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Application News

No. B75

Imaging Mass Microscope

Drug Imaging on a Model Hair Sample
- Toward the Observation of Drug Use History -

Imaging mass spectrometry is increasingly being utilized in various fields. When hair grows, trace amounts of drugs that a person has used are deposited in the structure. Due to this characteristic, hair is gaining attention as a sample which can provide a historical record of an individual's drug use. Currently, drugs extracted from hair are analyzed such as by liquid chromatograph mass spectrometry. However, the loss of information on the drug distribution in hair due to extraction processes has been a problem. If imaging mass spectrometry on longitudinal sections of hair is made possible, the distribution of drug deposits according to the growth of the hair can be visualized, thereby showing the drug usage history of an individual. The utilization of such a technology is greatly anticipated in fields such as forensic medicine, clinical medicine, medication administration and forensic investigation.

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Preparation of a Model Hair Sample with Drug Use

Methoxyphenamine (MOP), a component of commercially available cough medicine which has a structure similar to a stimulant drug (methamphetamine) (Fig. 1), was selected as a model compound (analysis target). Observation should be done using a hair sample in which an orally taken drug is deposited in the hair by being carried to hair roots and the scalp through the blood system. Instead, we prepared a model hair sample with MOP added according to the protocol shown in Table 1. We took hair of an individual who has not taken any drugs and soaked the hair in an MOP solution of varying concentrations to make the hair absorb MOP. We thereby prepared model hair samples with a high concentration (A) and a low concentration (B). A negative control sample (C) was also prepared by soaking in water that does not contain MOP. Analysis was done using these three samples.

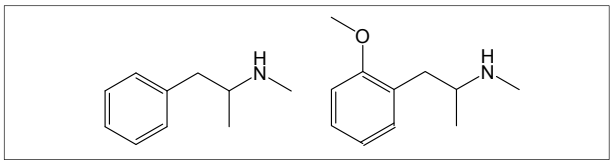

Fig. 1 Structural Formula of Methamphetamine (Left) and Methoxyphenamine (MOP, Right)

Table 1 Protocol for Model Hair Preparation

Adjust MOP (methoxyphenamine hydrochloride aqueous solution) to the following concentrations.

Sample A:	100 µg/mL
Sample B:	10 µg/mL
Sample C:	0 µg/mL (negative control)

Cut hair into segments approx. 2 cm long.
↓
Soak 50 to 100 pieces of hair in the above solutions.
↓
Stand at 37 °C for 24 hours.
(Mix 1 or 2 times during this time.)
↓
Wash these samples according to the test method*

* "7-2-4 Testing Using Hair Samples (Tests for methamphetamine and its metabolites)" in "Testing Methods and Annotations for Toxic Substances & Pharmaceuticals 2006 - Analysis, Toxicity, and Coping Methods"

Pretreatment and Quantitative Evaluation of Model Hair Samples

After washing the surfaces of model hair samples A and B, MOP was extracted according to the test method* and analyzed by liquid chromatography. As a result, the MOP content of the model hair samples was quantified to be 83.1 ng/mg for sample A and 20.0 ng/mg for sample B. These values roughly fall within the range of concentrations reported to be detected from hair samples of humans who have actually taken MOP or stimulant drugs.

Analysis of Methoxyphenamine Standard

Mass spectrometry was performed on droplets of MOP standard solution (50 pmol/mL) (Fig. 2) in advance to the analysis of the model hair samples. Measurement was done employing CHCA, which is widely used as a matrix, and a peak was observed at *m/z* 180.14 (▼ in Fig. 2 top panel), which corresponds to the $[M+H]^+$ ion. The bottom panel in Fig. 2 shows the spectrum obtained by performing MS/MS measurement using the above peak as a precursor. Taking into consideration the application of this method to actual specimens in the next step, we performed imaging mass spectrometry by MS/MS measurement (*m/z* 180.14 > 149.10) which features high specificity.

