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Comparing Two Seized Drug Workflows for the Analysis of Synthetic Cannabinoids, Cathinones, and Opioids

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Dr. Micheal Peat, Editor-in-Chief
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Re: Manuscript ID. JOFS-21-682, "Comparing Two Seized Drug Workflows for the Analysis of Synthetic Cannabinoids, Cathinones, and Opioids"

Dear Editor and Referees,
Thank you very much for your letter concerning the above referenced manuscript and the enclosed referees' comments. We found the referees' comments to be very constructive, and we appreciate the effort they put into thoughtfully reviewing our paper. We have heeded the advice of the reviewers, associate editor, and editor and have substantially shortened the manuscript and resubmitted it as a technical note. The specific actions we have taken to address the referees' questions and concerns, keyed to their specific comments, are described in the attached summary of revisions.

We have uploaded the revised manuscript and this revisions letter to the *Journal of Forensic Sciences* website. Please convey our thanks to the reviewers for their helpful comments and efforts on behalf of the review process. Thank you again for your review and consideration of our manuscript for publication.

Manuscript ID. JOFS-21-682

SPECIFIC RESPONSES TO THE REVIEWERS

Responses to the reviewer can be found in red text

We would like to thank both the referees for their suggestions and comments.

"The reviewers' comments highlight concerns with the inclusion of GC-FID in the workflow, the comparison of DART-MS with color tests, and the potential to miss substances in samples with the targeted GC-MS approach. Both reviewers provide additional details and comments that should be addressed in preparing the revised version of the manuscript."

We believe we have address these concerns by revising the introduction, conclusion, and other portions of the manuscript. The scope of the study was also clarified to demonstrate that the experimental workflow here has known limitations and therefore would not be an ideal workflow to adopt in full. The comparison, however, does provide insight into how different approaches could effect the time, data quality, and safety of the a workflow.

"Delete "comparison" and include with "analytical workflow" (i.e., "analytical workflow comparison")"

Completed.

"Original line numbers included - please exclude

Completed.

Reference 11 is a book - please add the relevant page numbers.

Reference 12 incomplete citation - please complete

Corrected.

Reference - journal title not abbreviated - please abbreviate according to Index Medicus

Corrected.

Highest academic degrees - please add this information for all authors included on the Title Page."

Please also note that when loading figures into Manuscript Central it is important to Insert FIG. X before each figure legend. In addition, you must load each figure as a separate file.

Corrected.

Please include the tables and their headers in the Main Document as separate pages after the References segment when uploading the revision.

This was done in the initial submission.

Reviewer(s)' Comments to Author:

Reviewer: 1

Line 9: This sentence reads that each chemist analyzed 50 samples using one workflow - needs to be clearer on what was actually done as stated below in Study design section.

Updated to "Four chemists were asked to analyze a total of fifty mock case samples across the two workflows."

Line 59: recommend moving this, spelled out, up to where abbreviation DART-MS is first used in body of article.

Completed.

Line 127: Using Mayer's or Mayers, should be consistent throughout.

Completed.

Line 131: Formatting: change to Existing Workflow - GC-FID
Completed.

Line 157: Suggested re-write: "as well as the library downloaded from the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) website(Ref#)." (and then add SWGDRUG.org to References)
Also include library version - same as the targeted GC-MS?
Completed.

Line 238: Suggested re-write: "..only two samples, Sample 3 (heroin and MDMA) and Sample 42 (heroin, etc), failed to produce..."
Completed.

Line 274: Using DART-MS, both chemists were able to correctly identify...
Completed.

Line 347: blanks (negative controls) included?
Added.

Reviewer: 2

The manuscript provided detailed procedures and data to demonstrate the pros and cons of two workflow used in their laboratory. But I would like to see the purpose/benefits/logics stated clearly about the switching flow at the beginning of the article. More concerning is the use of targeted GC-MS for drug confirmation which will have a potential to misidentify substances not monitored by the laboratory.
The use of targeted GC-MS methods does not preclude detection of compounds outside of the panel, because full-scan spectra are still used. While the compound outside of the panel may not be able to be confirmed, it would, likely, still be detected. This point has been clarified in the conclusion section of the manuscript. Additionally, the use of the targeted methods would certainly only be possible if used in conjunction with high-fidelity screening tools and/or in combination with a well characterized general method, as a tool to separate species that cannot be confirmed with general methods. These points have also been clarified in the conclusion section of the manuscript..

Page 1, Abstract: As stated in the abstract, one of the challenges for forensic drug analysis is the presence of emerging drugs. The experimental workflow, with the combination of DART and targeted GC-MS, may miss new drugs or drugs not monitored by the laboratory due to the lack of resolving power of DART (no separation and an in-house library with 600 compounds) and comprehension of targeted analysis of GC-MS. For instance, methamphetamine was not confirmed by the targeted GC-MS in sample #2.
While more discussion on this topic has been added to the body of the manuscript, due to the word count limit in the abstract the following sentence was added: “While the experimental workflow requires modifications and answering of additional research questions, this study shows how rethinking analytical workflows for seized drug analysis could reduce turnaround times, backlogs, and standards consumption.”

Page 2, Highlights: Are the first two sentences repetitive?
Second highlight has been replaced with “ Compared color tests, GC-FID, and GC-MS to DART-MS and targeted GC-MS”.

Page 3, Introduction: suggest to reword the first two paragraphs to be more precise and concise. In addition, adding a paragraph on analytical requirements for seized drug analysis and how the analytical workflows fulfil the requirements can be informative.
The introduction has been modified to be more concise, incorporate the requirements for seized drug analysis, and highlight the practical limitations of the study that was conducted.

Page 4, line 88: Can the authors specify the normal range of LOD instead of stating “reasonable detection”?
A normal range of LODs has been added, “on the order of 0.01 mg/mL to 0.1 mg/mL”.

Section of materials and methods: Should the source of chemicals be provided?

Table 1 does provide information on whether the samples were created using standards, adjudicated case samples, or a combination of both.

Page 5, in case samples: Was there any concern about the amount/concentration of each drug in a sample?
Concentration was not controlled for, as the majority of samples were adjudicated case samples, but those that were created from standards were created using roughly equal amounts of materials. A sentence has been added to this section to clarify this.

Page 6, Line 136-138: Were the samples prepared in a GC vial with shaking then transferred to another vial? It sounds tricky.
This is how sample preparation is typically done in the laboratory. This line has been modified to make it explicit that the vial was capped prior to shaking the vial.

Page 9, line 249: I doubt that the inconsistency results between two chemists of the color test were due to the inhomogeneity of the samples since consistent DART results were produced. The false negative or positive results of color tests can be from its low sensitivity and low selectivity/septicity.
The text has been updated to include this as a possible cause for the differences that were observed.

Page 10-11: Though both workflows used GC-MS for peak confirmation, different approaches were employed. Can the authors provide the reason why GC-MS was switched from general analysis to targeted mode? In current workflow, GC-MS was in general screening mode which was designed to detect nearly all the components in a sample with a tradeoff of lower sensitivity. On the other hand, in the experimental workflow, GC-MS in targeted mode was used with higher sensitivity but a tradeoff of detecting only selective compounds and that is the reason why substances were not confirmed in sample 2 and 42. Can it be risky to rely DART to detect everything a sample? When analyzing a sample with more diversified classes of substances, the targeted GC-MS can be time consuming in the experimental flow because more GC-MS methods (for each class) need be run for a better selectivity.
We hope that the new discussion that was added to the introduction and conclusion sections makes the driver for the investigation of the targeted methods clearer. To further this discussion, the following motivation was also added to the Targeted GC-MS section in the Methods & Materials section of the manuscript:

"The general purpose GC-MS methods can present challenges with the analysis of NPS samples, including the inability to separate spectrally similar compounds and subjective decision points. To address these challenges targeted GC-MS methods for specific compound classes were implemented using a previously published framework (4). These methods were developed to enhance separation of spectrally similar compounds and provide objective, data-driven decision points."

The reviewer is correct that the use of targeted methods, depending on the complexity of the sample, could add increased analysis time because of the need to run multiple methods (though this was not observed in this study). A more ideal approach would be to develop a general purpose method that was developed using the targeted method framework and therefore provides enhanced separation of commonly observed compounds along with objective, data-driven limitations. This general purpose method could then be complimented with class-specific targeted methods for instances where the general purpose method is not sufficient for confirmation. This approach is currently under development.

Page 12-13: Can authors explain why GC-FID was considered as a confirmation tool in the current workflow?
Under the workflow used at the laboratory the combination of GC-FID and GC-MS is used for confirmation. Retention times are confirmed using GC-FID and mass spectral similarity is confirmed using GC-MS. This is specified in the Study Design section of the manuscript.

Page 13, line 412: Can a dual injection GC be coupled with both FID and MS as a detector?

Yes on most current instruments this can be completed. This is the focus of current research efforts for the development of a general analysis method that includes dual locked retention times and mass spectral data.

Page 13, Line 413-414: Did the authors refer 2DxGC? 2DxGC can be the choice for a better resolving power.

A reference to this possibility has been added to the conclusion: “Two-dimensional GC is another potential tool that could provide multiple datapoints to increase confidence in identification.”.

Comparing Two Seized Drug Workflows for the Analysis of Synthetic Cannabinoids, Cathinones, and Opioids

As the challenges faced by drug chemists persist, due to the presence of emerging drugs, laboratories continue to look for new solutions, ranging from existing methods to implementation of entirely new technology. A common barrier for making workflow changes is a lack of pre-existing data demonstrating the potential impact of these changes. In this study, we compare, qualitatively and quantitatively, an existing workflow for seized drug analysis to an experimental workflow. Four chemists were asked to analyze a total of fifty mock case samples across the two workflows. The existing workflow employed color tests for screening alongside general purpose GC-FID and GC-MS analyses for confirmation. The experimental workflow combined DART-MS screening with class-specific (targeted) GC-MS analysis for confirmation. Comparison of the workflows showed that screening by DART-MS required the same amount of time as color tests but yielded more accurate, and specific, information. Confirmation using the existing workflow required more than twice the amount of instrument time and data interpretation time while also presenting other analytical challenges that prevented compound confirmation in select samples. Targeted GC-MS methods simplified data interpretation, reduced consumption of reference materials, and addressed almost all limitations of general purpose methods. While the experimental workflow requires modifications and answering of additional research questions, this study shows how rethinking analytical workflows for seized drug analysis could reduce turnaround times, backlogs, and standards consumption. It also demonstrates the potential impact of being able to investigate workflow changes prior to implementation.

Keywords: Seized Drug Analysis; Analytical Workflow Comparison; Mass Spectrometry; DART-MS; GC-MS

Highlights

- A comparison of two seized drug workflows was completed, measuring time and data quality
- Compared color tests, GC-FID, and GC-MS to DART-MS and targeted GC-MS
- Screening with DART-MS was found to produce more specific results in the same amount of time as color tests
- Targeted GC-MS analyses were found to greatly reduce standards consumption and instrument time

Backlogs and analytical challenges continue to be major bottlenecks for forensic seized drug analysis (1,2). This has been compounded by the continued emergence of novel psychoactive substances (NPSs) which has led to over 80 % of laboratories reporting limited analytical tools as a major challenge (3). To address these challenges laboratories may seek out modifications to existing technologies, such as the adoption of new gas chromatography mass spectrometry (GC-MS) methods (4), or implementation of completely new technologies, such as direct analysis in real time mass spectrometry (DART-MS) (5,6) or Raman spectroscopy (7) to replace or compliment tools in their toolkit. When implementing new approaches or technologies, laboratories must estimate the improvements (*i.e.*, changes to throughput or accuracy) of new workflows while also considering upfront and recurring costs and the time required for procurement, method development, validation, and training. Oftentimes, the decision to change must be made without being able to tangibly measure the potential benefits or drawbacks of shifts in workflow, due to time and resource constraints. In some forensic disciplines, such as DNA analysis, the efficacy of different workflows has been studied, providing ability to make data-driven decisions (8,9).

