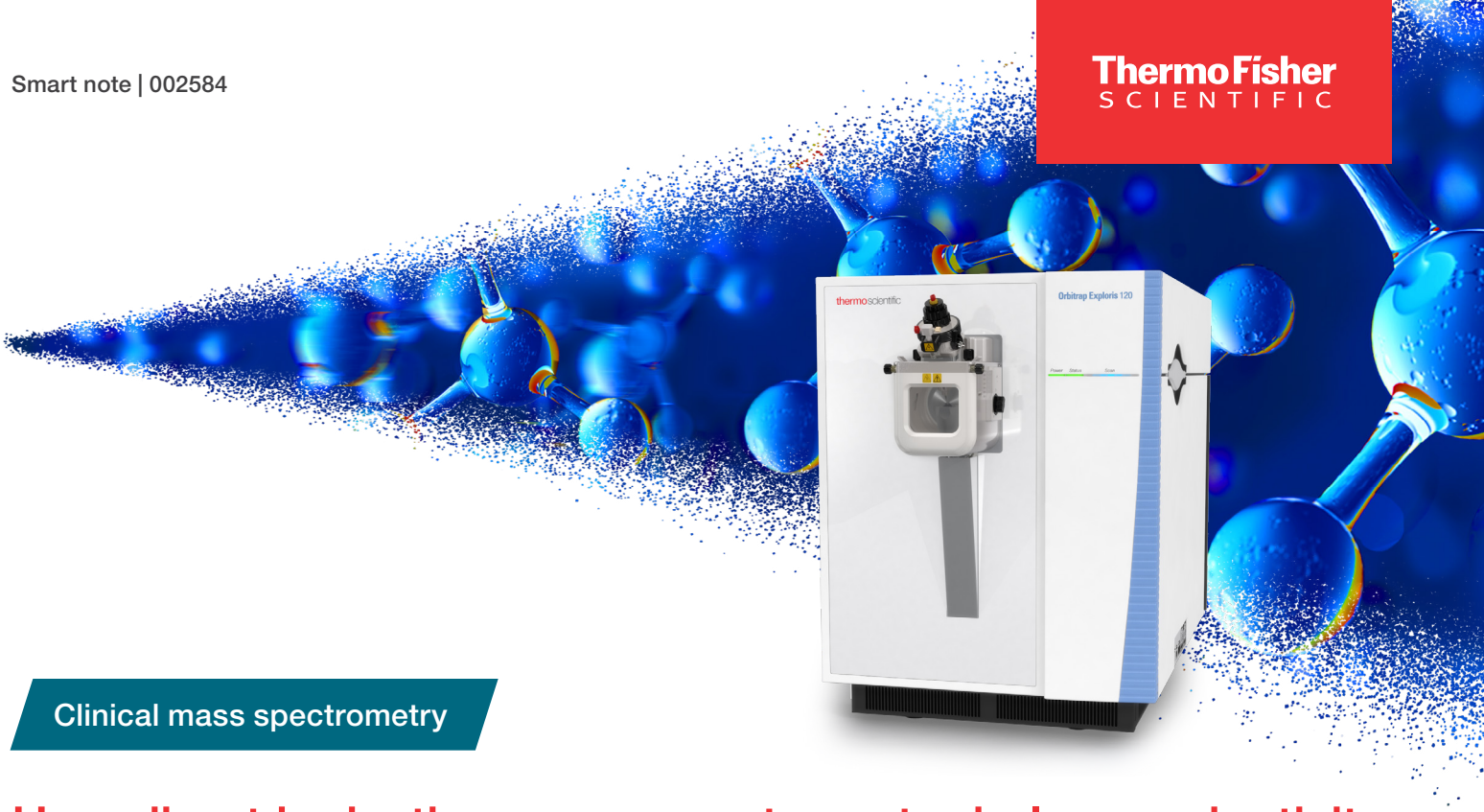

Clinical mass spectrometry

How direct ionization mass spectrometry helps productivity in toxicology

Overview: What is direct ionization mass spectrometry?

Direct ionization mass spectrometry benefits:

- Directly ionizes samples at atmospheric pressure
- Typically does not use chromatography, making it a “greener” choice due reduced solvents needed
- Requires little to no sample preparation
- Consumes a minimal amount of sample
- Maintains sample integrity and is nondestructive with some methods
- Supports quick analysis and sample turnaround time

What are the most common direct ionization methods used in toxicology?

Direct analysis in real-time (DART)

Direct analysis in real-time (DART) is a common direct ionization technique that utilizes an atmospheric pressure ionization source. The JumpShot™ ionization source (made by IonSense and acquired by Bruker, Figure 1) is compatible with Thermo Fisher Scientific’s newest instrumentation, the Thermo Scientific™ TSQ™ Plus series triple quadrupole and the Thermo Fisher™ Orbitrap™ Exploris™ series mass spectrometers, in addition to other legacy Thermo Fisher Scientific mass spectrometers. DART requires no sample preparation. Ionization by DART occurs via a chemical ionization method using an ionizing gas from the ion source that contains excited state species, which ultimately results in ionization of the sample. Samples can be run by DART as a single sample, a 12-sample strip, or a 384-well plate depending on setup.

