# Intelligent System Emulation Technology Transfers Methods from Binary to Quaternary LC

## Technical Overview


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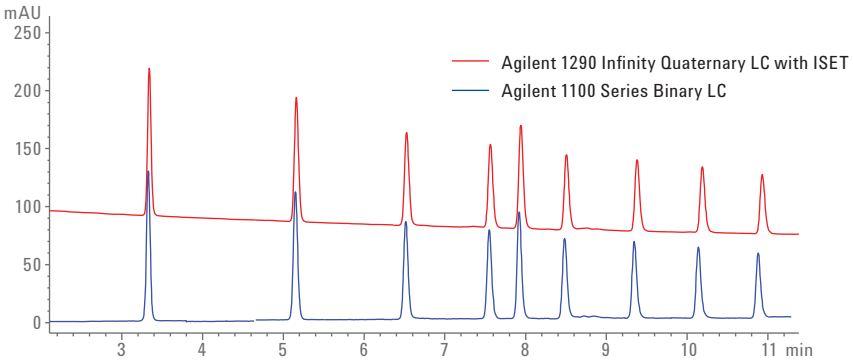
### Abstract

The Intelligent System Emulation Technology (ISET) in combination with Agilent 1290 Infinity Binary or Quaternary LC systems enables the emulation of Agilent 1100/1200 Series LCs for seamless instrument-to-instrument method transfer. This Technical Overview shows the successful emulation of demanding gradient analysis from the 1100 Series Binary LC systems to the 1290 Infinity Quaternary LC using ISET.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## Introduction

Many industries have a strong demand for seamless instrument-to-instrument method transfer. Changing validated or established methods is expensive and time-consuming, and a comparison with reference data is difficult. Old equipment has to be replaced from time to time. The Agilent 1290 Infinity Quaternary LC, in combination with the Intelligent System Emulation Technology (ISET), can emulate older Agilent LC systems, for example the Agilent 1100 Series Binary LC[1].

Typically, method transfer from a binary LC system to a quaternary LC system is not recommended due to the different delay volume and gradient formation behavior. However, by using ISET and the 1290 Infinity Quaternary LC, it is possible to transfer even demanding gradients from an 1100 Series Binary LC system to the 1290 Infinity Quaternary LC.

## Experimental

An Agilent RRLC checkout sample (p/n 5188-6529) comprised: acetanilide, acetophenone, propiophenone, butyrophenone, benzophenone, valerophenone, hexanophenone, heptanophenone, and octanophenone. Caffeine, theophylline, and testosterone were purchased from Sigma-Aldrich, Corp., Germany.

An Agilent 1290 Infinity Quaternary LC was used, with:

- Agilent 1290 Infinity Quaternary Pump (G4202A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector with10-mm flow cell (G4212A)

The Agilent 1100 Series Binary LC comprised:

- Agilent 1100 Series Degasser (G1379A)
- Agilent 1100 Series Binary Pump (G1312A)
- Agilent 1100 Series Autosampler (G1313A)
- Agilent 1100 Series Thermostated Column Thermostat (G1316A)
- Agilent 1100 Series Diode Array Detector with a 10-mm flow cell (G1315B)

Agilent OpenLAB CDS ChemStation Rev.C.01.05 software was used for data acquisition and evaluation.

### Experimental conditions

| Conditions for all samples | |
|---|---|
| Column | Agilent ZORBAX Eclipse Plus C18, 4.6 x 100 mm, 5 µm (p/n 959996-902) |
| Temperature | 30 °C |
| Solvent | A) water<br>B) acetonitrile |
| **RRLC checkout sample** | |
| Gradient | 15 to 90 % B in 10 minutes |
| Stop time | 11.5 minutes |
| Post time | 3 minutes |
| Flow rate | 1.2 mL/min |
| DAD | 245/10 nm, ref 400/100 nm, 10 Hz |
| **Caffeine and theophylline analysis** | |
| Gradient | 5 to 12 % B in 10 minutes |
| Stop time | 11.5 minutes |
| Post time | 3 minutes |
| Flow rate | 1.2 mL/min |
| DAD | 254/10 nm, ref: 400/100 nm, 10 Hz |
| **Testosterone analysis** | |
| Gradient | 80 to 95 % B in 10 minutes |
| Stop time | 11 minutes |
| Post time | 3 minutes |
| Flow rate | 1 mL/min |
| DAD | 254/10 nm, ref: 400/100 nm, 10 Hz |

