This Technical Note describes advantages, the use, configuration and installation of the Agilent 2D-LC Active Solvent Modulation (ASM) Solution.

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Active solvent Modulation (ASM)

Introduction to Active Solvent Modulation (ASM)

In conventional 2D-LC, 1D solvent in the sample loop is injected to the second dimension column. If the 1D solvent has high elution strength in respect to the 2D column, it impairs separation in the second dimension. This results in unretained elution, broad and distorted peaks, and loss of separation (see Figure 2 on page 3).

Active Solvent Modulation (ASM) dilutes the content of the sampling loop (sample and 1D solvent) with weak 2D solvent before it reaches the 2D column and therefore improves the separation in the second dimension (see Figure 3 on page 3).

Different ASM capillaries allow optimizing the dilution for different applications (see “Understanding the ASM factor” on page 14).

The ASM solution is primarily designed for 2D-LC modes multiple heart-cutting and high-resolution sampling. The 2D-LC Valve ASM is backward compatible to the standard 2D-LC valve G4236A. If ASM is not needed or for use in comprehensive 2D-LC, the ASM functionality can be disabled.

ASM is based on the 2D-LC Valve ASM G4243A and requires the InfinityLab 2D-LC solution and 2D-LC Software A.01.04 or later.
Example: ASM with HILIC in 1D and reversed phase in 2D

In this example, a HILIC separation was run in the first dimension and a reversed phase separation in the second dimension. If sample cuts are transferred to the second dimension, 40 μL of high organic solvent are brought to a reversed phase column.*

![1D Chromatogram(s)](image)

**Figure 1** Analysis of pesticides using a HILIC separation with high organic solvent composition in 1D

**2D resolution with conventional valve**

The high elution strength of 1D solvent causes bad separation with broad and distorted peaks in the left 2D chromatogram.

![Conventional analysis of Cut#3 using a reversed phase separation in 2D](image)

**Figure 2** Conventional analysis of Cut#3 using a reversed phase separation in 2D

**2D resolution with ASM valve**

In the right 2D chromatogram a 2D-LC Valve ASM was used instead of a conventional 2D-LC valve. Peaks are resolved and the sensitivity is increased.

![ASM analysis of Cut#3 using a reversed phase separation in 2D](image)

**Figure 3** ASM analysis of Cut#3 using a reversed phase separation in 2D

* 1D analysis of pesticides using: 1D: Zorbax RX-SIL (150 x 2.1 mm ID, 5 µm), A = 10 mM NH₄Ac in H₂O; B = ACN, Gradient: 100 to 95% acetonitrile in 5 min, 500 µL/min. MHC with 40 µL loops. 2D: Bonus RP (50 x 2.1 mm, 1.8 µm), H₂O/acetonitrile gradient (0.2% formic acid), weak solvent 3% acetonitrile, 400 µL/min, EICs from conventional 2D-LC (undiluted)
Active solvent Modulation (ASM)
Operating Principle

Figure 4 Operating principle with sample loop in flow path (schematic view)

1D solvent in the sample loop is partially diluted by 2D solvent from the 2D pump.

Figure 5 Operating principle with sample loop and ASM capillary in parallel flow path (schematic view)

Introducing a parallel flow through an ASM capillary strongly dilutes 1D solvent with weaker 2D solvent. These solvent conditions focus the sample on the head of the 2D column and therefore enable a good separation.

red: 2D solvent from 2D pump, blue: sample with 1D solvent in sample loop

Figure 6 Operating principle with sample loop and ASM capillary in parallel flow path

This is how the same flow path looks inside the 2D-LC valve ASM. The flow coming from the 2D pump splits up at valve port 10. One part goes through the sample loop in deck A and carries parked sample cuts and 1D solvent. The other part of 2D solvent goes through the ASM capillary between valve ports 9 and 6. Flows unite at port 5 and 1D solvent is diluted before it arrives at the 2D column head.
Once the ASM phase has finished, which is a settable method parameter, the analytical gradient starts. As opposed to a dilution with a permanent by-pass, the ASM capillary is no longer in the flow path, such that fast 2D gradients are possible through the sample loop only.
Active solvent Modulation (ASM)
Operating Principle

1. Classic 2D-LC: deck B in 2D

2. ASM position: dilute deck A

3. ASM position: dilute deck B

4. Classic 2D-LC: deck A in 2D

**Figure 8** Switching cycle of the ASM valve (countercurrent mode)

1. Cuts are parked in deck A.
2. 2D solvent flows through deck A and the ASM capillary.
3. ASM capillary leaves flow path, normal analysis with flow passing deck A. Further cuts are meanwhile parked in deck B.
4. Cuts in deck B are analyzed with ASM.
5. Cuts in deck B are further analyzed without ASM, new cuts are parked in deck A.

