

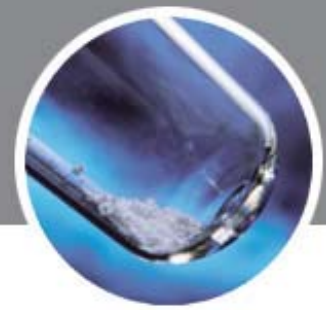
Recovery collection – complete sample recovery in preparative HPLC



Presented by

Udo Huber
Senior Application Chemist





- **Introduction**

- Recovery collection – Why?

- Recovery collection – How?

- Recovery collection with the Agilent 1100 Series purification system

- **Basic solutions**

- 12-position/13-port valve

- Third-party fraction collector and BCD board

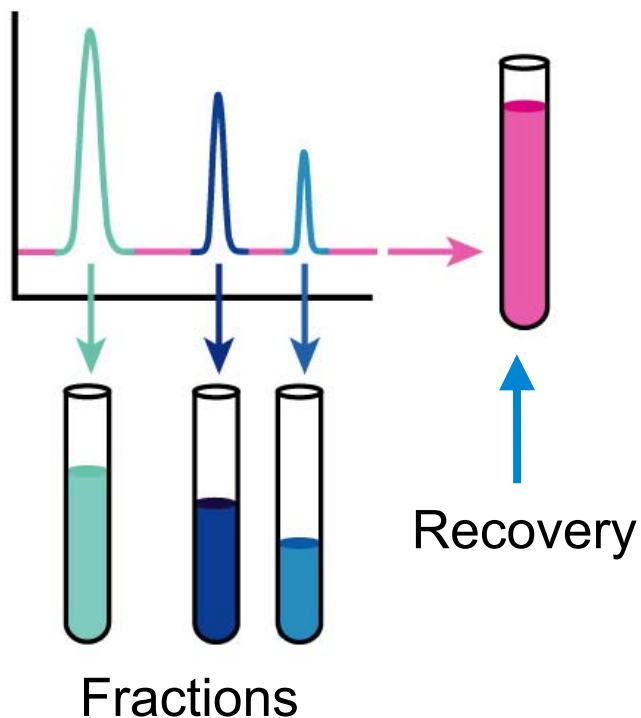
- **Agilent solution**

- Agilent 1100 Series recovery collector

- Funnel tray

- Recovery location setup and tracking

Recovery collection



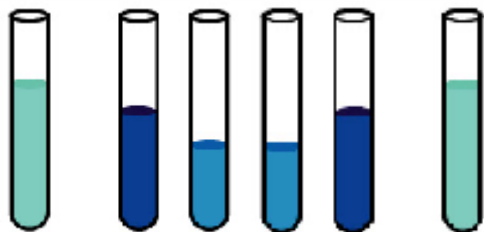
Recovery collection:

Everything that is not collected as a fraction will not go into waste but into a sample-specific container.

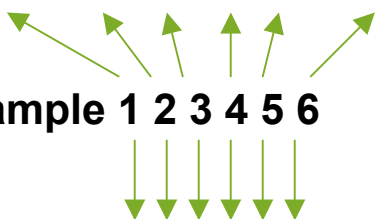
Applications:

- Walk-up system, chemists get fractions + recovery = everything back
- For very valuable samples

Recovery collection



Recovery of sample 1 2 3 4 5 6



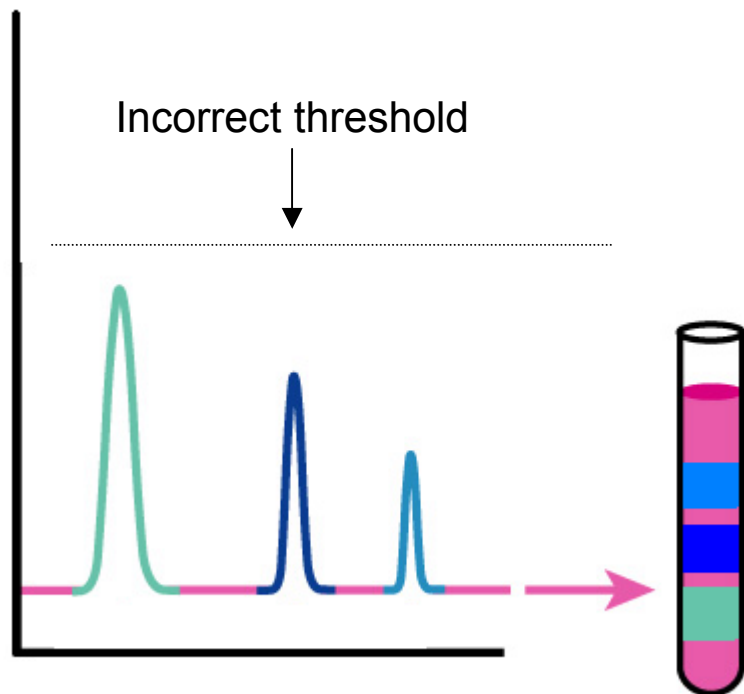
Or



Recovery collection:

- Gives each sample a dedicated waste container.
- If required, recovery solution can be evaporated and sample re-dissolved and re-injected.
- Eliminates sample entering general waste container from which it is not recoverable.

