Agilent 1290 Infinity LC the ideal partner for MS – Part 3

Complimentary sensitivity of UV and MS detection

Application Note

Pharmaceutical and Chemical

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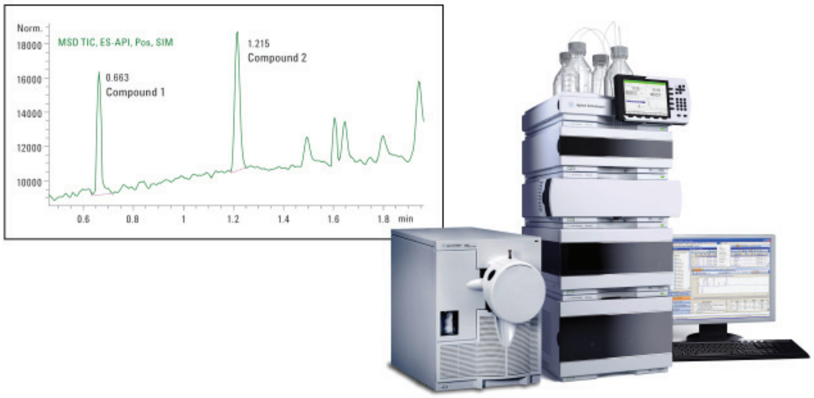

Abstract

This Application Note shows a comparison of sensitivities that can be achieved in typical fast LC/MS analysis with a single quadrupole mass spectrometer and diode array detectors for UV detection in different configurations. The limits of quantification are compared and the precision of peak areas as well as the range for detection by UV and MS together is determined for different DAD configurations.

Introduction

Typical single quadrupole LC/MS systems contain a diode array detector (DAD) for additional UV detection. To achieve the best sensitivity for compound quantification, the MS is operated in selected ion monitoring (SIM) mode which is the most sensitive detection in such a system. On the DAD side, improvements were made to achieve higher sensitive detection, which enables better comparability to the MS SIM detection and quantification. The Agilent 1290 Infinity DAD is best suited for use in such a single quadrupole UHPLC/MS system for fast LC/MS analysis because of its high detection sensitivity. This can be achieved using different DAD flow cells, in particular with the Max-Light flow cell.

This Application Note compares the sensitivities that can be achieved in typical fast UHPLC/MS analysis between a single quadrupole mass spectrometer in SIM mode and a diode array detector (DAD) for UV detection using different flow cells. The limits of quantification are compared, and the overlapping range is determined for different DAD configurations. In addition, a comparison to an Agilent 1200 Series DAD SL detector is included.

Experimental

Equipment:

Agilent 1290 Infinity LC system containing an Agilent 1290 Infinity Binary Pump, Agilent 1290 Infinity High Performance Autosampler, Agilent 1290 Infinity Thermostatted Column Compartment, Agilent 1290 Infinity DAD and Agilent 6140 Single Quadrupole Mass Spectrometer.

Column:	Agilent ZORBAX SB C18, 50 × 2.1 mm, 1.8 µm
Software for data acquisition and data analysis:	ChemStation B.04.03

HPLC Method:

Solvent A:	Water + 0.1% formic acid
Solvent B:	Acetonitrile + 0.1% formic acid
Flow rate:	1.0 mL/min
Gradient:	0 min 10% B – 2.0 min 50% B – 2.01 min 95% B – 2.5 min 95% B
Stop time:	2.5 min
Post time:	1 min
Injection volume:	1 µL
Needle wash:	6 s in MeOH
Column temperature:	40 °C
Diode array detector:	10 mm standard Max-Light cartridge cell, 60 mm high sensitivity Max-Light cartridge cell, wave length 280/4 nm, Ref. 360/16 nm, slit 8 nm, data rate 20 Hz.

