Performance comparison applying ternary and quaternary gradients on the Agilent 1260 Infinity Quaternary LC System

Application Note

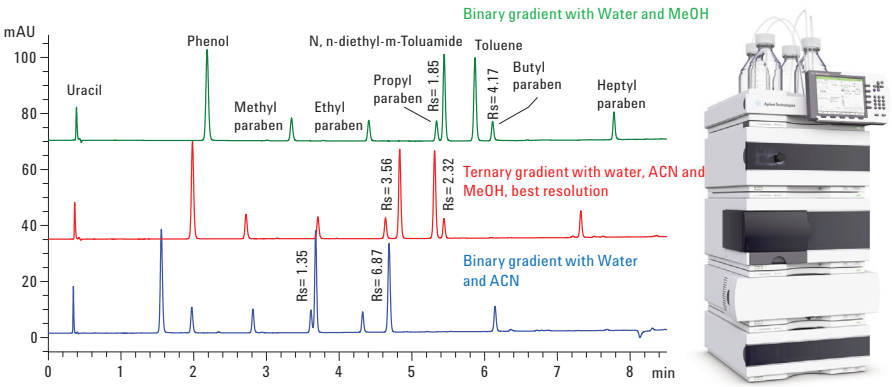
Pharmaceuticals

Author

A.G.Huesgen

Agilent Technologies

Waldbronn, Germany



Abstract

Ternary and quaternary gradients are used infrequently in reversed phase chromatography. However, these types of gradients can be very helpful in finding optimized conditions for a separation during method development. In this Application Note, binary, ternary, and quaternary gradients are applied and compared. Performance data, including resolution and precision of retention time are evaluated and the results show that these gradients are a useful tool.

Agilent Technologies

Introduction

Ternary and quaternary gradients are often used to keep the ionic strength of a mobile phase mixture constant even though the percentage of one mobile phase component without buffer or modifier is increased during a run. Another application is the variation of ionic strength or the modifier concentration using a ternary gradient to optimize a method without the need to prepare several mobile phase sets. Ternary mixtures of three organic solvents are used to a certain extent in normal phase chromatography and size exclusion chromatography. In reversed phase chromatography, the use of more than one organic phase is not common. The reason might be that without computer modeling/prediction software packages the optimization of a method is difficult and often accompanied by trial and error.[1]

This Application Note is a comparison between simple binary and ternary gradients. For one separation example, the ionic strength was kept constant using a ternary and a quaternary gradient.

Experimental

Instrumentation

An Agilent 1260 Infinity LC System with the following configuration was used (Table 1).

Configuration of the Agilent 1260 Infinity LC system
Quaternary pump with integrated degassing unit (G1311B)
Column compartment (G1316A)
Diode array detector (G4212B) with 10 mm path length cell
Autosampler (G1367E) and sample cooling unit (G1330B) at 10 °C
Software: ChemStation B.04.02

Table 1
Instrument configurations.

Results and Discussion

The first application example used the following compounds containing acidic, basic and neutral compounds (Figure 1).

Two binary gradients with acetonitrile and methanol as the organic components were compared to a ternary gradient using a mixture of methanol and acetonitrile as organic mobile phase (Figure 2).

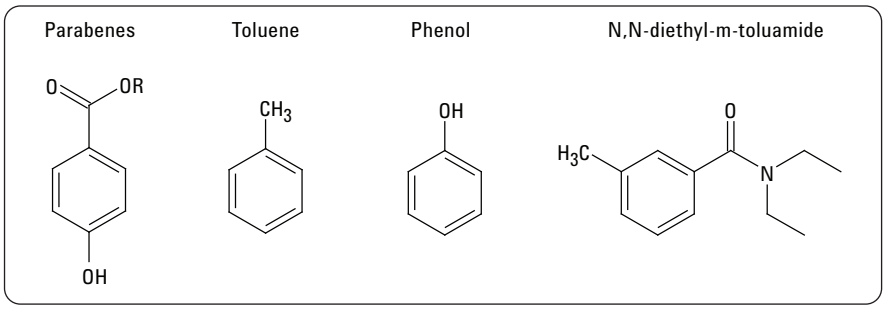


Figure 1
Chemical structure of first application example.

