Universal Analytical Method Development for Various HPLC Systems Using the Agilent 1290 Infinity II Method Development Solution

On-The-Fly Target System Emulation Using Intelligent System Emulation Technology – ISET

Application Note

Small Molecule Pharmaceuticals

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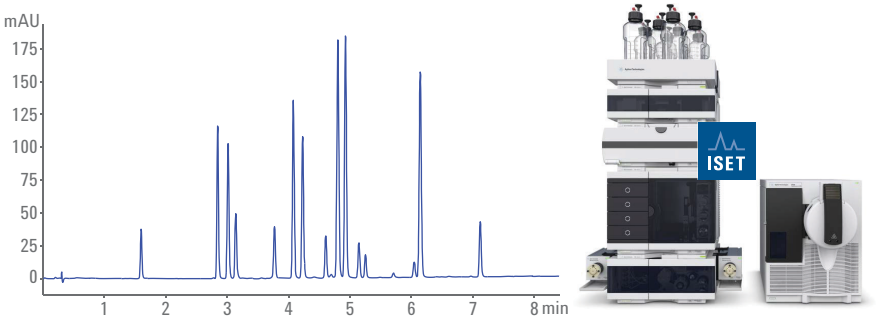
Abstract

This Application Note describes a combined approach to analytical method development using the Agilent ChemStation Method Scouting Wizard, and method transfer using Agilent Intelligent System Emulation Technology (ISET) for direct emulation of target HPLC systems. Based on the demonstrated success for highly dissimilar target systems such as the Agilent 1100 Series LC and Waters Acquity UPLC H-Class, the proposed workflow presents a general approach to develop analytical methods with the need for only one parent analytical method development system.



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Introduction

Today, analytical method development facilities are facing the challenge to develop LC methods for a high diversity of target systems used across different departments, or even within one analytical laboratory, because systems differ in manufacturer or LC generation. To overcome the need for a large number of method development systems, this Application Note presents a workflow that combines analytical method development with on-the-fly target system emulation using Agilent Intelligent System Emulation Technology (ISET). Ideally, the Agilent 1290 Infinity II Method Development Solution is used as a parent system, which develops analytical methods for different target systems without the need for manual system changes or dedicated analytical method development systems that can only address a limited number of target LCs.

In a previously published workflow, a UHPLC method was developed by mobile phase and column screening with subsequent transfer to standard HPLC conditions and ISET emulation of the target LC system[1]. In contrast, the workflow described in this Application Note directly develops the target system's analytical method using the 1290 Infinity II Method Development Solution and ISET emulation of the target LC system. Figure 1 shows a schematic overview of the workflow. First, columns, solvents, and temperatures are screened for suitable methods under ISET conditions. The initial screening is followed by a refinement campaign, which further optimizes the methods that showed the best separation regarding resolution and run time. After identification of a suitable separation analytical method, the method is transferred to the target system, and method robustness is tested over multiple injections.

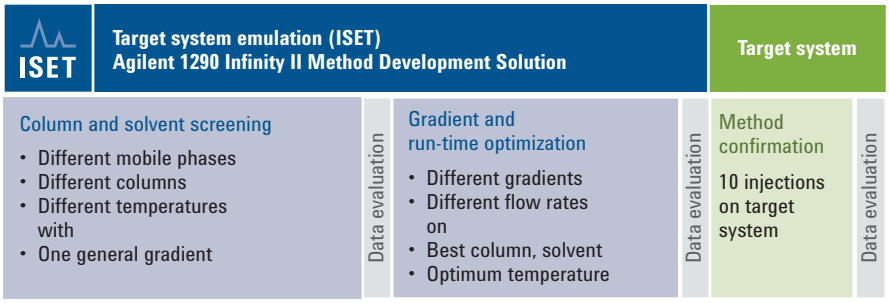

Figure 1. General workflow for the development of a chromatographic method directly towards a chosen target system by a combination of the Agilent ChemStation Method Scouting Wizard and Agilent Intelligent System Emulation Technology (ISET).

