Explanation of Anomalies in Business Process Event Logs with Linguistic Summaries

Chapter 12

Mass Spectrometry Imaging of Drugs of Abuse in Hair

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Abstract

Hair testing is a powerful tool routinely used for the detection of drugs of abuse. The analysis of hair is highly advantageous as it can provide prolonged drug detectability versus that in biological fluids and chronological information about drug intake based on the average growth of hair. However, current methodology requires large amounts of hair samples and involves complex time-consuming sample preparation followed by gas or liquid chromatography coupled with mass spectrometry. Mass spectrometry imaging is increasingly being used for the analysis of single hair samples, as it provides more accurate and visual chronological information in single hair samples.

Here, two methods for the preparation of single hair samples for mass spectrometry imaging are presented.

The first uses an in-house built cutting apparatus to prepare longitudinal sections, the second is a method for embedding and cryo-sectioning hair samples in order to prepare cross-sections all along the hair sample.

Key words MALDI-MSI, MetA-SIMS, Cocaine, Longitudinal sectioning, Cross-section

1 Introduction

Hair testing is a powerful tool routinely used for the detection of drugs of abuse in toxicology and forensic applications [1–3]. The analysis of hair is highly advantageous as it can provide prolonged detection and chronological information about drug intake or chemical exposure in contrast to the analysis of biological fluids [4]. However, current methodology requires large amounts of hair samples and involves complex and time-consuming sample preparation, which includes homogenization, derivatization, sample-cleanup, and extraction techniques followed by gas or liquid chromatography coupled with mass spectrometry (GC-MS or LC-MS).

The use of matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) for the detection and imaging of drugs and pharmaceuticals in tissues is well established.

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However, it is increasingly being used for the analysis of drugs of abuse in hair. The feasibility of using matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) for the analysis of drug of abuse in hair was initially explored in order to detect cocaine and its metabolites in hair, following pulverization and extraction [5, 6]. Following this, the detection and imaging of cocaine and its metabolites in single intact user hair samples were demonstrated using a MALDI-triple quadrupole linear ion trap instrument [7]. Some preliminary results on the determination of cocaine and cannabinoids in single intact hair samples using a MALDI-LTQ Orbitrap XL instrument have also been presented [8]. MALDI-tandem mass spectrometry (MALDI-MS/MS) imaging has been used to monitor the distribution of pharmaceuticals, such as the synthetic opioid painkiller tilidine in intact hair samples of children obtained from a forensic intoxication case [9]. The detection of zolpidem in single intact hair samples was performed by MALDI-MS and MALDI-Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) imaging [10]. The effect of hydrogen peroxide treatment on both cocaine users and externally contaminated hair samples has also been investigated using MALDI-MSI [11].

While the analysis of intact hair samples has been demonstrated by MALDI-MS imaging, one of the issues often raised is the extraction efficiency of the embedded drugs by the matrix solution. As the drugs are considered to be bound to melanin inside the core of the hair, it remains difficult to know whether the drug is completely extracted out of the hair by the MALDI matrix especially through the impermeable outer surface. The preparation of longitudinal sections has been proposed as a solution to address this issue. This also opens the possibility of analyzing drug user hair samples with surface analysis techniques such as secondary ion mass spectrometry (SIMS).

MALDI-MS and MALDI-FTICR-MS imaging were used to measure methamphetamine in longitudinally sectioned hair samples [12, 13]. MALDI-FTICR-MS imaging was also used to monitor the distribution of ketamine in longitudinal scraped scalp hairs from chronic users [14]. MALDI-MS/MS imaging has also been used for the detection of nicotine in longitudinally sectioned hair from heavy smokers [15].

Recently, MALDI-MS/MS imaging was employed to investigate the incorporation of methoxyphenamine (an analogue of methamphetamine) into hair. The analysis was performed at various time points on single longitudinal sectioned hairs from volunteers after a single oral administration [16]. The consequences of different washing procedures on the distribution of cocaine in hair were recently investigated using MALDI-MS/MS and metal-assisted secondary ion mass spectrometry (MetA-SIMS) imaging [17].

In this chapter, we present two methods for the preparation of longitudinal and cross-sections of single hair samples for the analysis of drugs of abuse by MALDI-MS/MS and MetA-SIMS imaging.

