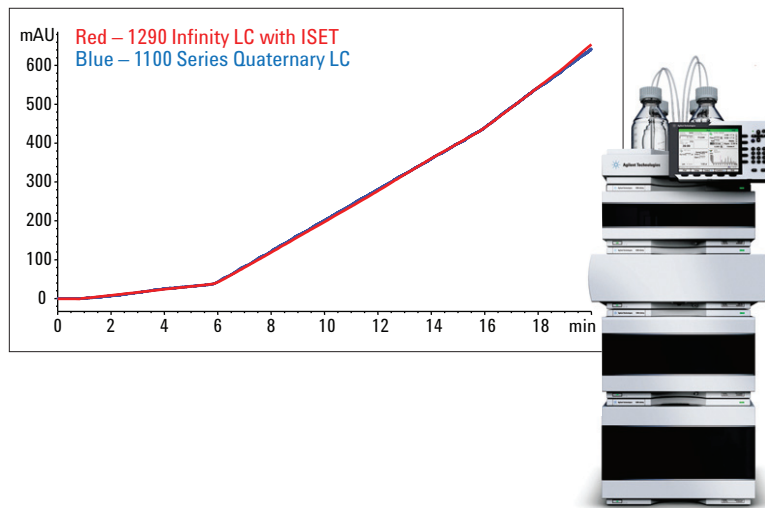


# Seamless transfer of elution gradients from Agilent 1100/1200 Series LCs to an Agilent 1290 Infinity LC using ISET

## Technical Overview

### Author

A.G.Huesgen  
Agilent Technologies, Inc.  
Waldbronn, Germany



### 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, the same chromatographic results can be achieved without any modifications to the instrument or changes to the original method.

In this Technical Overview, we demonstrate that gradients run on an Agilent 1100 Series LC System, including typical linear gradients as well as gradients with different slopes, steps, and isocratic parts, correlate almost 100% to gradient profiles created on the Agilent 1290 Infinity LC System with ISET.



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## Introduction

Instrument-to-instrument method transfer is often problematic, especially in highly regulated environments, because critical parameters such as retention times and resolution might change due to differences in system delay volume and gradient mixing behavior. Agilent provides seamless method transfer, for example, from Agilent 1100/1200 Series LCs and Agilent 1220/1260 Infinity LCs to the Agilent 1290 Infinity LC with ISET<sup>1</sup>. Because ISET is a feature that can be simply switched on and off, you can run legacy methods unchanged.

Of special interest is whether typical gradient profiles obtained from a conventional LC can be transferred to an Agilent 1290 Infinity LC System with ISET with high correlation. High correlation implies that retention times and resolution will correlate to a high extent, even for peaks that react sensitively to small composition changes.

In this Technical Overview, tracer experiments were selected to demonstrate that ISET provides optimum results for a wide range of gradient applications. Tracer experiments give more detailed and complete results than injection of arbitrarily selected compounds.

In this Technical Overview, we demonstrate the transfer of several typical gradient profiles from an Agilent 1100 Series Quaternary LC to an Agilent 1290 Infinity LC System with ISET using tracer experiments.

## 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 following chromatographic conditions were used for all gradients:

Compound: Uracil as tracer,  
10 mg/L in methanol  
Column: Restriction capillary  
Mobile phases: Water (A)/methanol  
with tracer (B)  
Column temperature: 30 °C  
Detection with DAD: 254/4 nm,  
Ref. 360/100 nm, 10 Hz

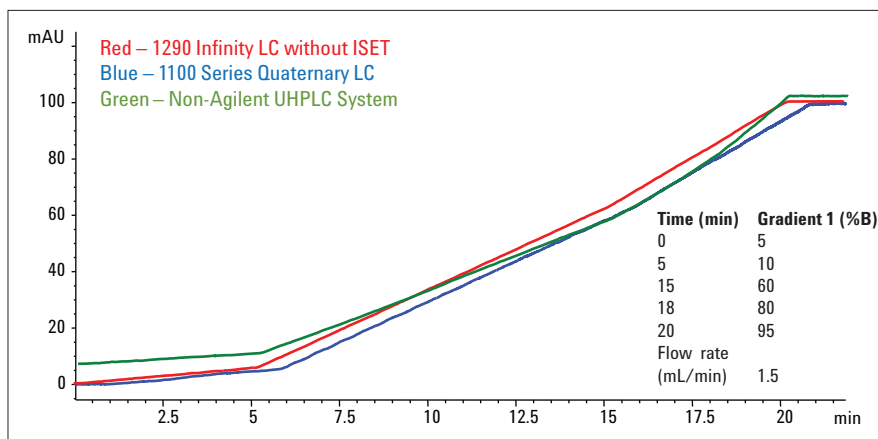
Module	Agilent 1100 Series LC	Agilent 1290 Infinity LC with ISET
	Product number	Product number
Pump	G1311A manufactured in 2003	G4220A
Autosampler	G1313A	G4226A
Thermostat	none	G1330B
Column Compartment	G1316A	G1316C
Diode Array Detector	G1315B	G4212A

