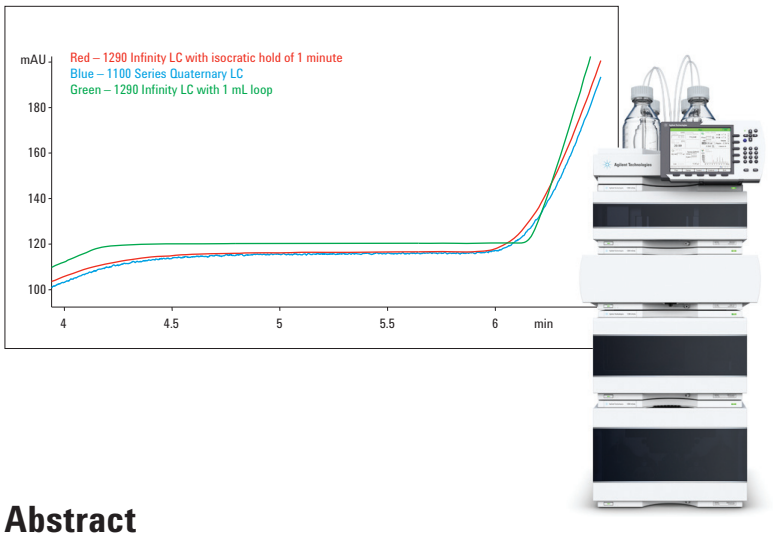
Comparing gradient transfer of isocratic hold and delay volume addition using the Agilent 1290 Infinity LC with ISET

Technical Overview



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Abstract

Agilent Intelligent System Emulation Technology (ISET) is a function within the Agilent 1290 Infinity LC System that allows to emulate other systems for seamless transfer of methods between LC instruments. Using ISET, retention times and resolution obtained on the Agilent 1290 Infinity LC are the same as obtained on the Agilent 1100 Series Quaternary LC system.

In this Technical Overview, we demonstrate that a gradient run on an Agilent 1100 Series LC correlates nearly 100% with the gradient profile of an Agilent 1290 Infinity LC System with ISET. Neither the addition of an isocratic step, nor the installation of an additional delay volume can deliver the same good correlation results.

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Introduction

Instrument-to-instrument method transfer is often problematic, especially in highly regulated environments, because any modifications of the original method should be avoided. Agilent Intelligent System Emulation Technology (ISET) provides seamless method transfer, for example, between earlier Agilent 1100/1200 Series LCs and Agilent 1220/1260 Infinity LCs to the Agilent 1290 Infinity LC System.[1] Legacy methods can run unchanged, retention times and resolution with the same as obtained from the emulated LC.

An important criterion for a seamless method transfer is whether gradient profiles obtained from a conventional LC can be transferred to the Agilent 1290 Infinity LC with ISET with high correlation. High correlation ensures that retention times and resolution will correlate to a high extent.

Another issue of interest is whether the addition of an isocratic step or the addition of an additional delay volume can compete.

Tracer experiments were chosen because they can deliver more complete correlation information than the injection of arbitrary selected peaks.

This Technical Overview details the following experiments:

- Transfer of a gradient from an Agilent 1100 Series Quaternary LC to an Agilent 1290 Infinity LC without ISET
- Applying the same gradient on an Agilent 1290 Infinity LC with an isocratic hold at the beginning of the run
- Applying the same gradient on the Agilent 1290 Infinity LC with an additional delay volume installed
- Applying the same gradient to the Agilent 1290 Infinity LC with ISET

All gradient curves were compared and the advantage of the ISET function is shown.

Experimental

Instrumentation

Table 1 shows the configurations of the instrumentation used for the tracer experiments.

Software

Agilent Chemstation revision C 01.03 and ISET revision 1.0.

