Performance of the Agilent 1290 Infinity Quaternary Pump using Trifluoroacetic Acid (TFA) as the mobile phase modifier

Technical Overview

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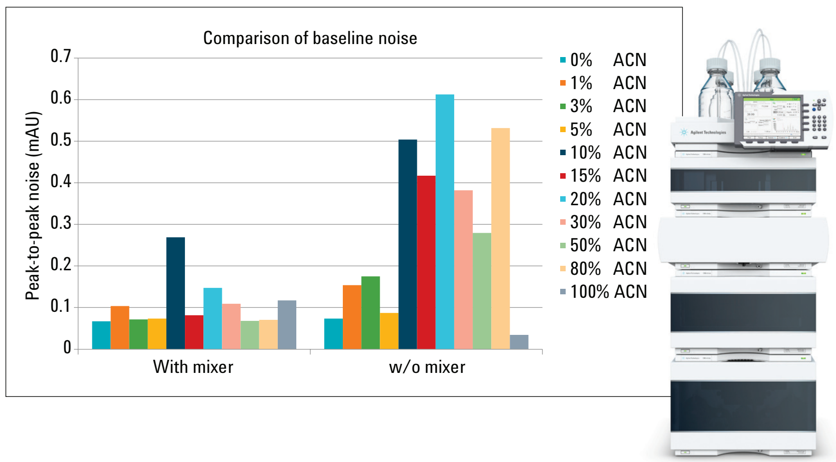

Abstract

In liquid chromatography, baseline noise should be as low as possible to provide an optimum signal-to-noise ratio and to enable reliable integration of peaks at trace level. Baseline noise has different sources. One source is the detector, and another is the mixing performance of the pump, which is especially important if, for example, trifluoroacetic acid (TFA) is used as modifier in the mobile phase.

This Technical Overview shows the mixing performance of the Agilent 1290 Quaternary Pump using TFA as the modifier in the mobile phase. Mixing performance depends strongly on the mixing volume. Typically, larger mixing volumes are better for higher baseline performance. Therefore, the influence of an additional mixer (V380 Jet Weaver) on the baseline noise of the 1290 Infinity Quaternary Pump is evaluated.

The results obtained using the Agilent 1260 Infinity Quaternary Pump are compared with results obtained using a non-Agilent quaternary UHPLC system.

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Introduction

Modifiers for mobile phases are frequently used for HPLC and UHPLC applications. Some of these modifiers, such as TFA have a strong influence on the baseline performance.[1-3] These phenomena lead to increased drift and baseline noise especially at low UV wavelengths, such as 214 nm. To reduce drift and baseline noise, good mixing performance of the LC pump is mandatory. This can be achieved either by large mixing volumes or by effective mixing in a small volume. With the introduction of UHPLC quaternary LCs, pump volumes have been significantly reduced, for example, down to 350 µL for the 1290 Infinity Quaternary Pump. This influences the mixing performance and, when using critical modifiers such as TFA, an additional mixer might be necessary.

This Technical Overview shows that an additional mixer, the V380 Jet Weaver for the 1290 Infinity Quaternary Pump, improves the baseline performance significantly when TFA is used as the modifier. The mixer is based on the Jet Weaver design, which provides the highest mixing performance at the lowest volume.

The results obtained on the 1260 Infinity Quaternary Pump are compared to results obtained on a quaternary UHPLC system of a different LC manufacturer.

Experimental

Instruments

The following instruments were used, see Table 1.

Software

Agilent OpenLAB CDS ChemStation C.01.04

Chromatographic conditions

Column:	Agilent Poroshell 120 SB-AQ, 100 × 3.0 mm, 2.7 µm
Mobile phases:	Water + 0.05%, 0.1%, TFA (A), Acetonitrile + 0.045%, + 0.05%, + 0.09%, + 0.1%, TFA (B)
Flow:	1 mL/min
Column temperature:	45 °C
DAD:	214, 254, 280/4 nm, Ref 400/100 nm, 20 Hz
Step gradient:	0%, 1%, 3%, 5%, 10%, 15%, 20%, 30%, 50%, 80%, 100%B, each held for 10 minutes
Stop time:	130 minutes
Post time:	5 minutes

	Agilent 1290 Infinity Quaternary LC System	Agilent 1260 Infinity Quaternary LC System	Non-Agilent Quaternary UHPLC System
Module	**Product number**	**Product number**	
Quaternary pump	G4204A	G1311B	Quaternary pump
Autosampler	G4226A	G1329B	Autosampler
Autosampler thermostat	G1330B	G1330B	Autosampler cooler
Thermostatted column compartment	G1316C	G1316C	Column compartment
Detector	Diode array detector		
G4212A	Diode array detector		
G4212B	Variable wavelength Detector		

Table 1
Instrumentation used.

