A Multipurpose Preparative System for the Detection and Collection of Active and Non-Active UV/VIS Compounds

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Abstract:

- Versatility is a major concern for researchers today. It's advantageous to have a single automated system to accomplish both NP and RP in preparative HPLC with the collection of fractions. This type of system increases its usefulness without significantly increasing the cost of a single preparative HPLC system. UV/Vis is used to detect a substantial number of compounds; although other modes of detection are beneficial or required. One such mode is ELSD, commonly used in analytical HPLC; rarely used in preparative work as an alternative to MS. The principle requirement for ELSD is that the sample is less volatile than the mobile phase not its ionization potential. The system presented has be designed to work under a wide range of mobile phase conditions and preparative flow rates. The ELSD has been uniquely designed to work in preparative HPLC, and can accommodate sample masses > 1 milligram without overloading the detector, while still maintaining excellent analytical capabilities. The Liquid Handler which accomplishes both injection and fraction collection has a "syringeless" system based on a state of the art "rotary piston technology" which accommodates a larger dynamic volume and flow rate range. This combined with unique Liquid Handling techniques and various injection capabilities allows a virtually limitless dynamic volume range and an increased flow rate range. Data will be presented to support this novel approach to preparative HPLC and fraction collection.

Outline:

- Concept
- System: Hardware and Software
- Data and Results
- Conclusion and Summary

Concept:

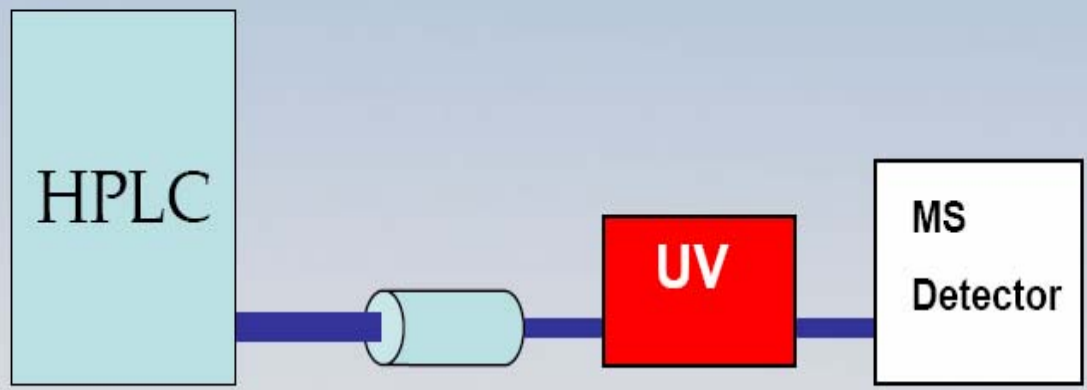
- What is the “Core” issue that a preparative HPLC-MS addresses?
  - Easily obtain enhanced information about fractions collected
  - Reduce the number of fractions collected
  - Reach into a “dirty” sample and pull out a peak of interest

Analytical HPLC-MS

- Robust and Stable technique
- Compounds are separated by the HPLC column and mobile phase conditions the effluent from the column passes through a UV/VIS detector and MS which provides the signal and molecular ion (s) for the individual compounds
- This technique, especially incorporating MS, has become extremely useful; providing valuable information to the researcher

Analytical HPLC-MS:

- Many MS Options
  - Single Quad
  - Dual Quad
  - Triple Quad
  - MS^n
  - TOF
  - Q-TOF



Simple Plumbing, Simple System

Preparative HPLC-MS

- Preparative HPLC-MS is a newer technique
- The fundamentals are the same; however, the details are dramatically different from analytical HPLC-MS

Preparative vs. Analytical

- Again, lets take a look at the goal of the two techniques
- Analytical HPLC-MS: Information
- Preparative HPLC-MS: Purify Sample
- In preparative HPLC-MS preservation of the sample is imperative

Preserving the Sample

- **Why is this an issue in preparative HPLC?**
  - Unlike UV detection, MS and ELSD are destructive techniques
  - MS detectors are also very sensitive
  - On-Line HPLC-MS requires a small amount of the effluent being split of for the MS detector
    - Mass directed fractions can be collected
    - Requires complex plumbing and precise splitting schemes