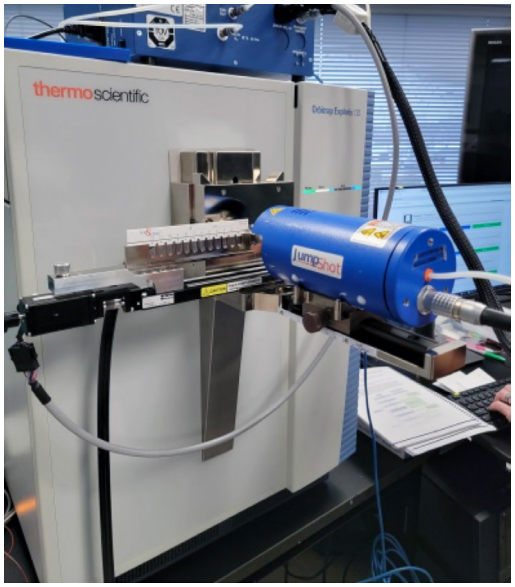

Figure 1. JumpShot ionization source mounted on an Orbitrap Exploris 120 mass spectrometer

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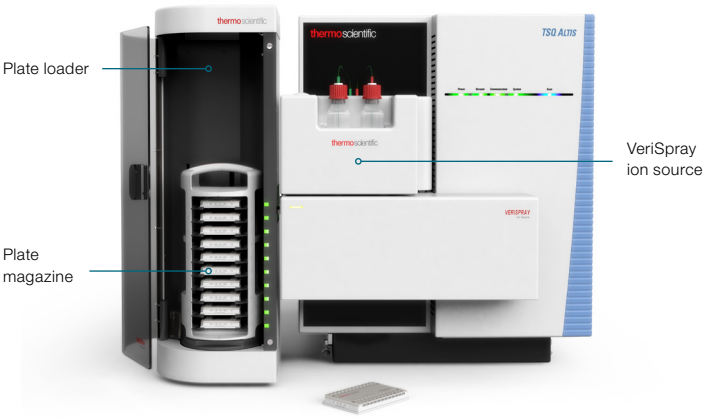


Figure 2. VeriSpray system showing the plate loader with ten-plate magazine and the VeriSpray ion source installed on the TSQ Altis mass spectrometer

VeriSpray PaperSpray ion source

The Thermo Scientific™ VeriSpray™ PaperSpray ion source provides paper spray-based direct ionization at atmospheric pressure and is compatible with several Thermo Scientific mass spectrometers, such as the TSQ Plus series triple quadrupole mass spectrometers (Figure 2). Minimal sample preparation is required with the VeriSpray ion source. It utilizes an electrospray (ESI) ionization method in which a high voltage is applied to paper spotted with sample and solvent. The VeriSpray ion source has the capacity for high throughput using a 24-spot sample strip plate, and up to 10 plates can be loaded per run. Using the VeriSpray ion source, there is little solvent consumption, a 1–2 minute analysis time per sample, and no liquid waste. Data acquisition is automated without user intervention.

Laser diode thermal desorption (LDTD)

Laser diode thermal desorption (LDTD) is another direct ionization technique that utilizes an atmospheric pressure ionization source. The Luxon Ion Source™, made by Phytronix in partnership with Thermo Fisher Scientific, is compatible with TSQ Plus series triple quadrupole mass spectrometers as well as the Orbitrap Exploris series mass spectrometers (Figure 3). LDTD requires minimal sample preparation. Ionization by LDTD occurs by initial sample desorption from the sample plate followed by ionization of the sample by atmospheric pressure chemical ionization (APCI). LDTD is a high-throughput system that can accommodate 96-, 384-, or 1536-well plates.

Figure 3. The Luxon Ion Source

What is the ionization mechanism?

DART

DART uses a gas-phase ionization mechanism that is based on a chemical ionization method at atmospheric pressure to ionize analyte molecules (Figure 4). In short, this process utilizes an ionizing atmospheric gas, which is generated from excited state gas species (typically He or N), that subsequently ionizes and desorbs analyte molecules.

The steps involved in this process involve He (or N) gas entering the ionization source where a voltage is applied to an electrode needle to create excited metastables (long-lived, excited-state neutral gas molecules). Only the neutral metastable species are allowed to exit the ion source. A grid voltage helps define the polarity of the ionization of analyte molecules. The positive or negative ions generated from interacting with atmospheric water outside the source are attracted back to grid electrode in the source depending on voltage setting.

In positive ion mode, proton transfer occurs. Using a Penning ionization reaction, the excited He metastables react with atmospheric water clusters outside the ion source to create protonated water clusters. The protonated water clusters then directly protonate the analyte sample, which is also located in the open air near the inlet of the mass spectrometer. This process typically results in $[M+H]^+$ analyte ions. In the negative ion mode, electron capture generates negative ions. The same Penning reaction takes place, producing a positive ion and an electron. Electrons ionize atmospheric oxygen by capture of an electron and O_2^- ionizes the sample. Finally, the sample is then desorbed and enters the inlet of the mass spectrometer.

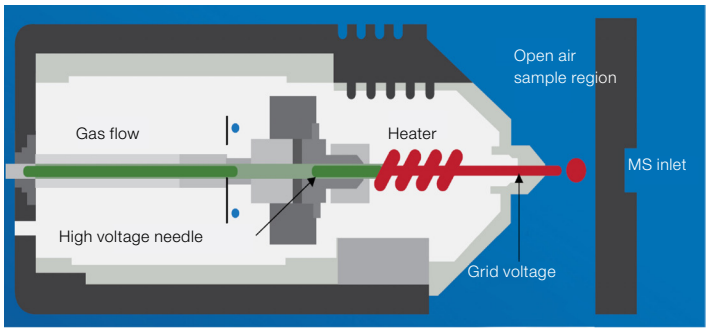


Figure 4. DART mechanism

VeriSpray PaperSpray ion source

The VeriSpray ion source uses filter paper and an electrospray ionization-based method to ionize samples (Figures 5 and 6). A small amount of sample is applied to the paper (one end is cut into a triangle-like sharp point) within the multi-sample plate and allowed to dry. A wetting/extraction agent and spray solvent is added. High voltage is then applied to the paper, causing a spray stream to emerge from the tip of the paper (Taylor cone), which contains droplets of electrically charged ions from the sample. These ions then enter the mass spectrometer inlet for analysis.

