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Rapid analysis of forensic-related samples using two ambient ionization techniques coupled to high-resolution mass spectrometers

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RATIONALE: This paper highlights the versatility of interfacing two ambient ionization techniques, Laser Diode Thermal Desorption (LDTD) and Atmospheric Solids Analysis Probe (ASAP), to high-resolution mass spectrometers and demonstrate the method's capability to rapidly generate high-quality data from multiple sample types with minimal, if any, sample preparation.

METHODS: For ASAP-MS analysis of solid and liquid samples, the material was transferred to a capillary surface before being introduced into the mass spectrometer. For LDTD-MS analysis, samples were solvent extracted, spotted in a 96-well plate, and the solvent was evaporated before being introduced into the mass spectrometer. All analyses were performed using Atmospheric Pressure Chemical Ionization in positive mode.

RESULTS: Seven consumer "Spice" packets were combined and analyzed by both ASAP and LDTD, which identified 11 synthetic cannabinoids/cathinones by full MS and MS/MS experiments. To further show the usefulness of these techniques, black tar heroin was analyzed, which resulted in the identification of heroin and its impurities (monoacetylmorphine, papaverine, and noscapine). These experiments were performed on the LTQ-Orbitrap to demonstrate the ability to perform both parallel and serial MS and MSⁿ experiments.

CONCLUSIONS: Interfacing LDTD and ASAP to high-resolution mass spectrometers allows for expeditious analysis of a wide range of samples, with minimal or no sample preparation. Both allow for rapid full scan, MS/MS, and/or MSⁿ experiments from a single sample introduction. Published in 2017. This article is a U.S. Government work and is in the public domain in the USA.

Over the past several decades, mass spectrometry (MS) has become an indispensable tool for analytical chemists of various disciplines. This is due in part to the evolution of new ionization techniques, which have greatly expanded the use of MS. Perhaps most notable was the introduction of electrospray ionization (ESI), which increased the scope of MS to include larger, more polar molecules.^[1] Within the past decade, there has been an escalation in the development of ambient ionization techniques, which are easily adaptable for small molecules, and in some cases have considerably reduced sample preparation time. Ambient ionization techniques can be grouped into two major categories: ESI-based techniques, which include desorption electrospray ionization (DESI),^[2–7] electrospray laser desorption ionization (ELDI),^[8–10] matrix-assisted laser desorption electrospray ionization (MALDESI),^[11–13] and paper spray ionization (PSI),^[17,14,15] and atmospheric pressure chemical ionization (APCI)-based ambient ionization techniques, which include atmospheric solids analysis probe (ASAP),^[16–19] desorption atmospheric pressure chemical ionization (DAPCI),^[20–22] direct analysis in real time (DART),^[23–26] and laser diode thermal desorption (LDTD),^[27–30] among others.^[31]

Ambient techniques offer several advantages over more traditional ionization techniques such as ESI. These advantages include ultrafast analysis (as little as 8 seconds

per sample) due to the elimination of chromatographic separation. Other benefits include an increase in ionization efficiency^[27] attributable to the elimination of mobile phases, which causes increased suitability for smaller nonpolar and less polar compounds, a lack of solvent clusters and metal adducts,^[28] and a decrease in sample preparation time. Specifically, LDTD and ASAP have been applied to a variety of fields including environmental,^[29] polymers,^[32] food,^[27] toxicology,^[33] and proteomics,^[34] among others. Coupling any of these ionization techniques with a high-resolution mass spectrometer, such as an Orbitrap, offers enormous advantages. In addition to the rapid analysis obtained with ASAP and LDTD, an Orbitrap provides high mass accuracy and high resolving power, and, as a result, increases the specificity of the procedure. The benefit of high mass accuracy becomes important when analyzing small molecules, because molecules with minor mass differences can be resolved. In a 2015 study, Jagerdeo and colleagues demonstrated the effectiveness of coupling ASAP with a linear ion trap to analyze forensic samples.^[18] In that study, the researchers established a protocol that allowed for method development with a single sampling. That methodology serves as the basis for the ASAP experiments presented here. This new work expands the applications to two separate approaches, wherein both ASAP and LDTD probes were interfaced with an Orbitrap mass spectrometer to analyze drugs of abuse.

Laser diode thermal desorption and atmospheric solids analysis probe are two methods that offer complete data acquisition of synthetic cannabinoids and cathinones in a

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matter of seconds. LDTD uses a specially designed 96-well plate with stainless steel alloy inserts. The sample is thermally desorbed from the stainless steel well by an infrared laser, which forms neutral gas-phase molecules.^[35] These gas-phase molecules are carried into the mass spectrometer inlet by compressed air. Just prior to entering the mass spectrometer inlet, the neutral molecules are ionized by a corona discharge needle. This LDTD-APCI-MS method results in a completely automated analysis with low sampling times, at approximately 8 s per well, and high-throughput capabilities of running up to 10 plates sequentially.^[35]

The ASAP ambient ionization technique offers similar advantages to the LDTD technique. ASAP can be interfaced with any mass spectrometer that has an APCI source, and it does not require any additional software or hardware modifications. In ASAP, a melting point capillary tube is used to introduce the sample into a stream of heated nitrogen gas, which results in the sample being desorbed from the capillary.^[18] The desorbed sample is then ionized by a corona discharge needle. This is an attractive technique for rapid, high-throughput screening of a wide range of samples, including both volatile and nonvolatile compounds, drugs, and fungi.^[16,36] For this study, the ASAP was interfaced to a Thermo Ion Max source, which allowed for ease of switching between APCI and ESI with minimal modification. Switching from APCI to ESI is as simple as moving the voltage supply lead from the corona discharge needle to the ESI probe. However, one can also remove the corona discharge needle when the system is being used in the ESI mode. No modification to the ASAP interface is required, but if a capillary is in place, it should be removed.

Over the past decade a new class of designer drugs referred to as Spice has become a phenomenon throughout the world. Spice, which can be purchased at convenience stores and on the internet, is advertised as herbal incense, and is not intended for human consumption. However, Spice is marketed as a 'legal high' and contains synthetic cannabinoids. Manufacturers of these deceptively 'harmless' herbal incense products dissolve synthetic cannabinoids in a solvent and then spray the solution directly on dried plant material.^[37] The first-generation Spice products contained the synthetic cannabinoids CP-47, 497-C8, JWH-018, and JWH-073, so named for the structure of the compound, cyclohexylphenol (CP), and after John W. Huffman (JWH) who first synthesized several of the compounds.^[38] Shortly after these compounds were discovered in Spice, the US Drug Enforcement Agency (DEA) designated them, as well as two others, as Schedule I controlled substances. However, this did not put an end to the Spice trend as manufacturers developed structurally similar compounds to avoid the regulations.

