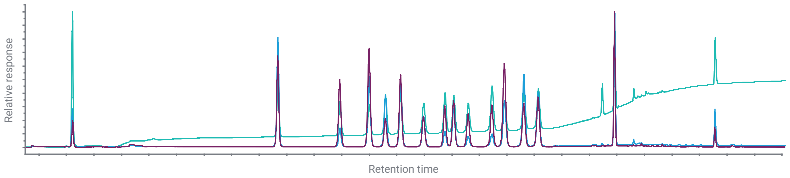
Application Note

Environmental

Determination of Explosives in Soil Using the Agilent 1260 Infinity III LC System

Injector workflows for reliable calibration without manual work



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Abstract

Both military and certain civil activities can lead to explosive residue found in the environment. A new regulation in Germany requires the determination of six explosives in soil. Hexyl, one of these six compounds, is not part of established analysis methods. This application note demonstrates the analysis of 16 explosives and related compounds, including Hexyl, in soil within a single separation. Injector workflows available with Agilent InfinityLab autosamplers allow automating the preparation of calibration solutions.

Introduction

Residues of nitroaromatics and nitramines used as explosives can be found in the environment due to military activities, as well as civil activities such as mining and construction. In Germany, a new regulation (Bundes-Bodenschutz- und Altlastenverordnung (BBodSchV)) came into effect in 2023 and requires the determination of the explosives 2,4-dinitrotoluene, 2,6-dinitrotoluene, Hexyl, RDX, PETN, and TNT.[1] Hexyl is not included in established methods, such as US EPA method 8330B.[2] According to the BBodSchV, the above-mentioned explosives in soil must be determined using the procedure described in DIN ISO 11916-11, which describes the determination of selected explosives and related compounds using HPLC with UV detection.[3] Figure 1 shows the explosives and related compounds covered in DIN ISO 11916-1.

As seen in Figure 1, some of the explosives and related compounds are structurally very similar, which makes it difficult to separate them chromatographically.[4] Accordingly, the US EPA method 8330B recommends analysis employing a C18 phase as a primary column and an additional analysis for confirmation employing a CN or phenyl-hexyl phase.[2,4,5] The Agilent InfinityLab Poroshell 120 PFP (pentafluorophenyl) stationary phase provides extra retention and selectivity for positional isomers of nitro-substituted benzenes[6], allowing complete separation of the explosives and related compounds shown in Figure 1.

This application note demonstrates analysis of the explosives and related compounds covered in DIN ISO 11916-1 in soil samples, employing the Agilent 1260 Infinity III LC and an InfinityLab Poroshell 120 PFP column. Preparation of calibration standards is performed using the injector workflows available with Agilent InfinityLab autosamplers. The automated preparation of calibration standards avoids manual liquid handling steps, saving time and cost, and achieving results independent of operator skills.[7] The 1260 Infinity III LC is equipped with Agilent InfinityLab Assist and Agilent InfinityLab Level Sensing. The InfinityLab Assist allows for automation of system preparation and flushing, thereby avoiding manual work and saving time. InfinityLab Level Sensing facilitates accurate tracking of HPLC solvent levels, preventing the LC from running dry.

