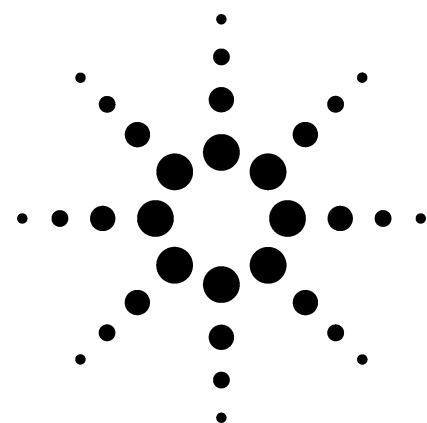


Analysis of Trace Residues of Explosive Materials by Time-of-Flight LC/MS

Application

Forensics



Authors

Russell Kinghorn and Courtney Milner
Baseline Separation Technologies Pty Ltd
41 Greenway Street
Bulleen, VIC 3105
Australia

Jerry Zweigenbaum
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

A key technique used in trace explosives analysis is HPLC with UV detection, following the guidelines set out in USEPA method 8330. Although sensitive for many target explosives, the method is limited by a lack of detector selectivity. This application note outlines the benefits and limitations of the use of liquid chromatography/time-of-flight mass spectrometry (LC/TOFMS) for the detection and quantitation of trace levels of these explosive residues.

Introduction

The identification of explosive residues in crime scene forensic investigation, environmental site remediation, and homeland security is an analysis of major significance to both public and regulatory authorities. The traditional and most commonly accepted method for the analysis of the nitroaromatic class of explosives is USEPA

Method 8330. This method provides a sensitive UV-based analysis of 14 nitroaromatics and nitramines. However, due to the lack of selectivity provided by UV detection, confirmation of the species present requires the analysis to be performed on two analytical columns with different stationary phases.

The terrorist attacks on 9/11/2001, and subsequent attacks around the world have brought a new focus onto the identification and quantitation of explosive residues in crime scene investigation and homeland security. One of the front lines of homeland security, airport departure gates, uses sophisticated screening devices such as ion mobility spectrometers. These devices, though sensitive, face selectivity limitations in that they cannot determine the explosive species present. Additionally, terrorists are becoming increasingly erudite, as was seen in the attempt by Richard Reid in late December 2001. He used a peroxide-based explosive within his shoes, which was not detectable at trace levels using the analytical techniques commonly used for explosives analysis.

Inherent to the nature of explosive compounds is their instability, and propensity to breakdown. One of the best known and most common explosive compounds, trinitrotoluene (TNT) is reduced by bacteria to 2-amino-4,6-dinitrotoluene, (2-AMDNT) and 4-amino-2,6-dinitrotoluene (4-AMDNT); a metabolism that occurs also in plants and animals. Both of these compounds are markers for the former presence of TNT, and are also known to show severe toxicity and mutagenicity, making them important environmental markers.



An extensive search of the literature found several articles detailing the analysis of explosive materials using liquid chromatography (LC) [1-4]. However, of the 14 explosive materials of interest, very few, in particular TNT and RDX, were readily identified using mass spectrometry (MS) [5-7].

There still exists the requirement for a reliable and sensitive confirmatory technique of analysis for these explosive residues that can be performed on samples from a wide variety of sources. Liquid chromatography/mass spectrometry (LC/MS) provides an excellent tool for this analysis with the ability to couple the mass spectrometer to existing instruments performing USEPA method 8330. Furthermore, the choice of a mass selective detector (MSD) can provide confirmatory information previously required through the use of a second analytical column.

Accurate mass measurement, such as provided by the Agilent LC/MSD TOF time-of-flight mass

spectrometer (LC/MSD TOF), greatly increases the confidence of identification because it inherently limits the possible number of candidate compounds. The better the precision and accuracy of the mass measurement, the fewer compounds are theoretically possible. This is particularly useful when needing to analyze samples from a variety of sources, each with their own potential interferences such as those encountered with explosives residues.

This application note demonstrates the utility of the LC/MSD TOF for the determination of low level explosives. The LC/MSD TOF provides accurate mass determination (better than 3 ppm) and linearity to three orders of magnitude, and thus is an excellent tool for the detection, confirmation, and quantitation of explosive compound residues.

The explosives studied are shown in Tables 1 and 2, including the chemical structure and theoretically calculated exact mass.

Table 1. Names, Abbreviations and Molecular Formulae of Explosives Studied

| # | Name | Abbreviation | CAS no. | Molecular formula |
|----|--|--------------|-------------|---|
| 1 | Hexamethylenetriperoxidediamine | HMTD | NA | C ₆ H ₁₂ N ₂ O ₆ |
| 2 | Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine | HMX | 2691-41-0 | C ₄ H ₈ N ₆ O ₈ |
| 3 | Hexahydro-1,3,5-trinitro-1,3,5-triazine | RDX | 121-82-4 | C ₃ H ₆ N ₆ O ₆ |
| 4 | 1,3,5-triamino-2,4,6-trinitrobenzene | TATB | 3058-38-6 | C ₆ H ₆ N ₆ O ₆ |
| 5 | Ethylene glycol dinitrate | EGDN | 628-96-6 | C ₂ H ₄ N ₂ O ₆ |
| 6 | 1,3,5-Trinitrobenzene | 1,3,5-TNB | 99-35-4 | C ₆ H ₃ N ₃ O ₆ |
| 7 | 1,3-Dinitrobenzene | 1,3-DNB | 99-65-0 | C ₆ H ₄ N ₂ O ₄ |
| 8 | Methyl-2,4,6-trinitrophenylnitramine | Tetryl | 479-45-8 | C ₇ H ₅ N ₅ O ₈ |
| 9 | 4-amino-2,6-dinitrotoluene | 4A-DNT | 1946-51-0 | C ₇ H ₇ N ₃ O ₄ |
| 10 | Nitrobenzene | NB | 98-95-3 | C ₆ H ₅ NO ₂ |
| 11 | Nitroglycerin | NG | 55-63-0 | C ₃ H ₅ N ₃ O ₉ |
| 12 | 2-amino-4,6-dinitrotoluene | 2A-DNT | 355-72-78-2 | C ₇ H ₇ N ₃ O ₄ |
| 13 | 2,4,6-Trinitrotoluene | TNT | 118-96-7 | C ₇ H ₅ N ₃ O ₆ |
| 14 | 2,6-Dinitrotoluene | 2,6-DNT | 606-20-2 | C ₇ H ₆ N ₂ O ₄ |
| 15 | 2,4-Dinitrotoluene | 2,4-DNT | 121-14-2 | C ₇ H ₆ N ₂ O ₄ |
| 16 | Hexanitrostilbene | HNS | 19138-90-0 | C ₁₄ H ₆ N ₆ O ₁₂ |
| 17 | 2-Nitrotoluene | 2-NT | 88-72-2 | C ₇ H ₇ NO ₂ |
| 18 | 4-Nitrotoluene | 4-NT | 99-99-0 | C ₇ H ₇ NO ₂ |
| 19 | Pentaerythritol tetranitrate | PETN | 78-11-5 | C ₅ H ₈ N ₄ O ₁₂ |
| 20 | 3-Nitrotoluene | 3-NT | 99-08-1 | C ₇ H ₇ NO ₂ |
| 21 | Triacetone triperoxide | TATP | NA | C ₉ H ₁₈ O ₆ |
| 22 | Carbamite | Carbamite | NA | C ₁₇ H ₂₀ N ₂ O |

NA Not applicable

Table 2. Molecular Structures and Calculated Accurate Masses of Explosives Studied

