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Application News

No. HPLC-023

High Performance Liquid Chromatography

Using Peak Deconvolution of Photodiode Array Data to Attain Faster, Easier Performance of EPA Method 8330B

■ Introduction

Explosive ordinance and its residues can be found throughout the globe, not just at ordinance manufacturing sites, but also at military testing grounds and warzones. The presence of unexploded ordinance and its manufacturing or decomposition byproducts can lead to harmful impact on the soil and waterways in those areas. Therefore, much of the world is at risk of having some environmental contamination from these materials.

US Environmental Protection Agency Method 8330B, "Nitroaromatics, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography" provides conditions for the low-level detection of certain explosive and propellant residues in water, soil, and sediment. It seems like a very simple method to perform. However, actual practice of this method shows that there are factors affecting the chromatography that lead to difficulty in getting good separation on some of the analytes.

The method describes the analytical HPLC procedure for the analysis of the following list of compounds shown in Table 1.

Table 1: List of Analytical Target Compounds

Analyte	Abbreviation
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX
1,3,5-Trinitrobenzene	1,3,5-TNB
1,3-Dinitrobenzene	1,3-DNB
Methyl-2,4,6-trinitrophenylnitramine	Tetryl
Nitrobenzene	NB
2,4,6-Trinitrotoluene	2,4,6-TNT
4-Amino-2,6-dinitrotoluene	4-Am-DNT
2-Amino-4,6-dinitrotoluene	2-Am-DNT
2,4-Dinitrotoluene	2,4-DNT
2,6-Dinitrotoluene	2,6-DNT
2-Nitrotoluene	2-NT
3-Nitrotoluene	3-NT
4-Nitrotoluene	4-NT
Nitroglycerin	NG
Pentaerythritol tetranitrate	PETN
3,5-Dinitroaniline	3,5-DNA

The general HPLC analytical concepts are to use isocratic elution on a primary column (C-18 or C-8) with tentative peak identification confirmation performed on a secondary column (Cyano or Phenylhexyl). In lieu of using a confirmation column (and the time involved with a second analysis of the same sample), confirmation can be performed with a photodiode array or mass spectral detector. The method utilizes the 254 nm detection band for quantitation of most of the analytes, but 210 nm for the nitrate esters (NG and PETN).

The chromatographic performance of this method is both easy and frustrating, as the separation of all peaks is not always possible. Furthermore, the separation changes, sometimes very noticeably, between columns, even columns with the same type of phase (C-18) but from different column manufacturers. Trying to optimize one area of the chromatogram often leads to co-elution of peaks in another.

UHPLC and mass spectral detection are two options that might provide much better results, but both are more expensive hardware configurations that some laboratories might not be able to accommodate.

