

Agilent 1260 Infinity II Prime LC with ISET

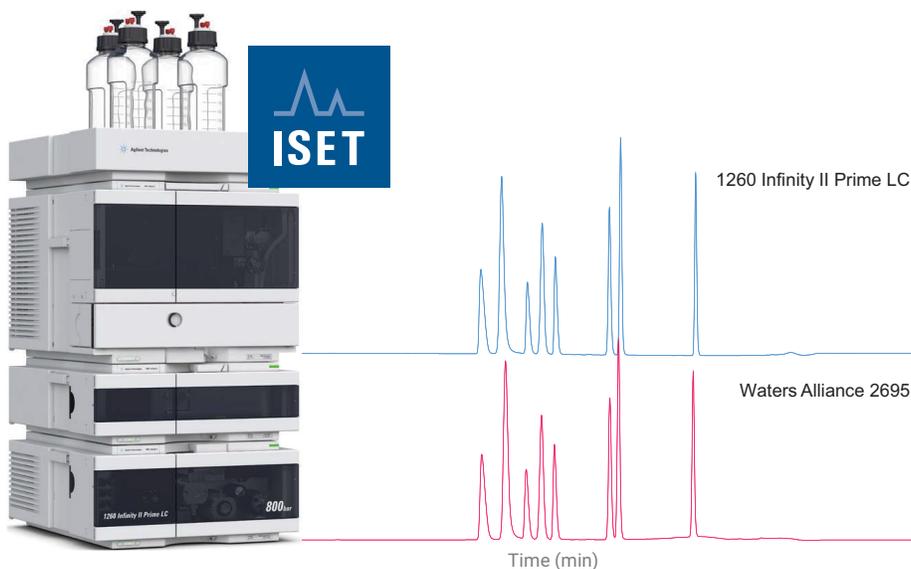
Emulation of a Waters Alliance LC for the Analysis of Metoclopramide and its Impurities

Author

Melanie Metzloff
Agilent Technologies, Inc.

Abstract

Instrument-to-instrument method transfer is a crucial topic for many different industries, particularly for the regulated pharmaceutical industry. Agilent Intelligent System Emulation Technology (ISET) facilitates the seamless transfer of analytical methods from conventional LC systems to an Agilent UHPLC system. This Application Note demonstrates the straightforward transfer of an analytical method from the Waters Alliance 2695 LC to a 1260 Infinity II Prime LC by using ISET. Metoclopramide and its impurities were analyzed on both systems. Retention times and resolution of the different experiments were evaluated and compared.



Introduction

The transfer of an analytical method from a conventional HPLC system to a UHPLC system such as the 1260 Infinity II Prime LC is an important and often critical topic for many laboratories, especially those in highly regulated environments. Changing established analytical methods can require revalidation, which is time-consuming and expensive. The 1260 Infinity II Prime LC facilitates the emulation of legacy LC systems such as the Waters Alliance 2695 LC through ISET¹.

For most LC methods, linear solvent strength gradients are used. However, the Alliance 2695 LC allows different gradient profiles, such as concave and convex gradient curves. The 1260 Infinity II Prime LC with ISET can emulate these gradient curves.

This Application Note describes the analysis of metoclopramide, an antiemetic drug, and seven different impurities. For the separation of the drug mixture, a concave gradient was applied using the Waters Alliance 2695 LC. The analytical method was further transferred to a 1260 Infinity II Prime LC with ISET. Retention times and resolution from both systems were evaluated and compared.

Experimental

Instrumentation

For the analysis of metoclopramide and its impurities, the following instruments were used:

Waters Alliance 2695 HPLC system with Dual Absorbance Detector VWD 2487

Agilent 1260 Infinity II Prime LC:

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- Agilent 1260 Infinity II Vialsampler (G7129C), equipped with an integrated sample cooler (option #100) and with an integrated column compartment (6 μ L heater; option #066)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with a standard 10 mm flow cell (G1315-60022)

Software

Agilent OpenLab CDS 2.2 (M8413A), to control the Waters Alliance 2695 LC by OpenLab CDS 2.2, Waters DP 2017R2 was installed.

Sample

For the experiments the following compounds were used:

- Metoclopramide hydrochloride
- **Impurity B:** Methyl 4-(Acetylamino)-5-chloro-2-methoxybenzoate
- **Impurity C:** 4-Amino-5-chloro-2-methoxybenzoic acid
- **Impurity D:** Methyl 4-(Acetylamino)-2-methoxybenzoate
- **Impurity F:** 4-Amino-5-chloro-N-[2-(diethylamino) ethyl]-2-hydroxybenzamide
- **Impurity G:** N'-(4-Amino-5-chloro-2-methoxybenzoyl)-N, N-diethylethane-1,2-diamine N-oxide
- **Impurity H:** 4-(Acetylamino)-2-hydroxybenzoic acid
- **Impurity 9:** Methyl 4-amino-2-methoxybenzoate

Every compound was dissolved in 100 % methanol to a concentration of 2 mg/mL and further diluted with water to a final concentration of 20 ng/ μ L.

