# Agilent InfinityLab LC with ISET

User Manual




Agilent Technologies

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# In this book

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

### 1 What is ISET?

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

### 2 Installing and Configuring ISET

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

### 3 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.

### 4 Understanding ISET Functionality

This chapter explains in detail how ISET works.

### 5 Application and Technical Notes

This chapter gives an overview on additional literature.

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# 1 What is ISET?

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

# 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 7
- 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

## 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.

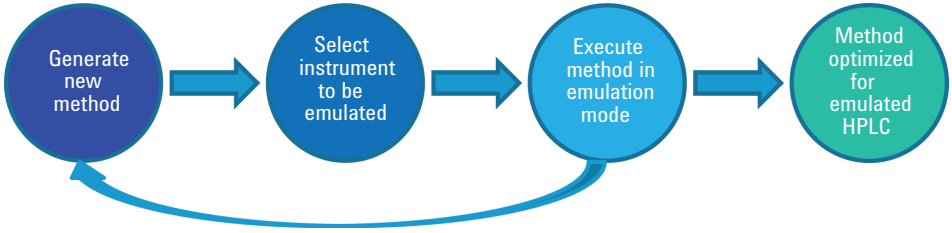



**Figure 2** Method development

## Method robustness testing

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

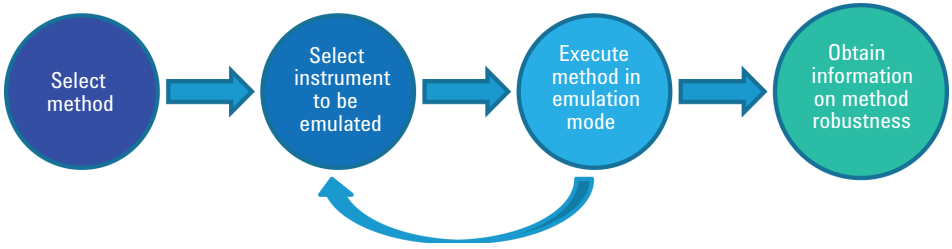



**Figure 3** Method robustness testing

## 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.

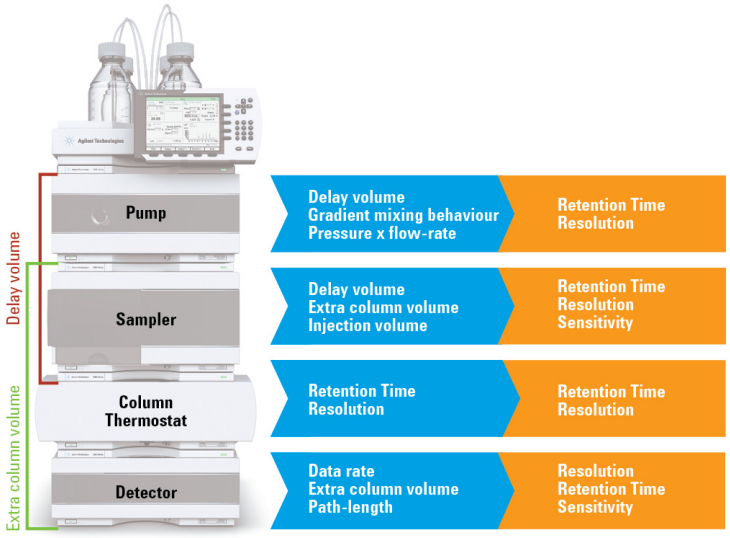



**Figure 4** Parameters affecting method transfer

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.

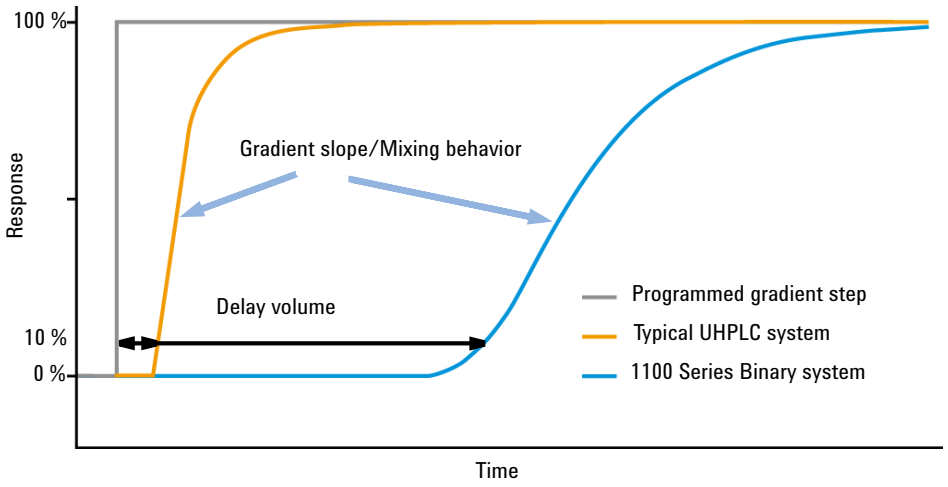



**Figure 5** Comparison of delay volumes and gradient mixing behaviors between a typical UHPLC system and an 1100 Series Binary LC using a tracer experiment. On a typical UHPLC system the mixed solvents reach the column much earlier, and the set composition is also achieved earlier due to the steeper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

