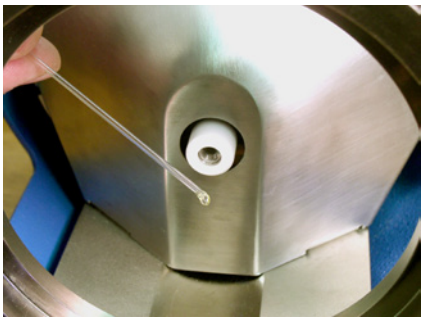
Direct Analysis in Real Time (DART™) Mass Spectrometry



Robert B. Cody†, James A. Laramée††, J. Michael Nilles†††, and H. Dupont Durst††††

†JEOL USA, Inc.
††EAI Corporation
†††Geo-Centers Inc.
††††Edgewood Chemical Biological Center

Introduction

Mass Spectrometry (MS) is one of the fastest-growing areas in analytical instrumentation. The use of mass spectrometry in support of synthetic, organic, and pharmaceutical chemistry is well established. Mass spectrometry is also used in materials science, environmental research, and forensic chemistry. It has also evolved into one of the core methods used in biotechnology. However, currently available ion sources place extreme restrictions on the speed and convenience of sample analysis by mass spectrometry. Here we report a method for using mass spectrometry to instantaneously analyze gases, liquids, and solids in open air at ground potential under ambient conditions.

Traditional ion sources used in mass spectrometry require the introduction of samples into a high vacuum system. Traditional ion sources operated in vacuum include electron ionization (EI)[1], chemical ionization (CI)[2], fast atom bombardment (FAB)[3], and field desorption/field ionization (FD/FI)[4]. These techniques have been used successfully for decades. However, the requirement that samples be introduced into a high vacuum for analysis is a severe limitation. Gas or liquid samples must be introduced through a gas chromatograph or a specially designed inlet system. Solid samples must be introduced by using a direct insertion probe and a vacuum lock system. Direct insertion probes can result in vacuum failure and/or contamination of the ion source if too much sample is introduced.

Atmospheric pressure ion sources such as atmospheric pressure chemical ionization (APCI)[5], electrospray ionization (ESI)[6-8], matrix-assisted laser desorption ionization (MALDI)[9-10] and atmospheric pressure photoionization (APPI)[11] have broadened the range of compounds that can be analyzed by mass spectrometry. However, these ion sources require that samples be exposed to elevated temperatures and electrical potentials, ultraviolet irradiation, laser radiation, or a high-velocity gas stream. Safety considerations require that the ion source be fully enclosed to protect the operator from harm.

The new ion source reported herein overcomes these limitations. The new technique, referred to as Direct Analysis in Real Time (DART™), has been coupled to the AccuTOF-LC™ atmospheric pressure ionization mass spectrometer to permit high-resolution, exact mass measurements of gases, liquids, and solids[12,13]. DART successfully sampled hundreds of chemicals, including chemical agents and their signatures, pharmaceutics, metabolites, pesticides and environmentally significant compounds, peptides and oligosaccharides, synthetic organics, organometallics, drugs of abuse, explosives, and toxic industrial chemicals. These chemicals were detected on a variety of surfaces such as concrete, human skin, currency, airline boarding passes, fruits and vegetables, body fluids, cocktail glasses, and clothing. The composition of drug capsules and tablets was directly analyzed.

Background and Principle of Operation

DART grew out of discussions at JEOL USA, Inc. between two of the authors (Laramee and Cody) about the possibility of developing an atmospheric pressure thermal electron source to replace the radioactive sources used in hand-held detectors for chemical weapons agents (CWAs), drugs, and explosives. The discovery that DART could be used for positive-ion and negative-ion non-contact detection of materials on surfaces, as well as for detection of gases and liquids, led to the development of a commercial product.

DART is based on the atmospheric pressure interactions of long-lived electronic excited-state atoms or vibronic excited-state molecules with the sample and atmospheric gases. The DART ion source is shown in **Figure 1**. A gas (typically helium or nitrogen) flows through a chamber where an electrical discharge produces ions, electrons, and excited-state (metastable) atoms and molecules. Most of the charged particles are removed as the gas passes through perforated lenses or grids and only the neutral gas molecules, including metastable species, remain. A perforated lens or grid at the exit of the DART provides several functions: it prevents ion-ion and ion-electron recombination, it acts as a source of electrons by surface Penning ionization, and it acts as an electrode to promote ion drift toward the orifice of the mass spectrometer's atmospheric pressure interface.

