# Agilent 1290 Infinity Binary LC with ISET – Emulation of the Waters Alliance 2695 LC System Analyzing Analgesics

## Application Note

Small Molecule Pharmaceuticals & Generics


### Author

A.G.Huesgen

Agilent Technologies, Inc.

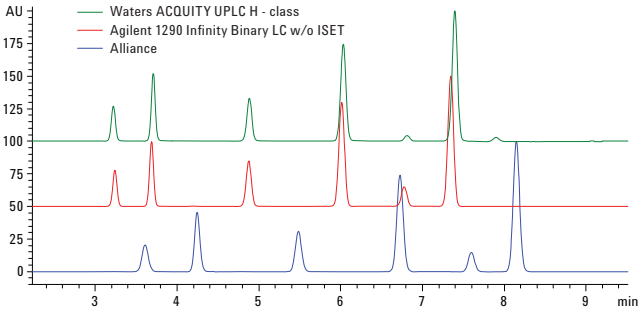
Waldbronn, Germany



### Abstract

Transfer of conventional methods to UHPLC systems typically needs a change of the method, due to the smaller system delay volume and gradient mixing behavior of these systems. In most cases, this is done by installing an additional delay volume or by adding an isocratic hold step at the beginning of the run.

This Application Note shows the emulation of the Waters Alliance 2695. The transfer of the analysis of analgesics onto the Agilent 1290 Infinity Binary LC using Intelligent System Emulation Technology (ISET) is shown, based on a Waters Application Note. Using ISET to enable a method transfer through simple mouse clicks was evaluated. The agreement of retention times and resolution was determined.



Agilent Technologies

## Introduction

Seamless instrument-to-instrument method transfer is often a strong demand for most industries because changing established methods is expensive and time consuming, and comparison with reference data is difficult. Nevertheless, old equipment has to be replaced occasionally. Seamless method transfer from the Agilent 1100 or 1200 Series LC System to the Agilent 1260 or 1220 Infinity LC System is possible without the need to change the chromatographic conditions[1,2]. Method transfer to an Agilent 1290 Infinity Binary LC system is possible by activating the ISET with a simple mouse click

The Agilent 1290 Infinity Binary LC, in combination with ISET, offers the possibility to emulate older non-Agilent instrumentation such as the Waters Alliance LC systems. A conventional method from the Waters Alliance LC system was transferred to the 1290 Infinity Binary LC without ISET and onto a Waters ACQUITY UPLC H-Class. In a second step, the method was transferred onto the 1290 Infinity Binary LC enabling ISET. The same results regarding retention times and typically better resolutions were received.

## Experimental

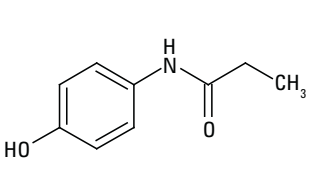
The following instruments were used (Table 1).

Table 1. Instrumentation used.

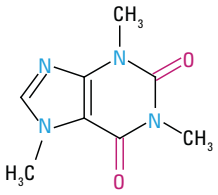
| | Agilent 1290 Infinity Binary LC | Waters ACQUITY UPLC H-Class | Waters Alliance |
|---|---|---|---|
| Binary Pump | G4220A | Quaternary Pump | 2695 |
| Autosampler | G4226A | Autosampler | |
| ALS cooler | G1330B | Autosampler cooler | |
| Column compartment | G1316C | Column compartment | |
| Diode array detector | G4212A | VWD (TUV) | VWD (Dual absorbance Detector) |

| Chromatographic conditions | |
|---|---|
| Column | Atlantis T3, 4.6 × 150, 5 µm |
| Mobile phases | A= 0.1 % formic acid pH = 2.3, B = ACN |
| Gradient | at 0 minutes 15 % B,<br>at 10 minutes 60 % B,<br>at 11 minutes 15 % B,<br>at 15 minutes 15 % B |
| Flow rate | 1mL/min |
| Injection volume | 10 µL |
| Column temperature | 30 °C |
| Detection | 260 nm, 5Hz |

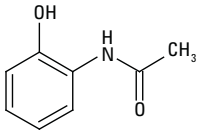
### Analyzed compounds



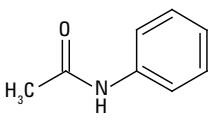
Paracetamol/acetaminophen



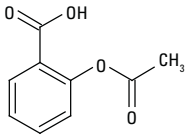
Caffeine



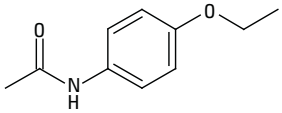
2-Acetamidophenol



Acetanilide



Acetylsalicyclic acid



Phenacetin

### Acquisition and evaluation software

OpenLAB CDS ChemStation version C.01.04 and ISET

Figure 1 shows the parameter that must be entered in the ISET parameter screen of the 1290 Infinity Binary LC to select the instrument to be emulated.