**Table 1**  
Instrumentation used for the experiments.

## Results and discussion

Method transfer from HPLC to UHPLC systems can be generally problematic due to the significant lower delay and different mixing behavior of UHPLC systems as shown in Figure 1. As a result, the gradient, the retention times, and the resolution of peaks will change.

There are two typical ways to overcome this problem: either an isocratic hold is added at the beginning of the gradient, or the delay volume is increased with additional tubing between the pump and autosampler. Both methods can only compensate for the smaller delay volume but not for the overall gradient behavior (transition volume, mixing performance) over the complete gradient and run time.



**Figure 1**  
Overlay of gradient 1, run on an Agilent 1100 Series Quaternary LC, an Agilent 1290 Infinity LC without ISET, and another vendors' UHPLC instrument.

## Transfer of gradients from an Agilent 1100 Series Quaternary LC System to an Agilent 1290 Infinity LC with ISET

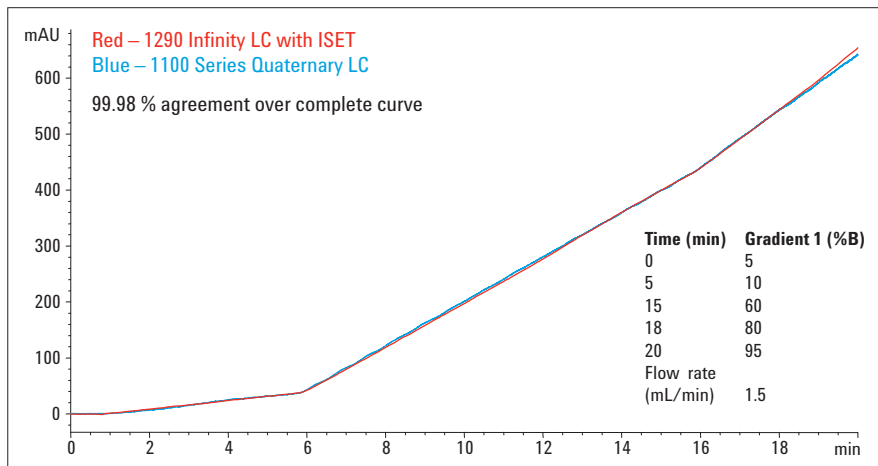
Manifold separation problems may cause HPLC gradients to contain different lengths, slopes, steps and isocratic parts in their profile. Therefore, six typical gradient profiles were run using an Agilent 1100 Series Quaternary LC and an Agilent 1290 Infinity LC with ISET. Three gradient profiles contained different slopes and isocratic steps. The other three gradient profiles were linear, but with different run times. The agreement of both curves was calculated using correlation factors that were converted into percentage values.

**Gradient 1** contained different slopes and the complete run time was 20 minutes. Figures 2 and 3 show overlays of the gradient profile from the Agilent 1100 Series Quaternary LC and from the resulting Agilent 1290 Infinity LC with ISET. In Figure 3, the view is enhanced to show the curve behavior after a slope change.

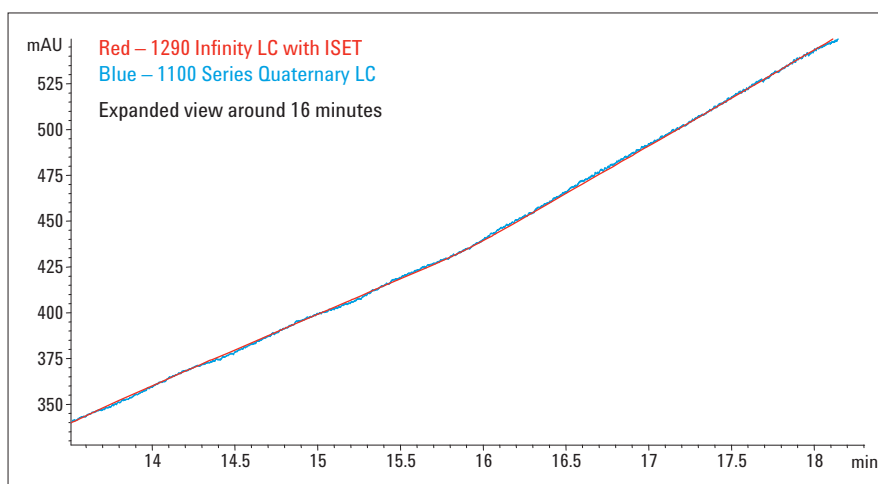
The emulated method of the Agilent 1290 Infinity LC with ISET provides perfect agreement of both curves.