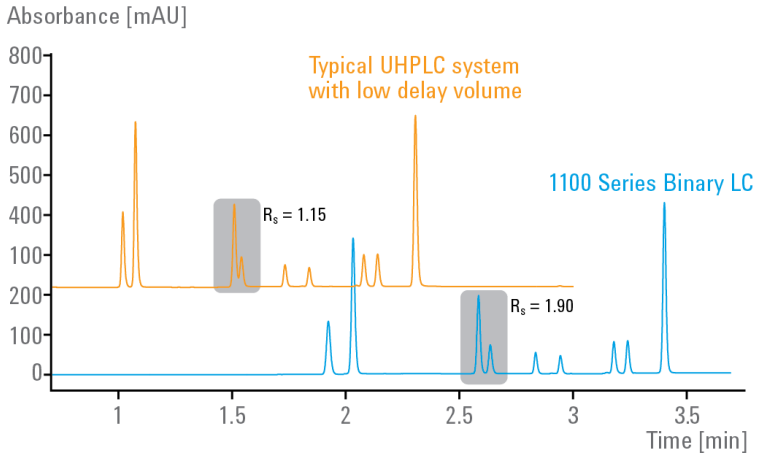



**Figure 6** Different solvent compositions at the column due to different delay volumes and gradient mixing behaviors result in different retention times and resolutions.

| | |
|---|---|
| Column: | Poroshell 120, 3.0 x 50 mm (2.7 µm). Flow rate: 0.85% mL/min |
| Mobile Phase: | Water, Acetonitrile |
| Gradient: | 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.

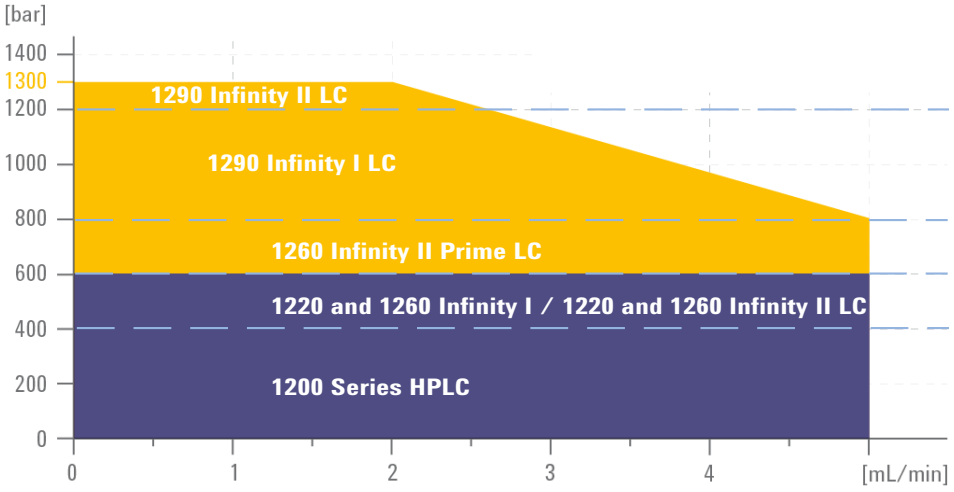



**Figure 7** Agilent InfinityLab Series Power Range

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. The results are similar retention times and similar chromatographic resolution.

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

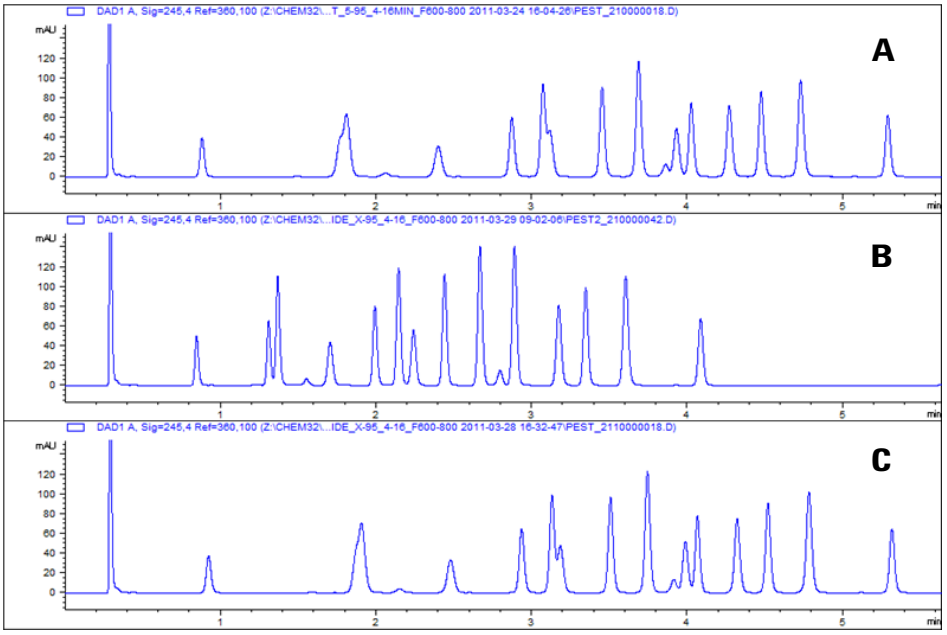



**Figure 8** Chromatograms of a gradient separation of a pesticide mixture run on (A) 1100 Series Quaternary Pump (G1311A) + Autosampler (G1367A), (B) 1290 Infinity II LC without emulation, (C) 1290 Infinity II LC in emulation mode

