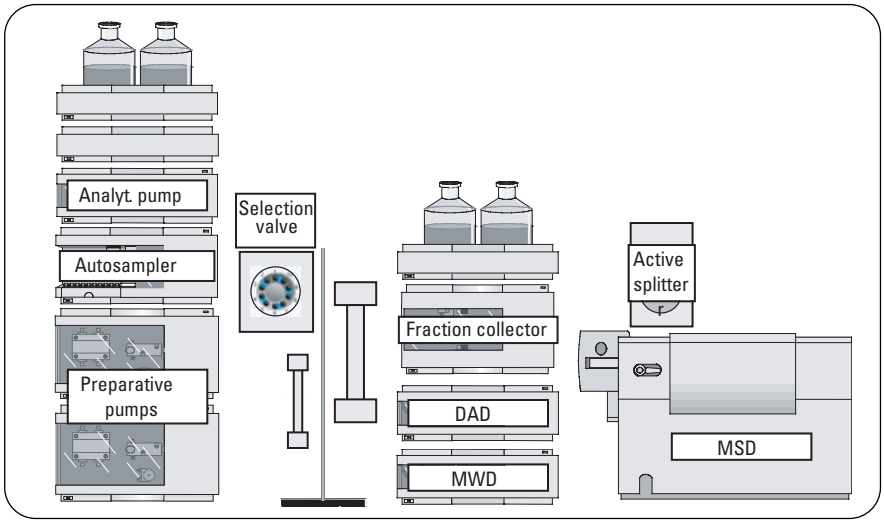
An optimized Agilent 1100 Series system for analytical and preparative work

Application

Udo Huber
Tony Brand



Abstract

This Application Note describes the configuration, setup and operation of an Agilent 1100 Series HPLC system for analytical and preparative work. The system is set up using a two 2-position/10-port valve[1], which is switched between preparative and analytical mode. The system is generically controlled using application-specific methods, which make it possible to operate it with the Agilent ChemStation software alone, with the Purification software or with the EasyAccess Plus software.

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Introduction

The typical workflow for purification of compounds in drug discovery includes not only preparative but also several analytical HPLC steps. First the purified compounds have to be analyzed for purity before they are sent to activity testing, and second a pre-preparative analysis is often performed to confirm the presence of the target mass and to give an indication of the amount of target compound in the reaction mixture. Very often preparative and analytical HPLC are carried out on dedicated systems. The advantage in using this approach is that analytical and preparative work can be done in parallel and that the valuable preparative system is not tied up with routine analytical work. On the other hand, two expensive mass-selective detectors (MSD) are required for sophisticated purification and analysis of the purified compounds. Therefore, if the number of samples to be purified per day is not too high, a single system could be used to perform both tasks.

A combined system for analytical and preparative work is always a compromise with regard to performance of the hardware, such as, the inner diameter of the capillaries. In this Application Note we will illustrate the setup of a system with the best possible configuration.

Experimental

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

- Two Agilent 1100 Series preparative pumps
- Agilent 1100 Series dual-loop autosampler PS
- Agilent 1100 Series quaternary pump
- Agilent 1100 Series column organizer
- Agilent 1100 Series multi-wavelength detector
- Agilent 1100 Series diode array detector
- Agilent 1100 Series fraction collector PS
- Agilent 1100 Series 2-position/10-port valve
- Agilent 1100 Series mass-selective detector
- Agilent active splitter

The system was controlled using the Agilent ChemStation software (rev. A.10.02). The dual-loop autosampler was equipped with a 2000-µL and a 50-µL loop. The multi-wavelength detector (MWD) was used for preparative work and was equipped with a 0.3-mm or 0.06-mm pathlength preparative flow cell. The diode-array detector (DAD) was used for analytical work and was equipped with a 10-mm pathlength flow cell. The configuration of the system is shown on the cover page, the required stainless steel capillaries are listed in table 1. PEEK tubing of the same lengths with appropriate inner diameters (0.5 mm, orange, 0.18 mm, yellow, 0.13 mm, red) together with PEEK fittings can also be used (for plumbing see figure 2).

