White Paper

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Forensic Laboratory Needs – Technology Working Group (FLN-TWG)

Evolving Approaches and Technologies for Seized Drug Analysis

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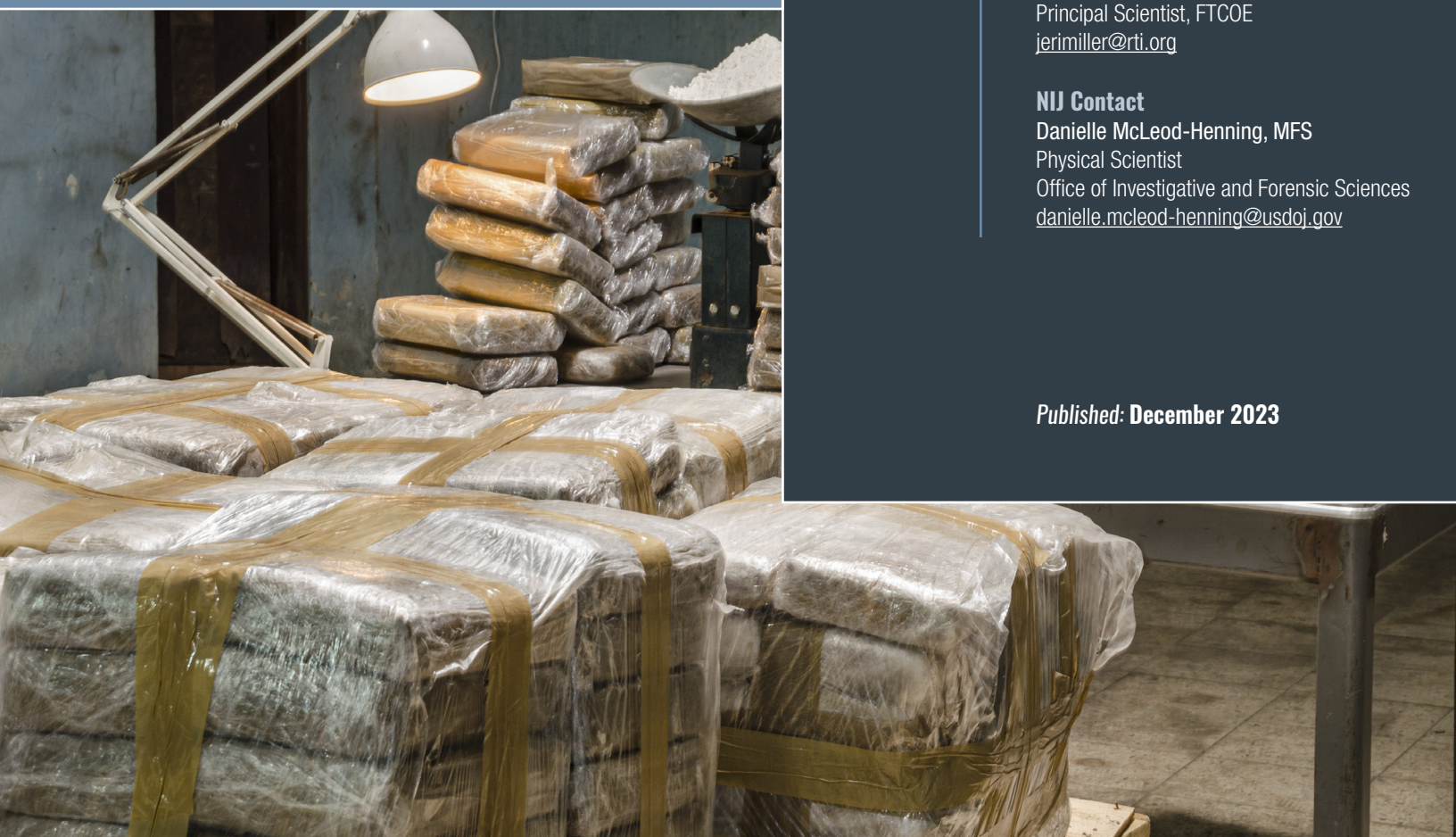