It is becoming ever more important to characterize the synthetic cannabinoids contained in Spice products. Since these compounds are not intended for human consumption, there have not been extensive studies into the clinical consequences stemming from their use and abuse. However, it has been reported that there is a rise in human fatalities due to Spice exposure.^[39] Studies have shown that these synthetic cannabinoids bind to cannabinoid receptors in the brain with affinities similar to or greatly exceeding that of cannabis.^[39,40] One significant difficulty in regulating these compounds is their ability to go undetected in routine drug screenings.

In addition to the analysis of synthetic cannabinoids, black tar heroin was examined using the same techniques. Heroin, a Schedule I substance under the Controlled Substances Act, is one of the most abused illicit drugs. Heroin is a semisynthetic drug produced by the acetylation of morphine, a naturally occurring alkaloid found in opium, and is a highly addictive, tolerance developing drug.^[41] Black tar heroin contains naturally occurring impurities, as well as impurities that arise from the synthesis process. Some of the major impurities found in heroin are morphine, codeine, noscapine, papaverine, and monoacetylmorphine,^[18] all of which can be identified in a single acquisition using the procedures described in this paper.

Drugs of abuse have been analyzed by various mass spectrometry techniques including LC/MS, GC/MS, and DART-MS.^[18,40,42,43] Herein, we report the analysis of Spice packets and black tar heroin by two ambient APCI techniques, LDTD and ASAP. The applications used in this study demonstrate the capabilities of the techniques when analyzing difficult forensic samples with minimal to no sample preparation providing a rapid screening procedure.

EXPERIMENTAL

Chemicals, reagents and materials

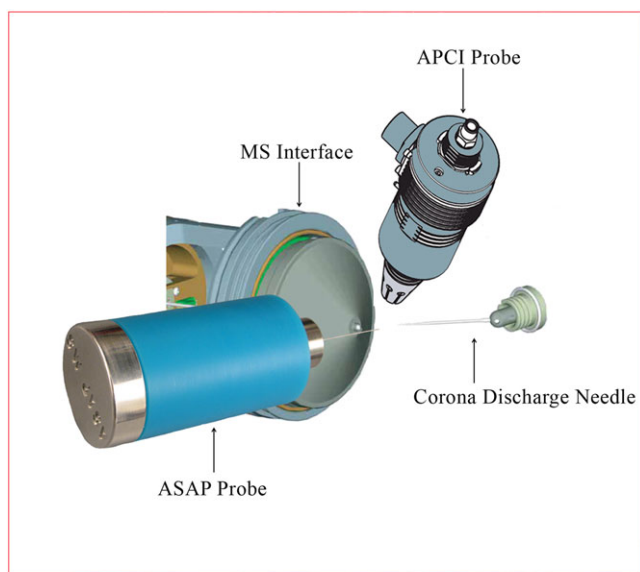
For confirmation of the chemicals present in the samples, certified standards of JWH-019, JWH-250, JWH-210, JWH-018, JWH-081, JWH-073, AM-2201, RCS-4, AM-694, and 3,4-methylenedioxypropylvalerone hydrochloride (MDPV) were purchased from Cayman Chemical Company (Ann Arbor, MI, USA) at a purity of >98%, except AM-694 which was obtained at >95% purity. Papaverine was purchased from Sigma Aldrich Inc. (Milwaukee, WI, USA) at a purity of 98%. Standards of heroin, noscapine, and 6-monoacetylmorphine were purchased from Cerilliant Corporation (Round Rock, TX, USA) at a purity of 100%, >98%, and 100%, respectively. Black tar heroin, 5-F-UR-144, and seven Spice packets were obtained from the DEA Special Testing Laboratory (Sterling, VA, USA). Purchased standards were diluted to 5 µg/mL with methanol. LC/MS-grade methanol (MeOH), centrifuge tubes, and pipette tips were purchased from Fisher Scientific (Pittsburgh, PA, USA). The LazWell™ 96-well plates used in the autosampler of the LDTD probe were purchased from Phytronix Technologies (Quebec, Canada). Samples analyzed directly on the ASAP source used a closed-end melting point capillary tube of 100 mm in length purchased from Altech Associates (Bannockburn, IL, USA). The mass spectrometer internal calibration was performed using a mixture of caffeine, MRFA (peptide), and Ultramark 1621 purchased from Sigma Aldrich (St. Louis, MO, USA).

Instrumentation and analytical conditions

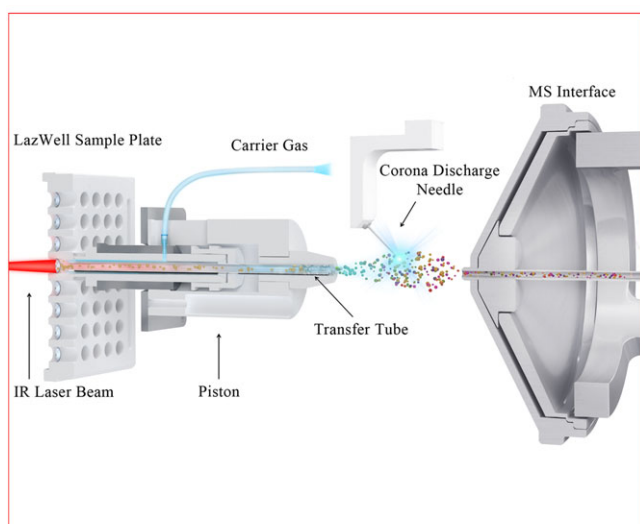
ASAP-HESI Q Exactive and ASAP-HESI-LTQ Orbitrap XL

Positive ion mass spectra were acquired using both a Q Exactive and LTQ-Orbitrap XL mass spectrometer that were configured with an Ion Max source, a Heated Electrospray Ionization (HESI II) probe (Thermo Fisher Scientific Inc., San Jose, CA, USA), and an Atmospheric Solid Analysis Probe (ASAP) (M&M Mass Spec Consulting, LLC, Harbeson, DE, USA). The ASAP source was interfaced with the Ion Max

source from the left side of the mass spectrometer, which is commonly used for an atmospheric pressure photoionization (APPI) probe, as shown in Fig. 1(A). The depth of the HESI II probe in the Ion Max source was set between the B and C markings located on the outer surface of the probe. The tip of the HESI II probe was aligned with the orifice of the mass spectrometer. Nitrogen was used as the auxiliary gas and set to an arbitrary flow rate of 5; the sheath gas and sweep gas were set to 0. The temperature and discharge voltage used for all experiments was 400°C and 4.5 kV, respectively. Data were acquired on the Q Exactive using Xcalibur software version 2.2 with Tune Page version 2.2 SP1, while data acquired on the LTQ-Orbitrap XL used Xcalibur 2.0.7 and Tune Page 2.5.5 SP1 (Thermo Fisher Scientific Inc.).



