

Agilent InfinityLab LC with ISET

User Manual



Notices

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1

In This Book

The manual describes the Agilent InfinityLab LC Intelligent System Emulation Technology (ISET). It contains the following:



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What is ISET?

This chapter gives a definition and brief overview of ISET and its intended use.

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What is Intelligent System Emulation Technology (ISET)

Instrument-to-instrument method transferability is an important topic for all laboratories throughout all industries, where HPLC and UHPLC methods are transferred between different departments and locations with different LC instruments. In the pharmaceutical industry, the transfer of analytical methodology between R&D, contract research organizations and manufacturing is an essential part in the development of a new pharmaceutical product. Several hundred observations from the FDA and a proposal for a new chapter in USP 1224 *Transfer of analytical procedures* emphasize the actuality and importance of this topic.

ISET Use Cases

ISET offers the following options for instrument to instrument method transfer :

- Perform legacy methods, see [Figure 1](#) on page 6
- Method development, see [Figure 2](#) on page 7
- Method robustness testing, see [Figure 3](#) on page 7

Perform legacy methods

With ISET it is possible to perform legacy methods on modern (U)HPLC instruments without changes in retention time and resolution.



Figure 1: Backward compatibility

What is ISET?

What is Intelligent System Emulation Technology (ISET)

Method development

With ISET it is possible to develop methods for a variety of instruments on one high performance instruments while maintaining individual instrument characteristics.

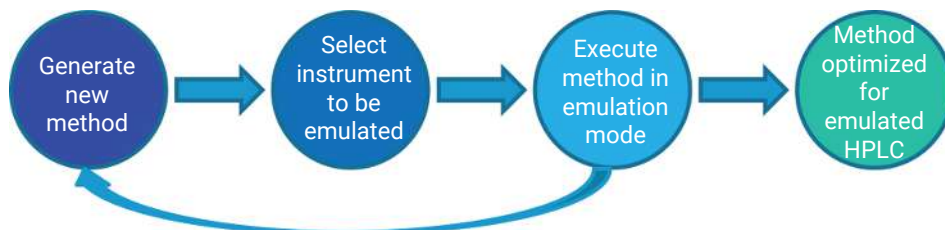


Figure 2: Method development

Method robustness testing

With ISET it is possible to test a method for robustness against variation in instrument characteristics using one single instrument.

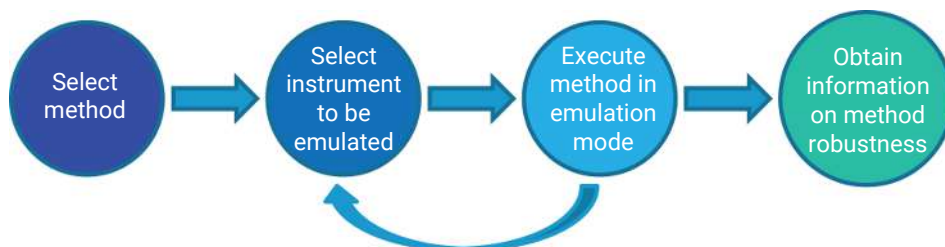


Figure 3: Method robustness testing

Which Parameters Affect Method Transfer?

Design differences between LC instrumentation – such as power range, delay volume, mixing behavior, temperature control, extra column volume and detector cell design – all affect the ability to transfer a method from one system to another. Therefore identical LC methods used on different LC instrumentation could result in different retention time and chromatographic resolution.

What is ISET?

What is Intelligent System Emulation Technology (ISET)

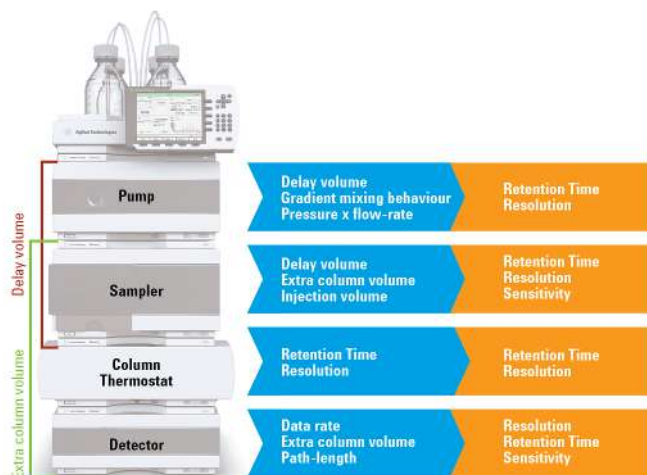


Figure 4: Parameters affecting method transfer

A wide range of instrument parameters have a direct impact on the retention times, resolution and sensitivity of a separation.

The Impact of Delay Volume and Gradient Mixing

The delay volume of an LC system determines how fast the gradient reaches the column. Further, the mixing behavior influences the gradient profile. Both these factors – delay volume and mixing behavior – are determined by the instrument design and the consequences for method transfer are differences in retention times and in resolution.

What is ISET?

What is Intelligent System Emulation Technology (ISET)

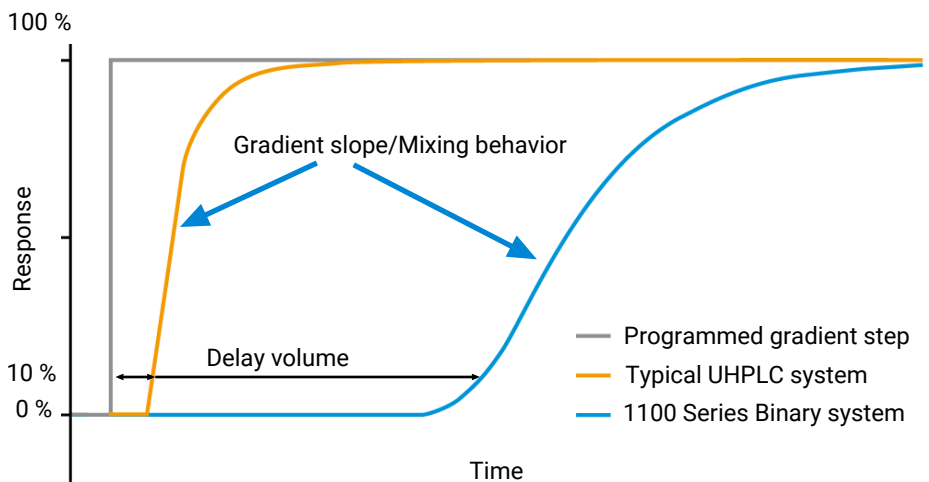


Figure 5: Comparison of delay volumes and gradient mixing behaviors between a typical UHPLC system and an 1100 Series Binary LC using a tracer experiment. On a typical UHPLC system the mixed solvents reach the column much earlier, and the set composition is also achieved earlier due to the steeper gradient slope.

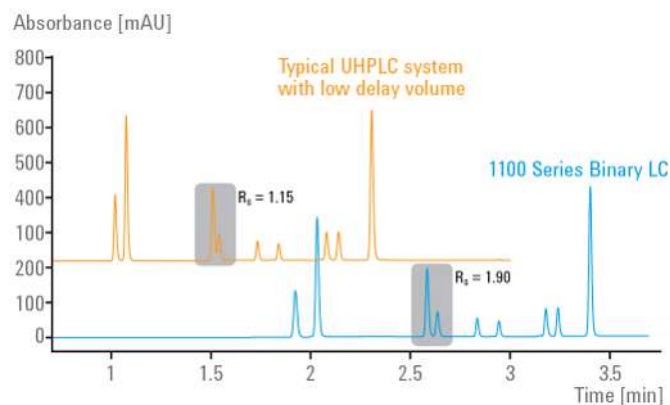


Figure 6: Different solvent compositions at the column due to different delay volumes and gradient mixing behaviors result in different retention times and resolutions.