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<br>1260 Infinity Quaternary Pump |
| | G1311C | 1260 Infinity Quaternary Pump VL |
| | G1312A | 1100 Series Binary Pump |
| | G1312B | 1200 Series Binary Pump SL<br>1260 Infinity Binary Pump |
| | G1312C | 1260 Infinity Binary Pump VL |
| | G4204A | 1290 Infinity Quaternary Pump |
| | G7104A | 1290 Infinity II Flexible Pump |
| | G7104C | 1260 Infinity II Flexible Pump |
| | G4220A[1] | 1290 Infinity Binary Pump |
| | G4220B[1] | 1290 Infinity Binary Pump VL |
| | G7120A[1] | 1290 Infinity II High Speed Pump |
| | G5611A | 1260 Infinity Bio-inert Quaternary Pump |
| Autosamplers | G1313A | 1100 Series Standard Autosampler |
| | G1329A | 1100 Series Thermostatted Autosampler<br>1200 Series Standard Autosampler |
| | G1329B | 1200 Series Standard Autosampler SL<br>1260 Infinity Standard Autosampler |
| | G1367A (100 µL syringe) | 1100 Series Well Plate Sampler |
| | G1367B (100 µL syringe) | 1100 Well Plate Sampler<br>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<br>1200 Series Micro Well Plate Autosampler<br>1260 Infinity High Performance Micro Autosampler |
| | G4226A (20, 40, 100 µL syringe) | 1290 Infinity High Performance Autosampler |
| | G7167A/B (40 µL / 100 µL / 900 µL syringe) | 1290 Infinity II Multisampler |

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

| | | |
|---|---|---|
| | G7129A (40 µL / 100 µL / 900 µL syringe) | 1260 Infinity II Vialsampler |
| | G7129B (40 µL / 100 µL / 900 µL syringe) | 1290 Infinity II Vialsampler |
| | G7129C (40 µL / 100 µL / 900 µL syringe) | 1260 Infinity II Vialsampler |
| | G5667A | 1260 Infinity High Performance Bio-inert Autosampler |
| 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 or G7120A only.

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

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

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

| System | Specifications | Comment |
|---|---|---|
| **Acquity UPLC**[1] | **Total system delay volume:**<br><120 µL<br>50 µL, 100 µL mixer<br>**Injection range:**<br>0.1 – 5 µL, up to 50 µL with optional sample loop.<br>Standard configuration with 5 µL sample loop and 100 µL syringe.<br>**Sample loops available:**<br>1, 2, 5, 10, 20, 50 µL | **Syringes available:**<br>100, 250 µL<br>(syringe volume does not contribute to dwell volume). |
| **Acquity UPLC H-Class** | **Total system delay volume:**<br><400 µL<br>100 µL mixer<br>**Injection range:**<br>0.1 – 10 µL, up to 1000 µL with optional sample loop.<br>Standard configuration with 10 µL sample loop and 100 µL syringe.<br>**Sample loops available:**<br>10, 50, 100, 250, 1000 µL | **Syringes available:**<br>100, 250, 500 µL<br>(syringe volume does not contribute to dwell volume). |

[1] Emulation of this instrument is possible with G4220A/B or G7120A only.

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

| | | |
|---|---|---|
| **Pumps** | **LC-20 AB**[1]<br>w/o mixer, 100 µL, 0.5 mL, 1.7 mL, 2.6 mL mixer | Binary High Pressure Mixing Pump |
| | **LC-20 AT**<br>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**<br>**Injection Range:**<br>0.1 – 100 µL, up to 2000 µL with optional sample loop.<br>Standard configuration with 100 µL sample loop.<br>**Sample loops available:**<br>50, 100, 500, 2000 µL | Sampler |

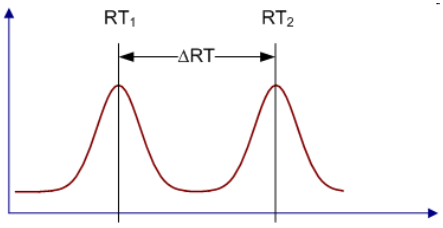
[1] Emulation of this instrument is possible with G4220A/B or G7120A only.

**NOTE** ISET supports the emulation of any LC instrument. For details see "Generic emulation using dwell volume" on page 39.

# Specifications

**Table 4** Retention Time (RT) Deviation

| | | |
|---|---|---|
| RT | ±5%<br>±0.3 min | (for RT > 6 min)<br>(for RT ≤ 6 min) |
| ΔRT | ±5%<br>±0.1 min | (for ΔRT > 2 min)<br>(for ΔRT ≤ 2 min) |




**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 emulation and generic solvents.

## Demo/Trial

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

- Agilent 1290 Infinity II Flexible Pump (G7104A with firmware revision B.06.56 and later)
- Agilent 1260 Infinity II Flexible Pump (G7104C with firmware revision B.07.20 and later)
- Agilent 1290 Infinity Quaternary Pump (G4204A with firmware revision B.06.56 and later)
- Agilent 1290 Infinity II High Speed Pump (G7120A) with firmware revision B.06.42 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)

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

**Table 5** Pictograms of ISET versions

| | Demonstration/trial | Registered |
|---|---|---|
| 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.

# 2
# Installing and Configuring ISET

Preconditions for ISET 20
Installing and configuring ISET 25
ISET Removal and Reinstallation 27

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

# Preconditions for ISET

You need one of the Chromatographic Data Systems specified in Table 8 on page 23 (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 ( ≥ A.02.04 – see Table 8 on page 23) is required.

