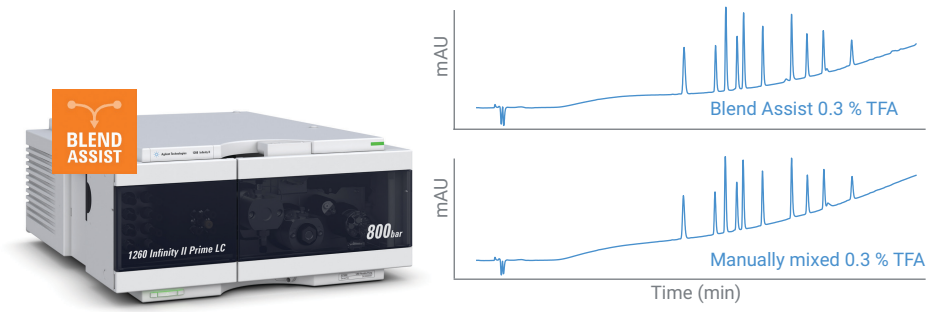
Technical Overview

Agilent 1260 Infinity II Flexible Pump with Blend Assist

Fast Optimization and Simplified Variation of Solvent Composition Using Blend Assist

Suitable for Agilent
1260 Infinity III LC



Authors

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Abstract

The Agilent 1260 Infinity II Flexible Pump with Blend Assist can be used to vary buffer or modifier concentration using ternary or quaternary gradients. This Technical Overview used Blend Assist for two different approaches:

- First, the Blend Assist functionality was used to test the effect of different trifluoroacetic acid (TFA) concentrations on the separation of a 10-peptide standard. The optimized method was evaluated regarding retention time precision, and compared to manually mixed mobile phases.
- Second was a multimethod experiment. In one sequence, three different groups of compounds were analyzed with varying concentrations of ammonium formate. For each group, the retention time precision demonstrated the excellent precision of the 1260 Infinity II Flexible Pump together with Blend Assist.

Introduction

Blend Assist is a software feature implemented in the driver of the Agilent 1260 Infinity II Flexible Pump. It uses the quaternary mixing capability of the 1260 Infinity II Flexible Pump for online dilution of a stock solution (buffer or modifier).

To optimize the separation of compounds by changing buffer or modifier concentration, Blend Assist can be used during LC method development. The software tool circumvents the elaborate, time-consuming mixing of various concentrations of a buffer or modifier. Using the quaternary LC system, one or two channels contain a high concentration of buffer/modifier (stock solution); by defining the desired concentration, Blend Assist dilutes the solvents[1].

Another area of application is a multimethod approach. For this approach, three sample mixtures and three methods with different modifier concentrations are included in one sequence. By enabling Blend Assist, the different methods can run without interrupting the system to exchange the mobile phases.

Experimental

Instrumentation

For the different experiments, the following modules were used:

- Agilent 1260 Infinity II Flexible Pump (G7104C) with V380 Jet Weaver Mixer (option #070)
- Agilent 1260 Infinity II Vialsampler (G7129C), equipped with an integrated sample cooler (option #100)
- Agilent 1260 Infinity II MCT (G7116A)
- Agilent 1260 Infinity II Diode Array Detector HS (G7117C), equipped with a Max-Light cartridge cell: 10 mm cell path length and σV = 1.0 µL

Software

Agilent OpenLAB CDS (M8413A)

Chemicals

All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak). Acetonitrile was purchased from Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA) and ammonium formate were purchased from Sigma-Aldrich (Steinheim, Germany).

Methods

Table 1. Chromatographic conditions for the analysis of a 10-peptide mixture.

Parameter	Value
Compounds	Agilent 10 peptide standard (p/n 5190-0583)
Column	Agilent AdvanceBio Peptide Map, 2.1 × 100 mm, 2.7 µm (p/n 655750-902)
Solvent	A) Water B) Acetonitrile C) Water + 1 % TFA (solvent A additive) D) Acetonitrile + 1 % TFA (solvent B additive)
Gradient	0 minutes 5 %B 4 minutes 65 %B
Stop time	5 minutes
Post time	3 minutes
Flow rate	1 mL/min
Injection	Injection volume: 2 µL with 3 seconds needle wash (60 % acetonitrile in water) Sample temperature: 10 °C
Column temperature	55 °C
DAD	220/4 nm, Ref. 360/80 nm, 20 Hz

Table 2. Chromatographic conditions for the analysis of sulfa drugs, a testosterone mix, and the Sigma Peptide Standard.

