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Development of an Open-Source Direct Analysis in Real Time Mass Spectrometry (DART-MS) Search Software and Library Building Tool for the Analysis of Complex Drug Mixtures

Award DJO-NIJ-20-RO-0012

Final Research Report

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Summary of Project

Statement of the Problem & Project Overview

Seized drug analysis is the most frequently requested forensic examination in the USA, accounting for approximately 33% (or over one million) submissions per year. Given the large number of submissions, laboratories are often challenged with trying to reduce turnaround times and backlogs. According to the 2019 National Forensic Laboratory Information System (NFLIS)-Drug Survey of Crime Laboratory Drug Chemistry Sections, nearly 60% of laboratories noted an increase in caseload over the last year and over 40% reported an increase in turnaround time. The average turnaround time for laboratories in the survey ranged from 49 days to 151 days.

One of the major drivers for increased case submissions and turnaround times is the emergence of new psychoactive substances (NPSs) and synthetic opioids. These compounds, especially synthetic cathinones and cannabinoids, present analytical challenges due to the continued creation of analogs and other structurally similar chemicals which can make identification and confirmation difficult. Also, many of the traditional presumptive tests (e.g., color tests and microcrystalline tests) are not well suited for these compounds. For synthetic cathinones, gas chromatography mass spectrometry (GC-MS) fragmentation patterns can often appear indistinguishable and the presence of a molecular ion may not occur. Other compounds, like synthetic opioids, are often present at low concentrations creating detection challenges not only in presumptive analysis but also in confirmatory analysis by GC-MS – where detection limits, co-eluting peaks, or tailing from large amounts of cutting agent may hinder accurate identification. The multi-component nature of many of these samples can present additional analytical difficulties if a complete chemical profile is desired. Due to these challenges over half of laboratories reported emerging drugs as a major contributor to their backlogs and over 80% reported limited analytical tools as a driver for emerging drug issues.

Direct analysis in real time mass spectrometry (DART-MS) is one of the analytical tools forensic chemists are utilizing to help tackle some of the issues posed by NPSs, synthetic opioids, and other emerging drugs. DART-MS is an appealing tool for drug screening applications because it can provide a sensitive, near-complete mass spectral profile of a sample within a matter of seconds. The application of DART-MS for drug analysis has been rigorously demonstrated over the last decade and has been shown to excel with analysis of traditional drugs, emerging drugs, synthetic opioids, pharmaceuticals, plant materials, and other compounds of interest. While DART-MS is traditionally used to obtain a simple presumptive mass spectrum, novel applications of the technique are beginning to emerge. These applications include using solid phase microextraction for sample clean-up, probing drug residues for investigative purposes, and...
psychoactive plant species identification\textsuperscript{15}. Advances in areas like thermal desorption (TD)-DART-MS have unlocked the ability to use nitrogen as a source gas with little to no impact on detection capabilities\textsuperscript{16,17}, relieving potential concerns about high helium consumption.

One of the main drawbacks to DART-MS for forensic laboratories is the difficulty of data processing and interpretation, especially for complex mixtures. Without chromatographic separation, spectra of mixtures can often be convoluted. Currently, data processing typically involves extraction of a mass spectrum from a case sample and its presumed molecular ion peak is compared to a look-up table of molecular ions for known drugs or compounds of interest. While useful, this approach does not provide the chemist with any additional information or confidence level that the presumed molecular ion peak from the mass spectrum is produced by the suspected drug. This limitation can be addressed by leveraging the fact that most mass spectrometer systems can produce a series of fragmentation spectra by utilizing in-source collision induced dissociation (is-CID), as shown in Figure 1. Combining the intact molecular ion from the low fragmentation spectra (the data that is commonly used) with data from one of more higher fragmentation spectra can begin to provide a probabilistic value to a drug's presence or absence in a sample. Development of a search algorithm that leverages the additional information provided by the fragmentation spectra would offer chemists increased information and confidence to make their determinations.

