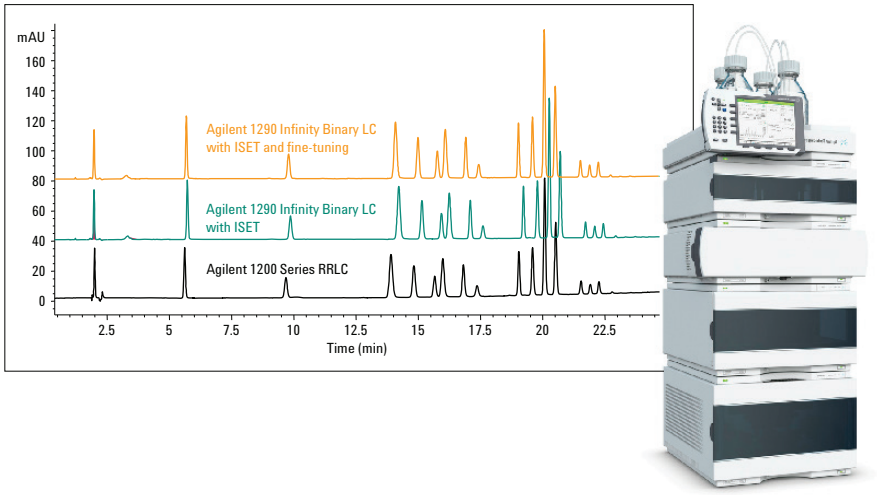
Seamless instrument-to-instrument method transfer of the EPA method 8330A/B for nitroaromatics from an Agilent 1200 Series LC to the Agilent 1290 Infinity Binary LC using ISET

Application Note

Environmental



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Abstract

This Application Note presents an Application Solution for the analysis of nitroaromatic compounds. The seamless transfer of the EPA method (8330A/B) from the Agilent 1200 Series Rapid Resolution LC (RRLC) to the Agilent 1290 Infinity Binary LC using Intelligent System Emulation Technology (ISET) is shown. The deviation of retention times from the original data is evaluated. Data based on ISET and data obtained without ISET are compared. Additional aspects regarding improvement of resolution and of the limit of detection using the 1290 Infinity Binary LC are discussed.

Verified for Agilent
1290 Infinity II LC

Agilent Technologies

Introduction

Explosives and other nitroaromatic compounds are known to be toxic and are monitored in areas where these compounds were used, for example, former military training grounds. The EPA and other environmental agencies have published methods for the analysis of nitroaromatics and related compounds.[1]

This Application Note performs an analysis of an explosive standard based on the EPA method 8330A/B. The chromatographic conditions were first applied onto a 1200 Series RRLC then transferred to the 1290 Infinity Binary LC with, and without using ISET.

With ISET, the chromatograms obtained on the 1200 Series RRLC and the 1290 Infinity Binary LC are expected to have similar retention times within a ±5% range and also the resolutions are expected to be nearly the same or better.

ISET compensates for different delay volumes and also for different gradient profiles caused by differences in mixing behavior of the pumps.

Using the 1290 Infinity Diode Array Detector (DAD) with the 10-mm or the 60-mm path length cell, an improved limit of detection (LOD) can be expected. This is based on the lower noise level and the lower post-column dispersion volume achieved through smaller detector cell volume and low volume connecting capillaries. The lower post-column dispersion volume results in lower peak widths and, consequently, in a better signal-to-noise ratio. Significantly better LODs are typically obtained if the 60-mm path length cell is used, due to the 6-times longer path length.

A further advantage of a DAD in general is the usage of spectral libraries which provides an additional identification tool for the analysis of explosives.

Experimental

Table 1 shows the instruments used.

Detector cells used in the 1290 Infinity DAD:

10-mm cell (G4212-60008) and 60-mm cell (G4212-60007)

Chromatographic conditions

Sample:	Dr. Ehrenstorfer Nitroaromatic-Explosive Mix no. 3 (p/n 08330300), 10 ng/µL each
Column:	Agilent Poroshell 120 C18, 4.6 × 150 mm, 2.7 µm (p/n 683975-902)
Mobile phase:	A = Water, B = Methanol
Gradient:	0 minutes 20% B, at 10 minutes 30% B, at 30 minutes 95% B, at 31 minutes 95% B
Flow rate:	0.8 mL/min , maximum backpressure of 125 bar
Stop time:	40 minutes
Post time:	5 minutes
Injection volume:	3 µL of undiluted sample 1 µL for LOD Experiment, 1:10 diluted sample was injected, 1 ng/µL
Column temperature:	35 °C
DAD:	254/214, 235/10 nm, Ref 400/80 nm,
Flow cell:	10 mm
Peak width:	< 0.013 minutes (20 Hz)