Parameter	Value		
Column	Agilent ZORBAX StableBond 80Å C18, 2.1 × 50 mm, 1.8 µm, 1200 bar (p/n 857700-902)		
Solvent	A) Water B) Acetonitrile C) 250 mM Ammonium formate (solvent A additive)		
Compounds	**Sulfa drugs:** Sulfanilamide, Sulfathiazole, Sulfachloropyridazine, Sulfamethazine (100 ng/µL each)	**Testosterone mix:** Testosterone c-IIIN 50 ng/µL and Testosterone acetate 25 ng/µL	Sigma Peptide Standard (Sigma H2016)
Blend Assist setting	200 mM Ammonium formate (channel A + C as additive)	50 mM Ammonium formate (channel A + C as additive)	25 mM Ammonium formate (channel A + C as additive)
Gradient	0 minutes 10 % B 0.5 minutes 10 % B 4 minutes 30 % B 4.2 minutes 50 % B	0 minutes 45 %B 3 minutes 95 %B	0 minutes 2 %B 0.5 minutes 2 %B 6 minutes 28 %B 7 minutes 95 %B
Stop time	5 minutes	4 minutes	7 minutes
Post time	3 minutes	3 minutes	3 minutes
Flow rate	0.5 mL/min	1 mL/min	0.5 mL/min
Injection	Injection volume: 1 µL with 3 seconds needle wash (60 % acetonitrile in water) Sample temperature: 10 °C	Injection volume: 5 µL with 3 seconds needle wash (60 % acetonitrile in water) Sample temperature: 10 °C	Injection volume: 2 µL with 3 seconds needle wash (60 % acetonitrile in water) Sample temperature: 10 °C
Column temperature	60 °C	30 °C	35 °C
DAD	254/8 nm, Ref. 360/100 nm, 20 Hz	254/8 nm, Ref. 360/100 nm, 20 Hz	220/8 nm, Ref 360/100 nm; 20 Hz

Results and Discussion

Changing TFA Concentration to Optimize the Separation of a 10-Peptide Mixture

To improve separation of the Agilent 10-peptide mixture, the 1260 Infinity II Flexible Pump with Blend Assist was used to test different TFA concentrations in the mobile phases.

By changing the TFA concentration from 0.05 to 0.3 %, the separation of the peptides was clearly improved (Figure 1). With 0.05 % TFA in the mobile phases, peak 4 (Neurotensin) and peak 5 (Angiotensin I) coeluted; with 0.2 % TFA, both peaks were baseline separated. By increasing to 0.3 % TFA, the resolution between these peaks could be further improved, from 1.77 to 2.25. Table 3 shows a detailed overview of the resolution for the different peptides under different modifier concentrations.

Using 0.3 % TFA in the mobile phases, the peptides of the mixture were well separated, with a resolution of above 2 for all compounds.

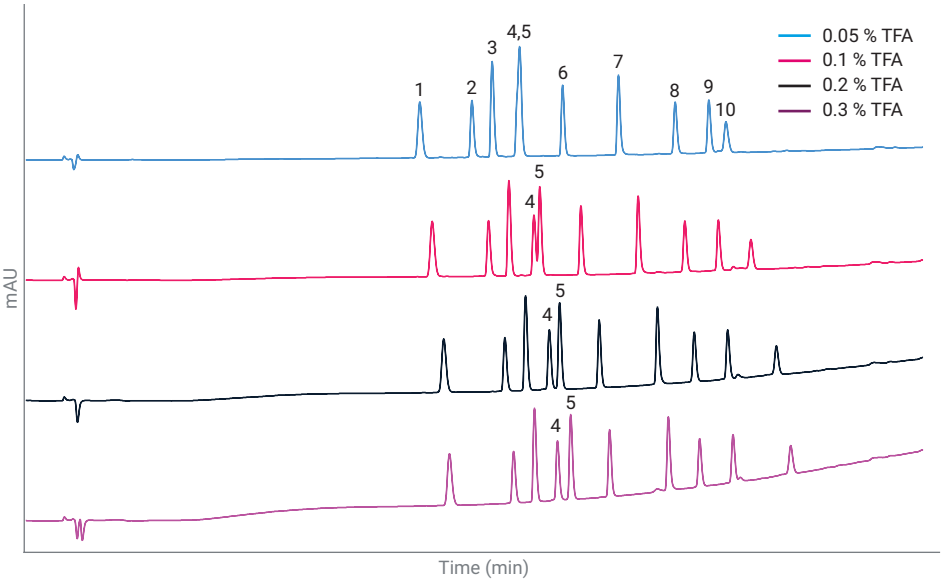


Figure 1. Impact of increasing TFA concentration for the separation of the Agilent 10 peptide standard. TFA concentration was changed using Blend Assist.

