

# Eliminating Delays Caused by Column Wash and Reconditioning to Increase Throughput for Gradient HPLC/UHPLC Methods

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## Key Words

UHPLC, HPLC, Gradient Method, Tandem Operation, x2 Dual, UltiMate 3000 LC System, Chromeleon 6.8 CDS Software, Smart Software, Smart Hardware, eWorkflow, Water Soluble Vitamins, Dual-Gradient

## Summary

Many fast UHPLC and traditional HPLC methods suffer under delays caused by the need of washing and equilibrating the column after the separation. Typically we see 20% – 40% of the runtime used up by these essential steps within a gradient method.

This document describes a solution utilizing a dual-gradient pump setup with column switching capabilities to overcome these delays without the need of redeveloping existing methods. The described “smart hardware and smart software solution” provides a turn-key approach for the everyday routine application as well as speeding-up research type of LC and UHPLC assays.

## Background

Washing and reconditioning HPLC and UHPLC columns after a successful separation is not only a “best practice”, but is simply essential. Doing adequate washing steps will not only extend the column life time significantly, but will also reduce column based sample carry over. Lastly and ultimately, it is very critical for maintaining stable retention times.

Column washing steps and column reconditioning though requires additional run time and causes avoidable delays before the next injection. Depending on the internal system delay volumes, matrix complexity and column volume, these delays can present a substantial part of the overall run time (20% – 40%).

Even for isocratic methods, the experienced chromatographer may decide to incorporate a wash step to protect his or her LC column from premature aging and excessive sample matrix accumulation.

## Solution

Tandem operation is a robust and fully automated UHPLC and LC setup which features 2 integrated, fully independently controllable gradient pumps. One 2-position 10-port column switching valve switches 2 identical separation columns between the active separation flow path and the “offline” reconditioning flow path. In this manner, one column is used for the ongoing separation, while the other column is switched offline from the detector, washed and thoroughly conditioned for the next injection.

The dual-gradient pumps always operate as conditioning (i.e. Left Pump) or separation pump (i.e. Right Pump). They have dedicated jobs so to speak. As some of the connecting tubing between valve and autosampler is part of both flow paths, there is also a brief wash step before switching of the columns.

Thermo Scientific™ Dionex™ Chromeleon™ 6.8 Chromatography Data System (CDS) software and the Thermo Scientific™ Dionex™ UltiMate™ 3000 LC Dual System fully automate the tandem operation and offer easy tools for employing quantitation alternatives, immediately traceable system suitability testing and an advanced method setup wizard. For easy and dead-volume free plumbing, dedicated Thermo Scientific™ Dionex™ Viper™ fingertight fitting system connection kits are available.

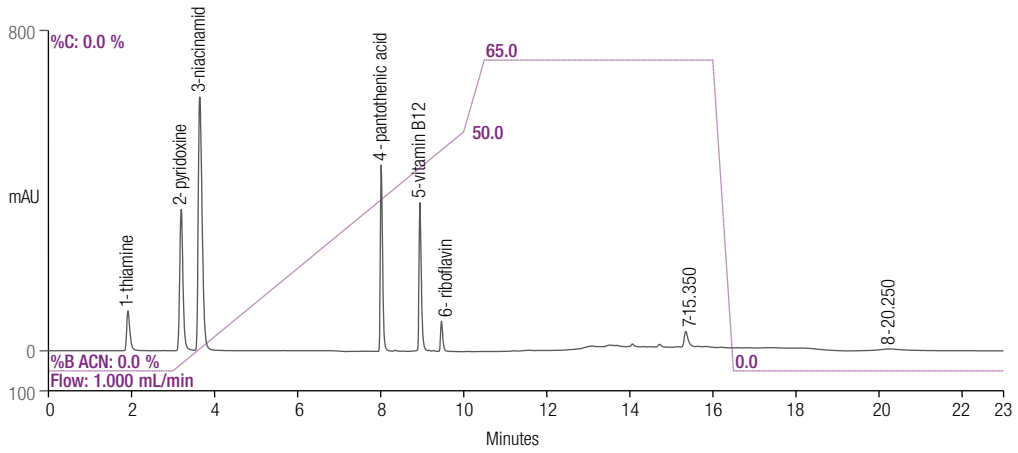


Figure 1. Original Method for Water Soluble Vitamins, Thermo Scientific™ Acclaim™ PA Column, 5 μm × 2.1mm ID × 150mm, pH 3.4.