Acquisition and Evaluation Software

Agilent OpenLAB CDS ChemStation Edition C.01.04

	Agilent 1290 Infinity Binary LC	Agilent 1200 Series RRLC
Binary pump	G4220A	G1312B
Auto sampler	G4226A	G1367E
ALS cooler	G1330B	G1330B
Column compartment	G1316C	G1316B
Diode array detector	G4212A	G1315C

Table 1
Instrumentation used.

Results and discussion

The analysis of explosives was done:

- Using conventional chromatographic HPLC conditions, according to US Environmental Protection Agency, EPA Method 8330B/A-29
- Applying these conditions on to the Agilent 1200 Series RRLC
- Transferring the chromatographic conditions on to the Agilent 1290 Infinity Binary LC with and without applying ISET and comparing the obtained data with the original data
- Applying an additional fine tuning step to further improve agreement of retention times compared to the original results obtained on the 1200 Series RRLC

Additional experiments entailed using the 60-mm path length cell of the 1290 Infinity DAD to improve the limit of detection and the usage of a spectral library for identification purposes.

Analysis of explosives with and without ISET

The explosive standard was best analyzed using water and methanol as mobile phases, two wavelengths and a 150-mm length column. Nitropenta was analyzed at 214 nm, the other compounds were analyzed at 235 nm, see marked area in Figure 1. The complete method was developed using the 1200 Series RRLC. Precision data, based on six consecutive runs, and resolution for all peaks were determined.

Having completed the method, it was transferred onto the 1290 Infinity Binary LC. During the initial analysis, ISET was not activated then, the analysis of the explosives was done with ISET enabled, see Figure 2.

Peak identification for Figures 1 and 2

1 Nitroguanidin
2 Octogen (HMX)
3 Hexogen (RDX)
4 1,3,5 Trinitrobenzene
5 2-Amino-6-Nitrotoluene
6 1.2-Dinitrobenzene
7 1,3-Dinitrobenzene
8 2-Amino-4-Nitrotoluene
9 Nitrobenzene
10 Tetryl
11 2,4,6-trinitrotoluene (TNT)
12 4-Amino-2,6-dinitrotoluene
13 2,6-Dinitrotoluene
14 2-Nitrotoluene
15 4-Nitrotoluene
16 3-Nitrotoluene
17 Nitropenta

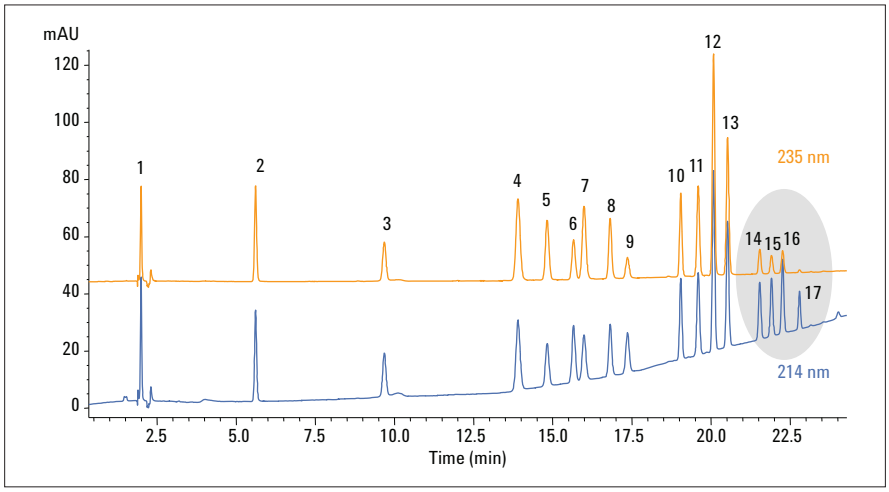


Figure 1
Analysis of explosive standard at two different wavelengths.

