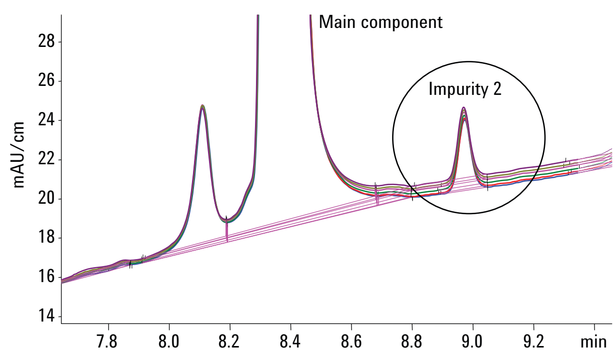
Reliable and Automatic Integration of Trace Compounds Using the Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector Solution

Technical Overview



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Abstract

The Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector (HDR DAD) solution expands the linear dynamic range by a factor of 30. By combining the signals from two diode array detectors with different path length Agilent Max-Light flow cells, the 1200 Infinity Series HDR DAD solution facilitates detection and quantification of main and trace components in a single run without exceeding the linear range of the HDR DAD solution. Another advantage is that trace compounds are integrated automatically with excellent area precision, without the need to set integration events manually.

Introduction

When using conventional diode array detectors in HPLC, the analysis of main and trace components often requires two separate analyses to be able to quantify all components with good area precision. Another problem is that trace compounds often need manual integration to obtain reliable area results, especially when the chromatogram shows baseline drift.

Both challenges can be solved by using the 1200 Infinity High Dynamic Range DAD (HDR DAD) solution. The detector design facilitates the analysis of main and trace components simultaneously in a single run and avoids the need for manual integration of the trace components. This is made possible by the improved signal-to-noise (S/N) ratios for measured trace components.

Experimental

Instrumentation

Table 1 shows the configuration of the Agilent 1260 Infinity Binary LC System used for the experiments.

Software

Agilent OpenLAB CDS ChemStation Edition, revision C.01.05

Samples

The sample contained one main component and four impurities with the following concentrations:

- Main compound: 10 mg/10 mL
- Impurities: 5 mg/10 mL; diluted 1:1,000 with the main component solution to give a resulting concentration of 0.05 %

Chromatographic conditions

Table 2 shows the chromatographic conditions.

Table 1. Configuration of the Agilent 1260 Infinity Binary LC System.

Description	Model number
Agilent 1260 Infinity Binary Pump	G1312B
Agilent 1260 Infinity Autosampler	G1329B
Agilent 1260 Infinity Thermostat (for sample cooling)	G1330B
Agilent 1260 Infinity Thermostatted Column Compartment	G1316A
Agilent 1260 Infinity Diode Array Detector with 3.7-mm path length flow cell	G4212B
Agilent 1260 Infinity Diode Array Detector with 10- and 60-mm path length flow cells	G4212B
Agilent 1100 Series Diode Array Detector (for comparison experiments)	G1315B

Table 2. Chromatographic conditions.

Parameter	Conditions
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 3.5 µm (p/n 959963-902)
Mobile phases	Water + 0.5 % TFA/acetonitrile + 0.045 % TFA = 90/10
Flow rate	1.2 mL/min
Gradient	10 % B at 0 minutes; 95 % B at 15 minutes
Stop time	20 minutes
Post time	4 minutes
Injection volume	10 µL
Detection	220/10 nm, Ref: 450/80 nm, 10 Hz
Column temperature	30 °C

Basic Principles of the 1200 Infinity Series HDR DAD Solution

The 1200 Infinity Series HDR DAD solution expands the linear dynamic range by a factor of more than 30. By combining the signals from two diode array detectors with different path length Max-Light flow cells, the HDR DAD solution enables detection and quantification of components with significantly different concentrations in a single run. The HDR DAD solution clusters two 1260 or 1290 Infinity DADs together, see Figure 1. Detector 1 is equipped with a 60-mm path length cell for analyzing low concentration components and Detector 2 is equipped with a 3.7-mm path length cell for analyzing high concentration compounds. The 60-mm cell must be installed in the first detector and the 3.7-mm cell as the second detector. The HDR DAD signal is one combined signal, normalized to a 10-mm path length. The HDR DAD linear range is typically as wide as 0.6 $\times 10^{-6}$ to 6.7 AU/cm. A conventional 1200 Infinity Series Diode Array Detector has a maximum linear range of 7 $\times$ 10^{-6} to 2 AU/cm.