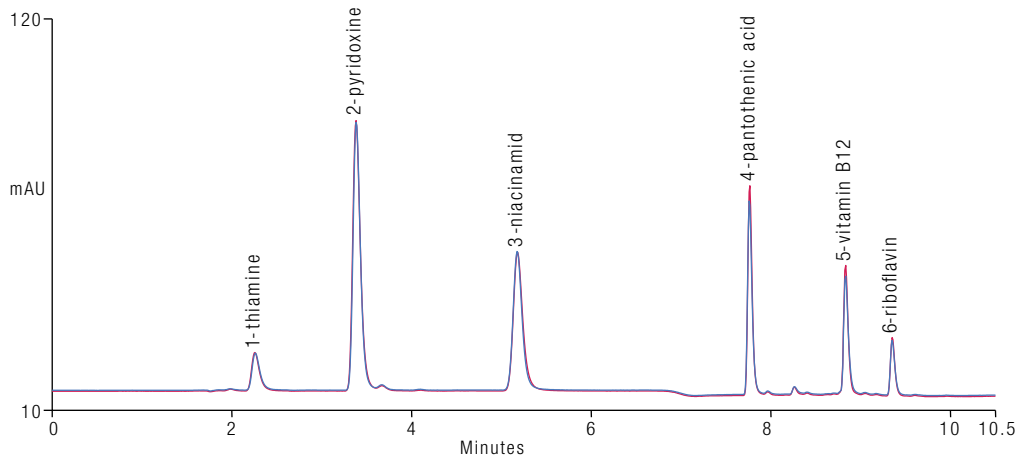


Figure 2. Tandem Method for Water Soluble Vitamins, Overlay of Two Consecutive Runs Using Column A and Column B, 10 × diluted.

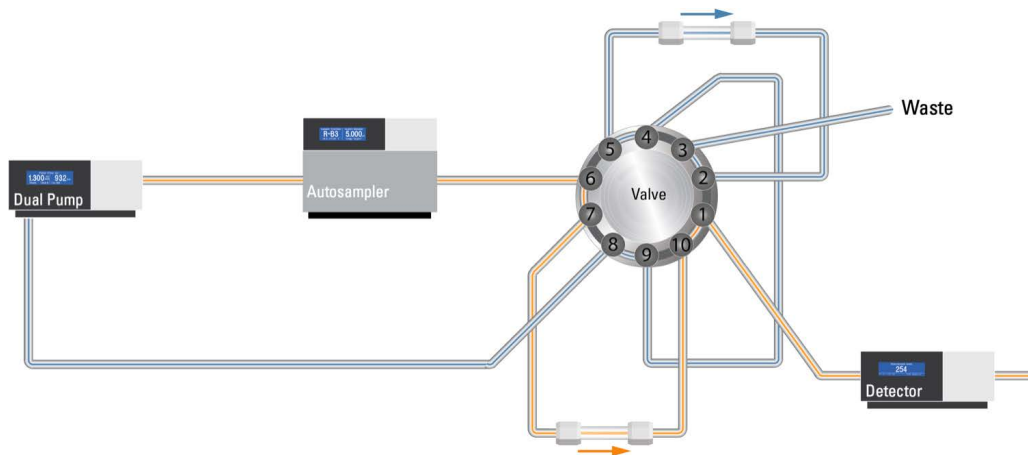
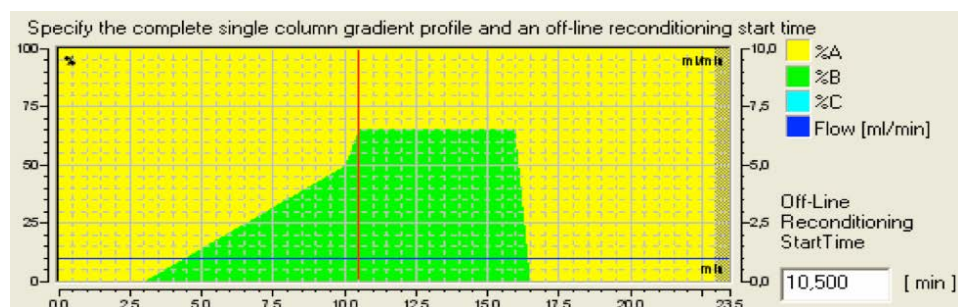