2 Materials

2.1 Chemicals and Materials

1. Gelatin from porcine skin.
2. Double-sided copper tape.
3. Double-sided tape.
4. Methanol (MeOH).
5. Dichloromethane (DCM).
6. Trifluroacetic acid (TFA)
7. α-Cyano-4-hydroxycinnamic acid (CHCA) matrix solution (10 mg/mL in 70:30 MeOH: H_2O with 0.2% TFA).
8. Cocaine base.
9. Indium tin oxide (ITO)-coated glass slides, 25 × 50 × 1.1 mm, 4–8 Ω resistance
10. Small magnets.
11. Plastic tubes.

2.2 Instruments

1. Cryo-microtome.
2. Bruker ImagePrep matrix application device.
3. Leica DMRX light microscope equipped with a digital camera.
4. Quorum Technologies sputter coater equipped with a gold target, quartz crystal microbalance, and film thickness monitor.
5. Waters MALDI HDMS Synapt mass spectrometer equipped with a 200 Hz, 335 nm neodymium-doped yttrium aluminum garnet (Nd:YAG) laser.
6. Physical Electronics TRIFT II TOF-SIMS mass spectrometer equipped with an Au liquid metal ion gun tuned for 22 keV Au^+ primary ions.

2.3 Software

1. BioMap 3.7.5.5 software.
2. Waters MALDI imaging pattern creator software.
3. Waters MALDI imaging converter software.
4. Waters MassLynx 4.1 software.
5. Physical Electronics WinCadence 4.4.0.17 software.
6. Physical Electronics Vacuum Watcher 3.1.4.3 software.

3 Methods

3.1 Sample Decontamination

1. Intact single hair samples were placed into a glass vial containing 10 mL of dichloromethane and shaken for 1 min (*see* **Notes 1** and **2**). Retain the wash solutions for further analysis, if required.
2. The hair samples were left to dry at room temperature before further preparation.

3.2 Longitudinal Sectioning

1. The in-house built cutting apparatus shown in Fig. 1a consists of a stainless-steel block (60 × 110 × 10 mm) with 20–80 μm grooves spaced 5 mm apart, which is based on previously reported specifications [18]. In addition to this is the cutting device, which is also made from stainless steel and holds half of a cryo-microtome blade at a 30° angle [19]. The schematics of the cutting apparatus are shown in Fig. 1b.
2. Following decontamination, place a single hair sample into one of the grooves of the cutting block and fix at one end with a small piece of double-sided tape, as shown in Fig. 2a (*see* **Notes 3–5**).
3. While holding the other end with a gloved finger, slowly run the cutting device along the length of the hair (Fig. 2b).
4. Place another piece of double-sided tape on the other end of the hair and transfer onto double-sided copper tape mounted on a ITO glass slide, press the hair into the tape using a clean glass slide (Fig. 2c).
5. Use a Leica DMRX microscope equipped with a digital camera to determine if the hair section has been successfully transferred onto the glass slide.

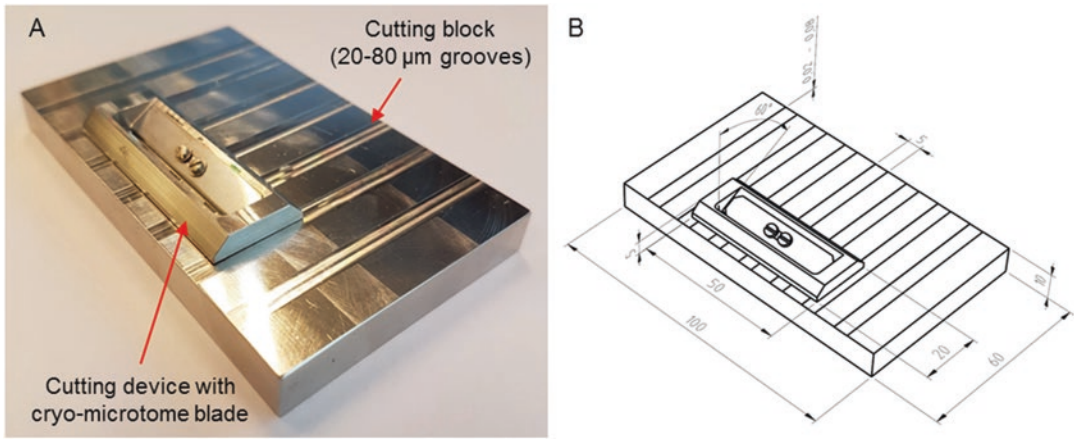

Fig. 1 Cutting apparatus used for longitudinal sectioning of hair samples. (**a**) Image of the in-house built cutting apparatus and (**b**) schematic of the cutting apparatus

