

EASY-nLC 1200 System – Peak Nano LC Performance at Your Fingertips

Stephan Meding, Chris Pynn, and Remco Swart
Thermo Fisher Scientific, Germering, Germany

Key Words

EASY-nLC 1200, EASY-Spray, Nano LC

Goal

To provide an overview of the hardware and software features of the Thermo Scientific™ EASY-nLC™ 1200 system.

Overview

The EASY-nLC 1200 is a dedicated system for proteomic analyses (Figure 1). It combines best-in-class 1200 bar performance with operational simplicity. It features fully integrated hardware with pumps, autosampler, valves, flow path, control PC, and touch screen interface in one single, compact housing. The EASY-nLC 1200 comes pre-configured for two analysis modes: direct injection onto a separation column and pre-concentration onto a trap column. Changing between analysis modes cannot be simpler, requiring just one click in the software and adding the trap column to the flow path. All components requiring maintenance or repair are easily accessible by removing the side panels of the housing. The software is designed to be intuitive, while giving the operator full control over the system via a touch screen interface. The EASY-nLC 1200 includes all popular features of previous generations, such as the interactive overview screen and the method wizard, while incorporating new features, such as intelligent maintenance to further improve the user experience. Furthermore, the EASY-nLC 1200 is fully integrated into Thermo Scientific™ Xcalibur™ software for seamless LC-MS system control. As such, method set-up and analyses can be conducted from a single software suite.



Figure 1. The EASY-nLC 1200.

Key Hardware Components of the EASY-nLC 1200

The EASY-nLC 1200 is designed with simplicity, reliability, and intuitive operation in mind. Its pre-configured set-up is optimized for proteomic workflows. The EASY-nLC 1200 can deliver system pressures up to 1200 bar. In order to achieve this, key hardware components were re-designed. All components of the high pressure flow path such as the pumps, pressure sensors, flow sensors, valves, and fluidic connections have been re-engineered to withstand the higher 1200 bar system pressure. Exploiting the higher pressure rating for LC-MS analyses yields two major benefits: (i) increased analytical depth by using longer separation columns and (ii) higher sample throughput by faster sample loading and column equilibration.

The entire flow path is now connected using Thermo Scientific™ Dionex™ nanoViper™ fittings. This minimizes dead volume and guarantees leak-tight connections that can be fitted or exchanged tool-free within seconds. Maintaining the instrument is easier than ever and provides the user with greater confidence in the quality of the connections. Proprietary Thermo Scientific

maintenance-free switching valves have been developed (Figure 2). As a result, the operational time of your LC-MS system will increase and the cost of ownership will significantly decrease. To assure good valve performance and lifetime, the valves are specified for $\leq 95\%$ acetonitrile in water (volume/volume) and can be used with nanoViper connections only.

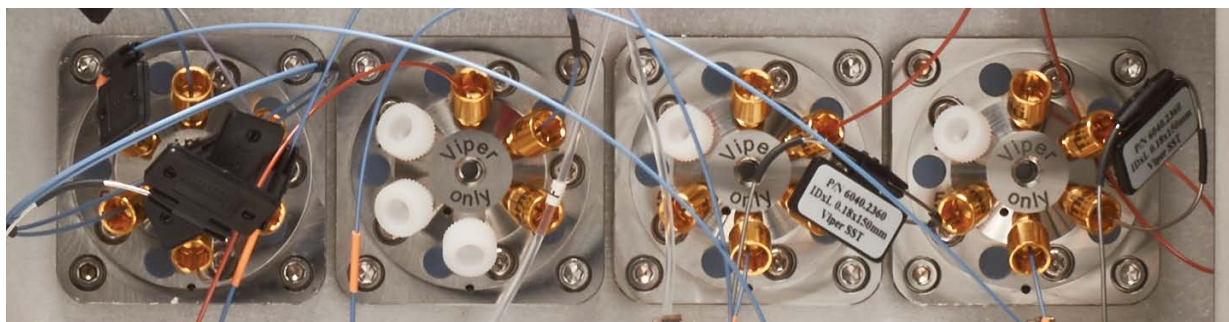


Figure 2. The Thermo Scientific switching valves with nanoViper connections.