A



B

Figure 1. (A) Schematic of the Atmospheric Solids Analysis Probe (ASAP) interfaced with an Ion Max source and the high-resolution mass spectrometers (Q Exactive and an LTQ-Orbitrap XL). (B) Schematic of Laser Diode Thermal Desorption (LDTD) probe interfaced with an Ion Max source and a high-resolution mass spectrometer (Q Exactive). [Color figure can be viewed at wileyonlinelibrary.com]

LDTD Q Exactive

All data were acquired in the positive ion mode on the Q Exactive mass spectrometer when coupled to a Phytronix model T-960 Laser Diode Thermal Desorption (LDTD) ion source. The LDTD source is comprised of a modified Ion Max source operated in APCI mode, a 20 W diode laser, and an autosampler that uses the Lazwell™ 96-well plate (Phytronix Technologies), as shown in Fig. 1(B). Nitrogen was used as the sweep gas and set to an arbitrary flow rate of 1; the sheath and auxiliary gas were set to 0. A discharge voltage of 5 kV, a capillary temperature of 250°C, and a vaporizer temperature of 31°C were used for all experiments. Compressed air was used for the gas flow to sweep the sample into the source and for the operation of the autosampler. Data acquired on the Q Exactive used Xcalibur version 2.2 and the Tune Page version 2.2 SP1 software (Thermo Fisher Scientific Inc.).

Sampling procedure and experiments

ASAP-HESI Q Exactive and ASAP-HESI LTQ-Orbitrap XL

Black tar heroin, "Spice" products, and drug standards were analyzed directly without any sample preparation. For solid samples, the capillary was rubbed on the material a few times to transfer the compounds to the capillary, and any excess particulates were removed with a tissue wipe before the capillary was placed into the stream of hot nitrogen gas supplied by the HESI II probe. For liquid samples, the capillary was dipped into the solution to form a coating on the surface and any solvent was allowed to evaporate at room temperature before analysis. The optimal position of the capillary was found to be in the region above the corona discharge needle and between the HESI II probe and the orifice of the mass spectrometer. If an Ion Max source is used, the probe is interfaced from the left side that is normally used for an APPI probe, and this will ensure that the optimal position is achieved. When the capillary was introduced into the source, it was initially retracted from the optimal position, so as to give the user the opportunity to start the acquisition and then slowly moved into its ideal location. Rubbing the capillary on the sample allowed at least the first few centimeters of the capillary to be covered with the material. As sample was desorbed from the capillary, the response was monitored and the position of the capillary was slowly adjusted, prolonging the analysis time. The mass accuracy of the data was based off the instrument's internal calibration, and no additional calibration was needed.

LDTD-Q-Exactive

Equal portions of seven different commercial "Spice" products were mixed to create an analytical sample. From this combined sample, 0.1295 g was extracted with 3 mL of methanol by vortexing for 3 min and then left to settle for 15 min. A 1:3 dilution of the supernatant with MeOH was performed. A 5 µL aliquot of the diluted solution was added to a Lazwell™ 96-well plate equipped with stainless steel wells. The Lazwell™ plate was placed in a hood until the liquid evaporated from the wells and was then loaded into the LDTD autosampler and analyzed as outlined above. The instrument was operated in a data-dependent mode where a full-scan

mass spectrum was acquired over a mass range of 225–500 m/z followed by eleven MS/MS spectra in positive ion mode with a total runtime of 0.38 min. The full MS section of the experiment was performed at a resolution of 35 K in centroid mode with an AGC setting of $1e6$ and a max IT time of 50 ms. The dd-MS/MS portion of the experiment was performed at a resolution of 35 K in centroid mode with an AGC setting of $2e4$, a max IT of 50 ms, an isolation width of 3 Da, a Normalized Collision Energy (NCE) of 30, and a stepped NCE of 20%.

Several laser patterns were used to control the laser and then evaluated to determine the optimal conditions where the response of the analyte was maximized while minimizing the release of potentially interfering matrix residues. The laser pattern ultimately chosen was as follows: 2.0 s at 0%; ramp to 45% over 6 s (2–8 s); hold for 4.5 s (8–12.5 s), drop to 0% in 1 s, then hold at 0% for 2 s, using a 20 W diode laser. Total analysis of the sample was achieved within 18 s from sample to sample, which included the movement of the 96-well plate to position the sample well in front of the laser.

RESULTS AND DISCUSSION

The contents of seven "Spice" packets were combined and analyzed with both ASAP and LDTD coupled to a high-resolution mass spectrometer. In total, ten synthetic cannabinoids and one synthetic cathinone were detected, all of which are listed in Table 1. For analysis with the ASAP-HESI-Q Exactive, the signal lasted long enough to perform a full scan and eleven MS/MS experiments, permitting acquisition of an MS/MS spectrum for each cannabinomimetic identified, as shown in Fig. 2. Figure 2(A) shows the total ion current chromatogram for all the experiments, identifying the time dedicated to complete each MS/MS experiment. Figure 2(B) shows the full scan mass spectrum of the extract of the mixed "Spice" products with the m/z values of the identified compounds marked. The individual MS/MS spectrum of each compound is shown in Figs. 2(C)–2(L). Different collision energies were selected for each compound to provide adequate fragmentation while retaining reasonable signal for the precursor. With the exception of 5-F-UR-144 (Fig. 2(L)), the MS/MS spectra did not show any significant interference. The abundance of the interfering ion in the spectrum for 5-F-UR-144 was so low that it did not impact the MS/MS spectrum significantly, as shown in Fig. 2(N). However, in the event of a significant interference, the result would be a mixed spectrum of the analyte of interest and the interfering compound, especially when the compounds are very close in mass and abundance.