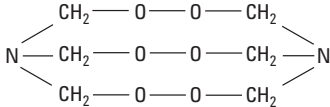
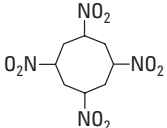
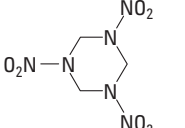
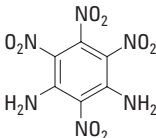

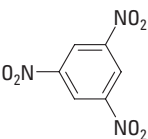
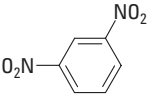
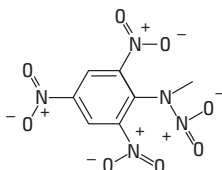
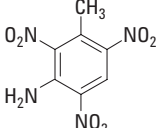
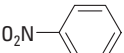
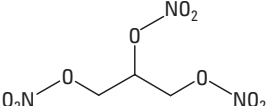
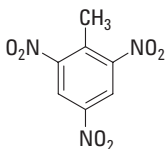
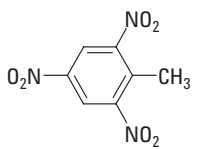
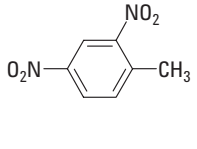
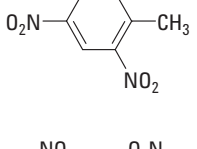
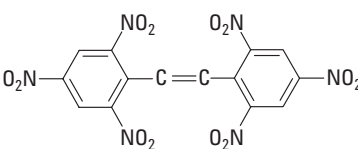
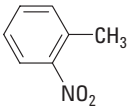
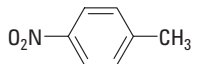
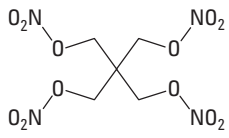
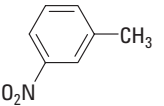
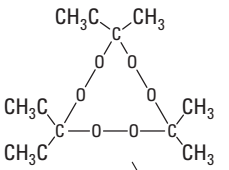
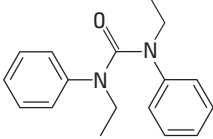
| # | Name | Molecular formula | Structure | Molecular weight |
|----|-----------|-------------------|---|------------------|
| 1 | HMTD | $C_6H_{12}N_2O_6$ |  | 208.0695 |
| 2 | HMX | $C_8H_8N_8O_8$ |  | 296.0465 |
| 3 | RDX | $C_3H_6N_6O_6$ |  | 222.0349 |
| 4 | TATB | $C_6H_6N_6O_6$ |  | 258.0349 |
| 5 | EGDN | $C_2H_4N_2O_6$ |  | 152.0069 |
| 6 | 1,3,5-TNB | $C_6H_3N_3O_6$ |  | 213.0022 |
| 7 | 1,3-DNB | $C_6H_4N_2O_4$ |  | 168.0171 |
| 8 | Tetryl | $C_7H_5N_5O_8$ |  | 287.0138 |
| 9 | 4A-DNT | $C_7H_7N_3O_4$ |  | 197.0437 |
| 10 | NB | $C_6H_5NO_2$ |  | 123.0320 |
| 11 | NG | $C_3H_5N_3O_9$ |  | 227.0026 |

Table 2. Molecular Structures and Calculated Accurate Masses of Explosives Studied (continued)

| # | Name | Molecular formula | Structure | Molecular weight |
|----|-----------|---|---|------------------|
| 12 | 2A-DNT | C ₇ H ₇ N ₃ O ₄ |  | 197.0437 |
| 13 | TNT | C ₇ H ₅ N ₃ O ₆ |  | 227.0178 |
| 14 | 2,6-DNT | C ₇ H ₆ N ₂ O ₄ |  | 182.0328 |
| 15 | 2,4-DNT | C ₇ H ₆ N ₂ O ₄ |  | 182.0328 |
| 16 | HNS | C ₁₄ H ₆ N ₆ O ₁₂ |  | 450.0044 |
| 17 | 2-NT | C ₇ H ₇ NO ₂ |  | 137.0477 |
| 18 | 4-NT | C ₇ H ₇ NO ₂ |  | 137.0477 |
| 19 | PETN | C ₅ H ₈ N ₄ O ₁₂ |  | 316.0139 |
| 20 | 3-NT | C ₇ H ₇ NO ₂ |  | 137.0477 |
| 21 | TATP | C ₉ H ₁₈ O ₆ |  | 222.1103 |
| 22 | Carbamite | C ₁₇ H ₂₀ N ₂ O |  | 268.1576 |

Methodology

The work undertaken in this study was performed on an Agilent 1100 LC system consisting of: binary pump, autosampler, thermostatted column compartment, and the LC/MSD TOF.

LC Conditions

| | | | |
|--------------------|--|-------------------|----------------|
| Solvents | Methanol and water | | |
| Flow rate | 0.9 mL/min | | |
| Gradient | Time (min) | % Methanol | % Water |
| | 0 | 60 | 40 |
| | 1 | 60 | 40 |
| | 15 | 92 | 8 |
| | 16 | 100 | 0 |
| | 18 | 100 | 0 |
| | 19 | 60 | 40 |
| Post time | 5 minutes | | |
| Total run time | 24 minutes | | |
| Injection volume | 10 μ L, with needle wash | | |
| Column temperature | 40 $^{\circ}$ C | | |
| Column | ZORBAX Extend-C18 4.6 mm \times 250 mm, 5 μ m p/n 770450-902 | | |

MS Detection conditions

| | |
|-----------------------|------------------|
| Ionization | APCI |
| Gas temperature | 350 $^{\circ}$ C |
| Vaporizer temperature | 325 $^{\circ}$ C |
| Drying gas flow | 5 L/min |
| Nebulizer pressure | 40 psig |
| PCI Corona current | 4 μ A |
| PCI Capillary voltage | 4000 V |
| NCI Corona current | 10 μ A |
| NCI Capillary voltage | 1500 V |
| Scan m/z range | 70–1000 |
| Fragmentor voltage | 100 V |
| Storage mode | Profile |
| Skimmer | 60 V |
| Oct RF | 250 V |

The experimental conditions listed above were optimized for sensitivity. Vaporizer temperature, drying gas temperature and flow rate, corona current, capillary voltage, and fragmentor voltage were all optimized.

A large increase in signal was observed by reducing the drying gas flow rate from 6 L/min to 5 L/min. This resulted in a 30% increase in signal area for more than 80% of the explosives under investigation.

Reference Mass Introduction with LC/MSD TOF

The Agilent LC/MSD TOF uses a reference mass in the generation of reliable accurate masses. The electrospray source for the LC/MSD TOF is a unique dual spray assembly that allows the simultaneous and constant introduction of a reference mass solution. When using APCI (atmospheric pressure chemical ionization), the reference masses must be introduced into the mobile phase post-column.

This was achieved via a low dead-volume tee connected prior to the APCI source with PEEKTM tubing. An isocratic pump was used to deliver the reference mix at a flow rate of 50 μ L/min in positive ion mode and flow programmed from 70 μ L/min to 150 μ L/min in negative ion mode over the run time of 1 to 15 minutes. In order to ensure pulse-less reference mass introduction a rapid resolution column (ZORBAX SB-C18, 30 mm \times 2.1 mm, 5 μ m, part number 873700-902) was installed in the flow path, providing backpressure for the isocratic pump.

The reference mix was modified to suit the methodology, 25 μ L of purine and 250 μ L of HP-0921 was added to 250 mL of 90:10 methanol:water.

This enabled the use of the following reference masses:

Positive ion mode: 121.050873 and 922.009798
Negative ion mode: 119.036320, 966.000725, and 980.016375

In negative ion mode, with increasing organic mobile phase strength, the reference masses 966.000725 and 980.016375 decrease in intensity. By using the custom reference mass mix outlined above and the use of flow programming, sufficient abundance of the reference mass ions is maintained throughout the analytical run.

