Fast and flexible optimization of buffer/modifier concentrations using ternary gradients with the Agilent 1260 Infinity LC Quaternary System

Technical Overview

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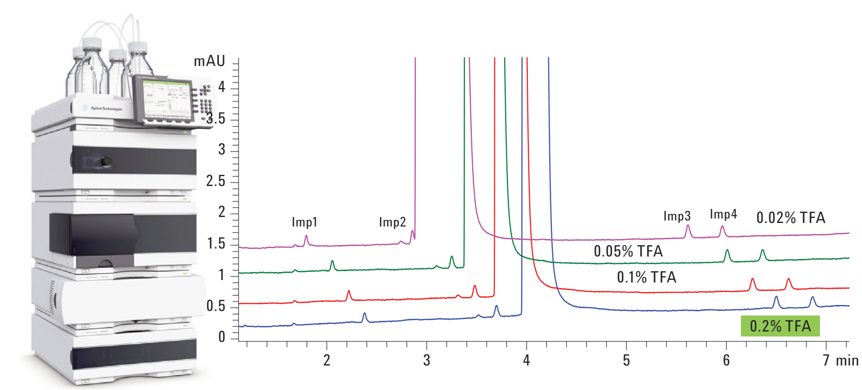

Abstract

The Agilent 1260 Infinity Quaternary LC offers:

- Ability to run both standard bore UHPLC and conventional HPLC applications on the same system configuration
- Up to 600 bar pressure range to support sub-2-µm particle columns
- Up to four different solvents to form ternary and quaternary gradients
- Optimization of buffer and modifier concentrations using ternary gradients without the need to prepare different buffer or modifier concentrations manually

In this Technical Overview we demonstrate that the Agilent 1260 Quaternary LC is best suited to automatically optimize buffer and modifier concentrations by using ternary gradients.

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Introduction

Ternary and quaternary gradients are often used to keep buffer and modifier concentration of a mobile phase mixture constant while using two other channels to run a water/organic gradient. This workflow is extremely helpful for optimizing a method without preparing several mobile phase sets.

Equipment

The Agilent 1260 Infinity Quaternary LC system used included the following:

- Agilent 1260 Infinity Quaternary Pump (G1311B)
- Agilent 1260 Infinity Autosampler (G1367E)
- Agilent 1260 Infinity Thermostatted Column Compartments (G1316C)
- Agilent 1260 Infinity Diode Array Detector (G4212B)
- Agilent ZORBAX columns of different lengths, internal diameters and chemistries
- Agilent ChemStation B04.02

Results and discussion

Ternary gradient to vary TFA concentration

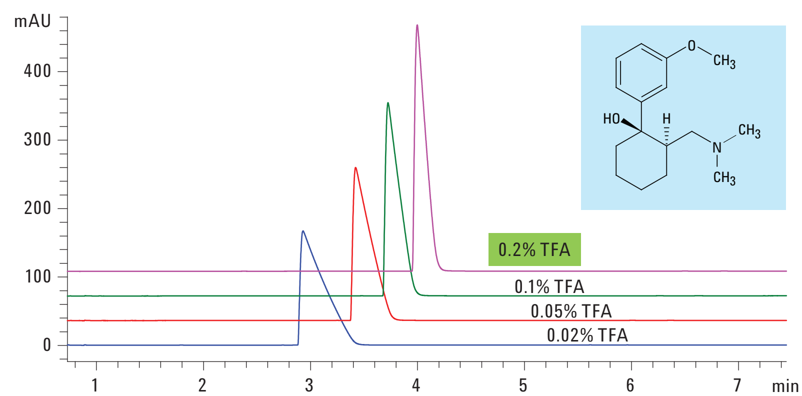
In the following examples we used a ternary gradient to investigate the influence of various TFA concentrations on the separation of impurities from one main compound.

In Channel C a solution of 1% TFA in water was set up. The total combined flow from channels A, B and C was 1.5 mL/min.

The following experiments were performed:

1. Experiment: Flow rate for Channel C = 260 µL/min = 20%C, resulting in 0.2 % TFA concentration
2. Experiment: Flow rate for Channel C = 130 µL/min = 10%C, resulting in 0.1 % TFA concentration
3. Experiment: Flow rate for Channel C = 65 µL/min = 5%C, resulting in 0.05 % TFA concentration
4. Experiment: Flow rate for Channel C = 26 µL/min = 2%C, resulting in 0.02 % TFA concentration

Figure 1 shows the influence of the TFA concentration on the peak shape of the main peak. The effect in this case is based on the ion pairing capabilities of TFA rather than on differences in pH. The highest concentration of TFA showed the best peak shape for the main peak.



