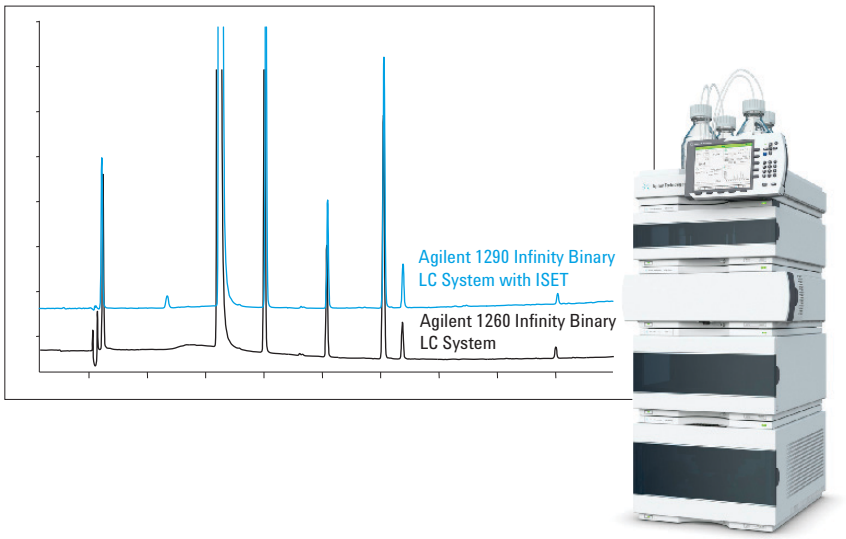
Transferring methods to the Agilent 1290 Infinity LC System using Intelligent System Emulation Technology (ISET)

Analysis of paracetamol and its impurities

Technical Overview



Author

A.G.Huesgen

Agilent Technologies, Inc.

Waldbronn, Germany

Abstract

Agilent's Intelligent System Emulation Technology (ISET) offers seamless transfer of methods from conventional LC systems such as the Agilent 1100 Series Quaternary LC System, which has a higher delay volume and different mixing behavior, to the Agilent 1290 Infinity LC System. When the ISET function is enabled on the 1290 Infinity LC System, almost the same retention times and resolution can be obtained. This Technical Overview describes the transfer of a conventional HPLC method from an Agilent 1260 Infinity Binary LC System to the 1290 Infinity LC System. Paracetamol and its impurities were analyzed using the 1290 Infinity LC System both with and without ISET enabled. Retention times and resolution of the different experiments were evaluated and compared with the original data obtained using the 1260 Infinity Binary LC System.

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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. Agilent provides seamless method transfer from the Agilent 1100/1200 Series LC systems, as well as from the Agilent 1220/1260 Infinity LC systems to the Agilent 1290 Infinity LC System using the Agilent Intelligent System Emulation Technology (ISET)[1]. ISET is implemented in the method screen of the pump. In this screen, ISET can be simply switched on to enable legacy methods to be run unchanged, or switched off to run fast or high-resolution UHPLC methods. ISET compensates not only for the different system delay volumes, but also for different mixing behavior of, for example, the Agilent 1260 Infinity Binary LC Pump and the Agilent 1290 Infinity Binary Pump. Using ISET, nearly the same retention times and resolution can be achieved when a method is transferred to the 1290 Infinity LC System.

In this Technical Overview a conventional method was transferred from the 1260 Infinity Binary LC System to the 1290 Infinity LC System. Paracetamol and six impurities were analyzed. The application was transferred to the 1290 Infinity LC System with and without ISET. Retention times and resolution were evaluated and compared with the 1260 Infinity Binary LC System data.

Experimental

Instrumentation and software

The Agilent 1290 Infinity LC System used for the experiments consisted of the following modules:

- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity Autosampler with Thermostat (G4226A, G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1260 Infinity Diode Array Detector (G4212A)

The Agilent 1260 Infinity Binary LC System was used for the experiments consisting of the following modules:

- Agilent 1260 Infinity Binary Pump (G1312A)
- Agilent 1260 Infinity Autosampler with Thermostat (G1329B, G1330B)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1260 Infinity Diode Array Detector (G4212B)

Agilent ChemStation revision C.01.03 and ISET revision 1.0 were used for the experiments. All LC modules had firmware revisions A.06.32 or B.06.32 or B.06.41 or higher, and all modules had RC.Net drivers.

