Trace analysis of explosives in soil samples using the Agilent 1290 Infinity LC System equipped with an Agilent Max-Light 60-mm cartridge cell

Application Note

Environmental

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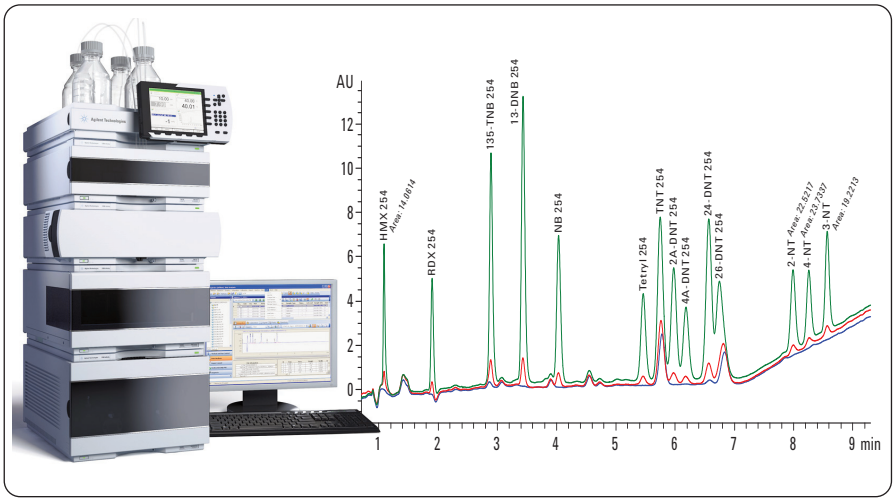

Abstract

This Application Note describes how the Agilent 1290 Infinity LC system equipped with the Agilent Max-Light 60 mm Diode Array Detector cartridge cell was used for residue analyses of nitroaromatic explosives in soil. Excellent sensitivity can be achieved with detection limits below 1 µg/L for standard solutions. Chromatographic selectivity for the structurally related compounds was obtained using an Agilent Poroshell 120 EC-C18 column operated at 44 °C. The developed method is validated and applied to a set of soil samples.

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Introduction

Explosive residues are found in groundwater, sediment, and soil that have been contaminated by military or terrorist activities and civil activities such as mining and construction. Trinitrotoluene (TNT) and its metabolites and hexogen (RDX) are the most commonly used explosives[1,2]. Bacteria in the soil transform TNT to the toxic and mutagenic metabolites 2-amino-4,6-dinitrotoluene (2A-DNT) and 4-amino-2,6-dinitrotoluene (4A-DNT).

The United States Environmental Protection Agency has published EPA Method 8330 for the analysis of nitroaromatics and nitramines[3,4]. The structures of the 14 compounds studied in this work are shown in Table 1. The determination of explosives in environmental samples is a challenging task. The inherent limited thermal stability of some of the explosives makes them unsuitable for GC analysis. Consequently, LC is the method of choice for these compounds. LC/MS using atmospheric pressure chemical ionization (APCI) is applicable for explosive residue analysis[2] because the sensitivity for certain explosives is as low as 1 to 10 ng/kg or ng/L. However, for some compounds the sensitivity is not within standards and the instrumental cost is high.

Explosive residues can be detected at relatively low levels with UV or DAD detectors. One of the disadvantages of using UV-based detection compared to mass selective detection is the lack of selectivity. This can be problematic because some of the compounds are structurally very similar, which makes it difficult to separate them chromatographically. The EPA Method 8330, therefore, recommends the use of two columns. A C18 phase is used as the primary column while an additional analysis on a CN phase is required for confirmation purposes. Coupling columns in series produces a combination of both stationary phases in the same analyses[5]. However, sample matrixes can vary significantly and produce interferences during sample analyses. Nevertheless, LC with UV detection (EPA 8330) is still the method of choice for this analysis.

This Application Note shows the advantages of using state-of-the-art equipment combined with a sensitive detector for the analysis of all explosives, especially those in soil samples. The analyses were performed using the Agilent Poroshell 120 column.

