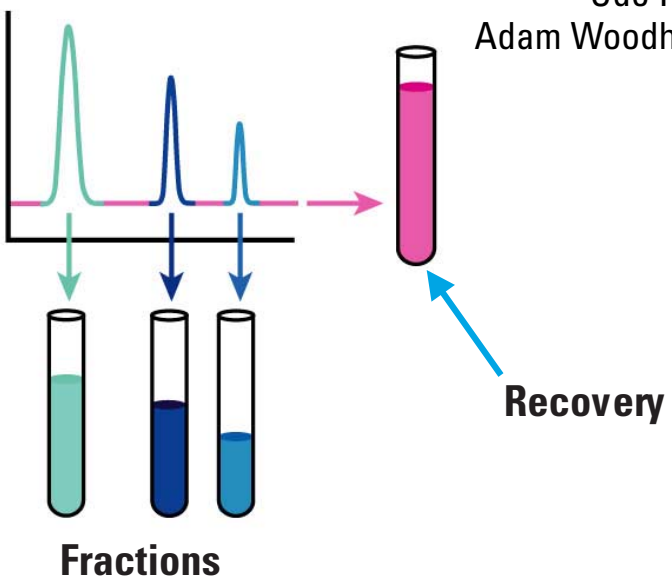
Recovery collection with the Agilent 1100 Series purification system

Application

Udo Huber
Adam Woodhouse



Abstract

In this Application Note we show three system configurations for recovery collection with the Agilent 1100 Series purification system[1,2]. One configuration uses a 12-position/13-port valve [3] in the waste line of the fraction collector, which offers up to twelve recovery locations. Another possibility is to use a third-party fraction collector, controlled by the ChemStation through external contacts using a BCD board. The most sophisticated approach, however, is to use a dedicated Agilent 1100 Series fraction collector, which gives complete recovery location tracking with the graphical user interface of the software or in the reports.

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Introduction

Preparative HPLC is nowadays the method of choice for sample purification in the discovery of active compounds in the pharmaceutical industry but also in other areas such as crop science. Peak-based fraction collection on the signal from a UV detector leads to several fractions per sample; by using a mass-selective detector (MSD) the number of collected fractions can usually be reduced to one fraction per sample containing the target compound. Regardless of the triggering mechanism it is sometimes desirable to collect not only the compounds of interest but also everything else from the sample in a dedicated vessel, the so-called recovery location (see front cover). The recovery solution can be evaporated, redissolved and re-injected to recover anything of interest that was in the sample but was missed in the first purification run.

