# Injection of large sample volumes using the Agilent 1100 Series purification system with an injection pump

## Application

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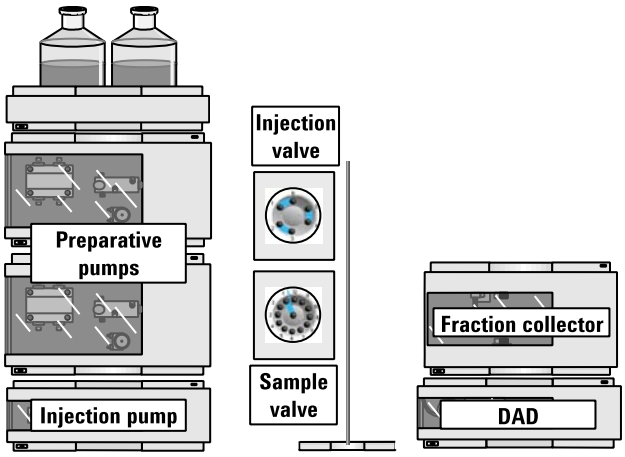


**Abstract**

Column loading and overloading in preparative HPLC can be performed either by concentration overloading through increased sample concentration at constant sample volume, or by volume overloading through applying more sample volume of the same concentration onto the column. While small sample volumes are usually injected by an autosampler, higher sample volumes above 10 mL up to the liter range are usually applied to the column using an injection pump. In this Application Note we show how a simple valve solution[1] can be set up and configured into an Agilent 1100 Series purification system[2, 3], using an isocratic pump as injection pump. The system is controlled via pump and valve timetables. System enhancements, for the injection of high-concentrated samples and simple time-based fraction collection, are decribed. Further, the mass-based purification of a real-life, drug discovery sample on an Agilent 1100 Series purification system equipped with an injection pump is shown.

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## Introduction

For high-throughput purification it is important to purify as much compound as possible in a single run. Therefore, the preparative column has to be overloaded, which means that much more sample is applied per weight of packing material than in an analytical run. Overloading is usually done as concentration overloading where a small sample volume with a high concentration is injected. The limiting factor is the solubility of the compound in the sample solvent and the necessity to avoid precipitation during the injection cycle. If the maximum sample concentration is reached, further overloading can only be achieved by volume overloading where only the sample volume is increased. Depending on the solubility of the sample compound, sample injection volumes can vary from a few microliters up to several liters. While small sample volumes up to 5 mL can easily be injected using an autosampler, larger sample volumes are usually injected using an injection pump. If a low-pressure injection pump is used, for example, a peristaltic pump, the sample has to be pumped into a sample loop, which is then switched into the high-pressure flow path to the column. If a high-pressure injection pump is used, the sample can be pumped directly onto the column. This latter process has the advantage that the sample is preconcentrated on the column head. Such a purification system, equipped with an Agilent 1100 Series isocratic pump as high-pressure injection pump, is described in this Application Note.

## Equipment

The experiments were performed on an Agilent 1100 Series system containing the following modules:

- Two Agilent 1100 Series preparative pumps
- Agilent 1100 Series isocratic pump (as injection pump)
- Agilent 1100 Series column organizer
- Agilent 1100 Series diode-array detector (DAD)
- Agilent 1100 Series fraction collector PS
- Agilent 1100 Series 12-position/13-port valve
- Agilent 1100 Series 2-position/6-port valve

The system was controlled via the Agilent ChemStation (rev. A.10.02).

The cover page shows the system configuration and table 1 lists the required stainless steel capillaries. PEEK tubing of the same lengths with appropriate inner diameters (0.5 mm, orange) together with PEEK fittings can also be used (for plumbing see figures 1 and 2).