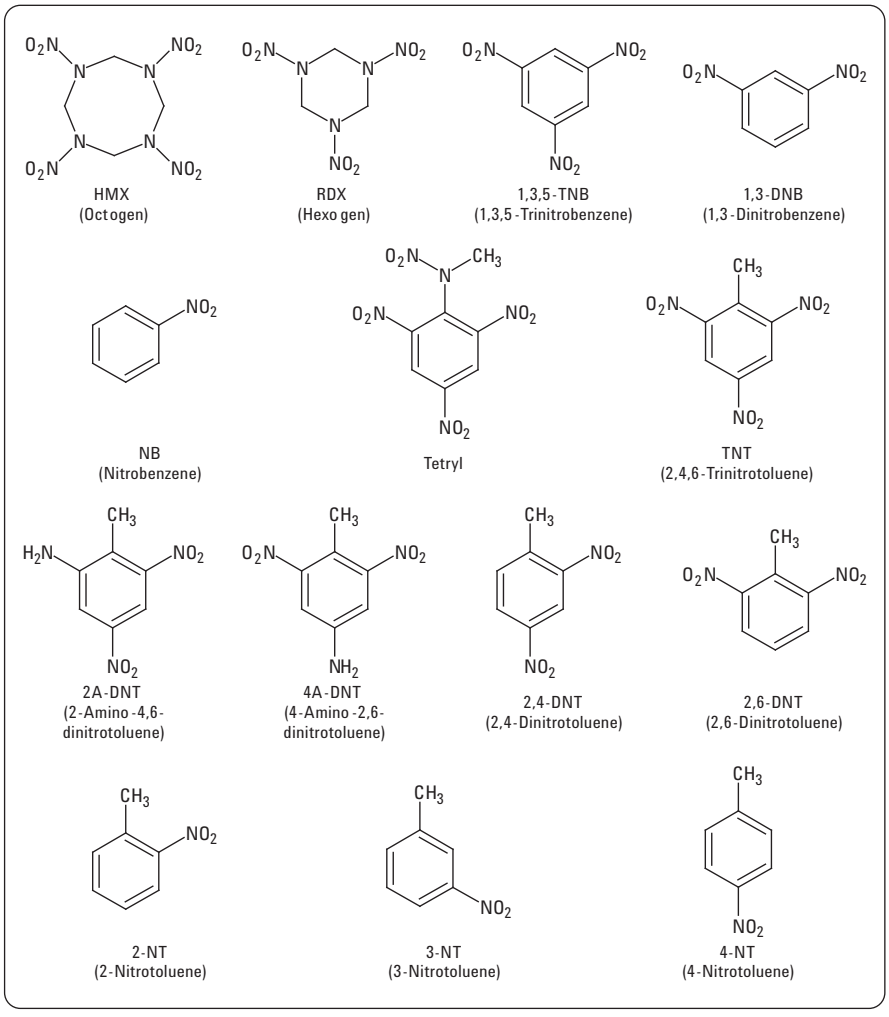

Table 1
Compounds listed in US EPA Method 8330.

Experimental

Chemicals and solutions

The explosive standard mixtures were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The mixtures were prepared as 100 ng/mL of each in acetonitrile (Table 2).

All dilutions and sample solutions were acidified with 0.1% (v/v) formic acid to prevent degradation of tetryl. The final solvent for injection was a mixture of 0.1% formic acid in water/acetonitrile 75/25 (v/v).

Sample preparation

Dry soil samples were extracted as follows:

1. Weigh 2 g of soil in a 15 mL centrifuge tube.
2. Spike with explosives (optional).
3. Add 5 mL 0.1% formic acid in acetonitrile.
4. Shake for 10 s.
5. Ultrasonicate at room temperature for 15 min.
6. Store in refrigerator for 2 h.
7. Ultrasonocate at room temperature for 15 min.
8. Centrifuge.
9. Dilute 1 mL of the liquid with 3 mL of 0.1% formic acid in water.
10. Mix and filter through membrane filter (regenerated cellu lose, 0.45 µm).

