

Simultaneous UHPLC/MS Analyses of Explosive Compounds

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Key Words

- MSQ Plus Mass Detector
- Explosives
- Library Spectra
- Sensitivity
- UHPLC

Introduction

Explosive compounds, which are recognized as four major categories, nitroaromatics, nitroamines, nitrate esters and peroxides according to their chemical structures, are widely used in warfare, mining industries, terrorist attacks and civil constructions. Explosive contaminated soils are mostly found on firing points, impact areas and training ranges. Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a primary explosive found on the training ranges, as well as 2,4,6-trinitrotoluene (TNT), 2,6-dinitrotoluene (2,6-DNT) and 2,4-dinitrotoluene (2,4-DNT). The explosive contaminates in soil are possible sources for surface and ground water contaminations, posing the environmental and public health risks due to the compounds' toxicity, carcinogenicity and mutagenicity.^{1,2} The increased terrorism activities have brought the world's attention on explosive compounds, especially peroxide explosives. Triacetone triperoxide (TATP) became a well known peroxide explosive after its use by a terrorist in 2001. The analyses of explosive compounds are demanded by the environmental monitoring and protection agencies, crime scene investigations and homeland securities. Explosive analyses are challenging processes because most of the explosive materials degrade quickly after their explosion and the sample matrices vary from one to the other. Furthermore, the peroxide explosives are not suitable for UV detection because of their lack of chromophores and their instability under the illumination of UV light.

The U.S. Environmental Protection Agency (USEPA) method 8330 is the current standard method for the identification of explosive compounds, which uses HPLC separation and UV detection of nitroaromatic and nitroamine compounds. However, the lack of selectivity of UV detection makes compound identification in complicate matrices ambiguous. Mass spectrometry has been employed in TATP detection with Agilent LC/MSD TOF instrument; however, the Agilent instrument and method demonstrated poor sensitivity with limit of quantitation (LOQ) at 1 mg/L.³

In this application, we developed an ultra high performance liquid chromatography/mass spectrometry (UHPLC/MS) method to efficiently separate, detect and quantitate all four classes of explosive compounds, including eight nitroaromatics, two nitroamines, five nitrate esters and two peroxides. The explosives were separated on a Thermo Scientific Hypersil GOLD PFP, 1.9 μ m, 2.1 x 100 mm column and detected by selected ion monitoring (SIM) on a Thermo Scientific MSQ Plus Mass Detector – a fast scanning, single-quadrupole mass spectrometer.



Experimental Conditions

Standard Preparation

Hexamethylenetriperoxidediamine (HMTD), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), ethylene glycol dinitrate (EGDN), diethylene glycol dinitrate (DEGDN), 1,3,5-trinitrobenzene (1,3,5-TNB), 1,3-dinitrobenzene (1,3-DNB), methyl-2,4,6-trinitrophenylnitramine (Tetryl), 4-amino-2,6-dinitrotoluene (4A-DNT), 2-amino-4,6-dinitrotoluene (2A-DNT), nitroglycerin (NG), 2,4,6-trinitrotoluene (TNT), 2,6-dinitrotoluene (2,6-DNT), 2,4-dinitrotoluene (2,4-DNT), pentaerythritol tetranitrate (PETN), trimethylolethane trinitrate (TMETN), and triacetone triperoxide (TATP) were purchased from AccuStandard® (New Heaven, CT, USA) as 100 mg/L standard solution in acetonitrile or in solid form. The stock solutions of 1000 mg/L of RDX, TNT, Tetryl and PETN standard were prepared by dissolving accurately weighed solids in acetonitrile or methanol. The calibration standards were prepared by diluting the 100 mg/L stock solutions with water to 0.010, 0.032, 0.160, 0.800, 4.00, and 20.00 mg/L.

Sample Preparation

Blank soil sample (San Jose, CA) was dried and homogenized. Each 2.0 g of the dried blank soil sample was amended with 0.04 μ L, 0.2 μ L, 1 μ L, 2 μ L and 10 μ L standard solution containing 100 mg/L RDX, TNT, Tetryl and PETN, which corresponded to 2, 10, 50, 100 and 500 μ g/kg for each analyte in soil. The amended soil samples (2.0 g) were added to 5 mL of acetonitrile. The solutions were capped and sonicated for 15 min. The supernatants (3.5 mL) were transferred to a clean vial, evaporated at 37 °C to dryness under nitrogen. The residues were reconstituted with 200 μ L acetonitrile as samples for LC/MS analyses.