Recent research efforts have demonstrated how new approaches can address the ability to keep pace with the changing landscape, ensure adequate standards are available, provide methodologies for differentiating isomeric or isobaric species, and develop tools for sensitive detection of small amounts of highly toxic compounds (10). While most of these approaches have been demonstrated on a subset of neat samples or case samples, the studies often do not consider how they may be implemented into casework. For this, it is important to consider the specifications set forth by documentary standards such as ASTM E2329 (11) or the Scientific Working Group for Seized Drug Analysis (SWGDRUG) recommendation (12). In summary, multiple analytical approaches that measure different chemical properties must be utilized to come to an identification. Commonly this is accomplished using a screening tool such as color tests, DART-MS or microcrystalline tests coupled with confirmatory tools such as gas chromatography mass spectrometry (GC-MS).

In this study, two different analytical workflows for seized drug analysis were compared to measure differences in time, data quality, safety, and simplicity. The workflows were compared using mock and adjudicated samples that were given to four different practicing forensic chemists who analyzed all samples using one of the two workflows. The first workflow modeled existing practices at the Maryland State Police Forensic Sciences Division (MSP-FSD) and employed a combination of color tests, general purpose gas chromatography flame ionization detection (GC-FID), and general purpose GC-MS. The second workflow was developed to address many of the known limitations in the first workflow by leveraging DART-MS for screening coupled with GC-MS methods developed for the targeted analysis of different drug classes. While these methods utilized full scan mode, and therefore could detect a wide range of compounds, they were developed to enhance separation of structurally similar compounds and provide examiners with a more objective analysis. To focus the study, samples were limited to three compound classes – synthetic cannabinoids, synthetic cathinones, and opioids. This study yielded tangible data to allow for direct

comparison of the two workflows and better understand how changes to the existing laboratory protocols influence data quality, turnaround times, and requirements on the chemists. However, because of the limited classes of compounds examiner, additional work and research questions would need to be addressed for wide-scale implementation of a new workflow.

Materials & Methods

Study Design and Analytical Workflows

For this study, the goal was to identify and quantify the differences in two analytical workflows for seized drug analysis, specifically targeting synthetic cannabinoids, synthetic cathinones, and opioids. To do this, 50 samples, (described in more detail in the next section) were created that span the range of complexities and compounds within the three drug classes that are commonly observed at MSP-FSD. A portion of each of the 50 samples was provided to four different chemists at MSP-FSD who were asked to analyze the samples using one of the two workflows – referred to hereafter as the existing workflow and the experimental workflow. Each chemist analyzed half of the samples using the existing workflow and the remaining half using the experimental workflow. To simplify the process of recording times, samples were batched into groups of five and chemists analyzed one batch at a time. For each step in the workflow, chemists recorded the amount of time required to prepare, analyze, and interpret the data for the batch of samples. Chemists were also asked to provide their interpretation of the results after each analysis as well as an overall result of the controlled substance(s) present in each sample.

Schematics of the existing and experimental workflows are provided in Figure 1. For the existing workflow, which reflects current procedures at MSP-FSD, a batch of samples was first screened using three color tests (Mayers, cobalt thiocyanate, Marquis(13)) to provide an indication of the type, or types, of compounds that may be present in the sample. Two separate methanolic extracts were then created for each sample, one for GC-FID analysis and the other for GC-MS analysis. Details regarding these methods are provided below. To confirm a controlled substance in a sample, the resulting GC-FID data was used to compare retention times of compounds in the samples to known standards while the resulting GC-MS data was used to obtain mass spectra of compounds in a sample to compare to spectra of standards previously collected on the instrument. The methods used for GC-FID and GC-MS were general purpose methods designed to achieve reasonable detection, on the order of 0.01 mg/mL to 0.1 mg/mL, of a wide range of controlled substances.

In the experimental workflow, screening was completed using direct analysis in real time mass spectrometry (DART-MS) and was chosen because it produces more information-rich results than most other commonly deployed screening tools. It can often provide a near-complete chemical profile of a mixture and can identify the specific compounds, or group of isomeric compounds, in a sample. To leverage the higher fidelity screening information, confirmation was completed using a suite of targeted GC-MS methods. The methods were created to maximize retention time differences of similar compounds to reduce the number of pairs of

compounds that could not be differentiated. Individual methods were created for synthetic cannabinoids, synthetic cathinones, and opioids. To investigate an approach to reduce consumption of reference materials, all methods were retention-time locked (where the carrier gas flow rate is adjusted to maintain consistent retention times of a lock column over the column's lifespan). This allowed for the analysis of only the lock compound with each batch, eliminating the need to run individual standards which were required for GC-FID analysis. For samples that contained compounds in multiple classes (*i.e.*, dibutylone and fentanyl), analysis by multiple targeted methods was required. In addition, samples that were found by DART-MS to contain no controlled substances were concentrated, through the addition of more powder to the solution, and re-analyzed by DART-MS. If the concentrated sample also returned a negative result, the sample was reported as no controlled substances and no further analysis was completed.

Case Samples

For this study, a total of 50 samples were analyzed, the identities of which are provided in Table 1. Samples were created from either adjudicated case samples or standards purchased from Cayman Chemical (Ann Arbor, MI, USA) and Sigma-Aldrich (St. Louis, MO, USA). Samples were, largely, representative of commonly seen mixtures and ranged in complexity from simple, single compound samples to complex mixtures with drugs from multiple classes. The relative concentration of substances in the mixtures was not controlled. Eight of the 50 samples contained no controlled substances. A total of 27 samples contained a single controlled substance, 10 contained two controlled substances (8 of which contained substances from multiple drug classes), and 5 contained three or more controlled substances. A total of 11 samples contained at least one synthetic cannabinoid, 19 samples contained at least one synthetic cathinone, and 22 samples contained at least one opioid. Once created, samples were divided into 2 mL GC-MS vials, each containing between 10 mg and 50 mg of powder. A set a vials was given to each chemist for analysis. Vials were labelled with only a number and the identity of the contents provided until the study was complete.

Existing Workflow - Color Tests

Three color tests were completed (Mayers, cobalt thiocyanate, and Marquis) in disposable well plates according to the standard operating procedures at MSP-FSD. To complete a test, several drops of the appropriate reagent(s) were added to the well followed by a small amount (several milligrams) of sample powder after which the color change, if any, was observed. In addition to noting the color changes that occurred, chemists were also asked to provide an interpretation of each result, and record the time it took to complete the entire process for every batch of five samples.

The Marquis reagent was created by combining 10 mL of 37 % formaldehyde with 100 mL of concentrated sulfuric acid. Cobalt thiocyanate reagent was created by dissolving 6.0 g of cobalt thiocyanate in 240 mL of water mixed with 360 mL of 0.1 M hydrochloric acid. The Mayers reagent was created by dissolving 6.0

g of mercuric chloride in 600 mL of water followed by the addition of potassium iodide to dissolve the red precipitate.

Existing Workflow – GC-FID

GC-FID was employed to compare retention times of the controlled substances in the samples to reference materials. Analyses were completed on one of two Agilent GC systems (Agilent Technologies, Santa Clara, CA, USA) using methods that were validated for casework. Parameters for both methods are provided in Supplemental Table 1.

Samples were prepared by dissolving 1 mg to 2 mg of material into approximately 1.5 mL of methanol. The solution was capped, shaken by hand for several seconds, and then allowed to sit for several minutes so any undissolved particulates could settle. The supernatant was then transferred to another GC vial for analysis.

All samples were analyzed with a single injection. Once compounds were preliminarily identified, reference materials (solutions containing known drugs) were analyzed using the same method to establish retention times for comparison. In addition to the suspected controlled substance, all isomers and similar compounds (compounds that have similar retention times) were also run. For each batch, reference materials were only run once, even if they were required for multiple samples. A list of reference materials run for each of the controlled substances in the study is provided as Supplemental Table 2. For a positive identification of a substance, the retention times of the sample and the reference material needed to be within ±1 % of one another and none of the other required reference materials, if applicable, had retention times within ±1 % of the sample. Overall identification of a substance required a positive identification from the GC-FID data and the GC-MS data, discussed in the next section.

Existing Workflow – GC-MS (General Purpose)

General purpose GC-MS was the second component of the confirmation process and was used to compare mass spectra from compounds in samples to those previously collected from reference materials. Analysis was completed on one of two Agilent GC-MS systems. There were three casework validated methods that chemists could use depending on which laboratory they were in as well as their preference and the suspected compounds in the sample. Method parameters for the three methods are provided in Supplemental Table 3. Sample preparation for GC-MS was identical to GC-FID.

All samples were analyzed as a single injection. A cocaine positive control was run with each batch of samples for each method used. After analysis, all peaks in the chromatogram were searched against mass spectral libraries created in house, as well as the library downloaded from the SWGDRUG website (v 3.6)(14). Positive identification criteria included having an abundance of 200,000 counts or greater in the chromatogram along with an acceptable mass spectral match to a library entry. If any of these criteria were not met, or the GC-FID criteria were not met, an “insufficient” finding was made.

Experimental Workflow – DART-MS

Sample screening using the experimental workflow was completed using DART-MS. The protocols used here have been discussed in detail elsewhere(15). Briefly, samples were prepared by dissolving approximately 1 mg of material into 1 mL of methanol containing tetracaine as an internal standard. Data was collected using a sequence-based approach with individual, 1 min data files collected for each sample. Within the 1 min datafile, the internal standard solution was analyzed once by itself followed by three analyses of the sample combined with the internal standard. All analyses were completed by dipping a clean glass microcapillary into the solution and placing it in the open-air sampling region. Measurements were made on one of two systems using identical methods. The systems consisted of DART-SVP ion sources (IonSense, Saugus, MA, USA) coupled to JEOL AccuTOF 4G-LCplus mass spectrometers (JEOL USA, Peabody, MA, USA). Helium was used as the DART gas source with a gas stream temperature of 400 ºC and operation in positive ionization mode. The mass spectrometer was also operated in positive ionization mode with an orifice 1 voltage of +30 V, a ring lens voltage of +5 V, an orifice 2 voltage of +5 V, and an ion guide voltage of +800 V. Spectra were collected from *m/z* 80 to *m/z* 800 at a rate of 0.4 s/scan.

Upon completion of the sequence, the datafiles were automatically mass drift compensated using the *m/z* value for the protonated molecule of tetracaine (the internal standard). For each sample, an averaged mass spectrum of the three analyses was extracted, background subtracted, and saved as a centroided datafile. The centroided spectra were then analyzed using the "Search From List" feature within Mass Mountaineer (Diablo Analytical, Antioch, CA, USA) using an in-house created search list containing information for over 600 compounds of interest to seized drug analysis. Search parameters for peak identification included a minimum peak height threshold of 5 % relative abundance and a maximum *m/z* drift of ±0.005 Da (5 mDa) which was based on the mass tolerance of the instrument. For instances where multiple compounds produce the same *m/z* value, fragment ions were used to differentiate compounds, if possible. The tetracaine internal standard was used as a quality control compound, where the presence and correct *m/z* value of the protonated molecule was required for a datafile to be used. The time required to analyze every batch of five samples was also noted.

