

Determination of Trace Nitroaromatic, Nitroamine, and Nitrate Ester Compounds for EPA Method 8330B

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Introduction

The purpose of this application note is to demonstrate the capabilities of the Horizon Technology SPE-DEX® 4790 Automated Extractor System, when used for the analysis of nitramine, nitroaromatic, and nitrate ester compounds in surface/ground water. The SPE-DEX® 4790 is a fully-automated, solid-phase extraction system that provides fast extraction, and improves the quality and consistency of results.

EPA Method 8330B provides high performance liquid chromatographic (HPLC) conditions for the detection of parts per billion (ppb) levels of certain explosives residues in surface or ground water. Nitroaromatic compounds are usually associated with explosive manufacturing and ordinance facilities. Common nitroaromatic compounds include trinitrotoluene (TNT) and dinitrotoluene (DNT). Nitramines are the most recently introduced class of organic nitrate explosives and include hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX).

All laboratories are responsible for operating a formal quality assurance program. EPA Method 8330B minimum requirements include an initial demonstration of laboratory capability (IDC) which consists of the Initial Precision and Recovery (IPR) samples and a Method Detection Limit (MDL) study.

The capability of the Horizon Technology SPE-DEX® 4790 Automated Extractor System for the extraction of nitroaromatics and nitramines in surface/ground water was demonstrated by performing the IPR and MDL studies with a 3M Empore™ 47mm SDB-RPS disk.

Instrumentation

- Horizon Technology SPE-DEX® 4790 Automated Extractor System
- Horizon Technology Envision™ Platform Controller
- 3M Empore™ (47mm) SDB-RPS Disk and Disk gasket
- ICS-3000 HPLC with UV-Vis Detector



SPE-DEX® 4790 Automated Extraction System and Envision™ Platform Controller.

Method Summary

- 1) 1000 mL of DI water was used.
- 2) Spike surrogate and spikes into samples.
- 3) Set the water to waste vacuum at - 25 in.Hg and the solvent waste vacuum at -10 in.Hg.
- 4) Prepare the extractors, purge twice to flush the system.
- 5) With each extractor use one Empore SDB-RPS disk with gasket in disk holder.
- 6) Start extraction method 8330B, collect extract at high vacuum at - 25 in.Hg.
- 7) Collect extract approximately 20 mLs.
- 8) Add 30mLs of acetonitrile to the extract and concentrate to 1 mL.
- 9) Add 1mL of reagent water so final volume is 2 mLs.
- 10) Transfer a portion of the extract to a GC vial.
- 11) Analyze by HPLC.

HPLC Method Summary

Primary Column HPLC Conditions:

- Mobile Phase: 50 % H₂O / 49 % MeOH / 1 % ACN
- Isocratic Flow Rate: 1.2 mL/min
- Run Time: 25 min
- UV/Vis Channel: 254 nm
- Injection Volume: 100 uL
- Column Oven Temp: 36° C
- Column: Hydro-RP (Supplier: Phenomenex)

Confirmation Column HPLC Conditions:

- Mobile Phase B: 50% H₂O/49% MeOH/0.1% ACN
- Mobile Phase C: 40% H₂O/40% MeOH/20% ACN
- Gradient Flow Rate: 1.2 mL/min
- Run Time: 25 min
- UV/Vis Channel: 254 nm
- Injection Volume: 100 uL
- Column Oven Temp: 36° C
- Column: Phenyl Hexyl (Supplier: Phenomenex)

106 %. Figure 3 is a LCS spiked at 12.5 ng using the conditions on the confirmation column. The recovery ranges are 75 % - 103 % Figure 4 is a LCS spiked at 125.0 ng using the condition on the confirmation column. The recovery range is 84 % - 96 %.

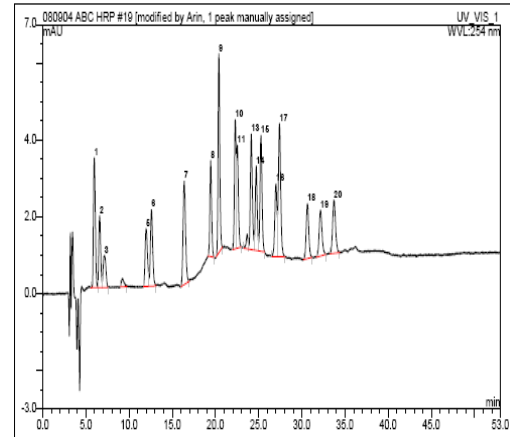
Table 1: Gradient Table for HPLC

Gradient Time Min.	% Mobile Phase B	% Mobile Phase C
0.0	50	50
10.0	50	50
10.2	100	0
16.0	50	50
18.0	50	50