Chromatographic Conditions

Instruments:	Thermo Scientific Accela pump Thermo Scientific Accela Autosampler			
Columns:	Hypersil GOLD PFP, 1.9 μ m, 100 x 2.1 mm			
Flow Rate:	0.5 mL/min			
Mobile Phase:	A: water, 1 mM ammonium formate B: methanol			
Gradients:	Time (min)	A(%)	B(%)	μ L/min
	0.0	80.0	20.0	500
	10.0	45.0	55.0	500
	12.0	20.0	80.0	500
	12.1	5.0	95.0	500
	12.9	5.0	95.0	500
	13.0	80.0	20.0	500
	15.0	80.0	20.0	500
Injection Volume: 2 μ L partial loop injection, 25 μ L loop size				

Mass Spectrometer Conditions

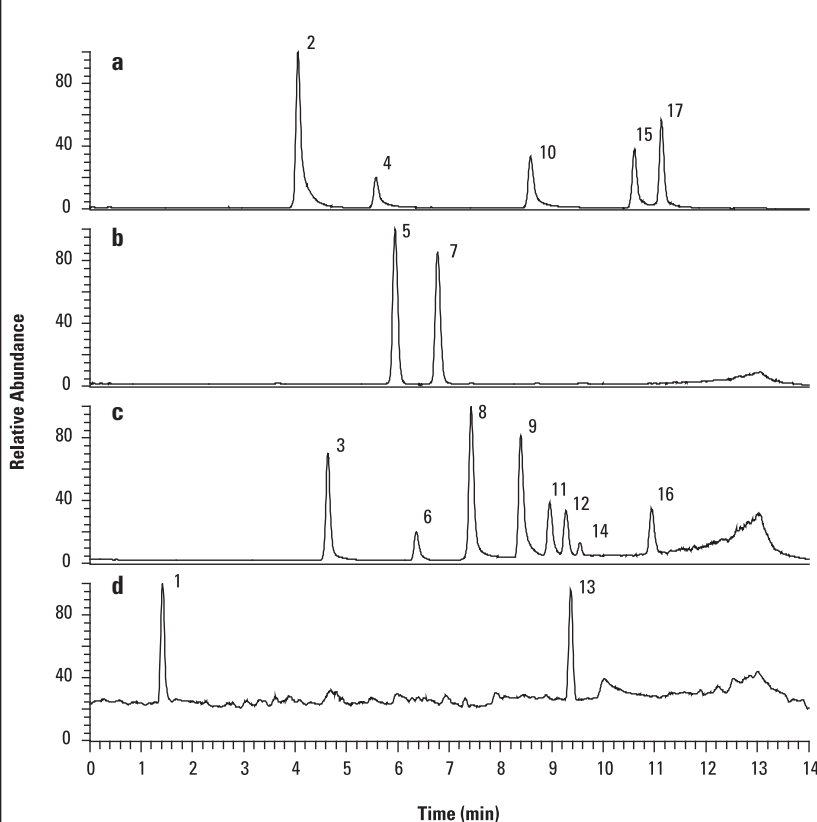
Instrument:	MSQ Plus Mass Detector
Ionization:	Atmospheric Pressure Chemical Ionization (APCI)
Polarity:	Positive and Negative
Probe Temperature:	350 $^{\circ}$ C
Cone Voltage:	60.0 V
Scan Mode:	Full scan with mass range of 50-400 amu or selected ion monitoring (SIM)
Corona Current:	30 μ A
Scan Time:	0.5 s for full scan, 0.25 s for SIM

Results and Discussion

UHPLC Separation and MS Detection

USEPA 8330 method provides sensitive UV detection for nitroaromatic and nitroamine explosives. However, two analytical columns with different stationary phases are required to separate and identify the isomers, 2,4-DNT and 2,6-DNT, 4-A-2,6-DNT and 2-A-4,6-DNT, which make this method time consuming and results in low sample throughput.

The simultaneous separation and detection of seventeen explosive compounds was achieved through UHPLC/MS, using the Thermo Scientific Accela system with a fast scanning, single quadrupole mass spectrometer (Figure 1). Water and methanol were used as the mobile phases and the optimized gradient is shown in the Chromatographic Conditions. The elution order of the compounds and their retention times are shown in Figure 1. Hypersil GOLD™ PFP has a fluorinated phenyl group in the stationary phase which improves selectivity towards aromatic compounds. It also provides better resolutions for polar compounds containing hydroxyl, carboxyl, nitro or other polar groups. Eight nitroaromatic compounds, two nitroamine compounds, five nitrate ester compounds and two peroxides were separated with baseline resolution on a Hypersil GOLD PFP, 1.9 μ m, 100 x 2.1 mm column. The isomer pairs, 2,4-DNT and 2,6-DNT, 4-A-2,6-DNT and 2-A-4,6-DNT, were separated with the peak resolution of 2.8 and 7.3 respectively (Peaks 9 and 11, 12 and 16).



Peak	Compound	Retention Time (min)
1	HMTD	1.42
2	EGDN	4.06
3	TNB	4.64
4	DEGDN	5.58
5	HMX	5.95
6	1,3-DNB	6.36
7	RDX	6.77
8	TNT	7.43
9	2,6-DNT	8.40
10	NG	8.58
11	2,4-DNT	8.96
12	4-A-2,6-DNT	9.28
13	TATP	9.37
14	TETRYL	9.55
15	TMETN	10.60
16	2-A-4,6-DNT	10.94
17	PETN	11.13

Figure 1: UHPLC/MS separation and detection of the 17 explosives standard with negative APCI (a-c) and positive APCI (d) ionizations. a) Extracted ion chromatogram at m/z of 61.96; b) Extracted ion chromatogram at m/z of 102.05; c) Extracted ion chromatogram at m/z of 213.02, 168.09, 227.01, 182.07, 197.04 and 241.02; d) Extracted ion chromatogram at m/z of 209.04 and 348.08.

