Agilent InfinityLab 2D-LC Solution with mass spectrometric detection and diverter valve

Technical Note

This Technical Note describes the purpose, installation, and configuration and use of a diverter valve for the Agilent InfinityLab 2D-LC solution with a combination of UV and mass spectrometric (MS) detection.

Purpose of using a diverter valve

In two-dimensional liquid chromatography (2D-LC), the second dimension can be used as an effective desalting tool to allow online coupling of chromatographic methods using MS-incompatible mobile phases to MS detection. A diverter valve can be used to automatically divert salt or buffers coming from the first-dimension (^{1}D) mobile phase to waste at the beginning of every second-dimension (^{2}D) analysis. This is shown in Figure 1 on page 1*.

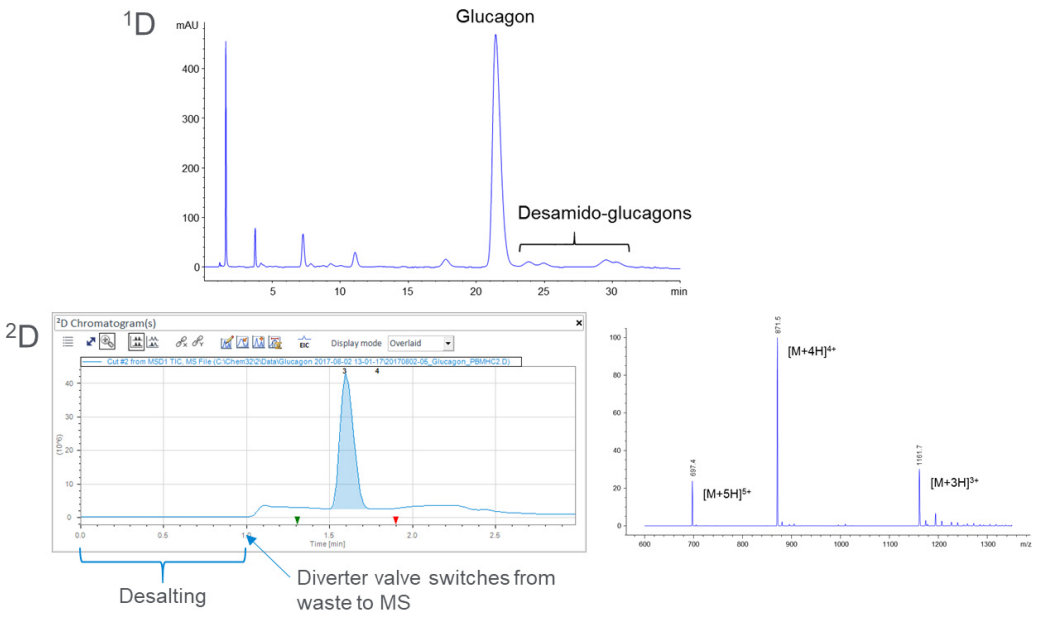

Figure 1 Peak-based multiple heart-cutting 2D-LC analysis of the peptide glucagon.

* Data from Agilent Application Note 5991-8437EN: 2D-LC as an Automated Desalting Tool for MSD Analysis - Direct Mass Selective Detection of a Pharmaceutical Peptide from an MS-Incompatible USP Method.
Chromatographic conditions: ^{1}D: Column: Agilent ZORBAX Eclipse Plus C18, 3.0 × 150 mm, 3.5 µm, Solvent: A) 16.3 g KH2PO4 in 800 mL water adjusted to pH 2.7/200 mL acetonitrile, B) Water/acetonitrile (60/40), Flow rate: 0.5 mL/min, Temperature: 45 °C, Detection: VWD, 214 nm, Heart-cutting of the glucagon peak; ^{2}D: Column: Agilent AdvanceBio Desalting-RP, 2.1 × 12.5 mm, Solvent: A) Water + 0.1 % formic acid, B) Acetonitrile + 0.1 % formic acid, 1 min desalting time at 5% B, Flow rate: 0.4 mL/min, Detection: MSD.

Diverter valve solution for a combination of UV and MS detection

CAUTION

Switching a (diverter) valve while the flow is on generates pressure pulses.

When using a diverter valve downstream to the flow cell of a UV detector (^{2}D UV detector), this may damage the flow cell.

This is no specific 2D-LC issue but the valve switches more frequently for 2D-LC, once per cut analyzed in ^{2}D.

