



Published December  
2023

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The [Forensic Laboratory Needs Technology Working Group \(FLN-TWG\)](#), formed by the National Institute of Justice (NIJ) in partnership with the Forensic Technology Center of Excellence (FTCOE) at RTI International, created this document in support of NIJ's mission to improve knowledge and understanding of federal, state, local, and tribal forensic science service providers' (FSSPs') technology needs.



## TECHNICAL NOTE

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

## Introduction

Direct analysis in real time (DART) is a rapid noncontact ambient ionization source that was first described in 2005<sup>1</sup> and allows for the direct analysis of solid, liquid, or gas samples without extensive sample preparation.<sup>1, 2</sup> DART itself is only an ionization source and must be coupled with a mass spectrometer to detect the ionized species generated in the open-air gap between the DART source and the mass spectrometer inlet. DART ionization occurs based on gas phase reactions of electronic or vibronic excited-state species, typically helium or nitrogen, with atmospheric reagent molecules (such as water or solvent, and target analytes) through a process that is closely related to atmospheric pressure chemical

ionization.<sup>1,3</sup> DART is a soft ionization technique that provides easily interpreted mass spectra that are dominated by the formation of molecular ions or protonated/deprotonated molecules depending on the nature of the source gas, analyte, concentration, and ion source polarity.<sup>3,4</sup> The ability to gather real-time results with little to no sample preparation makes ambient ionization mass spectrometry (MS) techniques such as DART-MS an attractive option for rapid screening of seized drug evidence.<sup>5</sup>

The DART source consists of a discharge chamber where the metastable species are generated through the formation of a plasma.<sup>3</sup> A series of perforated electrodes are used to remove charged species from the gas stream before a heater. The heater



increases the temperature of the metastable gas stream to assist with thermal desorption of the target analytes. The last component of the DART source is a grid electrode that removes ions of the opposite polarity to prevent signal loss caused by ion-ion recombination and to help reduce the noise in the background spectrum.<sup>1, 6</sup> The polarity of the DART source is controlled based on the voltages applied to the electrodes, which allows for rapid switching between positive and negative ion mode. Positive ion mode works best for basic molecules that easily protonate, whereas negative ion mode works best for acidic molecules that readily deprotonate. The sample is introduced between the exit of the DART source and the entrance of the mass spectrometer inlet with sample introduction techniques ranging from direct analysis of the material itself to deposition onto sample introduction matrices such as glass capillaries or wire mesh.

According to the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) recommendations<sup>7</sup> and ASTM E2329-17 Standard Practice for Identification of Seized Drugs,<sup>8</sup> MS is a Category A technique because of the ability to provide the highest level of selectivity or discriminating power through structural information. However, because DART is a soft ionization technique, an additional collisional activation technique is required to fragment the ionized molecules and generate structural information. Ambient ionization sources such as DART can be coupled with different mass analyzers, such as single quadrupoles, ion traps, triple quadrupoles, and time-of-flight mass analyzers. When only a single-stage mass spectrometer is available, in-source collision-induced dissociation (IS-CID) is used to generate fragment ions through collisions with atmospheric background gases as the precursor ions transition from the atmospheric pressure source region to the high vacuum of the mass analyzer.<sup>9</sup> This approach was used by the National Institute of Standards and Technology (NIST) to develop the DART-MS Forensic Database for which low, medium, and high IS-CID activation conditions were used to gather both

molecular weight and structural information to assist with the identification of seized drugs.<sup>10</sup> The database can be leveraged using a new library search algorithm for mixture analysis using DART-MS and IS-CID mass spectra.<sup>11</sup>