Notice how the ISET function is able to follow the original gradient profile after a significant composition change such as the slope change at 15 minutes. Figure 3 shows that the original curve and the ISET curve correlate to nearly 100%, even after a slope change.

Due to the high correlation, similar retention times and resolution can be expected even for more demanding gradients.



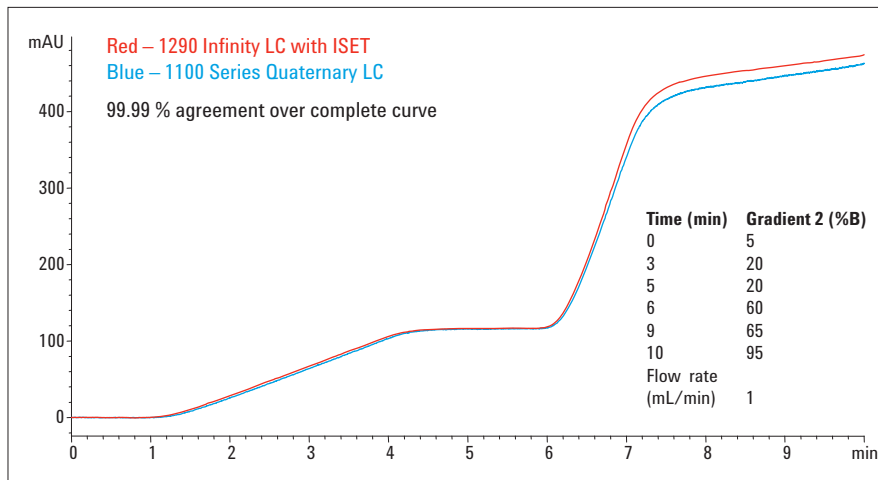
**Figure 2**  
Overlay of gradient 1 using an Agilent 1100 Series Quaternary LC and an Agilent 1290 Infinity LC with ISET.



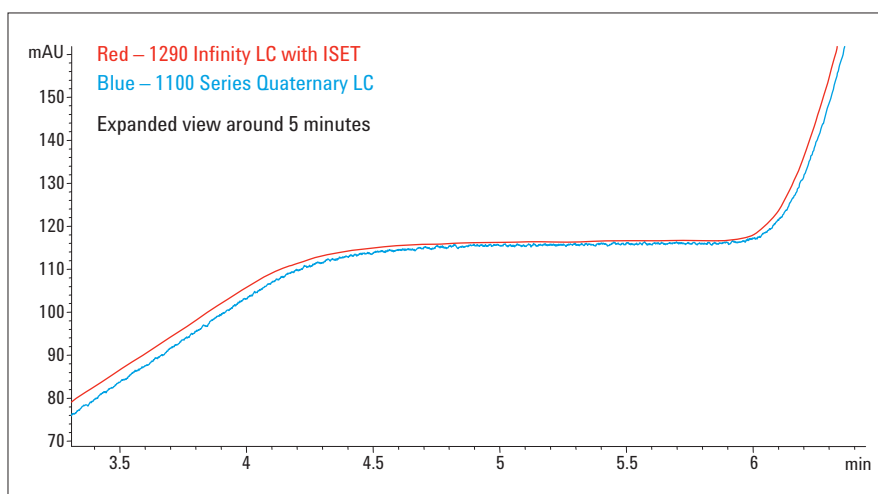
**Figure 3**  
Expanded view around 16 minutes after change of slope at 15 minutes.

**Gradient 2**, with a run time of 10 minutes, contained different slopes and one isocratic section in the middle of the chromatogram. These gradients are frequently used if the resolution for closely eluting peaks should be improved. Figures 4 and 5 show overlays of the gradient profiles from an Agilent 1100 Series Quaternary LC System and an Agilent 1290 Infinity LC System with ISET.