Several ionization mechanisms are possible, depending on the polarity and reaction gas, the proton affinity and ionization potential of the analyte, and the presence of additives or dopants. The simplest process is Penning ionization [14] involving transfer of energy from the excited gas M^* to an analyte S having an ionization potential lower than the energy of M^*. This produces a radical molecular cation $S^{+\cdot}$ and an electron (e^-).

$$M^* + S \rightarrow S^{+\cdot} + M + \text{electron}^-$$

Penning ionization is a dominant reaction mechanism when nitrogen or neon is used in the DART source. Nitrogen or neon ions are effectively removed by the electrostatic lenses and are never observed in the DART back-

†11 Dearborn Road, Peabody, Massachusetts, USA.
††1308 Continental Drive, Suite J, Abingdon, MD 21009
†††Box 68 Gunpowder Branch, APG, MD 21010
††††Aberdeen Proving Grounds, Maryland USA

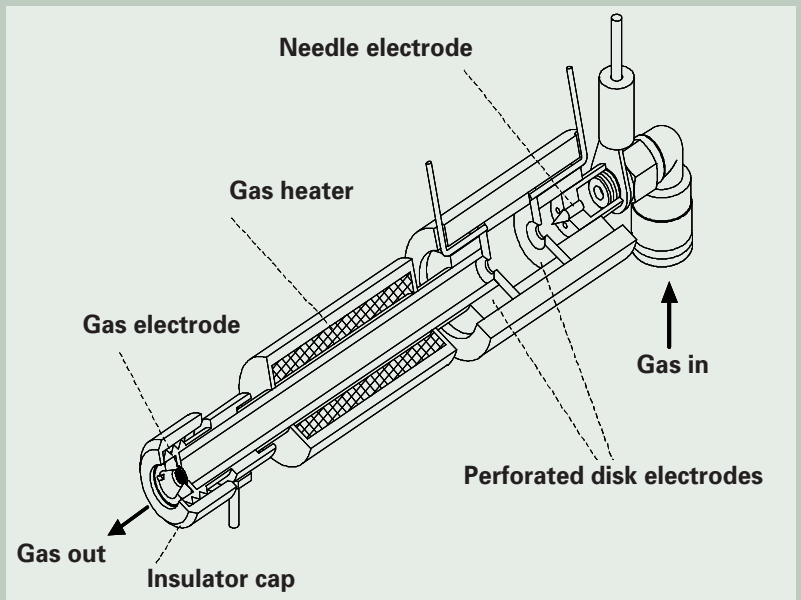


Fig.1. Schematic diagram of the DART ion source.

and are never observed in the DART background mass spectrum.

When helium is used, the dominant positive-ion formation mechanism involves the formation of ionized water clusters followed by proton transfer reactions:

$$He(^2 3S) + H_2O \rightarrow H_2O^{+\bullet} + He(^1 1S) + electron^-$$
$$H_2O^{+\bullet} + H_2O \rightarrow H_3O^+ + OH^\bullet$$
$$H_3O^+ + n\ H_2O \rightarrow [(H_2O)nH]^+$$
$$[(H_2O)nH]^+ + M \rightarrow MH^+ + nH_2O$$

The helium 2^3S state has an energy of 19.8 eV. Its reaction with water is extremely efficient [15] with the reaction cross section estimated at 100 A^2. Because of this extraordinarily high cross section, DART performance is not affected by humidity.

Negative-ion formation occurs by a different mechanism. Electrons (e^-) are produced by Penning ionization or by surface Penning ionization:

$$M^* + surface \rightarrow M + surface + e^-$$

These electrons are rapidly thermalized by collisions with atmospheric pressure gas

$$e^-_{fast} + gas \rightarrow e^-_{slow}$$

Thermal electrons undergo electron capture by atmospheric oxygen

$$e^-_{slow} + O_2 \rightarrow O_2^-$$

to produce O_2^-, which reacts with the analyte to produce anions. The DART negative-ion reagent mass spectra are virtually identical for nitrogen, neon, and helium. However, negative-ion sensitivity increases for DART gases in the following order:

nitrogen < neon < helium

This is due to the increased efficiency in forming electrons by Penning ionization and surface Penning ionization as the internal energy of the metastable species increases.

The polarity of the DART ion source is switched between positive-ion mode and negative-ion mode by changing the polarity of the disk electrode and grid. The polarity of the discharge needle is not changed, so the plasma is not interrupted. This permits rapid switching between positive and negative modes.