For detection of trace components, the HDR DAD signal is based on the signal acquired by the 60-mm cell. The HDR DAD signal is always normalized to 10-mm path length. The peak height, the peak area of the impurities, and the noise level obtained from the signal of the 60-mm cell is divided by a factor of six to give the HDR DAD signal. The 3.7-mm cell is used to provide the HDR DAD signal for the main component, which is typically out of the linear range of the 60-mm cell. For peaks between trace and main component absorbance range, a combination of both signals is used combined by a weighting function.

The high dynamic range (HDR) tool is configured during instrument configuration. Both detectors are clustered and the delay volume of the capillary connecting both detectors is filled in. In the user interface, both detectors appear as one detector, see Figure 2.

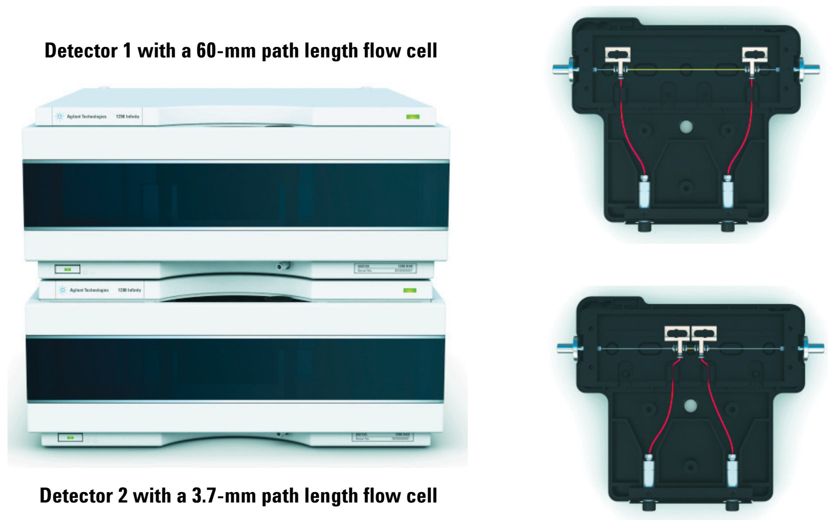


Figure 1. Agilent 1200 Infinity Series HDR DAD solution with two clustered detectors.

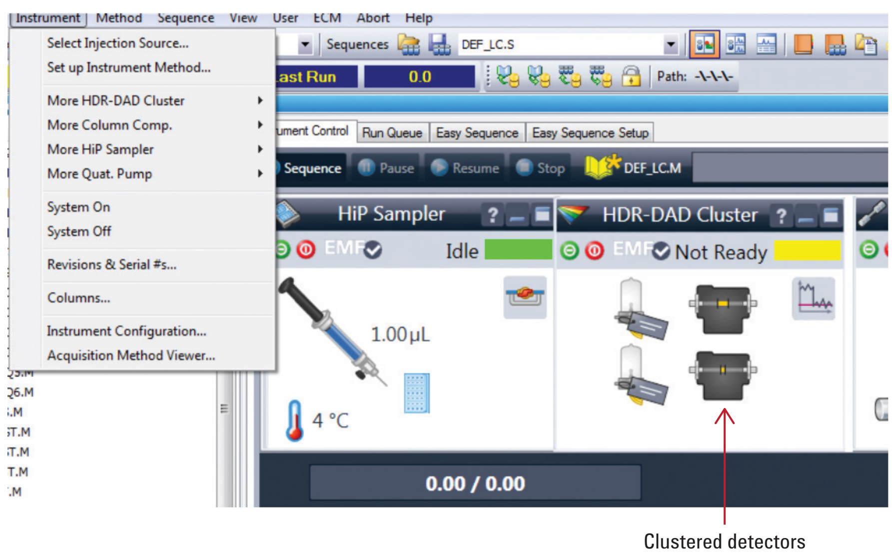


Figure 2. Configuring the Agilent 1200 Infinity Series HDR DAD tool in the Agilent ChemStation.

