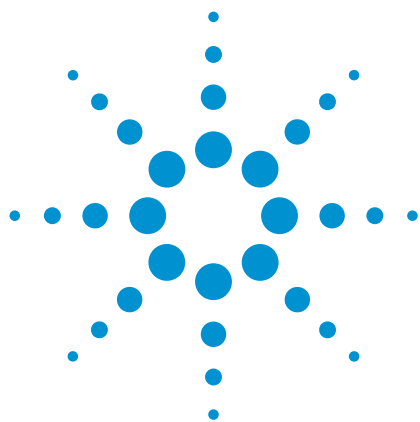


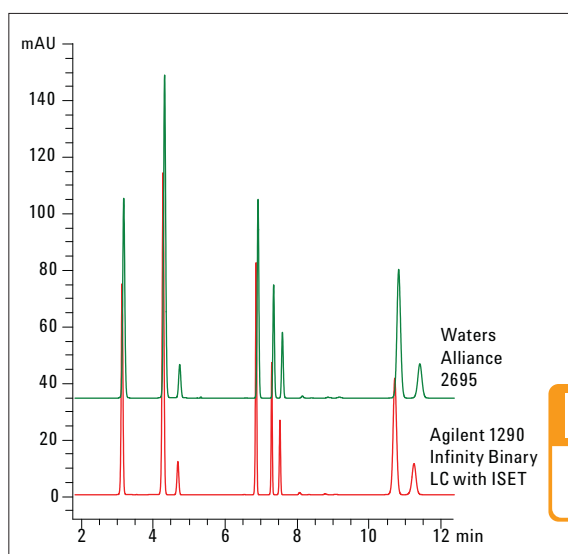


Agilent 1290 Infinity Binary LC with ISET - Emulation of the Waters Alliance 2695 LC system analyzing antioxidants

Application Note

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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. A second step, 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 antioxidants 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 the food industry and official control labs because changing established methods is expensive and time consuming and comparison with reference data is difficult. Equipment has to be replaced eventually, and the 1290 Infinity Binary LC, in combination with ISET, offers the possibility to emulate older non-Agilent instrumentation, like the Waters Alliance LC systems. Old methods from non-Agilent equipment can be transferred to the 1290 Infinity Binary LC with ISET and the same results regarding retention times and resolution are received. With the 1290 Infinity Binary LC, an UHPLC method can be applied to be prepared for the future

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

Chromatographic conditions

Column: Atlantis T3 4.6 × 150, 5 µm
 Mobile phases: A = water + 0.1% formic acid, B = ACN/MeOH (50/50) + 0.1% formic acid
 Gradient: at 0 minutes 35% B, at 4 minutes 55% B, at 6 minutes 81% B, at 15 minutes 81% B, at 16 minutes 35% B, at 20 minutes 35% B
 Flow rate: 2 mL/min
 Injection volume: 10 µL
 Column temperature: 30 °C
 Detection: 280 nm, 10 Hz

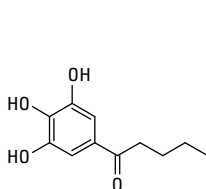
Experimental

The following instruments were used, see Table 1.

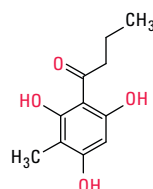
	Agilent 1290 Infinity Binary LC	Waters Alliance LC
Binary pump	G4220A	2695
Auto sampler	G4226A	
ALS cooler	G1330B	
Column compartment	G1316C	
Diode array detector	G4212A	Dual absorbance detector

Table 1
Instrumentation used.

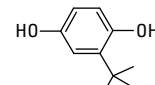
Analyzed compounds



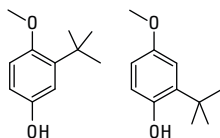
PG



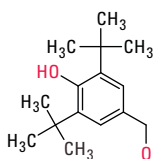
THBP



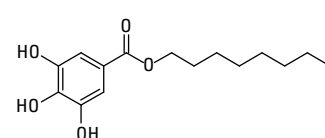
TBHQ



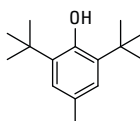
BHA



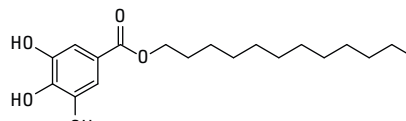
IONOX 100



OG



BHT



LG

PG = Propyl gallate, THBP = 2,4,5-trihydroxybutyrophenone, TBHQ = Tert-butylhydroquinone, BHA = Butylated hydroxyanisole, IONOX 100 = 2,6-di-tert-butyl-4-hydroxymethylphenol, OG = Octyl gallate, BHT = Butylated hydroxytoluene, LG = Lauryl gallate

Acquisition and Evaluation Software

OpenLAB CDS Chemstation version C.01.04 and ISET

Results and Discussion

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

- Analysis of antioxidants on the Alliance 2695, based on a Waters Application Note¹
- 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 $< \pm 5\%$
- Determination of the resolution, typically better on the 1290 Infinity Binary LC, specified maximum deviation $< -5\%$

In the 1290 Infinity Binary LC ISET set up screen, the instrumentation which should be emulated has to be selected, 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. The organic mobile phase for the analysis of antioxidants was a mixture of 50% methanol and 50% acetonitrile. For the following experiments in the selection of mobile phases section, methanol was chosen. Acetonitrile was also evaluated, but no difference was observed in this instance.