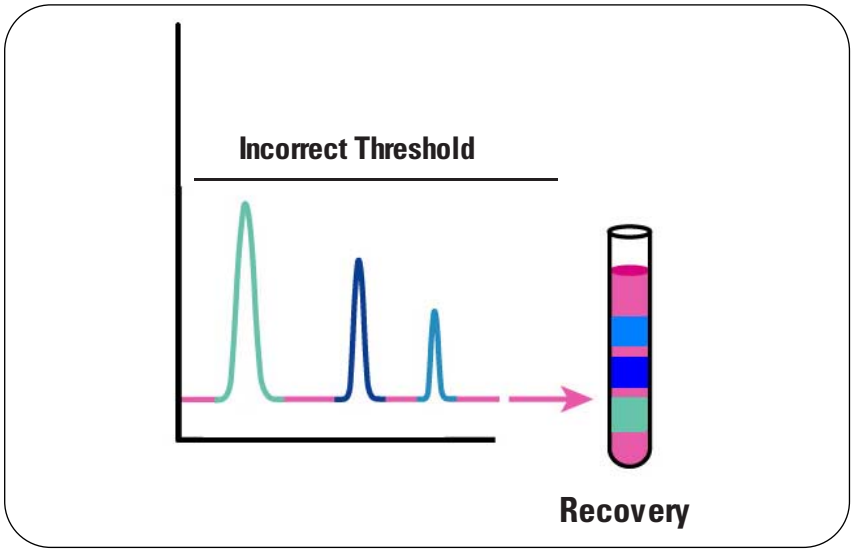


Figure 1

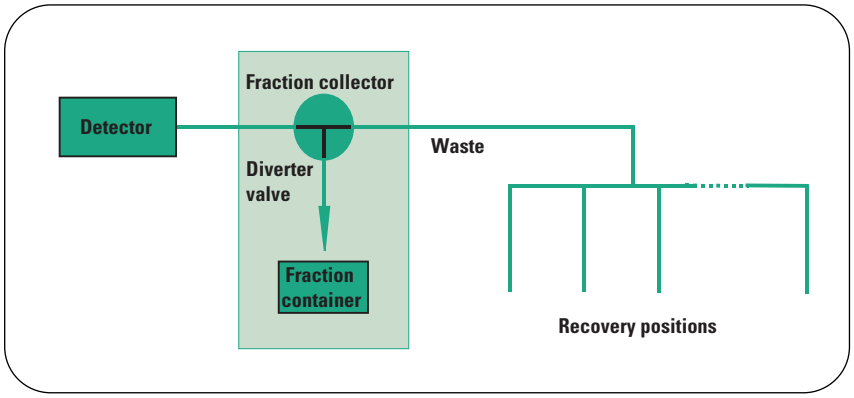


Figure 2
Recovery collection from the waste line of the fraction collector

Discussion

Recovery collection – Why?

The recovery solution can be used to recover anything from the sample that was not triggered as a fraction in the first purification run. But it is also a safety feature to recover, for example, the target compound if the purification system did not collect fractions as expected. Possible reasons for non-collection could be:

- User inputs, wrong target mass or formula. For mass-based fraction collection the mono-isotopic mass must always be entered, not only the average molecular mass.
- User selects inappropriate generic method.
- Peaks fail to cross trigger threshold of generic methods (figure 1).
- Sample has poor ionization Compound must ionize to give a signal in the MSD.
- Mechanical or software failure.

Recovery collection – How?

Collection of the recovery solution must be done from the waste line of the fraction collector as shown in figure 2. If more than one fraction collector is configured in the system the waste lines must be combined before going to the recovery collection.

Recovery collection with the Agilent 1100 Series purification system

With the Agilent 1100 Series purification system recovery collection can be done in three different ways:

- 12-position/13-port valve in the fraction collector waste line
  - 12 recovery locations
  - Basic recovery location tracking by software
- Third-party fraction collector and BCD board
  - Number of recovery locations depends on fraction collector
  - No recovery location tracking by software
- Agilent 1100 Series fraction collector
  - Up to 120 recovery locations with funnel tray
  - Complete recovery location tracking

12-position/13-port valve

The recovery collection using a 12-position/13-port valve was described in another Application Note[4]. Basically the waste line of the fraction collector is connected to the inlet position of the valve and the twelve outlet positions are connected to the recovery containers. For each injection the valve is automatically switched to the next position. Tracking of the recovery positions is simply done by reporting the valve position with the sample information in a report as shown in figure 3.