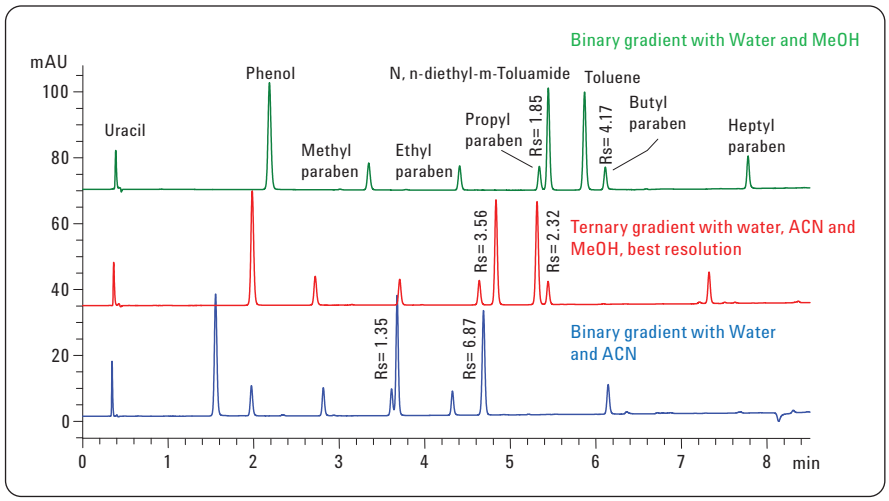


Figure 2
Optimization of resolution using a ternary gradient.

Chromatographic conditions	
Column	Agilent ZORBAX Eclipse Plus C18, 3 mm × 100 mm, 3.5 µm
Mobile Phase	A = Water
	B = Acetonitrile
	C = Methanol
Gradient binary ACN	0 min 20% B
	8 min 95% B
Gradient binary MeOH	0 min 20% B
	8 min 95% B
Gradient ternary	0 min 10% ACN, 10% MeOH
	8 min 47.5% ACN, 47.5% MeOH
Flow rate	1.2 mL/min
Stop time	8.5 min
Post time	3 min
Inj vol	3 µL
Column temp	40 °C
DAD	254/10 nm, Ref 360/100 nm,
Flow cell	10 mm
Peakwidth	> 0.013 min (20 Hz)

As illustrated, the modifications of the organic mobile phase composition has an impact on the selectivity of toluene and butylparaben. The binary acetonitrile gradient shows the fastest elution of all the peaks but the resolution of N,N-diethyl-m-toluamide from propylparaben is not sufficient. Better results are obtained for the methanol binary gradient, but elution of all peaks is prolonged. The best compromise between resolution and run time is obtained for the ternary gradient with acetonitrile and methanol. The percentage of both organic components was always 50% over the complete run. This means at the start of the run the total organic percentage was 20% and the percentage of acetonitrile and methanol was 10% each. At the end of the gradient the percentage of organic phase was 95% and the percentage of acetonitrile and methanol was 47.5% each. The performance results of the three gradient applications are combined in Table 2.

The best resolution for both critical pairs is obtained for the ternary gradient.

Tramadol and related impurities were analyzed in another example (Figure 3). Impurities A, B, C and D are UV-active impurities and are present in a 0.02–0.03% range of the main compound (Figure 4). These impurities and the main compound tramadol were analyzed using ternary and quaternary gradients.