## Results and Discussion

The 1290 Infinity Quaternary Pump with mixer has a delay volume approximately 300 µL smaller than that of the 1100 Series Binary Pump. The following experiments were done to evaluate the agreement of retention times and resolution when gradients were transferred from the 1100 Series Binary LC to the 1290 Infinity Quaternary LC:

- A gradient from 15 to 90 % organic phase with an RRLC checkout sample
- A gradient from 5 to 12 % organic phase with a mixture of caffeine and theophylline
- A gradient from 80 to 95 % organic phase with a testosterone sample

In the first experiment, a more common gradient was chosen and applied for the separation of nine peaks. Figure 1 shows the overlaid chromatograms. Without ISET, all peaks eluted earlier than on the 1100 Series Binary LC due to the lower delay volume of the 1290 Infinity Quaternary LC.

Figure 2 summarizes the results for the deviation of retention time. The deviation range permitted for retention times was ± 5 %. Without ISET, the retention times deviated from –1.4 to –6.1 %. With ISET, the deviation was less than 0.5 % for all peaks.

Another important parameter is resolution, which should be the same or better after emulation. The deviation range permitted was –5 %. Figure 3 reveals that the resolution was between ~2 and 5 % better after emulation. Without emulation, the resolution was between ~4.5 and 9.2 % better.

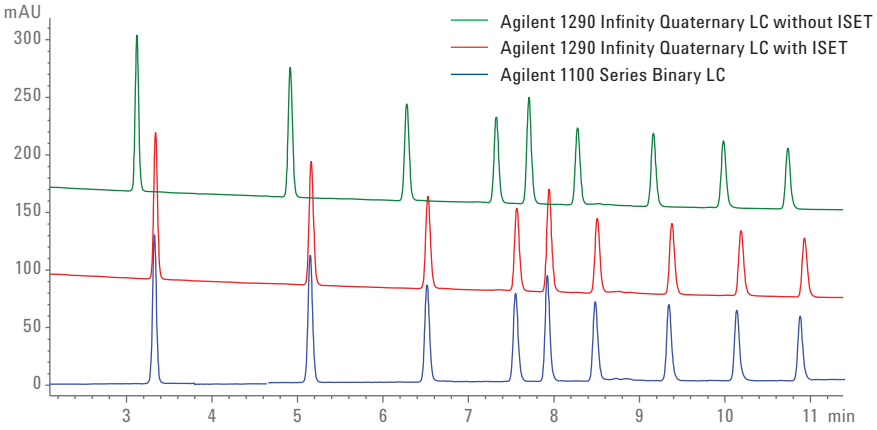


Figure 1. Overlay of the Agilent 1100 Series LC chromatogram with Agilent 1290 Infinity Quaternary LC chromatograms with and without applying ISET.

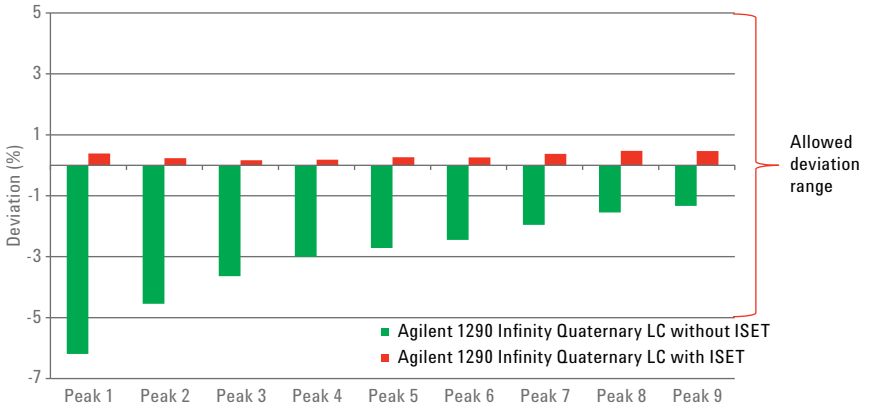


Figure 2. Deviation of retention times of the Agilent 1290 Infinity Quaternary LC with and without ISET.