The MSQ™ Plus Mass Detector was employed for the detection of the explosive compounds. Full scan mode with a mass range of 50-400 amu was employed for the compound identification and confirmation, while SIM mode was used for the sensitivity and quantitation studies.

The mass spectra for some explosive compounds are difficult to be predicted because of their reactivity. An array of the ions, such as additive adducts and decomposing ions, is observed in the LC/MS analyses of explosives.⁴ The observed ion signals vary depending on many factors, for example, the ionization sources, analytes concentrations, additive concentrations, impurities in the mobile phases and the contaminations of the LC/MS system.

APCI was used in the MS detection of the explosives because it gave better sensitivities than ESI. Nitroaromatics, nitroamines and nitrate esters were detected using APCI negative mode, while peroxides were detected using APCI positive ionization (Figure 2). Some explosive standards, including TNB, 1,3-DNB, TNT, 2,6-DNT, 2,4-DNT, 4-A-2,6-DNT and 2-A-4,6-DNT, showed both molecular ion signals ($[M]^-$ or $[M-H]^-$) and decomposing ions ($[M-30]^-$ and/or $[M-17]^-$) in their MS spectra. Other explosive standards showed only decomposing ions: the nitrate esters, including EGDN, DEGDN, NG, TMETN and PETN, showed decomposing ions of $[NO_3]^-$ at m/z 61.95; the nitroamines, including RDX and HMX, showed decomposing ions at m/z 102.05 and 129.16. TATP formed adduct ions with its decomposing ions and ammonium, $[M+NH_4 + H(OOC(CH_3)_2OOH)]^+$ at m/z of 348.08. In this case, the addition of 1 mM ammonium acetate in the mobile phase A was critical, providing the sources of ammonium ions to facilitate the formation of the ammonium adduct.

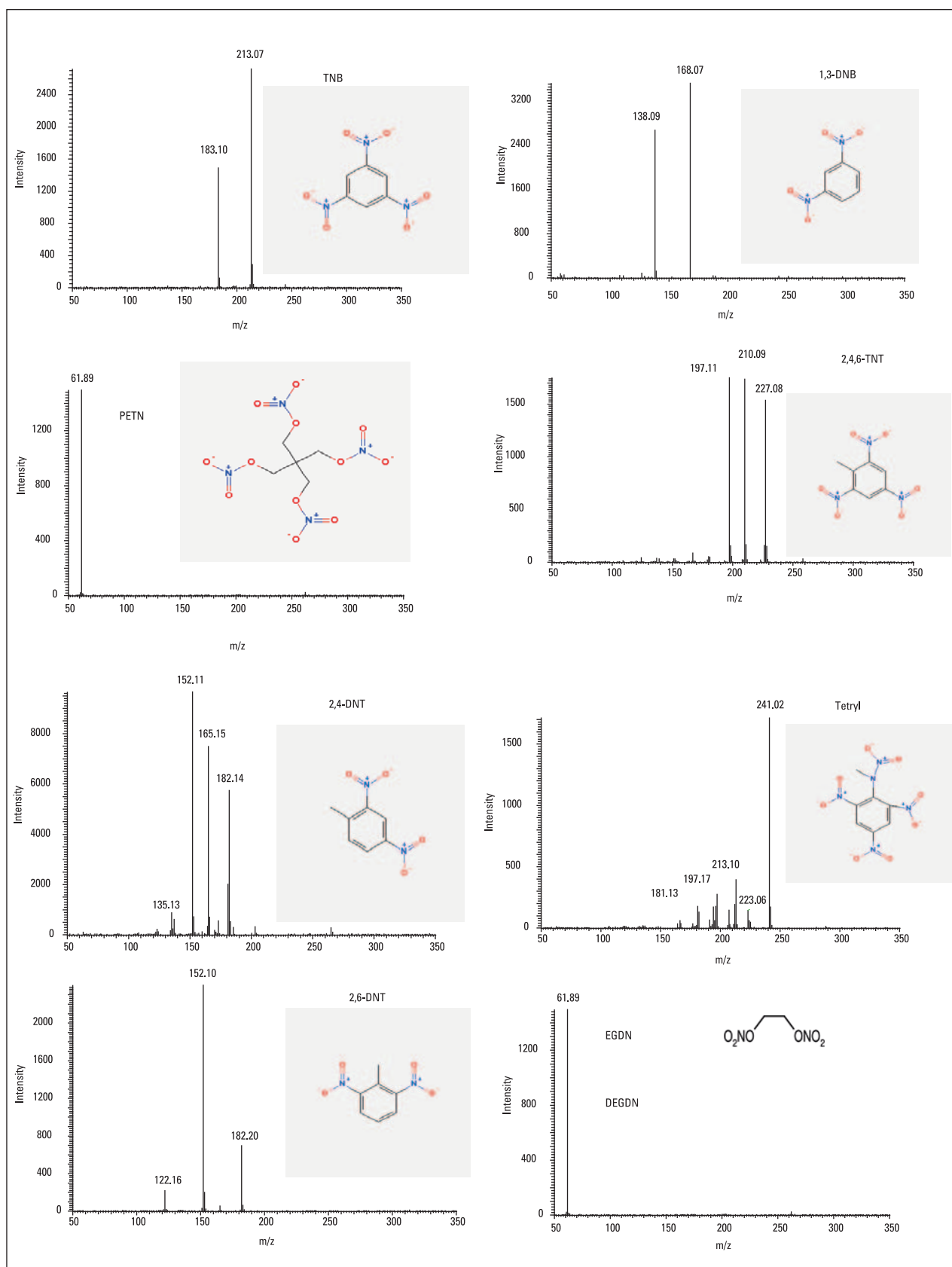
The two isomer pairs, 2,6-DNT and 2,4-DNT, 4-A-2,6-DNT and 2-A-4,6-DNT, demonstrated significant differences in their fragmentation MS spectra with the source induced fragmentation (SID) of the MSQ Plus Mass Detector. The spectrum of the 2,6-DNT showed one major fragmentation ion $[M-30]^-$ at m/z 152.10, while 2,4-DNT gave two major fragmentation ions $[M-30]^-$ at m/z 152.11 and $[M-17]^-$ at m/z 165.15. 4-A-2,6-DNT showed one major fragmentation ion $[M-30]^-$ at m/z 167.09, while 2-A-4,6-DNT gave two major fragmentation ions $[M-30]^-$ at m/z 167.10 and $[M-17]^-$ at m/z 180.16. Thus, the identification of these isomers was strengthened with the single quadrupole MS detector.