Nitroaromate-Explosive Mix 1 (08330100)	Nitroaromate-Explosive Mix 2 (08330200)
1,3-DNB	2A-DNT
2,4-DNT	4A-DNT
RDX	2,6-DNT
NB	2-NT
HMX	3-NT
1,3,5-TNB	4-NT
TNT	Tetryl

Table 2
Sample solutions.

Chromatographic conditions

Method parameters:

Column	Agilent Poroshell 120 EC-C18, 100 mm x 2.1 mm, 2.7 µm (p/n 695775-902)	
Mobile phase	A=0.01% (v/v) formic acid in water B=methanol	
Flow rate	0.55 mL/min	
Gradient	0–2.5 min 2.5–6.5 min 6.5–11.5 min 11.5–13 min	20% to 28% B 28% to 30% B 30% to 70%B 20% B
Column temperature	44 °C	
Injection	20 µL, with needle wash (flushport, 5 s, water/methanol 1/1)	
Sample temperature	15 °C	
DAD	Signal A: 254/8 nm (quantification) Signal B: 234/8 nm (qualifier wavelength for confirmation) Peakwidth > 0.025 min	

Equipment

An Agilent 1290 Infinity LC system with the following configuration was used:

- 1290 Infinity Binary Pump with integrated vacuum degasser (G4220A)
- 1290 Infinity Standard Autosampler (G4226A)
- 1290 Infinity Thermostat (G1330B)
- 1290 Infinity Thermostatted Column Compartment (G1316C)
- 1290 Infinity Diode Array Detector (G4212A)
- Max-Light Cartridge High Sensitivity Cell (60 mm optical path length) (G4212-60007)
- Max-Light Cartridge Standard Cell (10 mm optical path length) (G4212-60008)

Results and discussion

Method optimization

The separation of the 14 compounds in a reasonable time is not straightforward because of structural similarities. Consequently, the choice of the stationary and mobile phase is important. The final chromatographic method is based on the method described in Technical Overview 5990-5552EN[6], but a gradient is used instead of the original isocratic method to analyze the soil matrix. A shallow gradient is applied for separation and elution of the explosives and a column rinsing step is incorporated at the end of the run. The total analysis time including column re-equilibration is 13 min.

Control of the column temperature is crucial for the separation of explosives 6 to 11[3,4,7]. A temperature of 44°C was best to achieve the desired selectivity. A small amount of formic acid was added to mobile phase A to ensure the stability of tetryl. The addition of the acid does not affect the retention of the investigated compounds but has an effect on the baseline shape during the gradient. Therefore, a low concentration of 0.01% (v/v) of formic acid was finally selected to obtain a relatively flat baseline.

Since extraction of the soil samples is carried out with acetonitrile, it is impossible to inject large volumes of the extract without sacrificing chromatographic efficiency and peak shape. The injection volume should be kept below 2 µL under the given analytical conditions. When larger volumes must be injected to increase sensitivity the acetonitrile is diluted with a weaker solvent, such as water. Diluting the samples three times with 0.1% formic acid in water permits the use of injection volumes of 20 µL and higher. For a 20 µL-injection of a diluted sample, the amount loaded on the column is increased 2.5 times compared to an injection of undiluted samples and solutions. An example of the influence of sample solvent and injection volume is shown in Figure 1.

Efforts were made to further concentrate the extract by evaporation under nitrogen and reconstitution in a smaller volume of injection solvent. Unfortunately, some of the more volatile explosives (such as nitrobenzene and nitrotoluenes) were lost during this step.