Capillary	From	To	Part number
0.5 x 600 mm	Preparative pump	Selection valve port 2	G2260-87300
0.5 x 280 mm	Selection valve port 3	Dual-loop autosampler	5022-6510
0.5 x 400 mm	Dual-loop autosampler	Selection valve port 10	G2260-87301
0.5 x 150 mm	Selection valve port 1	Preparative column	5022-6509
0.17 x 600 mm	Quaternary pump	Selection valve port 4	G1312-67305*
0.17 x 280 mm	Selection valve port 9	Analytical column	5021-1818
0.17 x 600 mm	Analytical column	Diode-array detector	5065-9933
0.17 x 700 mm	Diode-array detector	Selection valve port 6	5065-9932
0.17 x 800 mm	Selection valve port 5	Active splitter	01048-87303
0.12 x 200 mm	Active splitter	MSD sprayer	5065-9935

* included in G1311A

Table 1
Required capillaries

The capillaries included in the 0.3-mm or 0.06-mm preparative flow cell were used to connect the preparative column to the multi-wavelength detector and the multi-wavelength detector to the active splitter. The inlet capillary of the fraction collector was used to connect the active splitter to the fraction collector enhanced by an appropriate delay volume as described in the Purification System User's Guide[2].

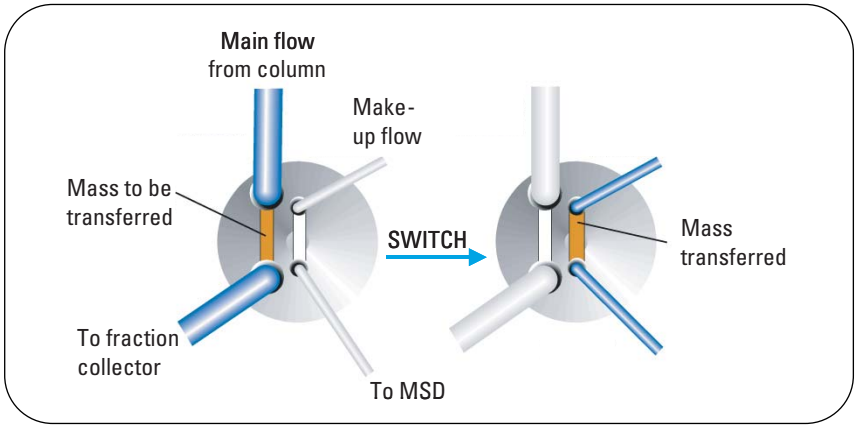

Figure 1
Operating principle of the active splitter

Results and discussion

Basic ideas

Mode selection

The analytical or preparative mode is chosen by selecting a method. There is at least one method for analytical work and one for preparative work. The selection valve is used to switch between analytical and preparative mode guiding the flow either from the quaternary or from the preparative pump through the dual-loop autosampler. In the preparative mode the quaternary pump is used as a make-up pump. In this case the 2-position/10-port valve switches the analytical column and the diode-array detector out of the flow path.

Active splitter

A flow splitter is required for preparative work with mass-based fraction collection – in the Agilent 1100 Series purification system the Agilent active splitter[3] is used. The operating principle is shown in figure 1 – the two flow paths, the main flow from the preparative pumps to the fraction collector and the make-up flow from the make-up pump to the MSD, are completely separate. The splitter is a rapidly switching valve collecting the mobile phase from the main flow and transferring it into the make-up flow. During a preparative session the splitter is switched on and the quaternary pump is used to deliver the make-up flow. During an analytical session the splitter is switched off. The flow from the quaternary pump is directed through the dual-loop autosampler, the analytical column, diode array detector, and the make-up flow section of the Agilent active splitter to the MSD. The *RunSync* command from the *Active splitter* menu in the Agilent ChemStation software is used to make sure the active splitter is turned on in a preparative method but not turned on in an analytical method. If *RunSync* is selected the splitter is automatically turned on when the method is started and turned off when it is finished. If *RunSync* is not selected the splitter remains off during the complete run.

