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

Application

Forensics

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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	$C_6H_{12}N_2O_6$
2	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0	$C_4H_8N_8O_8$
3	Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4	$C_3H_6N_6O_6$
4	1,3,5-triamino-2,4,6-trinitrobenzene	TATB	3058-38-6	$C_6H_6N_6O_6$
5	Ethylene glycol dinitrate	EGDN	628-96-6	$C_2H_4N_2O_6$
6	1,3,5-Trinitrobenzene	1,3,5-TNB	99-35-4	$C_6H_3N_3O_6$
7	1,3-Dinitrobenzene	1,3-DNB	99-65-0	$C_6H_4N_2O_4$
8	Methyl-2,4,6-trinitrophenylnitramine	Tetryl	479-45-8	$C_7H_5N_5O_8$
9	4-amino-2,6-dinitrotoluene	4A-DNT	1946-51-0	$C_7H_7N_3O_4$
10	Nitrobenzene	NB	98-95-3	$C_6H_5NO_2$
11	Nitroglycerin	NG	55-63-0	$C_3H_5N_3O_9$
12	2-amino-4,6-dinitrotoluene	2A-DNT	355-72-78-2	$C_7H_7N_3O_4$
13	2,4,6-Trinitrotoluene	TNT	118-96-7	$C_7H_5N_3O_6$
14	2,6-Dinitrotoluene	2,6-DNT	606-20-2	$C_7H_6N_2O_4$
15	2,4-Dinitrotoluene	2,4-DNT	121-14-2	$C_7H_6N_2O_4$
16	Hexanitrostilbene	HNS	19138-90-0	$C_{14}H_6N_6O_{12}$
17	2-Nitrotoluene	2-NT	88-72-2	$C_7H_7NO_2$
18	4-Nitrotoluene	4-NT	99-99-0	$C_7H_7NO_2$
19	Pentaerythritol tetranitrate	PETN	78-11-5	$C_5H_8N_4O_{12}$
20	3-Nitrotoluene	3-NT	99-08-1	$C_7H_7NO_2$
21	Triacetone triperoxide	TATP	NA	$C_9H_{18}O_6$
22	Carbamite	Carbamite	NA	$C_{17}H_{20}N2_{O}$

NA Not applicable

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

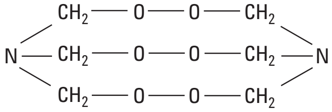
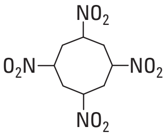
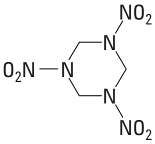
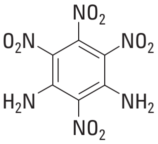
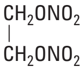
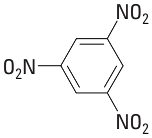
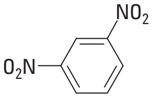
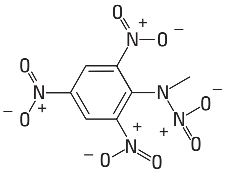
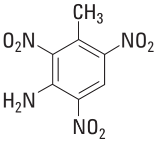
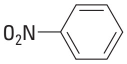
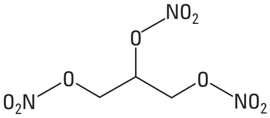
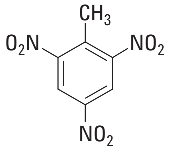
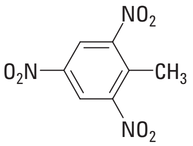
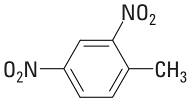
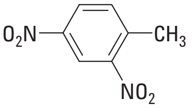
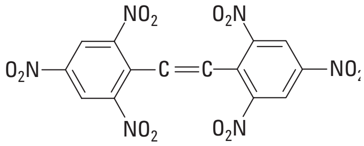
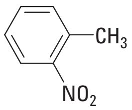
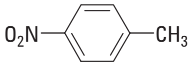
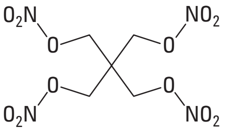
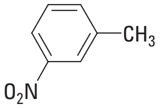
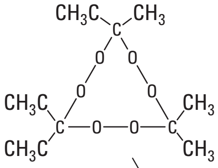
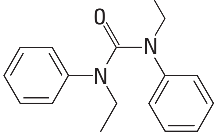
#	Name	Molecular formula	Structure	Molecular weight
1	HMTD	$C_6H_{12}N_2O_6$		208.0695
2	HMX	$C_4H_8N_8O_8$		296.0465
3	RDX	$C_3H_6N_6O_6$		222.0349
4	TATB	$C_6H_6N_6O_6$		258.0349
5	EGDN	$C_2H_4N_2O_6$		152.0069
6	1,3,5-TNB	$C_6H_3N_3O_6$		213.0022
7	1,3-DNB	$C_6H_4N_2O_4$		168.0171
8	Tetryl	$C_7H_5N_5O_8$		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	$C_7H_7N_3O_4$		197.0437
13	TNT	$C_7H_5N_3O_6$		227.0178
14	2,6-DNT	$C_7H_6N_2O_4$		182.0328
15	2,4-DNT	$C_7H_6N_2O_4$		182.0328
16	HNS	$C_{14}H_6N_6O_{12}$		450.0044
17	2-NT	$C_7H_7NO_2$		137.0477
18	4-NT	$C_7H_7NO_2$		137.0477
19	PETN	$C_5H_8N_4O_{12}$		316.0139
20	3-NT	$C_7H_7NO_2$		137.0477
21	TATP	$C_9H_{18}O_6$		222.1103
22	Carbamite	$C_{17}H_{20}N_2O$		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 µL, with needle wash		
Column temperature	40 °C		
Column	ZORBAX Extend-C18 4.6 mm × 250 mm, 5 µm p/n 770450-902		

