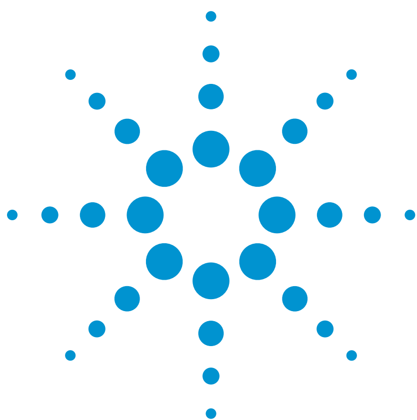

Transferring and optimizing the determination of explosives to fast and high resolution chromatography

Application Note

Environmental

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Abstract

This Application Note describes the method for detection of nanogram levels of explosive constituents in seawater samples, after explosion of ordnance devices. Sample preparation is done by enrichment on highly cross-linked polystyrene-divinylbenzene (PS-DVB) cartridges such as the Agilent SampliQ PS-DVB.

The improved chromatographic method was developed using the Method Development System from Agilent Technologies in combination with the Agilent 1290 Infinity LC System. For the screening experiments, a series of RRHD- and RRHT columns with different selectivities were used. The column with the best selectivity for explosives was the Agilent ZORBAX Stable Bond CN where the separation of eight explosives and the internal standard 2,3-Dinitrodimethylbutane was achieved in 7.0 minutes. Detecting capabilities improved by a factor of 4 with the new 60-mm cell for the Agilent 1290 Infinity Diode Array Detector are shown. The retention time precision was better than 0.5% RSD, and linearity was better than 0.999 over the range from 0.5 to 50 ng/µL.

Agilent Technologies

Introduction

Unexploded ordnance devices have been found in the ground and near the Baltic sea coasts. These old munitions are often corroded and cannot be deactivated. Sometimes the only way for deactivation is a controlled detonation. After these explosions, residues can be found in the water and soil. The most widely found explosives are trinitrotoluene (TNT), its metabolites and hexogen (RXD). The residues must be monitored due to the toxicity. TNT promotes liver damage and anaemia found in chronically exposed munitions workers[1].

According to the limited thermal stability of some of the explosives, for example, hexogen (RXD), octogen (HMX), tetryl or nitropenta only an HPLC method is useful. Early references showed successful separation of explosives in soil samples[2].

This Application Note describes a method for detection of nanogram levels of explosive constituents in seawater samples after explosion of ordnance devices in 7 minutes. Sample preparation was done by enrichment on highly cross-linked polystyrene-divinylbenzene (PS-DVB) cartridges like the Agilent SampliQ PS-DVB.

The chromatographic method was developed using the Method Development System in combination with the Agilent 1290 Infinity LC system. For the screening experiments, RRHD- and RRHT columns with different selectivities were used.

Experimental

Instrumentation

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

G4220A	Agilent 1290 Infinity Binary Pump with integrated vacuum degasser and different solvent mixers
G4226A	Agilent 1290 Infinity Autosampler
G1316C	Agilent 1290 Infinity Column Compartment
G4212A	Agilent 1290 Infinity Diode Array Detector
Software:	ChemStation C.01.01

Table 1
Configuration of the Agilent 1290 Infinity LC system.

Sample preparation

The reference sample used to check the separation was prepared by mixing 50 µL of the 1 mg/mL stock solution of each compound (Figure 1) and diluting with acetonitrile to a concentration of 50 µg/mL in 1 mL sample volume. These solutions can be stored at 6 °C protected from light for six months. The stock solution of the internal standard 2,3-dinitrodimethylbutane was prepared by weighing 10 mg into a 100 mL flask and dissolving it with acetonitrile.

The calibration samples were prepared by mixing aliquots of each stock solution and acetonitrile in a 10 mL flask to yield the final concentration of 0.5, 1, 5, 20, 50 µg/mL. The internal standard was added to a final concentration of 50 µg/mL. As an example of the calibration samples, the solution used for calibration of the 20 µg/mL point was prepared by mixing 200 µL of each stock solution in a 10 mL volumetric flask and diluting it to the final volume with acetonitrile. Calibration points used to evaluate the correlation were: 0.5, 1. 5, 20, 50 µg/mL with the Agilent ZORBAX Stable Bond CN, 2.1 × 100 mm, 1.8 µm column at 0.5 mL/min.

If necessary, the samples can be filtered through borosilicate glass fibre filter with a porosity of 0.75–1.5 µm.

