Analytical Methods

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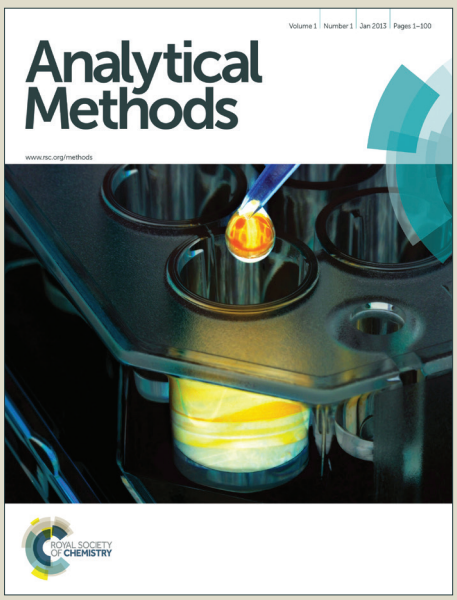

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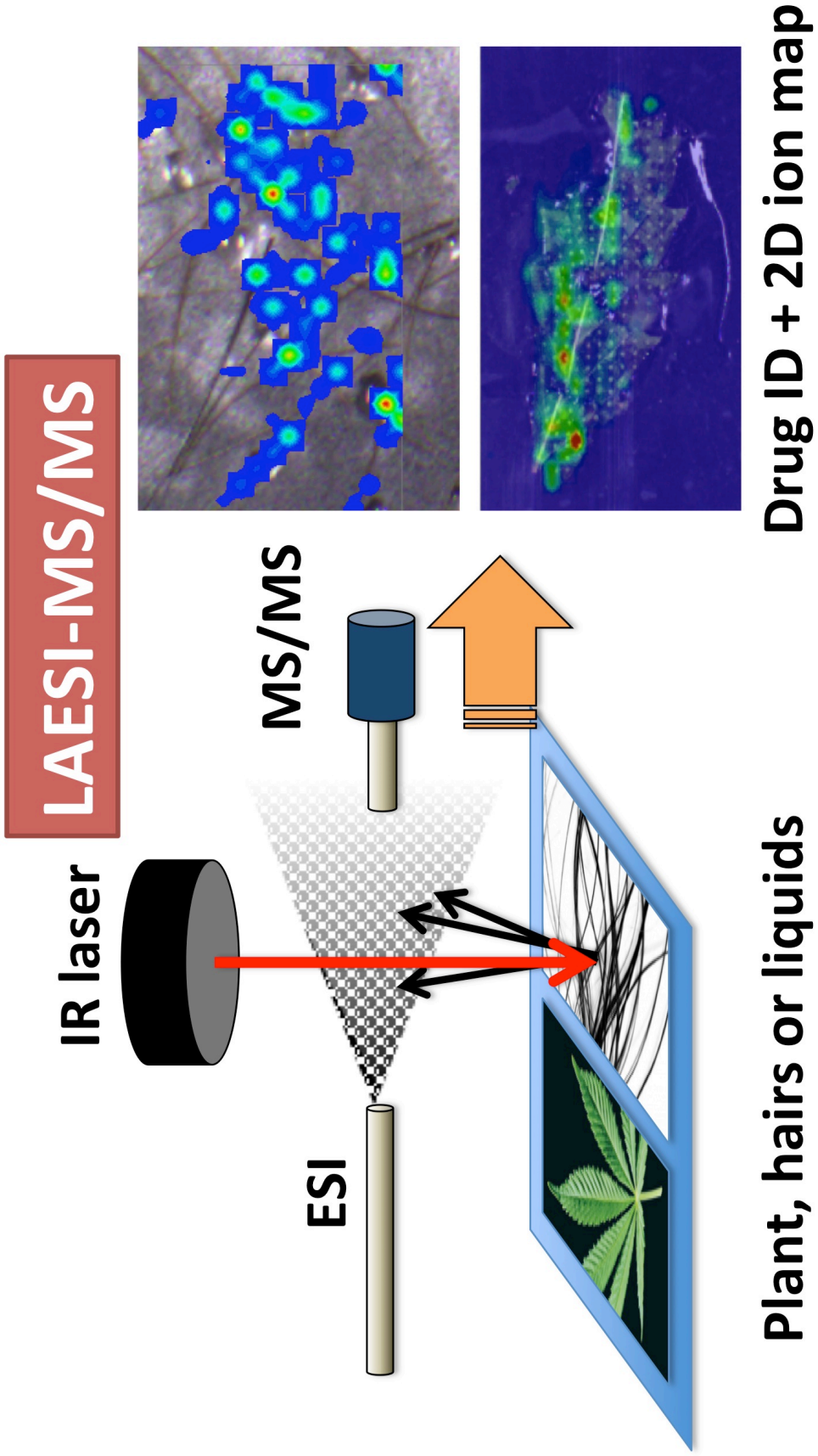

Direct Analysis of Drugs in Forensic Applications using Laser Ablation Electrospray Ionization-Tandem Mass Spectrometry (LAESI-MS/MS).

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ABSTRACT: Laser ablation electrospray ionization tandem mass spectrometry (LAESI-MS/MS) was applied to the analysis of scheduled drugs in a variety of forensically relevant media including solutions, hair and botanic matter. LAESI-MS/MS was generally able to identify unreacted drugs directly from solutions in which common presumptive color tests had been performed. A significant correlation of 0.7 was found between the pKa of the drugs and the frequency of a positive identification in the solutions indicating that basic drugs are more favorably ionized. Basic drugs` like amphetamine and methamphetamine were readily identified

at 0.01 mg/mL, well below the normal limits of detection of the color test results. For hair analysis, LAESI-MS/MS could directly identify the presence of morphine, codeine and cocaine in human hair samples at biologically relevant levels of ~10 ng/mg of drug in hair. This detection was possible without any hydrolysis, extraction, derivatization, or separation of the drugs. LAESI-MS/MS could also identify the presence of tetrahydrocannabinol (THC) or cannabidiol (CBD) in cannabis leaves, in addition to mapping the spatial abundance of THC/CBD across the different leaves. The simplicity and lack of sample preparation for hair and plant analyses are noteworthy benefits, but the current detection limits are close to biologically relevant levels. These preliminary studies indicate that with some additional optimization and validation, LAESI-MS/MS could provide a direct confirmation of color spot test results at an average analysis time of 20 seconds per sample, which is considerably faster than any GC or LC run and could be a major benefit for large caseloads or backlog reduction.