Poroshell 120, 3.0 x 50 mm (2.7 μ m). Flow rate: 0.85 mL/min

Water, Acetonitrile

0 min (10% Acetonitrile), 3 min (90% Acetonitrile)

The Solution for Instrument to Instrument Method Transfer: ISET

Intelligent System Emulation Technology allows to run HPLC or UHPLC methods from other instruments and delivers almost the same chromatographic results without any changes in the instrument setup or the original method - all by a few mouse clicks.

Performance Specification

The InfinityLab LC Series delivers the key requirement for the implementation of ISET technology.

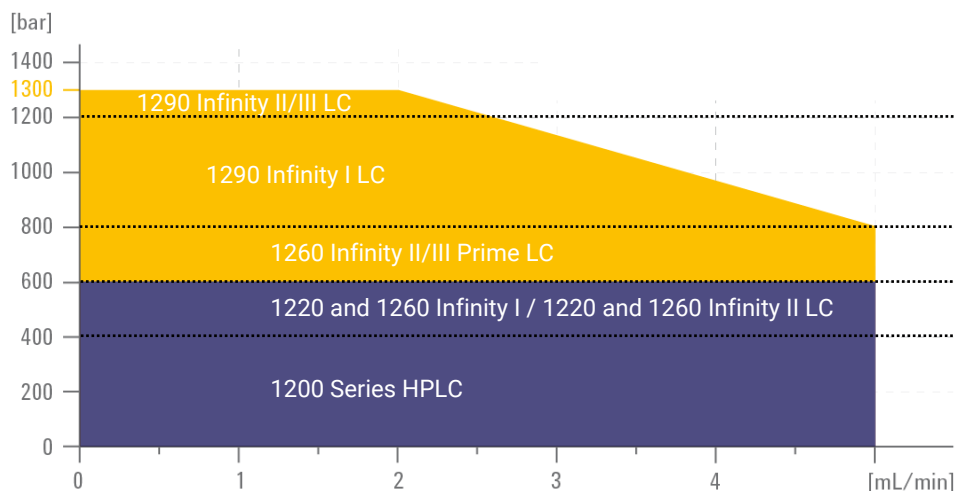


Figure 7: Agilent InfinityLab Series Power Range

The wide power range of the InfinityLab LC Series ensures that both HPLC and UHPLC methods from narrow bore to standard bore columns can be executed.

The ISET Emulation Algorithm

With the detailed knowledge about the system behavior of the target LC instrument and the high accuracy of the InfinityLab LC Series instruments, ISET is able to create an emulation function, which delivers similar gradient conditions as the selected instrument. This results in similar retention times and similar chromatographic resolution.

The InfinityLab LC Series instruments with ISET deliver a gradient similar to the 1100 Series Binary LC by emulating the delay volume and mixing behavior.

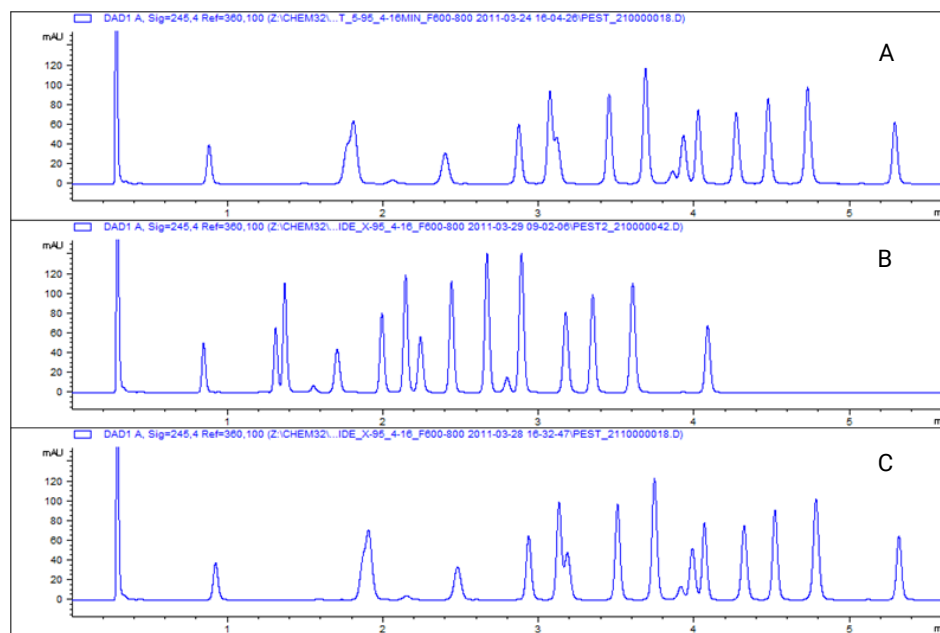


Figure 8: Chromatograms of a gradient separation of a pesticide mixture run on (A) 1100 Series Quaternary Pump (G1311A) + Autosampler (G1367A), (B) 1290 Infinity II LC without emulation, (C) 1290 Infinity II LC in emulation mode

The result: similar retention times and similar resolution, without modifying the instrument or the original method.

NOTE

The sample and method conditions in the figure above are used to display a critical transfer situation. The method is not optimized, and not meant to be a good example for best resolution. For ISET, it is key to always reproduce the original resolution. It delivers a good separation in C if the separation in A is good, and a compromised separation in C if the separation in A is compromised. ISET truly reproduces the original separation pattern.

ISET Support for Emulation

Table 1: List of modules, which can be emulated by ISET (Agilent)

Pumps	G1311A	1100 Series Quaternary Pump
	G1311B	1200 Series Quaternary Pump 1260 Infinity Quaternary Pump
	G1311C	1260 Infinity Quaternary Pump VL
	G1312A	1100 Series Binary Pump
	G1312B	1200 Series Binary Pump SL 1260 Infinity Binary Pump
	G1312C	1260 Infinity Binary Pump VL
	G4204A	1290 Infinity Quaternary Pump
	G4220A ¹	1290 Infinity Binary Pump
	G4220B ¹	1290 Infinity Binary Pump VL
	G5611A	1260 Infinity Bio-inert Quaternary Pump
	G7104A	1290 Infinity II/III Flexible Pump
	G7104C	1260 Infinity II/III Flexible Pump
	G7111A	1260 Infinity II/III Quaternary Pump VL
	G7111B	1260 Infinity II/III Quaternary Pump
	G7112B	1260 Infinity II/III Binary Pump
	G7131A	1290 Infinity II/III Bio Flexible Pump
	G7131C	1260 Infinity II/III Bio Flexible Pump
	G7132A	1290 Infinity II/III Bio High-Speed Pump
	G7120A ¹	1290 Infinity II/III High-Speed Pump
Autosamplers	G1313A	1100 Series Standard Autosampler

	G1329A	1100 Series Thermostatted Autosampler 1200 Series Standard Autosampler
	G1329B	1200 Series Standard Autosampler SL 1260 Infinity Standard Autosampler
	G1367A (100 µL syringe)	1100 Series Well Plate Sampler
	G1367B (100 µL syringe)	1100 Well Plate Sampler 1200 Series High Performance Autosampler
	G1367C (100 µL syringe)	1200 Series High Performance Autosampler SL
	G1367D (100 µL / 40 µL syringe)	1200 Series High Performance Autosampler SL+
	G1367E (100 µL / 40 µL syringe)	1260 Infinity High Performance Autosampler
	G1377A	1100 Micro Well Plate Sampler 1200 Series Micro Well Plate Autosampler 1260 Infinity High Performance Micro Autosampler
	G4226A (20, 40, 100 µL syringe)	1290 Infinity High Performance Autosampler
	G5667A	1260 Infinity High Performance Bio-inert Autosampler
	G7129A (40 µL / 100 µL / 900 µL syringe)	1260 Infinity II/III Vialsampler
	G7129B (40 µL / 100 µL / 900 µL syringe)	1290 Infinity II/III Vialsampler
	G7129C (40 µL / 100 µL / 900 µL syringe)	1260 Infinity II/III Vialsampler
	G7137A	1290 Infinity II/III Bio Multisampler
	G7167A (40 µL / 100 µL / 900 µL syringe)	1260 Infinity II/III Multisampler
	G7167B (40 µL / 100 µL / 900 µL syringe)	1290 Infinity II/III Multisampler
LC Systems	G4288A, G4289AA, G4290AA	1120 Compact LC
	G4288C, G4290C	1220 Infinity II LC VL / 1220 Infinity LC VL
	G4288B, G4290B, G4294B	1220 Infinity II LC / 1220 Infinity LC

¹ Emulation of this module is possible with G4220A/B, G7120A, or G7132A only.