Add 5 g sodium chloride to the (sea) water sample (1000 mL). The SampliQ PS-DVB cartridges should be conditioned with 3 mL methanol followed by 3 mL acetonitrile and 10 mL of water. Afterwards, the sample should flow with a flow rate of approximately 20 mL/min through the cartridge. The salt can be washed out with 5 mL water. The elution is done with 2 aliquots of 2.5 mL of a 50/50 v/v mixture of methanol and acetonitrile. The eluate is evaporated by nitrogen gas at 40 °C. The residue is reconstituted with 1 mL of a 50/50% mixture of methanol and acetonitrile.

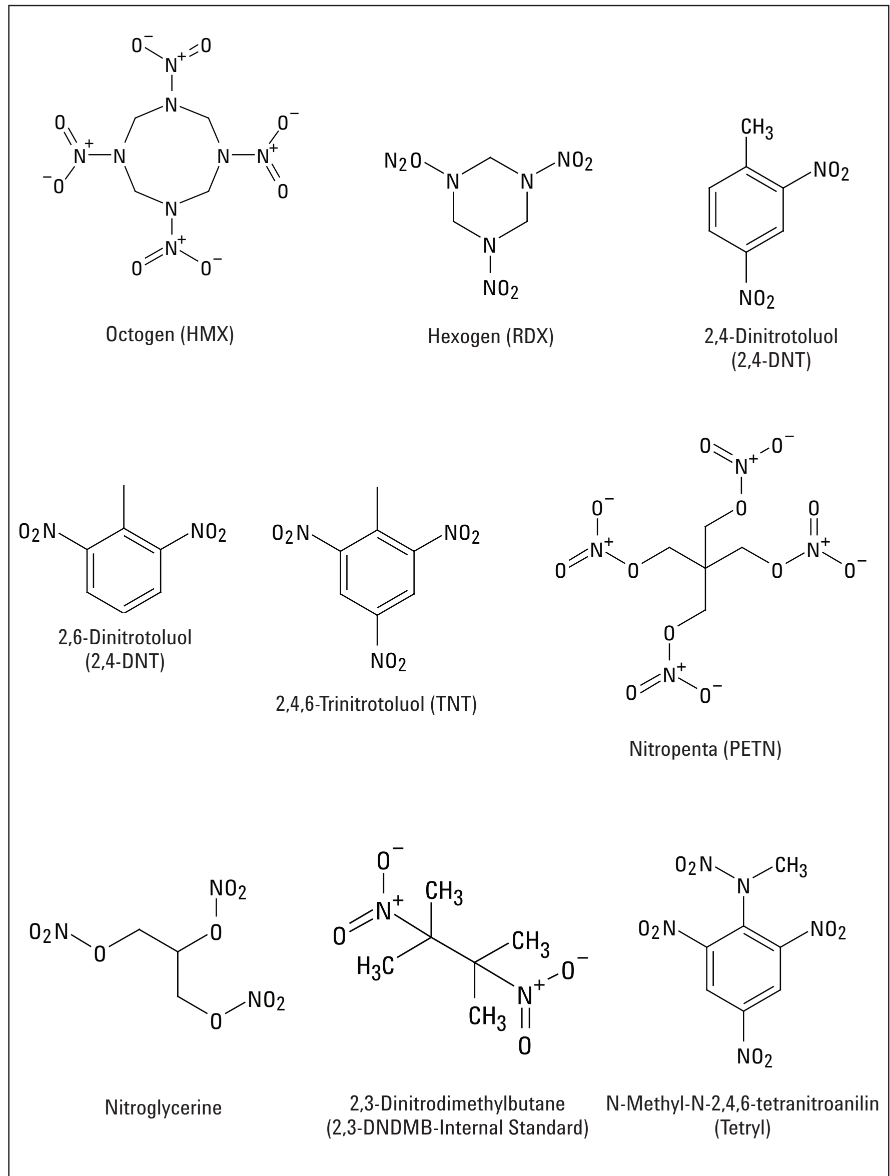

Figure 1
Formulae of explosives.

Results of column screening

With the Agilent Method Development Solution for the Agilent 1290 Infinity LC system, up to seven columns with one flushing channel in two column compartments can be used. It is possible to combine RRHD columns (up to 1200 bar) with RRHT columns (up to 600 bar) with automatic setup of the maximum pressure in the system, according to the pressure limits of the columns. If switched to the next column, the pressure of the actual combination will be reduced down to 100 bar before switching to the next column and loading the new parameters for the next column. Both RRHD and RRHT columns were used (see list on right). Optimization of gradients, temperatures, solvents and columns are possible and could be combined. All results are achieved overnight.