MS Detection conditions

Ionization	APCI
Gas temperature	350 °C
Vaporizer temperature	325 °C
Drying gas flow	5 L/min
Nebulizer pressure	40 psig
PCI Corona current	4 µA
PCI Capillary voltage	4000 V
NCI Corona current	10 µA
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 PEEK™ tubing. An isocratic pump was used to deliver the reference mix at a flow rate of 50 µL/min in positive ion mode and flow programmed from 70 µL/min to 150 µL/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 × 2.1 mm, 5 µm, 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 µL of purine and 250 µL 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 (µg/L)
HMTD	208.0695	$[M\text{-}H]^+$	207.0611	207.0612	0.18	30
HMX	296.0465	$[M+CHO_2]^-$	341.0447	341.0446	–0.11	10
RDX	222.0349	$[M+CHO_2]^-$	267.0330	267.0328	–1.07	0.5
TATB	258.0349	$[M\text{-}H]^-$	257.0276	257.0276	–.02	5
EGDN	152.0069	No response by TOFMS				
1,3,5-TNB	213.0022	$[M]^-$	213.0027	213.0026	–0.63	15
1,3-DNB	168.0171	$[M]^-$	168.0176	168.0175	–0.92	10
Tetryl	287.0138	$[M\text{-}NO_2]^-$	241.0214	241.0214	–0.24	5
4A-DNT	197.0437	$[M\text{-}H]^-$	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	$[M\text{-}H]^-$	196.0363	196.0364	0.92	5
TNT	227.0178	$[M]^-$	227.0183	227.0178	–2.6	4
2,6-DNT	182.0328	$[M]^-$	182.0333	182.0331	–1.1	8
2,4-DNT	182.0328	$[M]^-$	182.0333	182.0331	–1.1	4
HNS	450.0044	$[M]^-$	450.0049	450.0042	–1.6	1
2-NT	137.0477	$[M\text{-}H]^-$	136.0404	136.0406	1.5	100
4-NT	137.0477	$[M\text{-}H]^-$	136.0404	136.0407	2.2	50
PETN	316.0139	$[M\text{-}NO_2 + CH_2O_2]^-$	316.0269	316.0267	–0.94	250
3-NT	137.0477	$[M]^-$	137.0482	137.0480	–1.7	5000
TATP	222.1103	Unassigned	ND	89.0597	ND	1000
Carbamite	268.1576	$[M+H]^+$	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 $[M\text{-}1]^+$ 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 $[M\text{-}H]^+$ 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.

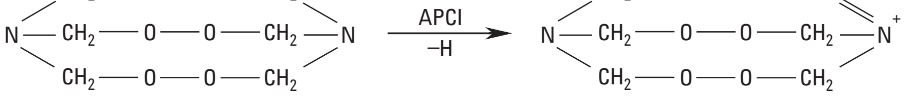


Figure 1. The theoretical positive ion formed from HMTD using APCI.

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.

