

# Fast Screening for Explosives at Ultra-High Resolution: Utilization of Simple Method Development Using an Orbitrap Mass Spectrometer

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## Overview

**Purpose:** To develop a single liquid chromatography mass spectrometry (LC-MS) method for screening 21 explosives and related compounds (Table 1) utilizing ultra-high resolution and minimal mass spectrometry method development.

**Methods:** A set of explosives and related compounds was mixed and diluted in a serial manner in 50% MeOH to target concentrations. A river water sample was spiked with the compounds mixture to final concentrations of 1, 10, and 100 ng/mL. All samples were analyzed with 3 replicate injections. Chromatographic separation was achieved on a reversed-phase HPLC column. A Thermo Scientific LTQ Orbitrap XL hybrid FTMS (Fourier Transform Mass Spectrometer) operated under negative ion atmospheric pressure chemical ionization (APCI) conditions was used as a detector. Ion source parameters were simply adjusted using direct infusion of NG, NB, and 4-NT. Data were acquired in full scan mode at ultra-high resolution and processed with a 5 ppm mass tolerance filter. Diagnostic ions for each compound (Table 1) were chosen after experiments. The major body of work was obtained on the LTQ Orbitrap XL™ hybrid FTMS. In addition, preliminary experiments were also performed on a benchtop standalone orbitrap, the Thermo Scientific Exactive.

**Results:** Limits of Detection (LODs) in range of 0.025 – 1 ng/mL and Lower Limits of Quantitation (LLOQs) in range of 0.05 – 2.5 ng/mL were achieved for most of the investigated compounds in neat solvent. (Table 2, Figure 1) Most of the compounds were detected in spiked river water at concentration of 1ng/mL. (Figure 2, Table 2)

## Introduction

Demand for a simple and fast screening of environmental pollutants, including explosives, is on the increase. Under atmospheric pressure ionization, most of the compounds used as explosives (nitrate esters, nitroaromatic compounds, and nitramines) get ionized very well in negative detection mode. Depending on their molecular structure, they tend to form a variety of ions such as [M]<sup>-</sup>, [M-H]<sup>-</sup>, adduct ions ([M+Cl]<sup>-</sup>, [M+AcO]<sup>-</sup>), and decomposition-related ions (e.g. [M-NO<sub>2</sub>]<sup>-</sup>).<sup>1,2,3</sup> Formation of those ions also depends on various factors, especially ionization technique [APCI, electrospray ionization (ESI), atmospheric pressure photoionization (APPI)], LC solvents, and LC additives. Very often multiple ions are simultaneously formed for a particular compound which can significantly complicate and prolong LC-MS method development. Since the orbitrap mass spectrometer can operate in full-scan mode at ultra-high resolution, the best ions for the monitoring of particular compounds can be chosen after data acquisition. This significantly simplifies method development, without compromising data integrity. Here we present a fast LC-MS screening method of 21 explosives and related compounds – including those from USEPA 8330 method.

## Methods

**Mass Spectrometry:** LTQ Orbitrap XL; preliminary data were obtained on the Exactive™ benchtop LC/MS

**Ionization Mode:** Negative ion APCI  
**Corona Needle Current:** 80 µA  
**Vaporizer:** 200° C  
**Ion Transfer Tube:** 125° C  
**Scan Mode:** FT Full MS 100-500 amu; external calibration  
**Mass Resolution Setting:** 30,000

**Liquid Chromatography:** Thermo Scientific Accela liquid chromatography system  
**Analytical Column:** Thermo Scientific Hypersil GOLD PFP 2.1x100 mm, 1.9 µm  
**Injection Volume:** 10 µL  
**Mobile Phase:** (A) H<sub>2</sub>O (90%) + MeOH (10%) + CH<sub>2</sub>Cl<sub>2</sub> (0.15%) (v/v/v); (B) MeOH + CH<sub>2</sub>Cl<sub>2</sub> (0.15%) (v/v)  
**Flow Rate:** 300 µL/min  
**Gradient:**