Table 2: List of instruments, which can be emulated by ISET (Waters)

System	Specifications	Comment
Alliance 2690, 2695	Total system delay volume: < 650 µL Injection range: 0.1 – 100 µL (standard), up to 2000 µL with optional sample loop standard configuration with 100 µL sample loop and 250 µL syringe. Sample loops available: 2000 , 200 , 193 , 100 µL	Syringes available: 25 , 250 , 2500 µL (syringe volume does not contribute to dwell volume).
Alliance 2790, 2795, 2796	Total system delay volume: < 400 µL Injection range: 5 – 50 µL (standard), up to 1500 µL with optional sample loop standard configuration with 50 µL sample loop and 500 µL syringe. Sample loops available: 5 , 20 , 50 , 100 , 500 , 2000 µL	Syringes available: 100 , 250 , 500 , 1000 , 2500 µL (syringe volume does not contribute to dwell volume).
Acquity UPLC ¹	Total system delay volume: <120 µL 50 µL, 100 µL mixer Injection range: 0.1 – 5 µL, up to 50 µL with optional sample loop. Standard configuration with 5 µL sample loop and 100 µL syringe. Sample loops available: 1, 2, 5, 10, 20, 50 µL	Syringes available: 100, 250 µL (syringe volume does not contribute to dwell volume).
Acquity UPLC H-Class	Total system delay volume: <400 µL 100 µL mixer Injection range: 0.1 – 10 µL, up to 1000 µL with optional sample loop. Standard configuration with 10 µL sample loop and 100 µL syringe. Sample loops available: 10, 50, 100, 250, 1000 µL	Syringes available: 100, 250, 500 µL (syringe volume does not contribute to dwell volume).

¹ Emulation of this instrument is possible with G4220A/B, G7120A, or G7132A only.

What is ISET?

The Solution for Instrument to Instrument Method Transfer: ISET

Table 3: List of instruments, which can be emulated by ISET (Shimadzu)

Pumps	LC-20 AB w/o mixer, 100 µL, 0.5 mL, 1.7 mL, 2.6 mL mixer	Binary High Pressure Mixing Pump ¹
	LC-20 AT w/o mixer, 100 µL, 0.5 mL, 1.7 mL, 2.6 mL mixer	Quaternary Low Pressure Mixing Pump
Sampler	LC-20 SIL A/AC Injection Range: 0.1 – 100 µL, up to 2000 µL with optional sample loop. Standard configuration with 100 µL sample loop. Sample loops available: 50, 100, 500, 2000 µL	Sampler

¹ Emulation of this instrument is possible with G4220A/B, G7120A, or G7132A only.

NOTE

ISET supports the emulation of any LC instrument. For details see [Generic Emulation Using Dwell Volume](#) on page 38.

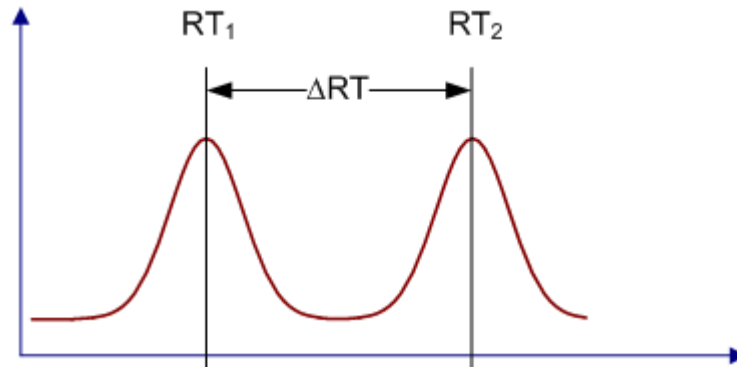
Specifications

Table 4: Retention Time (RT) Deviation

RT	±5% ±0.3 min	(for RT > 6 min) (for RT ≤ 6 min)
ΔRT	±5% ±0.1 min	(for ΔRT > 2 min) (for ΔRT ≤ 2 min)

What is ISET?

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NOTE

For other vendor systems these specifications are only valid for column operation at 40 °C. These specifications limits are not valid for the generic emulsion and generic solvents.

Demo/Trial

A 700-hour demonstration/trial version of ISET is available with the following pumps:

- Agilent 1290 Infinity Quaternary Pump (G4204A with firmware revision B.06.56 and later)
- Agilent 1290 Infinity Binary Pump (G4220A with firmware revision B.06.42 and later)
- Agilent 1290 Infinity Binary Pump VL (G4220B with firmware revision B.06.42 and later)
- Agilent 1290 Infinity II/III Flexible Pump (G7104A with firmware revision B.06.56 and later)
- Agilent 1260 Infinity II/III Flexible Pump (G7104C with firmware revision B.07.20 and later)
- Agilent 1290 Infinity II/III High-Speed Pump (G7120A) with firmware revision B.06.42 and later)
- Agilent 1290 Infinity II/III Bio Flexible Pump (G7131A with firmware revision B.07.34 and later)
- Agilent 1260 Infinity II/III Bio Flexible Pump (G7131C with firmware revision B.07.34 and later)

What is ISET?
The Solution for Instrument to Instrument Method Transfer: ISET

- Agilent 1290 Infinity II/III Bio High-Speed Pump (G7132A with firmware revision B.07.33)

A pictogram in the controller software indicates the use of the demonstration/ trial version.

Table 5: Pictograms of ISET versions

	Demonstration/ trial	Registered trial
Active		
Inactive		

Unless the demonstration/trial version is upgraded to a registered version, the ISET function will be deactivated when the trial period has elapsed.

NOTE

The demonstration/trial version can be deactivated via Agilent Lab Advisor to avoid any need for reconfiguration using 3rd Party Data Systems after the demonstration/trial version has elapsed.



3

Installing and Configuring ISET

This chapter gives detailed step-by-step instructions for installing a new system and upgrading an existing system with ISET.

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Preconditions for ISET

You need one of the Chromatographic Data Systems specified in [Table 8 Software compatibility ISET](#) on page 22 (OpenLAB CDS, OpenLAB CDS ChemStation Edition, OpenLAB CDS EZChrom Edition, or Mass Hunter).

Alternatively, you need one of those 3rd party CDS systems that support the Agilent Instrument Control Framework (ICF), plus you need this ICF installed in the specified revision.

In addition to the CDS the installation of the Agilent LC and CE drivers (\geq A.02.04 – see [Table 8 Software compatibility ISET](#) on page 22) is required.