Figure 3. Tandem Setup for a Dual Pump HPLC or UHPLC System.

Program your currently established gradient method as you would on any standard system setup. Choose the switching point after the last peak of interest has eluted either by moving the red line or inserting the time in the editing field.



Chromleon CDS automatically calculates and generates individual gradient tables for the separation pump (here Left Pump) or the reconditioning pump (here Right Pump). The user can choose higher or lower conditioning flows to either extend column washing or save mobile phase (buffer B).

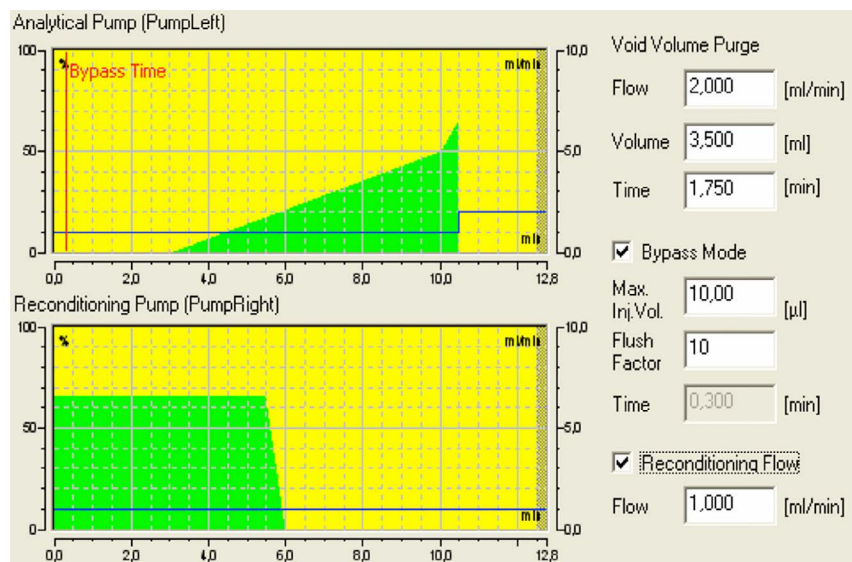


Figure 4. Chromleon 6.8 CDS Tandem Gradient Table Wizard.

Simply choose calibration strategy. Select calibration curve to be calculated using both columns in one data set or generate a separate calibration curve for each individual column used (Dual-Column Separate Calibration).

Title:  Unidentified peaks...

Retention time settings:  
 Use recently detected retention times  
of last

Peak retention time determination:  
 Use absolute greatest signal value  
 Use relative greatest signal value over the baseline