Experimental Workflow – GC-MS (Targeted Analysis)

The general purpose GC-MS methods can present challenges with the analysis of NPS samples, including the inability to separate spectrally similar compounds and subjective decision points. To address these challenges targeted GC-MS methods for specific compound classes were implemented using a previously published framework (4). These methods were developed to enhance separation of spectrally similar compounds and provide objective, data-driven decision points.

Preparation of samples for analysis was identical to that for the GC-FID and GC-MS methods described in the existing workflow above. A more in-depth discussion on the development of the targeted methods is provided elsewhere (4,16,17), and the actual instrument methods are provided in Supplemental Table 4.

All analyses were completed using an Agilent 7890/5977B GC-MS with helium as the carrier gas. The targeted methods were developed to maximize retention time differences between similar compounds within a reasonable runtime in order to minimize the number of compound pairs with overlapping retention time acceptance windows. The methods employed retention time locking to decrease consumption of reference materials. Using this approach, prior to running a batch of samples, the method was re-locked by analyzing the lock compound. A positive control was run with the batch of samples to confirm the locking was successful. If a sample contained compounds from multiple classes, repeat analyses were completed for all appropriate targeted methods.

After analysis, the resulting data was interpreted by comparing both the retention time and the mass spectra for all peaks within a chromatogram. A retention time acceptance window of ±2 % for all methods and a ±1 % window for the retention time agreement of the lock compounds were used. A positive identification was defined as a chromatographic peak with a signal to noise ratio greater than 5:1 within the ±2 % acceptance window of the previously run reference material and with a minimum mass spectral match factor of 85 a.u. when compared to mass spectral libraries created in house or provided in the SWGDRUG Library (v 3.6).

Results & Discussion

Comparison of Color Test to DART-MS for Compound Screening

Analysis of the 50 samples by four examiners produced a total of 100 results per workflow to compare while also providing two independent analyses of each sample on each workflow. Comparison of the two screening techniques initially proved to be difficult because of the lack of comparable data. To address this challenge, a scoring system, outlined in Table 2, was created. Scores ranged from -1 to 4 and attempted to capture both the accuracy and specificity of the result, with more accurate and specific results receiving higher scores. For DART-MS, the result was the identified compound(s) that met the identification criteria. For color tests, the result was the chemists' interpretation of the color changes that occurred based on their expert knowledge and prior experience. If the result was inconsistent with the actual contents of the sample, a score of -1 was given. If the result was inconclusive (*i.e.* it could not be determined whether or not a controlled substance was present in the sample), a score of 0 was given. For results that were consistent with the contents of the sample, positive scores were given. A score of 1 was given to results that were accurate but the least specific, defined as those where only a class identification (*i.e.* the sample contains an opioid, synthetic cannabinoid, etc.) was possible for at least one of the controlled substances in the mixture. The next level of specificity was defined as the sub-class (*i.e.* fentanyl) or isomer group (*i.e.* AB-FUBINACA or one of its isomers). If the sub-class was identified for at least one controlled substance in a sample with multiple controlled substances, a score of 2 was given. A score of 3 was given if the sub-class was correctly identified for a sample containing a single controlled substance or for a sample where the sub-class or isomer group was correctly identified for all compounds in a sample containing multiple controlled substances. The most specific level of information was identification of the specific compound, which was given a score of 4. For samples containing multiple controlled substances, all controlled

substances needed to be identified to obtain a score of 4. A score of 4 was also given when a sample that did not contain any controlled substances produced a result consistent with the absence of controlled substances.

This system was used to score all colorimetric and DART-MS results obtained by each of the four chemists. A complete list of scores is provided in the Supplemental Table 5 while the summary results are provided in Figure 2. As expected, DART-MS was able to provide a more complete chemical profile of the samples resulting in both more accurate and more specific results. The average score for DART-MS was 3.4 (±0.6) compared to 1.2 (±1.6) for color tests. This was not surprising since color tests usually only provide class-level information whereas DART-MS can provide more specific information in nearly all instances. Out of all the DART-MS results, only two samples, Sample 3 (heroin and MDMA 3) and Sample 42 (heroin, with an indication of fentanyl, acetyl fentanyl, FIBF, cocaine, and noscapine) failed to produce isomer group or compound identifications for all components in the sample. These missed identifications were the result of the concentrations of the compounds in the sample being below the detection limit of the technique, resulting in a score of 2.

The poor specificity and irreproducibility of the color tests results for this set of samples was unexpected. Color tests produced an inconclusive result nearly one third (n = 32) of the time and produced an inconsistent result on twelve separate occasions. Additionally, 18 % of the samples produced differing results when analyzed by the two chemists, resulting in different scores for the same sample. It is unclear what the driver of this observation was, but it may have been due to low selectivity or specificity of the color tests or heterogeneric samples. The twelve inconsistent results (score = -1) were spread across eight samples, four samples where both chemists had inconsistent results and four sample where only one chemist had an inconsistent result. Of the three samples where the color test produced results that led to an inconsistent identification by both chemists, two were samples without a controlled substance that contained significant fractions of quinine (Samples 20 and 45). These samples both produced responses consistent with the presence of heroin or another opiate. The third instance was a sample which contained JWH-018 but elicited a response consistent with a cathinone (Sample 24) and the fourth was a sample containing tramadol that produced a response consistent with a fentanyl (Sample 31). The four samples where one chemist got an inconsistent result included two instances where a synthetic cathinone produced a response consistent with a fentanyl (Samples 28 and 5), one instance where a methamphetamine response resulted from a sample containing a cathinone and fentanyl (Sample 12), and one instance where a heroin response resulted from a sample containing fentanyl (Sample 39).

For DART-MS, consistent results across chemists were obtained in all instances, except for Sample 42 where only one of the two chemists were able to detect low levels of FIBF and noscapine. There were no instances of a false positive or false negative identification. As expected, there were many instances where DART-MS produced only sub-class or isomer group information because of the fact isomeric compounds have identical base peaks and often have similar fragment ions. Given the lack of chromatographic

separation, DART-MS is unable to differentiate these compounds from one another. When sub-class or isomer group information was obtained, it frequently consisted of a narrow of candidate compounds (five or fewer), though for the cathinones, the sub-class list (*i.e.* Cathinone at *m/z* 192) can encompass more than ten compounds. Given DART-MS is being used as a screening tool, this is not an issue as the chemist now has confidence in the type and class of compound(s) present in the sample. Chemists should be aware, however, that low-level compounds, especially those with low proton affinity, may be missed in a DART-MS analysis because of competitive ionization, as was the case in Samples 3 and 42, where heroin was not identified above 5 % relative intensity.

Using DART-MS, both chemists were able to correctly identify all eight of the samples that did not contain controlled substances as negative while color tests produced two false positives (discussed above) along with a single inconclusive result for one chemist (Sample 41). Confirmation of negative samples by DART-MS, completed by analyzing a concentrated sample, did not introduce any complications or produce any measurable signatures of carryover or contamination. The use of the internal standard eliminated the potential of false positive identification of noise peaks in spectra from samples that do not contain controlled substances or other easily desorbed and ionized species by providing a substantial base peak in all spectra. The lack of a base peak leading to false positive identification of noise peaks (because peak searching above a relative intensity threshold is often employed) is a common limitation in spectra that do not contain controlled substances.

In addition to establishing the differences in accuracy and specificity produced by these two techniques, the time required for analysis was also measured. For both techniques, the time required for sample preparation, sample analysis, and data interpretation (for DART-MS), was noted by the chemists for each batch of five samples. For color tests, the average time per batch was 18.6 min while for DART-MS it was 20 min. This DART-MS analysis time was split up, roughly, as 5 min for sample preparation, 2 min for sequence preparation, 5 min for analysis of samples, and 8 min for data workup. In terms of sample consumption, color tests typically required more sample for analysis (approximately 5 mg versus 1 mg to 2 mg for DART-MS); though for most samples this difference would be negligible. From a potential exposure viewpoint, DART-MS presented a lower overall risk as handling of bulk powder is limited to only one transfer of material, unlike color tests which require multiple transfers of material. DART-MS only requires methanol to dissolve the sample, while color tests require the use of other, more hazardous, chemicals like formaldehyde and concentrated acids.

While DART-MS provides a more information-rich, more accurate, possibly safer, analysis in roughly the same amount of time as color tests, it does require a large upfront investment in the technology which could present a barrier for adoption. However, color tests were found to be inconsistent and prone to differing results given the set of samples tested. The lack of class or compound specific results and the high frequency of inconclusive results obtained using color tests indicates that this approach would be ill-suited for inclusion in a workflow that utilized targeted or class-specific confirmation methods. The ability to obtain

more granular and correct compound information from DART-MS is critical for use of targeted or class-specific confirmation methods. The benefits of DART-MS are not specific to the experimental workflow investigated here and can be realized when used alongside general purpose confirmation methods as well.

Comparison of General GC-MS and GC-FID to Targeted GC-MS

Because the technique used for confirmation in both workflows was identical, comparison of results was simplified. Overall, as expected, the results obtained from the existing workflow and the experimental workflow were largely similar. Because of differences in confirmation criteria between the two approaches, there were some differences regarding which compounds could be confirmed versus which compounds were identified but produced data that was insufficient for confirmation. Table 3 shows the summary of results obtained for the two workflows. Both workflows were found to have analytical limitations which presented as insufficient identifications. The existing workflow had ten samples with insufficient identifications while the experimental workflow had three samples. Insufficient identifications were caused by several factors including low chromatographic peak intensity, co-elution, and lack of inclusion on target compound panels.

For the existing workflow, using general purpose GC-FID and GC-MS methods, there were several samples that had co-eluting peaks – namely acetyl fentanyl and FIBF – which precluded the ability to confirm either when both were present in the sample. These two compounds were not sufficiently separated on the GC-FID method and did not provide sufficient separation to obtain clean mass spectra with the general purpose GC-MS methods. With the experimental workflow that used a targeted method developed specifically for opioid analysis detection and separation of these two compounds was readily achieved. An example of this is shown in Figure 3 for Sample 19. In addition to this, there was one sample (Sample 35) where co-elution of tramadol and mannitol precluded confirmation of tramadol for both workflows.

Another limitation with the existing workflow was the inability to confirm dibutylone. When analyzing dibutylone on both GC-FID and GC-MS, there were other isomeric compounds that eluted well within the ±1 % retention time window of dibutylone and had mass spectra that were too similar to allow for differentiation. Using the targeted methods in the experimental workflow, however, provided sufficient separation to allow for confirmation of dibutylone. The general purpose GC-MS methods in the existing workflow use a minimum of 200,000 count peak abundance in the chromatogram for confirmation which lead to inability to confirm the identifies of compounds in seven samples (resulting in an insufficient identification). This limitation could be addressed by concentrating the sample, though care must be taken to ensure the major components in the sample do not saturate the detector.

For the targeted method approach, there were two instances (Sample 2 and Sample 42) where controlled substances were present in the sample that were not part of the panels for any of the targeted methods and therefore could not be confirmed. While this resulted in incomplete confirmation of all substances in these two samples, it can be addressed by simply adding additional compounds to the panel(s). This

process does require some time due to the need to complete replicate measurements of standards but is straightforward. This also highlights the potential need for a catch-all method that incorporates compounds outside of the classes that have targeted methods.

