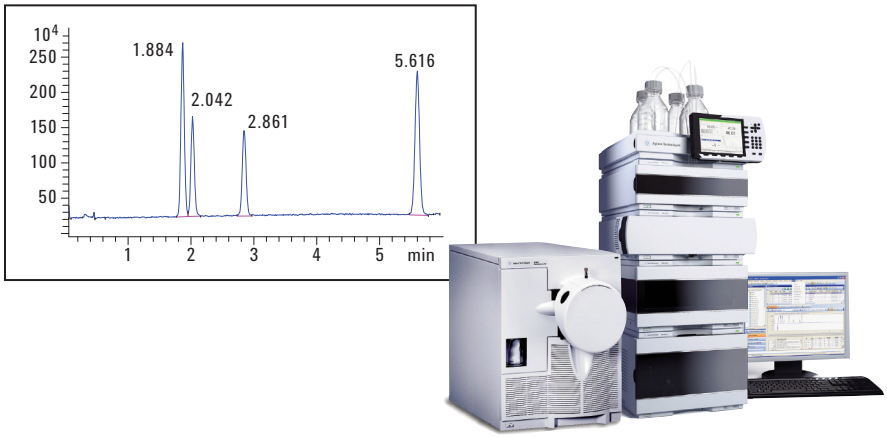
Agilent 1290 Infinity LC
The ideal partner for MS – Part 1

Influence of delay volume and system dispersion on resolution and sensitivity

Application Note

Pharmaceutical and Chemical



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Abstract

This Application Note demonstrates the influence of system delay volume and system dispersion on the resulting resolution and sensitivity achieved for LC/MS experiments. The advantages of using a low delay volume LC system and optimized connection of the LC to the MS are shown. The study illustrates that the Agilent 1290 Infinity LC system is an ideal partner for LC/MS instruments.

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Introduction

In LC/MS measurements, the achieved resolution and sensitivity of separated compounds heavily depend on the delay volume of the HPLC system and the connection between the LC system and the mass spectrometer.

The delay volume is responsible for the delay time, which is the time it takes a change in gradient to reach the column at a given flow rate. The delay volume also has an influence on the separation because it can cause isocratic elution and band broadening, especially for early eluting compounds.

The band broadening caused by the connection of the column outlet to the source of the mass spectrometer follows the Aris-Taylor equation, which gives the peak dispersion in cylindrical tubing:

$$\sigma^2_{v,ext} = \frac{\pi\, d^4\, L_{cap}\, u_{cap}}{96 D_m}$$

$\sigma^2_{v,ext}$	is the volume variance
d	is the tubing diameter
L	is the tubing length
u	is the linear velocity of the liquid
D_m	is the molecular diffusion coefficient

This equation clearly shows that the dispersion is proportional to the length of the tubing but more important, proportional to the fourth power of the inner diameter of the tubing.

This Application Note demonstrates the influence of system delay volume and system dispersion on the resulting resolution and sensitivity achieved for LC/MS experiments. The advantages of using a low delay volume LC system and optimized connection of the LC to the MS are shown. It is also shown that the Agilent 1290 Infinity LC system is the ideal partner for LC/MS instruments.

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 B04.03
HPLC Method:	
Solvent A:	Water + 0.1% formic acid Solvent B: Acetonitrile + 0.1% formic acid
Flow rate:	0.5 mL/min
Gradient A 0 min 12% B 5 min 20% B 5.01 min 95% B 6 min 95% B	Gradient B 0 min 10% B 5 min 20% B 5.01 min 95% B 6 min 95% B
Stop time:	6 min
Post time:	3 min
Injection volume:	1 µL
Needle wash:	6 s in MeOH
Column temperature:	40 °C
Diode array detector:	10 mm standard cell, wavelength 260/4 nm, ref. 360/16 nm, slit 4 nm, data rate 80Hz
MS Method:	
Source:	Gas temperature: 350 °C, nebulizer pressure: 45 psi, gas flow: 11 L/min, positive polarity
Scan:	100 – 1000 *m/z*
SIM:	271.0 *m/z*, 279.0 m/z, 285.5 *m/z*, 311.0 m/z
Sample:	Solution of Sulfamethizole (first peak, *m/z* 271.0), Sulfamethazine (second peak, *m/z* 279.0), Sulfachloropyridazine (third peak, *m/z* 285.0), Sulfadimethoxine (fourth peak, *m/z* 311.0) each at a concentration of 100 ng/µL.