LDTD

LDTD ionizes samples using an APCI-based method (Figure 7). A liquid or extracted sample is applied to a stainless steel plate and the sample is dried. The sample is then thermally desorbed from the stainless steel well by an infrared laser that heats the back of the plate. The heat releases neutral gas phase molecules which are directed towards the mass spectrometer inlet via a transfer tube. A carrier gas directs neutral molecules toward the mass spectrometer inlet via transfer tube. A corona discharge needle is located at the exit of the transfer tube, just prior to the mass spectrometer inlet and ionizes the sample. The ionized gas-phase molecules then enter the mass spectrometer inlet.

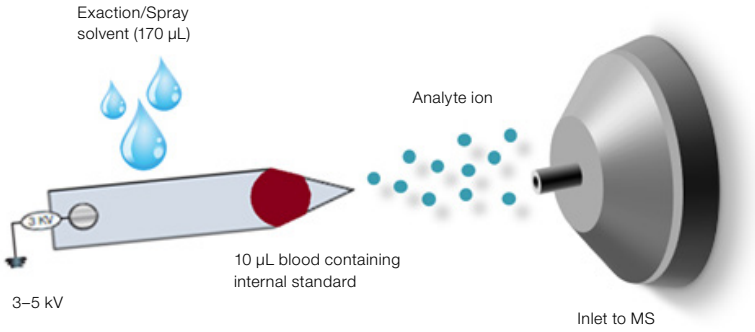


Figure 5. VeriSpray mechanism

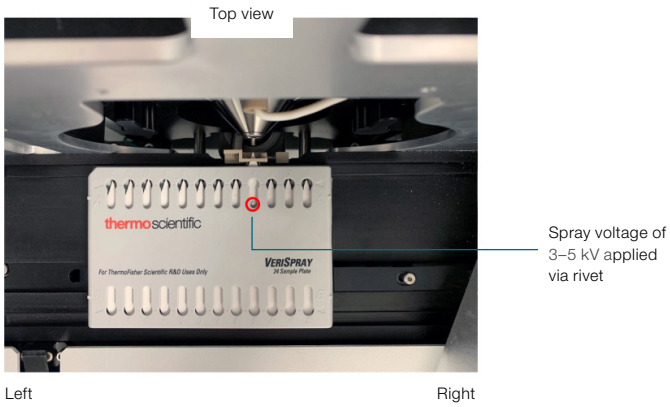


Figure 6. VeriSpray cartridge

The entire process takes less than 1 second!