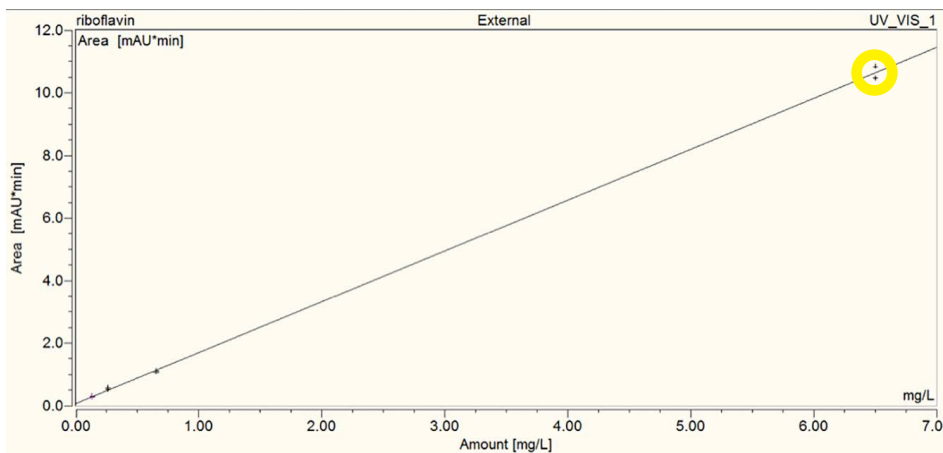
Amount interpretation:  
Dimension of amounts:   
Reference inject volume:  
 Use inject volume of first standard  
 Fixed:   $\mu$ l

Global calibration settings:  
Mode:   
 Auto Recalibrate   
Curve Fitting:   
 Dual-Column Separate Calibration

Dead/Delay time(s):  
Dead time:  min  
2nd Detector:  min  
3rd Detector:  min

Blank Run & Matrix Blank Subtraction:  
 No blank run subtraction  
 Subtract recent blank run sample in corresponding sequence  
 Subtract a fixed sample:   
  
 Enable matrix blank subtraction

Example A => Both Columns On One Calibration Curve



Example B => Individual Columns with Separate Calibration Curve

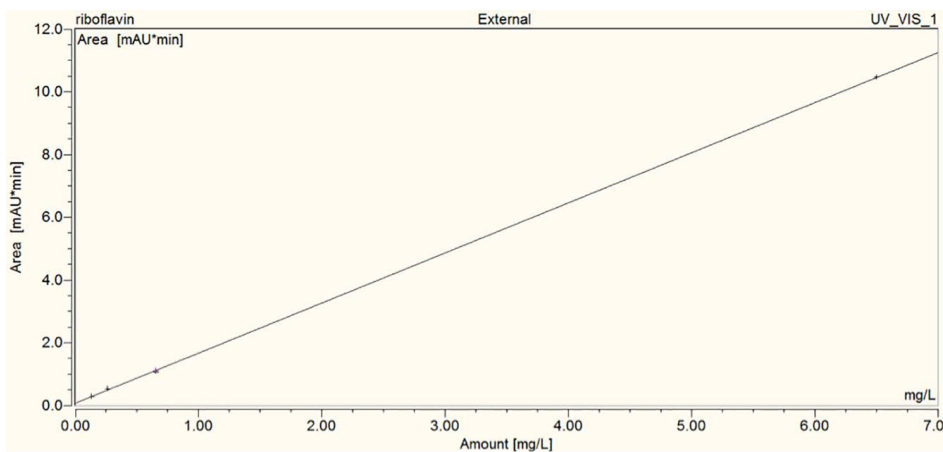


Figure 5. Chromeleon 6.8 CDS Processing Method, Choose Calibration Strategy.

Table 1. UltiMate 3000 SD Dual LC System (62 MPa, 9,000 psi, 520 bar).

