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### Introduction

As the efficiency of the purification process has increased, so have the expectations. Now, chemists are not only looking to recover the main synthetic product, but possibly also the starting materials, intermediates and impurities. In other instances, specifically with very complex mixtures like natural product extracts or biological fluids, there is often a desire to collect both a specific target and all the other significant peaks for any potential further analysis.

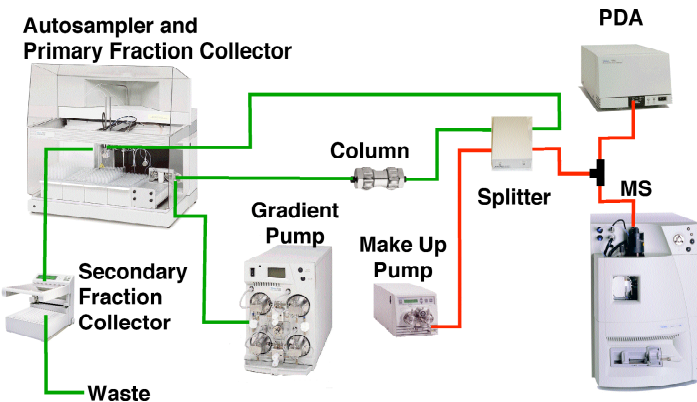
These are not unreasonable expectations since these components have to be separated as part of the process. However, until recently, there is no easy and satisfactory way to fulfill these expectations within the structure of the entire purification process. The simplest option was to collect all the targets on the same bed. This approach rapidly fills the fraction bed with a mixture of primary and secondary targets, which makes sorting, culling and reformatting a challenge. Though this approach will work, it adds an unnecessary level of complexity to the process. A more desirable approach would be to collect the primary targets in one location and everything else, the secondary targets, in another. This is now possible using Waters® Fractionlynx™ Application Manager, which allows secondary fractions to be easily collected and tracked for further use.

This poster shows the versatility of the software for this new approach to secondary fraction collection. Examples illustrated will use mass-directed purification of a primary targets and mass-directed or ELSD (Evaporative Light-Scattering Detection) of the secondary targets to separate fraction collectors.

Also highlighted in this poster are some of the additional configurations now possible with multiple fraction collectors. These include an additional collector for waste collection, additional collectors with a collector selector valve for high capacity collection, and multiple collection systems in parallel (Waters Purification Factory™) for high-throughput purification.

### Equipment

Waters® Mass-Directed Purification System: 2525 Binary Gradient Module, 2767 Sample Manager, Column Fluidics Organizer, 2996 Photodiode Array Detector, ZQ™ Mass Detector and an WFCIII Fraction Collector controlled by Masslynx™ Version 4.0 with Fractionlynx™, XTerra® C18 5 µm Column 19 x 50 mm.



Flow diagram of the mass-directed purification system with secondary collection.

### Software Control and Setup

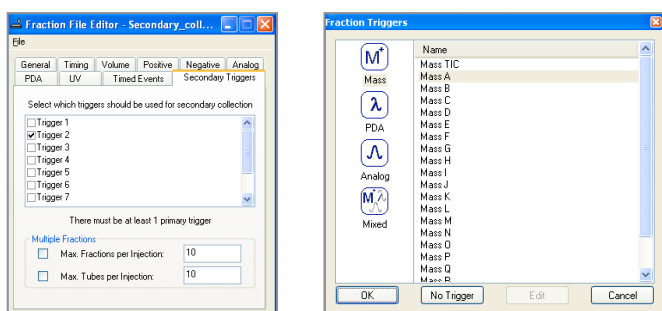
- Primary and secondary targets are entered into the sample list.
- Active triggers are selected.

- Primary and secondary triggers can be any possible combination of MS/UV(PDA)/Analog/or mixed triggers.

- Secondary trigger(s) are selected in the Fraction File.