Results and discussion

The influence of the LC system delay volume

The delay volume of an HPLC system influences the separation performance which is determined with the detector directly connected to the LC column outlet. Typically, this is a diode array detector (DAD) for the detection of compounds absorbing UV light. This influence on the separation performance continues to an associated detector, typically a mass spectrometer. The first experiment was done with an Agilent 1290 Infinity LC system with a delay volume of about 125 µL. For the simulation of a system with a higher delay volume, two mixing devices with a combined volume of about 700 µL were connected in series to the Agilent 1290 Infinity LC system between the Jet Weaver mixer inherent in the Agilent 1290 Infinity Pump and the Agilent 1290 Infinity Autosampler. This produced an overall delay volume of about 1000 µL, which is similar to a standard HPLC system for 3 or 4.6 mm id columns.

The measurement of the separation of four compounds with the unchanged Agilent 1290 Infinity LC system is shown in Figure 1A. There are two peaks very early in the gradient separated with a resolution of about 2; one in the middle and the last one at the end of the gradient time. The retention

times, peak widths, areas and heights are summarized in Table 1. Figure 1Aa shows the peaks in the DAD chromatogram as an inherent detector, Figure 1Ab shows the chromatogram in MS scan mode and Figure 1Ac shows the chromatogram in MS SIM mode as and associated detector. In comparison, the peaks are delayed by a short time of about 0.015 minutes (0.9 s) between detection with DAD and MS due to the transfer tubing. The same is true for the small differences in peak width of about 0.012 minutes (0.7 s).

Figure 1B shows the same separation but on a system with a larger delay volume of about 800 µL. In the comparison of the UV and MS chromatograms for the last peak, a shift in the retention time of 1.45 minutes is seen. This is nearly the theoretically expected retention time shift caused by the added delay volume for the given flow rate of 0.5 mL/min (Figure 1Ba). There are additional effects such as additional peak broadening of about 0.9 seconds and a loss in peak height of about 20% which compromises the sensitivity. The same effects can be found in the associated MS detector for the last peak (Figures 1Bb and 1Bc). More impressive are the effects on the first and second peak, here the delay time is only about 0.1 minutes (6 s). This cannot be explained by the retention time shift due to the added delay volume but it can be explained by the fact that those early eluting peaks start to elute under isocratic conditions caused by the delay volume before the gradient hits the column. For these peaks, a reduction in peak height of about 20% can be seen with the DAD and about 10% by MS TIC detection, therefore a reduction in sensitivity. A similar behavior can be seen for the MS SIM data but on a higher level of sensitivity.

These results demonstrate the advantage of a system with the lowest delay volume for the detection of separated compounds with an inherent associated detector. There are two advantages: shorter run time and higher sensitivity.

