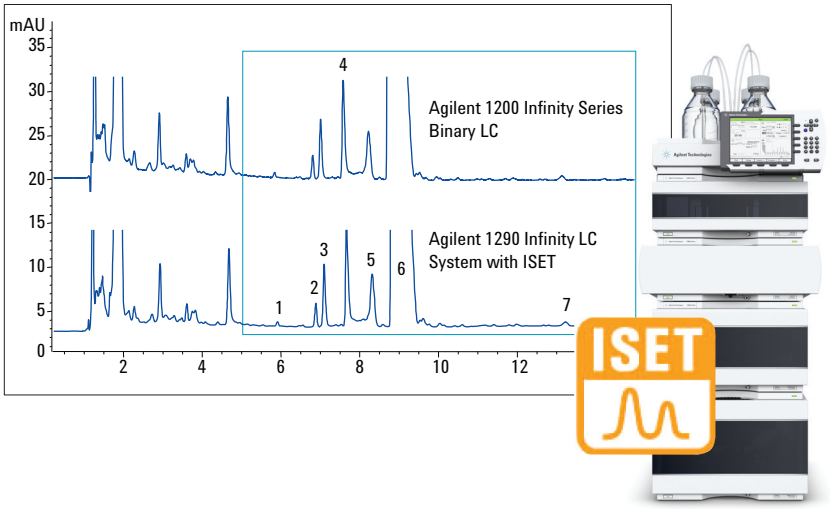
Fast screening of mobile and stationary phases with the Agilent 1290 Infinity LC and seamless method transfer to an Agilent 1200 Series LC using ISET

Application Note

Pharmaceutical QA/QC



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Abstract

This Application Note describes how the Agilent 1290 Infinity LC System Method Development Solution was used to find optimum separation conditions for degradation products of a pharmaceutical preparation. Having evaluated the optimum combination, a transfer to conventional conditions was performed. Finally, the Agilent Intelligent System Emulation Technology (ISET) of the 1290 Infinity LC was used to simulate the target Agilent 1200 Infinity Series LC. The method was then transferred to an Agilent 1200 Series LC to prove the functionality of the ISET tool.

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Introduction

In the pharmaceutical industry, method development, method validation, and transfer of the validated method to the QA/QC laboratories is a common workflow. Using UHPLC equipment for method development enables fast and effective development of optimum chromatographic conditions. However, many QA/QC departments do not have UHPLC equipment. Consequently, the UHPLC method has to be transferred to a conventional method using the LC instrumentation available. The availability of short sub-2 µm columns with a 2.1-mm id are nowadays used for rapid method scouting providing highly efficient separations with lowest solvent consumption.

Method development instrumentation like the Agilent 1290 Infinity Method Development Solution not only fully supports this mode of operation but offers additional tools for method development such as the Agilent Method Scouting Wizard. The Method Scouting Wizard automates the setup of methods and sequences to screen available combinations of columns, solvents, redefined gradients, and temperatures. In combination with the Agilent OpenLAB CDS Intelligent Reporter, chromatograms can be evaluated and presented in report templates which facilitate the finding of optimum stationary and mobile phase combinations.

Conversely, many laboratories still use older LC systems. In this case, the fast UHPLC methods have to be transferred to conventional methods, which do not exceed the power range of the existing LC system. The 1290 Infinity LC System in combination with ISET enables the simulation of the target instrumentation and consequently ensures a save and seamless transfer, for example, to an Agilent 1200 Series LC.

This Application Note shows how a method for the analysis of degradation products of metoprolol was developed using UHPLC conditions. This method was then transferred to a method which was compatible with a 1200 Series LC using the 1290 Infinity LC System in combination with ISET.

Method scouting was performed using different 2.1 × 50 mm, 1.8-µm particles columns and a 5-minute gradient. This saved a lot of time compared to using long conventional columns and conventional chromatographic conditions. As a result, the consumption of mobile phases was significantly reduced.