In the application for the analysis of black tar heroin using the ASAP-HESI-LTQ-Orbitrap XL, the signal lasted for more than 8 min, which allowed time for a full scan and six MS/MS experiments to be performed in the Orbitrap, as shown in Fig. 3. Figure 3(A) shows the total ion current chromatogram for all the experiments, identifying the time dedicated to complete each MS/MS experiment. Figure 3(B) shows the full scan mass spectrum of the black tar heroin with m/z values labeled for all the compounds and degradation products. The degradation products observed at m/z 268.133, 286.144, and 310.144 are potentially from degradation or in-source breakdown of monoacetylmorphine and heroin. The

Table 1. List of compound names, elemental compositions, CAS#, theoretical masses, theoretical $[M + H]^+$ masses, observed masses and the mass errors (mDa)

Compound name	CE (V)	Precursor ion (m/z)	Product ions (m/z)
JWH-019	30	356.200	356.200, 228.138, 155.049
JWH-250	30	336.195	336.195, 200.143, 121.0649
JWH-210	30	370.216	370.216, 214.122, 183.080
JWH-018	30	342.185	342.185, 214.122, 15.049
JWH-081	30	372.196	372.196, 214.122, 185.060
JWH-073	30	328.169	328.169, 200.107, 155.049
AM-2201	30	360.175	360.175, 232.112, 155.049
AM-694	30	436.056	436.056, 309.152, 230.930
RCS-4	30	322.179	322.179, 214.122, 135.044
5-F-UR-144	30	330.222	330.222, 232.113, 125.096
MDPV	30	276.159	276.159, 205.086, 175.075, 126.128
Heroin	30	370.166	370.166, 328.155, 310.145, 268.134
Monoacetyl-morphine	35	328.155	328.155, 268.134, 211.076, 193.065, 165.070
Papaverine	35	340.154	340.154, 324.124, 202.087, 171.068
Noscapine	25	414.156	414.156, 396.145, 365.103, 220.097

CE: Collision energy

MS/MS spectra of the degradation products at m/z 268.133 and 310.144 are shown in Figs. 3(C) and 3(D), respectively. The MS/MS spectra of monoacetylmorphine, papaverine, heroin, and noscapine are shown in Figs. 3(E)–3(H), respectively. In addition, three fragmentation experiments (MS^2 , MS^3 , MS^4) of noscapine were performed in the LTQ, and detected in the Orbitrap, as shown in Figs. 3(J)–3(L). The combination of experiments was performed in serial mode, where a full scan was acquired in the Orbitrap to determine the accurate mass of the analytes, followed by MS^n experiments performed in the LTQ and acquired in the Orbitrap. However, in parallel mode, while the full scan accurate mass of the analyte is acquired in the Orbitrap, the MS^n experiments are performed in the LTQ. Serial mode is always slower than parallel mode, so the experiments were deliberately performed in serial mode to demonstrate that the signal lasted long enough to perform such a complex set of experiments. The Q Exactive mass spectrometer uses quadrupole isolation to filter the ions prior to undergoing fragmentation in the HCD cell or analysis in the Orbitrap; therefore, this instrument is only capable of performing full MS and MS/MS experiments, and can only do so in serial mode. On the other hand, a LTQ-Orbitrap XL mass spectrometer has an ion trap that performs isolation prior to analysis in the Orbitrap when performing high-resolution analysis. Additionally, fragmentation and analysis can occur in the ion trap for a MS/MS experiment while the full scan spectra is being analyzed in the Orbitrap for parallel analysis. The LTQ-Orbitrap XL has the ability to perform both serial and parallel experiments. Since the experiments shown in Figs. 2 and 3 were performed directly from the Tune Page software, the time between experiments varied because the mass

