Flexible HPLC instrument setups for double usage as one heart-cut-2D-LC system or two independent 1D-LC systems

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Goal

Showcase several heart-cut 2D-LC setups with elution strength reduction implemented with the Thermo Scientific™ Vanquish™ UHPLC platform and highlight the benefit of the Thermo Scientific™ Vanquish™ Dual Split Sampler to build a flexible system, which can be used either as one heart-cut 2D-LC system or as two independent 1D-LC systems

Benefits

- Heart-cut 2D-LC with analyte trapping during fraction transfer enables straightforward peak identity and purity confirmation by MS detection despite MS incompatible mobile phases in the first-dimension separation.

- The Vanquish Dual Split Sampler in a 2D-LC setup greatly improves the instrument usability and value.

- Flexible, software-aided switching between the application options as a heart-cut 2D-LC system or as two independent 1D-LC systems enables more efficient instrument operation and increases the return on investment of the LC instrument as well as of associated MS detectors.

Introduction

Conventional liquid chromatography (LC) separations are referred as one-dimensional (1D) as the sample is subjected to one single separation process. However,

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despite considerable improvements in the commercially available LC hardware (instrumentation as well as column technology), 1D-LC sometimes cannot meet the demands in separation and resolution capabilities of the analytical task.[1,2] Sometimes a sufficient solution is the analysis of one sample by dual or multiple complementary techniques.[3] Another recent trend goes towards two-dimensional (2D) LC techniques, meaning in general that fractions eluting from the first dimension ([1]D) are further analyzed by a second dimension ([2]D) separation. Herein, the selection of orthogonal dimensions increases the available separation space and thus the probability to resolve the compounds of interest.[2] The interface connecting both dimensions usually consists of a combination of fluidic valves and storage loops or trap columns. Such setups are referred to as online 2D-LC as the transferred fractions do not leave the fluidic system. There are also offline 2D-LC setups, where fractions from the [1]D are collected externally into vials for a later reinjection into the [2]D.[2,4] The technique heart-cut 2D-LC refers to the transfer of a single fraction; multi-heart-cut 2D-LC refers to the transfer of several fractions; and comprehensive 2D-LC refers to the transfer of the whole [1]D eluate in a large number of fractions.[4] Comprehensive 2D-LC is a powerful tool in achieving separation of highly complex samples with 100–1000 components as it is needed, for example, in omics research areas.

However, comprehensive 2D-LC also often overshoots the actual analytical task, when only one or a few fractions are of greater interest. For example, one frequently requested application relates to the unambiguous identification and purity confirmation of single peaks in quality control (QC) labs. Commonly utilized phosphate and other mass spectrometry (MS) incompatible buffers in the mobile phase of LC-UV methods usually prevent their direct transfer to MS detection. Thus, if further confirmation by MS is needed, an adjustment of the 1D-LC method is required, such as the use of MS compatible eluent additives. Resulting differences in the elution pattern compared to the original method cause extra effort and documentation for justification, especially in a regulated environment. A preferred solution in such cases is a direct transfer of the respective peak (heart-cut) from the [1]D LC-UV run to an MS compatible [2]D LC-MS separation, preferably

combined with a desalting step for the removal of residual salts from the [1]D mobile phase.[5] The current technical note summarizes several possibilities to set up heart-cut 2D-LC systems implemented with the Thermo Scientific™ Vanquish™ UHPLC platform, provides strengths of each setup, and highlights the benefit of employing the Thermo Scientific™ Vanquish™ Dual Split Sampler to create a flexible system, which can be used either as one heart-cut 2D-LC system or as two independent 1D-LC systems. Software method setup is illustrated as well.

Experimental
Chemicals
- Deionized water, 18.2 MΩ·cm resistivity or higher

- Fisher Scientific™ Acetonitrile, Optima™ LC/MS grade (P/N A955-212)

- Fisher Scientific™ Methanol, Optima™ LC/MS grade (P/N A456-212)

- Fisher Scientific™ Formic acid, Optima™ LC/MS grade (P/N A117-50)

- Fisher Chemical™ ortho-Phosphoric acid (85%) (P/N O/0515/PB08)

- Desmedipham and Phenmedipham were provided by a reputable vendor

Sample handling
- Vials (amber, 1.5 mL) (P/N 6000.0072)

- Thermo Scientific™ SUN-SRi 11 mm Orange Snap Caps PTFE/Sil (P/N 501 382)

Instrumentation
- Thermo Scientific™ Vanquish™ Flex UHPLC system consisting of:

  – Vanquish System Base (P/N VF-S01-A-02)

  – Vanquish Quaternary Pump F (P/N VF-P20-A-01) [for [1]D]

  – Vanquish Binary Pump F (P/N VF-P10-A-01) [for [2]D]

– Vanquish Split Sampler FT (P/N VF-A10-A-02)

– or Vanquish Dual Split Sampler FT (P/N VF-A40-A-01)

– 1× or 2× Vanquish Column Compartment H
  (P/N VH-C10-A-02) + conversion kit for 2 column
  compartments VH-C10-A-02 (P/N 6732.0007)

– Vanquish Diode Array Detector FG (P/N VF-D11-A-010)
  with Flow cell, standard biocompatible path length
  10 mm (13 µL, PEEK) (P/N 6083.0540) [for [1]D]

– Vanquish Variable Wavelength Detector F
  (P/N VF-D40-A-01) with Flow cell, Semi-micro,
  7 mm (2.5 µL, SST) (P/N 6077.0360) [for [2]D]

– 2× or 3× Biocompatible 2-Position/6-Port Column
  Switching Valve, 150 MPa, VH-C (P/N 6036.1560)*

– Workflow Kit, Vanquish for online 2D LC
  (P/N 6036.2030)

– Stack Stabilizer for stacks of ≥5 modules:

  • Ion Bench with stack mounting kit (P/N 6036.1720) or

  • Stack Stabilizer Kit for benchtop installation
    (P/N 6036.1710)

– MS Connection Kit for Vanquish (P/N 6720.0405)

• Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-
  Orbitrap™ Mass Spectrometer (P/N 0726030) [for [2]D]

*2× Biocompatible 2-Position/6-Port Column Switching Valve,
150 MPa, VH-C are part of the Workflow Kit, Vanquish for online 2D LC
(P/N 6036.2030)

The number of required modules depends on the
target fluidic setup (see following sections). The given
configuration should be considered as an example.
However, the module selection can be adapted to the
user's needs, e.g. a Vanquish Dual Pump F (VF-P32-A-01)
could be used instead of the two pumps above. Details on
the capillary setups are given in the figures.

Sample preparation

A 10 µg/mL solution of each desmedipham and
phenmedipham was prepared in acetonitrile/water
(30/70; v/v).

Chromatographic conditions

Table 1. Chromatographic conditions of flow path 1 or [1]D

Property	Setting		
Column	Thermo Scientific™ Hypersil GOLD™, 2.1 × 100 mm, 1.9 µm, 175 Å (P/N 25002-102130)		
Mobile phase	A: 0.1% Phosphoric acid in water (v/v) B: Acetonitrile		
Gradient 1	Time (min)	A	B
	0	90	10
	3.0	10	90
	4.0	10	90
	4.1	90	10
	8.0	90	10
Gradient 2	Time (min)	A	B
	0	90	10
	5.0	10	90
	5.9	10	90
	6.0	90	10
	12.0	90	10
Flow rate	0.6 mL/min (Gradient 1) 0.3 mL/min (Gradient 2)		
Column temperature	40 °C		
Autosampler temperature	4 °C		
Injection volume	5 µL		
Detector settings	210 nm; 10 Hz 0.5 s response time		

Table 2. Chromatographic conditions of flow path 2 or ²D

Property	Setting
Column	Thermo Scientific™ Accucore™ PFP, 2.1 × 50 mm, 2.6 µm, 80 Å (P/N 17426-052130)
Trap column	Thermo Scientific™ Hypersil™ Javelin BDS C18 Javelin guard column, 3 × 20 mm, 3 µm (P/N 28103-023006)
Mobile phase	A: 0.1% formic acid in water B: 0.1% formic acid in methanol
Gradient	Time (min)* A B 0 70 30 3.5 30 70 4.0 10 90 6.0 10 90 6.1 70 30 8.0 70 30 *Absolute gradient times may change if used as ²D
Flow rate	0.6 mL/min
Column temperature	40 °C
Autosampler temperature	4 °C
Injection volume	5 µL
Detector settings	210 nm; 10 Hz 0.5 s response time

MS settings

Table 3. Instrument and scan settings for the mass spectrometer

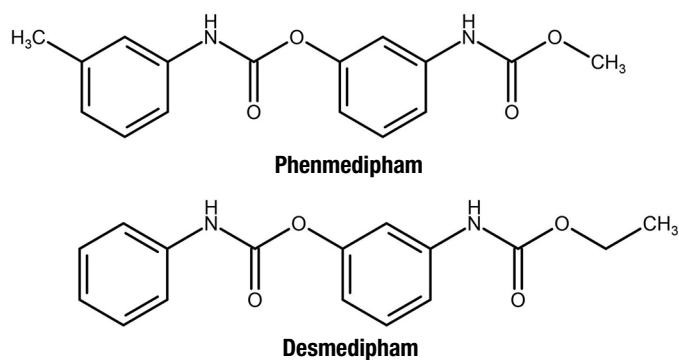| Property	Setting
Capillary temperature	350 °C
Sheath gas flow rate	35 Arb
Aux gas flow rate	8 Arb
Sweep gas flow rate	0 Arb
Spray voltage	3.7 kV
Aux gas heater temperature	250 °C
Scan settings	
Scan type	Full MS
Scan range	100–1000 m/z
Resolution	70,000
Polarity	Positive

Chromatography data system

Data acquisition and analysis is shown with Thermo Scientific™ Chromeleon™ CDS software, version 7.2.9, as well as with Thermo Scientific™ Xcalibur™ 4.0 software in combination with Thermo Scientific™ SII for Xcalibur 1.4 software.

Results and discussion

Background

The sample prepared for the current experiments serves as a proof-of-principle mixture to showcase the advantages of a 2D-LC separation. The two compounds, desmedipham and phenmedipham (Figure 1), are isomeric agrochemicals generally used as herbicides, which are hardly separable by C18 stationary phases and acetonitrile based mobile phases.

Heart-cutting, elution strength reduction, and trap washing

To ensure an appropriate mixing and trapping of transferred analytes, the idle ²D pump flow through the T-piece and trap column is set to 100% A. Just before the heart-cut, the flow rate is increased to 2 mL/min (if gradient 1 in Table 1 is used in the ¹D) or to 1 mL/min (if gradient 2 in Table 1 is used in the ¹D). Flow ramps (up and down) are set to 6 mL/min². The high flow rate is held during the heart-cut and for a short additional interval to wash the trap column and ramped down again before the ²D analysis starts. Heart-cut times were selected up to 50% broader than the peak base width to ensure complete peak transfer. Additional details are given in the appendix.

Phenmedipham

Desmedipham

Figure 1. Molecular structures of sample test compounds

Under the conditions given in Table 1 they co-elute in a single sharp peak (Figure 2). These conditions, utilizing non-volatile buffers (here phosphoric acid) in the mobile phase, reflect very common LC-UV conditions applied in QC labs. In comparison, Figure 3 shows the separation obtained by a direct 1D-LC-UV injection of the same sample under the conditions given in Table 2, which employ volatile, MS-compatible formic acid. Figure 4 displays the corresponding extracted ion chromatogram of the [M+H]+ ions of the two isomers and their peak apex spectra, which give slightly different fragmentation. Assuming an analysis according to the method in Table 1 runs in a QC lab, a transfer to MS compatible conditions like in the method in Table 2 would be required for MS based peak purity analysis or identity confirmation. The difference of the two methods, however, not only consists in the type of mobile phase buffer, but also in the stationary phase and organic modifier. They provide good orthogonality, essential in 2D-LC to increase the chance of chromatographic separation. Thus, they are also a good selection for a 2D-LC approach, providing chromatographic separation of the two isomers.