Step by step, the following workflow was processed:

- Fast method scouting to find optimum column and mobile phases conditions using the Agilent 1290 Infinity Method Development Solution and the Method Scouting Wizard
- Transfer of the fast method conditions to conventional conditions using the Agilent Method Translator
- Fine tuning of the conventional method using the Agilent 1290 Infinity Method Development Solution and the Method Scouting Wizard
- Simulating the target 1200 Series Binary LC using the ISET on the 1290 Infinity LC
- Transfer of the final method to a 1200 Series LC and evaluation of the difference of retention times and resolution compared to results of the 1290 Infinity LC with ISET

Experimental

Instruments

Table 1 shows the instruments used.

The 1290 Method Development Solution included:

- Two Thermostatted Column Compartments (G1316C with valve drive option #058)
- Two Solvent Selection Valves (G1160A)
- Agilent Method Development Valve Kit, high pressure (G4230B)
- Agilent Method Development Capillary kit, low dispersion, for short columns (Valve G4230B option # 001)

Software

Agilent OpenLAB CDS ChemStation Edition C.01.04 and Agilent Method Scouting Wizard A.02.02

Sample preparation

Two tablets containing 50 mg metoprolol each were pulverized and heated to 80 °C for 3 hours. The residue was dissolved in 3 mL of water. Centrifugation was used to remove all nondissolved particles. A 0.3-µL solution of the resulting clear liquid was injected into the LC system.

Results and discussion

Several different stationary phases and mobile phases were applied to find the optimum combination for the separation of degradation products of metoprolol. Tested column and mobile phase combinations are shown in Table 2. The Method Scouting Wizard was used to set up the required sequence with all run, flush, and equilibration methods. All 73 sequence lines were set up with a few mouse clicks.

	Agilent 1290 Infinity LC System with ISET	Agilent 1200 Series Binary LC
Binary pump	G4220A	G1312A
Autosampler	G4226A	G1329A
Autosampler cooler	G1330B	G1330A
Column compartment	G1316C	G1316A
Degasser		G1322A
Diode array detector	G4212A	G1315B

Table 1
Instrumentation used.

Start method for setting up sequences using the Method Scouting Wizard

Column dimensions:	Agilent ZORBAX RRHT columns with 2.1 × 50 mm, 1.8-µm, stable up to 600 bar
Stationary phases:	SB-C8, SB-CN, SB-Phenyl, Extend-C18, SB-C18
Flow:	0.25 mL/min
Mobile phases:	Water + 0.1% trifluoroacetic acid (TFA), pH 2.1, 0.02 M phosphate buffer pH 3.35, 0.02 M phosphate buffer pH 6.4, 0.02 M acetate buffer pH 4.52, 0.001 M triethylamine (TEA) pH 11, ACN + 0.09% TFA, ACN
Gradient:	5 to 65% organic in 5 minutes
Stop time:	10 minutes
Post time:	5 minutes
Column temperature:	40 °C
DAD:	230, 254, 280 nm/10 nm, ref = off, 20 Hz

Column	Aqueous phase	Organic phase
SB-C8, SB-CN, SB-Phenyl, Extend-C18, SB-C18	Water + 0.1% Trifluoroacetic acid (TFA), pH 2.1	ACN+0.09%TFA
SB-C8, SB-CN, SB-Phenyl, Extend-C18, SB-C18	Phosphate buffer pH 3.35	ACN
SB-C8, SB-CN, SB-Phenyl, Extend-C18, SB-C18	Phosphate buffer pH 6.4	ACN
SB-C8, SB-CN, SB-Phenyl, Extend-C18, SB-C18	Acetate buffer pH 4.52	ACN
Extend C 18	Triethylamine (TEA)pH 11	ACN

Table 2
Combination of columns, organic and aqueous phases.

The Method Scouting Wizard created 73 sequence lines including 42 sample runs and 31 flush and equilibration runs. The completion of all runs took 13 hours and 50 minutes. Two injections per column and pH combination were programmed. Figure 1 shows the chromatograms at different pH applied on the SB-C18 column. It is obvious that pH 2.1 provided the best separation and peak shape.

Analyzing the sample at pH = 11 using the Extend-C18 column did not deliver satisfactory results (Figure 2).

