

Agilent 1290 Infinity II LC with ISET – Emulation of the Agilent 1100 Series LC Through Waters Empower Software

Analysis of an Analgesic Mixture

Suitable for Agilent
1290 Infinity III LC

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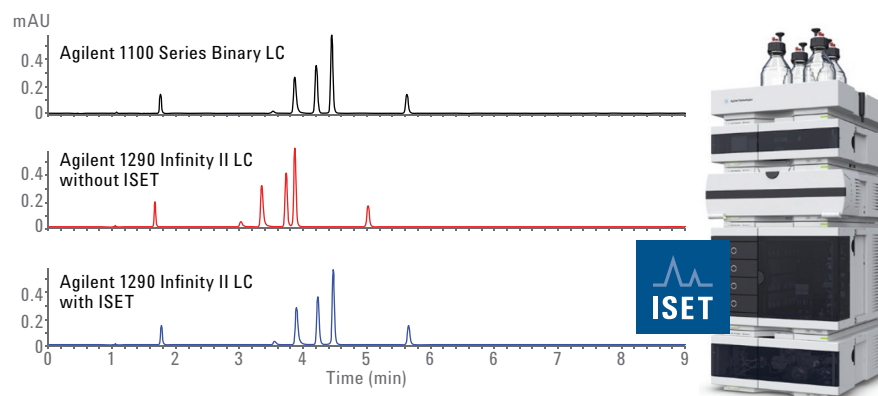
Application Note

Small Molecule Pharmaceuticals

Abstract

The Agilent Intelligent System Emulation Technology (ISET) enables seamless transfer of methods from HPLC systems such as the Agilent 1100 Series Binary LC to the Agilent 1290 Infinity II LC. With ISET, the same retention times and peak resolution can be obtained without any method or hardware changes.

This Application Note demonstrates the use of ISET through Waters Empower software with Agilent Instrument Control Framework for the analysis of five analgesics. The mixture was analyzed using an 1100 Series Binary LC and a 1290 Infinity II LC with ISET enabled. On both systems, the achieved retention times and resolution values showed excellent agreement.



Agilent Technologies

Introduction

Agilent Instrument Control Framework (ICF) is a software component that enables third-party software, such as Waters Empower, to fully control Agilent LC systems. With ICF, the control of the LC system will have the same look and feel in any third-party LC or LC/MS data system, and provide full functionality for each LC module¹. The Agilent Intelligent System Emulation Technology (ISET), for example, can easily be enabled by Waters Empower software with ICF. ISET, together with an Agilent 1290 Infinity or an Agilent 1290 Infinity II LC, enables seamless LC method transfer from a conventional HPLC system to a UHPLC system without changing the original method². Legacy methods can run unchanged, and retention times and resolution of the analytes will remain the same. Another advantage of ISET is that method development labs can increase the speed for method development by using the UHPLC performance, which enables fast screening of columns and solvents. The newly developed method can be further emulated for the target system to be confident that the method will run as intended³.

In this Application Note, a mixture of five analgesic drugs were analyzed on an Agilent 1100 Series Binary LC and further transferred to a 1290 Infinity II LC equipped with a high-speed pump. Deviation in retention time and resolution were calculated for the original method and the transferred method.

Experimental

Instrumentation

For the analysis of an analgesic mix, the following instruments were used:

Agilent 1100 Series Binary LC:

- Agilent 1100 Series Binary Pump (G1312A)
- Agilent 1100 Series Micro Vacuum Degasser (G1379A)
- Agilent 1100 Series Autosampler (G1313A)
- Agilent 1100 Series Thermostatted Column Compartment (G1316A)
- Agilent 1100 Series Diode Array Detector (G1315B)

Agilent 1290 Infinity II LC:

- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- 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

Solvents and samples

All solvents were LC grade. Acetonitrile and methanol were purchased from Merck, and the analgesic compounds from Sigma-Aldrich. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak).

For the experiments, the following mixture of compounds was used:

- Acetaminophen (20 µg/mL)
- Antipyrine (10 µg/mL)
- Hydroxyantipyrine (170 µg/mL)
- Acetanilide (10 µg/mL)
- Phenacetin (30 µg/mL)

For all compounds, a stock solution of 1 mg/mL in 20 % acetonitrile in water + 0.1 % formic acid was prepared and further diluted to the final concentration using 0.1 % formic acid in water.