**Table 6** Modules needed for ISET

| Module | P/N | Firmware Revision | Comments |
|---|---|---|---|
| 1290 Infinity Binary Pump<br>1290 Infinity Binary Pump VL<br>1290 Infinity Quaternary Pump<br>1290 Infinity II Flexible Pump<br>1260 Infinity II Flexible Pump<br>1290 Infinity II High Speed Pump | G4220A<br>G4220B<br>G4204A<br>G7104A<br>G7104C<br>G7120A | ≥B.06.42<br>≥B.06.42<br>≥B.06.56<br>≥B.06.70<br>≥B.07.20<br>≥B.06.70 | You need one of the pumps listed with either no mixer or one of the specified mixers (see Table 7 on page 22).<br><br>**NOTE**<br><br>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.<br><br>**NOTE**<br><br>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.<br><br>Currently ISET supports following resulting gradients: Water vs. Acetonitrile, Water vs. Methanol or Aqueous vs. Organic.<br><br>ISET in Pump Valve Clusters is supported since ISET 2. |
| 1290 Infinity Autosampler | G4226A | ≥B.06.42 | With 20 µL, 40 µL, 100 µL loop |
| 1290 Infinity II Multisampler[1] | G7167B | ≥D.06.70 | |
| 1260 Infinity II Multisampler[1] | G7167A | ≥D.06.75 | |
| 1290 Infinity II Vialsampler | G7129B | ≥D.06.75 | |
| 1260 Infinity II Vialsampler | G7129A | ≥D.06.75 | |
| 1260 Infinity II Vialsampler | G7129C | ≥D.07.20 | |

[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 | • V35 JetWeaver<br>• V100 JetWeaver<br>• V380 JetWeaver |
| 1290 Infinity Binary Pump VL | G4220B | • V35 JetWeaver<br>• V100 JetWeaver<br>• V380 JetWeaver |
| 1290 Infinity II High Speed Pump | G7120A | • V35 JetWeaver<br>• V100 JetWeaver<br>• V380 JetWeaver |
| 1290 Infinity Quaternary Pump | G4204A | • V35 JetWeaver<br>• V100 JetWeaver<br>• V380 JetWeaver |
| 1290 Infinity II Flexible Pump | G7104A | • V35 JetWeaver<br>• V100 JetWeaver<br>• V380 JetWeaver |
| 1260 Infinity II Flexible Pump | G7104C | • V35 JetWeaver<br>• V100 JetWeaver<br>• V380 JetWeaver |

**Table 8** Software compatibility ISET

| ISET Revision | Driver Revision | Emulated LC Instruments | OpenLAB CDS | OpenLAB CDS ChemStation Edition | OpenLAB CDS EZChrom Edition | Mass Hunter | ICF[1] |
|---|---|---|---|---|---|---|---|
| ISET 1 | A.02.04 | Agilent 1100 and 1200 Series,<br>Agilent 1260 Infinity LC | | CS B.04.03 SP1<br>≥ CS C.01.03 | ≥ EE A.04.03 | Q/TOF B.05.00<br>QQQ B.05.00 | A.01.04 |
| ISET 2 | A.02.06 | Scope of ISET 1 plus<br>Waters Alliance,<br>Agilent 1220 Infinity LC,<br>Agilent 1120 Compact LC,<br>Generic emulation | | CS B04.03 SP2<br>CS C01.03 SP1<br>≥ CS C.01.04 | CS B04.03 SP2<br>EE A04.03 SP1<br>≥ EE A.04.04 | Q/TOF ≥ B.05.01 (September 2012)<br>QQQ ≥ B.06.00 (October 2012) | A.01.05 |
| ISET 3 | A.02.09 | Scope of ISET 2 plus<br>Agilent 1290 Infinity Quaternary Pump<br>Waters H-Class<br>Waters Acquity<br>Shimadzu LC 20 | | ≥ CS C.01.05 | ≥ EE A.04.05 | Q/TOF ≥ B.06.00<br>QQQ ≥ B.07.00 | A.02.01 |
| ISET 4 | A.02.11 | Scope of ISET 3 plus<br>Agilent 1290 Infinity Binary Pump<br>Agilent 1290 Infinity II Flexible Pump<br>Agilent 1290 Infinity II High Speed Pump | | ≥ CS C.01.07 | ≥ EE A.04.07 | Q/TOF ≥ B.06.01<br>QQQ ≥ B.07.00 SP2 | A.02.03 DU2 |

**Table 8** Software compatibility ISET

| ISET Revision | Driver Revision | Emulated LC Instruments | OpenLAB CDS | OpenLAB CDS ChemStation Edition | OpenLAB CDS EZChrom Edition | Mass Hunter | ICF[1] |
|---|---|---|---|---|---|---|---|
| ISET 4.1 | A.02.12 | Scope of ISET 4 plus<br>Agilent 1260 Infinity Autosampler<br>Agilent 1290 Infinity II Vialsampler | | ≥ CS C.01.07 | ≥ EE A.04.07 | Q/TOF<br>≥ B.07.01<br>QQQ<br>≥ B.08.00 | A.02.03 DU2 |
| ISET 4.2 | A.02.18 | Scope of ISET 4.1 plus<br>1260 Infinity II Flexible Pump<br>1260 Infinity II Vialsampler | ≥ 2.2 | ≥ C.01.07 SR4 | ≥ A.04.08 SR1 | QTOF<br>≥ B.09.00<br>QQQ<br>≥ B.09.00<br>Ultivo<br>≥ C.01.00 | A.02.05 |

[1] Agilent Instrument Control Framework

# Installing and configuring ISET

**Hardware required** Pump G4204A, G7104A/C, G4220A/B or G7120A

**The installation of ISET in the trial version is already complete if the prerequisits 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.