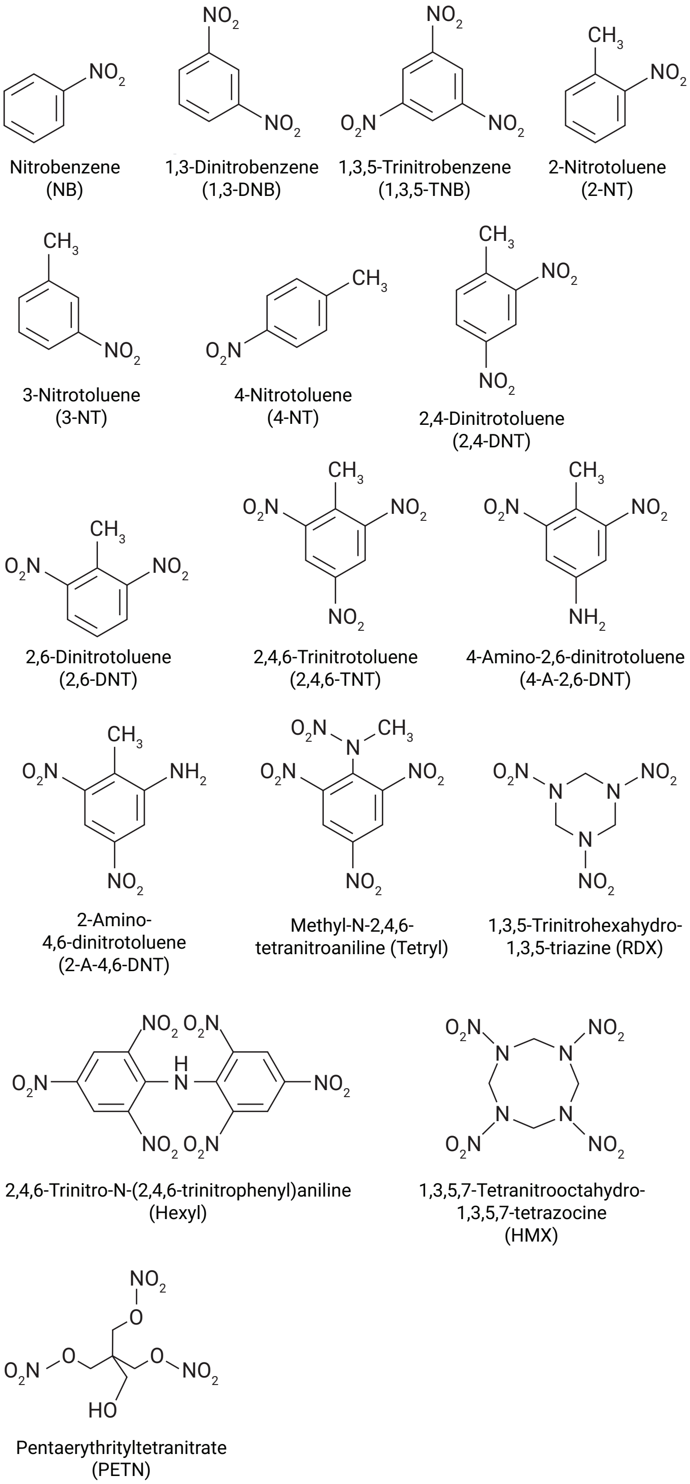

Figure 1. Explosives and related compounds covered in DIN ISO 11916-1.

Experimental

Equipment

The Agilent 1260 Infinity III LC System comprised the following modules:

- Agilent InfinityLab Assist (G7180A)
- Agilent InfinityLab Level Sensing (G7175A)
- Agilent 1260 Infinity III Quaternary Pump (G7111B)
- Agilent 1260 Infinity III Vialsampler (G7129C)
- Agilent 1260 Infinity III Multicolumn Thermostat (G7116A)
- Agilent 1260 Infinity III Diode Array Detector HS (G7117C) with Agilent InfinityLab Max-Light Cartridge Cell 10 mm, (G4212-60008)

Software

Agilent OpenLab CDS, version 2.8 update 07 (or later versions)

Columns

Agilent InfinityLab Poroshell 120 PFP, 3.0 × 150 mm, 2.7 µm (part number 693975-308)