This Application Note demonstrates a workflow that combines the Agilent ChemStation Method Scouting Wizard and ISET for direct analytical method development towards a chosen target system. It shows that analytical method development for highly different target systems such as the Agilent 1100 Series LC and Waters Acquity UPLC H-Class is possible with one hardware setup. To test the proposed workflow, a complex sample comprising 15 compounds were used, and the resulting methods were compared for equivalency on the Agilent 1290 Infinity II Method Development Solution and the chosen target system.

Experimental

Instrumentation

The Agilent 1290 Infinity II Method Development Solution comprised the following modules:

- Agilent 1290 Infinity II Flexible Pump (G7104A) with ISET enabled
- Agilent 1290 Infinity II Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B)
- Agilent 6140 Single Quadrupole LC/MS (G6140B)

In addition, the following parts are required to run the 1290 Infinity II Method Development Solution for automated method development:

- Agilent InfinityLab Quick Change 8-column selection valve (G4239C)
- Agilent 1290 Infinity Valve Drive (G1170A) with InfinityLab Quick Change 12-position/13-port valve (G4235A)
- Low dispersion capillary kit, 0.12 mm id, p/n 5067-4248

Instrumental setup

The 1290 Infinity II Flexible Pump was clustered with an InfinityLab Quick Change 12-position/13-port valve for solvent selection in the Agilent OpenLAB CDS ChemStation Edition instrument configuration. The solvents were defined in the ChemStation pump setup dialog. The Agilent 1290 Infinity II Multicolumn Thermostat (MCT) was equipped with the InfinityLab Quick Change 8-column selection valve, and clustered in the ChemStation instrument configuration. All columns were used with column ID tags (p/n 5067-5917) for automated column recognition in ChemStation and assigned in the ChemStation MCT dialog. Methods necessary for column and gradient screening as well as instrument flushing and column equilibration were automatically created using of the Method Scouting Wizard. The emulation of the target systems was done using ISET.

The Agilent 1100 Series LC comprised the following modules:

- Agilent 1100 Series Quaternary Pump (G1311A)
- Agilent 1100 Series Degasser (G1379A)
- Agilent 1100 Series Standard Autosampler (G1329A)
- Agilent 1100 Series Thermostatted Column Compartment (G1316A)
- Agilent 1100 Series Diode Array Detector (G1315B)

The Waters Acquity UPLC H-Class comprised the following modules:

- Acquity UPLC H-Class bio-Quaternary Solvent Manager
- Acquity UPLC bio-Sample Manager FTN
- Acquity UPLC Column Manager
- Acquity UPLC TUV Detector

Software

- Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, version C.01.07 with Agilent ChemStation Method Scouting Wizard, version A02.06
- Agilent OpenLab CDS version 2.1 for control of Waters Acquity H-Class

Columns

For Agilent 1100 Series LC as target system:

- Agilent InfinityLab Poroshell EC-C18 USP L1, 4.6 × 150 mm, 2.7 µm, p/n 683975-902
- Agilent InfinityLab Poroshell EC-C8 USP L7, 4.6 × 150 mm, 2.7 µm, p/n 683975-906
- Agilent InfinityLab Poroshell Bonus-RP USP L60, 4.6 × 150 mm, 2.7 µm, p/n 693968-901
- Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 3.5 µm, p/n 959963-902

For Waters Acquity UPLC H-Class as target system:

- Agilent InfinityLab Poroshell EC C18, 2.1 × 100 mm, 1.9 µm, p/n 695675-902
- Agilent InfinityLab Poroshell EC PFP, 2.1 × 100 mm, 1.9 µm, p/n 695675-408
- Agilent InfinityLab Poroshell EC Phenyl-Hexyl, 2.1 × 100 mm, 1.9 µm, p/n 695675-912
- Agilent InfinityLab Poroshell EC C8, 2.1 × 100 mm, 1.9 µm, p/n 695675-906