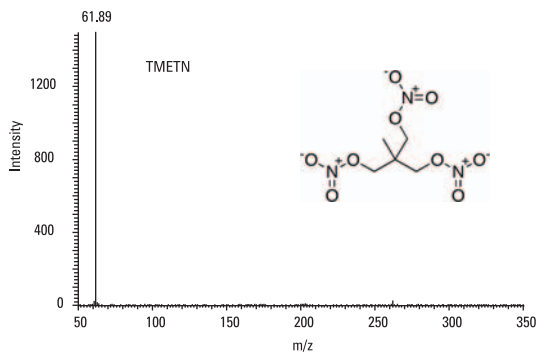
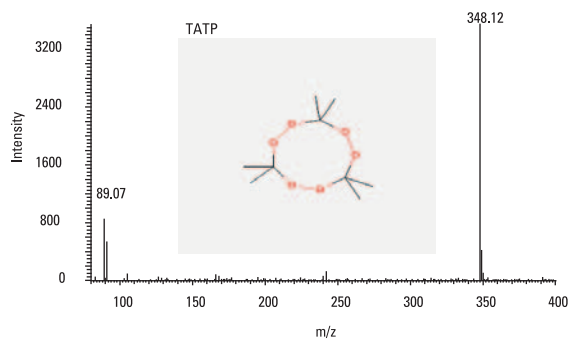
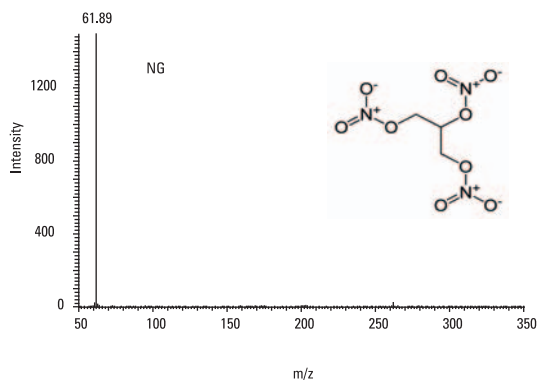
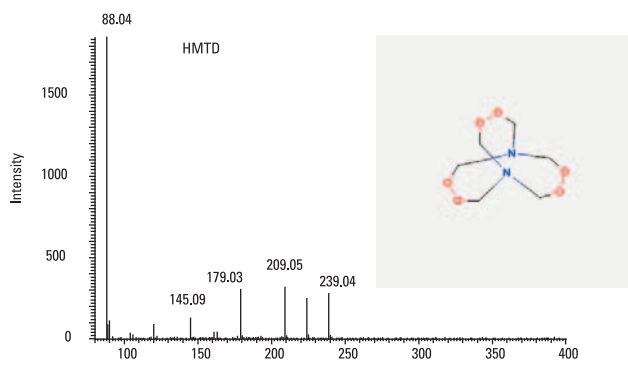
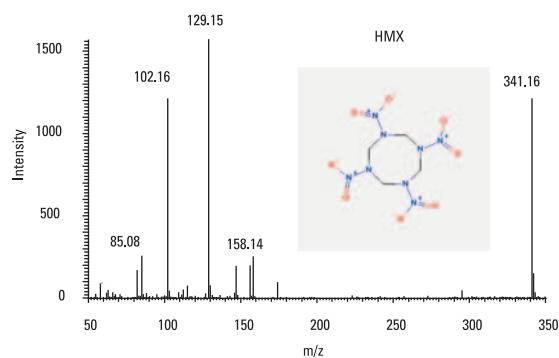
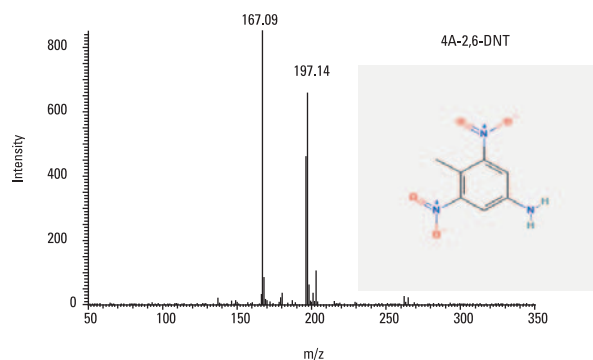
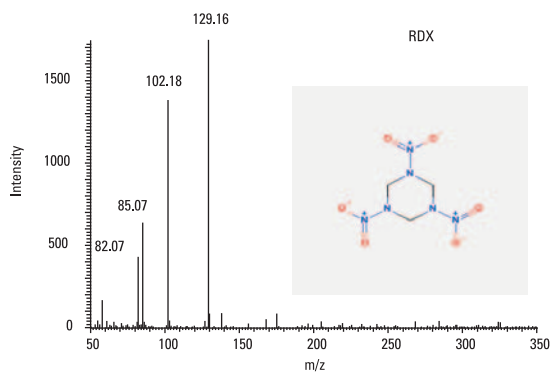
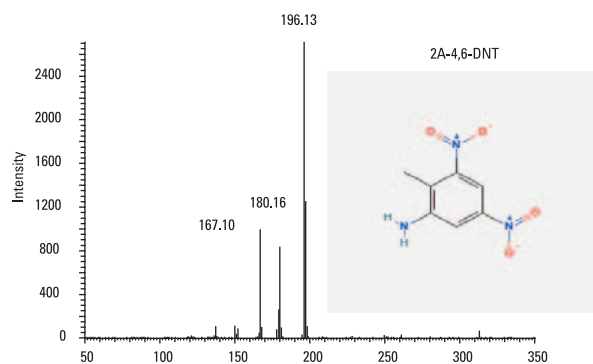
The identification of the explosive compounds with EPA 8330 method is based solely on the retention times of LC separations. The interference of the sample matrices alters the retention times of target compounds and causes false identifications. With the current UHPLC/MS method, target compounds are identified and confirmed by matching the APCI mass spectra against the MS spectra library. Figure 3A showed a total ion chromatogram (TIC) of a customer sample collected by this method. TNT and 2,4-DNT were easily identified by library spectra search against more than 20 explosive compounds (Figure 3). The Thermo Scientific Xcalibur software displayed the searching result with a list of compounds ranked by their matching scores. The implementation of the MS spectra library in compound identification provided more confirmative results compared to EPA 8330 method.

