Fast and Flexible Optimization of Modifier Concentrations Using an Agilent 1290 Infinity LC System with Blend Assist

Technical Overview

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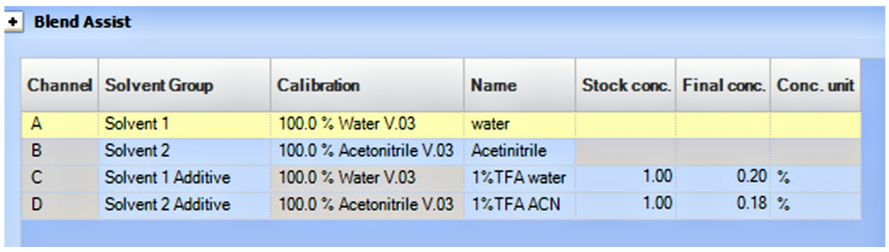
Abstract

During LC method development, varying buffer or modifier concentrations is frequently done to optimize the separation of compounds. Using the Agilent 1290 Infinity Quaternary Pump with Blend Assist simplifies and accelerates method development. Blend Assist is software implemented in the driver for the 1290 Infinity Quaternary Pump and can be used through any data system that controls the pump. Consequently, the 1290 Infinity Quaternary Pump can be integrated into any Agilent 1200 Infinity LC system providing optimum method development capability.

This Technical Overview shows how Blend Assist, in combination with the Agilent 1290 Infinity Quaternary LC System, is used to vary buffer/modifier concentration using ternary or quaternary gradients. Having optimized the separation on the 1290 Infinity Quaternary LC System with Blend Assist, the developed optimum conditions are applied directly onto an Agilent 1290 Infinity Binary LC to prove agreement of retention times and separation.



Blend Assist

Channel	Solvent Group	Calibration	Name	Stock conc.	Final conc.	Conc. unit
A	Solvent 1	100.0 % Water V.03	water			
B	Solvent 2	100.0 % Acetonitrile V.03	Acetinitrile			
C	Solvent 1 Additive	100.0 % Water V.03	1%TFA water	1.00	0.20	%
D	Solvent 2 Additive	100.0 % Acetonitrile V.03	1%TFA ACN	1.00	0.18	%

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Introduction

During LC method development, varying buffer or modifier concentrations is frequently done to optimize the separation of compounds. Using, for example, a 1290 Infinity Binary LC system, different mobile phases have to be mixed manually, which is not very convenient, time consuming, and error prone. Using a quaternary LC system, one or two channels can contain a high concentration of a modifier or a buffer. By varying the flow of this channel and applying ternary or quaternary gradients, the resulting modifier/buffer concentration can be changed between runs to find the optimum conditions.

Experimental

The following instruments were used, see Table 1.

Table 1. Instrumentation used.

Agilent 1290 Infinity LC Systems	
Pumps used	
G4204A	Agilent 1290 Infinity Quaternary Pump + Blend Assist
G4220A	Agilent 1290 Infinity Binary Pump used in the binary LC system
Modules used in both LC systems	
G4226A	Agilent 1290 Infinity Autosampler
G1330B	Agilent Autosampler cooler
G1316C	Agilent 1290 Infinity Thermostatted Column Compartment
G4212A	Agilent 1290 Infinity DAD with 10-mm path length cell

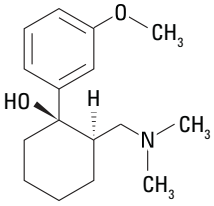
Chromatographic conditions for Experiment 1

