

Agilent 1290 Infinity with ISET

User Manual



Agilent Technologies

Notices

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In this book

The manual describes the Agilent 1290 Infinity Intelligent System Emulation Technology (ISET) V1.0 Revision. 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.

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What is ISET?

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

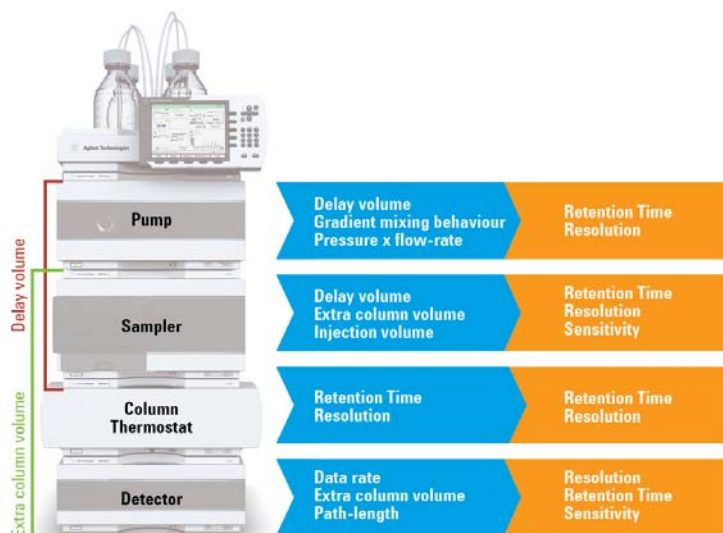


What is Intelligent System Emulation Technology (ISET)

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.

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.



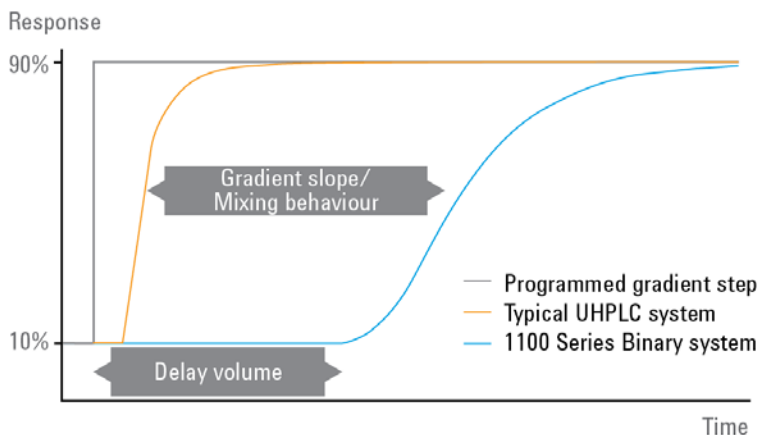
A wide range of instrument parameters have a direct impact on the retention times, resolution and sensitivity of a separation.

The impact of delay volume and gradient mixing

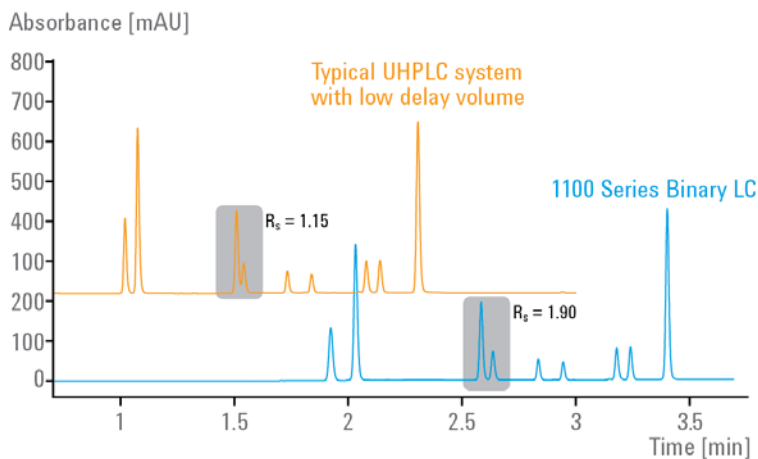
The delay volume of an LC system determines how fast the gradient reaches the column. Further, the mixing behavior influences the gradient profile. Both these factors – delay volume and mixing behavior – are determined by the instrument design and the consequences for method transfer are differences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

1 What is ISET?

What is Intelligent System Emulation Technology (ISET)



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.