MS Method:

Source:	Gas temperature: 350 °C, nebulizer pressure: 60 psi, gas flow: 12 L/min, capillary: 4000 V, positive polarity		
Signal 1:	SIM, peak width: 0.04 min, cycle time: 0.24 s, gain: 1.0		
	Ion (m/z)	Fragmentor (V)	Dwell time (msec.)
	279.10	120	114
	311.10	120	114
Sample:	Stock solution of: 1) Sulfamethazine (MW 278.0), 2) Sulfachloropyridazine (MW 310.0) each at a concentration of 10 ng/µL. Dilution: (pg/µL): 10,000; 5,000; 2,000; 1,000; 500; 200; 100; 50; 20; 10; 5; 2; 1; 0.5; 0.2; 0.1.		

Results and discussion

In a quantitative HPLC/single quadrupole MS analysis, typically the mass spectrometer has the higher sensitivity compared to the diode array detector and therefore delivers the lower limits of detection (LOD) and lower limits of quantification (LOQ).

This work shows calibration curves acquired with an Agilent 1290 Infinity LC single quadrupole LC/MS system for quantification and discusses the comparison of achieved limits of quantification from a single quadruple MS and a DAD in different cell configurations and models. As a definition for the limit of detection (LOD), a signal-to-noise ratio ≥ 3 was used, and for the limit of quantification (LOQ) a signal-to-noise ratio of ≥ 10 was used.

With the Agilent 6140 Single Quadrupole Mass Spectrometer a LOQ of 1 pg (on column) for both tested compounds was found with a S/N ≥ 10. The calibration was continued up to 10,000 pg (on column) with good correlation factors (Figure 1).