Introduction

In 1970, the US government passed into law the Controlled Substances Act, which helped to identify and rank substances of abuse and provide a scale of punishments associated with the manufacture, sale and use of such substances. The list of scheduled drugs is regularly updated at the state and federal levels. Despite these controls, drug abuse is a very common crime and drug analyses comprise the major workload of most forensic laboratories.[1] As a result, many laboratories have large backlogs and new, faster methods of confirmation are necessary to help laboratories keep pace with the number of evidence submissions.

Typically, the analysis of suspected drugs is performed using a sequence of increasingly selective tests. The first test is usually a presumptive test and includes polarized light

microscopy, immunoassays or color tests.[2, 3] Presumptive tests do not require much training or chemical expertise, help determine which samples are most likely to contain an illicit substance, and guide the conditions for subsequent methods of instrumental analysis. . However, positive results in this type of test are usually not specific enough to confirm the presence of a particular drug, which is why seized samples are always sent to a crime laboratory for confirmatory tests. Currently, the most common confirmatory tests are Gas Chromatography/Mass Spectrometry (GC/MS) and Fourier transform infrared spectroscopy (FTIR) although High Performance Liquid Chromatography Mass Spectrometry (HPLC/MS) is also gaining popularity in toxicology laboratories.[4] These techniques are popular due to their sensitivity, ability to identify multiple components at once, and their ability to be easily automated. However, a typical GC or LC run is 20 to 30 minutes long, not including sample preparation. This time requirement is a major reason for backlogs. Fast GC[5-8] is a potential approach for decreasing analysis times, but this approach does not reduce sample preparation time. Therefore, chromatography-free mass spectrometric methods of analysis like Direct Analysis in Real Time (DART)[9] and Desorption Electrospray Ionization (DESI) are gaining interest in various forensic laboratories.[10]

DESI was introduced in 2004 by Cooks and co-workers[10] and can analyze semi-volatle and non-volatile samples from a nonconductive surface under ambient conditions.[11, 12] DESI has been coupled with a variety of mass spectrometers including ion traps,[11] Orbitraps,[13] Fourier transform ion cyclotron resonance (FT-ICR),[14] and ion mobility time of flight (IMS-TOF)[15] mass spectrometers and has been used in a variety of forensic applications including detection of illicit drugs,[16-18] explosives,[19-21] alkaloids in plant matter[22] and imaging and the analysis of latent fingerprints.[23] DART was first reported in 2005 by Cody and Laramee and normally forms singly charged, protonated or deprotonated species in either the positive or negative mode,

respectively, but can form radical ions in some cases.[9,24.25] Unlike DESI, DART is typically limited to analytes with a molecular weight below 800 Daltons [11, 24, 26, 27]. DART has also been used for a variety of forensic applications including chemical warfare agents[9], explosives,[28] drugs,[29, 30] and ignitable liquids.[9] There are now dozens of variations of ambient sampling technologies coupled with ambient ion sources and each approach has its own merits.[11, 31-40] Several of these new ambient ionization methods have been studied in medicolegal and forensic applications requiring the identification of drugs. For example, techniques like paper-spray ionization and low temperature plasma (LTP) ionization have been used to successfully analyze both therapeutic and illegal drugs directly in biofluids like blood.[41-44]

LAESI was first presented by Nemes and Vertes in 2007 and addresses some of the problems associated with other ambient ionization techniques, such as the requirement of an external matrix in matrix assisted laser desorption electrospray ionization (MALDESI), an ill-defined sampling area (DESI), or a limited mass range (DART).[45] LAESI functions by ablating the sample with a pulsed mid-infrared Er:YAG laser that is tuned with an optical parametric oscillator (OPO) crystal to 2940 nm, which matches the vibrational state of the -OH bonds in water and thereby enhances ablation.[34] By using conventional ESI-based ionization, LAESI-MS also enables an extended mass range and can analyze samples up to 66 kDa.[45] An additional advantage of LAESI-MS over other techniques is its ability to perform high throughput automated analyses of liquid samples. Example applications of LAESI-MS are molecular imaging,[46] imaging of metabolites in plants,[47] and rat brain tissue sections.[47] Direct studies have also been performed using LAESI-MS on cyanobacteria[48] and individual plant cells.[49]

In addition to bulk drug samples described above, many workplaces and the criminal justice system are also concerned with the identification of drugs and drug metabolites

in biological fluids and matrices such as hair and urine. Although urine is a reliable and well-studied matrix for drug screening, it is normally collected without direct supervision and donors have been known to tamper with the sample through the use of surrogate urine bags or adulterants like bleach or vinegar.[50, 51] Other types of biological samples, such as saliva or human hair, can be collected with direct supervision. Hair samples have a major advantage of storing a longer chronological record of past drug use than most biological matrices. Hair samples are also more difficult to falsify or contaminate and are easy to collect and store. Despite these advantages, institutions have been slow to rely on hair testing because of the lengthy extraction procedures that are required to efficiently remove, concentrate, derivatize, separate and detect analytes in hair. A recent exception has been the direct analysis of drugs in hair down to 5 ng/mg using MALDI-mass spec imaging using a triple quadrupole linear ion trap.[52]

In this manuscript, we seek to expand the application of LAESI-MS into the realm of forensic science through the analysis of controlled drugs in solution, plant matter and human hair. These experiments demonstrate the feasibility of incorporating LAESI-MS/MS into the existing workflow under which most crime laboratories currently operate, to act as an alternative to GC-MS for the identification of drugs of abuse.[3] We show that common drugs of abuse can be detected quickly and accurately in a variety of media and with minimal sample preparation (i.e. just wetting).

Methods and Instrumentation

Reagents

All drugs utilized in this experiment were purchased from Sigma-Aldrich (St. Louis, MO). The cannabis leaves were obtained from Ohio Bureau of Criminal Investigation (BCI) in London,

Ohio. The deuterated methamphetamine standard (Cat# M-093) was obtained from Cerilliant (Round Rock, TX). The drug-laced hair standards (Drugs of Abuse in Human Hair Segments, SRM # 8448 and Drugs of Abuse in Human Hair II, SRM# 2380) were purchased from the National Institute of Standards and Technology (Gaithersburg, MD). The Mandelin, Marquis, Dille-Koppanyi and Cobalt Thiocyanate reagents were prepared in house[2] and required cobalt thiocyanate, cobalt acetate dehydrate, and ammonium vanadate which came from Sigma-Aldrich (St. Louis, MO) as well as methanol, glacial acetic acid, isopropylamine, sulfuric acid and 40% formaldehyde which were purchased from Fisher Scientific (Hampton, NH). The hydrochloric acid and sodium hydroxide used were also purchased from Fisher Scientific (Hampton, NH).