Time	A%	B%	Time	A%	B%
0.00	100	0	7.55	0	100
0.10	100	0	8.00	0	100
0.15	78	22	8.05	100	0
7.50	90	10	9.00	100	0

## Results

- Chromatographic separation of 21 explosives and related compounds in neat solvent is shown in Figure 1. Diagnostic ions for each compound were chosen after experiments. (Table 1)
- Calibration curves for the tested compounds were fit either linear or quadratic regression. (Table 2 A) Quantitation dynamic ranges were defined from the LLOQ – 500 ng/mL. (Table 2 A) The LLOQs were defined as concentrations with relative errors and relative CVs ≤20% for 3 replicate injections.
- Compared to other tested compounds, the sensitivity for EGDN and PGDN (structural analogs) was significantly lower. This observation is consistent with data published elsewhere.<sup>2</sup> Due to that fact, concentrations of EGDN and PGDN were increased (10 folds higher than the nominal concentrations) in each sample for the LC-MS experiments.

TABLE 1. Explosives and related compounds used in the LC-MS assay. Included types of ions with related accurate masses were used as diagnostic ions for particular compounds

Chemical name	Abbreviation	Molecular Formula	Nominal Mass	Quantifier Ions				
				[M-H] <sup>-</sup>	[M] <sup>-</sup>	[M+ <sup>35</sup> Cl] <sup>-</sup>	[M+ <sup>37</sup> Cl] <sup>-</sup>	[M-NO <sub>2</sub> ] <sup>-</sup>
1-Mononitroglycerin	1-MNG	C <sub>3</sub> H <sub>5</sub> NO <sub>2</sub>	137			172.0018	173.9989	
1,2-Dinitroglycerin	1,2-DNG	C <sub>3</sub> H <sub>3</sub> N <sub>2</sub> O <sub>2</sub>	182			216.9869	218.9840	
1,2-Ethanediol dinitrate	EGDN	C <sub>2</sub> H <sub>4</sub> N <sub>2</sub> O <sub>6</sub>	152			186.9763	188.9734	
Cyclotetramethylene tetranitramine	HMX	C <sub>4</sub> H <sub>8</sub> N <sub>4</sub> O <sub>8</sub>	296			331.0159	333.0130	
1,3,5-Trinitrobenzene	1,3,5-TNB	C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>6</sub>	213	213.0027				
Cyclotrimethylene trinitramine	RDX	C <sub>3</sub> H <sub>5</sub> N <sub>3</sub> O <sub>6</sub>	222			257.0043	259.0013	
Nitrobenzene	NB	C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub>	123		123.0326			
1,3-Dinitrobenzene	1,3-DNB	C <sub>6</sub> H <sub>4</sub> N <sub>2</sub> O <sub>4</sub>	168		168.0177			
1,2-Propanediol dinitrate	PGDN	C <sub>3</sub> H <sub>6</sub> N <sub>2</sub> O <sub>6</sub>	166			200.9920	202.9890	
2,4,6-Trinitrotoluene	TNT	C <sub>7</sub> H <sub>5</sub> N <sub>3</sub> O <sub>6</sub>	227	226.0106	227.0184			
Nitroglycerin	NG	C <sub>3</sub> H <sub>5</sub> N <sub>3</sub> O <sub>9</sub>	227			261.9720	263.9690	
2-Nitrotoluene	2-NT	C <sub>7</sub> H <sub>7</sub> NO <sub>2</sub>	137	136.0404	137.0482			
4-Nitrotoluene	4-NT	C <sub>7</sub> H <sub>7</sub> NO <sub>2</sub>	137	136.0404	137.0482			
3-Nitrotoluene	3-NT	C <sub>7</sub> H <sub>7</sub> NO <sub>2</sub>	137		137.0482			
2,6-Dinitrotoluene	2,6-DNT	C <sub>7</sub> H <sub>5</sub> NO <sub>4</sub>	182		182.0333			
2,4-Dinitrotoluene	2,4-DNT	C <sub>7</sub> H <sub>5</sub> NO <sub>4</sub>	182	181.0255	182.0333			241.0215
2,4,6-Trinitrophenyl-N-methyltrinitramine	Tetryl	C <sub>8</sub> H <sub>9</sub> N <sub>3</sub> O <sub>6</sub>	287					
2-Amino-4,6-Dinitrotoluene	2A-4,6-DNT	C <sub>7</sub> H <sub>6</sub> N <sub>2</sub> O <sub>4</sub>	197	196.0364	197.0442	232.0131	234.0101	
4-Amino-2,6-Dinitrotoluene	4A-2,6-DNT	C <sub>7</sub> H <sub>6</sub> N <sub>2</sub> O <sub>4</sub>	197	196.0364	197.0442	232.0131	234.0101	
1,2,4-Butanetriol trinitrate	BTTN	C <sub>4</sub> H <sub>8</sub> N <sub>3</sub> O <sub>9</sub>	241			275.9876	277.9847	
Pentaerythritol tetranitrate	PETN	C <sub>5</sub> H <sub>8</sub> N <sub>4</sub> O <sub>12</sub>	316			350.9833	352.9803	