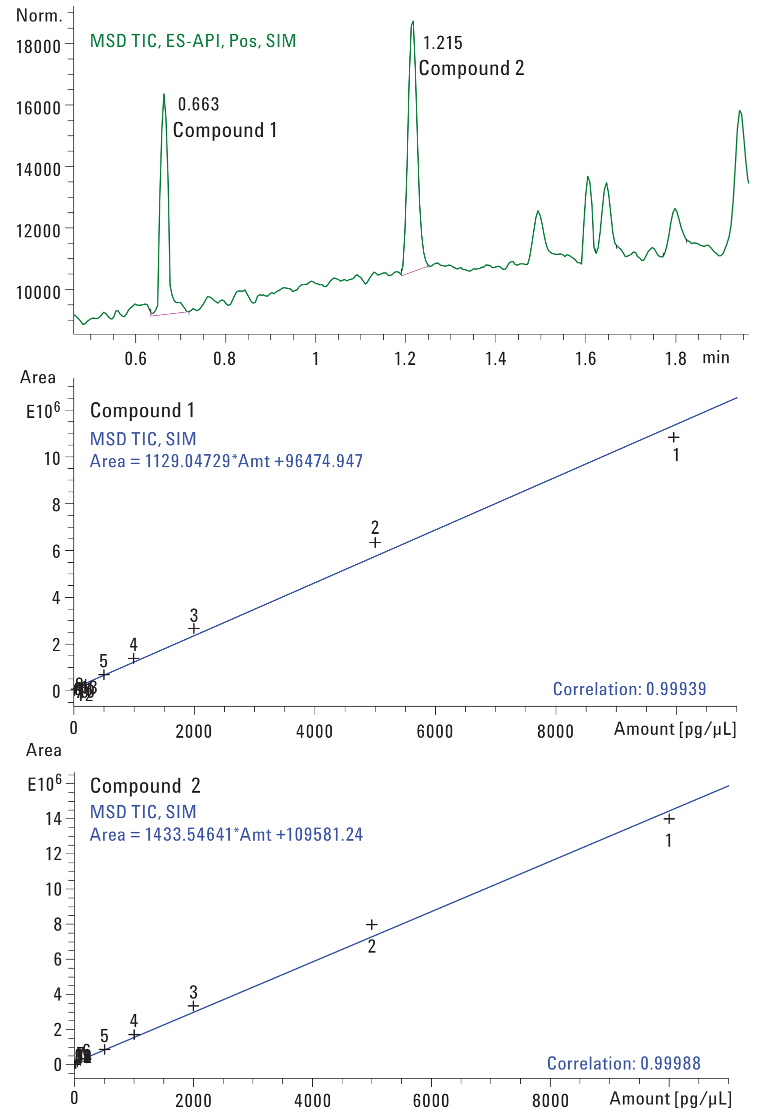

Figure 1
Calibration of compounds 1 and 2 with an Agilent 6140 Single Quadrupole Mass Spectrometer in SIM mode.
Compound 1: LOQ 1 pg/µL (S/N=10), Correlation: 0.99939.
Compound 2: LOQ 1 pg/µL (S/N=13), Correlation: 0.99988.

For the Agilent 1290 Infinity DAD equipped with the 10-mm Max-Light cell, the experiment delivered a LOQ of 100 pg (on column) with good correlation factors for a calibration up to 10,000 pg (on column) (Figure 2) which is a factor of 100 above the LOQ of the mass spectrometric detection. For DAD detection with higher sensitivity the 60-mm Max-Light cell is available for the Agilent 1290 Infinity DAD.