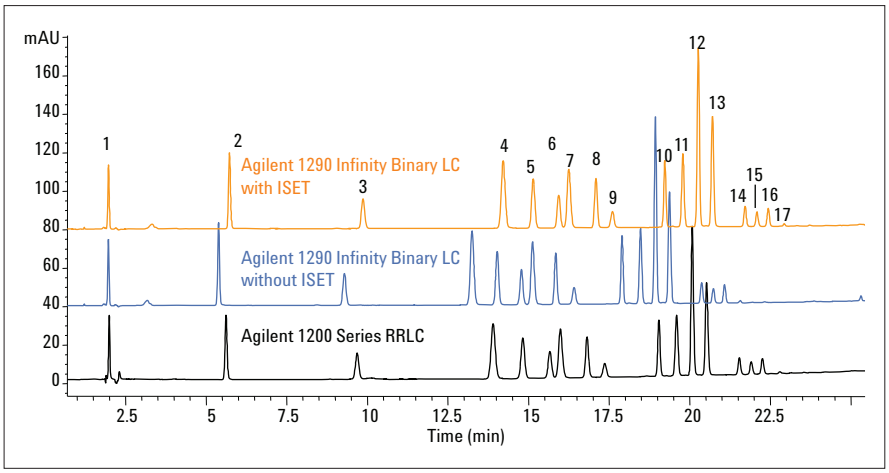


Figure 2
Comparison of the original chromatogram with chromatograms obtained on the 1290 Infinity Binary LC with and without ISET.

Without ISET, the retention times shifted to lower values due to the lower delay volume of the 1290 Infinity Binary LC system compared to the 1200 Series RRLC. With ISET, the agreement of the retention times was very good. To improve the agreement even further, a fine tuning step was applied. The delay volume offset of the 1290 Infinity Binary LC set up was decreased by 167 µL (Figure 3).

The result was that the retention time difference for all peaks was below 1.3% using ISET and the fine-tuning step. Without the fine-tuning step of ISET, the deviation of retention times was below 2.3%. Without ISET, the deviation of the retention times exceeded the allowed limit of ±5% (Figure 4).

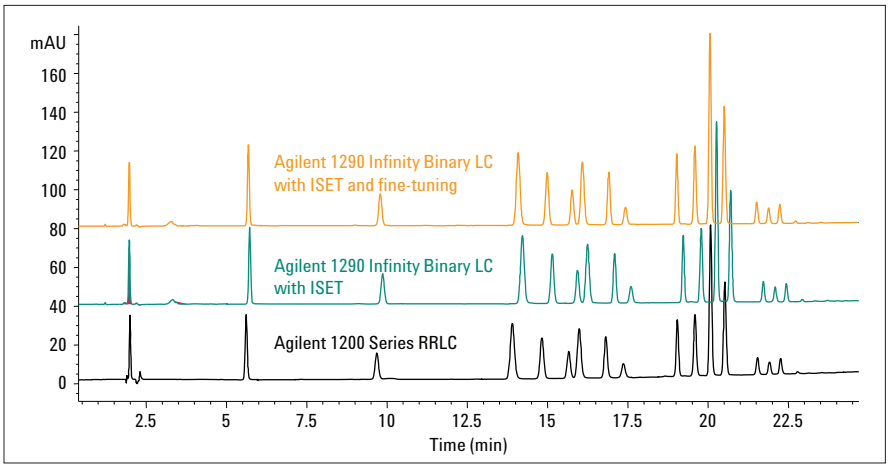


Figure 3
Overlay of original chromatogram with the chromatograms obtained with ISET and obtained with ISET and fine-tuning.