Splitter Technology:

- Passive Splitters
  - Ok for semi preparative work up to 50 mL/min
    - 1000:1 splitter
    - Problematic for preparative
    - 10,000:1 splitter
    - Clogging issues

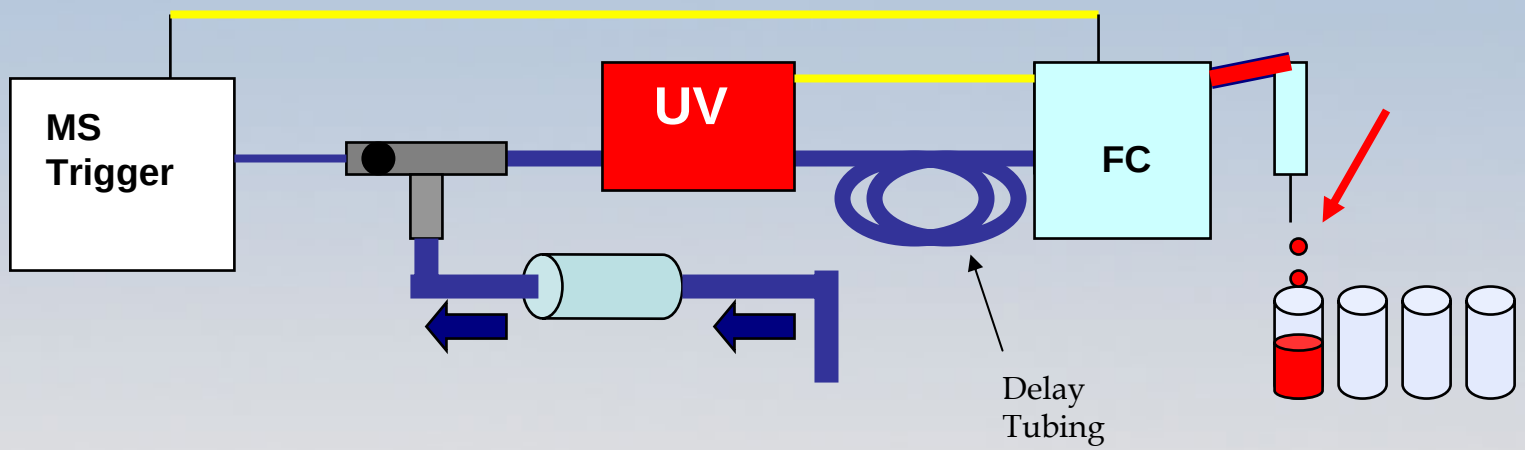
- Active Splitters

  -More expensive

  -At high split ratios for high flow rates can produce spiking

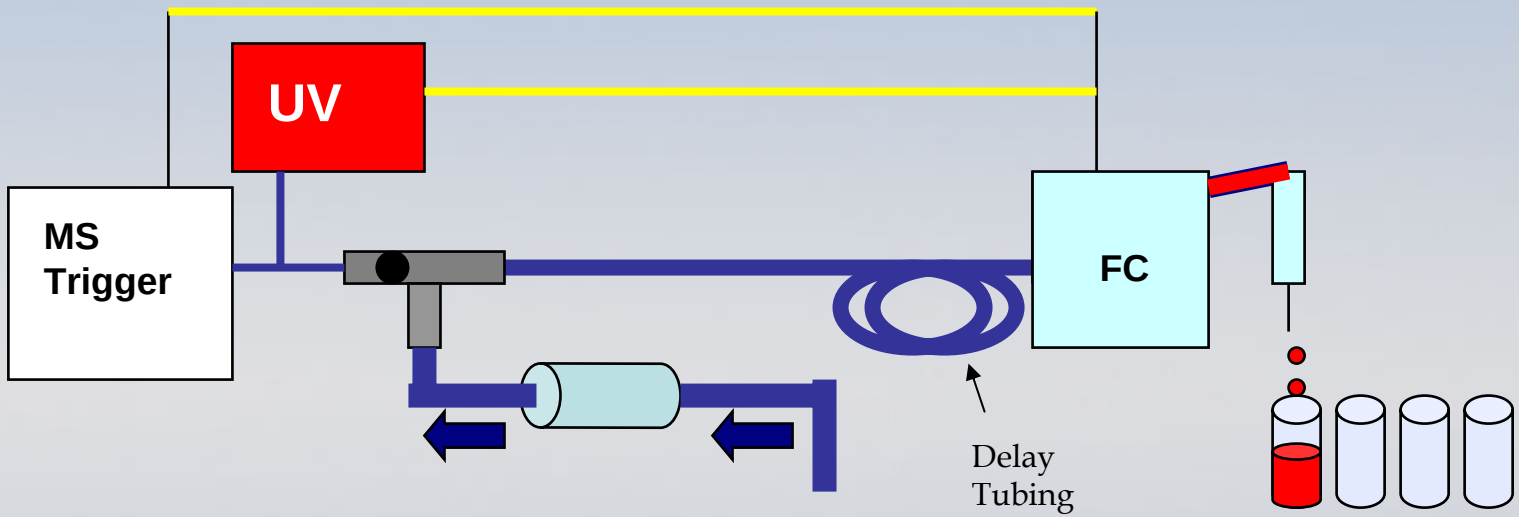
Method 1 Plumbing Scheme for On-Line Mass Directed Purification

