LC Troubleshooting Series
Peak Broadening

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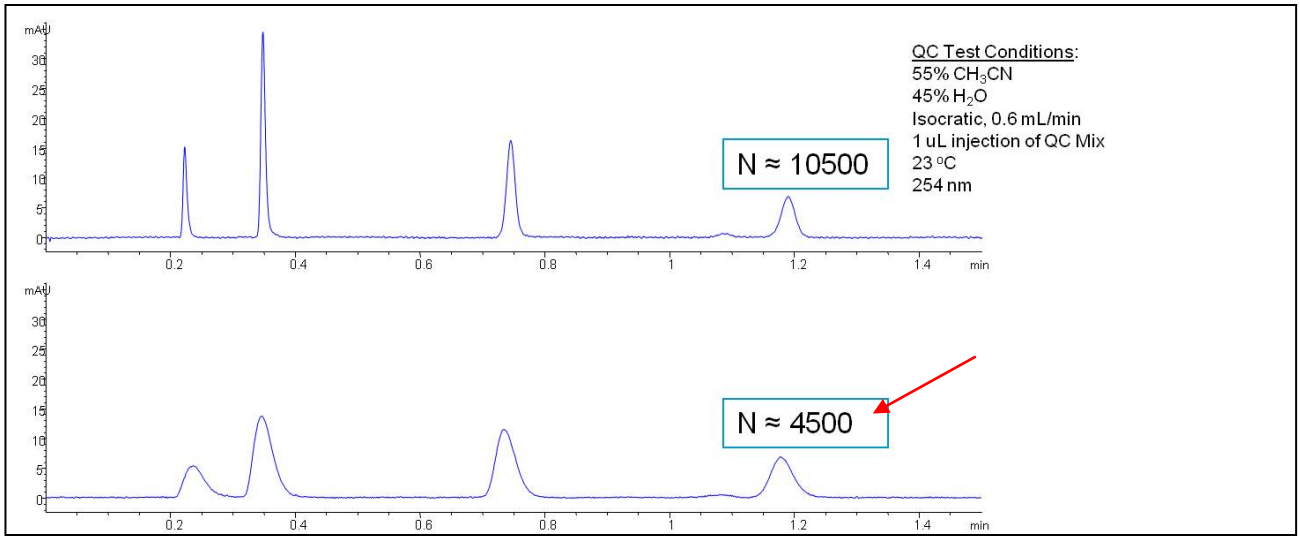
Why Peak Broadening Occurs

Peak broadening can occur for a lot of reasons. Some of the possible causes are:

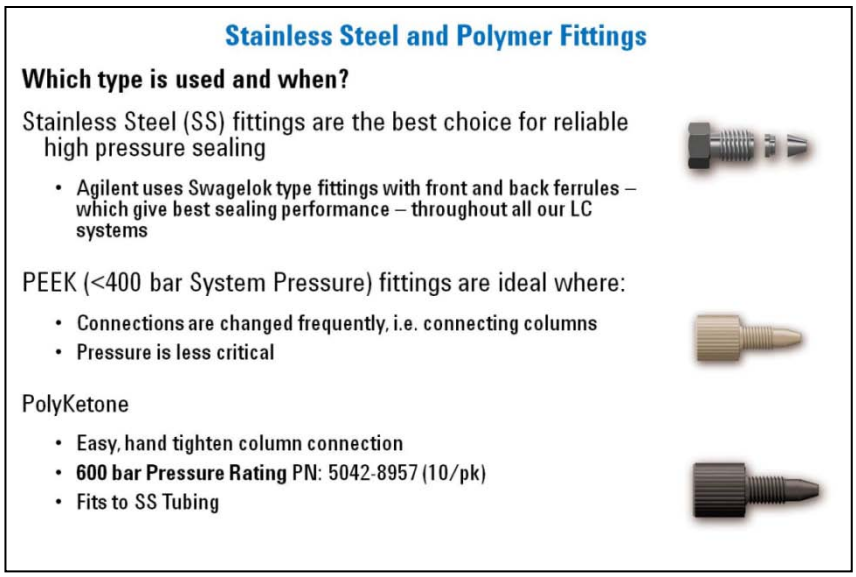
- Improper fittings / connections
- Improperly stacked instruments requiring excessive tubing length(s)
- Extra tubing volume
- Mobile phase issues
- System settings (data collection rate)



The chromatograms above represent a test mix containing naphthalene. The second chromatogram demonstrates peak broadening.