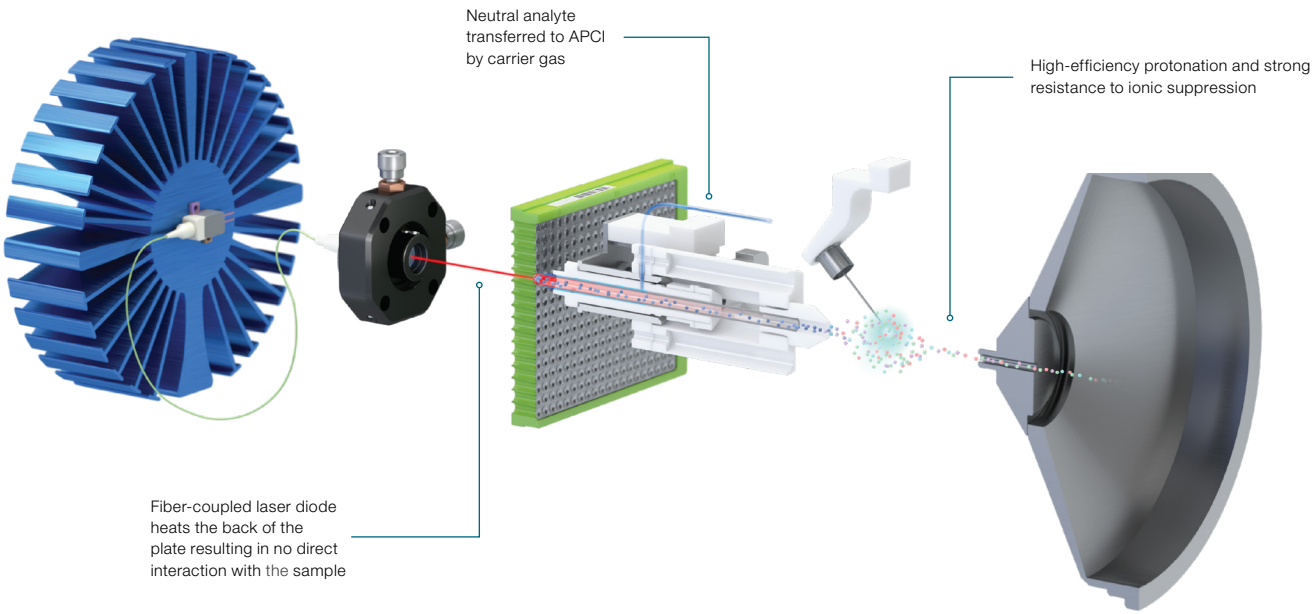


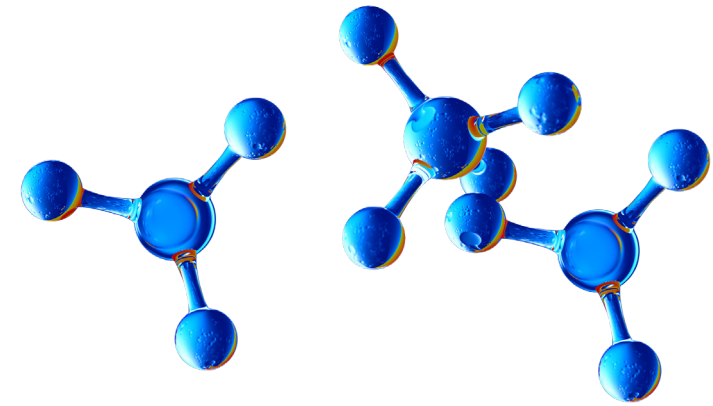
Figure 7. LDTD mechanism

How is direct ionization used for sample analysis?

DART

As with many other direct ionization methodologies, no chromatography or sample preparation is needed with DART. DART can ionize samples in solid, powder, liquid, or gas format or when the sample is absorbed onto paper. DART is non-destructive and does not come into contact with the sample, which maintains sample integrity. DART is typically used to ionize small (<1,000 Da), volatile molecules due to its chemical ionization-based method. DART can be a quantitative analysis technique when using an internal standard as well as a semi-qualitative technique.

DART can be used with a TSQ Plus series triple quadrupole mass analyzer in a targeted analysis or on the Orbitrap Exploris series mass analyzer in an untargeted analysis using its high-resolution accurate mass (HRAM) capabilities. DART can be performed in a single-use run, using the 12-sample QuickStrip™ sample card (Figure 8), or using a 384-well sample plate. DART is easy to use with a short analysis time of only a few seconds per sample or less than 25 min for 384 samples.



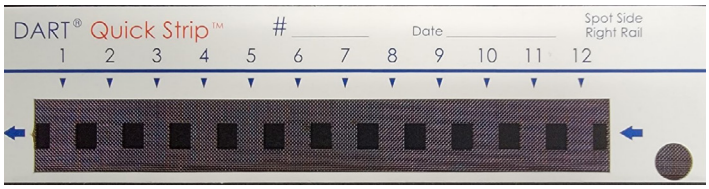


Figure 8. DART QuickStrip sample card

VeriSpray Paper Spray ion source

The VeriSpray ion source does not use chromatography and has minimal sample preparation. Samples can be directly spotted onto paper if they are in liquid form or using a liquid extraction method first if they are a solid. A wetting solvent is applied to the dried sample spot on the paper strip to facilitate ionization and then the spray solvent is added. Small molecules and larger macromolecules, such as peptides, can be analyzed using quantitative analysis if an internal standard is added. Samples can be run individually, or 24 samples can be run on a strip sample plate (Figure 9). The system has a removable magazine that holds up to 10 sample plates with the ability to run 240 total samples in an 8-hour run.

A

B

Figure 9. (A) VeriSpray strip sample plate and (B) removable magazine holderr

LDTD

There is no chromatography and minimal sample preparation with LDTD. Samples in the liquid state, or solvent spotted solid samples, are deposited into a stainless steel sample plate and then dried. Since LDTD uses an APCI ionization method, smaller- to medium-sized molecules are best for analysis. Due to its high sample capacity, LDTD is ideal for screening analysis. LDTD can also be quantitative when used with an internal standard.

LDTD is easily coupled to TSQ Plus series triple quadrupole mass spectrometers and can be run in a targeted analysis methodology. LDTD can also be used with Orbitrap Exploris series mass spectrometers in an untargeted, HRAM method. The Luxon Ion Source uses the LazWell™ plate, which is available in a 96-, 384-, and 1536-well format (Figure 10).

Table 1 provides a summary of the features of DART, VeriSpray, and LDTD direct ionization methods used in toxicology analysis.