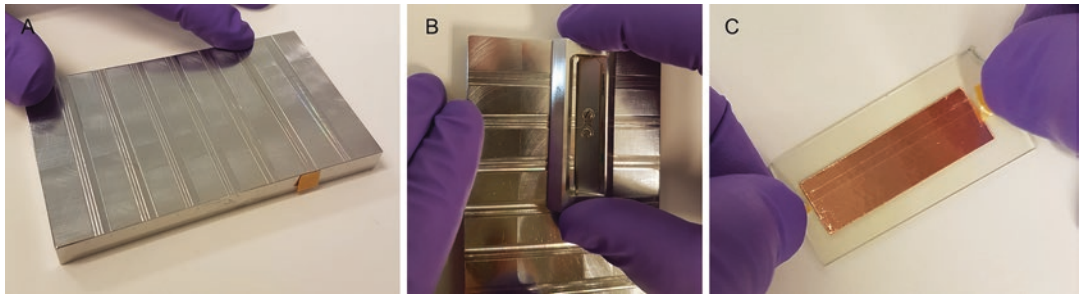

Fig. 2 Method for preparing longitudinal sections of single hair samples. (**a**) Hair sample attached to the cutting block with double-sided tape, (**b**) preparing longitudinal sections of single hair samples using in-house built cutting apparatus, (**c**) longitudinal sections mounted on conductive glass slide with double-sided copper tape

3.3 Preparation of Cross-Sections

1. Following decontamination, thread the hair sample through the lid of the tube and using a paper clip, attach two small magnets at the end of the single hair sample. Attach two more magnets at the opposite end of the hair sample.
2. Place the hair sample into a plastic tube that contains a 10% solution (w/v) of gelatin, as shown in Fig. 3b (*see* **Note 6**).
3. Snap-freeze the contents by placing the plastic tube in liquid nitrogen for 30 s (Fig. 3c).
4. Remove the embedded hair from the plastic tube and cut into 1 cm blocks (the average growth of hair being 1 cm/month), mount one of the blocks onto a cryo-microtome stage using a drop of water.
5. Section the embedded hair samples at −20 °C using a cryo-microtome to produce 12 μm thick sections, thaw mount sections onto a clean indium tin oxide (ITO) glass slide (*see* **Note 7**).
6. Inspect the sections using a Leica DMRX microscope equipped with a digital camera to determine the position of the hair cross-section and mark the location on the opposite side of the glass slide using a permanent marker pen.

3.4 Matrix Deposition for MALDI-MS/MS Imaging

1. Place the sample in the ImagePrep matrix application device.
2. Fill the bottle with the matrix solution.
3. Select the manufacturer's method for spraying CHCA and start the sequence.
4. Use a Leica DMRX microscope equipped with a digital camera to inspect the crystal coverage.

3.5 Metal Deposition for MetA-SIMS Imaging

1. Place the sample in the chamber of the Quorum Technologies sputter coater and close the lid.
2. Select the density of the metal (19.30 g/cm^3 in the case of gold) and the desired thickness (1 nm) on the film thickness monitor.

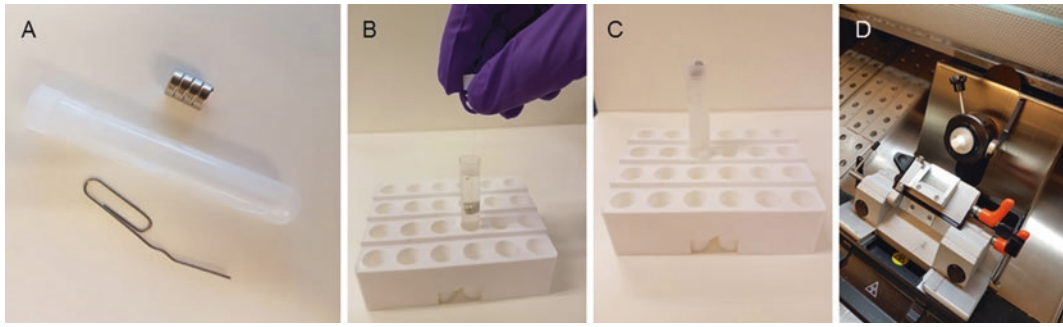

Fig. 3 Method for embedding single hair samples. (**a**) Materials required for the embedding of single intact hair samples, (**b**) single hair sample clipped between magnets and placed in a plastic tube filled with embedding material, (**c**) embedded hair sample following snap freezing, and (**d**) cryo-sectioning of embedded hair samples

3. Set the discharge voltage to 1.5 kV and the plasma current to 25 mA in order to achieve a homogenous coating.
4. Start the sequence (operate in automatic mode), once the sequence is complete vent the instrument and remove the sample (*see* **Note 8**).