Conventional loop-based heart-cut 2D-LC setups

The most conventional way to set up single- or multi-heart-cut 2D-LC systems is depicted in Figure 5 for the Vanquish platform. Both setups utilize sampling loops in the interface connecting the 1D and 2D. The 1D pump and column are in-line with the injector; the upper two-position valve switches the 1D eluate to waste or to the loop. As soon as the target fraction is placed in the loop, the 2D flow path can be switched in-line with the loop to initiate the 2D analysis. For single heart-cut there is only one loop (between port 3 and 6 in the upper valve in Figure 5, left side) to cut a fraction to be analyzed in the 2D. If more fractions need to be collected, the setup can be upgraded to a multi-heart-cut system by replacing the single loop by several loops linking two selection valves (Figure 5, right side).
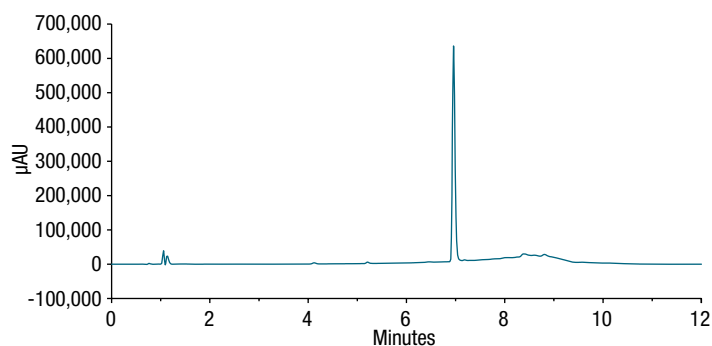

Figure 2. 1D-LC-UV chromatogram of the sample under MS-incompatible conditions given in Table 1 (gradient 2) recorded with setup 2 (see Figure 10)
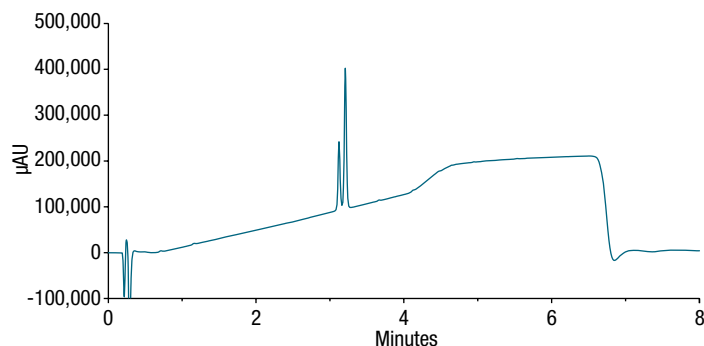

Figure 3. 1D-LC-UV chromatogram of the sample under MS-compatible conditions given in Table 2 recorded with setup 2 (see Figure 10)
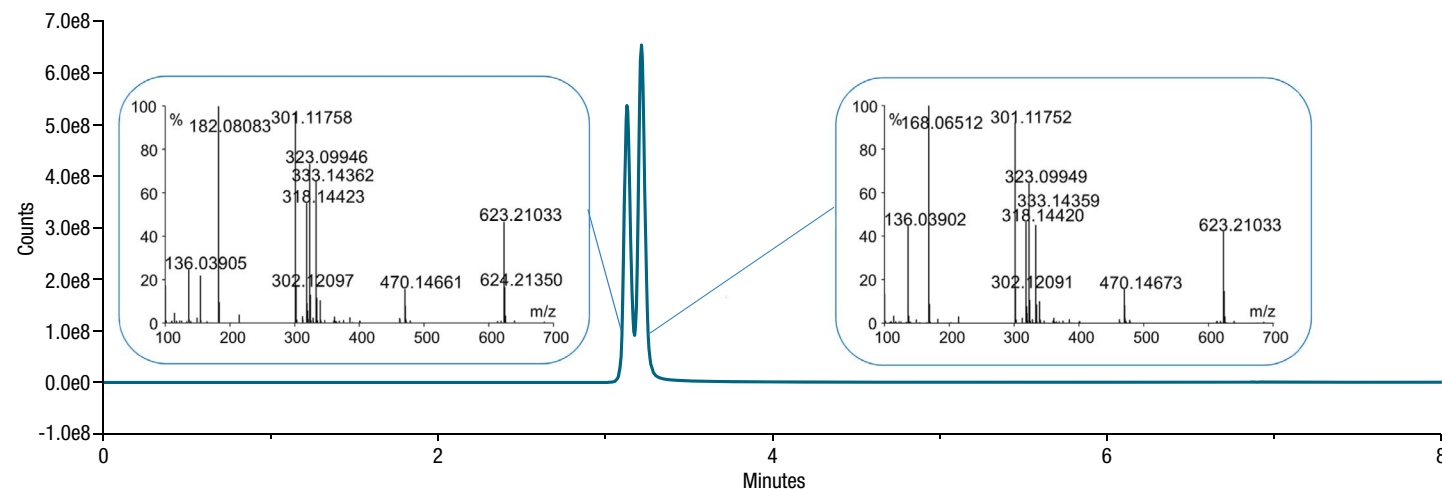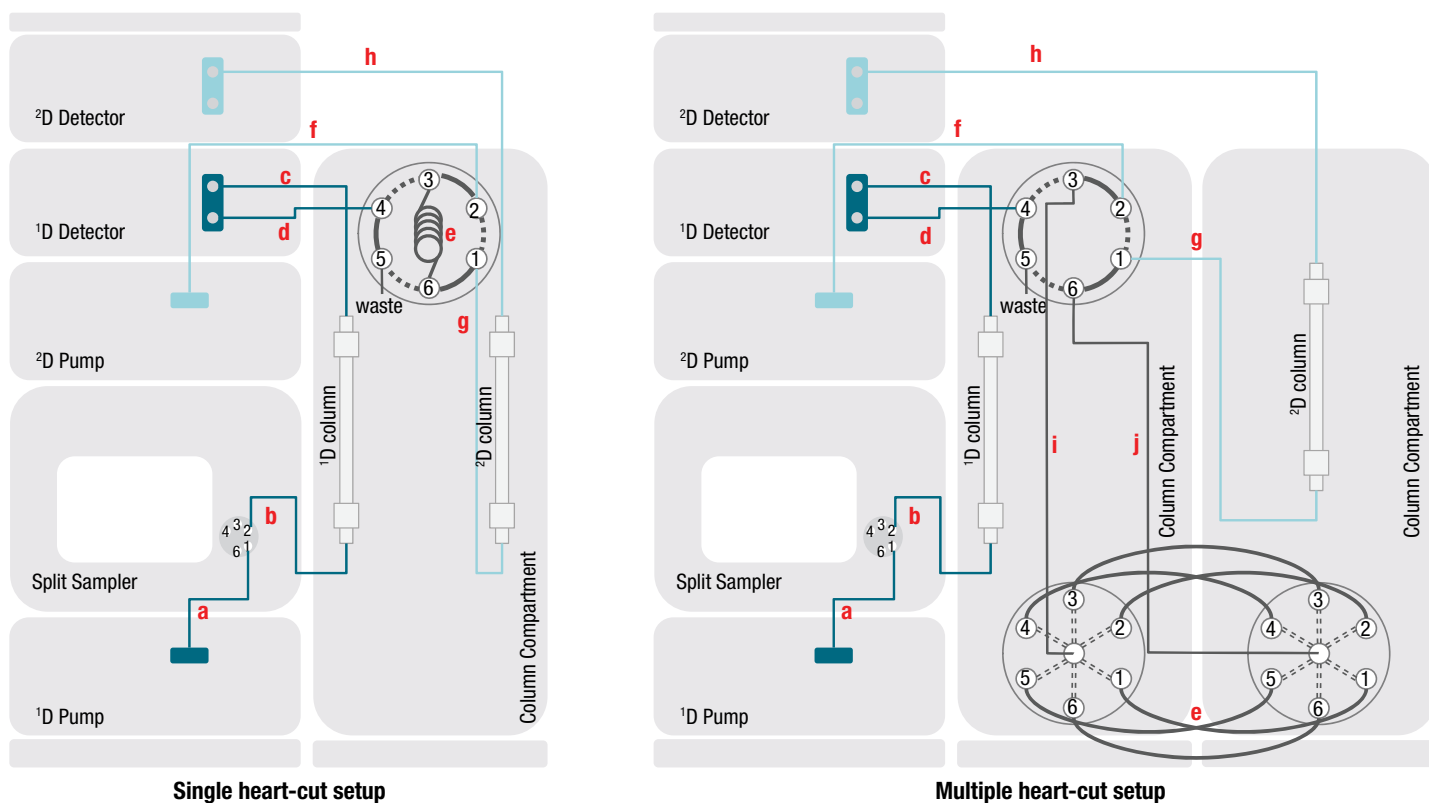

Figure 4. Extracted ion chromatogram of the [M+H]+ ions (mass range 301.11–301.12 m/z) and apex spectra of the sample under MS-compatible conditions given in Table 2 and Table 3 recorded with setup 2 (see Figure 10)

| Single heart-cut setup | | Multiple heart-cut setup | |

#	Connection	Part Number	#	Capillary	Part Number
a	Viper Capillary, ID × L 0.10 × 350 mm, MP35N	6042.2340	f	Viper Capillary, ID × L 0.1 × 450 mm, MP35N	6042.2350
b	Active Pre-heater, ID × L 0.1 × 380 mm, MP35N	6732.0110	g	Active Pre-heater, ID × L 0.1 × 610 mm, MP35N	6732.0150
c	Post-column Cooler 1 µL, ID × L 0.10 × 590 mm, MP35N	6732.0520	h	Post-column Cooler 1 µL, ID × L 0.10 × 590 mm, MP35N	6732.0520
d	Viper Capillary, ID × L 0.10 × 350 mm, MP35N	6042.2340	i	Viper Capillary, ID × L 0.1 × 650 mm, MP35N	6042.2370
e	Loops; size depends on the application; i.e.: Sample loop 200 µL, Viper Sample loop 20 µL, Viper	6830.2418 (200 µL) 6826.2420 (20 µL)	j	Viper Capillary, ID × L 0.1 × 650 mm, MP35N	6042.2370
	Waste fluidic, VH-D1	6083.2425			

Note: a and b capillaries are part of the module ship kits. Waste fluidics for connection to the detector are part of the detector ship kit.

Figure 5. Conventional, loop-based 2D-LC setups for single heart-cut and multi-heart-cut

Although these setups provide valuable 2D-LC capabilities, they come with one drawback frequently reported in 2D-LC: the fraction is transferred exactly as it exits the ^{1}D, including the peak(s) of interest as well as all constituents of the ^{1}D mobile phase that might impact the further process. On the one hand, the composition of the transferred solvent plug may affect the retention in the ^{2}D, if its elution power is strong and the volume is substantial.[3,6] On the other hand, mobile phase modifiers may have unwanted effects, like non-volatile salts that enter an MS detector.[5]

In the example of the peak in Figure 2, a fraction volume of around 35 µL (according to peak width and flow rate)

with an organic solvent content of >55% would have to be transferred on a small volume RP column like that given in Table 2. The result would be a very distorted peak shape if not a complete breakthrough. The trade-off in loop-based 2D-LC systems thus often consists in low flow rates and small diameter columns in the ^{1}D, high flow rates and wider diameter columns in the ^{2}D and small fraction volumes. However, a dilution of the fraction with solvent of weaker elution power is a helpful tool to overcome the described issue.[6] The same solvent can also be used to wash off salts before the MS detection. Systems that provide these advantages are presented in the following sections.

## Trap-based heart-cut 2D-LC setups with elution strength reduction

The utilization of a trap column instead of a sampling loop in the 2D-LC interface is not a solution by itself to the issue described above. Breakthrough still can occur, if strong solvent fractions are transferred to the trapping material, which usually is akin to the ^{2}D column phase. Instead, a reduction of the elution strength of the ^{1}D solvent plug provides an effective way to focus the analytes either on a trap column or at the head of the ^{2}D column directly. To achieve an adequate elution strength reduction, the cut fraction from the ^{1}D eluate needs to be thoroughly mixed with a solvent of weaker elution strength (e.g. highly aqueous phase in reversed-phase chromatography) before entering the respective column. This can be achieved with a high flow coming from a second pump. After the cut and trap process, the flow can optionally help to wash off non-volatile salts from the trap column before the MS detector is switched in-line for the ^{2}D analysis. Three different ways to set up and use Vanquish systems with such capabilities are presented here and discussed in detail. In summary, the configurations include the basic setups 1a and 1b employing a trap column, the setups 2a and 2b employing a trapping process on the ^{2}D column head and optional dual 1D-LC utilization, and the advanced setup 3, which covers all benefits of a trap column and the optional dual 1D-LC usage of the system.

Setups 1a and 1b – Basic setups with a trap column

Figure 6 shows two options of a simple setup *(setup 1a and 1b)* employing two pumps, two 6-port/2-position switching valves, a T-piece, and a trap column. Both options differ only in the position of the ^{1}D detector with respect to the heart-cutting valve with the rest of the functionality being identical as seen in Figure 7. The figure shows the four-step procedure: A) After the ^{1}D separation the upper switching valve controls the heart-cut of the ^{1}D fraction. When the valve is in position 1_2 the ^{1}D operates as a normal 1D-LC flow path. B) When it switches to position 6_1 the ^{1}D flow is combined and mixed with the ^{2}D flow in a T-piece and transferred to the trap column until C) the valve goes back to 1_2. The lower switching valve then controls the direction of the ^{2}D flow through the trap column. It is

either in position 1_2 for forward direction during idle time, fraction loading/mixing, and trap washing or D) in position 6_1 for the backward elution to the ^{2}D column and ^{2}D analysis. A configuration with both valves at position 6_1 at the same time should be avoided, to prevent non-volatile salts of the ^{1}D mobile phase from entering the MS.