Other reactions are possible. The presence of traces of dopants such as ammonium (e.g. from ammonium hydroxide headspace vapor) or chloride (e.g. from methylene chloride vapor) can modify the chemistry allowing the chemist to tailor the experiment for specific analyses.

DART produces relatively simple mass spectra characterized by M^+; and/or $[M+H]^+$ in positive-ion mode, and $M^{-\cdot}$ or $[M-H]^-$ in negative-ion mode. Fragment ions are observed for some compounds. The degree of fragmentation can be influenced by the choice of gas, the temperature, and the AccuTOF orifice 1 potential. Alkali metal cation attachment and double-charge ions are not observed.

The mechanism involved in desorption of materials from surfaces by DART is less well characterized. Thermal desorption plays a role if the gas stream is heated. However, the analysis by DART of inorganic materials such as sodium perchlorate or organic salts having little or no vapor pressure is evidence of other processes. It is postulated that the transfer energy to the surface by metastable atoms and molecules facilitates desorption and ionization.

In contrast with other ion sources that use metastable species [16-23], the DART ion source does not operate under reduced pressure, apply a high electrical potential to the analyte, or expose the analyte directly to the discharge plasma. Argon, used in many of these ion sources, is not well suited for use with DART because argon metastables are rapidly quenched in the presence of water vapor [20] by a reaction involving homolytic cleavage of the water bond without concomitant ion formation. None of these ion sources are designed for direct analysis of gases, liquids, and solids in open air under ambient conditions.

Experimental

A DART[tm] source [24] was installed on a JEOL AccuTOF-LC[tm] time-of-flight mass spectrometer. The DART source replaces the standard electrospray ionization (ESI) source supplied with the AccuTOF. No vacuum vent is required. The ion sources can be exchanged and made operational within minutes.

The mass spectrometer operates at a constant resolving power of approximately 6000 (FWHM definition). Typical atmospheric pressure interface conditions are: orifice 1 = 30V, and both orifice 2 and ring lens are set to 5V. The AccuTOF ion guide voltage is varied as needed depending on the lowest *m/z* to be measured. Orifice 1 temperature is typically kept warm (80 degrees C) to prevent contamination. Although there is some electrical potential on the exposed orifice 1, the voltage and current are so low that there is absolutely no danger to the operator, even with prolonged direct contact.

The DART source is operated with typical gas flows between 1.5 and 3 liters per minute. Gas temperature is programmable from ambient temperature up to approximately 350 degrees C (gas heater temperature from OFF to a maximum of 550 degrees C). Typical potentials are: discharge needle 2kV to 4 kV, electrode 1: 100V, grid: 250 V. Gas, liquid, or solid samples positioned in the gap between the DART source and mass spectrometer orifice 1 are ionized.

Because the mass spectrometer orifice is continually bathed in hot inert gas, the DART source is remarkably resistant to contamination and sample carryover. Mass scale calibration is easily accomplished by placing neat poly-

ethylene glycol average molecular weight 600 (PEG 600) on a glass rod or a piece of absorbent paper in front of the DART source. In positive-ion mode, this produces a series of $[M+H]^+$ and $[M+H-H_2O]^+$ peaks from *m/z* 45 up to beyond *m/z* 1000. By including background peaks, the calibrated mass range can be extended down to *m/z* 18 or 19. Negative-ion spectra of PEG are characterized by $[M+O_2-H]^-$ and $[(C_2H_4O)_n+O_2-H]^-$ ion series.

The reference spectrum can be acquired within seconds. There is no memory effect or carryover of the reference compound -- the PEG peaks do not persist after the reference standard is removed. For these reasons, a full reference mass spectrum can be quickly and easily included in each data file, and accurate mass measurements are routinely acquired for all samples.

Applications

The DART ion source has been used to analyze an extremely wide range of analytes, including drugs (prescription, over-the-counter, veterinary, illicit, and counterfeit) in dose form or in body fluids or tissues, explosives and arson accelerants, chemical weapons agents and their signatures, synthetic organic or organometallics compounds, environmentally important compounds, inks and dyes, foods, spices and beverages. An important benefit of DART is that materials can be analyzed directly on surfaces such as glass, TLC plates, concrete, paper, or currency without requiring wipes or solvent extraction.