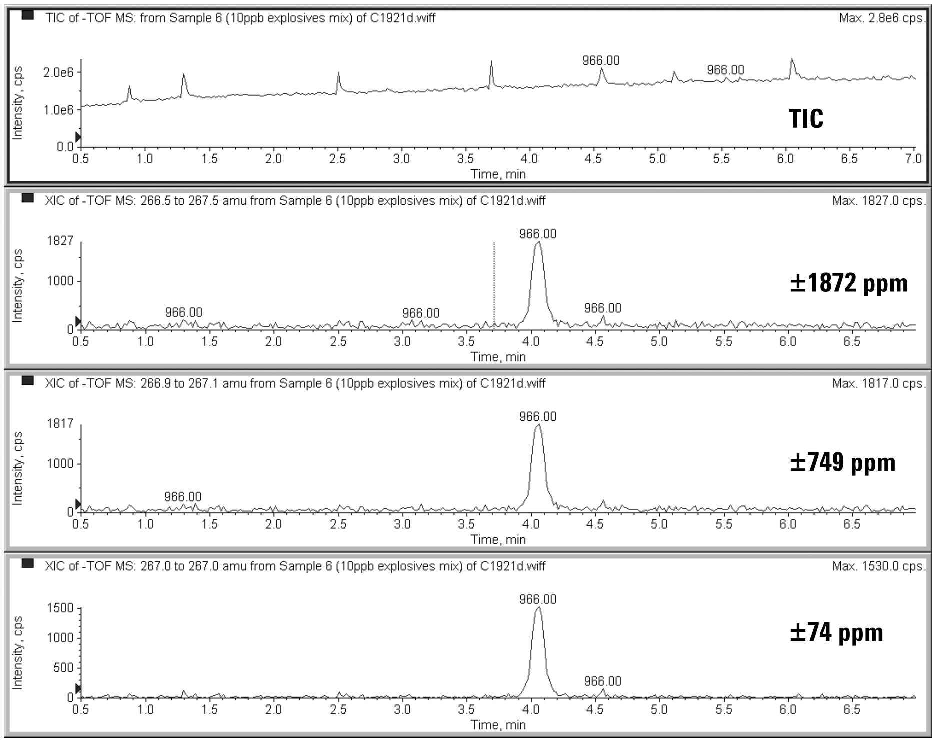


Figure 2. Effect of extracted ion range on noise of 10 µg/L RDX. The value given in each panel is the mass range extracted in parts per million (ppm) of expected exact mass of RDX.

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 (µg/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 (µg/L)

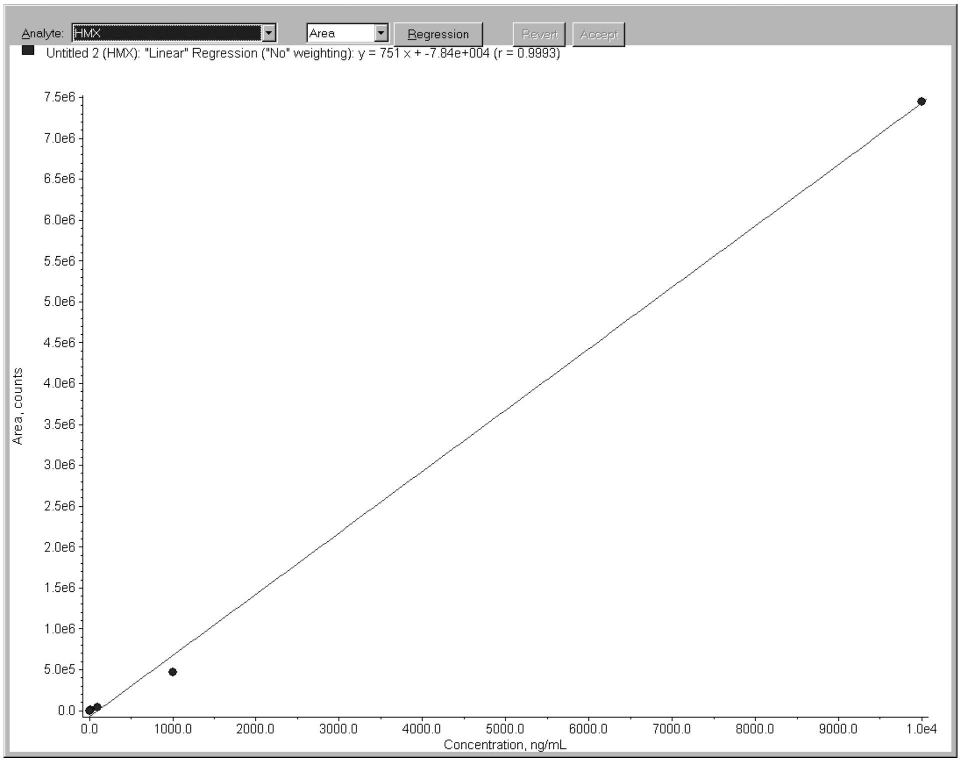
Replicate	1	10	100	1,000	10,000	100,000
1	4760	16400	127000	1730000	20700000	74800000
2	4330	16600	134000	1700000	20600000	73300000
3	4490	16500	134000	1840000	20900000	71600000
4	4200	16200	134000	1790000	20400000	71300000
5	3990	16100	132000	1830000	19600000	71200000
Average	4354	16360	132200	1778000	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 (µg/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

