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RESEARCH PAPER

Mass imaging of ketamine in a single scalp hair by MALDI-FTMS

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Abstract Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) coupled with mass spectrometry imaging (MSI) is a rapidly emerging technology that produces distribution maps of small pharmaceutical molecules in situ in tissue sections. Segmental hair analysis provides useful information regarding the state and history of drug use. A preliminary MALDI-Fourier transform ion cyclotron resonance (FTICR)-MSI method was developed for direct identification and imaging of ketamine in hair samples. After decontamination, the scalp hair samples from ketamine users were scraped gently and were fixed onto a stainless steel MALDI plate using double-sided adhesive tape. A Bruker 9.4 T solariX FTICR mass spectrometer with continuous accumulation of selected ions function was used in the positive ion mode. Four single hairs from the same drug abuser were analyzed. Three of four single hairs demonstrated ketamine spatial distribution, while only traces of ketamine were identified in the other one. The platform could provide detection power of ketamine down to the 7.7 ng/mg level in hair. MALDI-FTICR-MSI demonstrated the drug distribution over the whole hair length with higher spatial resolution compared with the traditional LC-MS/MS method after scissor cutting. Greater caution is needed in the interpretation of a single hair result because of the considerable variations in the growth rate and sample collection.

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Introduction

Hair has become a good supplemental matrix to blood and urine [1, 2]. Hair is a more stable matrix and has a substantially longer detection window, ranging from weeks to years. Segmental hair analysis, in particular, provides useful information concerning the state and history of drug use [2, 3].

Ketamine (K), also called K powder in China, is a rapid-acting dissociative anesthetic used on both animals and humans [4]. It is abused by an increasing number of young people, especially teenagers, as an illicit drug, and its harmful effects are disturbing. Therefore, it is crucial to establish a rapid and highly sensitive method to substantiate drug abuse.

The pre-preparation of hair samples involves a number of steps, including washing, segmentation, incubation, and extraction [5]. Separation and detection of the analytes of interest is achieved using chromatographic methods combined with mass spectrometry, e.g., gas chromatography-mass spectrometry (GC-MS) [6, 7] and liquid chromatography-mass spectrometry (LC-MS) [8, 9]. These steps are time-consuming, which is why it is so important to develop a fast and simple hair sample preparation technique.

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) coupled with mass spectrometry imaging (MSI) is a rapidly emerging technology that produces qualitative distribution maps of small pharmaceutical molecules and their metabolites in situ in tissue sections [10–12]. For hair, the nanogram per milligram level of drugs and the embedding of drugs in the hair matrix makes it difficult to detect with MALDI-MSI. Until now, only a few investigations have used MSI to detect stimulants in a single hair. Miki [13] was the first to report the direct detection of methamphetamine

in hair and its imaging by MALDI-time-of-flight (TOF), with careful optimization of sample preparation. Porta et al. [14] imaged the distribution of cocaine and its metabolites in intact single-hair samples from chronic users. Musshoff et al. [15] detected cocaine, cocaine metabolites, and cannabinoids from identical positions along the hair strand. There has been no report of mass imaging of ketamine in a single hair.

Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) offers high mass resolution for resolving closely spaced ions, as well as high mass accuracy for confident molecular formula assignment [16, 17]. In the low mass range (m/z<500), the higher performance of FTICR-MS instruments can effectively overcome the signal interferences from matrixes and other impurities. This is the most outstanding advantage of MALDI-FTICR in the analysis of small molecular compounds. MALDI-FTICR-MSI has also been used to spatially map small molecules from biological tissue sections [11, 17].

In this study, a MALDI-FTICR-MSI method was developed for the direct identification and imaging of ketamine in hair samples from chronic users by performing microsegmental analysis and evaluating the application.

Experimental

Chemicals and reagents

Methanolic solution (1.0 mg/mL) of ketamine was purchased from Cerilliant (Round Rock, TX, USA). Methanol and acetonitrile, both high-performance liquid chromatography (HPLC) grade, were bought from Sigma-Aldrich (St. Louis, MO, USA). MALDI matrixes a-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB) were obtained from Bruker Daltonics (Bremen, Germany). Formic acid was obtained from Fluka Chemical Co. (Buchs, Switzerland). Other reagents were all of analytical reagent grade, and no further purification was undertaken. Deionized water was purified using a Milli-Q system (Millipore, Billerica, MA, USA).

Standard and matrix solutions

Separate solutions were prepared containing 100 ng/mL and 10 μg/mL ketamine in MeOH/H_2O (70:30, *v*/*v*). CHCA and DHB were separately dissolved at concentrations of 10 mg/mL in a solvent mixture (acetonitrile/acetone/HCOOH, 50:50:0.1, *v*/*v*/*v*). The matrix solutions were kept in amber glass bottles to prevent their degradation by light and were stored at 4 °C.