	ACN binary	MeOH Binary	ACN/MeOH ternary
Resolution N,N-diethyl-m-toluamid	1.35	1.85	3.56
Resolution Butylparaben		4.17	2.32
Resolution Toluene	6.87		
Peakwidth (HH) Heptylparaben	0.0312 min	0.0300 min	0.0304 min
Peakwidth (HH) Phenol	0.0312 min	0.0379 min	0.0342 min

Table 2
Performance data of binary and ternary gradients.

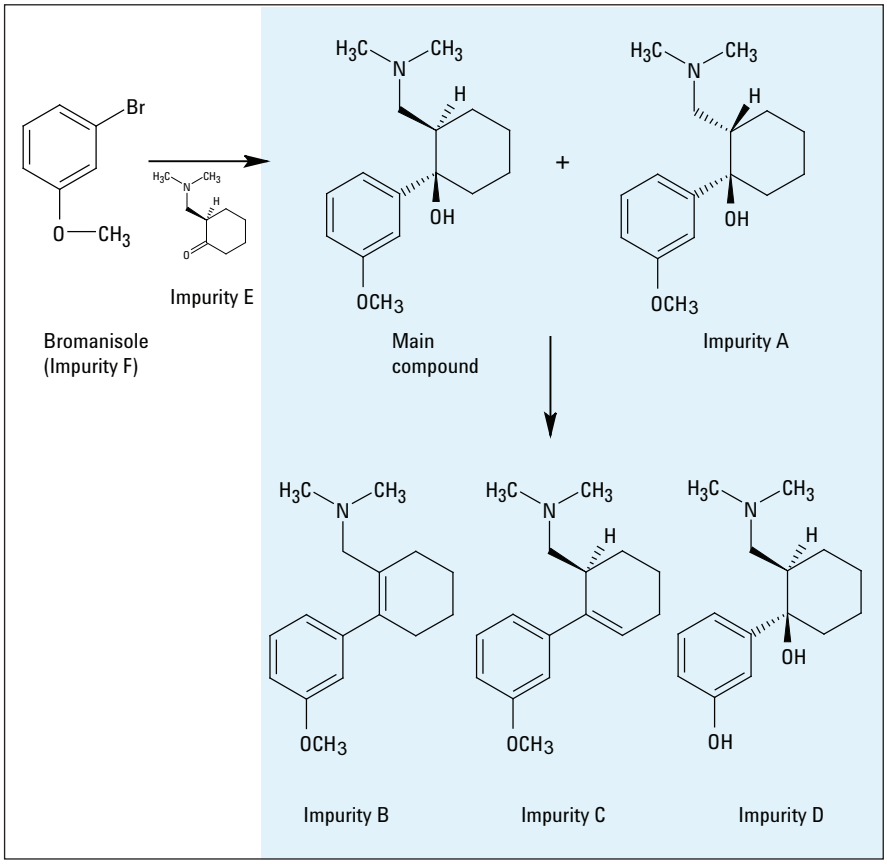


Figure 3
Chemical structure of tramadol and related impurities.

In the first experiment, the ionic strength was kept constant by adding 5% of a 2% TFA solution in water to a binary gradient of either acetonitrile/water or methanol/water resulting in a ternary gradient (Figure 5). Adding 5% of a 2% TFA solution means that the resulting concentration in the total mobile phase is 0.1% TFA. The total flow is 1.2 mL/min. The addition of 5% means that 0.06 ml/min of the TFA solution is mixed to the total flow rate.