Chromatographic conditions

The chromatographic conditions used for all gradients were as follows:

Compound:	Uracil as tracer, 10 mg/L in methanol
Column:	Restriction capillary
Mobile phases:	Water (A)/methanol with tracer (B)
Flow rate:	1 mL/min
Column temperature:	30 °C
Diode Array Detector:	254/4 nm, Ref. 360/100 nm, 10 Hz
Gradients:	Table 2

	Agilent 1100 Series Quaternary LC	Agilent 1290 Infinity LC with ISET
Module	**Product number**	**Product number**
Pump	G1311A manufactured in 2003	G4220A
Autosampler	G1313A	G4226A
Thermostat	none	G1330B
Column Compartment	G1316A	G1316C
Detector	G1315B	G4212A

Table 1
Instrumentation used for the experiments.

Tracer (%)	Time (min) *	Additional 1 mL loop on an Agilent Infinity LC	Isocratic hold 1 min on an Agilent Infinity LC Time (min)	Isocratic hold 0.95 min on an Agilent Infinity LC Time (min)
5	0	0	0	0
5	n/a	n/a	1	0.95
20	3	3	4	3.95
20	5	5	6	5.95
60	6	6	7	5.95
65	9	9	10	9.95
95	10	10	11	10.95

*** applied on 1100, 1290 Infinity LC with and without ISET.**

Table 2
Applied gradient on an Agilent 1100 Series LC and on an Agilent 1290 Infinity LC with and without ISET. Also applied on an Agilent 1290 Infinty LC with additional delay volume and two different isocratic holds.

Results and discussion

Typically, the transfer of a gradient from a conventional LC instrument to an UHPLC system will not deliver a good correlation (Figure 1).

Due to the significantly lower delay volume of the Agilent 1290 Infinity LC System, the gradient is shifted to shorter elution times. In addition, the mobile phase changeovers before and after the isocratic section show a sharper profile, which is characteristic of the Agilent 1290 Infinity LC with a delay volume of about 140 µL.

To overcome the transfer problems shown in Figure 1, two solutions are typically used. Either an isocratic hold is added at the beginning of the gradient, or the delay volume is increased by adding physical delay volume. Both methods can only compensate the smaller delay volume but not the overall gradient behavior (transition volume, mixing performance) over the complete gradient and run time. To compensate the different delay volumes, a 1-minute isocratic hold (simulates 1 mL of additional delay volume) was added to the method. In a second experiment, a 1-mL loop was installed between the pump and the autosampler on the Agilent 1290 Infinity LC. The resulting gradient curves from those two experiments were compared with the original gradient curve from an Agilent 1100 Series Quaternary LC (Figure 2).

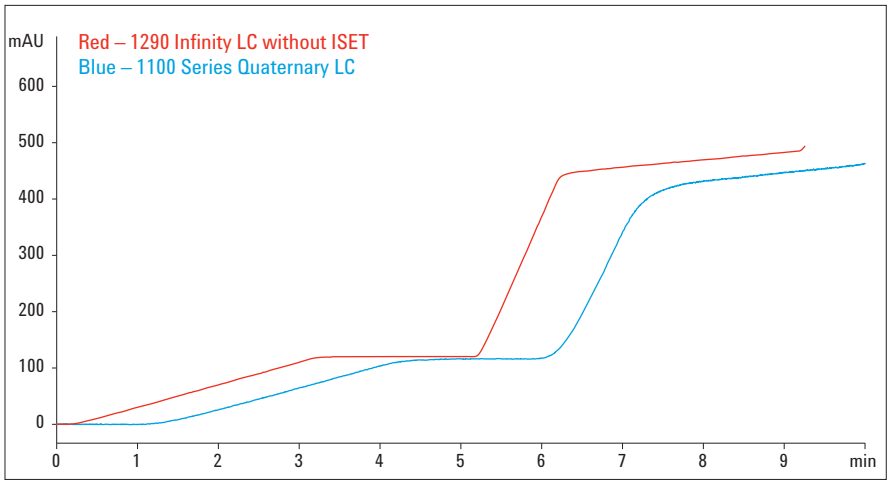

Figure 1
Transfer of gradient from the Agilent 1100 Series Quaternary LC to the Agilent 1290 Infinity LC without ISET.