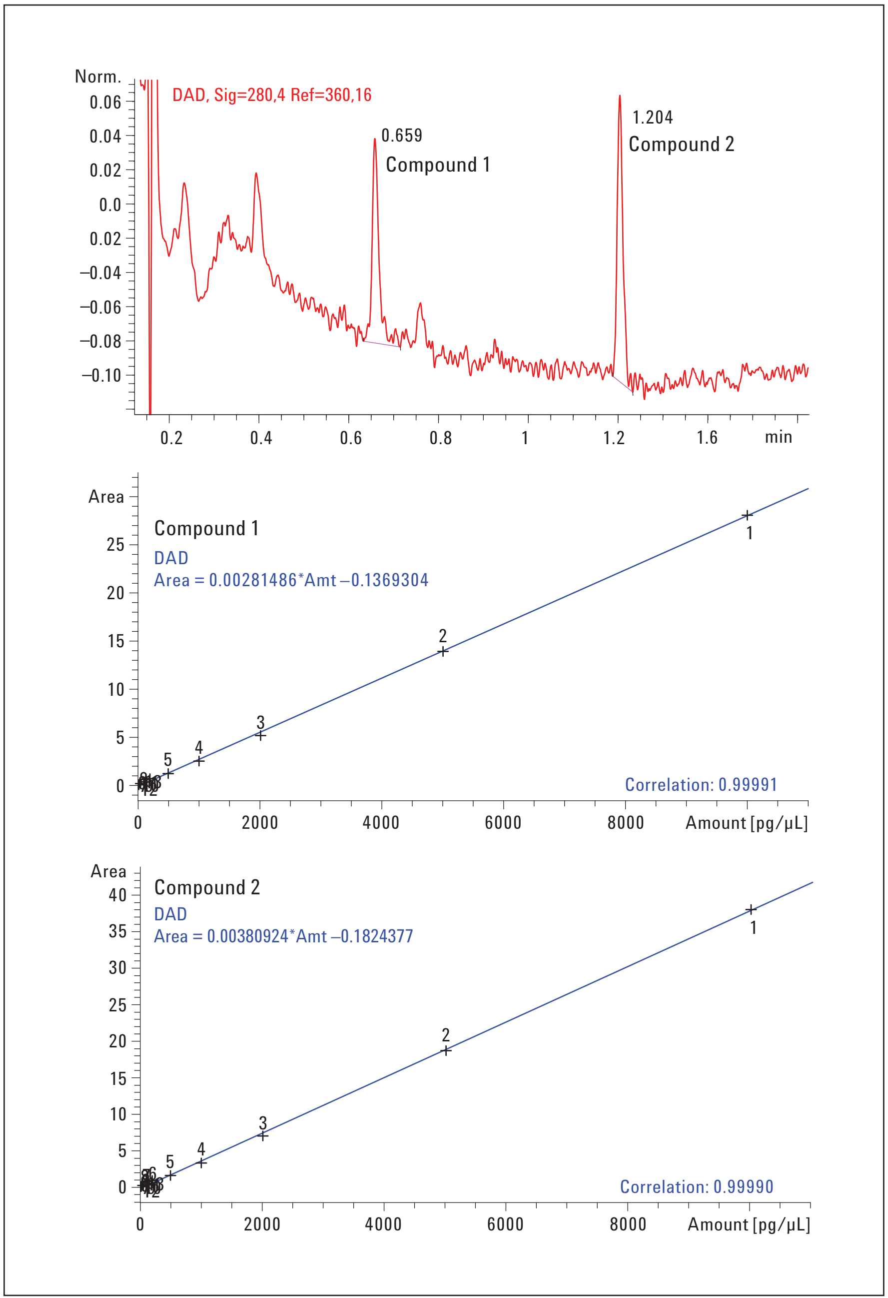

Figure 2
Calibration of compounds 1 and 2 with an Agilent 1290 Infinity DAD with 10 mm Max-Light cell.
Compound 1: LOQ 100 pg/µL (S/N=10), Correlation: 0.99991.
Compound 2: LOQ 100 pg/µL (S/N=11), Correlation: 0.99990.

This DAD configuration provides a LOQ of 20 pg (on column) for both compounds which reduces the gap between mass spectrometric detection and UV detection by a factor of 5 (Figure 3).

The used configuration comprising a 2.1-mm id column together with the 60-mm DAD flow cell works well under the chosen conditions but achieves best performance together with typically 3.0-mm id and 4.6-mm id columns at higher flow rates.