A full switching cycle of the ASM valve has 4 positions. Positions 1 and 3 are the same as for the standard 2D-LC valve G4236A. The ASM valve has two additional positions in step 2 and 4. In both steps, the ASM capillary is in the second dimension and dilutes solvent in deck A and B, respectively.
**Configuration**

**Adjusting the split ratio**

Different ASM capillaries are available for adjusting the split ratio and therefore the dilution.

The method can therefore be optimized either for optimum resolution (strong dilution) or lowest cycle time (weak dilution).
Configure the ASM Valve

**Figure 9**  ASM Valve configuration (overview)

1. Select a topology for using the ASM Valve.
2. Choose the ASM Valve as 2D-LC Valve. This is usually done automatically based on installed valves.
3. Choose an ASM capillary. This defines the split ratio.

**Preparations**

All modules including the ASM Valve are configured in OpenLAB CDS ChemStation Edition.

1. Select a valve topology using an ASM Valve.

**HINT**

For minimum carry-over, please use counter-current installation for the ASM Valve.

2. Select the ASM Valve as 2D-LC Valve (which is usually pre-selected).
3 Define the ASM capillary.
   a To configure capillaries, click on Capillaries... (see Figure 9 on page 8).
   b Select any of the pre-defined ASM capillaries.

![Figure 10](image) Figure 10 Configuration of the ASM valve with predefined capillaries

OR

If you are using a different capillary, you can choose **Generic Capillary**

![Figure 11](image) Figure 11 ASM valve configuration (overview)

In this case, you need to enter two of following three parameters: length, diameter or volume. These parameters are required for calculating the flush volume and back pressure, see “Understanding the ASM factor” on page 14

The ASM factor is calculated and displayed based on selected capillaries.

4 Install capillary connections as displayed in figure **Valve topology** in the UI, see Figure 9 on page 8.

**NOTE** Please note that ASM capillaries are labeled with ASM (in contrast to transfer and other capillaries).

**NOTE** Please note that port positions given in the MHC valve configuration in 2D-LC Software A.01.04 refer to standard 2D-LC valves, not ASM. Please use correct ports displayed figure **Valve topology**. This will be corrected in 2D-LC Software A.01.04 SR1.
Method development

ASM method development helps finding the optimal dilution of 1D solvents in the sample loop for best 2D resolution at lowest cycle time.

After switching on the ASM functionality (see “Method parameters” on page 10), execute the steps in the following order:

1. “Optimizing the dilution by using ASM capillaries” on page 11
2. “Optimizing the sample loop flush” on page 11
3. “Including the ASM phase to the 2D gradient” on page 12
4. “Optimizing dilution through method settings” on page 13

Method parameters

Advanced settings of 2D-LC method parameters allow switching on and off the use of the ASM functionality.

- If this option is off, it works as a standard 2D-LC valve without dilution.
- If this option is on, the user can set how often he wants to flush the sample loop during the ASM phase.
Optimizing the dilution by using ASM capillaries

A choice of four different ASM capillaries is available for achieving best results. Longer capillaries reduce, shorter capillaries increase the dilution of $^{13}$D solvent in the sample loop.

Install and configure different ASM capillaries (see “Configure the ASM Valve” on page 8) for optimizing the results.

<table>
<thead>
<tr>
<th>Capillary p/n</th>
<th>Length (mm)</th>
<th>Inner diameter (mm)</th>
<th>Volume (µl)</th>
<th>ASM factor</th>
<th>Split ratio (loop:ASM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5500-1300</td>
<td>85</td>
<td>0.12</td>
<td>0.96</td>
<td>5</td>
<td>1:4</td>
</tr>
<tr>
<td>5500-1301</td>
<td>170</td>
<td>0.12</td>
<td>1.9</td>
<td>3</td>
<td>1:2</td>
</tr>
<tr>
<td>5500-1302</td>
<td>340</td>
<td>0.12</td>
<td>3.8</td>
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<td>1:1</td>
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<tr>
<td>5500-1303</td>
<td>680</td>
<td>0.12</td>
<td>7.7</td>
<td>1.5</td>
<td>1:0.5</td>
</tr>
</tbody>
</table>

Optimizing the sample loop flush

Activate ASM in the software and set Flush sample loop to 3.0 times.