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 μ l). Flow rate: 0.85 mL/min.

Mobile Phase: Water, Acetonitrile.

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

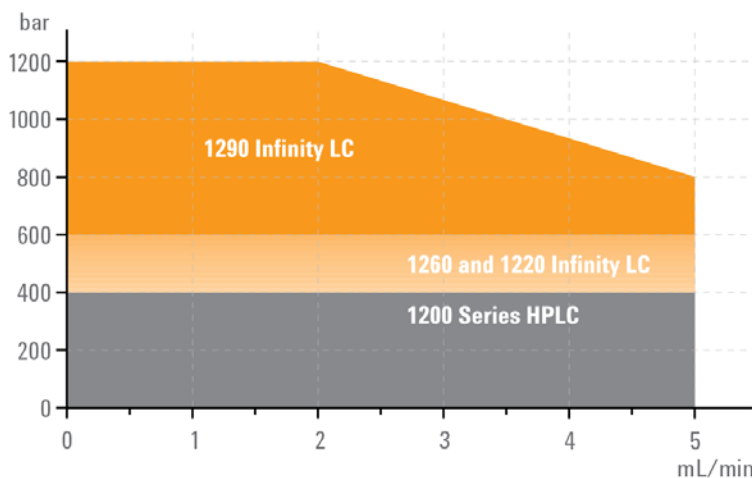
The solution for instrument to instrument method transfer: 1290 Infinity LC with ISET

The Intelligent System Emulation Technology enables the 1290 Infinity LC to execute other HPLC and UHPLC methods and deliver nearly the same chromatographic results without any change of the instrument or the original method – all by simple mouse click. This technology is based on two components: the 1290 Infinity performance specification, and the ISET emulation algorithm.

Agilent 1290 Infinity Performance specification

The 1290 Infinity LC with its broad power range, unmatched flow and composition accuracy, ultra-low delay volume, superior sensitivity, delivers the key requirement for the implementation of ISET technology.

1200 Infinity Series Power Range



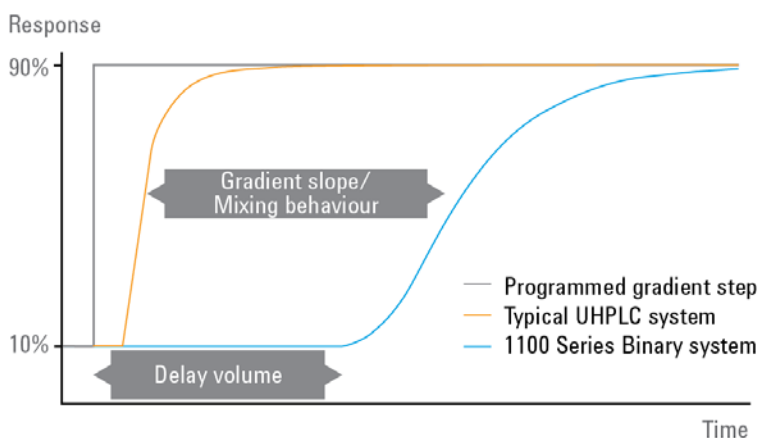
The wide power range of the 1290 Infinity LC ensures that both HPLC and UHPLC methods from narrow bore to standard bore columns can be executed

1 What is ISET?

The solution for instrument to instrument method transfer: 1290 Infinity LC with ISET

The ISET emulation algorithm

With the detailed knowledge about the system behavior of the target LC instrument and the high accuracy of the 1290 Infinity LC, 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 1290 Infinity LC with ISET delivers a gradient very similar to the 1100 Series Binary LC emulating the delay volume and mixing behavior.

The solution for instrument to instrument method transfer: 1290 Infinity LC with ISET