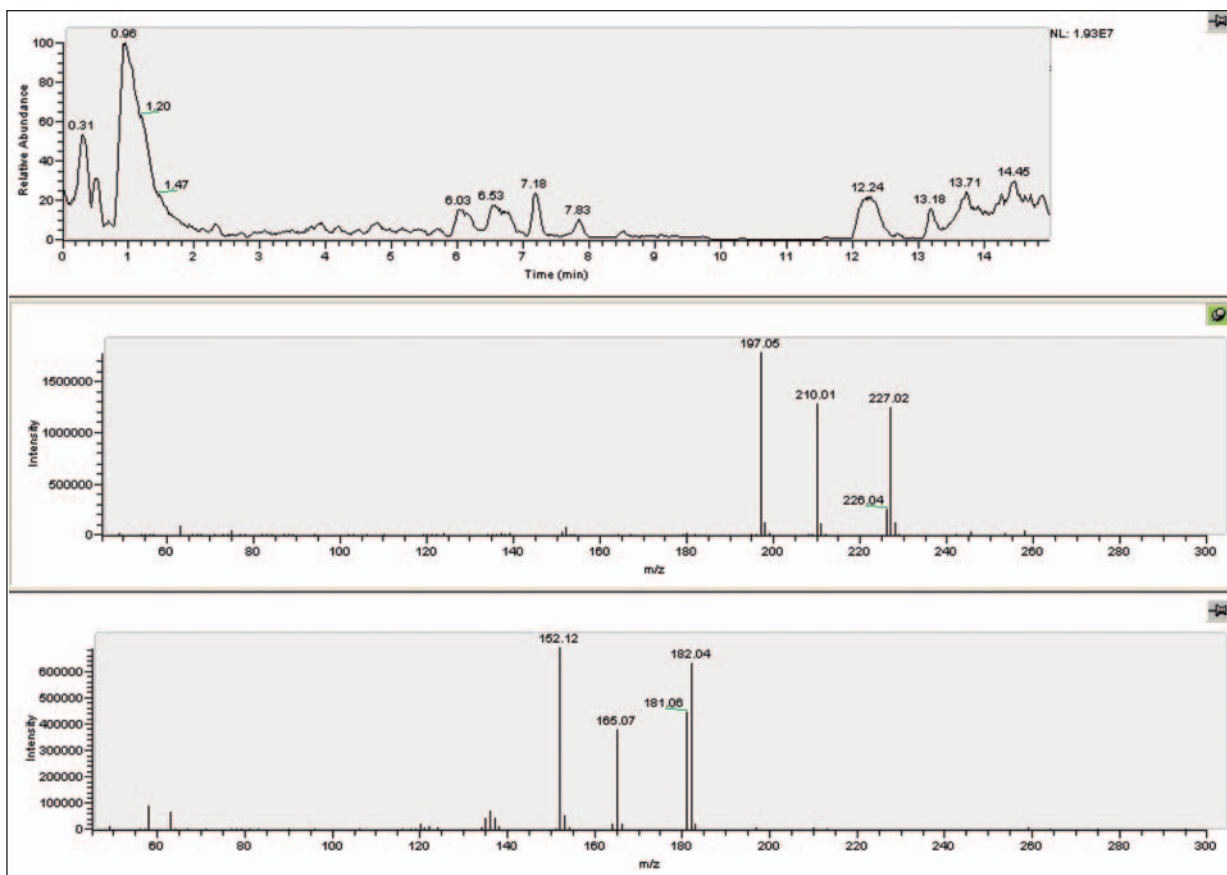
Detection Linearity and Sensitivity

The detection linearity of the UHPLC/MS system was investigated using the explosives standard. Calibration curves of seventeen standards were constructed over a concentration range of 10-100,000 ng/mL (ppb). Correlation coefficients of 0.999 or better were achieved for most of the standards (Table 1). The calibration curves for TNB, TNT, 2,6-DNT, 2,4-DNT and TETRYL showed linearity over four orders of magnitude working ranges (Table 1).

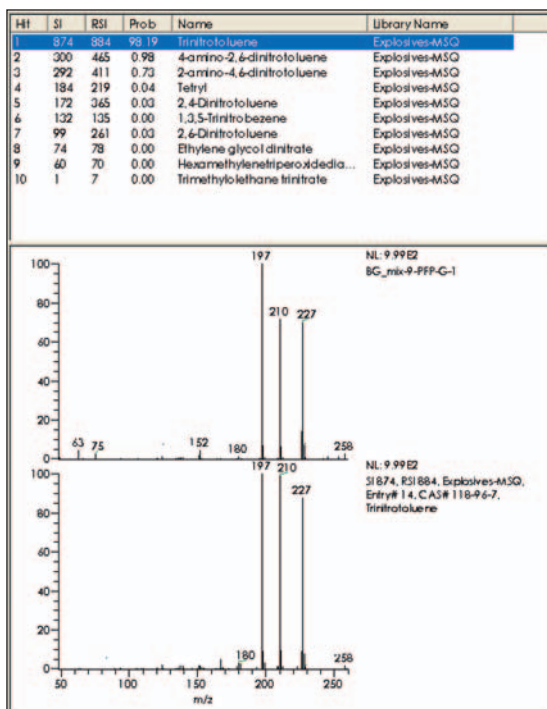
Improved sensitivities were observed by high throughput UHPLC because of the sharper and taller peaks produced by the sub-2 μ m particle columns. The SIM mode of the MSQ Plus Mass Detector further extended the detection sensitivity compared to the traditional UV detector. The limit of quantitation (LOQ) and the limit of detection (LOD) for seventeen standard explosive compounds were examined. The sensitivities were achieved at ppb level for TNB, 1,3-DNB, TNT, 2,6-DNT, 2,4-DNT, TATP and TETRYL (Table 1). This represents a thirty-five times improvement in the detection sensitivity for TATP relative to the detection sensitivity of the Agilent instrument and method. The detection sensitivities obtained by the UHPLC/MS method with library matching of APCI mass spectra was more than tenfold versus the EPA 8330 method.