Product ID	Type/Name	Part Number	Link/Comment
SRD-3600	Solvent Rack Degasser	5035.9230	<a href="http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/solvent-tray-degasser/rack-w-degasser/lp-72536.html">http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/solvent-tray-degasser/rack-w-degasser/lp-72536.html</a>
DGP-3600SD	Dual Pump	5040.0061	<a href="http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/pumps/dual-gradient-analytical/lp-72528.html">http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/pumps/dual-gradient-analytical/lp-72528.html</a>
WPS-3000TSL	Autosampler	5822.0020	<a href="http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/autosamplers-injectors/analytical/lp-72505.html">http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/autosamplers-injectors/analytical/lp-72505.html</a>
TCC-3000SD	Column Compartment	5730.0010	<a href="http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/column-compartments/thermo/lp-72512.html">http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/column-compartments/thermo/lp-72512.html</a>
Valve Actuator Kit HT	Valve Motor (left)	6730.0002	as above
Pod 2-pos 10-port, type HT	Valve Pod	6730.0026	up to 15000 psi
DAD-3000	Diode Array UV/VIS	5082.0010	<a href="http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/detectors/diode-array/lp-72513.html">http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/detectors/diode-array/lp-72513.html</a>
Analytical Flow Cell for DAD-3000, 13 $\mu$ L, 10mm path length	Flow Cell for DAD	6082.0100	as above
Viper UHPLC Fingertight Fitting and Capillary Kit	For Tandem Operation on UltiMate 3000 x2 Dual Standard LC Systems	6040.2804	<a href="http://www.dionex.com/en-us/products/accessories/reagents-accessories/viper-fingertight/lp-81335.html">http://www.dionex.com/en-us/products/accessories/reagents-accessories/viper-fingertight/lp-81335.html</a> 180 $\mu$ m ID

Table 2. UltiMate 3000 RS Dual LC System (103.4 MPa, 15,000 psi, 1034 bar).

Product ID	Type/Name	Part Number	Link/Comment
SRD-3600	Solvent Rack Degasser	5035.9230	<a href="http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/solvent-tray-degasser/rack-w-degasser/lp-72536.html">http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/solvent-tray-degasser/rack-w-degasser/lp-72536.html</a>
DGP-3600RS	Dual Pump	5040.0066	<a href="http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/pumps/dual-gradient-rslc/lp-82032.html">http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/pumps/dual-gradient-rslc/lp-82032.html</a>
WPS-3000TRS	Autosampler	5840.0020	<a href="http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/autosamplers-injectors/rslc/lp-72504.html">http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/autosamplers-injectors/rslc/lp-72504.html</a>
TCC-3000RS	Column Compartment	5730.0000	<a href="http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/column-compartments/rslc-thermo/lp-72511.html">http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/column-compartments/rslc-thermo/lp-72511.html</a>
Valve Actuator Kit HT	Valve Motor (left)	6730.0002	as above
Pod 2-pos 10-port, type HT	Valve Pod	6730.0026	up to 15000 psi
DAD-3000RS	Diode Array UV/VIS	5082.0020	<a href="http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/detectors/diode-array/lp-72513.html">http://www.dionex.com/en-us/products/liquid-chromatography/lc-modules/detectors/diode-array/lp-72513.html</a>
Semi-Micro Flow Cell for DAD-3000, 2.5 µL, 7mm path length	Flow Cell for DAD	6082.0300	as above
Viper UHPLC Fingertight Fitting and Capillary Kit	For Tandem Operation on UltiMate 3000 x2 Dual RS LC Systems	6040.2803	<a href="http://www.dionex.com/en-us/products/accessories/reagents-accessories/viper-fingertight/lp-81335.html">http://www.dionex.com/en-us/products/accessories/reagents-accessories/viper-fingertight/lp-81335.html</a> 130 µm ID

## Useful Links

Tandem Analysis for HPLC High Throughput

<http://www.dionex.com/en-us/products/liquid-chromatography/lc-solutions/dual-configuration/tandem-analyses/lp-80980.html>

Computer Assisted Design of Column Switching Instrument Methods for Reduced Chromatography Run Times

[http://www.dionex.com/en-us/webdocs/35679-LPN%201795-02\\_Computer%20assisted.pdf](http://www.dionex.com/en-us/webdocs/35679-LPN%201795-02_Computer%20assisted.pdf)

Increasing Analysis Throughput on HPLC Instruments—What is the Smartest Strategy?

[http://www.dionex.com/en-us/webdocs/40387-LPN%201849-01\\_Increasing%20analysis.pdf](http://www.dionex.com/en-us/webdocs/40387-LPN%201849-01_Increasing%20analysis.pdf)

Achieving Maximum Productivity by Combining UHPLC with Advanced Chromatographic Techniques

<http://www.dionex.com/en-us/webdocs/77378-PO-Pittcon-UHPLC-05Mar2010-LPN2421-01.pdf>

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