With the paucity of literature discussing the detection of explosives by LC/MS, the first step of development was evaluating component responses under both electrospray (ESI) and atmospheric pressure chemical ionization (APCI) in both positive and negative ion modes. Table 3 lists the response characteristics for many of the compounds tested in this study and it clearly shows that no one ionization and detection technique is universally applicable.

Table 3. Detection Modes for Various Explosives

| Compound | UV/Visible | ESI + | ESI - | APCI + | APCI - |
|---------------|------------|-------|-------|--------|--------|
| HMTD | √ | × | × | √ | × |
| HMX | √ | × | × | × | √ |
| RDX | √ | × | √ | × | √ |
| TATB | √ | × | × | × | √ |
| EGDN | × | × | × | × | P |
| 1,3,5-TNB | √ | × | √ | × | √ |
| 1,3-DNB | √ | × | × | × | √ |
| Tetryl | √ | × | × | × | √ |
| 4A-DNT | √ | × | × | × | √ |
| Nitrobenzene | √ | × | × | × | P |
| Nitroglycerin | P | × | × | × | P |
| 2A-DNT | √ | × | × | × | √ |
| TNT | √ | × | √ | × | √ |
| 2,6-DNT | √ | × | × | × | √ |
| 2,4-DNT | √ | × | √ | × | √ |
| HNS | √ | × | × | × | √ |
| 2-NT | √ | × | × | × | P |
| 4-NT | √ | × | × | × | P |
| PETN | P | × | √ | × | √ |
| 3-NT | √ | × | × | × | P |
| TATP | P | × | × | P | × |
| Carbamite | √ | √ | × | √ | × |

√ Good response

P Poor response

× No response

It was observed that negative APCI provided the best response for most explosives studied, and if run in positive APCI mode as well, additional components are detected. Negative APCI also has the advantage of being very selective, removing possible matrix interferences. The ability to couple UV detection prior to the mass spectrometer also provides a highly capable analysis for explosives. However, the major advantage of LC/MS over UV detection is the ability to detect the newer, more terrorist-friendly explosives such as TATP and HMTD in positive APCI mode. These peroxide explosives are reported to degrade when exposed to intense sources of UV light, such as what might be experienced in a UV detector.

A key parameter considered during the development of the method was the ability to transfer the HPLC method between different detectors. This precluded the use of nonvolatile buffers which would be detrimental to MS detection. Initial analyses investigated the use of buffers such as acetic acid, formic acid, ammonium acetate, and ammonium formate. While in many cases the chromatographic separation was improved, signal response was compromised. Using the high selectivity of the LC/MSD TOF, signal intensity was chosen as the key parameter to optimize. The addition of chloroform in APCI mode can also increase sensitivity with some explosive compounds; however, it was found in this study that the majority of compounds are best analyzed with no organic modifier present.

Various HPLC columns, mobile phase compositions, and gradients were also tested in this investigation. The conditions finally used were chosen for their selectivity, speed of analysis, and detection limits (DLs) attainable with MS.

Detection of Explosives Using the LC/MSD TOF

An overwhelming advantage of using the LC/MSD TOF for the trace level detection of any component is the confirmatory information that is provided through accurate mass measurement. An example of this mass accuracy is shown in Table 4, where observed masses and their deviations from the theoretical exact mass are shown.

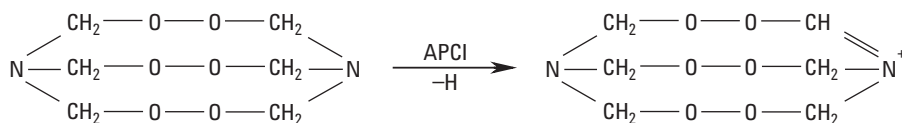
The ability to closely match the expected mass and the observed mass provides the analyst with a very high level of confidence in the assignment given to a chromatographic peak.

Table 4. Theoretical Exact Mass, Observed Mass, Mass Error, and Limit of Quantitation (LOQ) Using the LC/MSD TOF

| Compound | Monoisotopic mass | Adduct | Adduct mass | Observed mass | Mass error (ppm) | LOQ ($\mu\text{g/L}$) |
|-----------|-------------------|--|----------------------|---------------|------------------|-------------------------|
| HMTD | 208.0695 | $[\text{M}-\text{H}]^+$ | 207.0611 | 207.0612 | 0.18 | 30 |
| HMX | 296.0465 | $[\text{M}+\text{CHO}_2]^-$ | 341.0447 | 341.0446 | -0.11 | 10 |
| RDX | 222.0349 | $[\text{M}+\text{CHO}_2]^-$ | 267.0330 | 267.0328 | -1.07 | 0.5 |
| TATB | 258.0349 | $[\text{M}-\text{H}]^-$ | 257.0276 | 257.0276 | -0.02 | 5 |
| EGDN | 152.0069 | | No response by TOFMS | | | |
| 1,3,5-TNB | 213.0022 | $[\text{M}]^-$ | 213.0027 | 213.0026 | -0.63 | 15 |
| 1,3-DNB | 168.0171 | $[\text{M}]^-$ | 168.0176 | 168.0175 | -0.92 | 10 |
| Tetryl | 287.0138 | $[\text{M}-\text{NO}_2]^-$ | 241.0214 | 241.0214 | -0.24 | 5 |
| 4A-DNT | 197.0437 | $[\text{M}-\text{H}]^-$ | 196.0363 | 196.0362 | -0.92 | 10 |
| NB | 123.0320 | | No response by TOFMS | | | |
| NG | 227.0026 | | No response by TOFMS | | | |
| 2A-DNT | 197.0437 | $[\text{M}-\text{H}]^-$ | 196.0363 | 196.0364 | 0.92 | 5 |
| TNT | 227.0178 | $[\text{M}]^-$ | 227.0183 | 227.0178 | -2.6 | 4 |
| 2,6-DNT | 182.0328 | $[\text{M}]^-$ | 182.0333 | 182.0331 | -1.1 | 8 |
| 2,4-DNT | 182.0328 | $[\text{M}]^-$ | 182.0333 | 182.0331 | -1.1 | 4 |
| HNS | 450.0044 | $[\text{M}]^-$ | 450.0049 | 450.0042 | -1.6 | 1 |
| 2-NT | 137.0477 | $[\text{M}-\text{H}]^-$ | 136.0404 | 136.0406 | 1.5 | 100 |
| 4-NT | 137.0477 | $[\text{M}-\text{H}]^-$ | 136.0404 | 136.0407 | 2.2 | 50 |
| PETN | 316.0139 | $[\text{M}-\text{NO}_2 + \text{CH}_2\text{O}_2]^-$ | 316.0269 | 316.0267 | -0.94 | 250 |
| 3-NT | 137.0477 | $[\text{M}]^-$ | 137.0482 | 137.0480 | -1.7 | 5000 |
| TATP | 222.1103 | Unassigned | ND | 89.0597 | ND | 1000 |
| Carbamite | 268.1576 | $[\text{M}+\text{H}]^+$ | 269.1659 | 269.1665 | 2.1 | 10 |

ND Not determined

A powerful result of accurate mass measurement was the ability to assign the ion formed by positive APCI of HMTD. The paper by Xu et al [8] assigned the ion observed for HMTD as being the $[\text{M}-1]^+$ species. It was not clear what the ion was and thus it was identified only as a loss of one mass unit. Using the accurate mass data obtained from the Agilent LC/MSD TOF, this ion can be assigned as the $[\text{M}-\text{H}]^+$ species, as the likely result of the multiple peroxide linkages in close association with a nitrogen atom. Note that the measured mass in Table 4 shows a loss of hydrogen. This is shown in Figure 1 below.