## Capabilities

Ambient ionization techniques such as DART-MS provide a potential solution to ongoing challenges within the seized drug analysis discipline, including growing backlogs, difficult-to-analyze samples, and the identification of novel psychoactive substances (NPS) that continue to appear in seized drug casework.<sup>5</sup> The ability to analyze seized drug samples rapidly with little to no sample preparation provides laboratories with an additional resource to address these challenges. This suite of techniques is capable of directly analyzing powders,<sup>12</sup> pills,<sup>1</sup> plant material,<sup>13</sup> blotter paper,<sup>14</sup> solutions,<sup>15</sup> and even food matrices, such as edibles.<sup>16</sup> Currently, ambient ionization MS is viewed as a high-level screening technique because it incorporates MS detection and the ability to gather both molecular weight and structural information using IS-CID on single-stage mass spectrometers or CID on tandem mass spectrometers. The ability to gather information about a seized drug sample rapidly with only minimal analyst intervention provides increased analyst safety<sup>17</sup> and improved laboratory workflows because of greater confidence in the preliminary screening analysis.<sup>18</sup> This approach is a relatively robust emerging technology for seized drug analysis, with demonstrated success for the analysis of opiates,<sup>19</sup> opioids,<sup>20</sup> synthetic cathinones,<sup>18, 21-23</sup> synthetic cannabinoids,<sup>13, 24-26</sup> benzodiazepines,<sup>27</sup> and stimulants.<sup>28</sup> In addition to qualitative seized drug screening, quantitative capabilities, such as quantifying the amount of psychoactive compounds directly from *Mitragyna speciosa* (Kratom)<sup>29</sup> and peyote<sup>30</sup> have been demonstrated. There are also modified versions of DART-MS, such as thermal desorption DART-MS<sup>20, 31, 32</sup> and the use of nitrogen<sup>33-35</sup> rather than helium, that address specific limitations such as analyte volatility, reproducibility,

**Exhibit 1. Summary of DART-MS cost and analysis time considerations.**

Initial Cost	Cost/Analysis	Analysis Time	Cost-saving Considerations
(~\$30,000-\$50,000 for the DART source depending on the configuration) + mass spectrometer cost	Depends on sample introduction method	Approximately 30 s sample analysis and 1 min data analysis	Some vendors accept trade-ins




and sustainability. Finally, the additional resources required for the incorporation of ambient ionization MS technologies like DART-MS into forensic laboratories are publicly available through the [NIST DART-MS Forensics Database](#),<sup>10</sup> [data interpretation tool \(DIT\)](#),<sup>11</sup> and [implementation templates](#), including standard operating procedures for instrument operation, maintenance, and a validation plan.<sup>36</sup> **Exhibit 1** summarizes the approximate cost and analysis time of DART-MS for forensic laboratories that may be considering adopting this technology.

## Limitations

The main limitations of ambient ionization techniques like DART are caused by the lack of chromatographic separation before ionization. This can lead to difficulty in the analysis of complex samples because of issues with competitive ionization.<sup>27,37</sup> This is particularly true for minor components in mixtures that have lower proton affinities relative to the other components. Another issue caused by the lack of chromatographic separation is that the selectivity of the technique is shifted entirely to the mass spectrometer, which means that tandem mass spectrometry (MS/MS) and high-resolution mass spectrometry (HRMS) instrumentation are preferable. However, IS-CID with high-resolution single-stage mass spectrometers is the most often encountered form of DART-MS. The publicly available NIST DART-MS Forensics Database<sup>10</sup> and data interpretation tool (DIT)<sup>11</sup> provide resources to help analysts identify seized drugs based on the observed IS-CID fragment ion spectra. A final limitation associated with the lack of chromatographic separation is that most positional isomers cannot be readily differentiated, even with the use of IS-CID.<sup>38-40</sup> In addition, helium consumption can be an issue with DART-MS analysis because of the decreasing availability and increasing cost of ultra-high-purity helium. However, it should be noted that the DART-JumpShot source uses pulsed gas rather than a continuous gas flow, which may reduce helium consumption by up to 95% relative to previous models.<sup>5</sup>

## Installation needs

The DART-SVP or DART-JumpShot ionization source can be mounted onto most mass spectrometers designed for liquid chromatography, but interested parties should review the [compatibility chart](#)  provided by IonSense, a subsidiary of Bruker. For non-JEOL AccuTOF mass spectrometers, a