The difference between setup 1a and 1b is in the detection of the ^{1}D during the heart-cut. In setup 1a the ^{1}D detector is installed after the heart-cutting valve, meaning that there is no ^{1}D detection during the cut (see Figure 8). In setup 1b the detector is placed between the ^{1}D column exit and the valve, enabling a non-interrupted detection of the ^{1}D (see Figure 9). However, this comes with a certain limitation in selectable flow rates as the detector is in line during the mixing and trapping process. The back pressure generated by the two mixing flows, the tubing, and the trap column during the heart-cut must not exceed the specified pressure limits of the detector flow cell, which is, for example, up to 120 bar for most of the flow cells of the Vanquish DAD FG. The choice is up to the user, but a thorough check of pressure conditions during method development is explicitly recommended for setup 1b. Figure 8 and Figure 9 depict the data generated for a heart-cut 2D-LC analysis of the test sample with either fluidic setup. The transferred peak is cut from the ^{1}D UV chromatogram with setup 1a (Figure 8), but it is present in the UV chromatogram with setup 1b (Figure 9). The expected separation of the two compounds in the ^{2}D is evident. Run times are different as a gradient of lower ^{1}D flow rate had to be used with setup 1b compared to setup 1a due to the indicated flow cell backpressure limitations. More details on 2D-LC method creation are outlined in a later section and the appendix. The pressure trace in Figure 9 indicates the crucial point where the detector cell must not be stressed over its specification limits. The spectra shown in Figure 9 provide an unambiguous peak identity confirmation. The data in Figure 8 and Figure 9 nicely demonstrate the concept of heart-cut 2D-LC with elution strength reduction. Data generated with the other tested setups do not significantly differ from these and thus are not shown.
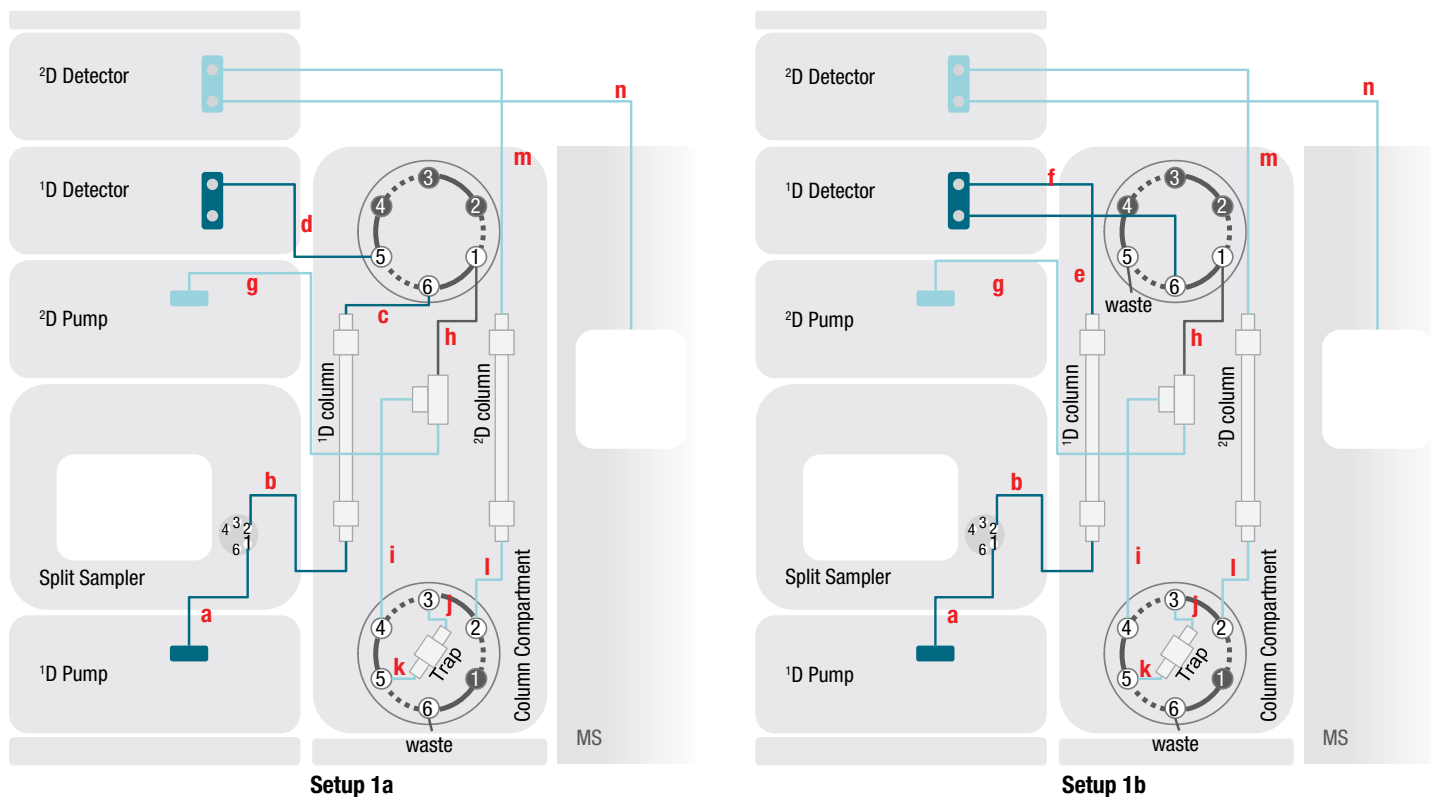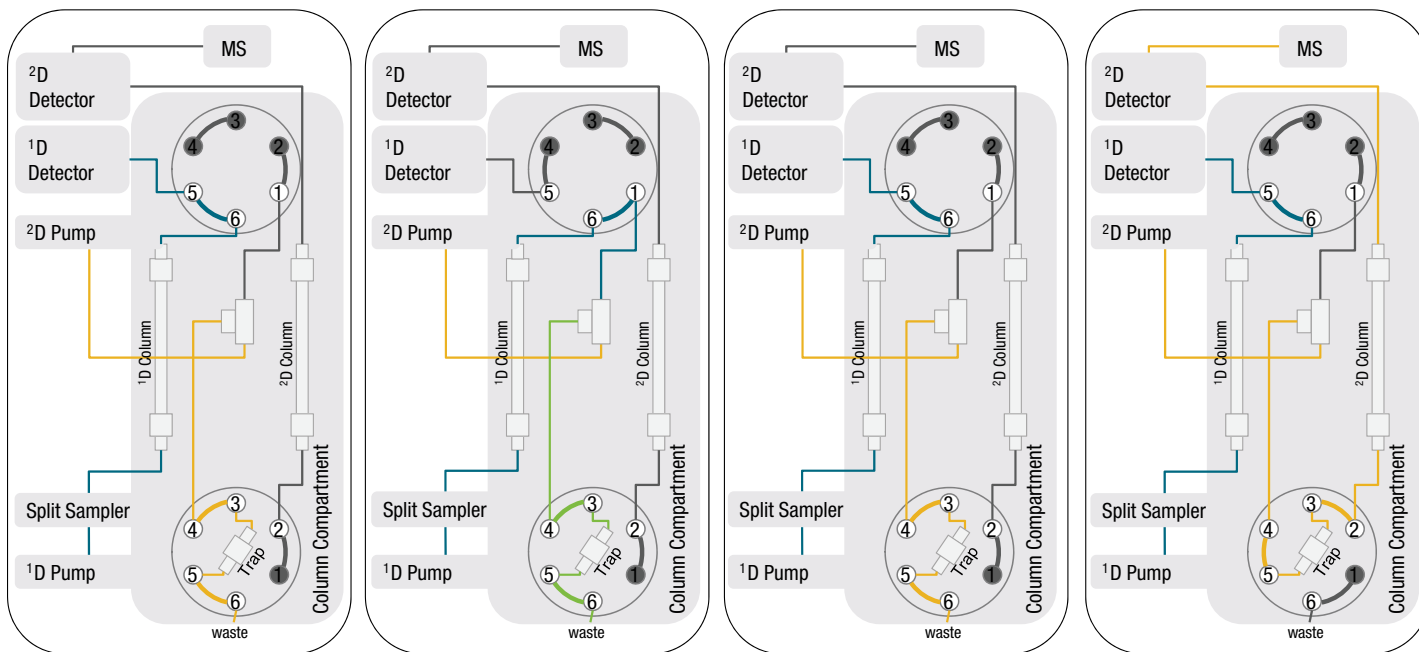
Setup 1a   **Setup 1b**

#	Connection	Part Number	#	Connection	Part Number
a	Viper Capillary, ID × L 0.10 × 350 mm, MP35N	6042.2340	h	Viper Capillary, ID × L 0.10 × 250 mm, MP35N	6042.2330
b	Active Pre-heater, 0.1 × 380 mm, MP35N	6732.0110	i	Viper Capillary, ID × L 0.18 × 450 mm, MP35N	6042.2365
c	Viper Capillary, ID × L 0.10 × 250 mm, MP35N	6042.2330	j	Viper Capillary, ID × L 0.10 × 150 mm, MP35N	6042.2320
d	Viper Capillary, ID × L 0.10 × 350 mm, MP35N	6042.2340	k	Viper Capillary, ID × L 0.10 × 250 mm, MP35N	6042.2330
e	Viper Capillary, ID × L 0.10 × 450 mm, MP35N	6042.2350	l	Active Pre-heater, 0.1 × 380 mm, MP35N	6732.0110
f	Viper Capillary, ID × L 0.10 × 350 mm, MP35N	6042.2340	m	Viper Capillary, ID × L 0.10 × 650 mm, MP35N	6042.2370
g	Viper Capillary, ID × L 0.10 × 550 mm, MP35N	6042.2360	n	Viper Capillary, ID × L 0.10 × 750 mm, MP35N	6042.2390
	Waste fluidic, VH-D1	6083.2425		Tee Piece ID 0.020'' for 1/16'' capillary	6263.0035
	6x Viper Blind plug	6040.2303			

Note: These configurations are supported by the Workflow kit, Vanquish for online 2D-LC (6036.2030). For more details, please refer to the Installation Guide, Vanquish for online 2D-LC. a and b capillaries are part of the module ship kits..

Figure 6. Trap-based heart-cut 2D-LC setups 1a and 1b with elution strength reduction
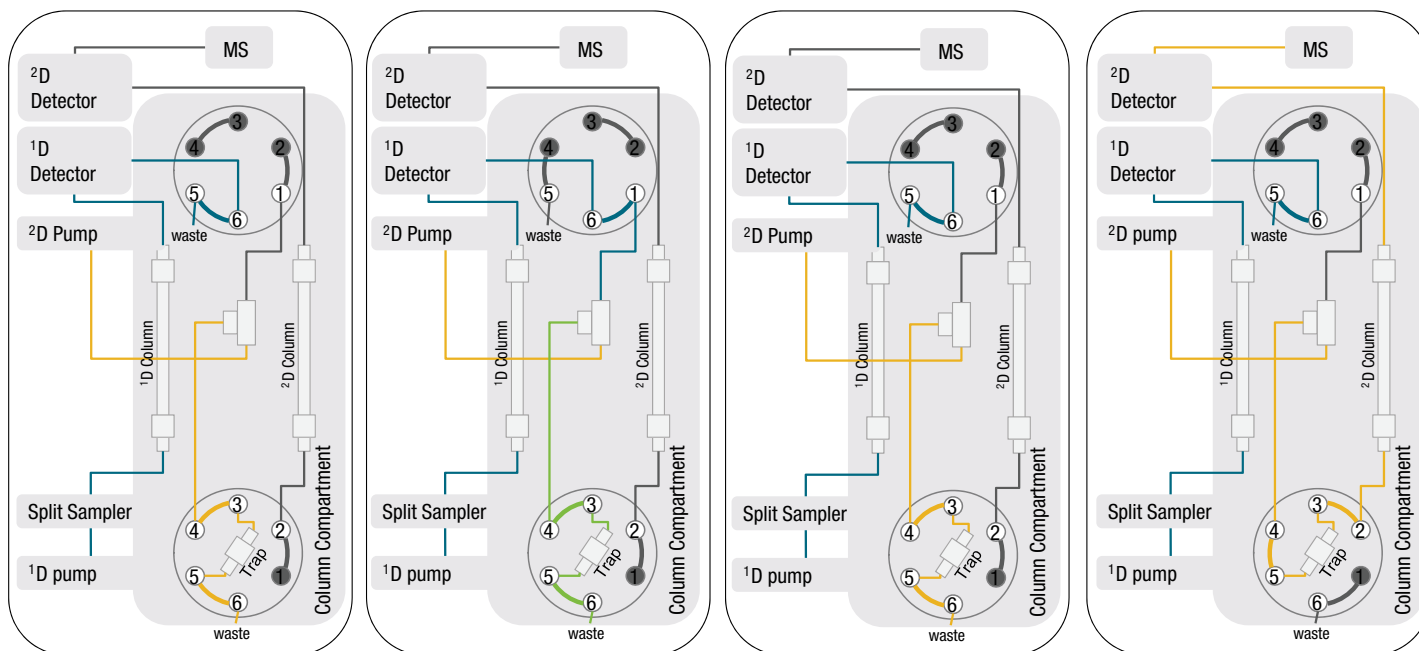
Setup 1a

A) ¹D analysis / ²D idle

B) Heart-cut with elution strength reduction and trapping

C) Trap washing (optional)

D) ¹D and ²D analysis

Setup 1b

A) ¹D analysis / ²D idle

B) Heart-cut with elution strength reduction and trapping

C) Trap washing (optional)

D) ¹D and ²D analysis

Action	Lower valve	Upper valve
A) ¹D analysis – ²D idle	1_2	1_2
B) Heart-cut + trapping	1_2	6_1
C) ¹D analysis + trap washing	1_2	1_2
D) ¹D and ²D analysis	6_1	1_2
AVOID	6_1	6_1

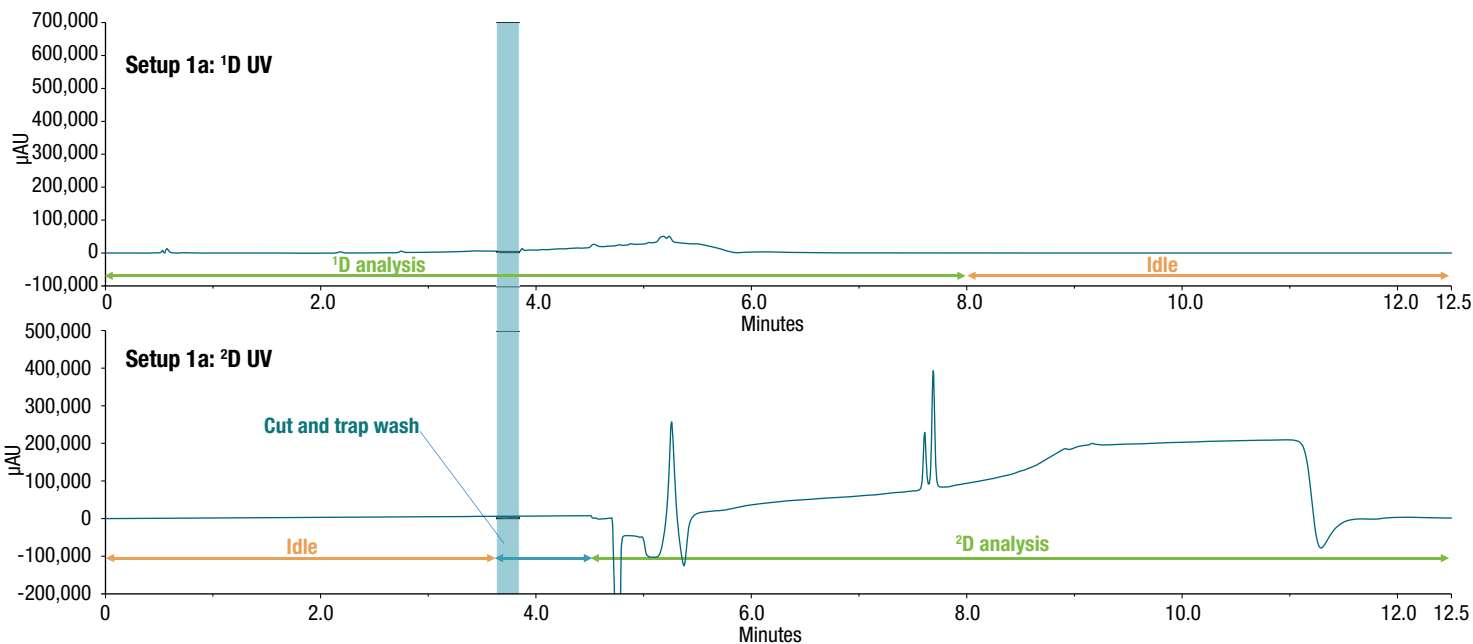Figure 7. The different stages of heart-cut 2D-LC with setups 1a and 1b and the respective valve positions