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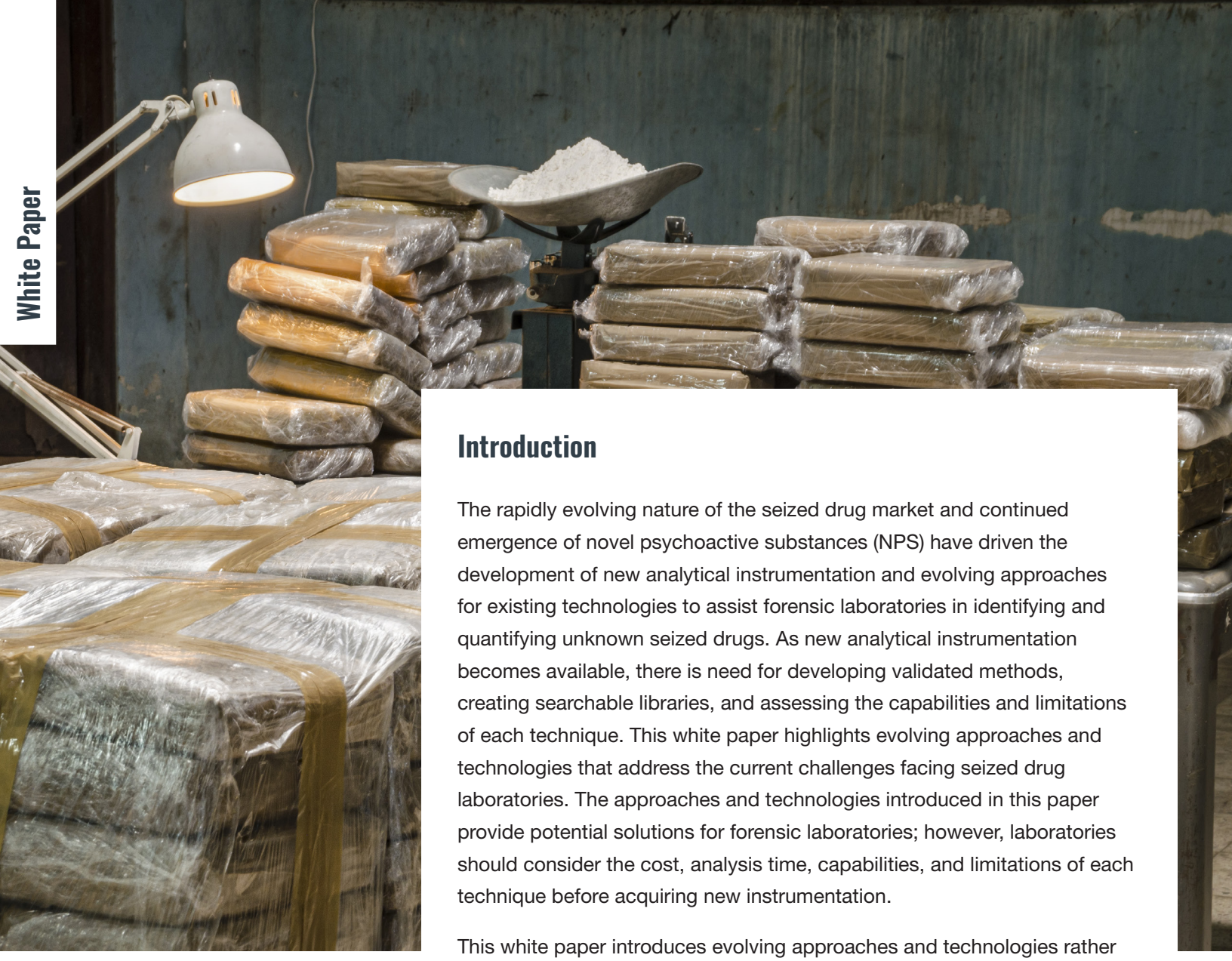

Introduction

The rapidly evolving nature of the seized drug market and continued emergence of novel psychoactive substances (NPS) have driven the development of new analytical instrumentation and evolving approaches for existing technologies to assist forensic laboratories in identifying and quantifying unknown seized drugs. As new analytical instrumentation becomes available, there is need for developing validated methods, creating searchable libraries, and assessing the capabilities and limitations of each technique. This white paper highlights evolving approaches and technologies that address the current challenges facing seized drug laboratories. The approaches and technologies introduced in this paper provide potential solutions for forensic laboratories; however, laboratories should consider the cost, analysis time, capabilities, and limitations of each technique before acquiring new instrumentation.