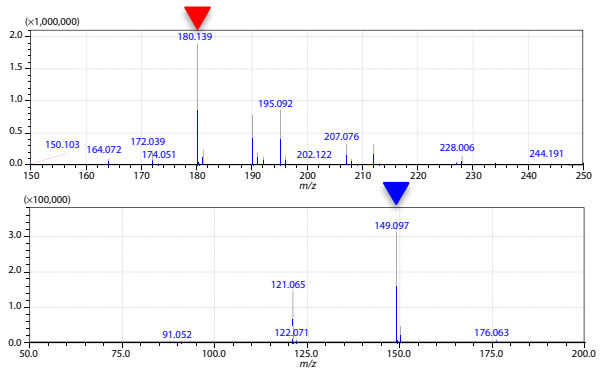

Fig. 2 Mass Spectra of Methoxyphenamine (MOP) Standard Obtained by MS (Top) and MS/MS (Bottom)

Fig. 3 iMScope *TRIO* (Left) and iMLayer (Right)

Table 2 Acquisition Parameters for Imaging Mass Spectrometry

Pitch (Spatial Resolution)	: 10 / 50 [µm]
Ion Polarity	: Positive ion
Mass Range	: Precursor *m/z* 180.14 → *m/z* 50 to 190
Accumulations	: 2 [times/pixel]
Sample Voltage	: 3.50 [kV]
Detector Voltage	: 2.0-2.1 [kV]
Number of Laser Shots	: 50-100 [shots]
Laser Repetition Rate	: 1000 [Hz]
Laser Diameter Setting	: 1 (approx. 10 µm) / 4 (approx. 50 µm)
Laser Intensity	: 21.7-30.0 / 56.4-63.0

Preparation of Hair Sections for Imaging Mass Spectrometry and Analysis Results

Longitudinal sections of the model hair were prepared by fixing model hair with electrically conductive double-sided tape and carefully slicing the hair using a microtome down to about half of the diameter of the hair. The sections were then vapor deposited with a CHCA matrix by sublimation using iMLayer (Fig. 3 right) and measured using iMScope *TRIO* (Fig. 3 left) under the conditions listed in Table 2.

Measurement was first performed using a relatively large laser beam diameter (approx. 50 µm) to obtain overall MS images (Fig. 4). The obtained MS images show that a peak is detected only at locations where hair can be seen in the optical images. We can also see that there are no signals originating from the drug for the negative control sample (Fig. 4 c). Hair has a three-layer structure comprising the cuticle, cortex, and medulla with the cuticle being the outermost layer. However, since the diameter of hair is between 50 µm to 150 µm, analysis of the local existence of drugs at each of the internal structures within hair is not possible with a spatial resolution of 50 µm. iMScope *TRIO* features a high spatial resolution (minimum laser beam diameter setting: approx. 5 µm) and it is expected to help visualize the detailed distribution (local existence) of drugs. Therefore, we next performed measurement with a laser beam diameter of 10 µm (Fig. 5). The results indicate that with the prepared hair samples, the drug is distributed outer part of hair and there is less distribution in the middle part.

The advantage of the sublimation method used above is that measurement is possible while maintaining a high spatial resolution. However, there are cases where the method is disadvantageous in terms of sensitivity (refer to Application News No. B62). We therefore, using the same samples, employed the two-step matrix coating method in which vapor deposition (by sublimation) is followed by spraying and performed imaging. The results are shown in Fig. 6. The detected signal intensity (after correcting based on a background peak) increased by three to six times and the drug distribution is more clearly imaged.

Discussion

In this research, we performed imaging mass spectrometry with a high spatial resolution on model hair samples and obtained detailed images showing the distribution of drugs within the hair samples.

Hair grows at a rate of approx. 1 cm per month while taking in substances including drugs from the blood system at its roots. Due to this characteristic, hair is known to provide a historical record of drug use and has been utilized in forensic medicine and investigation and in the future its application to other fields such as medication administration and doping tests is anticipated.