3.6 MALDI-MS/MS Imaging

1. Calibrate the instrument prior to analysis with either a standard mixture of polyethylene glycol (PEG 200-3000) in water mixed with matrix or a saturated solution of red phosphorus in acetone.
2. Spot 0.5 μL of a cocaine base standard (100 ng/μL in 70% MeOH) onto a MALDI target plate, followed by 0.5 μL of the matrix solution.
3. Optimize the instrumental settings using the cocaine base standard such as the laser power and collision energy (trap cell). The optimal settings were as follows: laser power 250 (200 Hz) and collision energy 10 eV (monitor the main product ion of cocaine at m/z 182 formed by neutral loss of benzoic acid).
4. Following method optimization, attach the sample onto a glass slide adaptor using double-sided tape and scan using a flatbed scanner to produce a digital image, ensure the image quality is around 600 dpi or better.
5. Import the digital image of the sample into the MALDI imaging pattern creator software, to define the area to be imaged and the spatial resolution (150 × 50 μm).
6. Set up the MS/MS imaging method in the MassLynx 4.1 software. The settings were as follows: positive ion mode, V-mode, mass range m/z 50–350, also use the previously optimized settings (*see* **step 3**).

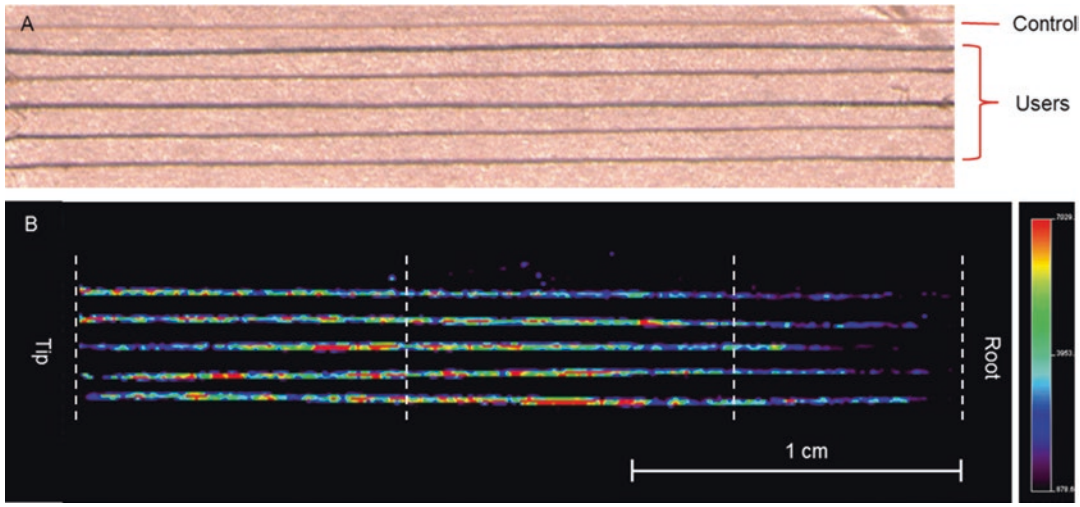

Fig. 4 MALDI-MS/MS imaging of longitudinal sectioned drug user hair. (**a**) Optical image of longitudinal sectioned control and drug user hair samples mounted on double-sided copper tape and (**b**) MALDI-MS/MS image showing the distribution of the cocaine product ion at m/z 182. The length of the hair samples analyzed was 2.7 cm, given that the average growth rate of human hair is around 1 cm per month this corresponds to a growth period of approximately 3 months. Since the spatial resolution along the hair is 150 μm, each pixel is equivalent to around 12 h of growth (reproduced from ref. 19 with permission from John Wiley & Sons, Ltd)

7. Following data acquisition, convert the raw data (.raw) into the Analyze file format (.img) using the MALDI imaging converter software.
8. Visualize the converted data using the BioMap 3.7.5.5 software (Fig. 4).