Table 6: Modules needed for ISET

Module	P/N	Firmware Revision	Comments
1290 Infinity Binary Pump	G4220A	\geq B.06.42	<p>You need one of the pumps listed with either no mixer or one of the specified mixers (see Table 7 ISET - supported mixers on page 21).</p> <p>NOTE: ISET emulation is not possible when the dwell volume of the system to be emulated is smaller than that of the emulating system. In this case an error message Dwell volume of emulated system too small to be emulated with current configuration! is shown in the driver and the method cannot be downloaded to the instrument.</p> <p>NOTE: In Method Scouting Wizard configuration either choose Generic as ISET solvent model which works always but leads to less accurate emulation results or make sure that the solvents combinations used in method scouting match with the solvent setting for the ISET solvent model. Currently ISET supports following resulting gradients: Water vs. Acetonitrile, Water vs. Methanol or Aqueous vs. Organic.</p> <p>ISET in Pump Valve Clusters is supported since ISET 2.</p>
1290 Infinity Binary Pump VL	G4220B	\geq B.06.42	
1290 Infinity Quaternary Pump	G4204A	\geq B.06.56	
1290 Infinity II/III Flexible Pump	G7104A	\geq B.06.70	
1260 Infinity II/III Flexible Pump	G7104C	\geq B.07.20	
1290 Infinity II/III High-Speed Pump	G7120A	\geq B.06.70	
1290 Infinity II/III Bio Flexible Pump	G7131A	\geq B.07.34	
1290 Infinity II/III Bio Flexible Pump	G7131C	\geq B.07.34	
1260 Infinity II/III Bio Flexible Pump	G7132A	\geq B.07.33	
1290 Infinity II/III Bio High-Speed Pump			
1290 Infinity Autosampler	G4226A	\geq B.06.42	With 20 μ L, 40 μ L, 100 μ L loop
1290 Infinity II/III Multisampler ¹	G7167B	\geq D.06.70	
1260 Infinity II/III Multisampler ¹	G7167A	\geq D.06.75	
1290 Infinity II/III Vialsampler	G7129B	\geq D.06.75	
1260 Infinity II/III Vialsampler	G7129A	\geq D.06.75	
1260 Infinity II/III Vialsampler	G7129C	\geq D.07.20	

Module	P/N	Firmware Revision	Comments
1290 Infinity II/III Bio Multisampler ¹	G7137A	≥D.07.33	

¹ single needle is supported, dual needle is not supported.

Table 7: ISET - supported mixers

Module	P/N	Supported mixers
1290 Infinity Binary Pump	G4220A	<ul style="list-style-type: none"> • V35 JetWeaver • V100 JetWeaver • V380 JetWeaver
1290 Infinity Binary Pump VL	G4220B	<ul style="list-style-type: none"> • V35 JetWeaver • V100 JetWeaver • V380 JetWeaver
1290 Infinity II/III High-Speed Pump	G7120A	<ul style="list-style-type: none"> • V35 JetWeaver • V100 JetWeaver • V380 JetWeaver
1290 Infinity Quaternary Pump	G4204A	<ul style="list-style-type: none"> • V35 JetWeaver • V100 JetWeaver • V380 JetWeaver
1290 Infinity II/III Flexible Pump	G7104A	<ul style="list-style-type: none"> • V35 JetWeaver • V100 JetWeaver • V380 JetWeaver
1260 Infinity II/III Flexible Pump	G7104C	<ul style="list-style-type: none"> • V35 JetWeaver • V100 JetWeaver • V380 JetWeaver
1290 Infinity II/III Bio High-Speed Pump	G7132A	<ul style="list-style-type: none"> • Bio V35 JetWeaver • Bio V100 JetWeaver • Bio V380 JetWeaver
1290 Infinity II/III Bio Flexible Pump	G7131A	<ul style="list-style-type: none"> • Bio V35 JetWeaver • Bio V100 JetWeaver • Bio V380 JetWeaver
1260 Infinity II/III Bio Flexible Pump	G7131C	<ul style="list-style-type: none"> • Bio V35 JetWeaver • Bio V100 JetWeaver • Bio V380 JetWeaver

Table 8: Software compatibility ISET

ISSET Revision	Driver Revision	Emulated LC Instruments	OpenLAB CDS	OpenLAB CDS ChemStation Edition	OpenLAB CDS EZChrom Edition	Mass Hunter	ICF ¹
ISSET 1	A.02.04	Agilent 1100 and 1200 Series, Agilent 1260 Infinity LC		CS B.04.03 SP1 ≥ CS C.01.03	≥ EE A.04.03	Q/TOF B.05.00 QQQ B.05.00	A.01.04
ISSET 2	A.02.06	Scope of ISET 1 plus Waters Alliance, Agilent 1220 Infinity LC, Agilent 1120 Compact LC, Generic emulation		CS B04.03 SP2 CS C01.03 SP1 ≥ CS C.01.04	CS B04.03 SP2 EE A04.03 SP1 ≥ EE A.04.04	Q/TOF ≥ B.05.01 (Sept ember 2012) QQQ ≥ B.06.00 (Octo ber 2012)	A.01.05
ISSET 3	A.02.09	Scope of ISET 2 plus Agilent 1290 Infinity Quaternary Pump Waters H-Class Waters Acquity Shimadzu LC 20		≥ CS C.01.05	≥ EE A.04.05	Q/TOF ≥ B.06.00 QQQ ≥ B.07.00	A.02.01
ISSET 4	A.02.11	Scope of ISET 3 plus Agilent 1290 Infinity Binary Pump Agilent 1290 Infinity II/III Flexible Pump Agilent 1290 Infinity II/III High-Speed Pump		≥ CS C.01.07	≥ EE A.04.07	Q/TOF ≥ B.06.01 QQQ ≥ B.07.00 SP2	A.02.03 DU2
ISSET 4.1	A.02.12	Scope of ISET 4 plus Agilent 1260 Infinity Autosampler Agilent 1290 Infinity II/III Vialsampler		≥ CS C.01.07	≥ EE A.04.07	Q/TOF ≥ B.07.01 QQQ ≥ B.08.00	A.02.03 DU2
ISSET 4.2	A.02.18	Scope of ISET 4.1 plus 1260 Infinity II/III Flexible Pump 1260 Infinity II/III Vialsampler	≥ 2.2	≥ C.01.07 SR4	≥ A.04.08 SR1	QTOF ≥ B.09.00 QQQ ≥ B.09.00 Ultivo ≥ C.01.00	A.02.05

ISET Revision	Driver Revision	Emulated LC Instruments	OpenLAB CDS	OpenLAB CDS ChemStation Edition	OpenLAB CDS EZChrom Edition	Mass Hunter	ICF ¹
ISET 4.3	A.02.18 with xml extension	Scope of ISET 4.2 plus Agilent 1260 Infinity II/III Quaternary Pump VL Agilent 1260 Infinity II/III Quaternary Pump Agilent 1260 Infinity II/III Binary Pump	≥ 2.3	≥ C.01.08	≥ A.04.08	QTOF B.09.00 QQQ B.09.00	A.02.05
ISET 4.4	3.4	Scope of ISET 4.3 plus Agilent 1290 Infinity II/III Bio High-Speed Pump Agilent 1290 Infinity II/III Bio Flexible Pump Agilent 1260 Infinity II/III Bio Flexible Pump	≥ 2.6	≥ C.01.10	≥ A.04.10	QTOF 11.0	3.0 U3

¹ Agilent Instrument Control Framework

Installing and Configuring ISET

Installation and configuration procedure for pumps G4204A, G7104A/C, G4220A/B, G7120A, G7131A/C, or G7132A.