Furthermore, the method described in this article for preparing a model hair sample has many points in common with the use of hair care products such as shampoo, treatment, hairstyling products, and hair dyes. We thus expect that the above analysis techniques can contribute to the development and evaluation of these products and in turn the enhancement of the beauty and health of hair.

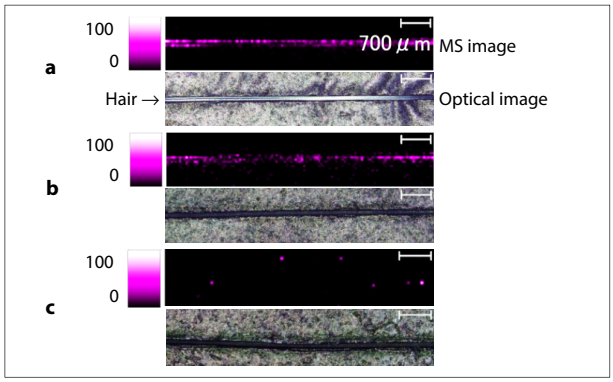

Fig. 4 Drug Imaging on Samples A (a), B (b), and C (c) Using a ϕ50 µm Laser Beam

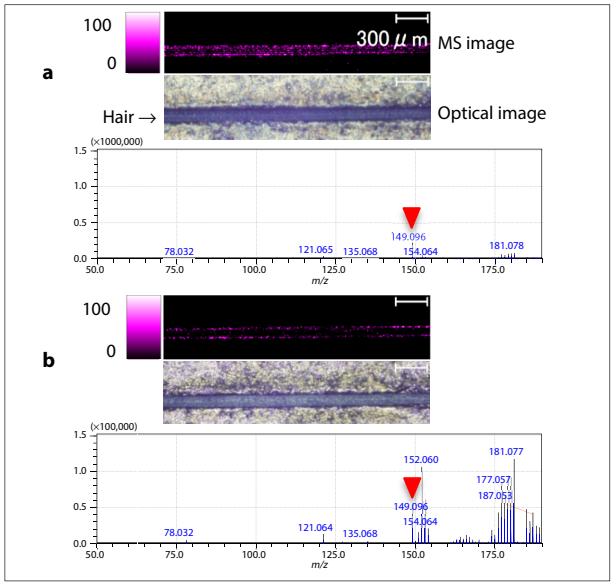

Fig. 5 Drug Imaging on Samples A (a) and B (b) Using a ϕ10 µm Laser Beam

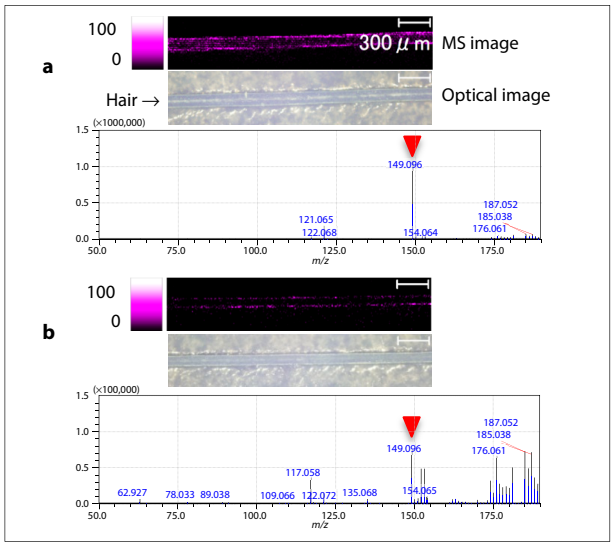

Fig. 6 Drug Imaging on Samples A (a) and B (b) Using the Two-step Method (Sublimation +Spraying) and a ϕ10 µm Laser Beam

First Edition: Mar. 2018

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