Detection and mapping of Cannabinoids in single hair samples through rapid derivatization- Matrix-Assisted Laser Desorption Ionization Mass Spectrometry

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Detection and Mapping of Cannabinoids in single hair samples through rapid derivatization - Matrix-Assisted Laser Desorption Ionization Mass Spectrometry.

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ABSTRACT: The sample preparation method reported in this work has permitted for the first time the application of Matrix Assisted Laser Desorption Ionization Mass Spectrometry Profiling and Imaging (MALDI-MSP and MALDI-MSI) for the detection and mapping of cannabinoids in a single hair sample. MALDI-MSI analysis of hair samples has recently been suggested as an alternative technique to traditional methods of GC-MS and LC-MS due to simpler sample preparation, the ability to detect a narrower time frame of drug use and a reduction in sample amount required. However, despite cannabis being the most commonly used illicit drug worldwide, a MALDI-MS method for the detection and mapping of cannabinoids in a single hair has not been reported. This is probably due to the poor ionization efficiency of the drug and its metabolites and low concentration incorporated into hair. This research showed that the *in situ* derivatization of cannabinoids through addition of an N-methylpyridium group resulted in improved ionization efficiency, permitting both detection and mapping of Δ9-tetrahydrocannabinol (THC), Cannabinol (CBN), cannabidiol (CBD) and the metabolites 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH), 11-Hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC) and 11-nor-delta(9)-carboxy-tetrahydrocannabinol glucuronide (THC-COO-glu). Additionally, for the first time an in-source re-arrangement of THC was observed and characterised in this paper thus contributing to new and accurate knowledge in the analysis of this drug by MALDI mass spectrometry.

The use of hair as an alternative biological sample in toxicological analysis is well documented. This is due to the fact that hair offers a longer time frame to detect drug use than the more traditional blood or urine. By measuring the length of the hair and approximating the rate of hair growth (1 cm a month on average) [1], it is possible to estimate when specific drug intake occurred, over a time period as long as the length of the hair allows (weeks, months, or even years) [2]. This is in stark contrast to blood and urine analysis where most drugs are not able to be detected beyond a few hours to days after intake [3]. Some important applications of hair samples for 'retrospective' detection of drug intake include investigating drug facilitated crime, workplace testing, child protection cases, and therapeutic monitoring.

Hair analysis is often used to identify cannabis consumption. Cannabis continues to be the most widely used illicit drug in England and Wales, with an estimated 6.7% of adults having used cannabis in the last year [4] a higher percentage than the European average of 5.7% [5]. Δ-9-tetrahydrocannabinol (THC) is the main psychoactive constituent of cannabis. THC undergoes a complex hepatic metabolism based on oxidation and subsequent glucuronidation [6]. Since this enzymatic pathway is only present *in vivo*, metabolite detection has been suggested as a solution to external contamination problems associated with solely analysing THC content in hair samples [1]. The main oxidative metabolites of THC are 11-Hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-delta-9-tetrahydrocannbinol (THC-COOH). This molecule then undergoes glucuronidation (phase II metabolism) to form 11-nor-delta(9)-carboxy-tetrahydrocannabinol glucuronide (THC-COO-glu) [7] as shown in figure 1. Other cannabinoids routinely analysed in hair samples include the *Cannabis sativa* plant degradation products, cannabinol (CBN) and cannabidiol (CBD) [8–13].

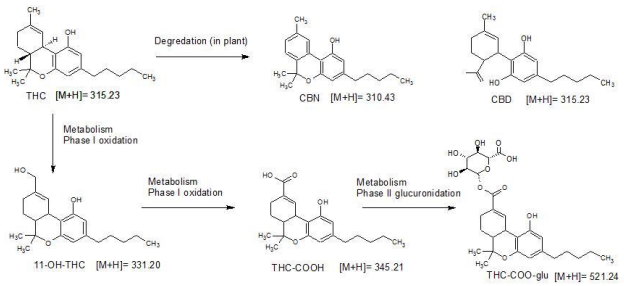

Figure 1 Degradation (ex-vivo) and metabolic (in vivo) pathway of THC

THC and associated cannabinoids and metabolites can already be detected in hair samples using standard analytical techniques such GC-MS [12,14–18] and LC-MS [19–23]. However, GC-MS requires multiple laborious and time consuming steps before chromatographic analysis can take place including digestion, extraction, sample clean-up and derivatization.