## Results and Discussion

The following experiments were done to prove the seamless method transfer from the Waters Alliance 2695 LC system to the Agilent 1290 Infinity Binary LC in combination with ISET.

### Experiments

- Analysis of analgesics on the Alliance 2695, based on a Waters Application Note[3]
- Transfer of the developed method onto the 1290 Infinity Binary LC without applying ISET and onto a Waters ACQUITY UPLC H-Class
- Analysis of analgesics on the 1290 Infinity Binary LC enabling ISET

### Evaluated parameters

- Determination of the deviation of retention times, specified deviation is < ± 5 %
- Determination of the resolution, typically better on the 1290 Infinity Binary LC. Specified maximum deviation < –5 %

### Transfer of method to the Agilent 1290 Infinity Binary LC without ISET and onto the Waters ACQUITY UPLC H-Class

Figure 2 shows the obtained chromatograms for the Water Alliance 2695 and both UHPLC systems overlaid. As expected, both UHPLC systems showed less retention for all peaks due to its significant lower delay volumes, (Figure 2).

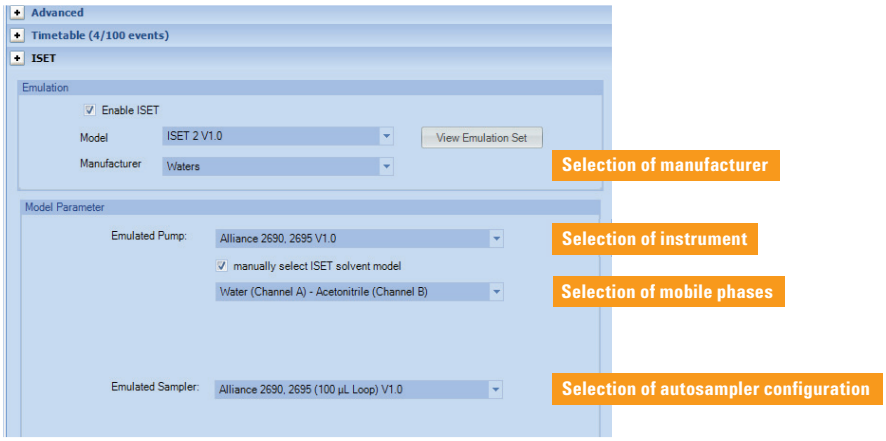



Figure 1. Selection of the instrumentation to be emulated.

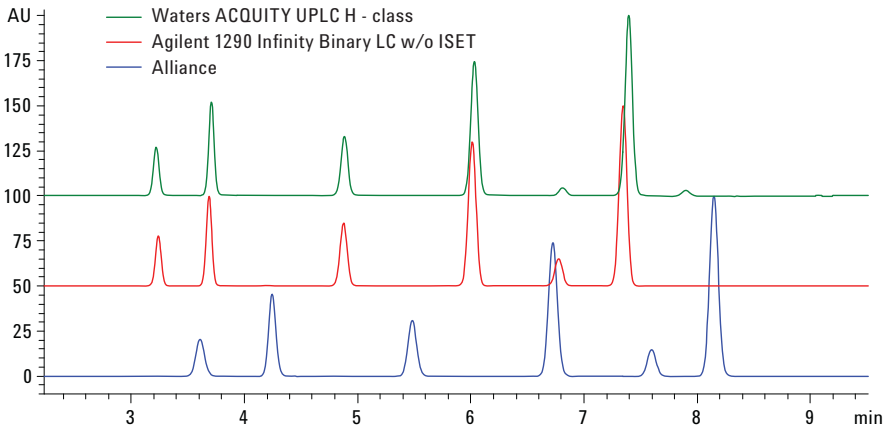



Figure 2. Overlay of chromatograms of the Waters Alliance 2695, an Agilent 1290 Infinity Binary LC without ISET, and the Waters ACQUITY UPLC H-Class.

Figures 3 and 4 show the comparison of the deviation of retention times and resolution. The retention times deviated from the original value significantly more than –5 % for both instruments.

The deviation of resolution was within the –5 % limit for both systems.