![Figure 1](image.png)

**Figure 1.** (A.) A graphical representation of how in-source collisionally induced dissociation (is-CID) occurs. Also shown is a series of increasing is-CID fragmentation spectra (B. – E.) for fentanyl. Note as the voltage of the orifice is increased the level of fragmentation also increases.

Currently, one commercial product is available that provides automated mixture analysis for DART-MS data. PIMISA (Primary Ion Middle Ion Spectral Analysis), created and marketed by IonSense (Saugus, MA), is a proprietary software that utilizes multiple is-CID fragmentation spectra to provide the user with potential identities and probabilities of drugs present in a sample. The software is designed as a “red-light / green-light” program that shows users if a compound of interest is detected. PIMISA is only available for the single quadrupole DART-QDa system which is designed for field-based applications and is not
commonly used in forensic laboratories. Forensic laboratories typically employ a time-of-flight (TOF) or quadrupole time-of-flight (Q-TOF) mass spectrometer for analysis.

The aim of this project was to develop and release a DART-MS Data Interpretation Tool (DIT), a free, flexible, vendor agnostic, and open-source DART-MS mixture interpretation software platform. The software provides laboratories with a means to interpret mixture spectra, utilize new NIST DART-MS spectral databases, and easily generate reports. While the initial development and application of this software was focused on seized drug analysis, the platform could be leveraged for any application where a spectral database exists. Additionally, laboratories that have adopted an ambient ionization mass spectrometry technique (AI-MS) other than DART-MS (such as Direct Sample Analysis or Atmospheric Sample Analysis Probe) can also use this software, provided they create an appropriate spectral database. Underpinning the software is the inverted library search algorithm (ILSA) which has been developed specifically for DART-MS data. Additionally, new databases have been designed to allow for seamless implementation into the software. The fourth update of the database was released in October of 2021, with other databases to follow. All databases will be continually updated at regular intervals.

Development of the DIT with community input allowed for the ILSA to be elevated from a research tool to a practical application. This work occurred concurrently with internally funded work to optimize the ILSA and continue to expand the database. Creation of the software and training materials occurred through collaboration with five practicing forensic laboratories that spanned local, state, and federal levels as well as spanned the range of instrumentation (single quad, TOF, and Q-TOF). These laboratories were given pre-release versions of the software to evaluate and provide feedback on to allow for continuous improvements throughout the duration of the project. The initial development of the DIT is the cornerstone to providing the community with a suite of tools to rapidly and reliably analyze complex DART-MS, or other AI-MS, spectra, easily create or amend spectral databases, identify unknowns, and generate reports.

**Major Goals and Objectives**

**Objective #1: Development of User Interface and Training Material**

Successful adoption of any software tool requires it be functional and usable. A functional program is able to robustly perform its specified tasks and reliably produce its specified outputs. A usable program is one with a clear interface (graphical or command line) and/or documentation that allows a user to operate the program to its fullest capabilities. The functionality of the DIT was developed through parallel research
optimizing underlying algorithms as noted previously. Developing the software with an emphasis on usability was the first objective of this proposal.

Objective #2: Software Evaluation by Forensic Laboratories

To ensure the developed software and documentation was in fact usable by the community for which it was intended, the second objective of this proposal was to design a formal software development and community testing strategy that allowed us to collect frequent and useful feedback throughout the development process.

Objective #3: Deployment of Software to Community

The final objective of the project was to ensure the open-source software was made widely available through a formal public release followed by a series of presentations and workshops.

Research Questions

The overarching objective of this research project was to transition a functional research algorithm into a usable software tool. To meet this objective, two specific questions were asked and answered through an iterative software development and testing procedure:

1) What features are necessary for laboratories to consider a new software tool in their workflow? For example, are there specific tasks the software must perform? Are there specific system requirements that must be met?

2) What documentation and training tools are necessary to ensure adoption of the software tool?