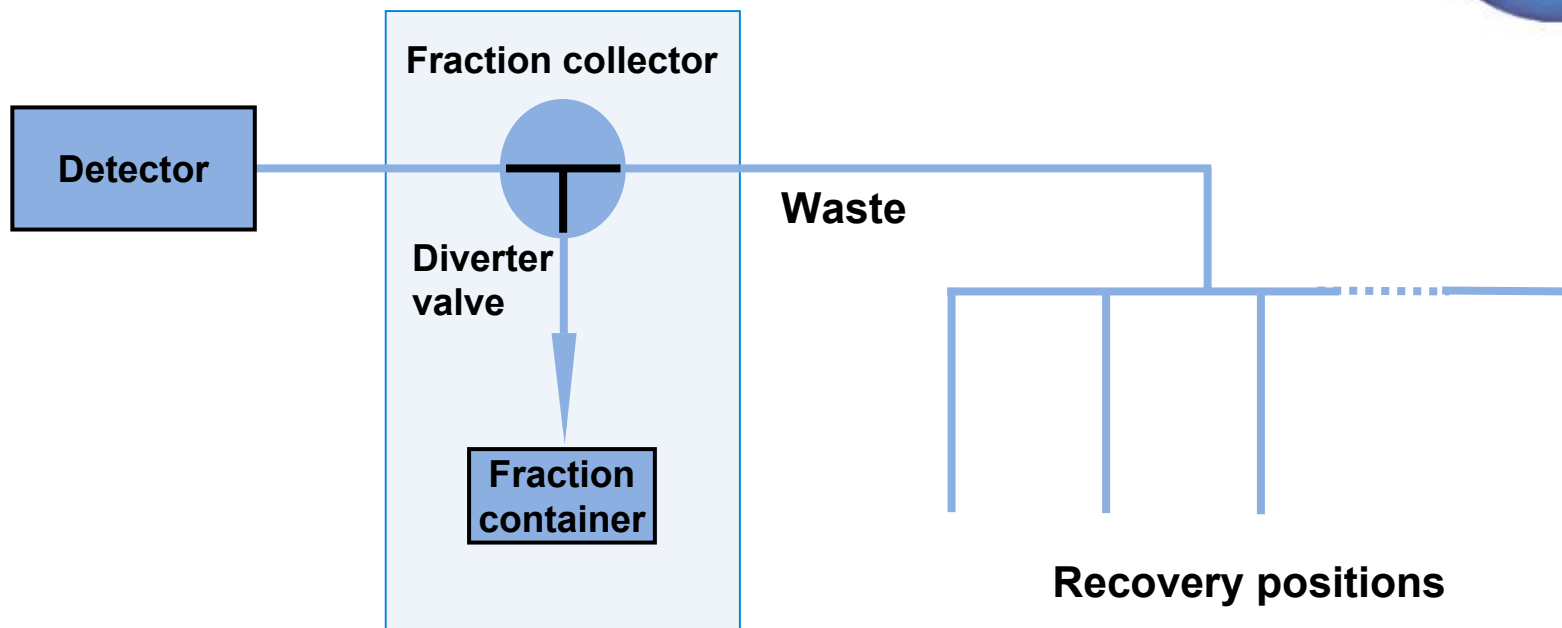
Recovery collection – Why?



Possible reasons for non-collection:

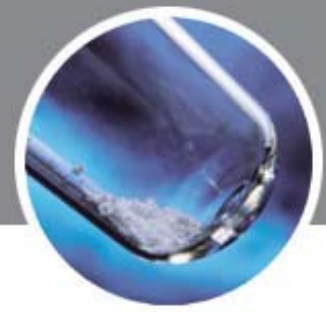
- User inputs wrong target mass/formula
- User selects inappropriate generic method
- Peaks fail to cross trigger threshold of generic methods
- Sample has poor ionization
- Mechanical/software failure

Recovery collection – How?

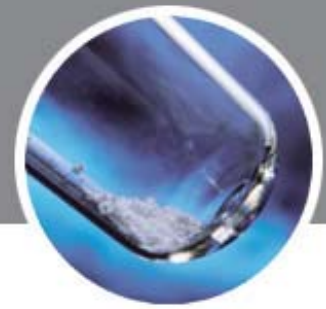


Recovery collection must be done from the waste line of the fraction collector. If more than one fraction collector is in the system, the waste lines must be combined before going to recovery collection.

Recovery collection with the Agilent 1100 Series purification system



- **12-position/13-port valve**
 - 12 recovery locations
 - simple recovery location tracking by software
- **Third-party fraction collector and BCD board**
 - Number of recovery locations depend on fraction collector
 - no recovery location tracking by software
- **Agilent 1100 Series fraction collector**
 - up to 120 recovery locations with unlimited volume
 - complete recovery location tracking



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Presentation

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Slide 4 of 4

Question for Presenter:

There are no questions pending.

