Agilent 1290 Infinity Binary LC with ISET, emulation of the Waters Alliance 2695 LC system analyzing aromatic acids

Application Note

Food Testing & Agriculture

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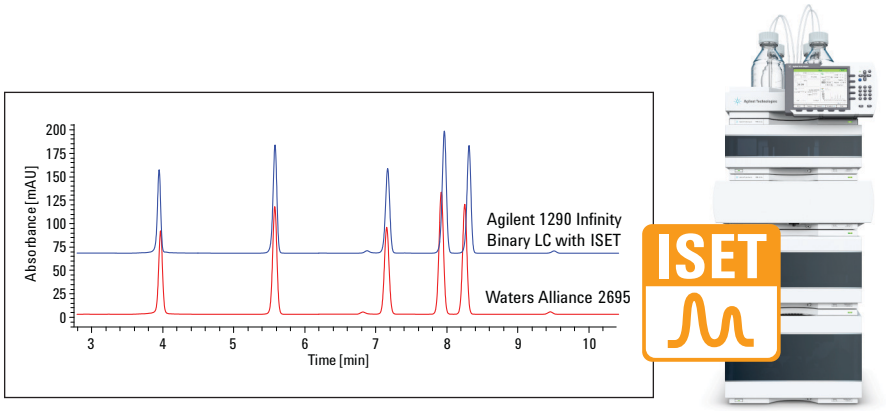

Abstract

The Intelligent System Emulation Technology (ISET) using the Agilent 1290 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 aromatic acids onto the 1290 Infinity Binary LC using ISET was evaluated. The agreement of retention times and resolution was determined.

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Introduction

Seamless instrument-to-instrument method transfer is often a strong demand for most industries. Changing established methods is expensive and time consuming and comparison with reference data is difficult, but equipment has to be replaced from time to time. The Agilent 1290 Infinity Binary LC, in combination with ISET, offers the possibility to emulate older non-Agilent instrumentation, like the Waters Alliance 2695 LC systems. Old methods from non-Agilent equipment can be transferred to the 1290 Infinity Binary LC with ISET maintaining the same retention times results and, typically, receiving better resolution. Using the 1290 Infinity Binary LC, UHPLC methods can be applied for future use.

This Application Note analyzes aromatic acids on the 1290 Infinity Binary LC using a chromatographic method developed on the Waters Alliance 2695 LC system. Seamless method transfer, with excellent correlation of retention times, and typically better resolution, is shown.

Experimental

The following instruments were used, see Table 1.

Acquisition and Evaluation Software

OpenLAB CDS ChemStation version C.01.04 and ISET

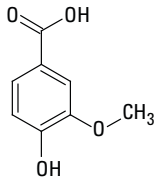
	Agilent 1290 Infinity Binary LC	Waters Alliance
Agilent 1290 Infinity Binary Pump:	G4220A	2695
Agilent 1290 Infinity Autosampler:	G4226A	
ALS cooler:	G1330B	
Column compartment:	G1316C	
Diode array detector:	G4212A	VWD (Dual absorbance detector)

Table 1
Instrumentation used

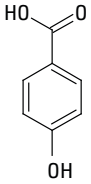
Chromatographic conditions

Column:	Atlantis T3 4.6 × 150, 5 µm
Mobile phases:	A = 10 mM NH_4COOH (0.771 g/L) pH = 3, B = ACN
Gradient:	at 0 minutes 0% B, at 10 minutes 25% B, at 11 minutes 0% B, at 15 minutes 0% B,
Flow rate:	2 mL/min
Injection volume:	10 µL
Column temperature:	30 °C
Detection:	285 nm, 5 Hz

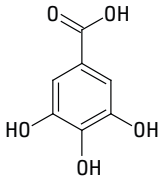
Analyzed compounds



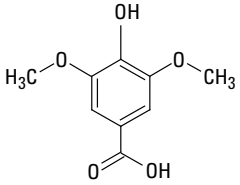
Vanillic acid



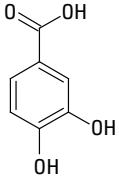
4-Hydroxybenzoic acid



Gallic acid



Syringic acid



Protocatechuic acid

The analyzed aromatic acids are plant ingredients used, for example, in traditional plant medicine.