LC-MS has gained in popularity over recent years as the aforementioned derivatization step is often not needed for successful analysis. However, both methods require a large amount of hair sample (10-50 mg). GC-MS and LC-MS analyses typically give a time of intake accuracy of one month due to the common practice of segmenting the hair into 1 cm pieces before analysis.

More recently Direct Analysis in Real Time (DART) has been proposed as method for the analysis of cannabinoids and cocaine in hair samples [2,24], however this method is not able to distinguish between the two isobaric species of THC and CBD, despite MS/MS analysis due to both compounds resulting in the same product ions. In addition to this, a large sample size is required and currently the method is only applicable to high levels of THC associated with chronic users due to the detection limit being approximately 5 ng/mg of hair. The authors stated that DART "should only be considered as a rapid pre-screening method", however, this could result in false negative results for lower-level users.

Matrix Assisted Laser Desorption Ionization-Mass Spectrometry (MALDI-MS) has been highlighted as a potential hair analysis method due several advantages over current techniques including improved chronological information [25], simpler sample preparation and ability to detect drugs on one single hair. Several drugs have already been analysed in hair samples using MALDI MSI including methamphetamine [26] and analogues [27], cocaine [28–31], ketamine [32], Zolpidem [33], and nicotine [34] by utilising an α-Cyano-4-hydroxycinnamic acid (CHCA) matrix without the need for analyte derivatization. Cannabis products were determined in the work of Musshof et al [29], but they were unable to determine the difference between the isobaric THC and CBD and did not look for any *in vivo* metabolites.

In this study, initial experiments suggested the occurrence of an in source-rearrangement of the THC molecule; in addition to low analyte ionization efficiency, this highlighted low probability of success in mapping cannabinoids in hair samples by MALDI MS Imaging (MALDI MSI). However, the final method developed included the novel use of 2-Fluoro1-Methylpyridinium *p*-tolunesolfonate (FMTPS) derivatization of hair samples *in situ* and showed greatly improved detection of cannabinoids and metabolites, allowing these species to be mapped using MALDI-MSI.

EXPERIMENTAL SECTION

Materials and Reagents.

α-Cyano-4-hydroxycinnamic acid (CHCA), trifluoroacetic acid (TFA), 2-Fluoro-1-methylpyridinium-*p*-toluene-sulfonate (FMPTS) and triethylamine (TEA) were purchased from Sigma-Aldrich, UK. Canabinol (CBN), cannabidiol (CBD) Δ9-tetrahydrocannabinol (THC), 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH) 11-Hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC), delta 9-tetrahydrocannabinolic acid A (THCA-A) and 11-nor-delta(9)-carboxy-tetrahydrocannabinol glucuronide (THC-COO-glu) were purchased as analytical references from Cerilliant (Sigma-Aldrich, UK) Acetonitrile (ACN) and Methanol were purchased from Fisher Scientific, UK.

Sample preparation.

CHCA was prepared at 5 mg/mL with the solvent composition being 70:30 ACN:0.2% Aqueous TFA.

Cannabinoid standards were mixed 1:1 with the matrix solution and deposited in triplicate on the MALDI target. The spots were left to dry at ambient temperature before analysis. Cannabinoid concentrations were 100 µg/mL.

Derivatization of standards for MALDI profiling analysis.

Derivatization was carried out according to Thieme *et al.* [35]. Briefly, 40µl FMPTS (10 mg/mL in acetonitrile) and 10µl of triethylamine were mixed using a vortex. This caused the colorless solution to turn "canary yellow" as previously reported [35,36]. 20µl of each cannabinoid standard (100 µg/mL) was then added and left at room temperature for five minutes. 1 µL of each solution was then spotted onto a target plate.

