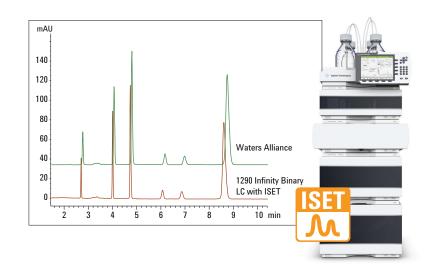


# Agilent 1290 Infinity Binary LC System with ISET - Emulation of the Waters Alliance 2695 LC system analyzing $\beta$ -blockers

# **Application Note**

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## **Abstract**

The Intelligent System Emulation Technology (ISET) using the Agilent1290 Infinity Binary LC was introduced to emulate Agilent 1200 Infinity Series and 1200 Series LCs, now the emulation of non-Agilent LCs has been introduced.

This Application Note shows the emulation of the Waters Alliance 2695. Based on a Waters Application Note, the transfer of the analysis of  $\beta$ -blockers onto the 1290 Infinity Binary LC using ISET was evaluated. The agreement of retention times and resolution was determined.



## Introduction

Seamless instrument-to-instrument method transfer is often an invariable demand in the pharmaceutical industry because changing validated methods is expensive and time consuming. Equipment has to be replaced from time to time, and the Agilent 1290 Infinity Binary LC, in combination with ISET offers a possibility to emulate older non-Agilent LC instrumentation, such as the Waters Alliance LC systems. Old methods from non-Agilent equipment can be transferred to the 1290 Infinity Binary LC System with ISET and receive the same results regarding retention times and resolution. Methods can be applied to be prepared for the future with the 1290 Infinity Binary LC UHPLC.

This Application Note analyzes  $\beta$ —blockers on the 1290 Infinity Binary LC System using a chromatographic method developed on the Waters Alliance 2695 LC system. Seamless method transfer with excellent correlation of retention times and resolution is shown.

# **Experimental**

Table 1 shows the instruments used.

# **Chromatographic conditions**

Column: Atlantis T3 4.6  $\times$ 150, 5  $\mu m$ 

Mobile phases:  $A = 10 \text{ mM NH}_4\text{COOH}$ 

(0.771 g/L) pH = 3,

B = ACN

Gradient: at 0 minutes 5 % B,

at 3 minutes 25 % B, at 10 minutes 25 % B, at 11 minutes 5 % B, at 15 minutes 5 % B

Flow rate: 2 mL/min Injection volume:  $10 \mu$ L Column temperature:  $30 \,^{\circ}$ C

Detection: 280 nm, 10 Hz,

filter response 0.2

# Acquisition and Evaluation Software

OpenLAB CDS Chemstation version C.01.04 and ISET

	Agilent 1290 Infinity Binary LC System	Waters Alliance LC system
Binary pump	G4220A	2695
Auto sampler	G4226A	
ALS cooler	G1330B	
Column compartment	G1316C	
Diode array detector	G4212A	Dual absorbance detector

Table 1 Instrumentation used.

# **Analyzed compounds**

$$0 \qquad 0 \qquad 0 \qquad H \qquad CH_3$$

Atenolol

$$\begin{array}{c|c} & OH & H \\ \hline \\ H_3CO & CH_3 \end{array}$$

Metoprolol

Pindolol 0 NH<sub>2</sub> OH OH

Labetalol

O N H

# **Results and Discussion**

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

 Analysis of β-blockers on the Alliance 2695, based on a Waters Application Note<sup>1</sup>

- Transfer of the developed method onto the 1290 Infinity Binary LC with and without applying ISET
- Determination of the deviation of retention times, specified deviation is < ±5%</li>
- Determination of the resolution, specified maximum deviation < -5%</li>

In the 1290 Infinity Binary LC ISET setup screen, select the instrumentation which is to be emulated, see Figure 1. With only four inputs, the emulation parameters are configured and can be saved together with the other chromatographic parameters, such as flow rate, temperatures, and more, in one method.

