

Computer-Aided Implementation of Instrument Methods for On-Line SPE-LC

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INTRODUCTION

Conventional HPLC methods for the analysis of low molecular weight compounds in complex sample matrices are often time-consuming, error-prone, and costly. Matrices such as urine, plasma, and surface water typically require manual sample pre-treatment to eliminate undesirable content, for better resolution of analytes such as drugs, endogenous substances, and polycyclic aromatic hydrocarbons (PAHs). Such samples are often treated using solid phase extraction (SPE) prior to analysis (off-line). Integrating the extraction processes into an HPLC system can increase sample throughput and enable automation, resulting in cost reductions and improvements in overall analytical quality.

On-line solid-phase extraction traps analytes on an extraction column while the matrix components are flushed to waste. The analytes are then transferred to the analytical liquid chromatography (LC) column for separation. Successful transfer requires definition of the correct valve switching times, and creation of two gradient control programs. This requires advanced knowledge of the analytical and applied data systems—a significant barrier to setting up an instrument control program.

The Dionex Chromeleon® On-Line SPE-LC wizard and editor remove that barrier by calculating the appropriate column switching times from method-dependent user inputs and generating a suitable instrument method.

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INSTRUMENT AND SOFTWARE REQUIREMENTS



Figure 1. Ultimate™ 3000 x2 Dual-Gradient system.

The On-Line SPE-LC wizard requires Chromeleon version 6.8 or higher and the following Dionex UltiMate 3000 modules (Table 1).

Table 1. Instrument Requirements	
Module	Module Type
Pump	One DGP-3600 Dual-Gradient pump (dual ternary) Or two of the following pumps: ISO-3100 Isocratic pump LPG-3400 Low-Pressure Gradient pump (quaternary) HPG-3200 High-Pressure Gradient pump (binary) HPG-3400 High-Pressure Gradient pump (binary with solvent selector)
Sampler	WPS-3000SL (in-line split loop autosampler) WPS-3000TSL (in-line split loop autosampler with thermostat)
Column Oven	TCC-3000 series (column oven with a six- or 10-port valve)
Detector	Any detector that can be controlled by Chromeleon or that has an analog output

SETUP FOR ON-LINE SPE-LC

Figure 2 shows the typical setup for on-line solid phase extraction liquid chromatography (SPE-LC). In the first step, sample fractionation (left), the sample is injected and transferred to the SPE column with the loading pump. The sample matrix is flushed to waste, while the analytes are retained on the column. At the same time the analytical LC column is equilibrated with the analytical pump. After the matrix is flushed to waste, the six-port valve is switched (middle) to a position that couples the SPE column with the analytical LC column, and the analytes are transferred. After the transfer, the six-port valve is returned to its original position (right). While the analytes are separated on the analytical LC column, the SPE column can be re-equilibrated with the mobile phase on the loading pump.

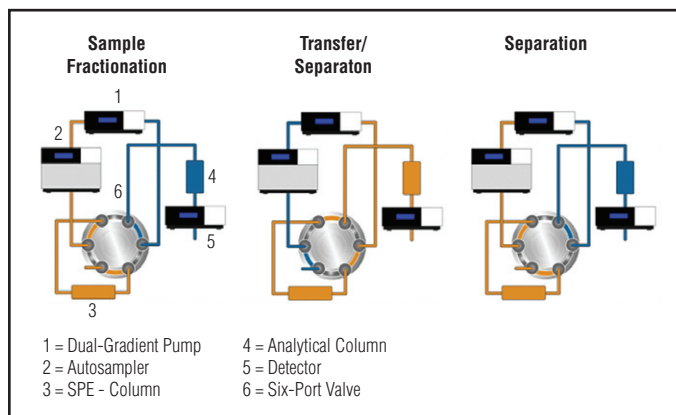


Figure 2. Typical instrument configuration for on-line SPE-LC.

Determining the proper switching times of the six-port valve (t_{v1} and t_{v2}) is important to successfully apply on-line SPE for fractionation, transfer, and separation. During method development, several partial steps must also be determined:

- Matrix depletion time (t_M): time for the matrix to elute from the SPE column
- Analyte break-through time (t_A): time for the target analytes to elute from the SPE column
- Transfer time (t_T): time for the analytes to completely elute from the SPE column and transfer to the analytical LC column

Based on this information, t_{v1} and t_{v2} can be calculated (Figure 3).

