to administering AAPH.

In drug discovery applications or basic medical research intended for clinical applications, it is essential to build an experimental system that is optimized for the given research objectives and sample characteristics. Therefore, various types of mass spectrometry technologies are used. Furthermore, multifaceted analysis using a new type of mass spectrometer, as reported in this article, is also expected to lead to discovering new information, and also to improve research efficiency.

Consequently, using imaging mass spectrometry, which enables directly using tissue section samples to measure the distribution of substances, in conjunction with other techniques has been gaining significant interest, not only for homogenate tissue samples, as reported in this article, but also for metabolomics, where research has been advancing at a remarkable pace in recent years.

### Cited References


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3) *Anal. Chem.* 84(4): 2048–54 (2012)


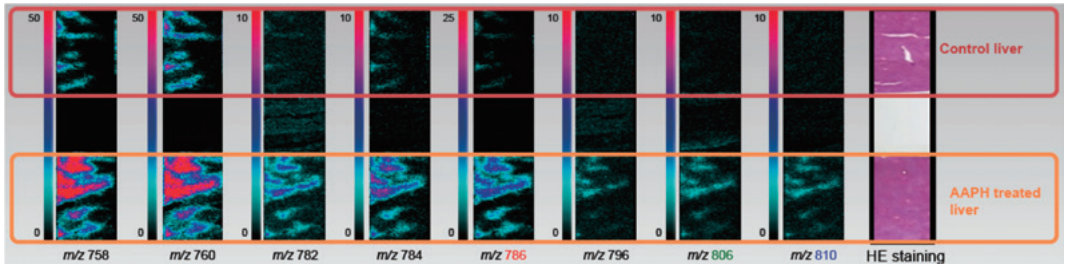


Fig. 7 Visualization of Localized Lipid Components Using the AXIMA Confidence

Fig. 8 iMScope *TRIO* Imaging Mass Microscope


Fig. 9 AXIMA Confidence

The iMScope, iMScope *TRIO*, AXIMA Confidence, and LCMS-IT-TOF are not approved or certified as medical devices. They can be used exclusively for research applications, and cannot be used for the purpose of treatment or diagnosis.


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