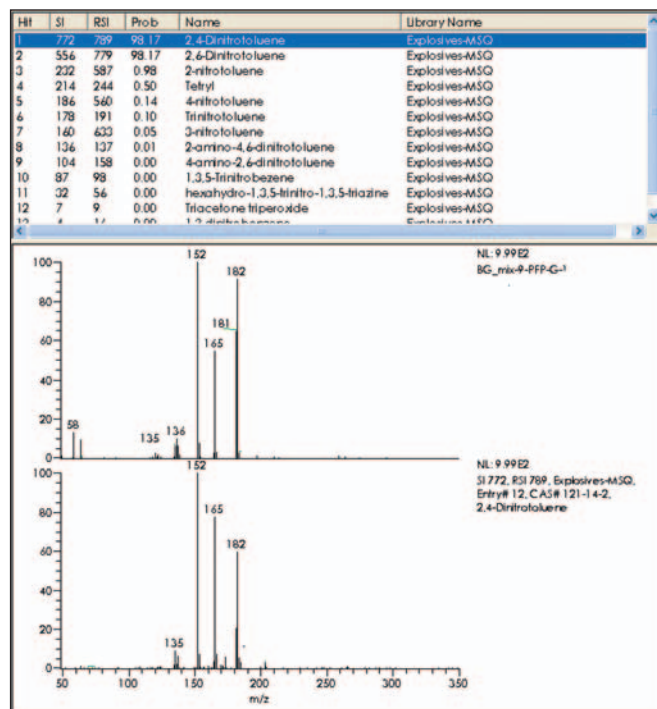




3a



3b



3c

Figure 3: The identifications of the explosives in customer sample using library spectra search: a) Total ion chromatography of the customer sample and the two MS spectra at 7.18 and 7.83 minute, respectively; b) The MS library search result for peak at 7.18 minute; c) The MS library search result for peak at 7.83 minute.

Compound	Monoisotopic Mass	Observed Mass	Linearity Range ng/mL	Correlation Coefficients	LOQ ng/mL	LOD ng/mL
HMTD	208.07	209.04	1000-100,000	0.9915	1136	341
EGDN	152.01	61.96	200-100,000	0.9997	79	24
TNB	213.00	213.00	10-100,000	0.9971	8	2
DEGDN	196.12	61.96	200-100,000	0.9991	617	185
HMX	296.05	102.05	225-100,000	0.9990	55	16
1,3-DNB	168.02	168.09	32-100,000	0.9950	16	5
RDX	222.03	102.05	225-100,000	0.9990	89	27
TNT	227.02	227.01	10-100,000	0.9977	8	2
2,6-DNT	182.03	152.07	10-100,000	0.9996	3	1
TATP	222.11	348.08	100-100,000	0.9964	28	8
NG	227.00	61.95	200-100,000	0.9994	265	79
2,4-DNT	182.03	152.07	10-100,000	0.9995	7	2
4-A-2,6-DNT	197.04	197.04	160-100,000	0.9998	91	27
TETRYL	287.01	241.02	10-100,000	0.9924	10	3
TMETN	255.14	61.95	200-100,000	0.9990	110	33
2-A-4,6-DNT	197.04	196.04	160-100,000	0.9965	75	22
PETN	316.01	61.95	200-100,000	0.9994	76	23

Table 1: LOQ and LOD of seventeen standard compounds

Analyses of Explosive Compounds in Soil Matrices

The explosive compounds, extracted from soil sample with acetonitrile, were analyzed using the UHPLC/MS method. Figure 4 showed the chromatography traces of RDX, TNT, Tetryl and PETN at 500 µg/kg, 10 µg/kg and the solvent extraction blank. The sample extraction recoveries from the soil matrices were evaluated. Four compounds, RDX, TNT, Tetryl and PETN, were tested at 500 µg/kg and 10 µg/kg levels (Table 2). Greater than 94% extraction recovery at 500 µg/kg level and more than 82% recovery at 10 µg/kg level were achieved for all the compounds tested. The method linearity and sensitivity were investigated for those compounds in soil matrices in the range of 2 to 500 µg/kg. Linear correlation coefficients of 0.996 or better were obtained (Figure 5). LOD of 0.2 to 0.6 µg/kg were achieved for TNT, Tetryl and PETN in soil matrices (Table 3).

Compound	Extraction Recovery %	
	10 µg/kg	500 µg/kg
RDX	89.7	96.2
TNT	92.1	98.5
Tetryl	90.6	95.4
PETN	82.3	94.3