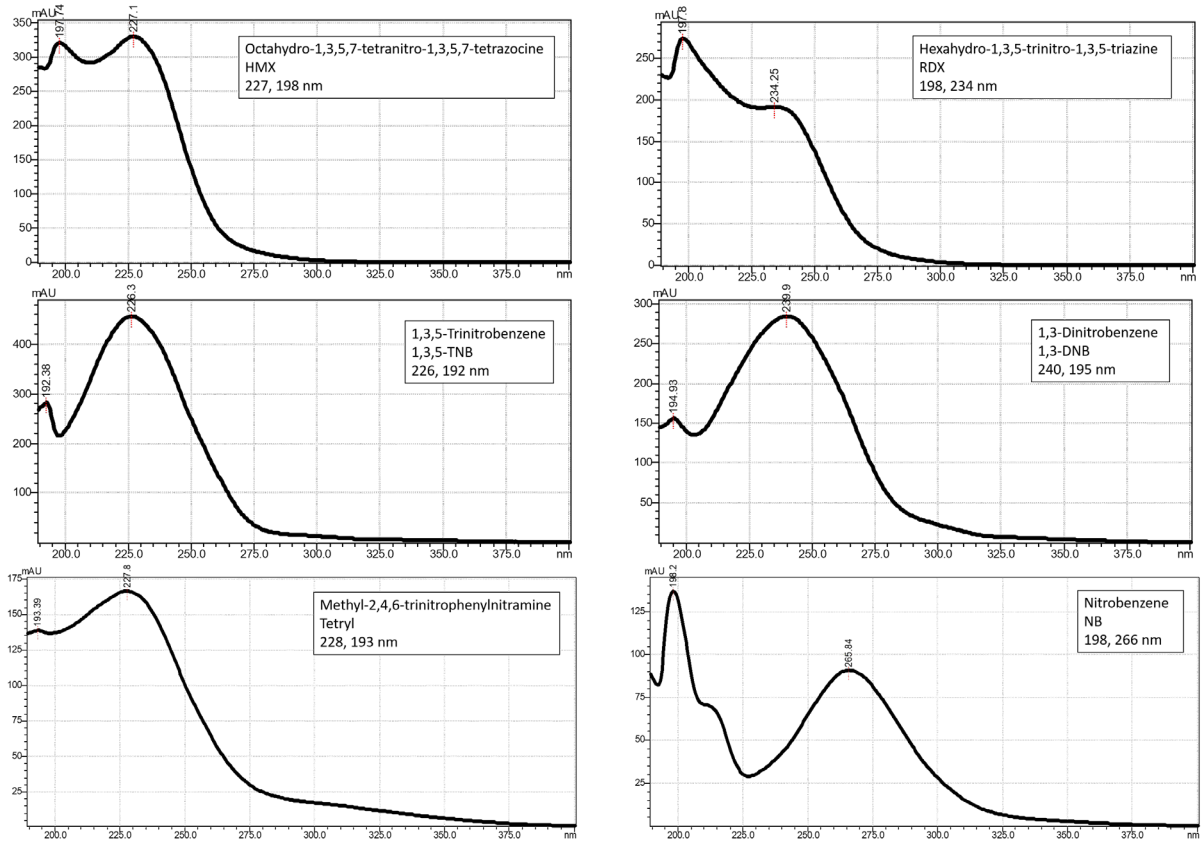
Intelligent Peak Deconvolution Analysis II (i-PDeA II) extracts target peaks from unseparated peak groups in photodiode array detector data using the chemometrics multivariate curve resolution alternating least squares (MCR-ALS) technique. It is possible to obtain fast, accurate quantitative analysis for partially co-eluting peaks, as well as spectral identification. This lessens method development time and increases the confidence in the analytical results.

■ Results

The data shown in this application note was obtained by using a Shimadzu Nexera-I MT 3D HPLC system, which includes the LC-2040 photodiode array detector (PDA). The method recommends using a C-18 (ODS) column as one of the primary columns for this analysis, with a methanol/water mobile phase. Several different C-18 columns (different manufacturers and column/particle dimensions) were tested, each with differing results. See Table 2, below. It is very difficult to get clean separation for all the analytes, as there are several positional isomers present that have very similar chromatographic characteristics. Depending on which column is selected, some peaks can switch elution positions. For the sake of gathering comparison data, a single C-18 column (2.1 x 200 mm, 5 µm) was selected, and separation conditions were developed on it.

Basic separation conditions were made with the use of methanol/water mobile phase combinations, but the addition of low percentages of acetonitrile were also tested for its effect on the chromatographic separation. The addition of a small percentage of acetonitrile to the mobile phase can help sharpen peak shapes, but also has a dramatic effect on the retention time of some peaks. This small amount of acetonitrile can cause some switching of elution order and is most profound in the elution order of PETN relative to the nitrotoluene peaks, as seen below.

Performance of the application was tested with different column temperatures, with 42°C being selected as having the best overall result.