Chromatographic conditions

Sample:	Tramdole and impurities; 4 mg Tramadole/mL
Column:	Agilent ZORBAX Eclipse Plus HT-C18, 100 mm × 4.6 mm, 1.8 µm
Mobile phase:	A = Water B = Acetonitrile C = 1% TFA in water
Gradient variation (Table 1)	
Flow rate:	1.5 mL/min
Stop time:	8 min
Post time:	3 min
Injection volume:	3 µL
Column temperature	40 °C
DAD:	270/10 nm, Ref 360/100 nm,
Flow cell:	10 mm
Peak width:	>0.013 min (20 Hz)

Figure 1
Influence of TFA concentration on peak shape.

Figure 2 shows the influence of TFA concentration on resolution. The separation of the impurity in front of the main peak is especially critical.

Table 2 combines the results of the experiments.

The peak shape of the main peak had significantly improved at 0.2% TFA as well as the resolution between Impurity 2 and the main peak, and slightly between Impurity 3 and 4. The retention times of all peaks are shifted to longer values with increasing TFA concentration.

	Start Conditions for Gradient			End Conditions for Gradient		
Resulting TFA concentration (%)	Water (%) channel A	ACN (%) channel B	TFA in water (%) channel C	Water (%) channel A	ACN (%) channel B	TFA in water (%) channel C
0.2	63	17	20	35	45	20
0.1	73	17	10	45	45	10
0.05	78	17	5	50	45	5
0.02	81	17	2	53	45	2

Table 1
Gradient variations.

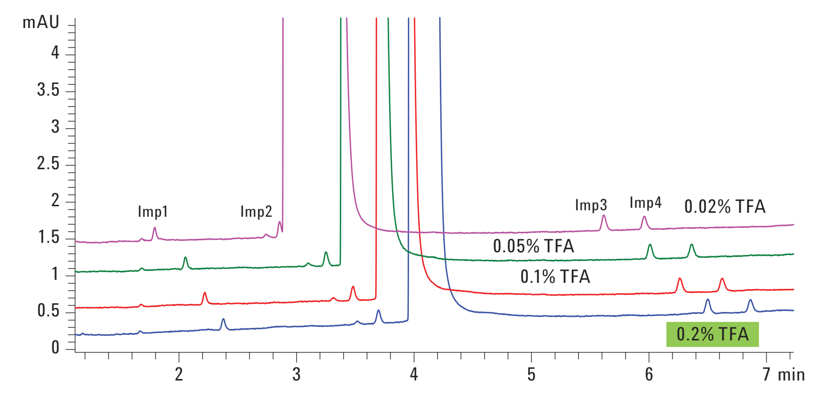


Figure 2
Influence of TFA concentration on resolution.

TFA concentration (%)	Rs (Tangent method) Main peak Impurity 2	Rs (half height) Impurity 4	PW (half height) (min) Main peak	RT (min) Impurity 1	RT (min) Impurity 4
0.02	0.409	5.00	0.2450	1.793	5.960
0.05	1.093	5.03	0.1850	2.054	6.363
0.1	1.765	4.89	0.1467	2.219	6.623
0.2	2.476	5.05	0.1142	2.377	6.864

Table 2
Results of the addition of different TFA concentration on peak width and resolution.

Ternary gradient to vary phosphate buffer concentration

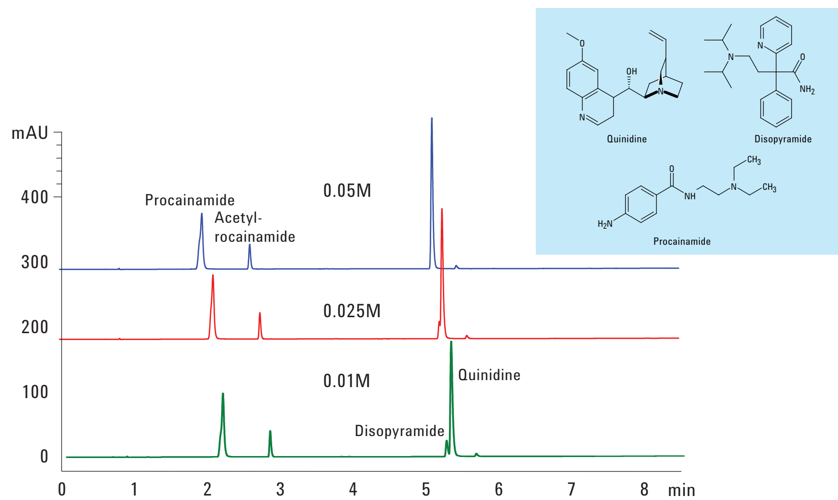
In the following experiments the phosphate buffer concentration was varied using ternary gradients. This demonstrates that varying the buffer concentration is helpful in finding additional peaks, even though the additional peak is partly coeluting in a peak with higher concentration.