Instrumentation

Experiments were performed using a Protea LAESI DP-1000 Direct Ionization System (Protea Biosciences, Morgantown, WV) attached to a Velos linear ion trap (LTQ) mass spectrometer (ThermoFisher Scientific, Waltham, MA). For all analyses described, ablation was carried out with a 2940 nm infrared laser operating in pulsed mode at 10 Hz. Drugs of abuse were analyzed from 96 well plates with 100 laser pulses per sample and with a delay of 4 seconds between wells. For the analysis of hair and cannabis leaves, 10 pulses were used from the same 10 Hz laser at ~200 μm to 500 μm intervals along the hair in a grid-like raster resulting in 280 to 900 analysis locations (pixels) per sample. The ESI spray solution consisted of a 0.1% acetic acid solution in 50% methanol flowing at a rate of 1 μL/min. Each analysis utilized data-dependant tandem mass spectrometry with the dynamic exclusion mode enabled. A 96 well plate can be analyzed as quickly as ~8 min for full-scan MS mode and in ~30 min using dynamic exclusion MS/MS mode. The MS/MS mode can be used to identify drugs in solution at an

average rate of 20 seconds per sample. An analysis time of ~30 minutes was required to generate an ion map of the plant leaf, but this varied based on the number of pixels required to fully map the leaf surface. Illustrations of the components of this system have been covered in elsewhere.[45, 53]

Method

The first set of analyses were performed on drugs that had been dissolved in different reagent solutions. The drugs used for this experiment included aspirin, cocaine, methamphetamine, amphetamine, phenobarbital, oxycodone, codeine and quinine—a common cutting agent. Solutions of each drug ranging from 0.01 mg/mL to 5 mg/mL were prepared from solid drug samples. Each drug was then dissolved in a variety of solvents and reagents before analysis, including water, 0.1 M hydrochloric acid, 0.1 M sodium hydroxide, and each color reagent (Scott's cobalt thiocyanate reagent, Marquis reagent, Mandelin reagent, and Dille-Koppanyi reagent).[2] These combinations were then placed in random order into six 96-well-plates. Two 50/50 mixtures of cocaine/quinine and cocaine/aspirin were also tested at a variety of concentrations. In summary, the color tests included a total of 252 solutions: nine different drug/drug combinations at four different concentrations, each in seven different reagents. Each well was analyzed on time for 4-6 seconds. One well plate also contained a series of methamphetamine samples ranging from 1 to 100 ppm that was used to test the dynamic range of the method. For the calibration curve, each well was analyzed three times. Samples were tested immediately after preparation. We have not yet tested the reliability of repeated freeze-thaw cycles on the analytical results but this could be an important factor for long-term storage of samples for implementation into casework laboratories.

The second type of matrix or media was human hair. These experiments were performed by first attaching several strands of hair to a microscope slide using removable, double-sided Scotch tape (3M). These hairs were then wetted with water and allowed to sit for a few minutes to absorb the water. The sample was then analyzed directly using LAESI. An ion map was generated using ProteaPlot software (Protea Biosciences Inc., Morgantown, WV) from the raw XCalibur files to show the abundance of the different drugs distributed across the group of hair strands attached to the sample slide.

For the third type of matrix, botanic matter, cannabis leaves were analyzed primarily to identify the presence of the psychoactive ingredient, tetrahydrocannabinol (THC), and secondarily to image the distribution of THC throughout the leaf. Different methods of attaching the leaf to the slide were tested in order to determine if any excess background signal was generated in LAESI from the different adhesive media. The leaf was attached to a microscope slide using either paraffin wax or Permount solution. After mounting, the leaf was then wetted using water and allowed to sit for several minutes before being placed into the instrument and analyzed directly using LAESI. An ion map was generated from the raw data file using ProteaPlot software.

Results and Discussion

Analysis of Drugs in Solution after a Presumptive Color Test

Liquid samples for LAESI-MS/MS analysis were prepared according to the method section. When some of the color reagents were combined with the drug, they reacted to produce a color indicative of the drug present. Some tests—like Marquis reagent—covalently modify the drugs to produce the observed color changes,[67] whereas reagents like cobalt thiocyanate instead

rely on the formation of a non-covalent or ionic complexes to produce the color changes.[68] The LAESI ion source is a relatively soft ion source and imparts little to no fragmentation during ionization, so covalent and non-covalent complexes ought to remain in-tact and observable in the resulting LAESI-MS spectra. Therefore, an unknown factor for consideration in the analysis of post-reaction color spot tests is whether or not the products or unreacted reagents of the color tests are observable after the color change is observed.

Some examples of color test results are shown in Figure 1. The two blue wells near the left and center of the plate show a positive test for cocaine with the Scott reagent (cobalt thiocyanate). These two wells show the different degrees of color that could develop with different drug combinations. The red square in Figure 1 shows a faint blue ring indicative of cocaine developing around the edge of the well. This well contained 2.5 mg/mL of cocaine and 2.5 mg/mL of aspirin. In contrast, the blue box shows a simple positive result for cocaine at 5 mg/mL. The Mandelin and Marquis reagents are two reagents commonly used to test for codeine and turn olive and dark purple, respectively, as shown in Figure 1. In all these cases, LAESI-MS/MS confirmed the results of the color tests.

Tandem MS spectra were compared with the NIST standard reference tandem MS spectra to confirm the presence of the expected drugs. Analyses were focused on the unreacted, unmodified drugs of a reaction, even though some of the color tests, such as Marquis, are known to involve covalently modified products. An example of a positive identification between the experimental and reference data for methamphetamine is shown in the supplemental material, Figure S-1. These analyses found that the target drugs could be identified most successfully at concentrations of 1 mg/mL and 5 mg/mL, as seen in Figure 2b. In this work, a positive

result/identification is defined as three or more spectra per well each containing three or more characteristic fragment ions with a signal to noise ratio of at least three.

As one might expect, the most reliable results are for basic dugs at high concentration in acidic media. For example, Figure 2 shows that the reagent 0.1 M HCl results in positive identifications by LAESI-MS/MS slightly above 50% for all the concentrations studied. However, for the water-soluble drugs with a pKa >7 at 1 and 5 mg/mL, the observation rate from the 0.1 M HCl solution was above 90%.