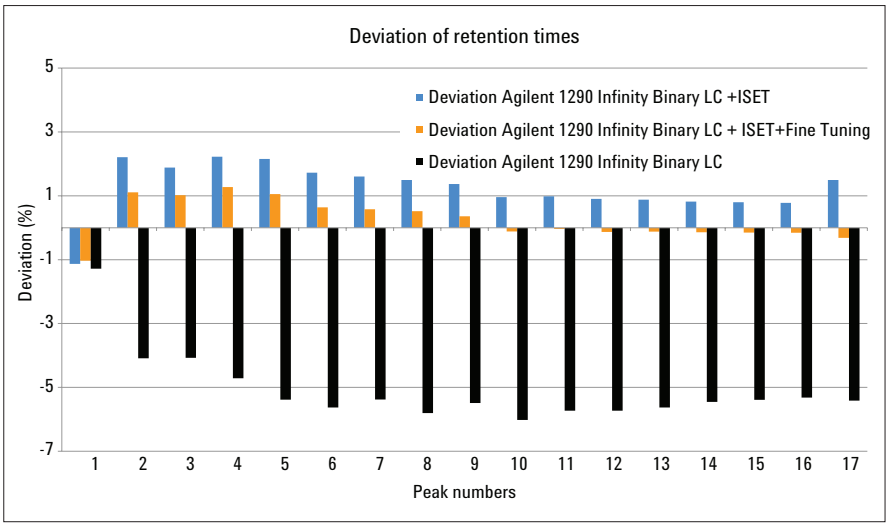


Figure 4
Deviation of retention times for different setups.

The resolution was also compared to the original data (Figure 5).

Better resolution was obtained on the 1290 Infinity Binary LC due to the lower dispersion volume of, for example, the detector cell and connecting capillaries. The improvement averaged approximately 6%.

Limit of detection (LOD)

The explosive were present in a concentration of 10 ng/µL each in the original solution. To determine the LOD, the original solution was 1:10 diluted resulting in a concentration of 1 ng/µL. A 1-µL amount was injected. Figure 6 shows an overlay of the results obtained on the 1200 Series RRLC DAD and the 1290 Infinity DAD with 10- and 60-mm path length cell. The noise level for the three instrument configurations is also shown.

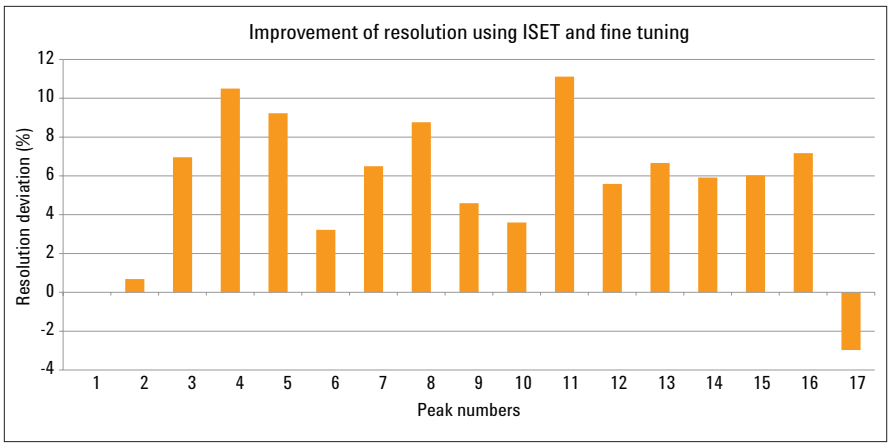


Figure 5
Deviation of resolution using ISET and fine–tuning.