Research Design, Methods, Analytical and Data Analysis Techniques

To address the aforementioned research questions, we needed a preliminary software tool as a starting point of discussion, and a strategy for collecting feedback from a community of users. The DIT was implemented as an R Shiny application, and a 2-month cycle of software development and community testing (feedback solicitation) was employed. Written feedback was solicited in the form of surveys, including a combination of rating scale and open-ended questions. Additionally, each testing cycle included 30 min to 60 min in-person or virtual follow up meetings with each of the collaborating labs to address concerns that were not easily shared via the survey. In total, two development/testing cycles were completed in the year, followed by a public software release.
**Expected Applicability of the Research**

The initial application of the DIT was focused on the analysis of seized drugs for presumptive screening, due to the increased use of DART-MS, and other AI-MS techniques, for this purpose. Use of the DIT in the space will allow drug chemists to more easily, accurately, and confidently, interpret complex mass spectra from chromatography-free mass spectrometry techniques. Use of the DIT also simplifies report generation and provides a freely available platform for data analysis. While the initial application of the DIT is for seized drug analysis, its use can be easily expanded to other relevant areas, only requiring the generation of a relevant spectral library. Investigations in the application of the DIT for trace evidence, ignitable liquids, and toxicology are currently underway.

Additionally, as the DIT is an open-source tool, interested software developers will be able to expand or modify all underlying functionality to suit particular applications, or even incorporate ideas from the DIT into their existing software systems.

**Participants & Other Collaborating Organizations**

Creation of the software tool was completed at NIST and involved Edward Sisco, Arun Moorthy, Ruthmara Corzo, and Stephen Tennyson (a student from University of Maryland hired on to assist in software design and coding). The five collaborating labs that provided feedback throughout the development process were Virginia Department of Forensic Science (POCs: Juli Cruciotti and Ryan Labor), Maryland State Police Forensic Sciences Division (POCs: Amber Burns and Elizabeth Schneider), Harris County Institute of Forensic Science (POCs: Juila Daszkiewicz and Jesse Zavala), US Food and Drug Administration Forensic Chemistry Center (POC: Sara Kern), and US Custom and Border Protection INTERDICT Laboratory (POCs: Natalie Borga, Chantelle Beachum, and Dennise Montero).

**Changes in Approach**

There were no changes in approach to note.
Outcomes

Activities & Accomplishments

The following are a list of the activities and accomplishments that occurred for each of the three goals of the project.

Objective #1: Development of User Interface and Training Material

- Developed DIT—a user-friendly interface to simplify usability of the ILSA and DART-MS library
- Developed additional functionality for the software tool, including report generation, ability to search less than three is-CID spectra, ability to search unit mass resolution spectra, and ability to save settings
- Created “How-to” guide to assist chemists in using the software tool

Objective #2: Software Evaluation by Forensic Laboratories

- Completed two iterations of software evaluation with collaborating forensic laboratories
- Incorporated many of the identified modifications or critical features into the publicly released software tool
- Identified additional “nice-to-have” features for future updates to the software

Objective #3: Deployment of Software to Community

- Made the software tool publicly, and freely, available
- Presented an FTCOE webinar to publicly release the software tool
- Presented at additional regional forensic conferences to disseminate the availability of the tool

Results and Findings

Objective #1: Development of User Interface and Training Material

The DART-MS Data Interpretation Tool (DIT) was developed as an R Shiny Application for several reasons. (1) The underlying library search algorithm, the ILSA, is a multi-step method where no single step requires the computational power afforded by compiled languages. (2) The DART-MS library is relatively small and thus does not require the computational power afforded by compiled languages. And most importantly, (3) the R programming language is well-known for its human-interpretability and thus interested users will be able to expand on the tool with a lower barrier to entry than a compiled programming language.
Significant efforts were placed in ensuring the DIT interface was user-friendly and intuitive, thus a simple user-manual was deemed sufficient documentation (through survey and conversation). The final user-manual was 17 pages and is primarily screen captures of utilities of interest to most forensic chemists.

In addition to the user-manual, a 12-page “software details” document was created to support users interested in exploring the source code that underlies the application.

The software and all documentation are available at https://data.nist.gov/od/id/mds2-2448.