The biggest difference between the two confirmatory approaches occurred when comparing the time for analysis, summarized in Table 4. As expected, sample preparation for each of the instrumental techniques was almost identical, with GC-FID, general GC-MS, and targeted GC-MS all requiring approximately 10 min to prepare a batch of samples. However, because the existing workflow requires both GC-FID and GC-MS, the net time for sample preparation per batch is roughly twice as long. Instrument time was drastically different for the workflows, with the existing workflow requiring a total of 7728.8 min (128.8 hours) while the experimental workflow required only 2853.5 min (47.6 hours) – inclusive of all samples, reference materials, positive controls, and negative controls. Using the experimental workflow resulted in a 63 % reduction in time. A major driver for this difference is the large number of reference materials that are required for GC-FID analysis using the existing workflow due to lack of retention time locking, retention indices, or relative retention times. As shown in Table 4, the existing workflow required an average of 25.5 runs per batch, 19.0 of which, on average, came from GC-FID. GC-FID accounted for 68 % of the instrument runtime for the existing workflow.

If GC-FID were removed from the existing workflow, the time comparison between the two approaches becomes more similar. Comparing general purpose GC-MS runs to targeted GC-MS runs resulted in similar instrument runtimes per batch (116 min vs. 143 min, or 1.9 hours vs. 2.4 hours) and a similar number of runs (6.5 average vs. 7.4 average). These values are closer than were expected since samples containing multiple controlled substances needed to be analyzed on multiple targeted methods and because the opioid targeted method was significantly longer than the most commonly used general GC-MS method (35 min compared to 12.67 min). Part of what balanced the runtimes was that samples where no controlled substances were identified by DART-MS were not run on targeted GC-MS methods in the experimental workflow. It should be emphasized that using DART-MS as a stopping point for negative samples is something that would need to be thoroughly investigated prior to implementation in a real-world setting and may have too many limitations to be practical.

In terms of data analysis, the general purpose GC-MS analysis and targeted method GC-MS analysis required a similar amount of analyst time, though the targeted method analysis was slightly faster. This is likely due to the use of a locked retention time lookup table where chemists entered the retention time of a peak in a sample and the possible compound(s) that fell within 2 % of that time were shown. Adding in the need to manually compare retention times to standards using GC-FID, the data interpretation component for the existing workflow was found to be almost twice as long as the experimental workflow.

In terms of the amount of sample consumed and the risks to chemists, both confirmatory workflows were nearly identical. The existing workflow does require slightly more material since separate samples are created for GC-FID and GC-MS, but this difference is likely negligible for almost all cases. One potential

challenge with the targeted method approach is that it requires different stationary phases (DB-200 and DB-5) which means laboratories would need at least two instruments to leverage such an approach. Alternatively, new methods would need to be developed.

Conclusions

The results of this study demonstrate qualitative and quantitative gains that could be achieved by altering a seized drug workflow. Given the two workflows used here, it was found that screening of samples using color tests and DART-MS required approximately the same amount of time; however, the accuracy and specificity of the data obtained by DART-MS, on average, was superior. The use of DART-MS also eliminated false positives, which were observed with the color tests, and eliminated the need for toxic chemicals and acids. Though DART-MS was studied in combination with targeted GC-MS methods, the improved data quality and results it offers could benefit the existing confirmation workflow as well. While implementation of DART-MS has obvious advantages, the upfront and recurring costs as well as the time required to implement the technique should be considered.

In terms of the confirmation processes studied, major improvements in analysis time were observed alongside some notable gains in analytical capabilities. Temporal benefits were largely driven by the use of a single confirmation tool (targeted GC-MS) in the experimental workflow instead of a dual-technique confirmation. The use of locked retention times provided further instrument time reductions due to the reduced analysis of reference materials. Ongoing work includes investigating the potential benefits of other approaches, such as relative retention times and retention indices, that could reduce the frequency in which reference materials are run. Interestingly, even with the need to analyze a sample on multiple targeted methods, instrument time of the experimental workflow was not substantially greater than the GC-MS analysis of the existing workflow.

An obvious downside to the use of targeted methods is the need to have a panel of compounds, which for this study, was limited to only compounds within the particular drug classes. Adding more commonly co-observed compounds to the method is simple though it does require some time. The targeted methods also highlighted how class-specific methods designed for enhancing separation can address limitations presented by general purpose methods. This was observed for multiple compounds (acetyl fentanyl, FIBF, dibutylone, and α-PVP) in the sample set. The use of different chromatographic thresholds for confirmation can also lead to differences in the number of compounds that can be identified.

While implementation of targeted methods may be appealing, they do require the use of an information-rich screening tool. Success of the targeted methods was largely due to the fact that DART-MS provided comprehensive and specific results to enable accurate identification of nearly all controlled substances in the samples. This approach would not have been successful had color tests been used as the screening tool. Another possible use for targeted GC-MS would be to supplement existing general purpose confirmation methods in cases where sufficient separation of compounds is not observed (such as acetyl

fentanyl and FIBF). The use of targeted methods requires minimal additional cost and effort beyond the purchase of consumables and method validation; however, depending on the class of compounds of interest, systems with different stationary phases may be required, which could be problematic for laboratories with only one or a few instruments. It should also be noted that even though targeted methods are designed to specifically detect a suite of compounds, because they are full-scan methods they are able to detect compounds outside of the panel. An example of this is the case that contained methamphetamine, whereby methamphetamine was able to be detected, just not confirmed. Another interesting possibility, which was not examined here, is the use of dual-injection methods that would allow for analysis of a sample by GC-FID and GC-MS simultaneously, on two separate stationary phases. Combining two different retention times and mass spectral data may provide additional instances of compound discrimination over any of the above-mentioned approaches. Two-dimensional GC is another potential tool that could provide multiple datapoints to increase confidence in identification.

This study highlights some of the strengths and limitations of two specific analytical workflows. Though there are limitations in the experimental workflow, it does highlight some reasons why laboratories may want to consider changes to their protocols. The experimental workflow described here is certainly not the ideal workflow for seized drug analysis, as several research questions still need to be answered. A workflow that contains both a combination of general analysis and targeted methods may be best. The ideal workflow would also certainly look different across laboratories and would be dependent on factors such as: caseload, personnel, types of cases frequently examined, jurisdictional requirements, and access to instrumentation. While it may not be practical to measure all gains and drawbacks prior to implementing changes to analytical protocols, the ability to test these changes, on a small scale, may prove consequential and may limit instances where new techniques are procured but never implemented into casework. Additional studies investigating different analytical workflows are still ongoing and are the focus of current research.

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Table 1. List of the 50 samples used in this study. Non-controlled substances in the samples are also listed, in italics. Sample numbers with a dagger (†) were created using one or more adjudicated case samples and sample numbers with an asterisk (*) were created using standards from Cayman Chemical. Some samples were created using a mixture of both (†*). Compound names with a double dagger (‡) are compounds that, when previously analyzed, were found to be insufficient concentrations to allow for confirmation.

Sample	Contents	Sample	Contents
1†	No Controlled Substance *Pill Binder*	26†	Eutylone *Caffeine*
2†	Methamphetamine	27*	No Controlled Substance *Caffeine*
3†	Heroin, MDMA *Mannitol, Quinine*	28†	4-Meththylethcathinone
4†	Fentanyl, Tramadol‡ *Levamisole, Mannitol, N-Phenylpropanamide, Procaine*	29†*	5-Fluoro-AKB48, α-PBP *Mannitol*
5†	MPHP *Dextromethorphan*	30*	Dibutylone, Fentanyl, JWH-250
6†	MDMA	31†	Tramadol *Dextromethorphan*
7†	No Controlled Substance *Mannitol*	32†	JWH-250
8†	Heroin *Papaverine*	33†	Fentanyl, Heroin, Acetyl Fentanyl‡, FIBF‡ *Caffeine, Quinine*
9†	Methyl Norfentanyl	34†	Eutylone
10†	4-Ethylmethcathinone	35†	Fentanyl, Tramadol‡ *Caffeine, Levamisole, Mannitol, N-Phenylpropanamide, Procaine*
11†*	Dibutylone *Caffeine*	36†	Methyl-AP-237
12†*	4-Ethylmethcathinone, Fentanyl, 4-Me-α-ethylaminopentiophenone	37†	Heroin
13†	FUB-AMB	38†	JWH-250, α-Methyl Fentanyl
14†	Cyclopropyl Fentanyl, Heroin, Phenyl Fentanyl *Caffeine, Mannitol*	39†	Fentanyl *Caffeine, Quinine, Xylazine*
15*	AB-FUBINACA 2-fluorobenzyl isomer	40†*	4-Chloroethcathinone, Cyclopropyl Fentanyl
16†	No Controlled Substance *Inorganic Compound*	41†	No Controlled Substance *Mannitol*
17†	Dibutylone	42†	Heroin, Acetyl Fentanyl‡, Cocaine‡, Fentanyl‡, FIBF‡, Noscapine‡ *Caffeine, Quinine*
18†	Acetyl Fentanyl, Fentanyl *Mannitol, Quinine*	43†	Methylone
19†	Heroin, Acetyl Fentanyl‡, Fentanyl‡, FIBF‡ *Caffeine, Lidocaine, Mannitol, Quinine*	44†	N-methyl Cyclopropyl norfentanyl
20*	No Controlled Substance *Guaifenesin, Quinine*	45*	No Controlled Substance *Lidocaine, Quinine*
21*	No Controlled Substance	46†	4-Methylethcathinone

	Acetaminophen, Citric Acid, Xylitol		
22†*	Fentanyl, XLR11	47†	JWH-018, 3,4-MDPV
23†	JWH-250	48†	N-Ethyl Pentylone
24†	JWH-018	49*	FUB-AMB
25†	α-PVP	50†*	α-PVP *Sodium Bicarbonate*

Table 2. Scoring system used to rank the colorimetric and DART-MS screening results.

Score	Outcome
-1	Identification of compound or compound class that is inconsistent with actual contents
0	Inconclusive Result
1	Correct identification of compound class for at least one compound
2	Correct identification of *at least* the sub-class or isomer group identified for *at least* one compound (mixtures only)
3	Correct identification of *at least* the sub-class or isomer group for all compounds
4	Correct identification of all compounds identified OR correct identification of a negative sample as negative for controlled substances

Table 3. Summary results for the confirmatory analysis of the fifty samples using the existing and experimental workflows. Only controlled substances are listed. Compounds that were detected but could not be confirmed are listed as insufficient, and the reason for the insufficient designation is provided. A double dagger (‡) indicates that the compound was not at a high enough abundance in the GC-MS chromatogram for confirmation, a superscript RT (^{RT}) indicates that there were multiple similar compounds with overlapping retention time windows which precluded confirmation, and compounds in parentheses indicate instances where co-elution precluded confirmation. A breakdown of these results is shown in Supplemental Table 6 and Supplemental Table 7.