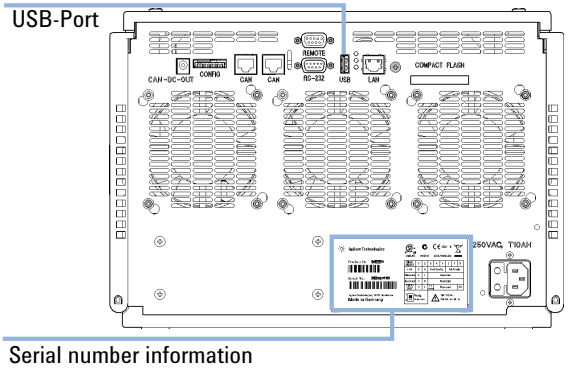
2 Plug the ISET USB dongle into the USB port on the back of the pump.

**NOTE**

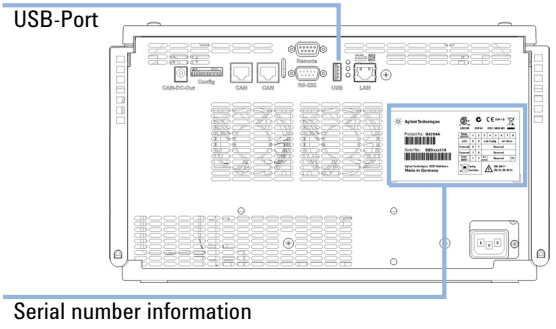
You must use the same ISET USB dongle for reinstallation as was originally used for installation.

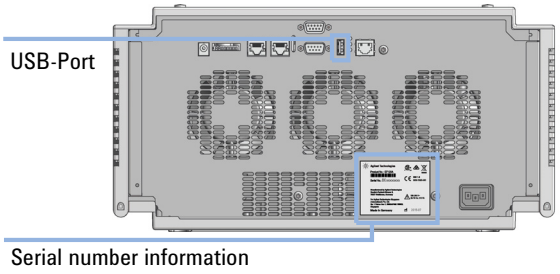
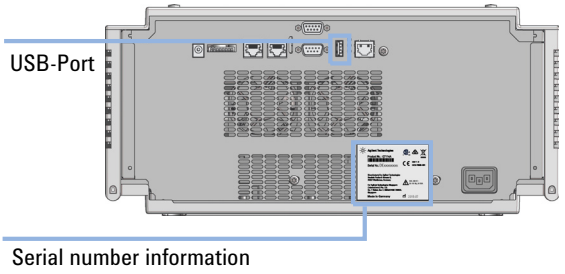
This, for example, is necessary after exchanging the mainboard of the pump. Thus it's advisable to keep the serial number information of the pump together with the ISET USB dongle.

**G4220A/B**




**G4204A**



| G7120A | G7104A/C |
| --- | --- |
|  |  |

3 Wait until blinking of USB dongle stops.

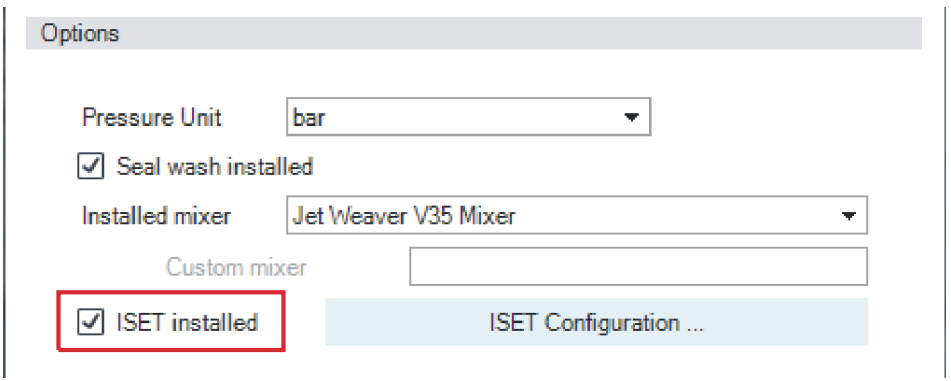
4 Switch off the pump.

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.




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.



# 3
# Setting Up ISET Parameters

Preparing the system for emulation 30
Setting up the basic ISET parameters 31
Fine-tuning the emulation 38
Generic emulation using dwell volume 39
Definition of dwell volume 40
Determination of dwell volume 40

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.

# Preparing the 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 differences in selectivity between similar types of column, for example, C18 from different manufacturers, is likely to be greater than differences caused to the separation by parameters that ISET controls.