Vapur<sup>®</sup> interface is required to alleviate the additional pumping load induced by the metastable gas stream emitted from the DART source. The DART controller module requires the input of ultra-high-purity helium and nitrogen gas with a 517 kPa to 551 kPa (75 psi to 80 psi) input pressure and output flow rates of 2 L/min to 4 L/min. The use of the DART-JumpShot can decrease the helium consumption relative to the SVP model. The DART source must be mounted on an existing mass spectrometer, which users should consider in terms of the required space, electrical, and gas needs. A fume extractor or snorkel, either fixed or portable, is highly recommended due to the desorption of potentially hazardous substances under ambient conditions.

## Vendor considerations

The only vendor for the DART source is IonSense (Saugus, MA, USA) who was recently acquired by Bruker Corporation (Billerica, MA, USA). However, the DART source can be combined with most mass spectrometer vendors. The DART-JumpShot is the currently available model, although the DART-SVP source is still heavily used in academic and forensic laboratories. The DART-SVP model provides users with standardized voltages and gas flows and automated sample introduction to simplify the analysis process. The DART-JumpShot provides pulsed gas capabilities to reduce background ion contribution and helium gas consumption.

## Space requirements

The DART controller module requires a physical footprint of approximately 15 cm × 30 cm × 30 cm (height × length × depth) and the DART source requires approximately 12 cm × 30 cm × 12 cm. The DART controller module also requires a single 100 VAC to 240 VAC, 50/60 Hz outlet, but a second outlet may be required if coupling the DART source to a non-JEOL AccuTOF mass spectrometer because of the addition of the Vapur<sup>®</sup> interface, which requires an auxiliary diaphragm pump. The DART controller module must be located within 75 cm of the DART source because of the length of the high-voltage cable. The auxiliary pump associated with the Vapur<sup>®</sup> interface should be placed on the floor by the instrument and must be within 2 m because of vacuum tubing restrictions. The last requirement is an ethernet connection to the computer operating the mass spectrometer and access to an internet browser to run the DART software. The computer does not need to be



connected to the internet, but the browser must be available to connect via an IP address.

### User training and skill level

The vendor offers training and installation. Installation typically only takes a few hours. Additional relevant training materials can be found through publicly available validated methods and standard operating procedures or additional training materials facilitated through NIST, such as the [DART-MS Implementation Webinar](#) presented in 2020.


### Implementation needs

Ambient ionization mass spectrometry technologies such as DART-MS have successfully been implemented in casework since 2008 by several forensic laboratories (see **Exhibit 2**). These laboratories have published validated methods and standard operating procedures that are available for other laboratories. Ongoing efforts are focused on the development of a [DART-MS Forensics Database](#) and [data interpretation software](#) to assist analysts with seized drug identifications.

### Validated methods

The seminal validation for forensic drug screening was published by Steiner and Larson at the Virginia Department of Forensic Science in 2009,<sup>19</sup> with a follow-up validation for the use of thin layer chromatography combined with DART-MS for pharmaceutical preparation identification in 2011.<sup>41</sup> The Virginia Department of Forensic Science has a publicly available validated [DART-MS method](#) and [MS acquisition standard operating procedure](#). Similarly, the [Harris County Institute of Forensic Sciences](#) also has a publicly available validated method for DART-MS. [NIST and the Maryland State Police Forensic Sciences Division](#) have developed a series of templates for the implementation of ambient ionization mass spectrometry technologies like DART-MS for seized drug analysis. They also recently published a method validation completed at the Maryland State Police Forensic Science Division Laboratory.<sup>36</sup> Finally, the U.S. Drug Enforcement Administration (DEA) has publicly available validated methods for DART coupled with tandem mass spectrometers (i.e., Q-TOF, QqQ, QIT, and Orbitrap) in the [Summary of Validated Qualitative Methods published](#)  through the DEA Office of Forensic Sciences.