Sample:	Tramadol and impurities; 4 mg Tramadol/mL + 4 impurities each 0.05%
Column:	Agilent ZORBAX RRHT Eclipse Plus C18, 4.6 × 100 mm, 1.8 µm, (p/n 959964-902)
Mobil phase:	A = Water B = Acetonitrile C = 1% TFA in water linked with channel A D = 1% TFA in acetonitrile linked with channel B
Gradient quaternary:	17 to 45% B/D in 8 minutes
Flow rate:	1.5 mL/min
Stop time:	8 minutes
Post time:	3 minutes
Injection volume:	3 µL
Column temperature:	40 °C
DAD:	270/10 nm Ref 360/100 nm Flow cell: 10-mm pathlength Peak width: >0.013 minutes (20 Hz)

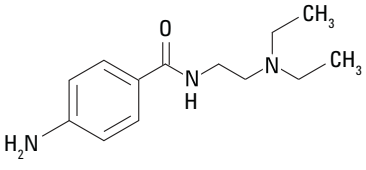
Compounds analyzed

Experiment 1

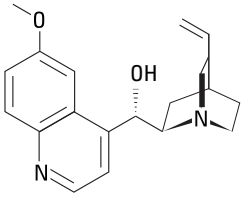
Tramadol + 4 impurities



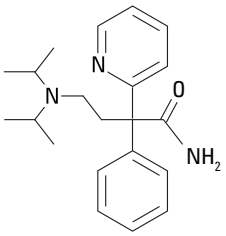
Experiment 2



Procainamide and acetyl procainamide



Quinidine



Disopyramide

Chromatographic conditions for Experiment 2

Column:	Agilent ZORBAX RRHT Eclipse plus C18, 4.6 × 100 mm, 1.8 µm (p/n 959964-902)
Mobil phase:	A = Water B = Acetonitrile/water: 80/20 C= 0.5 M NaH_2PO_4 (60 g/L) in water/ACN = 80/20, pH=4.35 linked with channel A
Gradient quaternary:	5% B to 60% B in 8 minutes
Flow rate:	1.5 mL/min
Stop time:	8.5 minutes
Post time:	3 minutes
Injection volume:	1 µL
Column temperature:	40 °C
DAD:	220/10 nm Ref 360/100 nm Flow cell: 10-mm pathlength Peak width: >0.013 minutes (20 Hz)

Acquisition and Evaluation Software

Agilent OpenLAB CDS ChemStation version C.01.04 and Blend Assist

Parameter setting and functionality of Blend Assist

Blend Assist is software implemented in the driver for the 1290 Infinity Quaternary Pump and applicable with any software which controls this pump. This offers optimum method development facilities for all 1200 Infinity LCs. All calculations regarding variation of buffer/modifier concentrations are done automatically. Figure 1 shows the parameter screen of Blend Assist.

After activating Blend Assist, the channels which should be linked together have to be determined. In this case, channel A, containing water, was linked with channel C containing a 1% TFA solution in water. Channel B, containing acetonitrile, was linked with channel D containing a 1% TFA solution in acetonitrile. Different methods could be set up by varying the final TFA concentration. Figure 1 shows that the final concentration for the linked channel A and C was 0.2%, and for the linked channels B and D the final TFA concentration was 0.18%. The concentration units can be set either to % or mM. In the example above, % was used.