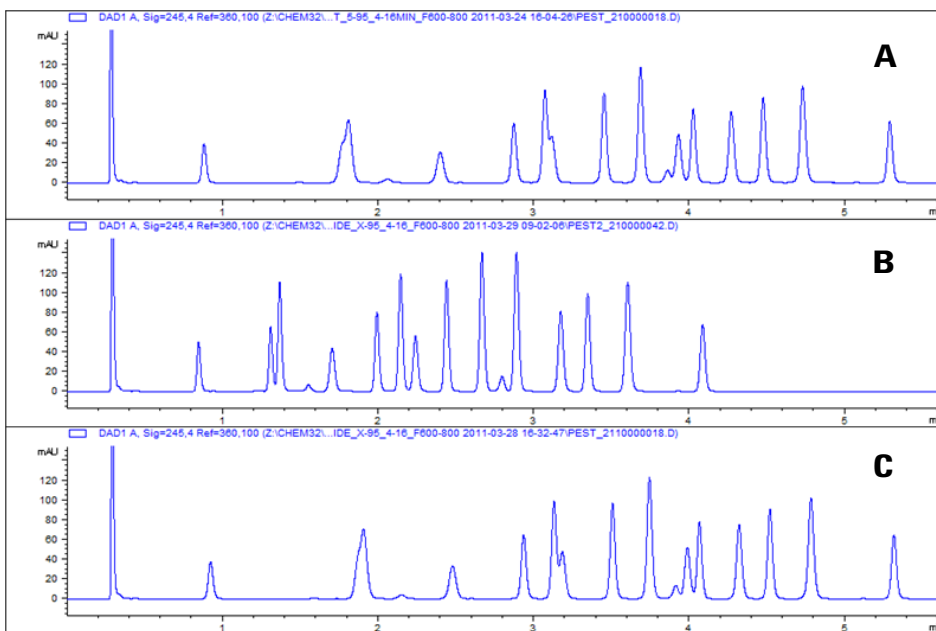


Figure 1 Chromatograms of a gradient separation of a pesticide mixture run on (A) 1100 Series Quaternary Pump (G1311A) + Autosampler (G1367A), (B) 1290 Infinity LC without emulsion, (C) 1290 Infinity LC in emulsion mode

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.

1 What is ISET?

The solution for instrument to instrument method transfer: 1290 Infinity LC with ISET

List of supported ISET modules

Revision 1 of ISET supports the emulation of the following modules:

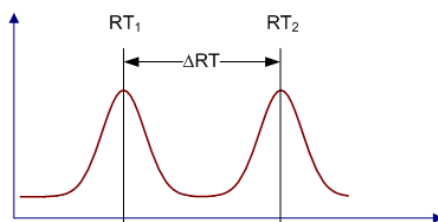
Pumps	G1311A	1100 Series Quaternary Pump
	G1311B	1200 Series Quaternary Pump 1260 Infinity Quaternary Pump
	G1311C	1260 Infinity Quaternary Pump VL
	G1312A	1100 Series Binary Pump
	G1312B	1200 Series Binary Pump SL 1260 Infinity Binary Pump
	G1312C	1260 Infinity Binary Pump VL
	G5611A	1260 Infinity Bio-inert Quaternary 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
	G5667A	1260 Infinity High Performance Bio-inert Autosampler

Future revisions of ISET will also cover LC systems from other vendors.

Specifications

Table 1 Retention Time (RT) Deviation

RT	$\pm 5\%$ ± 0.3 min	(for RT > 6 min (for RT $\leq$ 6 min
Δ RT	$\pm 5\%$ ± 0.1 min	(for Δ RT > 2 min (for Δ RT $\leq$ 2 min



Demo/Trial

A 700-hour demonstration/trial version of ISET is available with G4220A/B pumps with firmware revision B.06.42 and later. Unless the demonstration/trial version is upgraded to a registered version, the ISET function will be deactivated when the trial period has elapsed.

1 What is ISET?

The solution for instrument to instrument method transfer: 1290 Infinity LC with ISET



