Chapter 9
Recent Technological Developments in MALDI-MSI Based Hair Analysis

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Abstract Hair is a common piece of trace evidence found at a crime scene, however, often it is not possible to obtain DNA (due to the lack of a follicular root). These hair samples could potentially provide other intelligence, based on the molecular history of an individual that it contains. Currently, this type of analysis is performed using traditional hyphenated techniques gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS). However, these techniques require a large amount of hair, not a few single strands such as those typically found at a crime scene and also involve extensive sample preparation. Recently new technologies such as matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) have been used to monitor the distribution of drugs of abuse in single hair strands. Using this technology it is possible to reveal the distribution of compounds in the hair more accurately and in single strands as opposed to milligram quantities required by traditional hyphenated methods. The use of MALDI-MSI could provide law enforcement agencies with lifestyle information on an individual and help to narrow down the pool of suspects.

Keywords Hair · MALDI-MSI · Drugs of abuse · Medication

9.1 Incorporation of Compounds into Hair

Hair is a fibre that covers a large portion of the body in mammals and originates from the follicle, which is located 3–4 mm below the dermis. The follicles are surrounded by a network of capillaries that supply blood to the growing hair. Hair grows in

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three phases; firstly it is in the anagen phase (active growing), then it moves into the catagen phase (transition) and finally the telogen (resting) phase. During the anagen phase hair grows from the bulb and this phase can last several years. The hair stops growing during the catagen phase and a keratinized club is formed in the follicle. This is then ready to fall out in the telogen phase and the process may then start again [1, 2]. During the growth of the hair, compounds are incorporated into the hair shaft. There are several ways in which this can occur, the most common route is via the bloodstream during the anagen phase of hair growth. Other methods include incorporation via sweat/sebum after hair formation and from environmental exposure (e.g. via contact with contaminated hands or smoking). It is also possible that the compounds can be incorporated into the hair from the surrounding tissue [3, 4]. These processes are illustrated in Fig. 9.1.

The hair shaft consists of three parts: (i) the cuticle, which makes up the exterior of the hair and is made up of overlapping scale-like cells, (ii) the cortex, consisting

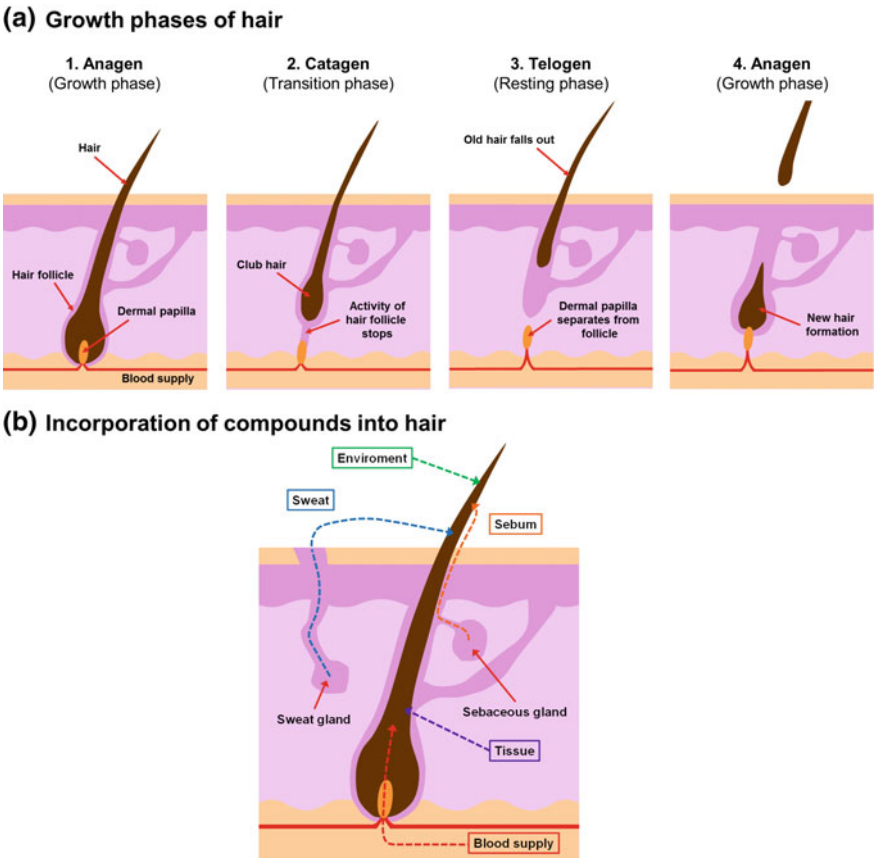

Fig. 9.1 Diagrams showing **a** the growth phases of hair and **b** incorporation routes of compounds into the hair