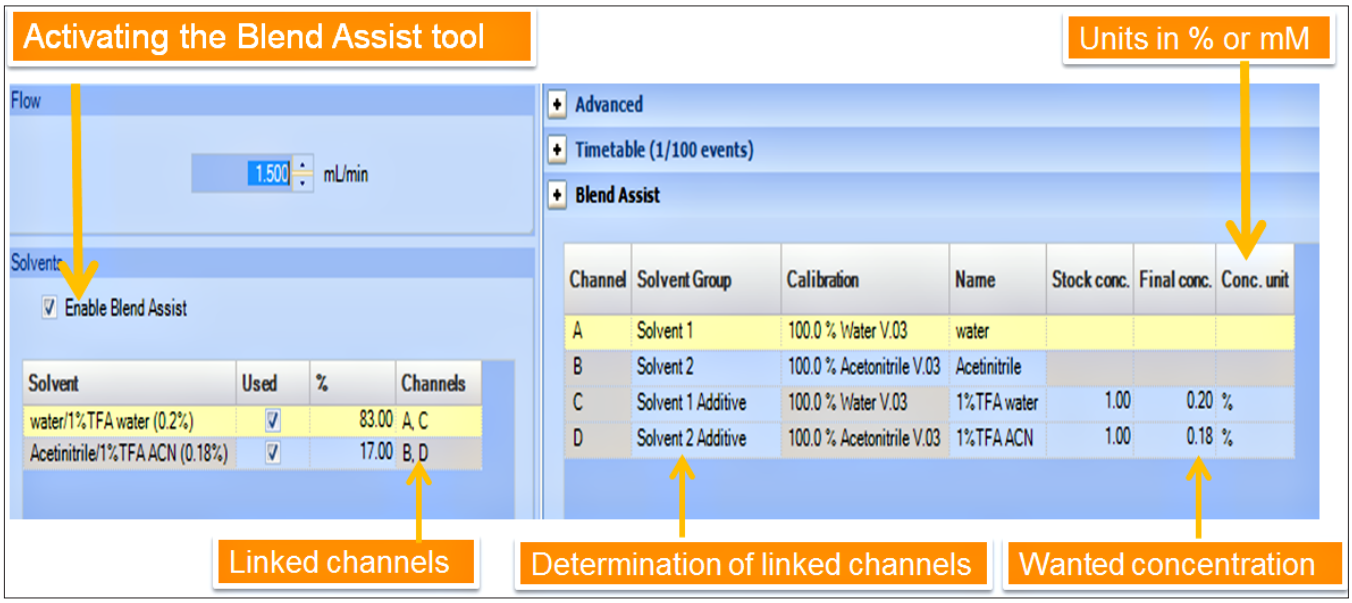


Figure 1. Blend Assist parameter screen.

The gradient is set up the same way as usual, see Figure 2.

Figure 3 shows the slopes for the different channels during progression of the applied gradient. The linked channels A and C show a down-slope, whereas the linked channels B and D show an up-slope.