Final methods

System	Agilent 1100 Series LC	Waters Acquity UPLC H-Class
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 3.5 µm	Agilent InfinityLab Poroshell EC PFP, 2.1 × 100 mm, 1.9 µm,
Temperature	40 °C	40 °C
Solvent	A) Water, 0.1 % (v:v) formic acid B) Acetonitrile, 0.1 % (v:v) formic acid	A) Water, 0.1 % (v:v) formic acid B) Acetonitrile, 0.1 % (v:v) formic acid
Flow Rate	1.7 mL/min	0.85 mL/min
Gradient	10 %B at 0 minutes 49 %B at 11.5 minutes 55 % B at 17 minutes	10 %B at 0 minutes 47 %B at 7.5 minutes 10 %B at 7.6 minutes
Stop time	17 minutes	9.5 minutes
Post time	3 minutes	None
UV Detection	254/10 nm, reference 360/100 nm, data rate 20 Hz	

Sample

As a test sample, a complex mixture of 15 pesticides and pharmaceuticals was used. The individual compounds were dissolved in acetonitrile (1 mg/mL) and finally mixed in equal amount. Table 1 outlines the compounds, their formulae, and masses.

Chemicals

All solvents were HPLC grade, and purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with an LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak). All Chemicals were purchased from Sigma-Aldrich (Germany).

Result and Discussion

Target system: Agilent 1100 Series LC

The initial method development campaign for the separation of a complex sample on the Agilent 1100 Series LC as the target system was done as a column, solvent, and temperature screening on the Agilent 1290 Infinity II Method Development Solution using the Method Scouting Wizard and ISET. In this screening campaign, four standard HPLC columns (see Experimental section), two solvents (methanol and acetonitrile), and three temperatures (30, 40, and 50 °C) were used. The initial generic gradient went from 5 to 70 % organic solvent in 30 minutes. Figure 2 shows the best possible separation of the complex test sample, obtained after the initial Method Scouting Wizard screening campaign.

Table 1. Composition of the test sample (mixture of 1 mg/mL solutions of each compound in acetonitrile).

Name	Chemical formula	*m/z* [M+H⁺]
Atrazine-desethyl	$C_6H_{10}ClN_5$	188.06
Metoxuron	$C_{10}H_{13}ClN_2O_2$	229.07
Hexazinone	$C_{12}H_{20}N_4O_2$	253.16
Terbuthylazine-desethyl	$C_7H_{12}ClN_5$	202.08
Methabenzthiazuron	$C_{10}H_{11}N_3OS$	222.06
Chlorotoluron	$C_{10}H_{13}ClN_2O$	213.08
Atrazine	$C_8H_{14}ClN_5$	216.10
Diuron	$C_9H_{10}Cl_2N_2O$	233.02
Metobromuron	$C_9H_{11}BrN_2O_2$	259.00
Metazachlor	$C_{14}H_{16}ClN_3O$	278.10
Nifedipine	$C_{17}H_{18}N_2O_6$	347.10
Sebuthylazine	$C_9H_{16}ClN_5$	230.11
Terbuthylazine	$C_9H_{16}ClN_5$	230.11
Linuron	$C_9H_{10}Cl_2N_2O_2$	249.02
Nimodipine	$C_{21}H_{26}N_2O_7$	419.18

