



Agilent 1290 Infinity Binary LC System with ISET – Emulation of a Waters Alliance 2695 LC Applying Concave, Convex, and Linear Gradients

Suitable for Agilent
1290 Infinity III LC

Author

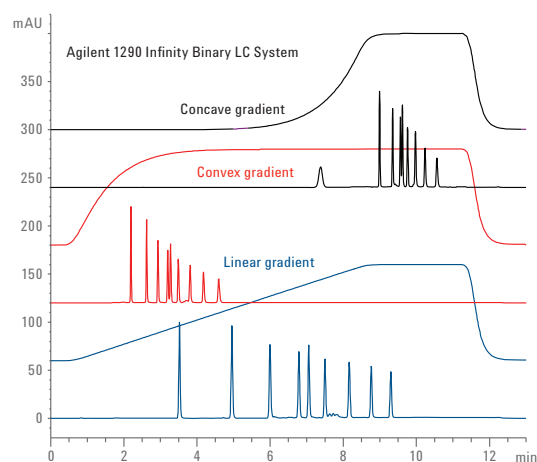
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Technical Overview

Abstract

The Agilent 1290 Infinity Binary LC System equipped with Intelligent System Emulation Technology (ISET) can emulate any Agilent 1200 Infinity Series LC, as well as Agilent 1100 and 1200 Series LCs and non-Agilent LCs, enabling seamless instrument-to-instrument method transfer.

This Technical Overview shows the successful emulation of a Waters Alliance 2695 LC applying concave, convex, and linear gradients.



Verified for Agilent
1290 Infinity II LC



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Introduction

Seamless instrument-to-instrument method transfer is often a strong demand for most industries. Changing established methods is expensive and time consuming, and comparison with reference data is difficult. Equipment has to be replaced from time to time. The Agilent 1290 Infinity Binary LC System with ISET allows emulating legacy Agilent LC systems as well as many 3rd-party LC instruments, such as the Waters Alliance 2695 LC systems^{1,2,3}.

Gradients in HPLC are, in most cases, linear. The Alliance 2695 LC also allows concave and convex gradients, even though these gradients are rarely used.

The 1290 Infinity Binary LC System with ISET is able to emulate these gradient curves.

In the following experiments, one concave, one convex, and one linear gradient is applied using the 1290 Infinity Binary LC System with ISET emulating a Alliance 2695 LC system. The same gradients were applied to the Alliance LC, and results of both systems are compared.

Experimental

Compounds

RRLC checkout sample, (p/n 5188-6529)

Instrumentation

The following instruments were used:

Agilent 1290 Infinity Binary LC System with:

- Agilent 1290 Infinity Binary Pump G4220A

- Agilent 1290 Infinity Autosampler G4226A
- Agilent 1290 Infinity Thermostat G1330B
- Agilent 1290 Infinity Thermostatted Column Compartment G1316C
- Agilent 1290 Infinity Diode Array Detector G4212A
- Waters Alliance 2695 with Dual Absorbance Detector VWD 2487

Chromatographic conditions

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm (p/n 959996-902)
Temperature	30 °C
Solvent A1	Water
Solvent B2	ACN
Flow rate	1.2 mL/min
Gradient	10 to 90 % ACN using linear (6), concave (10), and convex (2) gradients
Injection	3 µL, sample temperature 10 °C
Detection	Diode Array Detection 245/10 nm, Ref: 400/100 nm, 10 Hz
Acquisition and evaluation software	OpenLAB CDS, ChemStation Rev.C.01.05

Results and Discussion

The following experiments proved that linear, convex, and concave gradients applied to the 1290 Infinity Binary LC System with ISET can emulate the Alliance 2695 LC:

- Analysis of RRLC checkout sample on the 1290 Infinity Binary LC System with ISET; emulating an Alliance 2696 LC using the linear, the most concave, and the most convex gradient available on the Alliance LC system.
- Analysis of the RRLC checkout sample on the Alliance LC system using the same three chromatographic conditions.

Figure 1 shows the applied gradient curves, and the related chromatograms for both instruments.

The chromatograms are overlaid with the percentage of the organic phase (1B).

Using ISET on the 1290 Infinity Binary LC System enabled the formation of concave and convex gradients, and resulted in chromatograms with high similarity for both instruments.