The Dille-Koppanyi reagent gave the fewest positive results and LAESI-MS/MS was only able to identify the drug in the Dille-Koppanyi reagent in 21% of the cases studied. The Dille-Koppanyi reagent is made by combining cobalt (II) acetate dehydrate, glacial acetic acid, methanol, and isopropylamine, so is devoid of water and is quite volatile. Whereas the methanol present is ordinarily a reliable matrix replacement for water, the relatively long overall analysis time for the 96-well plate results in significant evaporative losses and concomitant decrease in signal yield for this particular reagent because of the laser focusing/ablation effects, despite the expected increase in concentration during evaporation. The latest commercial LAESI source is now equipped with a chiller to help prevent evaporation and maintain frozen tissue samples, but this was not available at the time of the experiments. The Dille-Koppanyi reagent therefore could prove more reliable when well plates are properly filled and chilled to prevent evaporation.

Figure 2a shows that some drugs, like amphetamine and cocaine, are identified most of the time and other drugs, like phenobarbital, are rarely identified by LAESI-MS/MS. The difference in observation frequency is attributed largely to the pKa of the drugs and their relative degree of protonation. Negative ion mode was not explored with these samples because of the general lack of negatively charged Brønsted-Lowry basic sites. Figure 3 shows a plot the pKa of

each drug versus the total number of identifications of that drug in all reagents at all concentrations. The pKa values used in this plot were taken from the literature.[54-60] The square of the linear correlation coefficient (R^2) of 0.69 means that the pKa accounts for 69% of the variance of the identifications. The main discrepancies in this plot are cocaine and quinine.

Based on pKa alone, one would expect an equal degree of ionization and an equal number of identifications for cocaine and quinine. Despite their similar pKa values (cocaine = 8.6 and quinine = 8.7), cocaine has a greater number of observations than expected and quinine has a fewer number of observations than expected. Quinine has a second, much weaker, Brønsted-Lowry basic site—with a conjugate pKa of 4.3—so this site was not considered relevant. In hindsight, we learned that the solubility of quinine is only around 0.10 mg/mL, whereas the solubility of cocaine is around 2500 mg/mL,[61, 62] so it is likely that quinine actually exceeded its saturation limit in many of the solutions, even though we did not observe any precipitates in the preparation flasks or wells plates.

As a general rule, most of the color tests studied were almost as effective as simple acid or water at providing positive identifications for the unreacted reagent drugs. Presumably, this implies that very few of the color tests goes to completion and that, typically, some detectable portion of each drug remains unaltered after each of the color tests. We did look for molecular ions consistent with drug-reagent complexes in each of the color spot tests, but did not find sufficient evidence for the identification of any complexes. In summary, the ability to identify drugs from solutions appeared to be more effected by the pH and pKa than by the presence of any of the color test reagents.

Two separate mixtures of drugs were also tested alongside the pure drugs, which included a mixture of cocaine and quinine and a mixture of cocaine and aspirin. In this case both quinine

and aspirin were selected because they are examples of cutting agents found in authentic street samples and could potentially interfere with the color test results or the LAESI ionization process. Both cocaine and quinine could be identified in a mixture at 1 mg/mL and 5 mg/mL (although, as discussed earlier, the quinine effectively saturates at approximately 0.1 mg/mL). Figure S-2 shows an example of the tandem MS spectra for the identification of the components of the cocaine and quinine mixture in cobalt thiocyanate reagent, the most commonly used presumptive test for cocaine. For the cocaine and aspirin mixture, only cocaine could be observed, presumably because the primary active ingredient in aspirin, acetylsalicylic acid, is not particularly soluble in water and does not carry a formal positive charge when protonated. Negative ion mode was not explored for aspirin, but would be expected to be more applicable because of the carboxylic acid site.

Out of 252 possible positive drug identifications, including all four concentrations of drugs in all seven reagents and solutions, LAESI-MS/MS identified drugs in 83 cases, or with a 37% success rate. Under more favorable conditions, such as with analytes at or above 1 mg/mL in neutral or acidic solutions, the percentage of true positives was greater than 90%, or fewer than 10% false negatives. Many of the false negative results include 36 wells (14% of the analyses) that contained the Dille-Koppanyi reagent, which in general provided very few positive LAESI spectra. The false negatives also include: 1) reagents containing a strong base and therefore hindering detection in positive mode; 2) phenobarbital, which gave very poor response in general; and 3) analytes at concentrations at or below the detection limits. We did not find any false positives in the color spot tests of LAESI-MS/MS analyses, so carryover or cross-contamination was successfully avoided. Some early experiments with a very concentrated

solution of flunitrazepam resulted in carryover for tens of seconds, but such problems are generally avoidable under more controlled conditions.

To explore the ability to quantify analytes in an aqueous medium using LAESI-MS/MS, a calibration curve for methamphetamine was constructed ranging in concentration from 1-100 ppm. Deuterated (D_{14}) methamphetamine was also added as an internal standard at a constant concentration of 50 ppm to each solution. The tandem mass spectra corresponding to non-deuterated and deuterated methamphetamine were integrated and the integrated peak areas plotted as a function of concentration, as shown in the supplemental material (Figure S-3). The linear range for methamphetamine analysis using LAESI-MS/MS extends from at least 1 to 100 ppm with R^2 values ranging from 0.999 for the absolute peak areas and 0.987 for the normalized peak areas. Although internal standards usually enhance quantitation with DESI ion sources relative to conventional external calibration,[19] the use of an internal standard here could not have been expected to improve the excellent correlation in this case. We anticipate that a more thorough validation study with different analytes would show that the use of deuterated internal standards would not be inferior to quantitation using absolute signals, but would either match or improve the correlation scores.

Analysis of Drugs in Human Hair Sample

Strands of spiked hair were studied using LAESI-MS/MS to determine if this system could identify drugs of abuse directly from the hair matrix with minimal sample preparation. Ion mapping was also used in order to identify areas within the hair samples that may contain high abundances of a particular drug.

This series of experiments was performed on two different NIST hair strand reference samples (NIST SRM 8448 and SRM 2380) that contained several drugs ranging in concentration from 0.99 ng/mg to 11.9 ng/mg. NIST SRM 8448 contained cocaine, benzoylecgonine, morphine and codeine and NIST SRM 2380 contained codeine, morphine, 6-monoacetylmorphine and tetrahydrocannabinol. Several strands of hair from either sample were attached to a microscope slide using double-sided Scotch tape, wetted with water and then analyzed using LAESI-MS without any further sample preparation. The adhesive on the tape did not significantly alter the normal LAESI background or analyte signals. LAESI-MS was able to identify the presence of morphine, codeine, and cocaine in the hair samples but not the other drugs (see supplemental Figure S-4 for MS/MS spectra). The use of an internal database of known drug standards would obviously improve spectral comparisons because of the known variance in product ion spectra with different instruments and conditions.[63-65] Tetrahydrocannabinol, benzoylecgonine, and 6-monoacetylmorphine were unable to be identified directly from hair using LAESI-MS, presumably because they appeared at lower concentrations than the drugs that were observed. All three unidentified drugs were present in the hair at concentrations lower than 2.10 ng/mg whereas all the identified drugs were present at concentrations above 6.7 ng/mg. Further work will be necessary to lower the detection levels and widen the coverage, but the ability to identify at least three drugs of abuse at biologically relevant levels, without any extraction, separation or concentration, is a potential benefit over conventional methods of hair analysis.