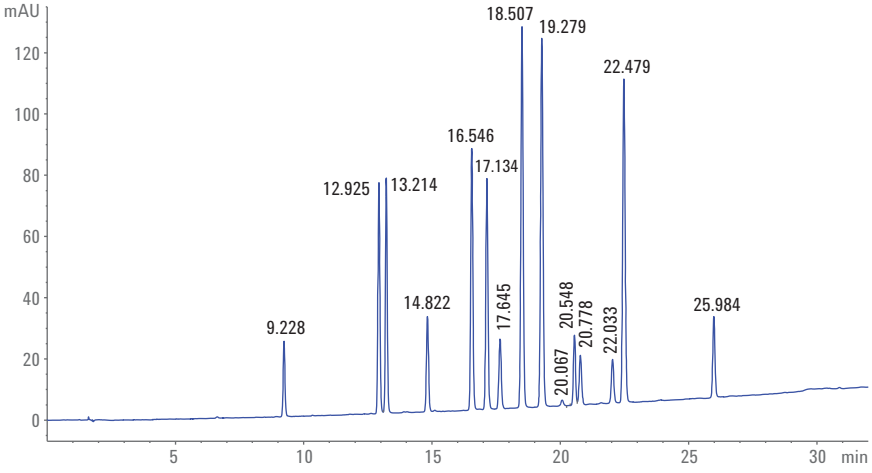

Figure 2. Best possible separation of the complex test sample, which was obtained after the initial Agilent ChemStation Method Scouting Wizard screening campaign. This screening campaign was run under ISET conditions set to the chosen target LC system, the Agilent 1100 Series LC.

To optimize this method, the initial percentage of organic solvent was set to 10 %, and the stop time and composition was set to 30 seconds after the last eluting compound. This method was optimized by a second campaign using gradients with increasing steepness and flow rates in fixed rates of 10 %, respective to flow rate and gradient time. To optimize the resolution of the critical pair of compounds, which eluted at 12.037 and 12.156 minutes in the final chromatogram, the slope of the gradient was decreased between 11.5 minutes and the end of the run at 17 minutes. In the final method, a compromise between speed and resolution of the critical pair was accepted (Figure 3).

To identify the compounds during the process of method development and optimization, their masses were tracked by the single quadrupole mass spectrometer. The method achieved after the final optimization was transferred directly to the target system, the 1100 Series LC, and 10 replicate injections of the sample were run (Figure 4).

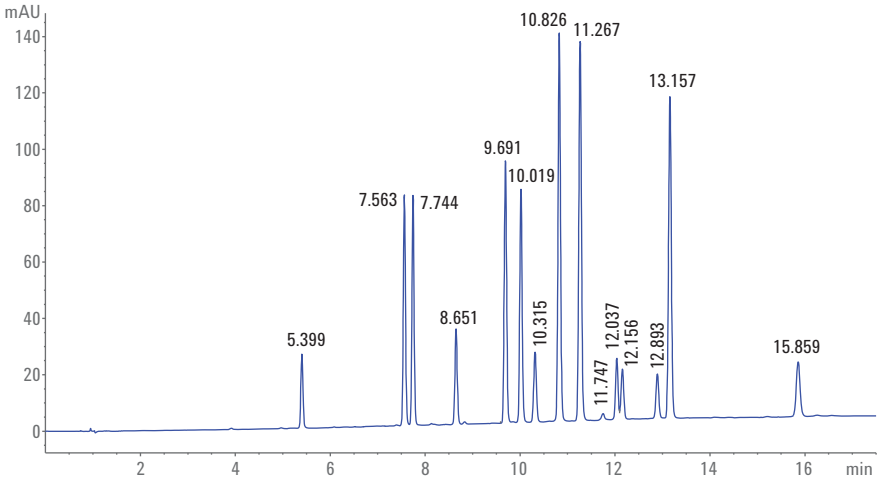

Figure 3. Best possible separation of the complex test sample, which was obtained after the refinement Agilent ChemStation Method Scouting Wizard screening campaign. This screening campaign was run under ISET conditions of the chosen target LC system, the Agilent 1100 Series LC.