2 Installing and Configuring ISET

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

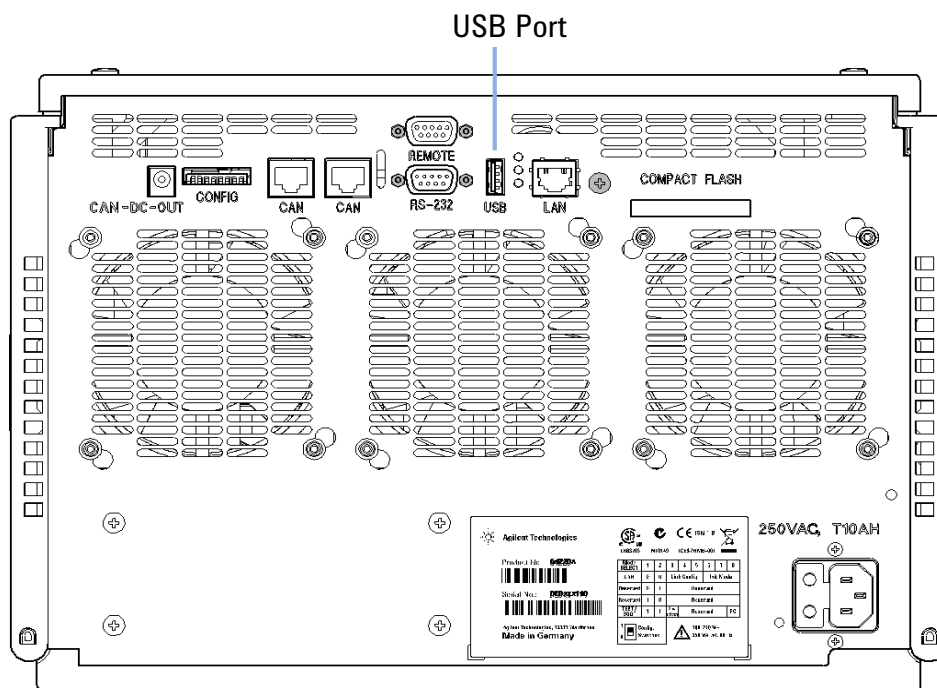


Installing and Configuring ISET

- ISET USB dongle
- G4220A/B pump with firmware rev. B.06.42 or later
- RC.Net drivers rev. A.02.04 or later
- Either OpenLAB CDS ChemStation Edition c.01.03 or OpenLAB CDS EZChrom Edition A.04.04

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 Switch off the G4220A/B pump.
- 2 Plug the ISET USB dongle into the USB port on the back of the G4220A/B pump.

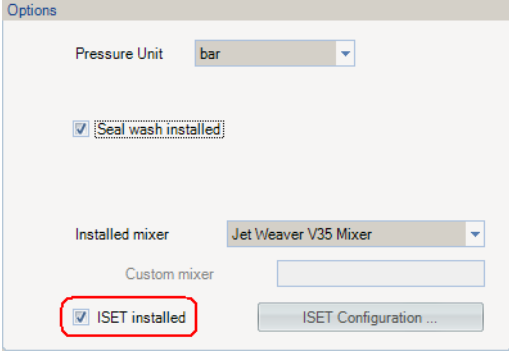


- 3 Switch on the G4220A/B pump to start the activation procedure.

NOTE

Do not remove the ISET USB dongle at this stage.

The pump configuration screen is displayed; the **ISET installed** check box in the **Options** section is marked to indicate that the installation is complete. You can now remove the ISET USB dongle.



The screenshot shows the 'Options' window of the ISET software. It contains the following elements:

- Pressure Unit:** A dropdown menu currently set to 'bar'.
- Seal wash installed:** A checked checkbox.
- Installed mixer:** A dropdown menu currently set to 'Jet Weaver V35 Mixer'.
- Custom mixer:** An empty text input field.
- ISET installed:** A checked checkbox, which is highlighted with a red rectangular border.
- ISET Configuration ...:** A button located at the bottom right of the window.

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 main board of 1290 Infinity pump 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; after this, the ISET USB dongle is no longer usable.



3

Setting Up ISET Parameters

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Setting up the basic ISET parameters 21

Fine-tuning the emulation 26

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 1290 Infinity System for emulation

- 1 Set up the 1290 Infinity 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 1290 Infinity method in the normal 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.

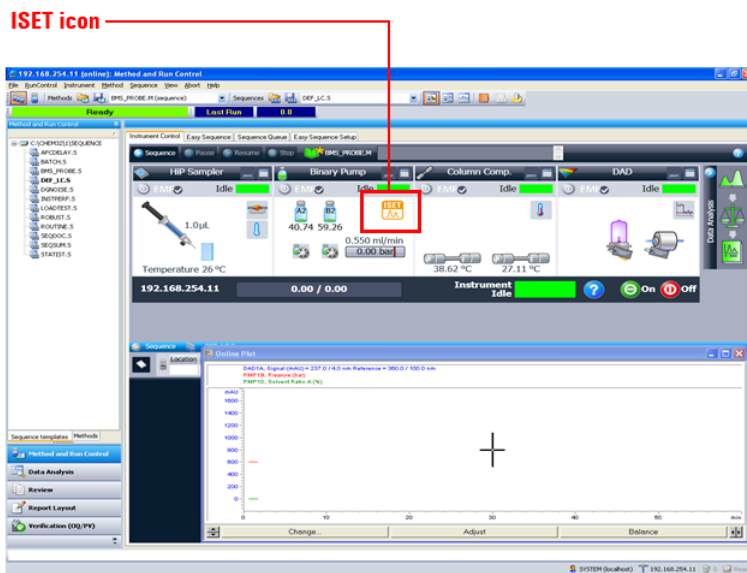
Setting up the basic ISET parameters

In this section, you will transfer your original method to the 1290 Infinity system and activate ISET by selecting the original hardware configuration. All known differences in the behavior between the original LC instrument and the current 1290 Infinity target system will be addressed by ISET.

