Agilent 1290 Infinity II LC with ISET–Emulation of the Waters Acquity H-Class for Ternary Gradients

Application Note

Biopharmaceuticals

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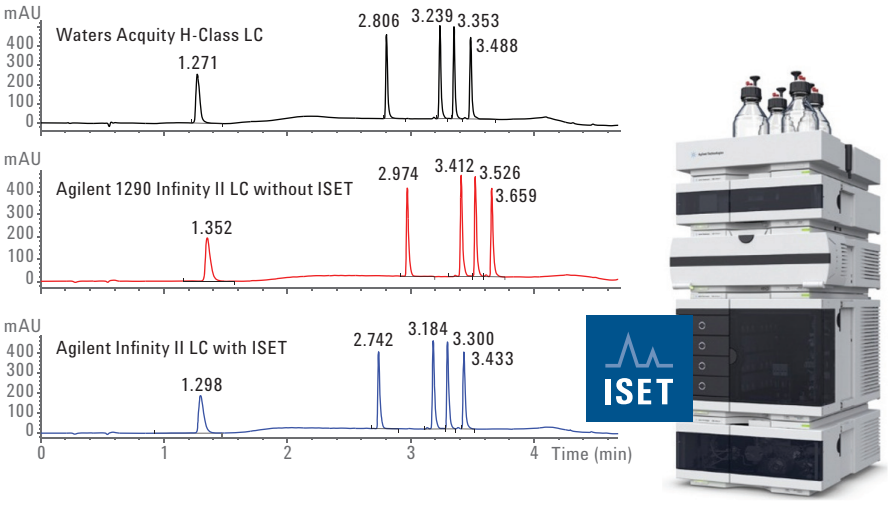
Abstract

Agilent Intelligent System Emulation Technology (ISET) within the Agilent 1290 Infinity II LC facilitates the transfer of methods from Agilent and non-Agilent HPLC and UHPLC systems. This Application Note shows how the 1290 Infinity II LC with ISET emulates the ternary gradient of a Waters Acquity H-Class system for the analysis of peptides. Over 97 % agreement is achieved using the 1290 Infinity II LC with ISET, in contrast to without ISET.



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Introduction

Modifiers such trifluoroacetic acid (TFA) are commonly used in different separation techniques to improve peak shape and chromatographic resolution. Marginal changes in the modifier concentration can have considerable effects on the retention time as well as the resolution of the analytes. Usually, such chromatographic methods are carried out isocratically or using a binary gradient with a predetermined amount of TFA in the mobile phases. An allocation of TFA to an independent solvent line can be helpful to optimize a method without preparing several mobile phase sets[1].

Generally, method transfer from different HPLC or UHPLC systems can be problematic due to different mixing behavior as well as variances in delay and transition volumes. Agilent Intelligent System Emulation Technology (ISET) enables the emulation of different liquid chromatography systems for seamless method transfer from one instrument to another[2,3]. Seamless LC method transfer is possible without changing the original methods – resulting in the same chromatographic results. This allows the user to run legacy methods without the need for any modifications to instrument and original methods, making the Agilent 1290 Infinity II LC a truly universal system to execute other HPLC and UHPLC methods. In addition, the user still has the option to benefit from the wide power range and the superior performance of the 1290 Infinity II LC.

This Application Note describes the emulation of a ternary gradient on the Waters Acquity H-Class system using the 1290 Infinity II LC with ISET for a separation of peptides using a water/acetonitrile mobile phase with TFA used as modifier. The chromatograms are compared, and the agreement of retention times (RT) was evaluated for the analysis on the 1290 Infinity II LC with and without ISET versus the Waters Acquity H-Class system.

Experimental

Instrumentation

All experiments were carried out on a Waters Acquity H-Class system and on an Agilent 1290 Infinity II LC comprising the listed modules.

Agilent 1290 Infinity II LC

- Agilent 1290 Infinity II Flexible Pump (G7104A)
- 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 path length Max-Light standard cartridge flow cell

Waters Acquity Bio H-Class system

- Waters BioQuaternary Solvent Manager
- Waters BioSample Manager FTN
- Waters Column Manager
- Waters TUV Detector

Chromatographic conditions

Table 1. Chromatographic conditions.

Parameter	Description
Mobile phase	A) Water B) ACN C) 1 % TFA
Flow rate	0.5 mL/min
Gradient	0 minutes – 85 %A, 5 %B, 10 %C 0.5 minutes – 85 %A, 5 %B, 10 %C 3 minutes – 50 %A, 40 %B, 10 %C 3.5 minutes – 10 %A, 80 %B, 10 %C 4 minutes – 10 %A, 80 %B, 10 %C 4.1 minutes – 85 %A, 5 %B, 10 %C 6.5 minutes – 85 %A, 5 %B, 10 %C
Stop time	6.5 minutes
Needle wash mode	Standard wash
Injection volume	0.2 µL
Column temperature	50 °C
Diode array detection	214/4nm, reference 360/100 nm Peak width >0.013 minutes (0.25 seconds response time) Data rate 20 Hz

Column

Agilent Poroshell 120, EC-C18, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS systems, Rev. C.01.07 [22 with ISET 4]

Sample

HPLC peptide standard mixture

Solvents

All solvents used were LC grade. Acetonitrile (ACN) was purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak). Trifluoroacetic acid (TFA) and HPLC peptide standard mixture were purchased from Sigma-Aldrich, St. Louis, Missouri, US.