Results and Discussion

The detector was changed for each experiment, but the same Agilent 1260 Infinity LC System setup was used. The experiments proved that manual setting of integration events was not necessary for the 1200 Infinity Series HDR DAD solution in order to achieve excellent area precision for the main and trace components. To evaluate the performance differences between the Agilent 1100 Series DAD compared to the 1200 Infinity Series HDR DAD solution, the following experiments were performed:

1. The sample containing one main component and four impurities at a concentration range of 0.05 % was injected in the 1260 Infinity LC connected to the 1100 Series DAD.
    - The data was evaluated using a set of integration parameters without manual integration events.
    - The data was evaluated using a set of integration parameters with manual integration events to improve the area precision.
2. The 1100 Series DAD was replaced by the 1260 Infinity DAD equipped with the 60-mm path length cell and the same amount of sample was injected using the same chromatographic conditions.
    - The data was evaluated with a set of integration parameters without manual integration events.
3. The 1260 Infinity DAD was replaced with the 1260 Infinity HDR DAD solution and the same amount of sample was injected using the same chromatographic conditions.
    - The data was evaluated with a set of integration parameters without manual integration events.

Figure 3 shows the chromatograms for all three experiments overlaid.

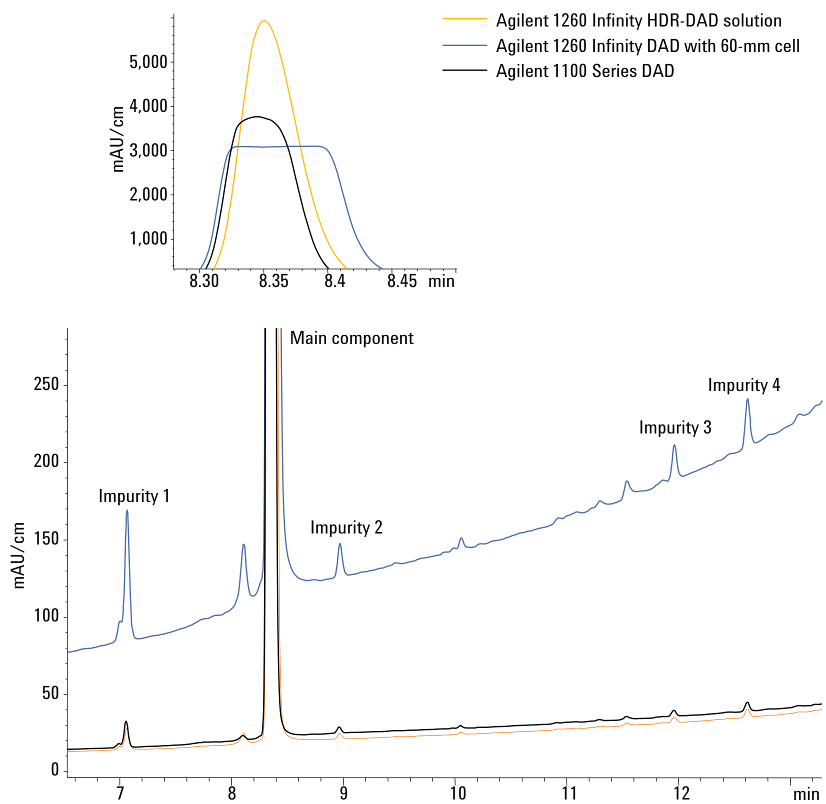


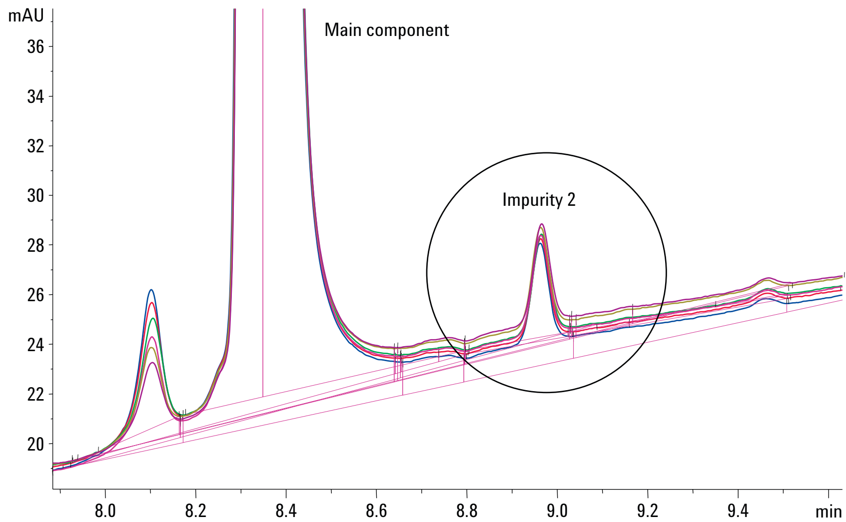
Figure 3. Overlay of resulting chromatograms obtained using the Agilent 1100 Series DAD (black), the Agilent 1260 Infinity DAD with a 60-mm cell (blue) and the Agilent 1260 Infinity HDR DAD solution (orange).