Chemicals

All solvents used were LC grade. Agilent InfinityLab Acetonitrile for LC/MS, gradient grade (part number 5191-5100)* and Agilent InfinityLab Methanol for LC/MS, gradient grade (part number 5191-5110)* were obtained from Agilent. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak). The nitroaromatic/nitramine mix containing 1,3,5-trinitrobenzene (1,3,5-TNB), 1,3-dinitrobenzene (1,3-DNB), 2,4,6-trinitrotoluene (2,4,6-TNT), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), 2-amino-4,6-dinitrotoluene (2-A-4,6-DNT), 2-nitrotoluene (2-NT), 3-nitrotoluene (3-NT), 4-amino-2,6-dinitrotoluene (4-A-2,6-DNT), 4-nitrotoluene (4-NT), 1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine (HMX), nitrobenzene (NB), 1,3,5-trinitrohexahydro-1,3,5-triazine (RDX) and N-methyl-N-2,4,6-tetranitroaniline (Tetryl), 1,000 µg/mL each in acetonitrile (part number NAIM-833E-1) was from Agilent. 2,4,6-Trinitro-N-(2,4,6-trinitrophenyl) aniline (Hexyl), 100 µg/mL in acetonitrile (HPC Standards), pentaerythrityltetranitrate (PETN), 1,000 µg/mL in methanol (Restek), potassium dihydrogen phosphate, and formic acid were purchased from VWR (Darmstadt, Germany). Phosphoric acid was obtained from Merck (Darmstadt, Germany).

Standard solutions

From the above-described stock solutions of the explosives and related compounds, a standard solution containing 10 µg/mL of each compound in acetonitrile with 0.1% formic acid, as well as a stock solution containing 80 µg/mL of each compound in acetonitrile with 0.1% formic acid for preparation of calibration solutions were prepared. Formic acid was added to prevent degradation of tetryl.[4]

Sample and sample preparation

A soil sample was obtained from a local garden and prepared following a modification of the procedure described in DIN ISO 11916-1 as follows: Coarse impurities were removed, and the sample was homogenized by sieving. An aliquot of approximately 5 g of the homogenized sample was weighed into an extraction vessel. 10 mL of acetonitrile were added to the extraction vessel. The extraction vessel was sealed, shaken briefly by hand, and ultrasonic extraction was applied in a bath at 50 °C for four hours. The soil particles were allowed to settle for 30 minutes, and an aliquot of the supernatant was filtered using a 0.2 µm Agilent Captiva Premium Syringe Filter (part number 5190-5088). An aliquot of the soil extract was spiked to a concentration of 2 µg/mL employing the 80 µg/mL stock solution.

Preparation of calibration standards and LC analysis

Calibration standards were automatically prepared by the autosampler from the 80 µg/mL stock solution employing a serial four-fold dilution with acetonitrile, with 0.1% formic acid using the injector workflow shown in Figure 2. This results in calibration solutions in the concentration range from 0.08 to 80 µg/mL. Agilent Amber 2 mL Screw Top Vials (part number 5182-0716) with Agilent 400 µL Glass Inserts (part number 5181-3377) and Screw caps (part number 5190-7024) were used. The vial holding the dilution solvent was capped with a preslit septum (part number 5185-5824). The LC method for the analysis of the explosives and related compounds is shown in Table 1.