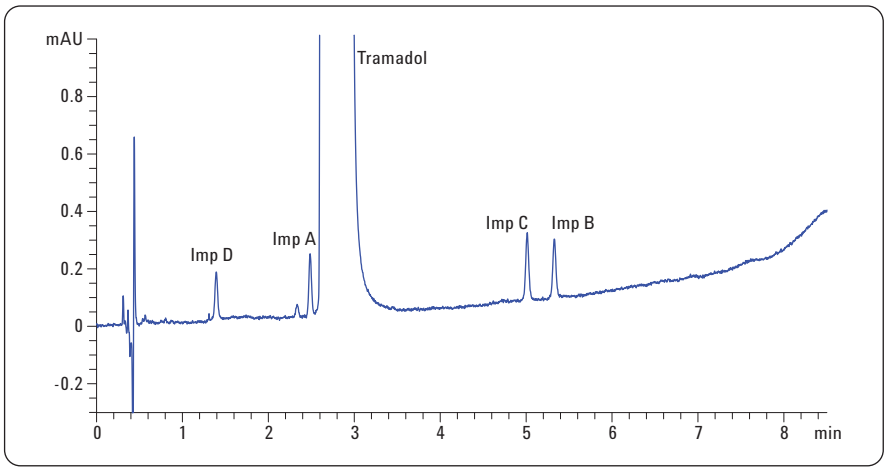


Figure 4
Tramadol and related impurities in a 0.02% to 0.03% range.

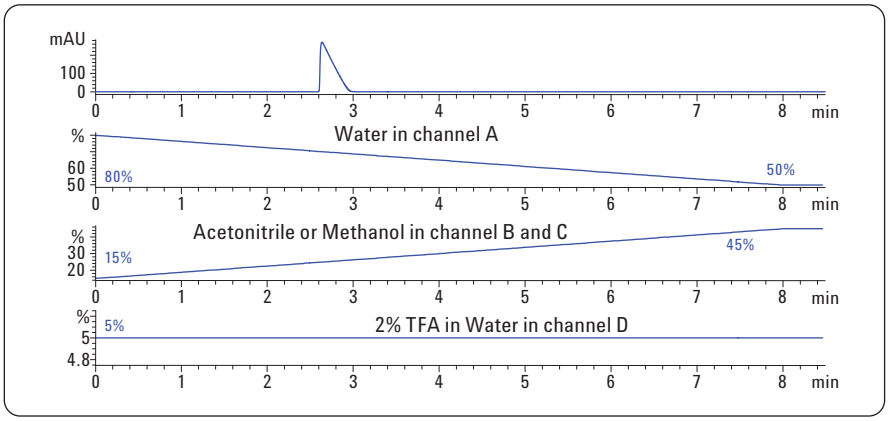


Figure 5
Keeping ionic strength constant for water/acetonitrile or water/methanol binary gradient.

Five percent of the aqueous TFA solution was added over the complete run. The upper trace shows the chromatogram of tramadol full scale, the second trace shows the percentage of the water phase. The third trace represents the percentage of organic phase and the fourth trace the percentage of the TFA solution. Figure 6 shows an overlay of three chromatograms obtained by adding the TFA solution as the third mobile phase.

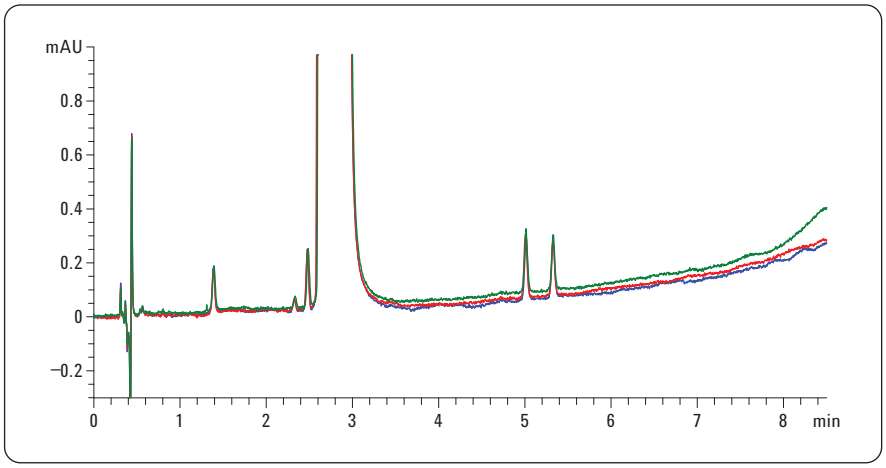


Figure 6
Overlay of three runs using a ternary gradient with acetonitrile. The ionic strength was kept constant by adding 5% of a 2% TFA aqueous solution using a third solvent channel.