The installation of ISET in the trial version is already complete if the prerequisites are met and the components have been properly installed. To upgrade to the registered version, you need to follow these steps:

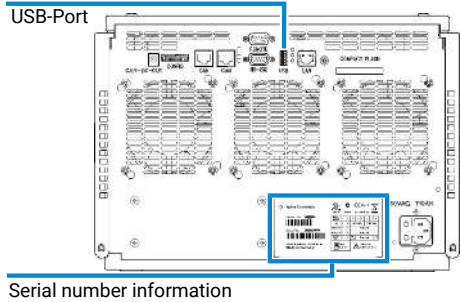
- 1 Power up the pump.
- 2 Plug the ISET USB dongle into the USB port on the back of the pump.

NOTE

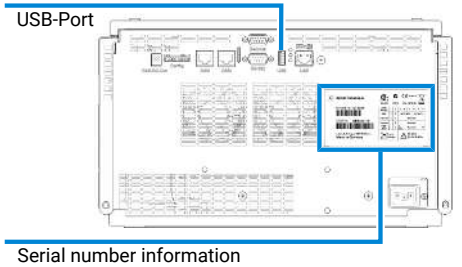
You must use the same ISET USB dongle for reinstallation as was originally used for installation.

This, for example, is necessary after exchanging the mainboard of the pump. Thus it's advisable to keep the serial number information of the pump together with the ISET USB dongle.

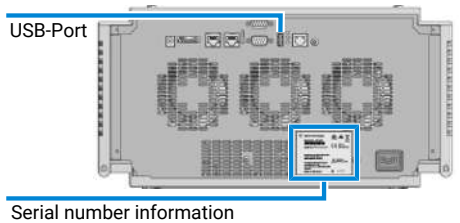
G4220A/B



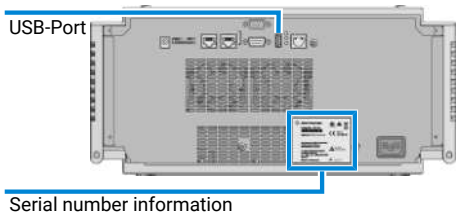
G4204A



G7120A or G7132A



G7104A/C or G7131A/C



- 3 Wait until blinking of USB dongle stops.
- 4 Switch off the pump.
- 5 Switch on the pump to finalize the activation procedure.

NOTE

Do not remove the ISET USB dongle at this stage.

- 6 Go to instrument configuration to display the pump configuration screen.

The **ISET installed** check box in the **Options** section is marked to indicate that the installation is complete.

Options

Pressure Unit

☒ Seal wash installed

Installed mixer

Custom mixer

☒ ISET installed

- 7 Remove the USB dongle.

ISET Removal and Reinstallation

ISET functionality will not be affected by a firmware update. If ISET was present before the update, it will remain active after the procedure.

Replacement of the pump main board will completely remove the ISET functionality.

Reinstalling ISET

In the case of a replacement of the pump's main board you will need to reinstall ISET.

To reinstall ISET, follow the installation procedure (see above).

NOTE

You must use the same ISET USB dongle for reinstallation as was originally used for installation.

The ISET USB dongle has a counter that tracks the number of times it has been used. You can install and reactivate ISET a maximum of five times.



4

Setting Up ISET Parameters

This chapter gives detailed step-by-step instructions for setting up the standard ISET parameters, and for setting up and using a verification method to confirm that ISET is functioning as expected. It also contains explanations of the advanced ISET parameters, with step-by-step instructions for setting them up.

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Preparing the 1290 Infinity System for Emulation

- 1 Set up the system with the correct column and mobile phases for the method to be transferred.

NOTE

If possible, transfer the column from the original system.

The difference in selectivity between similar types of columns (for example, C18 columns from different manufacturers) is likely to have a greater impact on the separation as changes to parameters controlled by ISET.

- 2 Download the method parameters for all modules from the original method.
Or enter the method parameters into the new method in the regular way.

NOTE

Ensure that all values are correctly transferred, and that appropriate values are provided for the data collection rate (detector **PeakWidth** setting).

- 3 Allow the system to equilibrate.

Setting Up the Basic ISET Parameters

In this section, you will transfer your original method to the system and activate ISET by selecting the original hardware configuration. All known differences in the behavior between the original LC instrument and the current target system will be addressed by ISET.

Prerequisites

- ISET is installed (see [Installing and Configuring ISET](#) on page 18).

NOTE: When ISET is installed but not enabled, the grey pictogram is displayed in the Pump Dashboard panel; the colored pictogram indicates that ISET is enabled.

NOTE

To create a new method, the latest ISET revision is always the best choice. For use of already established emulation methods, previous ISET versions are still available for backward compatibility.

Setting Up ISET Parameters

Setting Up the Basic ISET Parameters

- 1 Right-click in the Pump Dashboard panel and select **Method** from the context menu.

The method setup dialog box is displayed.

Advanced

Timetable (1/100 events)

☐ function centric view

Time [min]	A [%]	B [%]	C [%]	D [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	100.00	0.00	0.00	0.00	0.000	1200.00
1.00	10.00	90.00	0.00	0.00	---	---

Add Remove Clear All Clear Empty

Cut Copy Paste Shift Times 0.00 min

ISET

- 2 Expand the **Advanced** section of the pump method parameters and ensure that the following check boxes are marked:

Setting Up ISET Parameters
Setting Up the Basic ISET Parameters

Table 9: Advanced pump method parameters

1290 Infinity II/III High-Speed Pump (G7120A)
1290 Infinity Binary Pump (G4220A)
1290 Infinity Binary Pump VL (G4220B)
1290 Infinity II/III Bio High-Speed Pump (G7132A)

NOTE: ISET only works under the following preconditions:

- Minimum Stroke : Radio button Automatic and check box Synchronized are selected.
- Compressibility : Check box Use Solvent Types is selected.

Advanced

Minimum Stroke

Channel A: ☒ Automatic ☐ 20.00 µL

Channel B: ☒ Automatic ☐ 20.00 µL

☒ Synchronized

Compressibility

☒ Use Solvent Types

1290 Infinity II/III Flexible Pump (G7104A)
1260 Infinity II/III Flexible Pump (G7104C)
1290 Infinity Quaternary Pump (G4204A)
1290 Infinity II/III Bio Flexible Pump (G7131A)
1260 Infinity II/III Bio Flexible Pump (G7131C)

NOTE: ISET only works under the following preconditions:

- Minimum Stroke : Radio button Automatic is selected.
- Compressibility : Check box Use Solvent Types is selected.
- Primary Channel : Drop-down list Automatic is selected

Advanced

Minimum Stroke

☒ Automatic ☐ 20.00 µL

Compressibility

☒ Use Solvent Types

Maximum Flow Gradient

Flow ramp up: 100.000 mL/min² Flow ramp down: 100.000 mL/min²

Primary Channel

Automatic

Mixer Selection

Use Mixer if installed

NOTE

For ISET to work correctly, it is vital to ensure best accuracy of both flow and composition of the mobile phase. The critical performance factors are the stroke settings and the solvent correction. You can expect correct ISET results only when using the built-in solvent libraries.

- 3 Verify that the following method parameters for the pump show the correct values as given in your original method:

Setting Up ISET Parameters

Setting Up the Basic ISET Parameters


- solvent flow rate
- G4220A/B, G7120A, G7132A: Solvents A and B and solvent composition
G4204A, G7104A/C, G7131A/C: Solvents A, B, C, and D and solvent composition

NOTE: G4204A, G7104A/C, G7131A/C:

- Binary gradients with a constant addition of an additive in water are fully supported in ISET.
- Quaternary and ternary gradients or the simulation of binary gradients, which are composed with more than one organic solvent, are only supported by the generic model. ISET will still compensate delay volume differences.
- pressure limits
- stop- and post-times
- gradient timetable

NOTE: Dependent on selected manufacturer a gradient curve parameter can be defined in the time table. The default is a linear gradient.