Table 2: Extraction EPA Method 8330B programmed into the Envision Controller

STEP	SOLVENT	SOAK TIME	DRY TIME
Prewet #1	Methanol	1:00 min	45 sec
Prewet #2	Methanol	1:00 min	45 sec
Prewet #3	Methanol	1:00 min	45 sec
Prewet #4	Reagent Water	1:00 min	45 sec
Prewet #5	Reagent Water	1:00 min	45 sec
Sample Process			
Air Dry			2:00 min
Rinse Step #1	Methanol	2:00 min	1 min
Rinse Step #2	Methanol	1:00 min	45 sec
Rinse Step #3	Methanol	1:00 min	45 sec

Figure 1: LCS spiked at 12.5 ng and run on the primary column



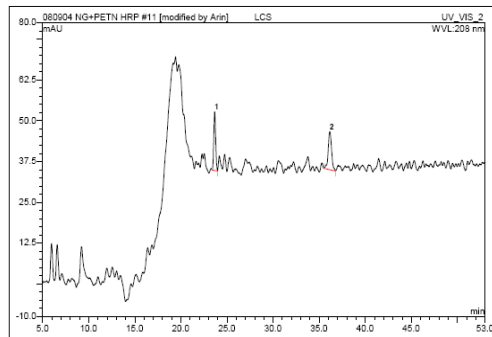
No.	Ret.Time min	Peak Name	Time	Area mAU*min	Height mAU	Amount ng	% Difference %
1	5.00	26DANT	09.05.08 02:11	0.856	3.373	12.0938	n.a.
2	6.59	24DANT	09.05.08 02:11	0.459	1.803	11.2701	n.a.
3	7.15	HMX	09.05.08 02:11	0.308	0.829	11.4232	n.a.
4	9.24	n.a.	09.05.08 02:11	0.078	0.217	n.a.	n.a.
5	11.08	RDX	09.05.08 02:11	0.488	1.503	12.3907	n.a.
6	12.58	PIORIC	09.05.08 02:11	0.810	1.085	12.2407	n.a.
7	16.38	1,3,5-TNB	09.05.08 02:11	0.903	2.888	11.7128	n.a.
8	19.44	1,2-DNB (Surrogate)	09.05.08 02:11	0.593	2.497	11.6560	n.a.
9	20.39	1,3-DNB	09.05.08 02:11	1.197	5.169	11.4875	n.a.
10	22.29	NB	09.05.08 02:11	0.764	3.355	10.7811	n.a.
11	22.83	Tertryl	09.05.08 02:11	0.828	2.894	14.5169	n.a.
12	23.87	n.a.	09.05.08 02:11	0.094	0.385	n.a.	n.a.
13	24.14	2,4,6-TNT	09.05.08 02:11	0.810	2.998	11.3324	n.a.
14	24.72	4-Am-2,6-DNT	09.05.08 02:11	0.570	2.195	10.7434	n.a.
15	25.27	2-Am-4,6-DNT	09.05.08 02:11	0.823	3.012	10.6947	n.a.
16	27.01	2,6-DNT	09.05.08 02:11	0.555	1.895	10.6853	n.a.
17	27.41	2,4-DNT	09.05.08 02:11	1.103	3.466	11.7130	n.a.
18	30.08	2-NT	09.05.08 02:11	0.487	1.427	9.9878	n.a.
19	32.18	4-NT	09.05.08 02:11	0.490	1.164	10.9962	n.a.
20	33.70	3-NT	09.05.08 02:11	0.946	1.994	10.3957	n.a.

Results

The samples were analyzed using the conditions on the primary column. Analytes Tertryl and 1,3-DNB co-eluted on the primary column, and were analyzed on the confirmation column for chromatographic separation of the two. All the samples with detectable levels of any compound on the primary column must be analyzed on the confirmation column. The confirmation is achieved when the results between the two columns have a RPD of less than 40% (results corrected for co-elution on the second column). The IPR and MDL results are presented in Tables 3-6. Figure 1 is a LCS (Laboratory Control Sample) spiked at a concentration of 12.5 ng, using the conditions on the primary column.

