Comparison of Sensitivity and Linearity of the Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector Solution and the Conventional Agilent 1290 Infinity DAD

Technical Overview

Author

A.G.Huesgen

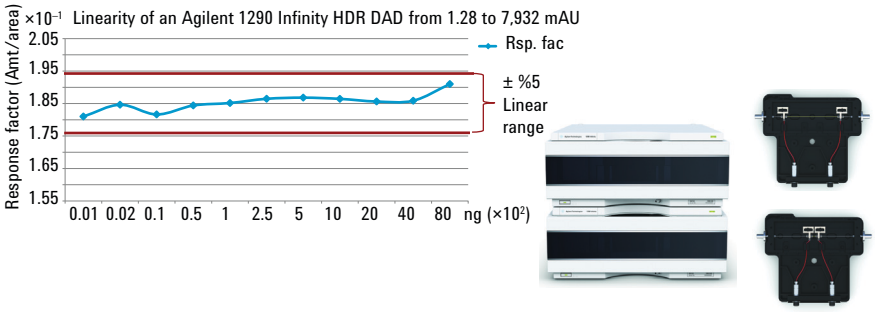
Agilent Technologies Inc.

Waldbronn, Germany

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 detector.

This Technical Overview evaluated linearity and limit of detection (LOD) for the 1290 Infinity HDR DAD solution and the 1290 Infinity DAD with the 10-mm path length flow cell. In addition, quantitative results of the 1290 Infinity HDR DAD and a 1200 Series DAD were compared for confirmation of results.



Agilent Technologies

Introduction

The Agilent 1200 Infinity Series HDR DAD solution combines the signals from two diode array detectors with different path length Agilent Max-Light flow cells, see Figure 1. 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. For detection of trace components, the HDR DAD signal was based on the signal acquired by the 60-mm cell. The 3.7-mm cell was 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 was used, combined by a weighting function. The HDR DAD signal was normalized to 10-mm path length.

The benefits of this design are:

- The 1200 Infinity Series High Dynamic Range Diode Array Detector (HDR DAD) solution expands the linear dynamic range by a factor of 30.
- The limit of detection (LOD) is typically improved by a factor of three to six compared to a 10-mm path length cell.
- Quantitative results agree with results obtained on conventional UV detectors like a 1200 Series DAD.

In the first set of experiments, linearity and LOD were compared using the 1290 Infinity HDR DAD and the 1290 Infinity DAD with a 10-mm path length detector cell. In the second set of experiments, quantitation results were confirmed by data obtained on a conventional DAD such as the Agilent 1200 Series DAD (G1315B).

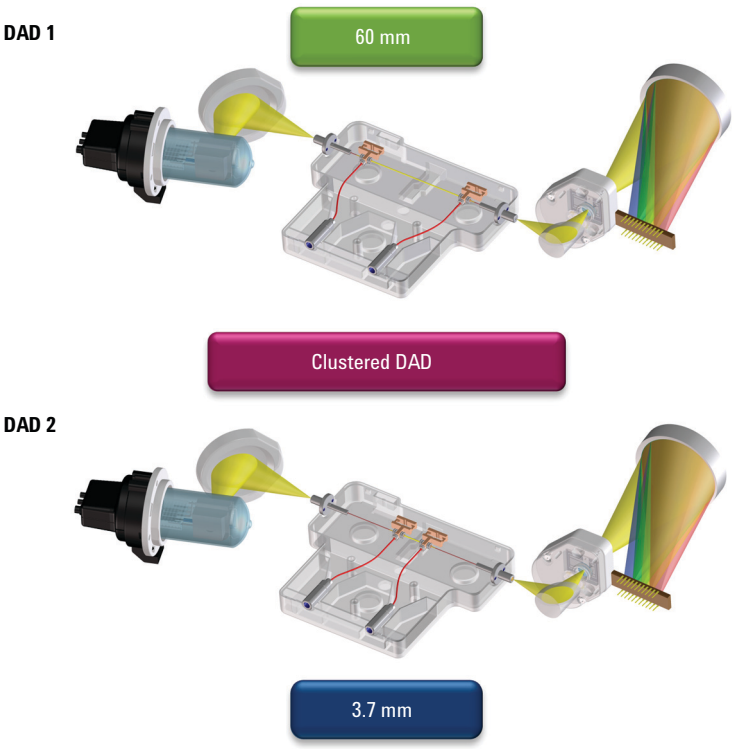


Figure 1. Clustered Agilent 1200 Infinity Series DADs.