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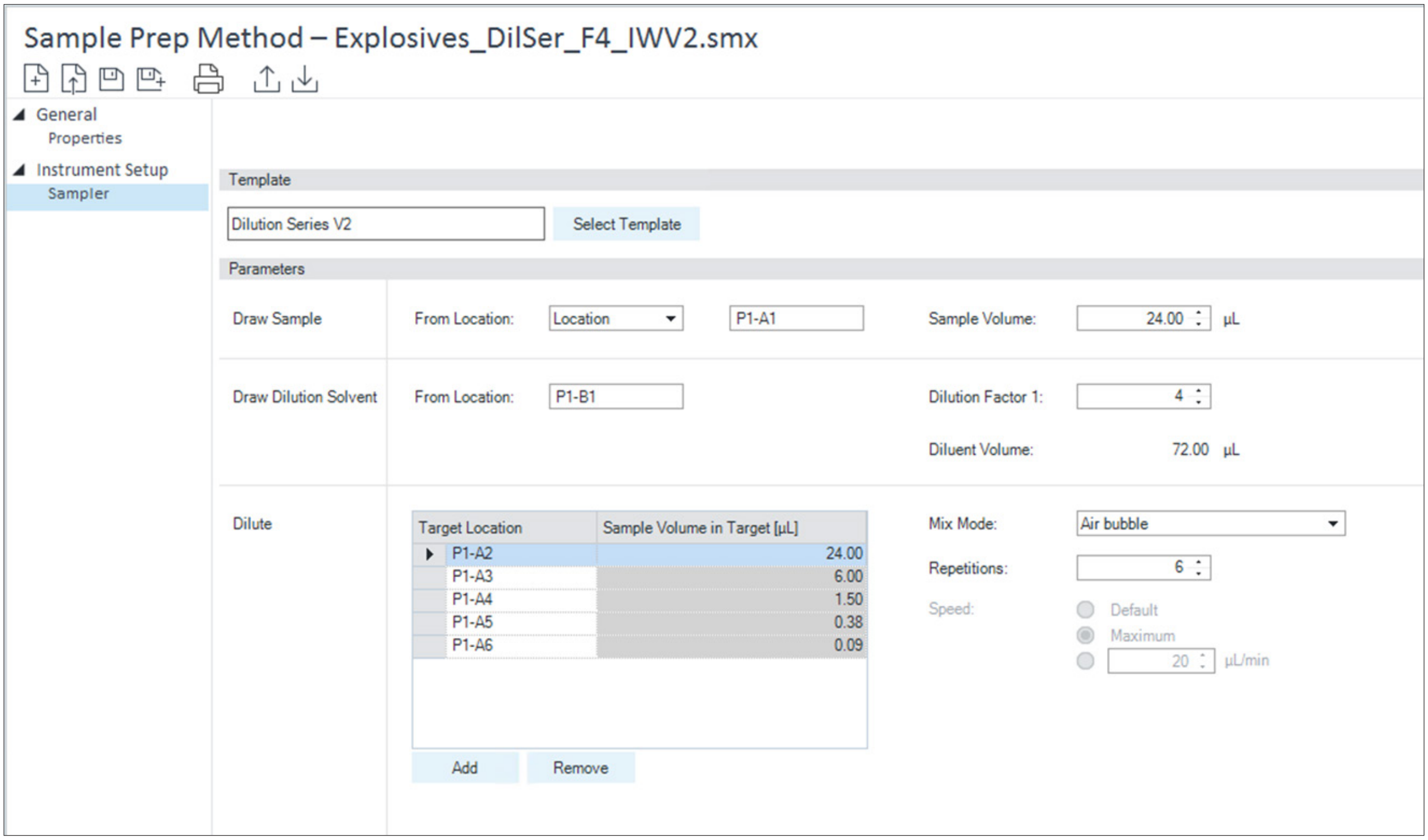
* Only available in select countries



Sample Prep Method – Explosives_DilSer_F4_IWV2.smx

Template: Dilution Series V2

Parameters

Draw Sample — From Location: Location, P1-A1 — Sample Volume: 24.00 µL

Draw Dilution Solvent — From Location: P1-B1 — Dilution Factor 1: 4 — Diluent Volume: 72.00 µL

Dilute

Target Location	Sample Volume in Target [µL]
P1-A2	24.00
P1-A3	6.00
P1-A4	1.50
P1-A5	0.38
P1-A6	0.09

Mix Mode: Air bubble

Repetitions: 6

Speed: ○ Default ● Maximum ○ 20 µL/min

Figure 2. Sample preparation method for automated preparation of the calibration standards.