All chromatograms show the 214 nm signal in order to detect 2,3-DNDMB and nitroglycerine. Higher selectivity is achieved at 235 nm for most substances. Identification and confirmation of each component is mandatory and can be done with the acquired DAD-spectra compared with spectra of a library (self created).

Chromatographic conditions

Columns for method development:

1. Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm, RRHD, (p/n 959758-906)
2. Agilent ZORBAX Eclipse Plus Phenyl-Hexyl, 2.1 × 100 mm, 1.8 µm, RRHT, (p/n 959764-912)
3. Agilent ZORBAX Stable Bond C18, 2.1 × 100 mm, 1.8 µm, RRHT, (p/n 828700-902)
4. Agilent ZORBAX Stable Bond AQ, 2.1 × 100 mm, 1.8 µm, RRHT, (p/n 828700-914)
5. Agilent ZORBAX Stable Bond CN, 2.1 × 100 mm, 1.8 µm, RRHT, (p/n 828700-905)
6. Agilent ZORBAX Bonus RP, 2.1 × 100 mm, 1.8 µm, RRHT, (p/n 828768-901)
7. Agilent Poroshell EC-C18, 2.1 × 100 mm, 2.7 µm, RRHT, (p/n 695775-902)

Mobile phases:

Gradient program

Mobile phase A:	Water
Mobile phase B:	Acetonitrile

Screening conditions for the Method Development System:

Components:	HMX, RDX, 2,3-DNDMB, 2,4-DNT, 2,6-DNT, TNT, Tetryl, PETN, and nitroglycerine
Flow:	0.35 mL/min
Gradient:	0 min 20% B, 4 min 95% B
Temperature:	30 °C
Injection volume:	1 µL
DAD:	Signal A: 214/4, Ref. 450/80
	Signal B: 235/4, Ref. 450/80
	Datarate: 20 Hz
	Cell: 10 mm Max-Light Cartridge Cell
	Spectra: full, 190–400 nm

The results of the screening/scouting experiments with a one-step gradient are presented in Figure 2, with the help of the facilities of the Agilent ChemStation with the integrated Intelligent Reporting. With the different selectivities of the columns, the separations differ remarkably. Great differences are seen between the unpolar phases such as the Agilent Eclipse Plus C18 column, the Agilent Eclipse Plus Phenyl-Hexyl column, the Agilent Poroshell EC-C18 column, and the Agilent ZORBAX Stable Bond C18 column (Figure 2: row 2, 1, 6, 3), respectively. Only the Eclipse Plus C18 column shows separation of more than four of the explosives (Figure 2: row 1). The polar RP-phases are more useful. The critical pair 2,4- and 2,6-dinitrotoluol is only completely separated at the Bonus RP and the SB-AQ (Figure 2: row 4, 5), with the disadvantage of coelution of TNT and Tetryl. Separation depending on retention time is best seen in Figure 3. By combining the results of Figures 2 and 3, the column with the best separation power can be chosen for further optimization.

Best separation with the simple gradient was achieved with the Stable Bond CN column (Figure 4, separately shown as real chromatogram) and therefore used for further optimization.

The final method separates all explosives within 7 minutes (Figure 5 and Table 1).

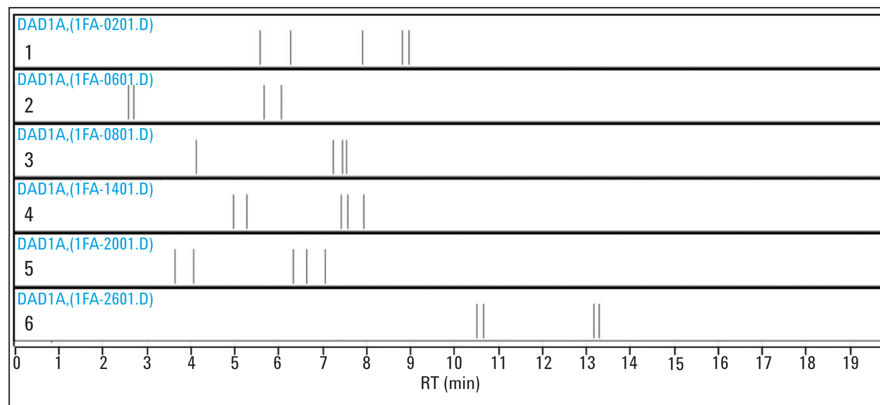

Figure 2
Scouting experiments with different columns.