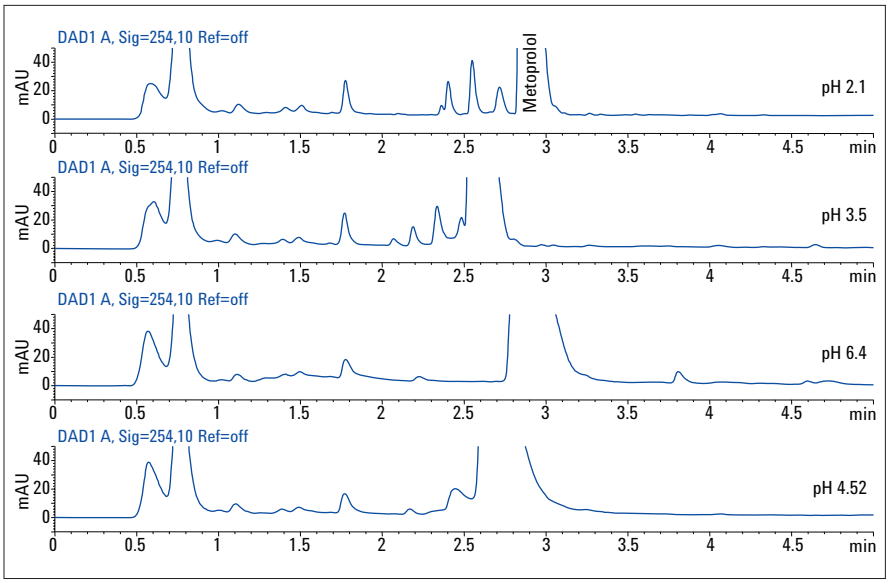

Figure 1
Evaluation of optimum pH on an Agilent ZORBAX RRHT SB-C18, 2.1 × 5, 1.8-µm column.

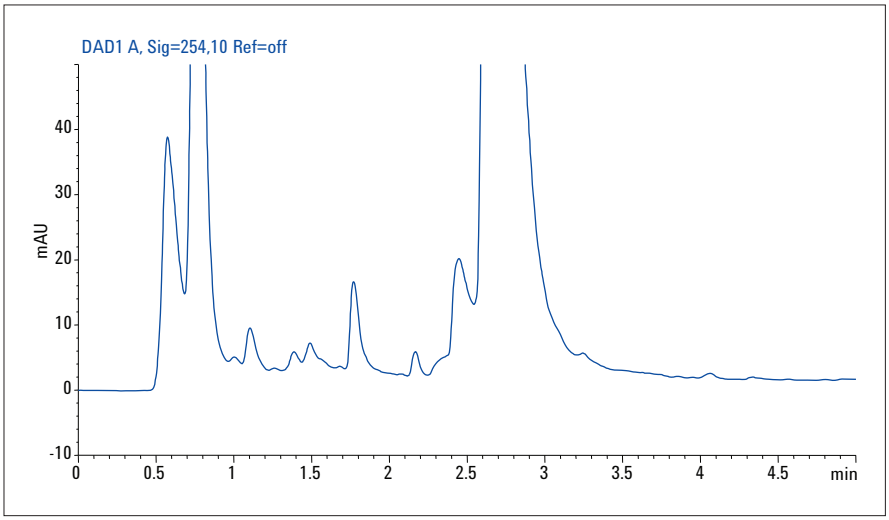

Figure 2
Evaluation at pH 11 on an Agilent ZORBAX RRHT Extend-C18 column.

The SB-C18 phase with pH 2.1 (TFA) provided the best separation and the best peak shape. The other stationary phases did not deliver better results (Figure 3).

The results shown in Figures 1 and 3 can also be presented in one bubble chart using the reporting possibilities of the of the OpenLAB CDS Intelligent Reporter (Figure 4). As mentioned above, two injections for each mobile and stationary phase combination were performed. For the bubble report, the second injection of each combination was selected for evaluation.