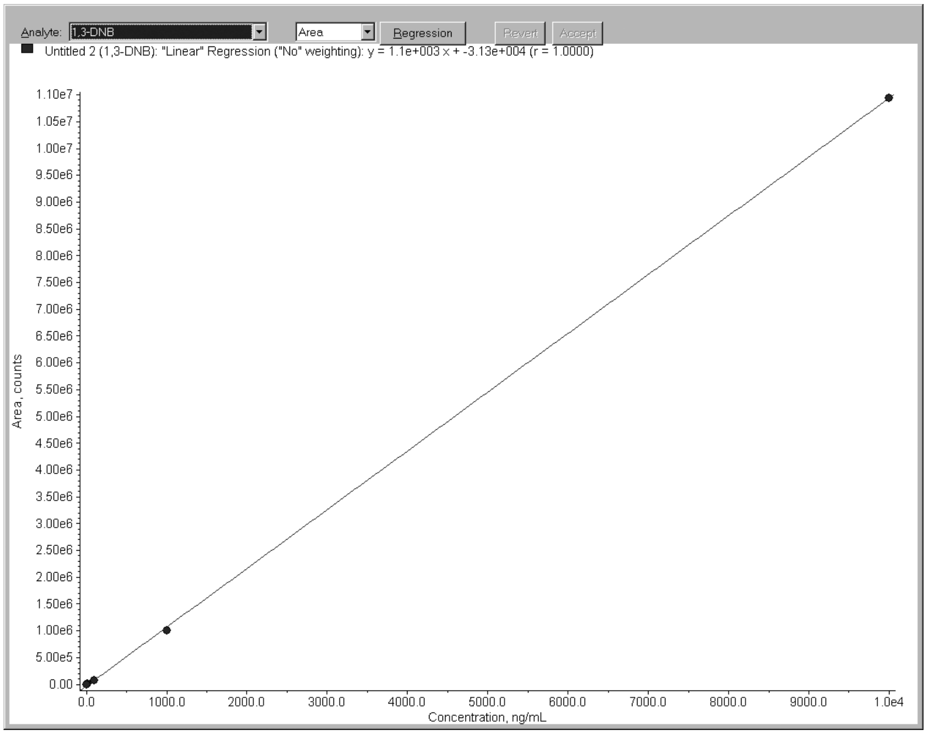
The linearity of the LC/MSD TOF was investigated for a range of the components in the mixture. Of the 10 components evaluated, most exhibited a linear regression coefficient of variation of greater than 0.998. Some of the compounds displayed excellent linearity across the four orders of magnitude. A 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.


Analyte: HMX

Area

■ Untitled 2 (HMX): "Linear" Regression ("No" weighting): y = 751 x + -7.84e+004 (r = 0.9993)