Column

Agilent ZORBAX RRHD SB C18, 4.6 × 150 mm, 3.5 µm (p/n 863953-902)

Software

Waters Empower 3 (build 3471) with Waters ICS 2.1 Hotfix 1 version and an Agilent ICF A.02.03 DU1 HF2 version to control the Agilent 1100 Series Binary LC and Agilent 1290 Infinity II LC system and ISET 4 V1.0.

Method

Table 1 shows method parameters.

Results and Discussion

A mixture of five analgesic drugs was analyzed using a conventional 4.6×150 mm HPLC column packed with $3.5 \mu\text{m}$ particles on an 1100 Series Binary LC. The LC system was controlled through Waters Empower 3 software. Figure 1 shows a chromatogram of the separated analgesic mix.

Table 1. Chromatographic conditions for the analysis of the analgesic mixture.

Parameter	Value
Mobile phase	A) water B) acetonitrile
Gradient	0 minutes – 15 %B 8 minutes – 50 %B 9 minutes – 80 %B
Stop time	10 minutes
Posttime	5 minutes
Flow rate	1.5 mL/min
Injection volume	5 μL with standard needle wash with 50 % methanol in water
Column temperature	30 $^{\circ}\text{C}$
Detection	254/4 nm, ref. wavelength 360/100 nm, 20 Hz

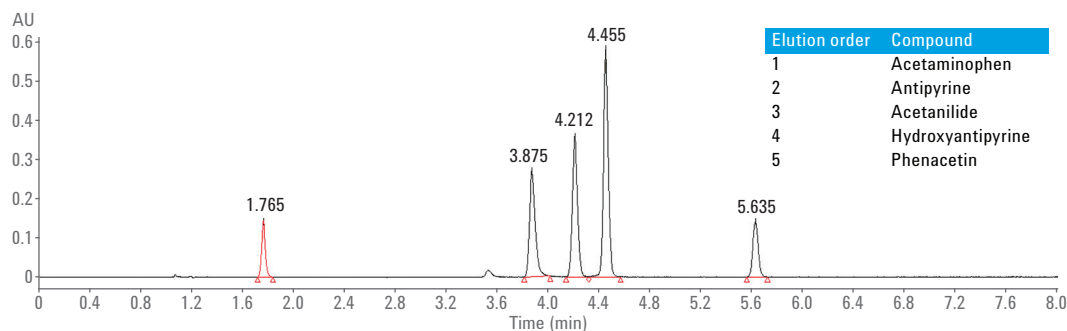


Figure 1. Chromatogram of an analgesic mixture acquired on an Agilent 1100 Series Binary LC.

The method was further transferred to a 1290 Infinity II LC. In general, UHPLC systems have significantly lower delay volumes compared to conventional HPLC systems. This will influence the retention time and resolution of the analytes. Two different solutions are typically used to compensate for the different delay volumes; an isocratic step can be programmed at the beginning of the gradient, or additional capillaries can be installed to increase the delay volume on the UHPLC system. A more

convenient way to transfer a method is by using ISET. No changes in the method or in the hardware are required. Figure 2 shows chromatograms for the analysis of the analgesic mixture performed on the 1100 Series Binary LC and on the 1290 Infinity II LC with and without ISET enabled.

As expected, all peaks shifted to lower retention times with the UHPLC system without ISET (Figure 2, blue trace). By enabling ISET (Figure 2 green trace),

the retention times fit well to the chromatogram acquired on the 1100 Series Binary LC.

In addition, the resolution changed by transferring the method to an UHPLC system. By using ISET, these values were in much better agreement with the original chromatogram. Table 2 shows a detailed overview. The obtained retention time precisions were excellent for both systems.

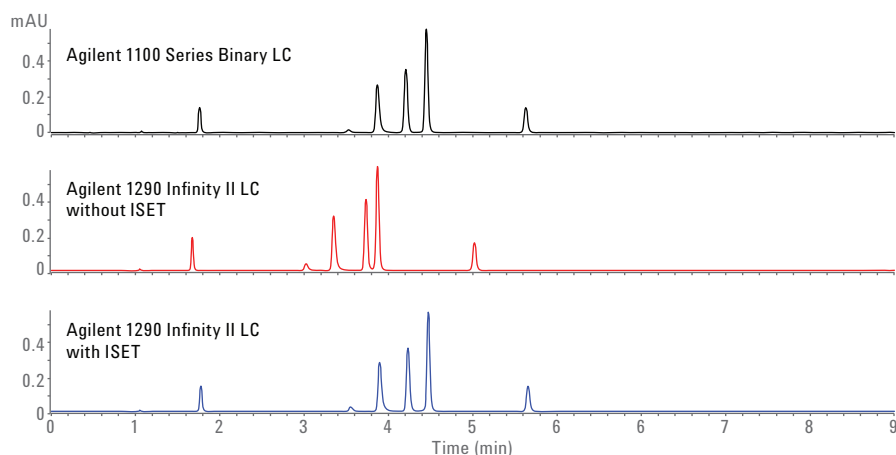


Figure 2. Overlay of chromatograms obtained on an Agilent 1100 Series Binary LC and on an Agilent 1290 Infinity II LC with and without ISET enabled.