The capillaries included in the 0.3-mm or 0.06-mm preparative flow cell were used to connect the preparative column to the diode-array detector and the diode-array detector to the fraction collector. The capillaries leading from the sample valve to the bottles containing solvent A, sample and wash solvent should be cut as short as possible after setting up the system.

| Capillary | From | To | Order number | Qty. |
|---|---|---|---|---|
| 0.6 × 400 mm | Preparative pump | Injection valve | G1361-67302* | 1 |
| 0.5 × 150 mm | Injection valve (port 3) | Injection valve (port 4) | 5022-6509 | 1 |
| 0.5 × 280 mm | Injection valve | Column | 5022-6510 | 1 |
| 0.5 × 600 mm | Injection pump | Injection valve | G2260-87300 | 1 |
| 1.5 × 600 mm | Sample valve | Injection pump/bottles | G1160-67300 | 2 |
| Adapter | Sample valve | | 0100-2298 | 2 |

* included in G1361A

**Table 1**
**Required capillaries**

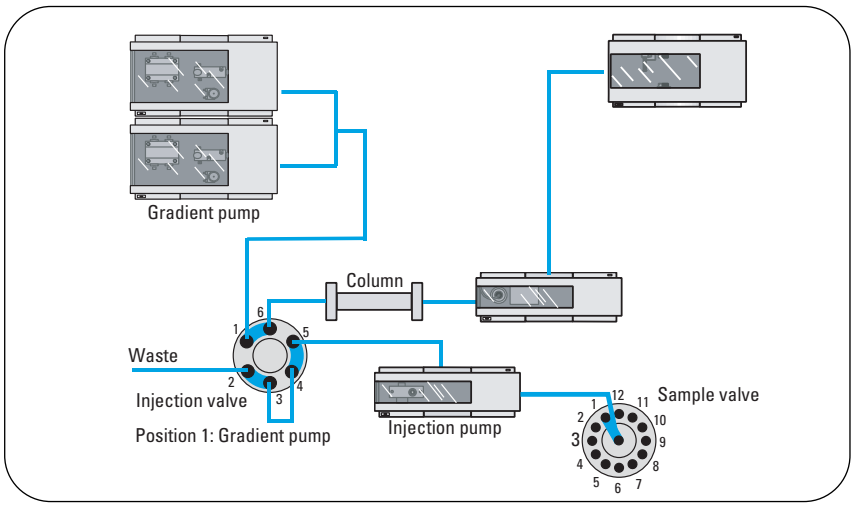


**Figure 1**
**System configuration**

## Results and discussion

### System configuration

The configuration of the system is shown in figure 1. A 2-position/6-port valve is used to switch between the preparative pumps, which generate the gradient for the purification run and the injection pump. The complete purification run consists of three steps:

1. Initially the injection valve is in position 1, in which the flow from the gradient pump goes to the column and the flow of the injection pump goes to waste.
2. When the run is started the injection valve switches to position 2 where the flow from the injection pump goes to the column. The injection pump transfers the sample onto the column.
3. After the sample is applied to the column the injection valve switches back to position 1 and the gradient is started. While the gradient is applied to the column, the injection pump and the injection valve are washed with an appropriate solvent.

### Sample injection system

The sample injection system consists of the injection pump and a 12-position/13-port valve where the outlet position of the valve is connected to the pump. Several containers with solvent of the gradient starting conditions (solvent A), the sample and wash solvent are connected in the following order to the inlet positions (figure 2):

- Position 1: Initial position, solvent A (gradient start composition)
- Position 2: Sample
- Position 3: Solvent A to flush sample through the valve and the injection pump completely onto column
- Position 4: Wash solvent to rinse injection valve and injection pump
- Position 5: Solvent A to remove wash solvent from injection pump and injection valve

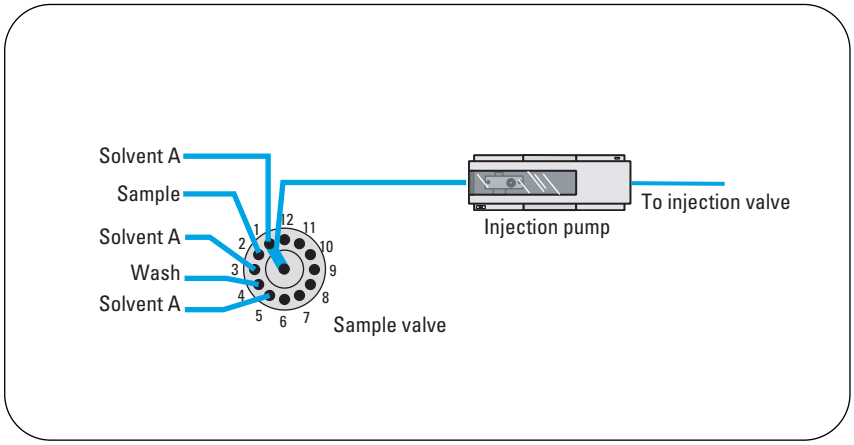


**Figure 2**
**Sample injection system**

The injection of the sample, flushing the sample onto the column and washing and re-equilibration of the sample valve and the injection pump is performed in seven steps:

1. At the start of the run the flow of the injection pump is zero.
2. After switching the injection valve to the injection pump the flow of the injection pump is increased. During the injection cycle the flow of the gradient pump can be lowered to save solvent.
3. When the injection cycle is started the sample valve is switched across the positions (sample, wash, solvent A) using the valve timetable.
4. The injected sample volume is determined by the flow rate of the injection pump and the time the valve remains in position 2. If a certain volume has to be injected completely the flow rate/time has to be optimized.
5. After the sample is injected solvent A from position 3 is used to flush the sample completely through the injection pump onto the column.
6. After the injection valve is switched into position 2 (gradient pump) the sample valve and injection pump must be washed thoroughly with an appropriated solvent from position 4.
7. After washing the injection valve and the sample injection system must be re-equilibrated with solvent A from position 5.

### Timing of pumps and valves

The workflow described under “System configuration” and “Sample injection system” is executed by setting up timetables for the gradient pump, injection pump, injection valve and sample valve. Since each module has its own timetable it is recommended to set

| Time | Gradient pump | | Injection | Inject. pump | Sample | Comment |
|---|---|---|---|---|---|---|
| | (% B) | Flow (mL/min) | valve pos. | Flow (mL/min) | valve pos. | |
| 0.0 | 5 | 20 | 1 | 0 | 1 | Starting conditions |
| 0.1 | | 20 | 2 | | | Injection valve to injection pump pos. |
| 0.2 | | 1 | | 0 | 2 | Sample valve to sample pos., lower gradient pump flow |
| 0.3 | | | | 10 | | Start injection pump |
| * 1.3 | | 1 | | | 3 | Sample valve to solvent A pos., transfer sample onto column |
| ** 1.8 | 5 | 20 | 1 | | | Injection valve to gradient pump pos., increase gradient pump flow |
| 1.9 | | | | | 4 | Sample valve to wash solvent pos. |
| ** 2.4 | | | | | 5 | Sample valve to solvent A pos., re-equilibrate |
| ** 2.9 | | | | 10 | | |
| 3.0 | | | | 0 | | Decrease flow of injection pump |
| 11 | 95 | | | | | End of gradient |

* timing depends on sample volume to be injected
** timing depends on injection system volume (capillaries, injection pump, valve)

**Table 2**
**General timetable**

up a general timetable first (table 2), for example in Microsoft Excel® and then extract the settings for each module.

This general timetable translates into the four timetables for the gradient pump, injection pump, injection valve and sample valve (figure 3). Other aspects have to be considered for a seamless operation of the injection pump system as follows:

- A blank run must be set up in the ChemStation because there is no autosampler in the system to give a start pulse.
- The run does not start with the injection, which is the switching of the injection valve back to the gradient pump position, but with the start of the injection cycle. The advantage is that if a compound of interest breaks through the column it will still be collected.
- For collection of fractions of large volumes the funnel tray can be used.

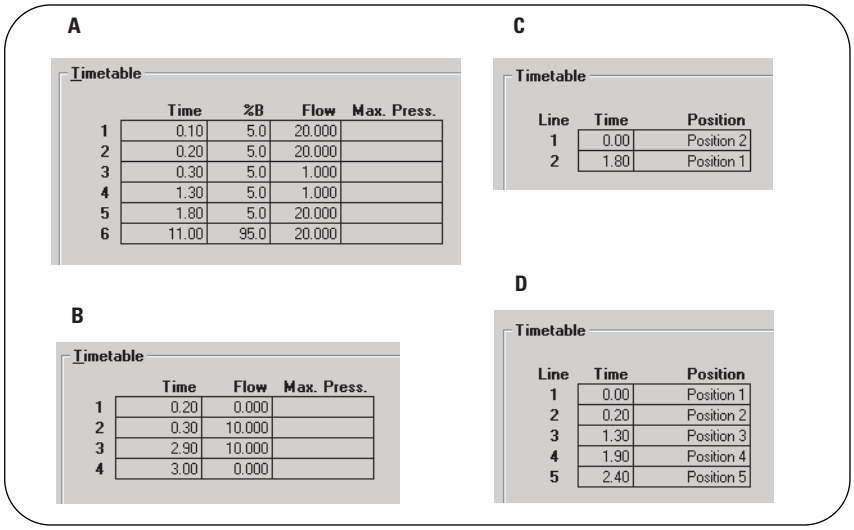


**Figure 3**
**A: Timetable gradient pump (Initial: 5 % B, 20 mL/min)**
**B: Timetable injection pump (Initial: 0 mL/min)**
**C: Timetable injection valve (Initial: position 1, gradient pump**
**D: Timetable sample valve (Initial: position 1, solvent A)**