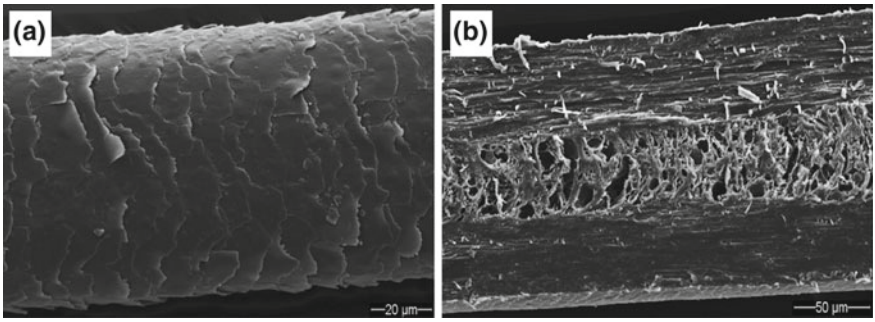

Fig. 9.2 Scanning electron microscope (SEM) images of hair samples. **a** Intact hair sample showing the exterior cuticle and **b** longitudinally sectioned hair sample showing the medulla surrounded by the cortex

of a fibrous structure and the location where the drug is bound and (iii) the medulla, at the centre of the hair consisting of a hollow honeycomb like structure [4]. Detailed images showing the different regions of hair are shown in Fig. 9.2.

Hair is common trace evidence found at a crime scene but usually in single strand quantities. In forensic science, nuclear DNA profiling of the follicular root of hair is often the best way to identify a suspect, however not all hairs found at a crime scene contain the root (falling out during the telogen phase). Mitochondrial DNA of the hair shaft is possible but cannot be used to definitively identify a suspect, unlike nuclear DNA and thus is of lower evidentiary value. Many forensic laboratories lack the facilities to perform this type of genetic test.

Microscopic comparative hair analysis is also a possibility, which involves comparing characteristics such as pigment distribution and scale patterns from hair of a suspect to those found at a crime scene. However this technique has come under a lot of scrutiny recently, which resulted in several convictions being overturned [5].

Hair testing for the detection of drugs of abuse is a powerful tool routinely used in toxicology and forensic applications as it offers several advantages of other biological samples such as prolonged detection window, which has clearly been demonstrated by the analysis of compounds such as cocaine in the hair of ancient mummies from civilizations dating back a millennia [6]. In forensic toxicology this gives us the opportunity to detect drugs in hair samples well after they have left the body, comparatively urine and blood only offer a short term exposure window (hours to days generally). In addition, hair sample collection is non-invasive and requires no special storage conditions like other biological matrices.

In this chapter, the feasibility of using MALDI-MSI in forensic analysis of hair is explored. The current applications, as well as the current limitations of the technique and its future in forensic hair testing are discussed.

9.2 Traditional/Current Methods of Analysis

Hair analysis is usually preceded by lengthy sample preparation procedures. Firstly, the hair sample may be segmented into the time period of interest; an estimation of 1 cm per month is used. Samples are often segmented into 1 or 3 month periods, but with the quantity required being 20–100 mg. Shorter hair samples require a thicker clump of hair consisting of hundreds of individual hairs. The general sample work up process involves: decontamination of the hair to remove external contamination, extraction of the drug from the hair to remove the drugs from the keratin matrix, sample clean up to preferentially extract the drugs of interest over matrix components (including steps such as derivatization) and finally analysis, which may involve reconstitution of the sample in the desired solvent. Vogliardi et al., conducted a comprehensive review of sample preparation methods for analysing drugs from hair samples [7]. To quantify the drugs present, a calibration curve is built by spiking known quantities of the drugs onto hair from non-drug users, to be extracted concurrently to the suspect hair samples. The use of internal standards is widespread, usually with deuterated analogues of the drugs of interest. Most drugs are present within the samples at levels of pg/mg to high ng/mg of hair, depending on the drug and its uptake into hair and the amount of drug used. The Society of Hair Testing has proposed some cut off values for commonly encountered drugs of abuse as well as some outline of procedures that should be followed in casework [8]. Hair analysis is used in a variety of applications amongst which: in child drug exposure cases, exploration of drug use trends, family court cases, pre-employment screening, following a driving ban and drug-facilitated offences [9–11].