FIGURE 1. Extracted ion(s) chromatograms (XICs) of the investigated compounds in neat solvent at concentrations of 5 ng/mL (500 ng/mL for EGDN and PGDN). Diagnostic ions used for each particular compound are listed in Table 1. All chromatograms are reconstructed with 5 ppm mass tolerance.

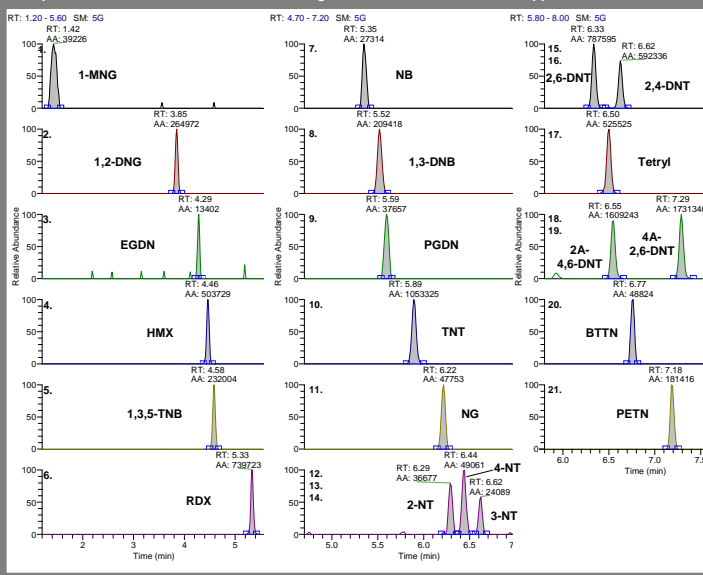


FIGURE 2. Extracted ion chromatograms (XICs) of the compounds spiked in river water to final concentrations of 1 ng/mL (except for EGDN, PGDN, and 2-, 3-, 4-NT). Mass tolerance = 5 ppm.