Drugs can be detected in pill form by placing the pill in front of the DART source for a few minutes. An example is shown below (**Figure 2**) for the rapid detection of illicit drugs in pills confiscated by a law-enforcement agency. The intact pills were simply placed in front of the DART source and analyte ions were observed within seconds. Exact mass and isotopic measurements confirmed the elemental compositions of the labeled components. All labeled assignments in the following examples were confirmed by exact mass measurements.

Drug counterfeiting is becoming a serious and widespread public health problem. Counterfeit drugs are not only illegal, but dangerous; they may contain little or no actual drug content, or they may contain completely different drugs with potentially toxic consequences.

DART can be used to rapidly screen for counterfeit drugs. An example is shown below in **Figure 3** where DART was used to analyze a sample of a real drug containing the antimalarial dihydroartemisinin, and a counterfeit drug containing no active ingredients.

DART has been applied to the direct detection of drugs and metabolites in raw, unprocessed body fluids, including blood, urine, perspiration, and saliva. An example is shown below in **Figure 4** for the negative-ion analysis of the urine of a subject taking prescription ranitidine. No extraction or other processing was used: a glass rod was dipped in raw urine and placed in front of the DART source.

For easy viewing, only abundant components are labeled in this figure. A more complete list of assignments is given in **Table 1**. Assignments are made for compounds com-

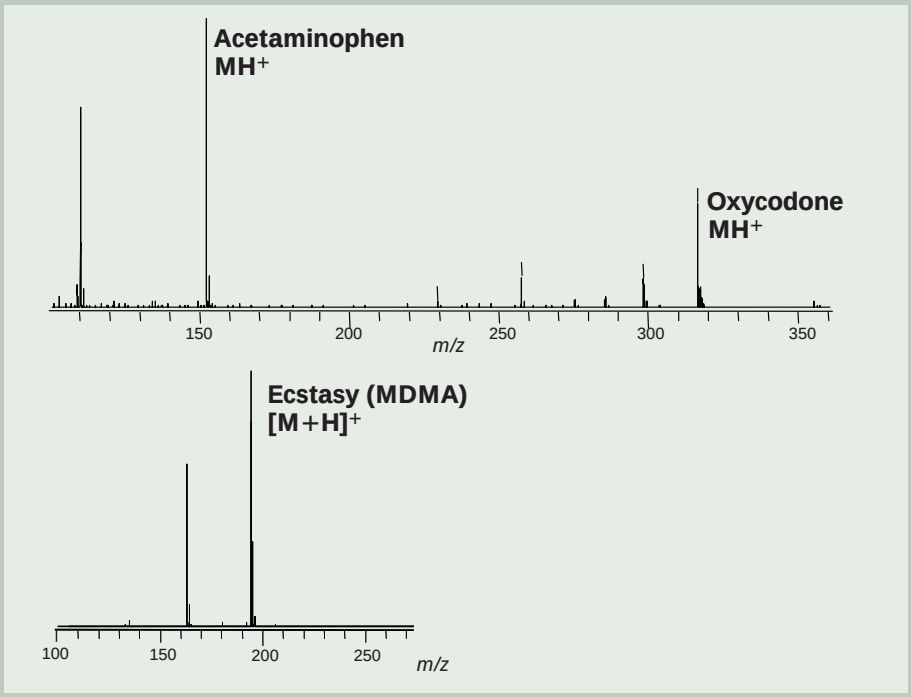


Fig. 2 DART mass spectra of two pills: An analgesic containing acetaminophen plus oxycodone (top) and methylenedioxyamphetamine ("ecstasy" ,bottom).

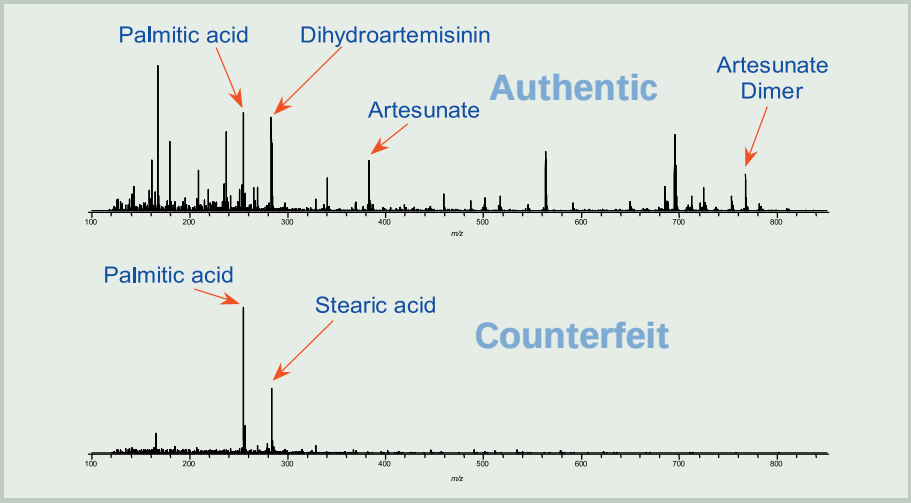


Fig. 3 Rapid detection of counterfeit drug. The top mass spectrum shows the authentic drug and the bottom mass spectrum shows the counterfeit drug.

