New fraction collection features with the Agilent 220 micro plate sampler and micro plate sampling software rev. A.03

Technical Note

Introduction

The Agilent 220 micro plate sampler (MPS) is an essential part of the Agilent 1100 Series system for combinatorial chemistry and high throughput LC analysis[1]. Besides flexibility, high sample capacity and robustness one of its strongest features is the fractionation capability. With the new micro plate sampling software revision A.03 it is possible to collect fractions based on time windows, peaks or masses. With the color-coded graphical user interface it is very easy to keep track of samples and related fractions. For purity control, collected fractions can be re-analyzed automatically. When using a mass selective detector the software calculates the purity of fractions and displays the result in an easy-to-read color-coded user interface.

Other new features include complete control over plate orientation, fill direction for fraction collection and the possibility to repeat a study up to ten times. This enables pooling of identical fractions from multiple injections of the same sample.

In a previous Technical Note[2] we described how to achieve optimum performance and precision with the Agilent 220 MPS. In this Technical Note we describe the hardware and software, how to optimize the software for fraction collection and how the new features of the micro plate sampling software helps with fraction collection tasks.

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Equipment

An Agilent 1100 Series system for combinatorial chemistry and high throughput LC analysis consists of an 1100 Series vacuum degasser, an 1100 Series binary pump, an 1100 Series thermostatted column compartement, a detector (1100 Series diode array detector, 1100 Series variable wavelength detector, 1100 Series fluorescence detector or any other detector connected via the Agilent 35900E analog-to-digital interface), an 1100 Series mass selective detector G1946B and an Agilent 220 micro plate sampler. The system is controlled using the Agilent ChemStation (revision A.07.01) and the micro plate sampling software (revision A.03.01).

Fraction collection features

Software revision A.03.01 offers three fraction collection features based on:

- time slices
- peaks, and
- masses

The features based on peaks and based on masses can also be combined using the Boolean logic combinations AND and OR.

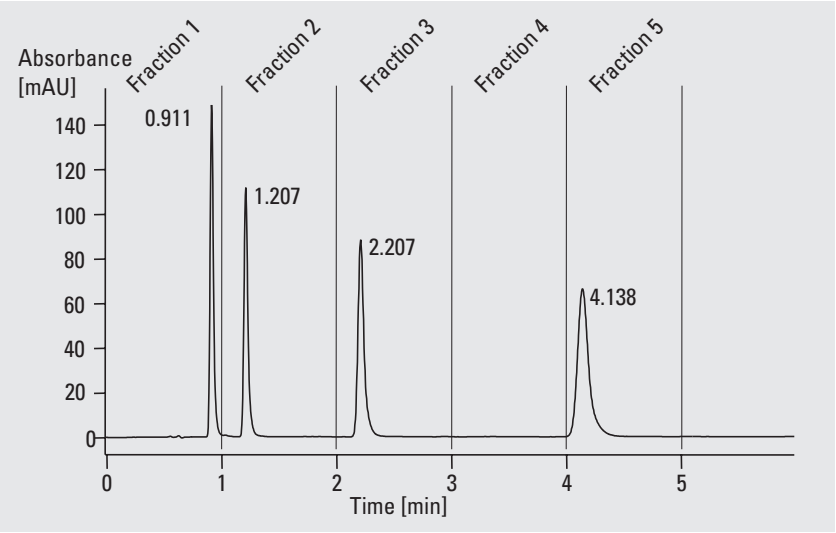
1. Fraction collection based on time slices

When *Enable Fraction Collection* is set up in the *Sampler Parameters* window the *Fraction Collection Table* is displayed after pressing the *Edit* button. In this table the commands *Time Based Collection*, *Start* and *Stop* can be set up for fraction collection based on time slices. Additionally, the time an event has to occur and a time slice *Width* has to be entered.

The latter command is used for *Time Based Collection*, where it specifies the duration of fractions collected beginning at the start time set up in the *Time Column*. The *Stop* command, which must always be the last command in the *Fraction Collection Table*, defines the end of the fraction collection based on time slices. In the example in figure 1 *Time Based Collection* starts at 0.00 minutes until five minutes with a *Width* of one minute. This leads to five fractions as shown in the chromatogram. The second possibility to collect fractions is by using the *Start Fraction* and *Stop* command, where *Start Fraction* defines the beginning of a fraction and *Stop* the end. In the example in figure 2 two fractions are collected this way.