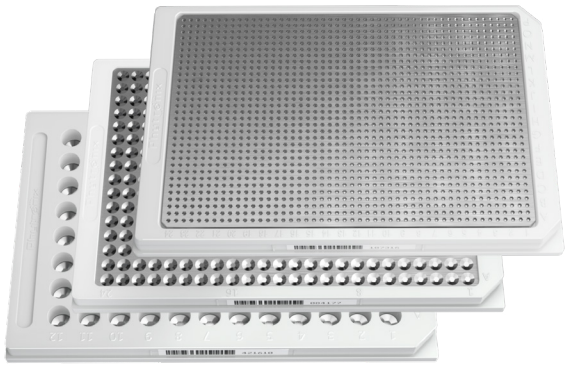


Figure 10. LazWell 96-, 384-, and 1536-well plates

Table 1. Characteristics of common direct ionization methods used in toxicology

	DART	VeriSpray	LDTD
High throughput	✔	✔	✔
Requires no sample prep	✔	None or minimal	Minimal
Run without chromatography	✔	✔	✔
Samples/run	Individually 12 on QuickStrip 384-well plate	Up to 24 samples/plate (up to 240 samples/run)	96/384/1536-well sample plates
Sample run time	Couple seconds/sample 15 minutes/96–384 samples <25 min for 384 samples	1–2 minutes/sample 8 hours/240 samples	0.9–18 seconds/sample 1 system can run 5,000 samples/day
Sample destruction	⊘	Wetting and spray solvent applied	Thermal desorption from well
Contact with sample	No	Yes: solvent, paper	Yes: solvent, well
Samples	Solid Liquid Gas Powder	Liquid (extracted solids)	Liquid (extracted solids)
Analyte size	Small molecules, typically <1,000 Da	Small to larger molecules	Small to larger molecules
Ionization mechanism	CI	ESI	APCI
Ion source	DART-OS JumpShot JumpShot-HTS	VeriSpray VeriSpray w/ Thermo Scientific™ FAIMS Pro™ interface	Luxon T-960 Luxon T-3840 Luxon HTS
Pairs with Thermo Scientific MS instruments	HRAM or targeted MS/MS (SRM)	Targeted MS/MS (SRM)	HRAM or Targeted MS/MS (SRM)
MS analysis method	✔	✔	✔
Quantitative	✔ (also semi-quant)	✔	✔
Typical samples analyzed	Drugs/pills/powder Explosives Propellants Ink Paint Lubricants Pesticides	Blood Urine/biofuids Drugs of abuse Antifungals Peptides Pesticides	Blood Saliva Urine Hair Lipids Pesticides Drug tests Explosives Food analysis

How are the data analyzed?

All the direct ionization methods—DART, VeriSpray ion source, and LDTD—can be used with the fully integrated Thermo Scientific™ Tox Explorer™ Collection, which has access to a database and library of over 1,700 compounds. Toxicology samples can be screened against the Tox Explorer HRAM MS/MS spectrum library as well as a database containing molecular formula, exact mass, and fragment ions. The Tox Explorer Collection is compatible with both HRAM and triple quadrupole mass spectrometry platforms such as the Orbitrap Exploris and TSQ Plus triple quadrupole mass spectrometers.

What are the benefits of direct ionization methods?

Applications of direct ionization methods include drugs of abuse, synthetic drugs screening/identification, novel psychoactive substances, beverage adulteration, steroids, gunshot residue, explosives, printer ink, and paint, among others. Highlights of the benefits of direct ionization methods include:

- None to minimal sample preparation
- Decreased evidence backlog (increased turnaround time)
- High throughput
- Decreased cost
- No LC or GC chromatography maintenance, troubleshooting, or operating/consumables costs
- Identification of substances previously not known

Benefits of the specific methods include:

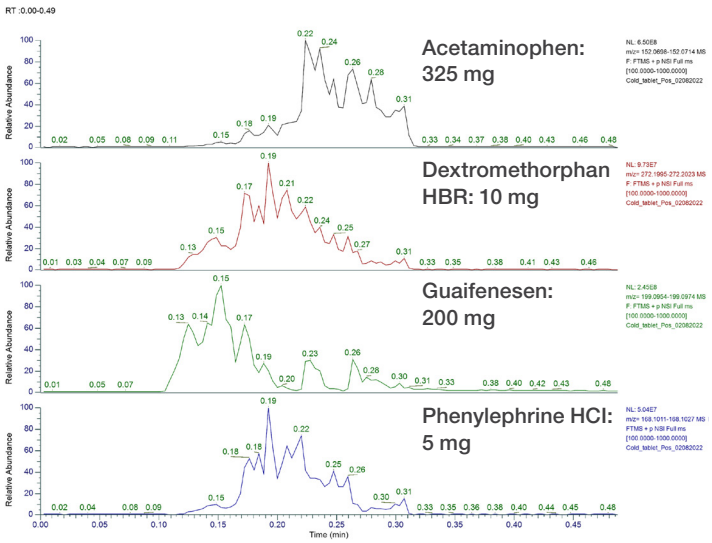
- **DART**
  - Selectivity
  - Maintenance of sample integrity of evidence (non-destructive)
  - Potential for field use
- **VeriSpray ion source**
  - High reproducibility
  - Low solvent consumption and no liquid waste
  - Sample volume for whole blood: 8–10 µL; urine: 5–12 µL
  - No carryover between samples
- **LDTD**
  - High-throughput screening
  - Minimal sample volume
  - Quantitative
  - Sensitivity

Are direct ionization methods applicable for toxicological sample analysis?

DART proof of concept

A DART ion source coupled to an Orbitrap Exploris 120 mass spectrometer was used to identify the active ingredients in an over-the-counter cold medicine. The cold medicine tablet was broken open and a small amount was applied directly to a business card. Each caplet contained acetaminophen, dextromethorphan HBR, guaifenesin, and phenylephrine HCl. Using HRAM, all four of the active ingredients in the cold medicine tablet were identified. The blank had no signal above background in the extracted ion chromatograms for each of the *m/z* corresponding to the four cold tablet active compounds (Figure 11).

A



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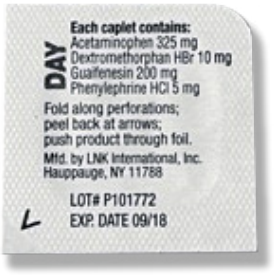


Figure 11. (A) Related extracted ion chromatograms and (B) cold medicine active ingredients

VeriSpray ion source proof of concept

A drugs of abuse panel was used to demonstrate the system sensitivity of the VeriSpray ion source system and determine the limit of quantification (LOQ). A VeriSpray ion source coupled to the TSQ Altis mass spectrometer was used. Run time per sample was 1.2 minutes. The system produced fast results with sufficient sensitivity without any sample preparation. For example, using the VeriSpray ion source with mass spectrometry, cocaine and benzoylecgonine had LOQs of 5 ng/mL (Figure 12 and Table 2).