**Objective #2: Software Evaluation by Forensic Laboratories**

After providing collaborating laboratories with each iteration of the DIT, a survey was sent asking the users to provide input on current functionality, desired future functionality, and identify the presence of any bugs within the program. Surveys consisted of a combination of multiple choice, ranking, and open-ended questions. The results of the surveys were used to drive and prioritize the next version of the software.

*Software & Survey Iteration #1*

The first version (v1) of the DIT provided to labs contained basic search functionality and basic database viewing capabilities. The survey provided to the laboratories focused on identifying critical needs and additions to the v1 DIT to improve usability for casework. A detailed description of the survey results is provided below.

The first question asked users to rate how impactful features that could be incorporated into future versions of the software would be. Options were “No Opinion”, “Not Impactful”, “Somewhat Impactful”, and “Highly Impactful / Needed Feature”. The results from this question are shown in Table 1.

**Table 1.** Feedback on the criticality of potential future features for the DIT after the first software iteration was provided to laboratories.

<table>
<thead>
<tr>
<th>Question</th>
<th>No Opinion</th>
<th>Not Impactful</th>
<th>Somewhat Impactful</th>
<th>Highly Impactful</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ability to search synonyms in the database viewer</td>
<td>--</td>
<td>--</td>
<td>57 %</td>
<td>43 %</td>
</tr>
<tr>
<td>Ability to save search settings</td>
<td>--</td>
<td>--</td>
<td>43 %</td>
<td>57 %</td>
</tr>
<tr>
<td>Ranking of search results by score (instead of alphabetically)</td>
<td>--</td>
<td>--</td>
<td>14 %</td>
<td>86 %</td>
</tr>
<tr>
<td>Ability to print spectra from the database viewer</td>
<td>14 %</td>
<td>--</td>
<td>14 %</td>
<td>71 %</td>
</tr>
<tr>
<td>Ability to batch samples</td>
<td>--</td>
<td>14 %</td>
<td>29 %</td>
<td>57 %</td>
</tr>
<tr>
<td>Ability to overlay / butterfly query spectra and database spectra</td>
<td>--</td>
<td>29 %</td>
<td>57 %</td>
<td>14 %</td>
</tr>
<tr>
<td>Ability for the search tool to not identify isotopic peaks as targets</td>
<td>14 %</td>
<td>14 %</td>
<td>43 %</td>
<td>29 %</td>
</tr>
<tr>
<td>Ability to upload less than 3 spectra</td>
<td>29 %</td>
<td>29 %</td>
<td>29 %</td>
<td>14 %</td>
</tr>
</tbody>
</table>
Ability to display target identities on the query spectra | 14 % | 14 % | 14 % | 57 %
Ability to display fragment ion structure in the database viewer when hovering over peaks | -- | 14 % | 57 % | 29 %
Ability to search only certain classes of compounds | -- | -- | 86 % | 14 %
Ability to edit database entries | -- | -- | 43 % | 57 %
A “Clear Search” button | 14 % | -- | 57 % | 29 %
Ability to display results as a peak table | 14 % | -- | 29 % | 57 %
Ability to simultaneously upload all 3 spectra | 14 % | -- | 14 % | 71 %

The second question asked what other features we should consider adding to the search portion of the software. Responses returned included:

- **Being able to batch and upload an entire set of case data into the program would be helpful** (ex. we will likely be analyzing cases with 29 tablets, so being able to upload the 30, 60, and 90V spectra centroid data for the runs in the sequence would help save time by tabbing through the data in the software rather than having to upload all three spectra individually for each item); Also being able to select and search multiple libraries at once may be helpful as well as possibly being able to select or de-select the libraries right on the search tab

- **Ability to select the name of the identified peak from the search matches in the table instead of the software defaulting it to what I think is the first one listed in the results table**

- **Would be nice if it was easy to upload the data into LIMS. Customizing the report would be nice too**

The third question asked what other features we should consider adding to the database viewer portion of the software. One response was returned.

- **CAS# could also be a helpful search feature (especially if multiple synonyms)**

The fourth question asked what data fields would be desired on the report that will be generated. The results of this question found in Table 2.