Table 3. Overview of the resolution for different TFA concentrations using Blend Assist.

Compound	Resolution			
	0.05 % TFA	0.1 % TFA	0.2 % TFA	0.3 % TFA
1. Bradykinin frag (1-7)	–	–	–	–
2. Bradykinin acetate	7.27	7.93	8.49	8.80
3. Angiotensin II	3.34	3.39	3.42	3.43
4. Neurotensin	3.37	4.21	4.05	3.92
5. Angiotensin I		0.99	1.77	2.25
6. Renin	6.02	7.07	6.87	6.74
7. [Ace-F-3,-2 H-1] Angiotensinogen (1-14)	9.88	10.11	10.15	10.24
8. Ser/Thr Protein Phosphatase (15-31)	9.68	8.01	6.26	5.33
9. [F14] Ser/Thr Protein Phosphatase (15-31)	5.51	5.59	5.60	5.57
10. Melittin	2.47	5.06	7.45	8.82

Next, the modifier concentration of 0.3 % TFA in the mobile phases were mixed manually and used for the analysis of the 10-peptide standard. Figure 2 shows a direct comparison of the chromatograms acquired using Blend Assist and using manually mixed mobile phases. Both chromatograms are similar. Table 4 shows a detailed comparison regarding retention time (RT), RT precision (RT RSD), and resolution. Both running conditions obtained excellent agreement for RT and resolution. The RT precision was slightly better for the runs using manually mixed solvents, however under both conditions the RT RSD was clearly below the specification of 0.15 %.

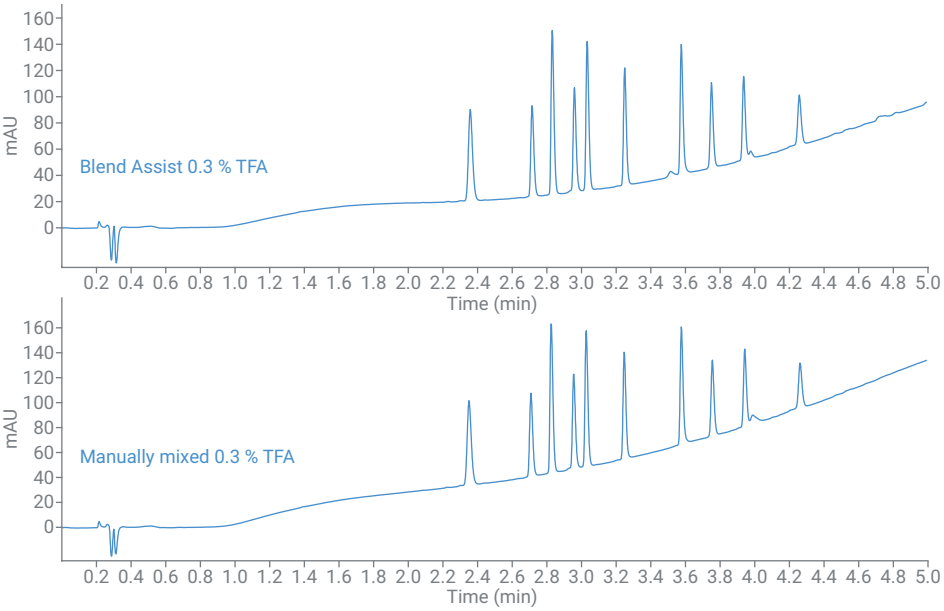


Figure 2. Comparison of the chromatograms for the analysis of a 10 peptide standard with and without Blend Assist.

Table 4. Comparison of the retention time and RT RSD for runs using Blend Assist, and runs using manually mixed 0.3 % TFA. Six consecutive runs in both setups were used for calculation.