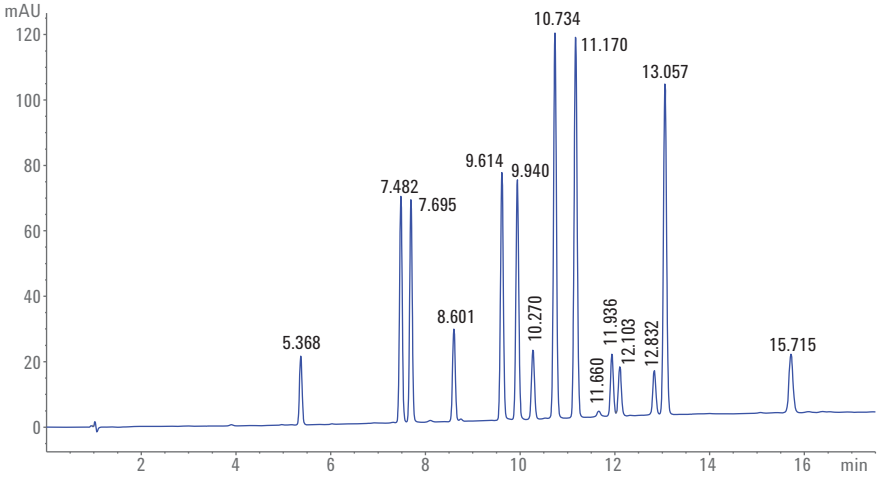

Figure 4. Final separation obtained on the target system, the Agilent 1100 Series LC.

After evaluation of the replicate runs on the target system, typical standard deviations of the retention times at or below 0.003 minutes could be found. The corresponding RSD values were typically below 0.03 %. The differences in retention time between the method development system and the target system were typically below 1 % (Table 2 and Figure 5). The complete method development time took approximately 35 hours for the first large screening campaign, approximately 8 hours for the optimization, and approximately 5 hours for the evaluation on the target system, which amounted to approximately 48 hours.

Table 2. Comparison of retention time, standard deviation and RSD values obtained on the Agilent 1290 Infinity II Method Development Solution and the target system, the Agilent 1100 Series LC (tr = retention time, $\bar{x}$ = average, σ = standard deviation, RSD = relative standard deviation).

No.	Compound	Agilent 1100 Series LC $\bar{x}$(tr) (min)	Agilent 1100 Series LC σ(tr) (min)	Agilent 1100 Series LC RSD (%)	Agilent 1290 Infinity II Method Development Solution $\bar{x}$(tr) (min)	Agilent 1290 Infinity II Method Development Solution σ(tr) (min)	Agilent 1290 Infinity II Method Development Solution RSD (%)	Δtr (%)
1	Atrazine-desethyl	5.365	0.002	0.035	5.401	0.002	0.037	0.7
2	Metoxuron	7.479	0.003	0.037	7.565	0.002	0.027	1.1
3	Hexazinone	7.692	0.003	0.037	7.745	0.001	0.016	0.7
4	Terbuthylazine-desethyl	8.599	0.002	0.027	8.653	0.002	0.022	0.6
5	Methabenzthiazuron	9.613	0.002	0.023	9.692	0.002	0.020	0.8
6	Chlorotoluron	9.939	0.002	0.023	10.021	0.002	0.019	0.8
7	Atrazine	10.269	0.002	0.020	10.316	0.002	0.016	0.5
8	Diuron	10.734	0.002	0.017	10.827	0.002	0.023	0.9
9	Metobromuron	11.170	0.002	0.020	11.269	0.003	0.024	0.9
10	Metazachlor	11.658	0.003	0.023	11.748	0.002	0.016	0.8
11	Nifedipine	11.935	0.003	0.025	12.039	0.002	0.017	0.9
12	Sebuthylazine	12.103	0.002	0.019	12.158	0.002	0.016	0.5
13	Terbuthylazine	12.833	0.002	0.019	12.895	0.002	0.016	0.5
14	Linuron	13.058	0.003	0.020	13.159	0.003	0.020	0.8
15	Nimodipine	15.718	0.004	0.027	15.861	0.003	0.019	0.9