Spiking of hair

Hair samples from an individual who reported not to have used any illicit drugs were collected by cutting and washed with methanol and water by vortexing. The samples were then cut into 5 cm sections and placed into the bottom of a well in a 24-well cell culture plate in order to keep the spiking solution volume to a minimum whilst still submerging the hairs. The limitation of five centimetres is due to the size of a MALDI target plate. Spiked samples were prepared by soaking in 300 µL of 0.5 µg/mL cannabinoid standard solution. Blank hair samples were prepared by soaking in 300 µL of methanol. The plate was sealed with tape to avoid evaporation of the standards. All hairs were soaked for two hours, removed and allowed to dry for one hour at room temperature.

User hair Sample

The hair sample collection was approved by the Sheffield Hallam University Research Ethics committee (SHU ethics number 13-2011). The hair sample was provided from a male volunteer who self-reported to smoke cannabis once a week. The hairs were less than 5 cm in length. To wash, the hairs were placed in a clean test tube with methanol (5 mL) and briefly vortexed, before being removed. This was repeated twice and the hairs were then left for two hours at room temperature to dry.

In situ derivatization of cannabinoids

The hair was placed on glass slide using double-sided Sellotape® Super Clear tape. 2.5 mL of derivatization reagent was then sprayed using a Neo for Iwata® air-brush at a pressure of 30 psi onto an area of 9 cm^2 with the sample in the centre of the area. This step was carried out in a fume hood due to hazards associated with the use of the tryethylamine catalyst.

Deposition of matrix for imaging

The hairs were coated in CHCA at 5 mg/mL with the solvent composition being 70:30 ACN:0.2% Aqueous TFA using the SunCollect autospraying system (SunChrom GmbH, Friedrichsdorf, Germany). Fifteen layers were sprayed at a flow rate of 2 uL/min.

INSTRUMENTATION

MALDI Instrumentation and analytical conditions

All data was acquired in positive ion mode on an Applied Biosystems/MDS Sciex hybrid quadrupole time-of-flight mass spectrometer (Q-Star Pulsar-*i*) with an orthogonal MALDI ion source (Applied Biosystems, Foster City, CA, USA) and a Neodymium-doped yttrium aluminium garnet (Nd:YAG) laser (355 nm, 1 KHz). The laser power was 30 % (1000 Hz, 3.2 µJ), which had an elliptical spot size of 100 × 150 µm [37]. Image acquisition was performed using the "raster image" mode [38]. Declustering potential 2 was set at 15 arbitrary units and the focusing potential at 20 arbitrary units, with an accumulation time of 0.999 sec. The MALDI-MS/MS images were obtained using argon as the collision gas; the declustering potential 2 was set at 15 and the focusing potential at 20, and the collision energy and the collision gas pressure were set at 20 and 5 arbitrary units, respectively.

Images were acquired using 'oMALDI Server 5.1' software supplied by MDS Sciex (Concord, Ontario, Canada) and processed using Biomap 3.7.5 software (www.maldi-msi.org) to generate black and white images for each *m/z* ratio of interest. Further Image analysis and processing was performed using the public domain software ImageJ (NIH; http://rsb.info.nih.gov/ij); where the previous black and white images were assigned different colors and overlaid to create one final image.

LC-MS Instrumentation and analytical conditions

All experiments were performed on a Thermo Finnigan LCQ™ 'classic' quadrupole ion trap liquid chromatography mass spectrometer with electrospray ionization (ESI) interfaced to a liquid chromatography system. The system used also consisted of an auto sampler and auto injector. The column used was a Phemonex Lunar® C18 (150 mm x 1mm, 5µm) with a corresponding guard column. LC-MS/MS Chromatographic separation was realised using gradient elution according to a previously published method by Roth *et al.* [39]. Briefly, 0.1% HCOOH in water was used as mobile phase A and ACN+ 0.1% HCOOH was used as mobile phase B. Mobile phase A was gradually reduced over time whilst mobile phase B was increased from 20 to 95%. The total run time was 15 minutes with the THC molecule eluting at 4 minutes.