Sample

The following mixture of compounds was used for the experiments:

Main:	Paracetamol
Impurity A:	2-Acetamidophenol
Impurity B:	N-(4-Hydroxyphenyl) propamide
Impurity F:	Nitrophenol
Impurity H:	4-(Acetylamino) phenyl Acetate (N,O-Diacetyl-4-aminophenol)
Impurity J:	4-Chloroacetanilide
Impurity K:	4-Aminophenol

Chromatographic conditions

Column:	Agilent ZORBAX Eclipse Plus C18, 100 × 4.6 mm, 3.5 µm
Mobile phase:	Water + 0.1% TFA, Acetonitrile + 0.09% TFA
Flow rate:	1.2 mL/min
Gradient:	5% ACN at 0 min, 5% ACN at 0.5 min, 90% ACN at 10 min
Stop time:	10 min
Post-time:	5 min
Injection volume:	5 µL (with needle wash for 6 s)
Column temp.:	30 °C
Detection:	220, 254, 270, 310/10 nm, Ref. 400/60 nm, 10 Hz, slit 4 nm

Results and discussion

The chromatographic method for paracetamol and its impurities was developed using the 1260 Infinity Binary LC System by deploying a conventional method with a 10 minute run time. The analysis of paracetamol and six impurities needs different wavelength settings to be able to quantify all peaks at maximum absorbance, (Figure 1). An isocratic step at low organic percentage at the beginning is needed to delay the elution of impurity K. To be able to elute impurity A in a reasonable time, the percentage of the organic phase was increased up to 90% within 10 minutes.

Compound	Detection wavelength
Impurity K	270 nm
Paracetamol	270 nm (linear)
Impurity B	254 nm
Impurity H	254 nm
Impurity F	310 nm
Impurity J	254 nm
Impurity A	220 nm

The original method was transferred to the 1290 Infinity LC without changing any parameters. As expected, the complete chromatogram shifted to lower retention times on the 1290 Infinity LC System due to the lower delay volume and different mixing behavior, (Figure 2).

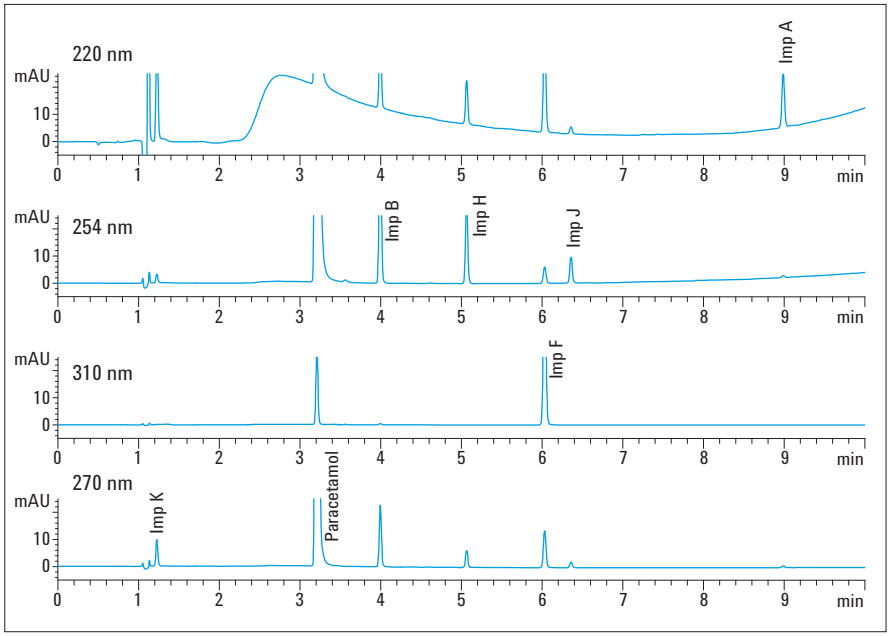

Figure 1
Analysis of paracetamol and six impurities at different wavelengths.