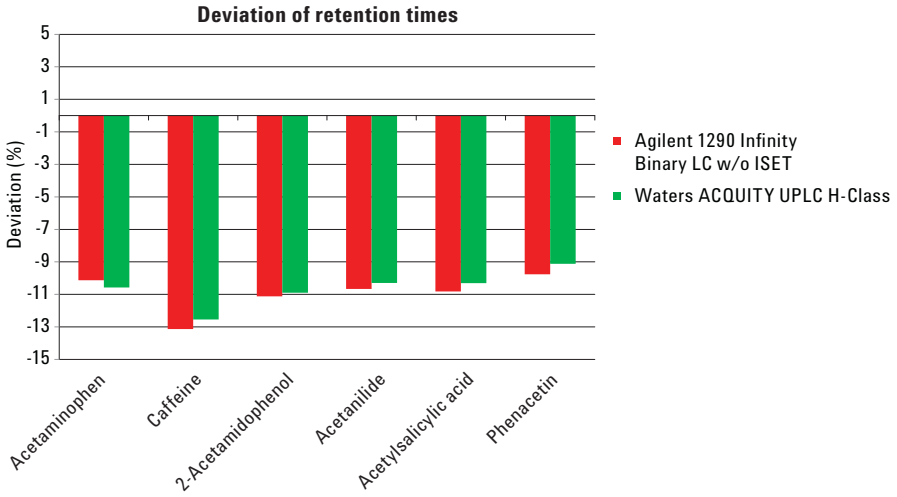



Figure 3. Deviation of retention times of the Agilent 1290 Infinity Binary LC without ISET and the Waters ACQUITY UPLC H-Class.

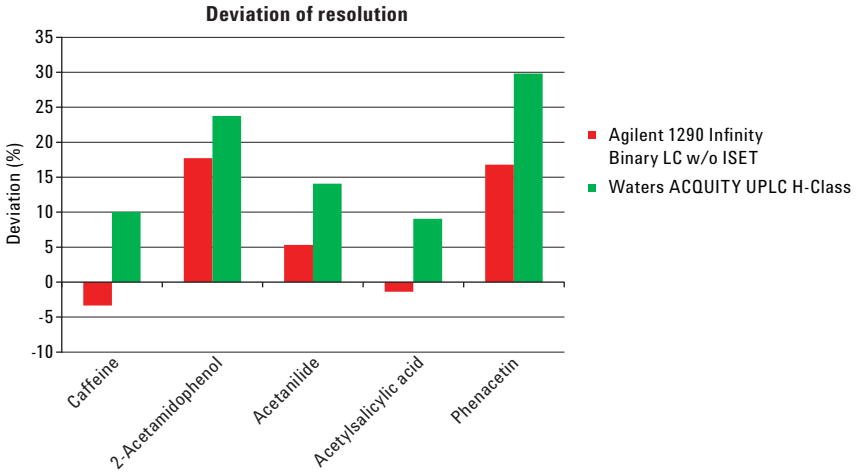



Figure 4. Deviation of resolution of the Agilent 1290 Infinity Binary LC without ISET and the Waters ACQUITY UPLC H-Class.

## Transfer of conventional method onto the Agilent 1290 Infinity Binary LC enabling ISET

The best correlation was obtained using the 1290 Infinity Binary LC with ISET and fine tuning, see the violet trace (Figure 6). Fine tuning means that an additional delay volume of 200 µL was added in the ISET parameter screen by enabling the manual fine tuning parameters (Figure 5). Without the fine tuning step, the agreement of retention times using the 1290 Infinity Binary LC was already close, see the red trace (Figure 6).

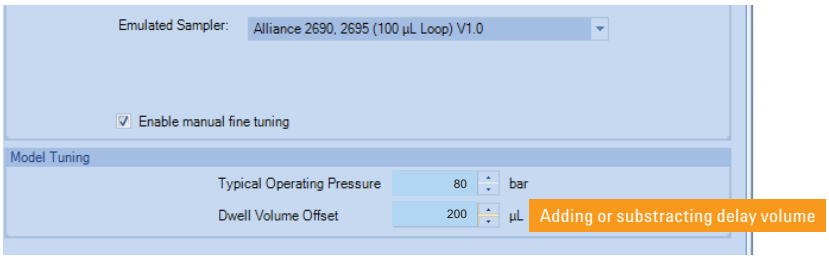



Figure 5. Fine tuning parameters within ISET.

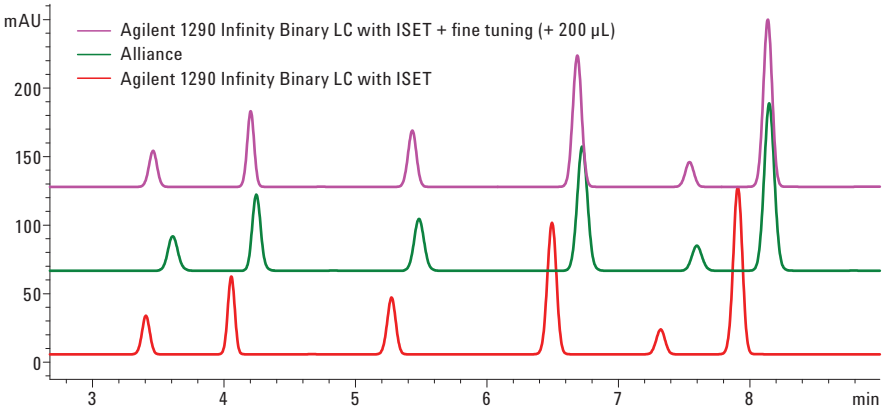



Figure 6. Transfer onto the Agilent 1290 Infinity Binary LC with ISET and with ISET and Fine Tuning.