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

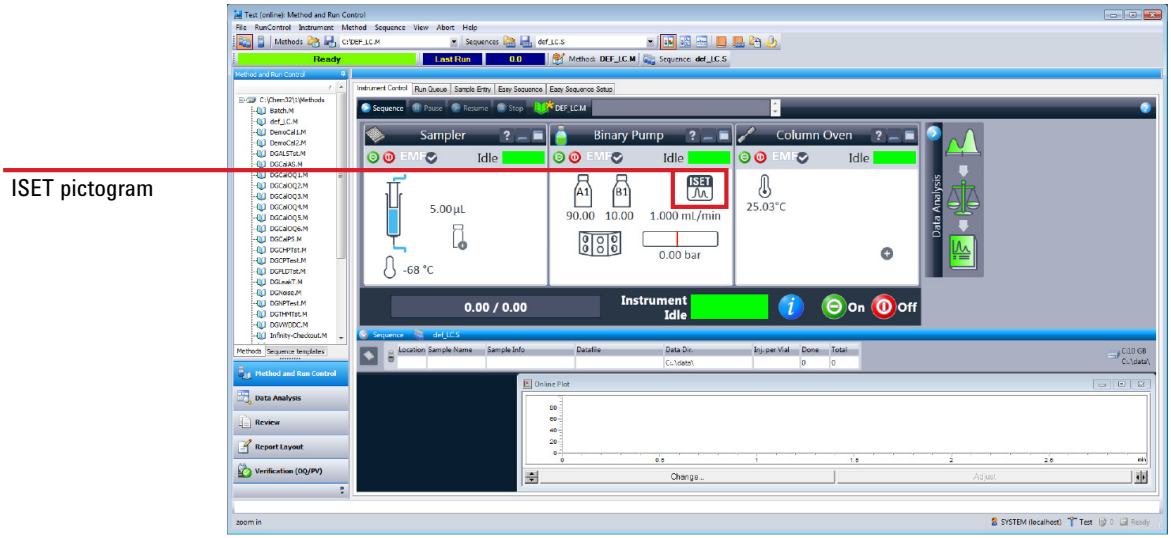
In this section, you will transfer your original method to the system and activate ISET by selecting the original hardware configuration. All known differences in the behavior between the original LC instrument and the current target system will be addressed by ISET.

**Prerequisites**

- ISET is installed (see "Installing and Configuring ISET" on page 19).

**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.

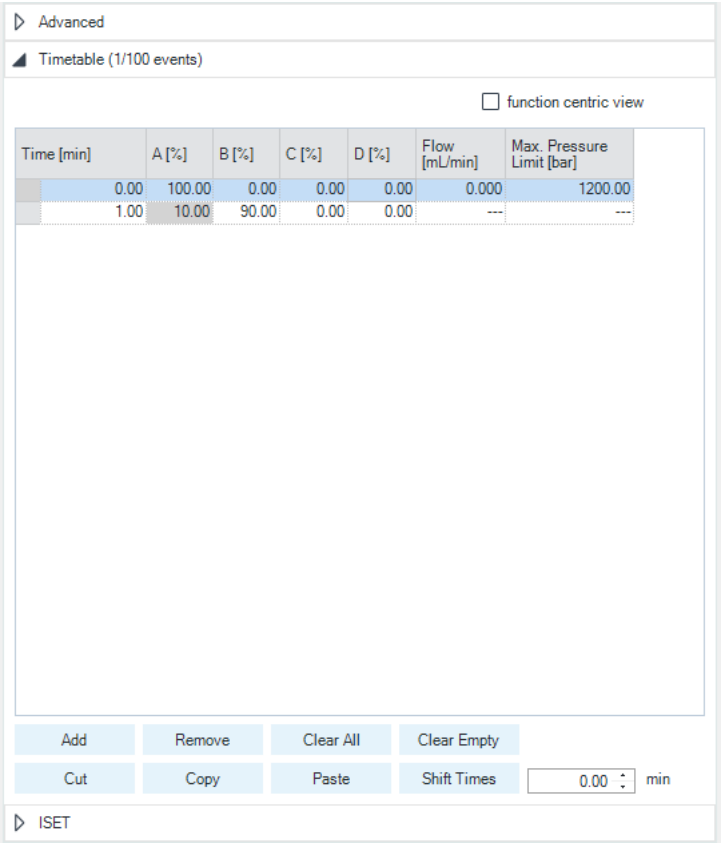



**NOTE**

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.

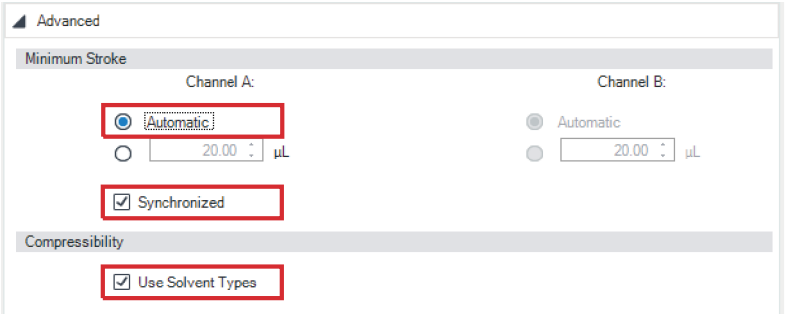
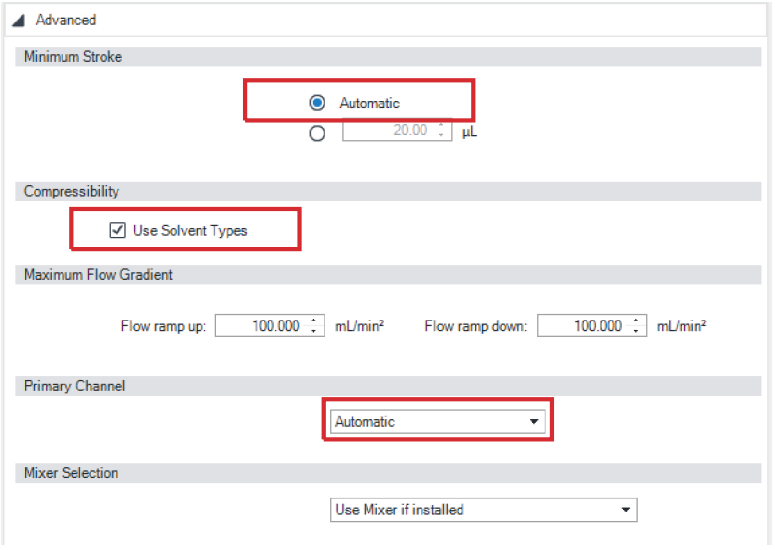
1 Right-click in the Pump Dashboard panel and select **Method** from the context menu.