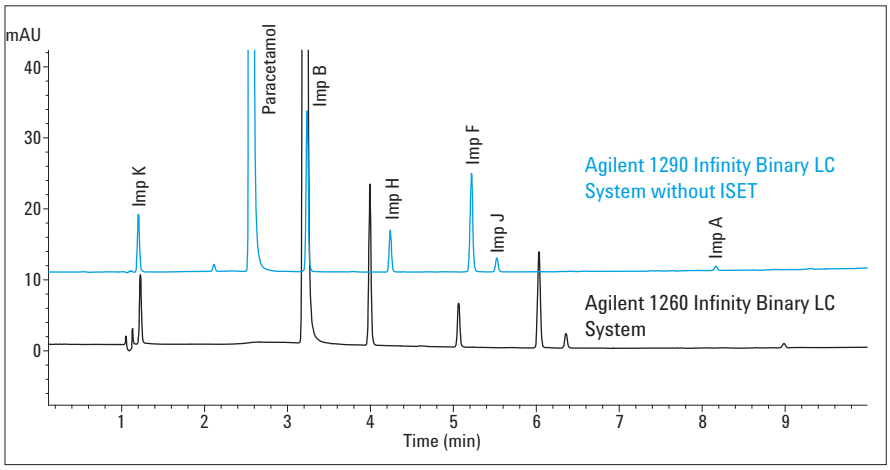

Figure 2
Overlay of chromatograms measured at 270 nm using the Agilent 1260 Infinity Binary LC System and the Agilent 1290 Infinity LC System without ISET enabled.

In the next experiment, the ISET function was enabled through the pump method screen of the software, (Figure 3). Here the originally used Agilent 1260 Infinity Pump and Autosampler had to be specified under *Module Parameter*. In our example, the original configuration of the 1260 Infinity Binary LC System included the pump (G1312B) and the autosampler (G1329B). All other parameters for detector, column thermostat, and autosampler remained the same. The new method was then saved under a new name and used in sequences or single runs.

The new method was transfered to the 1290 Infinity LC System and the resulting retention times and resolution evaluated. Figure 4 shows the original chromatogram was overlaid with a chromatogram obtained, and using the 1290 Infinity LC System with ISET.

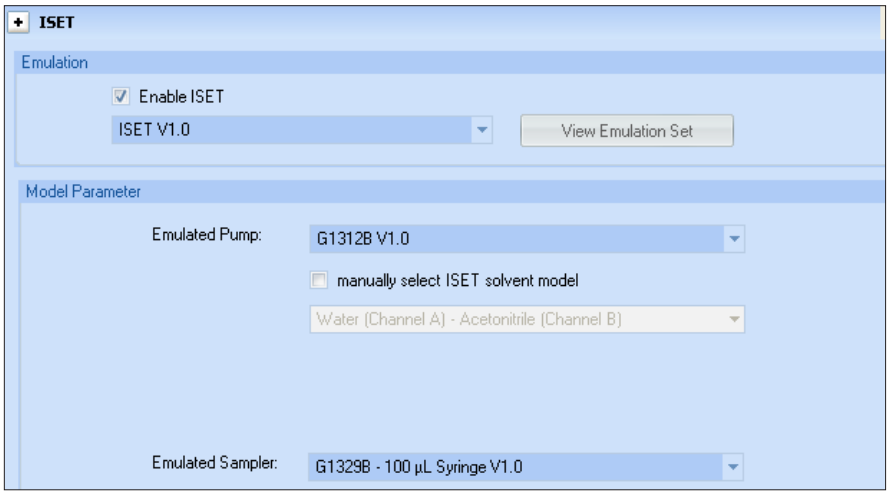

Figure 3
Software screen for enabling the ISET function.

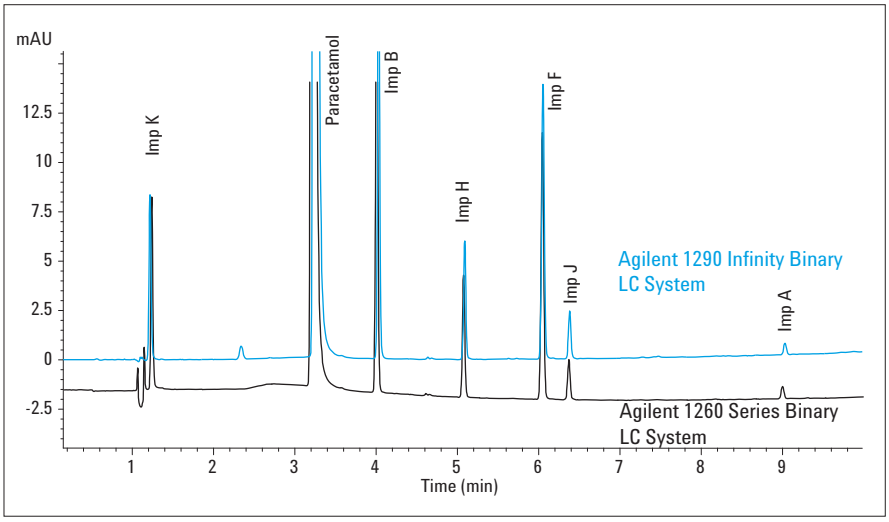

Figure 4
Overlay of chromatograms measured at 270 nm using the Agilent 1260 Infinity Binary LC System and the Agilent 1290 Infinity LC System with ISET enabled.

