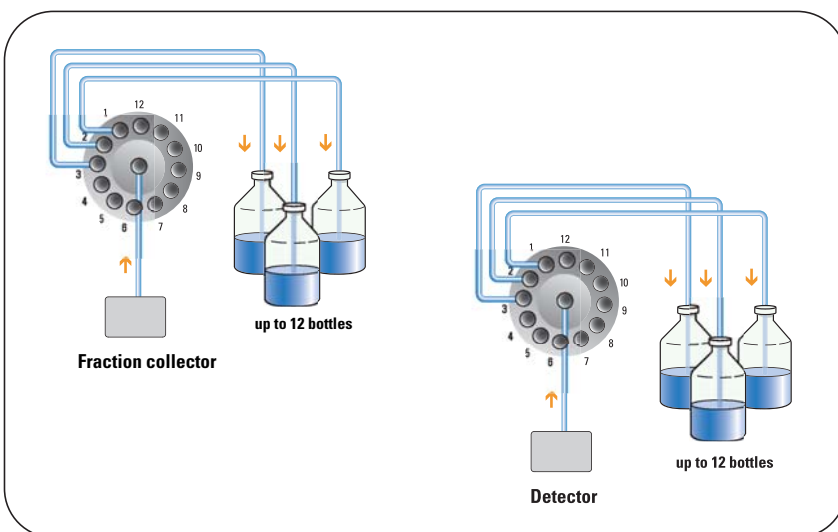


Recovery collection and time-based fraction collection — preparative HPLC with the Agilent 1100 Series valve solutions

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Application



Abstract

The Agilent 1100 Series purification system¹ is the ideal tool for compound isolation and purification in the pharmaceutical industry. The system has been optimized for high throughput, capacity and productivity. This Application Note demonstrates how the Agilent 1100 Series 12-position/13-port valve² can enhance further the purification system by adding easy recovery collection and simple time-based fraction collection.



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Introduction

In this Application Note we show two application examples using the Agilent 1100 Series 12-position/13-port valve in a purification system. First, it is used for recovery collection where the remainder of a sample that is not collected as a fraction is collected in a dedicated container. If something goes wrong during the purification run, e.g. due to incorrect parameter settings, the sample does not go to waste but to a location where it can be recovered easily. Second, the valve can be used in a simple purification system for time-based fraction collection.

Equipment

The system used comprised the following modules:

- Two Agilent 1100 Series preparative pumps
- Agilent 1100 Series preparative autosampler
- Agilent 1100 Series column organizer
- Agilent 1100 Series 12-position/13-port valve
- Agilent 1100 Series diode array detector
- Agilent 1100 Series fraction collector PS

The system was controlled using the Agilent ChemStation (rev. A.09.03) and the Purification/HighThroughput software (rev. A.01.02).

Results

Recovery collection

The Purification/HighThroughput software offers the capability of recovery collection. This means that everything that is not collected as a fraction goes into a dedi-

cated container where it can be easily recovered (figure 1). For recovery collection it is necessary to use the Agilent 1100 Series fraction collector AS with the funnel tray (G1364-84502). The needle of the 1100 Series fraction collector PS is too short to use the funnels.

Recovery collection can also be done by setting up the 12-position/13-port valve into the waste tubing of the fraction collector for both fraction collectors AS or PS, as shown in the cover illustration. The method settings for the valve are shown in the *Setup Valve* window in figure 2.