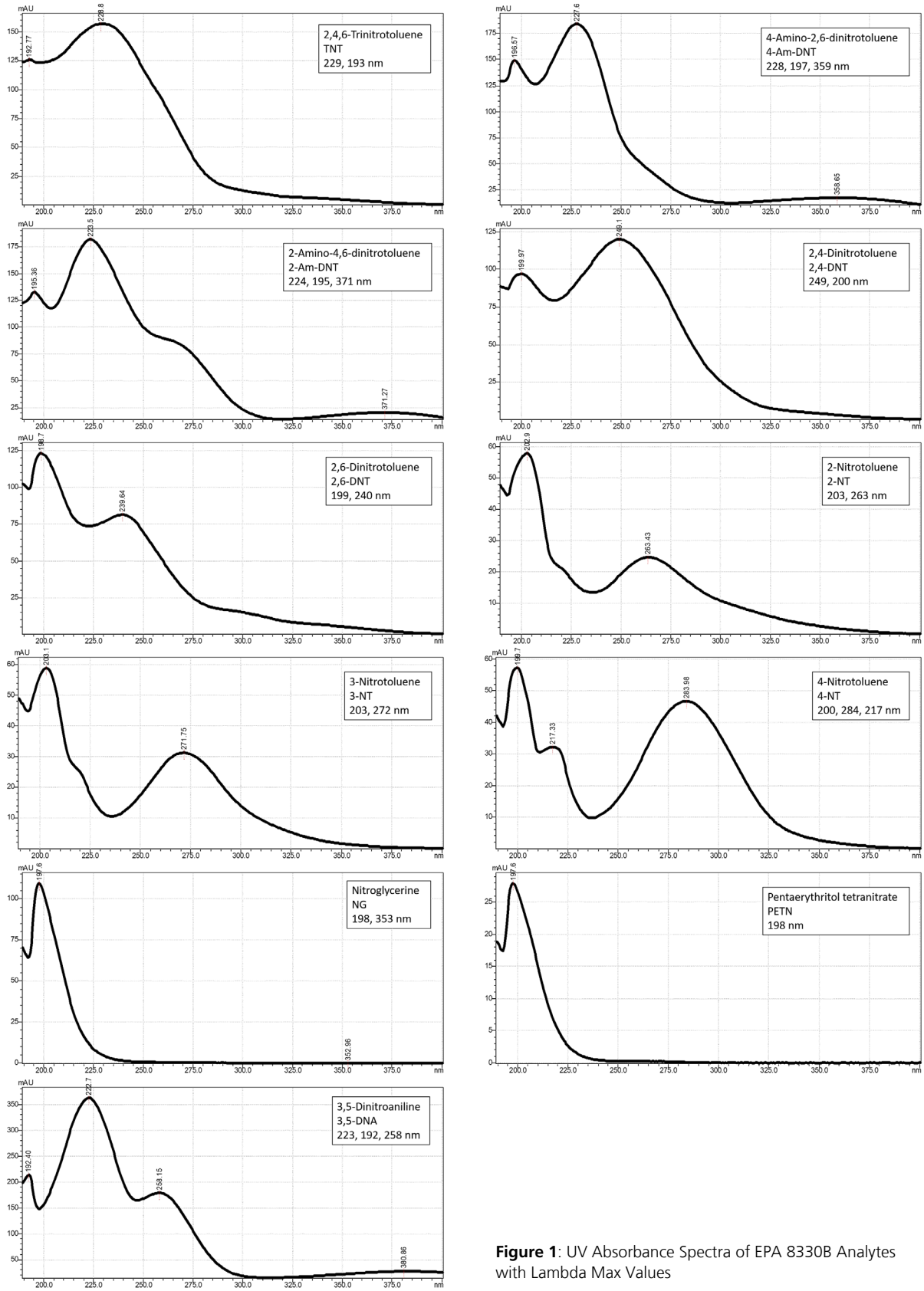


Figure 1: UV Absorbance Spectra of EPA 8330B Analytes with Lambda Max Values

Table 2: Elution Order of Analytes, Comparison of 6 Different C-18 Columns (Identical Mobile Phase and Column Temperature)