3.7 MetA-SIMS Imaging

1. Place the sample in the target holder and scan using a flat-bed scanner to produce a digital image, import the image into the stage control window.
2. Load the sample into the instrument and locate the sample using the scan in the stage control window and fine tune with the joy-stick.
3. Use a single 100 × 100 μm tile (containing 256 × 256 pixels) for imaging of hair cross-sections, images were acquired in positive ion mode for 15 min.
4. Use the mosaic mapping function to image a portion of the longitudinally sectioned hair sample. Images were acquired on a 2 mm portion of the longitudinally sectioned hair sample, which consisted of 32 × 7 tiles (each tile was 100 × 100 μm containing 256 × 256 pixels).
5. The results are calibrated using elements such as sodium (*m/z* 23), potassium (*m/z* 39), indium (*m/z* 115), or gold (*m/z* 197). Common hydrocarbon fragments such as CH_3^+ (*m/z* 15), $C_2H_3^+$ (*m/z* 27), and $C_4H_7^+$ (*m/z* 55) can also be used.

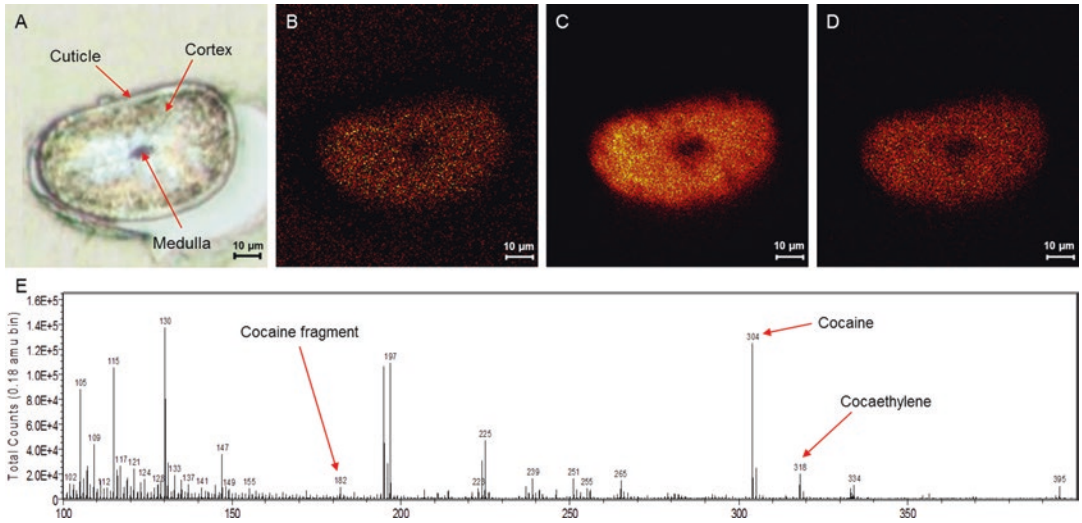

Fig. 5 MetA-SIMS imaging of cross-sections of drug user hair. (**a**) Optical image of hair cross section, (**b**) MetA-SIMS images showing the distribution of a cocaine fragment at m/z 182, (**c**) cocaine at m/z 304, (**d**) cocaethylene at m/z 318, and (**e**) average positive ion spectrum from drug users hair. The thickness of the cross-section was 12 µm, given that the average growth rate of human hair is around 1 cm per month this corresponds to a growth period of around 1 h

6. Select peaks of interest and visualize using the WinCadance 4.4.0.17 software (Figs. 5 and 6).

4 Notes

1. Decontamination of hair samples prior to analysis is an important step that serves two purposes. The first is to remove hair products, sweat, sebum, and other surface materials that can interfere with the analysis. The second is to remove any drug material deposited on the hair from sweat or environmental contamination [20].
2. The Society of Hair Testing (SOHT) currently recommends the use of organic solvents such as acetone or dichloromethane for decontamination, as aqueous solutions or methanol can cause the hair to swell causing extraction or incorporation of external contamination [20]. However, various laboratories and law enforcement agencies will have their own validated decontamination procedures.
3. Approximately 0.3 cm of the hair is covered with double-sided tape to fix the hair onto the cutting block and to transfer to the glass slide.
4. The minimum length of hair that can be prepared with the cutting apparatus is 1 cm, this is due to the maneuvering of the cutting device.