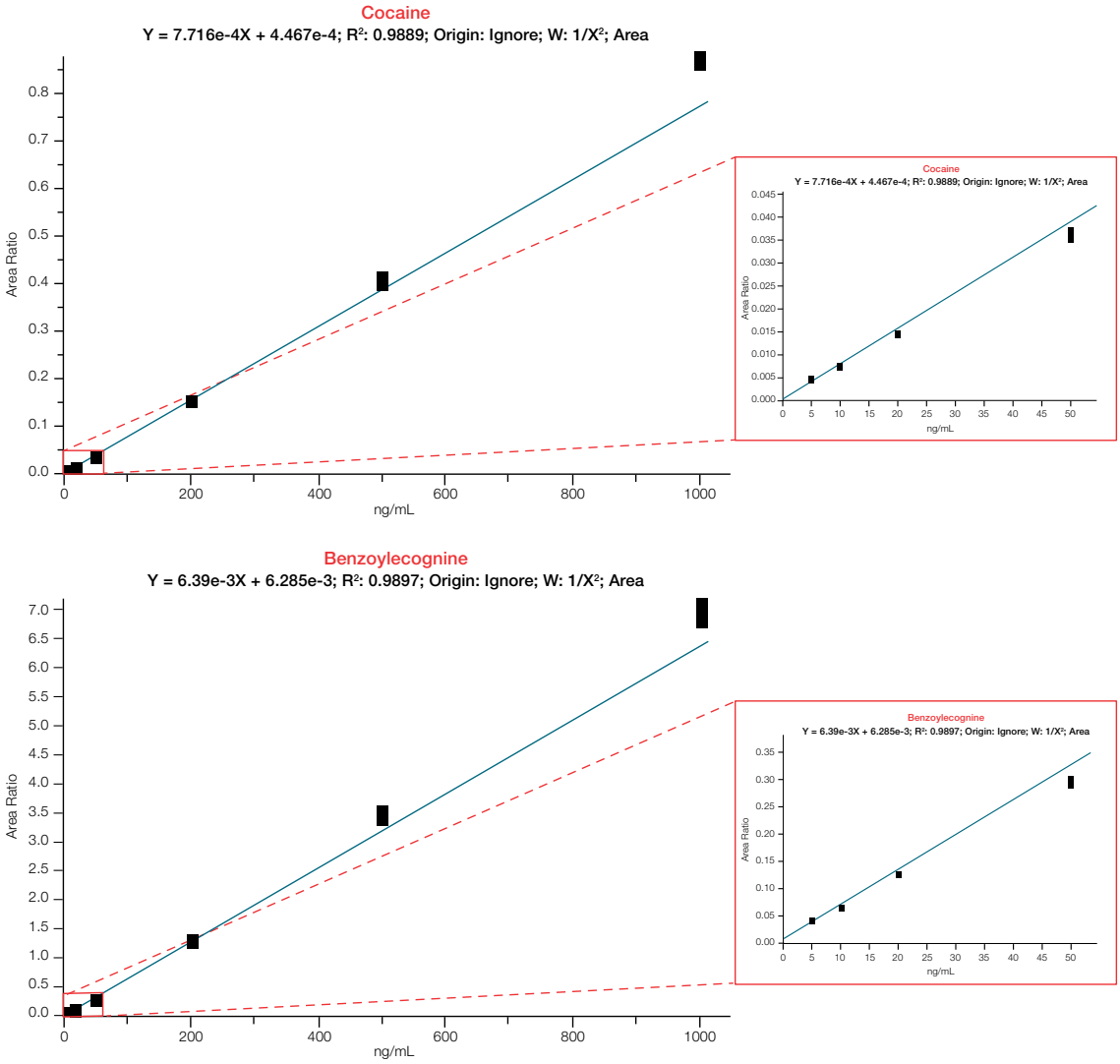


Figure 12. Drugs of abuse analysis using a VeriSpray ion source coupled to a TSQ Altis mass spectrometer with calibration curves for (A) cocaine and (B) benzoylecgonine. The LOQ for both compounds was determined to be 5 ng/mL based on the obtained precision and accuracy values for the calibrator samples, shown in Table 2.

Table 2. Precision and accuracy of calibrator samples.

Concentration (ng/mL)	% RSD		% Difference	
	Cocaine	Benzoylecgonine	Cocaine	Benzoylecgonine
5	5.6	2.9	<14.4	<10.2
10	2.1	1.5	<11.0	<10.9
20	1.1	0.8	<9.2	<6.9
50	2.8	2.1	<10.2	<11.6
200	1.4	2.0	<1.7	<2.7
500	2.5	2.4	<7.9	<11.2
1,000	0.8	2.2	<13.0	<11.1

LDTD proof of concept

Combining the Luxon Ion Source with the Thermo Scientific™ TSQ Vantage™ mass spectrometer, Vitamin A (Retinol) and E (Tocopherol) were evaluated for linearity, precision, and accuracy using the mass spectrometer in an MRM analysis mode. The run time was 8 seconds per sample. The correlation coefficients for both retinol and tocopherol were greater than 0.995. The obtained %CV was below 15% and the accuracy was within 15% of the nominal value. A summary of the data is show in Tables 3 and 4.[1]

Table 3. Inter-day calibration curve correlation coefficients

	Retinol	Tocopherol
Curve 1	0.9974	0.9958
Curve 2	0.9976	0.9972
Curve 3	0.9981	0.9954
Curve 4	0.9977	0.9953
Curve 5	0.9981	0.9950

Table 4. Inter-run precision and accuracy of retinol

Retinol	QC-L	QC-M	QC-H
Conc (ng/mL)	600	2,000	3,000
N	15	15	15
Mean (ng/mL)	658.6	1940.0	3018.8
SD	32.1	193.0	253.0
%CV	4.9	9.9	8.4
%Norm	109.8	97.0	100.6

How has direct ionization-MS been used to address real world toxicology applications?