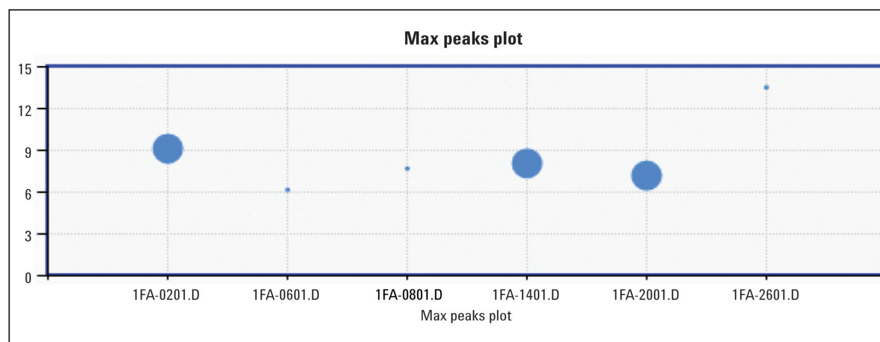

Figure 3
Maximum retention times of separations depending on columns, the bubble size corresponds to the number of separated peaks.

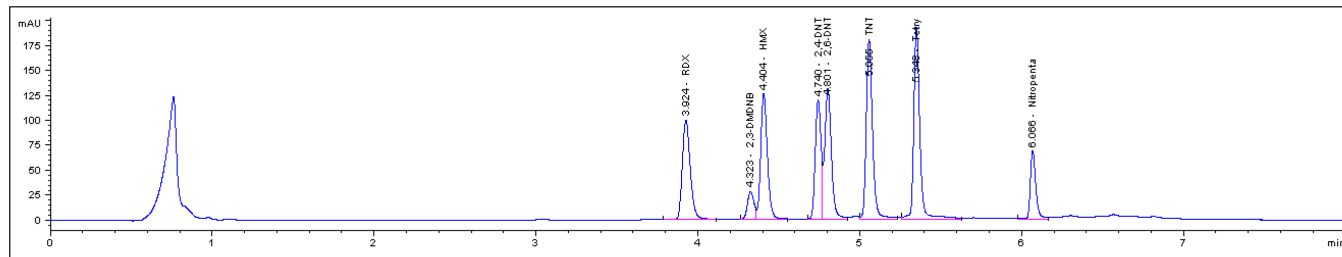

Figure 4
Separation of all explosives with a simple gradient on a Stable Bond CN phase.

Setup for testing precision and linearity

With the following setup for the reference sample the adapted method can be checked:

1. Set up of a chromatographic separation to compare the performance of different column types (Resolution > 2)
2. Precision of areas must be < 1 % RSD.
3. Precision of retention times must be < 0.5 % RSD.
4. Linearity should be given at least with $R^2 > 0.999$

Using these limits and settings for testing, the following samples were prepared and analyzed (Table 2).