RESULTS AND DISCUSSION

Profiling of THC

In preliminary MALDI MSP experiments analyses were carried out on the cannabinoid standard THC as purchased from the supplier. It was immediately observed a detection issue due to interference from matrix ion peak (*m/z* 315.10 as seen in Figure 1A, which is more apparent at concentrations lower than 100 µg/mL) in addition to a general low ionization yield in MALDI as previously reported [40]. For this reason, different matrix systems were trialled including type and amount of matrix 2,5-Dihydroxybenzoicacid (DHB), 6-Aza-2-thiothymine (ATT), 3-Hydroxycoumarin (3-HC) 2',4',6'-Trihydroxyacetophenone monohydrate (THAP), different solvent compositions, different amounts of Trifluoroacetic acid (TFA), and the addition of additives (Cetrimonium bromide (CTAB), Lithium salts and Aniline). In addition negative mode analysis was conducted with 9-Aminoacridine (9-AA) matrix. None of these experiments improved the detection of THC beyond that achieved with CHCA and will not be discussed further in this paper. Another observation from these MALDI profiling spectra was the presence of the *m/z* 313.22 and 315.23 (Fig 1A). While the *m/z* at 315.23 fitted the expected monoistopic *m/z* of THC, the *m/z* at 313.22 was unexplained. However, the lack of the peak at *m/z* 313.22 in the matrix blanks suggests that it is in fact associated with the THC molecule.

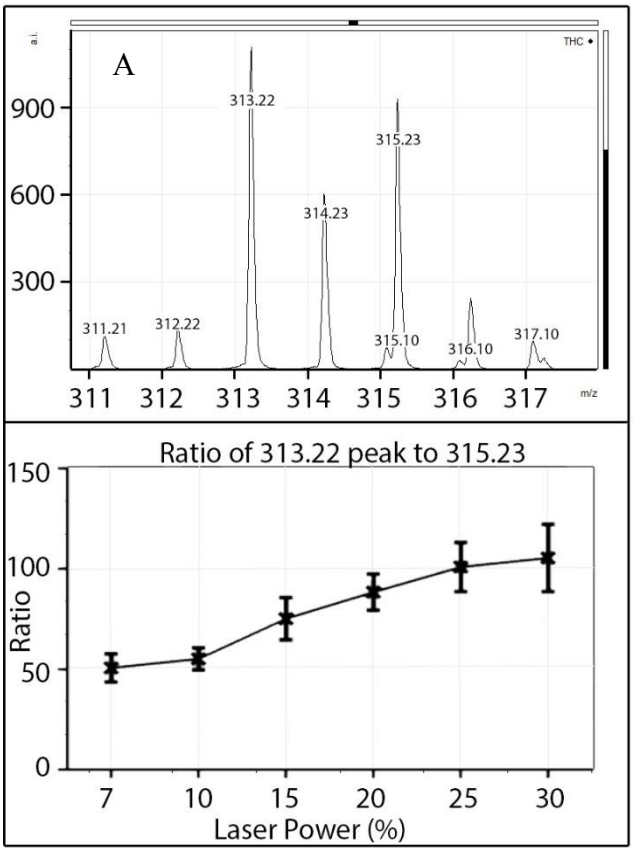

Figure 1. A- *m/z* region 311-317 of THC standard with CHCA matrix. B Ratio of signal intensity of *m/z* 313.22 to 315.25 at increasing laser energies.

In order to investigate this phenomenon further, LC-MS-MS analysis of the THC standard (100 ug/mL) was carried out. A single peak in the chromatogram confirmed the purity of the THC standard. Interestingly, the peak at *m/z* 313.22 was at 3% of the intensity of the *m/z* 315.23 peak (seen on MALDI at

approximately 110% (Figure 1A)) and the isotopic peak at *m/z* 314.23 was no longer detected. In addition to this, since this LC-MS system utilises Electrospray Ionization, it was reasonable to assume that the additional peak at *m/z* 313.22 is specific to the MALDI ionization process and was hypothesised that it could be dependent on the laser energy. In fact, experimentally it was observed that increasing laser power causes the ratio of *m/z* 313.22 to 315.23 signal intensity to increase (Figure 1B).