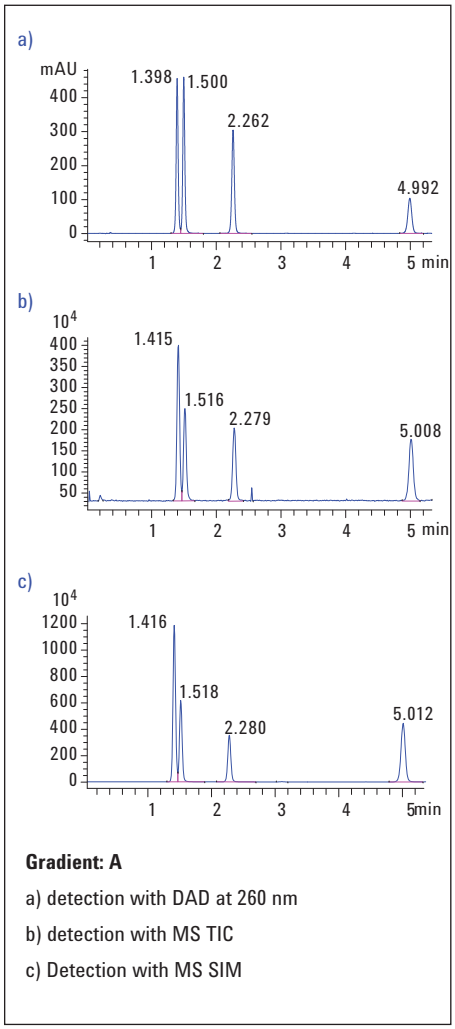

Figure 1A
Separation with lowest delay volume.

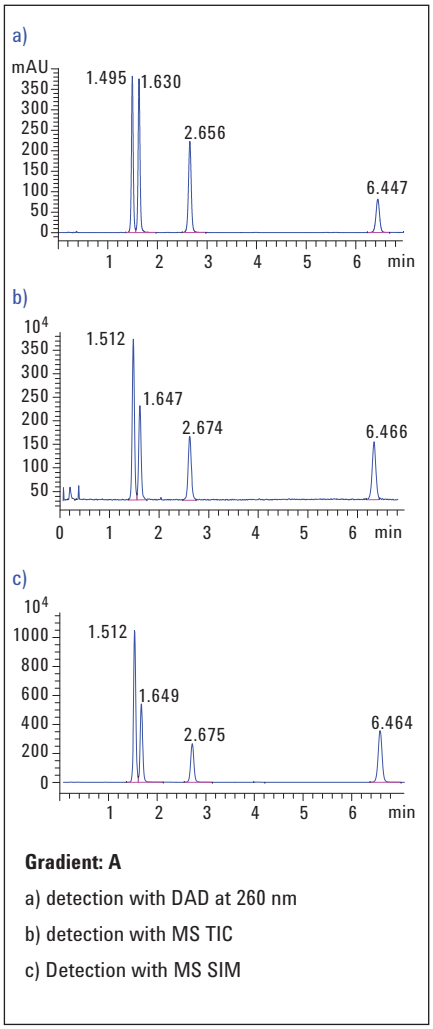

Figure 1B
Separation with high delay volume.

Peak	RT (min)	Width (min)	Height	Area
Standard delay volume - DAD				
1	1.398	0.0317	447.95	929.50
2	1.500	0.0327	451.27	956.05
3	2.262	0.0438	303.54	865.68
4	4.992	0.0622	103.46	445.63
Standard delay volume - MS TIC				
1	1.415	0.0442	3.59E+06	9.85E+06
2	1.516	0.0447	2.05E+06	5.70E+06
3	2.279	0.0551	1.72E+06	6.02E+06
4	5.008	0.0743	1.46E+06	6.99E+06
Standard delay volume - MS SIM				
1	1.416	0.0413	1.15E+07	3.06E+07
2	1.518	0.0431	5.71E+06	1.51E+07
3	2.280	0.0519	3.55E+06	1.12E+07
4	5.012	0.0763	4.45E+06	2.13E+07

Peak	RT (min)	Width (min)	Height	Area
Extended delay volume - DAD				
1	1.495	0.0382	377.67	946.35
2	1.630	0.0405	370.11	982.07
3	2.656	0.0608	221.65	879.79
4	6.447	0.0792	81.55	417.56
Extended delay volume - MS TIC				
1	1.512	0.0488	3.34E+06	1.05E+07
2	1.647	0.0510	1.91E+06	6.37E+06
3	2.674	0.0733	1.35E+06	6.38E+06
4	6.466	0.0749	1.21E+06	6.28E+06
Extended delay volume - MS SIM				
1	1.512	0.0486	1.03E+07	3.22E+07
2	1.649	0.0493	5.22E+06	1.66E+07
3	2.675	0.0695	2.66E+06	1.21E+07
4	6.464	0.0836	3.56E+06	1.99E+07

Table 1
Comparison of retention time, peak width, peak height and peak area for the low volume 1290 Infinity LC system with standard delay volume and extended delay volume. Data for the inherent DAD and the associated MS are shown. DAD height is given in mAU and DAD area is given in mAUs.