- ISET is installed (see “Installing and Configuring ISET” on page 15).

NOTE

When ISET is installed but not enabled, the grey icon is displayed in the Pump Dashboard panel; the orange icon indicates that ISET is enabled.



3 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.

Time [min]	A [%]	B [%]	Flow [ml/min]	Max. Pressure Limit [bar]
0.00	50.00	50.00	1.000	1000.00
1.00	10.00	90.00	---	---

- 2 Expand the **Advanced** section of the pump method parameters and ensure that the following check boxes are marked:
 - **Synchronized** in the **Minimum Stroke** section
 - **Use Solvent Types** in the **Compressibility** section

Channel A: Automatic 20.00 µL

Channel B: Automatic 20.00 µL

☒ Synchronized

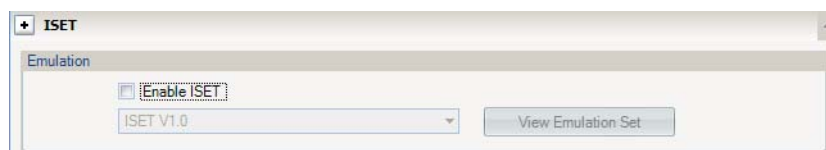
Compressibility

☒ Use Solvent Types

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
 - solvents A and B and solvent composition
 - pressure limits
 - stop- and post-times
 - gradient timetable
- 4 Expand the ISET section of the method parameters.



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

NOTE

Currently, only one version is available in the **Enable ISET** field. Future versions may come with updates that have either more aspects to emulate or a wider range of supported instrument configurations.

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

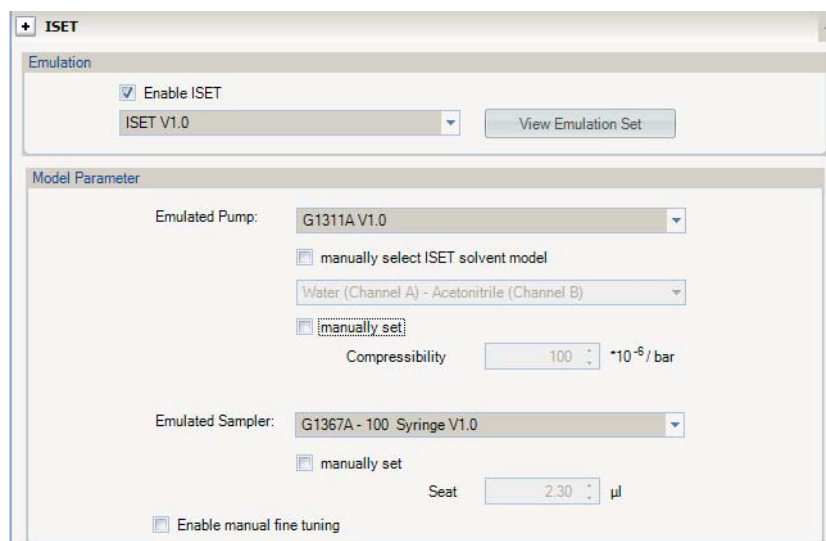


Figure 2 Model Parameter section

3 Setting Up ISET Parameters

Setting up the basic ISET parameters

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.

- 6 Click the **Emulated Pump** down arrow and select the pump used for the original method from the drop-down list, see [Figure 2](#) on page 23.

NOTE

The drop-down list shows the module number of the pump and the revision of its characterization. The current revision is V1.0. Future revisions of ISET might provide updated characterizations.

To determine a pump's name by its specific module number, please refer to [“List of supported ISET modules”](#) on page 12.

- 7 If the solvent system required by the method includes only water, methanol or acetonitrile, you need make no changes to the solvent model.

In ISET v1.0, water, methanol and acetonitrile are fully characterized for gradient compositions.