In Figure 5, the view is enhanced to show curve behavior after slope changes. The agreement of both curves is close to 100%. The gradient contained an isocratic step from 3 to 5 minutes. In Figure 5, the chromatogram is expanded at 3 to 7 minutes, to show that both gradient profiles correlate well, even at the changeovers from slope 1 to the isocratic step, and from the isocratic step, to the next gradient slope.



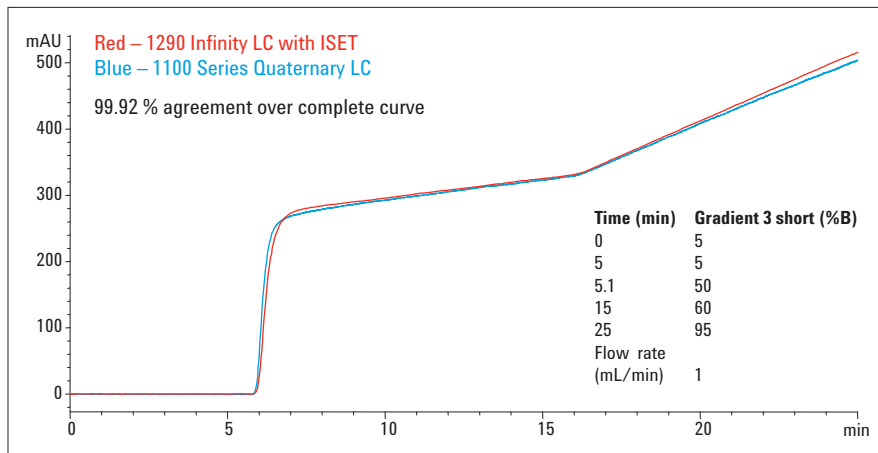
**Figure 4**  
Overlay of gradient 2 using an Agilent 1100 Series Quaternary LC and an Agilent 1290 Infinity LC with ISET.



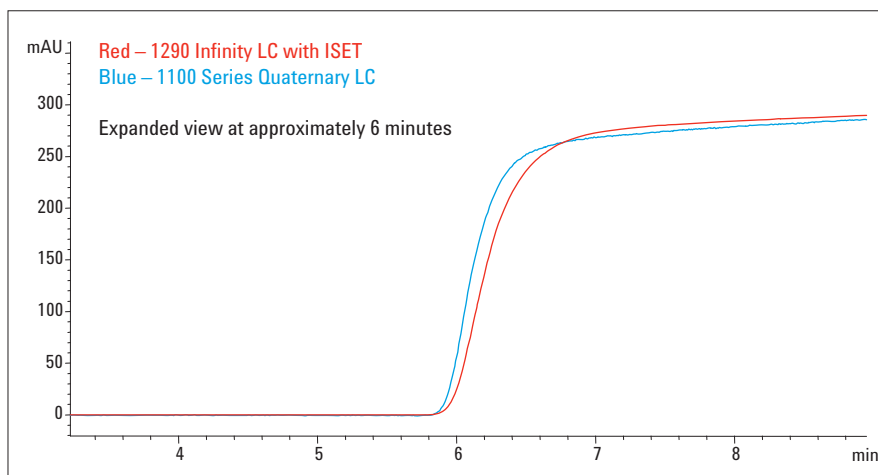
**Figure 5**  
Expanded view at approximately 5 minutes at two critical composition changeovers.

**Gradient 3**, with a run time of 25 minutes, contained an isocratic step at the beginning. This type of gradient is usually applied when hydrophilic and hydrophobic compounds must be analyzed in one run. At the beginning of the run, a low organic percentage is used and after the elution of the hydrophilic compounds the organic percentage is increased significantly to elute the hydrophobic compounds in a reasonable time frame (Figure 6). Both curves show an agreement of close to 100%.

In Figure 7 the chromatogram is enhanced at 3 to 9 minutes, to show that even at the steep increase from 5% to 50% organic in 0.1 minutes, the correlation is very good.



**Figure 6**  
Overlay of gradient 3 using an Agilent 1100 Series Quaternary LC and an Agilent 1290 Infinity LC System with ISET.