Some of the initial analyses of hair used immunoassays to detect drugs [12–14]. Immunoassays are still in use but mainly found in high throughput laboratories where they are employed as a screening tool for classes of compounds such as opiates or benzodiazepines, prior to confirmatory analysis using a hyphenated technique.

Gas chromatography-mass spectrometry (GC-MS) has been used extensively to detect drugs from hair samples, usually employing an electron ionization source coupled to a single or triple quadrupole mass spectrometer. Due to the varied sample preparation methods required for different types of drugs, methods are often developed for drugs, such as cocaine [15, 16] amphetamines [17], opiates [18] or cannabinoids [19]. There have also been many methods developed for a more comprehensive screening using GC-MS [20–22]. The volatility of some compounds is too low to be analyzed via GC so sample derivatization is often needed, thus adding an additional sample preparation step; cannabinoids are an example of drugs requiring such treatment. Derivatization for GC-MS may also be required for other objectives such as to decrease fragmentation or to improve isomer separation (both examples pertinent to analysis of amphetamines). The use of hair samples to detect markers of alcohol consumption and abuse has also been proposed, with methods for ethyl glucuronide [23] and fatty acid ethyl esters [24] being available for GC-MS. Again, the Society of Hair Testing has proposed some guidelines for the interpretation of these results which is not without difficulty or debate [25].

The use of liquid chromatography-mass spectrometry has developed more recently with improvements over GC-MS. The absence of a derivatization step reduces sample work up and enables the analysis of many compounds in a single run. The development of multi-analyte screens, with many more metabolites incorporated into the method or indeed several drug classes being detected in a single run, has made hair analysis more appealing and versatile [9, 26, 27]. A much larger number of drugs can be detected from a single screen (>100 compounds) with high resolution mass spectrometry [28–30]. Further extensive reviews of the current analytical methods for hair analysis are available in the literature [31].

9.3 MALDI-MSI and Current Applications in Hair Analysis

Matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) is a label free technique that can simultaneously monitor the distribution of drugs and metabolites. The technique also maintains the spatial localization of the compounds within a sample and can generate high spatial resolution images (<10 μm lateral resolution). The readers are directed to a series of review papers for a more detailed description of the technique and its various applications [32, 33]. A typical MALDI-MS imaging experiment of hair samples is illustrated in Fig. 9.3.

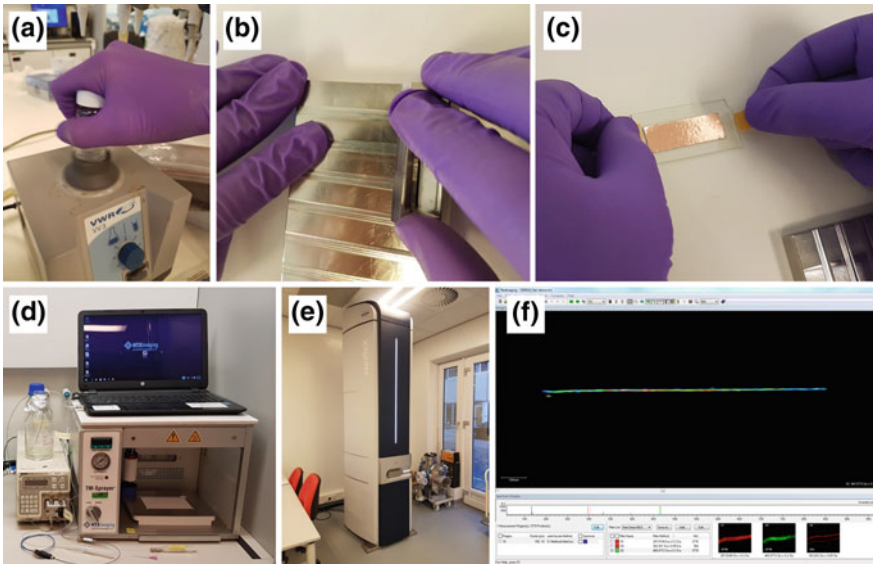

Fig. 9.3 Workflow for MALDI-MS imaging of hair samples. **a** Decontamination of hair samples, **b** longitudinal sectioning of hair samples, **c** mounting of hair samples, **d** matrix application, **e** MALDI-MS imaging and **f** data analysis and timeline visualization

As the workflow in Fig. 9.3 shows, the hair samples are first washed with one or a series of solvents (e.g. water, methanol or dichloromethane) to remove hair products, sweat and sebum. The washing step also removes other external contaminants such as any drug related compounds that could have been deposited from the environment. The hair samples are then longitudinally sectioned using a bespoke cutting device to expose the inside of the hair [34]. The hair samples are subsequently transferred and mounted onto a glass slide using double-sided conductive tape, after which the hair samples are coated with the MALDI matrix using an automated matrix sprayer (e.g. TM-sprayer, HTX technologies). Analysis is then performed using a MALDI-MS instrument (e.g. RapifleX, Bruker Daltonics) and images are constructed using vendor specific software (e.g. FlexImaging, Bruker Daltonics).