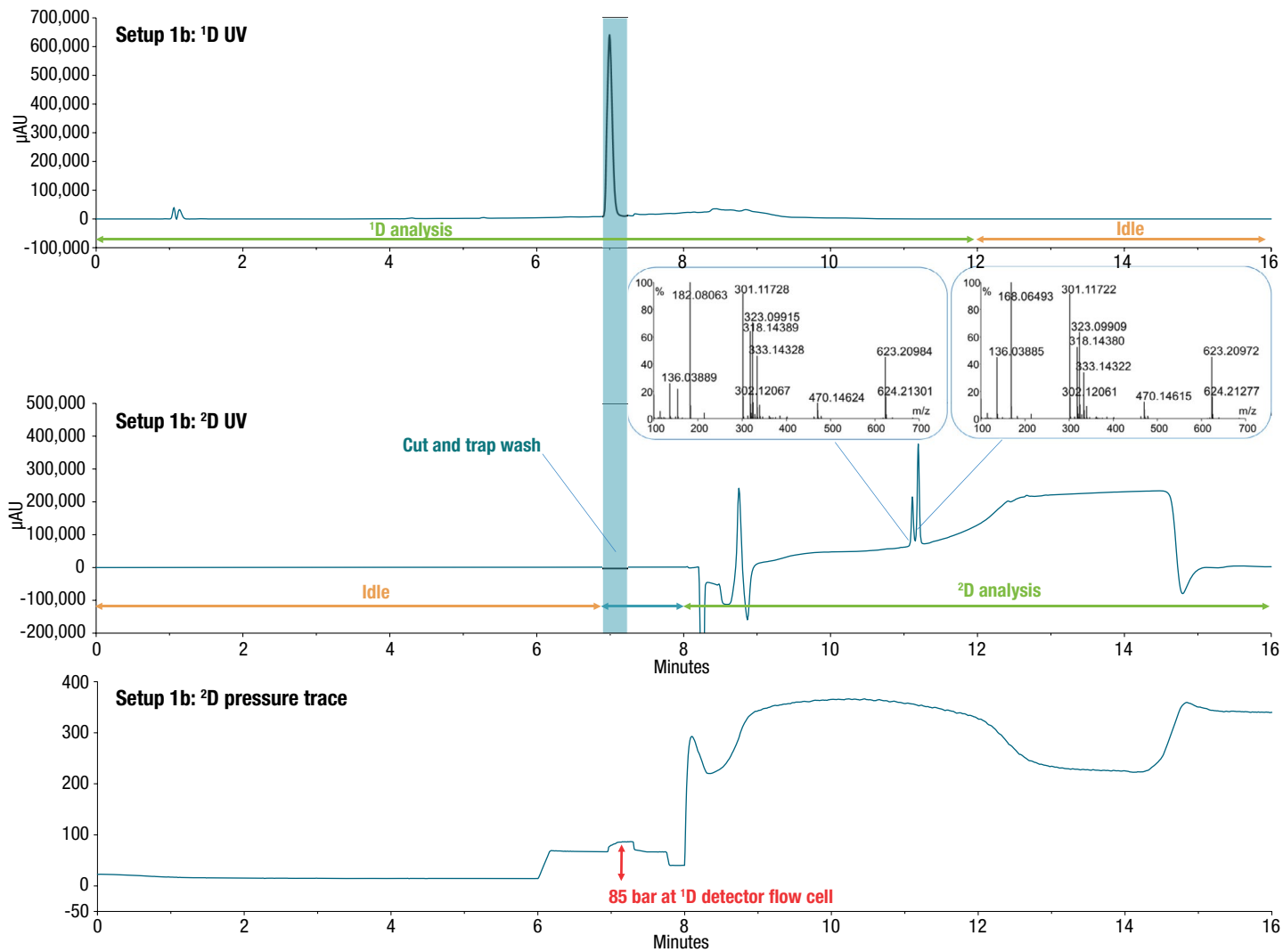Figure 8. Heart-cut ^{2}D-LC with setup 1a; ^{1}D and ^{2}D UV data

Figure 9. Heart-cut 2D-LC with setup 1b; ^{1}D and ^{2}D UV data, peak apex MS spectra and ^{2}D pressure trace

Setup 2 – Trapping on the ^{2}D column head

Another way to set up the fluidics of a system *(setup 2)* with the requested capabilities is shown in Figure 10 (left side), utilizing the same parts as before with one exception: no trap column is employed, but the trapping process is shifted directly to the ^{2}D column. The great advantage of this setup is the possibility to upgrade the system by replacing the Vanquish Split Sampler by a Vanquish Duo Split Sampler (Figure 10, right side). The second sampling unit of the dual sampler can be integrated into the ^{2}D fluidics converting the ^{2}D flow path into an individually usable, full-featured 1D-LC flow path. Thus, the LC system may be used either as a heart-cut 2D-LC system or as two independent 1D-LC systems without any need for manual replumbing. For example the 1D-LC-UV/MS data presented in the Figures 2 to 4 were recorded with the two independent flow paths of that setup.

Note: This upgrade would, in general, also be possible with setup 1a and 1b. However, due to the specific fluidic connections, a direct injection into the ^{2}D would always have the trap column in-line with the ^{2}D column and thus is not explicitly recommended.
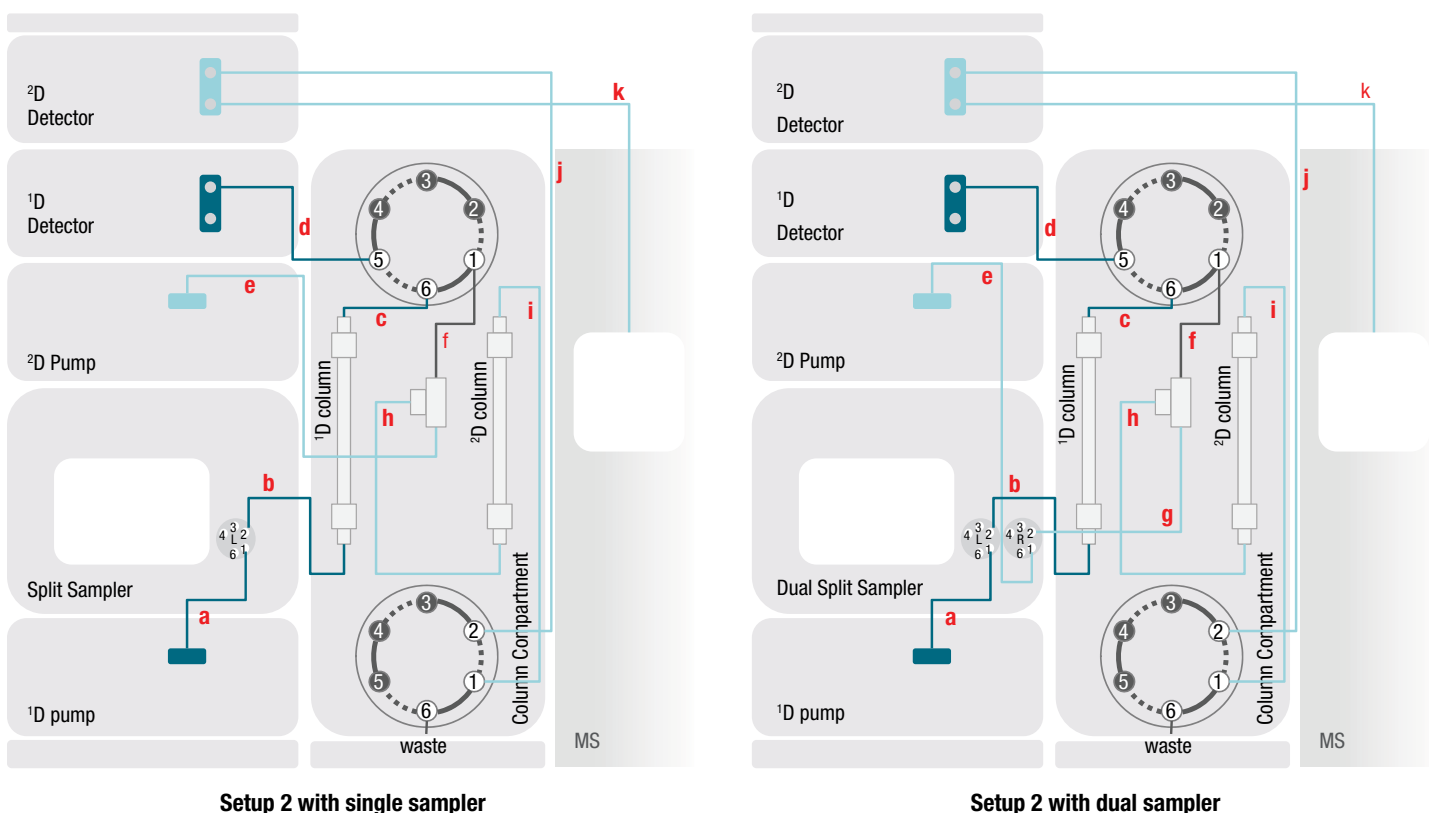
Setup 2 with single sampler

Setup 2 with dual sampler

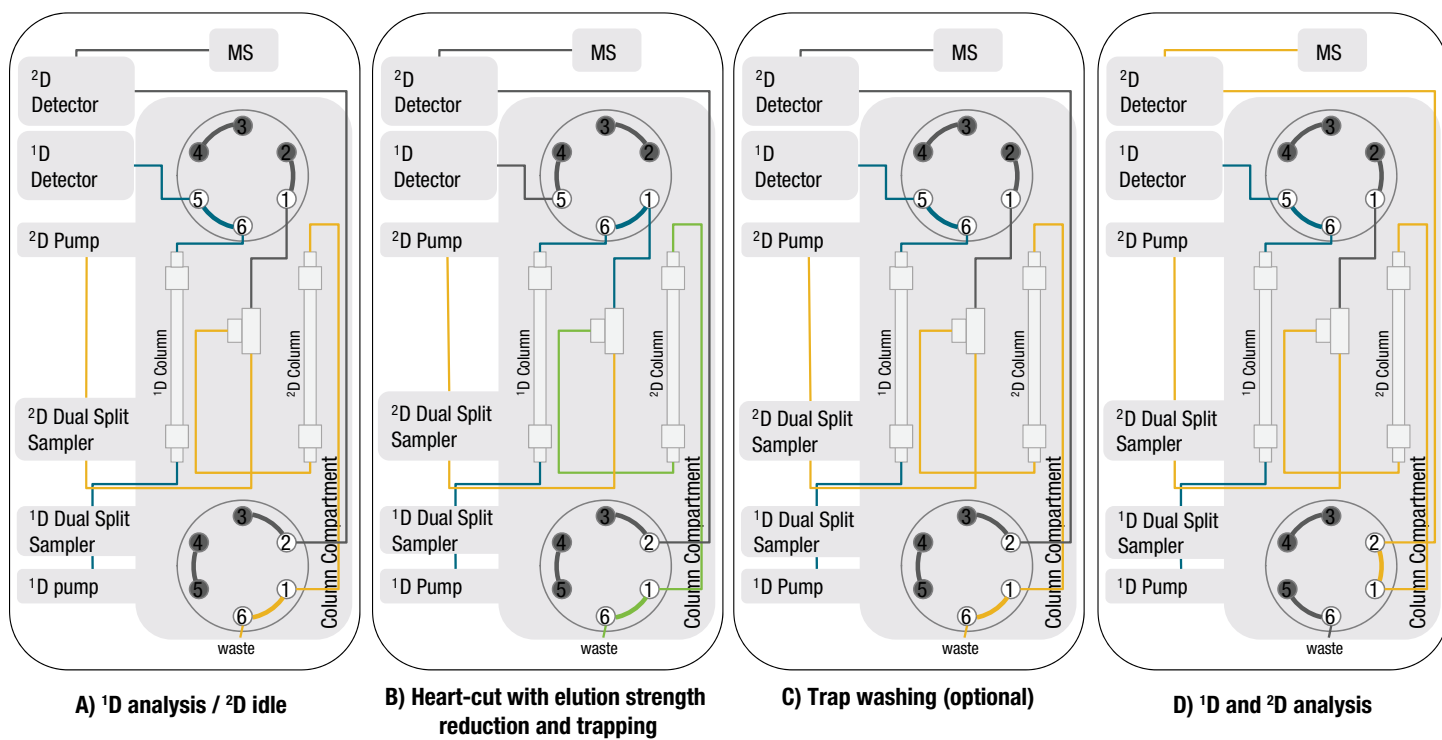| #	Connection	Part Number	#	Connection	Part Number
a	Viper Capillary, ID × L 0.10 × 350 mm, MP35N	6042.2340	h	Viper Capillary, ID × L 0.18 × 450 mm, MP35N *and* Active Pre-heater, 0.1 × 380 mm, MP35N	6042.2365 6732.0110
b	Active Pre-heater, 0.1 × 380 mm, MP35N	6732.0110	i	Viper Capillary, ID × L 0.10 × 250 mm, MP35N	6042.2330
c	Viper Capillary, ID × L 0.10 × 250 mm, MP35N	6042.2330	j	Viper Capillary, ID × L 0.10 × 650 mm, MP35N *and* Viper Capillary, ID × L 0.10 × 450 mm, MP35N	6042.2370 6042.2350
d	Viper Capillary, ID × L 0.10 × 350 mm, MP35N	6042.2340	k	Viper Capillary, ID × L 0.10 × 750 mm, MP35N	6042.2390
e	Viper Capillary, ID × L 0.10 × 550 mm, MP35N	6042.2360		Waste fluidic, VH-D1	6083.2425
f	Viper Capillary, ID × L 0.10 × 250 mm, MP35N	6042.2330		2× Viper Union	6040.2304
g	Viper Capillary, ID × L 0.10 × 250 mm, MP35N	6042.2330		Tee Piece ID 0.020'' for 1/16'' capillary	6263.0035
	6× Viper Blind plug	6040.2303			

Note: These configurations are supported by the Workflow kit, Vanquish for online 2D-LC (6036.2030). For more details, please refer to the Installation Guide, Vanquish for online 2D-LC. a and b capillaries are part of the module ship kits.