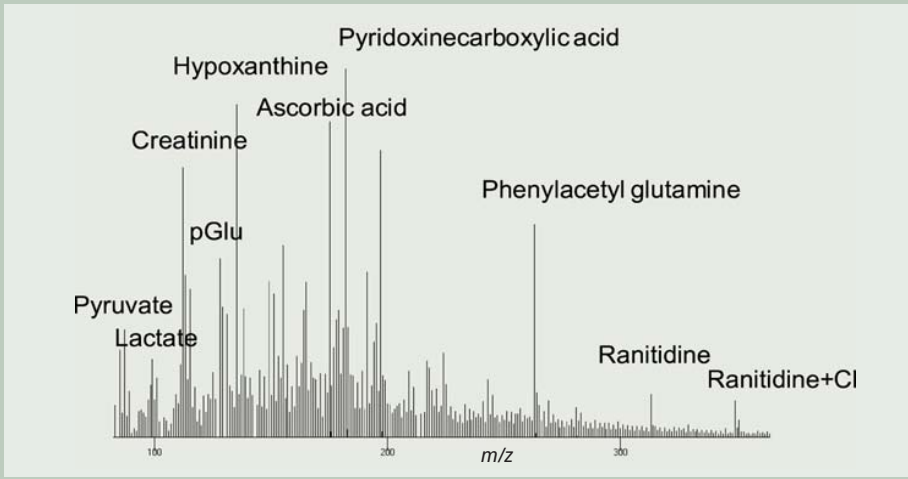


Fig. 4 Negative-ion DART analysis of the urine of a subject taking prescription ranitidine.

Table 1 Assignments for Compounds Detected in Negative-Ion DART Mass Spectrum of Raw Urine

Name	Meas.	Calc.	Diff(u)	Abund.
GBL	85.0295	85.0290	0.0006	11.0317
Pyruvic_acid	87.0084	87.0082	0.0002	7.1700
Lactic_acid	89.0236	89.0239	-0.0002	8.3658
Cresol	107.0492	107.0497	-0.0004	.9294
Uracil	111.0153	111.0195	-0.0041	14.3328
Creatinine	112.0513	112.0511	0.0002	81.6851
Purine	119.0354	119.0358	-0.0004	31.9510
Niacin	122.0277	122.0242	0.0035	3.1489
Dihydro_methyluracil	127.0486	127.0508	-0.0021	23.3773
pGlu	128.0353	128.0348	0.0006	59.2337
Methylmaleic_acid	129.0212	129.0188	0.0024	37.1191
Me_succinate/diMe_malonate	131.0368	131.0358	0.0010	19.3593
Deoxyribose	133.0489	133.0501	-0.0012	28.3521
Hypoxanthine	135.0306	135.0307	-0.0001	100.0000
Adipic_acid	145.0469	145.0501	-0.0032	11.7389
Methyl_hypoxanthine	149.0454	149.0463	-0.0009	37.5243
Hydroxymethyl_methyl_uracil	155.0453	155.0457	-0.0003	55.5832
a-aminoadipic_acid	160.0568	160.0610	-0.0042	9.5885
Methionine_sulfoxide	164.0419	164.0381	0.0037	11.7609
Methylxanthine	165.0408	165.0412	-0.0004	32.4341
Formiminoglutamic_acid	173.0536	173.0562	-0.0027	12.3531
Ascorbic_acid	175.0285	175.0243	0.0042	23.1998
Hippuric_acid	178.0513	178.0504	0.0009	66.4487
Glucose	179.0552	179.0556	-0.0004	39.7499
Dimethylxanthine	179.0552	179.0569	-0.0017	39.7499
Pyridoxinecarboxylic_acid	182.0479	182.0453	0.0026	34.7913
Hydroxyindoleacetic_acid	190.0542	190.0504	0.0037	5.4133
Dimethyluric_acid	195.0527	195.0518	0.0009	23.7577
AAMU (caffeine metabolite)	197.0667	197.0675	-0.0007	79.6617
Cinnamalidinemalonic_acid	217.0483	217.0501	-0.0017	60.5399
AFMU (caffeine metabolite)	225.0643	225.0624	0.0019	21.9092
Cytidine	242.0801	242.0777	0.0024	3.4545
Uridine	243.0641	243.0617	0.0024	21.1156
Phenylacetyl_glutamine	263.1033	263.1032	0.0001	48.9665
Adenosine	266.0861	266.0889	-0.0028	1.4869
Ranitidine	313.1321	313.1334	-0.0013	8.7459
Ranitidine+Cl	349.1113	349.1101	0.0011	11.7296