➔ Install the diverter valve as recommended in this Technical Note.

To avoid flow cells being damaged by pressure pulses, the installation of the diverter valve as shown in Figure 2 on page 3 for a 2-position/6-port valve is used. Coming from the ^{2}D detector, a T-piece is installed with connection to the MS and to the diverter valve.

The capillaries connecting the T-piece to the diverter valve and from diverter valve to waste have a large internal diameter and generate very little restriction compared to the restriction generated by the capillary between T-piece and MS and the MS sprayer. Therefore, most of the flow coming from the 2D detector goes to waste in diverter valve switching position 1. Depending on the back pressure ratio at the T-piece, a small flow will still go to the MS. In case of very high salt concentrations this should be considered. This diverter valve switching position is illustrated in Figure 2 on page 3 section A.

In switching position 2 of the diverter valve shown in Figure 2 on page 3 section B, the flow is blocked at the diverter valve by a blank nut such that the flow from the ^{2}D detector is let via the T-piece towards the MS. A pressure relieve valve is installed between the T-piece and the diverter valve, which protects the ^{2}D UV detector flow cell in case a blockage of the MS nebulizer occurs and the diverter valve is in position 2 (Figure 2 on page 3 section B).

The blank nut blocking the flow at port 2 of the diverter valve may be replaced by red PEEK tubing to generate a split between the MS and this tubing used as a (second) waste line. The ratio of the back-pressures generated in this flow path compared to the flow path towards the MS will determine the split ratio. This can be adjusted through cutting the length of the red PEEK tubing to a suitable length (Figure 2 on page 3 section C).

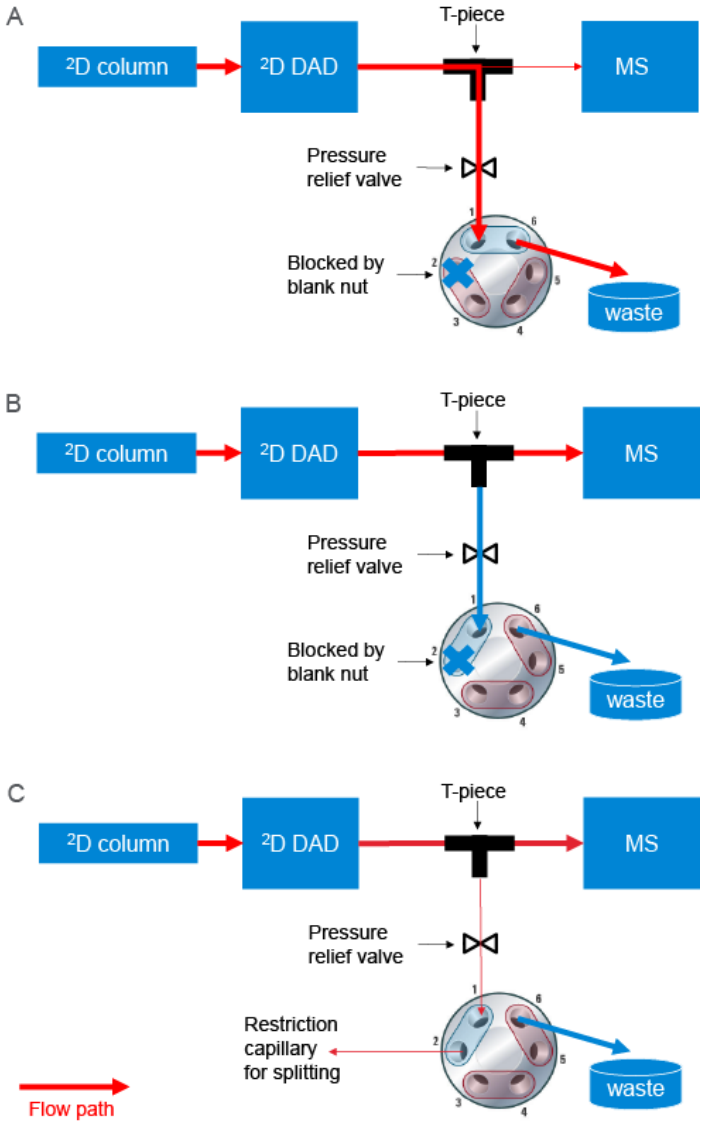

Figure 2 Diverter valve solution for a combination of UV and MS detection. (A) Position 1 during desalting, flow almost entirely goes to waste. (B) Position 2 during the analysis, flow goes to the MS. (C) The blank nut blocking the flow at port 2 of the diverter valve may be replaced by red PEEK tubing to generate a split between the MS and a waste line connected at this tubing. The ratio of the back-pressures generated in this flow path compared to the flow path towards the MS will determine the split ratio. This can be adjusted through cutting the length of the red PEEK tubing to the right length.