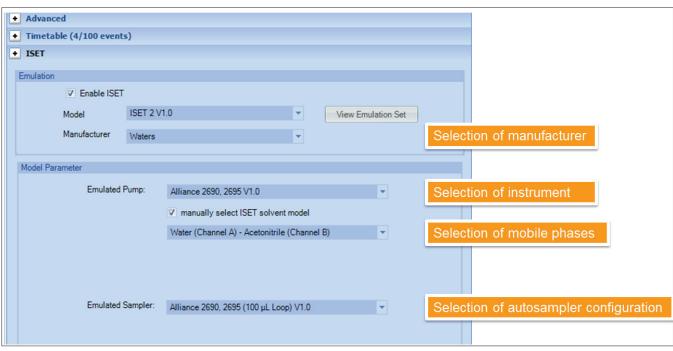


Figure 1
Selection of the instrumentation to be emulated.

Figure 2 shows the resulting chromatograms. Without ISET, all retention times shifted to lower values. With ISET, excellent correlation was obtained.

Figure 3 summarizes the results for the retention time differences. Without ISET, the deviation was -15.5%, maximum. With ISET, the deviation of retention times was <-2.5% overall. The specified allowed deviation is  $<\pm5\%$ .

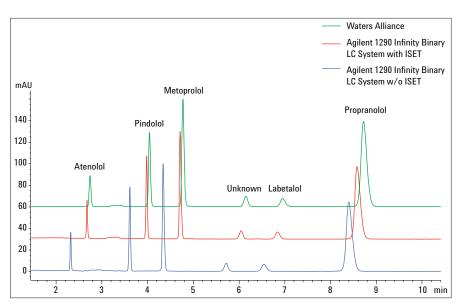


Figure 2
Overlay of chromatograms obtained on the Alliance and the Agilent 1290 Infinity Binary LC System with and without
ISET

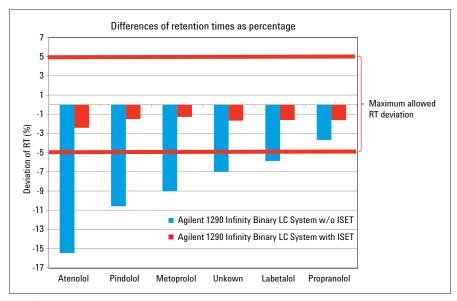


Figure 3
Deviation of retention times with and without ISET on the Agilent 1290 Infinity Binary LC System.

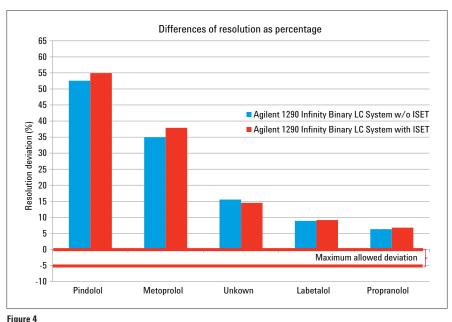
Figure 4 summarizes the results of the resolution differences. For all compounds, the resolution was better on the 1290 Infinity Binary LC System. This was mainly due to the small post column dispersion volume on the 1290 Infinity Binary LC System compared to the used configuration for the Waters Alliance 2695 LC. The lower dispersion volume resulted in lower peak widths at half height and, consequently, in improved resolution. The maximum allowed deviation is < –5%.

# Conclusion

The Agilent 1290 Infinity Binary LC System in combination with the Intelligent System Emulation Technology (ISET) enables the emulation of older non-Agilent LCs, such as the Waters Alliance LC system. The allowed maximum deviation of retention times is  $<\pm5\%$ . In this example, the deviation of retention times is <-2.5%. The resolution had improved for all analyzed compounds on the 1290 Infinity Binary LC System and fulfilled the maximum allowed deviation of <-5%.

# Reference

 Waters Application Note: Analysis of β-Blockers on Atlantis T3, January
 2009.



Resolution data with and without ISET.



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