The influence of the connection to an LC-associated detector

Even when the delay volume in the front end LC system is optimized, the connection to the mass spectrometer has to be optimized to get the best performance for sensitivity and peak resolution. Both depend on peak broadening effects due to capillary length and inner diameter. The inner diameter of a capillary has a large impact on peak broadening. Hence, the behavior of separated peaks was determined with capillaries of the same length but different inner diameter (Figure 2). As a basis for this comparison, the results obtained with the LC inherent DAD were used (a gradient (B) slightly different from the first experiment was applied, because it gave better baseline separation of the first two peaks, Figure 2 A). For this initial experiment, a connection capillary of 50 cm × 130 µm from the LC to the MS was used (Figures 2 Ba and 2 Bb). The observed shift in retention time (about 2 s) and peak width (about 1 s) between DAD and MS are in the same order as described in the experiment above for the low delay volume LC system configuration (Table 2). When the capillary was exchanged the situation was different. In the following experiment, the first capillary had the dimensions 50 cm × 180 µm. In this case, the retention time was shifted about 2 seconds and the peak width increased by about 300 ms. Both peak 1 and peak 2 are still well separated (Figures 2 Ca and Cb). The effect of changing the transfer tubing to this size is still small. By changing the connection to a capillary dimension of 50 cm × 250 µm, the retention time shift is about two seconds and the peak width at half height increases by about one second. Looking on the chromatograms (Figures 2 Da and Db), it can be seen how dramatic this effect falls on the separation especially for peak 1 and peak 2, they are now not baseline separated. Finally, by exchanging the connection capillary to the dimension 50 cm × 500 µm, the chromatographic resolution becomes completely destroyed (Figures 2 Ea and Eb).