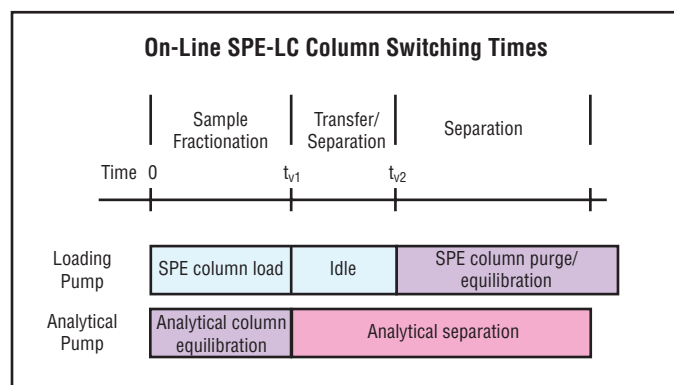


Figure 3. Schematic overview of an on-line SPE-LC method.

CREATING AN INSTRUMENT CONTROL PROGRAM FOR SPE-LC

UltiMate 3000 Dual-LC systems provide the technology to perform on-line SPE-LC. An on-line SPE-LC instrument method requires many modules and timed events. Table 2 shows the instrument commands and timed events for a typical on-line SPE-LC method.

Table 2. Steps Required for a Typical On-line SPE-LC Method

Starting Conditions	
0.000	PumpRight.Flow = 1.000 [ml/min] PumpRight.%B = 25.0 [%] PumpRight.%C = 0.0 [%] PumpLeft.Flow = 2.000 [ml/min] PumpLeft.%B = 0.0 [%] PumpLeft.%C = 0.0 [%] ValveLeft = 6_1
Gradient and Valve Switching	
2.000	ValveLeft = 1_2 PumpRight.Flow = 1.000 [ml/min] PumpRight.%B = 25.0 [%] PumpRight.%C = 0.0 [%]
5.000	ValveLeft = 6_1 PumpRight.Flow = 1.000 [ml/min] PumpRight.%B = 35.5 [%] PumpRight.%C = 0.0 [%] PumpLeft.Flow = 2.000 [ml/min] PumpLeft.%B = 0.0 [%] PumpLeft.%C = 0.0 [%] PumpLeft.Flow = 2.000 [ml/min] PumpLeft.%B = 100.0 [%] PumpLeft.%C = 0.0 [%]
12.000	PumpRight.Flow = 1.000 [ml/min] PumpRight.%B = 60.0 [%] PumpRight.%C = 0.0 [%] PumpRight.Flow = 1.000 [ml/min] PumpRight.%B = 90.0 [%] PumpRight.%C = 0.0 [%]
15.000	PumpLeft.Flow = 2.000 [ml/min] PumpLeft.%B = 100.0 [%] PumpLeft.%C = 0.0 [%] PumpLeft.Flow = 2.000 [ml/min] PumpLeft.%B = 0.0 [%] PumpLeft.%C = 0.0 [%]
20.000	PumpRight.Flow = 1.000 [ml/min] PumpRight.%B = 90.0 [%] PumpRight.%C = 0.0 [%] PumpLeft.Flow = 2.000 [ml/min] PumpLeft.%B = 0.0 [%] PumpLeft.%C = 0.0 [%]

The complexity of creating and editing such a method can present a barrier to adopting on-line SPE-LC technology. Chromeleon's new On-line SPE-LC wizard overcomes that barrier.

COMPUTER-AIDED IMPLEMENTATION: ON-LINE SPE-LC PROGRAM WIZARD

To simplify and accelerate creation and editing of on-line SPE-LC methods, a new instrument control wizard and a new method editor were introduced in Chromeleon version 6.8. The wizard guides the users through the creation process in an easy-to-use, step-by-step guide. The wizard provides schematic drawings of the system as a reminder during program generation. The editor allows users to visualize the method in an intuitive way.

To use the wizard, first make sure all required hardware is available (Table 1). Next, launch the wizard and choose the option to create a program for on-line SPE-LC (Figure 4, top). A short description of the procedure will appear, to explain the parameters to be entered during program generation (Figure 4, bottom). If desired, use the Help button to access further information about the on-line SPE-LC method.