- Gallic acid has antiviral and antifugal activities.
- Protocatechuic acid has antioxidant and anti-inflammatory activities.
- 4-Hydroxybenzoic acid is a metabolite of catechins after intake of green tea.
- Vanillic acid is used as flavoring agent.
- Syringic acid occurs in special plant oil.

In the ISET parameter screen of the 1290 Infinity Binary LC, enter the following parameter to select the instrument to be emulated, see Figure 1.

Results and Discussion

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

Experiments

- Analysis of organic acids on the Alliance 2695, based on a Waters Application Note[1]
- Transfer of the developed method onto the 1290 Infinity Binary LC with and without applying ISET and ISET with fine tuning

Evaluated parameters

- Precision of retention times for both LC systems
- Determination of the deviation of retention times; specified allowed maximum deviation is <±5%
- Determination of the resolution, typically better on the 1290 Infinity Binary LC; specified allowed maximum deviation is <–5%

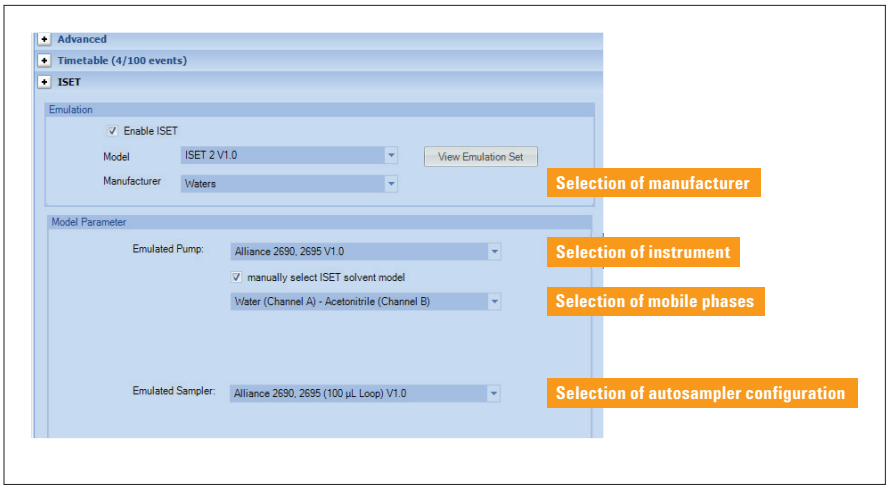


Figure 1
Selection of the instrumentation to be emulated.

Precision of Retention Times

Starting at 0% for the organic channel and at 100% for the aqueous channel are demanding gradient conditions for any pump. The precision, especially for the early eluting peaks, might be significantly lower than for the later eluting peaks. The 1290 Infinity Binary LC provided excellent precisions even for these demanding conditions, see Figure 2. The precision for the first peak was 0.02% RSD using the 1290 Infinity Binary LC and 0.23% RSD for the Alliance 2695 LC. The precision for all other peaks was ≤ 0.02% RSD for both instruments.

The 1290 Infinity Binary LC system performed approximately 10 times better for the first peak.

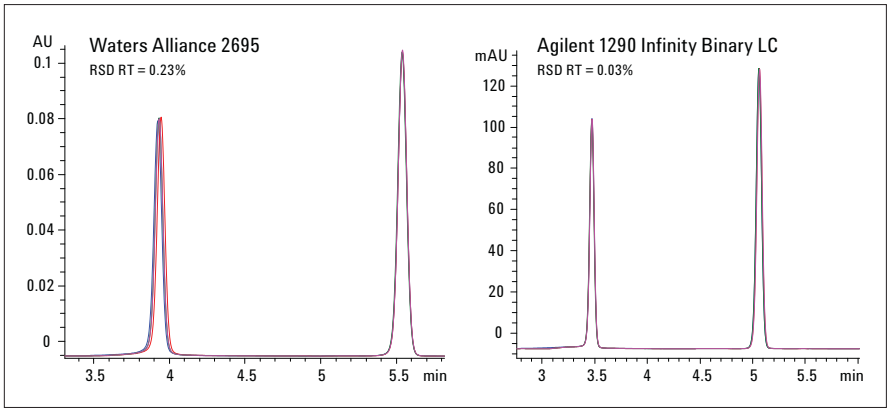


Figure 2
Precision of retention times applying demanding gradient conditions.

