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Should Your Autosampler Just Sit and Wait? Intelligent Instrument Control Allows Sharing HPLC Modules Across Systems

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INTRODUCTION

In traditional HPLC configurations, some of the modules are used inefficiently:

- The autosampler is idle for most of the time.
- The thermostatted column compartment (oven) could hold more than one column.

This inefficiency can be improved if these modules could be utilized in more than one system.

With a Dionex UltiMate™ 3000 Dual LC system, which consists of a dual-gradient pump (two independent pumps in one enclosure), an autosampler, a thermostatted column compartment, and two detectors, the capabilities of two traditional LC systems can be achieved—we call this "parallel chromatography" (Parallel LC). UltiMate 3000 Dual LC systems allow sharing of the autosampler and the column compartment. This sharing facilitates better utilization of the potential productivity of these modules.

Parallel LC with an UltiMate 3000 Dual LC system has several advantages:

- It nearly doubles the throughput at a cost that is substantially lower than two complete HPLC systems.
- This gain in productivity can be reached without modifying and revalidating the existing analytical method.
- It can be used for isocratic as well as gradient methods.
- A Dual LC system can analyze a sample set with two different (orthogonal) methods at the same time.

However, control of such a complex system requires powerful software.

In this presentation we show:

- Analytical scenarios that are especially suitable for Parallel LC.
- Intelligent software solutions for trouble-free control of the two HPLC channels incorporated in a single UltiMate 3000 Dual LC system.

DEFINITIONS

Parallel LC system: A Dionex UltiMate 3000 Dual LC system consisting of a dual-gradient pump (two independent pumps in one enclosure), two detectors, and a shared autosampler and column compartment, configured in a way that it behaves like two independent HPLC systems.

Sub-system: One of the two separation channels of a Parallel LC system, which corresponds to a complete HPLC system.

Module: A single instrument in the HPLC system, e.g. a pump or a detector.

Sub-module: A Dionex UltiMate DGP-3600 ×2 Dual-Gradient pump encloses two ternary low-pressure gradient pumps in a single housing. In this case each independent pump is called a "sub-module."

INSTRUMENTATION

Figure 1 shows the schematics of a traditional and a Parallel LC.

A traditional LC system consists of the following modules:

- One pump
- One injection device, in most cases an autosampler
- One thermostatted column compartment (oven) with:
  – one column
- One detector

A Parallel LC setup contains:

- Two pumps*
- One shareable autosampler
- One shareable thermostatted column compartment with:
  – Two columns
  – One motorized switching valve (two-position six-port)
- Two detectors

* Optimally the two pumps can be built into the same housing, as in the Dionex UltiMate DGP-3600 ×2 Dual-Gradient Pump, which encloses two ternary low-pressure gradient pumps in a single housing. In this solution the relative cost of a single system is further reduced.

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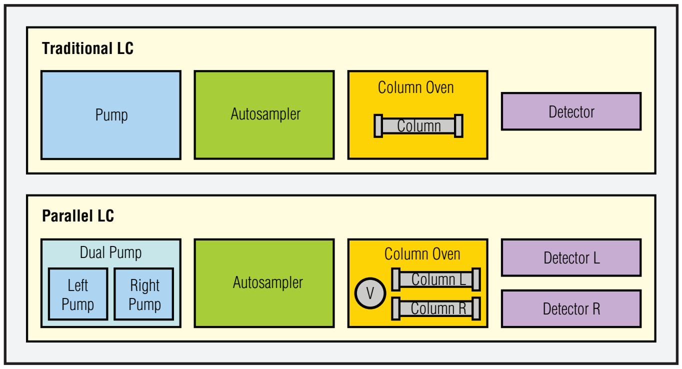

Figure 1. Schematic instrument setup for traditional and Parallel LC. Compared to a traditional setup, Parallel LC nearly doubles throughput by only adding a pump, a detector, and a switching valve (V). The autosampler and the column oven are shared between the two separation channels (sub-systems).