**NOTE**

Flushing the sample loop 3 times is typically enough and the recommended default. Less time may be sufficient and can be verified during optimization. The user interface displays how long this will take.

---

*Figure 13* Set Flush sample loop (example)
Including the ASM phase to the 2D gradient

Gradients that were programmed for the second dimension originally without ASM Valve must be shifted by the delay caused by this dilution during the ASM phase such that the analytical gradient starts after the ASM phase.

If the ASM phase takes for example 0.41 min (based on selected ASM capillary, flush factor and 2D flow rate), all times are shifted compared to a 2D gradient without ASM.

- Gradient ends later and the gradient stop time is increased by 0.41 min
- 2D Cycle time is increased accordingly
- One line is added to the gradient table for the ASM phase
- All times for the analytical gradient are shifted by 0.41 min.

This is true for shifted gradient steps as well (if applicable).

**Figure 14** Programming the 2D gradient table (example)
Optimizing dilution through method settings

For optimizing separation, you may use a lower percentage of B for the ASM phase and column equilibration phase compared to the original gradient for increasing dilution before the 2D column.

If for example the original analytical gradient started at 20 % B, you may use an ASM phase of for example 2 % B for diluting 1D solvent more strongly during the ASM phase by changing the gradient start condition and adding a line to the 2D gradient table for the ASM phase. The starting point for the analytical gradient does not change. The solvent composition of the equilibration phase is automatically reduced to the start condition.

Apply high-resolution sampling with small cut sizes. Small cut sizes reduce the transfer of solvent volume from 1D to 2D, which can further improve solvent compatibility and 2D resolution.
Understanding the ASM factor

The principle of ASM is diluting ¹D sample loop solvent with ²D solvent. The ASM solution achieves this dilution by a parallel flow of solvents via sample loop and ASM capillary.

\[
\text{Sample with } ^1\text{D solvent in sample loop}
\]

\[
\text{²D solvent from ²D pump}
\]

\[
\text{Parallel flow through ASM capillary}
\]

\[
\text{F}_{\text{loop}}, \text{P}_{\text{loop}}
\]

\[
\text{F}_{\text{ASM}}, \text{P}_{\text{ASM}}
\]

\[
\text{to ²D column}
\]

**Figure 16** Principle of active solvent modulation (schematic view)

The flow rates \( F \) through these parallel capillaries depend on the different backpressures \( p \) of the capillaries in use. The backpressure of a capillary depends on the capillary length \( l \), radius \( r \) to the power of 4, and the viscosity \( \eta \) of the solvent.

\[
p = \frac{8\eta l F}{\pi r^4} \quad \text{Hagen-Poiseuille equation}
\]

The Hagen-Poiseuille equation describes the relation of these parameters.

Different ASM capillary lengths have an effect on the following parameters:
- Capillary back pressure
- Dilution factor
- Optimum dilution for different applications
Example for calculation of split ratio and ASM factor.

**Figure 17** Backpressure of two flow paths in ASM

A longer capillary results in higher backpressure and therefore lower flow compared to a short capillary.

*Example:*

If the back pressure of the capillaries between ports 7 and 3 (2D-LC valve to sample loop and back) is twice as high as the back pressure of the ASM capillary between ports 9 and 6, twice as much solvent will run through the ASM capillary.

This will dilute 1D solvent in the sample loop by a factor of about 3, which is called the ASM factor.

Usage of the ASM capillary kit results in the following situation:

- The capillaries in ASM branch and transfer branch have the same inner diameter.
- The two transfer capillaries are equally long.
- The difference between $\text{ID}_{\text{loop}} = 0.35 \text{ mm}$ and $\text{ID}_{\text{capillaries}} = 0.12 \text{ mm}$ is large. Therefore the backpressure of the loops is negligible (this is, because the radius enters the Hagen-Poiseuille-Equation with the power of 4).
- Solvent composition and their viscosity in the parallel flowpaths are not predictable.