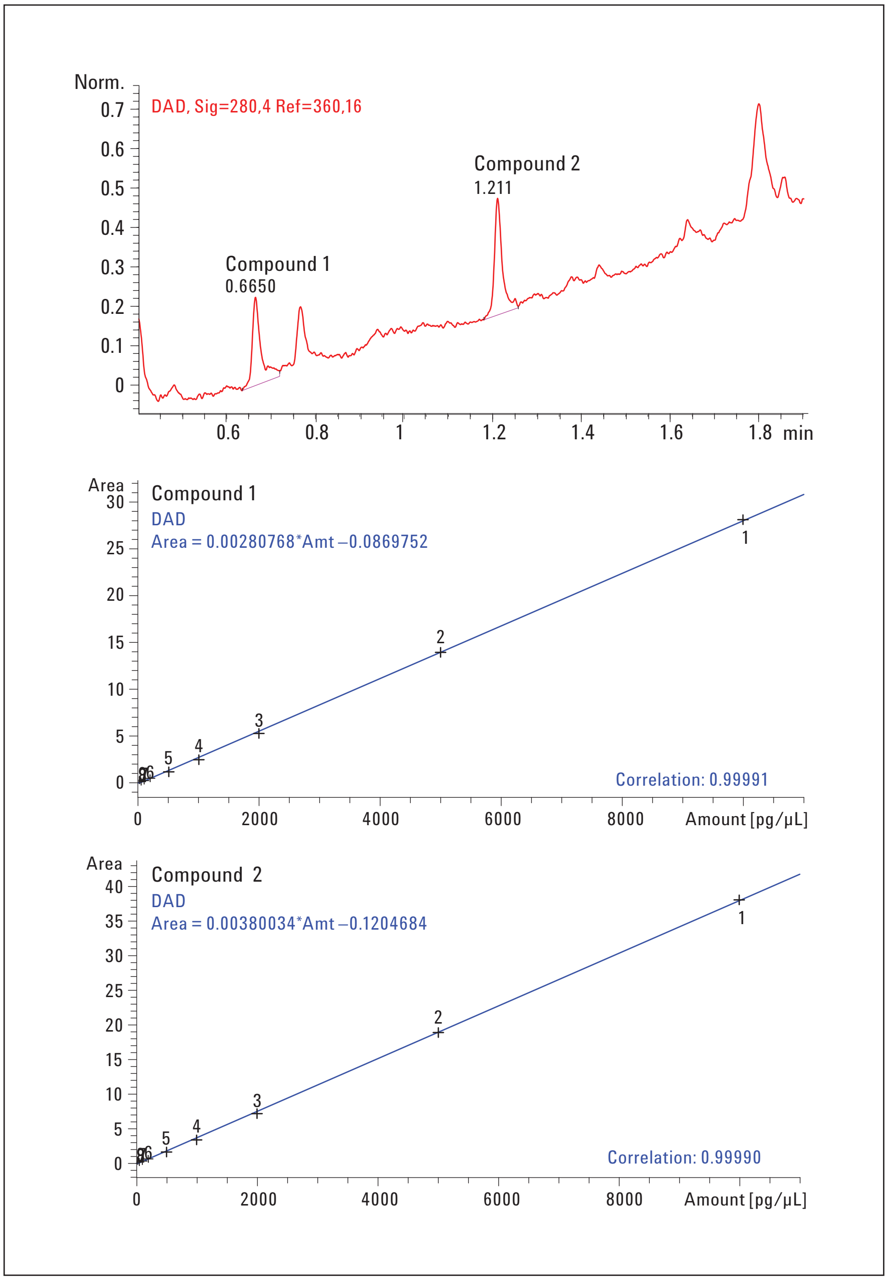

Figure 3
Calibration of compounds 1 and 2 with an Agilent 1290 Infinity DAD with 60 mm Max-Light cell.
Compound 1: LOQ 20 pg/µL (S/N=10), correlation 0.99991.
Compound 2: LOQ 20 pg/µL (S/N=12), correlation 0.99990.

Finally, a comparison to the Agilent 1200 Series DAD SL with a 10-mm cell was done, showing a LOQ of 200 pg (on column) which is far off from the mass spectrometric sensitivity (Figure 4).

The results for the achieved limits of detection are summarized in Table 1. This clearly shows the best overlap between mass spectrometric detection and UV detection is achieved with the Agilent 1290 Infinity DAD equipped with the 60-mm Max-Light cartridge cell. The Agilent 1290 Infinity DAD with the 60-mm cell provides a 5 times higher sensitivity compared to the 10-mm Max-Light cartridge cell and a 10 times higher sensitivity compared to the Agilent 1200 Series DAD SL with a 10-mm cell.