Hints for fraction collection based on time slices

- If possible add a “blank” fractionbefore your first fraction.
- If possible perform a *Rinse needle outside* after injection of the sample. A warning message appears but the first fraction can be collected after about 0.5 minutes without any problems.
- If possible do not cut peaks directly after the downslope is finished. Add some “baseline” at the end of the peak.
- Use the *Clean Needle* option when using the *Start Fraction* and *Stop* command. It is not valid for *Time Based Collection*.



Fraction Collection Table

	Time	Event	Width	Clean Needle
	0.00	Time Based Collection	1	☐
	5.00	Stop	n/a	☐

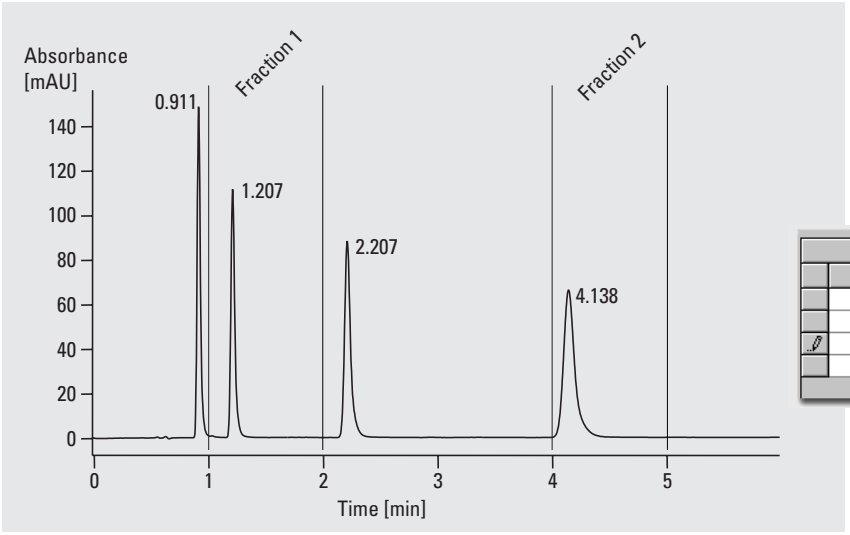
Figure 1
Time based collection

2. Fraction collection based on peaks

To collect fractions based on peaks it is important to set up the proper detector in the *System Configuration* window. This can either be the Agilent 1100 Series diode array detector, the 1100 Series variable wavelength detector, the 1100 Series fluorescence detector or any other detector connected via the Agilent 35900E A/D interface. To set up the fraction collection based on peaks the *Peak Detection* command has to be entered in the *Fraction Collection Table*. Three parameters, *Threshold*, *Peak Slope* and *Maximum Fraction Duration*, have to be entered in the *Enable Fraction Collection* window. While the parameters *Threshold* and *Peak Slope* determine whether a peak is collected or not *Maximum Fraction Duration* ensures that an end of the fraction is found for a strongly tailing peak. Depending on the detector the units for *Threshold* and *Peak Slope* are mAU, LU or mV. In figure 3 the *Peak Detection* command is entered at 1.60 minutes. Therefore, the first two peaks were not collected.

Hints for fraction collection based on peaks

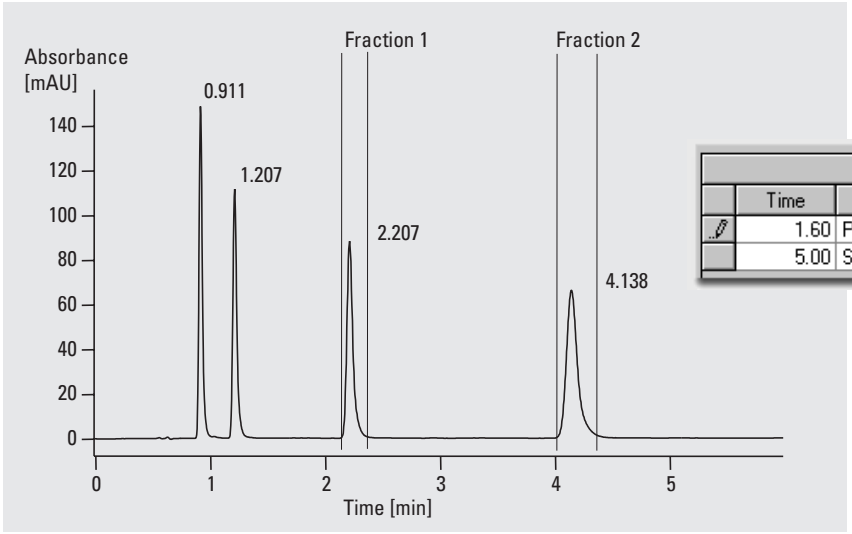
- Make sure to set up the right detector in the *System Configuration* window.
- Make sure the analog output of the control module is connected to output analog 1 of the detector. This ensures that signal A of the detector is used to trigger fraction collection.
- The analog output range of the detector must be set to 0.1 V
- If possible perform a *Rinse needle outside* after injection of the sample. A warning message appears but *Peak Detection* can be started after about 0.5 minutes without any problems.