Chemicals

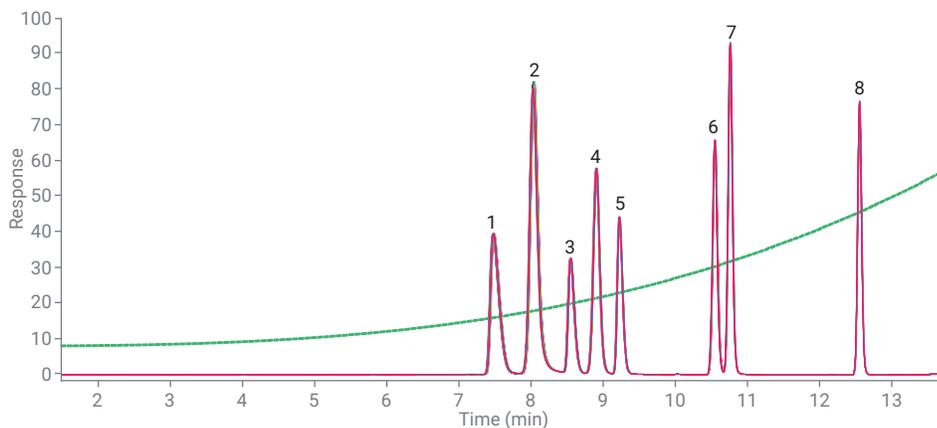
All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22- μ m membrane point-of-use cartridge (Millipak). Acetonitrile was purchased from Merck (Darmstadt, Germany) and formic acid (FA) from VWR (Darmstadt, Germany). Metoclopramide was obtained from Sigma-Aldrich (Taufkirchen, Germany), and the metoclopramide impurities were bought from LGC Standards (Teddington, United Kingdom).

Results and discussion

Metoclopramide and its impurities were analyzed with a conventional analytical method on a Waters Alliance 2695 LC applying a concave gradient and using a 4.6 × 150 mm column packed with 5 μm particles. Figure 1 shows a chromatogram overlay of 10 consecutive runs and a table with the corresponding retention times and resolution.

Table 1. Chromatographic parameters for analysis of metoclopramide and impurities.

Parameter	Value
Column	Agilent ZORBAX StableBond C18; 4.6 × 150 mm, 5 μm (p/n 883975-902)
Solvent	A) 0.1 % FA in water B) 0.1 % FA in acetonitrile
Gradient	0 minutes – 8 %B 14 minutes – 66 %B; curve 8 14.1 minutes – 8 %B; curve 6
Stop time	18 minutes
Flow rate	2 mL/min
Injection	Injection volume: 10 μL, 3 seconds needle wash (60 % acetonitrile in water)
Sample temperature	8 °C
Column temperature	25 °C
DAD	270/4 nm, Ref. off, 5 Hz



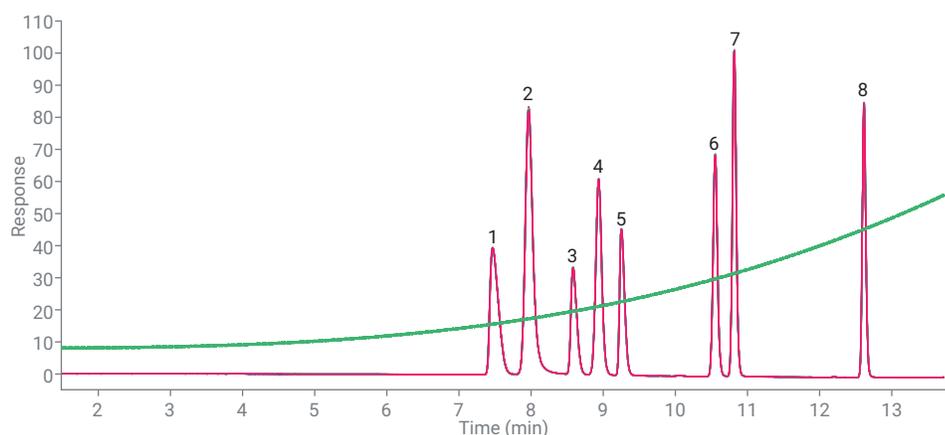
No.	Compound	RT (min)	RSD RT (%)	Resolution
1	Impurity F	7.50	0.12	–
2	Impurity H	8.05	0.12	2.58
3	Metoclopramide	8.57	0.07	2.84
4	Impurity 9	8.93	0.07	2.16
5	Impurity G	9.24	0.05	2.08
6	Impurity C	10.57	0.04	9.93
7	Impurity D	10.78	0.03	1.75
8	Impurity B	12.57	0.01	16.46

Figure 1. Analysis of metoclopramide and impurities. Acquired on a Waters Alliance 2695 LC system, overlay of 10 consecutive runs. The green curve in the chromatogram displays the solvent ration B.

The analytical method for the separation of metoclopramide and the impurities was further transferred to the 1260 Infinity II Prime LC with ISET enabled. Figure 2 shows the resulting chromatogram overlay together with the corresponding retention time and resolution.

By using ISET on the 1260 Infinity II Prime LC, the formation of concave and convex gradient profiles is possible. In OpenLab CDS and ChemStation, the numbering of the 11 different gradient profiles used is identical to the Waters numbering. For these experiments, curve 8 (concave) and curve 6 (linear) were used.