One possible explanation for this observation is a laser induced re-arrangement of the THC molecule. The loss of hydrogens as free radicals would increase the conjugation of the THC molecule, making the molecule more stable and so the rearrangement more favourable. MS/MS spectra of the *m/z* 313.1 and 315.1 obtained by direct infusion of the THC standard also support this theory; the MS/MS spectra of the parent ion at *m/z* 315.1 is shown in Figure 3A(i) and the MS/MS spectra of the re-arranged parent ion at *m/z* 313.1 is shown in Figure 2A(ii). The spectra are very similar with many fragments forming from common mass losses (peaks labelled with a star) demonstrating that these peaks refer to the same (THC) species. Both the parent ions and many of the product ions have a mass shift of -2 Th, suggesting the loss of two hydrogens from the THC parent ion.

Bijlsma *et al.* reported the fragmentation pathway of THC-COOH including fragments at *m/z* 193 and *m/z* 257 based on MS^E accurate mass data [41]. These fragments would be identical for THC-COOH and for THC due to the loss of the COOH group from the molecule. In this analysis the *m/z* 259 and 193 ions were also observed for the 315.1 MSMS, whilst we also observed a shift to *m/z* 257 in the MS/MS spectrum of the 313.1 parent ion. The 193 fragment was present in both of the MSMS spectra, indicating this fragment does not contain the proposed site of the rearrangement (See Figure 2B).

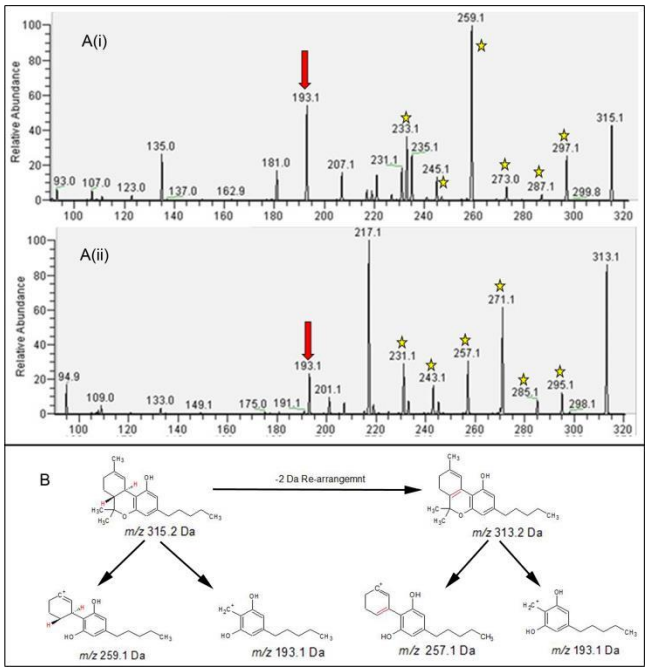

Figure 2. A. MS/MS spectra of THC. Panel A(i) and A(ii) show the Product ion mass spectrum of *m/z* 315 and of *m/z* 313 respectively. Both spectra were obtained through direct infusion on an LCQ instrument. Peaks with a star symbol denote a mass shift of 2 Th. Panel B shows the proposed rearrangement of the THC and structures of fragments present at *m/z* 259, 257 and 193 (257 and 193 structures as proposed by Bijlsma *et al.*).

Derivatization of cannabinoids

Once the nature of the peak at *m/z* 313.22 was elucidated, in order to avoid the rearrangement due to the laser energy a chemical modification of THC was carried out. Derivatization has previously been identified as a possible strategy to improve signal intensity and decrease matrix interference [42,43].