An advantage of MALDI-MS imaging of hair is the simpler and faster sample preparation, which can be accomplished in around 1 h. Traditional sample preparation time can run into days with the requirement to extract the drug from the hair and often perform lengthy clean up protocols. Another advantage is the improved time frame for compound detection that can achieved; the spatial resolution along the length of the hair determines the time frame. For example, a pixel size of 150 μm is equivalent to 11 h of growth, which is far more accurate than the current technique that provides this information per month. It is also of note that for MALDI-MSI the instrumental spatial resolution and sensitivity are continuously improving. The smallest pixel size that can be obtained with current state-of-the-art MALDI-MSI instrumentation (e.g. RapifleX, Bruker Daltonics) is ~5 μm [35], which corresponds to a growth time of approximately 0.36 h (22 min).

9.3.1 Drugs of Abuse

Over the last decade MALDI-MSI is increasingly being used for the analysis of hair and by far, the most common application is monitoring the distribution of exogenous compounds such as drugs of abuse in hair samples.

Vogliardi et al., initially explored the feasibility of using MALDI-MS for the analysis of drug of abuse in hair, in order to detect cocaine and its metabolites in authentic user hair samples. The authors used a sample preparation similar to that employed by the established analytical techniques, e.g. pulverization and extraction [36, 37]. The extract was then spotted onto a target plate treated with graphene and coated with the matrix α-cyano-4-hydroxycinnamic acid (CHCA) using an electrospray deposition system. Analysis showed the presence of cocaine and its metabolites benzoylecgonine and cocaethylene. Using this method a limit of detection of 0.1 ng/mg could be achieved for cocaine in the hair matrix. Whilst no imaging experiments were performed, and hence only limited temporal information was obtained, this work demonstrated the feasibility of detecting drugs of abuse from hair samples by MALDI-MS and laid the foundation for future imaging experiments.

Following this, Miki et al., monitored the distribution of methamphetamine in longitudinal hair sections obtained from a chronic user [38, 39]. Manual longitudinal

sections of hair samples were prepared to gain access to the inside of the hair, where the drug is bound. The authors also attempted to cut hair samples with the use of a laser microdissection system, however this resulted in poor mass spectral quality possibly due to thermal degradation. MALDI-time-of-flight (TOF)-MS imaging was performed on the hair samples. The resulting image showed, as the authors described it a "barcode-like" pattern, which arises from repeated drug administration. MALDI-Fourier transform ion cyclotron resonance (FTICR)-MS imaging was performed to provide further confirmation on the identity of methamphetamine. The images showed the same pattern as previously seen in the MALDI-TOF-MS images and the exact mass and tandem mass spectrometry (MS/MS) confirmed the identity.

Porta et al., monitored the distribution of cocaine and its metabolites benzoylecgonine, ethylcocaine and norcocaine in single intact hair samples from chronic users [40]. The hair samples were washed and mounted onto a MALDI target plate, then manually coated with CHCA using a TLC sprayer. A dilution series of cocaine and its metabolites was prepared and spotted next to the hair samples in order to provide quantitative information. Deuterated versions of these compounds were added to matrix solution to act as an internal standard. MALDI-MS/MS imaging was performed using a MALDI-triple quadrupole linear ion trap equipped with a high repetition rate laser.

Shen et al., monitored the distribution of ketamine in single hair samples obtained from a chronic user [41]. The surface of the hair samples was gently scraped off using a scalpel to expose the inside for analysis. The matrix was applied manually to the hair before analysis by MALDI-FTICR-MS. The images obtained from four hair samples taken from the same donor, showed the distribution of ketamine throughout three of the four hairs analyzed, however only trace amounts were found in the second hair sample.

Kamata et al., investigated the incorporation rates of methoxyphenamine (an analogue of methamphetamine) into hair using MALDI-MS/MS imaging [42]. The analysis was performed at various time points on single longitudinal sectioned hairs from volunteers after a single oral administration of the medication. The images showed the drug was localized in the root region below the scalp (4 mm) after 24 h; the images of the hair samples 3–7 days post administration showed that the drug then began to migrate from the root region to the scalp surface region of the hair. After 2 weeks the drug was localized in the hair above the scalp surface and absent from the root region.