DART real world application

DART was instrumental in the identification of six synthetic cannabinoids originating from seized material in various matrices from “crazy shops” in Slovakia using the DART-LTQ-Orbitrap XL system.[2] Seized solid herbal material (dried plant parts with suspected dried cannabinoid on the plant) was measured directly using DART by holding the seized sample between the DART ion source and the mass spectrometer inlet with tweezers. The synthetic cannabinoids, including AM-2201, were directly detected in the herbal matrices without sample preparation or solvent extraction and were compared to MeOH extracted samples for confirmation.

Simultaneous detection of several cannabinoids was achieved. The formula mass of the synthetic compound AM-2201, *m/z* 360.1758, was compared to the high-resolution mass, *m/z* 360.1759, and used for identification. The synthetic cannabinoid identity was confirmed by GC–MS mass spectra compared to a library (no analytical standards were available). The analysis time per sample was less than 3 minutes with a sampling time of 30 seconds. The limit of detection was approximately 1–2 ppm.

VeriSpray PaperSpray ion source real world application

Using a VeriSpray PaperSpray ionization source coupled to a Thermo Scientific™ TSQ Fortis™ triple quadrupole mass spectrometer and Thermo Scientific™ TraceFinder™ software, on-site quantitative drug checking for harm reduction and overdose prevention was utilized to overcome the limitations of current testing technologies.[3] Drug checking services at a supervised consumption site in Vancouver, BC, Canada, piloted the use of the VeriSpray ion source with mass spectrometry on-site to identify and quantitate actual street drugs.

Targeted quantitative analysis was conducted on 49 drugs, and untargeted analysis was used to detect unknown/unexpected components. Samples were collected, prepared, analyzed, and reported in approximately 5 minutes. In all, 113 samples with 17 internal standards were analyzed. Of these, the client-expected substance was identified in 88 (78%) of the submitted drug samples.

Fentanyl was the most commonly expected drug out of the 113 samples. Of the 59 expected fentanyl samples, only 45 of them contained fentanyl. The most common unexpected substance in fentanyl samples was the synthetic precursor of fentanyl, 4-anilino-N-phenethyl-piperidine (4-ANPP). 4-ANPP was found in 74% of fentanyl samples, suggesting a high degree of poor manufacturing. In the 36 samples containing both fentanyl and 4-ANPP, the median concentration of fentanyl was 3.3% (w/w%) and ranged from 0.4% to above the upper limit of quantitation (ULOQ). The median concentration of 4-ANPP was 2.2% and ranged from 0.3% to above the ULOQ.

The second most common unexpected substance in the fentanyl samples was etizolam. Etizolam is a sedative that resembles benzodiazepines. Etizolam was detected in 10 of the 59 samples expected to be fentanyl. Seven of these ten samples had only etizolam while three samples had both fentanyl and etizolam. Unknown contamination or substitution of etizolam for fentanyl is a cause for concern, especially when a fentanyl overdose is suspected. While naloxone aids in the prevention of a fatal fentanyl overdose, it has no effect on an overdose with etizolam. None of the clients submitting these samples had any knowledge that they had etizolam instead of fentanyl.

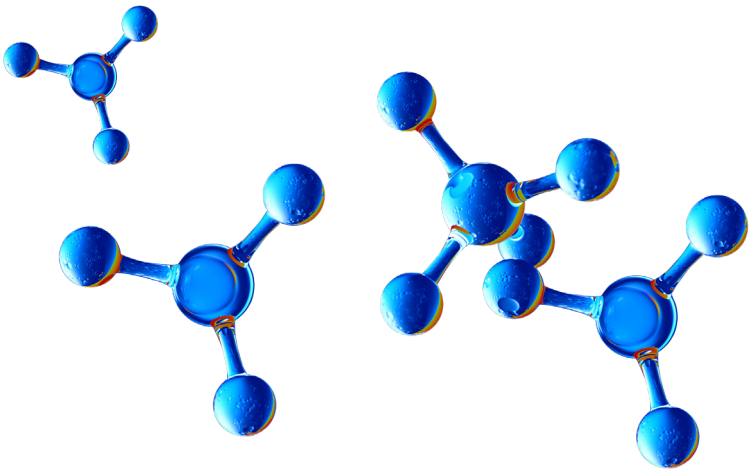
LDTD real world application

Using a Luxon T-960 LDTD on a Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap mass spectrometer, synthetic cannabinoids from "Spice" samples were identified.[4] Spice, a new class of designer drug, contains synthetic cannabinoids that are dissolved and sprayed on dried plant material. Seven Spice packets were obtained from the DEA Special Testing Laboratory. The contents of the Spice packets were combined, and a portion was extracted with methanol for analysis. The extract was analyzed using the Luxon LDTD ionization source with a LazWell 96-well plate that was interfaced to the Q Exactive high-resolution mass spectrometer. The mass spectrometer was operated using full scan followed by 11 data-dependent MS/MS scans. Sample analysis time took 18 seconds per sample. Using this methodology, ten synthetic cannabinoids and one synthetic cathinone were detected and identified from the Spice sample: JWH-019, AM-694, JWH-210, AM-2201, RCS-4, JWH-073, JWH-018, 5-F-UR-144, JWH-250, JWH-081, and MDPV.

