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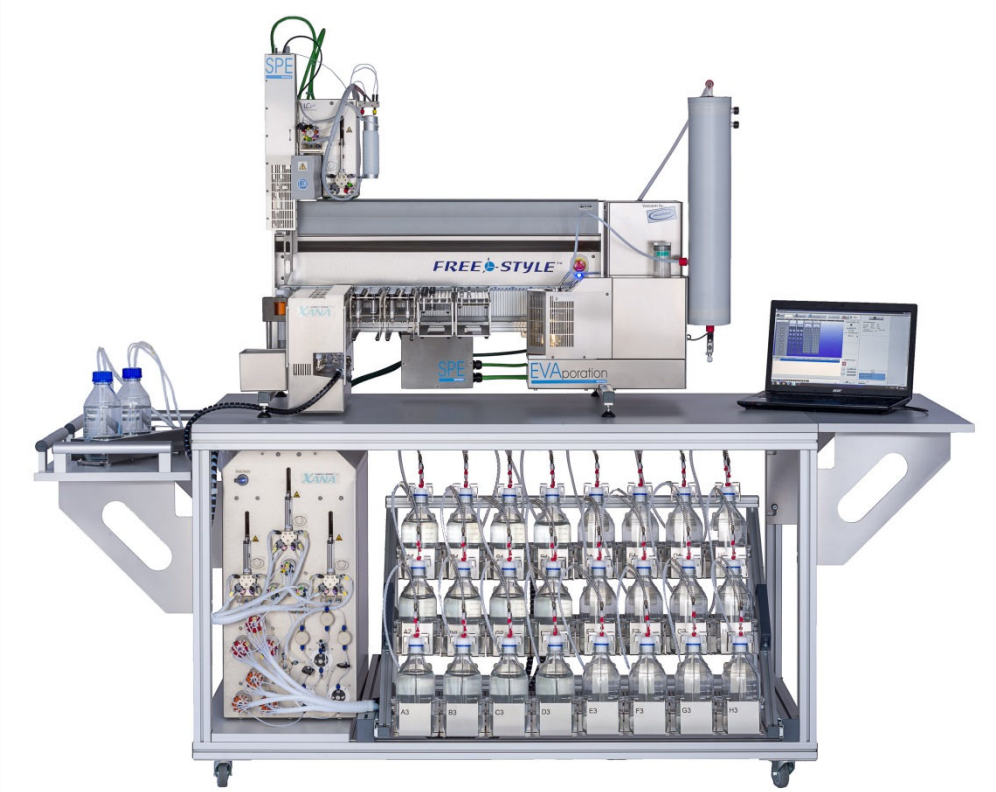

Automated Detection of Selected Explosives and Related Compounds

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Contents

Keywords: DIN EN ISO 22478:2006 (F21), German drinking water ordinance, TrinkwV; DepV – landfill ordiance, US EPA Method 8330, explosives and related substances, nitrotoluenes, nitramines, nitrite esters

1 Introduction

River water and ground water are regularly checked for their quality. For the purpose of this monitoring, samples are taken according to DIN EN ISO 22478:2006 and tested for explosives and related compounds using solid phase extraction. These highly toxic, aromatic components are largely relics from the two world wars and entered the ground and river water through various routes including destroyed ammunition factories. Even today, these substances find their way into the ground water stemming from military manoeuvres and training areas or shooting ranges, which necessitates continuous monitoring.

The following shows how samples are prepared by means of *S*olid *P*hase *E*xtraction (Solid Phase Extraction - SPE), both manually and automatically using the FREESTYLE XANA robotic system for subsequent analysis. Due to the option of using the system around the clock, parallelisation of individual processing steps as well as the simultaneous processing of several samples, a very high sample throughput can be achieved through automated processing. The user's workload is thereby considerably reduced, and more time for other tasks becomes available.