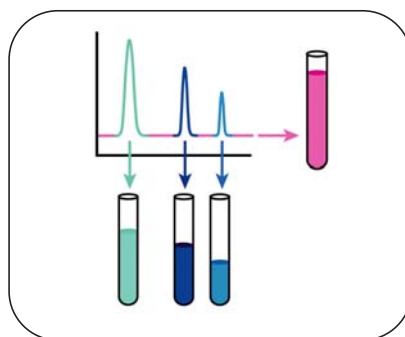


Figure 1
Recovery collection

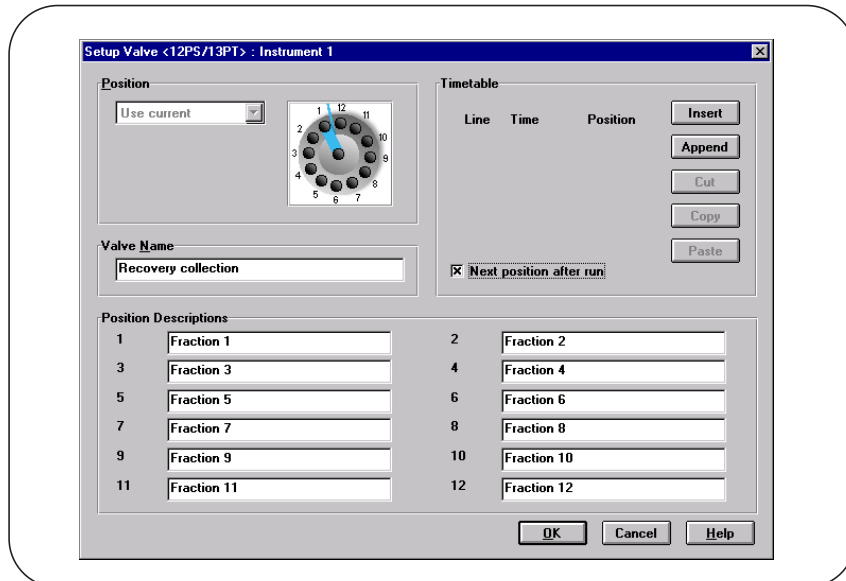


Figure 2
Setup Valve window

For recovery collection the *Next position after run* box must be checked. This automatically sets *Position to Use current*. With this setting the valve is automatically switched to the next recovery location after a run is finished and this position is kept when the next run starts. This means the recovery for sample one is collected into position one, for sample two into position two and so on. A prerequisite is that the valve is manually switched to position one before the first run is started. With the 12-position/13-port valve the recovery of up to twelve samples can be collected. If more recovery locations are needed, more 12-position/13-port valves could be attached to the outlets of the first

valve. The only restriction is the maximum number of valves that can be attached to the system and the more complex method setup for each of the connected valves. Because the valve switches to the next position after the run is finished and before the post run is started, it is recommended to include the post run time, for example, for column equilibration into the run time. Therefore, the mobile phase coming from the column during column wash and equilibration is still collected into the recovery location of the sample to which it belongs and not yet into the recovery location of the next sample. Example methods are shown in tables 1a and 1b.

Time-based fraction collection

The Agilent 1100 Series 12-position/13-port valve can also be used as a simple fraction collector, connected to the detector instead of the Agilent 1100 Series fraction collector. Fractions can only be collected in time slices by switching the valve using the *Timetable* (figure 3). The last position is used as waste position to collect the mobile phase when no analysis is running. Since a fixed position is used as starting position in the method, the valve will switch back to this position after the run is finished. Therefore, all mobile phase coming from the column during equilibration, injection cycle, etc., will go to the waste position and will not dilute

With post time

Gradient: at 0 min 10 % B
at 9 min 90 % B
at 10 min 90 % B
Stop time: 10 min
Post time: 3 min

Table 1a
Gradient run with post time for equilibration

Without post time

Gradient: at 0 min 10 % B
at 9 min 90 % B
at 10 min 90 % B
at 10.1 min 10 % B
at 13 min 10 % B
Stop time: 13 min
Post time: off

Table 1b
Equilibration included in run time

Figure 3
Time-based fraction collection with the 12-position/13-port valve

or contaminate any fraction. When using a method with these valve settings in a sequence it is possible to do pooling, even with injections from different sample vials. Time-based fraction collection with an Agilent 1100 Series 12-position/13-port valve works only with the ChemStation software — the Purification/HighThroughput software is neither required nor supported.

Conclusion

In this Application Note we showed how to use a 12-position/13-port valve in an Agilent 1100 Series purification system. It can be used for recovery collection when connected to the waste line of the fraction collector. It can also be used for time-based fraction collection in a simple purification system instead of a fraction collector.

References

1. “New perspectives in purification with HPLC and HPLC/MS”, Agilent Technologies Brochure, **2001**, publication number 5988-3673EN.
3. “New dimensions for HPLC applications”, Agilent Technologies Brochure, **2002**, publication number 5988-6707EN.

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