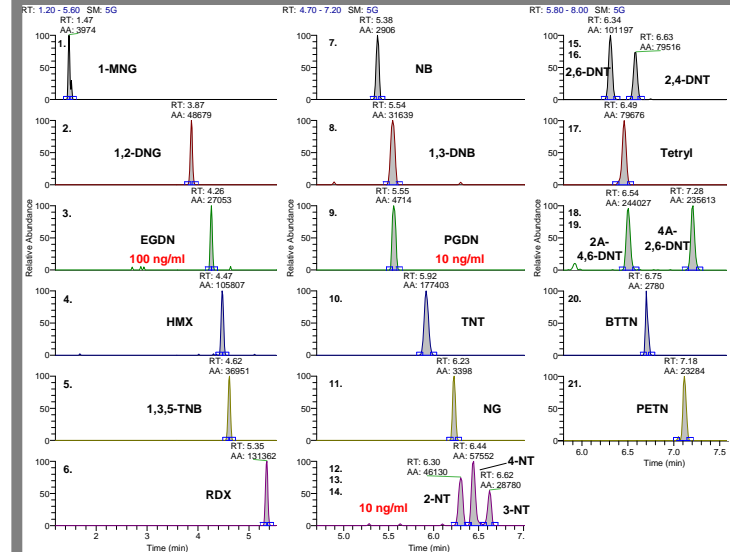


FIGURE 3. XICs for NB obtained on (A) LTQ Orbitrap XL and (B) Exactive benchtop LC-MS.

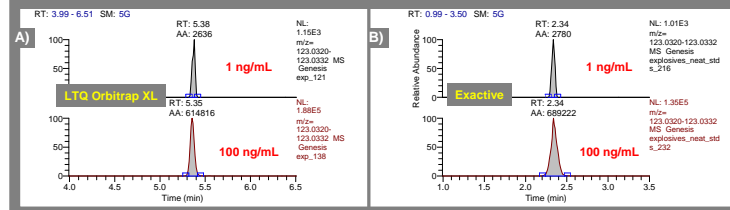


FIGURE 4. A) Number of scans in XIC for NB at LOQ, medium, and high levels. No smoothing applied. B) Comparison of mass accuracy for NB: the highest and lowest detected signal.

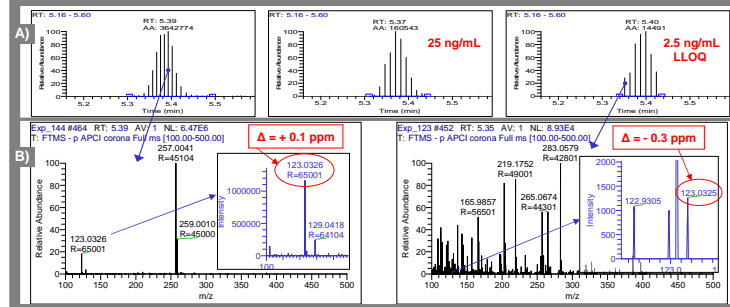


TABLE 2. A) Lower detection limit, quantitation dynamic range, and statistic data for the investigated compounds measured in neat solvent. Obtained values are based on three replicate measurements. B) Calculated concentrations of the compounds spiked in river water samples to final concentration levels of 1, 10, and 100 ng/mL. Obtained values are based on averages of three replicate measurements.