1.1 Analytes

Analytes
2,4,6-Trinitrophenylmethylnitramine (Tetryl)
Diethylene glycol dinitrate (DEGDN)
Cyclotrimethylene trinitramine (Hexogen)
Picric acid
Ethylene glycol dinitrate (EGDN)
1,3,5–Trinitrobenzene (1,3,5-TNB)
1,3-Dinitrobenzene (1,3-DNB)
Nitroglycerin (NG)
2,4,6-Trinitrotoluene (2,4,6-TNT)
Cyclotetramethylentetranitramine (Octogen)
2-Amino- 4,6-dinitrotoluene (2-A-4,6-DNT)
4 Amino-2,6-dinitrotoluene (4-A-2,6-DNT)
2,4-Dinitrotoluene (2,4-DNT)
2,6 Dinitrotoluene (2,6-DNT)
2-Nitrotoluene (2-NT)
4-Nitrotoluene (4-NT)
3-Nitrotoluene (3-NT)
Pentaerythritol tetranitrate (PETN)
Diphenylamine (DPA)
Hexanitrodiphenylamine (HNDPA)

2 Method Development

2.1 Chemicals

- Double distilled water, Milli-Q Integral
- Methanol: for analysis, Merck
- Ammonium acetate: Bio ultra ≥ 99 %, Fluka
- Acetic acid, 99 % p.a., Sigma Aldrich
- Acetonitrile, 99 % p.a., Merck
- Sodium chloride, p.a., Merck

2.2 Standards

- DIN 38407-21-A 2 x 1 mL 10 µg/mL in methanol, AccuStandard
- DIN 38407-21-B 2 x 1 mL 10 µg/mL in methanol: Acetonitrile (98:2), AccuStandard

2.3 Sample material

The sample material used consists of 1 L tap water, which is spiked with 1 µg/L of each analyte.

For its preparation, first dissolve 5 g sodium chloride in 1 litre of each sample.
The sample is then spiked with 100 µL of the standard mix (2.2) to obtain a concentration of 1 µg/L.

2.4 Solid phase extraction (manual)

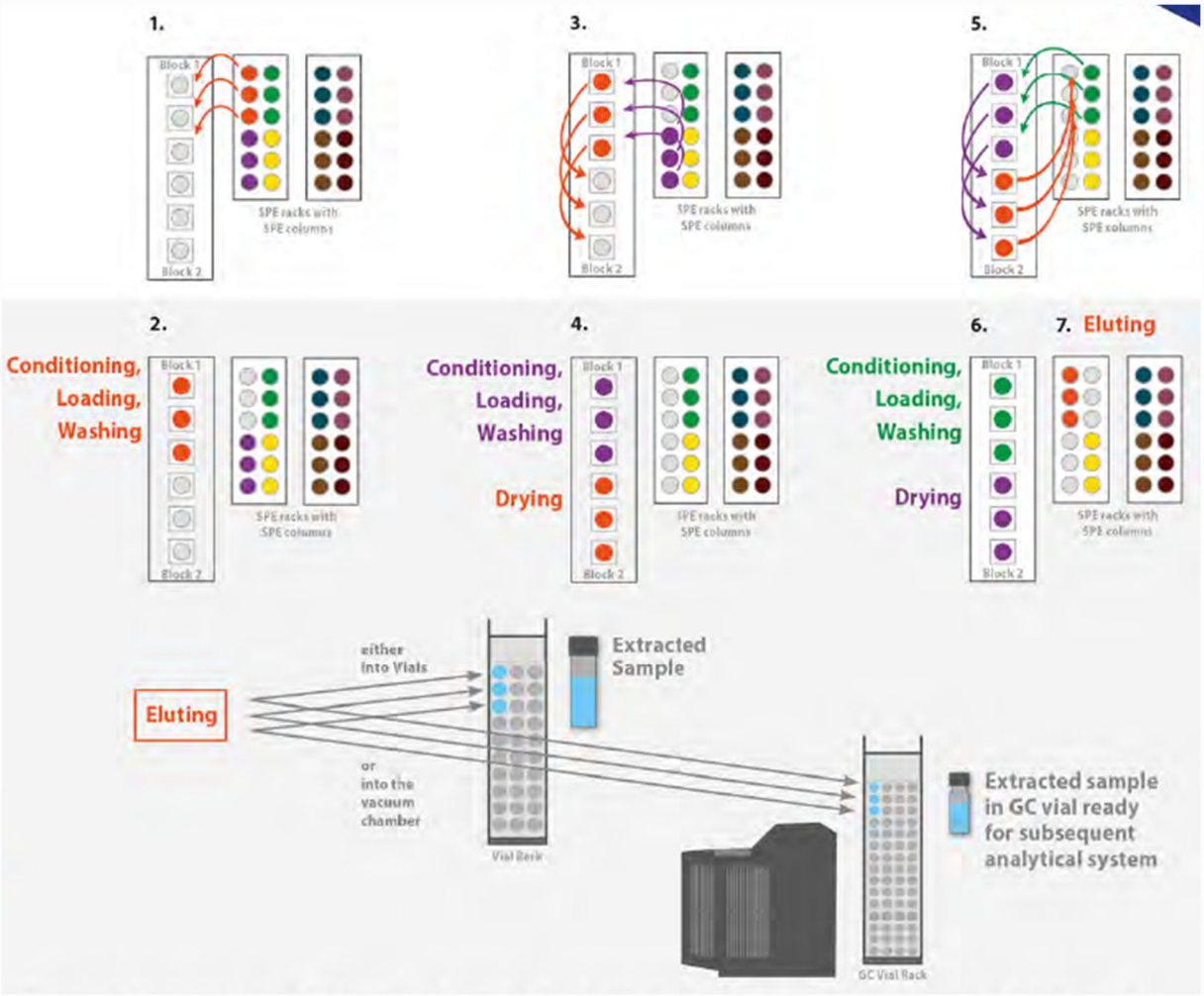
SPE - Steps	CHROMABOND® HR-X, 3 mL, 200 mg
Conditioning	3 mL methanol 3 mL acetonitrile 10 mL water
Loading	sample loading at 1000 mL/h
Washing	10 mL water
Elution	3 mL methanol - acetonitrile (1:1, v/v)