The initial solution was 0.5 M KH_2PO_4, pH = 4.6 (60 g/L) in Channel C. This solution was added to the total flow at different percentages. The buffer contained 20% acetonitrile and the organic phase contained 20% water to avoid precipitation problems. The total flow rate was 1.5 mL/min.

Figure 3 shows an overlay of the three chromatograms. The lowest buffer concentration provided the best resolution between disopyramide and quinidine.

The following experiments were performed:

1. Experiment: Flow rate Channel C = 130 µL/min = 10%C, resulting concentration 0.05 M buffer/20% ACN
2. Experiment: Flow rate Channel C = 65 µL/min = 5%C, resulting concentration 0.025 M buffer/20% ACN,
3. Experiment: Flow rate Channel C = 26 µL/min = 2%C, resulting concentration 0.01 M buffer/20% ACN



Chromatographic conditions

Column:	Agilent ZORBAX Eclipse Plus HT-C18, 100 mm x 4.6 mm, 1.8 µm
Mobile phase:	A = Water B = Acetonitrile/water = 80/20 C = 0.5 M NaH_2PO_4 in water/ACN = 80/20, pH=4.6
Gradient variation (Table 3)	
Flow rate:	1.5 mL/min
Stop time:	8.5 min
Post time:	3 min
Injection volume:	1 µL
Column temperature	40 °C
DAD:	270/10 nm, Ref 360/100 nm,
Flow cell:	10 mm
Peakwidth:	>0.013 min (20 Hz)

Figure 3
Influence of buffer concentration on resolution.

The performance results of the experiments are combined in Table 4.

The lowest buffer concentration resulted in a nearly baseline separation of the last two peaks. The retention time for all peaks was slightly prolonged.

Conclusion

The Agilent 1260 Infinity Quaternary LC provides high flexibility regarding automated generation of buffer and modifier concentrations by using ternary gradients. This eliminates the need to tediously prepare different buffer and modifier concentrations manually. For example, a moderately high concentration of the buffer or modifier can be placed in Channel C. During a run, a certain percentage of C is added to the total flow from channels A and B, forming the desired buffer and modifier concentration. Using the ternary gradients, a separation can be optimized quickly and conviently within the sequence and without changing solvents.

	Start Conditions for Gradient			End Conditions for Gradient		
Resulting buffer Concentration	Water (%) Channel A	ACN/water= 80/20 (%) Channel B	NaH_2PO_4, pH 4.6 in water/ACN= 80/20(%) Channel C	Water(%) Channel A	ACN/water= 80/20 (%) Channel B	NaH_2PO_4, pH 4.6 in water/ACN= 80/20(%) Channel C
0.05 M	85	5	10	30	60	10
0.025 M	90	5	5	35	60	5
0.01 M	93	5	2	38	60	2

Table 3
Gradient variation.

Buffer concentration	Rs (half height) Quinidine	PW (5 sigma) (min) Quinidine	RT (min) Procainamide	RT (min) Quinidine
0.05M	Co-elution with Disopyramide	0.068	1.959	5.115
0.025M	0.862	0.077	2.107	5.251
0.01M	1.188	0.080	2.145	5.279

Table 4
Results of different phosphate buffer concentration on resolution, peak width and elution times.

Appendix

Calculation example for resulting buffer/modifier concentration

The initial concentration in Channel C is 10%. The target resulting concentration of the buffer and modifier should be 1%. Total flow rate is 1000 µL/min = 100%.

- The dilution factor for Channel C is = 10%/1% = 10
- The flow rate (µL/min) for Channel C is = Total flow rate (µL/min) × dilution factor in percent (%)/100 (%)

  In this example, the flow rate for Channel C is = 1000 µL/min × 10%/100% = 100 µL/min
- The percentage for Channel C is = Calculated flow rate for channel C (µL/min) × 100%/total flow rate (µL/min)

  In this example the percentage for Channel C is: 100 µL/min × 100%/ 1000 µL/min = 10%

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