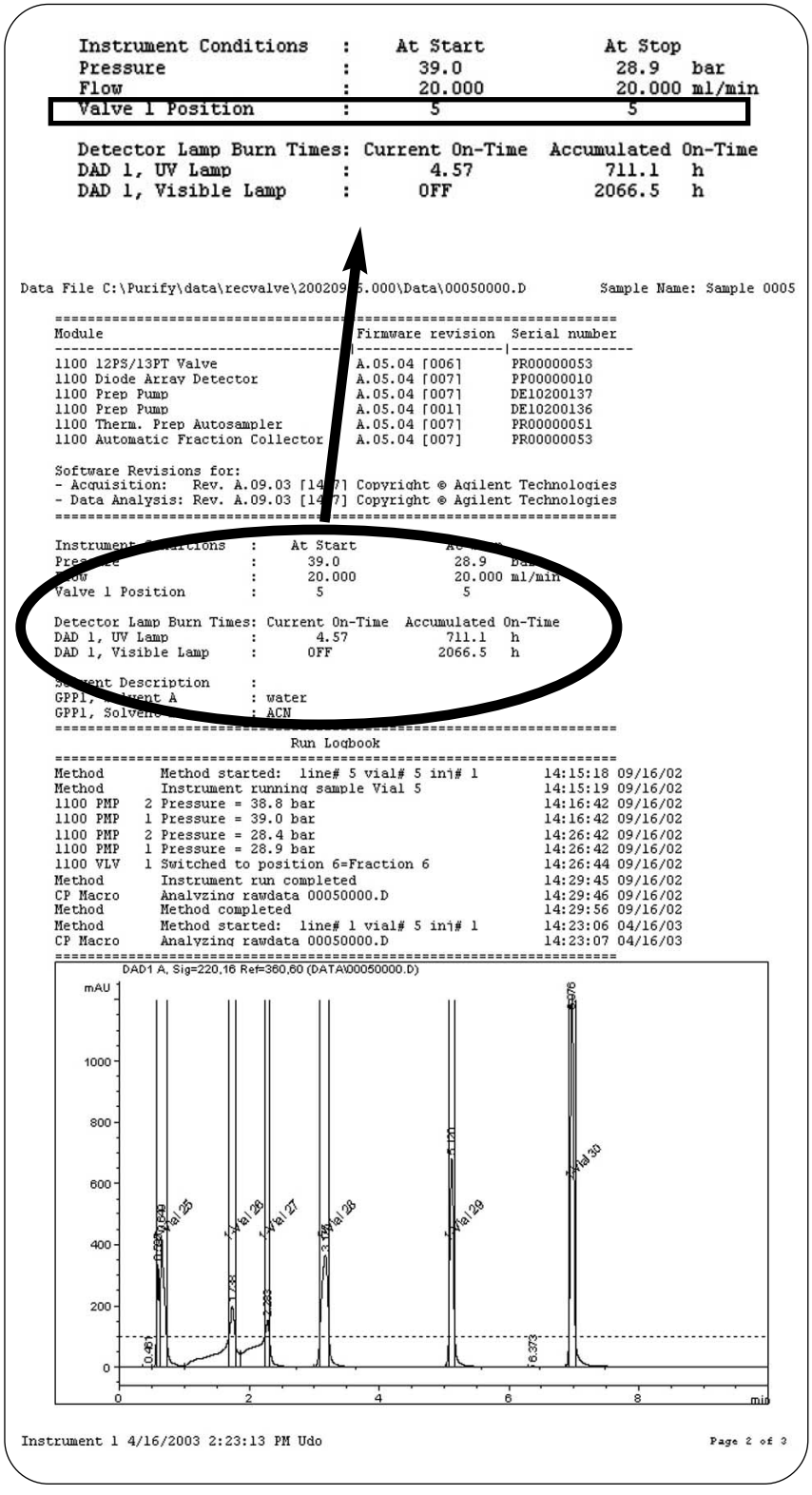


Figure 3
ChemStation report style: Full

Third-party fraction collector and BCD board

The BCD board, which can be installed in the autosampler, provides a BCD output for the bottle number of the autosampler and four external relay contacts. General-purpose cables are available to connect the BCD output and the external contacts to external devices. The BCD board can be configured for *Binary Output* in the *Injector Configuration* window in the Agilent ChemStation as shown in figure 4. After the configuration the four contacts can be closed and opened using an injector program (figure 5) in the ChemStation to move the third-party fraction collector to the next position after an injection or to switch the diverter valve. Tracking of the recovery location in the ChemStation software or in a report is not possible.