**Figure 1. The theoretical positive ion formed from HMTD using APCI.**

Further, a high degree of mass accuracy can increase the detection limit (DL), as noise is effectively reduced by narrowing the monitored mass range. This can be shown by observing the signal-to-noise (S/N) of RDX over a mass window of 0.1 amu (similar to what can be achieved on a single quadrupole system) and a mass window of 0.01 amu for a 1 µg/L (ppb) solution (Table 5).

Table 5. Calculated S/N for a 1 µg/L RDX at Different Mass Extraction Windows

| Extracted ion range | Noise time range | Mean noise | P-P noise | Peak height | S/N (P-P) |
|---------------------|------------------|------------|-----------|-------------|-----------|
| 267.0–267.1 | 3.509–3.692 | 21.4 | 57.0 | 285.5 | 5.0 |
| 267.03–267.04 | 3.509–3.692 | 5.2 | 21.0 | 245.5 | 11.7 |

A greater than two-fold increase in sensitivity is seen for these compounds. Figure 2 shows the reduction in noise that is observed with the extraction of a narrower mass range, a critical factor in confirmation when dealing with complex matrices.

An interesting observation that was made at higher concentrations was the dominance of a different adduct. This was a particular feature of HMX and RDX, whereby at high concentrations the adduct formed was $[M + CH_2O_2]^-$ instead of the otherwise observed $[M + CHO_2]^-$. This radical anion

adduct could be explained by a charge exchange catalyzed by the very high concentration of ions/molecules in the APCI source. This split of signal would also explain the highly accurate mass measurement in spite of the high concentration that typically causes detector saturation and loss of accuracy.

Mass Accuracy with Concentration

The mass accuracy of the LC/MSD TOF was evaluated for four of the explosive compounds over a concentration range of 100,000 µg/L (100 ppm) to 1 µg/L (1 ppb) and is shown in Tables 6–9. The mass accuracy data was obtained from observing the mass spectral data at the apex of a plus/minus 1 amu extracted window of the accurate mass. The % RSD for each mass is reported and the mass error from the average mass. It should be noted that the error for the 100,000 µg/L HMX solution is for the previously mentioned $[CH_2O_2]^-$ adduct. Saturation of the detector at high concentrations is known to cause a loss of mass accuracy as shown in the results. For HMX and RDX the low concentration and low signal intensity resulted in a reduced mass accuracy as well. Higher signal intensity for the two other compounds, TNT and 2A-DNT, resulted in mass accuracy less than 2 ppm at the 1 µg/L concentration.

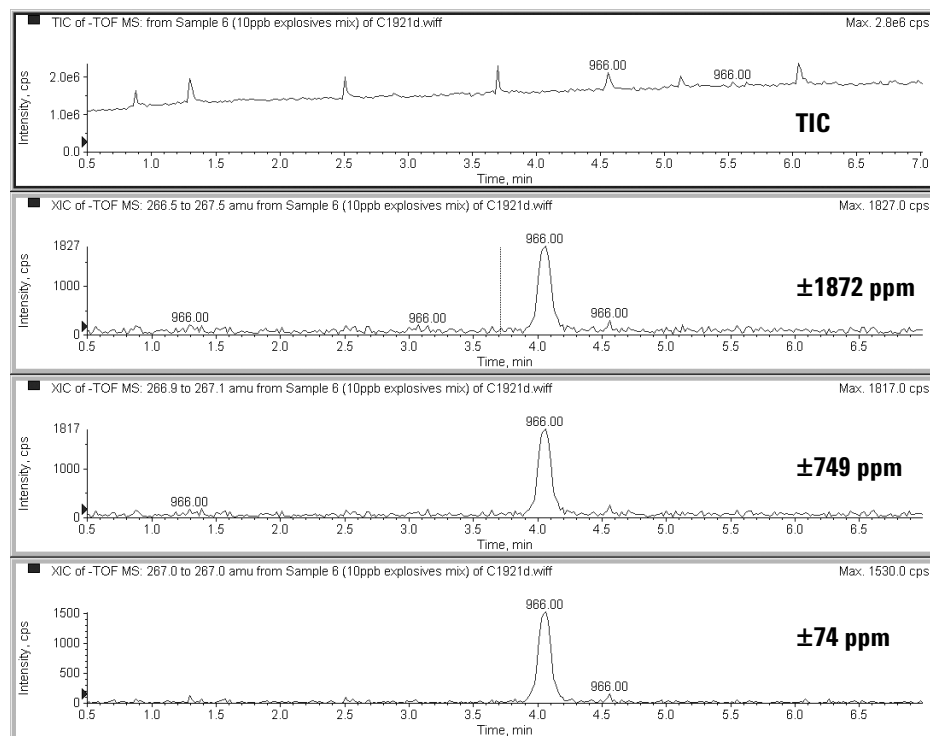


Figure 2. Effect of extracted ion range on noise of 10 µg/L RDX. The value given in each panel is the mass range extracted in parts per million (ppm) of expected exact mass of RDX.

Table 6. Mass Accuracy at Five Concentration Levels (1–100,000 µg/L) for HMX

| Replicate | 1 | 10 | 100 | 1,000 | 10,000 | 100,000 |
|--------------------|----------|----------|----------|----------|----------|----------|
| 1 | 341.0453 | 341.0444 | 341.0441 | 341.0449 | 341.0444 | 342.0668 |
| 2 | 341.0425 | 341.0461 | 341.0444 | 341.0445 | 341.0445 | 342.0645 |
| 3 | 341.0429 | 341.0446 | 341.0441 | 341.0446 | 341.0445 | 342.0628 |
| 4 | 341.0418 | 341.0445 | 341.0443 | 341.0444 | 341.0444 | 342.0651 |
| 5 | 341.0416 | 341.0457 | 341.0447 | 341.0443 | 341.0445 | 342.0600 |
| Average | 341.0428 | 341.0451 | 341.0443 | 341.0445 | 341.0445 | 342.0638 |
| SD | 0.0015 | 0.0008 | 0.0002 | 0.0002 | 0.0001 | 0.0026 |
| Error (ppm) | -5.6 | 1.14 | -1.2 | -0.62 | -0.62 | 34.61 |

Table 7. Mass Accuracy at Five Concentration Levels (1–100,000 µg/L) for RDX

| Replicate | 1 | 10 | 100 | 1,000 | 10,000 | 100,000 |
|--------------------|----------|----------|----------|----------|----------|----------|
| 1 | 267.036 | 267.0328 | 267.0345 | 267.0329 | 267.0324 | 267.0333 |
| 2 | 267.0357 | 267.033 | 267.0341 | 267.0331 | 267.0328 | 267.0336 |
| 3 | 267.0354 | 267.0314 | 267.0338 | 267.0331 | 267.0325 | 267.0335 |
| 4 | 267.0371 | 267.0326 | 267.033 | 267.0332 | 267.0327 | 267.0333 |
| 5 | 267.0297 | 267.0349 | 267.0334 | 267.0331 | 267.0322 | 267.0335 |
| Average | 267.0348 | 267.0329 | 267.0338 | 267.0331 | 267.0325 | 267.0334 |
| SD | 0.0029 | 0.0013 | 0.0006 | 0.0001 | 0.0002 | 0.0001 |
| Error (ppm) | 6.4 | -0.69 | 2.7 | -0.06 | -2.2 | 1.2 |