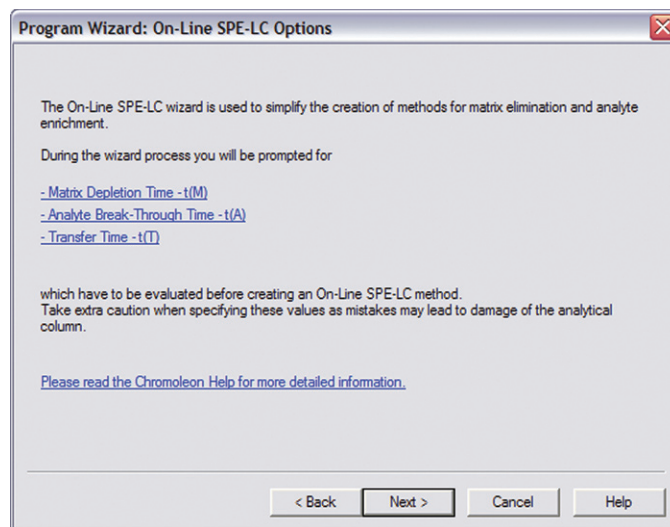
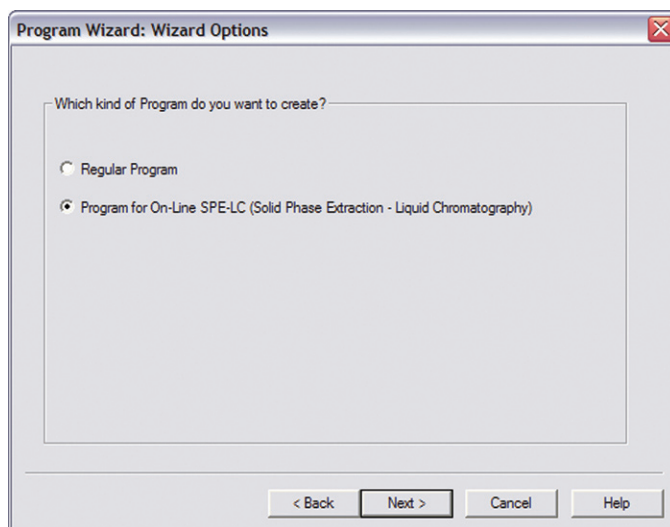


Figure 4. First steps in on-line SPE-LC program generation.

The next step provides options to define the system configuration, including selection of the Loading and Analytical pumps, switching valve, and the valve position used for sample fractionation (Figure 5).

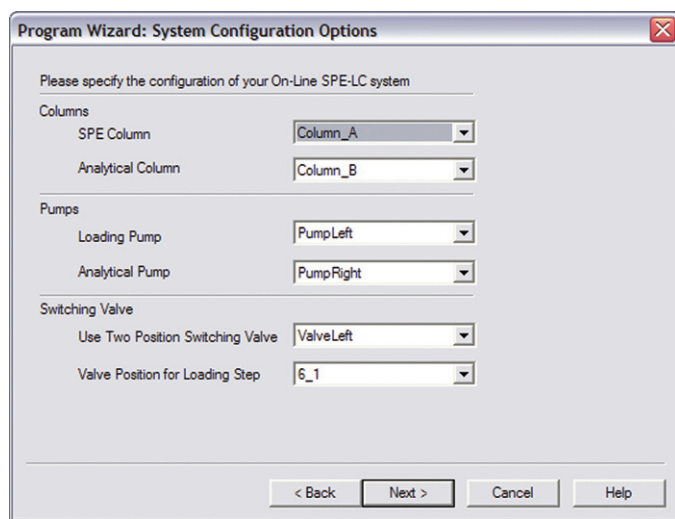


Figure 5. System Configuration Options.

Next, enter the matrix depletion time (t_M), analyte break-through time (t_A), and transfer time (t_T) determined during on-line SPE-LC method development. Based on these parameters, Chromeleon calculates the switching times of the six-port valve using the equations below (Figure 6).

$$t_{V1} = \frac{(t_M + t_A)}{2} \quad t_{V2} = t_{V1} + t_T + 1$$

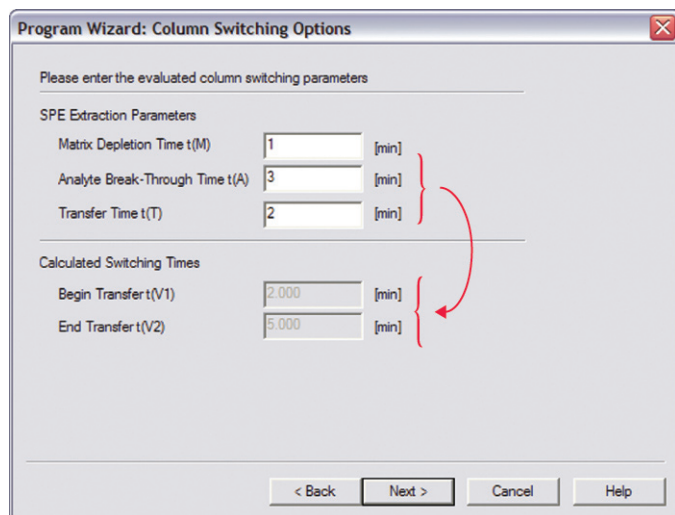


Figure 6. Column Switching Options.