File Name	HPLC Method	Vol	Inlet Volume	MS Method	Fraction File	Mass A	Mass B	Fraction Trigger 1	Fraction Trigger 2	Process	Parameter File
1 MS loggers_1	Generic1	4.31.A	1000	Generic1	Secondary_collect	300.2	118.1	Mass A	Mass B	AutoPurify	Auto_secondary.flt
2 MS loggers_2	Generic1	4.31.B	1000	Generic1	Secondary_collect	300.3	118.1	Mass A	Mass B	AutoPurify	Auto_secondary.flt
3 MS loggers_3	Generic1	4.31.C	1000	Generic1	Secondary_collect	274.1	118.1	Mass A	Mass B	AutoPurify	Auto_secondary.flt
4 MS loggers_4	Generic1	4.31.D	1000	Generic1	Secondary_collect	288.2	118.1	Mass A	Mass B	AutoPurify	Auto_secondary.flt
5 MS loggers_5	Generic1	4.31.E	1000	Generic1	Secondary_collect	311.1	118.1	Mass A	Mass B	AutoPurify	Auto_secondary.flt

Masslynx purification sample list.

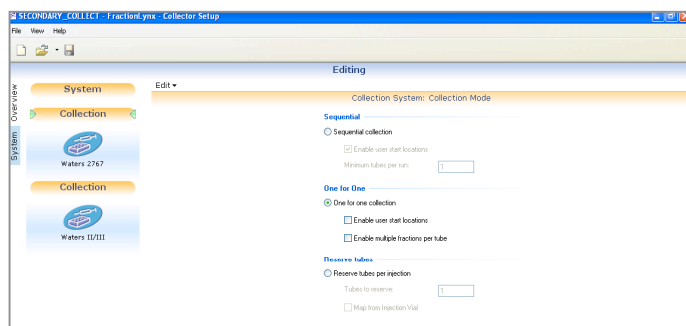


The Fraction File Editor window with the Secondary Triggers tab selected.

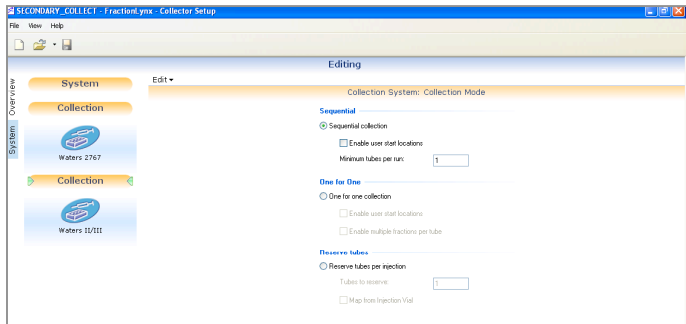
The Fraction Trigger window is displayed when double clicking on a trigger field.

### Collector Setup

- To setup secondary collection in Fractionlynx, a second collection system is added. The first collection system is the primary, the second is the secondary.
- Each collection system has a specific collection mode. This allows for 1 to 1 collection on one bed, with sequential collection on the other.



Fractionlynx Collector Setup screen with the primary collector's collection setting displayed.



Fractionlynx Collector Setup screen with the secondary collector's collection setting displayed.



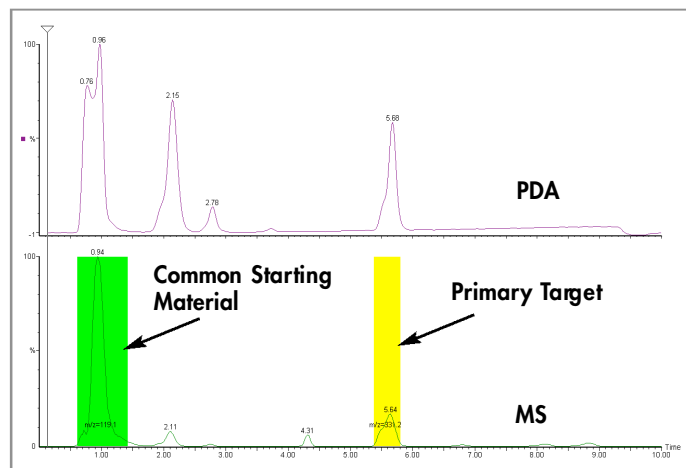
Waters Mass-Directed Purification System.

### Recovering Starting Materials

- In this example, it was desired to collect both the synthetic product and common starting material from a series of samples. The plan of action was to use mass-directed purification with secondary collection for the starting material. The primary targets are in the Mass A column and the secondary target, a common starting material, is in the Mass B column.