Table 1. LC method for the analysis of explosives.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120, 3.0 × 150 mm, 2.7 µm
Solvent	A) 10 mM Potassium phosphate pH 3.0 B) Methanol
Gradient	Time (min) %B 0.0 10 0.5 40 16.0 60 20.0 95 Stop time: 28 min Post time: 7 min
Flow Rate	0.5 mL/min
Temperature	25 °C
Detection	210/4 nm, 235/4 nm, 254/4 nm; Reference 480/20 nm 10 Hz
Injection	Injection volume: 5 µL 3 second needle wash in acetonitrile/water (50:50; v/v) Sample temperature: 6 °C

Results and discussion

The separation of explosives and related compounds was successfully accomplished employing the 1260 Infinity III LC and an InfinityLab Poroshell 120 PFP column, as can be seen in Figure 3. The combination of the InfinityLab Poroshell 120 PFP column and a methanolic gradient enabled baseline separation of all 16 compounds covered in DIN ISO 11916-1 within a single analysis. It is known that tetryl shows decomposition in methanol/water solutions as well as with temperatures at or above room temperature[2,3] and that acidic conditions help to prevent decomposition.[2,4] The employed 10 mM pH 3.0 potassium phosphate buffer successfully avoids decomposition of tetryl.

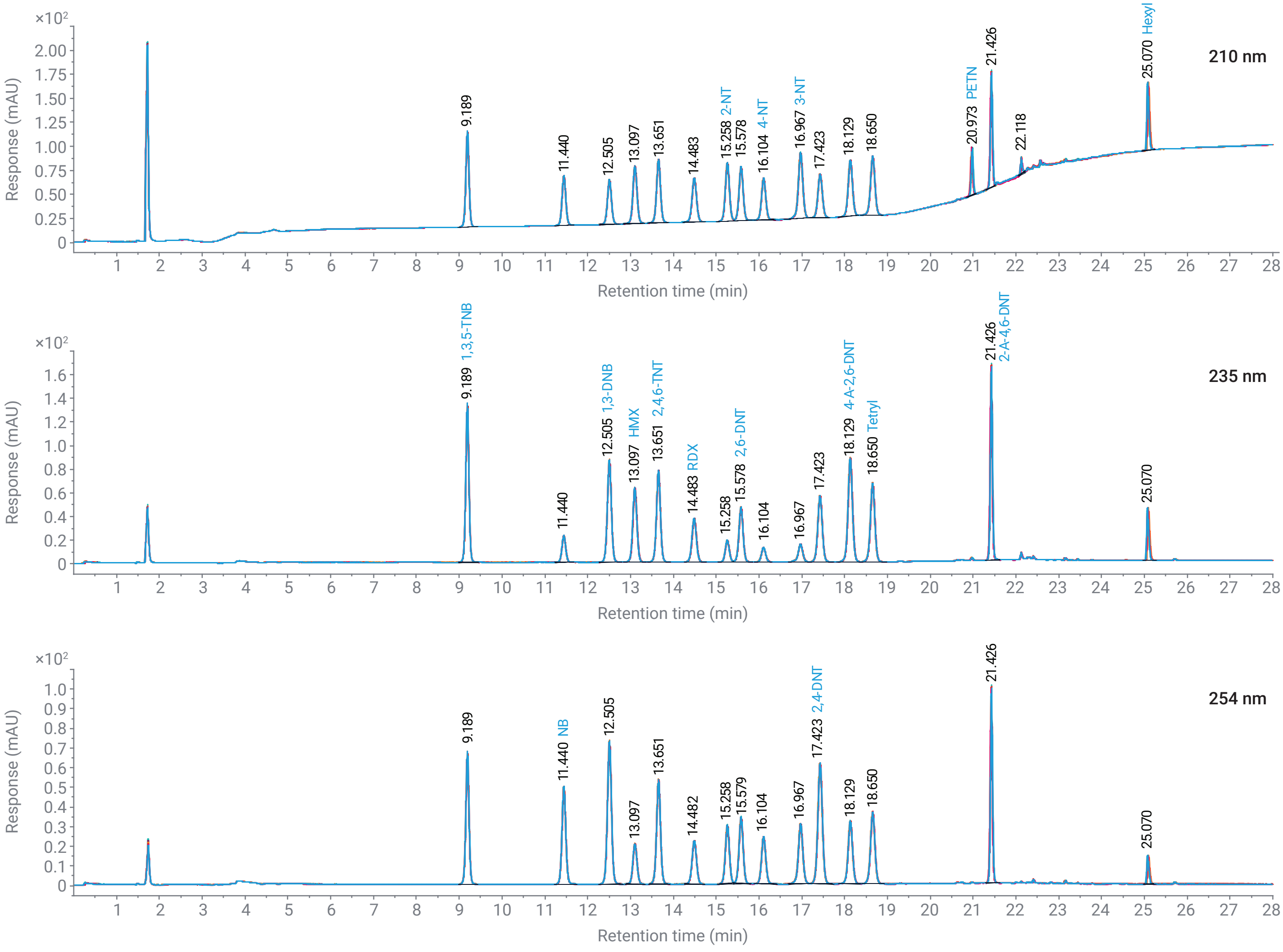


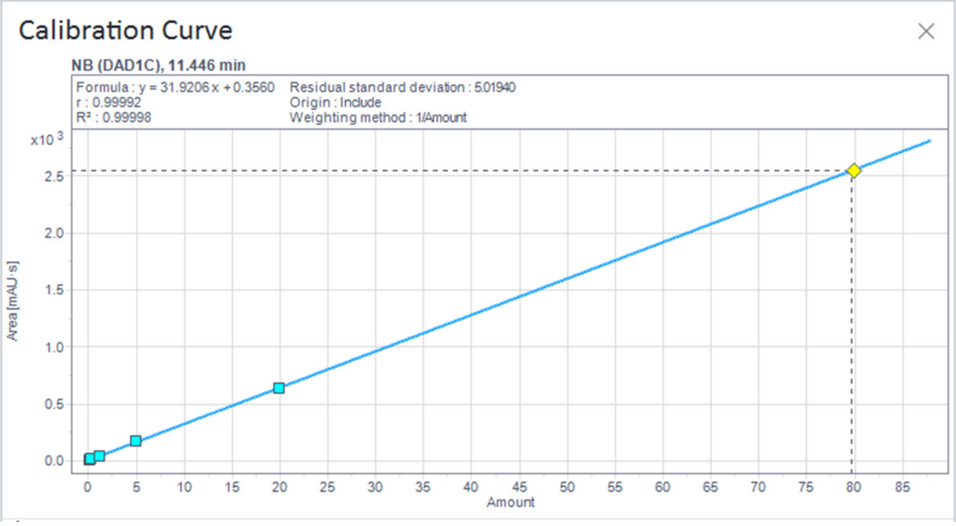
Figure 3. Separation of 16 explosives and related compounds employing the Agilent 1260 Infinity III LC and an Agilent InfinityLab Poroshell 120 PFP column. An overlay of six consecutive analyses of the 10 µg/mL standard solution is shown at different wavelengths.

Table 2 shows the retention time and peak area precision together with the selected detection wavelength for each compound. Excellent retention time and peak area precision is obtained.

InfinityLab autosamplers, in combination with the injector workflows, allow fast and easy automation of the preparation of calibration standards, reducing manual liquid handling steps, saving time and cost, and achieving results that are independent of operator skills.[7] The dilution series injector workflow (see Figure 2) was employed to obtain a serial four-fold dilution of the 80 µg/mL stock solution. The analysis of the resulting calibration solutions resulted in calibration curves with excellent linearity, as is shown for NB in Figure 4. A 1/amount weighting was used for calibration, as low concentrations of explosives and related compounds are expected in soil samples. Table 3 summarizes the calibration results.

Table 2. Retention time and peak area precision (n = 6). Analysis of the 10 µg/mL standard solution.