The bubble size represents the number of integrated peaks and, consequently, the best mobile and stationary phase combination. The integration parameters were the same for all analyzed chromatograms. The SB-C18 phase with pH 2.1 (TFA) provided the most integrated peaks together with the Extend–C18 column. The Extend-C18 column was not selected for further evaluation because with pH 2.1 the lower pH limit was reached. The SB-C18 column can be used from pH 1 to pH 8 and was therefore best suited for a pH of 2.1.

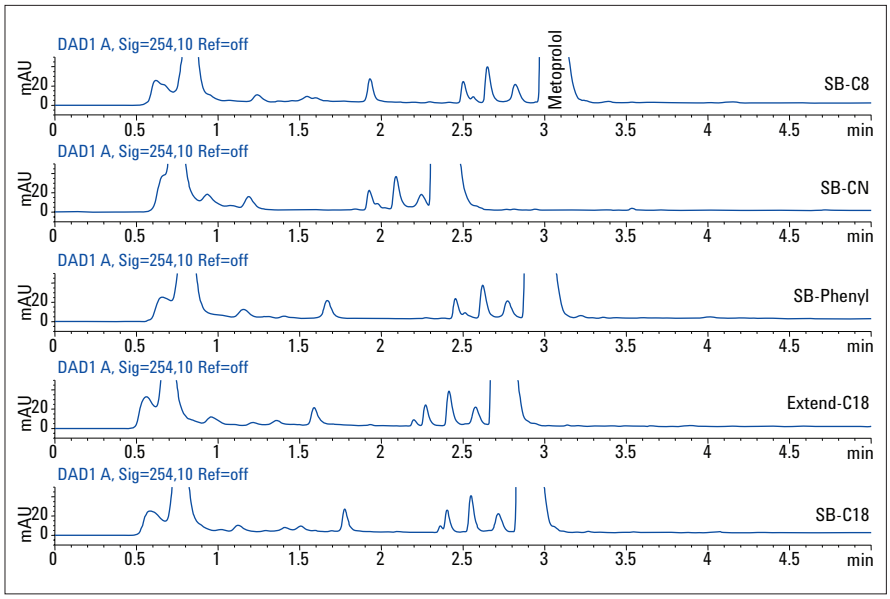

Figure 3
Evaluation of optimum stationary phase at pH 2.1.

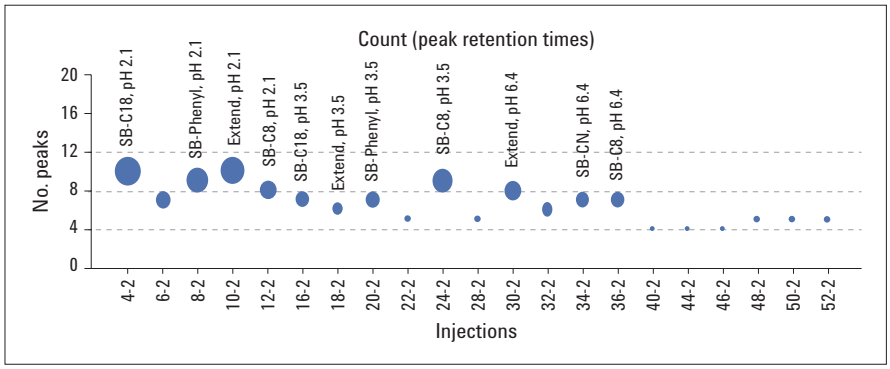

Figure 4
Combination of column and mobile phase evaluation.

Method translation from fast to conventional conditions

The UHPLC method was transferred to a conventional method to comply with the power range of the target LC, a 1200 Series Binary LC with a pressure limit of 400 bar and a delay volume of about 950 µL. The transfer of the UHPLC method to a conventional method started using the Method Transfer and Cost Savings Calculator tool (Figure 5). This tool is available online at: www.agilent.com/chem/hplc2uhplc.

Table 3 summarizes the transfer conditions.