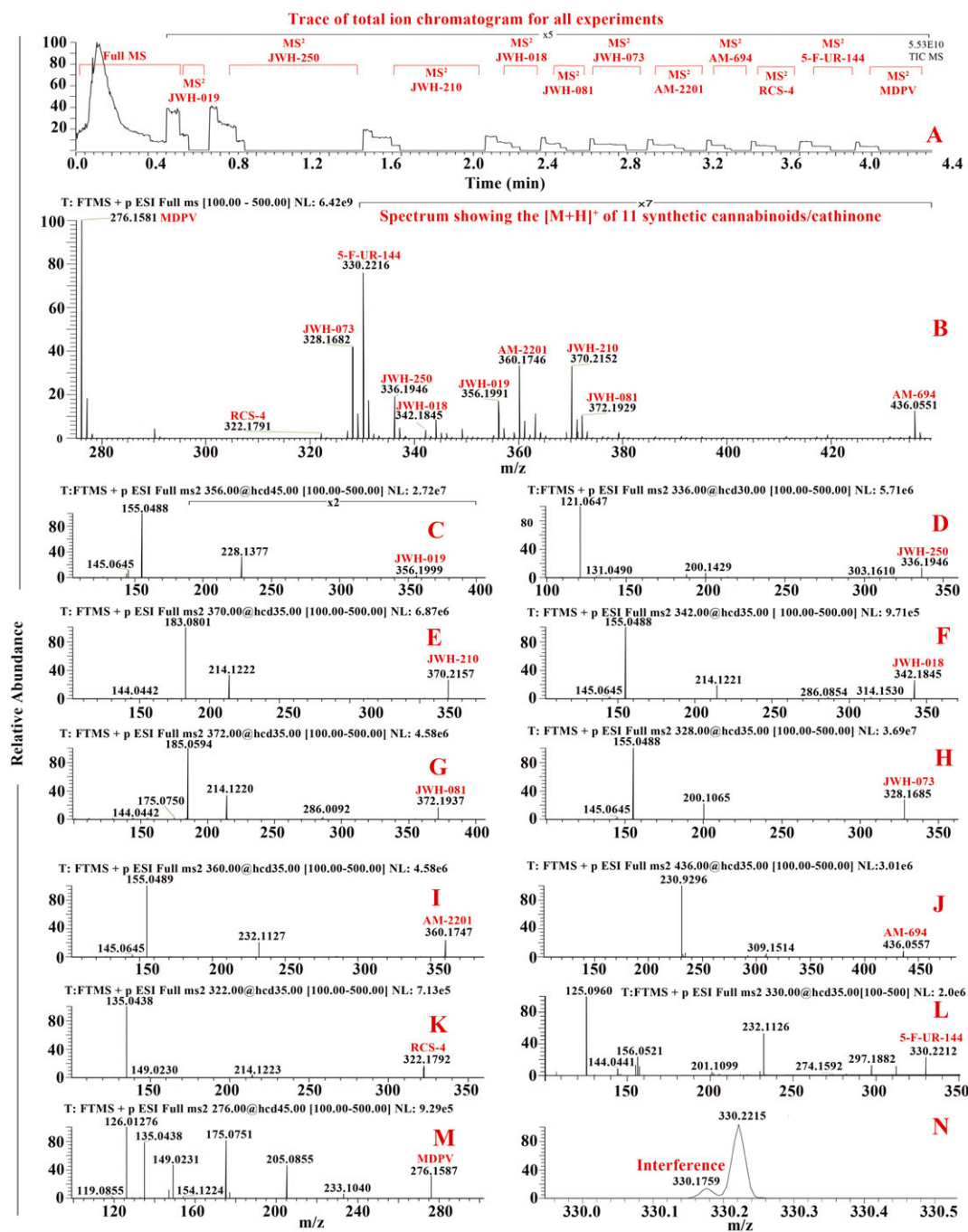


Figure 2. (A) Total analysis time for all experiments, and the total ion trace for 11 synthetic cannabinoids and/cathinone from a combined sample of seven "Spice" packets. (B) Full scan spectrum showing the $[M + H]^+$ ion of each analyte. (C–M) MS/MS spectra of each analyte. (N) Interference with the mass spectrum of 5-F-UR-144. [Color figure can be viewed at wileyonlinelibrary.com]

selection, collision energy, and choice of experiments had to be entered manually. Once these parameters are defined, an Xcalibur software method can be created, and, as a result, the actual time to perform an experiment could be reduced to less than 1 min.

For ASAP experiments the goal is to have a light coating of the material on the capillary, and any excess should be removed. If more material is needed to complete the entire experiment or combination of experiments, then two different approaches can be used: (1) for liquid samples, a 5 μ L aliquot

of sample can be added to the tip of the capillary or multiple 5 μ L aliquots can be added, after the solvent from the previous addition has evaporated; (2) for solid samples, an abrasive sandpaper can be used to roughen the surface of the capillary, so as to increase the surface area before the capillary is rubbed onto the sample. The roughened surface capillary could also be used for liquid samples. The conditions detailed in the instrumentation and analytical methods section were determined to be the optimal conditions for all of the experiments outlined in this paper.

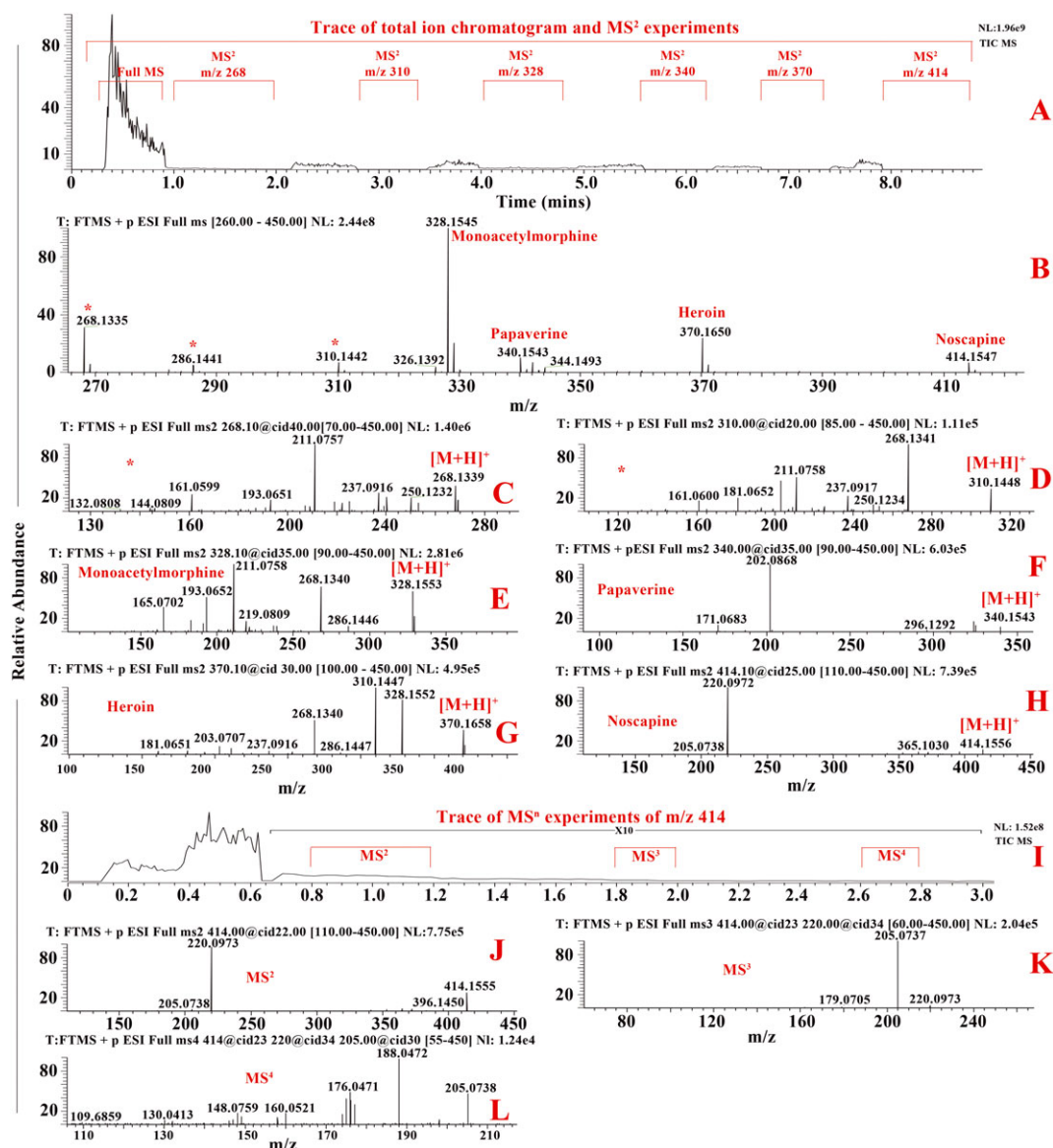


Figure 3. (A) Total analysis time for all experiments, and the total ion trace of black tar heroin and selected constituents. (B) Full scan spectrum of noscapine, heroin, papaverine, monoacetylmorphine, and degradation products (*). (C, D) MS/MS spectra of degradation products with $[M + H]^+$ ion of m/z 268 and 310, respectively. (E) MS/MS spectrum of monoacetylmorphine $[M + H]^+$ ion of m/z 328. (F) MS/MS spectrum of papaverine $[M + H]^+$ ion of m/z 340. (G) MS/MS spectrum of heroin $[M + H]^+$ ion of m/z 370. (H) MS/MS spectrum of noscapine $[M + H]^+$ ion of m/z 414. (I) Total analysis time and total ion trace for MS², MS³, and MS⁴ for noscapine. (J–L) MS², MS³, and MS⁴ spectra respectively for noscapine. [Color figure can be viewed at wileyonlinelibrary.com]