Figure 10. Trap-based heart-cut 2D-LC setup 2 with elution strength reduction with standard single sampler and dual sampler

The system operation of setup 2 with a dual sampler is depicted in Figure 11. Again, the upper switching valve controls the fractionation (A). With the valve in position 6_1 the ^{1}D eluate is directed to the T-piece to be diluted with the ^{2}D flow and trapped on the head of the ^{2}D column (B). The lower switching valve controls if the ^{2}D flow goes to waste or the detectors. During the trapping and washing process it is in position 6_1 to prevent non-volatile salts from entering the MS (B+C). For the ^{2}D analysis it switches to position 1_2 (D). The same valve configuration is used if both flow paths are utilized as independent 1D-LC paths.

Once more, a configuration with both valves in 6_1 must be avoided. One weak point of setup 2 is again a limitation in the applicable flow rates in 2D-LC methods. During the flow combination at the T-piece the pressure in both flow paths increases significantly, flow rates must be selected such that the pressure limits of the columns and the system are not exceeded. The installation of the ^{1}D detector in front of the fractionation valve, like shown with setup 1b, is not recommended for setup 2 because the generated pressure will easily exceed the flow cell limits.

Setup 2 with dual sampler

A) ^{1}D analysis / ^{2}D idle

B) Heart-cut with elution strength reduction and trapping

C) Trap washing (optional)

D) ^{1}D and ^{2}D analysis

| Action	Lower valve	Upper valve
A) ^{1}D analysis – ^{2}D idle	6_1	1_2
B) Heart-cut + trapping	6_1	6_1
C) ^{1}D analysis + trap washing	6_1	1_2
D) ^{1}D and ^{2}D analysis	1_2	1_2
D) Direct injection ^{1}D and ^{2}D	1_2	1_2
AVOID	1_2	6_1

Figure 11. The different stages of heart-cut 2D-LC or direct injection with setup 2 with a dual sampler and the respective valve positions

Setup 3 – Advanced setup with trap column and flexible usage as 2D-LC or two 1D-LC systems

The last setup *(setup 3)* to be presented here is seen in Figure 12. It employs one more column compartment and one more switching valve compared to the former setups but in return overcomes several shortcomings of those and makes use of the upgrade by the dual sampler. The functionality is outlined in Figure 13. Nothing changes for the ^{1}D, so the upper valve again controls the fractionation (A). The left lower valve controls if the ^{2}D flow goes to the T-piece or directly to the right lower valve. The right lower valve determines if the flow goes through the trap column or not. If a ^{1}D fraction is directed to the T-piece, both lower switching valves are in position 1_2 to mix it with the ^{2}D flow and load it onto the trap column in forward direction (B). For trap washing the position is kept (C), but for the ^{2}D analysis both lower valves switch to position 6_1 to backflush the trap column to the ^{2}D column (D). If a direct injection to the ^{2}D column is done, the left lower valve is in 6_1, but one usually would use the right lower valve in 1_2 (E). However, the trap column can also be switched into the flow to create conditions as present in a 2D-LC run.

Similar to setup 1a vs. 1b, it is possible to install the ^{1}D detector in front of the fractionation valve to detect the ^{1}D during the cut, but again this comes with a trade-off for
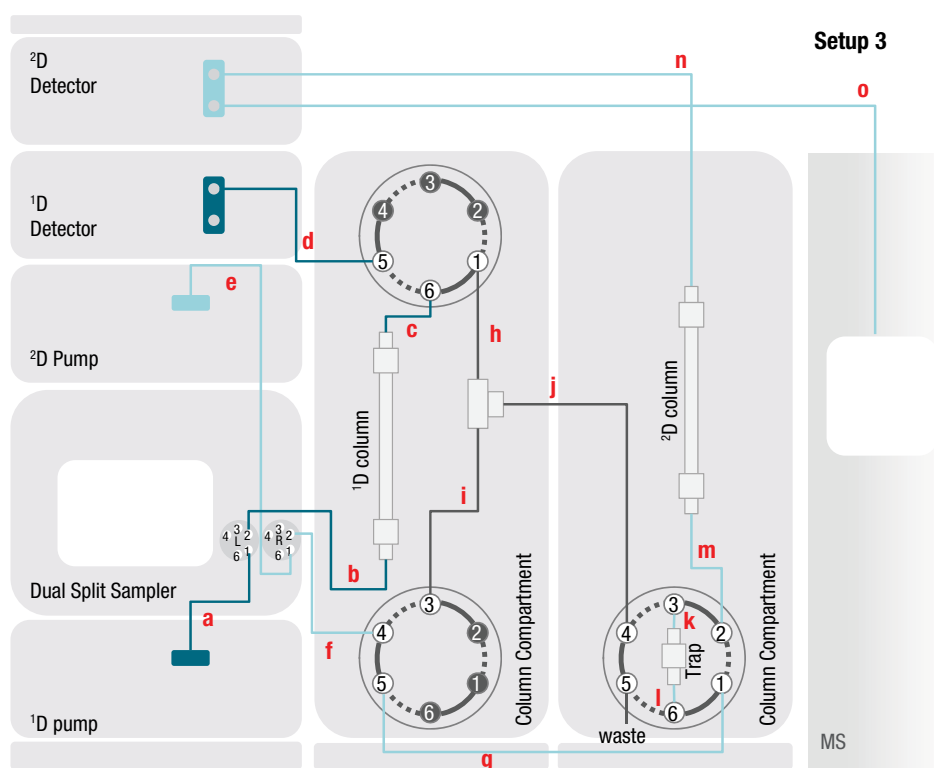
#	Connection	Part Number	#	Connection	Part Number
a	Viper Capillary, ID × L 0.10 × 350 mm, MP35N	6042.2340	h	Viper Capillary, ID × L 0.10 × 250 mm, MP35N	6042.2330
b	Active Pre-heater, 0.1 × 380 mm, MP35N	6732.0110	i	Viper Capillary, ID × L 0.10 × 350 mm, MP35N	6042.2340
c	Viper Capillary, ID × L 0.10 × 250 mm, MP35N	6042.2330	j	Viper Capillary, ID × L 0.18 × 450 mm, MP35N	6042.2365
d	Viper Capillary, ID × L 0.10 × 350 mm, MP35N	6042.2340	k	Viper Capillary, ID × L 0.10 × 150 mm, MP35N	6042.2320
e	Viper Capillary, ID × L 0.10 × 550 mm, MP35N	6042.2360	l	Viper Capillary, ID × L 0.10 × 250 mm, MP35N	6042.2330
f	Viper Capillary, ID × L 0.10 × 450 mm, MP35N	6042.2350	m	Active Pre-heater, 0.1 × 380 mm, MP35N	6732.0110
g	Viper Capillary, ID × L 0.10 × 250 mm, MP35N	6042.2330	n	Viper Capillary, ID × L 0.10 × 650 mm, MP35N	6042.2370
	Waste fluidic, VH-D1	6083.2425	o	Viper Capillary, ID × L 0.10 × 750 mm, MP35N	6042.2390
	2× Viper Union	6040.2304		Tee Piece ID 0.020'' for 1/16'' capillary	6263.0035
	6× Viper Blind plug	6040.2303			

Note: These configurations are supported by the Workflow kit, Vanquish for online 2D-LC. For more details, please refer to the Installation Guide, Vanquish for online 2D-LC. a and b capillaries are part of the module ship kits.

Figure 12. Trap-based heart-cut 2D-LC setup 3 with elution strength reduction and dual sampler

flexibility in flow rate selection as discussed above. Two major advantages of using a second column compartment and a third valve in setup 3 over the previously discussed setups are that the column temperatures of both dimensions can be set independently and there are no potential valve positions that accidentally feed the ^{1}D mobile phase to the ^{2}D column or MS. Thus, the setup is less prone to user errors.

Setup 3

A) ^{1}D analysis / ^{2}D idle

B) Heart-cut with elution strength reduction and trapping

C) Trap washing (optional)

D) ^{1}D and ^{2}D analysis

E) OR direct injection ^{1}D and ^{2}D

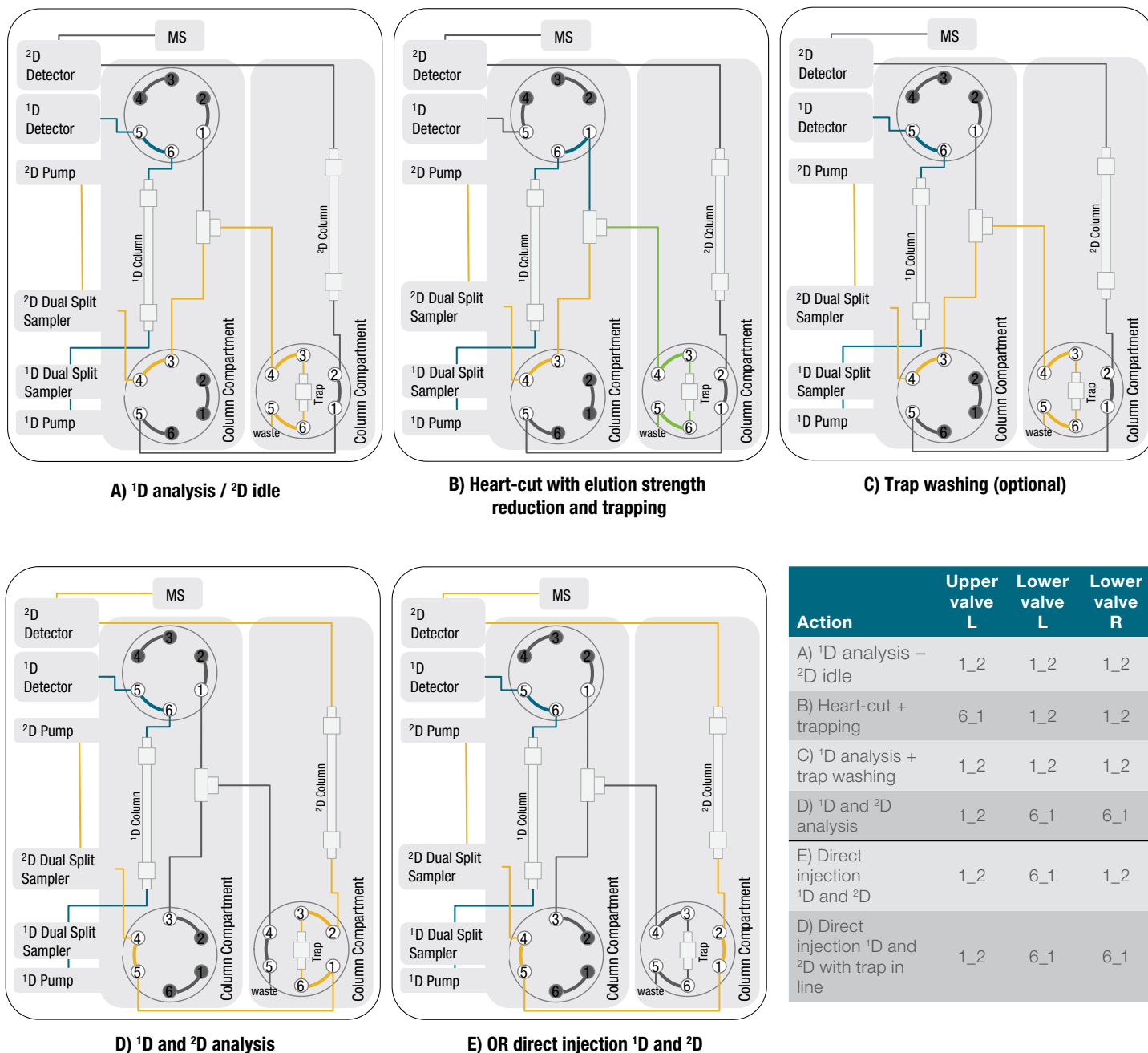| Action	Upper valve L	Lower valve L	Lower valve R
A) ^{1}D analysis – ^{2}D idle	1_2	1_2	1_2
B) Heart-cut + trapping	6_1	1_2	1_2
C) ^{1}D analysis + trap washing	1_2	1_2	1_2
D) ^{1}D and ^{2}D analysis	1_2	6_1	6_1
E) Direct injection ^{1}D and ^{2}D	1_2	6_1	1_2
D) Direct injection ^{1}D and ^{2}D with trap in line	1_2	6_1	6_1

Figure 13. The different stages of heart-cut 2D-LC or direct injection with setup 3 and respective valve positions