With ISET the retention times and the resolution showed a significantly better agreement with the original chromatogram. Figure 5 combines the results for the retention times with and without ISET.

The retention times without ISET differed up to -20%. The deviation of retention times on the 1290 Infinity LC with ISET was less than 1.3%. Figure 6 combines the results of the resolution data. The resolution without ISET differed up to -65%. The deviation of the resolution on the 1290 Infinity LC System with ISET was less than 2.3%.

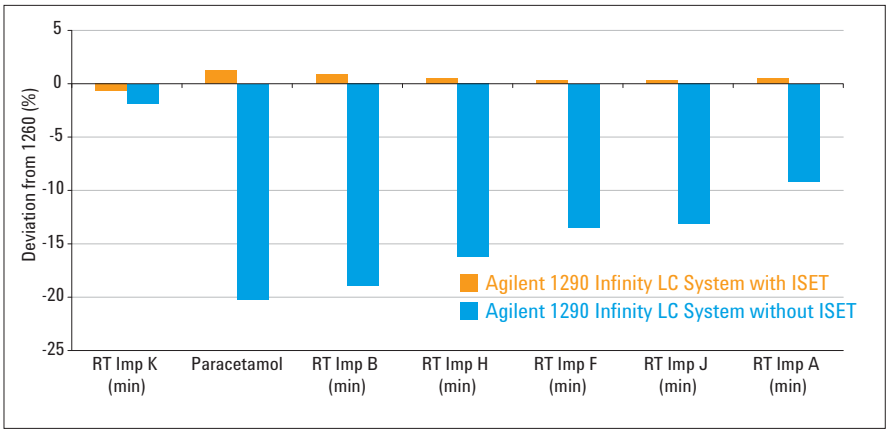

Figure 5
Deviation of retention times using the Agilent 1290 Infinity LC System with and without ISET.

Conclusion

The Agilent Intelligent System Emulation Technology (ISET) facilitates seamless transfer of conventional methods from an LC or another UHPLC system to the Agilent 1290 Infinity LC System. By specifying the pump and autosampler originally used, the 1290 Infinity LC System behaves like the original system and provides the same retention times and resolution. In the described experiments, a conventional method for the analysis of paracetamol and six impurities was transferred from the Agilent 1260 Infinty Binary LC System to the 1290 Infinity LC System with ISET enabled. Using ISET, the retention times did not differ more than 1.3% from the original data. The resolution did not differ more than 2.3%.

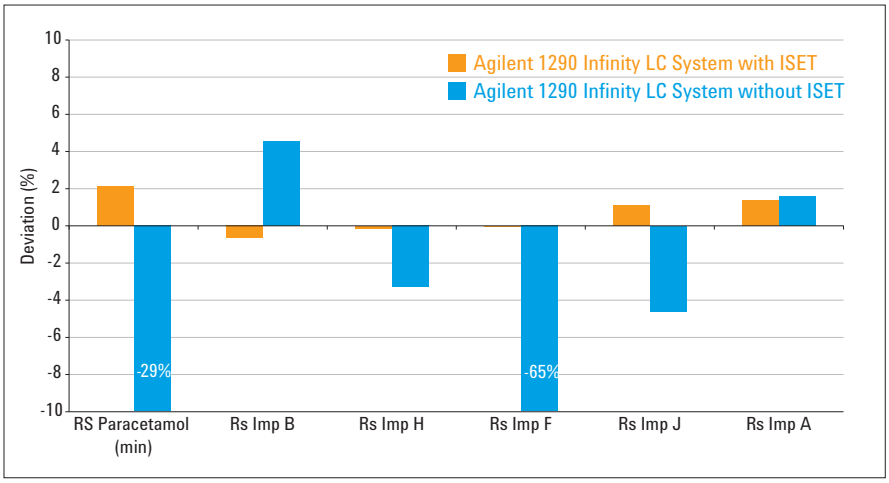

Figure 6
Deviation of resolution using the Agilent 1290 Infinity LC System with and without ISET.

Reference

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

www.agilent.com/chem/iset

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