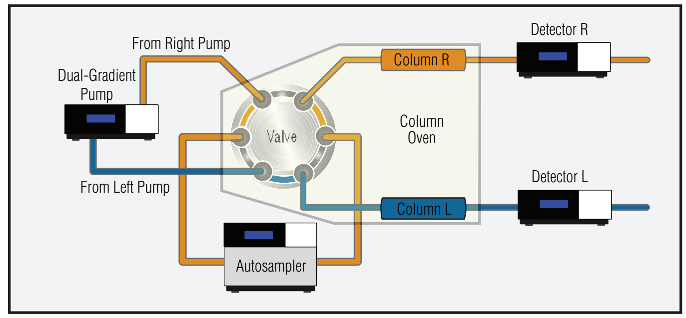

Figure 2. Flow scheme for UltiMate 3000 Parallel LC mode. The autosampler is currently in the flow path of the right pump. (A Dionex UltiMate DGP-3600 ×2 Dual-Gradient Pump encloses two ternary low-pressure gradient pumps in a single housing. The sub-devices are called left pump and right pump.)

In Parallel LC, two columns and detectors are used in parallel in two separate flow paths ("sub-systems"). The autosampler is utilized in both sub-systems; a two-position six-port valve switches between the sub-systems (Figure 2). The two independent pump modules (left pump and right pump) in the dual-gradient pump deliver the flow to their dedicated sub-systems. Figure 3 shows a photo of an UltiMate 3000 Dual LC system configured for Parallel LC and lists the comprising modules.

APPLICATION EXAMPLES

For successful implementation of Parallel LC, there are a few requirements on the analytical methods that will be run on the two sub-systems:

Temperature Requirements: Both analytical methods must operate at the same temperature, because the two columns are placed in the same column compartment and the samples in the same autosampler.

Eluent Requirements: Because switching the autosampler between the two flow paths can transfer eluent of different composition between the sub-systems, the eluents must be miscible and the initial eluent conditions for both methods should support fast flow path switching.

Considering the above requirements there are a few application areas that are especially suitable for Parallel LC.

Figure 3. Dionex UltiMate 3000 Dual LC system for Parallel LC. The modules are arranged in a way that ensures optimal plumbing. Modules from top left: SRD-3600: six-channel solvent rack with degasser, DGP-3600A: analytical dual-gradient pump, WPS-3000TSL Analytical: thermostatted in-line split-loop well plate sampler, VWD-3400: four-channel variable wavelength detector, TCC-3100 Thermostatted Column Compartment with an integrated two-position six-port switching valve, and a second VWD-3400 detector.

Doubling Sample Throughput for a Single Method

In this scenario the same analytical method is run on both sub-systems. Samples are injected alternately to the two flow paths. By running an UltiMate 3000 Dual LC System in Parallel LC mode, the throughput can be increased by close to 100% compared to a traditional HPLC. This gain in productivity can be achieved both for isocratic and gradient methods.

Isocratic Methods

For isocratic runs the implementation is relatively simple, as all the above mentioned method requirements are fulfilled during the entire separation time.

Gradient Methods

For gradient methods the Eluent Requirements must be considered: switching of the autosampler between the sub-systems must be timed properly during the equilibration phase. Figure 4 shows how the gradients, valve switches, and injections are synchronized. As discussed later, the software provides the necessary tools for avoiding eluent mixing at undesired times.

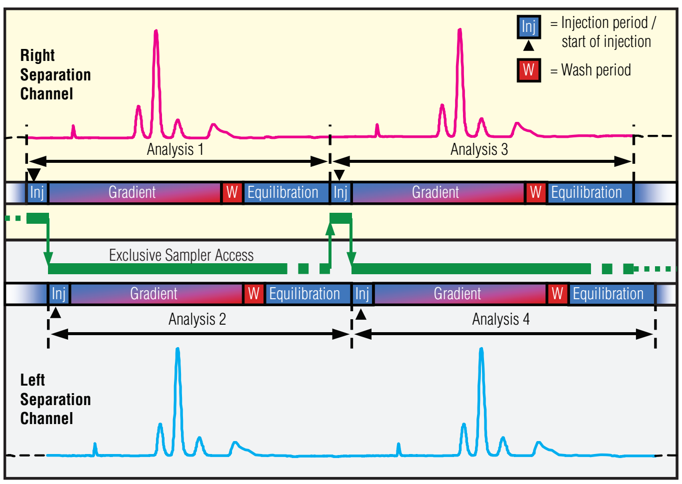

Figure 4. Running the same gradient method with Parallel LC. Proper timing of the valve switches ensures that a portion of the gradient does not interfere with the other separation channel during valve switching. The throughput is nearly doubled compared to a traditional LC system.