To image the hair strands, the software was programmed to raster across the hair sampling every 200 μm to 500 μm, depending on the size of the sample. The path of the laser could be directed to follow a single hair strand or to raster across an associated bunch of hair

strands. MS/MS data could then be used to determine the distribution of morphine using the selected reaction monitoring transition of *m/z* 286 to *m/z* 201.

An ion map generated for the abundance of morphine in the hair is shown in Figure 4. The color scale shows most abundant areas in red and the least abundant signals in blue or transparent. The distribution of cocaine was similar to that of morphine. The morphine signals were routinely larger from the hair shaft relative to the surrounding background, but the signals along any one shaft do not appear to be of consistent intensity. At this time, we suspect that this behavior is more representative of the variance in ablation and ionization efficiency than of the true morphine concentration, as there should be no reason why the hair (wet or before wetting) would have a heterogeneous distribution of drugs along a shaft. The one exception would be if certain portions of the hair shaft were wetted more thoroughly than others and therefore enabled more efficient photoabsorption and ablation. Indeed, early experiments with dry hair gave insufficiently weak signals. The regions of largest morphine signal tends to correlate with intersecting or protruding hairs, indicating that either the larger surface area of hair in these regions, or the excess water held between hair shafts by water tension, enhance the signals in these regions. Despite this effect, the ion map for morphine correlates well with the individual hair shafts.

Direct Analysis of Drugs in Plant Matter

Cannabis leaves were also studied using LAESI-MS to determine if this system could identify THC directly from the plant matrix without prior sample preparation. Ion mapping was also used to better understand how the psychotropic chemical THC is distributed throughout the leaf. In one case, a leaf was fixed onto a glass depression slide and the leaf was wetted with

water prior to analysis. After wetting, the leaves were analyzed directly by LAESI-MS/MS. The MS/MS results are shown in Figure S-5 wherein the data is compared to a reference spectrum for THC taken using another ambient technique, desorption atmospheric pressure photoionization (DAPPI).[66] In addition to DAPPI, other ambient techniques like DESI have also been used to analyze cannabis leaves.[19,67] The two MS/MS spectra in the supplemental material show similar fragment ions at *m/z* 259, *m/z* 233 and *m/z* 193 and *m/z* 135, which are characteristic of both THC and the non-psychoactive cannabinoid, cannabidiol (CBD),[19] which is also listed as a schedule I controlled substance in the USA.

During the analysis of the wetted leaves without fixing, the ablation process caused the leaf matter to move around in the well during analysis, which is undesirable. To prevent movement, the plant matter was subsequently secured with the commonly used mounting media Permount solution or paraffin wax. Neither matrix significantly altered the background ion signal (Figure S-6) and enabled adequate ion signal for MS/MS spectra of THC/CBD to be acquired (Figure S-7).

To image the leaf, the software was programmed to raster the leaf in a grid-like array every 500 μm in a 1 cm x 4 cm array. MS/MS data could then be used to plot a selected-reaction-monitoring (SRM) image for the transition of *m/z* 315 → *m/z* 259 for THC/CBD in the form of a relief map. THC and CBD cannot be distinguished via CID fragmentation of the protonated precursor ions, even though the NIST EI fragmentation patterns are significantly different. The ion maps generated for a leaf mounted in Permount and paraffin wax solution are shown in Figure 5. The color scale shows most abundant signal areas in red and the least abundant signals in blue. For the leaf held with paraffin wax, the areas of high signal abundance tend to correlate with both the leaf's vasculature structure and the areas that are darkened or better wetted. In this

particular leaf, it appears that the majority of the observed THC/CBD is contained in the top half of the leaf. A second leaf mounted with permount solution showed lower levels of THC/CBD, but the regions of abundant signal also correlate with the veins of the leaf. At this point, we have not established whether the THC/CBD content is actually higher at these locations, or whether wetting and subsequent ablation and ionization efficiency is better at these locations. From a forensic perspective, the distribution of THC/CBD in a leaf is less important than the ability to identify the psychoactive component itself, but follow-up studies could provide interesting biological information from the spatial distribution of compounds in the plant. Regardless of the reasons, these preliminary experiments show that the veins of the leaf seem to provide the largest signal responses compared to other locations in the leaf.

Conclusions

The national backlog of drug samples in crime laboratories is typically analyzed using a variety of methods that almost always includes GC/MS. Current GC/MS methods are typically 20 to 30 minutes long and—with the sample preparation and possible derivatization times included—severely limits the number of samples that can be analyzed per day. One way to relieve this backlog is to implement new instrumentation, which can quickly determine the identity of drugs on a presumptive test, without the need to prepare a new sample. Herein, we provide preliminary results to show that LAESI-MS/MS can analyze 96 solution-based color-test samples in 30 minutes at an average time of ~20 seconds each. LAESI-MS/MS is able to quickly and easily identify the presence of a drug in an unknown sample with more than 90% of the successful identifications occurring at a concentrations at or above 1 mg/mL in neutral or acidic conditions. LAESI-MS/MS was also shown to be able to identify mixtures of drugs in solution

and should be capable of identifying minor impurities, cutting agents or adulterants. LAESI-MS/MS can also identify drugs in hair and plant samples, although significant work lies ahead to validate and quantify drugs in these more difficult applications. Still, the ability to screen hair and botanical matter for drugs of abuse in a matter of seconds may complement existing techniques in forensic and first responder applications.