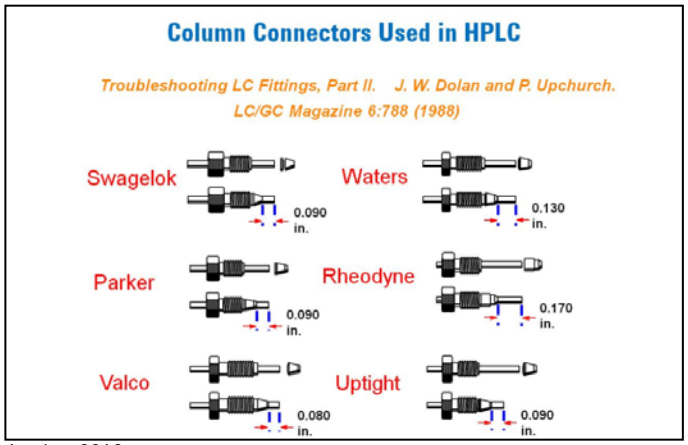
Making Good Connections

Good connections are a key to good results. Agilent recommends using Swagelok fittings or preferably fingertight fittings.



To make a good fitting:

1) Select a screw that is long enough for the fitting you'll be using.
2) Slide the screw over the end of the tubing.
3) Carefully slide the ferrule component(s) on after it and then finger-tighten the assembly.
4) Then, use a wrench to gently tighten the fitting, which forces the ferrule to crimp onto the tubing. Don't over-tighten it-- this will shorten the useful life of the fitting.
5) Once you believe you have the fitting complete, loosen the fitting and inspect it to be sure that the ferrule has the correct position on the tubing – just a bit over the top of the ferrule-- as shown below.



This chart to the left demonstrates the range of fitting types that can be used, depending on your provider.

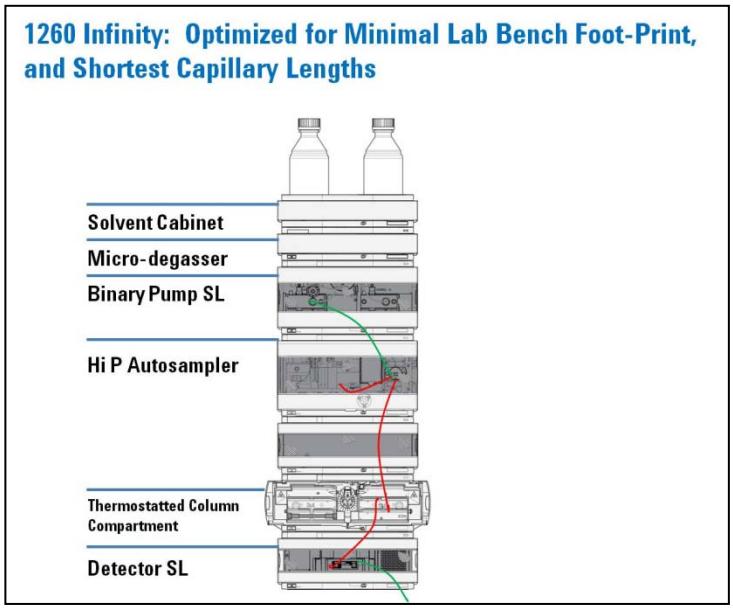
Ferrule setback is critical, so ideally, prepare your fitting in the system where you'll use it, so you can ensure the setback is correct for the instrument.