2.5 Solid phase extraction (automated)

2.5.1 FREESTYLE XANA

The LCTech robotic system FREESTYLE XANA is particularly suitable for automated sample preparation of large volume water samples of 1 to 10 L. A very high sample throughput can be achieved as a result of its innovative design, which enables parallelisation of individual processing steps and simultaneous processing of up to 3 samples.

Working mechanism of FREESTYLE XANA



1. The SPE gripper moves up to 3 columns from the SPE rack into block 1 of the working station:

2. Simultaneous conditioning, loading and washing of the 3 columns.

3. The SPE gripper transfers the 3 processed columns from block 1 into block 2 of the working station. Then the SPE gripper takes the next columns (up to 3) out of the SPE rack and places them into block 1 of the working station.

4. Block 1: Conditioning, loading and washing of up to 3 columns simultaneously.
   Block 2: Drying of the first 3 columns.

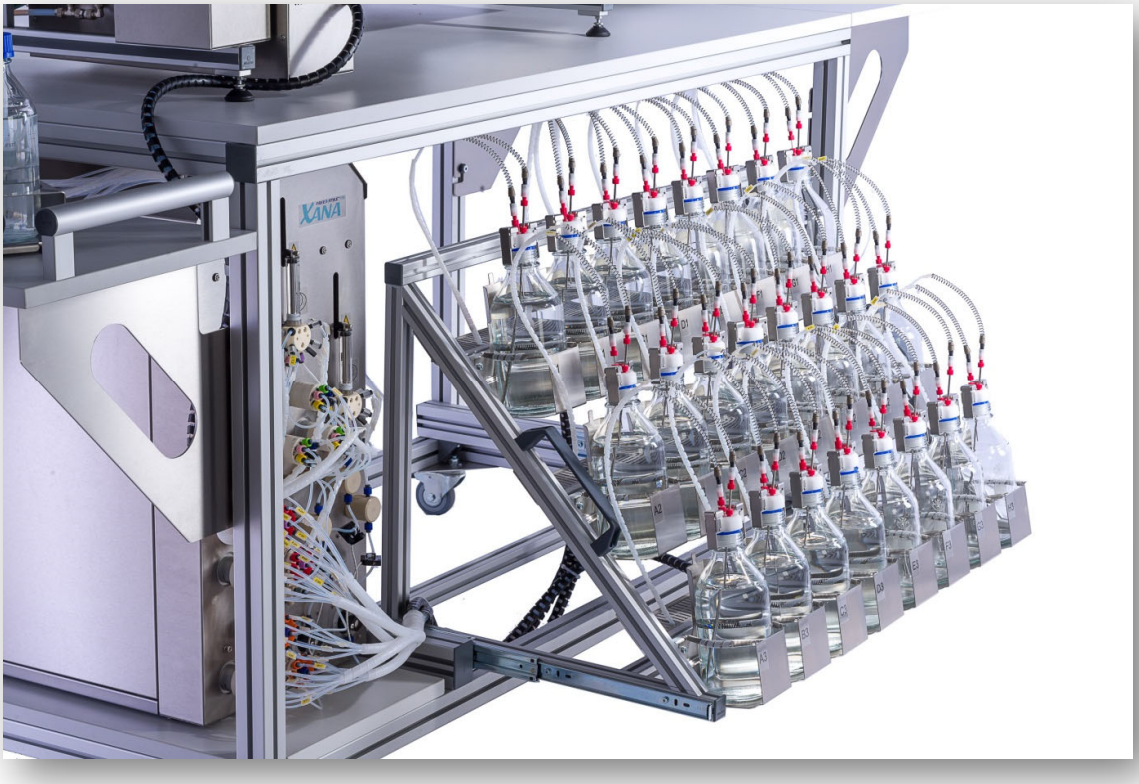
5. The SPE gripper returns the 3 dried columns of block 2 to the SPE rack.
Then, the SPE gripper moves 3 loaded and washed columns from block 1 into block 2 of the working station. The SPE gripper takes the next columns (up to 3) from the SPE rack and places them into block 1 of the working station.