Sample	Existing Workflow Results	Experimental Workflow Results
1	No Controlled Substances	Not Analyzed
2	Methamphetamine	*Methamphetamine Not Confirmed (Not in Targeted Methods)*
3	MDMA *Insufficient: Heroin‡*	Heroin, MDMA
4	Fentanyl *Insufficient: Tramadol‡*	Fentanyl *Insufficient: (Tramadol | Mannitol)*
5	MPHP	MPHP
6	MDMA	MDMA
7	No Controlled Substances	Not Analyzed
8	Heroin	Heroin
9	N-Methyl Norfentanyl	N-Methyl Norfentanyl
10	4-Ethylmethcathinone	4-Ethylmethcathinone
11	*Insufficient: Dibutylone^{RT}*	Dibutylone
12	4-Etylmethcathinone, Fentanyl, 4-Me-α-ethylaminopentiophenone	4-Ethylmethcathinone, Fentanyl, 4-Me-α-ethylaminopentiophenone
13	FUB-AMB	FUB-AMB
14	Cyclopropyl Fentanyl *Insufficient: Heroin‡, Phenyl Fentanyl‡*	Cyclopropyl Fentanyl, Heroin, Phenyl Fentanyl
15	AB-FUBINACA 2-fluorobenzyl isomer	AB-FUBINACA 2-fluorobenzyl isomer
16	No Controlled Substances	Not Analyzed
17	Insufficient: Dibutylone^{RT}	Dibutylone
18	Acetyl Fentanyl, Fentanyl	Acetyl Fentanyl, Fentanyl
19	Fentanyl, Heroin *Insufficient: (Acetyl Fentanyl | FIBF)*	Acetyl Fentanyl, Fentanyl, FIBF, Heroin
20	No Controlled Substances	Not Analyzed
21	No Controlled Substances	Not Analyzed
22	Fentanyl, XLR11	Fentanyl, XLR11
23	JWH-250	JWH-250
24	JWH-018	JWH-018
25	*Insufficient: α-PVP^{RT}*	α-PVP
26	Eutylone	Eutylone
27	No Controlled Substances	Not Analyzed
28	4-Methylethcathinone	4-Methylethcathinone
29	5-Fluoro-AKB48, α-PBP	5-Fluoro-AKB48, α-PBP
30	JWH-250 *Insufficient: Dibutylone^{RT}, Fentanyl‡*	Dibutylone, Fentanyl, JWH-250
31	Tramadol	Tramadol
32	JWH-250	JWH-250
33	Fentanyl, Heroin *Insufficient: (Acetyl Fentanyl | FIBF)*	Acetyl Fentanyl, Fentanyl, FIBF, Heroin

34	Eutylone	Eutylone
35	Fentanyl *Insufficient: (Tramadol‡ | Mannitol)*	Fentanyl *Insufficient: (Tramadol | Mannitol)*
36	Methyl-AP-237	Methyl-AP-237
37	Heroin	Heroin
38	JWH-250, α-Methyl Fentanyl	JWH-250, α-Methyl Fentanyl
39	*Insufficient: Fentanyl‡*	Fentanyl
40	4-Chloroethcathinone, Cyclopropyl Fentanyl	4-Chloroethcathinone, Cyclopropyl Fentanyl
41	No Controlled Substances	Not Analyzed
42	Fentanyl, Heroin *Insufficient: (Acetyl Fentanyl | FIBF), Cocaine‡*	Acetyl Fentanyl, Fentanyl, FIBF, Heroin *Insufficient: Cocaine (Not in Targeted Methods), Noscapine‡*
43	Methylone	Methylone
44	N-methyl Cyclopropyl norfentanyl	N-methyl Cyclopropyl norfentanyl
45	No Controlled Substances	Not Analyzed
46	4-Methylethcathinone	4-Methylethcathinone
47	JWH-018, 3,4-MDPV	JWH-018, 3,4-MDPV
48	N-Ethylpentylone	N-Ethylpentylone
49	FUB-AMB	FUB-AMB
50	*Insufficient: α-PVP^{RT}*	α-PVP

Table 4. Metrics for the GC-FID and GC-MS analyses for both workflows. A further breakdown of these results is shown in Supplemental Table 6 and Supplemental Table 7.

	Existing Workflow			Experimental Workflow
	GC-FID	**General GC-MS**	**Combined Total**	**Targeted GC-MS**
Average Sample Preparation per Batch (min)	9.0 (±2.0)	13.6 (±4.0)	22.6 (±6.0)	8.8 (±1.3)
Average Data Interpretation per Batch (min)	8.2 (±5.4)	22.7 (±10.4)	30.9 (±15.8)	16.5 (±1.5)
Average Instrument Time per Batch (min)	264.3 (±108.9)	116.3 (±43.2)	380.6 (±152.1)	142.7 (±50.0)
Cumulative Average Time per Batch (min)	281.5 (±116.3)	152.6 (±57.6)	434.1 (±173.9)	168 (±52.8)
# Runs per Batch (Samples + Standards)	19.0	6.5	25.5	7.4
Total Instrument Time (min)	5286.3	2442.5	7728.8	2853.5

FIG 1. Schematic of the existing and experimental workflows.

FIG 2. Histogram showing the distribution of scores for the color test results (blue, n = 100) and the DART-MS results (grey, n = 100).

FIG 3. Representative GC-MS chromatograms of Sample 19 analyzed using a general purpose method from the existing workflow (top) and the opioid targeted GC-MS method from the experimental workflow (bottom). Only the first ten minutes of the chromatograms are shown as there were no additional peaks past this point. The elution order was different for the two runs because the methods use different stationary phases.

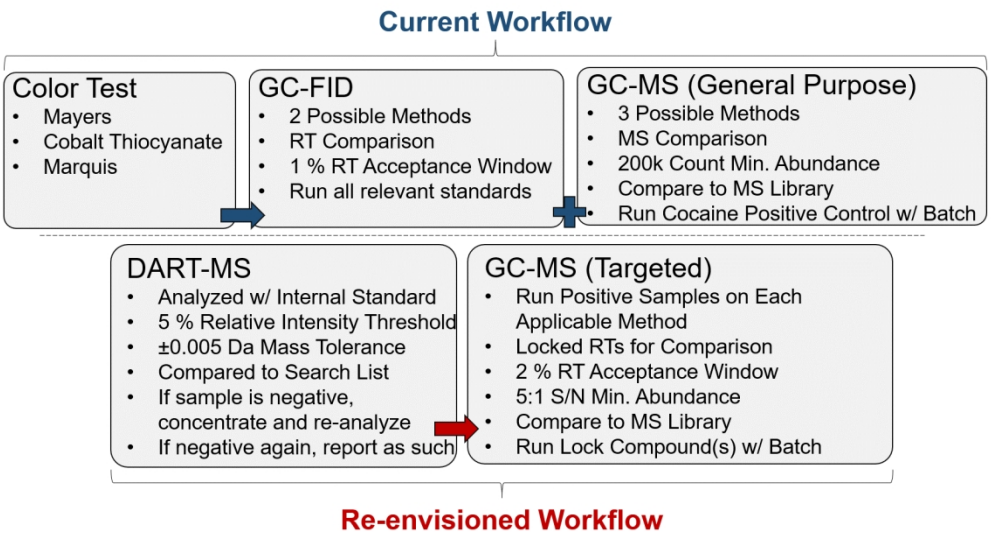

FIG 1. Schematic of the existing and experimental workflows.

152x82mm (300 x 300 DPI)

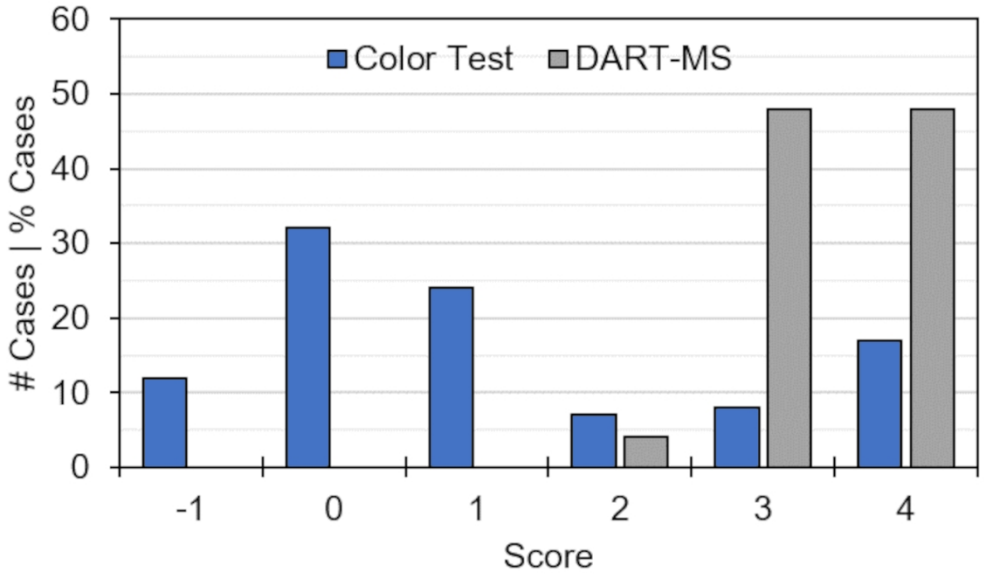

FIG 2. Histogram showing the distribution of scores for the color test results (blue, n = 100) and the DART-MS results (grey, n = 100).

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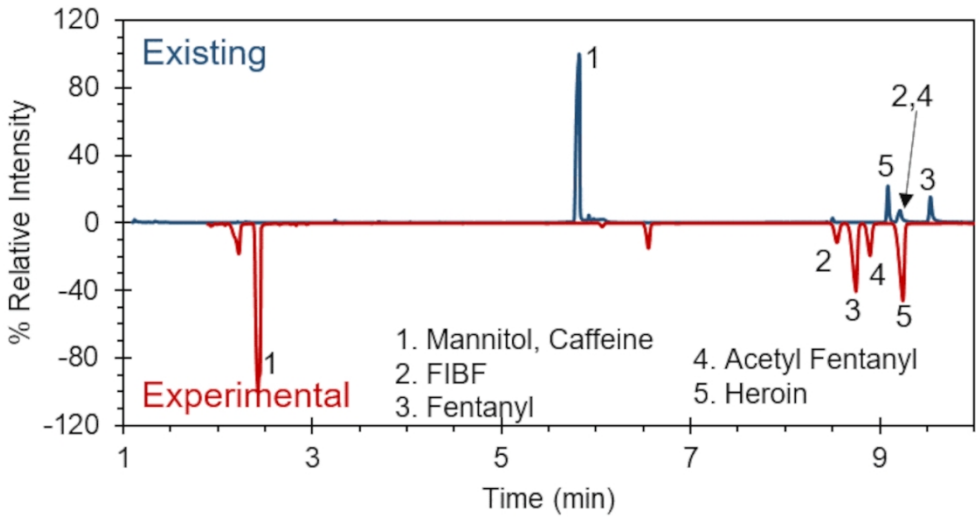

FIG 3. Representative GC-MS chromatograms of Sample 19 analyzed using a general purpose method from the existing workflow (top) and the opioid targeted GC-MS method from the experimental workflow (bottom). Only the first ten minutes of the chromatograms are shown as there were no additional peaks past this point. The elution order was different for the two runs because the methods use different stationary phases.

114x59mm (300 x 300 DPI)

Supplemental Information for:

Comparing Two Analytical Workflows for Seized Drug Analysis of Synthetic Cannabinoids, Cathinones, and Opioids

Supplemental Table 1. Method parameters for the two GC-FID methods used in the existing workflow.