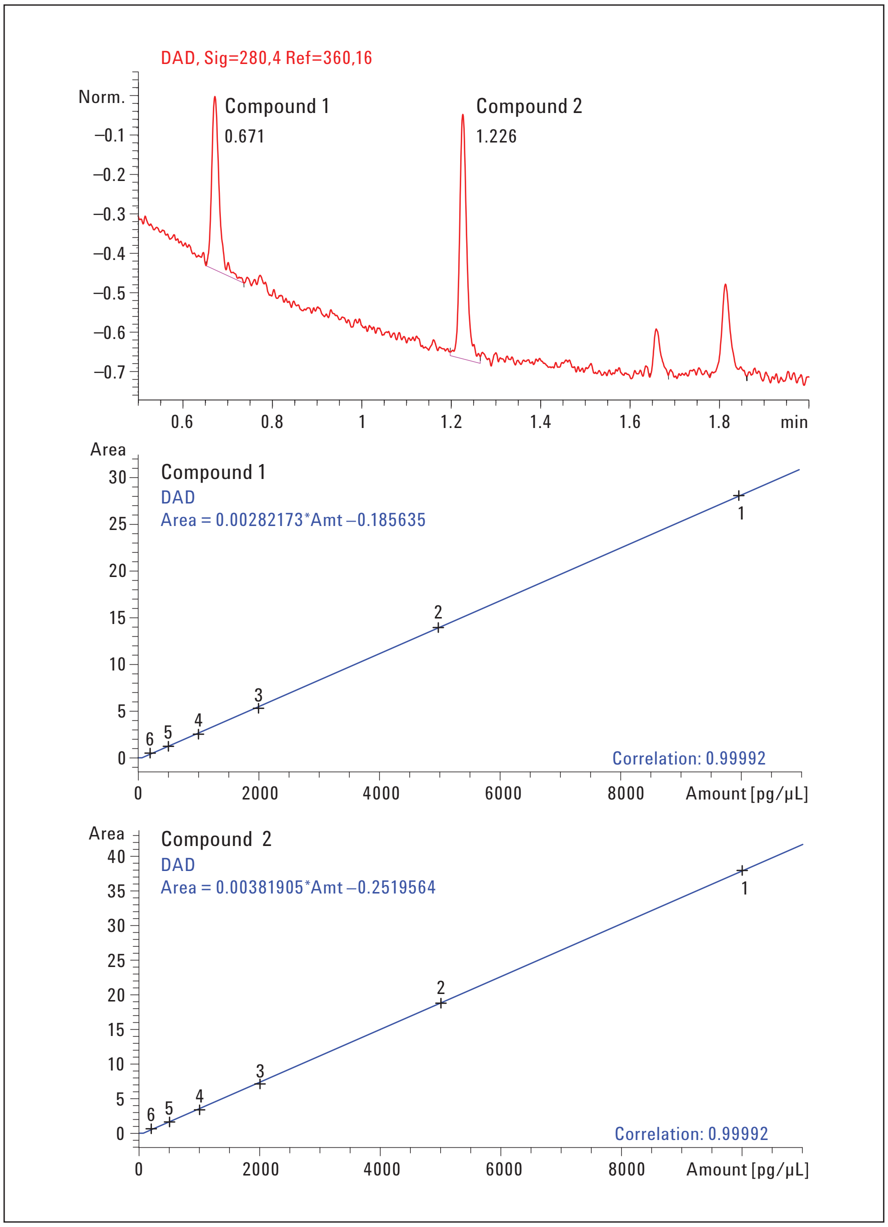

Figure 4
Calibration of compounds 1 and 2 with an Agilent 1200 Series DAD SL with 10 mm cell.
Compound 1: LOQ 200 pg/µL (S/N=10), correlation: 0.99992.
Compound 2: LOQ 200 pg/µL (S/N=15), correlation: 0.99992.

	Compound 1 [pg]		Compound 2 [pg]	
	LOD	LOQ	LOD	LOQ
Agilent 6140 MS SIM	0.2	1.0	0.2	1.0
Agilent 1290 Infinity DAD 60 mm Max-Light cell	10	20	10	20
Agilent 1290 Infinity DAD 10 mm Max-Light cell	20	100	20	100
Agilent 1200 Series DAD SL 10 mm cell	100	200	100	200

Table 1
LOD and LOQ for compound 1 and 2 measured with MS and DAD in different configurations.
LOD: S/N ≥ 3, LOQ: S/N ≥ 10.

In addition to LOQ, another important parameter for the quality of quantification is the precision of peak areas, which can be compared by the calculated relative standard deviations (RSD [%]). Therefore, all points used in the shown calibrations were done in five replicates and the RSD [%] was calculated from average area and standard deviation. For Compound 1, the area RSD [%] versus the concentration for mass spectrometric detection and detection with different DAD configurations and DAD models is shown in Figure 5. For the mass spectrometric detection, the relative standard deviation for the peak area at 5 pg (on column), is 5.70% (Figure 5 and Table 2). Towards the LOQ the RSD for the mass spectrometric detection goes up to about 10%. For higher concentrations above 20 pg (on column), the RSD for mass spectrometric detection is always between 1% and 2%. For the detection of Compound 1 with a DAD at the LOQ, the individual RSDs are between 4% and 5%. For higher concentrations, the RSDs for the detection with a DAD went down to about 0.2% for all DAD configurations and models. The comparison of the DAD detection to the mass spectroscopic detection for higher concentrations always shows a lower RSD value for the DAD detection and herewith higher precession. Comparison of the different DAD configurations and DAD models shows a lower RSD and herewith higher precession for the one with the higher sensitivity (lower LOQ) compared to the one with lower sensitivity (higher LOQ) at its LOQ. Similar results were found for Compound 2 (data not shown).