Experimental

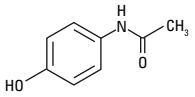
The following instruments, conditions and compounds were used.

Instrumentation

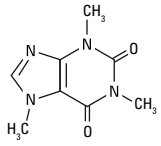
Instrument	Part no.
Agilent 1290 Infinity HDR Diode Array Detector with 60-mm and 3.7-mm flow cells in series	2x G4212A
Agilent 1290 Infinity Diode Array Detector with 10-mm flow cell	G4212A
Agilent 1290 Infinity Thermostatted Column Compartment	G1316C
Agilent 1290 Infinity HiP Autosampler	G4226A
Agilent 1290 Infinity Thermostat	G1330B
Agilent 1290 Infinity Binary Pump	G4220A
Agilent 1200 Series DAD (conventional DAD)	G1315B

Compounds analyzed

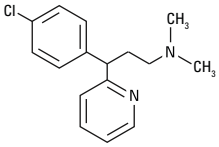
- Caffeine was used for experiments evaluating linearity of detectors.
- Anthracene was used for experiments evaluating the limit of detection of detectors.
- Comparison of quantitative results was done using a pharmaceutical preparation with:



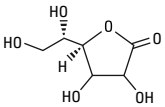
Paracetamol



Caffeine



Chlorphenamine



Vitamin C

Chromatographic conditions for evaluating linearity

Parameter	Value
Sample	Caffeine dissolved in water, 0.5 µg/mL to 500 µg/mL
Column	Agilent ZORBAX RRHT Eclipse Plus C18, 4.6 × 150 mm, 1.8 µm, (p/n 993967-902)
Mobile phases	A) water B) acetonitrile
Gradient	at 0 minutes 5 % B, at 5 minutes 20 % B
Flow	1 mL/min
Stop time	8.5 minutes
Post time	5 minutes
Injection volume	2 µL from 0.5 µg/mL to 500 µg/mL; 4, 8, and 16 µL for 2,000, 4,000, and 8,000 ng injection from 500 µg/mL solution
UV	273/10 nm, Ref: 380/80 nm, 20 Hz
Column temperature	30 °C

Chromatographic conditions for evaluating LOD

Parameter	Value
Sample	Anthracene dissolved in acetonitrile, 10 pg/µL
Column	Agilent ZORBAX RRHT Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm, (p/n 959741-902)
Mobile phases	A) water B) acetonitrile
Isocratic	65 % B
Flow	0.5 mL/min
Stop time	5 minutes
Injection volume	1 µL
UV	251/4 nm, Ref: 450/80 nm, 2.5 Hz
Column temperature	36 °C

Chromatographic conditions for comparing quantitation

Parameter	Value
Column	Agilent ZORBAX RRHT Eclipse Plus C18, 4.6 × 100 mm, 5 µm, (p/n 959996-902)
Mobile phases	A) water + 0.1 % TFA, B) acetonitrile + 0.09 % TFA
Gradient	at 0 minutes 5 % B, at 0.5 minutes 5 % B, at 6.1 minutes 40 % B, at 6.5 minutes 95 % B, at 8 minutes 5 % B
Post time	2 minutes
Flow	1 mL/min
Stop time	10 minutes
Injection volume	0.5, 1, 2 µL, for a 3-level calibration and 1.5 µL to compare quantitation, see Table 1
UV	254/10 nm, Ref: 380/80 nm, 10 Hz
Column temperature	40 °C

Sample preparation

1. Two capsules of the pharmaceutical preparation were opened and dissolved in 20 mL distilled water.
2. Extraction with ultrasonic bath for 5 minutes
3. Filtering with Agilent Captiva Premium Syringe Filter, regenerated cellulose, p/n 5190-5111
4. Clear liquid was filled and stored in 1.5-mL LC vials containing:
   - 250 ng/µL chlorphenamine
   - 20,000 ng/µL paracetamol
   - 2,500 ng/µL caffeine
   - 15,000 ng/µL vitamin C
5. Dilution 1:20 with water containing:
   - 12.5 ng/µL chlorphenamine
   - 1,000 ng/µL paracetamol
   - 125 ng/µL caffeine
   - 750 ng/µL vitamin C
6. This solution was injected using different injection volumes, see Table 1.

Software

OpenLAB CDS ChemStation C.01.05

The HDR software tool is configured in the instrument configuration screen like all other modules, see Figure 2.