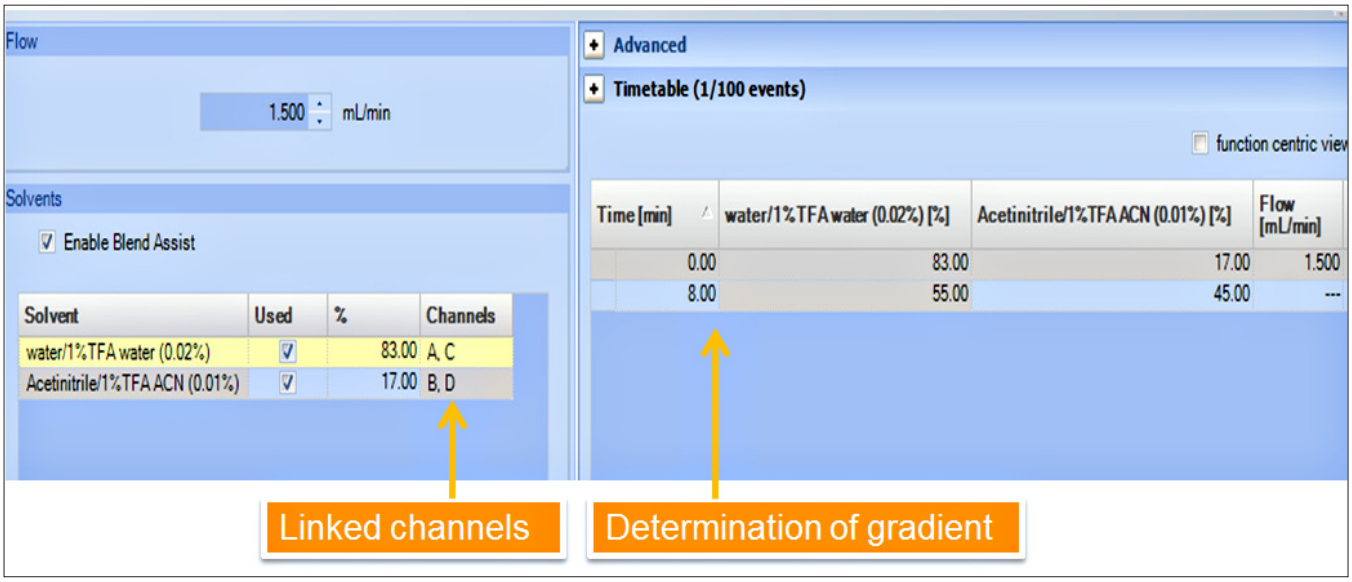


Figure 2. Determination of gradient.

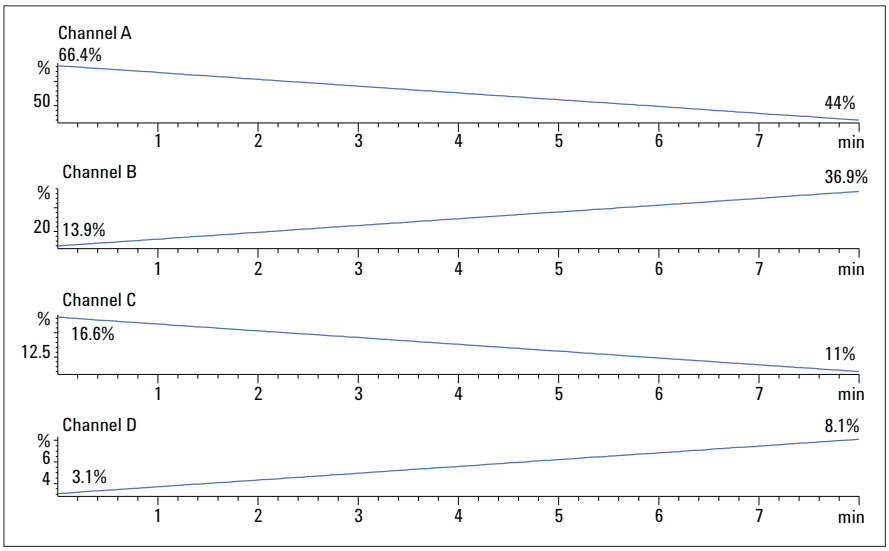


Figure 3. Up- and down-slopes of the different mobile phase channels.

Results and Discussion

Experiment 1

Variation of TFA concentration to optimize the separation of Tramadol and its four impurities

The 1290 Infinity Quaternary Pump and Blend Assist were used without the need to mix different TFA concentrations for the aqueous and organic channel.

The following experiments were performed using quaternary gradients:

- Variation of TFA concentration from 0.02, 0.05, 0.1, to 0.2% in water, and variation of the TFA concentration in the organic phase, but always 10% less than in the aqueous phase.

- Comparison of Blend Assist results with results based on manually mixed mobile phases using the 1290 Infinity Binary LC

Increasing the TFA concentration improved the peak shape of Tramadol significantly. The best peak shape for Tramadol was obtained at 0.2% TFA in the aqueous phase and 0.18%TFA in the organic phase. The comparison with manually mixed mobile phases applied onto the 1290 Infinity Binary LC showed excellent agreement with Blend Assist results, see blue and red trace in Figure 4.

Further the separation between Tramadol and Impurity 2 showed an optimum separation using a concentration of 0.2% TFA in the aqueous phase and 0.18% TFA in the organic phase, see Figure 5.