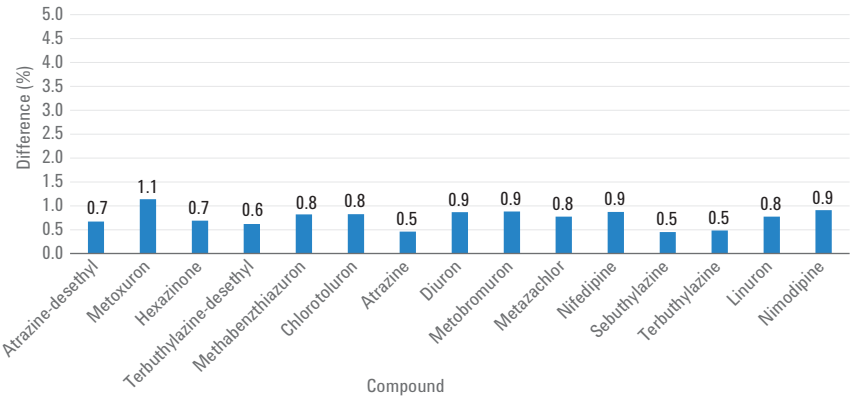

Figure 5. Retention time differences of the individual compounds in the comparison of the target system, the Agilent 1100 Series LC, with the Agilent 1290 Infinity II Method Development Solution.

Target system: Waters Acquity UPLC H-Class

For the development of a separation method suitable for the Waters H-Class as a target system, Agilent InfinityLab Poroshell columns with smaller particles (1.9 µm) were used. As a starting point, C8, C18, phenyl-hexyl, and pentafluoro-phenyl (PFP) phases were used. These columns had more typical dimensions used for UHPLC instruments (2.1 × 100 mm). The initial campaign was run with methanol and acetonitrile as organic solvents, and three different temperatures were tested. The initial generic gradient had a length of 20 minutes, and the organic solvent increased from 5 to 70 %. As expected, the columns with the C8 and C18 material showed a similar separation behavior as already obtained for the conventional LC method (data not shown). Surprisingly, the PFP stationary phase showed a dramatically earlier elution with slightly different selectivity compared to the C8 and C18 phases (Figure 6).

Because all the compounds were already separated, and the last peak eluted at a retention time of 12 minutes, this separation was taken for optimization. In a second campaign, different flow rates and gradients were tested to separate the pesticide sample on the PFP column in a shorter run time and with optimum resolution. Finally, the separation could be achieved in only 7.4 minutes applying a gradient from 10 to 47 % acetonitrile at a flow rate of 0.85 mL/min (Figure 7).

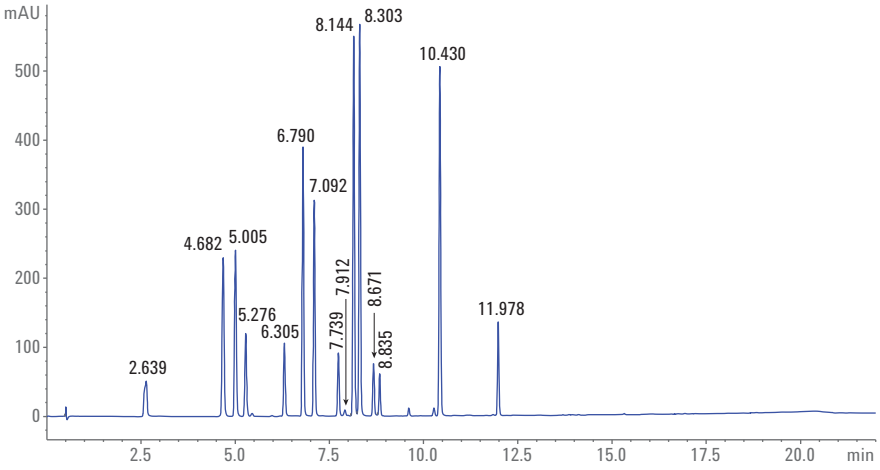

Figure 6. Separation of the pesticide sample on a PFP column with a gradient starting at 5 % acetonitrile and increasing to 70 % in 20 minutes at 40 °C. This screening campaign was run under ISET conditions set to the chosen target LC system, the Water H-Class.