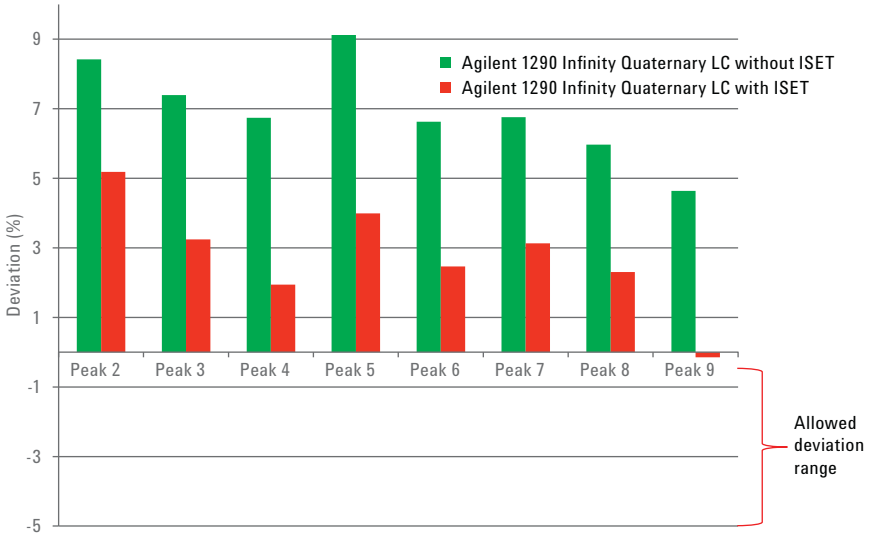


Figure 3. Deviation of resolution for the Agilent 1290 Infinity Quaternary LC with and without ISET.

In the second experiment, a more demanding gradient from 5 to 12 % organic was applied for the separation of two very hydrophilic compounds, theophylline and caffeine. Figure 4 is an overlay of the chromatograms obtained on the 1100 Series LC and the 1290 Infinity Quaternary LC with and without applying ISET.

The deviation of retention times was less than 8.1 % without ISET and less than 0.84 % with ISET, as shown in Figure 5.

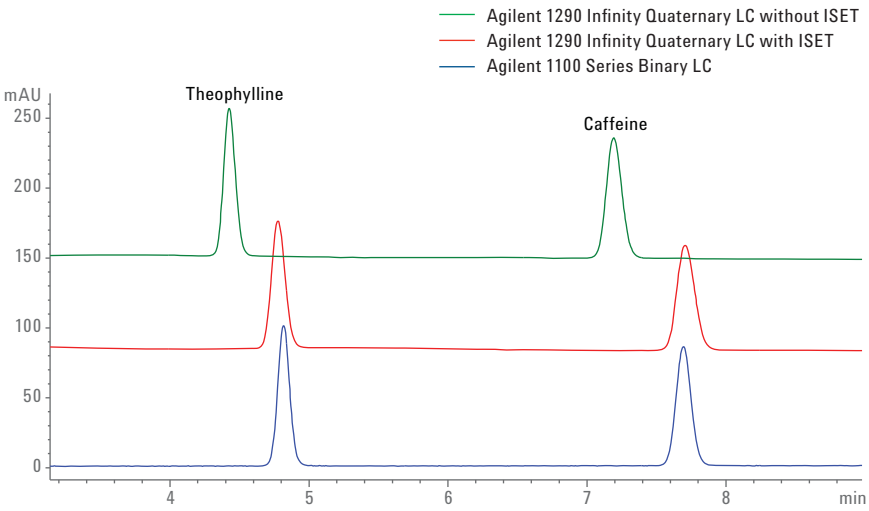


Figure 4. Overlay of chromatograms obtained on the Agilent 1100 Series LC and the Agilent 1290 Infinity Quaternary LC with and without applying ISET.

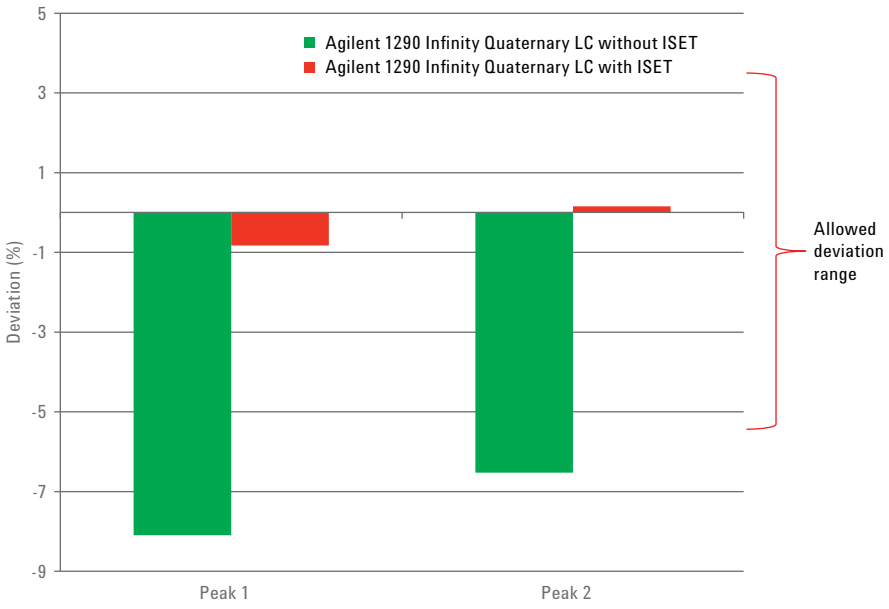


Figure 5. Deviation of retention times for the Agilent 1290 Infinity Quaternary LC with and without ISET.