The high dynamic range (HDR) tool is configured during instrument configuration. Both detectors are clustered, and the delay volume of the capillary conneting both detectors is filled in. In the user interface, the detectors appear as one detector, see Figure 3.

Table 1. Injected amounts.

Compound	Calibration level 1 (ng)	Calibration level 2 (ng)	Calibration level 3 (ng)	Unknown sample (ng injected)
Vitamin C	375	750	1,500	1,125
Paracetamol	500	1,000	2,000	1,500
Caffeine	62.5	125	250	187.5
Chlorphenamine	6.25	12.5	25	18.75

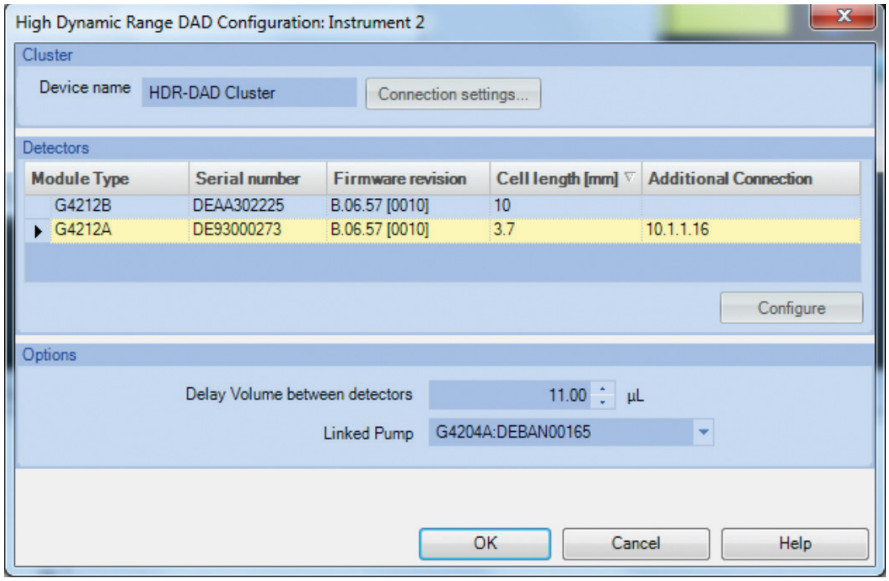


Figure 2. Configuring the DAD cluster.

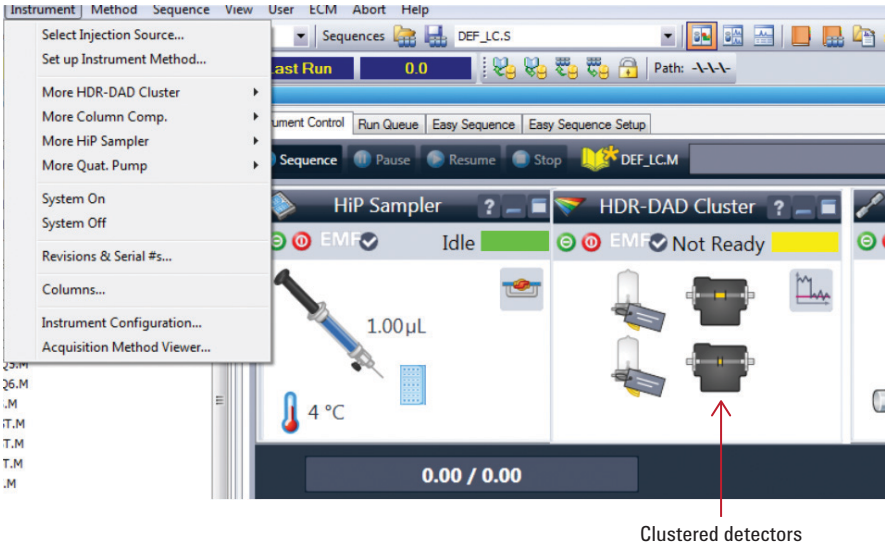


Figure 3. Activating the HDR tool within the Agilent ChemStation.

Results and Discussion

Comparison of linearity

The following amounts of caffeine were injected in the 1290 Infinity HDR DAD and the 1290 Infinity DAD with 10-mm path length cell:

- 1 ng, 2 ng, 10 ng, 50 ng, 100 ng, 250 ng, 500 ng, 1,000 ng, 2,000 ng, 4,000 ng, and 8,000 ng

A multilevel calibration was set up for both detectors, and linearity, based on response factors, was evaluated.