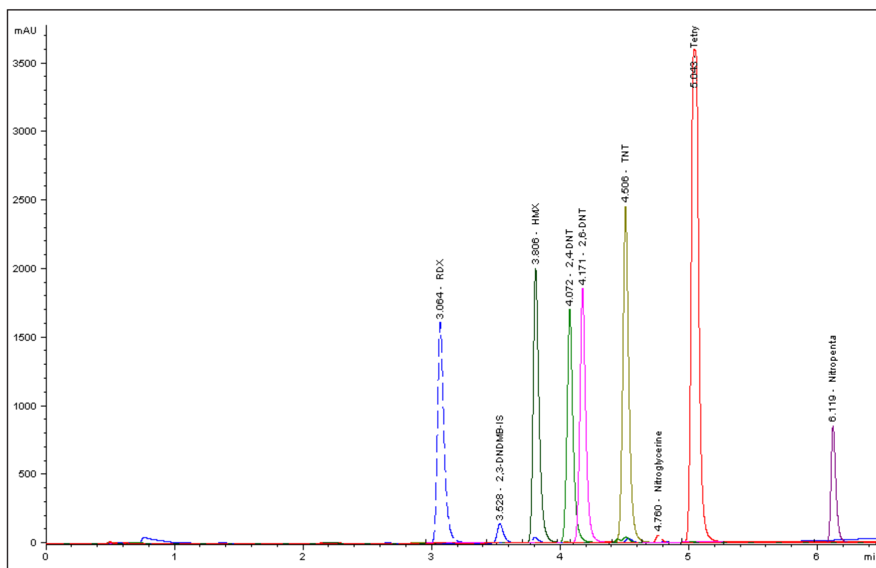

Figure 5
Agilent Stable Bond CN, 2.1 × 100 mm, 1.8 µm (final separation, 2 µL of 1 mg/mL of each, except 2,3-DNDMB and nitroglycerine each 1 µL of 0, 1 mg/mL).

	Stable Bond CN-RRHT, 2.1 x 100 mm, 1.8 µm, 600 bar
Flow rate	0.5 mL/min
Gradient	0–6 min 20% to 40% B
	6–7 min 40% B
Temperature	30 °C
Injection volume	1 µL
Detection	Signal A: 214/4, Ref. 450/80
	Signal B: 235/4, Ref. 450/80
Data rate	20 Hz
Maximum pressure	588 bar

Table 1
Final instrument conditions.

Sample	Purpose	Number of injections
Blank solution	Verify baseline stability and identify artifacts	3
Reference sample	Verify precision of areas and retention times for reference solution	10
Calibration	Verify linearity	3 for each level
Highest concentration and Blanc solution	Verify carry over	3 of each sample

Table 2
Sample analysis.

Results and discussion

Explosives are toxic components that have to be monitored especially after a controlled detonation.

The chromatographic properties result from the chemical structure; many of the components are very polar but not good water soluble substances. These aliphatic or aromatic nitro compounds (see Figure 1) are not well separated on typical columns with non-polar stationary phase material, polar column materials are more suitable.

Table 3 lists the results for resolution and symmetry of all explosives for the final method. For all peaks, the resolution is greater than 2.0.

Table 4 shows the data for the precision of the method according to the separation with the Agilent ZORBAX Stable Bond CN, 2.1 × 100-mm column (Figure 5) with a flow rate of 0.5 mL/min.

The precision data of the retention times prove the high precision and stability of the flow, even at high pressure and high flow rates. The data reflect the high efficiency of the low volume Agilent Jet Weaver as a gradient mixing tool. The data for precision of areas show the high performance of the Agilent 1290 Infinity Autosampler. This is also shown by correlation coefficients for all components greater than 0.999 (see Figure 6 with TNT as an example and reference).

A further improvement in sensitivity was achieved by using the 60-mm cell. Figure 7 shows the chromatograms of the 1 µg/mL standard with the 10-mm Max-Light Cartridge Cell and the 60-mm High Sensitivity Cell at 214 nm. The peak areas and heights are remarkably higher, improving the sensitivity, and the influence of the gradient at the 214-nm signal can be seen.

Compound	Retention time	Resolution	Symmetry
RDX	3.063	--	0.73
2,3-DNDMB	3.528	4.24	0.76
HMX	3.806	3.05	0.74
2,4-DNT	4.072	2.88	0.88
2,6-DNT	4.171	2.01	0.78
TNT	4.505	3.54	0.79
Nitroglycerine	4.760	3.05	0.73
Tetryl	5.042	2.88	0.87
Nitropenta	6.119	15.40	0.76

Table 3
Resolution and symmetries of the explosives of the final separation (see Figure 5).

Compound	Retention time		Areas (50 ng/µL)		Linearity
	Mean	RSD	Mean	RSD	R^2
RDX	3.078	0.214	565.30	0.285	0.9993
2.3-DNDMB	3.538	0.289	685.50	0.366	0.9991
HMX	3.815	0.207	652.20	0.351	0.9988
2.4-DNT	4.083	0.144	515.60	0.371	0.9999
2.6-DNT	4.183	0.259	631.88	0.441	0.9998
TNT	4.518	0.276	815.42	0.299	0.9999
Nitroglycerine	4.760	0.228	772.17	0.311	0.9993
Tetryl	5.053	0.219	882.19	0.289	0.9998
Nitropenta	6.130	0.239	344.66	0.498	0.9998

Table 4
Determination of the precision of areas and retention times for reference sample, linearity for 0.1–50 ng/µL calibration, 214 nm.