The recoveries range from 79 % - 120 %. Figure 2 is a LCS spiked at 125.0 ng for additional compounds and detected with a wavelength of 208 nm. The recovery range is 101 % -

Figure 2: LCS spiked at 125.0 ng and run on the primary column



No.	Ret.Time min	Peak Name	Time	Area mAU*min	Height mAU	Amount ng	% Difference %
1	23.66	NG	09.05.08 02:11	4.327	17.991	132.7286	n.a.
2	36.15	PETN	09.05.08 02:11	4.932	11.692	126.4853	n.a.

Figure 3: LCS spiked at 12.5 ng and run on the confirmation column

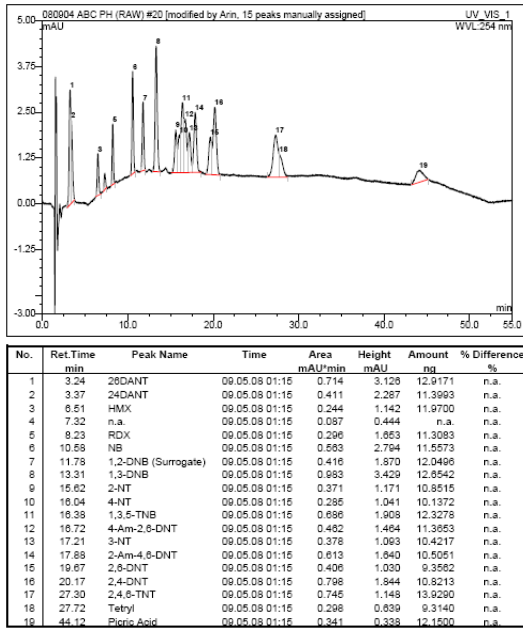


Figure 4: LCS spiked at 125.0 ng and run on the confirmation column

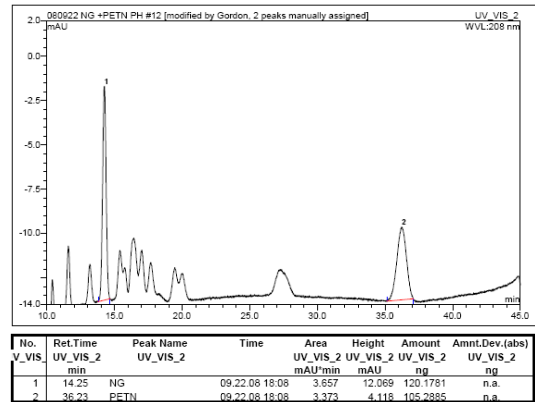


Table 3: Initial Precision and Recovery and Method Detection Limits (Note: IPR Data uses 4 of the MDL study injections)
Primary Column Data:

	Data File	MDL 1	MDL 2	MDL 3	MDL 4	MDL 5	MDL 6	MDL 7
	Target	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Rep-6	Rep-7
Analytes	Conc. (ng)	(ng)	(ng)	(ng)	(ng)	(ng)	(ng)	(ng)
HMX	5.0	3.593	3.864	3.867	3.385	3.842	3.314	3.365
RDX	5.0	4.253	3.981	4.088	4.142	4.550	4.006	4.358
1,3,5 TNB	5.0	4.249	4.043	3.898	4.100	3.783	3.816	3.875
1,2-DNB (Surrogate)	100.0	84.142	80.891	76.214	81.989	78.292	78.921	74.849
Tetryl/1,3-DNB	10.0	8.945	8.687	8.349	9.055	8.611	6.104	7.849
NB	5.0	4.193	4.047	3.650	3.930	4.117	3.773	3.281
2,4,6-TNT	5.0	4.053	4.040	3.630	3.927	3.822	3.872	3.567
4-Am-2,6-DNT	5.0	7.181	7.044	6.665	6.719	6.879	6.053	6.150
2-Am-4,6-DNT	5.0	4.160	4.119	3.673	4.035	3.980	3.753	3.530
2,6-DNT	5.0	3.139	4.556	4.260	4.535	4.204	4.256	3.899
2,4-DNT	5.0	3.476	4.342	3.927	4.391	4.066	4.117	3.911
2-NT	5.0	4.293	3.907	3.599	3.731	3.910	3.590	3.360
4-NT	5.0	4.254	4.220	3.805	3.802	3.668	4.005	3.309
3-NT	5.0	3.966	3.951	3.789	3.968	3.875	3.403	3.310