Figure 7 summarizes the retention time deviations obtained on the different 1290 Infinity Binary LC configurations. The allowed deviation of retention times for the 1290 Infinity Binary LC with ISET is ± 5 %. Optimum results were achieved using the ISET tool with fine tuning. With ISET and no fine tuning, only the first peak was slightly out of the specification. The resolution had improved for all peaks.

Figure 8 compares resolution data. Using ISET and ISET with fine tuning, resolution had improved for all peaks.

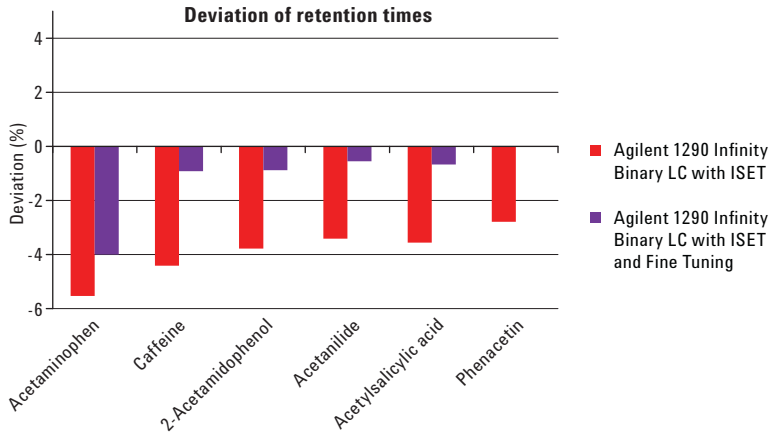



Figure 7. Deviation of retention times as percentage for all used Agilent 1290 Infinity Binary LC configurations.

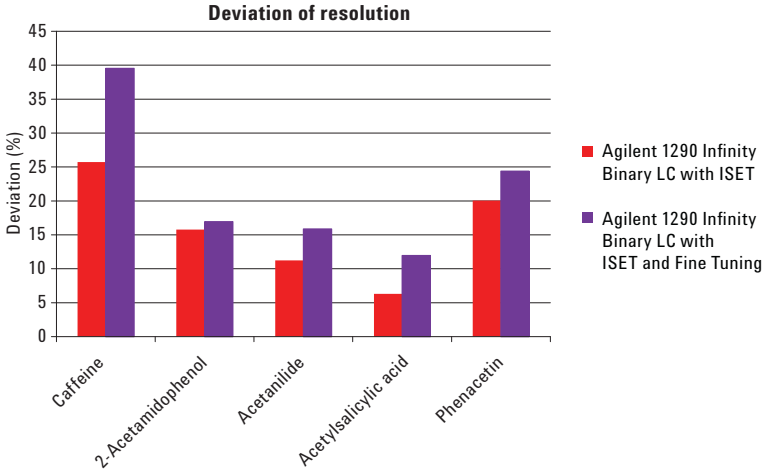



Figure 8. Deviation of resolution as percentage for all used Agilent 1290 Infinity Binary LC configurations.

## Conclusion

Method transfer using the Agilent 1290 Infinity Binary LC in combination with the ISET enables the emulation of older non-Agilent LCs, such as the Waters Alliance LC system, in a time-saving and convenient way. There is no need to change the original method by adding the additional delay volume of an isocratic hold step. With a few mouse clicks, the ISET is enabled and allows seamless method transfer. In this example, the deviation of retention times was < 4 % using the 1290 Infinity Binary LC with ISET and fine tuning. The allowed deviation of retention times is < ± 5 %. The resolution was improved for all peaks. The allowed maximum deviation for the resolution is < –5 %.

## References


1. A.G. Huesgen, "Seamless method transfer from the Agilent 1200 Series LC System to the Agilent 1260 Infinity LC System, Comparison of system suitability results according EP and USP regulations for the analysis of paracetamol (acetaminophen) and its impurities", Agilent Application Note, Publication Number 5990-6207EN, September 1, **2010**.

2. A.G. Huesgen, "Seamless method transfer from the Agilent 1200 Series to the Agilent 1220 and 1260 Infinity LC systems, Comparison of system suitability results according to USP method USP/NF 23 for the analysis of Paracetamol (Acetaminophen) and Aspirin in pain relievers", Agilent Technical Overview, Publication Number 5990-6206EN, July 1, **2010**.

3. Waters Application note, "Analysis of analgesics using Atlantis T3", WA60217 KK PDF, **2009**.

www.agilent.com/chem/ISET


Published in the USA, September 1, 2013
5991-2792EN

Agilent Technologies