Hair specimens collection

Drug-free hair was donated by healthy volunteers in the laboratory and preserved at room temperature. The authentic scalp hair specimens were obtained from ketamine abusers who went to a rehabilitation center. The study was conducted under the guidelines for the protection of human subjects, and the subjects agreed to participate in the study through oral informed consent.

Decontamination procedure

The hair strands were washed with different solvents as follows: 5 mL of 0.1 % sodium dodecyl sulfate for 5 min, twice with 5 mL of deionized water for 5 min, and twice with 5 mL of acetone for 5 min. The last wash was stored for further analysis.

Sample preparation for MALDI-MS

Single hair strands were put on a glass slide and was scraped gently using a scalpel to make the surface of the hair rough, respectively. After cleaning the surface with a rubber suction bulb, the hair shaft was fixed onto a stainless-steel MALDI plate using double-sided adhesive tapes. The length for each side of hair shaft covered by double-sided adhesive tape was about 1 cm. CHCA matrix solution was used to coat the surface of hair by manual spread using a self-made tip (Figs. 1 and 2).

MALDI-FTICR-MS and data analysis

To perform the MALDI imaging analysis, a Bruker 9.4 T solariX FTICR mass spectrometer equipped with SmartBeam™ laser optics and external ion accumulation was used in positive ion mode over a mass range of 100–

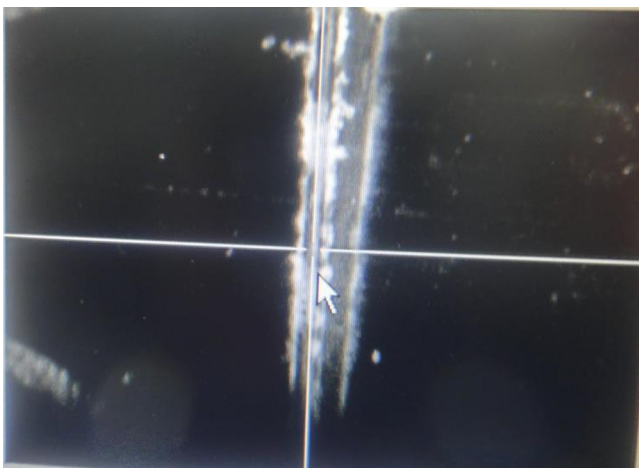

Fig. 1 Photo of hair stabilized on the MALDI target plate

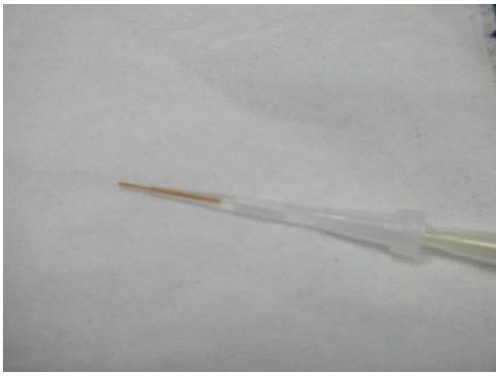

Fig. 2 Self-made tip for manually spotting the hair strands

700 *m/z* with a resolution of 400,000 at *m/z* 200. The laser beam diameter was 100 μm. Spot interval was set as 500 μm and the imaging resolution was 500 μm.

Before starting the experiments with the authentic hair, two drug-free hair samples were fixed onto a stainless steel MALDI plate using double-sided adhesive tapes. Two microliters of ketamine solution (100 ng/mL) directly spotted on a drug-free hair. After drying, CHCA matrix solution was manually spotted on the hair samples, respectively. Fourier transform mass spectrometry (FTMS) full-scan spectra (*m/z* 100–1,000) of drug-free hair and spiked hair were recorded (Fig. 3).

The MS/MS spectra of the peaks were acquired with the continuous accumulation of selected ions (CASI) function on the key parameter settings listed in Table 1.

Data were acquired using solariXcontrol software. Ketamine chemical formula was calculated by the SmartFormula calculator. Mass imaging was reconstructed using FlexImaging v3.0 software (Bruker, Bremen, Germany).

Results and discussion

Sample preparation and solvent matrix condition

It was effective when a hair shaft was scraped gently using a scalpel from the proximal to distal end one time (Fig. 1) because drugs are entrapped mainly in the core of the hair shaft. The sensitivity was very poor when the intact hair was coated and microspotted directly as described by Porta et al. [14]. But scraping the hair with a scalpel was not successful each time. To prevent contamination, the scalpel should not be scraped back and forth, and from the root to the distal side only one time. The damaged hair was broken into segments and was not selected for MSI.