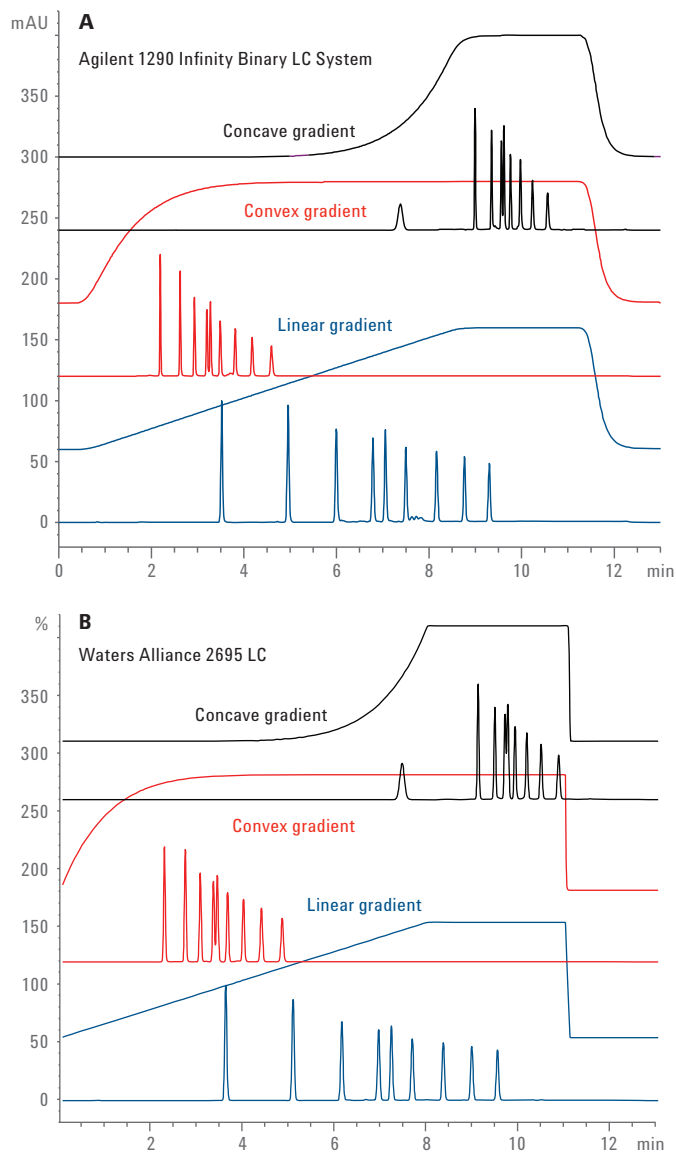


Figure 1. Overlay of % B and related normalized chromatograms on a Waters Alliance 2695 LC and an Agilent 1290 Infinity Binary LC System with ISET.

In Figure 2, the appropriate chromatograms of the 1290 Infinity Binary LC System with ISET and of the Alliance 2695 LC are overlaid representing the linear, convex, and the concave gradient.