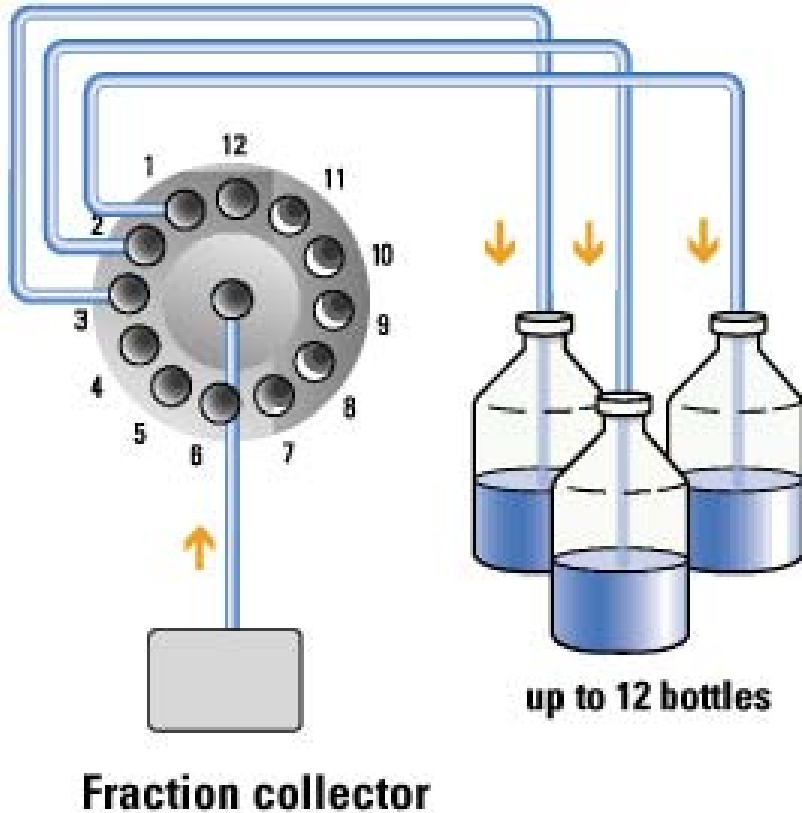
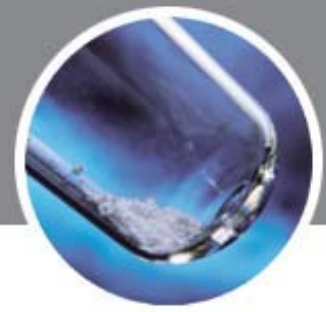
Question & Answer Session

Please type your question into the Question Box at any time during the presentation.

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12-position/13-port valve



Recovery collection:

- Waste line of fraction collector is connected to position 13 of the valve
- Valve positions 1 - 12 can be used for recovery

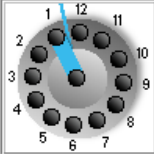
Software control



Setup Valve 2 <12PS/13PT> : Instrument 1

Position

Use current



Valve Name

Recovery collection

Timetable

Line	Time	Position

Next position after run

Position Descriptions

1	Recovery Sample 1	2	Recovery Sample 2
3	Recovery Sample 3	4	Recovery Sample 4
5	Recovery Sample 5	6	Recovery Sample 6
7	Recovery Sample 7	8	Recovery Sample 8
9	Recovery Sample 9	10	Recovery Sample 10
11	Recovery Sample 11	12	Recovery Sample 12

OK Cancel Help

Recovery collection:

- Valve switches to next recovery position after each run (*Next position after run*).
- After 12 samples system must be reset.

Reporting of recovery position



```

Data File C:\Purify\data\recvalve\20020916.000\Data\00050000.D      Sample Name: Sample 0005
=====
Module                Firmware revision  Serial number
-----
1100 12PS/13PT Valve  A.05.04 [006]    PR00000053
1100 Diode Array Detector A.05.04 [007]    FP00000010
1100 Prep Pump         A.05.04 [007]    DE10200137
1100 Prep Pump         A.05.04 [001]    DE10200136
1100 Thera. Prep Autosampler A.05.04 [007]    PR00000051
1100 Automatic Fraction Collector A.05.04 [007]    PR00000053

Software Revisions for:
- Acquisition: Rev. A.09.03 [1407] Copyright © Agilent Technologies
- Data Analysis: Rev. A.09.03 [1417] Copyright © Agilent Technologies
=====
Instrument Conditions : At Start      At Stop
Pressure              : 39.0         28.9 bar
Flow                  : 20.000      20.000 ml/min
Valve 1 Position      : 5         5

Detector Lamp Burn Times: Current On-Time  Accumulated On-Time
DAD 1, UV Lamp       : 4.57         711.1 h
DAD 1, Visible Lamp  : OFF         2066.5 h

Solvent Description :
GPP1, Solvent A     : water
GPP1, Solvent B     : ACN
=====
Run Logbook
=====
Method      Method started: line# 5 vial# 5 ini# 1      14:15:18 09/16/02
Method      Instrument running sample Vial 5            14:15:19 09/16/02
1100 PMP    2 Pressure = 38.8 bar                    14:16:42 09/16/02
1100 PMP    1 Pressure = 39.0 bar                    14:16:42 09/16/02
1100 PMP    2 Pressure = 28.4 bar                    14:26:42 09/16/02
1100 PMP    1 Pressure = 28.9 bar                    14:26:42 09/16/02
1100 VLV    1 Switched to position 6=Fraction 6    14:26:44 09/16/02
Method      Instrument run completed                      14:29:45 09/16/02
CP Macro    Analyzing rawdata 00050000.D                 14:29:46 09/16/02
Method      Method completed                             14:29:56 09/16/02
Method      Method started: line# 1 vial# 5 ini# 1        14:23:06 04/16/03
CP Macro    Analyzing rawdata 00050000.D                 14:23:07 04/16/03
=====
DAD1 A, Sig=220.16 Ref=300.60 (DATA00050000.D)
mAU
1000
800
600
400
200
0
0 2 4 6 8 10
min
0.257 0.374 1.126 1.278 1.383 1.530 1.677 4.121 6.375 8.370
Instrument 1 4/16/2003 2:23:13 PM Udo
Page 2 of 3
    
```