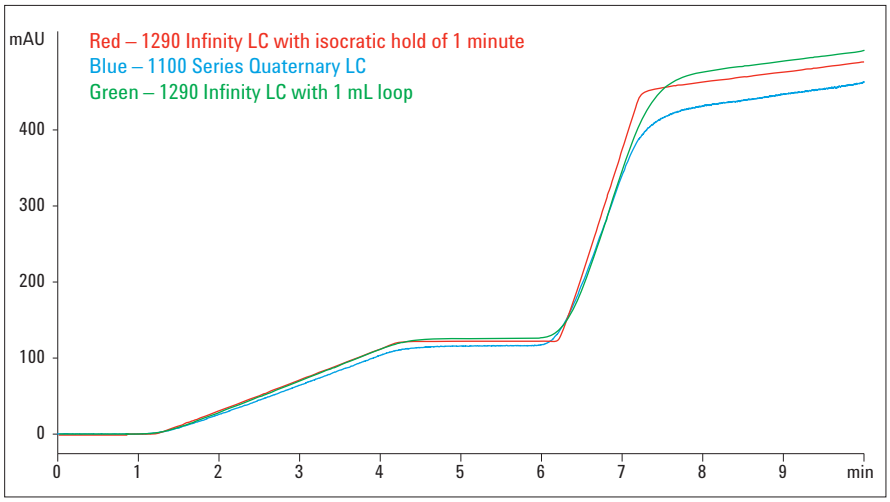

Figure 2
Overlay of the original curve with the curve obtained on the Agilent 1290 Infinity LC with isocratic hold of 1 minute and the addition of a 1-mL loop.

The most critical parts of a gradient are significant mobile phase changes. Figure 3 shows a more detailed view of before and after the isocratic range of the applied gradient, between 4 and 6.5 minutes.

Neither curve follows the original curve satisfactorily. However, using an isocratic hold allows the user to optimize the length of the isocratic hold at the beginning of the chromatogram. Figure 4 shows a better correlation by optimizing the isocratic hold and changing it to 0.95 minutes.

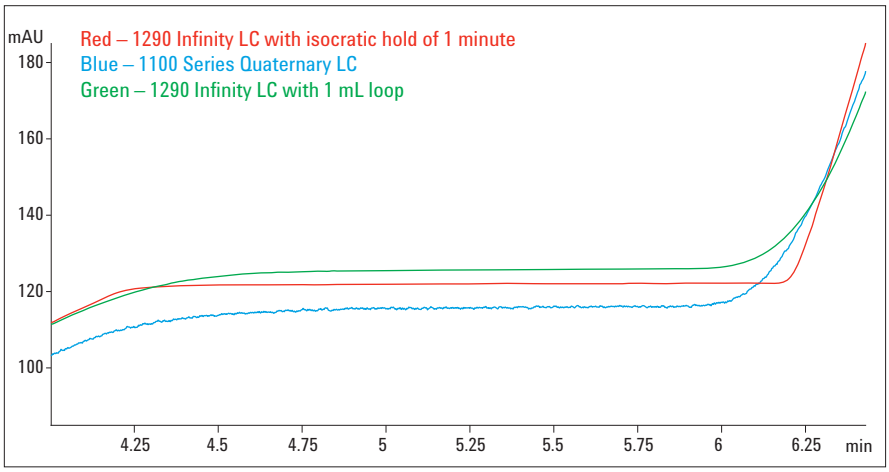

Figure 3
Enhanced part around the isocratic range of the gradient.