Comparison of the different setups

Figure 14 gives an overview on the chromatographic performance of all setups for the second flow path in 1D-LC mode as well as in heart-cut 2D-LC mode. The peak areas and widths were independent from the applied instrument setup as well as from the analytical mode. The consistent peak areas confirmed the transfer of complete peaks from the ^{1}D to the ^{2}D. No significant differences in peak widths were found, proving the concept of compound trapping and focusing. Thus, all presented setups are equivalent from a chromatographic point of view and just provide different ways to realize heart-cut 2D-LC with elution strength reduction assisted fraction trapping. The setup selection strongly depends on the application requirements and preferences of the user. All strengths and limitations herein discussed are again summarized in Table 4, highlighting setup 3 as the most favorable system.
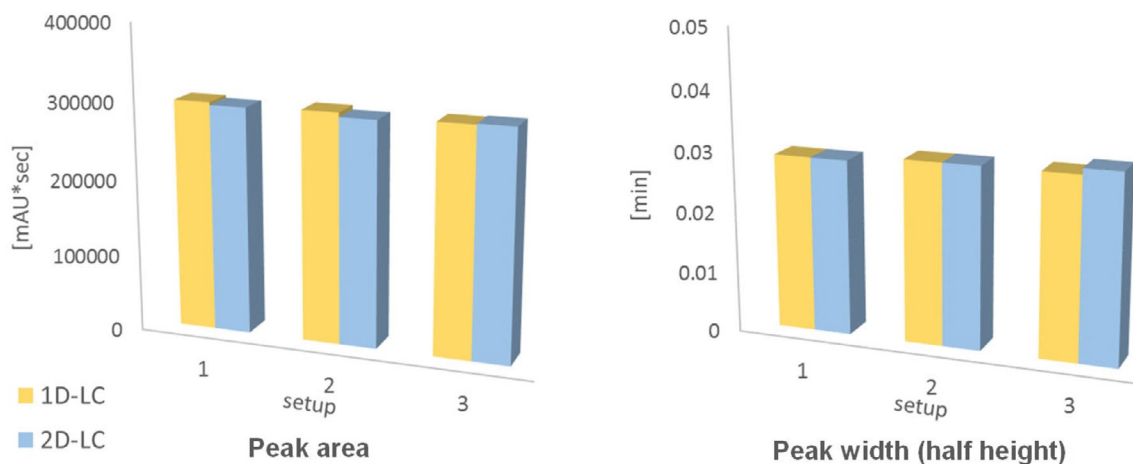

Figure 14. Comparison of chromatographic results (UV) for the desmedipham peak obtained by 1D-LC (direct injection into second flow path) and heart-cut 2D-LC with the three presented instrument setups (1D-LC in setup 1 was enabled by manual replumbing)

Table 4. Comparison of the heart-cut 2D-LC setups applying elution strength reduction

	Setup 1a	Setup 1b	Setup 2	Setup 3
Flexible flow rate settings	yes	no	no	yes
Direct injection ^{2}D without manual replumbing	no	no	yes	yes
Parallel 1D-LC in both flow paths	no	no	yes	yes
Trap column in line with ^{2}D column	always in line	always in line	no trap column	in- or off- line with ^{2}D column
^{1}D UV detection during heart-cut	no	yes	no	no (possible as in setup 1b)
All valve positions prevent ^{1}D eluate to contaminate into MS	no	no	no	yes
Equipment	1 column compartment, 2 valves	1 column compartment, 2 valves	1 column compartment, 2 valves	2 column compartments, 3 valves
Different temperature settings for ^{1}D and ^{2}D	no	no	no	yes

Moreover, the benefits that arise from the combination of a 2D-LC system with a Vanquish Dual Split Sampler are outlined here:

1. The system is flexible in its use either as two independent 1D-LC flow paths or as one 2D-LC flow path, depending on the current need of the user.

2. No manual modifications are required to switch between both application options.

3. Simplified method development for the ^{2}D in 2D-LC is provided. Due to enabled direct injection, the ^{2}D method can be developed in a 1D way.

4. More efficient instrument utilization is facilitated. If there is no analytical task pending that requires dedicated 2D-LC options, the system can be utilized for more routine work in 1D-LC. This also increases the return on investment (ROI) of the instrument.

5. Similarly, more efficient instrument utilization is possible for MS devices if used as detectors in the setup. Mass spectrometers usage can be increased by running 1D-LC applications if no 2D-LC tasks are pending. This increases the efficiency of the system and ROI.

6. The concept generally can be applied to all kinds of 2D-LC setups (online/offline/heart-cut/multi-heart-cut and comprehensive 2D-LC), depending on the exact fluidic setup.

7. Since the Vanquish Split Sampler can be substituted with a Vanquish Dual Split Sampler, the number of instrument modules required, and thus, the overall instrument size, is the same as for "2D-LC only" instruments. Therefore, the instrument offers more options but with the same instrument size.

8. The instrument size is more compact compared to a solution that utilizes two single autosamplers instead of one dual sampler. The samples for two methods can be stored in one location. This is a major advantage, for example, if a sample needs to be analyzed by complementary 1D-LC methods. It is not necessary to move samples from one location to another.

9. Even more sophisticated workflows are enabled, such as a trapped fraction can be spiked with an internal standard or reagent on the trap column before the ^{2}D analysis is started.

Instrument control

All presented heart-cut 2D-LC setups were tested under Chromeleon CDS version 7.2.9 as well as under Xcalibur 4.0 software in combination with SII for Xcalibur 1.4 software. Chromeleon is the recommended software for the highest flexibility in 2D-LC method setup due to the exclusive availability of custom columns, custom variables, and intelligent run control (IRC). Switchable instrument usage as one 2D-LC system or as two 1D-LC systems is available under Chromeleon CDS versions from 7.2.8, as the Vanquish Dual Split Sampler is not supported by earlier versions.

System operation is also possible under different software packages. Vanquish modules, including the Vanquish Dual Split Sampler, are supported from SII for Empower 1.0 (Empower 3FR4 required). SII for Xcalibur from version 1.4 supports the Vanquish Dual Split Sampler as well, but not in operation in dual mode, meaning a shared use by two independent 1D-LC instruments. Neither SII for Empower nor SII for Xcalibur provide custom variables or IRC

1D-LC methods in either flow path are set up as usual in Chromeleon CDS or SII software using the instrument method wizard. Depending on the selected configuration, 1D-LC injections may be available in only one or in both flow paths.

An important aspect during *2D-LC method* creation is to be aware of the temporal difference between the detection of a ^{1}D peak and its arrival at the cutting valve. The shift can be positive or negative depending on the position of the ^{1}D detector (in front of or behind the valve) and its magnitude may be estimated from the fluidic volumes between the points of detection and cutting and from the ^{1}D flow rate. However, the calculated delay should be seen as a first estimation and experimental confirmation and fine-optimization is recommended when the instrument is installed. If the ^{1}D resolution allows for it, a cutting window broader than the actual peak width could be considered to compensate for minor retention time shifts, especially if quantitative data evaluation is intended. For a dilution-assisted trapping, the flow through the trap column may be ramped up some time before the cut to ensure a sufficient dilution of strong solvent from the ^{1}D and kept up for some time after the cut to wash the trap column (see more in the appendix).

In general there are two options to create analytical *heart-cut 2D-LC methods:*

(1) ^{1}D and ^{2}D analyses are combined in one instrument method, leading to a single data file for each 2D-LC run (as in Figure 8).

(2) The instrument methods for ^{1}D and ^{2}D are separated. Each 2D-LC analysis comprises two steps in the sequence and results in two data files.

Moreover, the user may choose between a *hard-coded or smart-coded* method setup, if the instrument is under Chromeleon CDS control. The former meaning that the cut times are fixed, and a new instrument method is required for every change in the target heart-cut. This kind of 2D-LC method creation is available with all software packages mentioned above. However, Chromeleon uniquely provides some more extraordinary features that enable for a more flexible and intelligent method setup. These features comprise custom columns in the sequence, custom variables in the instrument method, and intelligent run control (IRC) settings in the processing method.

Although smart-coding appears more complex than hard-coding, it is seen as the superior way to set up heart-cut 2D-LC methods. However, a template created once may be re-used for many other cases. One template is also provided with the current Technical Note online on AppsLab (https://appslab.thermofisher.com/). One drawback compared to the hard-coded option is the longer run time, as the ^{2}D does not start directly after the cut but waits for the ^{1}D to finish. On the other hand, the method is completely flexible in cut selection and thus is applicable for each peak in the considered elution window. Which way is preferred is up to the user and also depends on the software package. Table 5 summarizes the capabilities of the different CDS systems.

Some examples on the addressed aspects and detailed instructions on how to set up the system, instrument methods, and sequences are outlined in the appendix.

Conclusion

- The Vanquish UHPLC platform offers several options to set up heart-cut 2D-LC with elution strength reduction assisted fraction trapping. Such instruments facilitate straightforward peak identity and purity confirmation by mass spectrometry from MS incompatible LC-UV methods in the ^{1}D dimension.

- The most favorable setups employ a Vanquish Dual Split Sampler and provide utilization as either one heart-cut 2D-LC system or as two independent 1D-LC systems without any manual intervention. The flexible operation tremendously increases the instrument's usability and value.

- Due to its multiple extraordinary features Chromeleon is the preferred CDS software to install and control the instrument. However, working under SII for Xcalibur as well as SII for Empower is possible.

References:
1. Guiochon, G. The limits of the separation power of unidimensional column liquid chromatography, *J. Chrom. A* **2006,** *1126,* 6–49.

2. Guiochon, G. et al., Implementations of two-dimensional liquid chromatography, *J. Chrom. A* **2008,** *119,* 109–168.

3. Grübner, M.; Steiner, F. Complementary Dual LC as a convenient alternative to multiple heart-cut 2D-LC for samples of medium complexity, Thermo Fisher Scientific Technical Note 73184, 2019, https://assets.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-73184-lc-multiheart-cut-alternative-tn73184-en.pdf (accessed October 22, 2019).

4. Stoll, D. R.; Carr, P. W., Two-dimensional liquid chromatography: A state of the art tutorial, *Anal. Chem.* **2017,** *89,* 519–531.

5. Petersson, P.; Haselmann, K.; Buckenmaier, S., Multiple heart-cutting two dimensional liquid chromatography mass spectrometry: Towards real time determination of related impurities of bio-pharmaceuticals in salt based separation methods, *J. Chrom. A* **2016,** *1468,* 95–101.

6. Stoll, D. R. et al., Evaluation of detection sensitivity in comprehensive two-dimensional liquid chromatography separations of an active pharmaceutical ingredient and its degradants, *Anal. Bioanl. Chem.* **2015,** *407,* 265–277.

Table 5. Comparison of the heart-cut 2D-LC setups applying elution strength reduction

	Chromeleon	Xcalibur with SII for Xcalibur	Empower with SII for Empower
Support of Vanquish Dual Split Sampler	✓	✓	✓
Support of Dual LC	✓	✗	✓
Heart-cut 2D-LC in one method	✓	✓	✓
Heart-cut 2D-LC in separated methods	✓	✓	✓
Custom columns/custom variables	✓	✗	✗
Hard-coded heart-cut 2D-LC	✓	✓	✓
Smart-coded heart-cut 2D-LC	✓	✗	✗
Intelligent Run Control (IRC)	✓	✗	✗

Appendix: Instrument configuration, method and sequence setup for Vanquish systems with flexible use in heart-cut 2D-LC or dual 1D-LC mode

Instrument configuration

2D-LC instrument

- For a heart-cut 2D-LC system all involved modules are configured as one instrument (Figure 15A).

- Avoid double assignments of module and channel names, e.g. rename default "PumpModule" to "PumpModule_1" and default "Pump_Pressure" to "Pump_Pressure_1". In the special case of two DADs used in one instrument to record UV spectra in both dimensions, the renaming of the channel "3DFIELD" needs to be implemented by a certified service technician.

- If two column compartments are used (setup 3), these must be added as two individual modules, not in combined mode, to ensure that more than two valves can be configured.

- If a dual sampler is installed and the system is to be used for 2D-LC as well as for 1D-LC injections, both sampling units are assigned to that single instrument. When setting up an instrument method with the instrument method wizard, the user selects the injection unit that needs to be active (Figure 15B). Thus 1D-LC analysis in both flow paths is feasible in that configuration, as long as only one sampling unit is active at the same time.

Dual 1D-LC instrument

- For full applicability of parallel dual LC, the instrument configuration has to be converted into two separate instruments, one for every flow path and the sampling units of the dual sampler are assigned to either of them (Figure 15C).

Configuration switch

- In practice it is most convenient to prepare both instrument configuration options and export and store them in an easily accessible place like the computer desktop. Depending on the current need, the user can import the required configuration with a few clicks without spending a lot of time on rearranging the configuration (Figure 15D).

Instrument method setup

1D-LC methods

1D-LC methods in either flow path are set up as usual in Chromeleon CDS or SII software using the instrument method wizard. If a single sampler is installed, 1D-LC is available for the first flow path only. If the dual sampler is installed, both flow paths are ready for 1D-LC injections. If the instrument is configured as dual LC instrument the correct injection unit is already defined prior to method setup. In a 2D-LC instrument configuration the injection unit has to be selected as a first step in the wizard (Figure 15B). The user needs to make sure that all valves are in their proper positions. Switching is usually not required during 1D-LC runs.