The method setup dialog box is displayed.




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

**Table 9** Advanced pump method parameters

| | |
|---|---|
| **1290 Infinity II High Speed Pump (G7120A)**<br>**1290 Infinity Binary Pump (G4220A)**<br>**1290 Infinity Binary Pump VL (G4220B)**<br><br>**NOTE**<br><br>ISET only works under the following preconditions:<br><br>• **Minimum Stroke**: Radio button **Automatic** and check box **Synchronized** are selected.<br>• **Compressibility**: Check box **Use Solvent Types** is selected. |  |
| **1290 Infinity II Flexible Pump (G7104A)**<br>**1260 Infinity II Flexible Pump (G7104C)**<br>**1290 Infinity Quaternary Pump (G4204A)**<br><br>**NOTE**<br><br>ISET only works under the following preconditions:<br><br>• **Minimum Stroke**: Radio button **Automatic** is selected.<br>• **Compressibility**: Check box **Use Solvent Types** is selected.<br>• **Primary Channel**: Drop-down list **Automatic** is selected |  |

**NOTE**

For ISET to work correctly, it is vital to ensure best accuracy of both flow and composition of the mobile phase. Critical performance factors are the stroke settings and the solvent correction. Only when the built-in solvent libraries are used is the operation accurate enough to expect correct results.

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

- solvent flow rate
- G4220A/B, G7120A: Solvents A and B and solvent composition

  G4204A, G7104A/C: Solvents A, B, C, and D and solvent composition

NOTE

**G4204A, 7104A/C:**

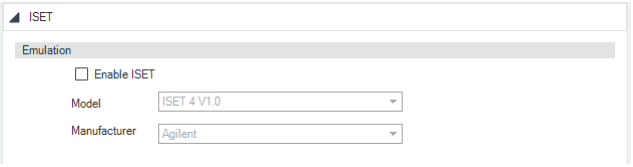
- Binary gradients with a constant addition of a additive in water are fully supported in ISET.
- Quaternary and ternary gradients or the simulation of binary gradients, which are composed with more than one organic solvent, are only supported by the generic model. ISET will still compensate delay volume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



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.

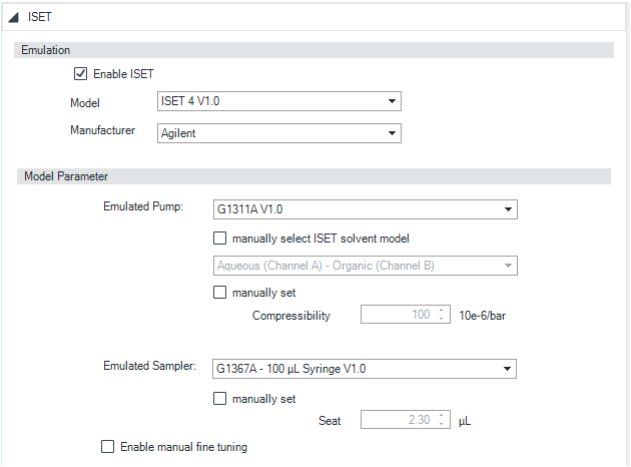



**Figure 9** Model Parameter section

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 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 39).

6 Click the **Emulated Pump** down arrow and select the pump used for the original method from the drop-down list, see Figure 9 on page 36.

**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 14.

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 38).

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 perfect match of the emulated method with respect to the original if there are still 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 Offset**s shift the gradient to a later position; negative **Dwell Volume Offset**s 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**.

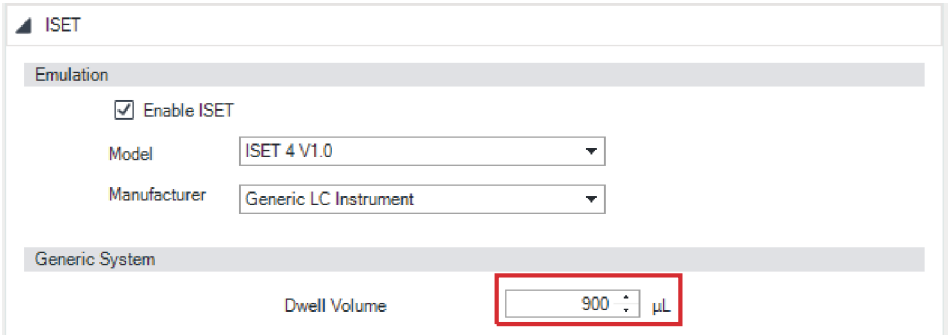



**Figure 10** Generic emulation using dwell volume

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 40).