Linearity was given from 1.28 mAU to 7,932 mAU peak height, which is outstanding for an UV detector. The correlation factor for the 8,000 ng injection was 0.99992, and for the 4,000 ng injection 0.99999. Figure 5 shows the chromatograms of all injections overlaid.

Equal amounts were injected into the same 1290 Infinity LC, now equipped with a 1290 Infinity DAD with a 10-mm cell. The linearity ranged from 1.44 mAU to 2726 mAU peak height, see Figure 6. The correlation factors for the high amount injections were:

- Correlation: 0.999995 up to 1,000 ng = 1,353 mAU height
- Correlation: 0.999954 up to 2,000 ng = 2,726 mAU height
- Correlation: 0.963008 up to 8,000 ng

This means excellent linearity was given up to 1,000 ng, and good linearity was given up to 2,000 ng injected amount. Above 2,000 ng, the detector ran into saturation, see Figure 7.

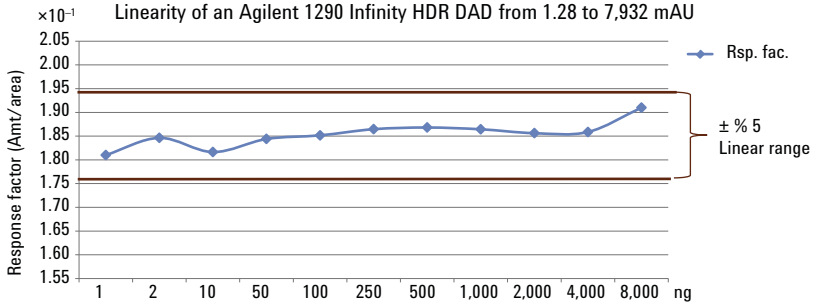


Figure 4. Linearity of an Agilent 1290 Infinity HDR DAD, based on response factors.

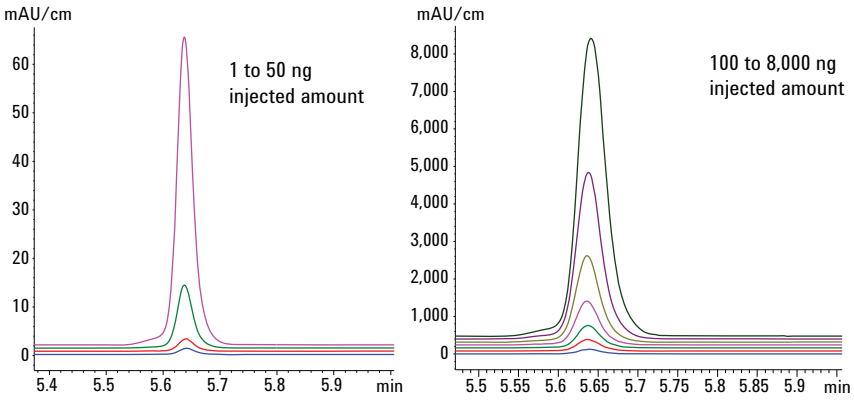


Figure 5. Overlay of all chromatograms representing injected amounts from 1 to 8,000 ng.

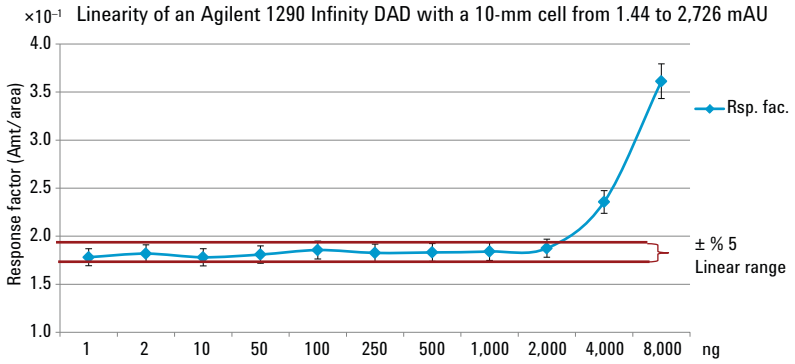


Figure 6. Linearity of an Agilent 1290 Infinity DAD equipped with a 10-mm path length cell, based on response factors.

Comparison of LOD for anthracene

A 10-pg amount of anthracene was injected in the 1290 Infinity HDR DAD and the 1290 Infinity DAD with 10-mm cell, see Figure 8. The LOD was evaluated based on peak height, peak-to-peak noise, and a signal-to-noise (S/N) ratio of two.