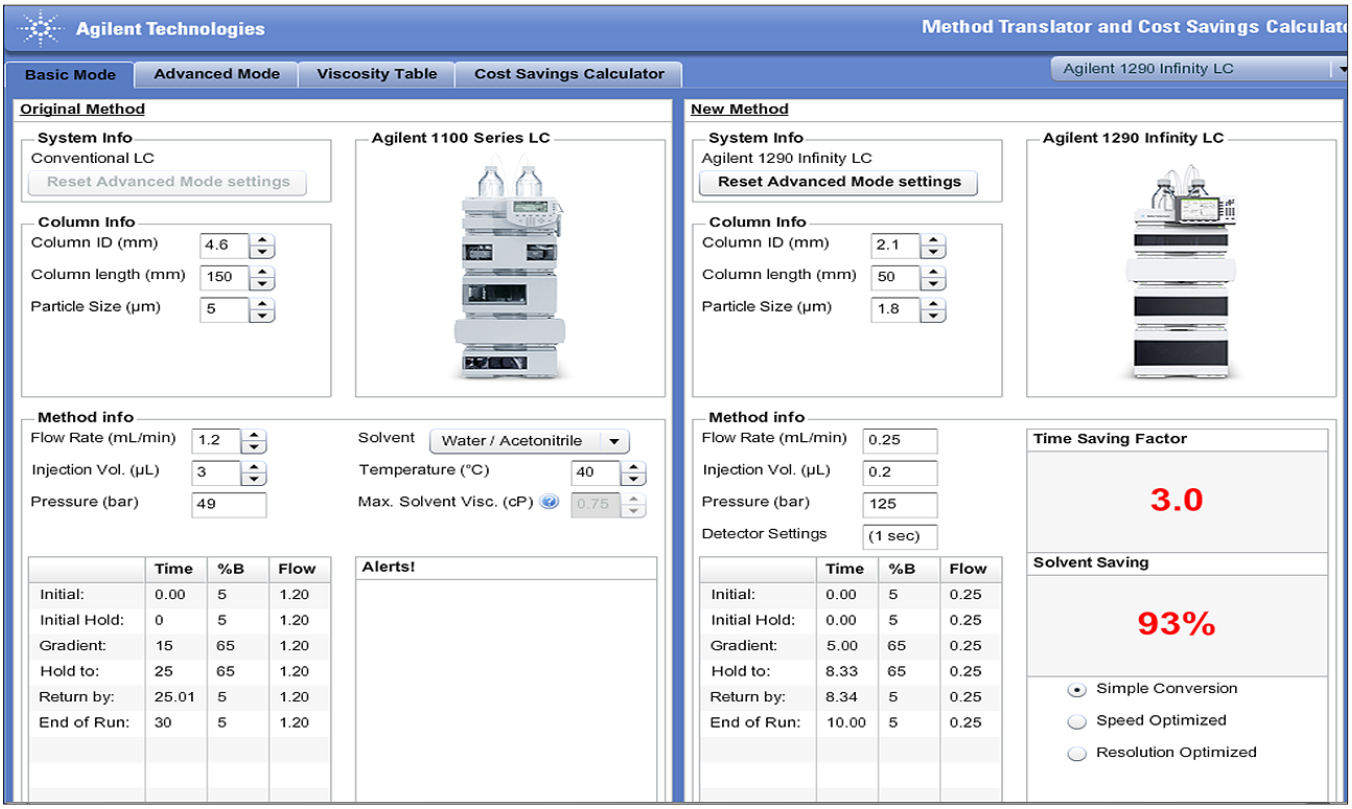

Figure 5
Transfer of the UHPLC method to a conventional chromatographic method

Parameter	Fast method development conditions	Conventional conditions
Column dimension	2.1 × 50 mm, 1,8 µm	4.6 × 150 mm, 5 µm
Flow (mL/min)	0.25	1.2
Gradient	At 0 minutes 5% organic At 5 minutes 65% organic	At 0 minutes 5% organic At 15 minutes 65% organic
Injection volume (µL)	0.3	3
Column temperature (°C)	40	40
Stop time	10	25
Post time	5	5

Table 3
Method transfer from 2.1-mm id column to 4.6-mm id column.

Fine tuning of conventional method

After having transferred the method to conventional conditions an additional fine tuning step was added. One additional column temperature and two additional gradients were tested. The Method Scouting Wizard was used to automatically develop the sequence with all needed lines. Table 4 summarizes the conditions for the fine tuning step together with the finally developed chromatographic conditions.