The target for the derivatization was the hydroxyl group since all cannabinoids of interest contain this functional group. After carefully reviewing the literature, the derivatization method using 2-Fluoro-1-Methylpyridinium *p*-tolunesolfonate (FMPTS) to form an N-methylpyridinium derivative, as reported by Quirke *et al.* for the detection of alcohols using electrospray ionization mass spectrometry, was chosen [36]. FMPTS derivatization has previously been reported to improve the detection of a range of compounds with alcohol moieties, in various sample types including surfactants [44], oestrogens [45] and the narcotic analgesic burprenorphine [35], using LC-MS analysis, and polyamides [42] and sterols [46] in MALDI profiling experiments.

This strategy was also selected due to the simplicity of the nucleophilic substitution reaction which occurs readily at room temperature [47], the stability of the products formed [44,48]and also the addition of a permanent charge to the analytes. This is of particular importance as it allows all cannabinoids to be analysed in positive mode analysis (despite the non-derivatized THC-COOH being theoretically more suited to negative mode).

Derivatization was successful for all cannabinoids of interest, with all peaks being observed and in agreement with the expected monoisotopic *m/z* values (Table 1). The derivatized species show an addition of 92 a.m.u. as first reported by Quirke *et al.* [36] and confirmed by others [45,46].

Cannabinoid	[M+H] Theoretical	[M+H] Experimental	Derivatized [M+92] Theoretical	Derivatized [M+92] Experimental
THC	315.23	315.23	406.27	406.28
CBN	311.20	311.20	402.24	402.24
CBD	315.23	315.23	406.27	406.28
11-OH-THC	331.23	331.23	422.27	422.26
THC-COOH	345.21	345.21	436.25	436.25
THC-COO-glu	521.24	521.25	612.28	612.28

Table 1- Theoretical and experimental *m/z* ratios for derivatized and non-derivatized cannabinoid standards.

After derivatization the ions corresponding to non-derivatized cannabinoids were not observed, suggesting that reaction went to completion, (or such that non-derivatized cannabinoids re-

mained present at concentrations below the limit of detection). The expected derivatized THC peak at *m/z* 406.28 was the most abundant in the spectrum. However, there was evidence that rearrangement still occurred as the peak at *m/z* 404.27 was observed, though it was present at only 6% of the intensity of the *m/z* 406.28 peak, as opposed to approximately 100% when run without derivatization. This suggests that the derivatization largely protects THC from the re-arrangement, possibly due to steric hindrance, or increasing the required amount of laser energy to re-arrange the molecule. The peak at *m/z* 406.28 was also observed in the mass spectrum of the derivatized CBD molecule. This was anticipated as THC and CBD are isobaric species, however an additional peak at *m/z* 483.32 was also detected in the CBD spectrum; CBD gains two N-methyl-pyridinium groups as it has one more hydroxyl group than THC. The peak at *m/z* 483.32 corresponds to the loss of a methyl group from the doubly-derivatized molecule expected to be observed at *m/z* 498.32 Theoretically there could be two additions of the derivatization group to 11-OH-THC and THC-COOH and up to five additions on the THC-COO-glu molecule as a result of multiple hydroxyl groups being present, though corresponding *m/z* values were not observed. THC-COO-glu was detected at *m/z* 612.28, corresponding to a single addition, in the mass spectrum though the peak at *m/z* 436.25 was much more abundant, suggesting the glucuronide group readily fragments from the parent molecule during analysis resulting in the detection of the THC-COOH. A further experiment increasing the laser power used for analysis showed that the ratio of THC-COO-glu to THC-COOH decreased with increasing laser power (data not shown). Another potential interferent in the assay was THCA-A, the biogenic prescursor to THC. This was analysed using the method and showed no trace of the ions relating to THC or derivatised THC (data not presented).

It was also noted that for all derivatized samples there was almost complete suppression of CHCA matrix-related peaks, as previously observed by Murgasova *et al.* [42].

Imaging of Cannabinoids in hair samples

Once the detection of cannabinoids through derivatization was optimised, this sample preparation method was adapted to permit mapping of these species in single hair samples using MALDI-MSI. Preliminarily, blank and cannabinoid spiked hairs were imaged to verify efficiency of the derivatization method for imaging purposes and were compared to hairs which had not gone through the derivatization step (Fig 4).