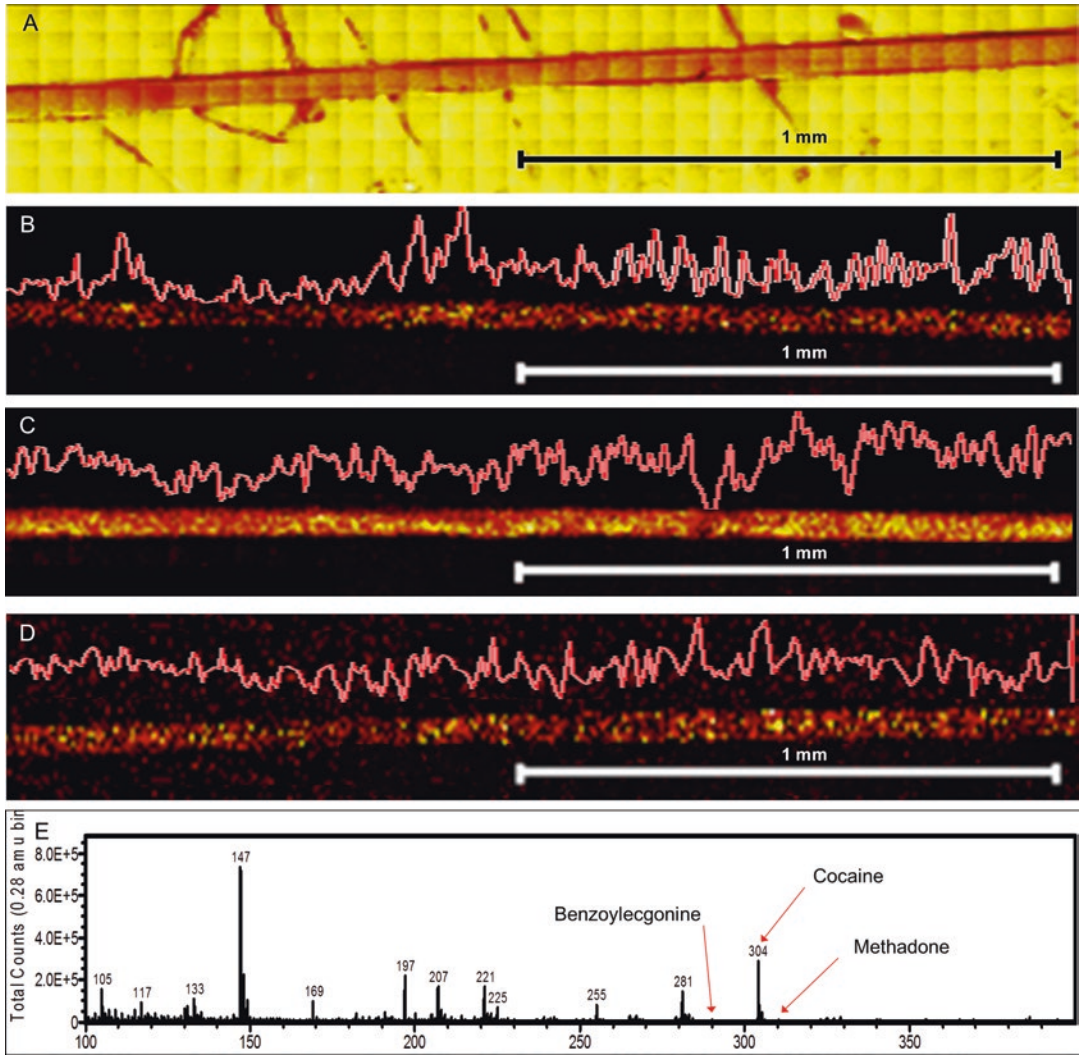

Fig. 6 MetA-SIMS imaging of longitudinal sectioned drug user hair. MetA-SIMS images of the (**a**) total ion current, (**b**) distribution of benzoylecgonine at m/z 290, (**c**) cocaine at m/z 304, (**d**) methadone at m/z 310, and (**e**) average positive ion spectrum from drug users hair. The area of the analyzed sample was 2 mm, given that the average growth rate of human hair is around 1 cm per month this corresponds to a growth period of 6 days. The smallest pixel size that can be achieved is 1 μm, which is theoretically equivalent to around 5 min of hair growth (reproduced from ref. 19 with permission from John Wiley & Sons, Ltd)

5. The maximum length of hair that can be prepared with the cutting apparatus is 6 cm, longer hairs need to be cut into segments and sectioned individually.
6. A 1% or 2% (w/v) solution of carboxymethylcellulose (CMC) or a 10% (w/v) solution of trehalose was also found to be suitable materials for embedding single intact hair samples.
7. As the ITO slides come wrapped in plastic it is necessary to clean them with hexane and ethanol, as polydimethylsiloxane

(PDMS)-related peaks can hinder the analysis of compounds in the low mass range with TOF-SIMS.

8. Gold coating has previously been shown to improve the secondary ion yield in SIMS, as well as to reduce surface charging on insulating samples [21].

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