**Table 2.** Feedback on the necessary fields required for report generation.

<table>
<thead>
<tr>
<th>Report Feature</th>
<th>% Yes</th>
<th>Report Feature</th>
<th>% Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filename</td>
<td>100 %</td>
<td>Time of Analysis</td>
<td>71 %</td>
</tr>
<tr>
<td>Date of Analysis</td>
<td>100 %</td>
<td>Peak Table</td>
<td>71 %</td>
</tr>
<tr>
<td>Case Number</td>
<td>100 %</td>
<td>Print all three spectra</td>
<td>57 %</td>
</tr>
<tr>
<td>Item Number</td>
<td>100 %</td>
<td>Annotated spectrum (peaks labelled with Target #)</td>
<td>57 %</td>
</tr>
<tr>
<td>Instrument Name / Serial #</td>
<td>100 %</td>
<td>Print only the 30 V spectrum</td>
<td>43 %</td>
</tr>
<tr>
<td>Search Parameters</td>
<td>86 %</td>
<td>Option to add laboratory name / logo</td>
<td>43 %</td>
</tr>
</tbody>
</table>
The fifth question asked if there were any additional things that users would like to see in a report. One response was returned:

- Ability to customize the report template; DART method name

The sixth question focused on future utility of the software and asked users which, if any, of eight proposed future features would be beneficial to have in later versions of the software. The results of the question are shown in Table 3.

Table 3. Feedback on desired future functionality of the DIT.

<table>
<thead>
<tr>
<th>Future Feature</th>
<th>% Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ability to create your own database</td>
<td>71 %</td>
</tr>
<tr>
<td>Tools to assist in the identification of unknown samples</td>
<td>71 %</td>
</tr>
<tr>
<td>Ability to save searches</td>
<td>57 %</td>
</tr>
<tr>
<td>Tools to cluster samples to assist in making associations for forensic intelligence purposes</td>
<td>57 %</td>
</tr>
<tr>
<td>A combined DART-MS &amp; GC-MS search algorithm</td>
<td>57 %</td>
</tr>
<tr>
<td>Ability to upload and track verification samples for QA/QC purposes</td>
<td>43 %</td>
</tr>
<tr>
<td>Ability to query previous searches</td>
<td>29 %</td>
</tr>
<tr>
<td>Ability to datamine previous searches when new compounds are added to the database</td>
<td>29 %</td>
</tr>
</tbody>
</table>

The seventh question asked if the user has encountered any bugs. One user reported experiencing bugs on occasion when using the DIT. The final question asked for the users overall rating of the current version of the software, on a scale of 1 to 5, with 5 being the highest ranking. A total of 14 % of users ranked it a 3, 43 % ranked it a 4, and 43 % ranked it a 5.

Updates to Software for Iteration #2

Based on the feedback provided in the survey, and conversations with the collaborating laboratories, a number of updates and additions were made to the second iteration of the software. These updates included:

Overall:
Database was updated to contain 72 new compounds
New ‘About’ tab was added to contain disclaimers and contact information

Libraries Tab:
New “Views” functionality added to allow users to either interact with or print library spectra
Compound name synonyms were added to assist in naming differences
Search the database, allowed for the searching of synonyms as well as just the compound name

Search Tool Tab:
Ability to search any combination of is-CID mass spectra (instead of having to search all three)
Potential matches were changed from alphabetical listing to listing by score
New “Views” functionality added to allow users to either interact with data or print reports
Changed default search mode set to ‘Mixture analysis’, from ‘Pure Compound analysis’
Incorporation of text boxes to input Date, Instrument Name, Case Number, Item Number, and General Comments for report generation
Ability for users to save and load presets
Inclusion of a “Clear Search” button
Inclusion of a “Restore Defaults” button
Inclusion of an ‘Update Search’
Inclusion of target peak annotations in the 30 V spectrum of the report
Ability to show all search parameters when printing a report

The second version (v2) of the DIT was then provided back to laboratories for additional testing and feedback. During this time, a user manual was also created and provided to laboratories for feedback. The user manual walked though all functionalities of the DIT, how to install, open, and interact with the DIT, and a brief overview of how the ILSA works.

