SHIMADZU

Enhanced Analysis of explosives listed in EPA Method 8330B using APCI-LC-MS/MS

ThP 105

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1. Introduction

Explosives are compounds widely used in military operations and the mining and construction industries. Explosive byproducts have adverse effects on the population as they contaminate soil, air, and water. They contribute to disease states such as cataracts, increased susceptibility to leukemia, and damage to the reproductive system. Explosives are thermolabile; therefore, quantitation of explosives by analytical techniques such as LC-MS/MS is generally more difficult due to the instability of molecular ions (i.e., in-source fragmentation of the compounds of interest). This analysis describes the quantitation of the explosives listed in EPA method 8330 by LC-MS/MS.

2. Methods

Explosives were separated using a Nexera LC and quantified by a Shimadzu LCMS-8050. The EPA 8330 explosives panel was run using an isocratic method consisting of 70% water and 30% methanol for 17 minutes using a Shim-pack Velox SP-C18 (2.7 µm, 2.1×100 mm) column. An 8330 mix of standards was purchased from Restek, and it was serially diluted using LCMS Water. The standards were prepared in concentrations from 0.001 µg/mL to 5 µg/mL for all compounds. All 17 explosives listed in EPA 8330 were analyzed by atmospheric pressure chemical ionization liquid chromatography mass spectrometry (APCI-LC-MS/MS). Table 1 lists the MRMs used for quantitation.

UHPLC conditions (Nexera system)

Column: Shim-pack Velox SP-C18
Mobile Phase A: H_2O
B: MeOH
Flow rate: 0.4 mL/min
Isocratic: 70%:30% (A:B)
Injection vol.: 2 µL
Column temperature: 30 °C

MS conditions (LCMS-8050)

Nebulizing gas: 3 L/min
Drying gas: 5 L/min

Cold Method

Interface temperature: 250 °C
DL temperature: 150 °C
Heat block temperature: 150 °C

Hot Method

Interface temperature: 500 °C
DL temperature: 300 °C
Heat block temperature: 300 °C

Table 1. MRM transitions and method conditions for analyzed explosives.

Chemical name (Polarity)	Abbreviation	Method Condition	Transition 1	CE	Transition 2	CE	Transition 3	CE
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (-)	HMX	Cold	102.00>46.1	24	N/A		N/A	
Hexahydro-1,3,5-trinitro-1,3,5-triazine (-)	RDX	Cold	129.00>82.05	19	129.00>84.95	12	129.00>46.05	11
1,3,5-Trinitrobenzene (-)	1,3,5-TNB	Cold	213.00>183.05	12	213.00>94.95	25	213.00>125	18
1,3-Dinitrobenzene (-)	1,3-DNB	Cold	168.02>138.10	14	168.02>46	20	168.02>108.05	16
3,5-Dinitroaniline (-)	DNA	Cold	183.03>46	23	183.03>153.10	13	183.03>123.20	18
Nitroglycerin (-)	NG	Cold	62.00>46.10	26	N/A		N/A	
Methyl-2,4,6-trinitrophenylnitramine (-)	Tetryl	Cold	241.02>213.05	8	241.02>181.05	9	241.02>196.1	17
2,4,6-Trinitrotoluene (-)	TNT	Cold	227.02>210.05	11	227.02>197.1	11	227.02>124.00	17
2-Amino-4,6-dinitrotoluene (-)	4-Am-DNT	Cold	196.04>46	27	196.04>136.1	19	196.04>150.05	21
4-Amino-2,6-dinitrotoluene (-)	2-Am-DNT	Cold	197.04>167.1	11	197.04>46	30	197.04>119.9	18
2,4-Dinitrotoluene (-)	2,4-DNT	Cold	181.03>135.1	20	181.03>46	31	181.03>116.05	16
2,6-Dinitrotoluene (-)	2,6-DNT	Cold	182.03>152.1	12	182.03>46	23	182.03>122.05	13
Nitrobenzene (-)	NB	Hot	123.03>45.95	23	N/A		N/A	
2-Nitrotoluene (+)	2-NT	Hot	108.00>91.05	-20	108.00>92.8	-21	108.00>65.05	-28
3-Nitrotoluene (+)	3-NT	Hot	108.00>90.95	-21	108.00>65.1	-29	108.00>93.15	-19
4-Nitrotoluene (-)	4-NT	Hot	136.04>46.05	23	N/A		N/A	
Pentaerythritol tetranitrate (-)	PETN	Cold	62.00>46.10	26	N/A		N/A	

3. Results

3.1. Method development

Flow Injection Analysis (FIA) was employed for the preliminary ionization assessment and MRM development of the explosives listed in Table 1. Ionization was evaluated through Q1 and Q3 scans conducted in both positive and negative ion modes. Any detectable precursor ions were further examined using MS/MS scans across a range of collision energies. Initial results indicated that compounds such as nitrobenzene (NB), 2-nitrotoluene (2-NT), 3-nitrotoluene (3-NT), and 4-nitrotoluene (4-NT) exhibited poor ionization under default source conditions, yielding minimal signal intensity. To address this, we varied the source temperature to enhance ionization efficiency for these analytes. Following the identification of viable MRM transitions, we proceeded to systematically optimize the source parameters to improve overall sensitivity and analytical performance.