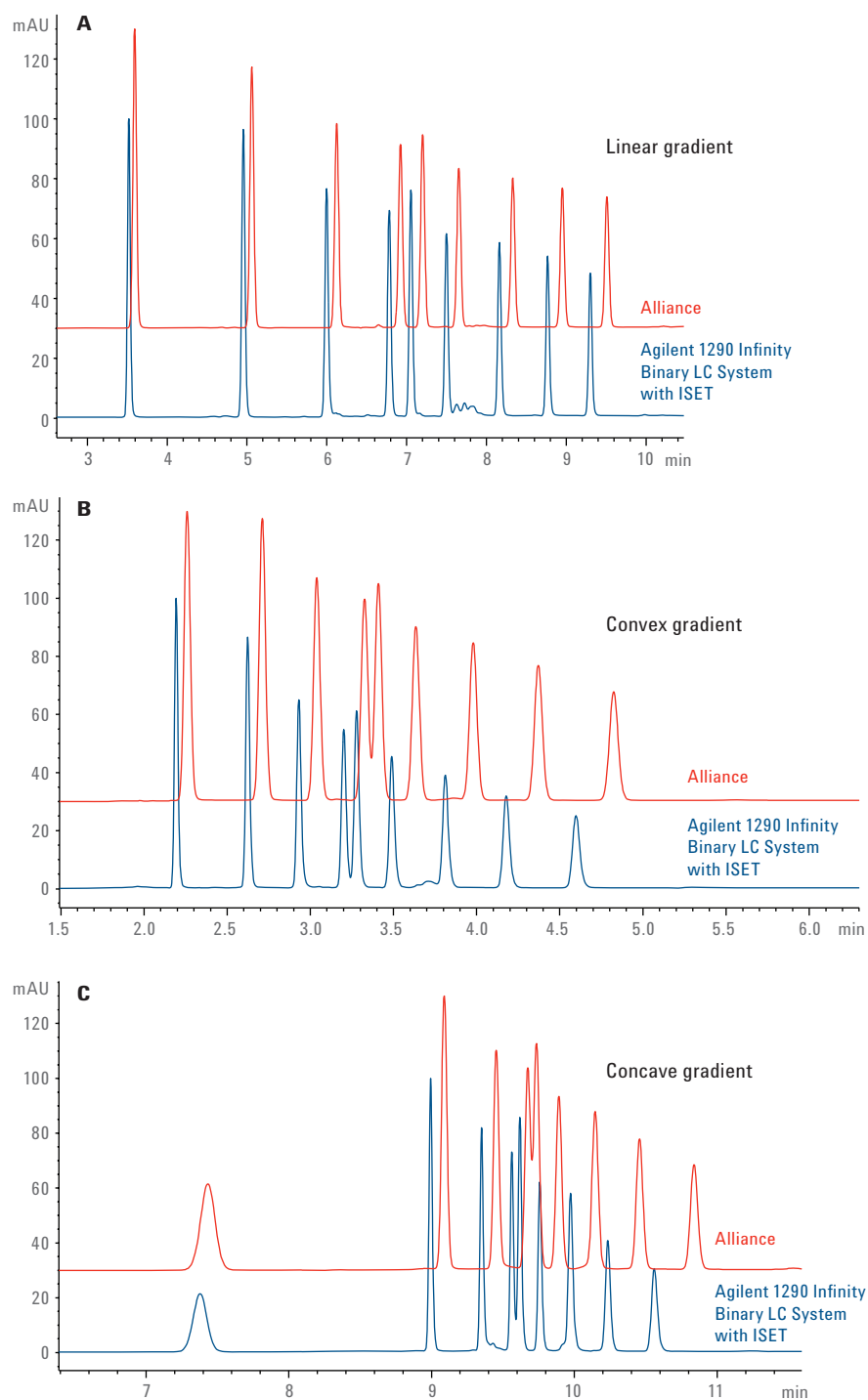


Figure 2. Overlay of normalized chromatograms obtained by the linear, convex, and concave gradient on both instruments.

Evaluated parameters

The deviation of retention times for the 1290 Infinity Binary LC System using ISET was evaluated. The specified allowed deviation is $< \pm 5\%$. Figure 3 shows the combined results.

The retention time deviation was within the specified limit. In all cases, the 1290 Infinity Binary LC System showed less retention while emulating the Alliance LC than the Alliance LC itself. The deviation for the linear gradient was $< 2.2\%$, for the convex gradient $< 4.7\%$, and for the concave gradient $< 2.6\%$.

In the ISET parameter screen, fine tuning parameters can be set to improve results. For example, additional fine tuning was applied to the convex gradient (Figure 4). An additional 100- μL delay volume for the 1290 Infinity Binary LC System was added in the fine tuning part of the ISET parameters screen.

Without fine tuning, the RT deviation was $< 4.7\%$; with fine tuning, the RT deviation was $< 2.25\%$.

In addition, the deviation of the resolution was determined. The specified allowed maximum deviation was $< -5\%$. Typically, the resolution was significantly better on the 1290 Infinity Binary LC System, as expected (Figure 5). The improved resolution was primarily due to the significantly reduced extra-column volume of the 1290 Infinity Binary LC System, which provided lower peak width and, consequently, better resolution.

Conclusion

The Agilent 1290 Infinity Binary LC System in combination with ISET, emulated concave and convex gradients that were used on the Waters Alliance LC system. The deviation of retention time without fine tuning was less than 4.7% , and less than 2.6% with fine tuning. The resolution on the 1290 Infinity Binary LC System was significantly improved.