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

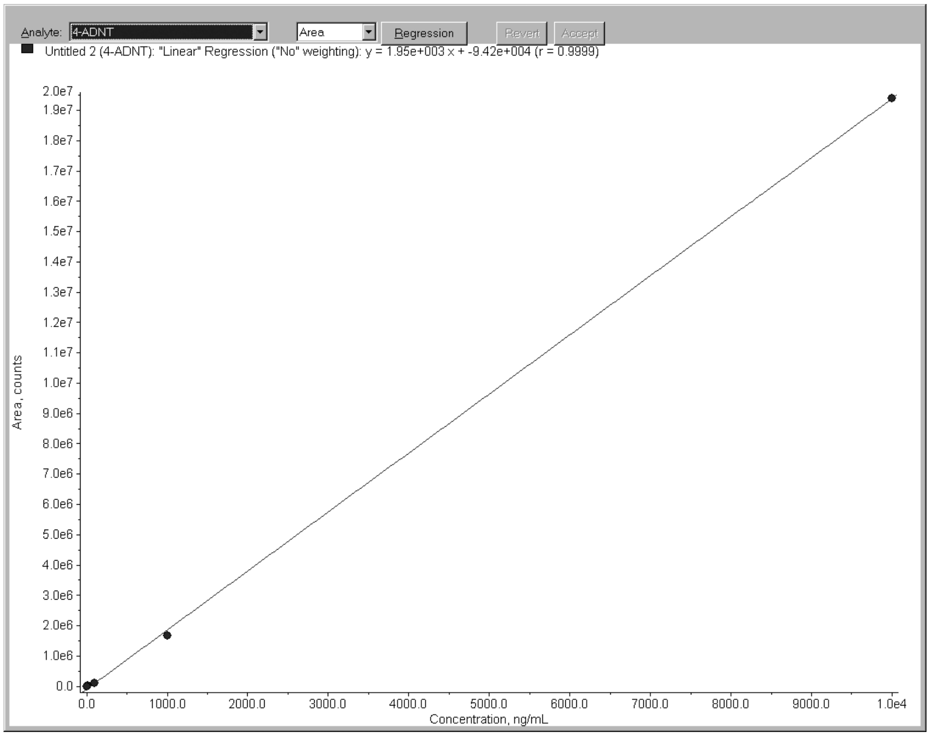


Analyte: 1,3-DNB

Area

Untitled 2 (1,3-DNB): "Linear" Regression ("No" weighting): y = 1.1e+003 x + -3.13e+004 (r = 1.0000)

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



Analyte: 4-ADNT

Area

Untitled 2 (4-ADNT): "Linear" Regression ("No" weighting): y = 1.95e+003 x + -9.42e+004 (r = 0.9999)

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.