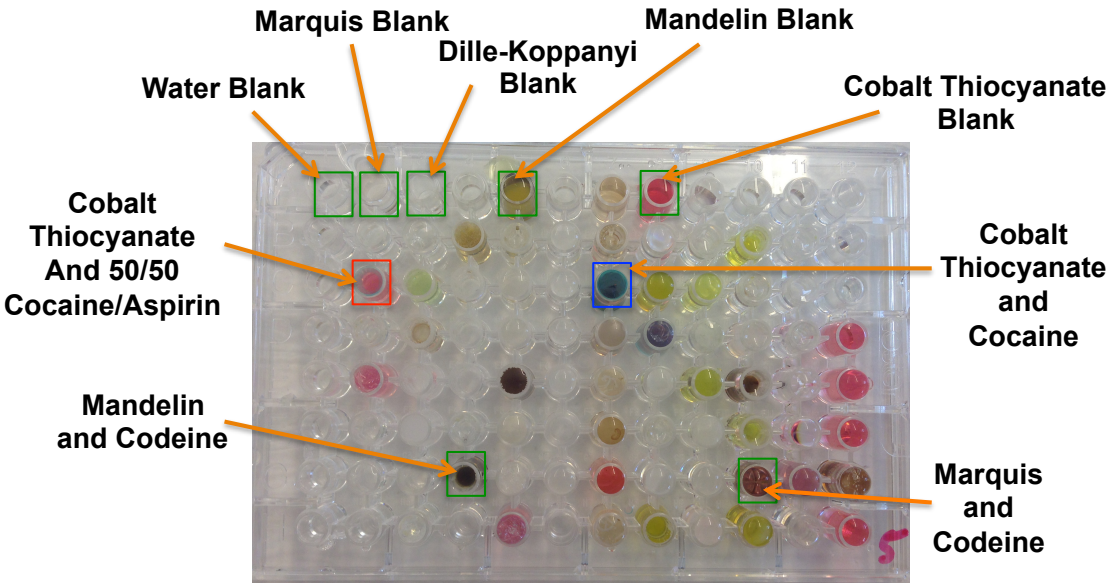
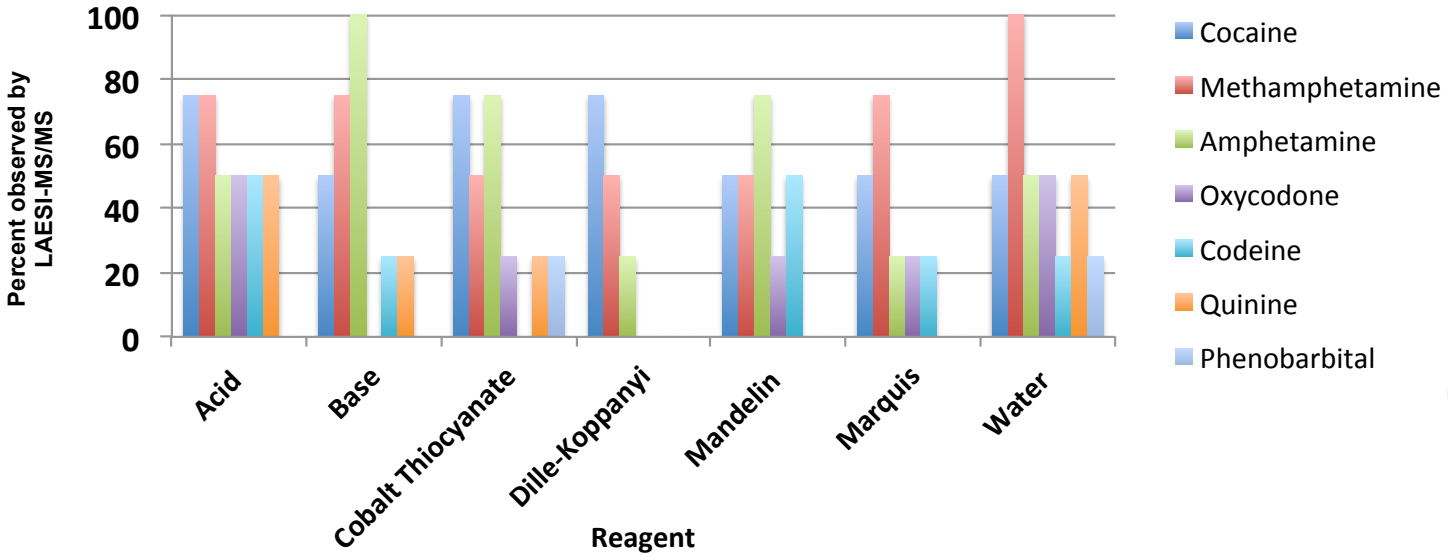


Figure 1. Photograph of a 96-well plate taken with Protea's built in camera prior to analysis using LAESI-MS/MS. The red square indicates the reaction of 2.5 mg/mL each of cocaine and aspirin with cobalt thiocyanate and the blue square indicates the reaction of 5 mg/mL cocaine with cobalt thiocyanate. Green squares indicate other wells of interest within the well-plate. All the wells contain solution, but not all are expected to be colored.

a)



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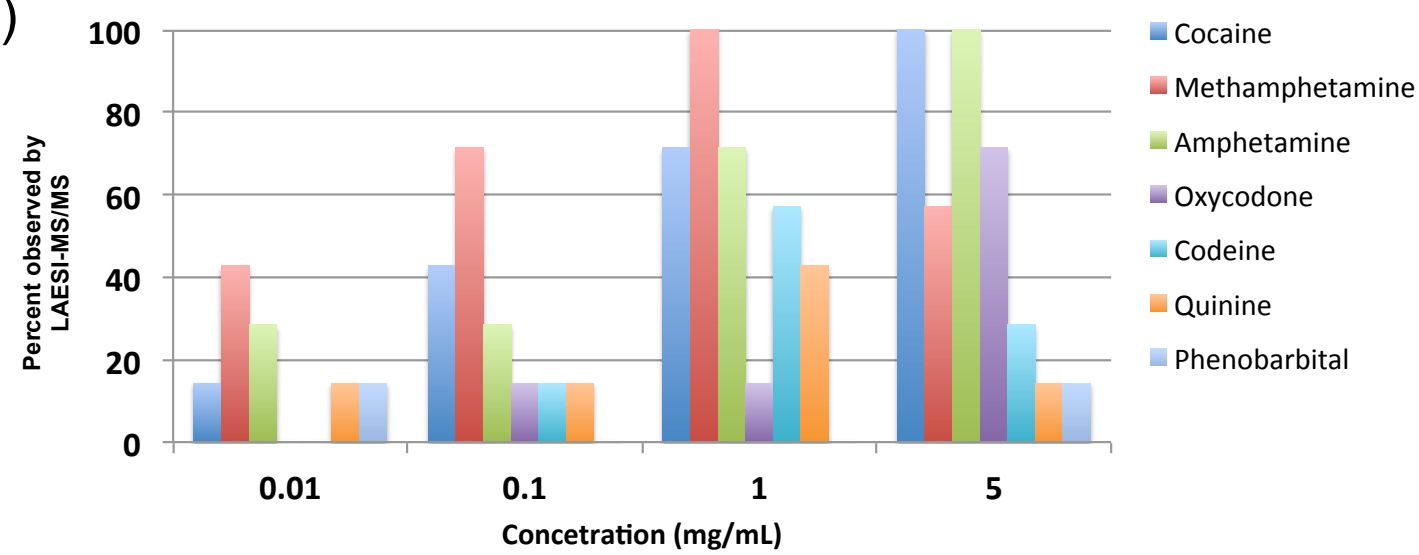

Figure 2. Bar graphs summarizing the LAESI-MS/MS positive identifications of the color spot tests a) separated by reagent and b) separated by concentration of drug.