Results and Discussion

A mix of five peptides was analyzed using a ternary gradient with 1% TFA as modifier on the separate solvent line C, continuously delivering 10 %, resulting in a total concentration of 0.1 % TFA throughout the gradient. The method was applied to a Waters Acquity H-Class system, to a 1290 Infinity II LC without ISET, as well as to a 1290 Infinity II LC with ISET. Figure 1 shows all three chromatograms and the retention time precision values (over six consecutive runs) for the separation on the 1290 Infinity II LC. The retention times as well as the retention time deviations for all five peaks are found in Table 2.

Without ISET, a clear retention time shift was visible on the 1290 Infinity II LC, resulting in a deviation of over 5 % for four of five peaks from the retention times found on the Waters Acquity H-Class system. After enabling ISET, the retention times agreed well with the retention times of the original chromatogram. In contrast to the retention times on the 1290 Infinity II without ISET, the deviation was below 2.3 % for all analyzed compounds (right column Table 2). A shift of less than 5 % is regarded as tolerable in accordance with the ISET specifications[4].

Conclusion

The emulation of other HPLC or UHPLC instruments with the Agilent 1290 Infinity II LC using ISET allows the user to run existing methods without modifications of instrument or methods, and achieve comparable chromatograms. This was shown for the analysis of five peptides with a ternary gradient having 1 % TFA in a separate solvent channel. The method was transferred from a Waters Acquity H-Class system to the 1290 Infinity II LC. By enabling ISET, a 97 % agreement of retention times was found, making ISET within the 1290 Infinity II LC an ideal solution for the emulation of ternary gradients of non-Agilent LCs.

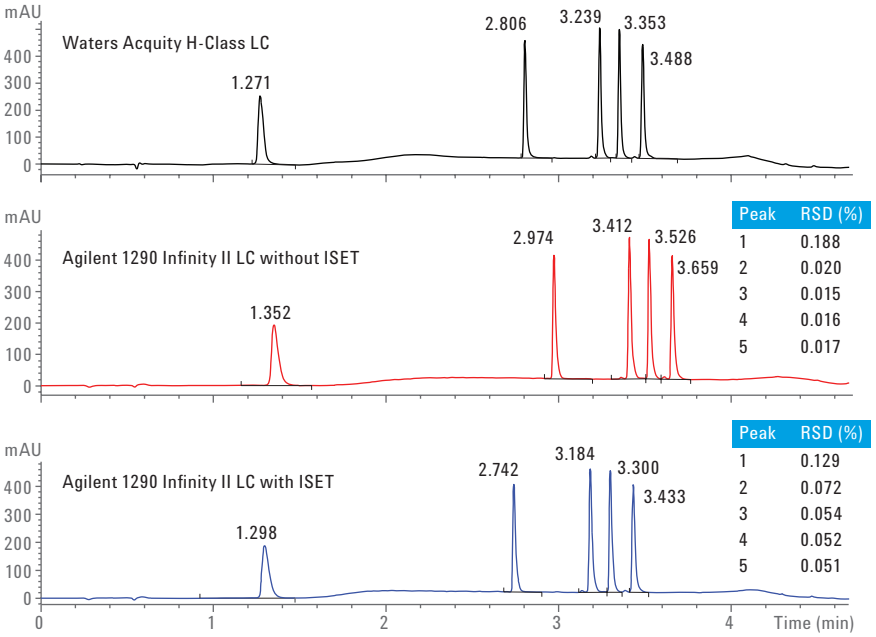

Figure 1. Transfer of a ternary gradient with 1 % TFA in a separate C solvent channel from a Waters Acquity H-Class system to an Agilent 1290 Infinity II LC System without and with ISET.

Table 2. Retention times of all systems with the emerging deviations (Dev.) after transfer from a Waters Acquity H-Class to an Agilent 1290 Infinity II LC System without and with ISET.

Peak	Waters Acquity H-Class RT	Agilent 1290 Infinity II LC			
		RT without ISET	RT with ISET	Dev. without ISET	Dev. with ISET
1	1.271	1.352	1.298	–6.37	–2.12
2	2.806	2.974	2.742	–5.99	2.28
3	3.239	3.412	3.184	–5.34	1.70
4	3.353	3.526	3.3	–5.16	1.58
5	3.488	3.659	3.433	–4.90	1.58

References

1. Gratzfeld-Huesgen, A., Fast and flexible optimization of buffer/modifier concentrations using ternary gradients with the Agilent 1260 Infinity LC Quaternary System, *Agilent Technologies Application Note*, publication number 5990-6864EN, **2010**.

2. Gratzfeld-Huesgen, A., Agilent 1290 Infinity Binary LC with ISET – Emulation of the Waters Alliance 2695 LC System Analyzing Analgesics, *Agilent Technologies Application Note*, publication number 5991-2792EN, **2013**.

3. Gratzfeld-Huesgen, A., 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 Application Note*, publication number 5990-9113EN, **2011**.

4. Agilent 1290 Infinity with ISET, *User Manual*, part number G4220-90314, **2015**.

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