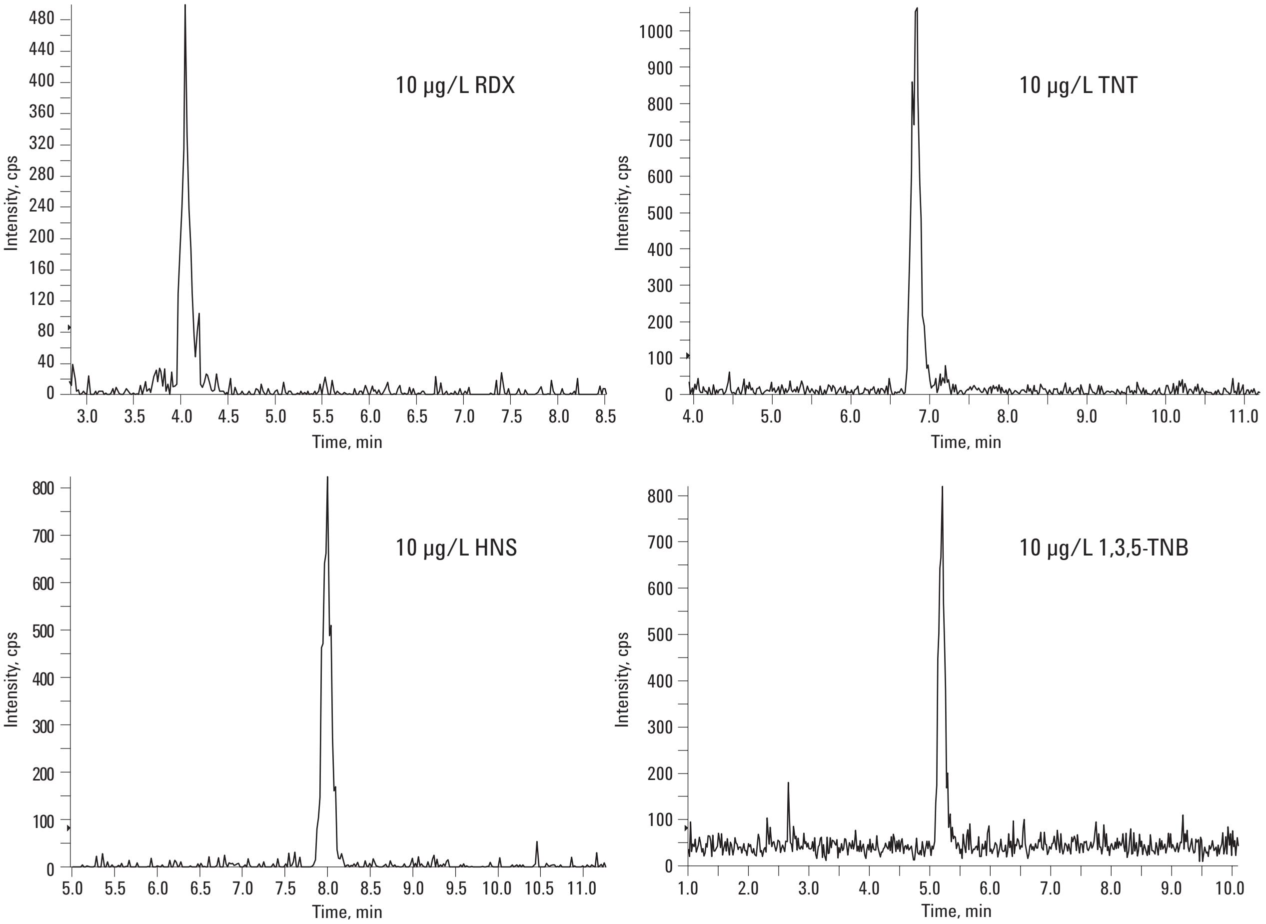


Figure 6. **Representative chromatographic responses for four of the explosive compounds at the 10 µg/L concentration.**