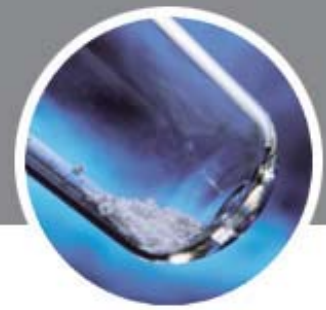
```

Instrument Conditions : At Start      At Stop
Pressure              : 39.0         28.9 bar
Flow                  : 20.000      20.000 ml/min
Valve 1 Position      : 5         5

Detector Lamp Burn Times: Current On-Time  Accumulated On-Time
DAD 1, UV Lamp       : 4.57         711.1 h
DAD 1, Visible Lamp  : OFF         2066.5 h
    
```

ChemStation report style: Full

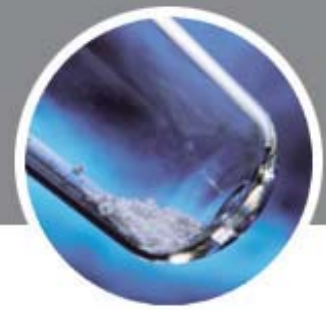
Third-party fraction collector and BCD board



BCD board:

- The BCD board provides a BCD output for the bottle number of the autosampler and **four external contacts**.
- The external contact closure contacts are relay contacts.
- There are general purpose cables available to connect the BCD output and the external contacts to external devices.

Third-party fraction collector and BCD board



Injector Configuration : Instrument 1 [X]

Volumes

Syringe: 900.0 μ l

Seat Capillary: 20.0 μ l

Max. Inj. Volume: 900 μ l

external Contacts

installed

use BCD port for

Vial Number

Binary Output

Trays

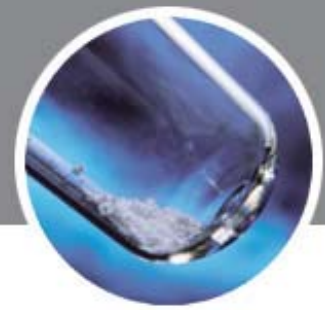
A:	15 Vials	B:	15 Vials
----	----------	----	----------

OK Cancel Help

Configuration:

- Installation in the autosampler
- Installation recognized automatically
- Set *use BCD port for* to *Binary Output*

Third-party fraction collector and BCD board



Setup Injector : Instrument 1 [X]

Injection

Standard Injection Injection Volume: 500.0 µl

Injection with Needle Wash Wash Vial: 0

Use Injector Program Total Lines: 4 **Edit ...**

Optimization: none [v] 0.00 min. after Injection

OK **Cancel** **Help**

Injector Program : Instrument 1 [X]

Function # Change

1 CONTACT A CLOSED Insert

Append

Program Table:

#	Command
1	CONTACT A CLOSED.
2	CONTACT A OPEN.
3	DRAW def. amount from sample
4	INJECT

Cut Copy Paste

OK **Cancel** **Help**

Third-party fraction collector and BCD board



Injector Program : Instrument 1

Function

1 CONTACT A CLOSED

Change
Insert
Append

Program Table:

#	Command
1	CONTACT A CLOSED.
2	CONTACT A OPEN.
3	DRAW def. amount from sample
4	INJECT

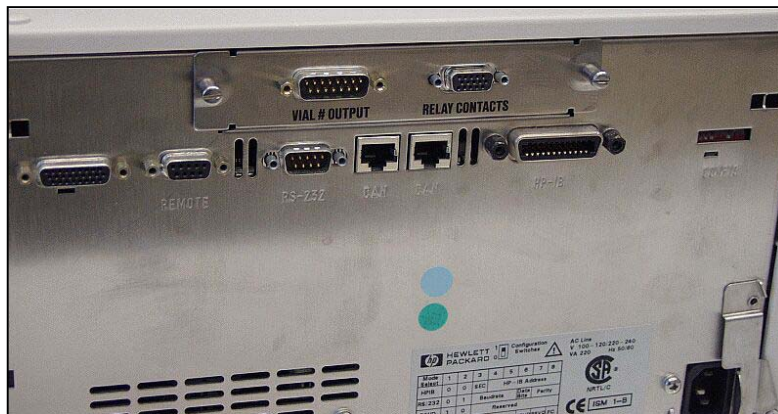
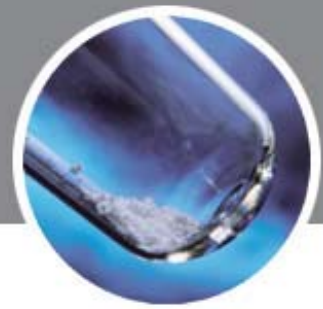
Cut
Copy
Paste

OK Cancel Help

Set up:

- Contact A closed/open to give pulse
- Contact A was used to move third-party FC to next position
- Contact B could be used to switch diverter valve of third-party FC

Hardware





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Presentation

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Question for Presenter:

There are no questions pending.

