# Agilent 1290 Infinity II LC with ISET – Emulation of Waters Alliance 2695 LC Through Waters Empower Software

## Analysis of Paracetamol and its Impurities

Suitable for Agilent
1290 Infinity III LC

## Application Note

Small Molecule Pharmaceuticals


### Author

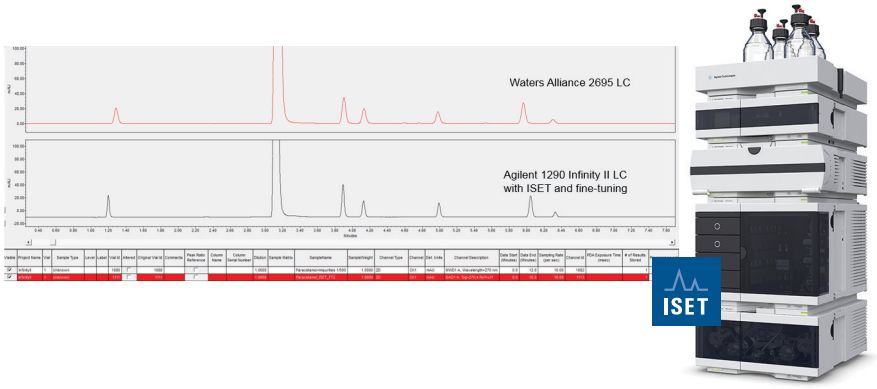
Melanie Metzlaff
Agilent Technologies, Inc.
Waldbronn, Germany



### Abstract

Instrument-to-instrument transfer of methods is a challenging topic for many laboratories in many industries. The challenging part is to take the individual instrument performance into account while maintaining retention times, and not losing resolution of the analytes during instrument transfer. Agilent Intelligent System Emulation Technology (ISET) enables seamless transfer of methods from an LC system with a higher delay volume and different mixing behavior to an Agilent 1290 Infinity and an Agilent 1290 Infinity II LC.

This Application Note shows the easy transfer of an LC method from the Waters Alliance 2695 LC to an Agilent 1290 Infinity II LC, with and without ISET enabled, while controlled through Waters Empower software. Paracetamol and its impurities were analyzed on both systems, and the resulting retention times and resolution values were compared.


Agilent Technologies

## Introduction

The transfer of methods from one instrument to another is an important and often problematic task for most industries. Especially in highly regulated environments, the transfer of already established and validated methods can be difficult and expensive because of changes in retention time and chromatographic resolution of the analytes[1]. Periodically, there is a requirement to change old equipment for new equipment.

The Agilent Intelligent System Emulation Technology (ISET), in combination with an Agilent 1290 Infinity LC or Agilent 1290 Infinity II LC, enables emulation of older non-Agilent instrumentation such as the Waters Alliance 2695 LC. ISET takes the higher delay volume and the different gradient mixing behavior into account and achieves similar retention times on both LCs.

In this Application Note, paracetamol and its impurities were analyzed on a Waters Alliance 2695 LC and then transferred to an Agilent 1290 Infinity II LC equipped with an Agilent 1290 Infinity II Flexible Pump. The transfer of the method to the UHPLC system was evaluated by disabling and enabling ISET, and the results were compared with the data from the Waters Alliance 2965 LC. By enabling ISET, excellent retention time correlation and resolution values were obtained.

## Experimental

### Instrumentation

For the analysis of paracetamol and its impurities, the following instruments were used.

Agilent 1290 Infinity II LC System:

- Agilent 1290 Infinity II Flexible Pump (G7104A)
- Agilent 1290 Infinity II Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B), equipped with a 10 mm Max-Light cartridge cell

Waters Alliance 2695 with Dual Absorbance Detector VWD 2487

### Solvents and samples

All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak).

The following mixture of compounds was used for the experiments:

**Main**
Paracetamol (5 mg/mL)

**Impurity A**
2-Acetamidophenol (10 µg/mL)

**Impurity B**
N-(4-Hydroxyphenyl) propamide (10 µg/mL)

**Impurity F**
Nitrophenol (10 µg/mL)

**Impurity H**
4-(Acetylamino) phenyl Acetate (N,O-Diacetyl-4-aminophenol) (10 µg/mL)

**Impurity J**
4-Chloroacetanilide (10 µg/mL)

**Impurity K**
4-Aminophenol (10 µg/mL)

### Column

Agilent ZORBAX RRHD Eclipse Plus C18, 4.6 × 100 mm, 3.5 µm (p/n 959961-902)