Gradient 3 and a column temperature of 40 °C provided the best separation (Figure 6).

The complete method development process using the Agilent Method Development Solution took approximately 21 hours including all runs. Three additional hours were needed to install all columns, to set up the mobile phases, to set up the columns in the column configuration screen, and to prepare the start method for the Method Scouting Wizard. Within 2 days, the completion of the final method was achieved.

Parameter	Fine tuning	Final conditions for 4.6 x 150 mm, 5-µm column
Column	Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm	Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm
Flow (mL/min)	1.2	1.2
Gradient 1	At 0 minutes 5% ACN At 15 minutes 65% organic	
Gradient 2	At 0 minutes 5% organic At 1 minutes 5% organic At 15 minutes 65% organic	
Gradient 3	At 0 minutes 5% ACN At 20 minutes 65% organic	At 0 minutes 5% ACN At 20 minutes 65% organic At 21 minutes 95% organic (cleaning step)
Injection volume (µL)	3	3
Column temperature 1 (°C)	40	40
Column temperature 2 (°C)	50	
Stop time	25	21
Post time	5	5

Table 4
Fine tuning with one additional column temperature and two additional gradient profiles.

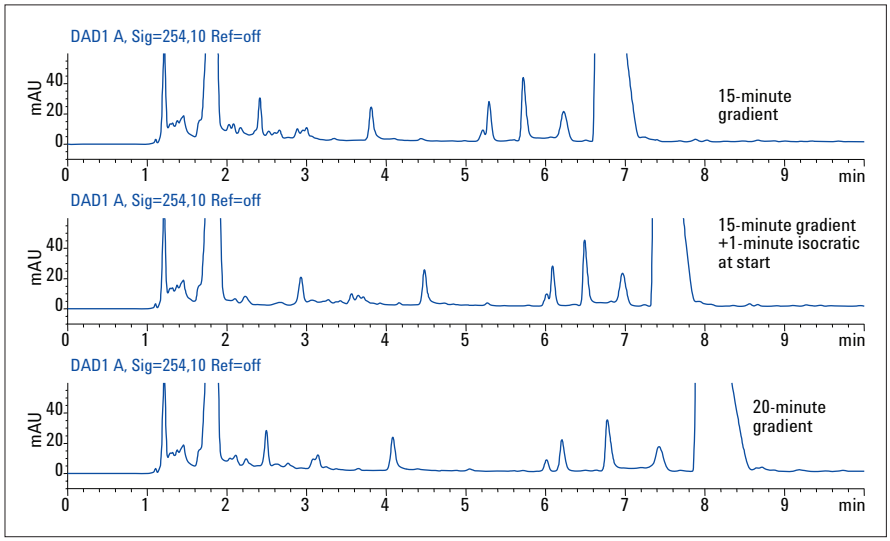

Figure 6
Fine tuning of method with different gradients.

Simulating the targeted LC system using the ISET tool on the 1290 Infinity LC System

The 1290 Infinity LC System in combination with the ISET tool was used to prove that the developed conventional method can be seamlessly transferred to the 1200 Series LC. The ISET tool enables the emulation of the target LC, in this case a 1200 Series Binary LC. In the pump set up screen of the 1290 Infinity LC, the ISET tool was enabled and the pump and autosampler of the target 1200 Series LC was selected (Figure 7).

The ISET tool compensated not only for differences in delay volume but also for differences in the mixing behavior[1]. Figure 8 shows the influence of the ISET tool on retention times and chromatogram appearance. Simultaneously, the chromatogram of the 1200 Series LC is shown, which was obtained after transfer of the final method to the 1200 Series Binary LC.