Chromatographic method	
Column	Agilent ZORBAX Eclipse Plus C18, 3mm × 100 mm, 3.5 µm
Mobile Phase	A = Water
	B = Acetonitrile
	C = Methanol
	D = 2% TFA in Water
Gradient binary ACN	0 min 15 % B, 5% D
	8 min 45 % B, 5% D
Flow rate	1.2 mL/min
Stop time	8.5 min
Post time	3 min
Inj vol	3 µL
Column temp	40 °C
DAD	270/10 nm, Ref 360/100 nm,
Flow cell	10 mm
Peakwidth	> 0.013 min (20 Hz)

In the second experiment, a quaternary gradient optimized the separation. These results were compared to those obtained using a ternary gradient with acetonitrile and methanol as the organic phase (Figure 7).

Table 3 combines the results for the three different chromatograms. The quaternary gradient is the best compromise between resolution, signal-to-noise and run time.

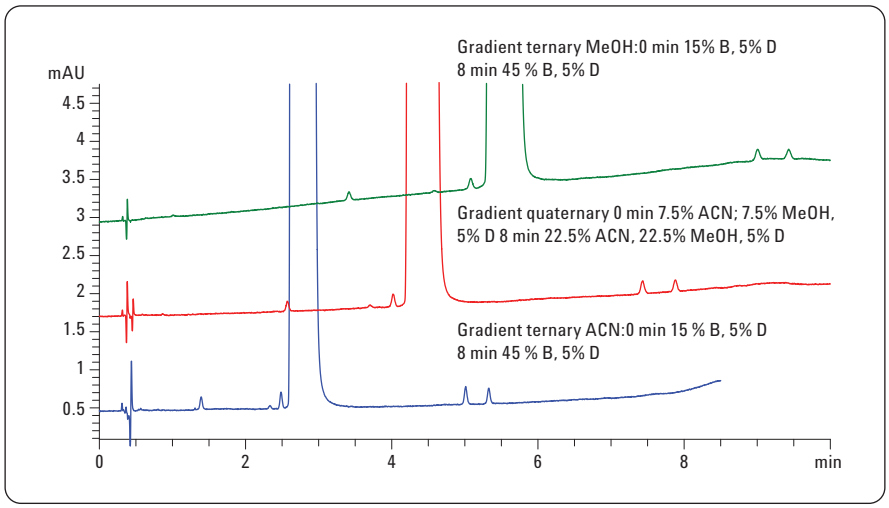


Figure 7
Optimizing the separation of tramadol and related impurities.

Chromatographic method	
Column	Agilent ZORBAX Eclipse Plus C18, 2mm × 100 mm, 3.5 µm
Mobile Phase	A = Water B = Acetonitrile C = Methanol D = 2% TFA in Water
Gradient ternary ACN	0 min 15 % B, 5% D 8 min 45% B, 5% D
Gradient tenary MeOH	0 min 15% B, 5% D 8 min 45 % B, 5% D
Gradient quaternary	0 min 7.5% ACN; 7.5% MeOH, 5% D 8 min 22.5% ACN, 22.5% MeOH, 5% D
Flow rate	1.2 mL/min
Stop time	8.5 min and 10 min
Post time	3 min
Inj vol	3 µL
Column temp	40 °C
DAD	270/10 nm, Ref 360/100 nm,
Flow cell	10 mm
Peakwidth	> 0.013 min (20 Hz)

	Ternary ACN	**Ternary MeOH**	**Quaternary ACN/MeOH**
Rs main	0.87	1.14	1.04
Rs Imp B	4.71	3.98	4.89
S/N Imp C (6*SD)	14.7	9	12.1
S/N Imp B (6*SD)	12.8	8.1	10.7

Table 3
Performance results of the ternary and quaternary gradients.