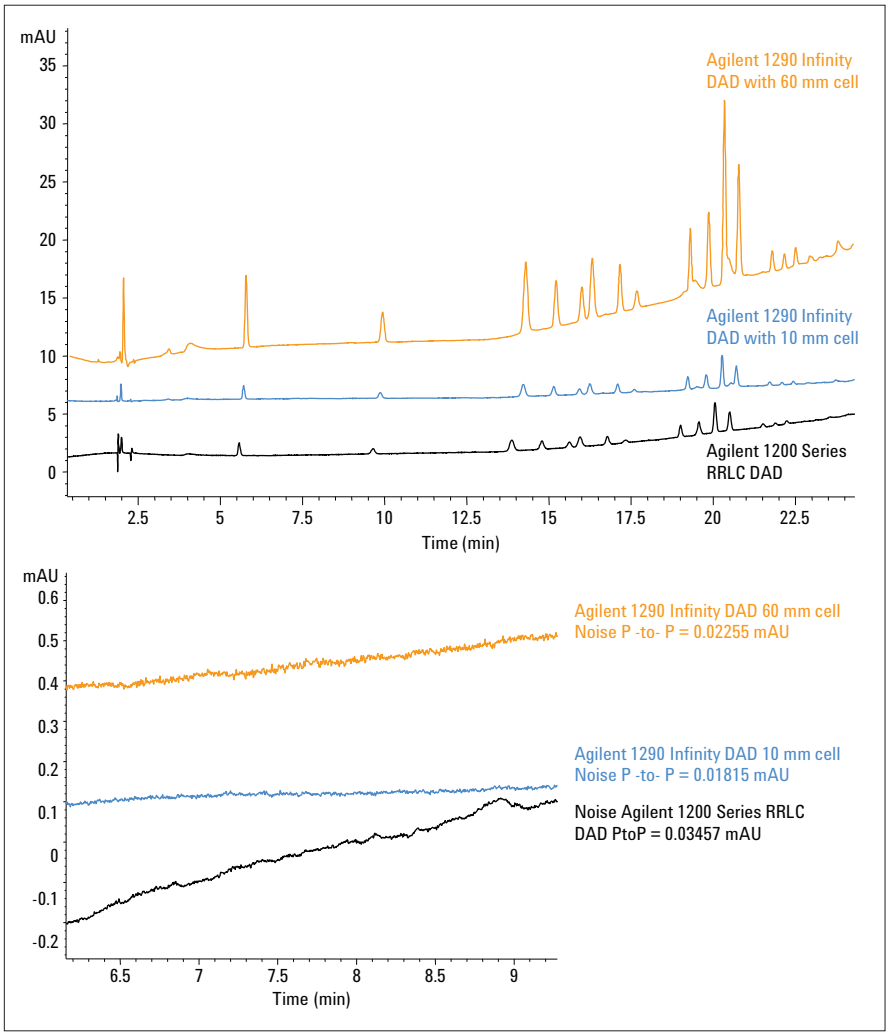


Figure 6
Peak height and noise level (7 to 7.5 minutes) at 1 ng injected amount for the three instrument configurations.

The LOD calculations for peaks 1-16 were based on 235 nm, and for peak 17 on 214 nm wavelength. The lowest noise level was obtained using the 1290 Infinity DAD 10-mm cell. The best signal-to-noise ratio was obtained using the 60-mm path length cell of the 1290 Infinity DAD. The LOD data are summarized in Figure 7.

The results show that lower LODs were obtained using the 10-mm path length cell and significantly better results were obtained using the 60-mm cell. On average, the 60-mm cell provided approximately 83% lower LODs and the 10-mm cell approximately 37% lower LODs.

Identification using a spectral library

Generating a spectra library was an additional identification tool. For the identification of the determined explosives, a spectral library containing about 30 compounds was used (Figure 8).

For example, the match factor of 999.108 together with the retention time ensured the identification of the peak at 16.808 minutes as 2-amino-4-nitrotoluene.