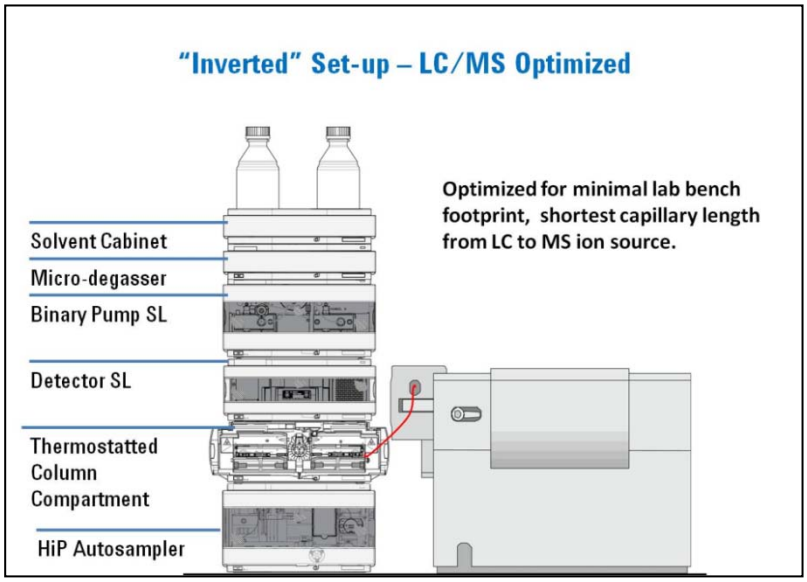
Instrument Stacking and Tubing Volume

Instrument set-up is important. Reducing extra tubing volume is critical to prevent peak broadening, particularly when working with high efficiency columns.

The LC Rack is a lab accessory that enables you to stack your instrument neatly and securely, and switch out modules more easily. It is part number 5001-3726.

The diagram to the right shows the recommended stacking structure for an LC, a 1260 Infinity LC in this case, equipped with low dispersion volume capillaries.





The diagram to the left details how to set up your instrument if you're using a mass spectrometer. Remember to reduce the amount of tubing that you're using to make connections.

Use of 0.12 mm Tubing instead of 0.17 mm Tubing – Reduces Tubing Volume by One-Half

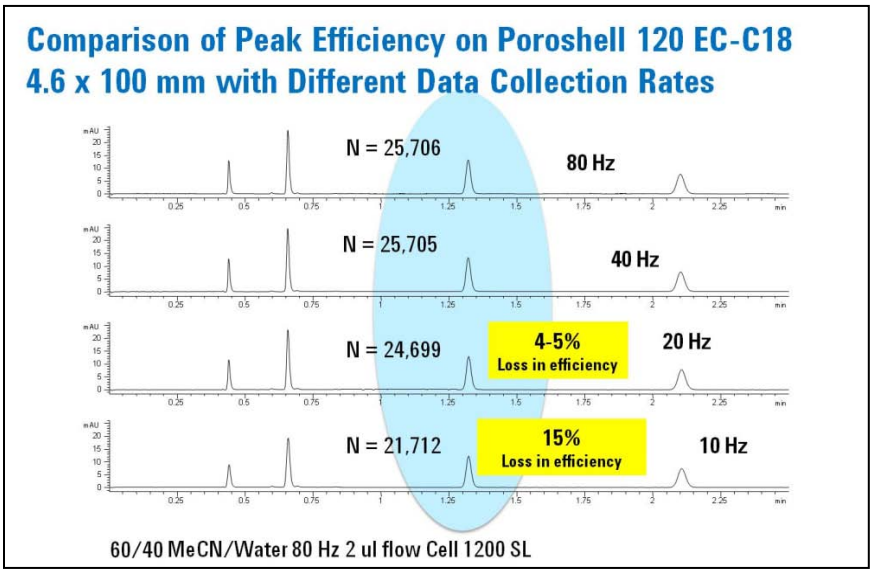
Inside Diameter (mm)	Length (mm)	Material	Color	Connections	Part Number	Volume (ul)
0.12	180	SS	Red	1 end pre-swaged	G1313-87304	2.0
0.12	280	SS	Red	1 end pre-swaged	01090-87610	3.2
0.12	105	SS	Red	1 end pre-swaged	01090-87611	1.2
0.12	150	SS	Red	pre-swaged	G1315-87312	1.7
0.12	105	SS	Red	Without fittings	5021-1820	1.2
0.12	150	SS	Red	Without fittings	5021-1821	1.7
0.12	280	SS	Red	Without fittings	5021-1822	3.2
0.12	400	SS	Red	Without fittings	5021-1823	4.5
0.17	180	SS	Green	1 end pre-swaged	G1313-87305	4.1
0.17	280	SS	Green	1 end pre-swaged	01090-87304	6.4
0.17	130	SS	Green	1 end pre-swaged	01090-87305	2.9
0.17	90	SS	Green	1 end pre-swaged	G1316-87300	2.0
0.17	105	SS	Green	Without fittings	5021-1816	2.4
0.17	150	SS	Green	Without fittings	5021-1817	3.4
0.17	280	SS	Green	Without fittings	5021-1818	6.4
0.17	400	SS	Green	Without fittings	5021-1819	9.1