Installation and configuration of the diverter valve solution

A CAN-based 2-position switching valve can be used as a diverter valve. The 2-position switching valves listed below are recommended examples.

Parts required	#	p/n	Description
	1	5067-4282	2ps/6pt valve head, 800 bar
OR	1	5067-4117	2ps/6pt ultra high pressure valve head, 1200 bar
OR	1	5067-4283	2ps/10pt valve head, 800 bar
OR	1	5067-4118	2ps/10pt ultra high pressure valve head, 1200 bar

Hardware required

The following valve hosts can be used:

- G1170A 1290 Infinity Valve Drive
- G7116B 1290 Infinity II Multicolumn Thermostat with valve drive installed
- G7116A 1260 Infinity II Multicolumn Thermostat with valve drive installed

Software required

Agilent OpenLAB 2D-LC Software A.01.04 or higher

1 Install the capillaries (see Table 1 on page 4).

Table 1 Parts required for installation of the diverter valve

Description	Required part	Part number
Diverter valve	e.g. 2ps/6pt Valve head, 800 bar	5067-4282
T-piece	Tee, 1/16 in, 316 SST, low dead volume	0100-0969
Pressure relief valve	Pressure relief valve	G4212-60022
Blank nut	1/16 in stainless steel blanking nut	01080-83202
PEEK fittings	Finger-tight PEEK fittings, 1/16 in (10/pk)	5063-6591
Capillary from 2D detector to T-piece	Stainless steel connecting capillary, 400 mm long, 0.12 mm id	5067-4606
Capillary from T-piece to MS	Tubing, PEEK, 1.6 mm od, 0.12 mm id, 1.5 m, e.g. cut to 400 mm length	0890-1915
Capillary from T-piece to pressure relief valve	Capillary ST 0.3x80 mm SL-SL	5500-1228
Capillary from pressure relief valve to diverter valve	Capillary ST 0.3x80 mm SL-SL	5500-1228

2 Configure the diverter valve in OpenLab CDS ChemStation Edition Instrument Configuration (Figure 3 on page 5, section A).