Compound	A) Calibration curve in neat solvent				B) Calculated concentrations in spiked river water (ng/mL)			
	LOD (ng/mL)	Dynamic Range (ng/mL)	CV(%) at LLOQ	R <sup>2</sup>	Cal. Curve Equation	1 ng/ml spiked	10 ng/ml spiked	100 ng/ml spiked
1-MNG	1	2.5-500	14.0%	0.9973	9533.87+1056.4*X	1.36	11.4	106
1,2-DNG	0.25	0.5-500	16.1%	0.9971	10598.9+5255.6*X	0.99	9.9	85
EGDN	500	500-5000	6.1%	0.9775	1960.66+36.4308*X+0.00259125*X <sup>2</sup>	N/D	N/D	878 **
HMX	0.1	0.5-500	6.1%	0.9986	17051.5+109655*X+25.2477*X <sup>2</sup>	1.04	11.5	90
1,3,5-TNB	0.1	0.25-500	7.6%	0.9962	4158.42+46379.9*X+11.6214*X <sup>2</sup>	0.88	9.0	83
RDX	0.05	0.25-500	5.3%	0.9984	4867.17+143878*X	0.94	9.8	93
NB	0.5	2.5-500	2.6%	0.9978	843.34+6021.53*X+2.95854*X <sup>2</sup>	0.42	7.5	83
1,3-DNB	0.1	0.25-500	8.9%	0.9978	2235.9+44219.4*X	0.82	8.2	81
PGDN	100	250-5000	9.6%	0.9973	4286.21+79.385*X+0.00672927*X <sup>2</sup>	N/D	102 *	806 **
TNT	0.05	0.1-500	9.8%	0.9990	6532.48+219955*X+77.2278*X <sup>2</sup>	0.88	8.3	76
NG	0.25	1-500	10.1%	0.9984	4370.09+10005.7*X+9.94908*X <sup>2</sup>	0.67	7.2	73
2-NT	1	2.5-500	4.1%	0.9972	4929.97+8249.13*X	1.03	6.7	63
4-NT	1	2.5-500	7.0%	0.9964	3098.64+9641.2*X	0.77 *	6.1	63
3-NT	1	1-500	1.8%	0.9981	643.073+4810.78*X+3.00785*X <sup>2</sup>	0.32 *	5.9	66
2,6-DNT	0.025	0.05-500	18.4%	0.9984	3728.22+136027*X	0.71	6.5	65
2,4-DNT	0.05	0.1-500	7.5%	0.9965	7114.54+120415*X+51.6008*X <sup>2</sup>	0.73	6.6	68
Tetryl	0.05	0.25-500	7.3%	0.9998	2561.81+65565*X+34.6845*X <sup>2</sup>	0.80	8.4	81
2A-4,6-DNT	0.05	0.05-500	6.6%	0.9981	6411.36+319564*X+314.997*X <sup>2</sup>	0.79	7.3	78
4A-2,6-DNT	0.05	0.1-500	15.5%	0.9968	14195.8+352400*X+370.302*X <sup>2</sup>	0.75	7.5	81
BTTN	0.25	1-500	5.4%	0.9979	5275.11+1465.8*X+16.1176*X <sup>2</sup>	0.75	7.1	73
PETN	0.25	0.25-500	20%	0.9930	4485.71+3295.7*X+27.6019*X <sup>2</sup>	0.80	8.9	84

\* Spiked at concentration level of 100 ng/mL. \*\* Spiked at concentration level of 1000 ng/mL.

\* Limited detection - response observed in 2 of 3 replicate injections.

- Although most of the work was performed on the LTQ Orbitrap XL, preliminary experiments were also performed on the Exactive benchtop LC/MS running at similar settings. The data obtained showed very similar response and selectivity on both instruments as demonstrated on nitrobenzene in Figure 3. (Shorter RT for the Exactive LC/MS data is due to the shorter LC column and faster solvent gradient.)
- Data obtained for river water samples spiked with the investigated compounds showed little to no chemical noise in chromatograms (due to the ultra-high resolution and narrow mass window) as shown in Figure 2. Calculated concentrations are generally lower than the nominal ones but do not show any dramatic drop of signal response. (Table 2B)
- Cycle time of the LTQ Orbitrap XL (<0.72 s) was sufficient to provide enough data points across a chromatographic peak as demonstrated for 3 different concentrations of Nitrobenzene in Figure 4A. The mass spectrometer, operated only with external calibration, exhibited very good mass stability and provided accurate data within the whole instrument dynamic range. (Figure 4B)

## Conclusions

An LC-MS method at ultra-high mass resolution was developed for 21 explosives and related compounds. For most of the compounds, LLOQs of 0.05 – 2.5 ng/mL was achieved for 10 µL sample volume in neat solvent. The compounds spiked into a river water sample showed good responses comparable to neat solvent samples. The mass spectrometer showed very good mass stability and accuracy which was beneficially utilized in applying of narrow mass tolerance filter for data processing. Preliminary data obtained on the Exactive LC/MS showed a response very similar to the LTQ Orbitrap XL, suggesting that the Exactive LC/MS may be the instrument of choice for this kind of experiment.

In future work, the Exactive LC/MS will be utilized for this assay. Further improvement of the LC/MS method can include addition of more compounds to the analysis, and chromatography optimization in order to achieve shorter run times. Also, the method robustness can be tested using other matrix samples, such as wastewater and/or soil.

## References

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