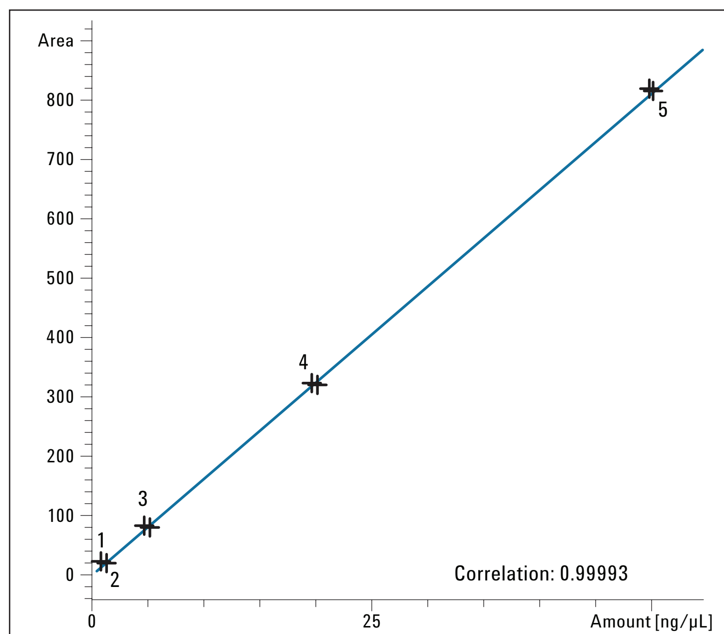

Figure 6
Calibration curve for TNT as an example for all explosives.

If the detection signal is changed to 235 nm, the influence of the eluent is minimized, the sensitivity for 2,3-DNDMB, nitropenta, and nitroglycerine are reduced, but detection at 214 nm is not needed and selectivity could be improved (Figures 7, 8) if the aliphatic components do not need to be detected.

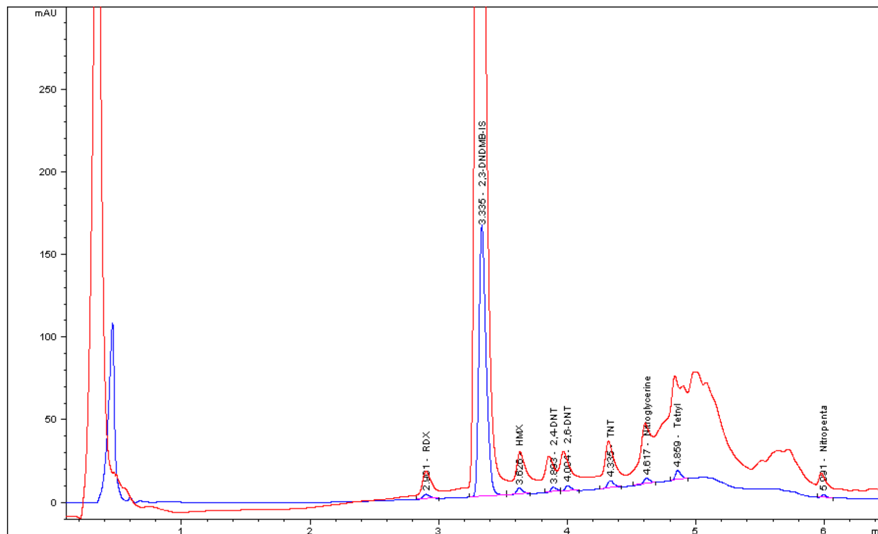

Figure 7
Detection of the 1 µg/mL standard with the 10 mm Max-Light Cartridge Cell (blue) and 60 mm High Sensitivity Cell (red) at 214 nm.