The solvent system used in the application of the matrix plays a very important role in sample preparation [17]. CHCA is the most commonly used matrix for the analysis of low-molecular-weight compounds. First, the CHCA solvent matrix (acetonitrile:H2O/HCOOH, 50:50:0.1, *v/v/v*) was applied to the hair. There was a significant lateral diffusion of ketamine across the hair shaft surface. Compared with tissue sections, hair is a keratinous fiber, which is strongly hydrophobic. The optimum results were attained by mixing with acetone instead of H_2O, and the crystal size was reduced to less than 250 μm. On the other hand, the shorter time for evaporation allows less time for the extraction of the analytes from the hair that would result in poor sensitivity. In the next step for research, more effective longitudinal sections of hair shafts and selected MALDI matrix sprayed automatically to get smaller-sized crystals will be able to improve sensitivity and spatial resolution.

MALDI-FTICR

Fourier transform ion cyclotron resonance (FTICR), which has the highest mass resolving power of current MS instrumentation (>100,000), as well as sub-part per million mass accuracy, offers a new strategy for imaging small molecules [18]. For FTICR, although the sensitivity is lower than for regular MALDI-TOF instruments [13], capabilities can be enhanced with the CASI mode. With the CASI mode, target ions are selected and enriched in the collision cell, which increases the signal intensity of the selected ions. The scheme of the CASI function is shown in Fig. 3.

Because the increase in spatial resolution negatively impacts sensitivity, there is a tradeoff between sensitivity and spatial resolution [17]. Typically, the laser spot size in MALDI-IMS ranges from 50 to 200 μm. In this study, the laser spot diameter was set at 100 μm. The purpose of our preliminary investigations was not to minimize the laser spot size. A sufficient amount of analyte ions must be absorbed to detect trace levels of ketamine in hair.

Imaging MS of ketamine in a single hair

Figure 4 shows a typical MALDI-MS/MS spectra from a ketamine abuser's hair shaft. The precursor ion $[M+H]^+$ ($C_{13}H_{16}NOCl$, *m/z* 238.0993) and one fragment ion ($C_{11}H_{12}Cl$, *m/z* 179.0622) for ketamine were monitored in MS/MS mode within an error of 3.0 ppm over the entire hair shaft.

Figure 5 shows the MS images of four different hair samples from the same individual. Although the length of the hair was originally approximately 6 cm, the imaging MS was conducted over the range of approximately 4 cm due to the fixed tape. Moniliform spot images with various intensities reflecting the concentrations of ketamine were observed on the hair sample. Only traces of ketamine were identified in hair 2. Hair 1, 3, and 4 analyzed demonstrate ketamine spatial