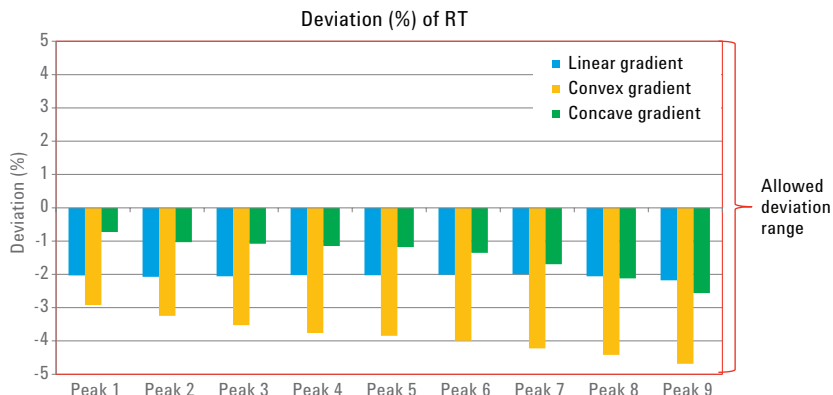


Figure 3. Deviation of retention times as percentage for the linear, convex, and concave gradient on an Agilent 1290 Infinity Binary LC System with ISET.

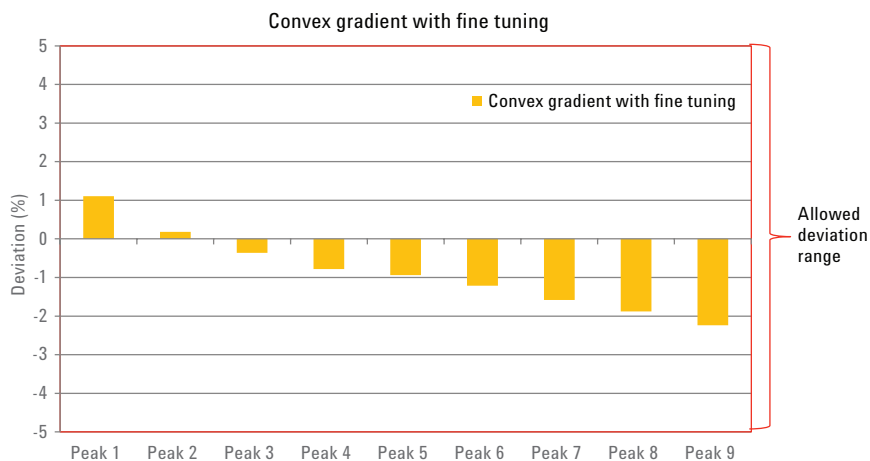


Figure 4. Influence of fine tuning on retention time deviation of the convex gradient.

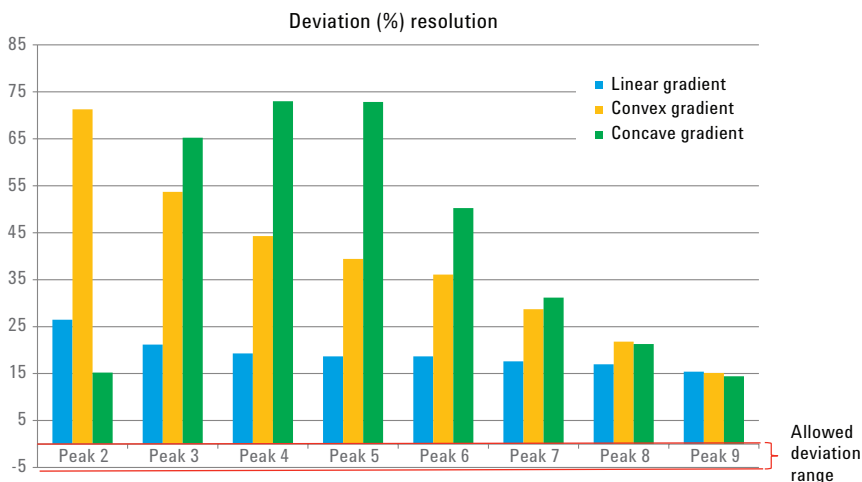


Figure 5. Improvement of resolution using an Agilent 1290 Infinity Binary LC System with ISET, emulating an Alliance LC.

References

1. Agilent 1290 Infinity Binary LC and 1290Infinity Quaternary LC with Intelligent System Emulation Technology, *Agilent Technologies* publication number 5991-3369EN, **2013**.
2. Huesgen, A.G. Seamless instrument-to-instrument method transfer from an Agilent 1100/1200 Series LC to an Agilent 1290 Infinity LC using Intelligent System Emulation Technology (ISET), *Agilent Technologies Technical Overview*, publication number 5990-9113EN, **2011**.
3. Huesgen, A.G. Agilent 1290 Infinity Binary LC with ISET - Emulation of the Waters Alliance 2695 LC system analyzing endocrine disruptors, *Agilent Technologies Application Note*, publication number 5991-1605EN, **2013**.

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