As expected, the 60-mm cell provided increased peak heights. The main peak is far beyond the linear range of the 60-mm cell and a reliable quantification is not possible. The 1100 Series DAD with 10-mm path length cell enables quantification of the trace components but not of the main component. The peak height is also beyond the linear range. Only the 1260 Infinity HDR DAD solution enables reliable quantification of the main and the trace components. The HDR DAD signal was normalized to 10-mm path length.

Integration Results of Experiment 1 for Trace Components Using the 1100 Series DAD

Figure 4 shows an overlay of six consecutive runs, including the integration start and stop marks and the resulting integration baseline.

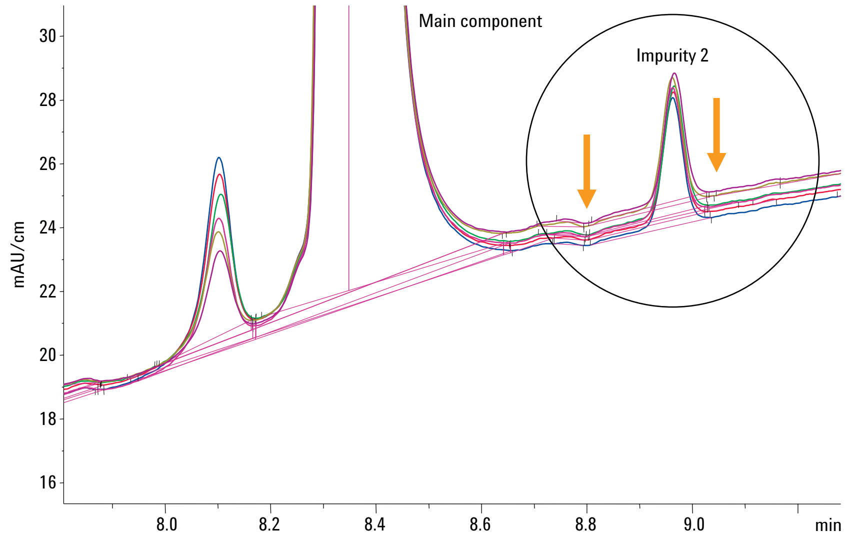
Figure 4 clearly shows that the automatic integration led to unreliable results for impurity 2. All other impurities were integrated correctly. To improve the integration, two manual integration events before and after impurity 2 were set.



	Area precision (% RSD)
	Agilent 1100 Series DAD without manual integration events
Impurity 1	0.300
Impurity 2	31.385
Impurity 3	1.120
Impurity 4	3.144

Figure 4. Integration of a trace component eluting shortly after the main peak.

A baseline-now event at 8.746 minutes before impurity 2 and at 9.125 minutes after impurity, see Figure 5. Using these manually-set integration events, impurity 2 was integrated reliably, and the area precision for all impurities was less than 3.2 % RSD.