This white paper introduces evolving approaches and technologies rather than providing an in-depth technical review. For those interested in a more in-depth review of each technique, please see the hyperlinked technical notes in **Table 1** that were developed specifically to address the types of questions that forensic laboratories need answered before acquiring new instrumentation. These topics include installation needs, vendor considerations, space requirements, user training, implementation needs, validated methods, searchable libraries, data interpretation, reporting and testimony, and consumables. We have also provided a list of early adopting laboratories that have offered to serve as points of contact for interested forensic laboratories that will help inform the seized drug community about the implementation readiness for each approach.

Challenges

The challenges discussed in this white paper were identified through discussion with representatives from local, state, and federal forensic laboratories and with academia and government organizations. The identified challenges are sub-divided into analytical and process challenges to more accurately represent the type of solution required. These challenges align with the existing seized drug research needs identified by the Forensic Science Research and Development Technology Working Group Operational Requirements[1] and National Institute of Standards and Technology Organization of Scientific Area Committees (NIST-OSAC) seized drugs subcommittee[2]. There are nuances within several of the identified challenges that may be dependent on specific jurisdictions. For example, differing values of total THC have been set to determine whether cannabis plant material is legal hemp or illegal marijuana. Each laboratory should consider the identified challenges within their own scope of testing and jurisdiction requirements.

Analytical Challenges

Analytical challenges relate to the detection, identification, differentiation, characterization, and quantitation of unknown analytes using validated techniques within operational forensic laboratories. Given the **rapidly evolving nature of the seized drug landscape** and the continued **emergence of NPS**, forensic laboratories are faced with the constant influx of challenging analytical determinations. Among these challenging analytical determinations is the necessity to **identify closely related chemical substances**, such as positional isomers and diastereomers. Given the potency of many NPS, there is an additional need for **minor component detection in mixtures** containing high quantities of adulterants, diluents, or less potent controlled substances. One example of this challenge is minute quantities of fentanyl or fentanyl-related substances present in mixtures of heroin, methamphetamine, or cocaine.

One of the largest analytical challenges and time-consuming analyses that forensic laboratories face today is the **differentiation of hemp and marijuana**. Although marijuana is a Schedule I controlled substance within the United States Controlled Substances Act[3], there are many states that have enacted legislation enabling medical use and recreational consumption or even decriminalizing the possession of small quantities of marijuana[4]. Federally, the Agricultural Improvement Act of 2018, commonly referred to as the Farm Bill, defines the legal threshold between hemp and marijuana based on a 0.3% Δ9-THC threshold on a dry weight basis[5]. The United States Department of Agriculture clarified in 2019 that the differentiation of hemp and marijuana is based on total THC content (i.e., Δ9-THC and Δ9-THCA)[6]. These legal definitions necessitate the qualitative identification of Δ9-THC and the determination of the total THC content from suspected marijuana plant materials. Meeting the legal definition of marijuana is even further complicated by the presence of **marijuana products and complex matrices**, such as brownies, gummies, oils, and vape cartridges. These samples require additional sample preparation to maximize analyte recovery and minimize analytical interferences but also raise questions about the determination of the total THC content based on a dry weight basis. Finally, the identification of **THC isomers and derivatives**, such as Δ8-THC and THC-O-acetate, presents another analytical concern when analyzing marijuana products. These THC isomers and derivatives have become increasingly popular in marijuana products because of their uncertain legal status from both a federal and state perspective. See the Marijuana and Marijuana Products Analysis Technical Note for a more in-depth review of the analysis of marijuana and marijuana products.

Process Challenges

Process challenges relate to limitations in the ability to process the quantity of casework samples submitted to a given agency within the desired turnaround time. **Screening multiple units** is a major process challenge for many forensic laboratories. Forensic casework often contains many items that require appropriate sampling and analysis before a final report can be submitted. Even with the application of appropriate statistical-based sampling schemes, there is still the need for alternative techniques or technologies enabling rapid screening or triaging of seized drug evidence. Another process challenge is addressing **seized drug backlogs**. Given the quantity of casework submissions and number of items within each submission, there is an inevitable backlog associated with seized drug evidence. Identifying strategies to decrease turnaround times within forensic laboratories is an ongoing need within the seized drug community.