## System scope and limitations

- The system is operated by the Agilent ChemStation (rev. A.10.02) only. It cannot be used with the Purification software.
- Fractions can be collected based on time or peak using the ChemStation sequence. Mass-based fraction collection is only possible if a single run (*Run Method* task) is set up.
- The injection cycle is controlled by gradient pump, injection pump, injection valve and sample valve timetables.

- The delay volume calibration procedure is not available because there is no autosampler in the system.
- The maximum flow rate of the isocratic pump is 10 mL/min. This is also the maximum flow rate for the sample valve. For higher flow rates a third preparative pump can be used without sample valve.
- For highly concentrated samples it is recommended to remove the PTFE frit from the purge valve of the isocratic pump (procedure described in the isocratic pump manual, part number G1310-90003).

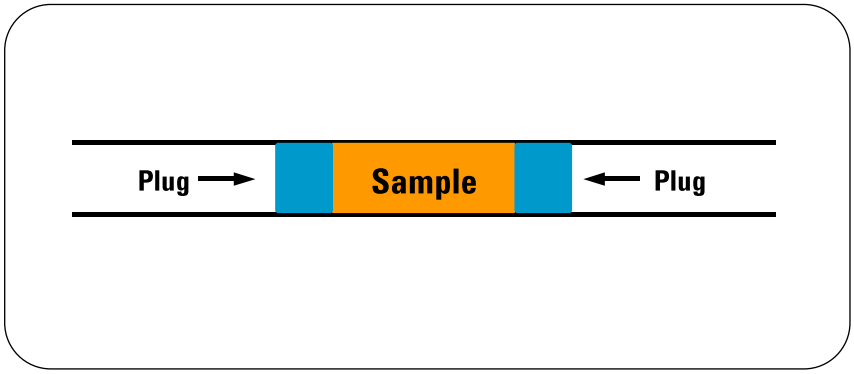


**Figure 4**
**Sandwich injection**

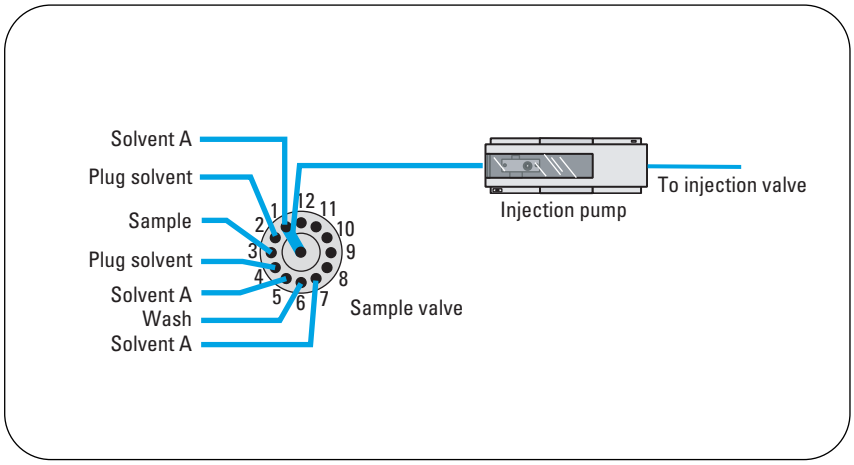


**Figure 5**
**Sample valve configuration for sandwich injection**

### System enhancement 1: Sandwich injection

For the injection of samples of high concentration[4] a technique called "sandwich injection" can be used. The idea is to protect the sample with a plug of pure sample solvent on either side to avoid mixing with the mobile phase, which could lead to precipitation (figure 4). With this technique, mixing with mobile phase occurs only at the beginning and the end of the plugs, where the sample concentration is zero. To perform sandwich injection with the injection pump system, two additional ports of the sample valve are used as shown in figure 5. The timetable for the pumps and valves must be adjusted accordingly.