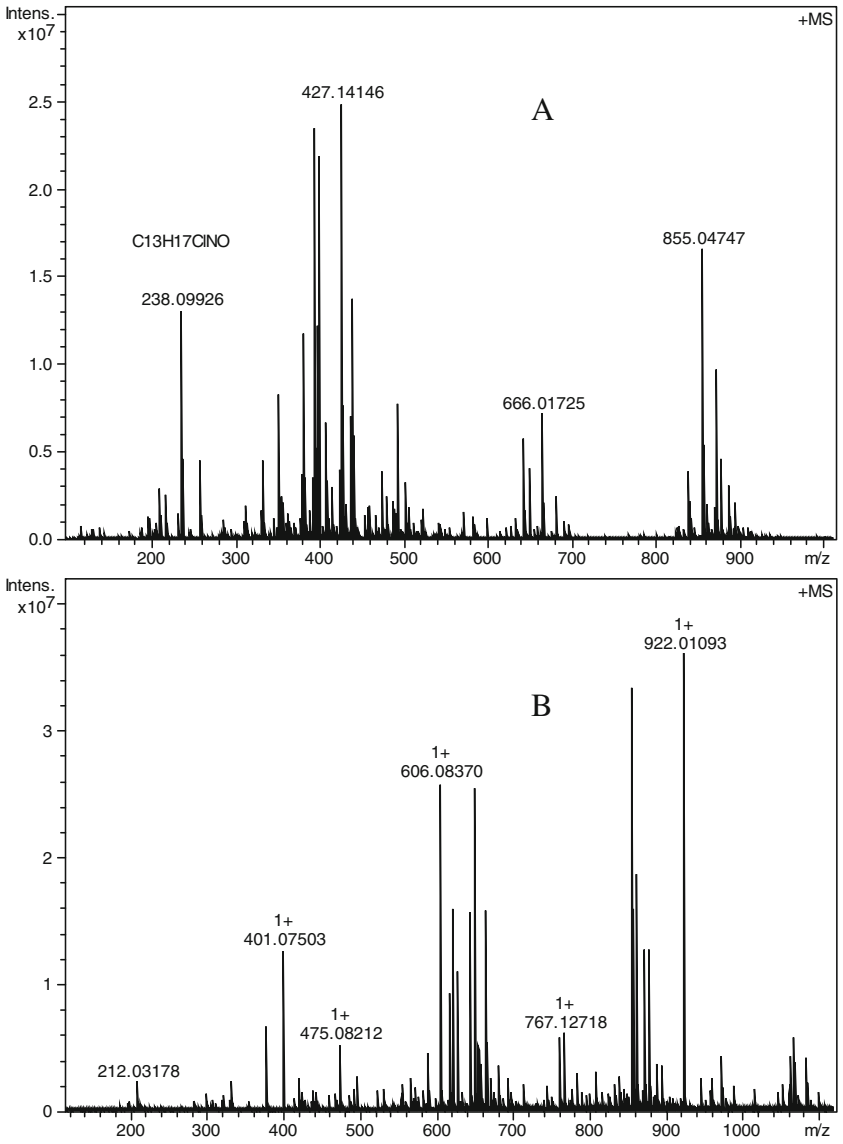

Fig. 3 Full-scan mass spectra of ketamine standard spotted on hair (**A**) and drug-free hair (**B**)

distribution. Ketamine concentrations near the roots were all significantly higher than that near the distals. But the highest intensity spots in hairs were inconsistent. There were few ketamine-positive spots detected at both sides of the hair shaft. This observed spatial profile means that the subject stopped consumption when he went to a rehabilitation center about 2 months before. The greater reduction of ketamine in the distal portion might be due to long-term cosmetic treatments [19, 20]. No ketamine was detected in the last acetone wash. Given the oral administration of ketamine and the washing procedure used, the external contamination factor can be excluded in our study.

Ketamine in hair samples from the same individual was already determined using LC-MS/MS method [4]. Ketamine

Table 1 Parameter settings for MALDI-imaging using Solarix FTMS

Acquisition parameters			
Acquisition mode	MALDI_Imaging	Acquired scans	1
Polarity	Positive	No. of cell fills	1
Broadband low mass	101.1 m/z	No. of laser shots	1,000
Broadband high mass	1,000.0 *m/z*	Laser power	30 %
Source accumulation	0.010 s	Laser shot frequency	1,000 Hz
Ion accumulation time	2.000 s	Laser beam diameter	100 μm