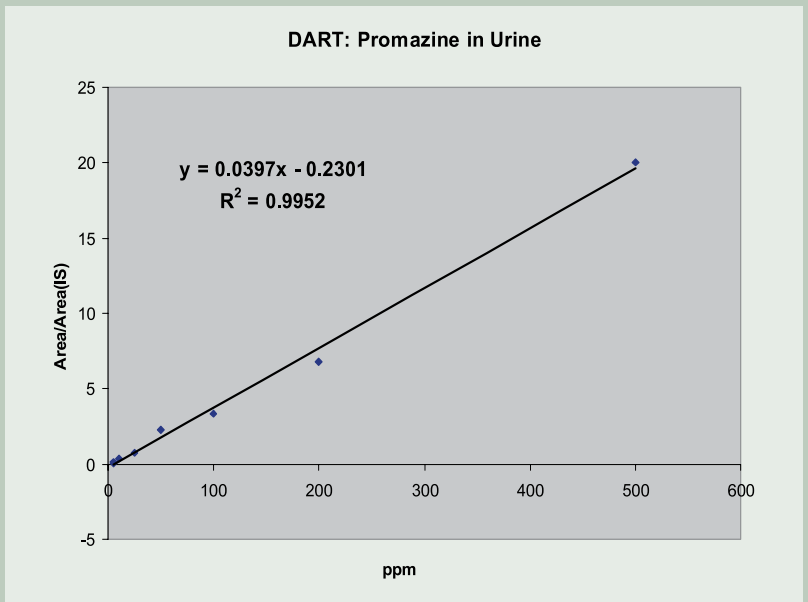


Fig.5. . Rapid quantitative analysis by DART of promazine in urine. Chlorpromazine was added as an internal standard.

monly encountered in urine that have elemental compositions that match the measured *m/z* values. It is interesting to note that the basic drug, ranitidine, is observed as an $[M-H]^-$ species in the negative-ion mass spectrum as well as an abundant $[M+H]^+$ species in the positive-ion mass spectrum. Ranitidine metabolites are also observed [11] in the positive-ion mass spectrum (not shown).

DART can be used for quantitative analysis. The absolute abundance of ions produced by DART depends on the positioning of the target in the gas stream. However, the use of an internal standard permits rapid quantitative analysis of drugs in urine, plasma, or other body fluids. **Figure 5** shows a working curve obtained for urine samples spiked with promazine at the 1 to 500 ppm level. Chlorpromazine (50 ppm) was added as an internal standard. Undiluted urine samples were applied to a glass rod. Each analysis was complete within seconds of placing the rod in front of the DART source. This approach has also been used to screen for the "date rape" drug gamma hydroxy butyrate (GHB) in urine [24] and for the rapid quantitative analysis of developmental drugs in plasma.

The detection of explosives is important for forensics and security. DART has been applied to the detection of nitro explosives such as nitroglycerine, TNT, and HMX, inorganic explosives such as ammonium nitrate, perchlorate and azide, and peroxide explosives such as TATP and HMTD. Examples are shown in **Figures 6** and **7**.

The high dynamic range of the DART-AccuTOF combination can permit the identification of trace-level impurities for quality control and similar applications. An example is shown in **Figure 8** and **Table 2** for the exact-mass analysis of 1% propazine and 0.2% simazine in a sample of the herbicide atrazine.

Conclusion

A new ion source has been developed that permits the analysis of gases, liquids, and solids in open air under ambient conditions. No solvents or high-pressure gases are used. The sample is not directly exposed to high voltages, laser beams or radiation or plasma. The combination of this source with a high-resolution time-of-flight mass spectrometer permits rapid qualitative and quantitative analysis of a wide variety of materials.