Collecting:
Off UV and MS
Fraction is not
lost



Method 2 Plumbing Scheme for On-Line Mass Directed Purification

- If splitter clogs, no signal reaches the fraction collector and therefore no fractions are collected
- Plumbing the system this way allows for easy switching between analytical and preparative



MS Limitations:

- Constant problem beyond the splitter is MS over-load
- For preparative flow rates and sample loads, even high split ratios can inject too much sample into the MS detector

New Solution to “Core” Problems:

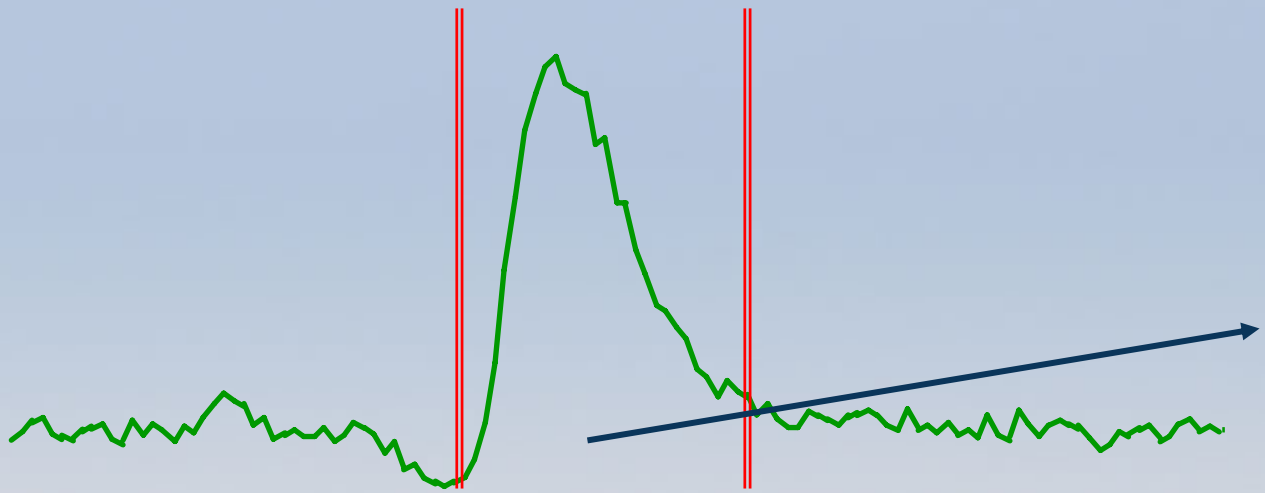
- Enables one to easily obtain enhanced information about fractions collected
- Enables one to reach into a dirty sample or a incomplete synthesis and pull out the compound of interest