6. Block 1: Conditioning, loading and washing of up to 3 columns simultaneously.
Block 2: Drying of up to 3 columns simultaneously.

7. The first 3 columns are sequentially eluted into vials or into the vacuum chamber of the EVAporation module. After EVAporation to the desired end volume, samples are filled into GC vials. The water samples are now prepared and ready for subsequent analysis.

Working station on the FREESTYLE platform

The sample rack of the FREESTYLE XANA offers space for 24 x 1 L sample containers. Loading of the pull-out sample rack with sample containers is effortless, since each position can be easily accessed.



Each sample container is fitted with a closure that is operated with one hand and holds the suction capillary at an angel. This capillary position in combination with an inclined position of the sample bottle in the rack maximises the sample load by minimising residues in the sample bottle.

2.5.2 Automated Processing with FREESTYLE XANA

The manual solid phase extraction can be easily transferred to the FREESTYLE XANA robotic system for automated sample preparation.

The easy-to-use system software offers a building set of ready-made functions, which make method development on the system fast and uncomplicated.

The following steps are fully automated processed by FREESTYLE XANA

SPE steps	Fully automated
Conditioning	3 mL methanol, 10 mL/min.
Conditioning	3 mL acetonitrile, 10 mL/min.
Conditioning	10 mL water, 10 mL/min.
Loading	1000 mL sample, 10 mL/min.
Washing	10 mL water, 8 mL/min.
Elution	3 mL methanol/acetonitrile 1:1, 3 mL/min.
Drying	10 mL air, 10 mL/min

The detailed method parameterization on the FREESTYLE XANA is shown in the method report below.

FREESTYLE

LCTech FreeStyle - Report on Methods: WAT Date: 28.10.2016 Time: 16:21:33

Name: Explosives_neu.wat

Column:	LCTech_3ml.col		Extension cannula:	yes
Conditioning 1: Volume: Suction Speed:	ON 3 ml 15 ml / min	 Dispensing Speed: Waiting time:	 10 ml / min 0 min	 Port : W2 Methanol
Conditioning 2: Volume: Suction Speed:	ON 3 ml 15 ml / min	 Dispensing Speed: Waiting time:	 10 ml / min 0 min	 Port : W3 Acetonitril
Conditioning 3: Volume: Suction Speed:	ON 10 ml 15 ml / min	 Dispensing Speed: Waiting time:	 10 ml / min 0 min	 Port : W4 H2O
Load 1: Volume: rinsing cycle included Rinsing volume: Rinsing volume:	ON Number of bottles: 15 ml 100 ml / min	 Transfer Speed Suction Speed:	 10 ml / min 20 ml / min	 Port : W4 H2O
Washing 1: Volume: Suction Speed:	ON 10 ml 15 ml / min	stay on actual position Dispensing Speed: Waiting time:	 10 ml / min 1 min	 Port : W4 H2O
Washing 2:	OFF			
Drying 1:	OFF			
Washing 3:	OFF			
Elution 1: Volume: Suction Speed:	ON 3 ml 10 ml / min	 Dispensing Speed: Waiting time: Vial Type:	 3 ml / min 0 min Type1@4	 Port : 7 MeOH/ACN 1:1
Drying 2: defined by volume	ON	 Drying volume: 10 ml	 Speed: 10 ml / min	
Washing 4:	OFF			
Drying 3:	OFF			
Elution 2:	OFF			
Drying 4:	OFF			
EVA :	OFF			
Tube filling volume before elution		1 ml		