Method validation

The detection limit with a 20-µL injection was found to be approximately 0.5 ppb (µg/L) in the injected solution when the 60 mm flow cell was installed. This means that the LOD in soil samples should be around 5 ppb (µg/kg). The Max-Light Cartridge high

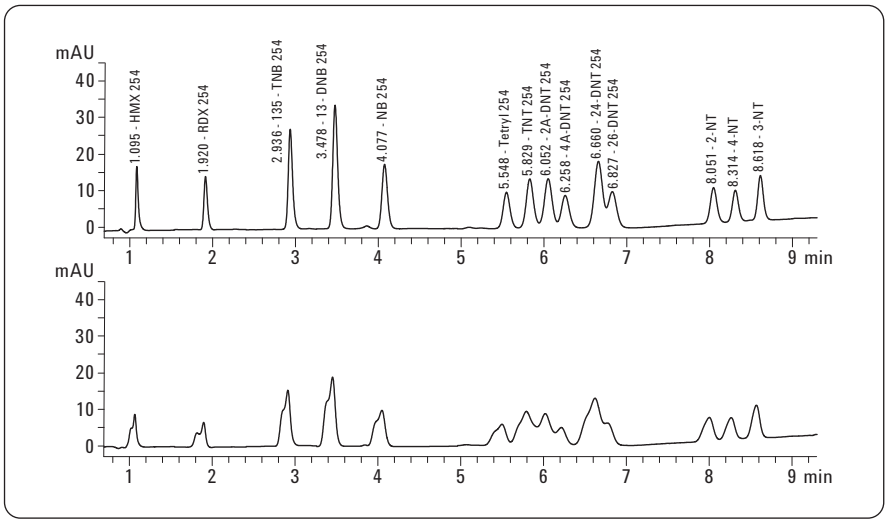

Figure 1
Comparison of an analysis of a standard solution in acetonitrile (500 ng/mL, 5 µL injected) – bottom chromatogram and in 0.1% formic acid in water/acetonitrile (125 ng/mL, 20 µL injected) – top chromatogram. In both analyses, the amount of each explosive on-column was 2.5 ng.

sensitivity flow cell has a significantly longer optical path length (60 mm) compared to the standard flow cell (10 mm) and should in theory improve the sensitivity by a factor of 6. The performance of both flow cells was compared and the results are summarized in Table 3. The signal-to-noise ratio is increased by a factor of approximately 4.5 for injection of a 5 ppb standard solution while the resolution remains unaffected. Consequently, the sensitivity will be about 5 times higher with the high sensitivity cell. This is confirmed in Table 3 when comparing the data for the analysis of a 5 ppb standard solution on a 10 mm flow cell and a 1 ppb standard solution on a 60 mm flow cell. With the standard 10 mm flow cell installed, several of the test compounds could not even be detected at the 1 ppb level. The influence of the increased path length on the signal for a 1 ppb standard solution is shown in Figure 2.

The repeatability of the method was investigated at three different levels. Standard solutions with a concentration of 1, 10, and 100 ppb were each injected eight consecutive times and the RSD was calculated. A calibration curve was constructed by single injections of the following standard solutions: 1, 2.5, 5, 10, 25, 50, 100, 250, and 500 ppb. The results are summarized in Table 4.

	10 mm, 5 ppb		60 mm, 5 ppb		60 mm, 1 ppb	
	S/N ratio	Resolution	S/N ratio	Resolution	S/N ratio	Resolution
Tetryl	4.5		19.5		3.5	
TNT	5.2	1.74	23.4	1.81	4.0	1.65
2A-DNT	5.5	1.30	24.3	1.36	4.7	1.32
4A-DNT	3.8	1.17	16.5	1.16	3.2	1.05
24-DNT	7.0	2.15	32.3	2.16	5.7	2.05
26-DNT	4.3	0.77	20.1	0.84	7.0	0.85

Table 3
Comparison of performance of the Agilent Max-Light 10 mm Cartridge and 60 mm flow cells.