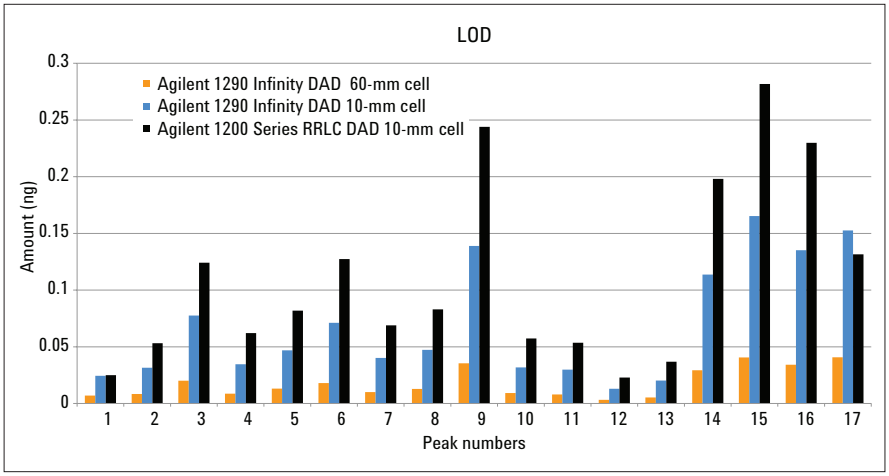
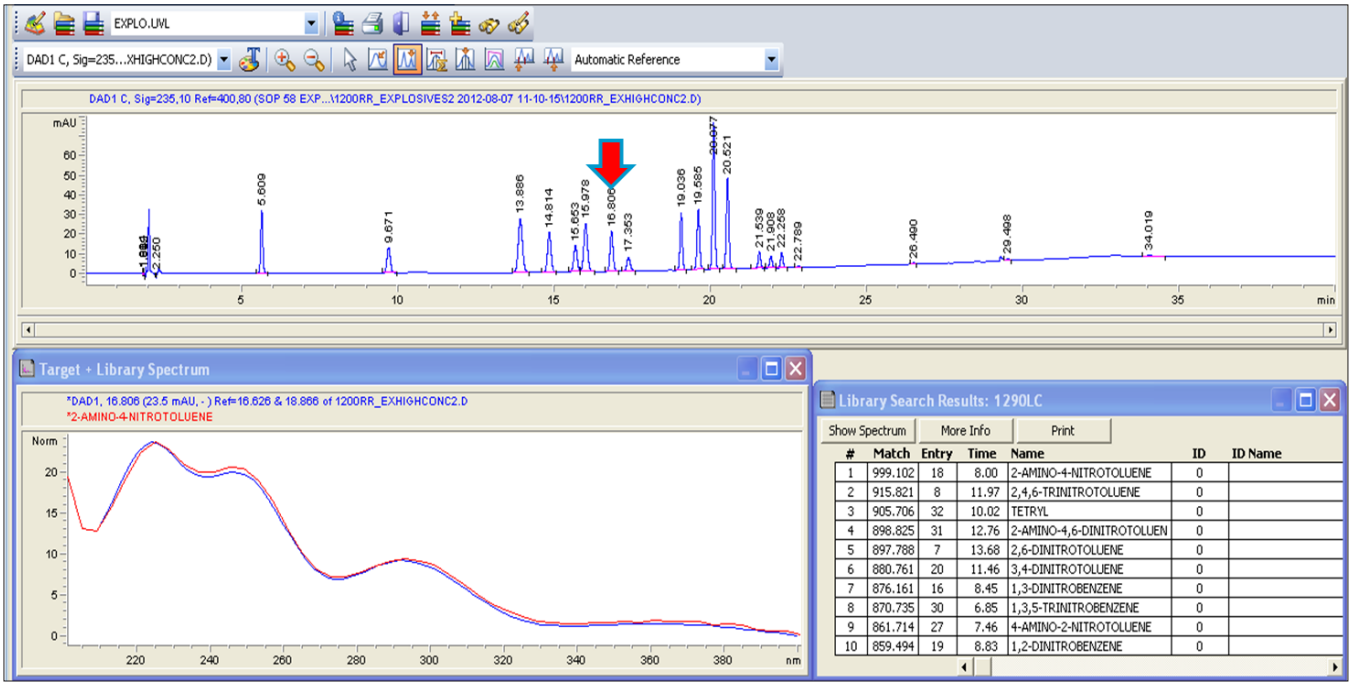


Figure 7
LOD data for the Agilent 1290 Infinity DAD with 10- and 60-mm path length cell and the 10-mm cell of the Agilent 1200 Series RRLC DAD.



#	Match	Entry	Time	Name	ID	ID Name
1	999.102	18	8.00	2-AMINO-4-NITROTOLUENE	0	
2	915.821	8	11.97	2,4,6-TRINITROTOLUENE	0	
3	905.706	32	10.02	TETRYL	0	
4	898.825	31	12.76	2-AMINO-4,6-DINITROTOLUEN	0	
5	897.788	7	13.68	2,6-DINITROTOLUENE	0	
6	880.761	20	11.46	3,4-DINITROTOLUENE	0	
7	876.161	16	8.45	1,3-DINITROBENZENE	0	
8	870.735	30	6.85	1,3,5-TRINITROBENZENE	0	
9	861.714	27	7.46	4-AMINO-2-NITROTOLUENE	0	
10	859.494	19	8.83	1,2-DINITROBENZENE	0	

Figure 8
Identification of 2-amino-4-nitrotoluene through spectral library.

Conclusions

A method for the analysis of an explosives standard was developed using the Agilent 1200 Series RRLC according to EPA 8330A/B. To prove the functionality of the ISET, the method was transferred to the 1290 Infinity Binary LC. The difference in retention times averaged <1.3%, the resolution was improved by about 6% on average. These data showed that the 1290 Infinity Binary LC in combination with ISET can emulate the target 1200 Series RRLC.

An additional benefit of using the 1290 Infinity DAD and the 10-mm detector was the improvement of the LOD by approximately 37%. Using the 60-mm detector cell the improvement for the LOD was about 80%.

To identify the compounds with higher confidence, a spectral library was used. Excellent match factors enabled the identification of the 17 compounds in addition to identification using retention times.

Reference

1.
US Environmental Protection Agency, EPA Method 8330B-A-29, revision 2, „Nitroaromatics and Nitramines by HPLC“, October **2006**.

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