Acknowledgment

Technical assistance and keen scientific insight were unselfishly provided by (in alphabetical order) Daniel Banquer, Ted Boileau, William Creasy, Daniel Evans, Drew McCrady, Michael McKie, Michael Nilles, Edward Owen, Gary Samuelson, Philip Smith, John Stuff, and Dean Tipple. The authors would like to thank Prof. Facundo Fernandez of Georgia Tech University for the dihydroartemisinin and counterfeit drug sample.

Additional information

Additional applications and digital videos showing DART analysis are available on the internet at http://www.jeolusa.com/ms/msprod-

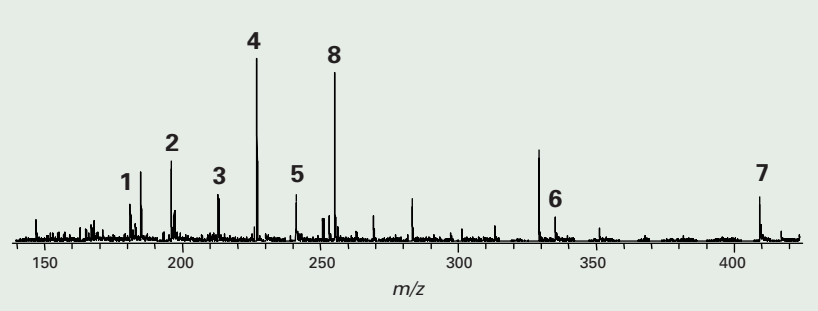


Fig.6. . 3 ppm explosives spiked into muddy water. 1=DNT, 2=amino-DNT, 3=trinitrobenzene, 4=TNT, 5=RDX+TFA, 6=Tetryl, 7=HMX+TFA, 8=palmitate in the water background (used as lock mass). Headspace vapor from a 0.1% aqueous solution of trifluoroacetic acid was used to produce TFA adducts.

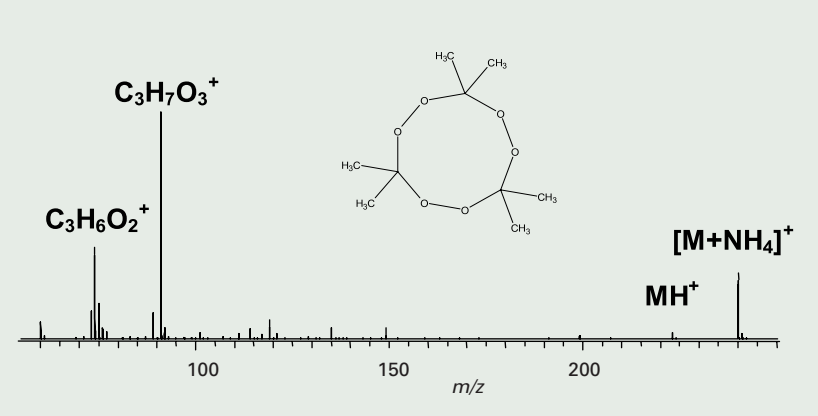


Fig.7. Positive-ion DART mass spectrum of triacetone triperoxide (TATP). Ammonium hydroxide headspace vapor provided a source of NH_4^+.

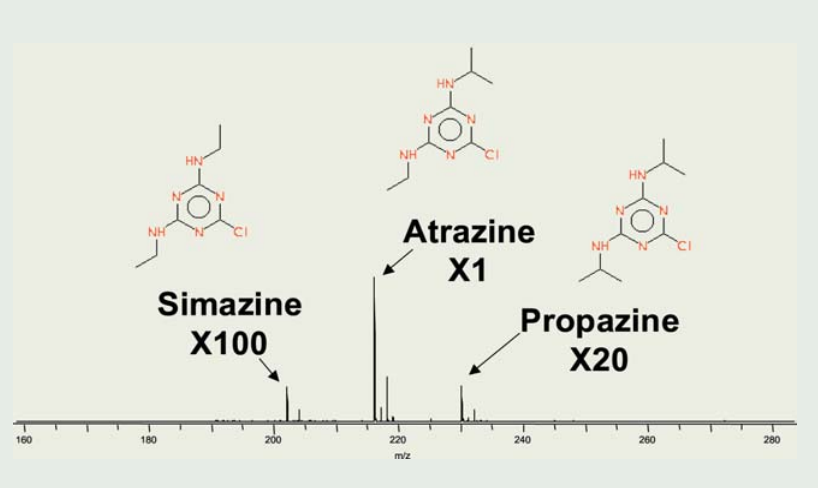


Fig.8. Exact-mass analysis of trace simazine and propazine in a sample of the herbicide atrazine.