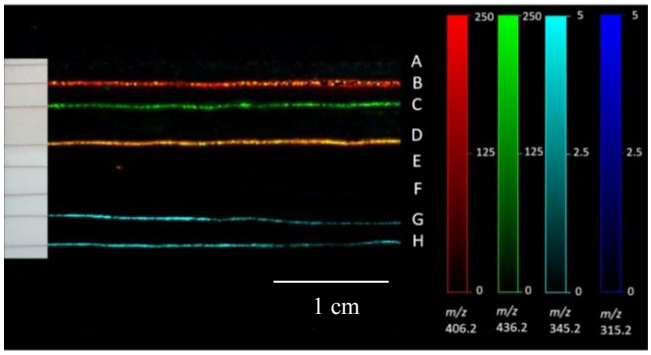

Figure 3. Comparsion between derivatized and non-derivatized hairs. Hair A soaked in methanol and derivatized. Hair B soaked in THC standard then derivatized. Hair C soaked in THC-COOH standard and derivatized. Hair D soaked in a 1:1 mixture of THC standard and THC-COOH standard and derivatized. Hair E soaked in methanol and not derivatized. Hair F soaked in THC standard and not derivatized. Hair G soaked in THC-COOH standard and not derivatized. Hair H soaked in a mixture of THC and THC-COOH and not derivatized.

Unless dramatic modifications are made to contrast and brightness, underivatized hairs soaked in THC standard could not be visualised in the 2D molecular map as the ion signals of the underivatized THC were of extremely low intensity. Interestingly THC-COOH could be visualised in the 2-D molecular ion map (cyan color) in hairs G and H which were soaked in THC-COOH standard and a mixture of THC and THC-COOH standard respectively, however this was also at relatively low intensity (Figure 4). The peak at *m/z* 406.2 corresponding to derivatized THC is clearly seen in the hair that was spiked with THC and subsequently derivatized (red in color). Similarly, the expected ion at *m/z* 436.2 was observed in the hair spiked with THC-COOH and subsequently derivatized (green color); the hair which was spiked with a mixture of THC and THC-COOH and then derivatized appears yellow in color as both THC and THC-COOH ions are present (a mixture of red and green gives yellow).

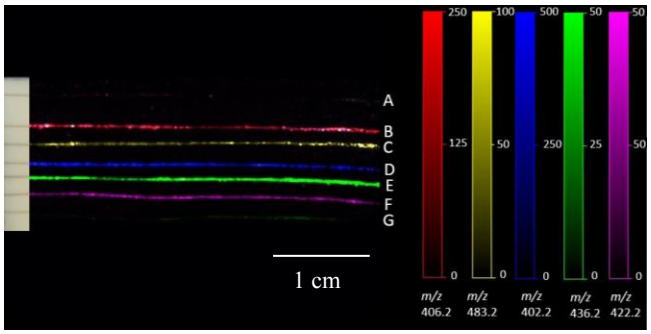

Figure 4. Simultaneous imaging of several cannabinoids of interest. Hair A soaked in Methanol. Hair B soaked in THC. Hair C soaked in CBD. Hair D hair soaked in CBN. Hair E hair soaked THC-COOH. Hair F soaked in 11-OH-THC . Hair G soaked in THC-COO-glu. All hairs were derivatized with FMTPS prior to analysis.