File Name	HPLC Method	Vol	Inlet Volume	MS Method	Fraction File	Mass A	Mass B	Fraction Trigger 1	Fraction Trigger 2	Process	Parameter File
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3 MS loggers_3	Generic1	4.31.C	1000	Generic1	Secondary_collect	274.1	118.1	Mass A	Mass B	AutoPurify	Auto_secondary.flt
4 MS loggers_4	Generic1	4.31.D	1000	Generic1	Secondary_collect	288.2	118.1	Mass A	Mass B	AutoPurify	Auto_secondary.flt
5 MS loggers_5	Generic1	4.31.E	1000	Generic1	Secondary_collect	311.1	118.1	Mass A	Mass B	AutoPurify	Auto_secondary.flt

Masslynx sample list for the purification of 5 synthetic products each with a common starting material.



Chromatographic results showing the primary and secondary triggering of the targets.

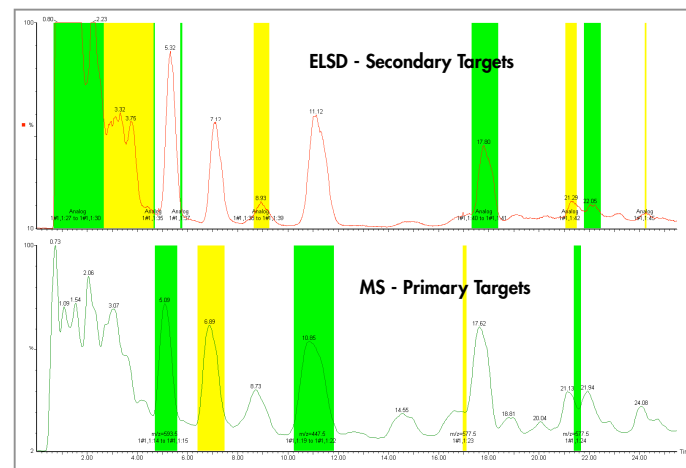
- The purified products and the recovered starting material are collected onto separate beds.
- For additional process simplicity, a funnel rack can be used on the secondary collector to automatically pool the common starting material.
- Reusing the collected starting materials can significantly reduce the overall library cost in terms of money and labor time, thus increasing the overall efficiency of the synthetic process.

### Non-Specific Secondary Collection

- In this example, there are 3 primary target masses for this natural product extract. However, their responses are unknown. Also, there is a limited amount of sample, enough for only 1 injection. The plan of action was to use the ELSD signal for secondary collection, while doing mass-directed purification for the primary target.

File Name	HPLC Method	Vol	Inlet Volume	MS Method	Fraction File	Mass A	Mass B	Fraction Trigger 1	Fraction Trigger 2	Process	Parameter File
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5 MS loggers_5	Generic1	4.31.E	1000	Generic1	Secondary_collect	311.1	118.1	Mass A	Mass B	AutoPurify	Auto_secondary.flt

Masslynx sample list for the purification of a natural product extract.

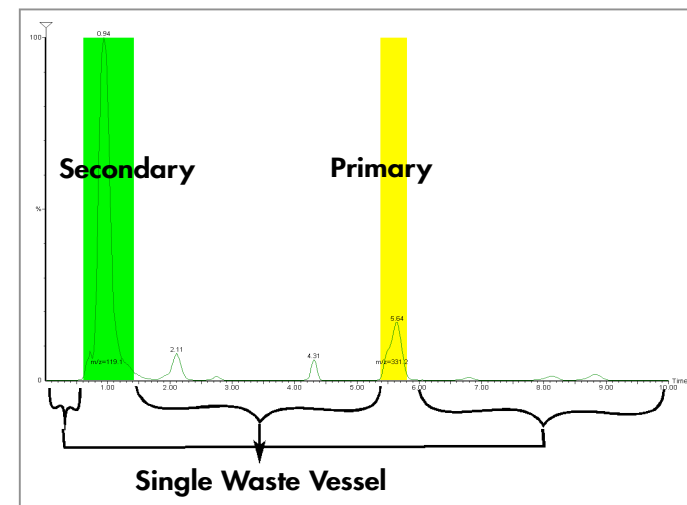


Chromatographic results showing the primary and secondary triggering of the targets.

- The mass-directed targets are collected into the primary bed, and all the other peaks detected by the ELSD are collected into the other bed.
- The ELSD fractions can be re-purified using an orthogonal chromatographic method to separate the co-eluting compounds which could have originally suppressed the ionization the main target.
- The secondary fractions could also be later used to screen the sample against other targets, reducing time and cost for extracting and preparing a new sample.