Analyses with Different Methods

Doubling throughput for a single method (see above) is perhaps the most obvious application of Parallel LC. However, Parallel LC is not limited to using the same method on both sub-systems. The methods can be different as long as the eluents are miscible at the time of the autosampler switching.

The samples that are injected on the two sub-systems can be different, or the same. The latter example is discussed in this presentation.

In many cases a single analytical run cannot resolve all the components in a complex sample and an "orthogonal separation" using a different column and/or eluent is necessary. Parallel LC provides great benefits for this situation:

- The injections on the two sub-systems can take place from the same vial. This decreases the sample preparation efforts.
- Chromeleon's reporting tools facilitate concise reporting of the combined results from the two methods. For example, presence of a compound can be confirmed based on presence (and spectrum) in both chromatograms; average amount can be calculated, etc.
- The complete result (i.e. combined results from the two methods) becomes available much faster for the single samples (compared to the scenario when the two analyses are performed sequentially, i.e. Method 2 is run on the same system after all samples have been analyzed with Method 1).

Isocratic Methods Using the Same Eluent

If both methods are isocratic and they use the same eluent (i.e. on two different columns) the implementation becomes relatively simple as eluent transfer between the flow paths is never an issue. Intelligent control and monitoring features ensure that the autosampler handles requests from both sub-systems to perform an injection at the same time. In such a case the software puts the second sub-system on hold until the first sub-system has completed the injection. This is shown in Figure 5.

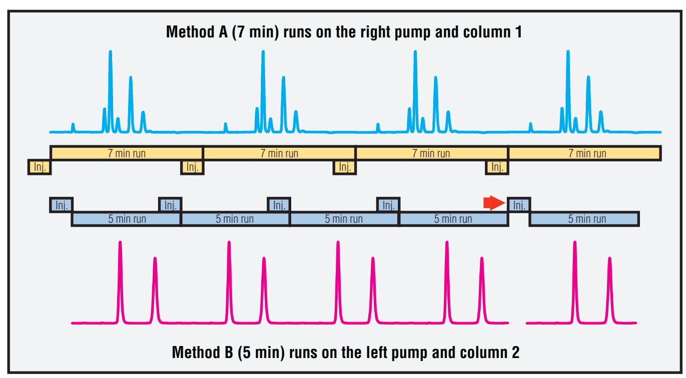

Figure 5. Running isocratic methods with the same eluents: The user does not need to worry about switching the autosampler between the sub-systems. In case of conflict (marked with red arrow) the software slightly delays the following injection and continues as soon as the autosampler becomes available.

Gradient Methods and Isocratic Methods Using Different Eluents

If the eluents are different or the methods use gradient elution the Eluent Requirements (see above) must be considered. As discussed later, the Chromeleon® Chromatography Management Software provides the tools for avoiding eluent mixing issues.

For a complete application example, see our presentation A Total Solution for Explosives Analysis by Reversed-Phase HPLC with a Parallel HPLC System.[1]

SOFTWARE SOLUTIONS

Control of a powerful solution such as Parallel LC requires powerful software.

In the presented solution, the instruments behave (from the user's point of view) as if two independent HPLC systems were present, despite the fact that some modules are shared.

The software has to ensure seamless cooperation of the two sub-systems and facilitate easy user interaction with them. Below we discuss the solutions Chromeleon provides for this.

System Configuration

First of all, the system must be configured with clear indication of which sub-system each module belongs to. Figure 6 shows the dialog boxes for configuring the shared modules.

Common and Specific Instrument Commands

A dual-gradient pump contains two independent pumps. There are however some instrument control commands that are valid for both sub-devices; for example, commands related to leak detection. The Chromeleon software ensures that common and specific commands are properly addressed during the runs.

Checking Method Parameters

Because the autosampler and the column compartment are shared, the two methods must use matching temperatures. If the user attempts to start methods that require different column oven or autosampler temperatures, the system will not start the second sample sequence and the user will be notified as shown in Figure 7.