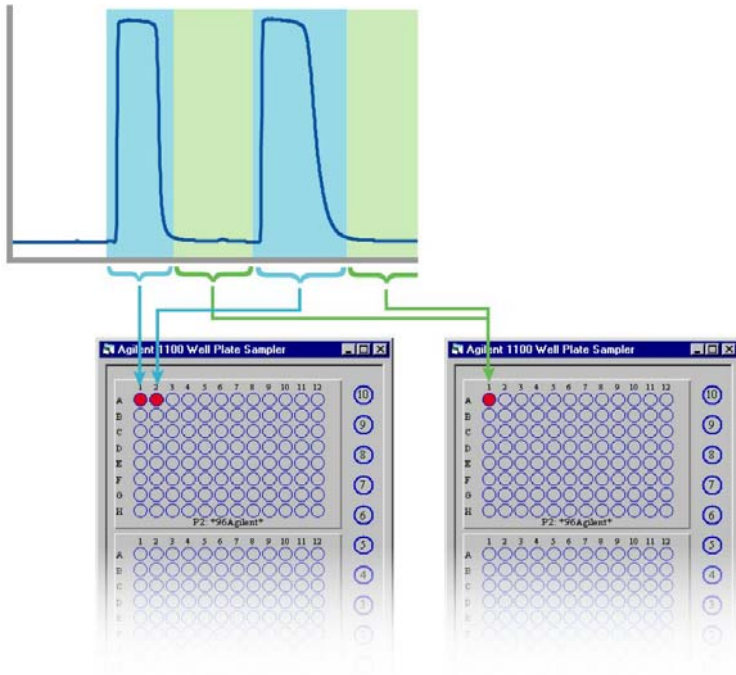
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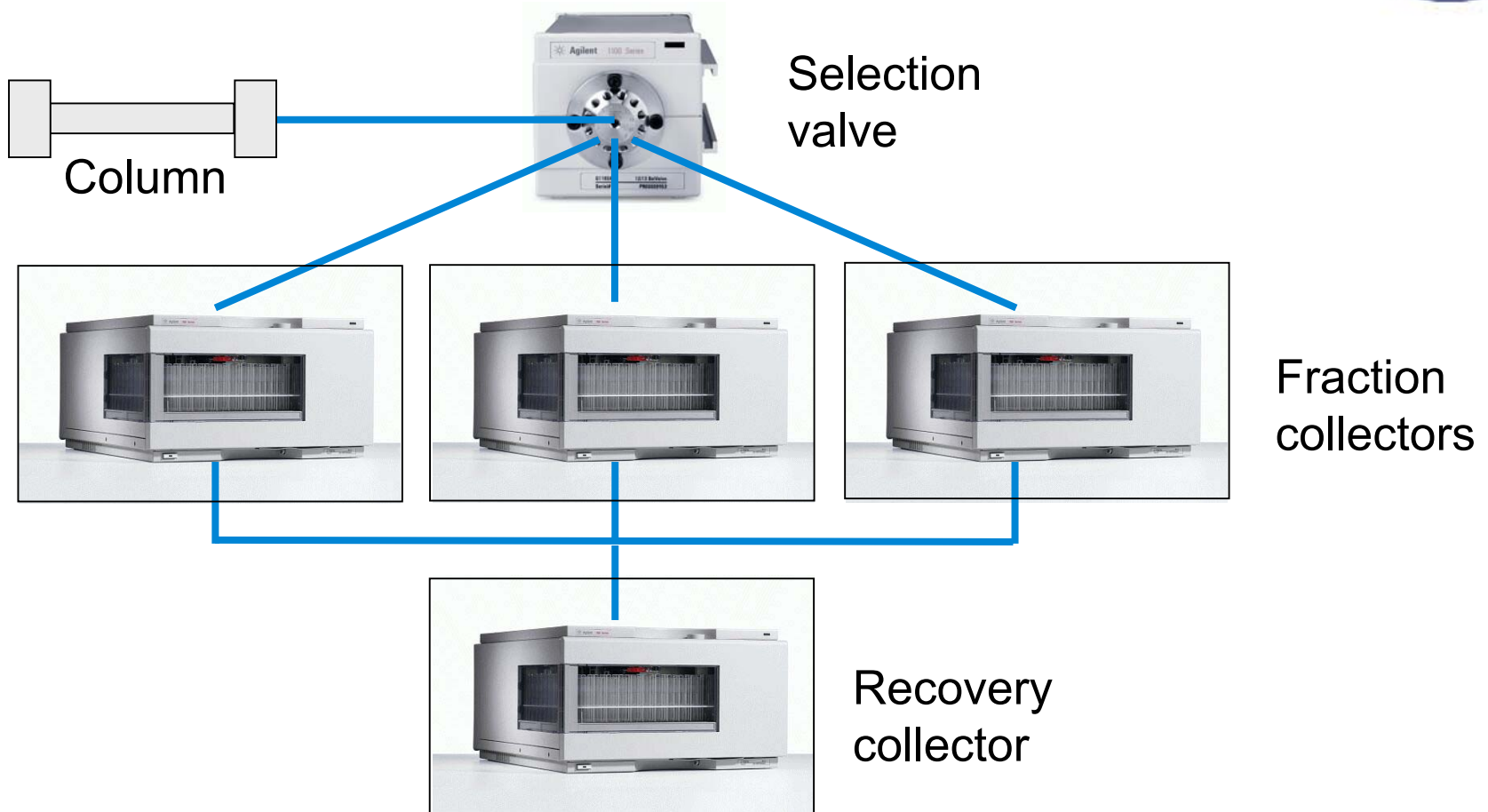
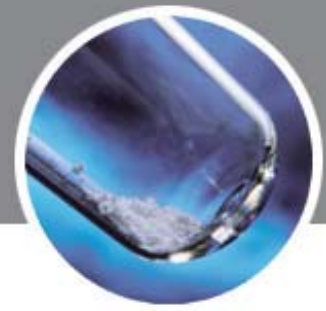
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Agilent 1100 Series fraction collector