Method	A	B
Instrument	Agilent 7890	Agilent 6890
Column	DB-5 15 m x 0.25 mm x 0.25 µm	DB-5MS 20 m x 0.18 mm x 0.18 µm
Temperature Program	160 ºC, Hold 1 min Ramp 20 ºC/min to 220 ºC Hold 1 min Ramp 30 ºC/min to 280 ºC Hold 7 min	150 ºC, Hold 1 min Ramp 30 ºC/min to 290 ºC Hold 7 min
Flow Rate	1.44 mL/min	0.8 mL/min 10 mL/min^2 to 1.8 mL/min at 3 min Hold 1.8 mL/min
Injection Volume	1 µL	1 µL
Inlet Temperature	250 ºC	250 ºC
Split Ratio	50:1	20:1
Detector Temperature	280 ºC	300 ºC
Data Collection Rate	50 Hz	50 Hz
Total Run Time	15 min	12.67 min

Supplemental Table 2. Reference material sets required to be run for GC-FID verification. Only compounds that required multiple reference materials to be run are listed. The number of reference materials required is listed in parenthesis.

Compound in Study	Reference Materials Run
AB-FUBINACA 2-fluorobenzyl isomer (6)	AB-FUBINACA AB-FUBINACA 2-fluorobenzyl isomer AB-FUBINACA 3-fluorobenzyl isomer AB-FUBINACA isomer 1 AB-FUBINACA isomer 2 AB-FUBINACA isomer 5
4-Chloroethcathinone (6)	2-Chloroethcathinone 3-Chloroethcathinone 3-Chloro-N,N-Dimethylcathinone 4-Chlorobuphedrone 4-Chloroethcathinone 4-Chloro-N,N-Dimethylcathinone
Crotonyl Fentanyl ***or*** Cyclopropyl Fentanyl (2)	Crotonyl Fentanyl Cyclopropyl Fentanyl
Dibutylone ***or*** Eutylone (9)	Dibutylone Eutylone 2,3-Eutylone 3,4-Methylenedioxy-α-methylamino-isovalerophenone 3,4-Methylenedioxy-N-isopropylcathinone 3,4-Methylenedioxy-N-propylcathinone N-Methylethylone Pentylone 2,3-Pentylone
4-Ethylmethcathinone (6)	2,3-Dimethylmethcathinone 2,4-Dimethylmethcathinone 3,4-Dimethylmethcathinone 2-Ethylmethcathinone 3-Ethylmethcathinone 4-Ethylmethcathinone
N-Ethylpentylone (7)	N,N-Dimethylpentylone N-Ethylpentylone 3,4-Methylenedioxy-N,N-Diethylcathinone 3',4'-Methylenedioxy-α-Ethylamino-isovalerophenone 3,4-Methylenedioxy-α-Dimethylamino-isovalerophenone 3,4-Methylenedioxy-α-Methylaminohexanophenone 3,4-Methylenedioxy-α-Methylaminoisohexanophenone
FIBF (3)	FIBF m-Fluoroisobutyryl fentanyl o-Fluoroisobutyryl fentanyl
JWH-250 (2)	JWH-250 JWH-302
3,4-MDPV (2)	2,3-MDPV 3,4-MDPV
Methamphetamine (2)	Phentermine Methamphetamine
4-Methyl-α-Ethylaminopentiophenone (3)	4-Methyl-α-Ethylaminopentiophenone 4-Methyl-N-Methylhexanophenone 4-Methyldiethcathinone
4-Methylethcathinone (6)	2-Methylethcathinone 3-Methylethcathinone 4-Methyl-N,N-Dimethylcathinone 3-Methylbuphedrone 4-Methylethcathinone 4-Methylbuphedrone
Methylone (2)	2,3-Methylenedioxymethcathinone Methylone
α-PVP (2)	α-PIPBP α-PVP

Supplemental Table 3. Method parameters for the three general purpose GC-MS methods used in the existing workflow.

Method	A	B	C
Instrument	Agilent 7890/5977B	Agilent 7890/5977B	Agilent 6890/5975B
Column	HP-5ms Ultra Inert 30 m x 0.25 mm x 0.25 µm	HP-5ms Ultra Inert 30 m x 0.25 mm x 0.25 µm	DB-5MS 20 m x 0.18 mm x 0.18 µm
Temperature Program	120 ºC, Hold 1 min Ramp 25 ºC/min to 280 ºC Hold 20 min	180 ºC, Hold 0 min Ramp 30 ºC/min to 280 ºC Hold 8 min	150 ºC, Hold 1 min Ramp 30 ºC/min to 290 ºC Hold 7 min
Flow Rate	1.6 mL/min	1.8 mL/min	0.8 mL/min 10 mL/min^2 to 1.8 mL/min at 3 min Hold 1.8 mL/min
Injection Volume	1 µL	1 µL	1 µL
Inlet Temperature	250 ºC	250 ºC	250 ºC
Split Ratio	50:1	50:1	30:1
Transfer Line	280 ºC	280 ºC	280 ºC
Quad Temperature	150 ºC	150 ºC	150 ºC
Source Temperature	230 ºC	230 ºC	230 ºC
Tune Mode	stune	stune	stune
Solvent Delay	1.4 min	1.15 min	1.2 min
Mass Scan Range	*m/z* 40 – *m/z* 550	*m/z* 40 – *m/z* 550	*m/z* 40 – *m/z* 550
Threshold	150 counts	150 counts	300 counts
Scan Speed	N = 2 [≈4 scan/s]	N = 2 [≈4 scan/s]	N = 2 [≈4 scan/s]
Total Run Time	27.4 min	11.33 min	12.67 min

Supplemental Table 4. Method parameters for the three targeted GC-MS methods used for the experimental workflow. All analyses were completed on an Agilent 7890/5977B.

Compound Class	Cannabinoids	Cathinones	Opioids
Lock Compound	AB-FUBINACA	Butylone	Fentanyl
Column	DB-200 30 m x 0.25 mm x 0.25 µm	DB-5 30 m x 0.25 mm x 0.25 µm	DB-200 30 m x 0.25 mm x 0.25 µm
Temperature Program	Isothermal at 290 °C	190 °C for 0.5 min Ramp 5 °C/min to 210 °C Ramp at 30 °C/min to 255 °C Hold 1.5 min	230 °C for 0.0 min Ramp at 2 °C/min to 290 °C Hold 5.0 min
Flow Rate	1.2 mL/min	1.9 mL/min	1.2 mL/min
Injection Volume	1.0 µL	1.0 µL	1.0 µL
Inlet Temperature	300 °C	300 °C	300 °C
Split Ratio	30:1	30:1	20:1
Transfer Line	300 °C	300 °C	300 °C
Quad Temperature	150 °C	150 °C	150 °C
Source Temperature	280 °C	280 °C	280 °C
Tune Mode	stune	stune	stune
Solvent Delay	1.4 min	1.15 min	1.3 min
Mass Scan Range	*m/z* 40 – *m/z* 550	*m/z* 40 – *m/z* 550	*m/z* 40 – *m/z* 550
Threshold	150 counts	150 counts	150 counts
Scan Speed	N = 2 [≈4 scan/s]	N = 2 [≈4 scan/s]	N = 2 [≈4 scan/s]
Total Run Time	12.0 min	7.5 min	35.0 min

Supplemental Table 5. Scores and results obtained for the color test (existing workflow) and DART-MS (experimental workflow) portions of the study. For each sample, scores are listed in the first row and the results listed in the second. For the color tests, results are shown in the following order: Mayers, cobalt thiocyanate, and Marquis from left to right represented by the color observed. A cell with an "X" indicates no reaction. DART-MS results for only the controlled substances are listed. DART-MS results were identical for both chemists except for Sample 42 where FIBF and noscapine were only identified by one chemist, as denoted with "(1)". In the Contents column, compound names with a double dagger (‡) are compounds in a sample that, when previously analyzed, were found to be at concentrations too low for confirmation.

Sample	Contents	Color Test		DART-MS	
		Score 1	Score 2	Score 1	Score 2
1	No Controlled Substance *Pill Binder*	4 X X X	4 X X X	4 No Controlled Substances	4
2	Methamphetamine	4 X	4 X	4 Methamphetamine	4
3	Heroin, MDMA *Mannitol, Quinine*	3	3	2 MDMA	2
4	Fentanyl, Tramadol‡ *Levamisole, Mannitol, N-Phenylpropanamide, Procaine*	1	1	4 Fentanyl, Tramadol	4
5	MPHP *Dextromethorphan*	-1	1	4 MPHP	4
6	MDMA	4 X	4 X	4 MDMA	4
7	No Controlled Substance *Mannitol*	4 X X X	4 X X X	4 No Controlled Substances	4
8	Heroin *Papaverine*	4	4	4 Heroin. 6-MAM	4
9	Methyl Norfentanyl	0 X	0 X	4 Methyl Norfentanyl	4
10	4-Ethylmethcathinone	0 X	0	3 Cathinone *m/z* 192	3
11	Dibutylone *Caffeine*	1	1	3 Cathinone *m/z* 236	3
12	4-Ethylmethcathinone, Fentanyl, 4-Me-α-ethylaminopentiophenone	-1 X	1	3 Fentanyl, Cathinone *m/z* 220, Cathinone *m/z* 192	3
13	FUB-AMB	0 X X	0 X X	4 FUB-AMB	4
14	Cyclopropyl Fentanyl, Heroin, Phenyl Fentanyl *Caffeine, Mannitol*	1	1	3 Cyclopropyl Fentanyl or isomer, Phenyl Fentanyl	3
15	AB-FUBINACA 2-fluorobenzyl isomer	0 X X X	0 X X X	3 AB-FUBINACA or isomer	3
16	No Controlled Substance *Inorganic Compound*	4 X X X	4 X X X	4 No Controlled Substances	4
17	Dibutylone	1	1	3 Cathinone *m/z* 236	3
18	Acetyl Fentanyl, Fentanyl *Mannitol, Quinine*	1	0 X	3 Acetyl Fentanyl or isomer, Fentanyl	3
19	Heroin, Acetyl Fentanyl‡, Fentanyl‡, FIBF‡ *Caffeine, Lidocaine, Mannitol, Quinine*	1	1	3 Acetyl Fentanyl or isomer, Fentanyl, FIBF or isomer, Heroin	3
20	No Controlled Substance	-1	-1	4	4