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
HMX	1	1	1	1	1	1
RDX	2	2	2	2	2	2
1,3,5-TNB	3	3	3	3	3	3
1,3-DNB	4	5	4	4	4	4
3,5-DNA	5	4	5	5	5	5
NB	8	9	6	6	6	8
Tetryl	6	6	7	7	7	6
NG	7	7	9	8	8	7
2,4,6-TNT	9	8	8	9	9	9
4-Am-DNT	10	10	11	11	11	10
2-Am-DNT	11	11	10	10	10	11
2,6-DNT	12	12	13	13	13	12
2,4-DNT	13	13	12	12	12	13
2-NT	14	15	14	14	14	14
4-NT	16	16	15	15	15	15
3-NT	17	17	16	16	16	17
PETN	15	14	17	17	17	16

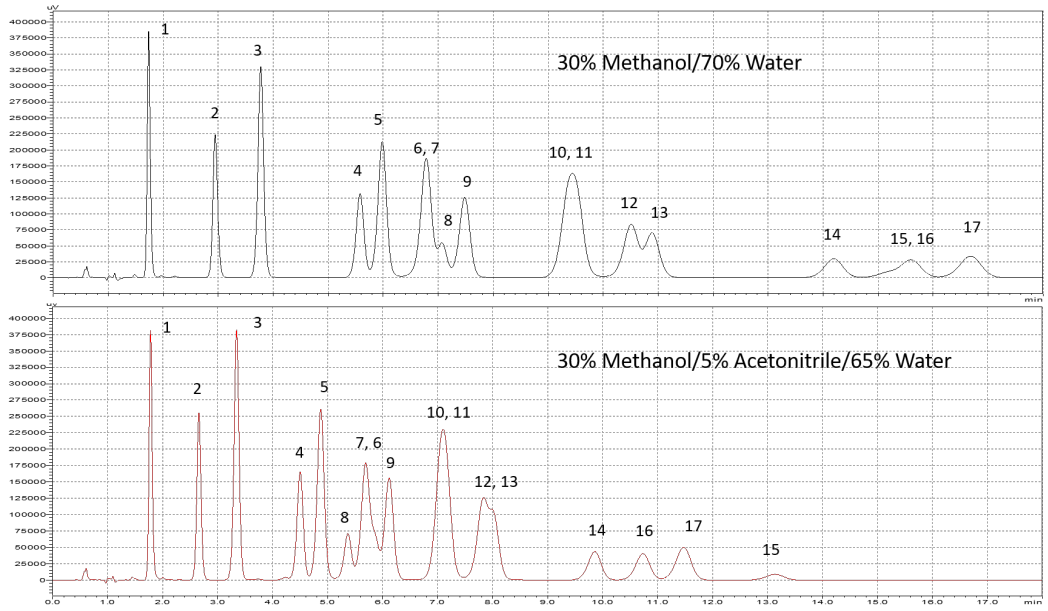


Figure 2: C-18 Separation, 0.500 mL/min, Column Temperature = 42°C, Detection at 210 nm

1 = HMX, 2 = RDX, 3 = 1,3,5-TNB, 4 = 1,3-DNB, 5 = 3,5-DNA, 6 = NG, 7 = Tetryl, 8 = NB, 9 = 2,4,6-TNT, 10 = 4-Am-DNT, 11 = 2-Am-DNT, 12 = 2,6-DNT, 13 = 2,4-DNT, 14 = 2-NT, 15 = PETN, 16 = 4-NT, 17 = 3-NT

In the chromatogram shown in Figure 2, there are some peaks that are poorly resolved (peaks 6, 7, 9, 12, and 13) or are co-eluting (peaks 10 and 11). Trying to quantitate these peaks with this degree of resolution would have the potential of giving inconsistent peak measurement and poor results.

The 5.1 – 6.5 minute and 6.5 – 8.5 minute time ranges in figure 2 were subjected to i-PDeA deconvolution analysis to get clean representation for the peaks.