The contents of seven Spice packets were combined, solvent extracted for 10 min, and analyzed by the LDTD-Q Exactive. This analysis identified the same set of compounds found in the ASAP-HESI-Q Exactive experiment. Since the hardware of the LDTD source is directly coupled to the mass spectrometer, and the software is integrated into the Xcalibur platform, it is much easier to automate the analysis, and, as a result, the analysis time to acquire a full scan and eleven MS/MS spectra was only 18 s. When compared to the analysis on the ASAP-HESI-Q Exactive, this analysis is significantly faster; however, when sample preparation is taken into account, the time per sample is roughly double. Figure 4(A)

shows the combined spectrum of all eleven compounds. Shown in Figs. 4(B)–4(L) are the extracted ion chromatograms and the associated MS/MS spectra for each of the compounds. This short LDTD acquisition time still allows for the acquisition of multiple MS/MS scans for each compound from a single sampling.

Method development for the LDTD source required a different approach than that taken for the ASAP counterpart. The most important aspect of method development for LDTD is the laser pattern. The LDTD probe introduces the sample into the mass spectrometer all at once; however, the signal from a sample will result in a chromatographic-like effect

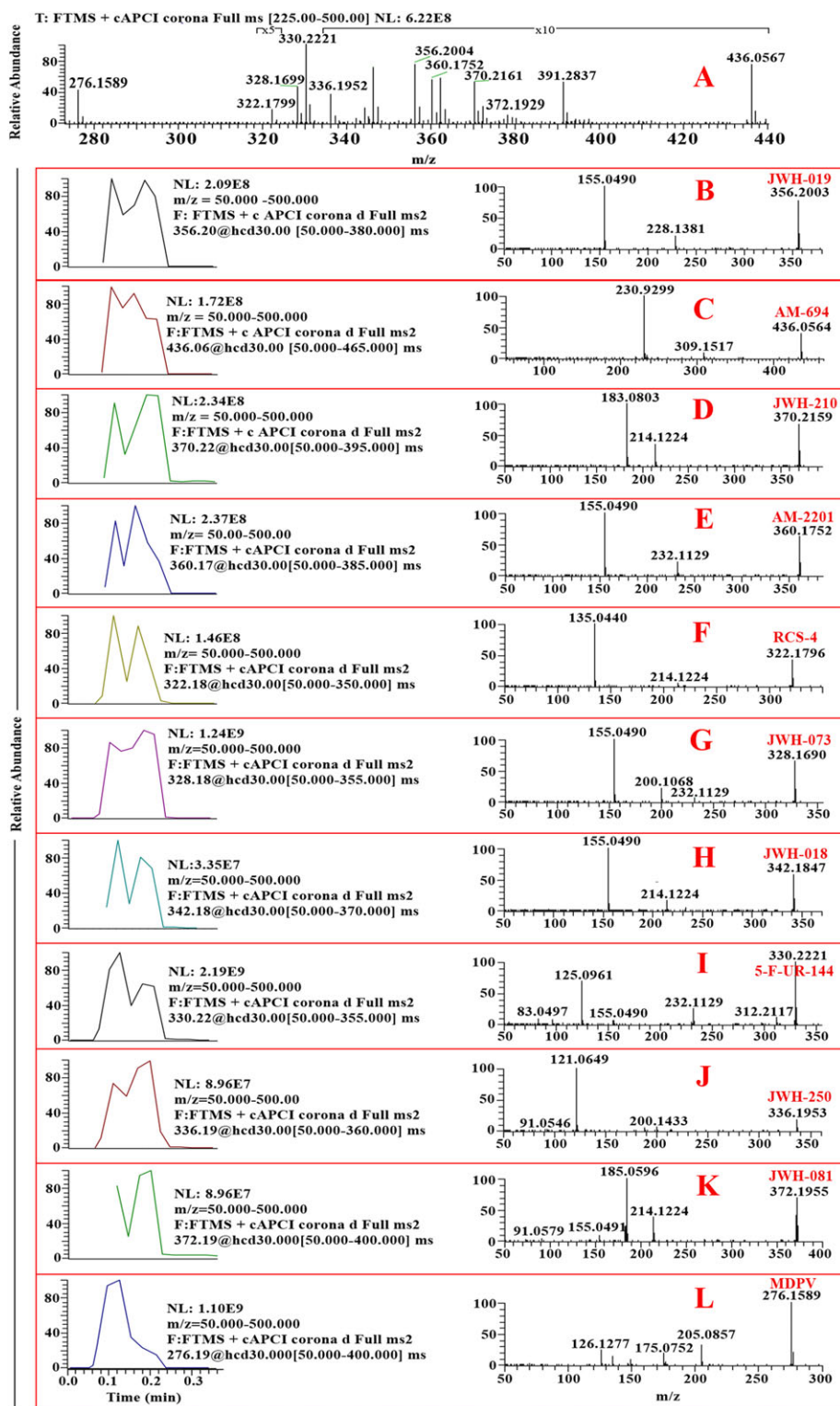


Figure 4. (A) Full scan spectrum of each analyte showing its $[M + H]^+$ ion for 11 synthetic cannabinoids/cathinone from a combined sample of seven Spice packets. (B–L) MS² spectra of JWH-019, AM-694, JWH-210, AM-2201, RCS-4, JWH-073, JWH-018, 5-F-UR-144, JWH-250, JWH-081, and MDPV. [Color figure can be viewed at wileyonlinelibrary.com]

attributable to the power gradient from the laser pattern. The response of an analyte can be optimized by adjusting the properties of the laser pattern, which includes the intensity

of the laser and the duration of each intensity, much like a chromatographic gradient. This effect is on a much faster timeframe than a traditional chromatographic separation, a

matter of seconds, as opposed to minutes. With appropriate internal standards, LDTD-MS can be utilized to quantitate samples.^[33,44] The laser pattern will determine the total experimental time, and, as a result, it could impact the resolution selected and the number of experiments performed. Sacrificing some resolution will improve the MS data acquisition rate. In contrast, these time constraints are not of the same importance for ASAP analysis. LDTD affords a quick sample screen in a matter of seconds, whereas ASAP is more suitable for optimizing experimental parameters on a sample-by-sample basis. For example, ASAP provides the opportunity to optimize collision energies for each individual sample, whereas, for LDTD, samples are analyzed in a data-dependent mode. One of the most common mistakes made when using LDTD occurs when the extracted matrix sample is overly concentrated before spotting the Lazwell™ 96-well plate with the intention of improving response. This approach leads to reduced signal when compared to a sample that is more diluted, because concentrating the sample also concentrates the matrix background as well and may lead to suppression of the signal of interest.