USE LOWER VOLUME RED TUBING WHEN POSSIBLE

GREEN TUBING HAS 2 x VOLUME OF RED TUBING FOR EQUAL LENGTH

Data Collection Rate and Flow Rate

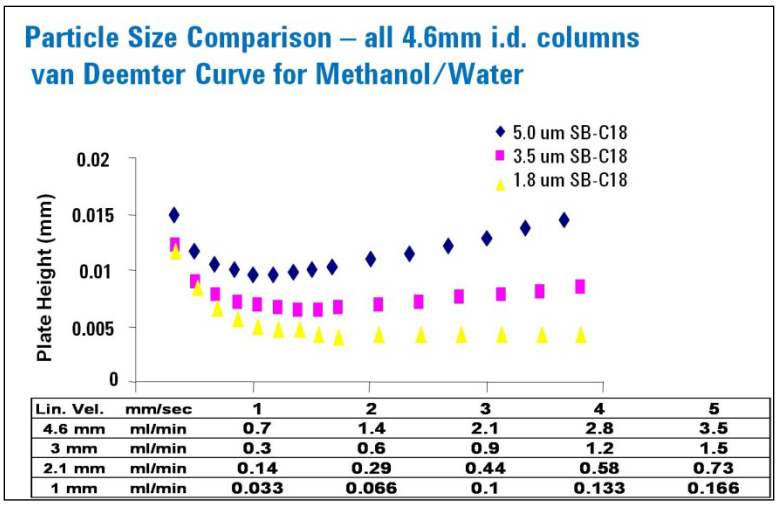
The data collection rate is a common source of peak broadening. You want to check your data collection rate to be sure it is optimized for the column and flow rate you are using.



This particular data to the left was run for an analysis using Poroshell 120 columns. We're measuring the efficiency here and, as you can see, our efficiency increases as the data collection rate used went higher.

Optimize your data collection rate by adjusting the detector setting and/or the time constant to the fastest possible value that does not compromise signal-to-noise. The Peak width control in ChemStation enables you to select the peak width, or response time, for your analysis.

Another setting to watch is your flow rate, or linear velocity. Each column particle size has an optimal linear velocity, which needs to be determined experimentally.



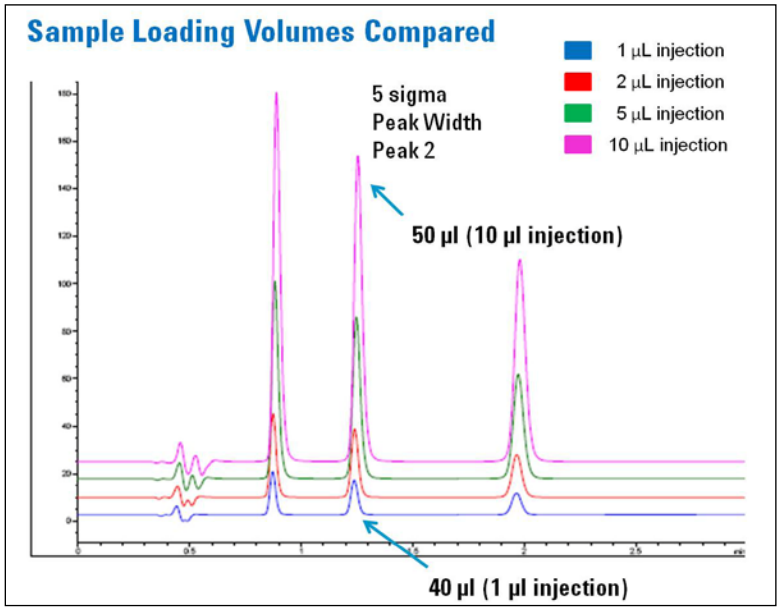
Lin. Vel.	mm/sec	1	2	3	4	5
4.6 mm	ml/min	0.7	1.4	2.1	2.8	3.5
3 mm	ml/min	0.3	0.6	0.9	1.2	1.5
2.1 mm	ml/min	0.14	0.29	0.44	0.58	0.73
1 mm	ml/min	0.033	0.066	0.1	0.133	0.166