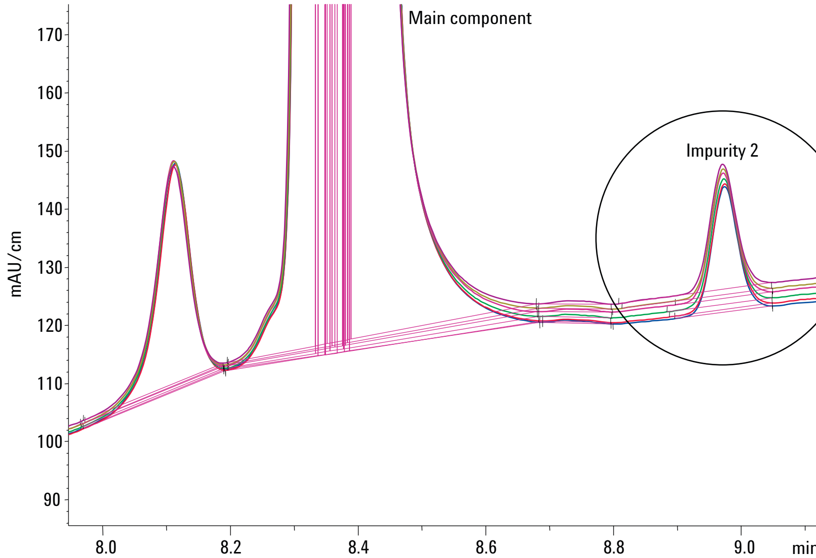


	Agilent 1100 Series DAD without manual integration events	Agilent 1100 Series DAD with manual integration events
Impurity 1	0.300	0.300
Impurity 2	31.385	1.697
Impurity 3	1.120	1.130
Impurity 4	3.144	3.144

Figure 5. Integration of impurity 2 applying two manual integration events.

Integration Results of Experiment 2 for Trace Components Using the 1260 Infinity DAD with 60-mm Cell

The 1260 Infinity DAD with a 60-mm path length flow cell provided reliable integration of the trace compounds mainly due to increased peak height and, consequently, five to six times more area counts, see Figure 6. This resulted in an area precision greater than 0.82 % RSD for all impurities.



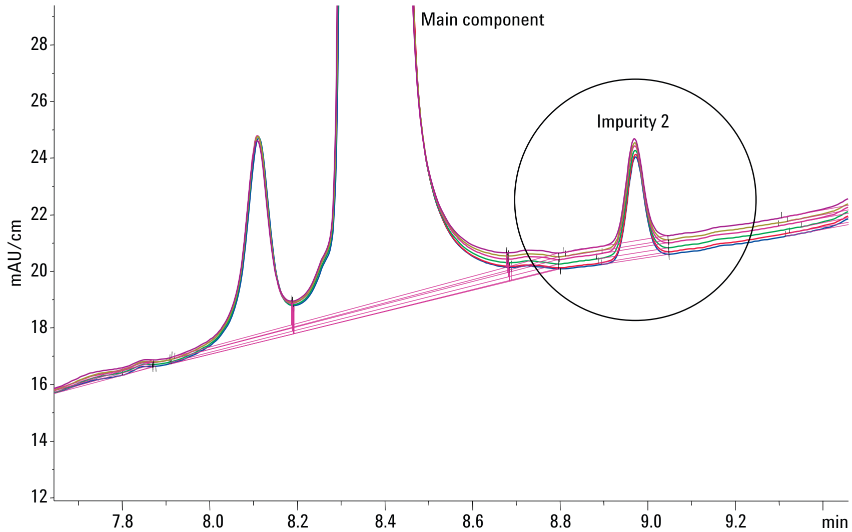
	Area precision (% RSD)		
	Agilent 1100 Series DAD without manual integration events	Agilent 1100 Series DAD with manual integration events	Agilent 1260 Infinity DAD with a 60-mm flow cell
Impurity 1	0.300	0.300	0.135
Impurity 2	31.385	1.697	0.296
Impurity 3	1.120	1.130	0.813
Impurity 4	3.144	3.144	0.405

Figure 6. Integration results and area precision using the Agilent 1260 Infinity DAD with a 60-mm flow cell.

Integration Results of Experiment 3 for Trace Components Using the 1260 Infinity HDR DAD Solution

The 1260 Infinity HDR DAD solution used the data from the 60-mm cell for the evaluation of the trace compounds. Consequently, similar results for the 1260 Infinity DAD with 60-mm cell were obtained, see Figure 7. The area precision for the 1260 Infinity HDR DAD signal was less than 0.8 % RSD.

Figure 8 summarizes the area precision data for all detectors and for all measured impurities.