Determination of the Deviation of Retention Times

The chromatographic conditions developed on the Alliance 2695 were transferred onto the 1290 Infinity Binary LC. The best correlation of retention times with the original data was obtained using the 1290 Infinity Binary LC with ISET and fine tuning, see the red trace in Figure 3. Fine tuning means in this case an additional delay volume of 400 µL was added in the ISET parameter screen by enabling the manual fine tuning parameter see Figure 4.

Without the fine tuning step, the agreement of retention times using the 1290 Infinity Binary LC was already close, see the orange trace in Figure 3.

Without enabling the ISET tool, the 1290 Infinity Binary LC showed less retention for all peaks as expected, see the blue trace in Figure 3.

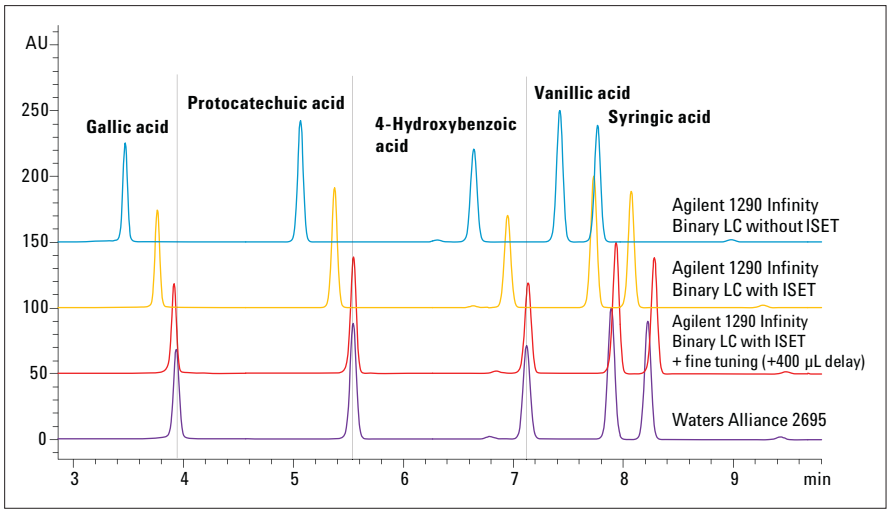


Figure 3
Overlay of chromatograms of all used LC instruments and Agilent 1290 Infinity Binary LC configurations.

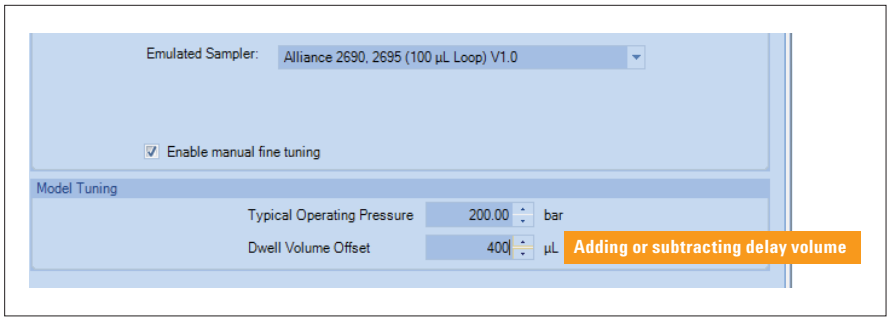


Figure 4
Fine tuning parameters within ISET.

Figure 5 summarizes the retention time deviations obtained on the different 1290 Infinity Binary LC configurations. Optimum results were achieved using the ISET tool with fine tuning. The maximum retention time deviation was +0.93%. With ISET and no fine tuning, the maximum deviation was 4.4%. This was also within the allowed specified range.

Without ISET, the retention time deviation on the 1290 Infinity Binary LC was 11.8%. This was mainly due to the significant lower delay volume of the 1290 Infinity Binary LC compared to the Alliance LC system. The allowed deviation of retention times for the 1290 Infinity Binary LC with ISET is ±5%.