Table 2. Comparison of the retention times (RT), RT precision and resolution between the Agilent 1100 Series Binary LC and the Agilent 1290 Infinity II LC with and without using ISET. RT precision was calculated from eight consecutive runs.

Compound	Agilent 1100 Series Binary LC			Agilent 1290 Infinity II LC			Agilent 1290 Infinity II LC with ISET		
	RT (min)	RSD RT (%)	Resolution	RT (min)	RSD RT (%)	Resolution	RT (min)	RSD RT (%)	Resolution
Acetaminophen	1.765	0.041	—	1.674	0.122	—	1.772	0.062	—
Antipyrine	3.875	0.031	31.69	3.354	0.052	27.8	3.892	0.022	31.68
Acetanilide	4.212	0.024	4.50	3.736	0.037	5.25	4.230	0.028	4.41
Hydroxyantipyrine	4.456	0.028	3.46	3.873	0.043	2.03	4.471	0.035	3.45
Phenacetin	5.635	0.023	16.60	5.022	0.039	16.56	5.652	0.021	16.61

By enabling ISET, the retention time deviation was well below 1 %, and so within the allowed range of ± 5 % deviation in retention time², see Figure 3A. Without using ISET, the deviation of retention time for most of the analgesic drugs was outside the specification range. The maximum retention time deviation was -13 %.

Comparing the resolution between the HPLC and UHPLC without enabling ISET, the resolution was outside the specification limit for some of the analgesic drugs, see Figure 3B. For the resolution, a deviation of maximum -5 % is allowed. The results improved by using ISET, and were close to the original data obtained with the 1100 Series Binary LC.

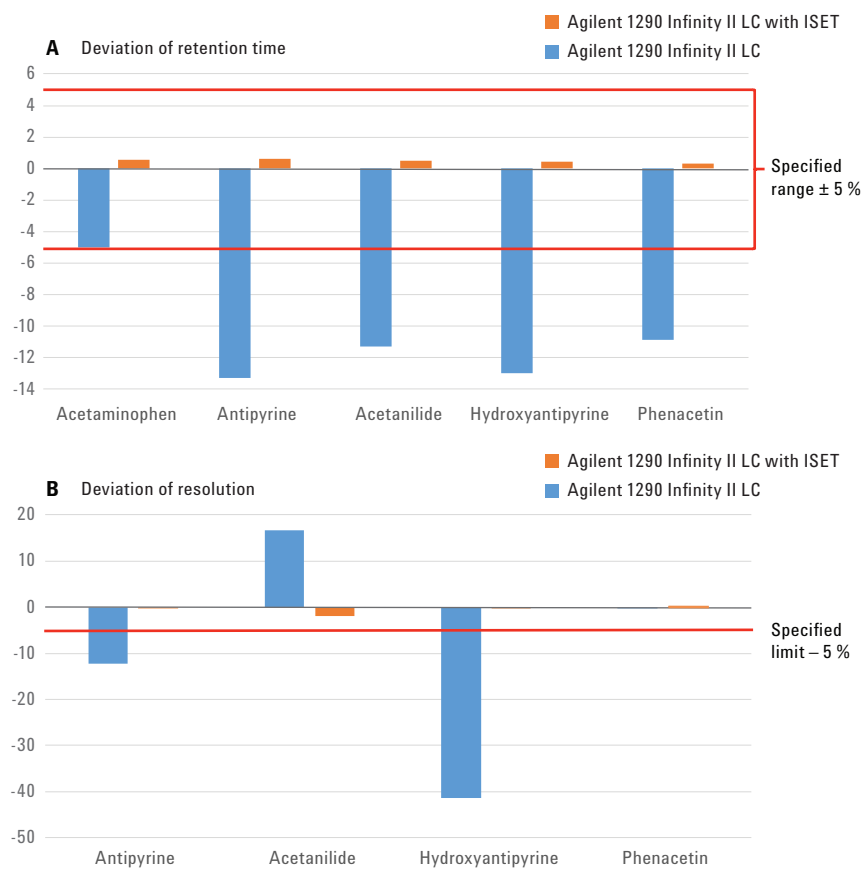


Figure 3. A) Deviation of retention time, B) Deviation of resolution for the Agilent1290 Infinity II LC with and without ISET enabled in comparison to the Agilent 1100 Series Binary LC.