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

Figure 3 summarizes the results for the retention time differences. Without ISET, the deviation was a maximum -7% . Using ISET, the deviation of

retention times was $< -1.7\%$ overall. The specified allowed deviation is $< \pm 5\%$.

Figure 1
Selection of the instrumentation to be emulated.

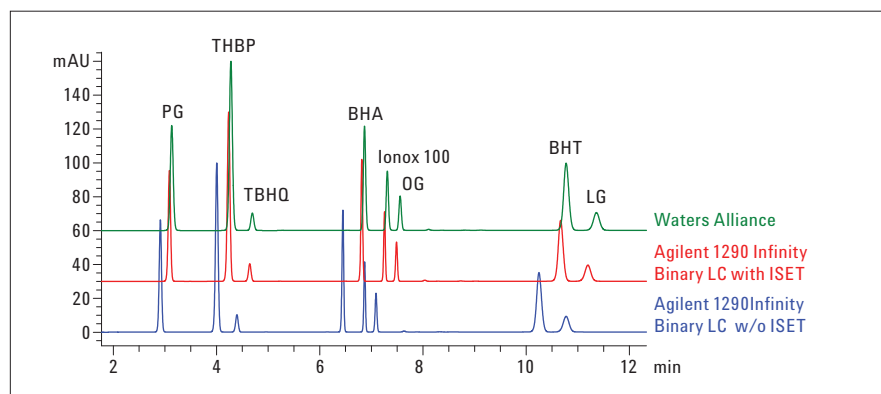


Figure 2
Overlay of chromatograms obtained on the Alliance 2695 and the Agilent 1290 Infinity Binary LC with and without ISET.

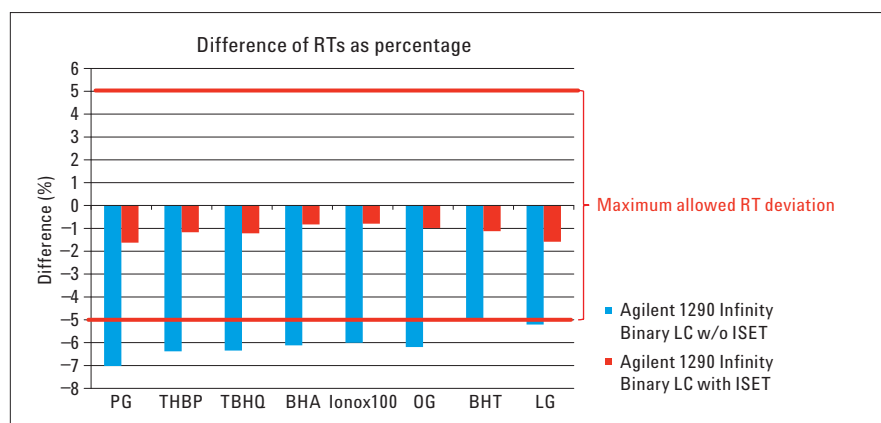


Figure 3
Deviation of retention times with and without ISET on the Agilent 1290 Infinity Binary LC.

Figure 4 summarizes the results for the resolution. For six compounds, the resolution was better on the 1290 Infinity Binary LC. This was mainly due to the small post column dispersion volume on the 1290 Infinity Binary LC compared to the used configuration for the Waters Alliance 2695 LC. The lower dispersion volume resulted in a smaller peak width at half height and, consequently, in improved resolution. For the last compound, the deviation was $< -3.3\%$ with ISET. This was within the allowed maximum deviation of $< -5\%$.

Conclusions

The Agilent 1290 Infinity Binary LC 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 deviation of retention times is $< \pm 5\%$. In this example, the deviation of retention times was $< -1.7\%$. The resolution had improved for six analyzed compounds on the 1290 Infinity Binary LC. For the last compound, the deviation was $< -3.3\%$ with ISET. This was within the allowed maximum deviation of $< -5\%$.

Reference

1. Waters Application note: Analysis of lipid soluble antioxidants using Atlantis T3, January 2009.

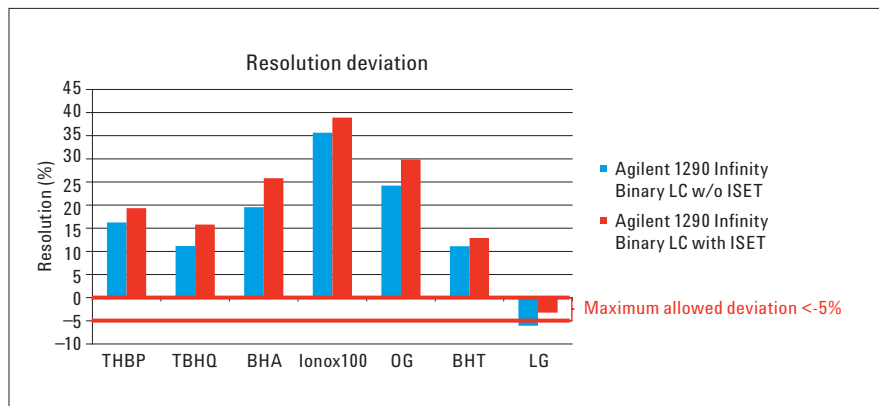


Figure 4
Resolution data with and without ISET.

www.agilent.com/chem/ISET

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