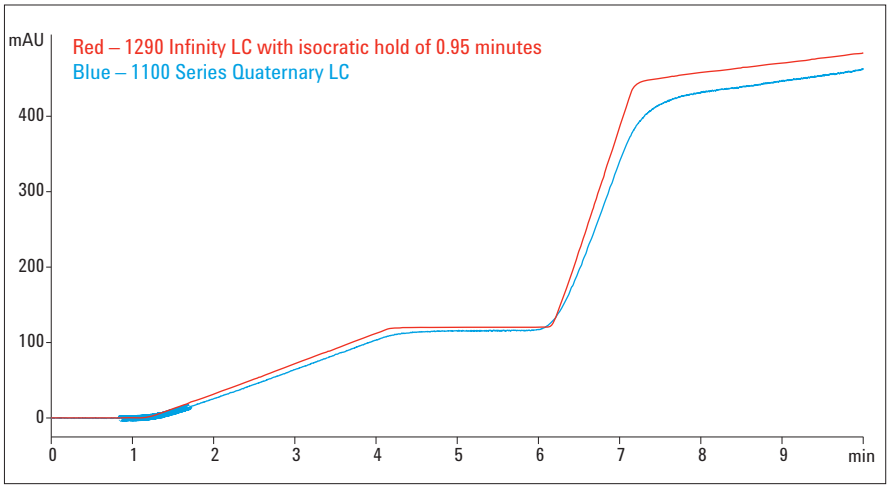

Figure 4
Overlay of gradient curve obtained on the Agilent 1100 Series Quaternary LC and an Agilent 1290 Infinity LC with an isocratic hold of 0.95 minutes.

Correlation of the gradients from an Agilent 1100 Series Quaternary LC and an Agilent 1290 Infinity LC with a 0.95-minute isocratic hold is better than a 1-minute isocratic hold, but still not as optimal as the one shown in the enlarged view in Figure 5.

The gradients on the Agilent 1290 Infinity LC with the isocratic hold are still different, in particular the changes to and from the isocratic and the gradient slopes.

In the last experiment, the ISET function of the Agilent 1290 Infinity LC was used to emulate the gradient of the Agilent 1100 Series Quaternary LC. Figure 6 shows the complete gradient of the 1100 Series LC (blue trace) the 1290 Infinity LC with ISET (red trace) and the 1290 Infinity LC with an isocratic hold of 0.95 minutes (green trace).

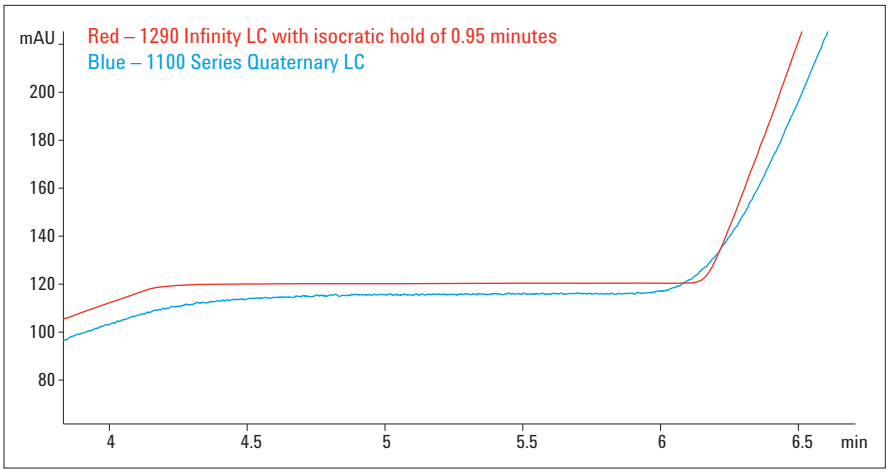

Figure 5
Overlay of an Agilent 1100 Series Quaternary LC gradient with curve obtained on an Agilent 1290 Infinity LC with an isocratic hold of 0.95 minutes (zoomed view around 5 minutes).