Results and discussion

To evaluate the mixing performance of the 1290 Infinity Quaternary Pump, a step gradient experiment was selected. The experiment contained 11 steps, each with a different acetonitrile percentage. Each step was held for 10 minutes. Different concentrations of TFA in the organic and aqueous phase were used. Three different wavelengths were applied for the Agilent 1290 Infinity and Agilent 1260 Infinity LCs. A wavelength of 214 nm was selected for the non-Agilent UHPLC system.

The following step gradient experiments were performed on the 1290 Infinity Quaternary LC. Three wavelengths, four different TFA concentrations, and two pump configurations with and without the additional mixer were selected.

1. TFA 0.1% in aqueous phase and 0.09% TFA in organic phase, no additional mixer
2. TFA 0.1% in aqueous phase and 0.09% TFA in organic phase, with additional mixer
3. TFA 0.1% in aqueous phase and 0.1% TFA in organic phase, no additional mixer
4. TFA 0.1% in aqueous phase and 0.1% TFA in organic phase, with additional mixer
5. TFA 0.05% in aqueous phase and 0.045% TFA in organic phase, no additional mixer
6. TFA 0.05% in aqueous phase and 0.045% TFA in organic phase, with additional mixer
7. TFA 0.05% in aqueous phase and 0.05% TFA in organic phase, no additional mixer
8. TFA 0.05% in aqueous phase and 0.05% TFA in organic phase, with additional mixer

The following step gradient experiments were performed on the 1260 Infinity LC and the non-Agilent UHPLC system:

1. TFA 0.1% in aqueous phase and 0.09% TFA in organic phase, no additional mixer
2. TFA 0.05% in aqueous phase and 0.045% TFA in organic phase, no additional mixer

The strongest influence on the mixing performance and, consequently, on the baseline noise was observed at a wavelength of 214 nm, as expected, see Figure 1.

The baseline noise at 214 nm with an average noise of 0.3 mAU over all 11 steps was approximately 15 times higher than at 254 or 280 nm with an average noise of approximately 0.02 mAU.

Therefore, only the signal at 214 nm was used for all following noise calculations. The baseline noise was calculated based on the peak-to-peak noise. The noise was measured for 1 minute in the middle of the 10-minute step for each acetonitrile concentration.

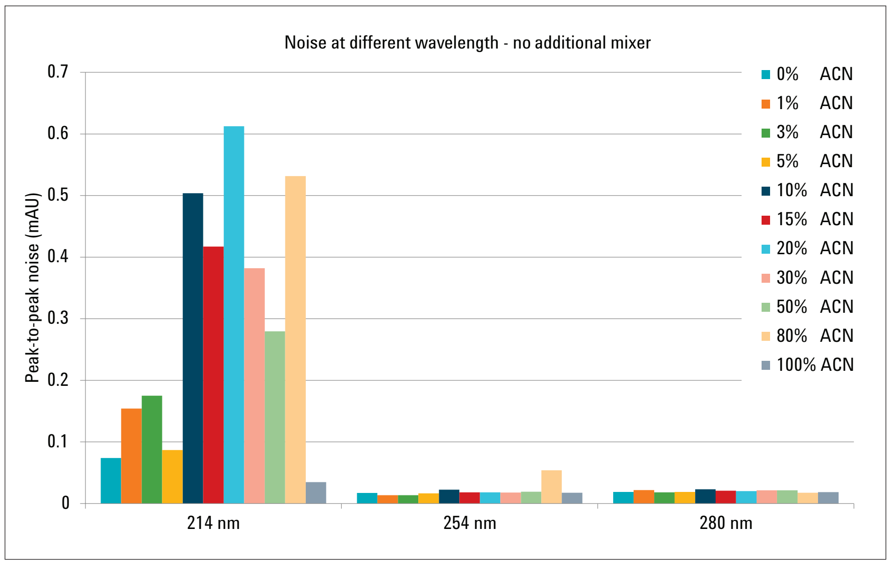

Figure 1
Influence of TFA on baseline performance at different wavelengths based on experiment 1.