In the third experiment, a gradient from 80 to 95 % organic was applied for the analysis of the hydrophobic compound, testosterone (Figure 6). At this high percentage of organic mobile phase, the deviation of retention times was very small. Even without ISET, the deviation was less than 1.6 % (Figure 7). With ISET, the deviation of the retention time was less than 0.55 %.

## Conclusions

Emulation of the Agilent 1100 Series Binary LC using the Agilent 1290 Infinity Quaternary LC was applicable, due to the low delay volume of the latter. Transferring conventional gradients and gradients with high and low percentage organic mobile phase was possible, with a retention time deviation of less than 0.9 %. Resolution was typically improved after emulation. Applying the same experiments using an Agilent 1200 Series Binary LC or an Agilent 1260 Infinity Binary LC, and emulating these LCs using the 1290 Infinity Quaternary LC, will deliver similar results.

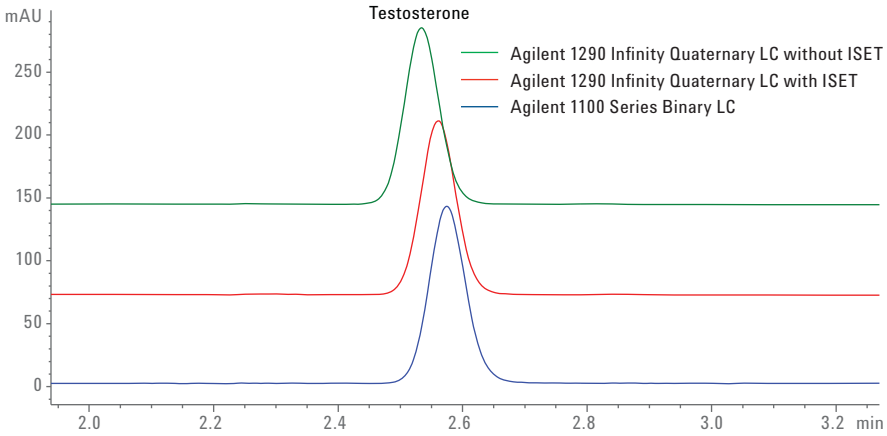


Figure 6. Overlay of chromatograms obtained on the Agilent 1100 Series LC and the Agilent 1290 Infinity Quaternary LC with and without applying ISET.

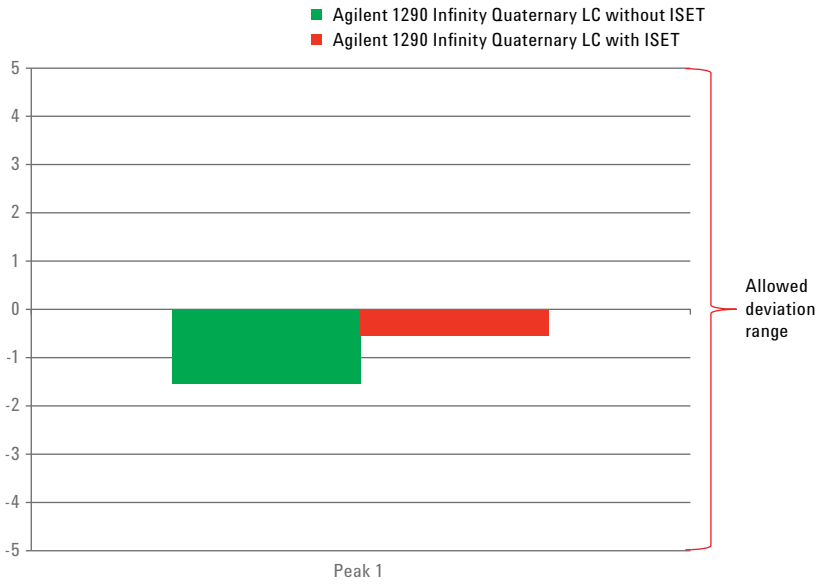


Figure 7. Deviation of retention time.

## Reference

1. 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**.

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