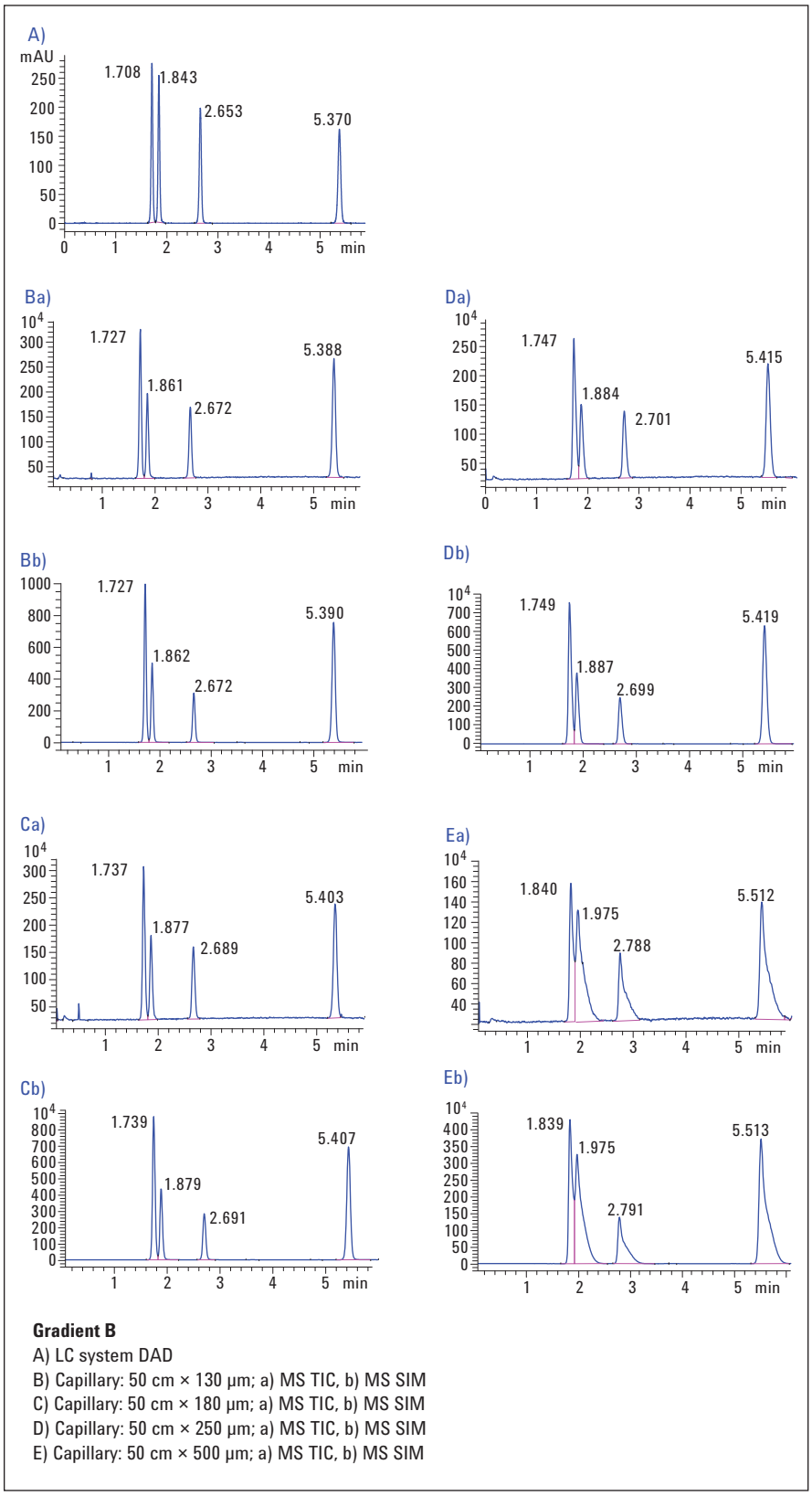

Figure 2
Influence of the connection from the LC system to the mass spectrometer.

1290 Infinity LC - DAD				
Peak	RT (min)	Width (min)	Height (mAU)	Area (mAU*s)
1	1.708	0.036	273.770	635.490
2	1.843	0.036	252.440	594.630
3	2.654	0.046	197.960	586.420
4	5.370	0.060	161.770	631.420

MS connection: 50 cm, 0.13 mm id - MS TIC				
Peak	RT (min)	Width (min)	Height (× 10^6)	Area (× 10^6)
1	1.727	0.046	2.984	8.691
2	1.861	0.046	1.694	4.843
3	2.672	0.055	1.422	5.007
4	5.388	0.069	2.389	10.571

MS connection: 50 cm, 0.18 mm id - MS TIC				
Peak	RT (min)	Width (min)	Height (× 10^6)	Area (× 10^6)
1	1.737	0.048	2.805	8.963
2	1.877	0.051	1.507	4.947
3	2.689	0.059	1.329	5.144
4	5.403	0.074	2.097	10.070

MS connection: 50 cm, 0.25 mm id - MS TIC				
Peak	RT (min)	Width (min)	Height (× 10^6)	Area (× 10^6)
1	1.747	0.051	2.310	8.539
2	1.884	0.061	1.112	4.407
3	2.701	0.068	1.149	5.295
4	5.415	0.082	1.936	10.570

MS connection: 50 cm, 0.13 mm id - MS SIM				
Peak	RT (min)	Width (min)	Height (× 10^6)	Area (× 10^6)
1	1.727	0.044	9.858	28.345
2	1.862	0.043	4.875	13.851
3	2.672	0.055	3.124	10.954
4	5.390	0.071	7.563	34.166