Off-Line Preparative LC-MS
An Alternative to On-Line LC-MS

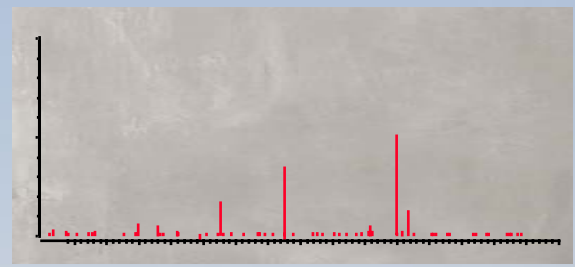
- Complete independent MS system onto standard preparative LC system

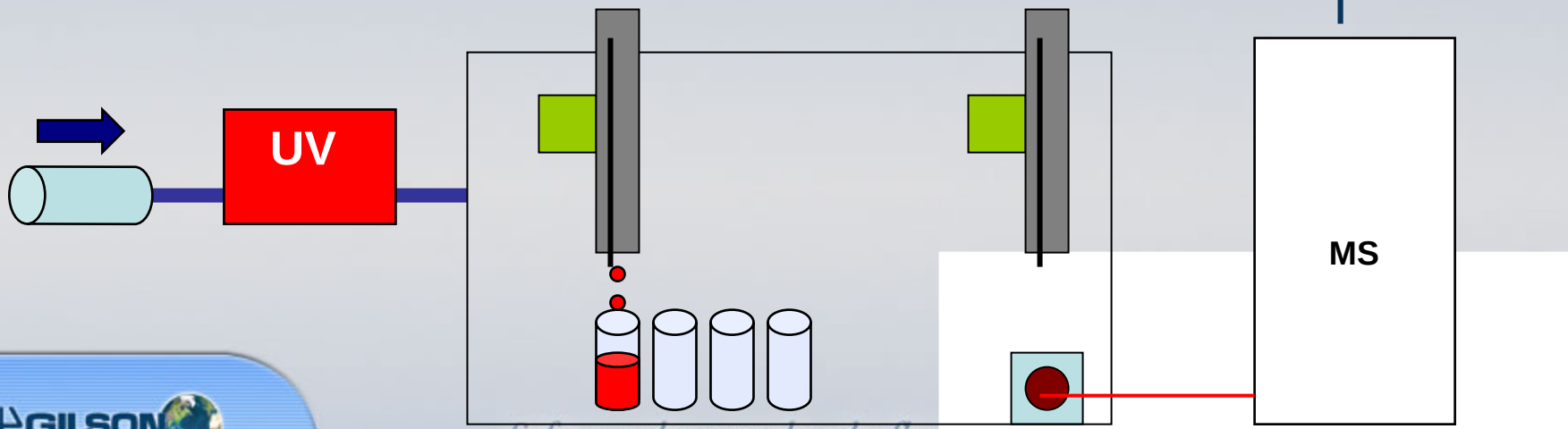
- System software will automatically re-inject a small aliquot of the collected fractions directly into the MS