Table 4: Primary Column Results

T value used for calculating MDL: 3.141						
Analytes	Mean (ng)	Mean Accuracy	Standard Deviation	Calc.MDL (ng)	Calc MDL (ug/L)	Reporting Limit (ug/L)
HMX	3.60	72%	0.25	0.79	0.016	0.25
RDX	4.20	84%	0.20	0.64	0.013	0.25
1,3,5 TNB	3.97	79%	0.17	0.53	0.011	0.25
1,2-DNB (Surrogate)	79.33	79%	3.26	10.23	0.205	n/a
Tetryl/1,3-DNB	8.23	82%	1.02	3.20	0.064	0.50
NB	3.86	77%	0.32	1.00	0.020	0.25
2,4,6-TNT	3.84	77%	0.19	0.59	0.012	0.25
4-Am-2,6-DNT	6.67	133%	0.43	1.34	0.027	0.25
2-Am-4,6-DNT	3.89	78%	0.24	0.76	0.015	0.25
2,6-DNT	4.12	82%	0.49	1.53	0.031	0.25
2,4-DNT	4.03	81%	0.31	0.97	0.019	0.25
2-NT	3.77	75%	0.30	0.95	0.019	0.25
4-NT	3.87	77%	0.33	1.04	0.021	0.25
3-NT	3.75	75%	0.28	0.87	0.017	0.25

Table 5: Confirmation Column Data*

	Data File	MDL 1	MDL 2	MDL 3	MDL 4	MDL 5	MDL 6	MDL 7
	Target	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Rep-6	Rep-7
Analytes	Conc. (ng)	(ng)	(ng)	(ng)	(ng)	(ng)	(ng)	(ng)
HMX	5.0	21.247	21.076	19.733	21.411	20.398	18.866	14.020
RDX	5.0	5.221	4.890	4.389	4.737	5.009	4.743	4.939
NB	5.0	4.680	4.943	4.570	5.012	4.626	4.580	4.226
1,2-DNB (Surrogate)	100.0	85.166	83.300	78.791	84.735	80.973	86.011	80.362
1,3-DNB	5.0	4.587	4.682	4.157	4.750	4.283	4.606	4.563
2-NT/4-NT/4-Am2,6-DNT	15.0	18.834	19.173	17.960	19.613	15.795	19.123	20.785
3-NT/2-Am4,6-DNT	10.0	9.391	11.221	10.385	11.062	9.329	10.274	10.882
1,3,5-TNB/2,6-DNT	10.0	8.304	8.133	7.692	8.369	7.844	7.879	8.149
2,4-DNT	5.0	4.425	3.852	3.751	4.522	3.6311	4.392	4.295
Tetryl	5.0	2.616	2.520	2.173	2.095	2.5325	2.074	2.167
2,4,6-TNT	5.0	4.126	4.927	4.675	5.417	4.2622	5.060	4.871

*Confirmation Column data includes separation of Tetryl and 13DNB

Table 6: Confirmation Column Results

T value used for calculating MDL: 3.141						
Analytes	Mean (ng)	Mean Accuracy	Standard Deviation	Calc.MDL (ng)	Calc MDL (ug/L)	Reporting Limit (ug/L)
HMX	19.54	391%	2.60	8.16	0.163	n/a
RDX	4.85	97%	0.26	0.82	0.016	0.25
NB	4.66	93%	0.26	0.82	0.016	0.25
1,2-DNB (Surrogate)	82.76	83%	2.75	8.63	0.173	n/a
1,3-DNB	4.52	90%	0.22	0.68	0.014	0.25
2-NT/4-NT/4-Am2,6-DNT	18.75	125%	1.56	4.89	0.098	0.75
3-NT/2-Am4,6-DNT	10.36	104%	0.77	2.40	0.048	0.50
1,3,5-TNB/2,6-DNT	8.05	81%	0.25	0.79	0.016	0.50
2,4-DNT	4.12	82%	0.37	1.15	0.023	0.25
Tetryl	2.31	46%	0.23	0.74	0.015	0.25
2,4,6-TNT	4.76	95%	0.45	1.42	0.028	0.25

Results

Data in Tables 3-6 meet the criteria for EPA Method 8330B and demonstrates that the Horizon Technology fully-automated extraction systems are capable of fully-automating EPA Method 8330B resulting in data that is both accurate and precise.

The Horizon Technology SPE-DEX 4790 Automated Extractor System, coupled with the Envision Platform, reduces analyst labor, solvent usage, turnaround time, and greatly improves accuracy and precision.

Acknowledgments

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