MS connection: 50 cm, 0.18 mm id - MS SIM				
Peak	RT (min)	Width (min)	Height (× 10^6)	Area (× 10^6)
1	1.737	0.051	8.659	28.700
2	1.879	0.050	4.210	13.740
3	2.691	0.060	2.830	11.070
4	5.407	0.075	6.925	33.910

MS connection: 50 cm, 0.25 mm id - MS SIM				
Peak	RT (min)	Width (min)	Height (× 10^6)	Area (× 10^6)
1	1.747	0.057	7.222	27.570
2	1.887	0.058	3.281	12.430
3	2.699	0.070	2.475	11.470
4	5.419	0.087	6.303	34.700

Table 2
Comparison of retention time, peak width, peak height and peak area for the different conecting capillaries from the Agilent 1290 Infinity LC system to the mass spectometer. DAD height is given in mAU and DAD area is given in mAUs.

A summary of the behavior of peak widths is given in Figure 3. It can be seen that the peak widths increase for all used capillaries compared to the DAD peak width and that peak widths increase with the inner diameter of the capillaries. From the data in Table 2 it can be seen that there is not only an influence on the peak width but also in the peak height and on the sensitivity.

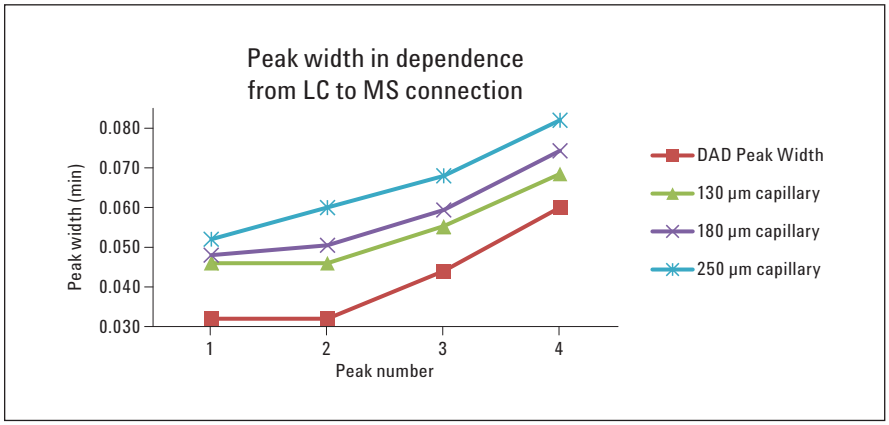

Figure 3
Peak width in relation to the inner diameter of the capillary connecting the LC to the MS.

A summary of the behavior of the peak height is given in Figure 4. It shows that the peak height decreases with the increase in the inner diameter of the connecting capillary and produces an accompanying loss in sensitivity. A similar behavior can be seen for the MS SIM data but on a higher level of sensitivity.

In a terminal experiment, the influence of the capillary length was determined. For this experiment, a capillary 500 cm × 130 µm was used. From the chromatograms, it can be seen that the peaks are well shaped and the early eluting peaks are well separated (Figure 5). The retention time is shifted by about 0.2 minutes compared to the DAD signals (Table 3 and Table 2). In theory, the peak width is most influenced by increased capillary inner diameter. In this case, the peak width is only increased by about 600 ms (Table 3 and Table 2). In comparison to Figures 3 and 4, the peak width performance for such a long capillary of 500 cm × 130 µm is in between the peak width performance of the 50 cm × 180 µm and the 50 cm × 250 µm capillary. The peak height performance is still between the 50 cm × 130 µm and the 50 cm × 180 µm capillary. A similar behavior can be seen for the MS SIM data but on a higher level of sensitivity. This demonstrates that the inner diameter is the much more critical parameter for the connection capillary from the LC system to the mass spectrometer than the length of the optimized capillary.