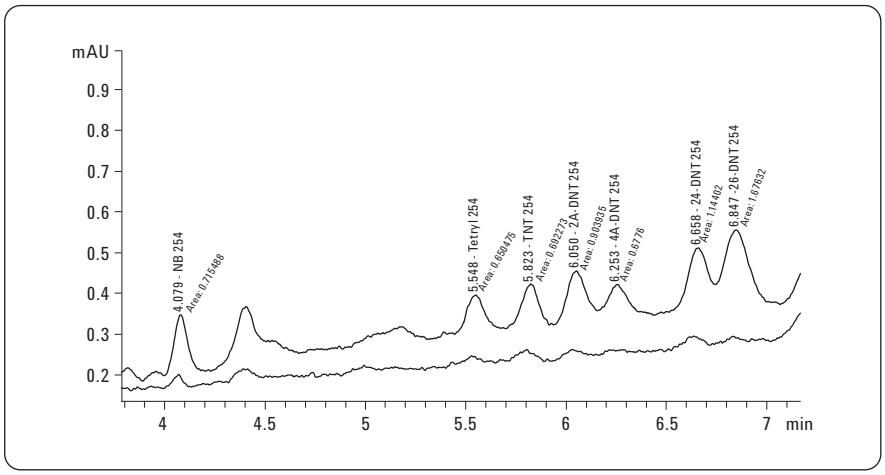

Figure 2
Detail of analysis of a 1 ppb standard solution with Max-Light Cartridge Standard Flow Cell (10 mm optical path length) – bottom chromatogram and with Max-Light Cartridge High Sensitivity Flow Cell (60 mm optical path length) – top chromatogram.

	Repeatability (n=8), %RSD				Linearity (1-500 ppb) R^2
Compound	Retention time 10 ppb	Area 1 ppb	Area 10 ppb	Area 100 ppb	
HMX	0.09	5.94	1.68	1.05	0.9994
RDX	0.11	5.96	1.91	0.88	0.9999
135-TNB	0.10	10.84	1.49	0.46	0.9995
13DNB	0.08	3.71	1.04	0.96	0.9995
NB	0.07	9.05	2.00	1.58	0.9996
Tetryl	0.09	10.85	1.63	1.64	0.9988
TNT	0.10	9.07	1.65	1.29	0.9992
2A-DNT	0.08	6.78	1.55	0.83	0.9994
4A-DNT	0.08	3.51	2.29	1.43	0.9992
24-DNT	0.08	6.78	1.84	1.17	0.9992
26-DNT	0.08	7.65	2.18	1.20	0.9996
2-NT	0.03	13.88	4.18	1.17	0.9997
4-NT	0.03	12.15	3.15	1.48	0.9998
3-NT	0.02	4.62	3.05	1.19	0.9995

Table 4
Method performance.

Sample analyses

Three different soil samples are analyzed before and after spiking with the explosives at the 50 and 500 ppb (µg/kg soil) level. After sample preparation and analysis, the result is compared to the injection of a 5 and 50 ppb (ng/mL) standard solution. The recovery for the spiked samples is calculated after the subtraction of peaks present in the non-spiked samples. The data are shown in Table 5. For most samples the recovery is within reasonable limits (for example, between 80 and 120%).

Some values, however, such as the 50 µg/kg spikes of TNT and 26-DNT are significantly higher than expected with interferences of the sample matrix. In the developed method, the DAD signal at 234 nm was additionally stored for confirmational purposes and the calibration was performed with this wavelength as well. When the detected peak corresponds with the respective explosive the calculated concentrations should be very similar for both wavelengths. This was not the case for the detected peaks in the soil samples in this study. Therefore it was concluded that these peaks are interferences of another nature.

This demonstrates the well known lack of selectivity of UV-based detectors compared to mass selective detection for very low concentrations of explosives. However, a DAD detector remains the first choice for expected concentrations in the range of approximately 100 µg/kg. An example of an analysis of nonspiked and spiked soil samples is shown in Figure 3.

	Spike 50 µg/kg			Spike 500 µg/kg		
Compound	**Soil 1**	**Soil 2**	**Soil 3**	**Soil 1**	**Soil 2**	**Soil 3**
HMX	80.7	72.0	69.2	91.9	95.3	90.3
RDX	106.8	95.0	94.2	90.0	89.6	89.6
135-TNB	79.5	100.2	104.5	93.3	99.2	100.5
13DNB	129.6	139.0	152.6	96.9	100.4	100.9
NB	104.8	110.7	107.0	104.3	111.2	108.3
Tetryl	95.1	95.4	94.7	92.7	94.2	96.6
TNT	137.5	248.9	191.1	100.6	97.6	93.1
2A-DNT	101.7	96.6	97.9	93.2	95.0	96.9
4A-DNT	102.4	91.5	96.5	90.5	93.1	95.3
24-DNT	121.0	89.9	88.8	98.3	96.7	97.9
26-DNT	157.7	230.5	202.0	99.3	93.8	93.6
2-NT	83.0	91.2	107.8	108.3	118.0	105.0
4-NT	115.7	103.2	97.2	108.8	104.9	99.1
3-NT	98.5	106.7	87.2	105.0	108.1	99.7