⚠ {ColumnOven} All samples scheduled for run on timebases UltiMate_L,UltiMate_R need to specify the same value for property (nominal) Temperature.
⚠ {Sampler} All samples scheduled for run on timebases UltiMate_L,UltiMate_R need to specify the same value for property TempCtrl.

Figure 7. Chromeleon's Ready Check function ensures that sequences, including the control programs, are syntactically and semantically correct. For Parallel LC systems it also confirms that there are no conflicts regarding the shared parameters. If this is not the case, the user gets a clear message.

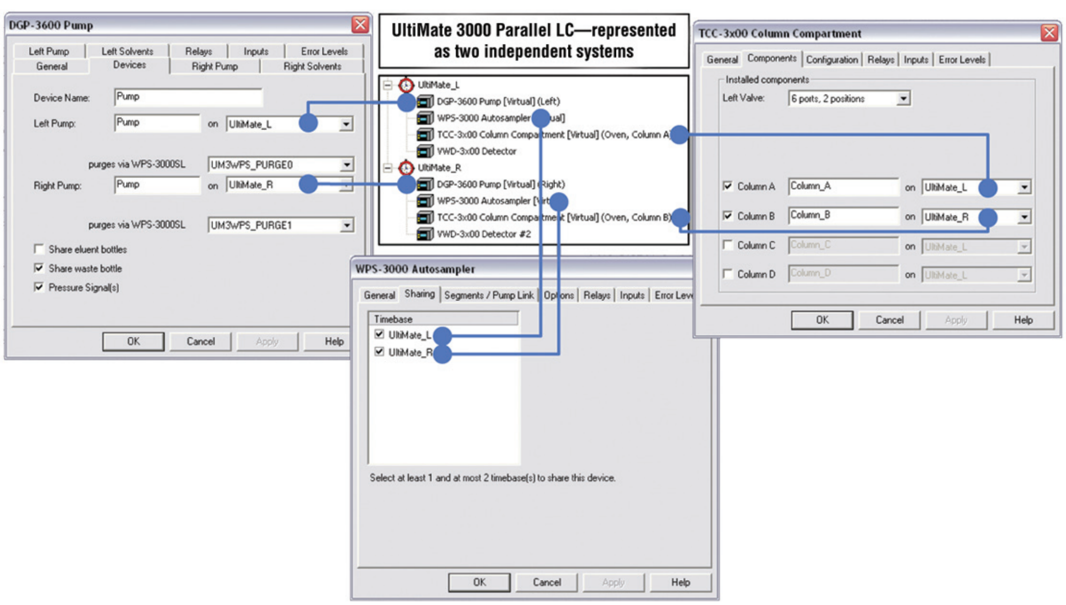

Figure 6. Configuring an UltiMate 3000 Parallel LC. In the shared dual-pump each of the independent sub-modules is assigned to its corresponding sub-system. For sharing the autosampler it is enough to indicate which sub-systems can access it. The column compartment is shared by assigning the two columns to the two different sub-systems.

Exclusive Access to the Autosampler

Correctly timed switching of the autosampler between the two sub-systems is an important consideration with Parallel LC. Two complications must be avoided:

- Sample mixing: Remainder of a sample enters a foreign flow path.
- Eluent mixing: Eluent with improper composition enters a foreign flow path.

Sample Mixing

The software automatically prevents sample mixing, as each sub-system takes exclusive access of the autosampler while it performs an injection. The Exclusive Access Time is automatically calculated during generation of the instrument method (Program) based on instrument and method parameters (such as Capillary Void Volume, Inject Volume, and Flow) and considering a user-defined Flush Out Factor (Figure 8). If an injection request arrives from one sub-system while the other one is already injecting, the software will simply put this sub-system on hold until the other one releases its exclusive access.