Table 2: Extraction recoveries in soil matrices

	LOQ µg/kg	LOD µg/kg
RDX	16.5	5.0
TNT	0.7	0.2
Tetryl	1.8	0.6
PETN	2.0	0.6

Table 3: The method LOQ and LOD for compounds in soil matrices

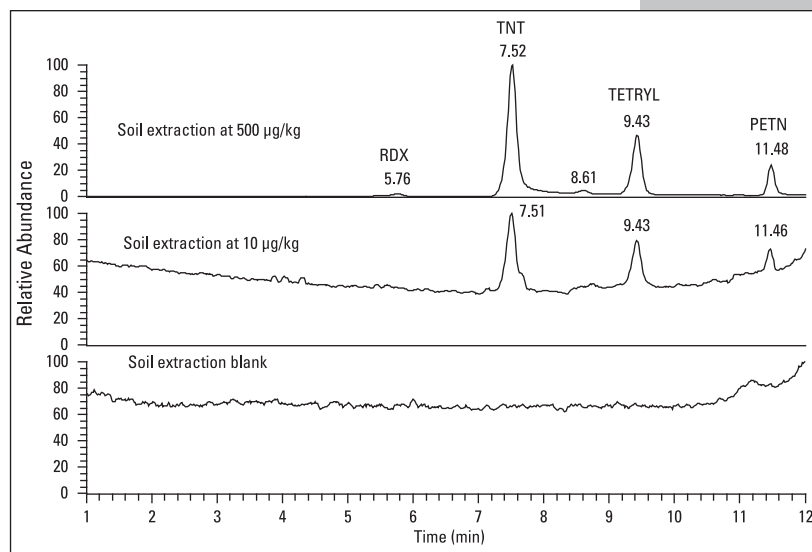


Figure 4: The UHPLC/MS analyses of the explosives in soil matrices

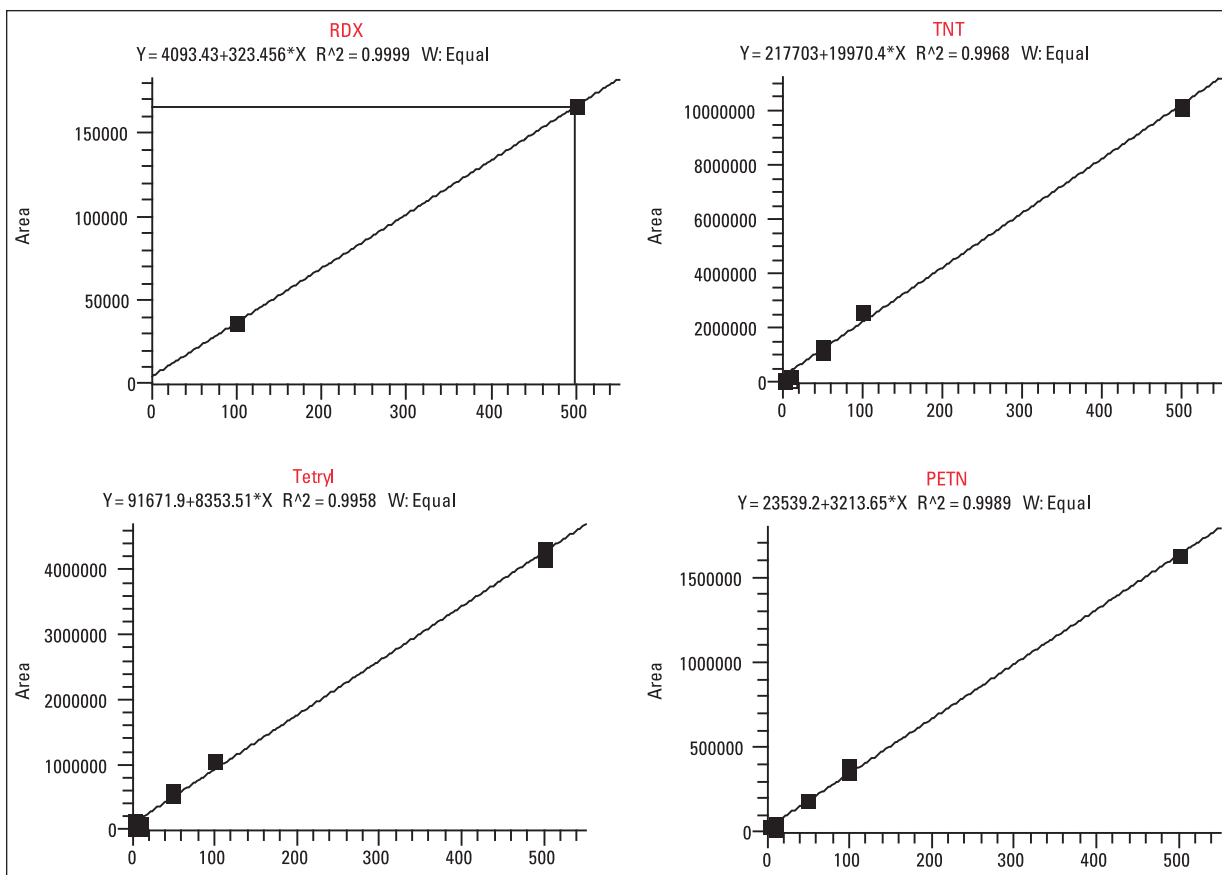


Figure 5: Linearity of the UHPLC/MS method for the analyses of explosives compounds in soil matrices

Conclusions

The simultaneous analyses of nitroamines, nitroaromatics, nitrate esters, and peroxide explosives by UHPLC/MS were accomplished. The UHPLC method, utilizing sub-2 μm particles, improved the separation efficiencies and resolutions. The MS detection method offered improved sensitivities, good selectivity and additional MS confirmations. The detection sensitivities were further increased by the pre-concentration step implemented in the sample preparation process. The more confirmative identifications of explosives were achieved by comparing of the collected APCI mass spectra to the comprehensive MS spectra library of the explosive residues. We demonstrated the improved separation performance, increased detection sensitivity and better selectivity, compared to the current USEPA 8330 method. We also achieved 35 times detection sensitivity for TATP compared to the Agilent instrument and method.

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