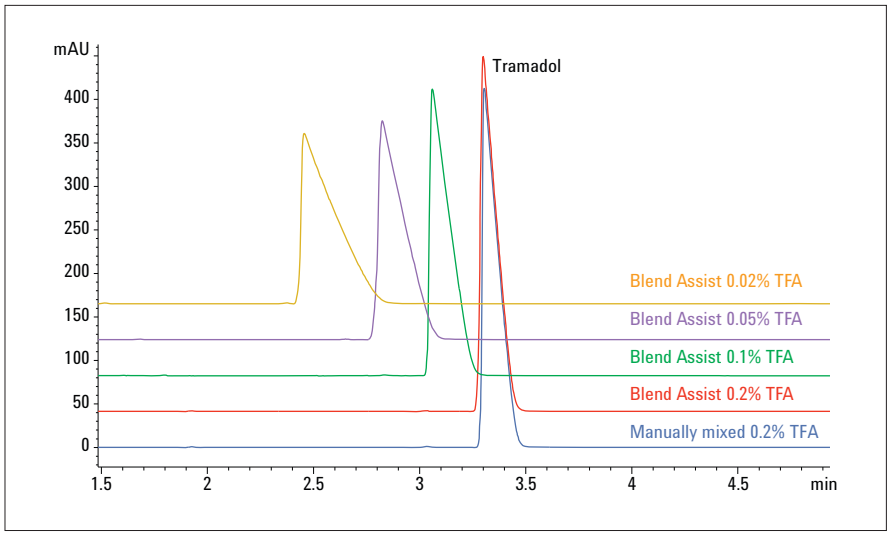


Figure 4. Influence of TFA concentration on peak shape and comparison of chromatograms obtained on the Agilent 1290 Infinity Quaternary LC with Blend Assist (red trace), and on the Agilent 1290 Infinity Binary LC (blue trace).

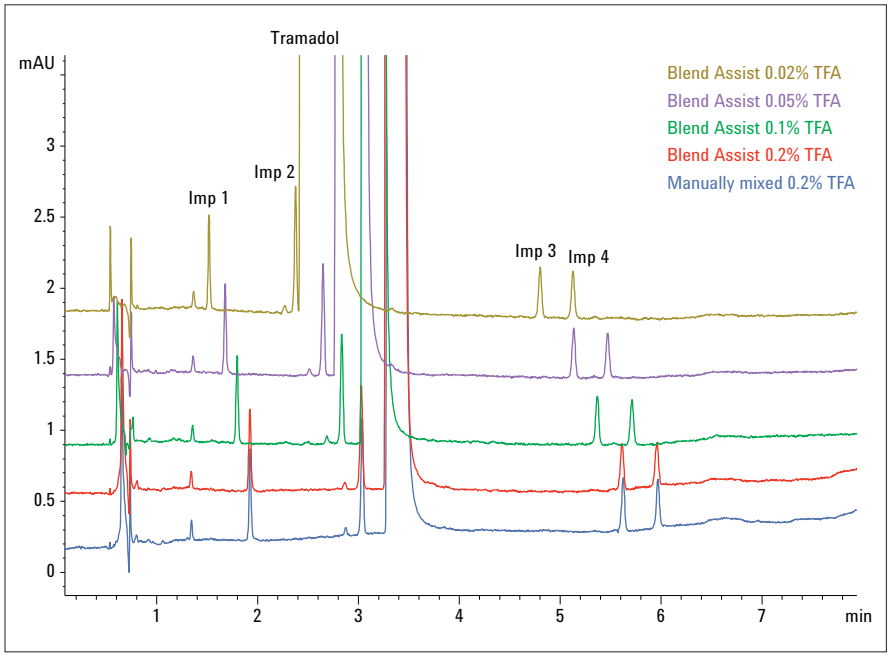


Figure 5. Influence of the TFA concentration on separation and comparison of chromatograms obtained on an Agilent 1290 Infinity Quaternary LC system with Blend Assist (red trace), and on the Agilent 1290 Infinity Binary LC (blue trace).