Table 2 summarizes the results of the LOD experiments. The 1290 Infinity HDR-DAD provided a LOD which had improved by a factor of five.

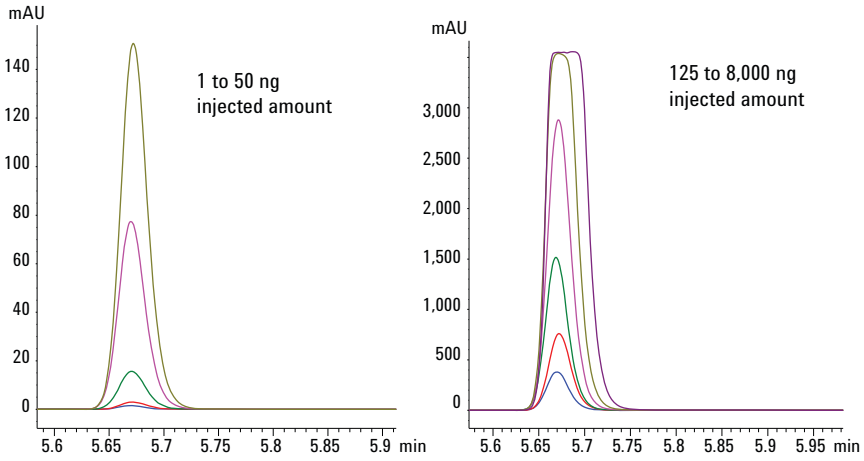


Figure 7. Overlay of all chromatograms representing the injected amounts from 1 to 8,000 ng.

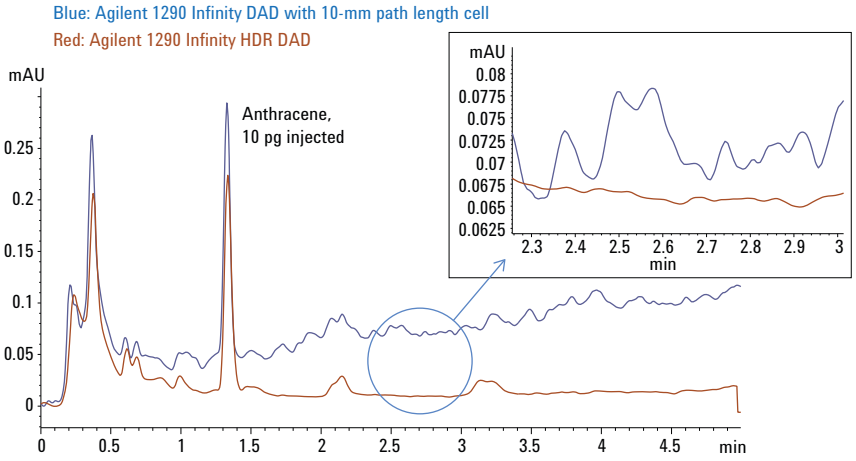


Figure 8. Overlay of chromatograms representing the 10-pg injection of anthracene on both detectors.

Table 2. Comparison of LOD.

Configuration	P to P noise	Peak height	S/N	LOD
Agilent 1290 Infinity HDR DAD	0.0006249 mAU	0.212098 mAU	339.4 (S/N = 2)	59 fg (factor 5 better)
Agilent 1290 Infinity DAD with a 10-mm cell	0.003916 mAU	0.251327 mAU	64.2 (S/N = 2)	310 fg

Comparison of quantitative results using a 1290 Infinity HDR DAD and a conventional UV detector, the 1200 Series DAD (G1315B)

A fixed-dose combination drug was used as the sample with the following ingredients:

Paracetamol and chlorphenamine with a ratio 80:1, other compounds were vitamin C and caffeine. A multilevel calibration was set up for both detectors, see Table 1. A solution with an unkown amount was injected, and the quantitative results were compared. Figure 9 shows an example chromatogram of the 1290 Infinity HDR DAD.

Figures 10 and 11 summarize the results of the comparison. The results show that both detectors gave comparable amounts and accuracy data.

Conclusion

The Agilent 1200 Infinity HDR DAD provided linearity from 1.28 to 7932 mAU peak height for Caffeine, whereas the 1290 Infinity DAD with a 10-mm cell provided linearity from 1.44 to 2,726 mAU peak height. This enabled the detection and quantitation of main and impurity compounds in a single run using the 1290 Infinity HDR DAD. The LOD was improved by a factor of five for anthracene using the 1290 Infinity HDR DAD. Quantitative results were completely comparable to those of a conventional detector.