CONCLUSIONS

The results reported herein demonstrate that LDTD and ASAP when combined with high-resolution mass spectrometers can be useful tools for forensic analysis. The LDTD software is integrated into the mass spectrometer data acquisition software, and, as a result, allows for automated sample analysis. The ASAP can be easily interfaced with any mass spectrometer, and the simplicity of its design allows it to be easily switched between API techniques. Coupling these ambient ionization techniques with high-resolution mass spectrometers allows for rapid full scan, MS/MS, MSⁿ, and possible positive/negative switching for a single sample introduction. Because of these features, these techniques are very useful for rapid, routine analysis or for unknown determination.

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REFERENCES

- [1] J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, C. M. Whitehouse. Electrospray ionization for mass spectrometry of large biomolecules. *Science* **1989**, *246*, 64.
- [2] Z. Takáts, J. M. Wiseman, B. Gologan, R. G. Cooks. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* **2004**, *306*, 471.
- [3] Z. Takáts, J. M. Wiseman, R. G. Cooks. Ambient mass spectrometry using desorption electrospray ionization (DESI): instrumentation, mechanisms, and applications in forensics, chemistry, and biology. *J. Mass Spectrom.* **2005**, *40*, 1261.
- [4] D. Calligaris, D. Caragacianu, X. Liu, I. Norton, C. J. Thompson, A. L. Richardson, M. Golshan, M. L. Easterling, D. A. Dillon, F. A. Jolesz, N. Y. R. Agar. Application of desorption electrospray ionization mass spectrometry imaging in breast cancer margin analysis. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 42, 15184.
- [5] Z. Takáts, I. Cotte-Rodriguez, N. Talaty, H. Chen, R. G. Cooks. Direct, trace level detection of explosives on ambient surfaces by desorption electrospray ionization mass spectrometry. *Chem. Commun.* **2005**, *15*, 1950.
- [6] T. J. Comi, S. W. Ryu, R. H. Perry. Synchronized desorption electrospray ionization mass spectrometry imaging. *Anal. Chem.* **2015**, *88*, 1169.
- [7] A. E. O'Leary, S. E. Hall, K. E. Vircks, C. C. Mulligan. Monitoring the clandestine synthesis of methamphetamine in real-time with ambient sampling, portable mass spectrometry. *Anal. Methods* **2015**, *7*, 7156.
- [8] M. Z. Huang, S. C. Cheng, S. S. Cheng, C. C. Chou, C. N. Cheng, J. Shiea, I. A. Popov, E. N. Nikolaev. Ambient molecular imaging of dry fungus surface by electrospray laser desorption ionization mass spectrometry. *Int. J. Mass Spectrom.* **2012**, *325*, 172.
- [9] M. Z. Huang, H.J. Hsu, C. I. Wu, S. Y. Lin, Y. L. Ma, T. L. Cheng, J. Shiea. Characterization of the chemical components on the surface of different solids with electrospray-assisted laser desorption ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 1767.
- [10] Y. Kao, S. Cheng, C. Cheng, J. Shiea. Depth profiling of inks in authentic and counterfeit banknotes by electrospray laser desorption ionization/mass spectrometry. *J. Mass Spectrom.* **2015**, *51*, 20.
- [11] J. S. Sampson, A. M. Hawkrige, D. C. Muddiman. Generation and detection of multiply-charged peptides and proteins by matrix-assisted laser desorption electrospray ionization (MALDESI) Fourier transform ion cyclotron resonance mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 1712.
- [12] J. S. Sampson, A. M. Hawkrige, D. C. Muddiman. Development and characterization of an ionization technique for analysis of biological macromolecules: liquid matrix-assisted laser desorption electrospray ionization. *Anal. Chem.* **2008**, *80*, 6773.
- [13] J. A. Barry, M. R. Groseclose, G. Robichaud, S. Castellino, D. C. Muddiman. Assessing drug and metabolite detection in liver tissue by UV-MALDI and IR-MALDESI mass spectrometry imaging coupled to FT-ICR MS. *Int. J. Mass Spectrom.* **2015**, *377*, 488.
- [14] R. D. Epsy, S. F. Teunissen, N. E. Manicke, Y. Ren, Z. Ouyang, A. van Asten, R. G. Cooks. Paper spray and extraction spray mass spectrometry for the direct and simultaneous quantification of eight drugs of abuse in whole blood. *Anal. Chem.* **2014**, *86*, 7712.
- [15] H. Wang, Y. Ren, M. N. McLuckey, N. E. Manicke, J. Park, L. Wheng, R. Shi, R. G. Cooks, Z. Ouyang. Direct quantitative analysis of nicotine alkaloids from biofluid samples using paper spray mass spectrometry. *Anal. Chem.* **2013**, *85*, 11540.