3 Select the diverter valve in the 2D-LC Configuration (Figure 3 on page 5, section B).

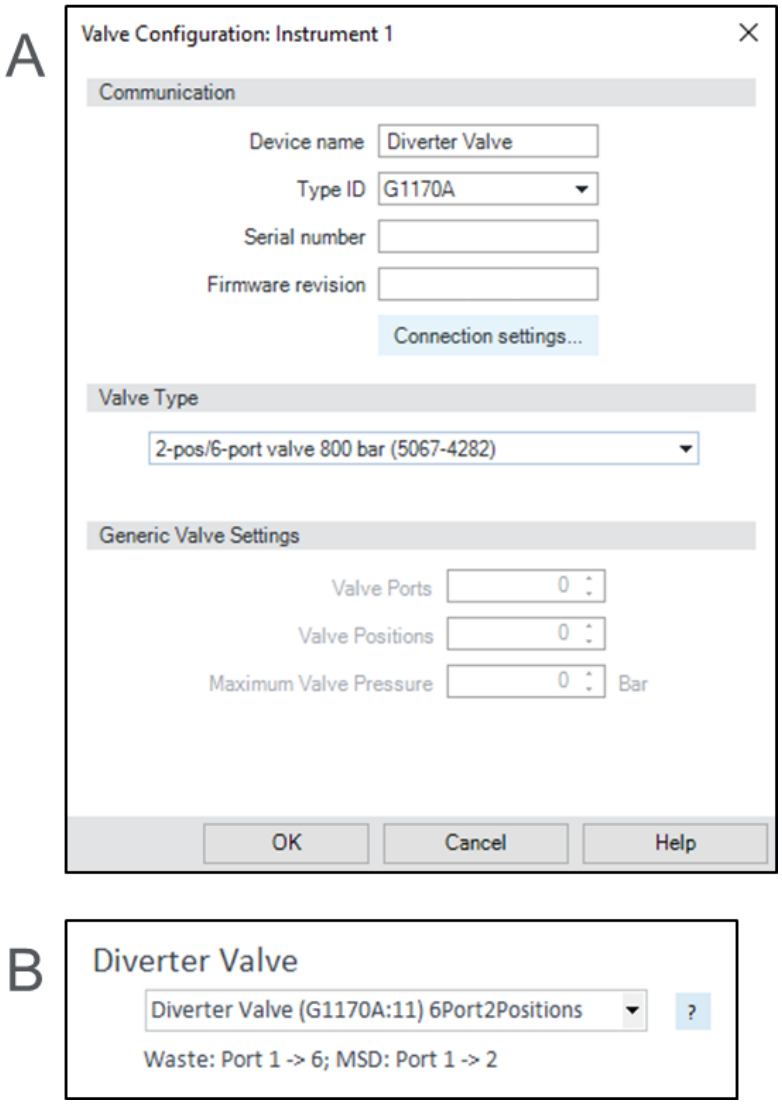

Figure 3 Configuration of the diverter valve in the ChemStation Instrument Configuration (A) and the 2D-LC Configuration (B).

Using the diverter valve

The diverter valve can be used to automatically divert salt or buffers coming from the ^{1}D mobile phase to waste at the beginning of every ^{2}D run. In the 2D-LC method, the ^{2}D time is defined after which the diverter valve switches to the MS (Figure 4 on page 6 section A). With the method setup shown in Figure 4 on page 6 section A, the diverter valve will automatically switch to waste for the first 1.00 minutes of every ^{2}D run. After 1.00 minutes, the diverter valve switches the flow to the MS.

The ^{2}D gradient needs to be programmed to allow trapping of the analytes on the ^{2}D column or desalting cartridge while salt or buffers from the ^{1}D mobile phase are eluted to waste. After the isocratic desalting phase, the actual ^{2}D gradient starts and trapped analytes are eluted to the MS. An example of a ^{2}D gradient used with a desalting cartridge is shown in Figure 4 on page 6 section B.

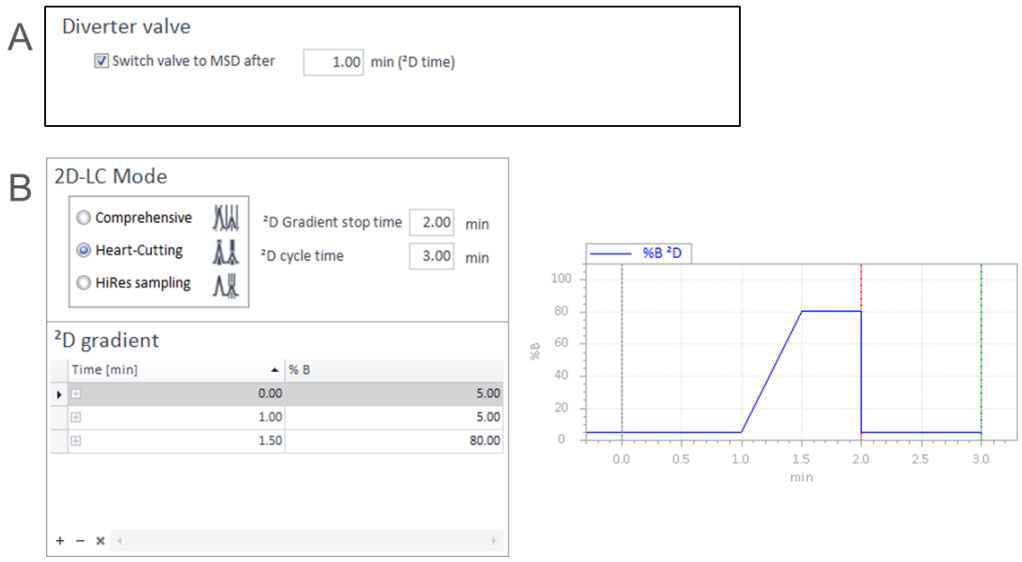

Figure 4 2D-LC method setup for diverting salt or buffers originating from the ^{1}D mobile phase to waste at the beginning of every ^{2}D run, (A) diverter valve setup in the **Advanced settings** tab of the 2D-LC method, (B) ^{2}D gradient setup in the **General settings** tab of the 2D-LC method.

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