Software control

Since the analytical or preparative mode is selected by loading a specific method the system can be used with the Agilent ChemStation software (rev. A.10.02), with the Purification software or with Easy Access Plus.

System plumbing

The system plumbing is shown in figure 2. The only large i.d. capillaries (0.5-mm i.d) that are used for analytical work are the ones connecting the dual-loop autosampler to the selection valve. All other capillaries used in the analytical flow path have a small i.d. (0.18-mm).

Valve setup

When a method is loaded in the Agilent ChemStation software the method parameters such as pump flow etc. are applied. The same is valid for the valve positions. If a method with a specific valve position is loaded the valve immediately switches to this position. Therefore the preparative method contains the valve settings for the selection valve as shown in figure 3. Valve position 2 is set up for the selection and the column valve in the analytical method.

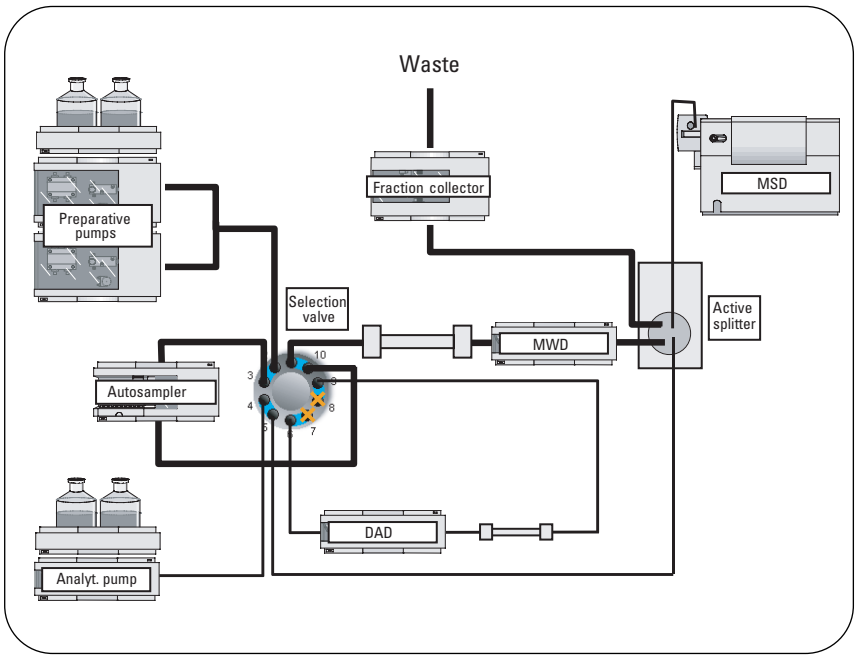

Figure 2
System plumbing diagram

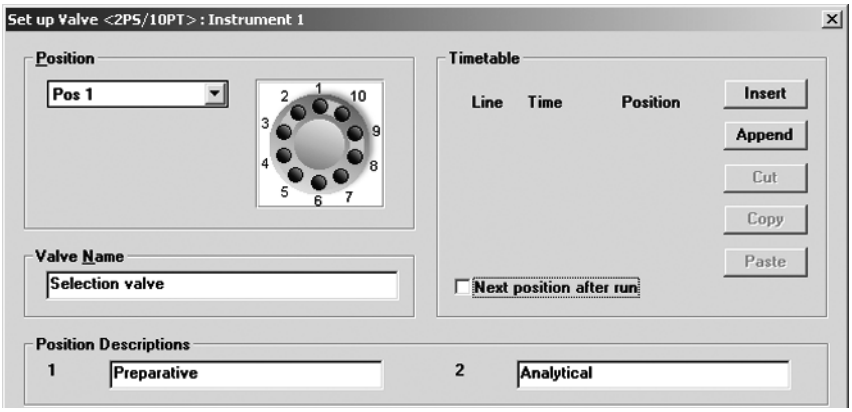

Figure 3
Preparative setting for the selection valve

Dual-loop autosampler

The dual-loop autosampler contains two injection loops. For preparative work a 2000-µL was configured and the partial loop fill technique was used [4]. For analytical work, a 50-µL loop was used either in partial or complete loop fill mode, depending on the concentration of the analytical sample.