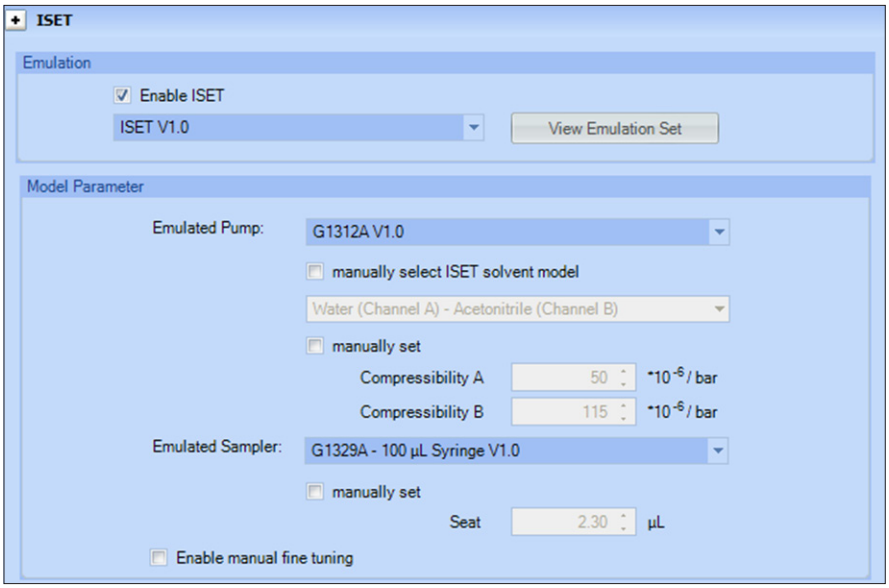

Figure 7
Enabling the ISET tool and selection of target pump and autosampler.

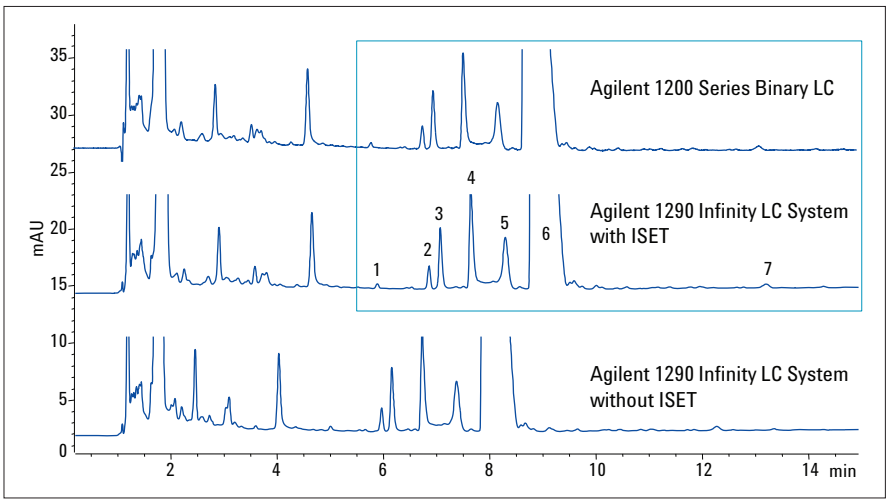

Figure 8
Comparison of chromatograms with and without ISET overlaid with the chromatogram finally obtained on the Agilent 1200 Series Binary LC.

Transfer of the final method to an 1200 Series LC

Figure 8 shows the 1290 Infinity LC chromatogram with ISET, overlaid with the chromatogram finally obtained on the 1200 Series LC. Both chromatograms correlated to a high extend. Figures 9 and 10 show the correlation of retention times and of the resolution of the peaks (numbering see Figure 8). The maximum deviation of the retention times between the 1290 Infinity LC System with ISET and the 1200 Series LC was 1.5% for peak 1 to 7. The maximum deviation of the resolution for peak 1 to 7 was 3%.