Fraction Collection Table

	Time	Event	Width	Clean Needle
	1.00	Start Fraction	n/a	☑
	2.00	Stop	n/a	☐
	4.00	Start Fraction	n/a	☑
	5.00	Stop	n/a	☐

Figure 2
Time-based collection uisng the *Smart Fraction* and *Stop* commands



Fraction Collection Table

	Time	Event	Width	Clean Needle
	1.60	Peak Detection	n/a	☑
	5.00	Stop	n/a	☐

Figure 3
Peak detection starting at 1.60 minutes

3. Fraction collection based on masses

The Agilent 1100 Series LC/MSD can also be used as part of an integrated system with the Agilent 220 micro plate sampler and the micro plate sampling software. When equipped with the mass based fraction collector kit (part number G1967B), the 1100 Series LC/MSD is capable of monitoring up to 16 *m/z* values to decide whether to trigger the Agilent 220 MPS for fraction collection. Since the complete solution is designed for synthetic chemists, the user will input target molecular weights and an adduct list. For example, if the chemist made a compound with molecular weight of 270, they would input a base mass of 270, and specify M+1 and M+Na(23) as adducts. If they were also doing negative ion mode, they could specify M-1 as an adduct. The 1100 Series MSD would actually monitor *m/z* 271, 293 and 269. If one adduct is specified, 16 base masses are possible, if 2 are specified, 8 masses are possible, and so on. Besides choosing the masses to monitor, it is also necessary to set several parameters in the *Fraction Collection* window (figure 4) to correctly collect peaks.

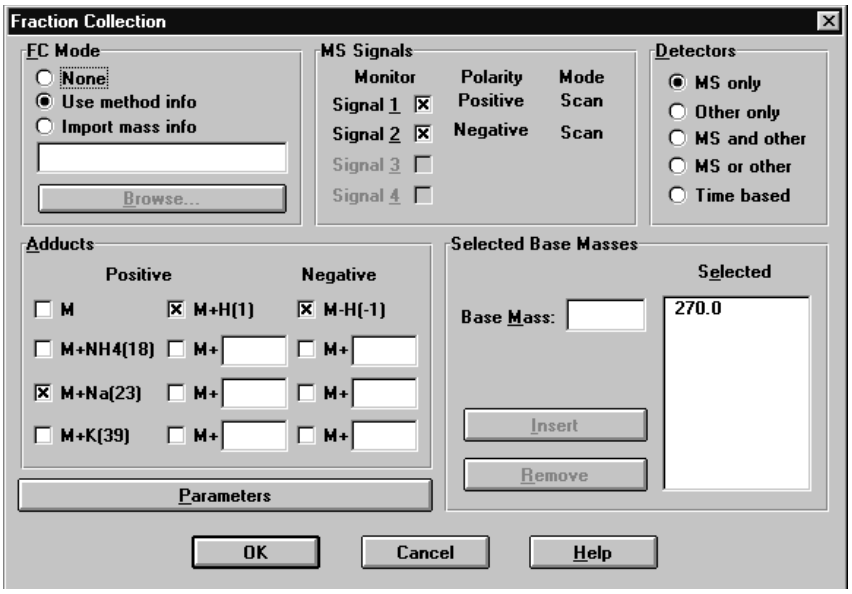


Figure 4
Time based collection

The best way to optimize the parameters is to do a trial run without actually collecting fractions. Then, in the *Data Analysis* part of the Agilent ChemStation, look at the data to determine the appropriate parameters. If we use the previous example, the ions at *m/z* 269, 271 and 293 have to be extracted. For best results, the ions have to be extracted in exactly the way they have to be monitored. For example, if a mass window of ±0.5 is to be used, the masses 268.5-269.5, 270.5-271.5 have to be extracted. Then a reasonable threshold value has to be determined so that most of the peak is collected but baseline noise does not trigger fraction collection. Similarly, the peak slope has to be examined. For example, if the peak is one million counts high and the peak width at half height is 0.10 minutes, the peak slope is 166,000 counts per second. A value of half to one quarter of that is reasonable.