Application example

The following sample applications were performed on a system setup and configured as described earlier. Fractions were collected based on a combination of UV-based (slope only, up slope 150 mAU/s, down slope 150 mAU/s) and mass-based fraction collection (threshold only, 150000 counts) using a logical AND connection[5] for the preparative run. The results are shown in figure 4.

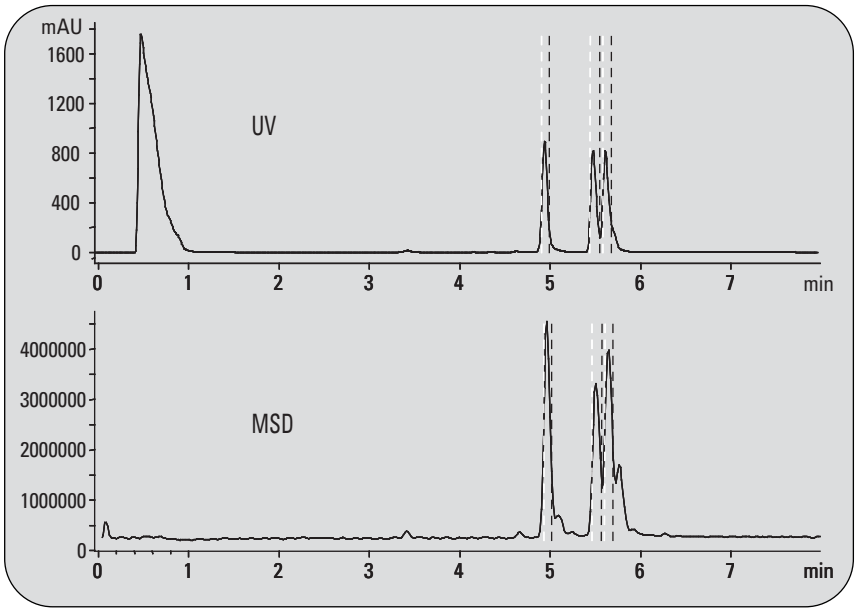

Figure 4
System plumbing diagram

Chromatographic conditions:		**MSD**	
Column:	ZORBAX SB-C18	Make-up flow:	1 mL/min
	21.2 x 50 mm, 5 µm	Make-up solvent:	water/acetonitrile.
Mobile phases:	water + 0.1 % HCOOH= A		50:50 + 0.1 % HCOOH
	acetonitrile + 0.1 %	Ionization mode:	API-ES positive
	HCOOH= B	Scan range m/z:	200 - 600
Gradient:	at 0 min 10 % B	Fragmentor:	20 Volt
	at 2 min 10 % B	Threshold:	250
	at 6 min 90 % B		
	at 7 min 90 % B	Drying gas flow:	13 L/min
	at 8 min 10 % B	Nebulizer pressure:	55 psig
Stop time:	8 min	Drying gas temp.:	350 ºC
Post time:	3 min	Cap. voltage:	3000 V
Flow:	25 mL/min		
Injection:	500 µL		
Column temp.:	ambient		
UV detector:	DAD 220 nm/16 (ref. off)		
	Prep. flow cell		
	(0.06-mm pathlength)		

The following runs (figure 5) using the collected fractions from the preparative run were performed in the analytical mode.