Potential Solutions

With the identified analytical and process challenges in mind, the Evolving Approaches and Technologies for Seized Drug Analysis subcommittee went in search of approaches that are either not widely known or possibly underused to find potential solutions that can be readily implemented to address the seized drug community's current needs. The potential solutions discussed here are not an exhaustive list but rather a starting point for further discussion about potential solutions to address current challenges within the seized drug community. Each approach was identified due to a specific capability to address one or more stated challenges faced by the seized drug community. In addition, the subcommittee addressed any associated limitations of the technique and barriers to implementation. A brief description for each technique is provided below, but more in-depth information can be found in the technical notes in Table 1.

Direct Analysis in Real Time-Mass Spectrometry (DART-MS)

DART-MS is a rapid, non-contact, ambient ionization approach that provides instantaneous mass spectral results for samples introduced in the gap between the DART source and the mass spectrometer inlet. DART-MS produces easily interpretable mass spectra through the formation of molecular ions or protonated/deprotonated molecules, depending on the nature of the analyte and mode of ionization. In-source collision-induced dissociation (IS-CID) can also be used to generate fragment ions for increased selectivity. Rather than traditional chromatographic separation before ionization, DART-MS enables rapid screening and high throughput analysis with little to no sample preparation, which can help address issues with screening multiple subunits of evidence and existing backlogs. In addition, there are available resources such as the NIST DART-MS Forensics Database and Data Interpretation Tool (DIT) to assist analysts with the data interpretation associated with DART-MS analysis[7,8].

Gas Chromatography-Vapor-Phase Infrared Spectroscopy (GC-VIR)

GC-VIR is a complementary instrumental technique to GC-MS where compounds are characterized based on the bending and stretching of chemical bonds at different wavelengths of infrared radiation in the gas phase. Characteristic absorption or transmittance data provide the unique ability to not only confirm identification but also to distinguish isomers without polymorphism concerns that can affect solid-phase IR measurements. GC-VIR overcomes some of the limitations of GC-MS when the analytes of interest produce no molecular ions or very weak molecular ions under electron ionization or when similar mass spectral fragmentation patterns are obtained for certain isomeric compounds. GC-VIR has been shown as a cost-effective technique useful for the differentiation between positional isomers and for the unambiguous identification of fentanyl-related substances, phenethylamines, cathinones, and synthetic cannabinoids.

Gas Chromatography-Vacuum Ultraviolet Spectroscopy (GC-VUV)

GC-VUV detection enables UV detection of analytes in the gas phase at wavelengths between 120 nm and 430 nm because of the transparency of the carrier gas. The analyte enters the VUV detector through a heated transfer line, where a make-up flow or purge gas is used to sweep the sample into the flow cell before reaching a holographic grating for diffraction onto a charge-coupled device detector. The result of VUV detection is enhanced $\eta \rightarrow \eta^*$ (double bond) transitions and $\sigma \rightarrow \sigma^*$ (single bond) transitions, which can provide unique chemical signatures for most analytes, including aromatic ring positional isomers and certain positional isomers differing in aliphatic substitution that can be problematic for traditional techniques such as GC-MS.

Ultra-High Performance Liquid Chromatography Photo Diode Array Ultraviolet Single Quadrupole Mass Spectrometry (UHPLC-PDA UV-MS)

UHPLC enables enhanced liquid-phase separations with simultaneous photodiode array ultraviolet and single quadrupole mass spectrometry detection. As a complement to GC-MS, this technique gives uncorrelated retention times, provides full-scan UV spectra, and enables the identification of molecular mass information (e.g., $[M+H]^+$ or $[M-H]^-$). This instrumental configuration is particularly useful for NPS analysis. This technique helps overcome limitations of GC-MS for NPS, including similar retention times, either very weak or a lack of molecular ions for several drug classes, or similar fragmentation patterns for diastereomers and certain positional isomers[9]. Photo diode array UV detection can distinguish between different classes of drugs and discriminate between positional isomers differing in substitution on an aromatic ring.