Ch#	Disp-lay	Type	Wavelength (nm)	Bandwidth +/-(nm)	Magnifi-cation
1	☑	Absorbance	254	4	1.00
2	☑	Absorbance	210	4	1.00
3	☑	Absorbance,N	210	4	1.00
4	☑	Absorbance,N	254	4	1.00
5	☐	Absorbance	254	4	1.00
6	☐	Absorbance	254	4	1.00
7	☐	Absorbance	254	4	1.00
8	☐	Absorbance	254	4	1.00
9	☐	Absorbance	254	4	1.00
10	☐	Absorbance	254	4	1.00
11	☐	Absorbance	254	4	1.00
12	☐	Absorbance	254	4	1.00
13	☐	Absorbance	254	4	1.00
14	☐	Absorbance	254	4	1.00
15	☐	Absorbance	254	4	1.00

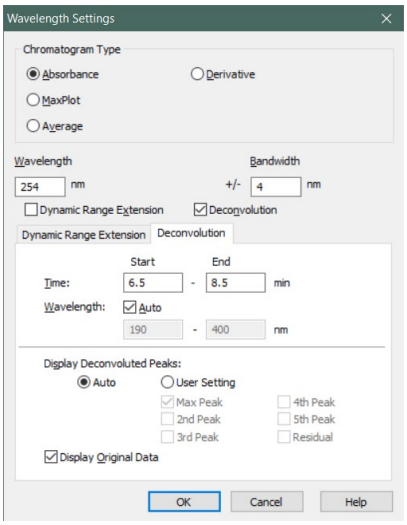


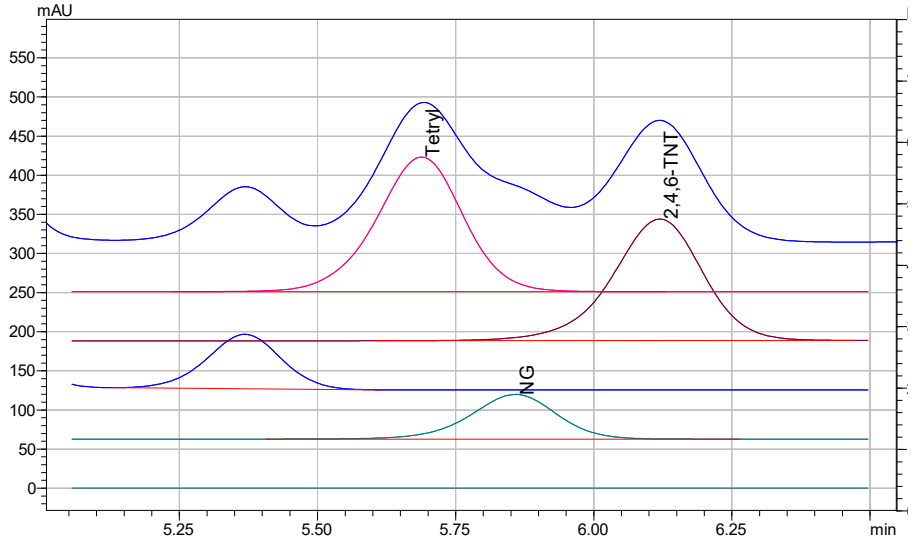
Figure 3: Setting Deconvolution Channels 3 and 4

The Compound Table of the software allows the user to designate which PDA channel will be used for quantitation for each entry. Also, by including a link for each analyte to a user-generated library spectrum file and setting a minimum spectra similarity, it is possible to perform automatic spectral confirmation of identity for all listed analytes.