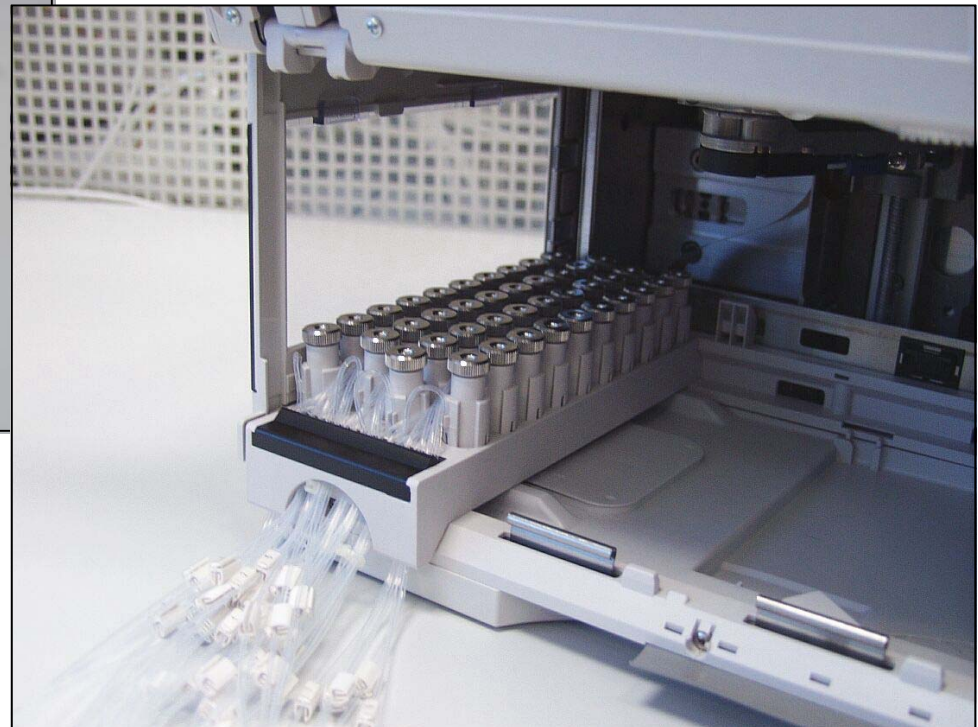
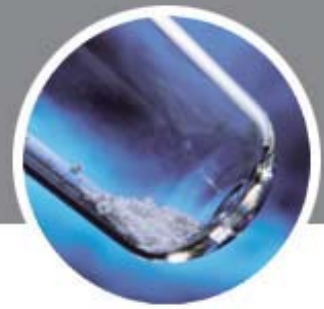


- Agilent fraction collector
- Complete software control
- Collection into any fraction container or into funnels
- Complete reporting of recovery locations (GUI, printed report)

Agilent 1100 Series fraction collector

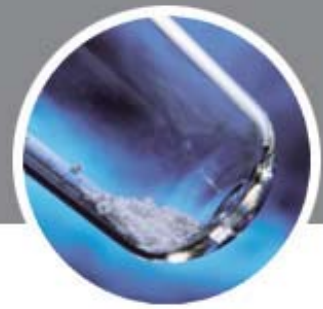


Funnel tray



The Agilent funnel tray for virtually unlimited recovery volume.

Fraction collector configuration



Fraction Collector 2 Configuration : Instrument 1

Trays

Tray A: 4 WellPlates Use for Sample Recovery

Tray B: --- Tube Volume [ml]: 0

Tray C: --- Tube Height [mm]: 0

Fraction Delay Volumes

Detector	Volume[μl]
1 DAD1	0

Collection Order

Row by row

Shortest path by column

Column by column

Shortest path by row

Collection Mode

Discrete fractions

Continuous flow

Needle Movement

into location Depth: 5.0 mm

above location Distance: 2.0 mm

drop setup Distance: 0.0 mm

Well-Plates

Plate Type: 24-well plate Treat as 4x96

Reserved Locations

Row(s):

Column(s):

Single Location(s):