Beasley et al., monitored the distribution of Δ^9-tetrahydrocannabinol (THC) in authentic user hair samples by MALDI-MS/MS imaging following derivatization [43]. The authors utilized 2-fluoro-1-methylpyridinium toluenesulfonate (FMPTS), a pre-charged reagent, to improve the sensitivity and detection of a range of cannabinoids and their metabolites. Recently Kernalléguen et al., monitored the distribution of synthetic cannabinoids in hair samples from self-reported users [44]. Hair samples were longitudinally sectioned and analyzed using MALDI-MS/MS imaging without any derivatization.

9.3.2 Medication

Within the management of chronic conditions, compliance to medications is crucial for the maintenance of the patient's health. The ability to monitor a patient's compliance to medication over a long period would be a great benefit to health care providers. This was demonstrated by Rosen et al., who monitored the distribution of the antiretroviral efavirenz, which is used in the treatment of HIV. The distribution of the drug was monitored in hair samples from three HIV infected patients using infrared-matrix-assisted laser desorption electrospray ionization-mass spectrometry imaging (IR-MALDESI-MSI). Normalization of the data was accomplished using pyrrole-2,3,5-tricarboxylic acid (PTCA), an oxidation product of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) which is a eumelanin biomarker to account for hair colour [45]. The advantages of this technique over conventional MALDI-MSI is that it does not require a UV MALDI matrix, which may interfere with analysis of low molecular weight compounds (<500 Da). A detailed description of the IR-MALDESI technique is reported elsewhere [46].

Poetzsch et al., monitored the distribution of tilidine (a synthetic opioid painkiller) in hair samples by MALDI-MS/MS imaging with complementary LC-MS/MS analysis [47]. Hair samples from children obtained from a forensic case involving suspected tilidine intoxication, as well hair from chronic users were analyzed. The images showed the compound was distributed throughout the hair samples even after a single dose, which led the authors to conclude this was most likely due to sweat contamination and incorporation into the hair matrix. Later the authors monitored the distribution of zolpidem in the hair of volunteers after a single dose using MALDI-MS imaging [47]. The images showed a band approximately 0.8 cm in length (~3.5 weeks of growth) a month after administration. Similarly Shima et al., monitored the distribution of zolpidem in moustache and head hair of volunteers after a single administration using MALDI-FTICR-MS imaging [48, 49]. The images showed the drug resided in the root area of the hair 24 h after zolpidem administration, after 35 days zolpidem was present further along the hair in a small band. Recently Wang et al., monitored the distribution of olanzapine in the hair of volunteers that had been taking the drug for 4 weeks [50]. The hair samples were collected and prepared for imaging by manually scraping the surface of the hair away. The authors used a novel matrix, esculetin, for analysis of the samples using MALDI-FTICR-MS imaging. A similar matrix was also used by the authors to monitor the distribution of methamphetamine [51].

An example illustrating the application of MALDI-MS imaging to detect medication in hair is shown in Fig. 9.4. Here, MALDI-FTICR-MS imaging was used to analyze hair samples obtained from an epilepsy patient, taking the anti-epilepsy medication carbamazepine (prescribed a daily dose of 800 mg). The image shows that the distribution of carbamazepine is consistent through the five hairs analyzed. This indicates that this patient is complying with their medication as prescribed. The image also shows the drug is absent from the control hair sample, which was obtained from a donor not using the medication. The slight variations in distribution