Software & Survey Iteration #2

The second survey consisted of predominantly open-ended questions and was designed to capture any outstanding issues, missing features, or bugs that needed to be addressed prior to public release while also asking for feedback on the updates that were made. Questions that were asked included:

Q1: Please provide any feedback on the new version of the software you have.
Q2: Are there other features that you would like to see added to the DART Search Tool prior to public release in October?
• Q3: Is there anything missing in the report (Expanded View) that you would like to see added?
• Q4: Is there anything specific you want to see, or think should be included, in a User Manual?
• Q5: Have you come across cases where you had incorrect identifications from the search tool?
• Q6: Have you come across any bugs in the software?
• Q7: How would you rate the overall usability of the DART Search Tool to date?

The results of this survey were extremely positive as users reported no bugs (Q6) and no incorrect identifications (Q5). Users reported there was no additional functionality required for an initial public release (Q2) and that the user manual contained all of the necessary information (Q4). One user requested the inclusion of a User ID section into the report functionality (Q3).

When asked for feedback on the second iteration of the software, feedback was highly positive and included:

• Being able to open just a 60V or 90V file vs opening all three 30V, 60V, and 90V in the search tool and the addition of synonyms are good.
• I love it!!!! There is so much information for each entry - having the structure, chemical formula, exact mass, and synonyms right there is very helpful. The expanded view and report printing capabilities are wonderful. This is ready for prime time!!! I would like to give it an 11/10.

When asked to rate the second iteration on the same scale of 1 to 5, all respondents rated the DIT either a 4 or 5.

Updates to Software Prior to Public Release

No significant changes were made to the software prior to public release. Minor changes included confirming all headings and displayed text were appropriately formatted, and additional commenting was added to source-code in preparation for public release.

Objective #3: Deployment of Software to Community

The software was made freely available for download on October 1, 2021. An FTCOE webinar that described the process of developing the DIT and demonstrated some of the important functionality was presented on October 21, 2021 (link to webinar: https://forensiccoe.org/dart-ms-seized-drug-analysis/). The DIT was added to the NIST program projects for methods, software tools and resources for forensics laboratories employing DART-MS or other AI-MS (link to program page: https://www.nist.gov/programs-projects/methods-software-tools-and-resources-forensics-laboratories-dart-ms-or-other-ai-ms).
Limitations

While the development cycle of the DART-MS Data Interpretation Tool has allowed us to address our underlying research questions and better comprehend the steps necessary to transition a functional research algorithm into a useable software tool, the tool itself has a few known limitations. Because the DIT is implemented as an R Shiny Application, it must be packaged with R-Portable for use on computers that do not already have an updated version of the R programming language installed on their device. Accordingly, the final program size is quite large (~300mb). Additionally, scale up studies have not been conducted and so it is unclear how the current implementation of the DIT will perform when using significantly larger DART-MS libraries or a significantly altered search algorithm.

Artifacts

The following section provides a list of artifacts that were produced and/or disseminated as a result of this award, or related parallel work.

List of Products

- The NIST/NIJ DART-MS Data Interpretation Tool:
- Manuscripts published as a result of parallel work:
- Manuscripts in preparation:
  - Evaluation of the Inverted Library Search Algorithm for Screening Seized Drug Evidence with DART-MS
  - The DART-MS Data Interpretation Tool
Datasets Generated

The following datasets were generated as a result of this award, or related parallel work.

- DART-MS Spectral Database (Parallel Work):
  - Sisco, E.; Moorthy, A.S.; NIST DART-MS Forensics Database (is-CID), 2020, National Institute of Standards and Technology, https://doi.org/10.18434/mds2-2313

Dissemination Activities


Disclaimer

Certain commercial products are identified in order to adequately specify the procedure; this does not imply endorsement or recommendation by NIST, nor does it imply that such products are necessarily the best available for the purpose.
References