4 Expand the ISET section of the method parameters.



The screenshot shows a software window titled "ISET" with a sub-section "Emulation". Inside the "Emulation" section, there is a checkbox labeled "Enable ISET" which is currently unchecked. Below this checkbox are two dropdown menus: "Model" and "Manufacturer". The "Model" dropdown is set to "ISET 4 V1.0" and the "Manufacturer" dropdown is set to "Agilent".

5 Mark the **Enable ISET** check box.

NOTE

Currently there are the following versions of ISET available:

ISET 4

Default - version of choice to establish a new method.

ISET 4 offers downward compatibility - version of choice to use methods, already available and established with **ISET 3**.

ISET 4 offers more flexibility (as more systems may be emulated) and bases on an optimized algorithm.

ISET 3

Offers downward compatibility - version of choice to use methods, already available and established with **ISET 2**.

ISET 3 offers more flexibility (as more systems may be emulated) and bases on an optimized algorithm.

ISET 2

Offers downward compatibility - version of choice to use methods, already available and established with **ISET 2**.

ISET 1

Offers downward compatibility - version of choice to use methods, already available and established with **ISET 1**.

The **Model Parameter** section of the method setup is displayed.

The screenshot shows the 'ISET' configuration window. It has two main sections: 'Emulation' and 'Model Parameter'. In the 'Emulation' section, the 'Enable ISET' checkbox is checked. Below it, 'Model' is set to 'ISET 4 V1.0' and 'Manufacturer' is 'Agilent'. The 'Model Parameter' section contains 'Emulated Pump' set to 'G1311A V1.0', an unchecked 'manually select ISET solvent model' checkbox with a dropdown set to 'Aqueous (Channel A) - Organic (Channel B)', an unchecked 'manually set' checkbox, a 'Compressibility' field set to '100' with a unit of '10e-6/bar', 'Emulated Sampler' set to 'G1367A - 100 µL Syringe V1.0', an unchecked 'manually set' checkbox, a 'Seal' field set to '2.30' with a unit of 'µL', and an unchecked 'Enable manual fine tuning' checkbox at the bottom.

Figure 9: Model Parameter section

The **Model Parameter** section enables you to configure the ISET emulation by selecting the original hardware module that you used during the development of your original method. Currently, the ISET library holds parameters only for

Setting Up ISET Parameters

Setting Up the Basic ISET Parameters

the pump and the sampler, since these are the major components that influence the mobile phase composition and its transient parameters as seen on the head of the column.

NOTE

With ISET 4, ISET 3, and ISET 2 under **Model** in the **Emulation** section there are different **Manufacturer** options.

If using the **Generic LC Instrument** option, the parameter in the **Dwell Volume** must be provided by the user (see [Generic Emulation Using Dwell Volume](#) on page 38).

- 6 Click the **Emulated Pump** down arrow and select the pump used for the original method from the drop-down list, see figure in [Mark the Enable ISET check box., step 5](#) on page 33.

NOTE

The drop-down list shows the module number of the pump and the revision of its characterization.

To determine a pump's name by its specific module number, please refer to [ISET Support for Emulation](#) on page 12.

- 7 If the solvent system defined in the method includes only water, methanol or acetonitrile, no changes to the ISET solvent model are required.

In ISET, water, methanol and acetonitrile are fully characterized for gradient performance.
- 8 If solvents other than water, methanol or acetonitrile are defined in the method, mark the **manually select ISET solvent model** check box, click the down-arrow and select an appropriate solvent system from the drop-down list:
 - select **Generic** when buffers or solvent mixtures are used.
 - select **Aqueous (Channel A) – Organic (Channel B)** or **Organic (Channel A) – Aqueous (Channel B)** for pure solvents as appropriate.

NOTE

You can also select Acetonitrile/Water or Methanol/Water if the solvents used are similar to these.

- 9 Mark the **manually set** check box for compressibility and enter the compressibility settings that were used in the original method.

NOTE

This is especially important if the compressibility values in the original method are different from the default values.

The parameters you set up to this step are those that ISET uses to correct for the behavior of the original pump module.

- 10 Click the **Emulated Sampler** down-arrow and select the sampler and configuration from the drop-down list.

ISET calculates delay volumes using the factory-installed values of capillaries, syringes and needle seats. If other capillaries are installed, fine-tune the delay volume (see [Fine-Tuning the Emulation](#) on page 36).

- 11 If a needle seat for higher injection volumes is installed, enter the correct needle seat volume in the **Seat** field.
- 12 When all method settings are finished, click **OK** to close the method setup-screen.

NOTE

During the run of an ISET method it is not possible to change the method parameters.

Fine-Tuning the Emulation

When the original system and the ISET system both use standard configurations, no offset setting should be required. As a rule of thumb, only the excess dwell volume of the system with respect to the standard configuration contributes to the offset. The total dwell volume offset setting is then composed of the original system's contribution minus the ISET system's contribution. If the emulated method was already run under ISET, the results of those previous runs may then be used to accomplish a match of the emulated method with respect to the original if there are remaining retention time differences.

In critical applications, where the gradient is too early or reaches the column head later than expected, the separation and selectivity are changed, and influence the resolution and retention times. For fine-tuning, the dwell volume may be adjusted to improve the emulation.

- 1 Mark the **Enable manual fine tuning** check box to display the fine-tuning parameters.

- 2 If necessary, enter a **Typical Operating Pressure**.

During the formation of gradients using Water/Methanol or Water/Acetonitrile, the system pressure changes. This change in pressure affects the volume of the damper of the pump, which results in a change in volume. The **Typical Operating Pressure** can be used to compensate for this change in volume.

- 3 If necessary, enter a **Dwell Volume Offset**.

The **Dwell Volume Offset** can be used to compensate for a difference in dwell volume between the original instrument and the ISET system caused, for example, by wider ID capillaries or an additional mixer (that is, non-standard system configurations).

Positive **Dwell Volume Offsets** shift the gradient to a later position; negative **Dwell Volume Offsets** shift the gradient to an earlier position

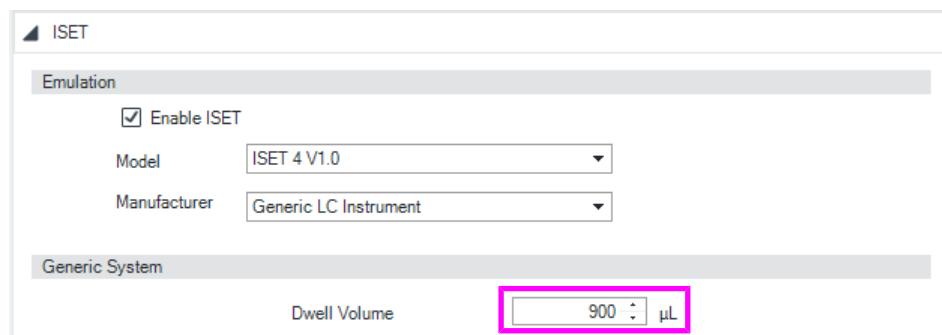
- 4 When all method settings are finished, click **OK** to close the method setup-screen.

NOTE

During the run of an ISET method it is not possible to change the method parameters.

Generic Emulation Using Dwell Volume

The generic emulation using dwell volume can be used for LC instruments not listed as ISET supported modules/systems. In these cases the dwell volume has to be entered into the field **Dwell Volume**.



The screenshot shows the ISET software interface. Under the 'Emulation' tab, the 'Enable ISET' checkbox is checked. The 'Model' dropdown is set to 'ISET 4 V1.0' and the 'Manufacturer' dropdown is set to 'Generic LC Instrument'. Below this, under the 'Generic System' section, the 'Dwell Volume' is set to '900 µL'. The '900 µL' input field is highlighted with a pink rectangular box.