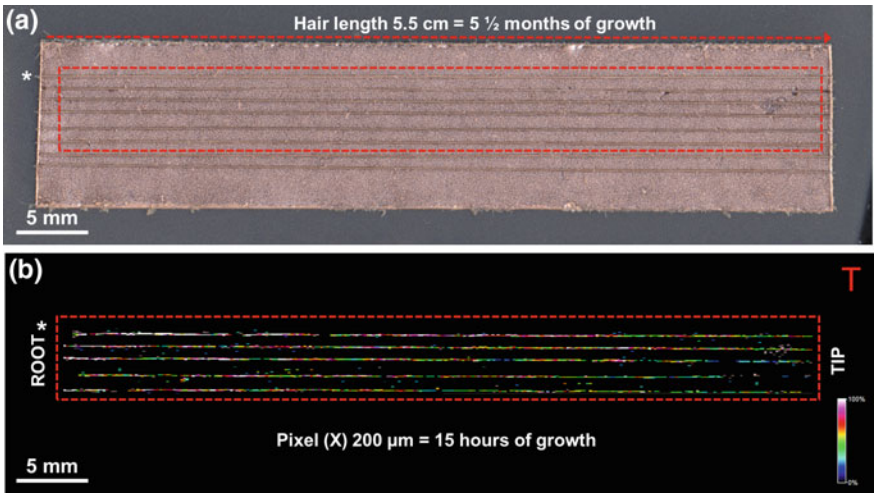

Fig. 9.4 MALDI-FTICR-MS imaging of carbamazepine user hair samples. **a** Optical image of longitudinally sectioned control and epilepsy patient hair samples following matrix application. **b** MALDI-FTICR-MS image showing the distribution of carbamazepine at *m/z* 237.1022. Analysis performed on the Bruker SolariX in continuous accumulation of selected ions (CASI) mode, spatial resolution of 200 × 20 µm and normalized with TIC (*control/drug free hair sample)

of the drug could be due to differences in growth phases or the preparation of the hair samples. Due to the use of a high mass resolving power instrument, the identity of the drug could also be confirmed.

9.3.3 Lifestyle Markers

Hair can also give an indication of personal lifestyle such as cigarette smoking. Nakanishi et al., demonstrated this opportunity by quantitatively monitoring the distribution of nicotine in single longitudinally sectioned hair from several heavy smokers [52]. Their work also shows that MALDI-MSI could be useful for confirming the cessation of smoking for health care practices.

9.3.4 Investigation into Forensic Procedures

MALDI-MS imaging has also been used to give insights into the effects of the decontamination procedure on the analysis of hair samples, as well as differentiate between external contamination and actual abuse. The consequences of different washing procedures on the distribution of cocaine in hair were investigated using MALDI-MS/MS and metal-assisted secondary ion mass spectrometry (MetA-SIMS)

imaging [53]. Intact hair samples that were externally contaminated with cocaine were washed with different solvents before analysis. The results showed that the most commonly used washing solvents, water and methanol, appeared to remove the most surface contamination. However, high spatial resolution imaging of longitudinal and cross-sections of contaminated hair samples showed the cocaine migrated into the hair giving rise to a false positive. If these samples had been analyzed by traditional methods they would have been called "a positive". This differentiation between external contamination and actual incorporation is only possible through the high spatial resolution analysis afforded by more advanced methods.

9.4 Current Challenges/Pitfalls of Hair Testing with MALDI-MSI

9.4.1 Growth Phases and Type of Hair

Whilst it has been demonstrated that the analysis of single hair samples is possible, one of the main challenges is the impact of the different growth phases on the interpretation of the results. As previously stated, hair undergoes cycles of growth and dormancy in three stages. The first is the anagen or growing phase which constitutes 85–90% of hairs, the catagen or transitional phase which is rare comprising only 1% of hairs and finally the telogen or resting phase consisting of 10–15% of hairs. The process then repeats and new hair begins to grow [4]. Variation in the distribution of a compound between hairs is often attributed to this but needs to be investigated more in depth. Another area that needs further investigation is the effect of the hair type on uptake and the distribution of a compound (e.g. arm, chest, leg or public hair), each having a different growth phase/rate. This is important as hair found at a crime scene may not always be scalp hair; for example in rape cases the most common hair type recovered is pubic hair. As such, the ability to interpret results from hairs other than head hair is very limited.

9.4.2 Sensitivity

There are several issues that can influence the sensitivity of detection of compounds in hair by MALDI-MSI. Unlike the established chromatography methodology, MALDI-MSI has no clean-up steps or separation prior to analysis. The latter can be compensated using ion mobility separation (IMS), which is accomplished immediately following ionization within the mass spectrometer. Several commercial instruments now have this analytical separation capability built into the instrument. The sensitivity is also associated with the efficiency of the extraction of the compounds from the hair. The sample preparation of currently established techniques

involves pulverization of the hair samples and the powder is then solvent-extracted with a procedure lasting from a few minutes to many hours. In contrast, MALDI-MS imaging of hair samples relies on local extraction of compounds directly from the hair, either through the cuticle of intact hairs or directly from the cortex of longitudinally sectioned hairs, which takes between 5 and 20 s and requires multiple passes or spray cycles using automated matrix sprayers prior to MALDI-MSI analysis. However, the preparation of the hair samples (e.g. intact vs. longitudinally sectioned hairs) for MALDI-MS imaging has an impact on the outcome. Longitudinal sectioning has been proposed to gain access to the centre of the hair, thus providing easier extraction of compounds [34, 38]. This is demonstrated in Fig. 9.5. As Fig. 9.5 shows, the exterior of the hair is coated with lauryl sulfate, an ingredient found in shampoos. The distribution of this species appears as two parallel lines in the longitudinally sectioned parts of the hair sample and clearly delineates the un-sectioned part of the hair. In contrast, the image of cholesterol sulfate (an endogenous metabolite) shows a higher intensity of the compound in the sectioned part, in comparison to the un-sectioned part. The overlay shows that these two species can discriminate between the interior and exterior of the hair sample. This example demonstrates the impact of the hair sample preparation on the detection of compounds and further illustrates the need for longitudinal sectioning of hair samples prior to the MALDI matrix application.