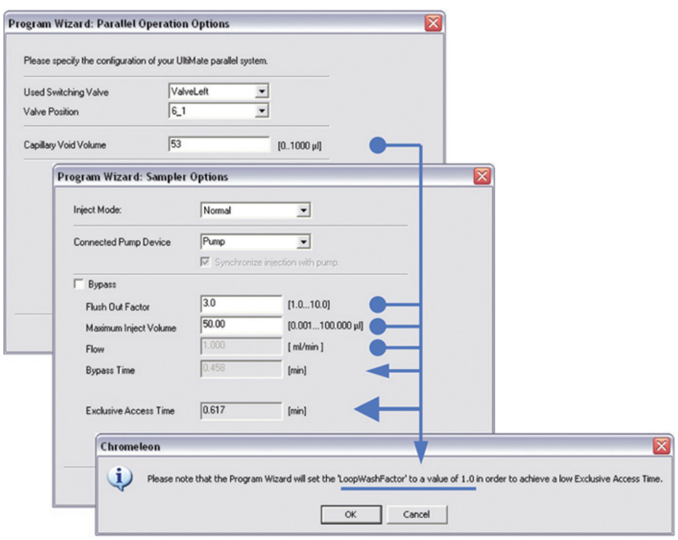

Figure 8. To prevent sample mixing (i.e. one sub-system taking control of the autosampler before the previous injection on the other sub-system is finished) each sub-system takes exclusive access of the autosampler throughout an injection. During generation of the instrument method (Program) the software automatically calculates the Exclusive Access Time. The calculation is based on instrument parameters (e.g. Capillary Void Volume) and method parameters (e.g. Flow, Inject Volume), and a user defined Flush Out Factor. Related parameters, like the Loop Wash Factor, are also optimized.

Eluent Mixing

Prevention of eluent mixing cannot be completely automated as the software is not aware of the chemical properties of the actual eluents in the two flow paths. However, the user can easily extend the period while one sub-system has exclusive access to the autosampler. This way the user can ensure not only that the injection has taken place, but also that the elution gradient has passed the autosampler before it is switched to the other flow path. This is illustrated in Figure 4.

Error Handling

The software ensures that a problem that only affects one sub-system does not (immediately) interfere with the analysis on the other one.

If there is a problem that affects a non-shared module, the other sub-system will continue to work without interruption. For example:

- If the pressure limit is exceeded on the left sub-system and it stops, the right sub-system can finish running its sequence of samples.

If the problem affects a shared module, the problem is handled in a way that ensures secure operation but influences the sub-systems as little as possible. For example:

- If there is a fatal problem with the shared column oven, like a leakage, this will stop both sub-systems. This is necessary for safe operation.
- If there is a problem with the shared autosampler while the left sub-system has exclusive access to it, the currently running sample can be finished on the right sub-system without interruption. The analyst can then either correct the problem, or deactivate the left sub-system and continue running exclusively on the right sub-system.

System Monitoring and Control

The software provides easy means of monitoring and controlling both sub-systems. Chromeleon's "Panel Tabsets" allow fast switching between user interface tabs. These allow immediate access to the control screens for any module (Figure 9).

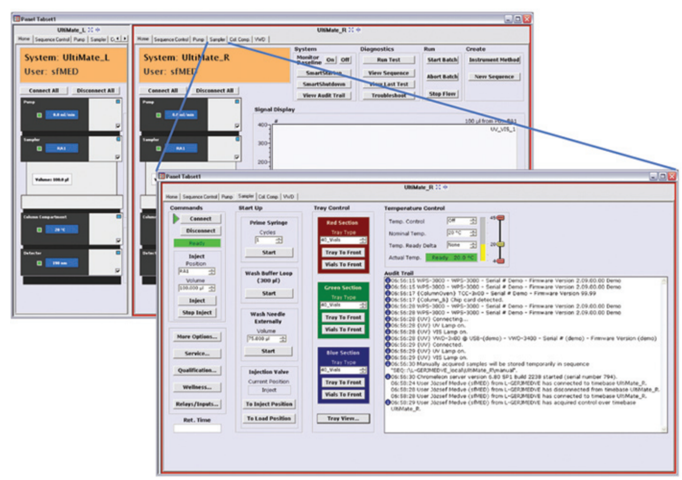

Figure 9. Panel Tabsets provide well structured overview and easy control possibilities for the two sub-systems. The screen can be shared between the sub-systems (as in the background) or it can be filled with the controls of a single module (as in the foreground).