The resolution between Impurity 2 and Tramadol increased from 0.48 at a TFA concentration of 0.02%, in the aqueous phase, to 2.83 at a TFA concentration of 0.2%. Slightly better resolution, with a value of 2.92, was achieved on the 1290 Infinity Binary LC (blue trace) compared to 2.83 obtained on the 1290 Infinity Quaternary LC with Blend Assist (red trace). The retention times on the 1290 Infinity Binary LC deviated from the values obtained on the 1290 Infinity Quaternary LC maximal +0.35%. Figure 6 combines the resolution results for Impurity 2 and Tramadol.

Experiment 2

Variation of buffer concentration to optimize the separation of procainamide, acetyl-procainamide, disopyramide and quinidine

The following experiments were done:

- Variation of phosphate buffer concentration; 10 mM, 25 mM, 50 mM
- Comparison with manually mixed mobile phases using the 1290 Infinity Binary LC

Channel C delivered the buffer solution at very low flow rates according to the wanted resulting buffer concentration in the mobile phase composition.

Flow rates for channel C

50 mM buffer: Flow rate = 130 µL/min

25 mM buffer: Flow rate = 65 µL

10 mM buffer: Flow rate = 26 µL/min

Even though the flow rates for channel C were very low, the precision of retention times was excellent. The precision of retention times using the 10 mM buffer concentration with 26 µL/min flow rate for channel C was between 0.24 and 0.03% RSD.

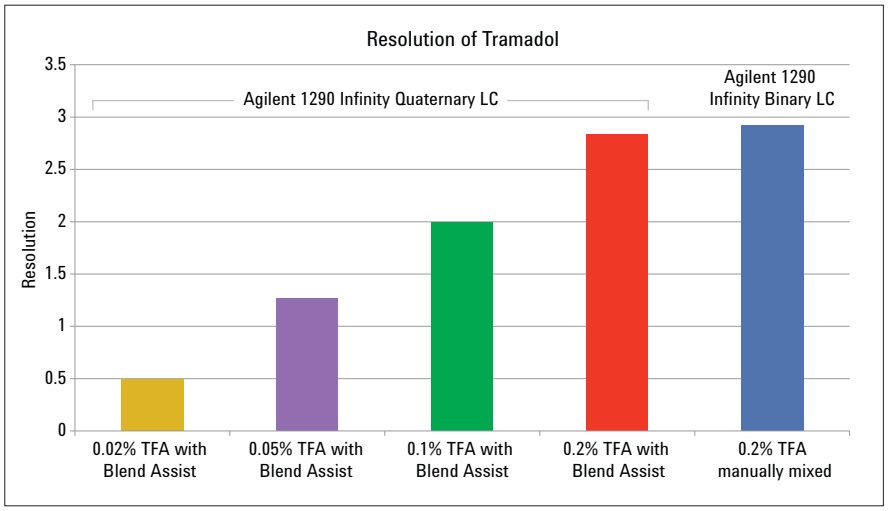


Figure 6. Resolution for different TFA concentrations and comparison with manually mixed mobile phases on the Agilent 1290 Infinity Binary LC.

The 10 mM phosphate buffer provided the best resolution between disopyramide and quinidine, see Figure 7. The transfer to a 1290 Infinity Binary LC with manually mixed phases (blue trace) showed excellent agreement with the results using a 1290 Infinity Quaternary LC with Blend Assist (red trace). The deviation of retention times is with maximal –0.4% negligible.

Figure 8 summarizes the resolution data for disopyramide and quinidine. In this case, the lowest buffer concentration provided the best separation with a resolution of 1.04. The manually mixed mobile phases used on the 1290 Infinity Binary LC provided a resolution of 1.15.