Spiked Recovery of Soil Samples

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

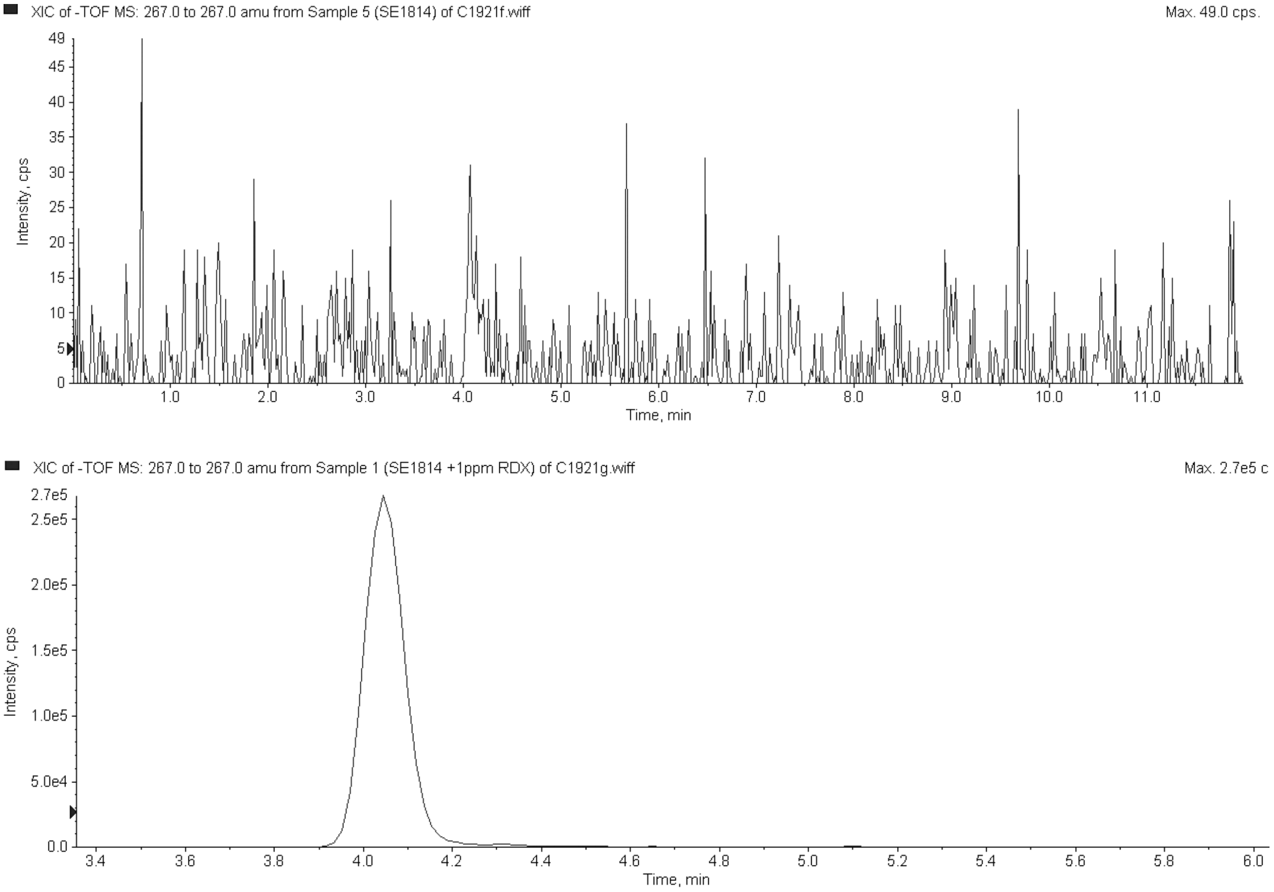


Figure 7. Soil sample SE1814 before and after spiking with 1 mg/L RDX. Extracted *m/z* 267.02-267.03

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 in 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 union exact mass of 227.0183 (1.3 ppm mass error). Note that the $[M\text{-}H]^-$ ion is also observed and its measured mass of 226.0106 is only 0.18 ppm from the expected exact mass of this ion.

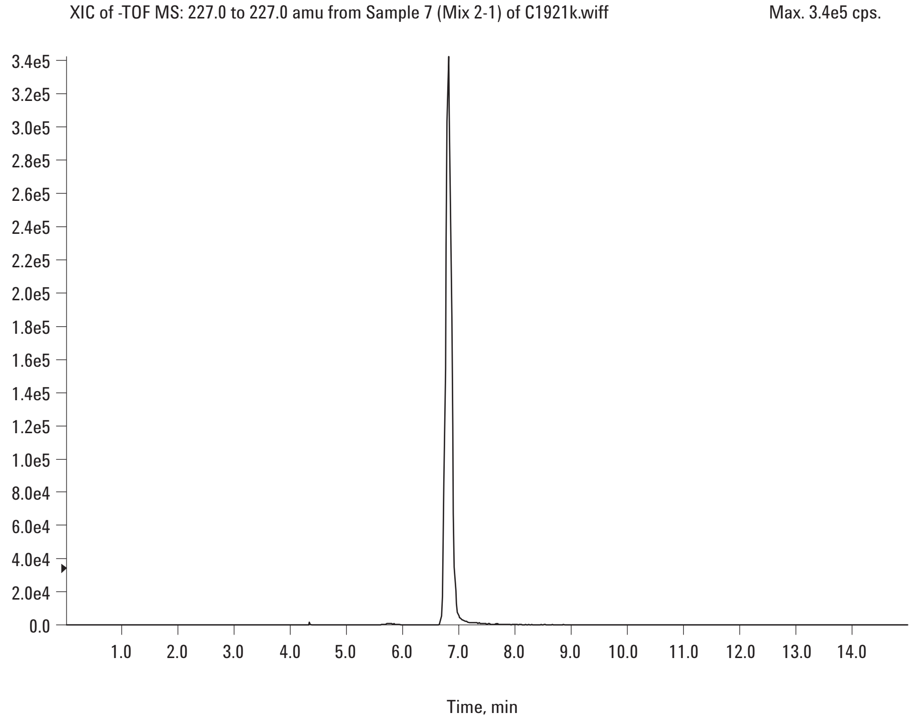


Figure 8. LC/MSD TOF Chromatogram of an unknown explosive material.

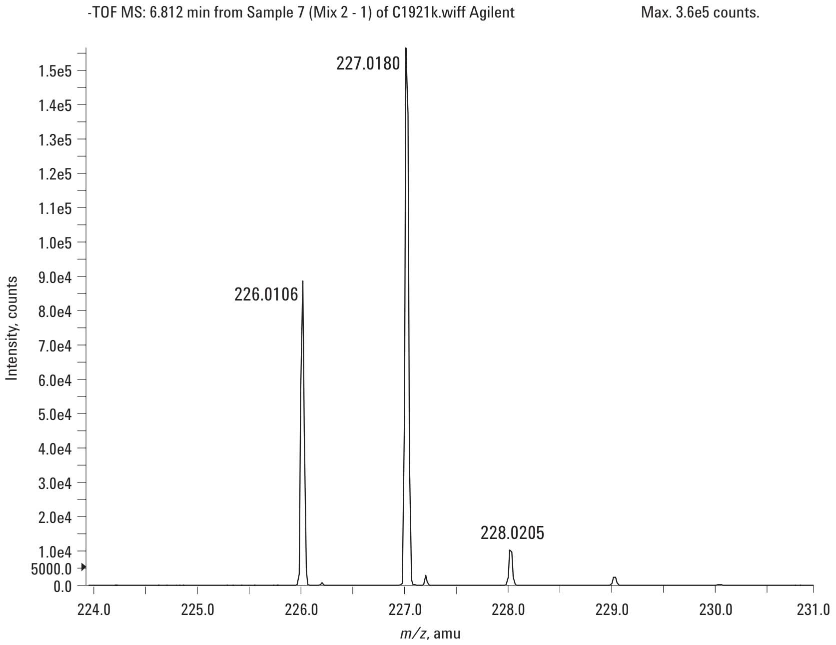


Figure 9. Mass Spectrum of an unknown explosive material.