**NOTE**

The generic emulation using dwell volume does not compensate for following aspects:

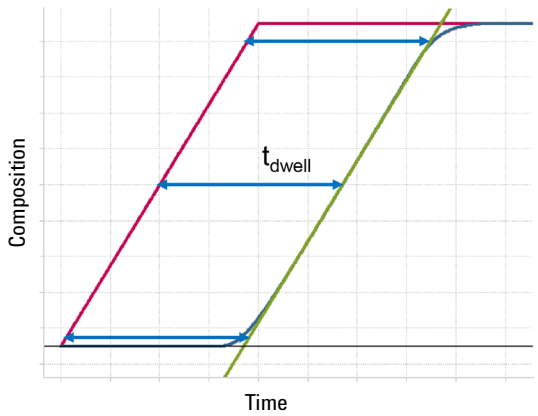
- Different mixing behaviors
- Composition errors

## 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 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:

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

# 4
# Understanding ISET Functionality

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ISET functionality 45

This chapter explains in detail how ISET works.

# 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 Infinity LC system pump.

This is the heart of the ISET system; however, the accurate performance of the 1290 Infinity 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 back-pressure 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 back-pressure 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 is dependent on the back-pressure 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 runnning conditions such as %B or pressure and, of course, 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 44.

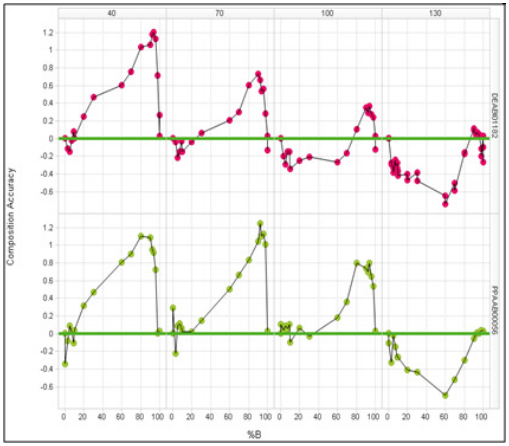



**Figure 11** Composition offset of an Agilent 1100 quaternary pump for acetonitrile-water at different compressibility settings.

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 44 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 45).

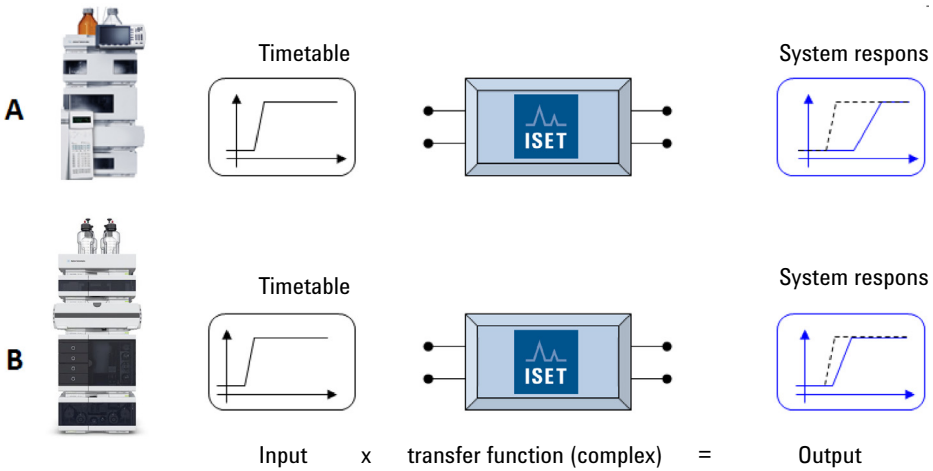



**Figure 12** Different gradient shapes resulting from the same time table for different instrument characteristics.

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 46).

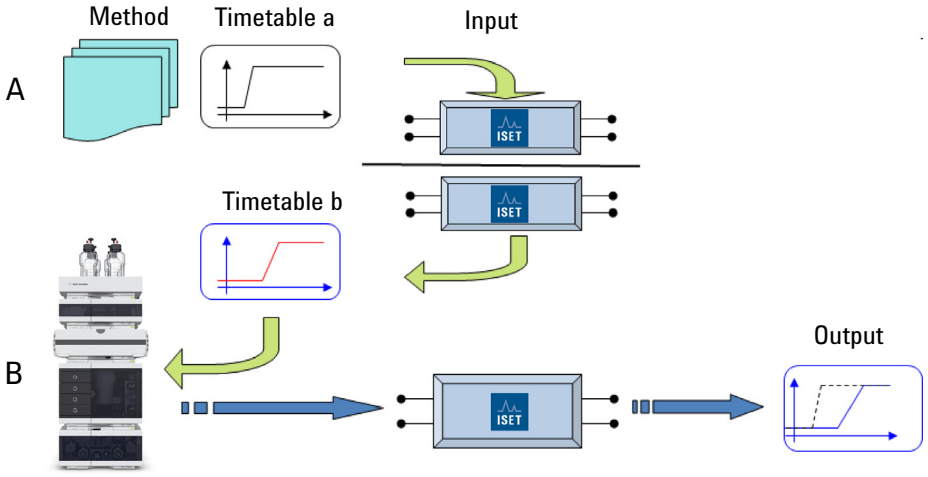



**Figure 13** Operation of the system in a mode that emulates the gradient response of a larger delay volume system.

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 12).

# 5 Application and Technical Notes

Application and technical notes 48

This chapter gives an overview on additional literature.

# 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 |

| p/n | Description |
|---|---|
| 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 |
| 5991-1603EN | Agilent 1290 Infinity Binary LC System with ISET - Emulation of the Waters Alliance 2695 LC system analyzing β-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) |

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

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# 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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