ID#	Name	Type	Channel	Ret. Time	Std. Spectrum	Min Similarity
1	HMX	Target	Ch1 254nm	1.782	C:\LabSolutions\UV	0.98000
2	RDX	Target	Ch1 254nm	2.614	C:\LabSolutions\UV	0.98000
3	1,3,5-TNB	Target	Ch1 254nm	3.287	C:\LabSolutions\UV	0.98000
4	1,3-DNB	Target	Ch1 254nm	4.377	C:\LabSolutions\UV	0.98000
5	3,5-DNA	Target	Ch1 254nm	4.729	C:\LabSolutions\UV	0.98000
6	NB	Target	Ch1 254nm	5.188	C:\LabSolutions\UV	0.98000
7	Tetryl	Target	Ch3 210nm	5.551	C:\LabSolutions\UV	0.98000
8	NG	Target	Ch3 210nm	5.735	C:\LabSolutions\UV	0.98000
9	2,4,6-TNT	Target	Ch3 210nm	5.960	C:\LabSolutions\UV	0.98000
10	4-Am-DNT	Target	Ch4 254nm	6.760	C:\LabSolutions\UV	0.98000
11	2-Am-DNT	Target	Ch4 254nm	6.880	C:\LabSolutions\UV	0.98000
12	2,6-DNT	Target	Ch4 254nm	7.533	C:\LabSolutions\UV	0.98000
13	2,4-DNT	Target	Ch4 254nm	7.705	C:\LabSolutions\UV	0.98000
14	2-NT	Target	Ch1 254nm	9.420	C:\LabSolutions\UV	0.98000
15	4-NT	Target	Ch1 254nm	10.253	C:\LabSolutions\UV	0.98000
16	3-NT	Target	Ch1 254nm	10.956	C:\LabSolutions\UV	0.98000
17	PETN	Target	Ch2 210nm	12.871	C:\LabSolutions\UV	0.98000

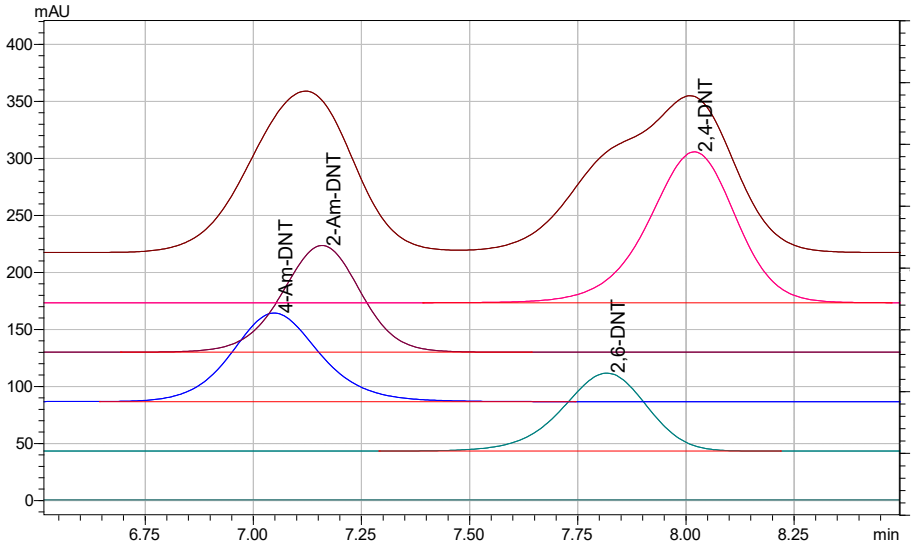
Figure 4: Compound Table Setting for Quantitation and Spectral Confirmation of Identity

Using the deconvolution function of the LabSolution software, it is possible to get accurate representation of each peak, with very good spectral confirmation (0 – 1.000000 scale).



ID#	Ret. Time	Name	Area	Similarity Index
	5.368		661644	0
7	5.689	Tetryl	1933975	0.999964
8	5.859	NG	637828	0.999221
9	6.120	2,4,6-TNT	1810921	0.999962

Figure 5: Peak Deconvolution, 210 nm, 5.0 - 6.5min.