System Control and Method Set-up

The EASY-nLC 1200 software is based on several fully interactive menus. Through them, one can obtain clear information on the system status, generate new methods, perform instrument maintenance, or configure the system.

System Status Overview

Key information about the system status is displayed on the overview screen in the home tab (Figure 3). The core components of the flow path can be controlled simply by touching them, for example, setting the flow rate of the pumps or switching the valve position. Maintenance procedures, such as flow sensor calibration or column equilibration, are directly accessible. Additionally, the overview screen shows which sequence and sample is currently running and which is next in the queue, along with which step in the analysis method is in progress.

New features added to the overview screen include the reminder buttons for overdue flow sensor calibration and solvent refreshment that will appear after six months of operation without flow sensor calibration or two weeks without solvent exchange (Figure 4). After solvent exchange, the solvent composition of solvent B needs to be entered and will be displayed below the solvent bottle B (Figure 3). These reminders help to keep track of required maintenance steps to assure consistent and reproducible system performance.

Another new feature is the ability to control the temperature of Thermo Scientific™ EASY-Spray™ columns. If the EASY-Spray source is installed, an interactive graphic representation appears in the top left corner of the main screen that allows setting the column temperature.

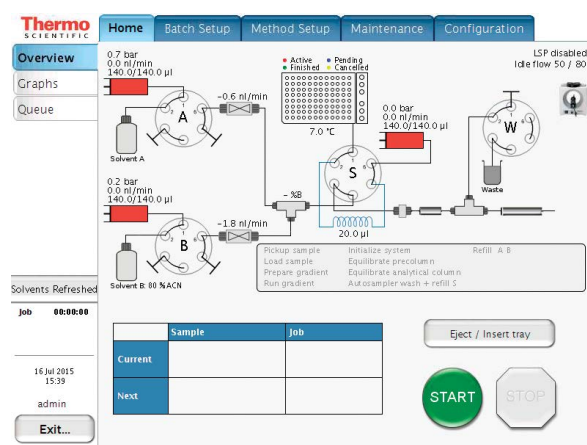


Figure 3. The main screen visualizes the flow-path (top), the current step being carried out in the method (middle), and the sample or script currently active (bottom).

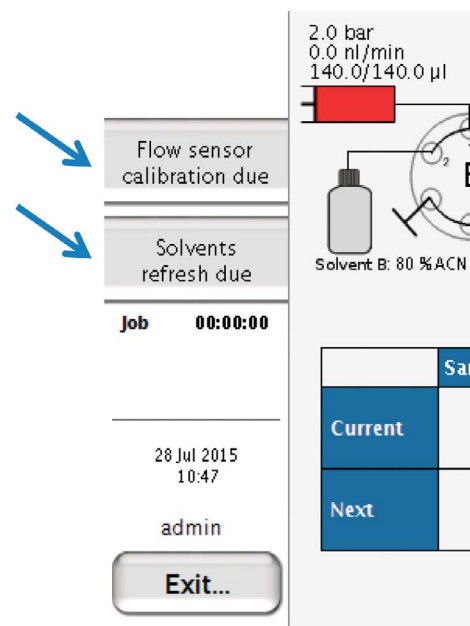


Figure 4. New reminder buttons for overdue flow sensor calibration and solvent refreshment.

Setting Up Your LC Method Using the Method Wizard

Method creation with EASY-nLC systems is simple. A five-step method wizard guides through the method set-up (Figure 5). The gradient step tab of the wizard graphically displays the programmed flow rate gradient profile to allow fast visual inspection (Figure 6). The last tab for

setting the column temperature is only available when the EASY-nLC 1200 is operated in combination with an EASY-Spray source. The method wizard is fully integrated into Xcalibur software, enabling comprehensive LC-MS method set-up within a single software solution. This makes the EASY-nLC 1200 an ideal nano LC system for Thermo Scientific mass spectrometers.

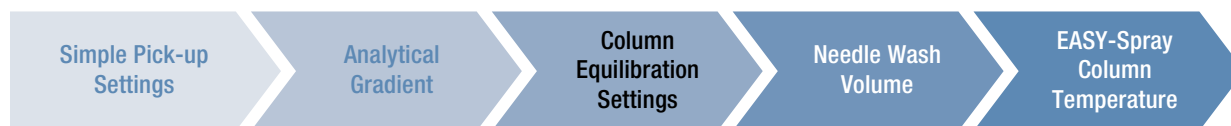


Figure 5. The five steps of the method wizard.