Reporting

One application area where Parallel LC is especially advantageous is analyzing the same sample with orthogonal methods (see Application Examples above). In this case Chromeleon can create reports where data from the two methods are consolidated. For example, presence of a compound can be confirmed if present in both chromatograms or the average amount can be calculated.

Explosives Analysis Report

Sample: Soil sample

Column:		Acclaim Explosives Column E1				Acclaim Explosives Column E2							
No.	Name	RT (min)	Peak Purity Match	Ref. Spectra Match	Amount ppm	RT (min)	Peak Purity Match	Ref. Spectra Match	Amount ppm	Confirmation	Peak Purity (Pure >= 950)	Average Amount	% Rel. Amount Deviation
2	RDX	8.217	1000	1000	1.15	11.183	1000	1000	1.15	Confirmed	Pure	1.15	0.00
7	2,4,6-Trinitrotoluene	21.433	1000	1000	1.57	21.333	1000	1000	1.47	Confirmed	Pure	1.52	6.37
8	4-Amino-2,6-Dinitrotoluene	23.658	1000	1000	0.99	34.692	999	1000	1.04	Confirmed	Pure	1.01	4.85
9	2-Amino-4,6-Dinitrotoluene	25.167	1000	1000	0.99	36.167	999	1000	1.00	Confirmed	Pure	0.99	1.17
10	2,6-Dinitrotoluene	26.158	960	994	0.10	24.583	881	988	0.11	Confirmed	Impure	0.11	7.44
11	2,4-Dinitrotoluene	27.458	906	956	0.05	n.a.	n.a.	n.a.	n.a.	Unconfirmed	Impure		
12	2-Nitrotoluene	29.833	1000	1000	3.01	28.350	1000	1000	2.78	Confirmed	Pure	2.89	8.20
13	4-Nitrotoluene	32.792	999	1000	1.30	30.875	999	1000	1.20	Confirmed	Pure	1.25	8.21
14	3-Nitrotoluene	34.792	922	994	0.11	33.200	919	980	0.10	Confirmed	Impure	0.11	5.76
1	HMX	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Not Present			
3	1,3,5-Trinitrobenzene	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Not Present			
4	1,3-Dinitrobenzene	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Not Present			
5	Nitrobenzene	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Not Present			
6	Tetryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Not Present			

Figure 10. Example of a consolidated Chromeleon report. The same soil sample was analyzed on two different columns (E1 and E2) in order to determine its contamination with explosives. Presence of a compound is confirmed if it is present in both methods. In this case the average amount is also calculated from the results given by the two methods.

CONCLUSION

Intelligent module sharing between HPLC systems can almost double the productivity of a traditional HPLC system, without the need for modifying and revalidating existing analytical methods.

Parallel LC is especially suitable in the following analytical scenarios:

- Doubling throughput: Analyzing different samples with the same isocratic or gradient* method on both sub-systems
- Orthogonal analysis: Analyzing the same samples with two different methods on the two sub-systems

Powerful Chromeleon software helps users while working with a Parallel LC system:

- Easy system configuration via dialog boxes
- Intelligent handling of exclusive access to the shared autosampler
- Prevention of typical user errors (syntax and logical errors as well)
- Intelligent error handling
- Easy system monitoring and control
- Powerful reporting, including consolidated data from the two sub-systems

*NOTE: For short gradient runs, Tandem LC can be an even more economical alternative.[2] Tandem LC is designed to operate with two identical columns running the same application. Compared to a traditional HPLC setup it needs an additional pump, but not an additional detector, as in Parallel LC.

REFERENCES

1. Maio, G.; Steiner, F.; Franz, H.; Tracy, M.; Liu, X.; Arnold F. A Total Solution for Explosives Analysis by Reversed-Phase HPLC with a Parallel HPLC System, Poster, Abstract #780-11P, Pittcon, Chicago, IL, February 25–March 1, 2007.
2. M. Karsten, B. Dolman, G. Maio, F. Steiner, H. Franz, F. Arnold and R. Swart: Increasing Throughput in LC and LC-MS with a Parallel HPLC System, Poster, Pittcon, Orlando, FL, March 12–17, 2006. (Available at www.dionex.com).

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LPN 1910-01 03/07