### Addition of Waste Collection

- For further safeguarding against losing a sample, another collector can be added to the system as a waste collector.



Example of waste collection with primary and secondary triggers.

- All column effluent that is not collected as a primary or secondary trigger is collected into a single waste vessel for each injection.

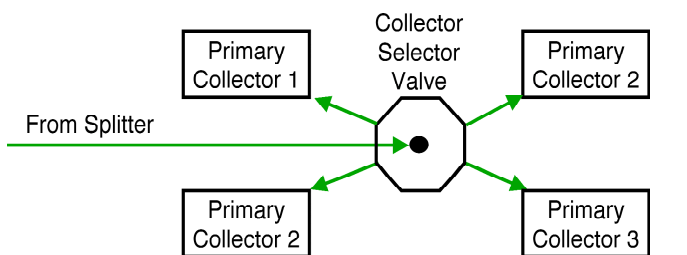


Collection flow path for primary, secondary and waste collection.

- Primary, secondary and waste collectors are connected serially.
- To setup Fractionlynx, a waste system is added to the system.

### Multiple Primary Collectors

- For instances where increased fraction tube capacity is necessary, multiple primary fraction collectors can be used.

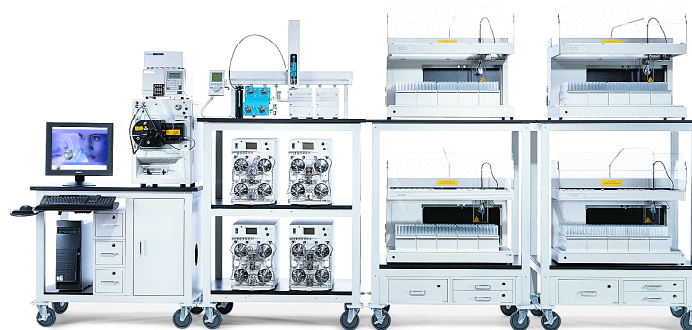


Collection flow path for multiple primary collectors with a collector selector valve.

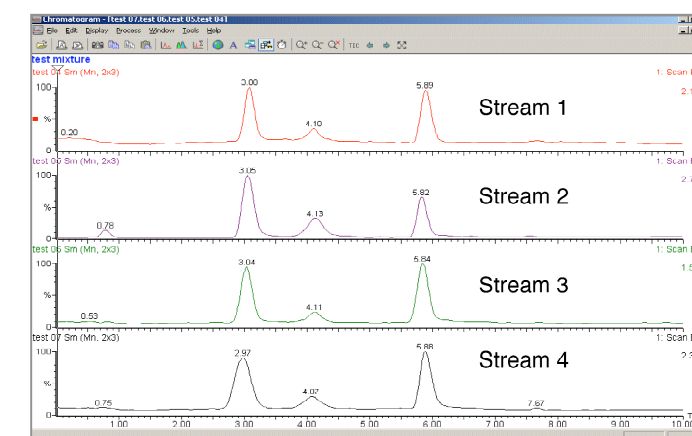
- A collector selector valve is used to keep the delay time to each collector constant
- To setup Fractionlynx, multiple collectors are added to the primary collection system
- Up to 8 primary collectors are supported.
- Each primary collector can have its own waste collector, for a total of 16 fraction collectors

### Multiple Systems—Purification Factory

- For high throughput applications, 4 simultaneous purifications can be run in parallel into 1 mass spectrometer.



Waters Purification Factory.



Chromatographic results from the 4 different streams.

- Fractionlynx is setup with 4 independent systems.
- Four complete collection systems controlled simultaneously, thus four samples are purified simultaneously.

### Conclusion

- Secondary collection is now a feasible option using Fractionlynx. It allows for different targets to be placed on different beds simplifying the process.
- Primary and secondary triggers can be any possible combination of MS/UV (PDA)/Analog/or mixed triggers.
- Secondary collection has many different applications, including starting material, intermediate or impurity collection and nonspecific collection while in conjunction with mass-directed purification.
- Advanced configurations with multiple collectors are now possible. These include:
  - A waste collector for additional safeguarding against sample loss.
  - Multiple primary collectors with a collector selector valve for high capacity collection.
  - Multiple collection systems in parallel (Waters Purification Factory™) for high-throughput purification.