Are there any additional considerations when using direct ionization methods?

DART

- While the use of chromatography may improve limit of detection, sensitivity, and specificity, DART has a quicker analysis time (a few seconds/sample or <25 min/384 samples), broad sample applicability, and can sufficiently detect low level drugs even in cutting agents with sufficient sensitivity and specificity needed for analysis. DART mass spectrometry has been shown to be more than sufficient for toxicology-based applications.
- DART is well suited for analysis of small molecules (<1,000 Da) due to the nature of the chemical ionization-based method it uses. However, if looking to analyze larger molecules, the VeriSpray ion source or LDTD ionization applications are alternative options.
- DART is also ideal for molecules that are volatile. If analysis of less-volatile molecules is desired, additional desorption of the sample can be achieved by heating the gas and metastables in the source to facilitate increased sample desorption.



VeriSpray PaperSpray ion source

- Mass spectrometry-based methods with no chromatography and no sample clean-up can have a higher background leading to lower limits of detection (LOD) due to a lower S/N ratio. The VeriSpray ion source can sufficiently achieve the low level of sensitivity needed for toxicology analysis. However, if increasing the LOD for your sample is needed, Thermo Fisher Scientific has developed the Thermo Scientific™ FAIMS Pro™ interface. Field asymmetric ion mobility spectrometry (FAIMS) is a technique that enhances selectivity by adding an additional dimension of separation based on ion mobility. By combining the VeriSpray ion source with the FAIMS Pro interface, background compounds are filtered out based on differences in ion mobility. This enhances signal by reducing background noise, thus increasing the S/N ratio.
- It may be a concern whether samples need to be rewetted during a paper spray run and/or spray solvent may run out quickly preventing further ionization of the sample. Fortunately, the VeriSpray ion source automates the addition of rewetting and addition of spray solvent to a sample at a rate of 10 µL/min for a continuous sample spray.
- Since the VeriSpray ion source is an ESI-based ionization method, it can accommodate the analysis of larger molecules. Peptides have been successfully analyzed using the VeriSpray technique.

LDTD

- When analyzing a large number of samples per run, to reduce sample preparation time if samples require solvent extraction prior to analysis, automation and multiplexing systems are available that can increase throughput.
- Automated sample spotters are available to reduce any inconsistencies that may happen with manual sample spotting.

Summary

The direct ionization sources described here, DART JumpShot, VeriSpray ion source, and Luxon LDTD, are easily integrated onto Thermo Scientific mass spectrometers including the TSQ Plus series quadrupole mass spectrometers and the Orbitrap Exploris series mass spectrometers. These combinations, used with the Tox Explorer workflow, can fit your toxicological analytical needs and improve the productivity of the laboratory. For example, the DART ionization source combined with a TSQ series mass spectrometer provides an analytical workflow characterized by rapid results, confident identification, high specificity with SRM spectra, and an easy-to-use system. Similarly, the Luxon Ion Source coupled to the Orbitrap Exploris mass spectrometer gives you the speed, resolution, and robustness to create the most efficient high-throughput workflows.

For all your drug screening needs please visit:

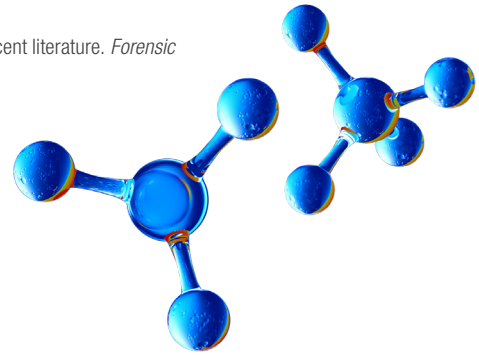
https://www.thermofisher.com/us/en/home/industrial/forensics/forensic-toxicology/drug-screening-confirmation.html

For additional information please visit these websites:

- VeriSpray PaperSpray Ion Source for Mass Spectrometry
- Orbitrap Exploris Mass Spectrometers
- TSQ Plus Triple Quadrupole Mass Spectrometers
- Clinical Research & Toxicology Applications

References

1. Picard, P. et al. Phytronix Technologies Application Note 2107: Analysis of Vitamin A and E in serum as a biomarker of clinical disorders: Vitamin A and E quantification in serum at 8 seconds per sample using LUXON-MS/MS.
2. Habala, L. et al. DART – LTQ ORBITRAP as an expedient tool for the identification of synthetic cannabinoids, *Legal Medicine* **2016**, *20*, 27–31.
3. Borden, S.A. et al. A new quantitative drug checking technology for harm reduction: Pilot study in Vancouver, Canada using paper spray mass spectrometry. *Drug Alcohol Rev.* **2022**, *41*(2), 410–418.
4. Jagerdeo, E. et al. Rapid analysis of forensic-related samples using two ambient ionization techniques coupled to high-resolution mass spectrometers. *Rapid Commun. Mass Spectrom.* **2017**, *31*, 782–790.
5. Sisco, E. et al. Forensic applications of DART-MS: A review of recent literature. *Forensic Chemistry* **2021**, *22*, 100294.



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