Figure 10: Generic emulation using dwell volume

Sometimes the dwell volume is specified by the vendor of the system to be emulated. If not, it can be determined (see [Determination of Dwell Volume](#) on page 39).

NOTE

The generic emulation using dwell volume does not compensate for following aspects:
Different mixing behaviors
Composition errors

Definition of Dwell Volume

The system dwell volume includes the volume of the system flow path from the point of mixing of the mobile phase components to the head of the column. It has an important effect on the gradient that the pump delivers because every gradient has an initial isocratic segment as the mobile phase must travel through

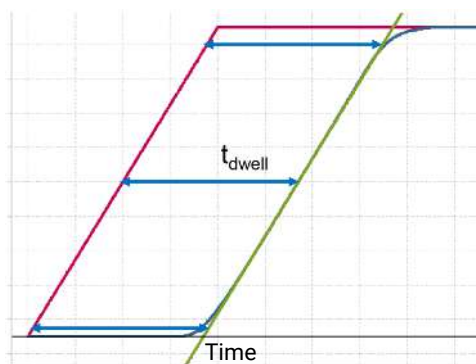
Setting Up ISET Parameters

Generic Emulation Using Dwell Volume

the dwell volume before any change made at the pump arrives at the head of the column. Any differences in dwell volume cause variations in retention times and often also in selectivity.

Determination of Dwell Volume

- 1 Replace column by a zero dead volume connection or low volume capillary.
- 2 Run a linear gradient from 0 – 10 min from 5 – 95 % B at a flow rate (F) of 1 mL/min with water (channel A) and water with 0.2 % acetone (channel B) at 263 nm detection wavelength.
- 3 Determine the difference in time (t_{dwell}) between the programmed and actual elution time of the gradient at 50 % of the composition.



- 4 V_{dwell} can be calculated from:

$$V_{\text{dwell}} = t_{\text{dwell}} \times F$$



5 Understanding ISET Functionality

This chapter explains in detail how ISET works.

Understanding ISET Functionality 41

Composition Differences 42

ISET Functionality 44

Understanding ISET Functionality

The transfer of a method from one liquid chromatography system to another usually involves a direct transfer of all instrument parameters, for example, flow rate, mobile phase composition and gradient timetable, injection volume, column temperature and UV detection wavelength.

However, there can also be subtle differences such as system delay (or dwell) volume (the volume of the system flow path from the point of mixing of the mobile phase components to the top of the column), the design of the autosampler and column compartment flow paths and temperature, detector cell design, and the extra-column volume of the system, detector data collection rates and response times and the sensitivity of the detector.

A closer investigation into the comparative performance of different designs of LC gradient pumps reveals that other hidden factors have an effect on how the pump delivers the gradient. Principally, this is characterized by the mixing behavior of the pump, which in turn is determined by the basic concept and design of the flow path, the volume and design of the mixer, the efficiency of the solvent mixing and the behaviour of the piston movement under different loads and compressibilities of solvents. This behaviour can be visualized by using tracer experiments (0.2 % acetone in channel B for example) to plot the profile of the pump as it delivers a stepwise gradient in the proportion of the solvents.

In practice, there is always a delay, and the step gradient is smoothed and transformed into a curve that is characteristic of the pump. Every model of pump has its own characteristic profile, and this leads to slight differences in nominally similar gradients on different pumps. Often, these are sufficient to cause problems in the transfer of some methods, and the challenge is to measure the characteristics of different pumps and reproduce them on the Agilent 1290 LC system pump.

This is the heart of the ISET system; however, the accurate performance of the 1290 is still needed to execute the settings.

ISET reveals and translates those parameters to provide an accurate method transfer from older Agilent systems to the system that is used for ISET, and vice versa.

Composition Differences

The primary and most visible aspect of compressibility settings is reduction of pressure ripple, but for predictable and accurate pumping, the flow accuracy is more critical. Additionally, with the high-pressure blending concept of binary pumps, this flow accuracy translates into compositional accuracy.

The compressibility of the solvents in use affect retention-time stability and predictability, especially when the backpressure in the system changes (for example, ageing of column). To minimize this effect, the pump provides a compressibility compensation feature that optimizes the stability of the flow accuracy according to the solvent type. The compressibility compensation is set to a default value and can be changed through the user interface.

Without compressibility compensation, the following happens during a stroke of the first plunger:

- The pressure in the plunger chamber increases, and the volume in the chamber is compressed depending on backpressure and solvent type.
- When dispensing a more compressible solvent against pressure, the displacement rate of the piston is reduced to compensate for the expansion of the solvent while it travels down the column.

When a compressibility value is set, the processor calculates a compensation volume that depends on the backpressure in the system and the selected compressibility. This compensation volume is added to the normal stroke volume and compensates for the loss of volume during the delivery stroke of the first plunger.

Composition differences are generated by the HPLC by imperfect compensation of the solvent compressibility. For example, due to mismatch of the compressibility settings and the actual solvent compressibility.

Accurate blending of mobile phase composition is vital for predictable retention. While historically the equipment was well-known for its reproducibility, in terms of accuracy of the mobile phase composition, offsets may have been allowed. However, in order to emulate the historic behavior, these systematic offsets, characteristic of the individual instrument classes, need to be taken into consideration.

As an example, consider volume contraction. When mixing water in an organic solvent, the basic pump concept, if it is low-pressure proportioning or high-pressure dispensing, may introduce a significant offset. This may even be different for various solvents, and may even change with running conditions such as %B or pressure and the compressibility settings that the original pump compensated for.

The composition differences generated by a 1100 quaternary pump for different settings of solvent compressibility are shown in [Figure 11](#) on page 43.

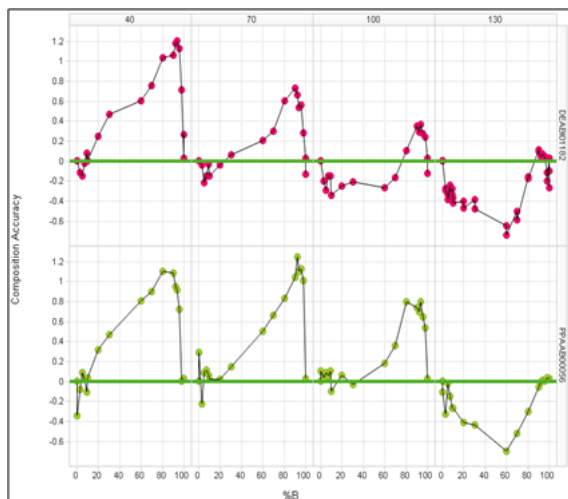


Figure 11: Composition offset of an Agilent 1100 quaternary pump for acetonitrile-water at different compressibility settings.

When a gradient timetable is executed on an (U)HPLC instrument, the shape of the resulting gradient formed at the top of the column depends mainly on

- the system volume and geometry between the point of mixing (usually in the pump) and the column head.
- the accuracy with which the programmed composition is delivered to the column.

When an HPLC system is characterized with regard to gradient shape, it is important to separate the effects of the geometric volumes from those of static composition errors produced by the pump. [Figure 11](#) on page 43 shows the composition offset generated by a 1100 quaternary pump for different settings of solvent compressibility.

ISET Functionality

ISET Functionality

The physical relationship between a programmed timetable and the system response can be described by a transfer function (Figure 12 on page 44).