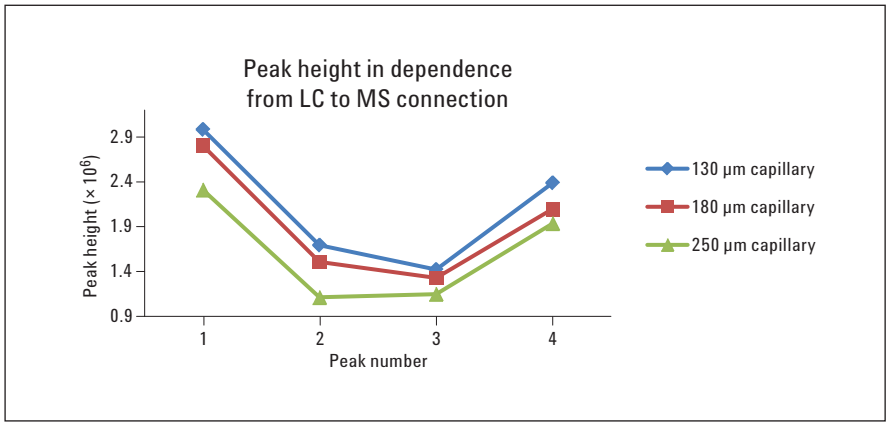

Figure 4
Peak height in relation to the inner diameter of the capillary connecting the LC to the MS.

MS connection: 500 cm, 0.13 mm id - MS TIC				
Peak	RT (min)	Width (min)	Height (× 10^6)	Area (× 10^6)
1	1.881	0.054	2.434	8.993
2	2.042	0.055	1.383	5.092
3	2.861	0.068	1.203	5.115
4	5.616	0.074	2.044	10.400
MS connection: 500 cm, 0.13 mm id - MS SIM				
Peak	RT (min)	Width (min)	Height (× 10^6)	Area (× 10^6)
1	1.883	0.061	7.671	29.210
2	2.041	0.060	3.857	14.290
3	2.862	0.064	2.594	11.120
4	5.617	0.082	6.398	33.590

Table 3
Retention time, peak width, peak height and peak area measured by MS TIC and MS SIM for a long 500 cm x 130 µm LC toMS connection capillary.

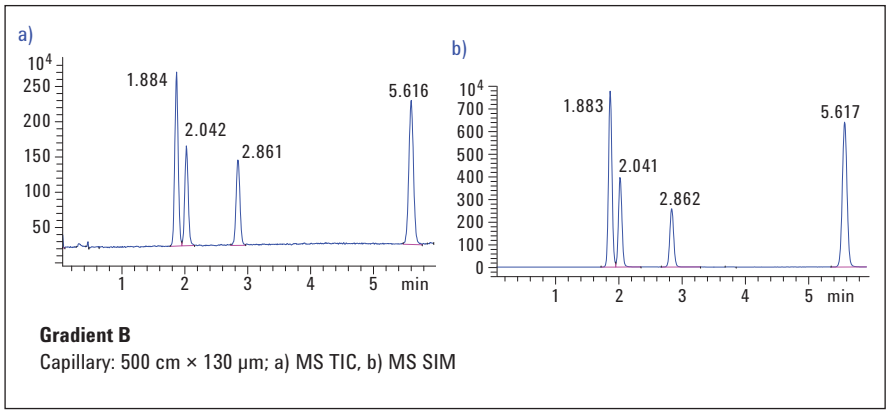

Figure 5
Influence of the connection from the LC system to the mass spectrometer.

Conclusion

This Application Note demonstrates the value of the Agilent 1290 Infinity LC system as a partner for mass spectrometry. It shows that the low delay volume of the Agilent 1290 Infinity LC system is beneficial for the separation by shortening elution times, degreasing peak width, and therefore increasing the peak height. In addition, the detection sensitivity is improved with the inherent DAD as well as the associated MS detectors. It is also shown that the connection from the LC system to the associated detector has to be optimized to achieve the highest performance in separation for peak width and peak height by selecting the connection capillary with the lowest possible inner diameter and length.

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