In a typical setup, the compounds elute from a column and then go through the flow cell of an UV detector. The output is connected to a splitter and a portion of the sample goes to the 1100 Series MSD while the majority goes back to the Agilent 220 MPS. The Agilent ChemStation method can be optimized to trigger the collection at the correct time. This is done using the *Collector Delay* values in the *Fraction Collection Parameters* screen (figure 5).

To determine *Collector Delay* for the Agilent 220 MPS with the micro plate sampling software, a delay sensor is mounted on the needle of the liquid handler. A green dye has to be injected in FIA mode (no column) and the time is measured for it to pass through the system. The sensor's output is monitored as a data curve during the run. The sampler task *Enable Fraction Collection Delay Sensor* is added to the *Task List* to open the low-pressure valve and park the needle over the rinse station. After the run is complete, the UV signal, the MS signal and the *Delay Sensor* instrument

curve are loaded in the *Data Analysis* view of the Agilent ChemStation. The differences in time between detection by the UV detector and the delay sensor and between the 1100 Series MSD and the delay sensor are measured and reported. These values are then used in the *Fraction Collection Parameters* screen.

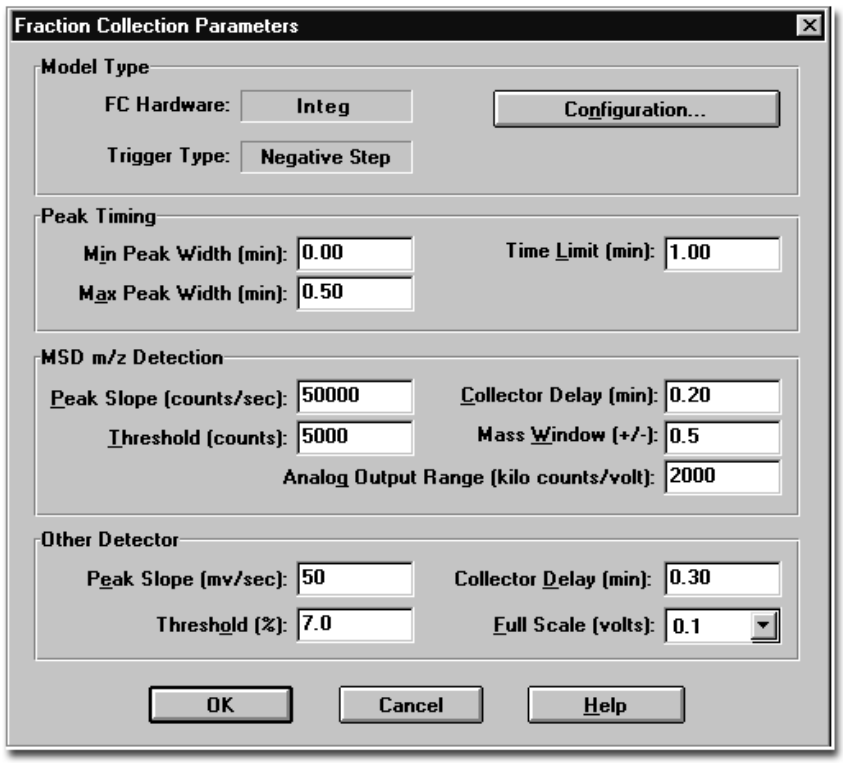


Figure 5
Fraction collection screen

Hints for fraction collection based on masses

- If possible, perform a trial run without collecting fractions. After the trial run optimize the fraction collection parameters for the actual run as described above.
- For flow rates below 1 ml/min use a make-up pump for acceleration and dilution of the flow from the splitter to the MSD.
- Make sure to optimize the collector delay time using the delay sensor and the sampler task *Enable Fraction Collection Delay Sensor*.
- Make sure the peak is detected by the MSD before it arrives at the fraction collector. This can be achieved by using a very short tubing from the splitter to the MSD and a longer tubing from the splitter to the Agilent 220 MPS.
- Consider that the base masses set up in the micro plate sampling software are used for purity calculations as well as for the selected base masses in the Agilent ChemStation. On the other hand, the adducts specified in the micro plate sampling-method are only used for purity calculations and the adducts for fraction collection have to be specified in the Agilent ChemStation method.
- If possible perform a *Rinse needle outside* after injection of the sample. A warning message appears but the first fraction can be collected after about 0.5 minutes without any problems.
- Too many peaks may also be collected if the ion of maximum abundance changes during peak elution. This could happen if both bromine isotopes are being monitored, for example. It may be necessary to reduce the number of adducts being monitored.