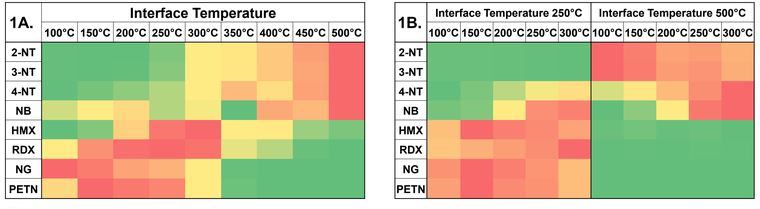

Figure 1. Source temperature optimization. **A.** Interface temperature optimization, **B.** Heat block and DL temperature optimization.

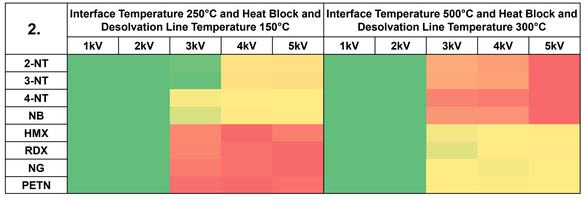

Figure 2. Interface voltage optimization.

Figures 1a, 1b, and 2 illustrate the source parameters optimized to enhance the ionization efficiency of nitroaromatic compounds such as nitrobenzene (NB), 2-nitrotoluene (2-NT), 3-nitrotoluene (3-NT), and 4-nitrotoluene (4-NT). While increased source temperatures significantly improved signal intensity for these analytes, a notable reduction in signal was observed for other explosives, including HMX, RDX, NG, and PETN. This compromise in performance necessitated the development of two separate LC-MS/MS methods for the analysis of the full EPA 8330 compound panel. Despite this division, both methods share identical chromatographic conditions. As a result, the transition between methods is fully automated and does not introduce downtime, allowing efficient and seamless analysis of the entire compound list.

3.2. Quantitative analysis

Table 2. Calibration curve of explosives analytes with the reported linear range, %accuracy, and the %RSD area of the lowest calibrator.

Compounds	LCMS-8050			
	Linear Range (µg/mL)	R^2	Accuracy (%)	LOQ %RSD (Conc.)
HMX	0.005-5.00	0.999	71.31-126.23	6.15
RDX	0.005-5.00	0.999	70.83-111.43	4.65
1,3,5-TNB	0.001-5.00	0.999	71.49-112.24	1.89
1,3-DNB	0.002-5.00	0.999	70.27-124.81	11.03
DNA	0.002-5.00	0.999	72.67-112.46	1.80
NG	0.20-5.00	0.997	79.37-111.29	11.55
Tetryl	0.002-5.00	0.999	71.98-109.61	1.60
TNT	0.001-5.00	0.999	74.67-125.73	3.28
2-Am-DNT	0.005-5.00	0.999	71.07-107.75	7.08
4-Am-DNT	0.01-5.00	0.999	70.52-109.68	10.24
2,4-DNT	0.01-5.00	0.999	71.46-114.61	26.91
2,6-DNT	0.02-5.00	0.999	76.42-115.24	8.80
NB	0.05-5.00	0.999	72.57-105.75	8.30
2-NT	0.05-5.00	0.999	73.52-111.88	6.82
3-NT	0.05-5.00	0.999	76.69-106.55	9.25
4-NT	0.05-5.00	0.999	77.64-106.15	17.38
PETN	0.50-5.00	0.961	83.16-106.5	18.23

The LOQ is defined as the lowest end of the linear range with S/N greater than 10. Quantitative analysis was done using MRM acquisition.

3.3. Sample preparation in River Water Matrix

A river water sample was obtained from the Little Patuxent River in Columbia, MD. The river water sample was filtered with 0.2 µm nylon filters before being analyzed. River water was spiked at different levels: 0.001µg/mL, 0.01 µg/mL, 0.1 µg/mL, and 1 µg/mL. Figure 3 shows chromatograms at different spike levels at or above LOQ listed in Table 2.

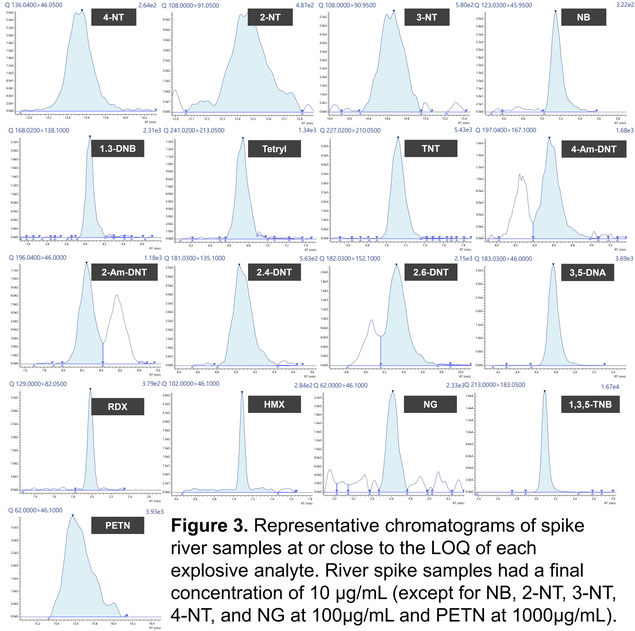

Figure 3. Representative chromatograms of spike river samples at or close to the LOQ of each explosive analyte. River spike samples had a final concentration of 10 µg/mL (except for NB, 2-NT, 3-NT, 4-NT, and NG at 100µg/mL and PETN at 1000µg/mL).

4. Conclusions

Two APCI-LC-MS/MS were developed and tested in river water for the analysis of all compounds listed in EPA 8330b. The use of LC-MS/MS allows for more sensitive and selective analysis in contrast to the traditional PDA; additionally, MRM analysis provides an extra layer of confirmation based on the unique fragmentation of each compound.