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).

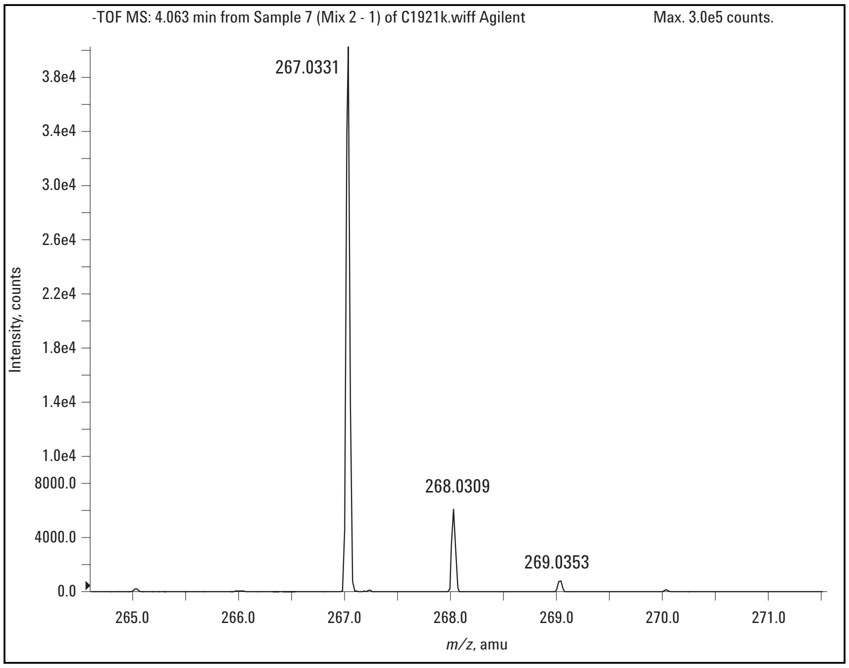


Figure 10. Confirmation of RDX in an explosive crime scene residue.

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

References

1. Determination of Concentration of Nitroaromatics and Nitramines by High-Performance Liquid Chromatography (HPLC). EPA Method 8330. Methods Manual for Detection of PEP Compounds. Environmental Protection Agency.

2 HPLC Analysis of Explosive Constituents in Soil Samples. Agilent Technologies, publication 5901-7626E www.agilent.com/chem

3 Reversed-Phase Separation. 15 Explosives from Soil Extract. Agilent Technologies, publication 5988-6342EN www.agilent.com/chem

4 Qualitative and Quantitative Analysis of Explosive and Related Compounds Using Polar and Nonpolar HPLC Columns. Agilent Technologies, publication 5988-6345EN www.agilent.com/chem

5 X. Zhao, J. Yinon, Characterization and origin identification of 2,4,6-trinitrotoluene through its by-product by liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry. (2002) *J Chrom. A.* **946** 125–132.

6. C. Sanchez, H. Carlsson, A. Colmsjo, C. Crescenzi, R. Batlle, Determination of Nitroaromatic Compounds in Air Samples at Femtogram Level Using C18 Membrane Sampling and On-Line Extraction with LC-MS. (2003) *Anal. Chem.* **75** 4639–4645.

7 A. Gapeev, M. Sigman, J. Yinon, Liquid chromatography/mass spectrometric analysis of explosives: RDX adduct ions. (2003) *Rapid Comm. Mass Spec.* **17** 943–948.

8 Xiaoma Xu, Anick M. van de Craats, Eric M. Kok, and Peter C.A.M. de Bruyn, Trace Analysis of Peroxide Explosives by High Performance Liquid Chromatography-Atomospheric Pressure Chemical Ionisation-Tandem Mass Spectrometry (HPLC-APCI-MS/MS) for Forensic Applications. (2004) *J Forensic Sci.* **49**, (6): 1–7.

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