### Searchable libraries

One of the major benefits of DART-MS for seized drug analysis is the presence of existing spectral libraries. The [NIST DART-MS Forensics Database](#) contains IS-CID mass spectra at multiple voltages for over 1,100 seized drugs or seized drug-related compounds collected on the JEOL AccuTOF mass spectrometer. Additional non-seized drug-related DART-MS libraries are available, such as the U.S. Fish and Wildlife Service Forest Database and the [Sexual Lubricant Database](#) .

### Data interpretation

NIST has developed a suite of data analysis and interpretation tools to facilitate the adoption of ambient ionization MS technologies into forensic laboratories. These resources include the [NIST DART-MS Forensics Database](#), [data interpretation software](#) to assist with mixture analysis, and [templates for the implementation of DART-MS](#) for seized drug analysis.

### Reporting and testimony

The points of contact from early adopting laboratories (**Exhibit 2**) may be able to provide details of their reporting and testimony for DART-MS analysis of seized drugs. In general, the process parallels existing electron ionization-mass spectrometry (EI-MS) spectral comparison. The DART-MS Forensics Database provides a library of DART-MS spectra collected at low, medium, and high activation conditions that allow for the comparison of both molecular weight and structural information. As with any library, the effectiveness is limited to the true positive compound being present in the library.

### Consumables

The DART-SVP and DART-JumpShot sources both require ultra-high-purity helium and nitrogen. The cost associated with these gases depends on the user's supplier and contract. In addition, there are consumables associated with sample introduction. IonSense, a subsidiary of Bruker, sells a suite of sample introduction consumables such as DIP-it® tips and QuickStrip™ sample cards, which are combined with a linear rail system for controlled sample introduction. The use of glass capillary tubes for sample introduction has also been demonstrated throughout the literature.





## Early adopting laboratories

**Exhibit 2. Point of contact information for early adopting laboratories that have implemented DART-MS into casework. This list may not be exhaustive but is intended to highlight points of contact for those interested in DART-MS implementation.**

Laboratory	Point of Contact	Email	Phone Number
Alabama Department of Forensic Sciences	Erin Shonsey	<a href="mailto:Erin.shonsey@adfs.alabama.gov">Erin.shonsey@adfs.alabama.gov</a>	205-982-9292
Harris County Institute of Forensic Sciences	Kay McClain	<a href="mailto:Kay.McClain@ifs.hctx.net">Kay.McClain@ifs.hctx.net</a>	832-927-5162
Maryland State Police Forensic Sciences Division	Amber McConnell	<a href="mailto:amber.mcconnell@maryland.gov">amber.mcconnell@maryland.gov</a>	443-357-1391
U.S. DEA Special Testing & Research Laboratory	Sandra Rodriguez-Cruz	<a href="mailto:sandra.e.rodriguez-cruz@dea.gov">sandra.e.rodriguez-cruz@dea.gov</a>	571-776-1854
U.S. FDA Forensic Chemistry Center	Sara Kern	<a href="mailto:sara.kern@fda.hhs.gov">sara.kern@fda.hhs.gov</a>	513-679-2700
Virginia Department of Forensic Sciences	Juli Cruciotti	<a href="mailto:juli.cruciotti@dfs.virginia.gov">juli.cruciotti@dfs.virginia.gov</a>	703-334-9726

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The FTCOE, led by RTI International, is supported through a Cooperative Agreement from the National Institute of Justice (15PNIJ-21-GK-02192-MUMU), Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this report are those of the author(s) and do not necessarily reflect those of the U.S. Department of Justice. Information provided herein is intended to be objective and is based on data collected during primary and secondary research efforts available at the time this report was written.

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## Suggested Citation

Davidson, J. T., Sisco, E., McConnell, A., & Hall A. (2023, December). *Direct analysis in real time mass spectrometry (DART-MS)*. Research Triangle Park, NC: RTI International.