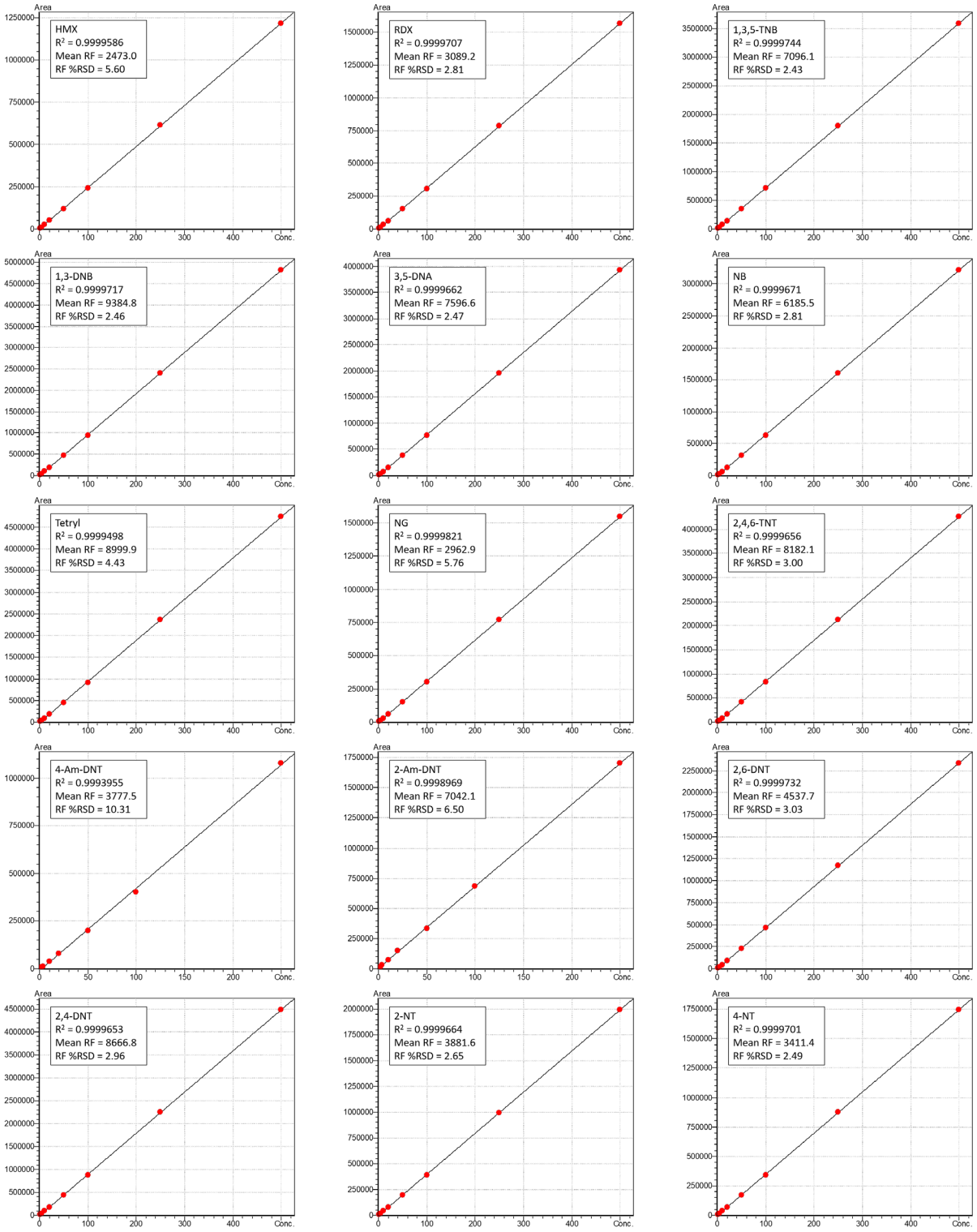


ID#	Ret. Time	Name	Area	Similarity Index
10	7.049	4-Am-DNT	1149961	0.999879
11	7.160	2-Am-DNT	1216223	0.999503
12	7.817	2,6-DNT	959707	0.999950
13	8.019	2,4-DNT	1922651	0.999917

Figure 6: Peak Deconvolution, 254 nm, 6.4 - 8.5 min.