- [16] C. N. McEwen, R. G. McKay, B. S. Larsen. Analysis of solids, liquids, and biological tissues using solids probe introduction at atmospheric pressure on commercial LC/MS instruments. *Anal. Chem.* **2005**, *77*, 7826.
- [17] C. Petucci, J. Diffendal. Atmospheric solids analysis probe: a rapid ionization technique for small molecule drugs. *J. Mass Spectrom.* **2008**, *43*, 1565.
- [18] E. Jagerdeo, J. A. Clark, J. N. Leibowitz, L. J. Reda. Rapid analysis of forensic samples using an atmospheric solid analysis probe interfaced to a linear ion trap mass spectrometer. *Rapid Commun. Mass Spectrom.* **2015**, *29*, 205.
- [19] E. J. Crevelin, F. H. Salami, M. N. R. Alves, B. S. De Martinis, A. E. M. Crotti, L. A. B. Moracs. Direct analysis of amphetamine stimulants in a whole urine sample by atmospheric solids analysis probe tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2016**, *27*, 944.
- [20] Y. Song, R. G. Cooks. Atmospheric pressure ion/molecule reactions for the selective detection of nitroaromatic explosives using acetonitrile and air as reagents. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 3130.
- [21] S. Yang, J. Ding, J. Zheng, B. Hu, J. Li, H. Chen, Z. Zhou, X. Qiao. Detection of melamine in milk products by surface desorption atmospheric pressure chemical ionization mass spectrometry. *Anal. Chem.* **2009**, *81*, 2426.
- [22] F. P. M. Jjunju, S. Maher, A. Li, S. U. Syed, B. Smith, R. M. A. Heeren, S. Taylor, R. G. Cooks. Hand-held portable desorption atmospheric pressure chemical ionization ion source for in situ analysis of nitroaromatic explosives. *Anal. Chem.* **2015**, *87*, 10047.
- [23] J. M. Nilles, T. R. Connell, H. D. Durst. Quantitation of chemical warfare agents using the direct analysis in real time (DART) technique. *Anal. Chem.* **2009**, *81*, 6744.
- [24] R. B. Cody, J. A. Laramée, J. M. Nilles, H. D. Durst. Direct analysis in real time (DARTtm) mass spectrometry, *JEOL News* **2005**, *40*, 8.
- [25] R. A. Musha, M. A. Domin, R. B. Cody, A. D. Lesiak, A. J. Dane, J. R. E. Shepard. Direct analysis in real time mass spectrometry with collision-induced dissociation for structural analysis of synthetic cannabinoids. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 2335.
- [26] T. Guo, P. Fang, J. Jiang, F. Zhang, W. Yong, J. Liu, Y. Dong. Rapid screening and quantification of residual pesticides and illegal adulterants in red wine by direct analysis in real time mass spectrometry. *J. Chromatogr. A* **2016**, *1471*, 27.
- [27] G. Blachon, P. Picard, P. Tremblay, S. Demers, R. Paquin, Y. Babin, P. B. Fayad. Rapid determination of chloramphenicol in honey by laser diode thermal desorption using atmospheric pressure chemical ionization-tandem mass spectrometry. *AOAC Int.* **2013**, *96*, 676.
- [28] M. Huang, C. Yuan, S. Cheng, Y. Cho, J. Shiea. Ambient ionization mass spectrometry. *Annu. Rev. Anal. Chem.* **2010**, *3*, 43.
- [29] P. B. Fayad, M. Prévost, S. Sauvé. Laser diode thermal desorption/atmospheric pressure chemical ionization tandem mass spectrometry analysis of selected steroid hormones in wastewater: method optimization and application. *Anal. Chem.* **2010**, *82*, 639.
- [30] N. D. Bynum, K. N. Moore, M. Grabenauer. Evaluation of laser diode thermal desorption tandem mass spectrometry (LDTD-MS-MS) in forensic toxicology. *J. Anal. Toxicol.* **2014**, *38*, 528.
- [31] D. N. Correa, J. M. Santos, L. S. Eberlin, M. N. Eberlin, S. F. Teunissen. Forensic chemistry and ambient mass spectrometry: a perfect couple destined for a happy marriage. *Anal. Chem.* **2016**, *88*, 2515.
- [32] M. J. P. Smith, N. R. Cameron, J. A. Mosely. Evaluating Atmospheric pressure Solids Analysis Probe (ASAP) mass spectrometry for the analysis of low molecular weight synthetic polymers. *Analyst* **2012**, *137*, 4524.
- [33] J. G. Swales, R. T. Gallagher, M. Denn, R. M. Peter. Simultaneous quantitation of metformin and sitagliptin from mouse and human dried blood spots using laser diode thermal desorption tandem mass spectrometry. *J. Pharm. Biomed. Anal.* **2011**, *55*, 544.
- [34] F. Zydell, S. Trimpin, C. N. McEwen. Laserspray ionization using an atmospheric solids analysis probe for sample introduction. *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 1889.
- [35] J. Wu, C. S. Hughes, P. Picard, S. Letarte, M. Gaudreault, J. F. Lévesque, D.A. Bateman, K. P. Nicoll-Griffith. High-throughput cytochrome P450 inhibition assays using laser diode thermal desorption-atmospheric pressure chemical ionization-tandem mass spectrometry. *Anal. Chem.* **2007**, *79*, 4657.
- [36] P. Wang, R. Wang, X. Su, Z. A. Ye. Novel and sensitive screening method for β -agonists in porcine urine by using atmospheric solid analysis probe source coupled tandem mass spectrometry. *Anal. Methods* **2012**, *4*, 4269.
- [37] M. Spaderna, P. H. Addy, D. C. D'Souza. "Spicing things up": synthetic cannabinoids. *Psychopharma* **2013**, *228*, 525.
- [38] S. Dresen, N. Fereiros, M. Putz, F. Westphal, R. Zimmermann, V. Auwärter. Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. *J. Mass Spectrom.* **2010**, *45*, 1186.
- [39] K. A. Seely, J. Lapoint, J. H. Moran, L. Fattore. Spice drugs are more than harmless herbal blends: a review of the pharmacology and toxicology of synthetic cannabinoids. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2012**, *39*, 234.
- [40] D. Zuba, B. Byrska, M. Maciow. Comparison of "herbal highs" composition. *Anal. Bioanal. Chem.* **2011**, *400*, 119.
- [41] Lesiak, A.D., Musah, R.A., Domin, M.A., Shepard, J.R.E. DART-MS as a preliminary screening method for "herbal incense": chemical analysis of synthetic cannabinoids. *J. Forensic Sci.* **2014**, *59*, 337.
- [42] S. Kneisel, V. Auwärter. Analysis of 30 synthetic cannabinoids in serum by liquid chromatography-electrospray ionization tandem mass spectrometry after liquid-liquid extraction. *J. Mass Spectrom.* **2012**, *47*, 825.
- [43] N. Uchiyama, R. Kikura-Hanajiri, J. Ogata, Y. Goda. Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. *Forensic Sci. Int.* **2010**, *198*, 31.
- [44] J. J. Lohne, W. C. Andersen, S. B. Clark, S. B. Turnipseed, M. R. Madson. Laser diode thermal desorption mass spectrometry for the analysis of quinolone antibiotic residues in aquacultured seafood. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 2854.