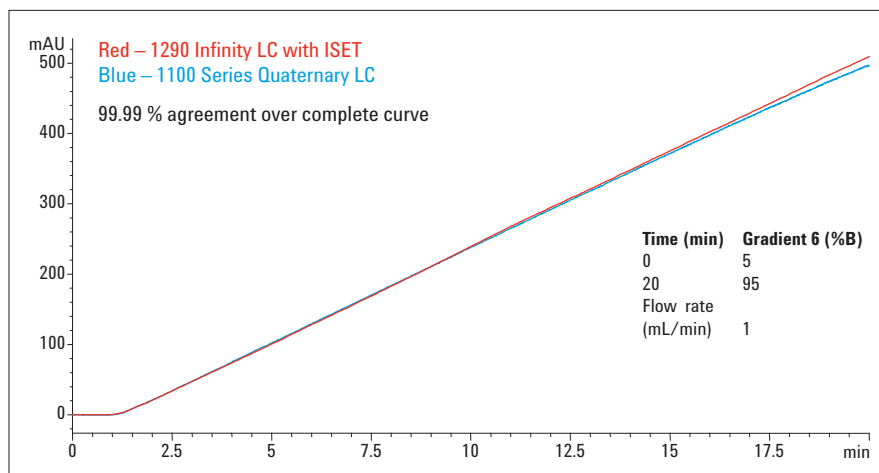
**Figure 7**  
Expanded view at approximately 6 minutes at critical ultrafast composition changes.

In the following, linear gradients of different lengths were applied to both instruments.

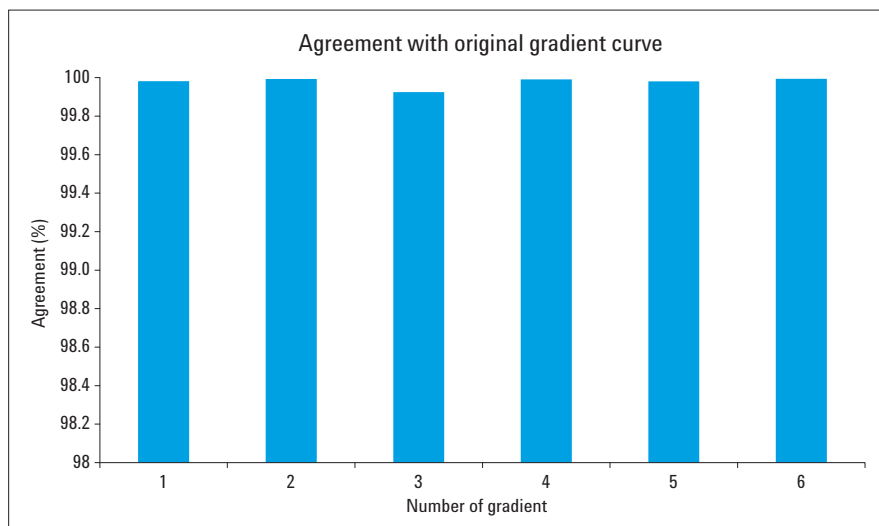
**Gradient 4, 5, and 6** were linear gradients from 5 to 95% organic with a run time of 3, 10, and 20 minutes. Figure 8 shows the linear gradient with a 20-minute run time as an example.

All linear curves show an agreement close to 100%. The agreement is close to 100% even for the gradient of 3 minutes, which is unusual for an Agilent 1100 Series Quaternary LC (Figure 9).

The performance results of the six gradients are summarized in Figure 9. Correlation factors were evaluated and transferred into percentage values. The agreement between the original gradient obtained from the Agilent 1100 Series Quaternary LC and the Agilent 1290 Infinity LC with ISET in all cases is close to 100%.



**Figure 8**  
Overlay of gradient 6 applied on an Agilent 1100 Quaternary Series LC and an Agilent 1290 Infinity LC with ISET.



**Figure 9**  
Agreement of gradients used and transferred from an Agilent 1100 Series Quaternary LC to an Agilent 1290 Infinity LC with ISET.

## Conclusion

Design differences between HPLC and UHPLC instruments such as power range, delay volume, and mixing behavior affect the ability to transfer a method from one system to another. Therefore, identical methods used on different LC instrumentation could result in different retention times and chromatographic resolution.

The Agilent 1290 Infinity LC with ISET allows users to emulate methods from conventional instruments with a simple mouse click.

Six different gradients were applied to the Agilent 1100 Series Quaternary LC and to the Agilent 1290 Infinity LC with ISET to prove that there is high correlation between the transferred methods using the ISET function. The resulting gradients from each instrument were monitored using a tracer experiment. An overlay of the obtained curves and the calculation of correlation factors show excellent correlation. These data demonstrate that retention times and resolution are similar to a large extent.

## Reference

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

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Published in USA, January 1, 2012  
5990-9545EN



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