Performing quantitative calibration on these standards shows very good linearity. All analytes are linear from 2 – 500 ng on column, except for 2-Am-DNT and 4-Am-DNT, which are linear from 2 – 250 ng on column.

All calibrations show a Pearson R^2 of 0.999 or higher over their linear range of responses.



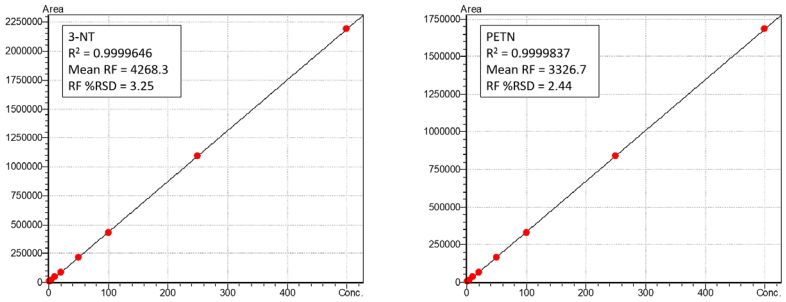


Figure 7: EPA Method 8330 Standard Mix, External Calibration

The spectral confirmation similarity index showed a 0.985 or better correlation to the stored library spectra throughout the calibration range.

Table 3: Spectral Comparison Similarity Indices, Calibration Standards

	Spectral Similarity Index, ng Analyte on Column							
Name	500 ng	250 ng	100 ng	50 ng	20 ng	10 ng	4 ng	2 ng
HMX	0.99964	0.99996	0.99999	0.99999	0.99999	0.99997	0.99997	0.99988
RDX	0.99990	0.99998	1.00000	1.00000	0.99999	0.99999	0.99996	0.99988
1,3,5-TNB	0.99962	0.99997	0.99996	0.99994	0.99991	0.99990	0.99985	0.99985
1,3-DNB	0.99989	0.99995	0.99996	0.99996	0.99996	0.99994	0.99979	0.99978
3,5-DNA	0.99975	0.99988	0.99989	0.99988	0.99986	0.99988	0.99951	0.99980
NB	0.99944	0.99978	0.99989	0.99991	0.99992	0.99988	0.99986	0.99955
Tetryl	0.99998	0.99999	0.99995	0.99989	0.99980	0.99976	0.99963	0.99933
NG	0.99957	0.99979	0.99988	0.99988	0.99985	0.99981	0.99868	0.99649
2,4,6-TNT	0.99995	0.99996	0.99997	0.99997	0.99997	0.99995	0.99983	0.99974
4-Am-DNT	0.99982	0.99984	0.99986	0.99986	0.99983	0.99975	0.99908	0.99914
2-Am-DNT	0.99981	0.99961	0.99969	0.99962	0.99955	0.99959	0.99944	0.99930
2,6-DNT	0.99987	0.99990	0.99990	0.99990	0.99987	0.99990	0.99917	0.99905
2,4-DNT	0.99991	0.99993	0.99994	0.99995	0.99993	0.99991	0.99956	0.99947
2-NT	0.99985	0.99993	0.99995	0.99995	0.99992	0.99989	0.99928	0.99562
4-NT	0.99982	0.99986	0.99987	0.99988	0.99987	0.99980	0.99896	0.99868
3-NT	0.99990	0.99994	0.99995	0.99995	0.99993	0.99990	0.99934	0.99828
PETN	0.99979	0.99988	0.99989	0.99993	0.99962	0.99879	0.99249	0.98528

■ Conclusion

By performing EPA Method 8330B analysis with Shimadzu photodiode array detection with LabSolution LC software and i-PDeA II, method performance is obtained with much less development time. Deconvolution of the poorly separated peaks significantly cuts the time required to attain a full separation.

Confidence in the quantitative result can be enhanced by being able to confirm spectral identity of the analyte peaks, even if the peaks are poorly resolved by the column and method.

First Edition: April 2019

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