### Software

- Waters Empower 3 (build 3471) to control the Waters Alliance 2695
- Waters Empower 3 (build 3471) with a Waters ICS 2.1 Hotfix 1 version and an Agilent ICF A.02.03 DU1 HF2 version to control the Agilent 1290 Infinity II LC

### Method

Table 1. Chromatographic conditions for the analysis of paracetamol and its impurities.

| Parameter | Value |
|---|---|
| Mobile Phase | A) 0.1 % TFA in water<br>B) 0.09 % TFA in acetonitrile |
| Gradient | 0 minutes – 5 %B<br>0.5 minutes – 5 %B<br>8 minutes – 70 %B<br>8.5 minutes – 5 %B<br>16 minutes – 5 %B |
| Flow rate | 1.2 mL/min |
| Injection volume | 3 µL with standard needle wash |
| Column temperature | 30 °C |
| Detection | 270/4 nm, ref. wavelength off, 10 Hz |

## Results and Discussion

To demonstrate easy and seamless method transfer from the Waters Alliance 2695 LC to the 1290 Infinity II LC, paracetamol and its impurities were analyzed, and the retention times and resolution values were evaluated.

First, the method was used on the Waters Alliance 2695 LC, and transferred to the 1290 Infinity II LC without ISET. Figure 1 shows the chromatograms obtained for both systems. Comparing the two chromatograms, all peaks elute earlier with the 1290 Infinity II LC system due to the significantly lower system delay volume.

To compensate for different delay volumes and gradient mixing behavior on both systems, ISET was enabled through simple clicks under the method tab of the pump. After enabling ISET, an orange icon appeared on the pump status dashboard, see Figure 2.

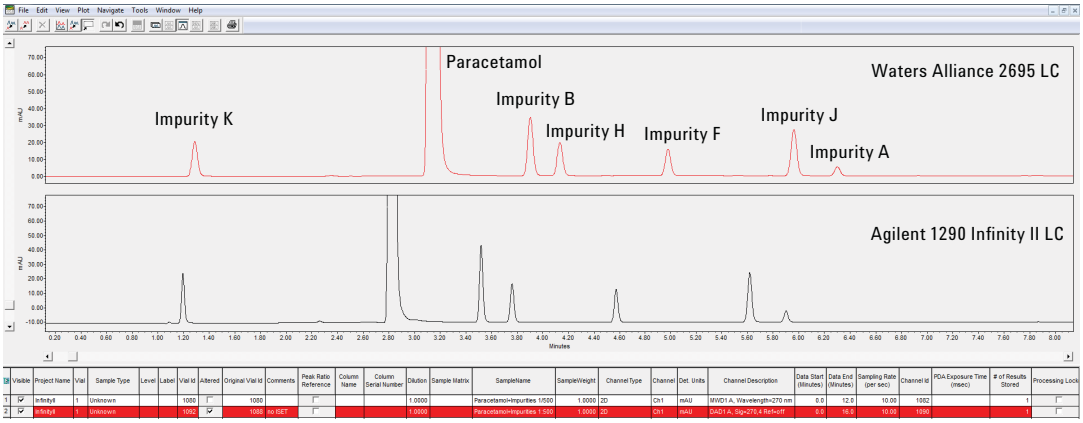



Figure 1. Chromatogram comparison of the Waters Alliance 2695 and the Agilent 1290 Infinity II LC without ISET for the analysis of paracetamol and its impurities.

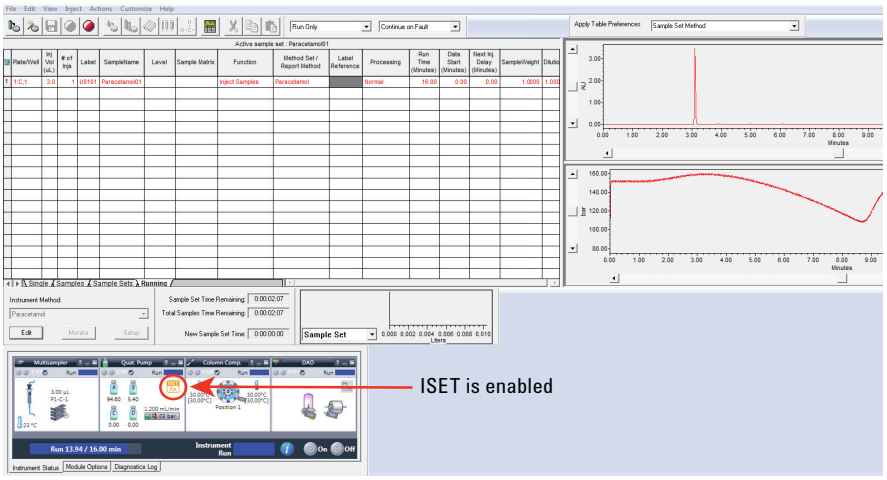



Figure 2. Example of the Agilent instrument status screen with ISET enabled under Empower 3 and ICF.