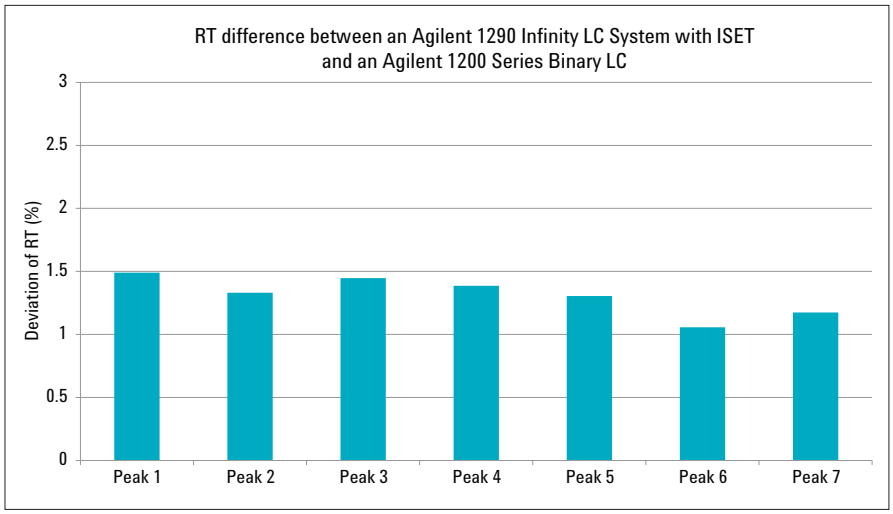

Figure 9
Deviation of retention times.

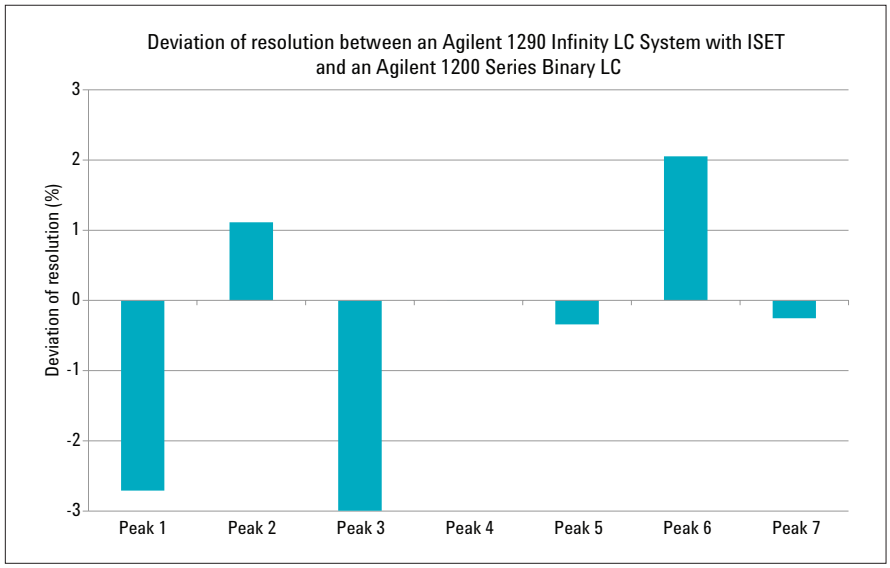

Figure 10
Deviation of resolution.

Conclusion

Method development is a demanding and time consuming task in the pharmaceutical industry. In this Application Note method development was done using the Agilent 1290 Infinity Method Development Solution in combination with the Method Scouting Wizard. The complete method development process was completed within two days using UHPLC chromatographic conditions.

The method was transferred to conventional conditions to comply with the power range of the 1200 Series Binary LC. To emulate the 1200 Series LC, a 1290 Infinity LC System in combination with ISET was used. The ISET tool compensated not only for differences in delay volume but also for differences in the mixing behavior. The same chromatograms were obtained on the 1290 Infinity with ISET and on the 1200 Series LC. This proves that the 1290 Infinity LC with ISET is best suited to simulate other LCs providing a seamless method transfer from an UHPLC system to a HPLC system.

In the example, the maximum deviation of the retention times between the 1290 Infinity with ISET and the 1200 Series LC was 1.5%. The maximum deviation of the resolution was 3%.

Reference

1.
A.G.Huesgen, "Method development on the Agilent 1290 Infinity LC using Intelligent System Emulation Technology (ISET) with subsequent transfer to an Agilent 1100 Series LC, Analysis of an analgesic drug", Agilent Application note, Publication number 5990-9715EN, **2012**.

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