Peptide	Blend Assist 0.3 % TFA			Manual mixed 0.3 % TFA		
	RT (min)	RT RSD (%)	Resolution	RT (min)	RT RSD (%)	Resolution
1. Bradykinin frag (1-7)	2.366	0.017	–	2.359	0.008	–
2. Bradykinin acetate	2.725	0.020	8.80	2.717	0.007	8.73
3. Angiotensin II	2.841	0.019	3.43	2.834	0.007	3.42
4. Neurotensin	2.970	0.018	3.92	2.965	0.008	3.92
5. Angiotensin I	3.044	0.016	2.25	3.037	0.009	2.18
6. Renin	3.261	0.013	6.74	3.258	0.009	6.75
7. [Ace-F-3,-2 H-1] Angiotensinogen (1-14)	3.590	0.018	10.24	3.588	0.010	10.22
8. Ser/Thr Protein Phosphatase (15-31)	3.764	0.019	5.33	3.768	0.005	5.42
9. [F14] Ser/Thr Protein Phosphatase (15-31)	3.591	0.016	5.57	3.956	0.005	5.63
10. Melittin	4.272	0.011	8.82	4.275	0.015	8.82

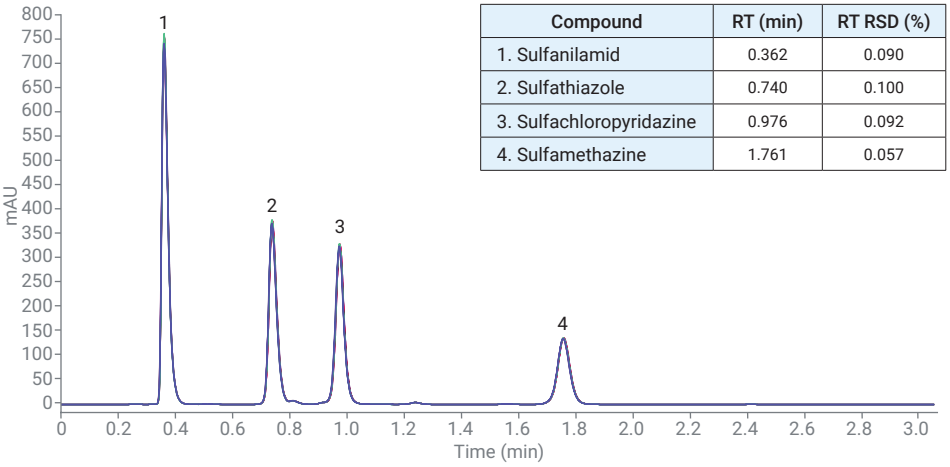
Analysis of Three Different Compound Mixtures by Varying the Buffer Concentration in a Multimethod Run

For this approach, three different sample mixtures were analyzed: sulfa drugs, a testosterone mix, and a Sigma peptide standard. All three types of analytes require a different concentration of ammonium formate in the aqueous phase for optimal separation. These analyses can be run in single sequences, exchanging the solvents for every method, or the analyses can be performed in one sequence, as performed in this Technical Overview. Using Blend Assist, the buffer concentration in the aqueous phase can be changed online for every method; therefore, running the three applications in one sequence is possible.

Figure 3 shows the analysis of four sulfa drugs: sulfanilamide, sulfathiazole, sulfachloropyridazine, and sulfamethazine. For the separation of the sulfa drugs, 200 mM ammonium formate was used as modifier. Excellent retention time precision was obtained for all peaks.

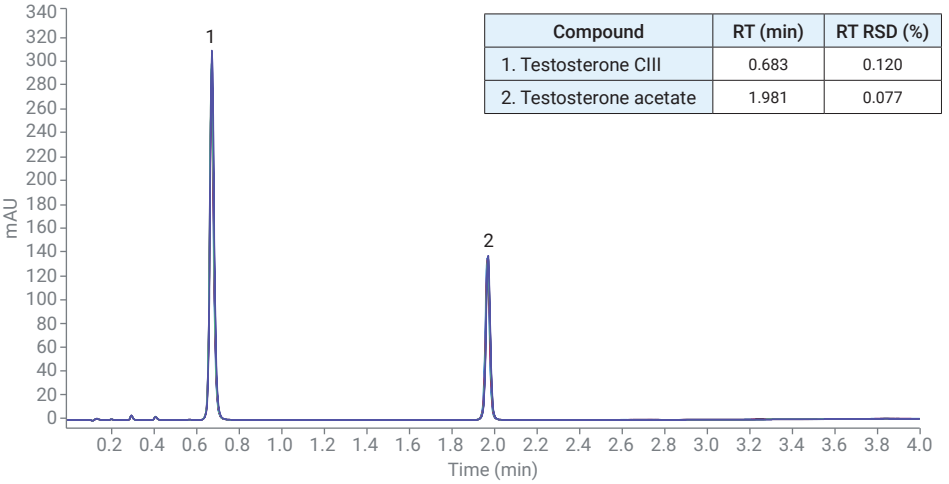
Figure 4 shows the analysis of testosterone CIII and testosterone acetate for six consecutive runs. Blend Assist was enabled to mix 50 mM ammonium formate into the aqueous phase. Excellent retention time precision was achieved for both peaks.