Method report

2.6 Eluent exchange

- The eluate (from manual or automated clean-up) is transferred into a vial (nano reaction vial, OD: 16 mm, 20 pcs. Item no.: 91680).
- Rinsing with 1 mL methanol.
- As a keeper, 0.5 mL of water (bidest, MilliQ-system) is added.
- The temperature on the thermoblock (Vario 4. Item no.: 919300) is set to 30° C.
- Sample concentration under a gentle stream of nitrogen to a volume of 0.5 mL, transfer with water to a filling volume of 1 mL.
- The eluate is then injected.

2.7 Measurement using HPLC-MS/MS

The HPLC measurement was performed on a Shimadzu Nexera 2 system with the following configuration:

Configuration	Designation
System controller	CBM-20A lite
Pump A	LC-30 AD
Pump B	LC-30 AD
Autosampler	SIL 30 AC
Oven	CTO-20 AC
PDA	SPD-M20 A

Column	EC 150/2 NUCLEOSHELL® RP 18, 2.7 µm, REF 763136.20
Eluent A	water-methanol – 25 mmol/L ammonium acetate pH 4.0 (76.5:13.5:10, v/v/v)
Eluent B	methanol – 25 mmol/L ammonium acetate pH 4.0 (90:10, v/v)
Gradient	0 % B to 100 % B in 40 min, hold for 10 min
Flow rate	0.5 mL/min
Injection volume	25 µL
Column temperature	40 °C
Detection	UV (210 nm, 230 nm, 254 nm, 360 nm)

Column	EC 150/2 NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm, REF 763736.20
Eluent A	water
Eluent B	methanol
Eluent C	methanol – 25 mmol/L ammonium acetate pH 4.0
Gradient	15 % B to 80 % B in 40 min, hold for 10 min at a flow rate of 0.45 mL/min, eluent C is added at a flow rate of 0.05 mL/min constantly over the total time.
Flow rate	0.5 mL/min
Injection volume	25 µL
Column temperature	60 °C
Detection	UV (210 nm, 230 nm, 254 nm, 360 nm)
Temperature:	for NUCLEOSHELL® RP 18: 40 °C for NUCLEOSHELL® Phenyl-Hexyl: 60 °C

In order to be able to properly separate the peaks of 2,4,6-TNT and Octogen, each of these analytes is also individually separated on the NUCLEOSHELL® Phenyl-Hexyl HPLC column and then measured.

2.8 Configuration FREESTYLE XANA

1.	FREESTYLE BASIC	P/N	12663-12
2.	FREESTYLE SPE	P/N	12668
3.	Rack for solvent delivery	P/N	13156
4.	FREESTYLE XANA	P/N	15082
5.	Column adapter for water extraction (3 mL)	P/N	14892
6.	Caps for columns (3 mL)	P/N	14862
7.	Frame for trays (100 mm)	P/N	11915
8.	Tray (4 mL)	P/N	11926
9.	Adapter for SPE-columns	P/N	13382
10.	Screw thread bottle (4 mL)	P/N	V0004

2.9 Consumables by MACHEREY-NAGEL

1.	EC 150/2 NUCLEOSHELL® RP 18, 2.7 µm	P/N	763136.20
2.	EC 150/2 NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm	P/N	763736.20
3.	CHROMABOND® HR-X PP-columns, 3 mL, 200 mg	P/N	730931