Table 8. Mass Accuracy at Five Concentration Levels (1–100,000 µg/L) for TNT

| Replicate | 1 | 10 | 100 | 1,000 | 10,000 | 100,000 |
|--------------------|----------|----------|----------|----------|----------|----------|
| 1 | 227.0174 | 227.0180 | 227.0176 | 227.0177 | 227.0185 | 227.0457 |
| 2 | 227.0178 | 227.0162 | 227.0179 | 227.0176 | 227.0184 | 227.0416 |
| 3 | 227.0184 | 227.0173 | 227.0180 | 227.0177 | 227.0183 | 227.0346 |
| 4 | 227.0173 | 227.0170 | 227.0181 | 227.0177 | 227.0183 | 227.0360 |
| 5 | 227.0197 | 227.0193 | 227.0181 | 227.0176 | 227.0184 | 227.0318 |
| Average | 227.0181 | 227.0176 | 227.0179 | 227.0177 | 227.0184 | 227.0379 |
| SD | 0.0010 | 0.0012 | 0.0002 | 0.0001 | 0.0001 | 0.0056 |
| Error (ppm) | -1.2 | -3.5 | -2.1 | -3.01 | 0.072 | 86 |

Table 9. Mass Accuracy at Five Concentration Levels (1–100,000 µg/L) for 2A-DNT

| Replicate | 1 | 10 | 100 | 1,000 | 10,000 | 100,000 |
|--------------------|----------|----------|----------|----------|----------|----------|
| 1 | 196.0375 | 196.0364 | 196.0361 | 196.0357 | 196.0399 | 196.0859 |
| 2 | 196.0371 | 196.0366 | 196.0361 | 196.0361 | 196.0397 | 196.0819 |
| 3 | 196.0360 | 196.0369 | 196.0364 | 196.0359 | 196.0397 | 196.0786 |
| 4 | 196.0358 | 196.0358 | 196.0368 | 196.0358 | 196.0390 | 196.0799 |
| 5 | 196.0368 | 196.0364 | 196.0364 | 196.0359 | 196.0394 | 196.0770 |
| Average | 196.0366 | 196.0364 | 196.0364 | 196.0359 | 196.0395 | 196.0807 |
| SD | 0.0007 | 0.0004 | 0.0003 | 0.0001 | 0.0004 | 0.0034 |
| Error (ppm) | 1.1 | 0.11 | 0.11 | -2.4 | 16 | 230 |

Area Repeatability

Time-of-flight mass spectrometers have traditionally had a reputation as being unsuitable for quantitation and the provision of repeatable areas.

The area repeatability for the LC/MSD TOF was investigated at multiple levels for three of the explosive components. Generally, the LC/MSD TOF showed repeatability across five runs of better than 5% RSD. However, sometimes when approaching the LOQ, this would increase to a larger error. The area repeatability for RDX, TNT, and 2A-DNT for five injections at each concentration level analyzed are shown in Tables 10–12.

Table 10. RDX Concentration ($\mu\text{g/L}$)

| Replicate | 1 | 10 | 100 | 1,000 | 10,000 | 100,000 |
|----------------|-------|-------|-------|--------|---------|----------|
| 1 | 426 | 1890 | 12300 | 154000 | 2540000 | 14100000 |
| 2 | 642 | 1780 | 13000 | 143000 | 2450000 | 15200000 |
| 3 | 541 | 1820 | 13300 | 146000 | 2460000 | 15300000 |
| 4 | 659 | 2620 | 14000 | 141000 | 2330000 | 14900000 |
| 5 | 508 | 2760 | 13600 | 149000 | 2130000 | 14700000 |
| Average | 555.2 | 2174 | 13240 | 146600 | 2382000 | 14840000 |
| SD | 96 | 475 | 642 | 5128 | 159593 | 477493 |
| %RSD | 17.42 | 21.86 | 4.85 | 3.5 | 6.7 | 3.22 |

Table 11. TNT Concentration ($\mu\text{g/L}$)

| Replicate | 1 | 10 | 100 | 1,000 | 10,000 | 100,000 |
|----------------|------|-------|--------|---------|----------|----------|
| 1 | 4760 | 16400 | 127000 | 1730000 | 20700000 | 74800000 |
| 2 | 4330 | 16600 | 134000 | 1700000 | 20600000 | 73300000 |
| 3 | 4490 | 16500 | 134000 | 1840000 | 20900000 | 71600000 |
| 4 | 4200 | 16200 | 134000 | 1790000 | 20400000 | 71300000 |
| 5 | 3990 | 16100 | 132000 | 1830000 | 19600000 | 71200000 |
| Average | 4354 | 16360 | 132200 | 1778000 | 20440000 | 72440000 |
| SD | 291 | 207 | 3033 | 61400 | 502991 | 1569394 |
| %RSD | 6.7 | 1.27 | 2.29 | 3.45 | 2.46 | 2.17 |

Table 12. 2A-DNT Concentration ($\mu\text{g/L}$)

| Replicate | 1 | 10 | 100 | 1,000 | 10,000 | 100,000 |
|----------------|------|------|-------|--------|----------|----------|
| 1 | 2300 | 7820 | 68400 | 779000 | 9720000 | 27600000 |
| 2 | 2440 | 9040 | 64500 | 807000 | 10400000 | 28800000 |
| 3 | 2340 | 8910 | 66200 | 862000 | 10400000 | 30800000 |
| 4 | 2250 | 8760 | 65900 | 849000 | 9690000 | 28400000 |
| 5 | 2350 | 7830 | 77800 | 940000 | 10100000 | 29600000 |
| Average | 2336 | 8472 | 68560 | 847400 | 10062000 | 29040000 |
| SD | 70 | 598 | 5350 | 61443 | 348310 | 1219836 |
| %RSD | 3.01 | 7.07 | 7.8 | 7.25 | 3.46 | 4.2 |

TOF Linearity

The linearity of the LC/MSD TOF was investigated for a range of the components in the mixture. Of the 10 components evaluated, most exhibited a linear regression coefficient of variation of greater than 0.998. Some of the compounds displayed excellent linearity across the four orders of magnitude. A linear dynamic range for this instrument is typically two-to-three orders of magnitude. As can be seen in the repeatability results for RDX, the area response is very linear between 10 and 10,000 µg/L. The 100,000 µg/L showed saturation and the 1 µg/L showed a less than 5x decrease in signal vs the nearly 10x for the other concentrations. Figures 3 to 5 show representative calibration curves for 3 of the 10 components evaluated.

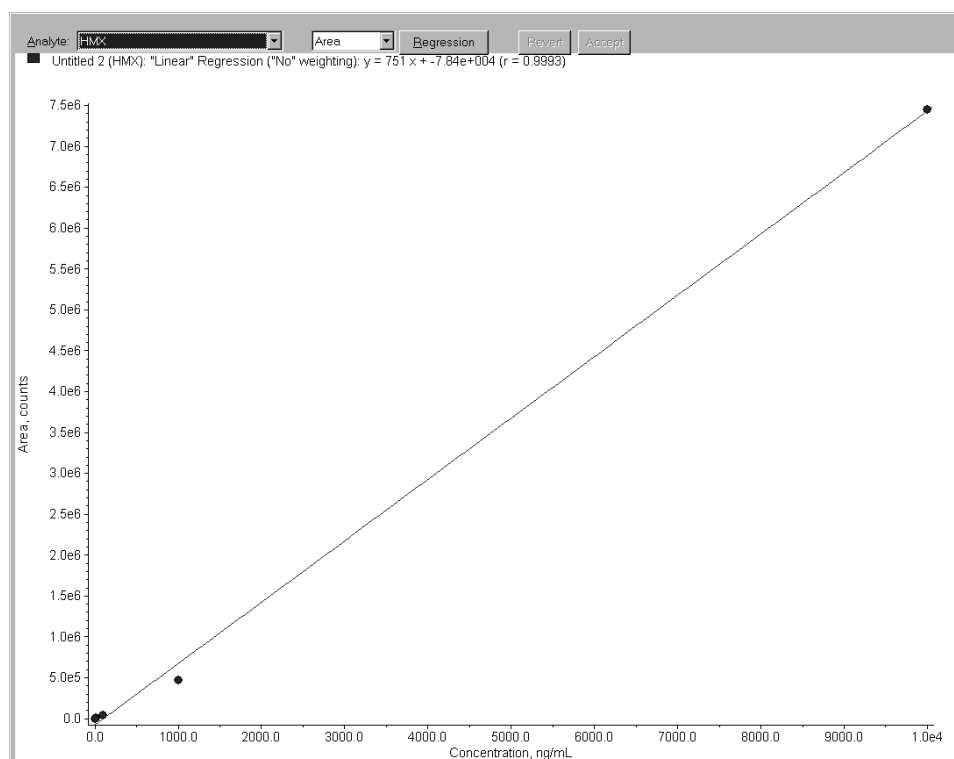


Figure 3. Calibration curve for HMX from 1 µg/L to 10,000 µg/L with MSD TOF.