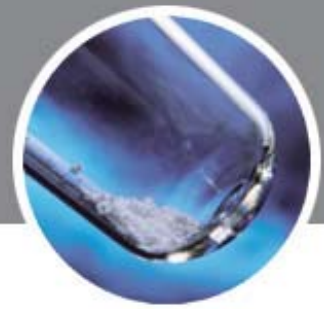
Installed Plates

Plate 1 (front left) Plate 3 (front right)

Plate 2 (back left) Plate 4 (back right)

OK Cancel Help More >>

Recovery location setup



1. ChemStation \Rightarrow next free recovery location is always used
2. Purify \Rightarrow recovery location can be specified

A. Setup Study Parameters screen

Setup Study Parameters

Study Information

ChemStore: <None>

Operator: Udo

Description: Test for recovery collection

Shut Down: <None>

Injection Order

Order injection by: Selection Location Method

Run Method If

Method: <None>

If: All masses found Some masses found No masses found

Default Sample Name Format

Format: Sample ?

Example: Sample 1A01
Sample 0103

Fraction Collection and Recovery

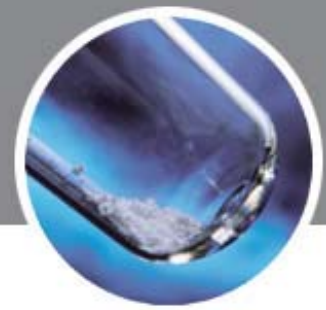
Fraction Start Loc.: 1-P1-A-01

Number of pools: <None>

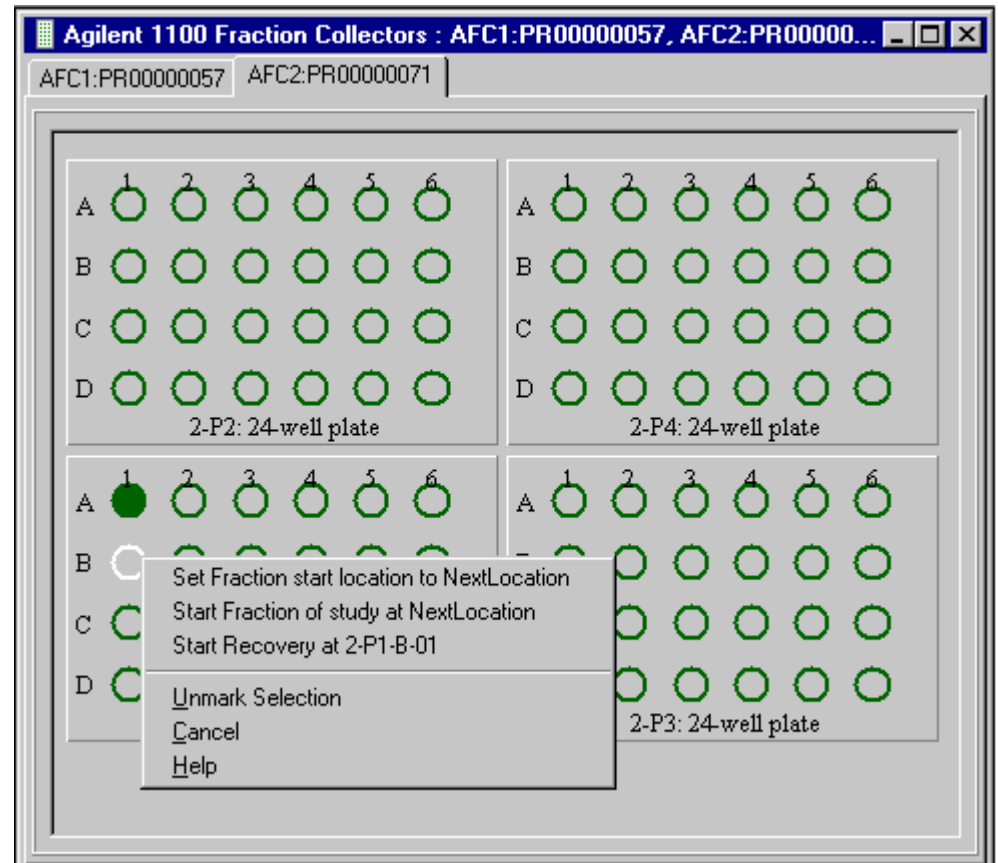
Recovery Start Loc.: 2-P1-A-01

Buttons: Help, Cancel, < Previous, Next >, Finish

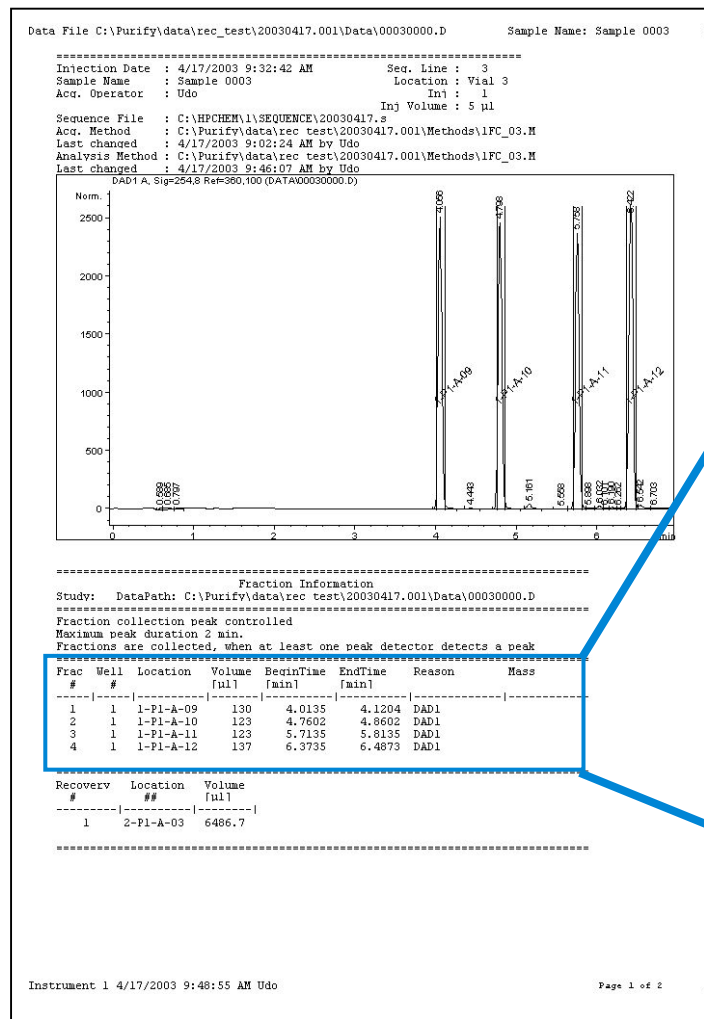
Recovery location setup



B. Graphical user interface



Recovery tracking



1. ChemStation report

```

=====
Frac Well Location Volume BeginTime EndTime Reason Mass
# # # [µl] [min] [min]
-----|-----|-----|-----|-----|-----|-----
1 1 1-Pl-A-09 130 4.0135 4.1204 DAD1
2 1 1-Pl-A-10 123 4.7602 4.8602 DAD1
3 1 1-Pl-A-11 123 5.7135 5.8135 DAD1
4 1 1-Pl-A-12 137 6.3735 6.4873 DAD1
=====
Recovery Location Volume
