

# Agilent 1290 Infinity II LC with ISET

Method Development for Transfer to an Agilent 1260 Infinity LC with Autosampler and Integrated Column Compartment

# **Technical Overview**

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# Abstract

Agilent Intelligent System Emulation Technology (ISET), with an Agilent 1290 Infinity LC or an Agilent 1290 Infinity II LC, facilitates seamless instrument-to-instrument method transfer. This Technical Overview describes a workflow for fast and automated method development using the Agilent 1290 Infinity II Method Development Solution. ISET enables the seamless transfer of the developed method to an Agilent 1260 Infinity LC equipped with an Autosampler with integrated column compartment (ICC).







# **Agilent Technologies**

### Introduction

Seamless instrument-to-instrument method transfer is an important topic for many laboratories in different industries. In the pharmaceutical industry, for example, the transfer of analytical methods from R&D to manufacturing is essential. Agilent Intelligent System Emulation Technology (ISET), in combination with an Agilent 1290 Infinity LC or an Agilent 1290 Infinity II LC, enables emulation of different Agilent and non-Agilent instrumentation, and facilitates seamless method transfer from one instrument to another<sup>1</sup>.

This Technical Overview describes a workflow for fast and automated method development on a 1290 Infinity II LC with ISET, and the seamless transfer to an Agilent 1260 Infinity LC equipped with an autosampler with an integrated column compartment (ICC). The Agilent 1290 Infinity II Method Development Solution provides for fast and automated scouting of stationary and mobile phases under ultrahigh performance LC conditions, as described in a previous Technical Overview<sup>2</sup>. The method developed under ultrahigh performance LC conditions is transferred to conventional LC conditions on the 1290 Infinity II LC and, using ISET, seamless transfer to a 1260 Infinity LC equipped with an autosampler with ICC is possible.

## **Experimental**

### Equipment

The Agilent 1290 Infinity II LC comprised the following modules:

- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B) with cooler (Option #100)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B) with 10 mm Max-Light cartridge cell (G4212-60008)

The Agilent 1260 Infinity LC comprised the following modules:

- Agilent 1260 Infinity Binary Pump (G1312B)
- Agilent 1260 Infinity Standard Degasser (G1322A)
- Agilent 1260 Infinity Autosampler (G7129A) with integrated column compartment, 3.0 μL heat exchanger (Option #063) and sample cooler (Option #100)
- Agilent 1260 Infinity Diode Array Detector (G4212B) with 10 mm Max-Light cartridge cell (G4212-60008)

#### Software

Agilent OpenLAB CDS ChemStation Edition rev. C.01.07 [27] with ISET 4 V1.0.

#### Columns

- Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 μm (p/n 959757-902)
- Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 μm (p/n 959993-902)

#### Chemicals

All solvents were LC grade. Acetonitrile was purchased from Merck (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA).

Sample HPLC standard mixture

#### **Methods**

Table 1. Chromatographic conditions for UHPLC analysis.

Parameter	Value
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 $\times$ 50 mm, 1.8 $\mu$ m
Solvent	A) H <sub>2</sub> 0
	B) Acetonitrile
Gradient	5 %B at 0 minutes,
	75 %B at 1.5 minutes,
	95 %B at 2.0 minutes
Stop time	2.5 minutes
Post time	2.0 minutes
Flow rate	1.000 mL/min
Temperature	60 °C
Injection volume	1.0 μL
Detection	254/4 nm, reference 360/100 nm, data rate 80 Hz

Table 2. Chromatographic conditions for HPLC analysis.