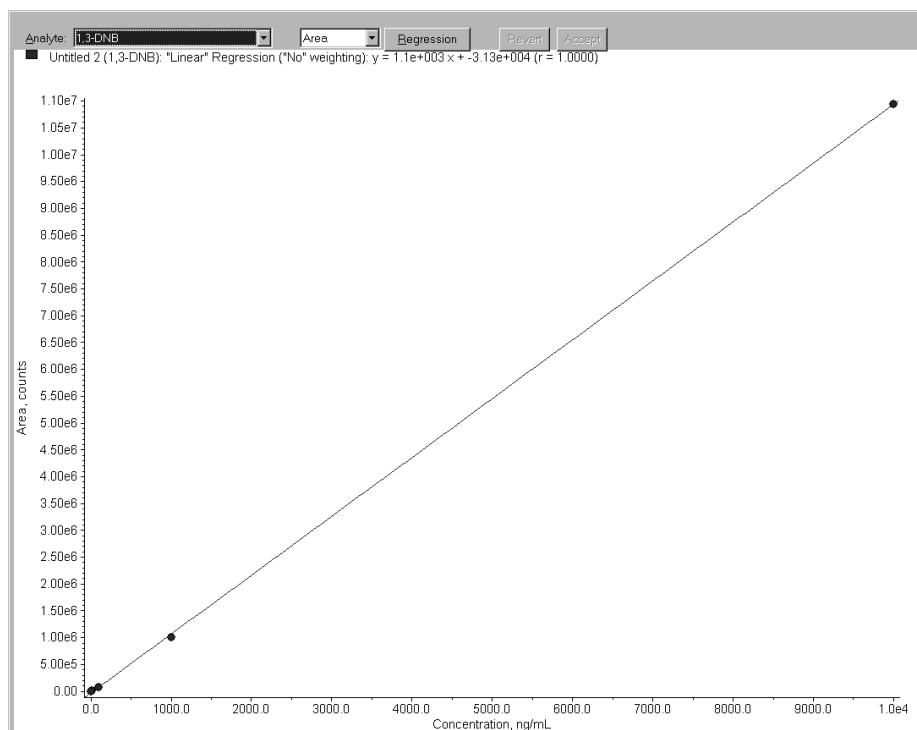


Figure 4. Calibration curve for 1,3-DNB from 1 µg/L to 10,000 µg/L with LC/MSD TOF.

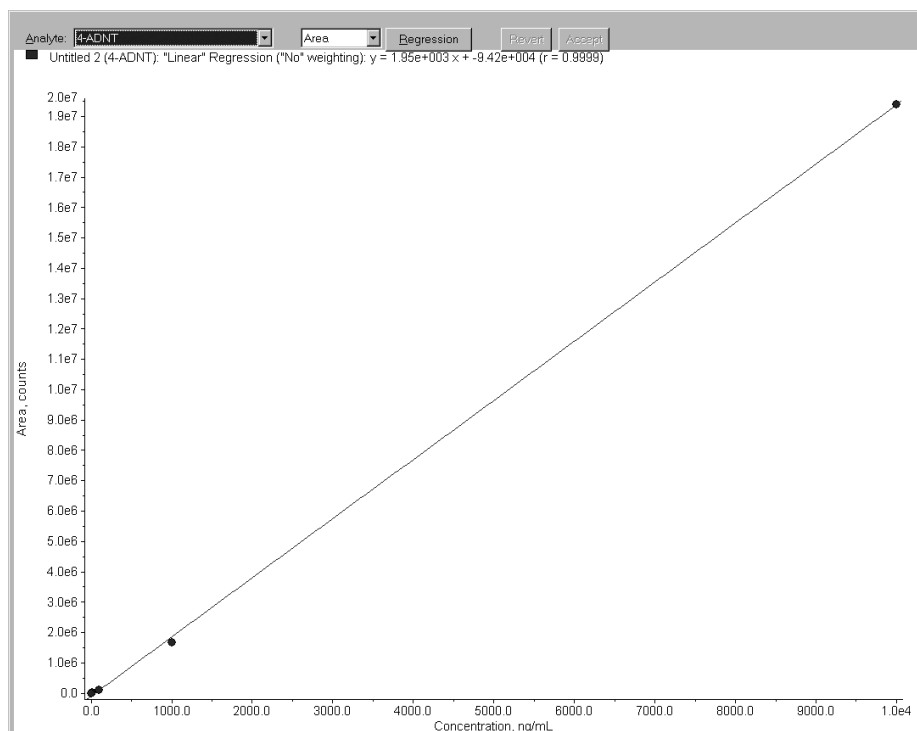


Figure 5. Calibration curve for 4A-DNT from 1 µg/L to 10,000 µg/L with LC/MSD TOF.

Chromatograms for four components are shown in Figure 6 at 10 µg/L with ±100-ppm extraction windows.