Some compounds have poor ionization efficiency, which limits the sensitivity of the method. In certain cases this can be overcome by derivatization prior to matrix

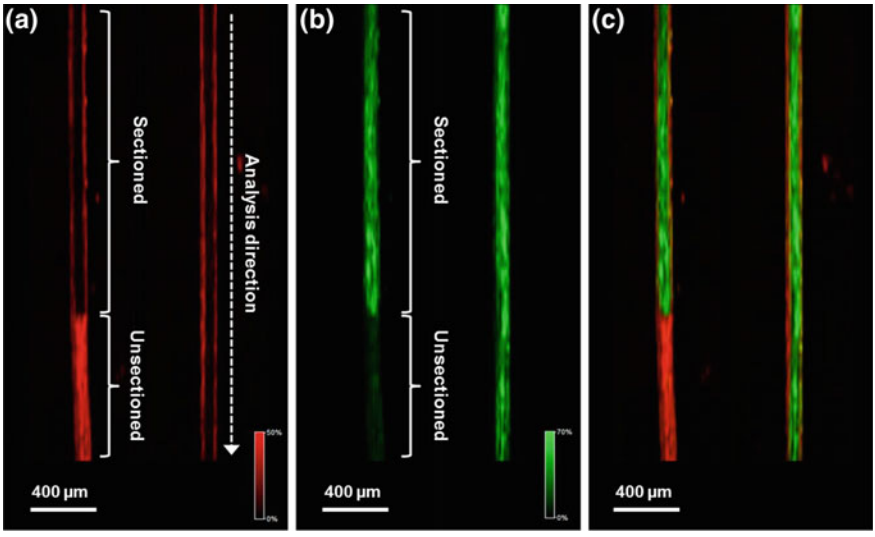

Fig. 9.5 MALDI-MS imaging of intact and longitudinally sectioned hair samples. MALDI-MS images showing the distribution of **a** lauryl sulfate at *m/z* 265.15, **b** cholesterol sulfate at *m/z* 465.30 and **c** overlay of selected species. Analysis performed on the Bruker RapifleX, spatial resolution of 100 × 10 µm and normalized with TIC

application. Beasley et al., recently demonstrated such a procedure with the analysis of cannabinoids in hair [43]. Interference from matrix peaks in the lower mass range (<500 Da) can also be an issue, however this can be overcome by targeted analysis such as tandem mass spectrometry (MS/MS), high mass resolving instruments (FTICR) or ion mobility separation.

9.4.3 Quantitation

Quantitation by MALDI-MSI is a topic of much debate in the field and as such, the methods used to provide the quantitative information vary across laboratories. All methods are typically complemented by established quantitation techniques. The most common quantitation method for MALDI-MSI involves spotting drug dilution series onto the glass slide next to the samples which are analyzed together [40]. However, this method does not accurately represent the response obtained from the sample, due to the difference in the matrix extraction efficiency of compound from the hair and the standards applied on the glass slide. Alternatively, spotting or spraying the dilution series onto control hair samples enables the calibration line to be matrix matched [54]. However, this method may not represent the matrix extraction efficiency. Ideally, the calibration curve should be matrix-matched and mimic the actual sample. Rosen et al., presented a method to prepare matrix matched standards, which involved incubating drug free hair samples in a dilution series of the drug [45]. The standards will also mimic the extraction of the drug from the actual hair samples with the MALDI matrix. However, the hair samples used to prepare the matrix matched standards need to be the same hair colour as the actual samples.

Hair can be considered a homogenous sample, the environment of which is not as complex as a tissue section. Poetzsch et al., demonstrated that the ionization of compounds is independent from the colour and the different structures of the hair. Therefore quantitation could be performed with or without an internal standard [55]. In contrast, Rosen et al., demonstrated that the hair colour does have an impact on the detection of compounds and developed a normalization method (using a melanin biomarker) to account for the variation [45]. This also correlates with the known phenomenon that darker hair bind compounds better than lighter coloured hair. However, the difference in findings is likely due to the different ionization methods employed in these studies. Regardless, if possible, the use of an internal standard is recommended to account for any signal variation and to normalize the images. Ideally, the internal standard should be an isotopically labelled version of the compound of interest or a structurally similar analogue. The internal standard can be incorporated into the MALDI matrix and homogenously applied onto the sample using automated matrix sprayers.