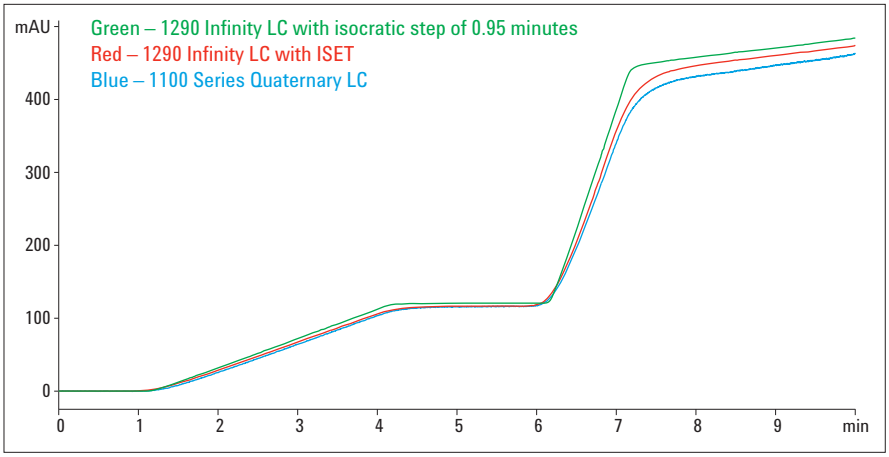

Figure 6
Overlay of gradients 1 applied on an Agilent 1100 Series Quaternary LC and an Agilent 1290 Infinity LC with ISET.

The gradient of the Agilent 1290 Infinity LC System with ISET follows the original gradient of the Agilent 1100 Series LC better than any of the gradients generated with additional delay volume or with an isocratic hold. This is even more obvious if the range around 5 minutes is expanded (Figure 7).

The red trace representing the gradient from the Agilent 1290 Infinity LC with ISET follows the gradient from the 1100 Series LC exactly, even at the changes to and from the isocratic step beween 4 and 6 minutes. The green curve representing the isocratic hold shows significantly less correlation.

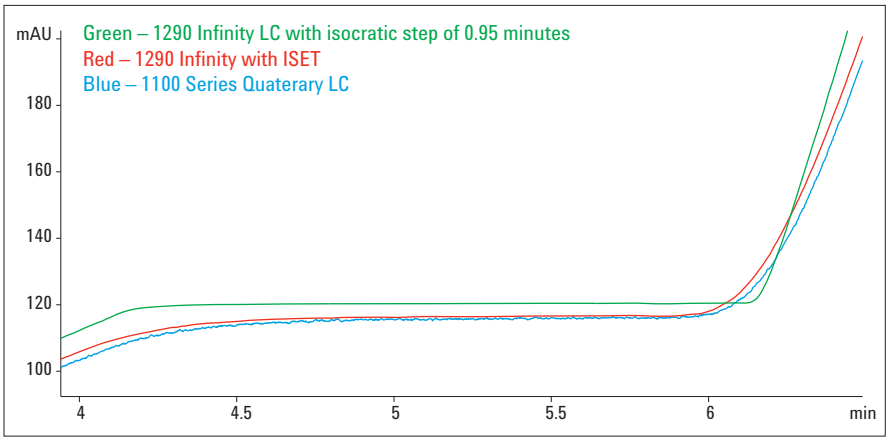

Figure 7
Overlay of gradient applied to the Agilent 1100 Series Quaternary LC System and the Agilent 1290 Infinity LC System with ISET (zoomed around 5 minutes).

Conclusion

One gradient was applied to the Agilent 1100 Series Quaternary LC using a tracer experiment. The same gradient was transferred to the Agilent 1290 Infinity LC with and without ISET, to the Agilent 1290 Infinity LC with additionally installed delay volume and to the Agilent 1290 Infinity LC with two different isocratic holds at the beginning of the run. The optimum correlation of the original curve was obtained using the Agilent 1290 Infinity LC with ISET. This ensures that retention times and resolution will both correlate to a high extent. Isocratic holds and the addition of delay volume did not deliver the same excellent performance.

Reference

1.
"Agilent 1290 Infinity LC with Intelligent System Emulation Technology", Agilent publication, Publication number 5990-8670EN, **2011**

www.agilent.com/chem/iset

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