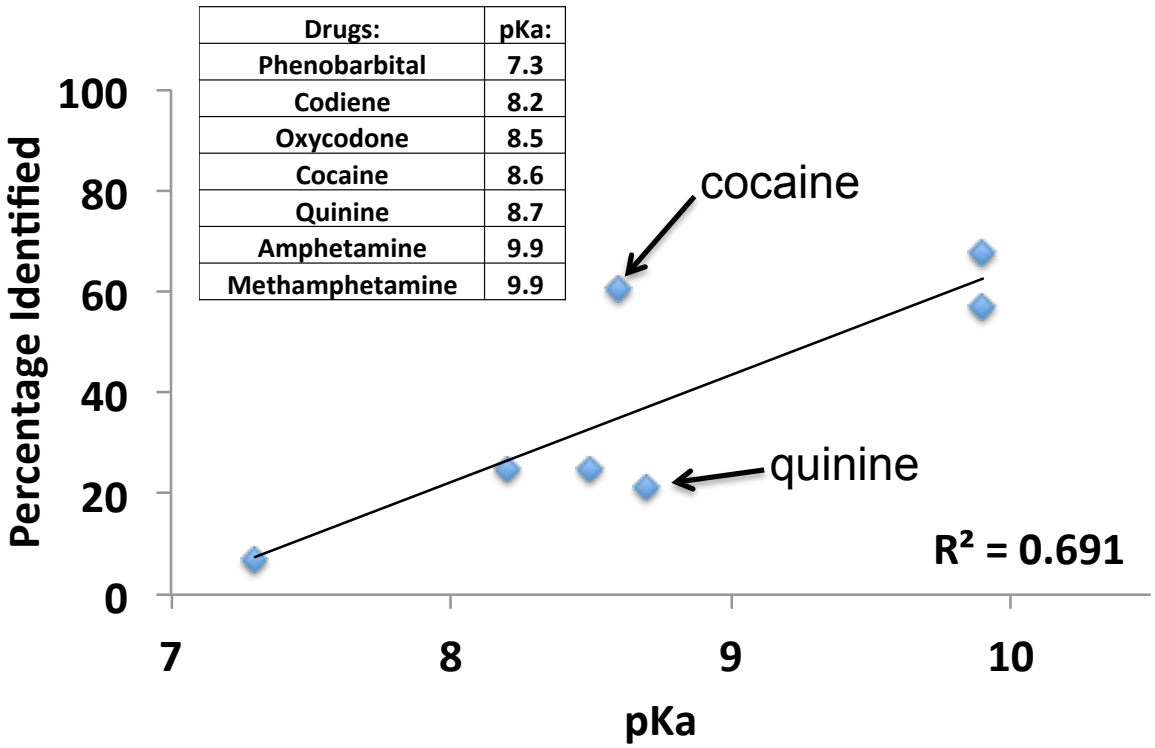

Figure 3. Plot of the number of positive drug identifications versus the pKa of the conjugate acid form of the individual drugs. Each drug and their corresponding pKa are identified in the inset table.