Since it was established that derivatization enhances both the THC and THC-COOH signal in imaging experiments (as shown in Figure 3), a second mapping experiment with the other cannabinoids shown in was carried out (Figure 4). The peak at *m/z* 406.2 corresponding to derivatized THC is clearly seen in the hair which was spiked with THC and then derivatized (red *m/z* map), the peak at *m/z* 483.2 was observed in the hair spiked with CBD and derivatized (yellow *m/z* map), the peak at *m/z* 402.2 was corresponding to the derivatized CBN was observed in the hair which was spiked with CBN and derivatized (blue *m/z* map), the peak at *m/z* 436.2 corresponding to the derivatized THC-COOH was observed in the hair which was spiked with THC-COOH and derivatized (green *m/z* map) and finally the peak at *m/z* 422.2 corresponding to the derivatized 11-OH-THC was observed in the hair which was spiked with 11-OH-THC and derivatized (magenta *m/z* map). As with the profiling experiments, THC-COO-glu fragmented to give THC-COOH at a *m/z* of 436.2 (green *m/z* map) and its image intensity reflect a 5X lower concentration compared to the other standards due to the concentration in which it is supplied. Users' hairs were investigated using the derivatization method coupled with MALDI MSI, employing this optimised method. In particular, MALDI MS/MS images were obtained of hairs collected from a volunteer who self-reported to use cannabis once a week and the transition *m/z* 406.2 derivatized THC parent ion compound to *m/z* 110.0 was monitored (Figure 5). The product ion at *m/z* 110.0 corresponds to the hydrated methylpyridinium fragment which is common to all FTMPS derivatives and has previously been used for confirmation [44].

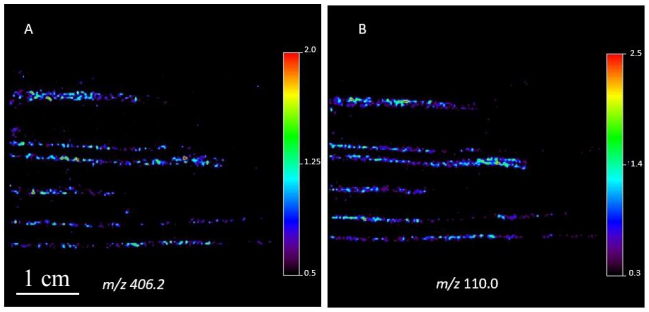

Figure 5. MS/MS image of user hairs. 6A shows derivatized THC parent ion at *m/z* 406.2 6B shows the map of the fragment ion at *m/z* 110.

CONCLUSIONS

The use of MALDI imaging and profiling to detect cannabinoids in hair samples following *in situ* derivatization is presented. The method shows for the first time potential to detect cannabinoids from a single hair.

During the development of this method an interesting, laser induced, THC rearrangement was observed. This caused increased fragmentation of the THC and hence low ability to detect the molecule without derivatization. The novel *in situ* derivatization, completed in minutes at room temperature using FMPTS, showed a greatly increased limit of detection over the non-derivatized analytes THC, CBD, CBN and THC metabolites. The ability to detect the metabolites of THC only formed *in vivo* THC-COOH, 11-OH-THC and THC-COO-Gluc will enhance the ability of the analyst to distinguish between use and unintentional exposure. During analysis the THC-COO-Gluc fragments to form THC-COOH, with the consequence that if the *m/z 436.2* is detected it cannot be determined which of the analytes was originally present. The *m/z* 612 is however unique to the THC-COO-Gluc. This is an advantage over traditional GC-MS methods where the glucuronide is not generally detected due to the common practice of hydrolysis or digestion of the hair sample, which converts it into the THC-COOH [49].

Prior to integration into a toxicology workflow a large sample of user hairs should be tested, from different levels of users and with different hair types. The comparison of levels of metabolites detected using traditional methods with the results from MALDI analysis will determine the limit of detection for hair samples and applicability to lower level users as well as the possibility of using the method quantitatively in the future. This will allow an assessment of the suitability of the method for users or whether it will be a screen for external contamination. The user hair tested here, from a regular but low level user, provides proof that the THC at least can be detected.

The method reported has a sample preparation workflow, not withstanding the derivatization step, which is less time consuming due to the lack of extraction step, than the traditional GC or LC methods. This method also gives the potential to simultaneously detect THC and metabolites in a single workup and analysis. An additional advantage is the potential of MALDI-MSI resolution allowing increased sensitivity to the time period of use, better than the traditional month by month history, although such an approach will require further validation. Analysis of hairs from a known cannabis user has shown applicability of the method to detect THC in real life samples.

ACKNOWLEDGMENT

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Graphical Abstract