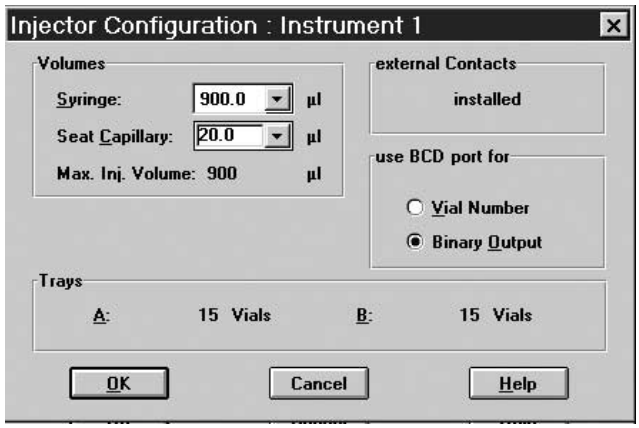


Figure 4
***Binary Output* configuration of the BCD board**

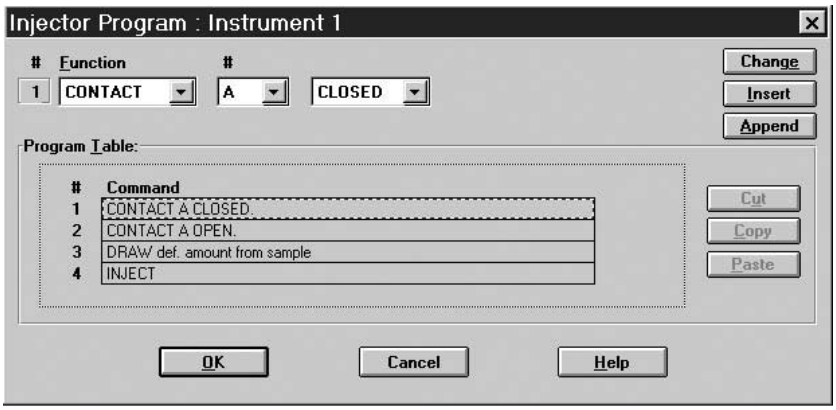


Figure 5
Injector program to close and open the external contacts

Agilent 1100 Series fraction collector

With ChemStation rev. A.10.01 it is possible to configure an additional Agilent 1100 Series fraction collector in the system for recovery collection. In this fraction collector all available trays for vials, wellplates and test tubes as well as the new funnel tray can be used. Each funnel tray consists of 40 funnels with tubing connected to the funnels (figure 6). This tubing can be placed in any vessel or container, for example, large glass bottles. This device makes it possible to collect virtually unlimited recovery volumes. Up to three funnel trays can be installed in the fraction collector, which gives up to 120 recovery locations as maximum. This tray can be installed in any fraction collector not only for

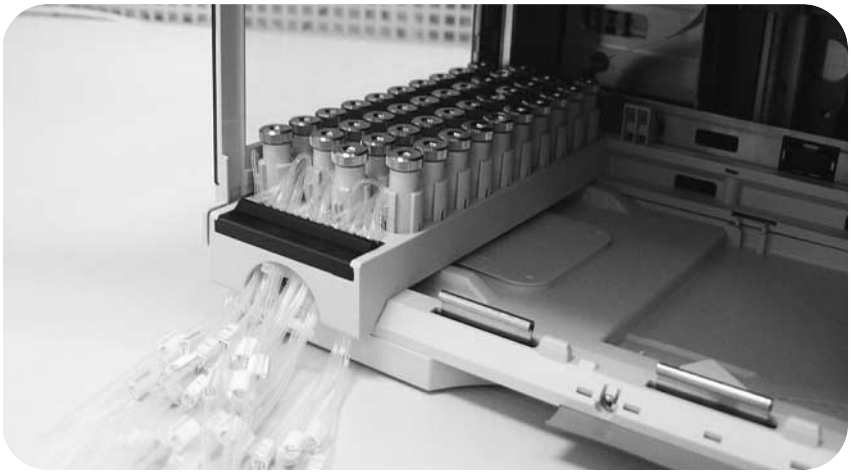


Figure 6
Funnel trays installed in a fraction collector

recovery collection but also for the collection of large-volume fractions. Tracking of the recovery locations can be done either in the graphical user interface (GUI) of the Purification software or in the ChemStation or Purification software reporting as shown in figures 7a and b.