Area precision (% RSD)				
	Agilent 1100 Series DAD without manual integration events	Agilent 1100 Series DAD with manual integration events	Agilent 1260 Infinity DAD with a 60-mm flow cell	Agilent 1260 Infinity HDR DAD solution
Impurity 1	0.300	0.300	0.135	0.135
Impurity 2	31.385	1.697	0.296	0.289
Impurity 3	1.120	1.130	0.813	0.795
Impurity 4	3.144	3.144	0.405	0.399

Figure 7. Integration of impurity 2 using the Agilent 1260 Infinity HDR DAD solution.

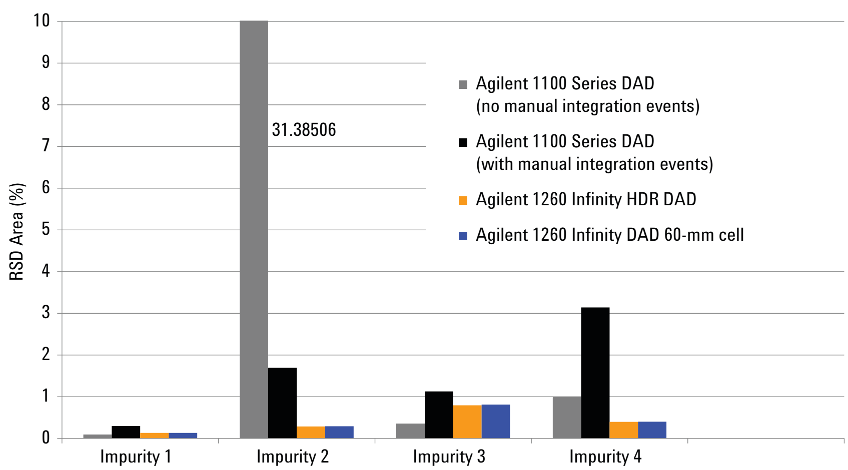


Figure 8. Area precision for all detectors and impurities.

Typically, the signals from the 1260 Infinity DAD with a 60-mm cell and the 1260 Infinity HDR DAD solution provided better area precision for trace components than the signal from the 1100 Series DAD. This is because the improved S/N ratio achieved by the 60-mm cell resulted in more reliable integration of trace components than was possible using the 1100 Series DAD, see Figure 9.

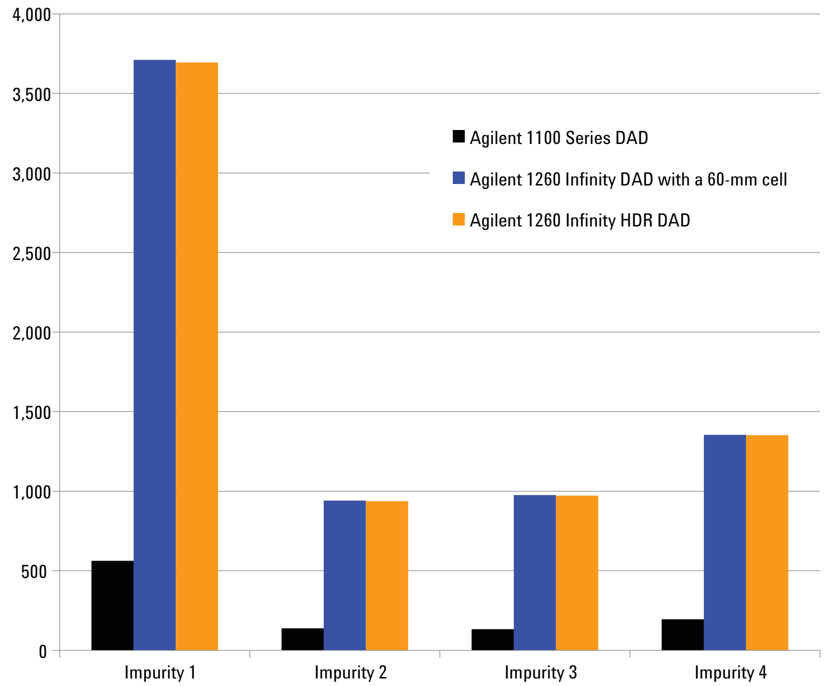


Figure 9. S/N ratio for all detectors and impurities.