- 8 If solvents other than water, methanol or acetonitrile are required by the method, mark the **manually select ISET solvent model** check box and 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 26).

- 11 If a needle seat for higher injection volumes is installed, enter the correct needle seat volume in the **Seat** field.

- 12 You can click **View emulation set** if you want to display the modifications to the gradient time table that ISET has calculated.

This gradient time table will be used to emulate the original method. Check that the stoptime of the original method and the emulation method are the same.

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

The ISET emulation method will then be prepared.

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 delay volume of the system with respect to the standard configuration contributes to the offset. The total delay 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 delay 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 **Delay Volume Offset**.

The **Delay Volume Offset** can be used to compensate for a difference in delay 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 **Delay Volume Offsets** shift the gradient to a later position; negative **Delay Volume Offsets** shift the gradient to an earlier position

4 You can click **View Emulation Set** to display the modifications to the gradient that ISET has calculated and will be used to emulate the original method.

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

The ISET emulation method will then be prepared.

NOTE

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

3 **Setting Up ISET Parameters**

Fine-tuning the emulation



4

Understanding ISET Functionality

Understanding ISET Functionality 30

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 1290 Infinity system, and vice versa.

System delay or dwell volume

The system delay (or dwell) volume includes the volume of the system flow path from the point of mixing of the mobile phase components to the top 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 delay volume before any change made at the pump arrives at the head of the column. Any differences in delay (or dwell) volume cause variations in retention times and often also in selectivity.

The ISET emulation considers the delay volume of both pump and autosampler. The delay volume from the autosampler through the column compartment to the detector is around 5 μl when red capillaries with 0.12 mm ID are installed and 14 μl when green capillaries with 0.17 mm ID are installed. These differences in volume are usually minor; they are not part of ISET, and can be neglected in most cases. If the volume is large enough to have an effect, the **Delay Volume Offset** function is available in ISET to compensate for the additional volume. The gradient delay resulting from geometric volume is shown in [Figure 3](#) on page 31.

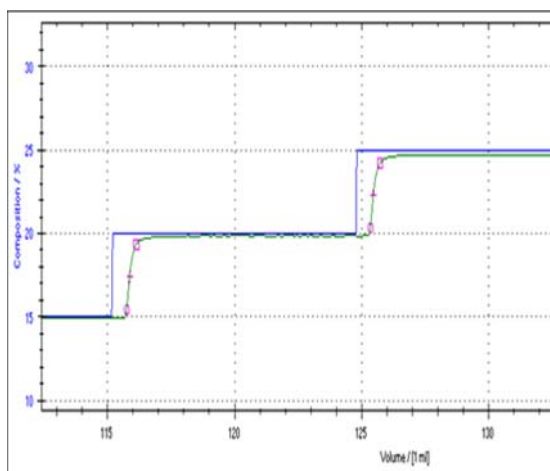


Figure 3 Step gradients are used to evaluate system delay volume and composition offset. The system delay volume is determined at 10 % of the step height, transition volume is from the 10% height to the 90% height, and the system dwell volume is determined at 50 % of the step height.

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 running 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 4](#) on page 33.

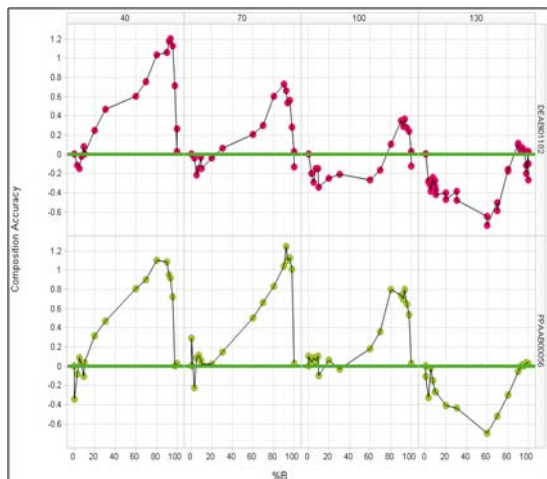


Figure 4 Composition offset of an Agilent 1100 quaternary pump for acetonitrile-water at different compressibility settings.

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 4](#) on page 33 shows the composition offset generated by a 1100 quaternary pump for different settings of solvent compressibility.

ISET functionality

The physical relationship between a programmed timetable and the system response can be described by a transfer function (Figure 5 on page 34).