Table 2. DART measured masses for $[M+H]^+$ from atrazine and trace impurities.

Compound	Composition	Measured	Calculated	Diff. (mmu)
Atrazine	$C_8H_{15}N_5Cl$	216.10159	216.10160	−0.01
Propazine	$C_9H_{17}N_5C$	230.11760	230.11725	+ 0.35
Simazine	$C_7H_{13}N_5Cl$	202.08440	202.08595	+1.60

saccutof_dart.html. Chemical agent data is available from the authors upon request.

References

[1] Dempster, A. J. *Phys. Rev*., **11**, 316-324, (1918).

[2] Munson, M. S. B.; Franklin, F. H. *J. Am. Chem. Soc*., **88**, 2621, (1966).

[3] Barber, M.; Bordoli, R. S.; Elliott, G. J.; Sedgwick, R. D.; Tyler, A. N. *J. Chem. Soc. Chem. Commun.*, **325**, (1981).

[4] Beckey H. D. *Research/Development*, **20**(11), 26-29, (1969).

[5] Horning, E. C.; Horning, M. E.; Carroll, D. I.; Dzidic, I.; Stilwell, R. N.; *Anal. Chem*., **45**, 936-943, (1973).

[6] Dole, M. Mack, L. L. Hines, R. L.; Mobley, R. C.; Ferguson, L. D. Alice, M. A. *J. Chem. Phys*., **49**, 2240, (1968).

[7] Aleksandrov, M. L.; Gall, L. N. Krasnov, N. V. Nikolaev, V. I. Pavlenko, V. A.; Shkurov, V. A. *Dokl. Akad. Nauk. SSSR*, **277**, 379-383, (1984).

[8] Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F. *Science*, **246**, 64-71, (1989).

[9] Tanaka, K.; Waki, H.; Ido, Y; Akita, S.; Yoshida, Y. *Rapid. Commun. Mass Spectrom*., **2**, 151-153, (1988).

[10] Karas, M.; Hillenkamp, F. *Anal. Chem*., **60**, 2299-2301, (1988).

[11] Robb, D. B.; Covey, T. R.; Bruins, A. P. *Anal. Chem*., **72**, 3653-3659, (2000).

[12] Cody, R. B.;Laramee, J. A. ; Durst, H. D. *Anal. Chem*., **77**(8), 2297 – 2302, (2005).

[13] Patents pending.

[14] Penning, F. M. *Naturwissenschaften*, **15**, 818, (1927).

[15] Mastwijk, H. C. Cold Collisions of Metastable Helium Atoms, Ph.D. Thesis, University of Utrecht, Netherlands, (1997).

[16] Faubert, D.; Paul, G.J.C., Giroux, J.; Bertrand, M. J. Int. J. Mass Spectrom. Ion Proc., 124, 69, (1993).

[17] Faubert, D.; L'Heureux, A.; Peraldi, O.; Mousselmal, M.; Sanchez, G.; Bertrand, M. J.; "Metastable Atom Bombardment (MAB) Ionization Source: Design, Optimization and Analytical Performances" in Adv. Mass Spectrom.: 15th International Mass Spectrometry Conference, Wiley: Chichester, UK, 431-432, (2001).

[18] http://www.jeol.com/ms/docs/map_note.pdf.

[19] Tsuchiya, M. Kuwabara, H.; *Anal. Chem*., **56**, 14, (1984).

[20] Tsuchiya, M. *Mass Spectrom. Rev*., **17**, 51, (1998).

[21] Tsuchiya, M. *Analytical Sciences*,, **14**, 661-676, (1998).

[22] Hiraoka, K.; Fujimaki, S.; Kambara, S.; Furuya, H.; Okazaki, S. *Rapid Commun. Mass Spectrom*., **18**, 2323-2330, (2004).

[23] McLuckey, S. A.; Glish, G. L.; Asano, K. G.; Grant, B. C., *Anal. Chem*., **60**, 2220, (1988).

[24] Guzowski, J. P., Jr.; Broekaert, J. A. C.; Ray, S. J.; Hieftje, G. M. J. *Anal. At. Spectrom*., **14**, 1121-1127, (1999).

[25] IonSense, Inc., 11 Dearborn Road, Peabody, MA USA 01960.

[26] Jagerdeo, E.; Cody, R. B. unpublished results.