Once the switching times of the six-port valve are determined, the gradients for the loading pump (Figure 7) and the analytical pump (Figure 8) can be programmed. The valve switching times appear in the gradient profile. Rows are prepared at the recommended switching times.

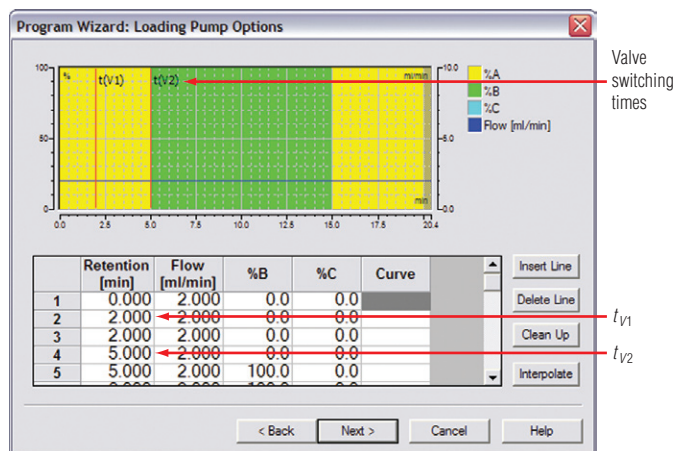


Figure 7. Programming the loading pump gradient.

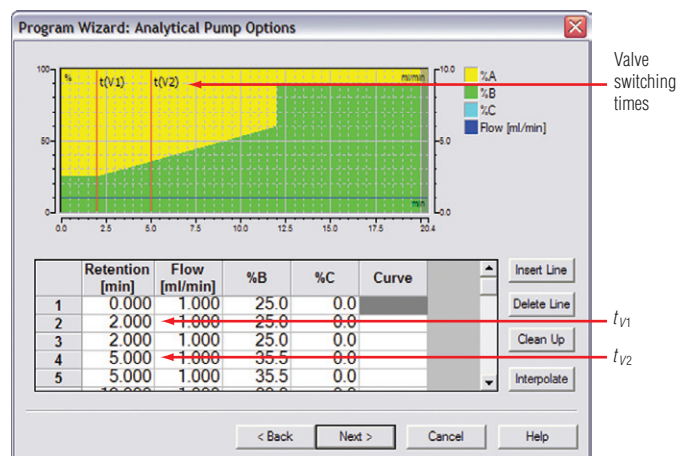


Figure 8. Programming the analytical pump gradient.

By entering the appropriate values into the wizard, users are able to quickly create on-line SPE-LC instrument control programs. The wizard does not allow entry of invalid values, ensuring that users can begin working with their instrument immediately.

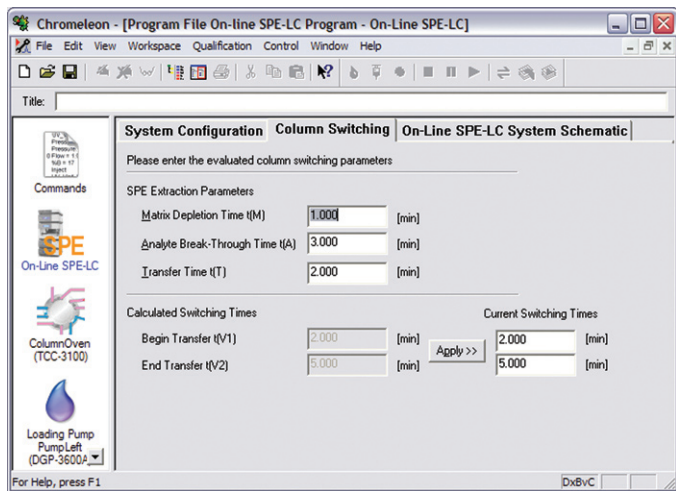


Figure 9. On-Line SPE-LC program editor.

Once an instrument control program is created, it is also easy to modify. The program editor provides an overview of all settings of the system (Figure 9).

CONCLUSION

Computer-aided implementation of instrument methods for on-line SPE-LC allows even inexperienced users to quickly create and run on-line solid phase extraction chromatography analysis, based on method-dependent parameters. This provides the following advantages:

- Faster on-line SPE-LC method development
- Fewer errors
- Improved reproducibility of the recovery
- Elimination of time-consuming off-line operations
- Enhanced operator safety due to a closed system
- Sophisticated automation possibilities, enabling unattended operation overnight or over the weekend
- Full instrument control, advanced diagnostics, and automated validation functions
- Complete documentation and data traceability of all sample cleanup and analytical separation steps

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