Figure 2 shows the baseline noise with and without mixer at different TFA concentrations in the mobile phases. The peak-to-peak noise was reduced by a factor of 2 to 5 over all experiments, when the additional mixer was used.

Figure 2
Baseline noise of the Agilent 1290 Infinity Quaternary Pump for different TFA concentrations applying the step gradient with and without an additional mixer.

Comparison with a 1260 Infinity Quaternary Pump and a Quaternary UHPLC System of a different LC manufacturer

Some experiments were repeated on a 1260 Infinity Quaternary LC and a quaternary UHPLC system of a different manufacturer. Figure 3 shows the results of one experiment, combined, as an example.

Adding the additional mixer to the 1290 Infinity Quaternary Pump provided the lowest baseline noise, even compared to the 1260 Infinity Quaternary LC with its larger mixing volume.

Further experiments showed that, at lower TFA concentrations in the mobile phases, the noise level was reduced for all instruments, see Table 2.

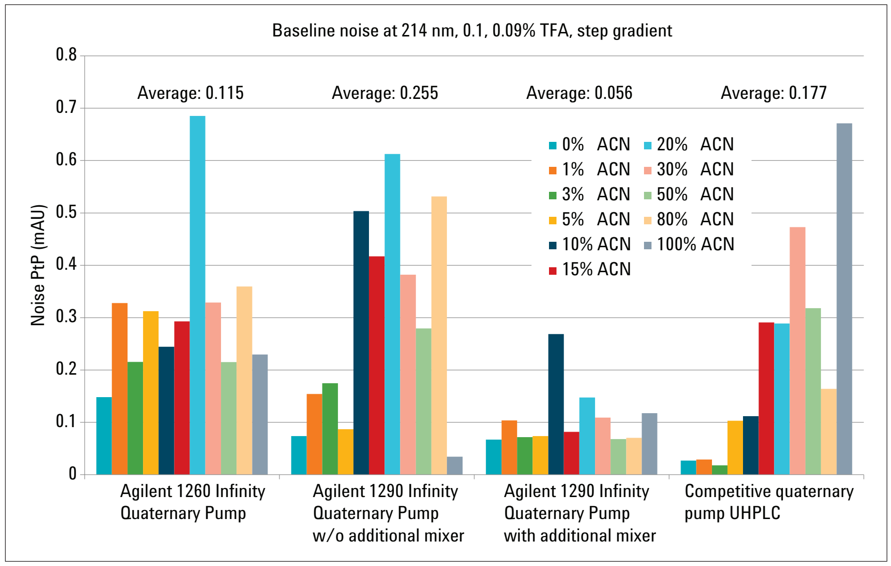

Figure 3
Comparison of peak-to-peak noise of the Agilent 1260 Infinity Quaternary LC, the Agilent 1290 Infinity Quaternary LC, and a non-Agilent quaternary UHPLC applying the step gradient at 0.1 and 0.09% TFA.

TFA concentration	Noise of Agilent 1260 Infinity Quaternary Pump (mAU)	Noise of Agilent 1290 Infinity Quaternary Pump with mixer (mAU)	Noise of Agilent 1290 Infinity Quaternary Pump without mixer (mAU)	Noise of non-Agilent quaternary pump (mAU)
0.1 and 0.09%	<0.7	<0.3	<0.62	<0.6
0.05 and 0.045%	<0.25	<0.11	<0.54	<0.4

Table 2
Noise at different TFA concentrations in the mobile phases.

Conclusion

Mobile phase modifiers, such as TFA, have a strong influence on the baseline performance, especially at a low wavelength such as 214 nm. Typically, TFA causes additional drift and noise. To minimize these phenomena, good mixing performance of the pump is needed. These experiments proved that the Agilent 1290 Infinity Quaternary Pump provided very good mixing performance, resulting in excellent noise behavior. Typically, the peak-to-peak noise was between 0.11 and 0.3 mAU. This was achieved by using an additional V380 Jet Weaver mixer, which provided the optimum mixing performance at the lowest mixing volume.

References

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