# # # [µl]
-----|-----|-----|-----
1 2-Pl-A-03 6486.7
=====

```


Recovery tracking



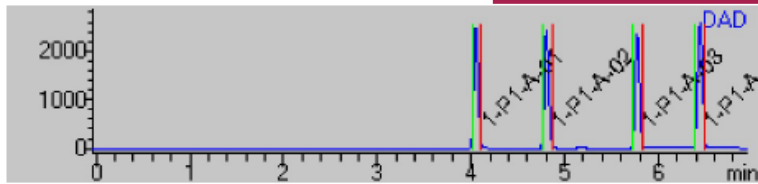
Fraction List

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	<None>
If			
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		

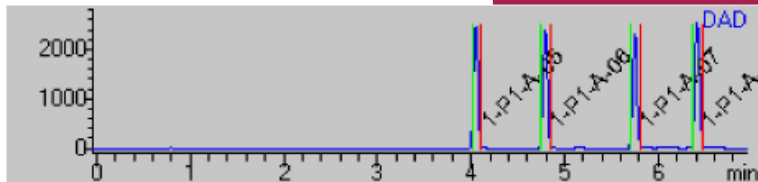
Fraction List of study 'rec_test 02 (StudyRun 1)'

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

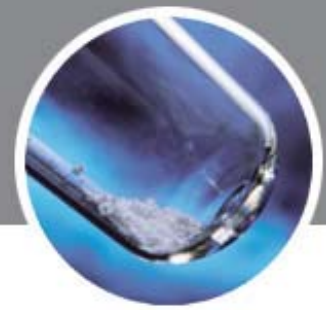


VIAL 2	Sample 0002	1fc_03.m	2-P1-A-02	6487.0						
					1	1	1-P1-A-05	4.01	4.11	DAD1
					2	1	1-P1-A-06	4.75	4.85	DAD1
					3	1	1-P1-A-07	5.71	5.81	DAD1
					4	1	1-P1-A-08	6.37	6.48	DAD1



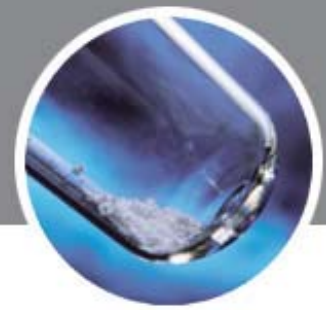
2. Purify report

Recovery tracking



3. Purify GUI

	Sample			Injection						
	Location	Name	Description	Injection	Pool	Method	Data File	Volume	Recovery	Recovery Vol.
	VIAL 1	Sample 0001		1	1	1fc_03.m	00010000.d	<Method>	2-P1-A-01	6480.63
▶	VIAL 2	Sample 0002		2	1	1fc_03.m	00020000.d	<Method>	2-P1-A-02	6487.08
	VIAL 3	Sample 0003		3	1	1fc_03.m	00030000.d	<Method>	2-P1-A-03	6486.67



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- Recovery collection – How?

- Recovery collection with the Agilent 1100 Series purification system

- **Basic solutions**

- 12-position/13-port valve

- Third-party fraction collector and BCD board

- **Agilent solution**

- Agilent 1100 Series recovery collector

- Funnel tray

- Recovery location setup and tracking