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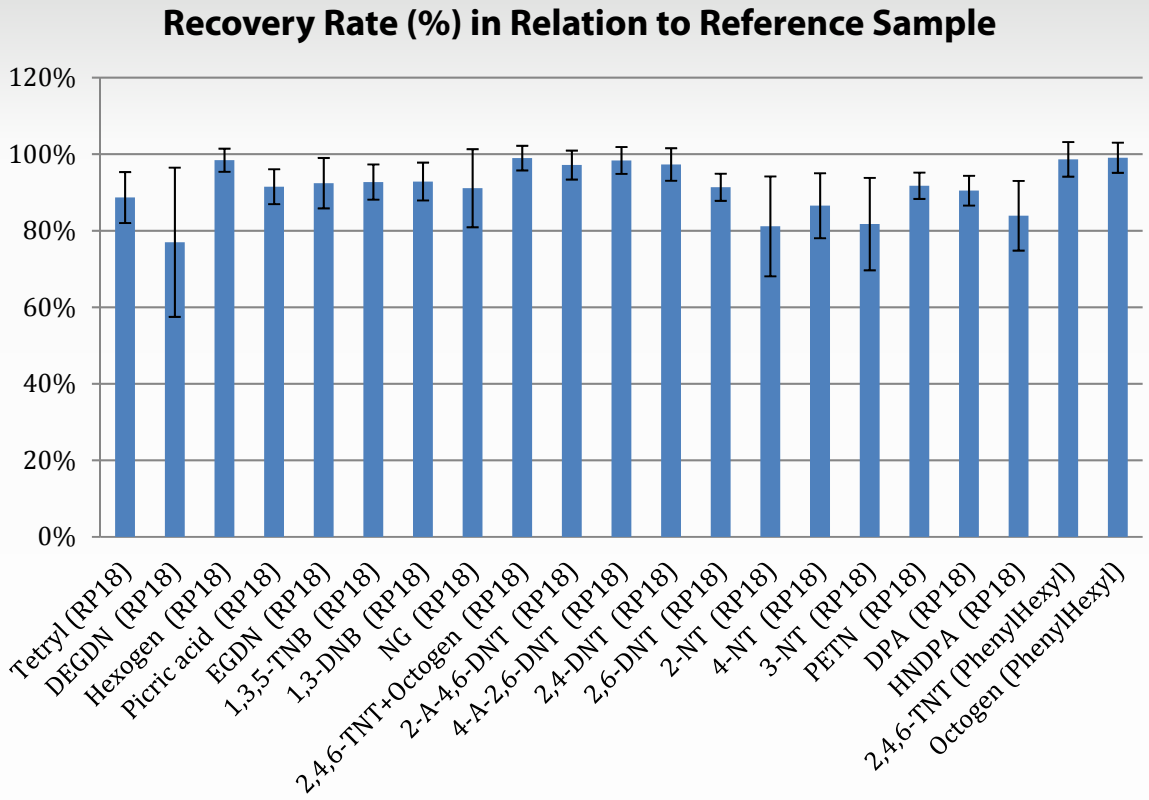
3 Results

3.1 Recovery rates (automated processing)

Analyte	Mean Value (%)	RSD (%)
Tetryl (RP18)	89 %	7 %
DEGDN (RP18)	77 %	20 %
Hexogen (RP18)	98 %	3 %
Picric acid (RP18)	92 %	5 %
EGDN (RP18)	92 %	7 %
1,3,5-TNB (RP18)	93 %	5 %
1,3-DNB (RP18)	93 %	5 %
NG (RP18)	91 %	10 %
2,4,6-TNT + Octogen (RP18)	99 %	3 %
2A-4,6-DNT (RP18)	97 %	4 %
4-A-2,6-DNT (RP18)	98 %	4 %
2,4-DNT (RP18)	97 %	4 %
2,6-DNT (RP18)	91 %	4 %
2-NT (RP18)	81 %	13 %
4NT (RP18)	87 %	8 %
3-NT (RP18)	82 %	12 %
PETN (RP18)	92 %	3 %
DPA (RP18)	90 %	4 %
HNDPA (RP18)	84 %	9 %
2,4,6-TNT (Phenyl Hexyl)	99 %	5 %
Octogen (Phenyl Hexyl)	99 %	4 %