In the third experiment the quaternary gradient was applied onto a column packed with 1.8 µm particles. The dimensions and the chemistry of the column was the same as that for the column packed with 3.5 µm particles (Figure 8).

The column packed with 1.8 µm particles produced a smaller peakwidth and better signal-to-noise ratio (Table 4).

When using ternary and quaternary gradients, it is important that the precision of retention times and areas are in the same range than usually expected for binary gradients. Table 5 contains the precision data for six consecutive runs using the column packed with 1.8 µm particles. The precision for retention times is excellent for all peaks. The precision of areas is also very good for peaks with heights of approximately 0.2 mAU.

Conclusions

Ternary and quaternary gradients can be used to optimize separation, while keeping the ionic strength constant. This is applicable during method development to quickly change mobile phase composition, ionic strength or modifier concentration. It can also reduce solvent consumption by avoiding the need for many different mobile phase compositions and changes in ionic strength. Finally, computer modeling software is available, the optimum separation can be found logically and quickly.

References

1.
Melvin R. Euerby,"Retention modelling in ternary solvent-strength gradient elution reversed-phase chromatography using 30 cm Columns", *J. of Chrom. A*, 1121 (2006) 219-227.

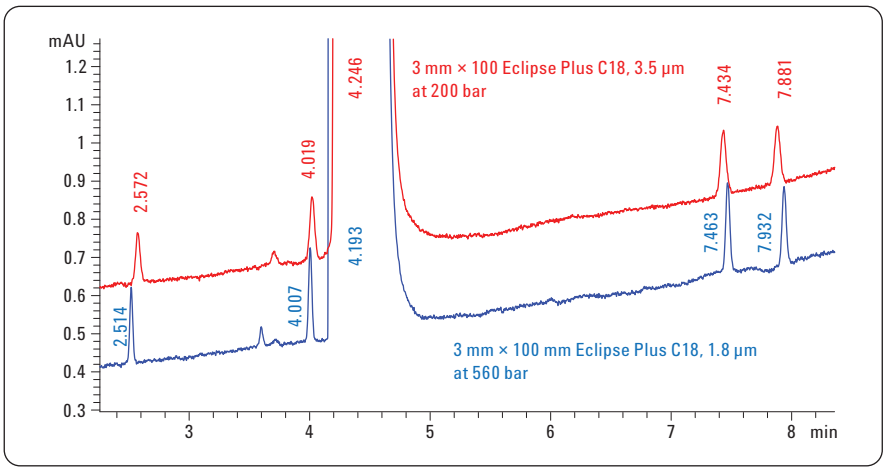


Figure 8
A 3.5 µm particle column versus 1.8 µm particle column for the separation of tramadol and related impurities using a quaternary gradient.

	Agilent ZORBAX Eclipse Plus C18, 3 mm × 100 mm, 1.8 µm	Agilent ZORBAX Eclipse Plus C18, 3 mm × 100 mm, 3.5 µm
S/N Imp C (6*SD)	14.97	12.1
S/N Imp B (6*SD)	13.1	10.7
Peak width HH Imp D	0.0283 min	0.0433 min
Peak width HH Imp B	0.0375 min	0.0550 mim

Table 4
Performance comparison of 1.8 µm particle column versus 3.5 µm particle column.

	RT %RSD	Area %RSD
Imp D	0.076	2.765
Imp A	0.049	2.662
Main (Tramadol)	0.065	0.192
Imp C	0.039	3.283
Imp B	0.055	2.909

Table 5
Precision data for a quaternary gradient analyzing impurities in the presence of a main compound.

www.agilent.com/chem/lc

September 1, 2010
Publication Number 5990-6439EN

Agilent Technologies