	Guaifenesin, Quinine							No Controlled Substances	
21	No Controlled Substance *Acetaminophen, Citric Acid, Xylitol*	4			4			4	4
		X	X	X	X	X	X	No Controlled Substances	
22	Fentanyl, XLR11	0			0			3	3
			X			X		Fentanyl, XLR11	
23	JWH-250	0			0			3	3
		X	X		X			JWH-250 or isomer	
24	JWH-018	-1			-1			4	4
		X	X		X	X		JWH-018	
25	α-PVP	0			0			4	4
				X			X	α-PVP	
26	Eutylone *Caffeine*	1			1			3	3
								Cathinone *m/z* 236	
27	No Controlled Substance *Caffeine*	4			4			4	4
		X	X	X	X	X	X	No Controlled Substances	
28	4-Methylethcathinone	0			-1			3	3
			X	X				Cathinone *m/z* 192	
29	5-Fluoro-AKB48, α-PBP *Mannitol*	0			0			3	3
			X	X			X	5-F-AKB, α-PBP or isomer	
30	Dibutylone, Fentanyl, JWH-250	1			1			3	3
								Cathinone *m/z* 236, Fentanyl, JWH-250 or isomer	
31	Tramadol *Dextromethorphan*	0			1			4	4
								Tramadol	
32	JWH-250	0			0			3	3
		X	X		X	X		JWH-250 or isomer	
33	Fentanyl, Heroin, Acetyl Fentanyl‡, FIBF‡ *Caffeine, Quinine*	0			2			3	3
		X						Acetyl Fentanyl or isomer, Fentanyl, FIBF or isomer, Heroin	
34	Eutylone	3			3			3	3
		X			X	X		Cathinone *m/z* 236	
35	Fentanyl, Tramadol‡ *Caffeine, Levamisole, Mannitol, N-Phenylpropanamide, Procaine*	2			2			3	3
								Fentanyl, Tramadol	
36	Methyl-AP-237	3			3			3	3
								Methyl-AP-237 or AP-238	
37	Heroin	3			3			4	4
								Heroin, 6-MAM	
38	JWH-250, α-Methyl Fentanyl	0			2			3	3
		X	X					JWH-250 or isomer, Methyl Fentanyl isomer	
39	Fentanyl *Caffeine, Quinine, Xylazine*	-1			0			4	4
					X	X	X	Fentanyl	
40	4-Chloroethcathinone, Cyclopropyl Fentanyl	2			1			3	3
								Cathinone *m/z* 212, Cyclopropyl Fentanyl or isomer	
41	No Controlled Substance *Mannitol*	4			0			4	4
		X	X	X		X	X	No Controlled Substances	
42	Heroin, Acetyl Fentanyl‡, Cocaine‡, Fentanyl‡, FIBF‡, Noscapine‡ *Caffeine, Quinine*	2			2			2	2
								Acetyl Fentanyl or isomer, Fentanyl, FIBF or isomer (1), Heroin, Noscapine (1)	
43	Methylone	1			1			3	3
			X			X		Cathinone *m/z* 208	

44	N-Methyl Cyclopropyl Norfentanyl	0			0			4	4
			X	X			X	N-Methyl Cyclopropyl Norfentanyl	
45	No Controlled Substance *Lidocaine, Quinine*	-1			-1			4	4
								No Controlled Substances	
46	Methylethcathinone	0			0			3	3
				X				Cathinone *m/z* 192	
47	JWH-018, 3,4-MDPV	1			1			4	4
		X						JWH-018, MDPV	
48	N-Ethyl Pentylone	1			1			3	3
					X			N-Ethylpentylone or isomer	
49	FUB-AMB	0			0			4	4
		X	X	X	X		X	FUB-AMB	
50	α-PVP *Sodium Bicarbonate*	0			0			4	4
				X			X	α-PVP	

Supplemental Table 6. Summary results for the GC-FID and GC-MS confirmatory analyses using the existing workflow broken down by batch. Method letters correspond to those listed in Supplemental Table 1 (GC-FID) and 3 (GC-MS). Reference materials required are based on Supplemental Table 2. Compounds that were detected but could not be confirmed are listed as insufficient, and the reason for the insufficient designation is provided. A double dagger (‡) indicates that the compound was not at a high enough abundance in the GC-MS chromatogram for confirmation, a superscript RT (^{RT}) indicates that there were multiple similar compound with overlapping retention time windows which precluded confirmation, and compounds in parentheses indicate instances where co-elution precluded confirmation.

Chemist 1 – Batch 1			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
1	A	A	No Controlled Substances
2	A	A	Methamphetamine
3	A	A	Heroin‡, MDMA
4	A	A	Fentanyl‡, Tramadol‡
5	A	A	MPHP
Cumulative Standards / + Controls	7	1	**FID:** Methamphetamine, Phentermine, MDMA, Heroin, Fentanyl, Tramadol, MPHP **MS:** Cocaine
Runtime (min)	180	164.4	
Chemist 1 – Batch 2			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
21	A	A	No Controlled Substances
22	A	A	Fentanyl, XLR11
23	A	A	JWH-250
24	A	A	JWH-018
25	A	A	*Insufficient: α-PVP^{RT}*
Cumulative Standards / + Controls	7	1	**FID:** XLR11, Fentanyl, JWH-250, JWH-302, JWH-018, α-PVP, α-PIPBP **MS:** Cocaine
Runtime (min)	180	164.4	
Chemist 1 – Batch 3			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
26	A	A	Eutylone
27	A	A	No Controlled Substabces
28	A	A	4-Methylethcathinone
29	A	A	α-PBP, 5-F-AKB48
30	A	A	Fentanyl, JWH-250 *Insufficient: Dibutylone^{RT}*
Cumulative Standards / + Controls	20	1	**FID:** Set of 6 4-Methylethcathinone compounds, Set of 9 Dibutylone/Eutylone compounds, α-PBP, 5-F-AKB48, Fentanyl, JWH-250, JWH-302 **MS:** Cocaine
Runtime (min)	375	164.4	
Chemist 1 – Batch 4			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
36	A	A	Methyl-AP-237
37	A	A	Heroin
38	A	A	JWH-250, α-Methyl Fentanyl
39	A	A	*Insufficient: Fentanyl‡*
40	A	A	4-Chloroethcathinone, Cyclopropyl Fentanyl
Cumulative Standards / + Controls	14	1	**FID:** AP-238, Heroin, α-Methyl Fentanyl, JWH-250, JWH-302, Fentanyl, Set of 6 4-Chloroethcathinone compounds, Cyclopropyl Fentanyl, Crotonyl Fentanyl **MS:** Cocaine

Runtime (min)	285	164.4	
Chemist 1 – Batch 5			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
41	A	A	No Controlled Substances
42	A	A	Fentanyl, Heroin
43	A	A	Methylone
44	A	A	N-Methyl Cyclopropyl Fentanyl
45	A	A	No Controlled Substances
Cumulative Standards / + Controls	5	1	**FID:** Heroin, Fentanyl, Methylone, 2,3-MDMC, N-Methyl Cyclopropyl norfentanyl **MS:** Cocaine
Runtime (min)	150	164.4	
Chemist 2 – Batch 1			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
6	A	B	MDMA
7	A	A	No Controlled Substances
8	A	B	Heroin
9	A	A	N-Methyl Norfentanyl
10	A	A	4-Ethylmethcathinone
Cumulative Standards / + Controls	9	A – 1 B – 1	**FID:** MDMA, Heroin, Methyl Norfentanyl, Set of 6 4-Ethylmethcathinone compounds **MS (A):** Cocaine **MS (B):** Cocaine
Runtime (min)	210	143.6	
Chemist 2 – Batch 2			
Case #	**GC-FID Method**	**GC-MS Method**	**Compound ID**
11	A	A	*Insufficient: Dibutylone^{RT}*
12	A	B	4-Ethylmethcathinone, Fentanyl, 4-Me-α-Ethylaminopentiophenone
13	A	B	FUB-AMB
14	A	A	Cyclopropyl Fentanyl, Heroin *Insufficient: Phenyl Fentanyl‡*
15	A	A	AB-FUBINACA 2-fluorobenzyl isomer
Cumulative Standards / + Controls	30	A – 1 B – 2	**FID:** Set of 6 AB-FUBINACA compounds, Set of 9 Dibutylone compounds, Set of 6 4-Ethylmethcathinone compounds, Fentanyl, Set of 3 4-Me-α-Ethylaminopentionphenone compounds, FUB-AMB, Heroin, Cyclopropyl Fentanyl, Crotonyl Fentanyl, Phenyl Fentanyl **MS(A):** Cocaine **MS(B):** Cocaine, Fentanyl (missing molecular ion)
Runtime (min)	525	154.9	
Chemist 2 – Batch 3			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
16	A	A	No Controlled Substances
17	A	A	*Insufficient: Dibutylone^{RT}*
18	A	A	Acetyl Fentanyl, Fentanyl
19	A	A	Fentanyl, Heroin *Insufficient: (Acetyl Fentanyl | FIBF)*
20	A	A	No Controlled Substances
Cumulative Standards / + Controls	17	1	**FID (A):** Acetyl fentanyl, Fentanyl, Set of 3 FIBF compounds, Heroin **FID (B):** Set of 9 Dibutylone compounds, Acetyl fentanyl, Fentanyl **MS:** Cocaine
Runtime (min)	330	164.4	

Chemist 2 – Batch 4			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
31	A	A	Tramadol
32	A	A	JWH-250
33	A	A	Fentanyl, Heroin *Insufficient: (Acetyl Fentanyl | FIBF)*
34	A	A	Eutylone
35	A	B	Fentanyl
Cumulative Standards / + Controls	19	A – 1 B – 1	**FID (A):** Acetyl Fentanyl, Fentanyl, Set of 3 FIBF compounds, Heroin **FID (B):** Tramadol, JWH-250, JWH-302, Set of 9 Eutylone compounds, Fentanyl **MS (A):** Cocaine **MS (B):** Cocaine
Runtime (min)	360	159.7	
Chemist 2 – Batch 5			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
46	A	A	4-Methylethcathinone
47	A	A	JWH-018, 3,4-MDPV
48	A	A	N-Ethylpentylone
49	A	B	FUB-AMB
50	A	A	*Insufficient: α-PVP^{RT}*
Cumulative Standards / + Controls	19	A – 2 B – 1	**FID:** Set of 6 4-Methylethcathinone compounds, 2,3-MDPV, 3,4-MDPV, JWH-018, Set of 7 N-Ethylpentylone compounds, FUB-AMB, α-PVP, α-PIPBP **MS (A):** Cocaine, MDPV (missing molecular ion) **MS(B):** Cocaine
Runtime (min)	360	187.1	
Chemist 3 – Batch 1			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
1	B	C	No Controlled Substances
3	B	C	MDMA
5	B	C	MPHP
7	B	C	No Controlled Substances
9	B	C	N-Methyl Norfentanyl
Cumulative Standards / + Controls	3	1	**FID:** MDMA, MPHP, N-Methyl Norfentanyl **MS:** Cocaine
Runtime (min)	101.4	76	
Chemist 3 – Batch 2			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
12	B	C	4-Ethylmethcathinone, Fentanyl, 4-Me-α-Ethylaminopentiophenone
14	B	C	Cyclopropyl Fentanyl *Insufficient: Heroin‡, Phenyl Fentanyl‡*
16	B	C	No Controlled Substances
18	B	C	*Insufficient: Acetyl Fentanyl‡, Fentanyl‡*
20	B	C	No Controlled Substances
Cumulative Standards / + Controls	15	3	**FID:** Set of 6 4-Ethylmethcathinone compounds, Set of 3 4-Me-α-Ethylaminopentiophenone compounds, Fentanyl, Crotonyl Fentanyl, Cyclopropyl Fentanyl, Heroin, Acetyl Fentanyl **MS:** Cocaine, 4-Ethylmethcathinone (missing molecular ion), Fentanyl (missing molecular ion)
Runtime (min)	153.4	101.4	