9.4.4 Limited Number of Compounds Detected

Despite the first demonstration of detecting compounds in hair being a decade ago and the increasing popularity of hair analysis with MALDI-MS imaging, only a selected number of compounds have been detected. In order to be implemented in forensic science, as well as other areas such as clinical screening to monitor patience compliance, the catalogue of compounds detected in hair needs to be expanded. A summary of the current compounds detected in hair is shown in Table 9.1.

As the table shows, the most commonly detected drug is cocaine, this is due to its high prevalence around the world, as well as its high ionization efficiency thus making it easy to detect. The table also shows that the most common MALDI matrix is CHCA, due to its versatility and sensitivity for the ionization of low to medium molecular weight compounds. Other MALDI matrices are being explored for hair analysis and as new matrices are being reported each year, the access to the detection new compounds is increasing. The table also shows that new compounds, such as synthetic cannabinoids, are now being detected in hair.

Table 9.1 List of compounds detected in hair by mass spectrometry imaging showing the matrix used and the limit of detection values as quoted by the authors (N/A indicates that no values were quoted in the referenced publication)

Compound	MALDI matrix	Instrument	Limit of detection	References
Cocaine and metabolites	CHCA	MALDI-MS/MS imaging	N/A	[54]
Cocaine and metabolites	CHCA	MALDI-MS/MS imaging	5 ng/mg	[40]
Cocaine	CHCA + graphite	MALDI-MS	0.1 ng/mg	[37]
Cocaine and metabolites	CHCA + graphite	MALDI-MS	N/A	[36]
Cocaine	CHCA	MALDI-MS/MS and MetA-SIMS imaging	N/A	[34]
Efavirenz	N/A	IR-MALDESI-MS imaging	1.6 ng/mg	[45]
Ketamine	CHCA	MALDI-FTICR-MS imaging	7.7 ng/mg	[41]
Methamphetamine	Umbelliferone	MALDI-FTICR-MSI	N/A	[51]

(continued)

Table 9.1 (continued)

Compound	MALDI matrix	Instrument	Limit of detection	References
Methamphetamine	CHCA	MALDI-MS and FTICR-MS imaging	N/A	[38, 39]
Methoxyphenamine	CHCA	MALDI-MS/MS imaging	100 fg/mm	[42]
Nicotine	CHCA	MALDI-MS/MS imaging	1.6 ng/mg	[52]
Olanzapine	Esculetin	MALDI-FTICR-MSI	N/A	[50]
Synthetic cannabinoids	CHCA	MALDI-MS/MS and MS^3 imaging	69 pg/mg	[44]
Tilidine	CHCA	MALDI-MSI	N/A	[47]
Zolpidem	CHCA	MALDI-FTICR-MSI	50 fg/mm	[48, 49]
Zolpidem	CHCA	MALDI-MS imaging	270 pg/mg	[55]
Δ^9-tetrahydrocannbinol	FMPTS + CHCA	MALDI-MS and MS/MS imaging	N/A	[43]

9.5 Conclusions and Future Prospective

The use of MALDI-MS imaging for hair analysis has been demonstrated with the currently reported work described in this chapter. MALDI-MS imaging has several advantages over the currently established techniques such as faster sample preparation, improved time frame of drug detection and visual representation of the data. The latter may become more and more important as it conveys a more accessible way for law enforcement agencies, court and juries to understand the data if presented as evidence. Even if MALDI-MS imaging may never replace the currently established techniques, it has been demonstrated that it can, at least, be a good complementary technique.

The application of MALDI-MSI to forensic hair analysis is now more feasible due to recent advances in matrix application devices that allow for fast and reproducible preparation of samples. Instrumental developments such as raster imaging [54] or new commercial instruments such as the RapifleX (Bruker Daltonics) [35] and more recently the uMALDI (Waters) source [56] now enable rapid analysis of samples. Whilst the cost of the instrumentation is an issue, this technology can be applied to other areas of forensic science, as shown in other chapters of this book. MALDI-MSI has already become routinely employed by other industries, such as the pharmaceutical industry for complementary analysis in drug discovery and development studies. Commercial screening of hair samples with MALDI-MS imaging is now available. This demonstrates a great interest in hair screening and a potential future market, which may branch out into forensics. Like all tools currently employed by forensic

scientists, MALDI-MSI will need to undergo further development and validation to mature into an invaluable and routinely used forensic tool.

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