Compound	Peak No.	Detection Wavelength (nm)	Retention time (min)	RT RSD (%)	Peak Area	Area RSD (%)
1,3,5-TNB	1	235	9.19	0.01	699.4	0.12
NB	2	254	11.44	0.01	311.3	0.13
1,3-DNB	3	235	12.51	0.01	579.2	0.19
HMX	4	235	13.10	0.02	430.8	0.23
2,4,6-TNT	5	235	13.65	0.01	507.6	0.29
RDX	6	235	14.49	0.02	273.7	0.35
2-NT	7	210	15.26	0.01	413.6	0.28
2,6-DNT	8	235	15.58	0.01	320.1	0.31
4-NT	9	210	16.11	0.01	307.2	0.37
3-NT	10	210	16.97	0.02	510.6	0.28
2,4-DNT	11	254	17.43	0.02	458.8	0.18
4-A-2,6-DNT	12	235	18.13	0.02	644.2	0.14
Tetryl	13	235	18.65	0.02	488.9	0.28
PETN	14	210	20.97	0.04	157.9	0.66
2-A-4,6-DNT	15	235	21.42	0.02	563.4	0.26
Hexyl	16	210	25.08	0.04	239.5	0.40


Calibration Curve

NB (DAD1C), 11.446 min

Formula : y = 31.9206x + 0.3560
r : 0.99992
R² : 0.99998
Residual standard deviation : 5.01940
Origin : Include
Weighting method : 1/Amount

Figure 4. Calibration curve for NB in the concentration range of 0.08 to 80 µg/mL.

Table 3. Calibration results for explosives and related compounds.

Compound	Detection Wavelength (nm)	Calibration Range (µg/mL)	R²	S/N at Lowest Calibration Level
1,3,5-TNB	235	0.08–80	0.99997	23.4
NB	254	0.08–80	0.99998	10.2
1,3-DNB	235	0.08–80	0.99998	15.6
HMX	235	0.08–80	0.99995	11.7
2,4,6-TNT	235	0.08–80	0.99996	14.0
RDX	235	0.31–80	0.99997	26.7
2-NT	210	0.31–80	0.99995	17.0
2,6-DNT	235	0.31–80	0.99996	33.1
4-NT	210	0.31–80	0.99993	12.4
3-NT	210	0.31–80	0.99997	20.5
2,4-DNT	254	0.08–80	0.99998	12.5
4-A-2,6-DNT	235	0.08–80	0.99996	15.6
Tetryl	235	0.08–80	0.99997	12.1
PETN	210	0.08–80	0.99994	12.5
2-A-4,6-DNT	235	0.08–80	0.99992	29.4
Hexyl	210	0.08–80	0.99990	22.8

A soil sample from a local garden was analyzed to demonstrate application of the method to a real sample. Figure 5 shows the chromatograms of the analysis of the soil extract, blank (see Figure 5A), as well as spiked with explosives and related compounds (see Figure 5B). In the analysis of the blank soil extract, no explosives or related compounds were detected. Matrix components eluted mainly in the time range between 21 and 26 minutes, showing no interference with the detection of explosives and related compounds.