2D-LC methods – cut delay

For the current example with setup 3 (see Figure 16), the experimental 1D-LC retention time of the ^{1}D peak was determined as 3.78 min with a peak start at 3.73 min and a peak end at 3.86 min. Due to the positioning of the ^{1}D detector after the cutting valve, a proper heart-cut needs to be executed before the peak reaches the detector but at the time it reaches the valve. The respective negative delay can be derived from the fluidic volumes between valve and detector and the ^{1}D flow rate. Here the most considerable volume contribution comes from the ^{1}D detector. From the specifications in the operating manual one can find a sum of ~50 µL illuminated and inlet volume for the utilized detector flow cell, translating into a delay of ~0.08 min for a flow rate of 0.6 mL/min. Thus, the heart-cut was set from 3.65 min to 3.78 min in order to capture the whole peak. Good results were achieved as the peak heights and areas in the ^{2}D did not differ from a direct 1D-LC injection. Nevertheless, the calculated delay should be seen as a first estimation and experimental confirmation and fine-optimization is recommended.
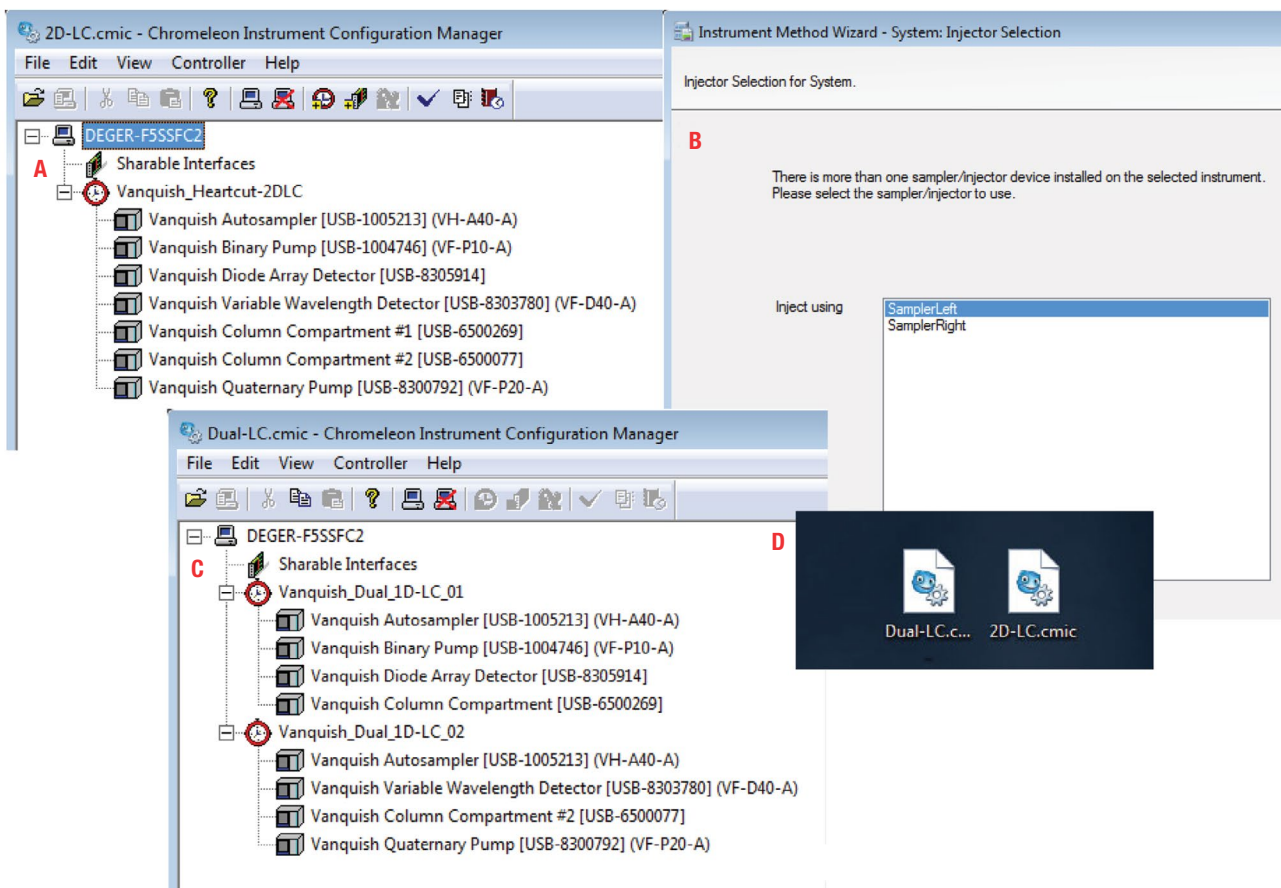
Figure 15. Instrument configurations (A, C), instrument method wizard (B), and desktop symbols (D)

2D-LC methods – Example 1: one instrument method comprising ^{1}D and ^{2}D with hard-coded cut
- Most important method aspects for setup 3 are shown in Figure 16.

- For the ^{1}D gradient there is no change except in the re-equilibration time.

- The switching times of the upper valve are set to 3.65 min and 3.78 min according to the previous cut delay calculation.

- The ^{2}D pump flow through the trap column is increased some time before the heart-cut (3.0 min) to ensure a sufficient dilution of strong solvent from the ^{1}D and is kept up for some time after the cut to wash the trap column (4.3 min).

- The ^{2}D pump flow ramp settings in the script editor should be considered carefully to make sure that the flow is up when the ^{1}D fraction arrives and down when the ^{2}D column is switched in line to start the ^{2}D run.

- The ^{2}D gradient starts at 4.5 min (pump gradient starts and both lower valves switch), thus the gradient times are shifted accordingly compared to Table 2.

- The cut times are fixed/hard-coded. A new instrument method is required for every change in the target heart-cut. This kind of 2D-LC method creation is available with all software packages mentioned above.
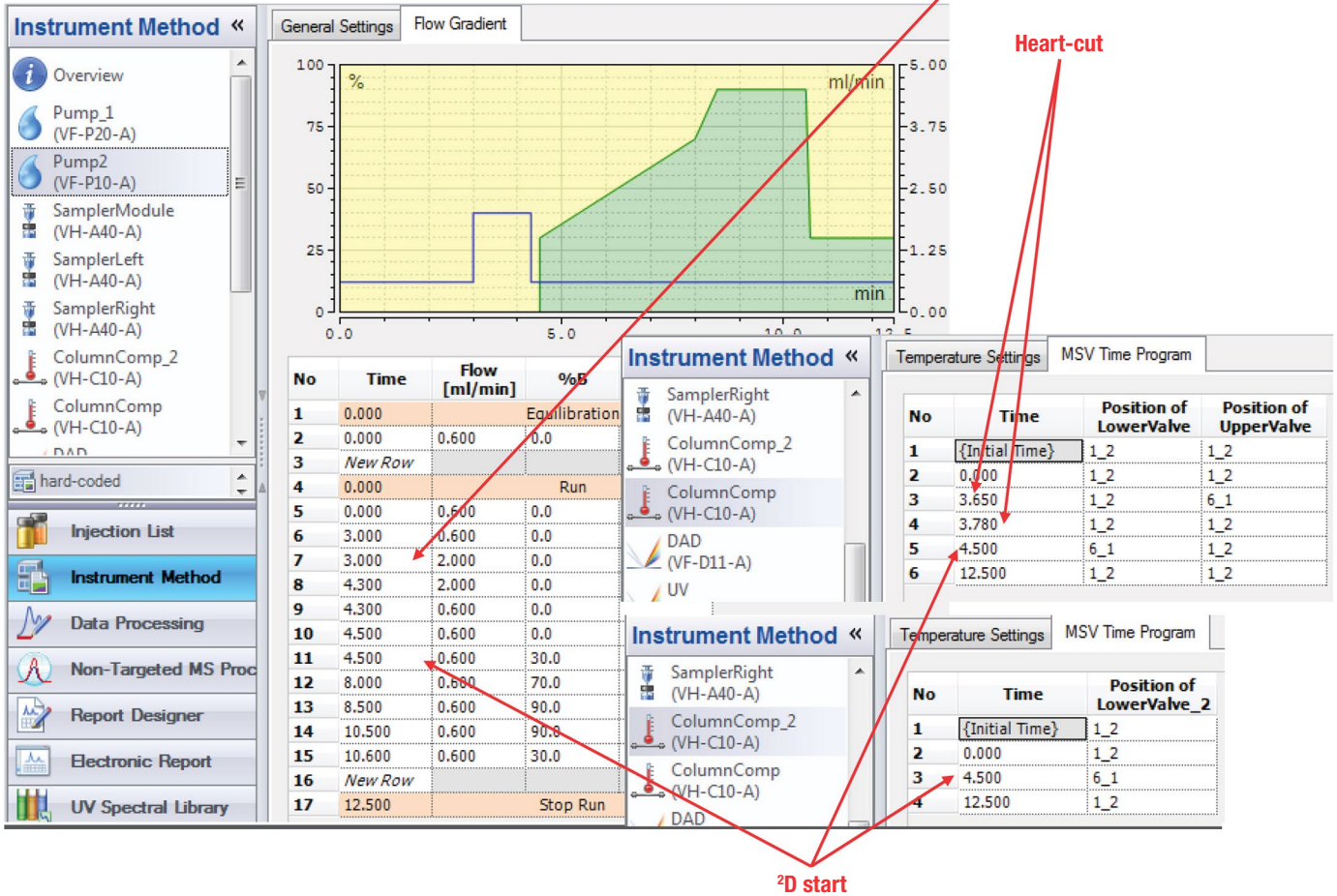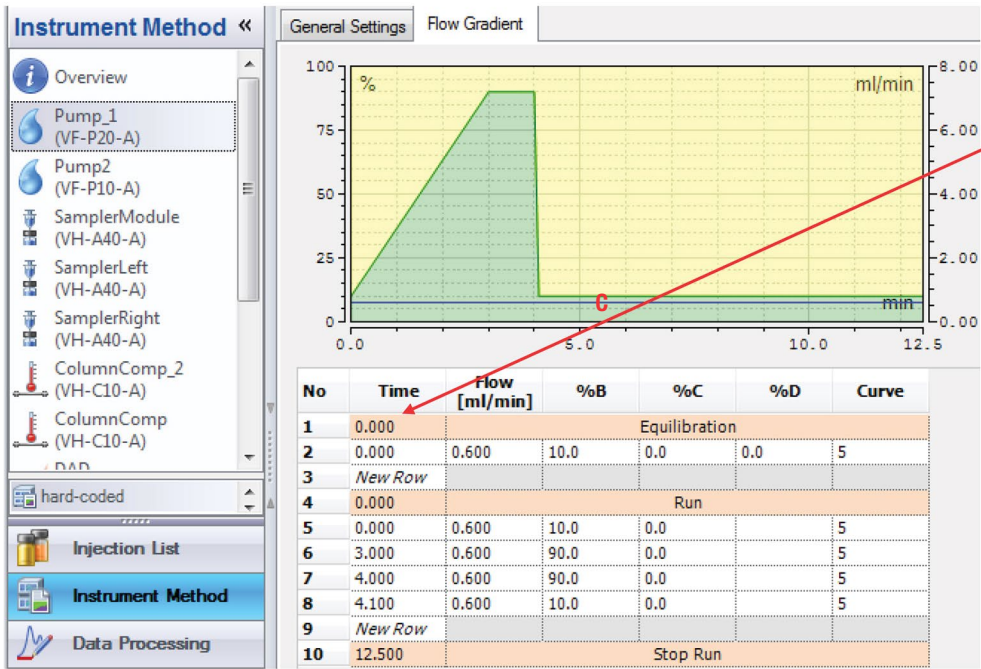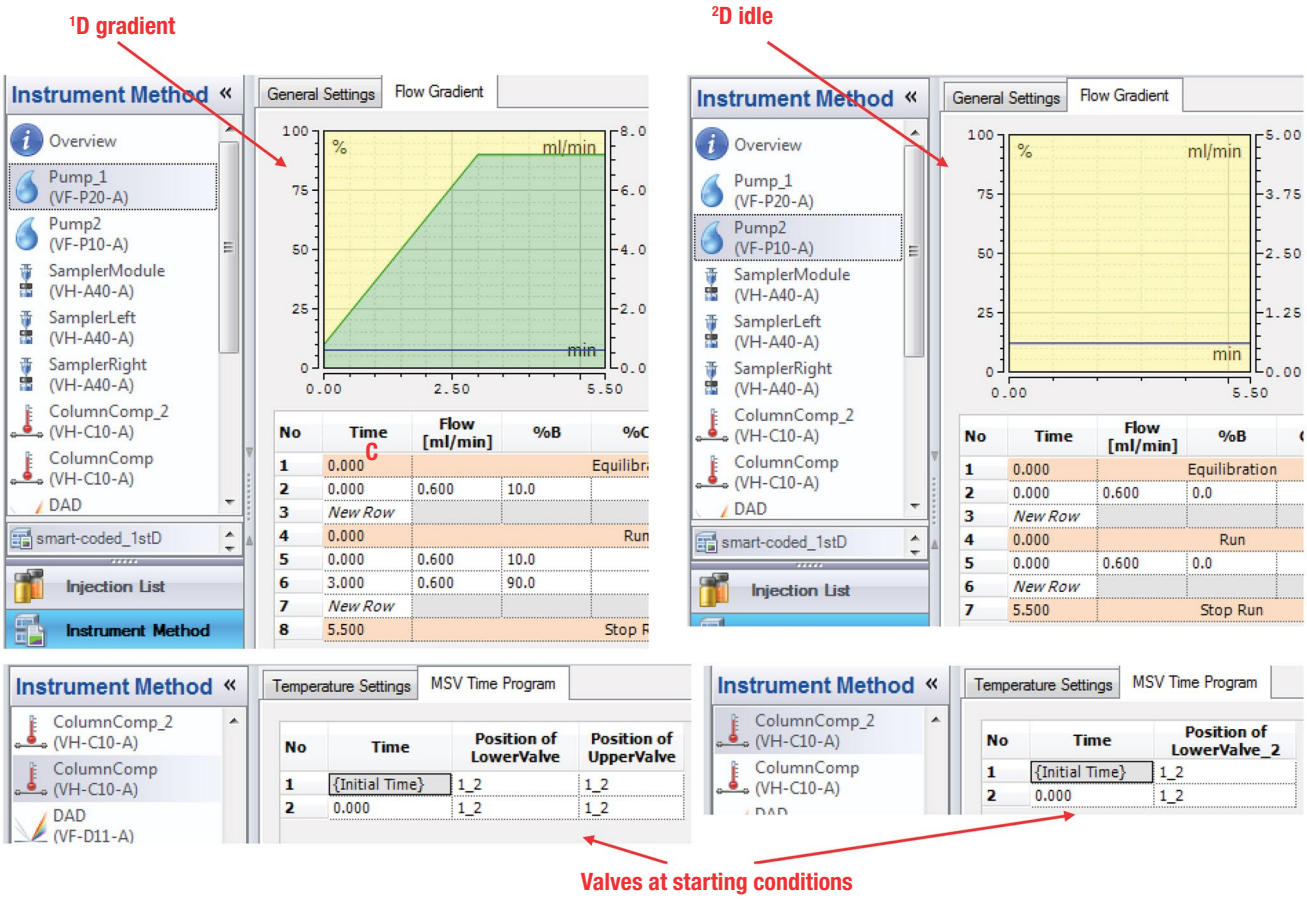
Figure 16. Important heart-cut 2D-LC instrument method aspects when programmed as one hard-coded instrument method for setup 3 (examplary methods are availiable with this Technical Note online at https://appslab.thermofisher.com/)
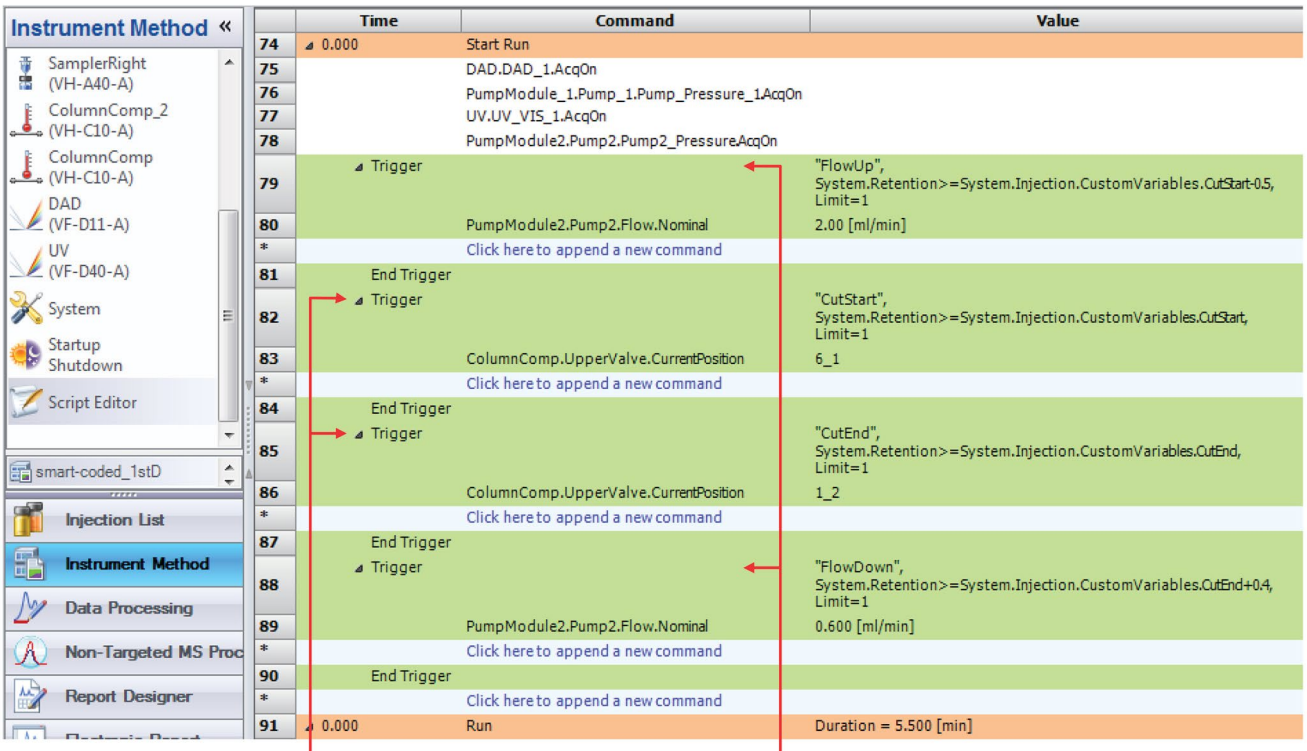
2D-LC methods – Example 2: separate instrument methods for ^{1}D and ^{2}D with smart-coded cut