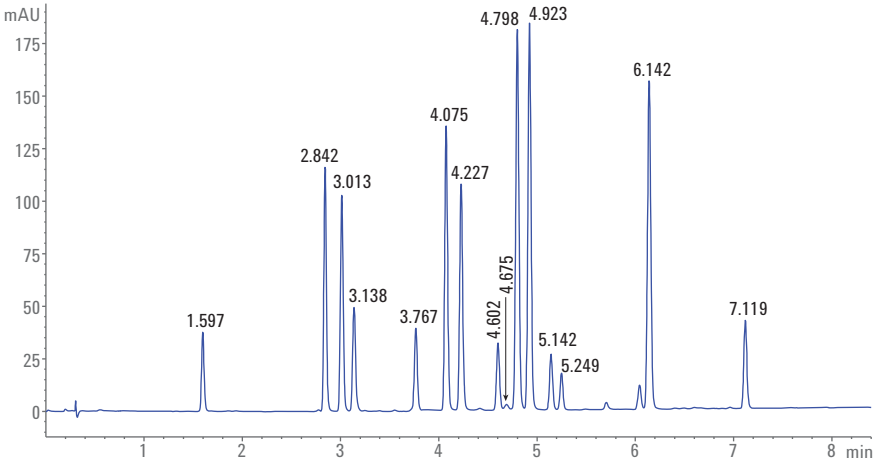

Figure 7. Final optimized separation of the pesticide sample on a PFP column with a gradient starting at 10 % acetonitrile and increasing to 47 % in 7.4 minutes at 40 °C. This screening campaign was run under ISET conditions set to the chosen target LC system, the Water H-Class.

This method was directly transferred to the target system, the Waters H-Class system, for evaluation. The identity of the retention times could be seen in the comparison of the chromatogram obtained on the 1290 Infinity II Method development system (Figure 7) and the Waters H-Class system (Figure 8).

For statistical evaluation, 10 replicate runs were done. As a result of this evaluation, typical standard deviations of the retention times below 0.01 minutes could be found. According to the short retention times, corresponding RSD values were typically below 0.2 %. The differences in retention time between the development system and the target system were typically below 2 % (Table 3 and Figure 9). The complete method development took approximately 37 hours.

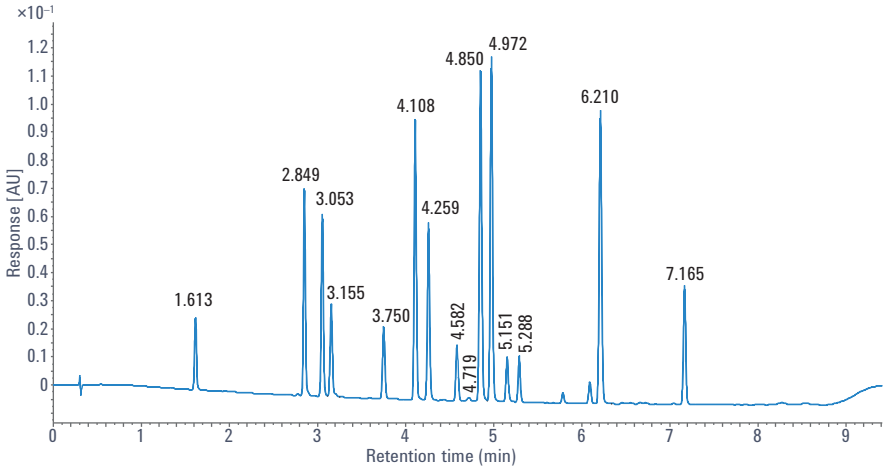

Figure 8. Final optimized separation of the pesticide sample on the Waters H-Class system using a PFP column and applying a gradient starting at 10 % acetonitrile and increasing to 47 % in 7.4 minutes with a flow rate of 0.85 mL/min, and a column temperature of 40 °C.

Table 3. Comparison of retention time, standard deviation and RSD values obtained on the Agilent 1290 Infinity II Method Development Solution and the target system, the Waters H-Class LC (tr = retention time, $\bar{x}$ = average, σ = standard deviation, RSD = relative standard deviation).