Table 5
Recovery of explosives in spiked soil samples (values are in % recovery).

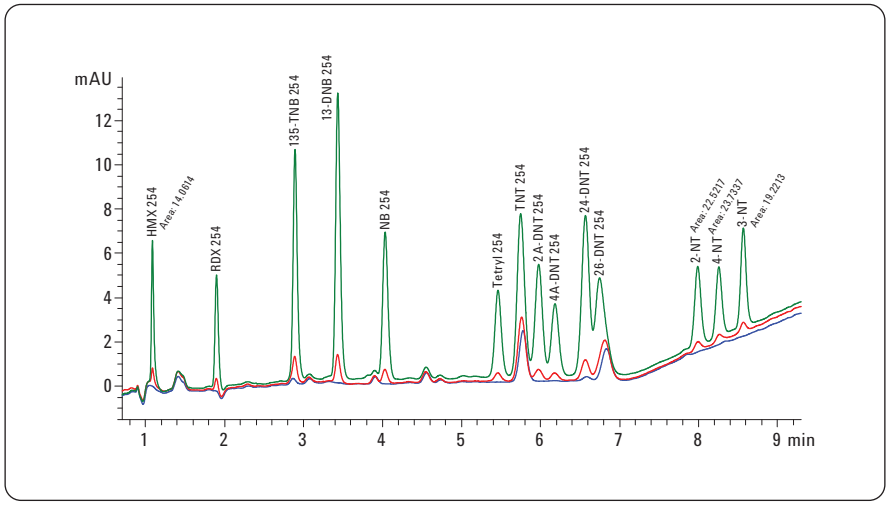

Figure 3
Overlay of a soil sample and spiked soil samples (50 and 500 µg/kg).

Conclusion

The developed method allows detection of the U.S. EPA 8330 explosives at sub-ng/mL levels in standard solutions. When applying the method to soil samples this corresponds to a detection limit of approximatly 5 ppb (µg/kg). Reaching these low levels is possible because the sensitivity of the new Agilent 1290 Infinity DAD in combination with a Max-Light Cartridge High Sensitivity Flow Cell. The results for repeatability and linearity in standard solutions and recovery in spiked soil samples demonstrate the applicability of this approach for routine analysis. Additionally the Agilent Poroshell 120 EC-C18 column proved its significance for supplying the necessary selectivity to separate these structurally similar compounds in a relatively short analysis time.

References

1.
R. Schuster, A. Gratzfeld-Huesgen, "HPLC Analysis of Explosive Constituents in Soil Samples," Agilent Technologies, publication, 5091-7626E, **1993**.

2.
R. Kinghorn, C. Milner, "Analysis of Trace Residues of Explosive Matrials by Time-of-Flight LC/MS," Agilent Technologies publication 5989-2449EN, March **2005**.

3.
U.S. Environmental Protection Agency, "EPA Method 8330, Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC), Revision 0," September **1994**.

4.
U.S. Environmental Protection Agency, "EPA Method 8330A, Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC), Revision 1," February **2007**.

[5.
M.J. Lang, "HPLC Analysis of Explosives using EPA Method 8330," Varian, LC Application Note nr. 24.

6.
"Isolation, Purification and Chromatography of Nitro Compounds and Explosives," Agilent Technologies publication 5990-5552EN, April **2010**.

7.
J.W. Henderson Jr., W.J. Long, "Separation of Explosives in EPA 8330: Column Choices Optimize Speed, Resolution, and Solvent Use," Agilent Technologies publication 5989-7632EN, November **2007**.

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