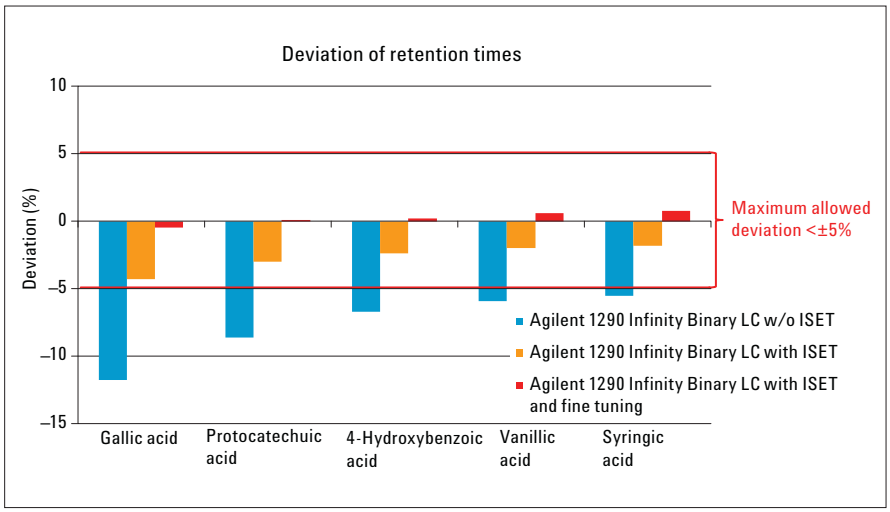


Figure 5
Deviation of retention times as percentage for all Agilent 1290 Infinity Binary LC configurations.

Determination of the Deviation of the Resolution Data

Figure 6 summarizes the resolution data for the different 1290 Infinity Binary LC configurations. With ISET and ISET and fine tuning, the resolution had improved for all peaks. Without ISET, the resolution for the second peak was decreased significantly.

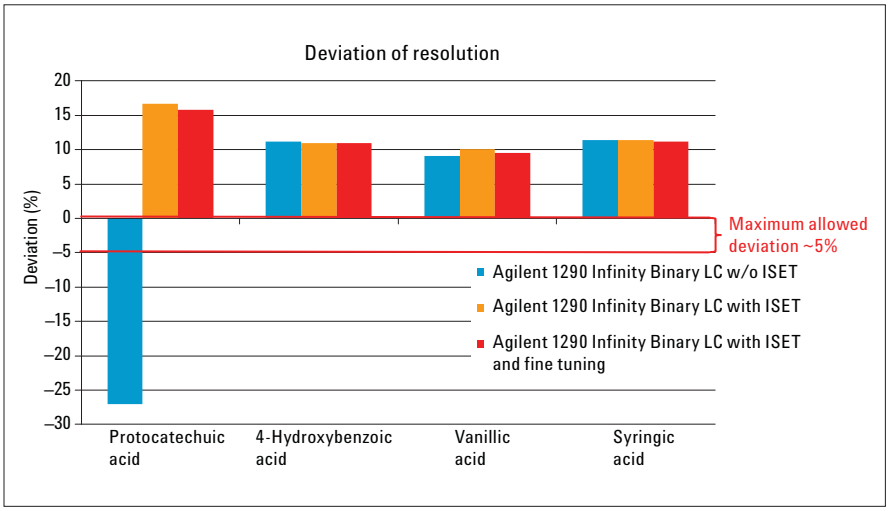


Figure 6
Deviation of resolution data for all Agilent 1290 Infinity binary LC configurations.

Conclusion

The Agilent 1290 Infinity Binary LC, in combination with the ISET, enables the emulation of older non-Agilent LCs, such as the Waters Alliance LC system, with excellent precision of retention times. The allowed deviation of retention times is <±5%. In this example, the deviation of retention times was <1% using the 1290 Infinity Binary LC with ISET and fine tuning. The resolution was improved for all peaks. The allowed maximum deviation for the resolution is <–5%

Reference

1.
Waters Application note, "Analysis of aromatic acids using Atlantis T3", **2009**.

www.agilent.com/chem/ISET

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