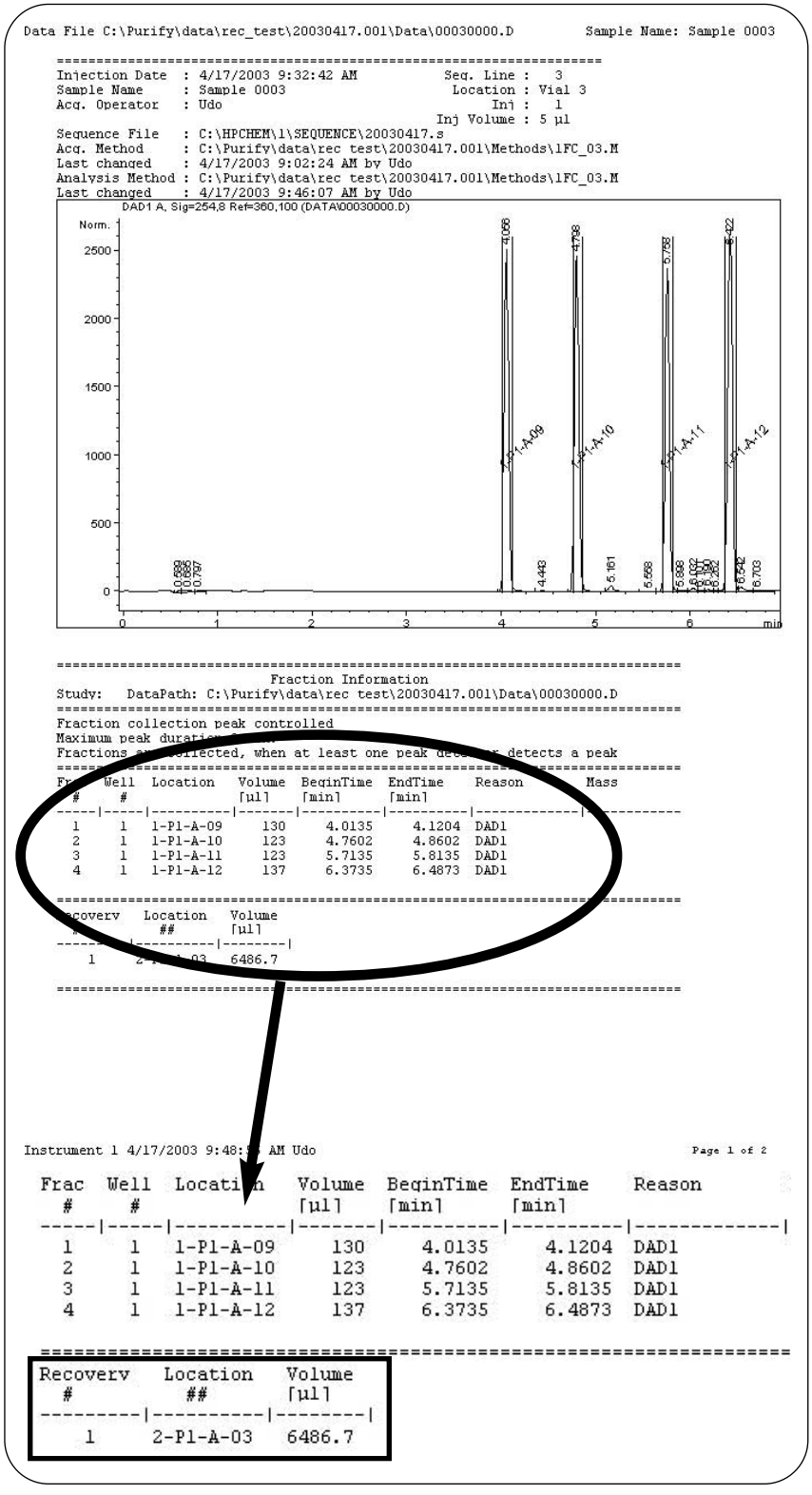


Figure 7a
ChemStation reporting of recovery locations, report style: Short

Fraction List

Study Name	rec_test 02	**Default Sample Name**	Sample ?
Study Path	C:\Purify\data\rec_test\20030417.0	**Injection order by**	Location
Study Run #	1	**Shutdown**	<None>
Description	Test for recovery collection	**Run Method If**	<None>
ChemStore	<Disabled>	**Fraction Start Loc.**	1-P1-A-01
Operator	Udo	**Number of Pools**	<None>
Start Date	17-Apr-03 9:05:40 AM	**Recovery Start Loc.**	2-P1-A-01
Stop Mode	Completed		

Fraction List of study 'rec_test 02 (StudyRun 1)'

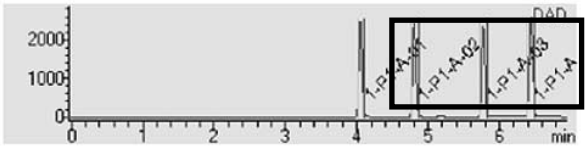
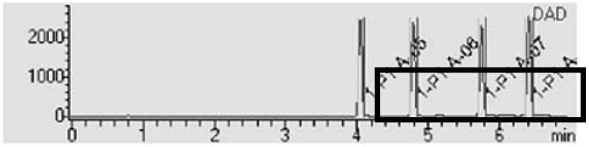
Location	Sample Name	Method	Rec. Loc.	Rec. Vol.	Fra	Wel	Location	Start	Stop	Reas
VIAL 1	Sample 0001	1fc_03.m	2-P1-A-01	6480.6						
					1	1	1-P1-A-01	4.01	4.11	DAD1
					2	1	1-P1-A-02	4.76	4.87	DAD1
					3	1	1-P1-A-03	5.73	5.83	DAD1
					4	1	1-P1-A-04	6.39	6.5	DAD1
VIAL 2	Sample 0002	1fc_03.m	2-P1-A-02	6487.0						
					1	1	1-P1-A-05	4.01	4.11	DAD1
					2	1	1-P1-A-06	4.75	4.85	DAD1
					3	1	1-P1-A-07	5.71	5.81	DAD1
					4	1	1-P1-A-08	6.37	6.48	DAD1

Figure 7b
Purification software reporting of recovery locations

Conclusion

The Agilent 1100 Series purification system offers three possibilities for recovery collection. The easiest way is to connect a 12-position/13-port valve into the waste line of the fraction collector. This offers the possibility of collection into up to twelve recovery positions with simple recovery location tracking of the valve position in the ChemStation report.

A third-party fraction collector can also be configured into the system controlled by the ChemStation injector program via external contacts of a BCD board. Tracking of recovery locations is not possible.

The most sophisticated approach is an additional Agilent 1100 Series fraction collector configured as recovery collector in the Agilent ChemStation. In addition to vials, wellplates or tubes the new funnel tray offers the possibility to collect virtually unlimited recovery volumes. The recovery tracking is done in the Purification software GUI as well as in the ChemStation and Purification software reporting.

References

1.
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2.
"Agilent 1100 Series Purification Platform – Customized system configurations", *Agilent Technologies Flyer, publication number 5988-3751EN*, **2001.**

3.
"New dimensions for HPLC applications", Agilent Technologies Brochure, *publication number 5988-6707EN*, **2002.**

4.
"Recovery collection and time-based fraction collection – preparative HPLC with the Agilent 1100 Series valve solutions", *Agilent Technologies Application Note, publication number 5988-8225EN*, **2002.**

Udo Huber is Application Chemist at Agilent Technologies, Waldbronn, Germany.
Adam Woodhouse is Product Specialist at Agilent Technolgies, Bracknell, UK.

www.agilent.com/chem/purification

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