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 $\times$ 150 mm, 5 $\mu$ m
Solvent	A) H <sub>2</sub> 0
	B) Acetonitrile
Gradient	5 %B at 0 minutes,
	75 %B at 18 minutes,
	95 %B at 24 minutes
Stop time	30 minutes
Post time	24 minutes
Flow rate	1.200 mL/min
Temperature	60 °C
Injection volume	4.8 μL
Detection	254/4 nm, reference 360/100 nm, data rate 20 Hz

# **Results and Discussion**

The 1290 Infinity II Method Development Solution enables fast and automated scouting of stationary and mobile phases under ultrahigh performance LC conditions, as described in a previous Technical Overview<sup>2</sup>. Figure 1 shows the chromatogram resulting from the chosen optimal ultrahigh performance LC conditions found after evaluation of the scouting campaign, and the retention time (RT) precision determined from eight consecutive runs. Excellent RT precision was obtained in the fast LC analysis on the 1290 Infinity II LC.



Figure 1. Fast UHPLC analysis on an Agilent 1290 Infinity II LC; RT precision determined from eight consecutive runs.

The developed ultrahigh performance method was transferred to conventional LC conditions and run on a 1290 Infinity II LC with ISET, as well as on a 1260 Infinity LC equipped with an autosampler and ICC. Figures 2 and 3 show the resulting chromatograms and the excellent RT precision determined from eight consecutive runs.

The 1290 Infinity II LC with ISET enables seamless transfer to an Agilent 1260 Infinity LC equipped with an autosampler with ICC. Figure 4 shows the conventional LC analysis on the 1260 Infinity LC and on the 1290 Infinity II LC with and without ISET. Table 3 shows the respective RT deviations.



Figure 2. Conventional LC analysis on an Agilent 1290 Infinity II LC with ISET; RT precision determined from eight consecutive runs.



Figure 3. Conventional LC analysis on an Agilent 1260 Infinity LC with an autosampler and ICC; RT precision determined from eight consecutive runs.

Using ISET, excellent agreement of RTs with deviations below 1 % between the 1290 Infinity II LC and the 1260 Infinity LC was achieved.



Figure 4. Conventional LC analysis on an Agilent 1260 Infinity LC with an autosampler and ICC and on an Agilent 1290 Infinity II LC with and without ISET.

Table 3. RTs and deviations for conventional LC analysis on an	Agilent 1260 Infinity	/ LC with an autosampler and ICC	, and on an Agilent 1290 Infinity II LC with
and without ISET.			

	Agilent 1260 Infinity LC	Agilent 1290 Infinity II LC without ISET			Agilent 1	Agilent 1290 Infinity II LC with ISET		
Peak	RT (min)	RT (min)	RT Deviation (min)	RT Deviation (%)	RT (min)	RT Deviation (min)	RT Deviation (%)	
1	1.292	1.286	-0.01	-0.5	1.286	-0.01	-0.5	
2	6.860	6.225	-0.64	-9.3	6.807	-0.05	-0.8	
3	8.133	7.392	-0.74	-9.1	8.100	-0.03	-0.4	
4	10.108	9.345	-0.76	-7.5	10.080	-0.03	-0.3	
5	11.979	11.210	-0.77	-6.4	11.955	-0.02	-0.2	
6	12.529	11.800	-0.73	-5.8	12.544	0.02	0.1	
7	13.663	12.896	-0.77	-5.6	13.644	-0.02	-0.1	
8	14.710	13.940	-0.77	-5.2	14.676	-0.03	-0.2	
9	17.952	17.187	-0.77	-4.3	17.933	-0.02	-0.1	

# Conclusions

The Agilent 1290 Infinity II Method Development Solution is ideal for fast and automated scouting of stationary and mobile phases under ultrahigh performance LC conditions. After transfer of the developed method to conventional LC conditions, ISET allows for seamless method transfer to an Agilent 1260 Infinity LC equipped with an autosampler with an ICC. Excellent agreement of RTs with deviations below 1 % between the Agilent 1290 Infinity II LC with ISET and the 1260 Infinity LC was achieved.

## References

- Agilent 1290 Infinity with ISET, Agilent Technologies User Manual, part number G4220-90314, 2015.
- Naegele, E.; Schneider, S. Automated Scouting of Stationary and Mobile Phases Using the Agilent 1290 Infinity II Method Development Solution, Agilent Technologies Technical Overview, publication number 5991-5934EN, 2015.

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