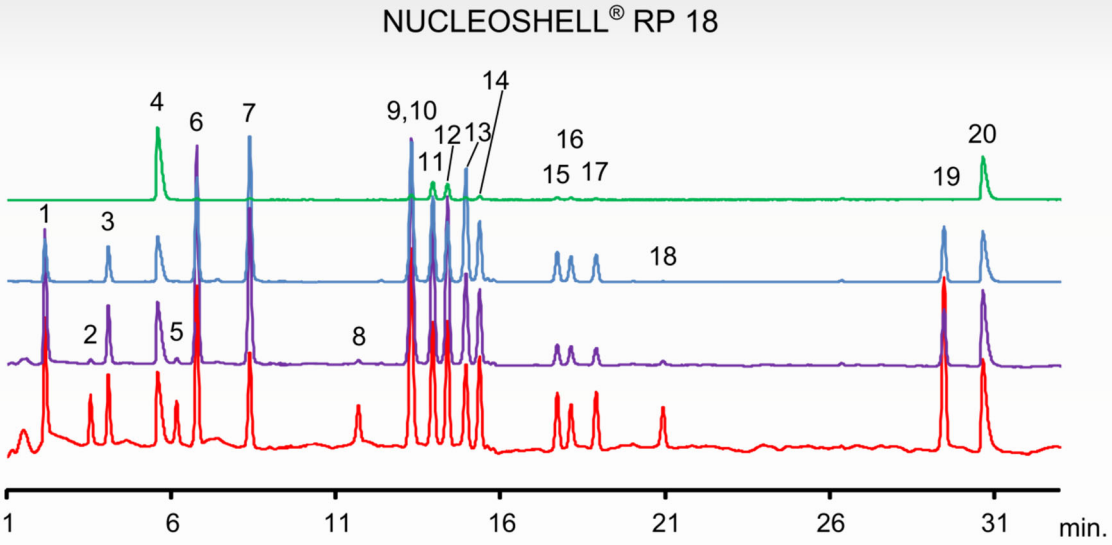


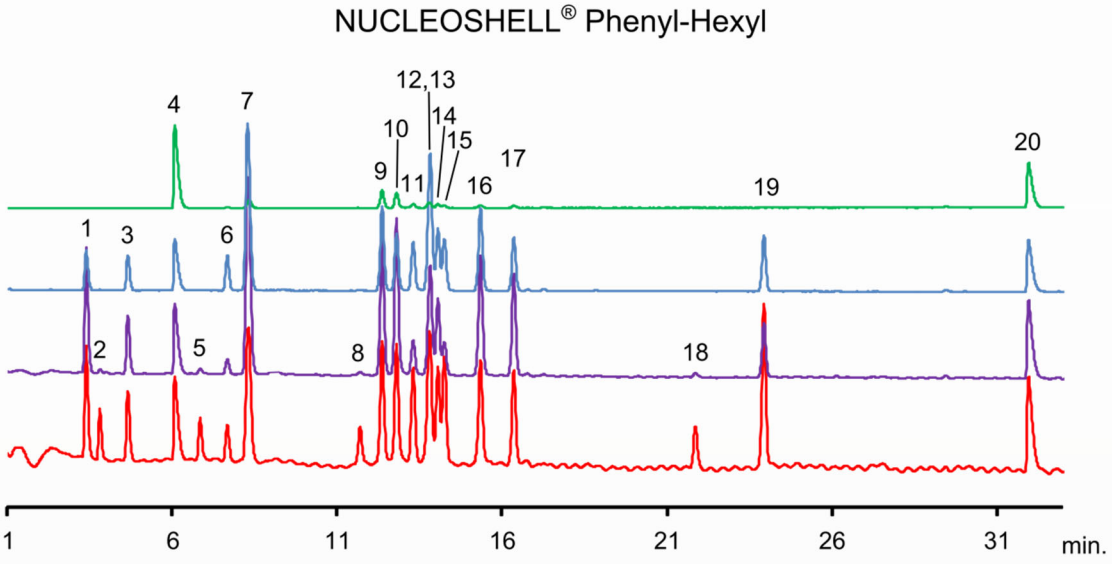
Summary

FREESTYLE XANA detects reliably and with high reproducibility explosives and their degradation products in water samples using fully automated and parallel SPE enrichment. Detection is carried out norm-conform to DIN EN ISO 22478:2006 with high reproducibility.

Unattended, the system processes samples 24 hours a day, 7 days a week.
Through parallelisation of individual processing steps and simultaneous processing of several samples, a very high sample throughput can be achieved.

3.2 Chromatograms





Peak allocation:

1 - Tetryl	11 - 2-A-4,6-DNT
2 - DEGDN	12 - 4-A-2,6-DNT
3 - Hexogen	13 - 2,4-DNT
4 - Picric acid	14 - 2,6-DNT
5 - EGDN	15 - 2-NT
6 - 1,3,5-TNB	16 - 4-NT
7 - 1,3-DNB	17 - 3-NT
8 - NG	18 - PETN
9 - 2,4,6-TNT	19 - DPA
10 - Octogen	20 - HNDPA

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