No.	Compound	Waters H-Class $\bar{x}$ (tr) (min)	Waters H-Class σ(tr) (min)	Waters H-Class RSD (%)	Agilent 1290 Infinity II Method Development Solution $\bar{x}$ (tr) (min)	Agilent 1290 Infinity II Method Development Solution σ(tr) (min)	Agilent 1290 Infinity II Method Development Solution RSD (%)	Δtr (%)
1	Atrazine-desethyl	1.616	0.004	0.220	1.587	0.003	0.164	-1.8
2	Hexazinone	2.852	0.003	0.105	2.833	0.004	0.159	-0.6
3	Metoxuron	3.056	0.003	0.112	2.997	0.005	0.161	-1.9
4	Terbuthylazine-desethyl	3.157	0.003	0.101	3.126	0.005	0.168	-1.0
5	Atrazine	3.753	0.003	0.070	3.764	0.005	0.132	0.3
6	Methabenzthiazuron	4.110	0.003	0.0.62	4.062	0.005	0.113	-1.2
7	Chlorotoluron	4.261	0.002	0.055	4.213	0.005	0.114	-1.1
8	Sebuthylazine	4.585	0.002	0.046	4.601	0.005	0.104	0.3
9	Metazachlor	4.734	0.041	0.871	4.676	0.005	0.104	-1.2
10	Metobromuron	4.864	0.038	0.788	4.781	0.006	0.122	-1.7
11	Diuron	5.992	0.055	1.101	4.908	0.005	0.097	-1.7
12	Terbuthylazine	5.166	0.043	0.824	5.137	0.005	0.100	-0.6
13	Nifedipine	5.340	0.150	2.806	5.240	0.005	0.089	-1.9
14	Linuron	6.212	0.001	0.024	6.121	0.006	0.105	-1.5
15	Nimodipine	7.167	0.001	0.016	7.108	0.005	0.066	-0.8

Conclusion

This Application Note demonstrates the use of the Agilent 1290 Infinity II Method Development Solution with the Agilent ChemStation Method Scouting Wizard for the direct development of analytical separation methods under ISET control for a chosen target system. The analytical method development for the separation of a complex sample was done for an Agilent 1100 Series LC and a Waters Acquity UPLC H-Class as target systems. Both instruments showed excellent correlation between the method, which was developed on the Agilent 1290 Infinity II Method Development Solution, and the target systems. The retention time deviation was typically below 2 %. The time needed for the development of the method on the 1290 Infinity II Method Development Solution and their evaluation on the target system typically took two days or less.

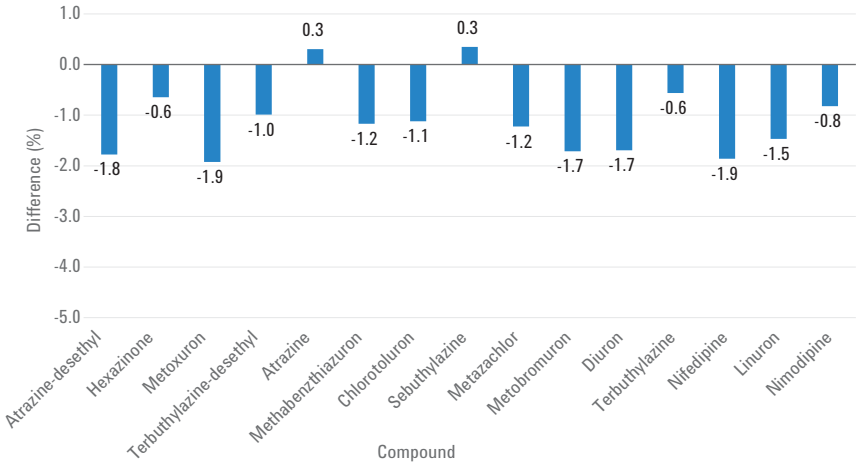

Figure 9. Retention time differences of the individual compounds in the comparison of the target system, the Waters H-Class, to the Agilent 1290 Infinity II Method Development Solution.

Reference

1. Huesgen, A. G. 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, *Agilent Technologies Application Note*, publication number 5991-0989EN, **2012**.

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