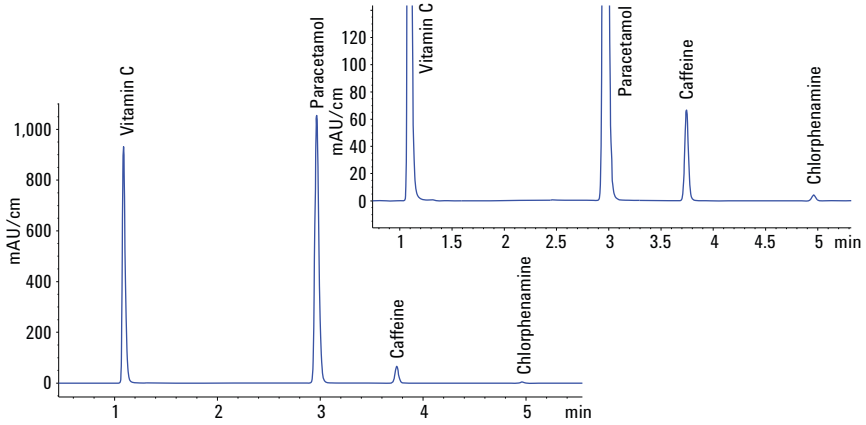


Figure 9. Example chromatogram of an Agilent 1290 Infinity HDR DAD.

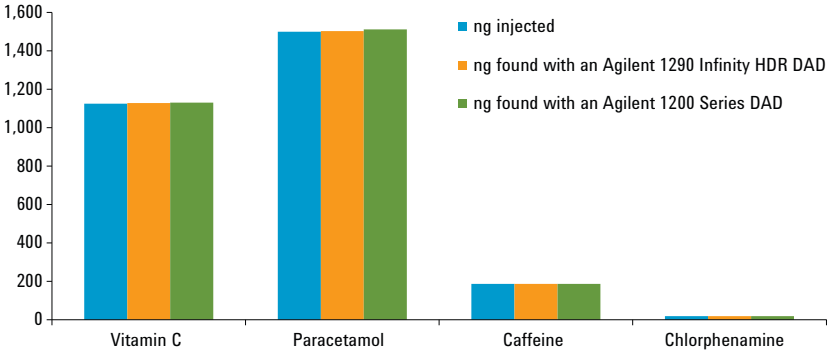


Figure 10. Comparison of amounts.

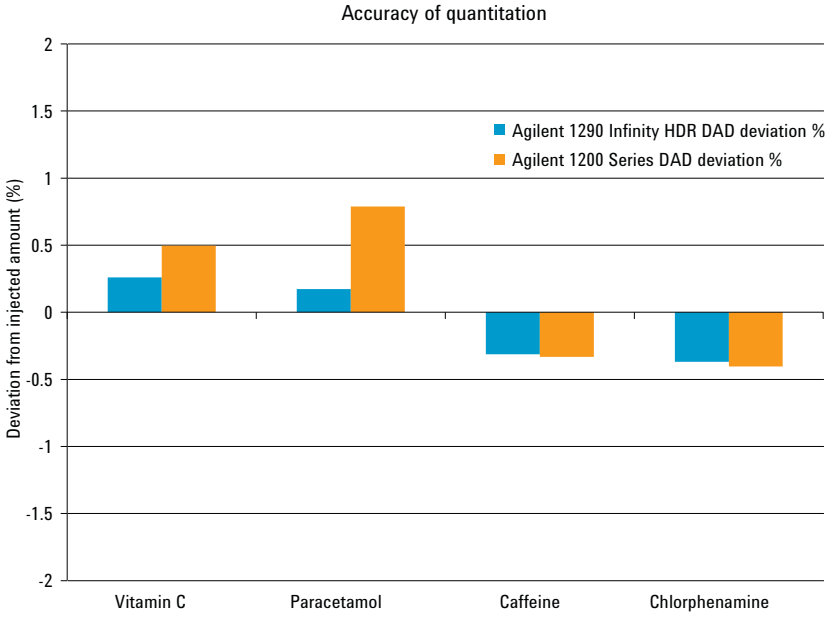


Figure 11. Comparison of accuracy.

www.agilent.com/chem/hdr

Published in the USA, March 1, 2014
5991-3877EN

Agilent Technologies