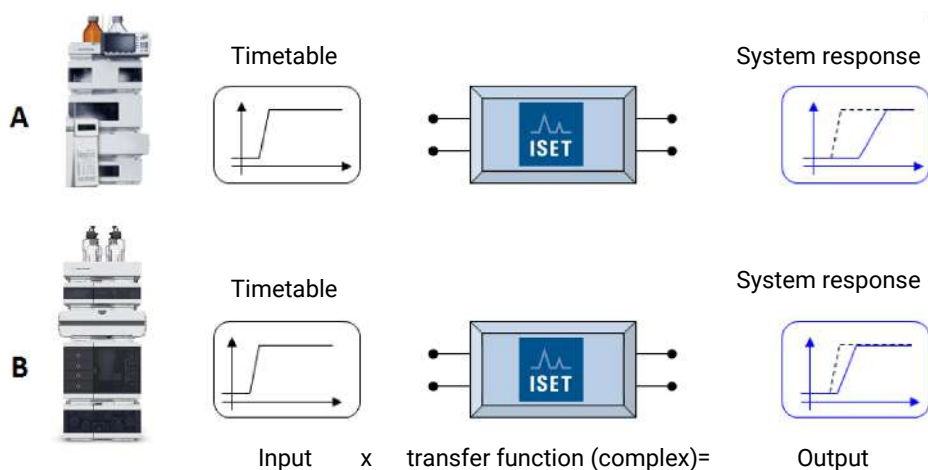


Figure 12: Different gradient shapes resulting from the same time table for different instrument characteristics.

For system B with a considerably lower delay volume than system A, it is possible to compute a (virtual) timetable from the transfer functions of the two instruments that corresponds to the programmed timetable of system A, and that generates a gradient response on system B that is equivalent to that of system A (Figure 13 on page 45).

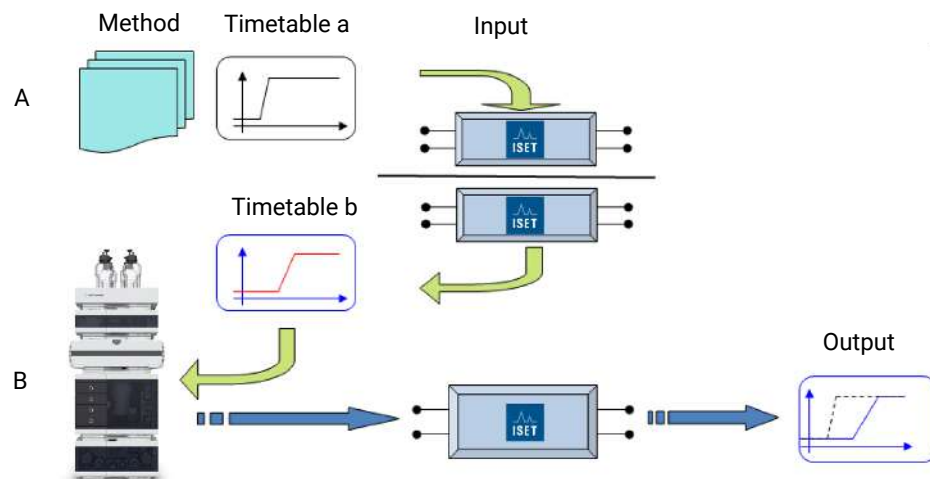


Figure 13: Operation of the system in a mode that emulates the gradient response of a larger delay volume system.

The transfer functions generated from thorough system characterizations are used by ISET to emulate another HPLC system. When the emulation mode is enabled, the system, emulating the original pump and auto-sampler can run the original gradient method and achieve a very similar separation to that produced on the emulated system (see [Figure 8](#) on page 11).






6 Application and Technical Notes

This chapter gives an overview on additional literature.

Application and Technical Notes 47

Application and Technical Notes

p/n	Description
 5991-7794EN	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
 5991-6890EN	Agilent 1290 Infinity II LC with ISET - Method Development for Transfer to an Agilent 1260 Infinity LC with Autosampler and Integrated Column Compartment
 5991-6715EN	Agilent 1290 Infinity II LC with ISET – Emulation of the Waters Acquity H-Class for Ternary Gradients
 5991-6541EN	Agilent 1290 Infinity II LC with ISET – Emulation of the Agilent 1100 Series LC Through Waters Empower Software
 5991-6408EN	Agilent 1290 Infinity II LC with ISET – Emulation of Waters Alliance 2695 LC Through Waters Empower Software
 5991-6289EN	Agilent 1290 Infinity II LC with ISET – Emulation of the Agilent 1100 Series Binary LC for Analysis of Tricyclic Antidepressant Drugs
 5991-5701EN	QbD Based Method Development on an Agilent 1290 Infinity UHPLC System Combined with a Seamless Method Transfer to HPLC Using Intelligent System Emulation Technology
 5991-5030EN	Intelligent System Emulation Technology Transfers Methods from Binary to Quaternary LC
 5991-4729EN	Agilent 1290 Infinity Binary LC System with ISET – Emulation of a Waters Alliance 2695 LC Applying Concave, Convex, and Linear Gradients
 5991-2792EN	Agilent 1290 Infinity Binary LC with ISET – Emulation of the Waters Alliance 2695 LC System Analyzing Analgesics
 5991-2275EN	Agilent 1290 Infinity LC with ISET under Waters Empower control – Emulation of Agilent 1100 Series LC
 5991-2019EN	Agilent 1290 Infinity Binary LC with ISET, emulation of the Waters Alliance 2695 LC system analyzing aromatic acids
 5991-1605EN	Agilent 1290 Infinity Binary LC with ISET - Emulation of the Waters Alliance 2695 LC system analyzing endocrine disruptors
 5991-1604EN	Agilent 1290 Infinity Binary LC with ISET - Emulation of the Waters Alliance 2695 LC system analyzing antioxidants

p/n	Description
 5991-1603EN	Agilent 1290 Infinity Binary LC System with ISET - Emulation of the Waters Alliance 2695 LC system analyzing b-blockers
 5991-1433EN	Seamless instrument-to-instrument method transfer of an USP/EP method from an Agilent 1220 Infinity LC to an Agilent 1290 Infinity Binary LC using Intelligent System Emulation Technology (ISET)
 5991-1194EN	Seamless instrument-to-instrument method transfer of the EPA method 8330A/B for nitroaromatics from an Agilent 1200 Series LC to the Agilent 1290 Infinity Binary LC using ISET
 5990-9715EN	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
 5990-9703EN	Transferring methods to the Agilent 1290 Infinity LC System using Intelligent System Emulation Technology (ISET) Analysis of paracetamol and its impurities
 5990-9692EN	Transferring methods to the Agilent 1290 Infinity LC using Intelligent System Emulation Technology (ISET) Analysis of metoclopramide hydrochloride and its impurities
 5990-9546EN	Comparing gradient transfer of isocratic hold and delay volume addition using the Agilent 1290 Infinity LC with ISET
 5990-9545EN	Seamless transfer of elution gradients from Agilent 1100/1200 Series LCs to an Agilent 1290 Infinity LC using ISET
 5990-9113EN	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)
 5991-9394EN	Agilent 1260 Infinity II Prime LC with ISET- Emulation of a Waters Alliance LC for the Analysis of Metoclopramide and its Impurities
 5991-8786EN	Agilent 1260 Infinity II Prime LC with ISET- Emulation of the Agilent 1200 Series Quaternary LC for the Analysis of Antihistaminic Drugs

Documents are available on the worldwide web site on the internet at (<http://www.agilent.com/en-us/promotions/applicationfinder?Focus=Intelligent%20System%20Emulation%20Technology>)

In This Book

The manual describes the Agilent Intelligent System Emulation Technology (ISET). It contains the following:

- Instructions for installing and configuring ISET,
- Details on setting up the ISET parameters,
- Detailed information about how ISET works.

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