In the recommended configuration with the ASM capillary kit (see note above) one can simplify the formulae for the calculation of split ratio and ASM factor as follows:

\[
\text{Split ratio} = \frac{l_{\text{ASM}}}{2l_{t1,2}}
\]

\[
l_{\text{ASM}} = \text{Length of ASM capillary}
\]

\[
l_{t1,2} = \text{Length of transfer capillary 1 or 2}
\]

\[
\text{ASM factor} = 1 + \left(\frac{1}{\text{Split ratio}}\right)
\]

**NOTE**

The ASM factor calculated by the software should not be considered to be a fix number but as a guiding value which is subject to method development.
Comprehensive 2D-LC and Active Solvent Modulation

The ASM Valve can also be used for improving comprehensive 2D-LC measurements, but it is primarily optimized for multiple heart-cutting and high-resolution sampling measurements.

The ASM phase contributes to the modulation cycle. When keeping the modulation time constant, this reduces available time for the separation phase of the cycle. Otherwise, increasing the modulation time may require reducing the 1D flow rate to fill the same sample loop volume. This would change 1D chromatography.

The ASM solution requires back pressure from capillaries between the 2D-LC valve to multiple heart-cutting valves. Therefore, comprehensive 2D-LC sample loops cannot be installed directly at the ASM valve. In addition, comprehensive 2D-LC sample loops have standard fittings, which do not fit to the M4 ports of the ASM valve.

Please note that ASM valves require twice as many switches as a standard 2D-LC valve. Comprehensive 2D-LC uses many valve switches and in combination with ASM, this may reduce the maintenance interval of the valve.

Software Compatibility

The Active Solvent Modulation requires 2D-LC Software A.01.04 minimum. 2D-LC Software A.01.04 requires OpenLAB CDS Chemstation Edition C.01.07 SR3, LC Drivers A.02.16 and firmware A/B/C/D.07.20.

For details please refer to release notes for 2D-LC Software and OpenLAB CDS ChemStation Edition.
Installation

Delivery checklist

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>G4243-90000</td>
<td>Agilent G4243A 2D-LC ASM Valve Guide Technical Note</td>
</tr>
<tr>
<td>5067-4266</td>
<td>2D-LC ASM Valve Head, 1300 bar</td>
</tr>
<tr>
<td>G4236-68000</td>
<td>2D-LC Easy Starter Kit</td>
</tr>
<tr>
<td>G1680-63721</td>
<td>Network LAN Switch</td>
</tr>
<tr>
<td>5500-1300</td>
<td>Capillary ST 0.12x85M/M ASM</td>
</tr>
<tr>
<td>5500-1301</td>
<td>Capillary ST 0.12x170M/M ASM</td>
</tr>
<tr>
<td>5500-1302</td>
<td>Capillary ST 0.12x340M/M ASM</td>
</tr>
<tr>
<td>5500-1303</td>
<td>Capillary ST 0.12x680M/M ASM</td>
</tr>
<tr>
<td>5500-1376</td>
<td>Capillary ST 0.12x170M/M transfer</td>
</tr>
<tr>
<td>5067-6171</td>
<td>Capillary Kit 2D-LC, Infinity Classic (optional)</td>
</tr>
<tr>
<td>5067-6585</td>
<td>Capillary Kit 2D-LC, 1290 Infinity II</td>
</tr>
</tbody>
</table>

For re-ordering parts, see “Replacement Parts” on page 23.
Installation Instructions

Setup

The installation of the valve depends on whether a co- or counter-current configuration shall be used. When working in ASM mode, Agilent recommends using a counter-current configuration. This section describes the setup for a counter-current configuration of the ASM Valve. For the co-current setup, please refer to “Co-current Configuration” on page 19.

The installation of a 2D-LC system depends on which modules you are using for which 2D-LC mode and is described in the 2D-LC Quick Installation Guide G4236-90020, which you can find on your 2D-LC Software DVD in folder documentation or on www.agilent.com using the guide part number.

In that documentation, the 2D-LC Valve ASM G4243A can be used in place of a standard 2D-LC Valve G4236A. For installation of connections to the system (1D column, 2D column, 2D pump and waste), please refer to the Quick Installation Guide.

The connection scheme is displayed in the graphical user interface of the 2D-LC Configuration as Valve Topology:

Please install following capillary connections:
Port Type Connection Capillary
---
1 10-23 to waste See "Quick Installation Guide"
2 M4 transfer capillary from MHC Valve, deck A 5500-1376
3 M4 transfer capillary from MHC Valve, deck B 5500-1376
4 10-23 to 1D column, 1D detector or pressure release kit See "Quick Installation Guide"
5 10-23 from 2D pump See "Quick Installation Guide"
6 M4 outlet to ASM capillary See list below
7 M4 transfer capillary to MHC Valve, deck B 5500-1376
8 M4 transfer capillary to MHC Valve, deck A 5500-1376
9 M4 inlet from ASM capillary See list below
10 10-23 to 2D column See "Quick Installation Guide"

List of ASM capillaries:
Which ASM capillary shall be used depends on the ASM factor, which is optimum for your application. You may choose from following capillaries:

<table>
<thead>
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</tbody>
</table>

**Co-current Configuration**

Co-current configuration may be used if the ASM valve is used as standard 2D-LC valve (set ASM mode off in 2D-LC method).