### System enhancement 2: Simple time-based fraction collection

If only a small number of fractions have to be collected based on retention time windows a second 12-position/13-port valve can be used instead of the fraction collector as described in another Application Note[5]. Control of time-based fraction collection is performed using the timetable of this valve, tracking of the fractions is only possible by the valve positions and switching times in the report. There are also no fraction tick-marks available in the data analysis or in the report.

### Applications

A system as described under "Equipment" was evaluated by Graham Foster and Richard O'Hanlon in the achiral/chiral HPLC large scale preparative laboratory at GSK Stevenage, UK. This application is a typical example for the purification of a compound in the upper milligram to lower gram scale in their drug discovery process. The sample provided by a chemist was 9 g of crude yellow oil, this was dissolved in 70 mL DMF. 0.5 mL TFA was added to 5 mL of the solution thus yielding 5.5 mL containing 650 mg crude product. This sample was injected onto the column. After 14 repetitive runs,

| | |
|---|---|
| Column: | C8 50 x 250 mm, 7 µm |
| Mobile phases: | water + 0.5 % TFA = A<br>acetonitrile + 0.5 % TFA = B |
| Gradient: | at 0 min 0 % B<br>at 40 min 75 % B<br>at 60 min 75 % B |
| Stop time: | 60 min |
| Flow: | 80 mL/min |
| Injection: | 5.5 mL |
| Column temp.: | ambient |
| UV detector: | DAD 280 nm/100<br>(ref. 550 nm/100)<br>Prep. flow cell<br>(0.06 mm pathlength) |
| **MSD** | |
| Ionization mode: | API-ES positive |
| Scan-range m/z: | 250 - 1000 |
| Drying gas flow: | 13 L/min |
| Nebulizer pressure: | 60 psig |
| Drying gas temp.: | 350 ºC |
| Cap. Voltage: | 3000 V |

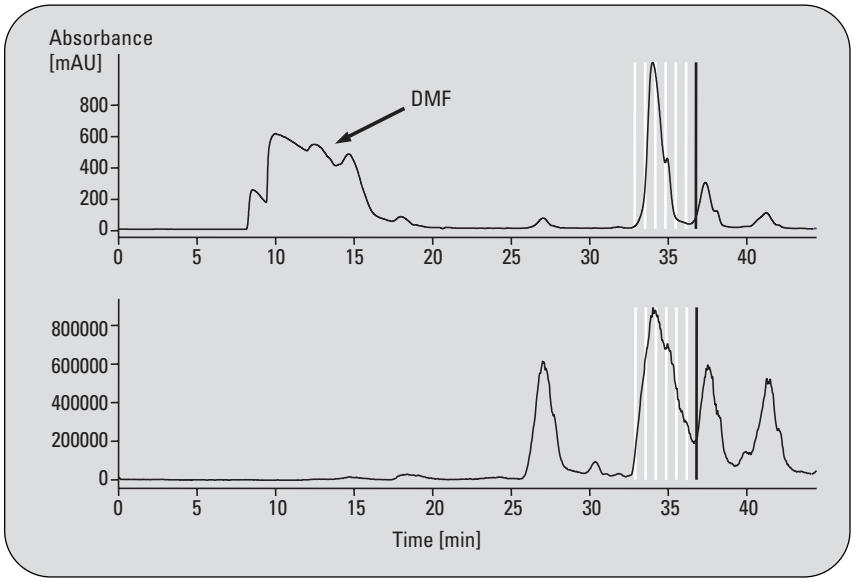


**Figure 6**
**Compound purification of 650 mg crude product**

removal of the solvent and the TFA, about 1g (the expected yield was 1.2 g) of a pure white powder was given back to the chemist. The chromatogram and the method are shown in figure 6.

## Conclusion

In this Application Note we showed the setup and configuration of an Agilent 1100 Series purification system with an isocratic pump and a simple valve solution for sample injection. The method setup using timetables for the pumps and valves were described and further system enhancements for the injection of high-concentrated samples and for simple time-based fraction collection were also shown. In the last part the purification of a real-life drug discovery sample by mass-based fraction collection on an Agilent 1100 Series purification system equipped with an isocratic injection pump was shown.

## References


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