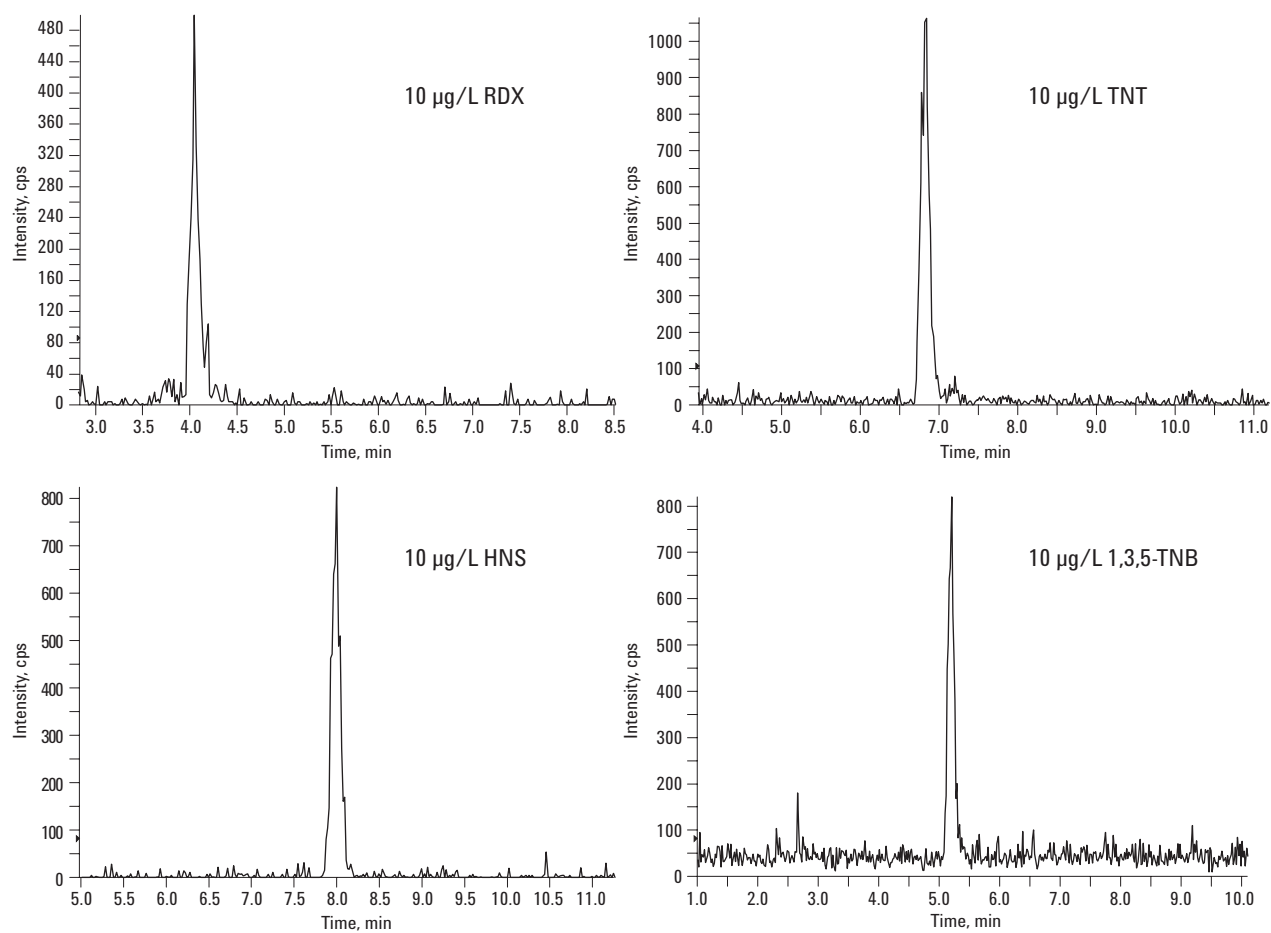


Figure 6. Representative chromatographic responses for four of the explosive compounds at the 10 µg/L concentration.

Spiked Recovery of Soil Samples

Figure 7 shows the results obtained from a soil spike of RDX.

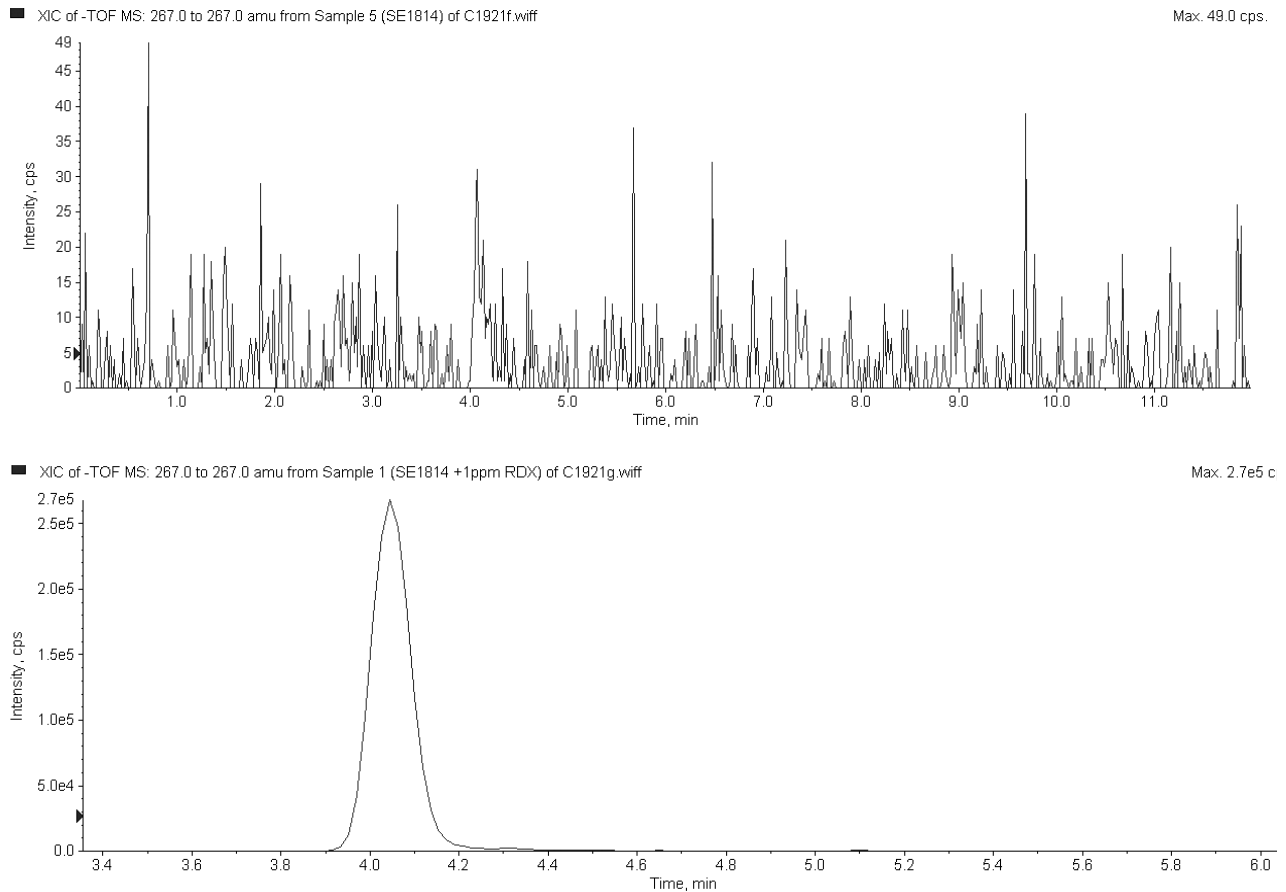


Figure 7. Soil sample SE1814 before and after spiking with 1 mg/L RDX. Extracted m/z 267.02-267.03

Table 13 gives the recoveries obtained when a dirty soil matrix is spiked with various explosives. The LC/MSD TOF provides a powerful tool in its ability to remove interference through the power of accurate mass measurements made at every scan.

Table 13. Spike and Recovery Levels for Three Soil Extracts

| Soil sample ID | Spiked compound (amount) | Recovery |
|----------------|---------------------------|----------|
| SE1814 | RDX (1ppm) | 115% |
| SE1814 | RDX (0.1ppm) | 120% |
| SE1816 | 2,4-Dinitrotoluene (1ppm) | 75% |
| SE1947 | 1,3-dinitrobenzene (1ppm) | 99% |

Analysis of Crime Scene Samples

Two blind samples from archived crime evidence were analyzed with the Agilent LC/MSD TOF using the methodology developed in this study.

The first sample was treated as an unknown explosive. A small amount of material was dissolved in methanol and the resulting chromatogram is shown in Figure 8. The retention time of 6.8 minutes results in either two possibilities by retention time match, TNT or 2A-DNT (a TNT metabolite). By measuring the accurate mass of 227.0180 (Figure 9), it is a match for TNT with a radical ion exact mass of 227.0183 (1.3 ppm mass error). Note that the $[M-H]^-$ ion is also observed and its measured mass of 226.0106 is only 0.18 ppm from the expected exact mass of this ion.