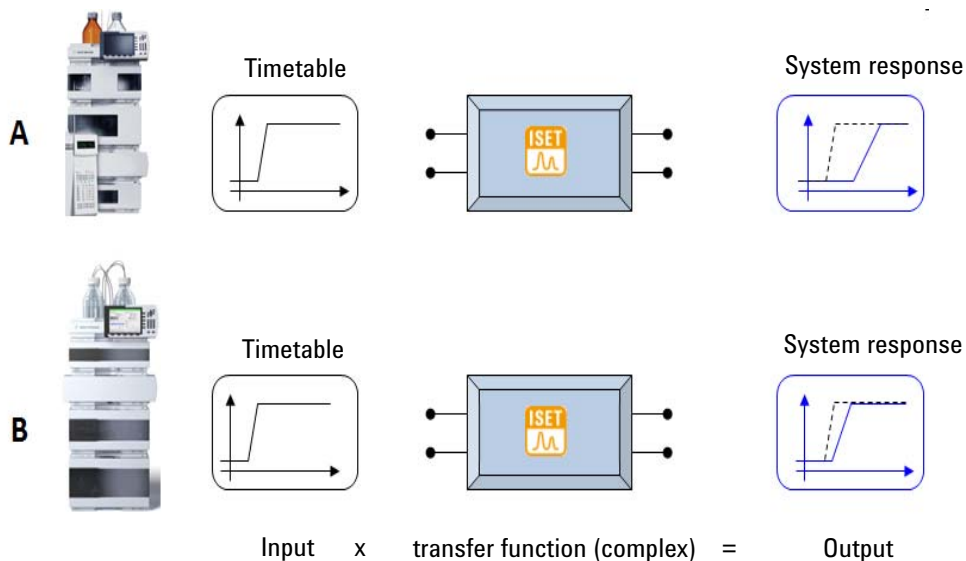


Figure 5 Different gradient shapes resulting from the same time table for different instrument characteristics.

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 6 on page 35).

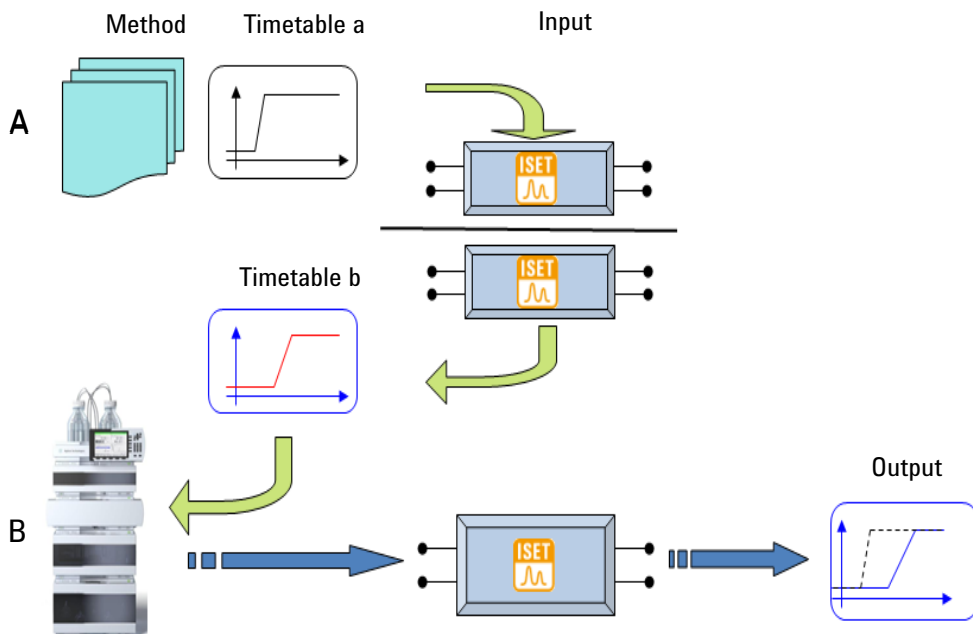


Figure 6 Operation of the 1290 Infinity system in a mode that emulates the gradient response of a larger delay volume system.

The transfer functions generated from thorough system characterizations are used by ISET, the instrument driver, to generate a (virtual) timetable that is executed by the 1290 Infinity pump to emulate another HPLC system. When the emulation mode is enabled, the 1290 Infinity, 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 1](#) on page 11).

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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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