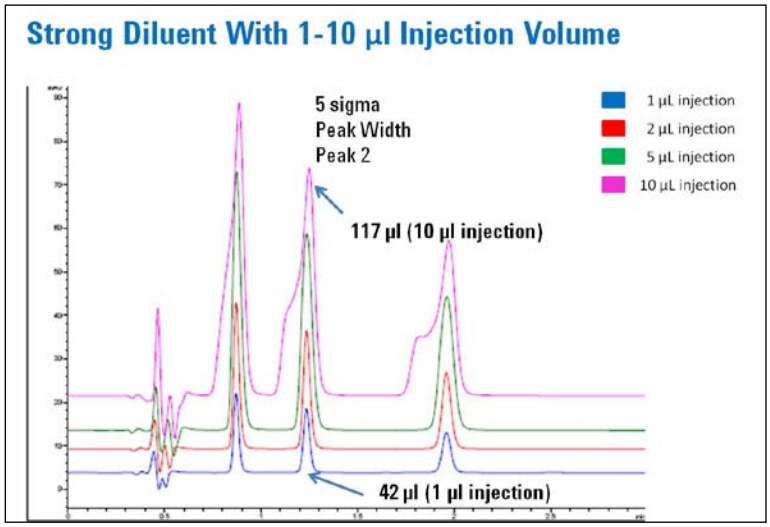
The van Deemter plots to the left illustrate how the velocity affects efficiency for several different columns.

Injection implications

Injections are another common trouble source for peak broadening. If your injection volume is too high, it can overload the column and cause peak broadening.

This series of chromatograms demonstrates peak broadening as a result of injection volumes that were increasingly too high.

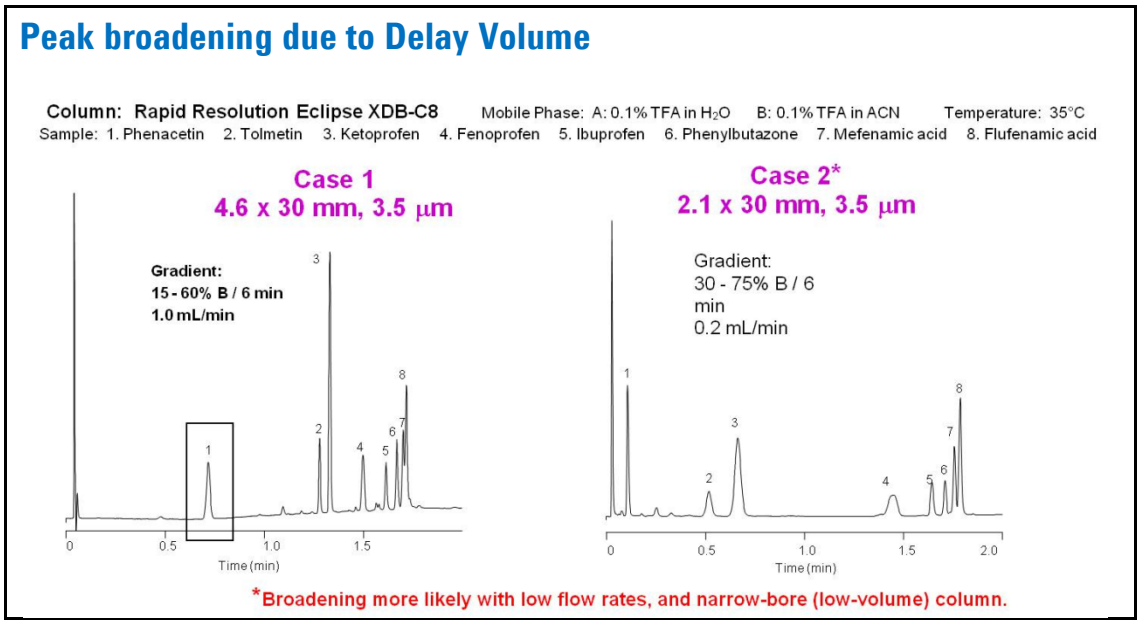




In the example to the left, there is evidence of band broadening due to the strong diluents at 5 µL on this small column operated in a low dispersion system configuration. But with 10 µL, the loss of peak symmetry is clearly a problem. This ultimately limits sensitivity unless an evaporation step is made to concentrate the analytes to support a smaller injection volume.

Gradients

When working with gradients, your gradient design can contribute to wider initial peaks, if the dwell volume is not accounted for in your method. See the example below.



Address this by reducing the initial gradient composition, so the peaks focus on the column, or use injector programming to start the gradient sometime before the sample injection is made.

Summary

Key points to troubleshoot peak broadening:

1) Ensure that instrument tubing volume is minimized on your instrument.
2) Optimize data collection rates for your column.
3) Scale injection volumes to avoid column overload.
4) For gradients, check programming to account for system dwell volume.

If you require additional assistance, you can always contact Agilent technical support by logging on to www.agilent.com/chem/contactus.