When the analytical method was transferred to a 1260 Infinity II Prime LC using ISET, highly similar chromatograms were obtained to those produced on the Alliance 2695 LC. Figure 3 shows a comparison of chromatograms acquired with the 1260 Infinity II Prime LC and with the Alliance 2695 LC.



No.	Compound	RT (min)	RSD RT (%)	Resolution
1	Impurity F	7.48	0.02	-
2	Impurity H	7.98	0.05	2.38
3	Metoclopramide	8.59	0.01	3.58
4	Impurity 9	8.95	0.01	2.29
5	Impurity G	9.26	0.03	2.19
6	Impurity C	10.56	0.03	10.51
7	Impurity D	10.83	0.01	2.46
8	Impurity B	12.63	0.01	18.93

Figure 2. Analysis of metoclopramide and its impurities. Acquired on a 1260 Infinity II Prime LC, overlay of 10 consecutive runs. The green curve in the chromatogram displays the solvent gradient B.

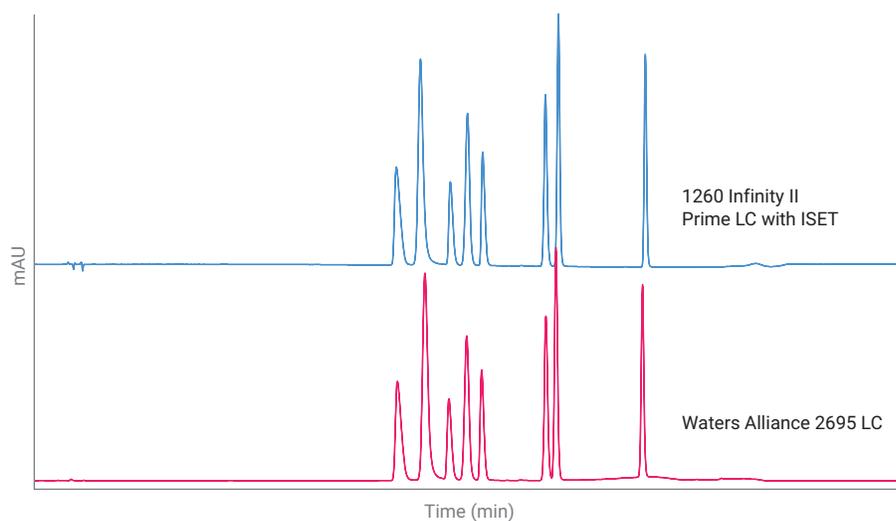


Figure 3. Overlay of a chromatogram acquired with the 1260 Infinity II Prime LC with ISET enabled and a chromatogram acquired with the Waters Alliance 2695 LC for the analysis of metoclopramide and impurities.

The retention times on both systems, with a maximum deviation of $\approx 0.9\%$ are in excellent agreement and clearly below the specified retention time deviation of $< \pm 5\%$. Furthermore, the deviation of resolution was determined. The resolution of most peaks was better for the runs using the 1260 Infinity II Prime LC, except for Impurity H. Table 2 shows a detailed overview for the deviation in retention time and resolution.

Conclusion

ISET allows the seamless transfer of legacy methods from a conventional HPLC to the 1260 Infinity II Prime LC. By enabling ISET, the emulation of a Waters Alliance LC is possible, even running concave and convex gradients.

In this Application Note, a method for the analysis of metoclopramide and its impurities was transferred from a Waters Alliance 2695 LC to a 1260 Infinity II Prime LC. Excellent retention time and resolution correlation were obtained. Furthermore, the resolution was increased for most of the peaks. This Application Note clearly demonstrates the ease-of-use of ISET. No modifications of the instrument or changes of the analytical method were necessary for the analysis of metoclopramide and its impurities on the 1260 Infinity II Prime LC.

Table 2. Comparison of retention time and resolution between a Waters Alliance 2695 LC and a 1260 Infinity II Prime LC.

Compound	Deviation RT (min)	Deviation RT (%)	Deviation resolution	Deviation resolution (%)
Impurity F	-0.02	-0.27	-	-
Impurity H	-0.07	-0.90	-0.20	-8.28
Metoclopramide	0.02	0.23	0.73	20.48
Impurity 9	0.02	0.22	0.13	5.58
Impurity G	0.02	0.23	0.12	5.25
Impurity C	-0.01	-0.09	0.58	5.51
Impurity D	0.05	0.46	0.71	28.76
Impurity B	0.06	0.48	2.47	13.06

References

1. Gratzfeld-Huesgen, A. Agilent 1290 Infinity Binary LC System with ISET – Emulation of the Waters Alliance 2695 LC system analyzing β – blockers, *Agilent Technologies Application Note*, publication number 5991-1603EN, **2014**.
2. Agilent InfinityLab LC with ISET, *Agilent Technologies User Manual*, part number G7120-90310, **2017**.

www.agilent.com/chem

This information is subject to change without notice.

© Agilent Technologies, Inc. 2018
Printed in the USA, July 1, 2018
5991-9394EN