By enabling ISET, the retention times on the 1290 Infinity II LC were shifted clearly to later retention times, and showed a significantly better agreement with the Waters Alliance 2695 LC results (Figure 3, blue trace). However, the best correlation with the original chromatogram was obtained using ISET with the fine-tuning option (Figure 3, black trace). By using the fine-tuning option, the dwell volume can be adjusted, and the emulation can be further improved[2]. For this Application Note, an additional delay volume of 100 µL was added in the ISET parameter screen (Figure 4).

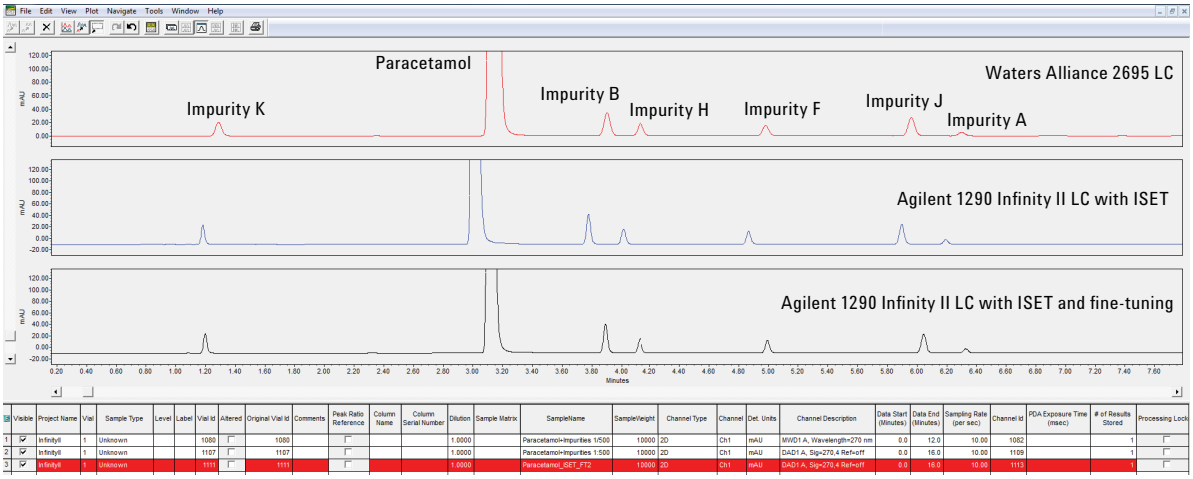



Figure 3. Chromatogram comparison of the Waters Alliance 2695 LC and the Agilent 1290 Infinity II LC with ISET and with ISET and fine-tuning for the analysis of paracetamol and its impurities.

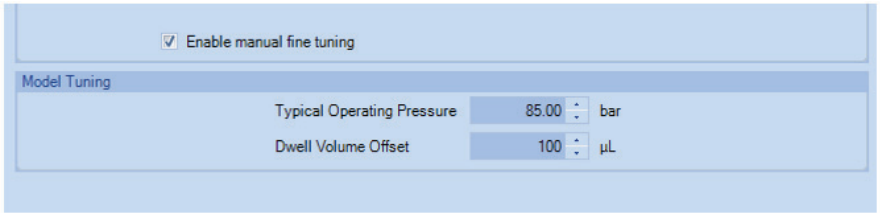



Figure 4. Fine-tuning parameters within the ISET screen.

A detailed comparison between the retention time deviations for the different used 1290 Infinity II LC settings and the original acquired data with the Waters Alliance 2695 LC can be seen in Figure 5. The ISET specification for the retention time deviation should be in a range of ±5 % (for RT > 6 minutes) or of ±0.3 minutes (for RT ≤ 6 minutes)[2]. Transferring the method to the UHPLC system without using ISET does not meet the specifications for most of the peaks. By enabling ISET, all peaks were within the specifications of ±0.3 minutes or ±5 %. A further optimization regarding the RT deviation was obtained by using ISET with the fine-tuning option. All peaks were below 0.1 minutes or 2 %.