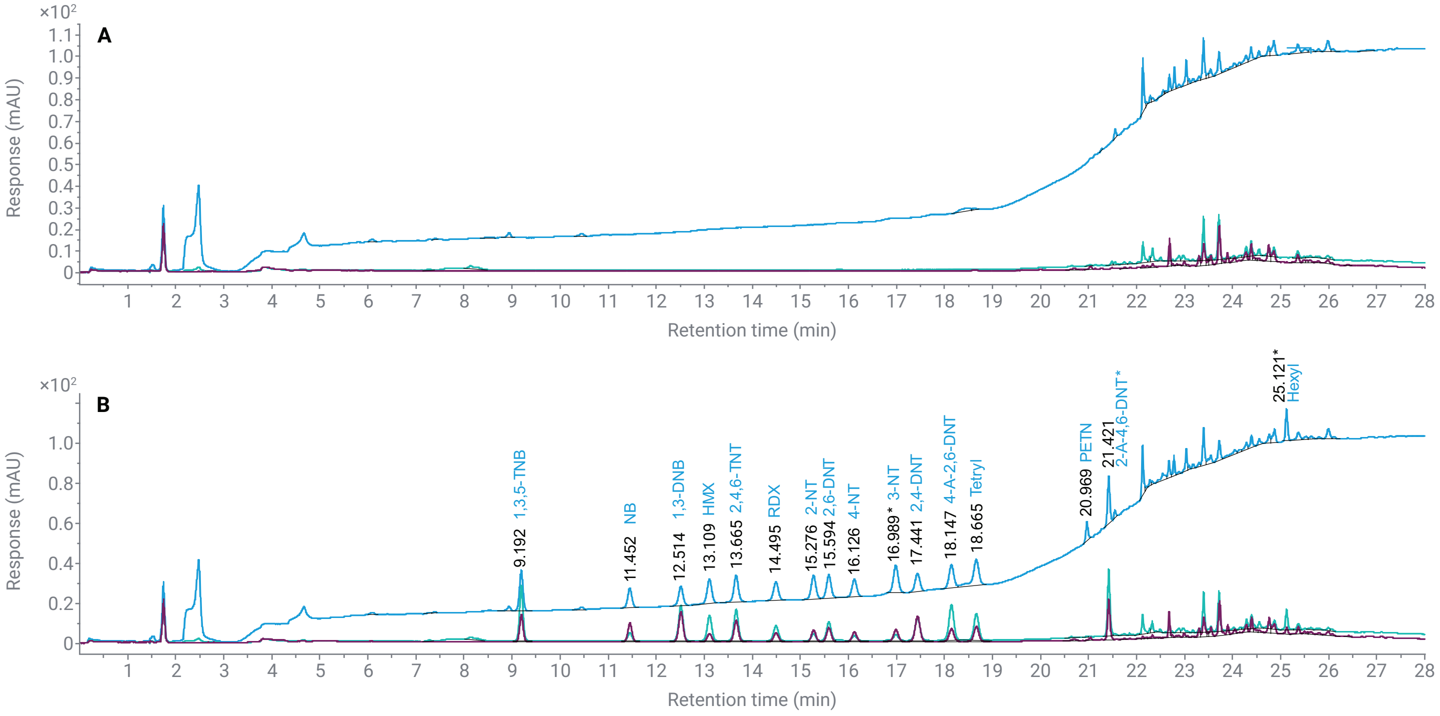


Figure 5. Analysis of a soil sample. (A) Blank soil extract. (B) Soil extract spiked to 2 µg/mL with explosives and related compounds.

Table 4 summarizes the determined concentrations of explosives and related compounds as well as the respective recoveries for the spiked soil extract.

Table 4. Recovery results for explosives and related compounds.

Compound	Soil Extract Spiked to 2 µg/mL	
	Concentration (µg/mL)	Recovery (%)
1,3,5-TNB	1.98	99
NB	1.83	91
1,3-DNB	2.00	100
HMX	1.97	99
2,4,6-TNT	1.98	99
RDX	1.89	95
2-NT	1.92	96
2,6-DNT	1.97	98
4-NT	1.97	99
3-NT	1.89	94
2,4-DNT	1.98	99
4-A-2,6-DNT	1.98	99
Tetryl	1.98	99
PETN	2.04	102
2-A-4,6-DNT	2.03	101
Hexyl	1.87	94

In the analysis of the spiked soil extract, excellent recoveries between 91 and 102% were determined.

Conclusion

Employing the Agilent 1260 Infinity III LC and an Agilent InfinityLab Poroshell 120 PFP column, all 16 explosives and related compounds covered in DIN ISO 11916-1 were successfully analyzed in soil samples within a single analysis. Excellent reproducibility is observed. The injector workflows available with Agilent InfinityLab autosamplers allow fast and easy automation of the preparation of calibration standards, resulting in calibration with excellent linearity while saving time and cost by avoiding manual liquid handling steps.

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