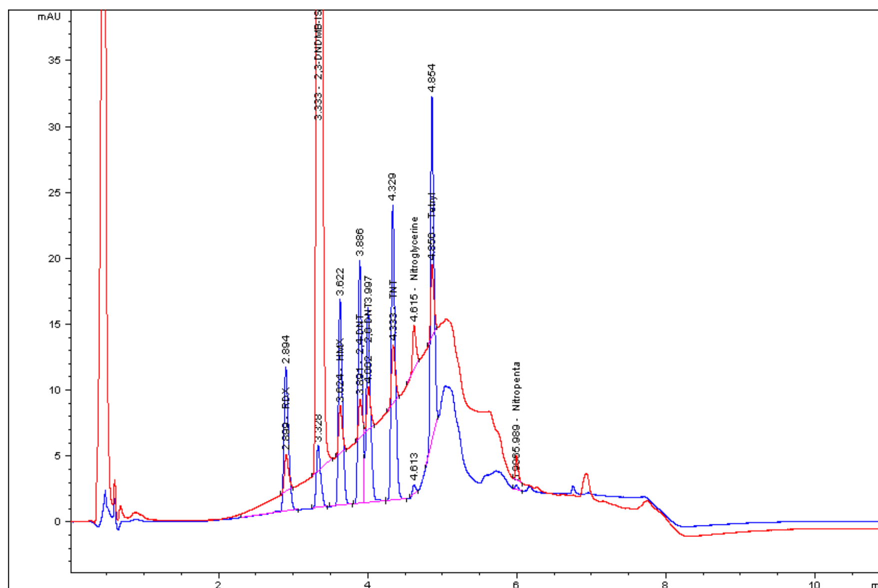

Figure 8
Detection of the 1 µg/mL standard with the 10 mm Max-Light Cartridge Cell (red) and 60 mm High Sensitivity Cell (blue) at 235 nm.

For detection of the contamination after detonation of an ordnance device in the Baltic sea, 1000 mL of the seawater were analyzed to detect any residues. TNT was found at a concentration of 8.8 ng/L (Figure 8), identified by retention time and the UV spectrum. No matrix interference could be detected.

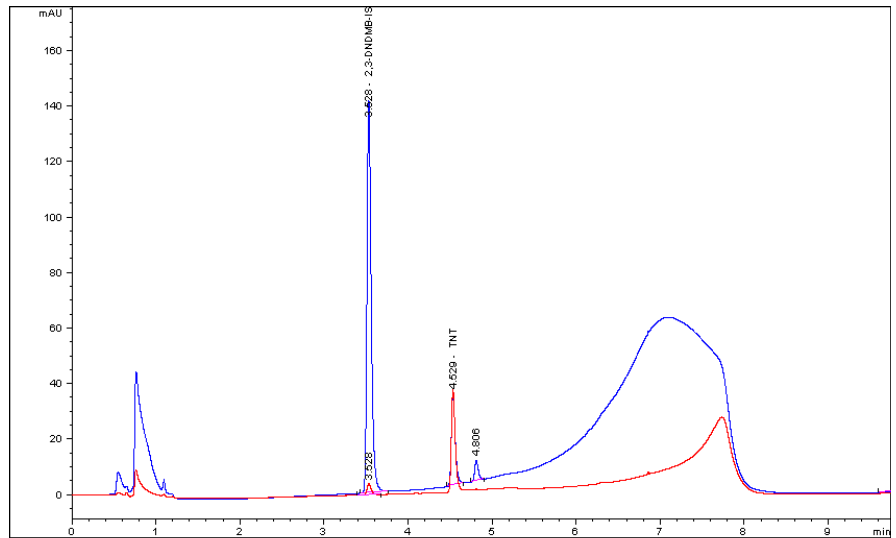

Figure 9
Determination of TNT in seawater after controlled detonation, 8.8 ng/L (214 nm-blue, 235 nm-red) with the 10 mm Max-Light Cartridge Cell.

Conclusion

The Agilent 1290 Infinity LC system is designed to provide highest speed, resolution and sensitivity.

The transfer of a determination of eight explosives and an internal standard from standard materials to RRHD or RRHT columns could be done overnight by selecting the best selectivity by using the Agilent Method Development system.

The results show, that the determination could be shortened to 7 minutes and is free of matrix influences. The overall runtime of the final method of 10 minutes (including backflushing) shows the multiple opportunities to establish high resolution and fast liquid chromatography.

Effective gradient mixing using the low volume Agilent Jet Weaver provides high precision gradient times.

The results in Tables 3 and 4 show that all criteria for the precision of the determination (areas, retention times) and resolution are fulfilled. The coefficients for linearity of all components are better than 0.999. All results show the applicability of the 1290 Infinity LC system for fast screening applications with high security for identification. Sensitivity can be improved by using the 60-mm High Sensitivity Cell for lowest detection limits.

References

1.
N.G. McCormick, F. E. Feeherry, H.S. Levenson, Environ. Microbiol. 176, 31, 949

2.
HPLC Analysis of Explosive Constituents in Soil Samples, Rainer Schuster and Angelika Gratzfeld-Huesgen, Agilent Application Note 12-5091-7626E, 1993

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