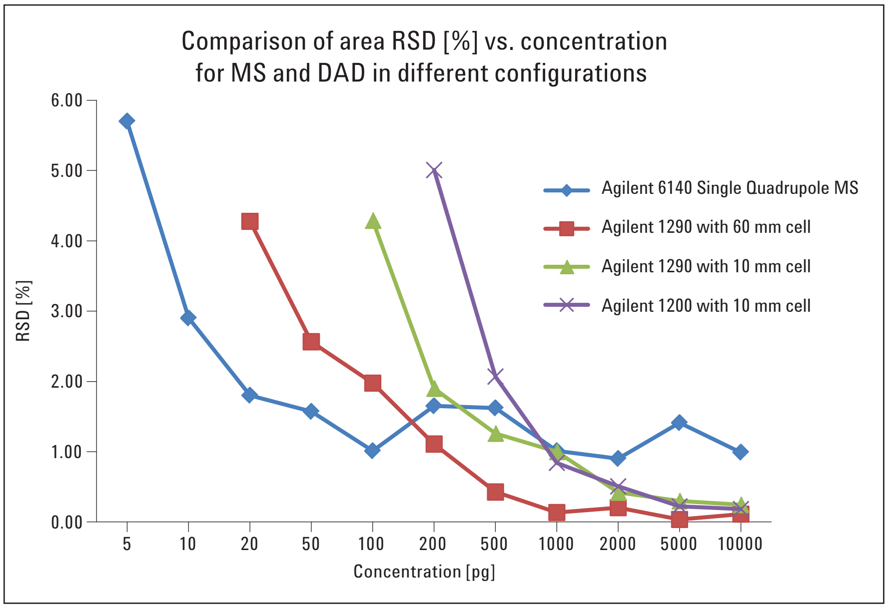

Figure 5
Area RSD [%] vs. concentration for mass spectrometric detection and detection with different DAD configurations and DAD models for Compound 1.

Compound 1											
Concentration [pg/µL]	5	10	20	50	100	200	500	1000	2000	5000	10000
Agilent 6140 SQ MS SIM	5.70	2.90	1.80	1.57	1.01	1.65	1.62	1.01	0.90	1.41	0.99
Agilent 1290 with 60 mm cell			4.28	2.56	1.97	1.11	0.43	0.14	0.20	0.04	0.11
Agilent 1290 with 10 mm cell					4.29	1.90	1.26	1.00	0.42	0.30	0.25
Agilent 1200 with 10 mm cell						5.01	2.07	0.84	0.51	0.22	0.19

Table 2
Area RSD [%] vs. concentration for mass spectrometric detection and detection with different DAD configurations and DAD models for Compound 1.

Conclusion

This Application Note shows a comparison of sensitivities that can be achieved in typical fast LC/MS analysis with a single quadrupole mass spectrometer and diode array detectors for UV detection in different configurations. The limits of detection and limits of quantification are determined with the result that the Agilent 1290 Infinity DAD equipped with a 60-mm Max-Light cartridge cell has the closest sensitivity to the mass spectrometric detection in an UHPLC system comprising an Agilent 1290 Infinity LC system and an Agilent 6140 Single Quadrupole Mass Spectrometer. Data are provided which show that the Agilent 1290 Infinity DAD configuration with the 60-mm Max-Light cartridge cell achieves a five times higher sensitivity than the configuration with the 10-mm Max-Light cartridge cell and a 10 times higher sensitivity than the Agilent 1200 Series DAD SL with 10-mm flow cell.

The displayed data and conclusion drawn from the discussion give verification that an LC/MS system with a DAD providing the necessary sensitivity and a single quadrupole mass spectrometer nicely fit together.

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