When using this configuration, please choose it as topology in the 2D-LC configuration. It will display all connections required. Please install capillaries accordingly.
The valve actuator contains sensitive optical parts, which need to be protected from dust and other pollutions. Pollution of these parts can impair the accurate selection of valve ports and therefore bias measurement results.

Always install a valve head for operation and storage. For protecting the actuator, a dummy valve head can be used instead of a functional valve. Do not touch parts inside the actuator.

The following procedure exemplarily shows a valve head installation. For correct capillary connections see **Valve topology** in the GUI.

For a correct installation of the valve head, the outside pin (red) must completely fit into the outside groove on the valve drive’s shaft (red). A correct installation is only possible if the two pins (green and blue) on the valve head fit into their corresponding grooves on the valve drive’s actuator axis. Their match depends on the diameter of the pin and groove.

The tag reader reads the valve head properties from the valve head RFID tag during initialization of the module. Valve properties will not be updated, if the valve head is replaced while the module is on. Selection of valve port positions can fail, if the instrument does not know the properties of the installed valve.

To allow correct valve identification, power off the module for at least 10 s.
1 Insert the valve head into the valve shaft.

OR
If the outside pin does not fit into the outside groove, you have to turn the valve head until you feel that the two pins snap into the grooves. Now you should feel additional resistance from the valve drive while continuously turning the valve head until the pin fits into the groove.

2 When the outer pin is locked into the groove, manually screw the nut onto the valve head.

NOTE
Fasten the nut with the 5043-1767 Valve Removal tool.
3 Install all required capillary connections to the valve.

4 Power on or power-cycle your module, so the valve head gets recognized during module initialization.
Valve head parts information

Replacement Parts

Table 1  ASM Valve Head

<table>
<thead>
<tr>
<th>Stator</th>
<th>Rotor</th>
<th>Bearing ring</th>
<th>Stator screws</th>
</tr>
</thead>
<tbody>
<tr>
<td>5068-0239</td>
<td>5068-0240</td>
<td>5068-0257</td>
<td>5068-0019</td>
</tr>
</tbody>
</table>

**NOTE**

Capillaries:

Agilent Technologies recommends replacing ASM and transfer capillaries at the same time.

The ASM Valve Capillary Replacement Kit (5067-6721) contains a set of capillaries with matching back pressures and volumes.

Table: 5067-6721 ASM Valve Capillary Replacement Kit

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5500-1300</td>
<td>Capillary ST 0.12x85M/M ASM</td>
</tr>
<tr>
<td>5500-1301</td>
<td>Capillary ST 0.12x170M/M ASM</td>
</tr>
<tr>
<td>5500-1302</td>
<td>Capillary ST 0.12x340M/M ASM</td>
</tr>
<tr>
<td>5500-1303</td>
<td>Capillary ST 0.12x680M/M ASM</td>
</tr>
<tr>
<td>5500-1376</td>
<td>Capillary ST 0.12x170M/M transfer</td>
</tr>
</tbody>
</table>
Valve Head Parts

The figure below illustrates replacement parts for the valve heads, with the 12ps/13pt selector valve as an example. The valves can vary in their appearance and do not necessarily include all of the illustrated parts. Neither, every spare part is available for each flavor of the valve.

Figure 18  Valve Head Parts (example)

1  Stator screws
2  Stator head assembly
3  Stator ring screws (not available)
4  Stator ring (available for service only)
5  Rotor seal
6  Bearing ring
7  Spanner nut (available for service only)

Technical specifications

Table 2  Technical specifications

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<tbody>
<tr>
<td>Max. Pressure:</td>
<td>1300 bar</td>
</tr>
<tr>
<td>Liquid Contacts:</td>
<td>Stainless Steel, PEEK</td>
</tr>
<tr>
<td>Connections:</td>
<td>Accepts 10-32 male threaded and M4 fittings</td>
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</table>