Standard System Operation During LC-MS Analyses

The EASY-nLC 1200 software is designed to deliver high performance while reducing cycle time. This is done in two ways: through step synchronization and intelligent flow control (IFC™). For synchronization, sample pick-up and column equilibration are done in parallel to speed up the analysis. Then the sample is loaded onto the column. During gradient delivery the autosampler wash is performed so that the system is ready for the next injection as soon as the gradient is finished. In order to minimize the duration of sample loading and column equilibration IFC™ can be used. The solvent volume and target pressure are set instead of the flow rate, thus the steps will be performed at accelerated flow rates, drastically reducing equilibration and loading times. During gradient delivery, automated flow control (AFC™) is used for delivering the flow and gradient composition with high precision and accuracy.

The EASY-nLC 1200 incorporates several features to help the operator to make the best use of the system's market-leading performance. For example, out-gassing following solvent mixing can result in accumulation of air in the system, especially in the pumps, thus compromising system performance. This can however be avoided with regular pump purges. By activating pre-run maintenance, the system will automatically check if the pumps have been purged within the last eight hours before starting a sequence, and if this was not the case, will automatically execute the purge air script before the first injection.

A new feature of the EASY-nLC 1200 is a check for trapped air in the pumps before each injection. If air is detected the injection will be stopped before sample is drawn from the vial. This test is executed in a matter of seconds and will prevent sample pick-up if the system is not functioning optimally.

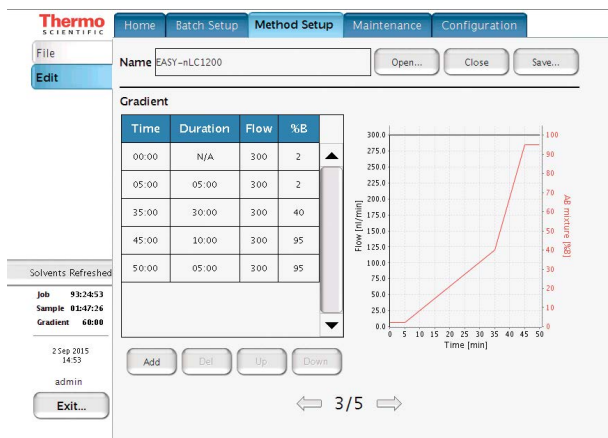


Figure 6. The gradient step of the method wizard.

Dedicated Procedures for System Set-up and Maintenance

Regular system maintenance is essential for long-term optimal system performance. To ensure optimal system performance, the EASY-nLC 1200 software comes with a set of methods called “scripts”, which check the system performance or carry out maintenance procedures such as initial system set-up, recalibration of flow and pressure sensors, and test for leaks and blockages. These tests also indicate the position of the leak or blockage in the flow path.

Additional scripts have been added to enable fast diagnosis when more in-depth troubleshooting is required. Constant flow and constant pressure scripts, for example, allow the system to be run at a user-defined flow rate or pressure level, while permitting manual control of the valves and pumps. Furthermore, a recording script has been implemented which will start recording the system parameters to the log file and enable visual display, for example of the flow rate or system pressure.

Finally, a column conditioning script has been added. This permits stepwise pressure ramping over a defined time span. This is particularly useful when preparing a separation column for first use. By slowly ramping up the pressure by increasing the flow rate, it can be assured that the packing material is well compacted within the column, thus assuring good column performance and longer column lifetime.

Conclusion

The EASY-nLC 1200 is designed to enable cutting-edge nano LC separations with very simple and straightforward method set-up. This is achieved by:

- Intuitive system operation and maintenance
- nanoViper connections and Thermo Scientific switching valves for improved system robustness
- 1200 bar pressure rating for faster sample analysis or better separations through the use of longer separation columns

Useful Links

Further information about the system is available on:
thermoscientific.com/nanoLCMS

For more information, visit: thermofisher.com/nanoLCMS

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