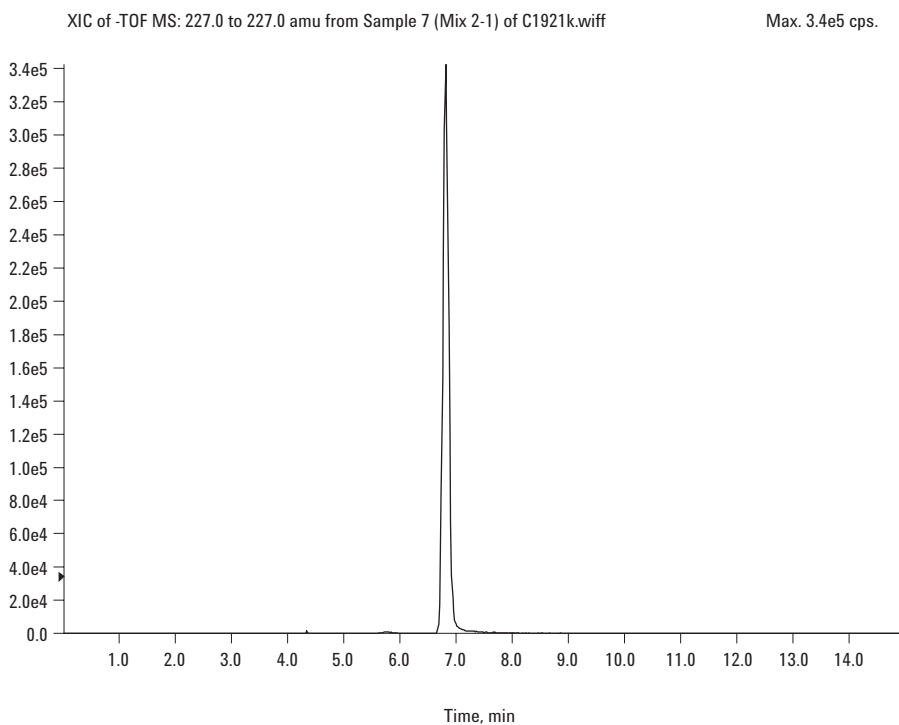


Figure 8. LC/MSD TOF Chromatogram of an unknown explosive material.

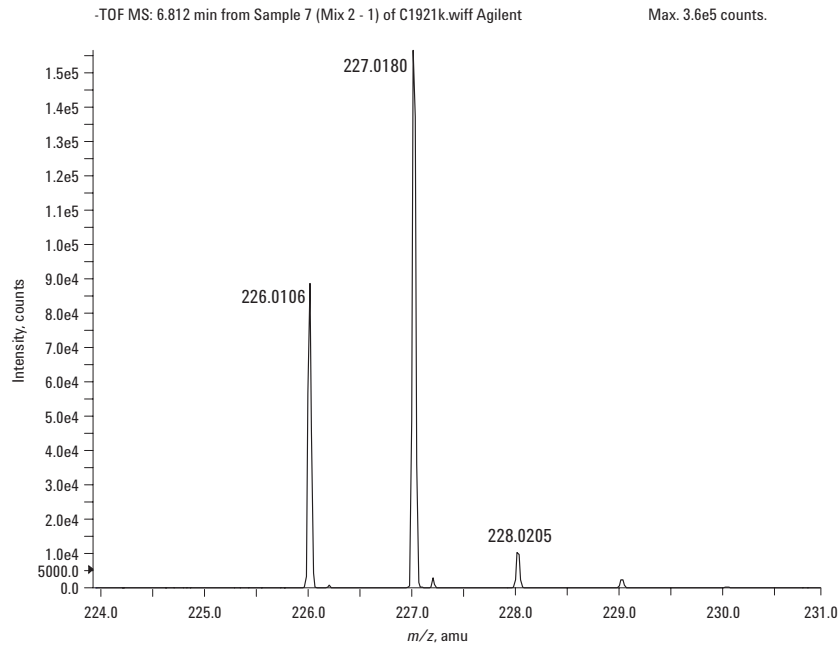


Figure 9. Mass Spectrum of an unknown explosive material.

The second sample was a soil extract to determine the possible presence of an explosive residue. By extracting all known accurate masses identified in this investigation within a 100 ppm mass window, one peak was identified at 4.0 minutes with a mass of 267.0331 m/z , which correlates to the presence of RDX with a mass error of 0.06 ppm for the formate adduct (Figure 10).

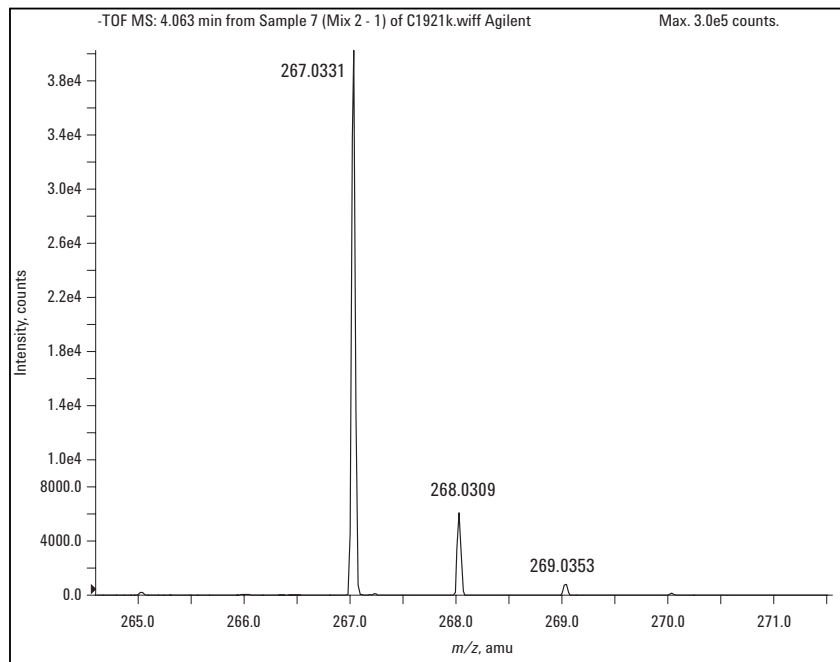


Figure 10. Confirmation of RDX in an explosive crime scene residue.

Summary

The detection of explosives has become a critical analysis in many countries from crime scene forensics to homeland security to environmental testing and remediation. The traditional method of analysis, USEPA method 8330 uses UV detection, which although for some components is sensitive, is nonselective and is prone to interference from the matrix.

The LC/MSD TOF, operated in APCI mode, has the advantage that all analyses take place in full scan mode, and hence any other components may be observed. This is coupled with a sensitivity that far exceeds UV detection as shown in Table 14. Additional confirmatory information and selectivity that is provided through the determination of the accurate mass provides a very powerful technique for the detection, identification and quantitation of explosive compounds.

This work has shown the Agilent LC/MSD TOF's ability to:

- Measure accurate masses within 3 ppm and often much better across a wide range of concentrations for many explosive compounds
- Obtain a high degree of selectivity, achieved with high resolution and accurate mass measurement at every scan
- Provide quantitative results
- Provide repeatability of response consistent with typical quantitative analysis
- Determines the identity of explosives in real samples with a high level of confidence

Table 14. LOQ for Explosives Using UV and LC/MSD TOF

| Compound | UV | LC/MSD TOF |
|-----------|--------|------------|
| HMTD | 10,000 | 30 |
| HMX | 1,000 | 10 |
| RDX | 100 | 0.5 |
| TATB | 1,000 | 5 |
| EGDN | 2,000 | N.D. |
| 1,3,5-TNB | 3,000 | 3 |
| 1,3-DNB | 500 | 2 |
| Tetryl | 500 | 5 |
| 4A-DNT | 500 | 10 |
| NB | 800 | N.D. |
| NG | 500 | N.D. |
| 2A-DNT | 500 | 5 |
| TNT | 200 | 4 |
| 2,6-DNT | 400 | 8 |
| 2,4-DNT | 400 | 4 |
| HNS | 500 | 1 |
| 2-NT | 300 | 100 |
| 4-NT | 200 | 50 |
| PETN | 1,000 | 250 |
| 3-NT | 300 | 5000 |
| TATP | 10,000 | 1000 |
| Carbamite | 500 | 10 |

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