**A**


**B**

| | Agilent 1290 Infinity II LC | Agilent 1290 Infinity II LC with ISET | Agilent 1290 Infinity II LC with ISET and fine-tuning |
|---|---|---|---|
| Imp K | 0.092 | 0.094 | 0.089 |
| Paracetamol | 0.322 | 0.131 | 0.022 |
| Imp B | 0.382 | 0.124 | 0.010 |
| Imp H | 0.373 | 0.121 | 0.006 |
| Imp F | 0.409 | 0.116 | -0.012 |
| Imp J | 0.341 | 0.067 | -0.083 |
| Imp A | 0.397 | 0.140 | -0.028 |

Figure 5. Deviation of retention time for the different Agilent 1290 Infinity II LC configurations. A) Retention time deviation in percentage. B) Retention time deviation in minutes

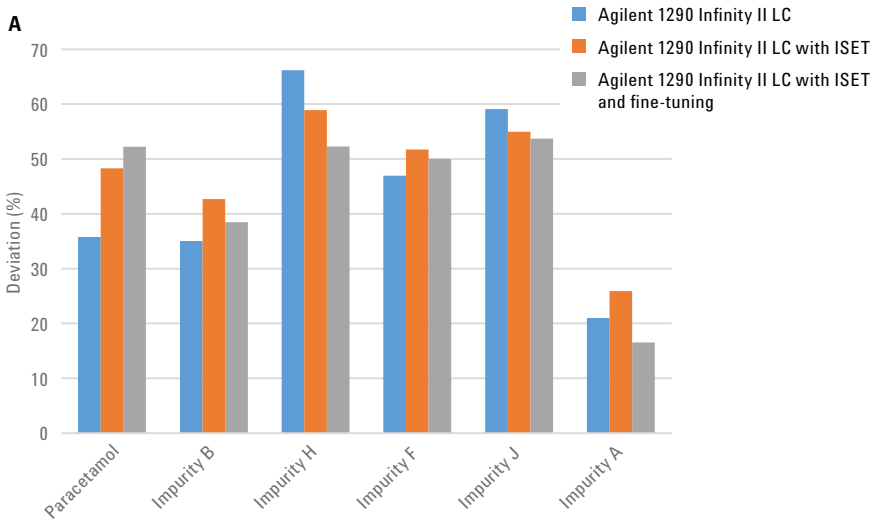
Another important chromatographic parameter to take into account for method transfer evaluation is the peak resolution. The maximum allowed loss in resolution is specified to be 5 %[3]. Figure 6 summarizes the obtained resolution deviation for the different 1290 Infinity II LC settings. By transferring the method to the UHPLC system, the resolution for all peaks was significantly increased.

## Conclusion

The Agilent Intelligent System Emulation Technology (ISET) enables seamless transfer of already established methods from a conventional HPLC system to an UHPLC system such as the Agilent 1290 Infinity II LC. By using ISET, the 1290 Infinity II LC can emulate the originally used HPLC system and deliver retention times close to the original data set. ISET can be activated by simple clicks, and no changes on the LC hardware or method is necessary.

In this Application Note, a method for the analysis of paracetamol and its impurities was transferred from a Waters Alliance 2695 LC to an Agilent 1290 Infinity II LC. By enabling ISET together with the fine-tuning option, excellent retention time correlation for all peaks was obtained. In addition, the resolution was increased for all peaks.

**A**




**B**

| | Waters Alliance 2695 LC | Agilent 1290 Infinity II LC | Agilent 1290 Infinity II LC with ISET | Agilent 1290 Infinity II LC with ISET and fine-tuning |
|---|---|---|---|---|
| Imp K | – | – | – | – |
| Paracetamol | 23.24 | 31.56 | 34.46 | 35.38 |
| Imp B | 9.51 | 12.84 | 13.57 | 13.17 |
| Imp H | 2.87 | 4.77 | 4.56 | 4.37 |
| Imp F | 10.5 | 15.43 | 15.93 | 15.75 |
| Imp J | 11.86 | 18.87 | 18.38 | 18.23 |
| Imp A | 4.05 | 4.9 | 5.1 | 4.72 |

Figure 6. A) Resolution deviation in percentage B) Resolution values for the Waters Alliance 2695 LC and an Agilent 1290 Infinity II LC with different settings.

## References


1. Huesgen, A. G., Agilent 1290 Infinity Binary LC with ISET, emulation of the Waters Alliance 2695 LC system analyzing aromatic acids, *Agilent Technologies Application Note*, publication number 5991-2019EN, **2013**.

2. Agilent 1290 Infinity with ISET, *Agilent User Manual*, part number G4220-90313, **2014**.

3. Huesgen, A. G., Agilent 1290 Infinity Binary LC with ISET - Emulation of the Waters Alliance 2695 LC System Analyzing Analgesics, *Agilent Technologies Application Note*, publication number 5991-2792EN, **2013**.

www.agilent.com

DE-003316

This information is subject to change without notice.


Published in the USA, January 1, 2025
5991-6408EN

Agilent Technologies