Six to seven-times better S/N for 1260 Infinity HDR DAD and 1260 Infinity DAD with a 60-mm cell was obtained. The better S/N ratio was a result mainly of increased peak height, see Figure 3 and, to a certain extent, of less baseline noise, see Figure 10.

Conclusion

The Agilent 1260 Infinity HDR DAD solution facilitated the analysis of main and trace components present at a concentration of 0.05 % in a single run by means of an increased linear dynamic range of greater than 30. Another advantage was that the integration of the trace component peaks did not require the manual setting of integration events for reliable quantification with excellent area precision. This was achieved through the increased S/N ratio and, consequently, increased area counts for all impurities.

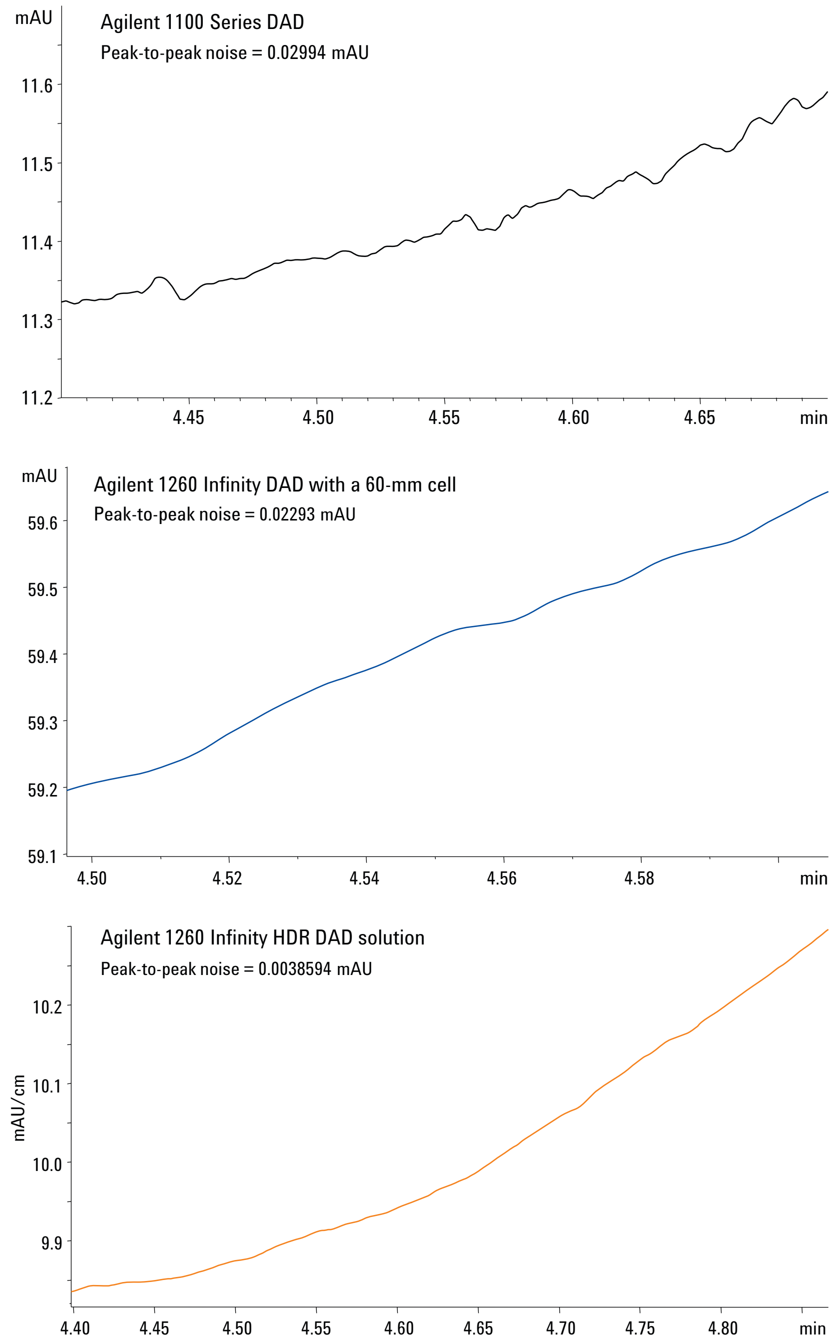


Figure 10. Baseline peak-to-peak noise for each detector deployed.

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