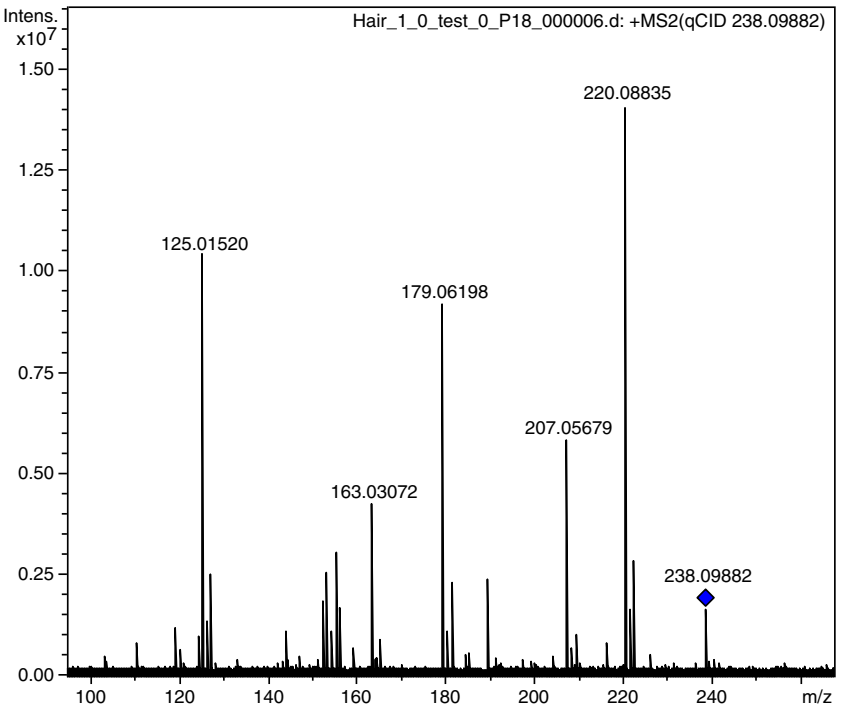

Fig. 4 MS/MS spectrum of ketamine directly from hair imaging analysis

in the 0–3 and 3–6 cm segments were at levels of 17.1 and 7.7 ng/mg, respectively. Although accurate contents of ketamine could not be estimated by imaging MS, it became clear that the limit of detection that can be achieved for ketamine in hair by MALDI-FTICR-MS was at the nanogram per milligram level. Porta et al. [14] used MALDI-SRM/MS to image the distribution of cocaine and its metabolites in intact single hairs down to a concentration of 5 ng/mg. Miki [13] demonstrated that methamphetamine could be visualized in hair at concentrations of 15–75 ng/mg. Sensitivity remains a never-ending challenge as hair analysis is applied in clinical and forensic toxicology. New methods for preparation of samples that are optimized for specific analytes, more sensitive mass spectrometers, and better instrumental techniques to perform targeted analysis or enrichment in the gas phase are needed to further improve the sensitivity of IMS [17].

Comparatively, MALDI-FTICR-MSI is a rapid method to image ketamine distribution in a single hair sample directly. It took about 3 h to prepare hair from washing to matrix application and MALDI-MS analysis. Although a typically LC-MS/MS method would likely be less than 10 min per injection, more segments to document the drug deposition in the hair clearly would take more time.

MALDI-FTMS imaging allowed obtaining a spatial resolution of 100 μm and thus chronological information about drug consumption over several months can be obtained. But

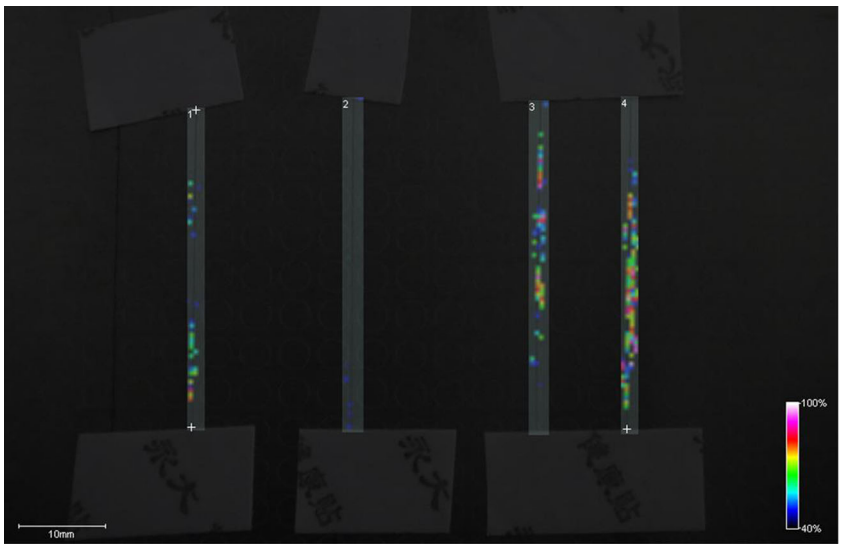

Fig. 5 Imaging results of ketamine abuser's hairs. Samples: hairs *1*, *2*, *3*, and *4*

from the results of four different hair samples from the same individual, using a single hair to investigate an individual's drug history is not accurate. We agree with Musshoff et al. [15] that hair analysis of single hairs can lead to misinterpretation. There are considerable variations in the growth rate of human head hair, as well as the inconsistent collection of hair [21, 22]. The growth rate of a single hair in a hair tuft can vary by up to 50 % of the mean value [23]. Approximately 10–20 % of the hair is in the catagen or telogen stage, which means that the hairs are not growing and that a part of the hairs have not grown in the last (up to 6) months [23]. There is potential for considerable variability in collecting hair from the scalp and sectioning the hair samples [21]. Until these mechanisms of drug incorporation into hair are better understood and the reasons for the intersubject variability clarified, caution is needed in the interpretation of single hair findings in forensic toxicology.

Summary

In a preliminary test, MALDI-FTICR-based imaging technique was developed for the rapid imaging of the ketamine distribution in a single hair sample. Three of four single hairs from the same individual analyzed demonstrate ketamine spatial distribution, while only traces of ketamine were identified in the other one. MALDI-FTICR-MSI demonstrated the drug distribution over the whole hair length with higher spatial resolution compared with the traditional LC-MS/MS method after scissors cutting. Greater caution is needed in the interpretation of a single hair result because of the considerable variations in the growth rate and sample collection.

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