Figure 5 shows the analysis of the Sigma peptide standard. The analysis was performed with 25 mM ammonium formate in the aqueous phase. Excellent retention time precision, well below the specification of 0.15 %, was achieved.



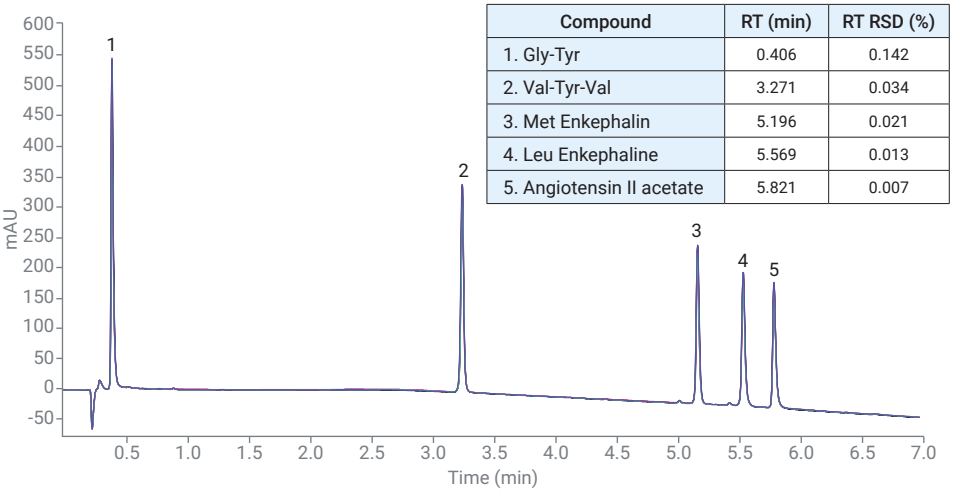
Compound	RT (min)	RT RSD (%)
1. Sulfanilamid	0.362	0.090
2. Sulfathiazole	0.740	0.100
3. Sulfachloropyridazine	0.976	0.092
4. Sulfamethazine	1.761	0.057

Figure 3. Separation of sulfa drugs: overlay of six consecutive runs.



Compound	RT (min)	RT RSD (%)
1. Testosterone CIII	0.683	0.120
2. Testosterone acetate	1.981	0.077

Figure 4. Separation of testosterone CIII and testosterone acetate: overlay of six consecutive runs.



Compound	RT (min)	RT RSD (%)
1. Gly-Tyr	0.406	0.142
2. Val-Tyr-Val	3.271	0.034
3. Met Enkephalin	5.196	0.021
4. Leu Enkephaline	5.569	0.013
5. Angiotensin II acetate	5.821	0.007

Figure 5. Separation of a Sigma peptide standard: overlay of six consecutive runs.

Conclusion

The Agilent 1260 Infinity II Flexible Pump with Blend Assist allows a straightforward variation of modifier/buffer concentration, and circumvents the need for manually mixing different concentrations. This Technical Overview demonstrates two different use cases for Blend Assist. In the first example, the TFA concentration in the mobile phases was changed to optimize the separation of a 10-peptide standard. Excellent agreement for retention and resolution was obtained by comparing the Blend Assist method with runs using manually mixed solvents.

In the second example, the concentration of the modifier ammonium formate was changed in three different methods for the analysis of sulfa drugs, a testosterone mix, and a peptide mix in one sequence. Excellent retention time precision was obtained for all three application examples.

Reference

1. Huesgen A.G. Fast and Flexible Optimization of Modifier Concentrations Using an Agilent 1290 Infinity LC System with Blend Assist, *Agilent Technologies Technical Overview*, publication number 5991-2169EN, **2013**.

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DE83399017

Printed in the USA, October 15, 2024
5991-9088EN

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