The 1100 Series Binary LC and the 1290 Infinity II LC were both controlled through Waters Empower software. Figure 4 shows the method screen of the Agilent 1290 Infinity II High-Speed Pump with the ISET setup. Through Waters Empower, enabling ISET is just as easy with the same functionalities as through Agilent OpenLAB CDS ChemStation Edition.

Conclusion

Agilent Intelligent System Emulation Technology (ISET) together with an Agilent 1290 Infinity II LC enables easy and straightforward transfer of methods from an HPLC to a UHPLC system. In this study, a mixture of five analgesics was separated on an Agilent 1100 Series Binary LC, and further transferred to a 1290 Infinity II LC. By enabling ISET, the retention times and resolution of the analytes were in excellent agreement

to the original HPLC method. Both LC systems were controlled through Waters Empower 3 software, and the pump function ISET was enabled within the software. The resulting chromatograms agreed over 99 % with respect to retention time. This Application Note clearly demonstrates the universal character of the 1290 Infinity II LC by emulating other LC systems, and controlled through third-party software such as Waters Empower.

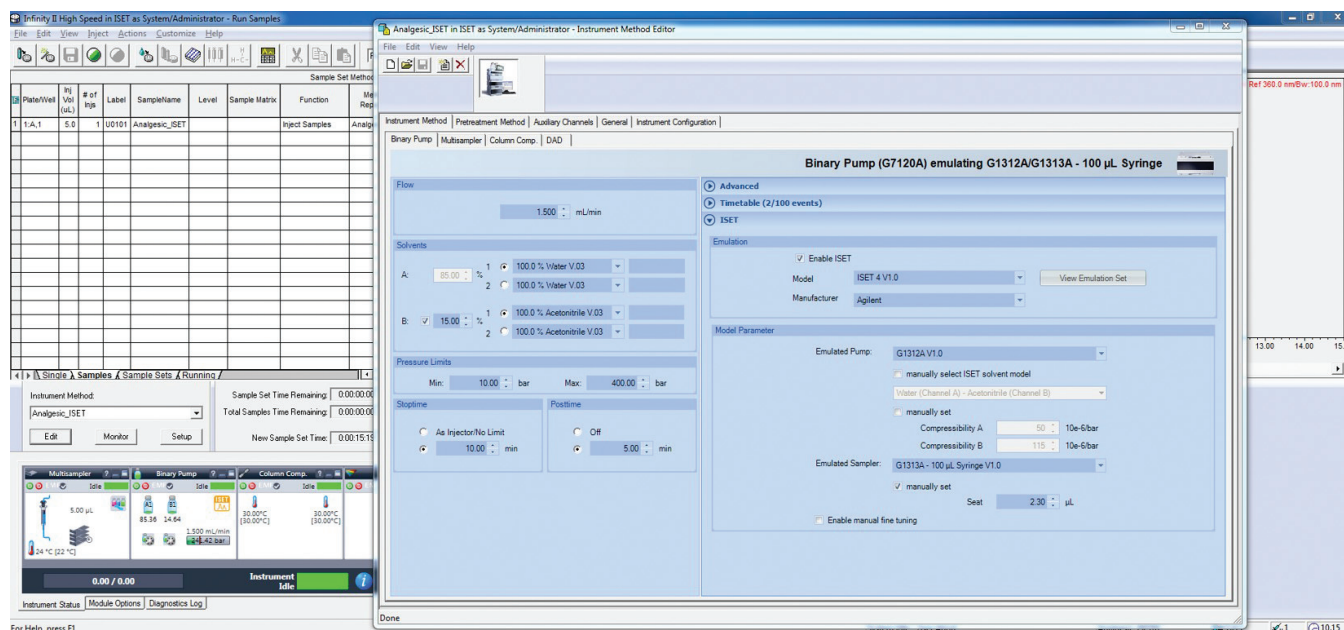


Figure 4. Example of the Agilent Instrument Status screen with enabled ISET and pump method screen under Empower 3 and ICF.

References

1. The Agilent Instrument Control Framework (ICF), *Agilent Technologies Customer Letter*, publication number 5990-5756EN, **2010**.
2. Agilent 1290 Infinity with ISET, *Agilent Technologies User Manual*, publication number G4220-90313, **2014**.
3. 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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