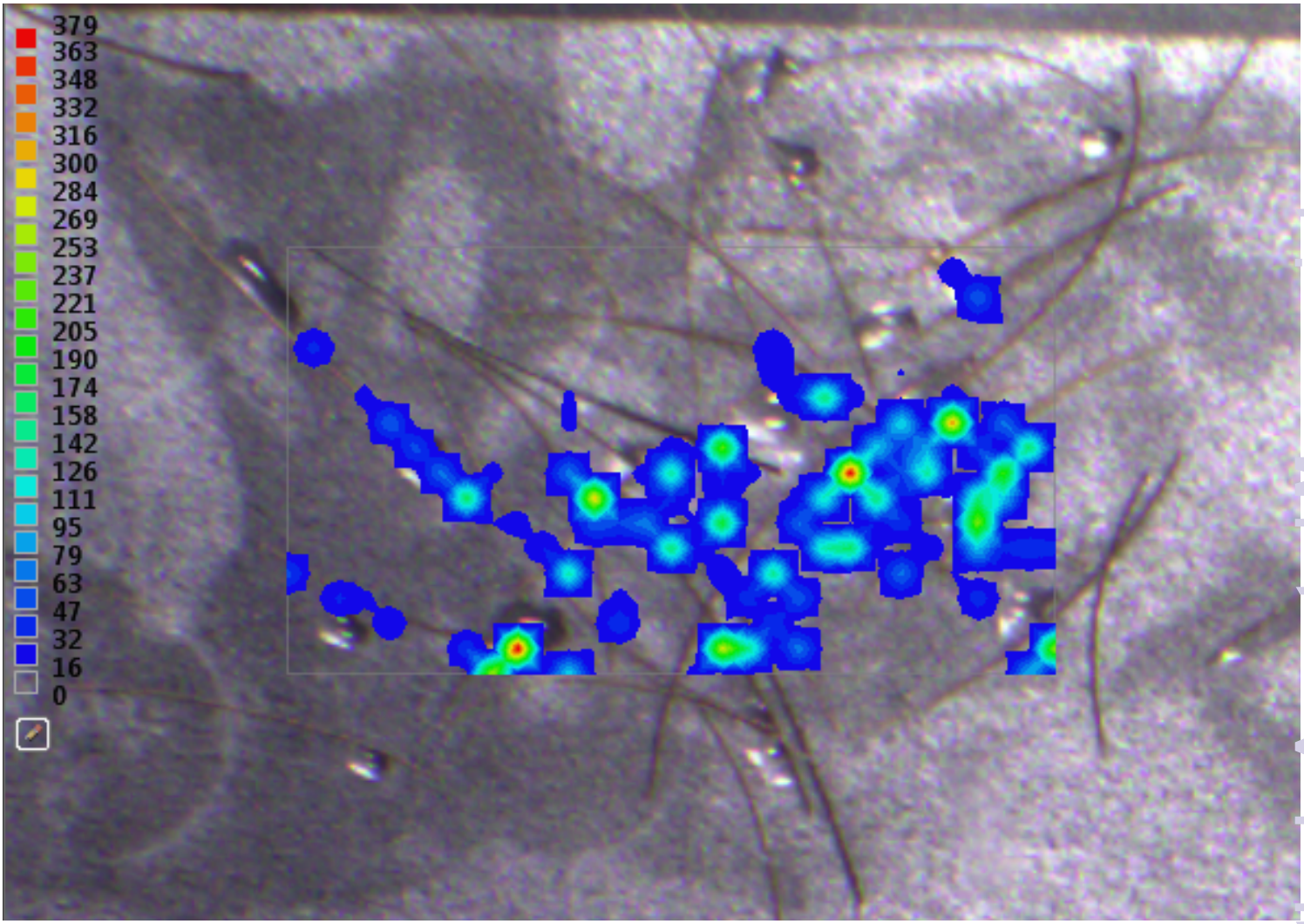

Figure 4. Overlay of the ion map of morphine onto the photographic image of the associated hair sample. The color scale associated with each of the ion maps indicates the absolute intensity of the product for the transition from *m/z* 286 → *m/z* 201.

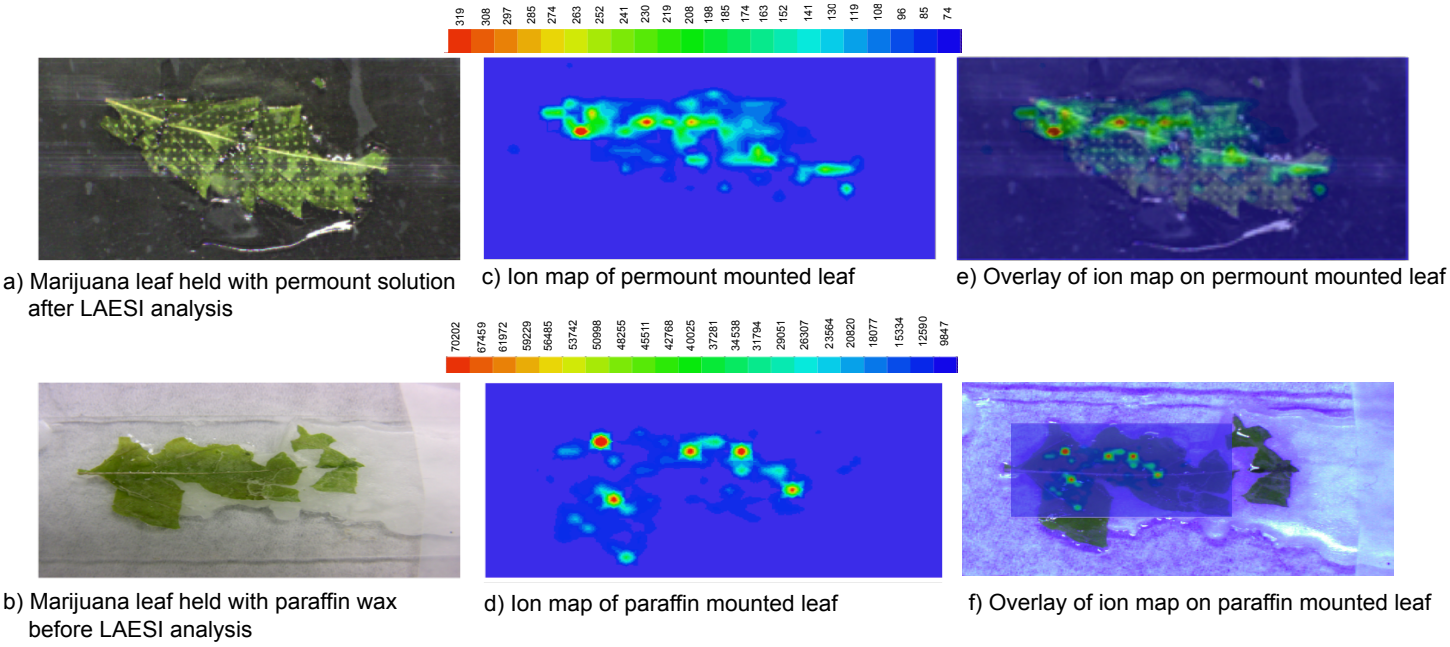

Figure 5. A cannabis leaf held onto a microscope slide with a) permount solution after LAESI analysis and b) paraffin wax before LAESI analysis, their associated ion maps c) for the permount mounted leaf d) for the paraffin mounted leaf and the overlay of the leaf image and its associated ion map for e) the Permount mounted leaf and f) the paraffin mounted leaf. The color scale associated with each of the ion maps indicates the absolute intensity of the product for the transition from *m/z* 315 → *m/z* 259, which is selective for THC & CBD.

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ASSOCIATED CONTENT

Supporting Information.

1) **Figure S-1.** An example of the comparison between the experimental and NIST reference data for a positive identification of amethamphetamine standard. The experimental data (top in red) is shown head-to-tail with the NIST reference spectrum (bottom in blue).

2) **Figure S-2.** Comparison of a) full-scan MS spectra of 50:50 quinine (m/z 325) and cocaine (m/z 304) as well as b) the tandem MS of the m/z 304 cocaine precursor ion and c) the tandem MS spectrum of the m/z 325 quinine precursor ion.

3) **Figure S-3**. Calibration curves generated from the peak area of the tandem mass spectrum of a) raw methamphetamine signal and b) the ratio of raw methamphetamine peak area to the raw peak area of the deuterated methamphetamine. Each calibration curve is shown with the 95% confidence interval of the linear regression line.

4) **Figure S-4**. Head-to-tail comparison of the tandem MS spectra obtained directly from human hair a) of codeine at ~7 ng/mg (precursor *m/z* 300) and b) morphine at ~12 ng/mg hair (precursor *m/z* 286). In each spectrum, the experimental data is shown in red while the NIST reference is shown in blue.

5) **Figure S-5.** MS/MS data for the analysis of THC in a cannabis leaf a) with LAESI and b) with DAPPI. (Reprinted with permission from reference 54.)

6) **Figure S-6.** The comparison of a) the MS data for paraffin wax and b) the MS data for permount solution with c) the typical background obtained during a LAESI analysis.

7) **Figure S-7.** MS/MS data for the analysis of THC in a cannabis leaf mounted using paraffin wax with LAESI.