Carry over

The dual-loop autosampler has several features to minimize carry over. After the sample is drawn into the needle the needle exterior can be cleaned either by dipping it into a wash vial or by actively washing the needle with an appropriate solvent delivered by a peristaltic pump. The carry over can be lowered even further by washing the needle interior, the needle seat and the injection valve after the injection using the wash solvent delivered by the metering device. Applying the rinse and flush features properly lowers the carry over usually to about 0.01 %. When using the dual-loop autosampler for preparative samples the amount and concentration are several orders of magnitude higher than for analytical samples. Therefore special care must be taken to avoid carry over from a preparative to a subsequent analytical run.

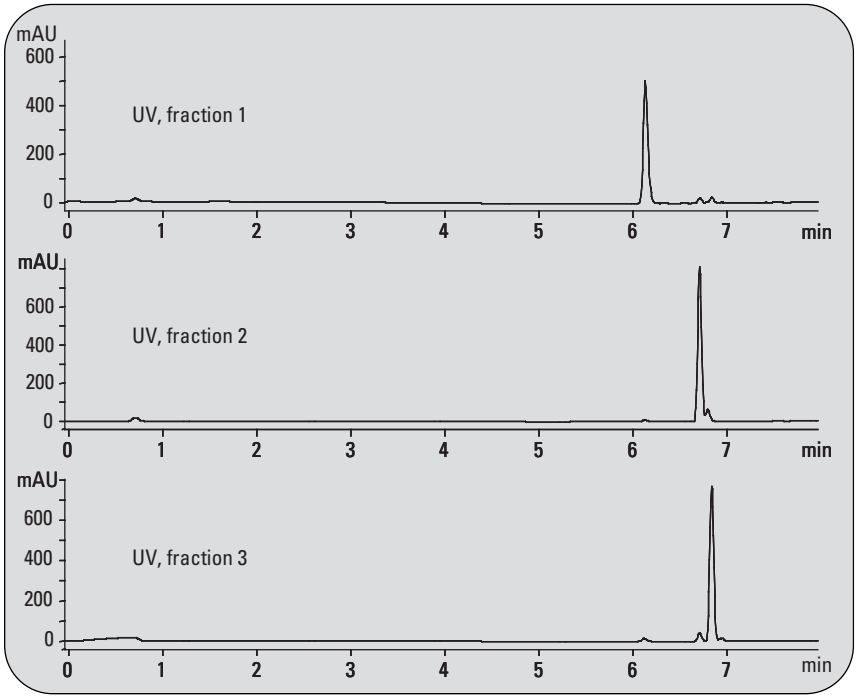

Figure 5
Analytical runs

Chromatographic conditions:		**MSD**	
Column:	ZORBAX SB-C18	Ionization mode:	API-ES positive
	4.6 x 50 mm, 5 µm	Scan range m/z:	200 - 600
Mobile phases:	Water + 0.1 % HCOOH= A	Fragmentor:	20 Volt
	Acetonitrile + 0.1 % HCOOH= B	Threshold:	250
Gradient:	at 0 min 10 % B		
	at 2 min 10 % B	Drying gas flow:	13 L/min
	at 6 min 90 % B	Nebulizer pressure:	55 psig
	at 7 min 90 % B	Drying gas temp.:	350 ºC
	at 8 min 10 % B	Cap. Voltage:	3000 V
Stop time:	8 min		
Post time:	5 min		
Flow:	1 mL/min		
Injection:	10 µL		
Column temp.:	ambient		
UV detector:	DAD 220 nm/16 (ref. off)		
	Prep. flow cell		
	(10-mm pathlength)		

Figure 6a illustrates the injection of a sample in the analytical mode with a concentration of 0.01 % of the preparative sample, equaling 0.01 % carry over. In this instance the peaks are well above 50 mAU. Figure 6b illustrates the injection of 10 µL of acetonitrile in the analytical mode immediately after a preparative run. Even with a carry over of slightly below 0.01 % the peaks are still visible. The only way to lower the carry over further is to perform a wash run after the preparative run and prior to the analytical run. As a result the carry over for this application could be lowered to about 0.0008 % as shown in figure 6c. The following method was applied for the wash procedure:

- Overfill loop by factor 1.0 (50-µL loop)
- Injector program:
  DRAW 500.0 µl from flush, def. speed
  WAIT 0.05 minutes
  EJECT 500.0 µl into seat, def. speed
  WAIT 0.05 minutes
- Needle wash in flush port for 10 s
- Rinse volume 20.0 times

The flush volume was set to 500 µL in the injector program, which equals ten times the loop size of 50 µL. The rinse volume was set to the maximum. The column valve was switched to the preparative position to bypass the analytical column. A short gradient was applied to wash the compounds through the system. A sample position must be set up and the wash method must be assigned to this sample position in the sequence table of the ChemStation or in the study of the Purification software. No blank run must be set up. However, the vial in the sample position is not used, which means no injection from the vial is done.

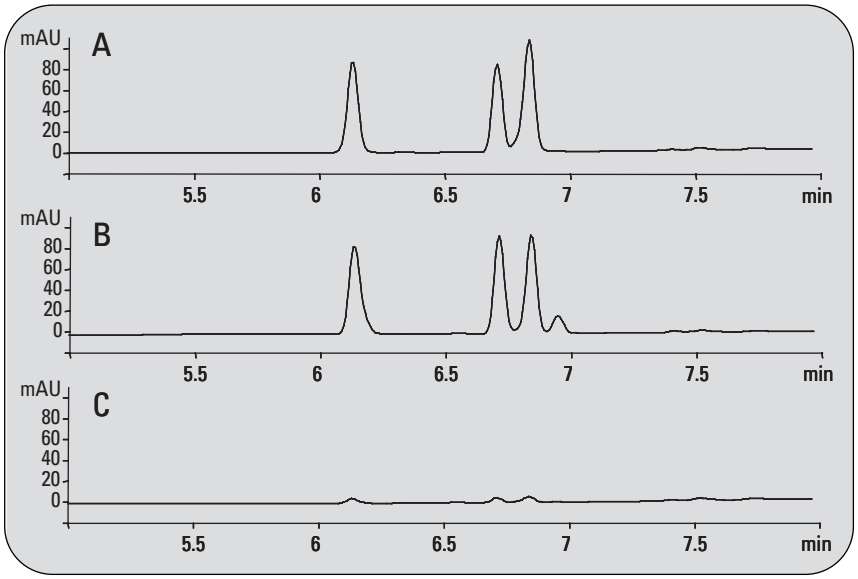

Figure 6
Carry over experiments

Conclusion

In this Application Note the configuration, setup and operation of a system suitable for preparative and analytical runs is described. The mode used is determined by applying a specific method to the sample. Due to the generic approach the system can be used for ChemStation-only operation (ChemStation rev. A.10.02), with the Purification software as well as with EasyAccess Plus. Nevertheless, a combined system for analytical and preparative work is always a compromise with regard to flow paths. The system described in this Application Note was set up with an the best possible configuration.

References

1.
"New dimensions for HPLC applications", *Agilent Technologies Brochure, publication number 5988-6707EN*, **2002.**

2.
"Agilent 1100 Series Purification System User's Guide", *Agilent Technologies Manual, publication number G2262-90003*, **2003.**

3.
"Optimizing Fraction Collection with LC/MSD Systems Using an Active Splitter and Delay Sensor", *Agilent Technologies Technical Note, publication number 5988-7610EN*, **2002.**

4.
"The Agilent 1100 Series dual-loop autosampler PS – Optimum performance when injecting large sample volumes", *Agilent Technologies Application Note, publication number 5989-0858EN*, **2004.**

5.
"Optimal Fraction Collecting in Preparative LC/MS", Ulrich Rosentreter, Udo Huber, *J. Comb. Chem., 6 (2), 159 -164*, **2004.**

Udo Huber is Senior Application Chemist at Agilent Technologies, Waldbronn, Germany.
Tony Brand is a Field Scientist at Agilent Technologies in Cary, North Carolina, USA.

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