Microcrystal Tests

Microcrystal tests are precipitation reactions between a reagent and a drug of interest, which form characteristic crystals with individualized size, shape, and optical properties. As a Category B technique[10,11], these tests can be used to screen samples quickly and inexpensively, reducing case backlogs and burden on instruments such as GC-MS. Although these tests are limited in scope because crystal property knowledge only exists for a few dozen compounds[12,13], these compounds include some of the most commonly seen drugs, allowing for a majority of the seized drugs encountered to be screened.

Table 1: Summary of evolving approaches and technologies for seized drug analysis.

Technique	Cost	Analysis Time	Capabilities	Limitations	Challenge(s) Addressed
Direct Analysis in Real Time-Mass Spectrometry (DART-MS)	• $30K–$50K for the DART source depending on the configuration • $100K–$500K for the mass spectrometer	• Approximately 30-s sample analysis and 1-min data analysis	• Rapid screening • Limited sample preparation	• Competitive ionization • Isomer differentiation	• Evolving drug landscape • Screening multiple units
Gas Chromatography-Vapor-Phase Infrared Spectroscopy (GC-VIR)	• $85K–$100K for the VIR detector alone coupled with GC or GC-MS • $120K–$150K for GC-VIR instrument	• Method dependent but on a similar timescale as existing GC-MS methods	• Isomer differentiation based on structural information • Eliminates concerns with polymorphism	• Relatively high limits of detection (tens of ng on column) • Spectra not directly comparable to solid-phase IR spectra	• Identifying closely related chemical substances • Evolving drug landscape
Gas Chromatography-Vacuum Ultraviolet Spectroscopy (GC-VUV)	• $39K–$99K for the VUV detector (higher costs are associated with the ability to collect full spectra) • $75K–$150K for the complete	• Method dependent but on a similar timescale as existing GC-MS methods	• Isomer differentiation • Rapid method development because of ease of spectral deconvolution	• Relative lack of structural information • For minor component analysis, relatively high limits of detection (high pg on column)	• Identifying closely related chemical substances • Evolving drug landscape
Ultra-High Performance Liquid Chromatography Photo Diode Array Ultraviolet Single Quadrupole Mass Spectrometry (UHPLC-PDA UV-MS)	• $150K–$230K for UHPLC-PDA UV-MS instrument	• Method dependent but typically quicker than GC-MS methods	• Good for thermally unstable, polar, and non-volatile analytes • Ability to generate protonated/deprotonated molecules	• Limited structural information from UV or MS (without IS-CID activation) • No universal UV library because of dependence on the mobile phase	• Identifying closely related chemical substances • Minor component detection in mixtures
Microcrystal Tests	• $30K–$40K for necessary materials	• 8–10 min/sample	• Rapid and relatively inexpensive • Reduces the burden on analytical instrumentation	• Limited scope of testing (~28 compounds) • Challenges obtaining reviewable data	• Seized drug backlogs

*Techniques displayed in alphabetical order.

Summary

This white paper introduces evolving approaches and technologies that are available to address existing challenges with the analysis of seized drugs. These challenges include the rapidly evolving nature of the seized drug landscape, including the presence of NPS; the identification of closely related chemical substances, such as positional isomers and diastereomers; minor component detection in mixtures; differentiation of hemp and marijuana, including the analysis of marijuana products, THC isomers, and THC derivatives; the screening of multiple subunits; and seized drug backlogs. The Evolving Approaches and Technologies for Seized Drug Analysis subcommittee developed five technical notes describing techniques that are potential solutions to the identified challenges faced by the seized drug community. These techniques include (1) DART-MS, (2) GC-VIR, (3) GC-VUV, (4) UHPLC-PDA UV-MS, and (5) microcrystal tests. In addition, the subcommittee developed a technical note describing best practices for the analysis of marijuana and marijuana products. Those who are interested in learning more about adopting the identified evolving approaches and technologies for seized drug analysis are encouraged to reach out to the technical points of contact listed within each technical note for additional information. Even though the identified techniques are not exhaustive, they do provide potential solutions to the ongoing challenges associated with seized drug analysis. There are also several other techniques beyond the scope of this white paper that forensic science service providers could consider to address existing challenges with seized drug analysis. Examples include lateral flow immunoassays (LFIA)[14-24], fast GC analysis using the QuickProbe[25-26], or field-portable instrumentation[27-35].

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