4. Other new fraction collection features

Maintain Sample-Fraction position 1:1
When *Maintain Sample-Fraction position 1:1* is checked the system collects fractions in the corresponding well position as the sample. Therefore, some restrictions apply:

- Only one fraction well per sample, that is, only one fraction per sample.
- *Overfill Action* is automatically set to *Put into Waste*.
- Shape of sample and fraction plate must be the same.
- There must be at least as many fraction plates as sample plates.

Overfill Action
Overfill Action determines what happens during fraction collection if a fraction well becomes full. If *Use Next Via*l is selected the fraction will continue in the next free vial. If *Put into Waste* is checked the remaining of the fraction will be put into waste.

Fill Order
Fill Order can be set to *Follow well number* or *Follow shortest path*. The resulting fill order is shown in figure 6.

Run Study n times
After starting a study the *Start Study* screen appears. In this screen it is possible to run the same study several times as specified in the *Run Study n times* field. This option can be used for fraction pooling. A study can be run not more than 10 times.

Fraction Analysis
When this function is selected all collected fractions are automatically injected after the study is finished. The fractions are analyzed using the method selected in the *Method for Fraction Analysis* drop down list box. Therefore, some restrictions apply:

- The selected method for re-analysis must not contain *Enable Fraction Collection* in the task list.
- Auto subdirectory generation must be selected.
- The method for *Fraction Analysis (Sampler Parameter* and so on) must be set up beforehand.

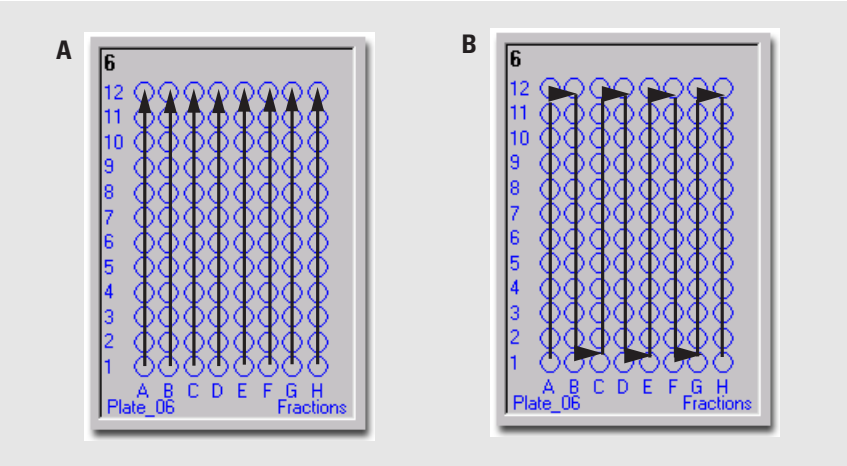


Figure 6
A: ***Follow well number,*** **B:** ***Follow shortest path***

Conclusion

In this Technical Note we described the new fraction collection features of the micro plate sampling software revision A.03. Fractions can be collected based on time slices by setting up a start time and a slice width or by setting up the start and stop time for the fraction. Fraction collection based on peaks can be done using the Agilent 1100 Series diode array detector, the Agilent 1100 Series variable wavelength detector, the Agilent 1100 Series fluorescence detector or any other detector connected using the Agilent 35900E A/D interface. For fraction collection based on masses the Agilent 1100 Series mass selective detector G1946B must be connected to the Agilent 1100 Series LC system. Fraction collection can be done for up to 16 masses and fragments.

The software contains additional features related to fraction collection. Using *Maintain Sample-Fraction position 1:1* the system collects fractions in the corresponding well position as the sample. *Overfill Action* controls what happens with the rest of the fraction in case a fraction well is filled completely. Options are to use the next free well or put into waste. *Fill Order* provides a choice of how fraction wells are filled, either according to well numbers or following the shortest path. In the *Start Study* window it is possible to enter how often the same study is repeated, which is useful for fraction pooling. With software revision A.03 it is also possible to automatically re-analyze the collected fraction.

Literature

1
"Agilent 220 Micro plate sampler: A solution for high-throughput HPLC analyses and purification" Agilent Brochure, **2000**, publication number 5968-9101E

2
"Optimizing the Agilent high throughput analysis system for high performance and precision" Agilent Technical Note, **2000**, publication number 5980-0494E

www.agilent.com/chem

Printed 10/2000
Publication Number 5988-0462EN

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