- Prior to the actual instrument method creation, create "custom columns" in the sequence table to enter individual cut times:

  – Right-click into the sequence table header, choose "Custom Columns" and "Insert Custom Variable". In the window that opens click "Create a new custom variable", select a name like "CutStart" or "CutEnd", type is "numeric", check "allow empty values", minimum is 0, maximum depends on the run time, unit is usually "min", precision should be not less than 2. Two custom columns are needed to define the cut start and end.

- Example instrument methods are depicted in Figure 17 and Figure 18. The first method contains the ^{1}D gradient, the cut and trap process, as well as the trap washing. The second method contains the ^{2}D gradient and the ^{1}D flushing and re-equilibration.

- The smart-coding only affects the ^{1}D method. Here, the ^{1}D gradient is programmed up to the time one can expect peaks to elute (gradient delay should be considered), the valves and the ^{2}D pump are just set to the starting conditions.

- All further events are implemented by triggers in the script editor (Figure 17). Four triggers, which refer to the previously created custom variables, are entered into the Start Run stage at 0 min: two to increase or decrease the flow rate of the ^{2}D pump ("FlowUp" and "FlowDown" in Figure 17) and two to start and end the fractionation ("CutStart" and "CutEnd" in Figure 17):

  – Click the line before where you want to insert a trigger block and then click in the "Insert" section of the ribbon "Conditional" and "Insert Trigger". For each trigger a unique name is entered in double quotation marks and a condition is compiled in the style of "System.Retention>=System.Injection.CustomVariables.[name e.g. CutStart or CutEnd]±X", the limit is set to 1 (maximum execution once per run) and finally the action is entered, i.e. flow rate is set or valve position is set. "X" in the condition can be 0 or for example -0.5 to increase the flow rate 0.5 min before the cut.

- "X" could also serve for the automated correction of the delay between detected peak start/end and required valve action (in the current case -0.08 min as above, not applied in Figure 17). With that the user could just enter the times detected in a 1D-LC analysis without manual calculation.

- The second method starts in the initial stage at the conditions at which the first method ends. At the 0 min stage both lower valves switch their position and the ^{2}D gradient starts (see Figure 18).

- A complete heart-cut 2D-LC analysis comprises an injection with the first method with target cut times entered into the created custom columns in the sequence table and a blank injection with the second method (Figure 19).

- The latter one can be either added to the sequence manually or automatically if a processing method using "intelligent run control" (IRC) is applied. This Chromeleon CDS feature facilitates, for example, the insertion of an injection as soon as a current injection is finished:

  – In the processing method enter the SST/IRC tab, click to the table to add a new test case and check "Create an unconditional test case". Enter a name. For the conditions check "Injection property" and enter "Inject InstrumentMethod", "=" and "[name of ^{1}D method]" (see Figure 19). In the action section add "Insert Injection" and browse for a source of an appropriate injection (must be a blank injection with the respective ^{2}D instrument method).

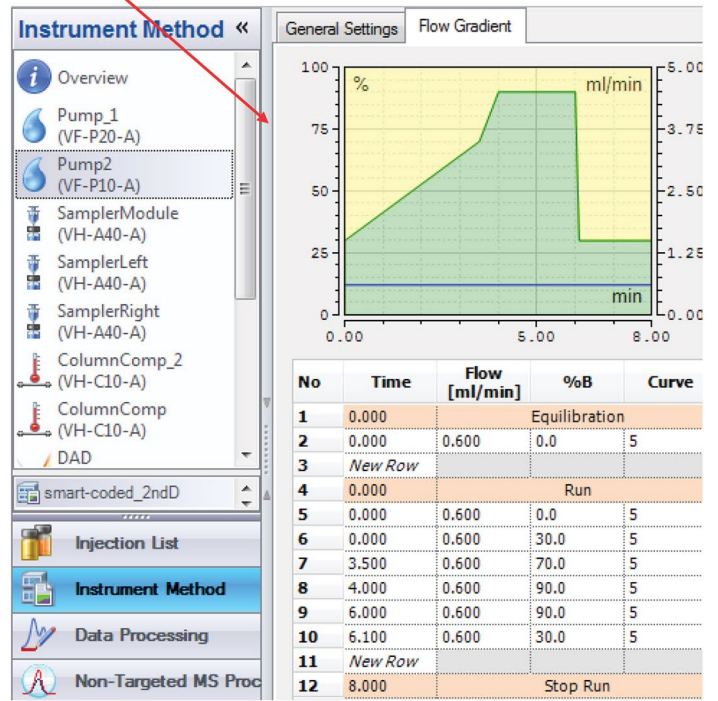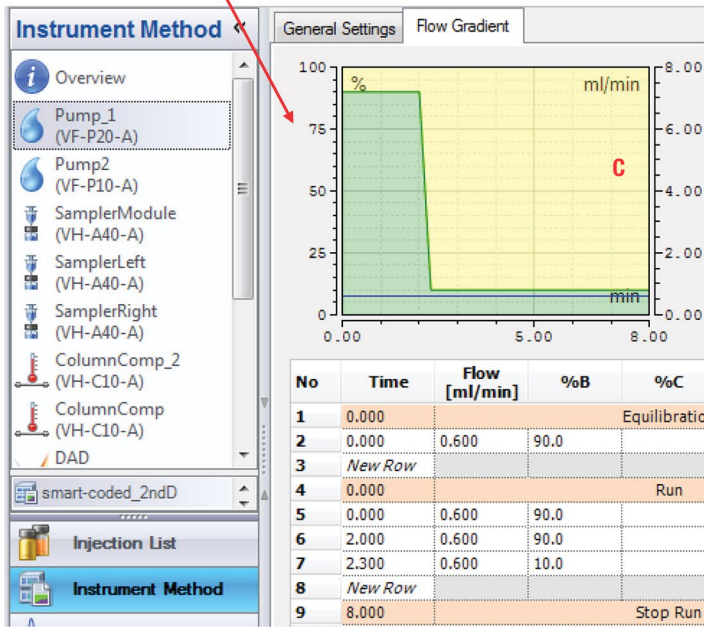¹D gradient

²D idle

Valves at starting conditions

Triggers for heart-cut

Triggers for trap flow

Figure 17. Important heart-cut 2D-LC instrument method aspects when programmed as separate instrument methods for setup 3; ¹D method with smart-coded heart-cutting (examplary methods are availiable with this Technical Note online at https://appslab.thermofisher.com/)

¹D flush and equilibration

²D gradient

Lower valves switch at start

Instrument Method

- Overview
- Pump_1 (VF-P20-A)
- Pump2 (VF-P10-A)
- SamplerModule (VH-A40-A)
- SamplerLeft (VH-A40-A)
- SamplerRight (VH-A40-A)
- ColumnComp_2 (VH-C10-A)
- ColumnComp (VH-C10-A)
- DAD

smart-coded_2ndD

Injection List

Instrument Method

General Settings | Flow Gradient

No	Time	Flow [ml/min]	%B	%C
1	0.000			Equilibratio
2	0.000	0.600	90.0	
3	New Row			
4	0.000			Run
5	0.000	0.600	90.0	
6	2.000	0.600	90.0	
7	2.300	0.600	10.0	
8	New Row			
9	8.000			Stop Run

Instrument Method

- Overview
- Pump_1 (VF-P20-A)
- Pump2 (VF-P10-A)
- SamplerModule (VH-A40-A)
- SamplerLeft (VH-A40-A)
- SamplerRight (VH-A40-A)
- ColumnComp_2 (VH-C10-A)
- ColumnComp (VH-C10-A)
- DAD

smart-coded_2ndD

Injection List

Instrument Method

Data Processing

Non-Targeted MS Proc

General Settings | Flow Gradient

No	Time	Flow [ml/min]	%B	Curve
1	0.000			Equilibration
2	0.000	0.600	0.0	5
3	New Row			
4	0.000			Run
5	0.000	0.600	0.0	5
6	0.000	0.600	30.0	5
7	3.500	0.600	70.0	5
8	4.000	0.600	90.0	5
9	6.000	0.600	90.0	5
10	6.100	0.600	30.0	5
11	New Row			
12	8.000			Stop Run

Instrument Method

- ColumnComp_2 (VH-C10-A)
- ColumnComp (VH-C10-A)
- DAD (VF-D11-A)

Temperature Settings | MSV Time Program

No	Time	Position of LowerValve_2
1	{Initial Time}	1_2
2	0.000	6_1
3	8.000	1_2

Instrument Method

- ColumnComp_2 (VH-C10-A)
- ColumnComp (VH-C10-A)
- DAD (VF-D11-A)

Temperature Settings | MSV Time Program

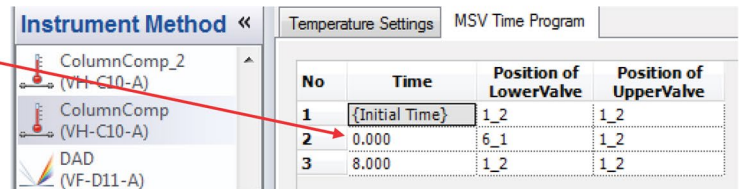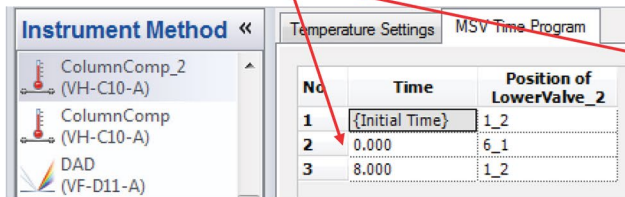| No	Time	Position of LowerValve	Position of UpperValve
1	{Initial Time}	1_2	1_2
2	0.000	6_1	1_2
3	8.000	1_2	1_2

Figure 18. Important heart-cut 2D-LC instrument method aspects when programmed as separated instrument methods for setup 3; ²D method

#	UV_VIS_1	Name	Type	Level	Position	Volume [µl]	*CutStart [min]	*CutEnd [min]	Instrument Method	Processing Method	Status
1	None	1st D: herbicides	Unknown		B:B1	5.00	3.65	3.78	smart-coded_1stD	Process with IRC	Idle
2	None	2nd D: herbicides	Blank			5.00			smart-coded_2ndD	Process with IRC	Idle

New Test Case

Injection Condition

Define the set of injections to which the test condition applies.

- ○ Apply to all injections
- ○ Injection type: Unknown ▼
- ● Injection property: Injection InstrumentMethod ▼   =

  smart-coded_1stD
- ○ Custom condition:

New Test Case

Actions

Select any actions which need to be performed.
Actions can be added more than once.

Available actions:
- Abort
- Arithmetic Combination
- Auto Dilution
- Copy Channel
- Derivative
- Extract From 3D Channel
- Extract MS Channel(s)
- Extract Opt. Int. Channel
- Insert Injection
- Pause
- Power Law
- Re-inject
- Smooth Channel

Selected actions:

	Insert Injection		
1	Source injection:	2nd D: herbicides	...
	Max. no. of insertions:	1 ⇕	

[Add] [Remove]

Figure 19. Sequence setup with two separated instrument methods and with IRC wizard

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