Chemist 3– Batch 3			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
21	B	C	No Controlled Substances
23	B	C	JWH-250
25	B	C	*Insufficient: α-PVP^{RT}*
27	B	C	No Controlled Substances
29	B	C	5-F-AKB48, α-PBP
Cumulative Standards / + Controls	6	1	**FID:** JWH-250, JWH-302, α-PVP, α-PIPBP, α-PBP, 5-F-AKB48 **MS:** Cocaine
Runtime (min)	139.4	76	
Chemist 3 – Batch 4			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
32	B	C	JWH-250
34	B	C	Eutylone
36	B	C	Methyl-AP-237
38	B	C	JWH-250, α-Methyl Fentanyl
40	B	C	4-Chloroethcathinone, Cyclopropyl Fentanyl
Cumulative Standards / + Controls	21	2	**FID:** JWH-250, JWH-302, Set of 9 Eutylone compounds, AP-238, α-Methyl Fentanyl, Cyclopropyl Fentanyl, Crotonyl Fentanyl, Set of 6 4-Chloroethcathinone compounds **MS:** Cocaine, Eutylone (missing molecular ion)
Runtime (min)	329.4	88.7	
Chemist 3 – Batch 5			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
41	B	C	No Controlled Substances
43	B	C	Methylone
45	B	C	No Controlled Substances
47	B	C	JWH-018, 3,4-MDPV
49	B	C	FUB-AMB
Cumulative Standards / + Controls	6	2	**FID:** Methylone, MDMC, 3,4-MDPV, 2,3-MDPV, JWH-018, FUB-AMB **MS:** Cocaine, 3,4-MDPV (missing molecular ion)
Runtime (min)	139.4	88.7	
Chemist 4 – Batch 1			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
2	B	C	Methamphetamine
4	B	C	Fentanyl, Tramadol
6	B	C	MDMA
8	B	C	Heroin
10	B	C	4-Ethylmethcathinone
Cumulative Standards / + Controls	12	1	**FID:** Methamphetamine, Phentermine, Tramadol, Fentanyl, MDMA, Heroin, Set of 6 4-Ethylmethcathinone compounds **MS:** Cocaine
Runtime (min)	215.4	76	

Chemist 4 – Batch 2			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
11	B	C	*Insufficient: Dibutylone^{RT}*
13	B	C	FUB-AMB
15	B	C	*Insufficient: AB-FUBINACA 2-fluorobenzyl isomer*
17	B	C	*Insufficient: Dibutylone^{RT}*
19	B	C	Fentanyl, Heroin *Insufficient: (Acetyl Fentanyl | FIBF)*
Cumulative Standards / + Controls	22	1	**FID:** Set of 9 Eutylone/Dibutylone compounds, FUB-AMB, Set of 6 AB-FUBINACA compounds, Acetyl Fentanyl, Fentanyl, Set of 3 FIB compounds, Heroin **MS:** Cocaine
Runtime (min)	342.1	76	
Chemist 4 – Batch 3			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
22	B	C	Fentanyl, XLR11
24	B	C	JWH-018
26	B	C	Eutylone
28	B	C	4-Methylethcathinone
30	B	C	Fentanyl, JWH-250 *Insufficient: Dibutylone^{RT}*
Cumulative Standards / + Controls	20	1	**FID:** XLR11, Fentanyl, JWH-018, Set of 9 Eutylone/Dibutylone compounds, Set of 6 4-Methylethcathinone compounds, JWH-250, JWH-302 **MS:** Cocaine
Runtime (min)	316.8	76	
Chemist 4 – Batch 4			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
31	B	C	Tramadol
33	B	C	Fentanyl, Heroin *Insufficient: (Acetyl Fentanyl | FIBF‡)*
35	B	C	Fentanyl *Insufficient: (Tramadol | Mannitol)*
37	B	C	Heroin
39	B	C	*Insufficient: Fentanyl‡*
Cumulative Standards / + Controls	7	1	**FID:** Tramadol, Acetyl Fentanyl, Fentanyl, Set of 3 FIBF compounds, Heroin **MS:** Cocaine
Runtime (min)	152	76	
Chemist 4 – Batch 5			
Sample #	**GC-FID Method**	**GC-MS Method**	**Controlled Substances Identified**
42	B	C	Fentanyl *Insufficient: (Acetyl Fentanyl | FIBF)*
44	B	C	N-Methyl Cyclopropyl Norfentanyl
46	B	C	4-Methylethcathinone
48	B	C	N-Ethylpentylone
50	B	C	*Insufficient: α-PVP^{RT}*
Cumulative Standards / + Controls	22	1	**FID:** Acetyl Fentanyl, Fentanyl Set of 3 FIBF compounds, Heroin, N-Methyl Cyclopropyl norfentanyl, Set of 6 4-Methylethcathinone compounds, Set of 7 N-Ethylpentylone compounds, α-PVP, α-PIPBP **MS:** Cocaine
Runtime (min)	342.1	76	

Supplemental Table 7. Batch results for the targeted GC-MS confirmation analyses. An "X" indicates that the sample was run on the targeted method listed ("Cath." indicates the synthetic cathinone method and "Cann." indicates the synthetic cannabinoid method). The total instrument time and number of runs for each batch are also provided along with the compounds that were confirmed in each sample. Compounds that were detected but could not be confirmed are listed as insufficient, and the reason for the insufficient designation is provided. A double dagger (‡) indicates that the compound was not at a high enough abundance in the GC-MS chromatogram for confirmation and compounds in parentheses indicate instances where co-elution precluded confirmation.

Chemist 1 – Batch 1					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
6		X		MDMA	
7				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
8	X			Heroin	
9	X			N-Methyl Norfentanyl	
10		X		4-Ethylmethcathinone	
+ Control	X	X		**Runtime:** 146.7 min	**# Runs:** 6
Chemist 1 – Batch 2					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
11		X		Dibutylone	
12	X	X		4-Ethylmethcathinone, Fentanyl, 4-Me-α-ethylaminopentiophenone	
13			X	FUB-AMB	
14	X			Cyclopropyl Fentanyl, Heroin, Phenyl Fentanyl	
15			X	AB-FUBINACA 2-fluorobenzyl isomer	
+ Control	X	X	X	**Runtime:** 163.5 min	**# Runs:** 9
Chemist 1 – Batch 3					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
16				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
17		X		Dibutylone	
18	X			Acetyl Fentanyl, Fentanyl	
19	X			Acetyl Fentanyl, Fentanyl, FIBF, Heroin	
20				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
+ Control	X	X		**Runtime:** 120 min	**# Runs:** 5
Chemist 1 – Batch 4					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
31	X			Tramadol	
32			X	JWH-250	
33	X			Acetyl Fentanyl, Fentanyl, FIBF, Heroin	
34		X		Eutylone	
35	X			Fentanyl *Insufficient: (Tramadol | Mannitol)*	
+ Control	X	X	**X**	**Runtime:** 214 min	**# Runs:** 9
Chemist 1 – Batch 5					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
46		X		4-Methylethcathinone	
47		X	X	JWH-018, 3,4-MDPV	
48		X		N-Ethylpentylone	
49			X	FUB-AMB	
50		X		α-PVP	
+ Control		X	X	**Runtime:** 73.5 min	**# Runs:** 8

Chemist 2 – Batch 1					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
1				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
2				Not Analyzed – No Targeted Method for Methamphetamine	
3	X	X		Heroin, MDMA	
4	X	X		Fentanyl *Insufficient: (Tramadol | Mannitol)*	
5				MPHP	
+ Control	X	X		**Runtime:** 127.5 min	**# Runs:** 6
Chemist 2 – Batch 2					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
21				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
22	X		X	Fentanyl, XLR11	
23			X	JWH-250	
24			X	JWH-018	
25		X		α-PVP	
+ Control	X	X	X	**Runtime:** 133 min	**# Runs:** 8
Chemist 2 – Batch 3					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
26		X		Eutylone	
27				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
28		X		4-Methylethcathinone	
29		X	X	5-F-AKB48, α-PBP	
30	X	X	X	Dibutylone, Fentanyl, JWH-250	
+ Control	X	X	X	**Runtime:** 143.5 min	**# Runs:** 10
Chemist 2 – Batch 4					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
36	X			Methyl-AP-237	
37	X			Heroin	
38	X		X	JWH-250, α-Methyl Fentanyl	
39	X			Fentanyl	
40	X	X		4-Chloroethcathinone, Cyclopropyl Fentanyl	
+ Control	X	X	X	**Runtime:** 249 min	**# Runs:** 10
Chemist 2 – Batch 5					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
41				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
42	X			Acetyl Fentanyl, Fentanyl, FIBF, Heroin *Insufficient: Cocaine (Not in Targeted Methods), Noscapine‡*	
43		X		Methylone	
44	X			N-Methyl Cyclopropyl Norfentanyl	
45				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
+ Control	X	X		**Runtime:** 120 min	**# Runs:** 5
Chemist 3 – Batch 1					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
2				Not Analyzed – No Targeted Method for Methamphetamine	
4	X			Fentanyl *Insufficient: (Tramadol | Mannitol)*	
6		X		MDMA	
8	X			Heroin	
10		X		4-Ethylmethcathinone	
+ Control	X	X		**Runtime:** 127.5 min	**# Runs:** 6
Chemist 3 – Batch 2					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
11		X		Dibutylone	
13			X	FUB-AMB	
15			X	AB-FUBINACA 2-fluorobenzyl isomer	
17		X		Dibutylone	
19	X			Acetyl Fentanyl, Fentanyl, FIBF, Heroin	
+ Control	X	X	X	**Runtime:** 128.5 min	**# Runs:** 8

Chemist 3 – Batch 3					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
22	X		X	Fentanyl, XLR11	
24			X	JWH-018	
26		X		Eutylone	
28		X		4-Methylethcathinone	
30	X	X	X	Fentanyl, JWH-250	
+ Control	X	X	X	**Runtime:** 183 min	**# Runs:** 11
Chemist 3 – Batch 4					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
31	X			Tramadol	
33	X			Acetyl Fentanyl, Fentanyl, FIBF, Heroin	
35	X			Fentanyl *Insufficient: (Tramadol | Mannitol)*	
37	X			Heroin	
39	X			Fentanyl	
+ Control	X			**Runtime:** 210 min	**# Runs:** 6
Chemist 3 – Batch 5					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
42	X			Acetyl Fentanyl, Fentanyl, FIBF, Heroin *Insufficient: Cocaine (Not in Targeted Methods), Noscapine‡*	
44	X			N-Methyl Cyclopropyl Norfentanyl	
46		X		4-Methylethcathinone	
48		X		N-Ethylpentylone	
50		X		α-PVP	
+ Control	X	X		**Runtime:** 135 min	**# Runs:** 7
Chemist 4 – Batch 1					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
1				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
3	X	X		Heroin, MDMA	
5		X		MPHP	
7				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
9	X			N-Methyl Norfentanyl	
+ Control	X	X		**Runtime:** 127.5 min	**# Runs:** 6
Chemist 4 – Batch 2					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
12	X	X		4-Ethylmethcathinone, Fentanyl, 4-Me-α-Ethylaminopentiophenone	
14	X			Cyclopropyl Fentanyl, Heroin, Phenyl Fentanyl	
16				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
18	X			Acetyl Fentanyl, Fentanyl	
20				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
+ Control	X	X		**Runtime:** 155 min	**# Runs:** 6
Chemist 4 – Batch 3					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
21				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
23			X	JWH-250	
25		X		α-PVP	
27				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
29		X	X	5-F-AKB48, α-PBP	
+ Control		X	X	**Runtime:** 58.5 min	**# Runs:** 6
Chemist 4 – Batch 4					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
32			X	JWH-250	
34		X		Eutylone	
36	X			Methyl-AP-237	
38	X		X	α-Methyl Fentanyl	
40	X	X		4-Chloroethcathinone, Cyclopropyl Fentanyl	
+ Control	X	X	X	**Runtime:** 198.5 min	**# Runs:** 10

Chemist 4 – Batch 5					
Sample #	**Opioid**	**Cath.**	**Cann.**	**Controlled Substances Identified**	
41				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
43		X		Methylone	
45				Not Analyzed – No Controlled Substances ID'ed in DART-MS	
47		X	X	JWH-018, 3,4-MDPV	
49			X	FUB-AMB	
+ Control		X	X	**Runtime:** 58.5 min	**# Runs:** 6