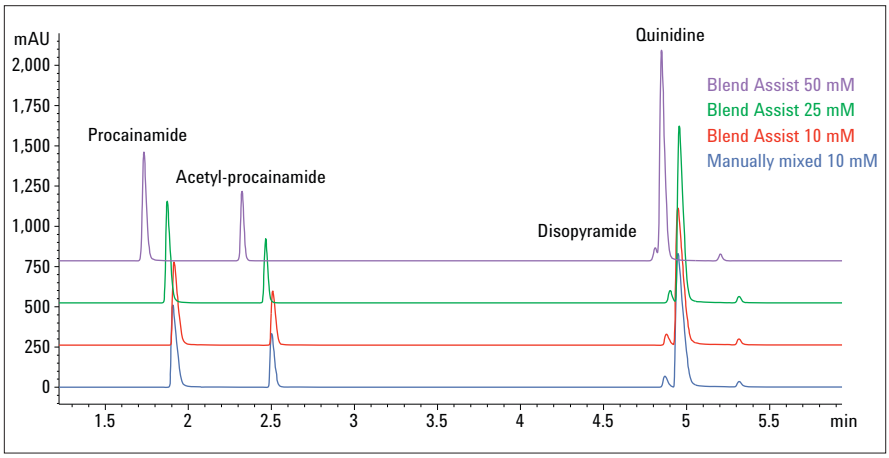


Figure 7. Influence of buffer concentration on separation; comparison of chromatograms obtained on the Agilent 1290 Infinity Binary LC (blue trace), and the Agilent 1290 Infinity Quaternary LC with Blend Assist (red trace).

Conclusion

Blend Assist software implemented in the driver of the Agilent 1290 Infinity Quaternary Pump facilitates method development for all Agilent 1200 Infinity LCs. Variation of modifier/buffer concentrations can be performed without the need to mix different concentrations manually.

In one example, the TFA concentration was varied on the 1290 Infinity Quaternary LC to improve peak shape of the main peak and resolution of impurities. Excellent agreement for retention and resolution was obtained by transferring the developed method onto the Agilent 1290 Infinity Binary LC. In a different example, the phosphate buffer concentration was varied on the 1290 Infinity Quaternary LC. Also shown in this example, excellent agreement for retention and resolution was obtained by transferring the developed method onto the 1290 Infinity Binary LC.

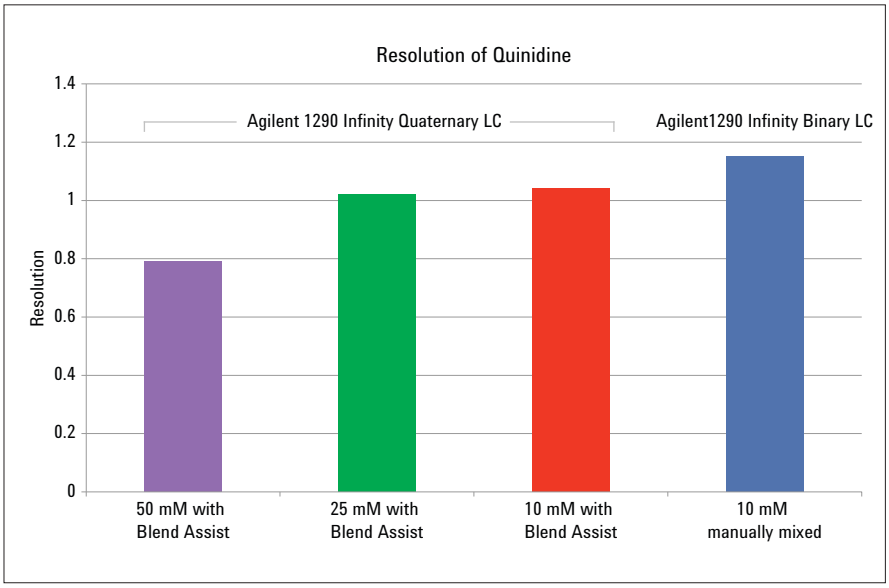


Figure 8. Resolution for different buffer concentrations and comparison with manually mixed mobile phases on the Agilent 1290 Infinity Binary LC.

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