Off-Line LC/MS



Mass Spectra of Entire Fraction





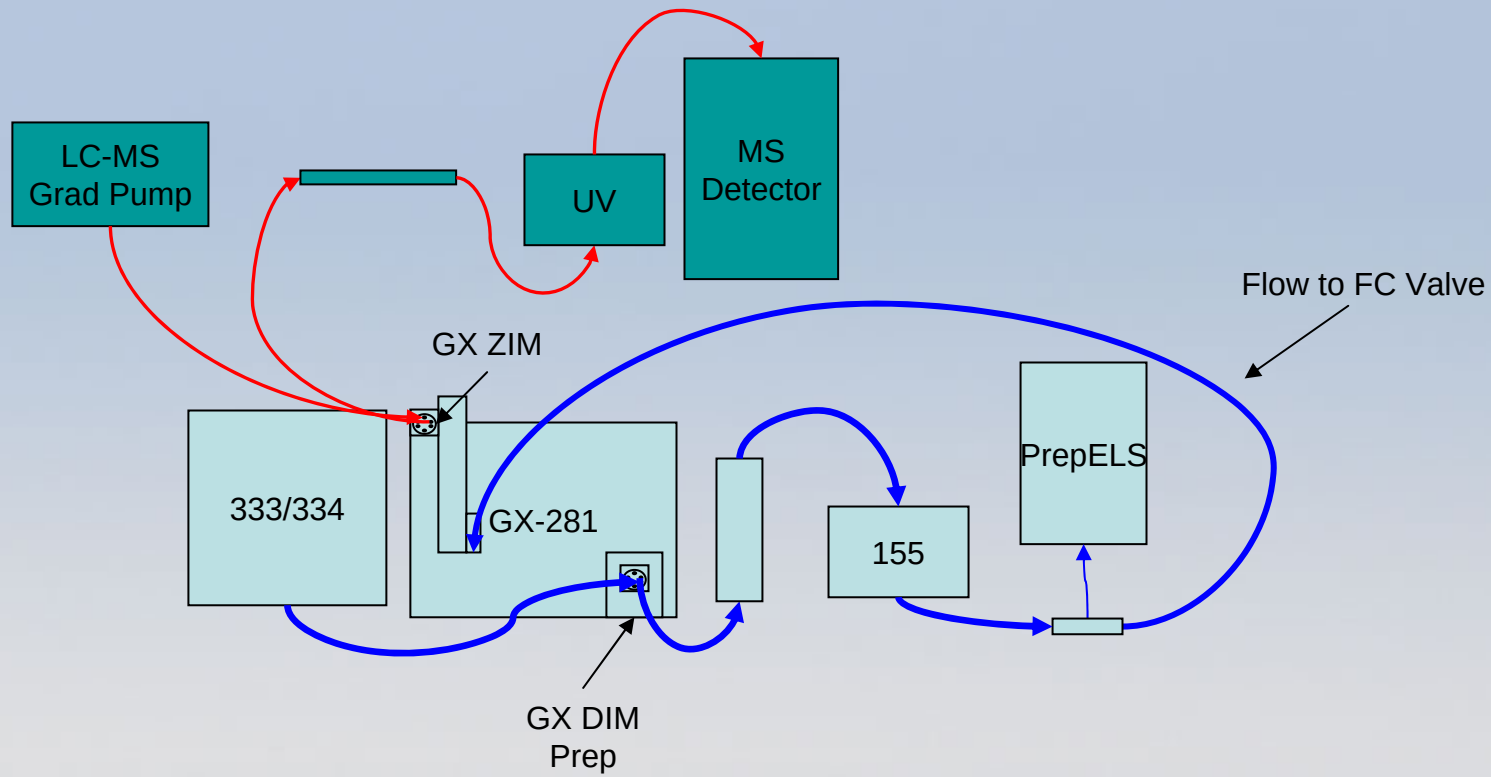
Off-Line LC/MS

- Offers a simple, robust system that will offer additional information about the sample
- Concerns about the safety and preservation of the sample
- MS data is available on the actual fraction collected, analytically
- No splitter involved and therefore concerns about clogging and loosing the sample are eliminated
- No complex plumbing
- Ability to use any MS, and other detector devices
  - e.g. ELSD

New Solution:

- Combine analytical LC/MS with a preparative UV/ELSD
- Run an analytical scout chromatogram under a basic gradient (5-95%) to determine the retention time of the compound/drug of interest
- Run an optimized preparative system gradient based on the data obtained from the analytical scout run to minimize the run time and separate interferents from the peak of interest in a narrower window for fraction collection without running extended gradients
- If needed, re-inject off-line for confirmation via MS

New Solution:



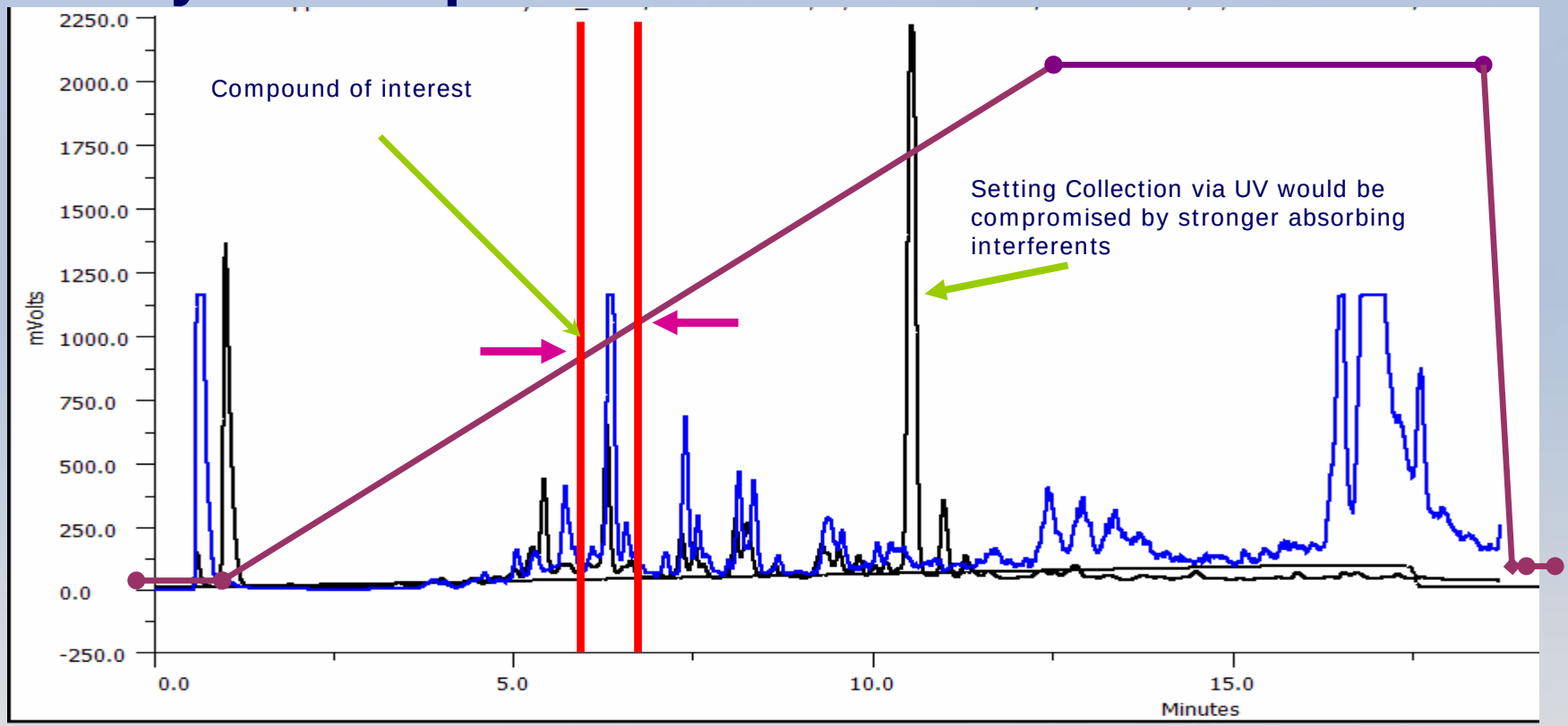
Optimization:

- Run analytical LC/MS first:
  - Determine elution conditions, optimum gradient percentage for the peak/analyte of interest, retention time relative to a small gradient window
  - Based on this information a preparative gradient run is activated to separate interferents from the peak of interest and collect the sample within one or a few fractions versus several fractions when a large drastic preparative gradient it employed

Review:

  - Therefore based on the data observed from the analytical scout run a very shallow preparative gradient is run via UV/ELSD that allows for the analyte of choice to be collected and all interferents to waste

Analytical Separation:

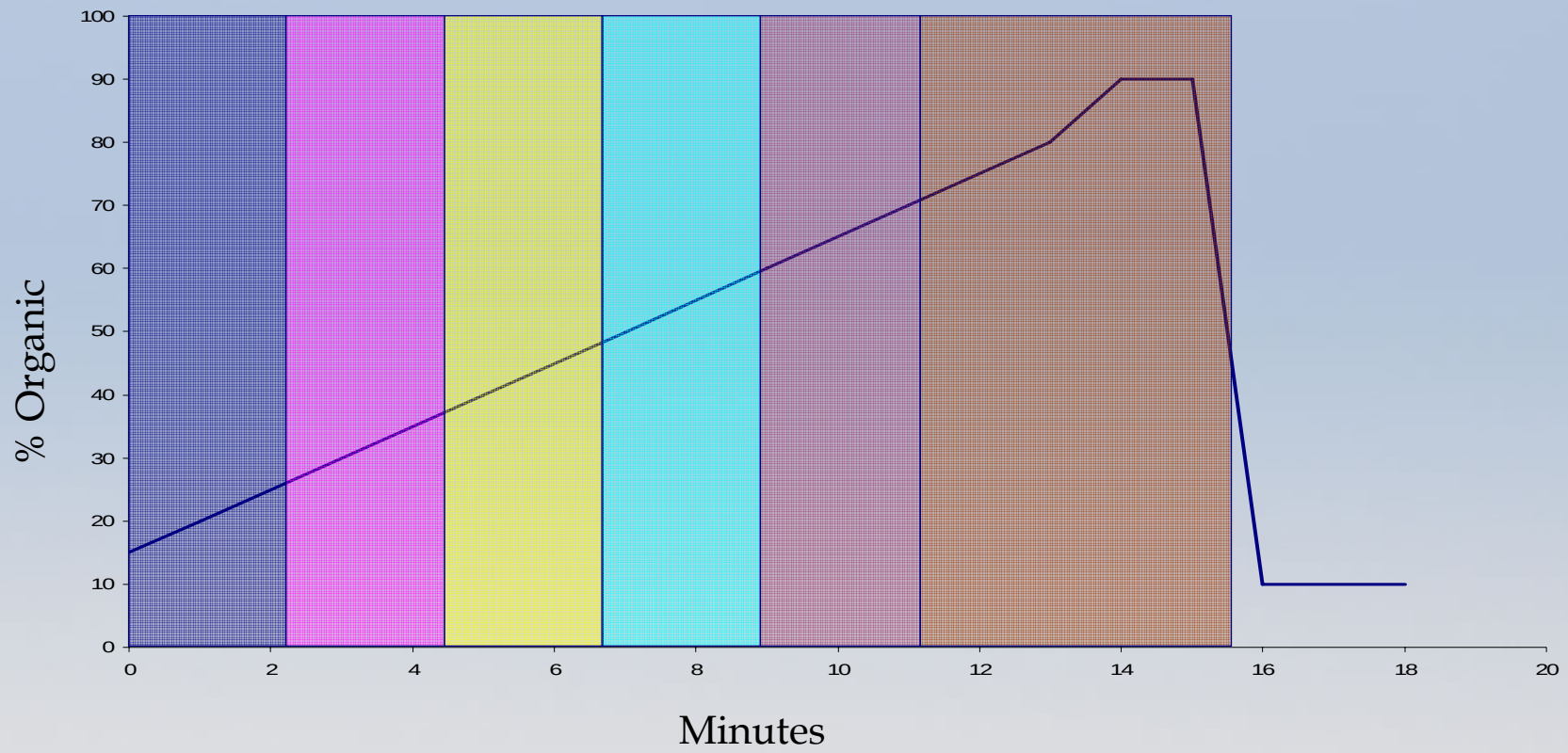


Graph 1: Example of an analytical scout for a synthesized compound, the black chromatogram is 254 nm, and blue represents the ELSD trace under analytical conditions, the ELSD although less specific is more universal and can see peaks that may not be observed by UV or MS, 4.6 x 150 mm Luna C18, 2 mL/min, 50 ul injection.

Preparative LC/ELSD:

- ELSD response does not depend on a sample's optical characteristics (like UV and fluorescence), therefore, any sample less volatile than the mobile phase can be detected
- ELSD response is proportional to sample mass, which is useful in determining sample purity or detecting unknown interferents, relative to MS
- ELSD offers full gradient capability, higher sensitivity and stable baselines with the capability of allowing collection of early eluting components
- The PREPELS detector is designed for preparative flow and preparative compound amounts, handling up to 5 mgs/mL of compound to the detector without off-scaling the detector signal
- Keeping in mind that a basic splitter is used in the system so only a small portion of the sample is diverted to the ELSD
- ELSD's in general are a very rugged and a easy drift tube design
- Advanced control over the PREPELS via a Thermal Split Technology offers temperature settings not only in the drift tube but also in the spray chamber

Analytical Gradient:



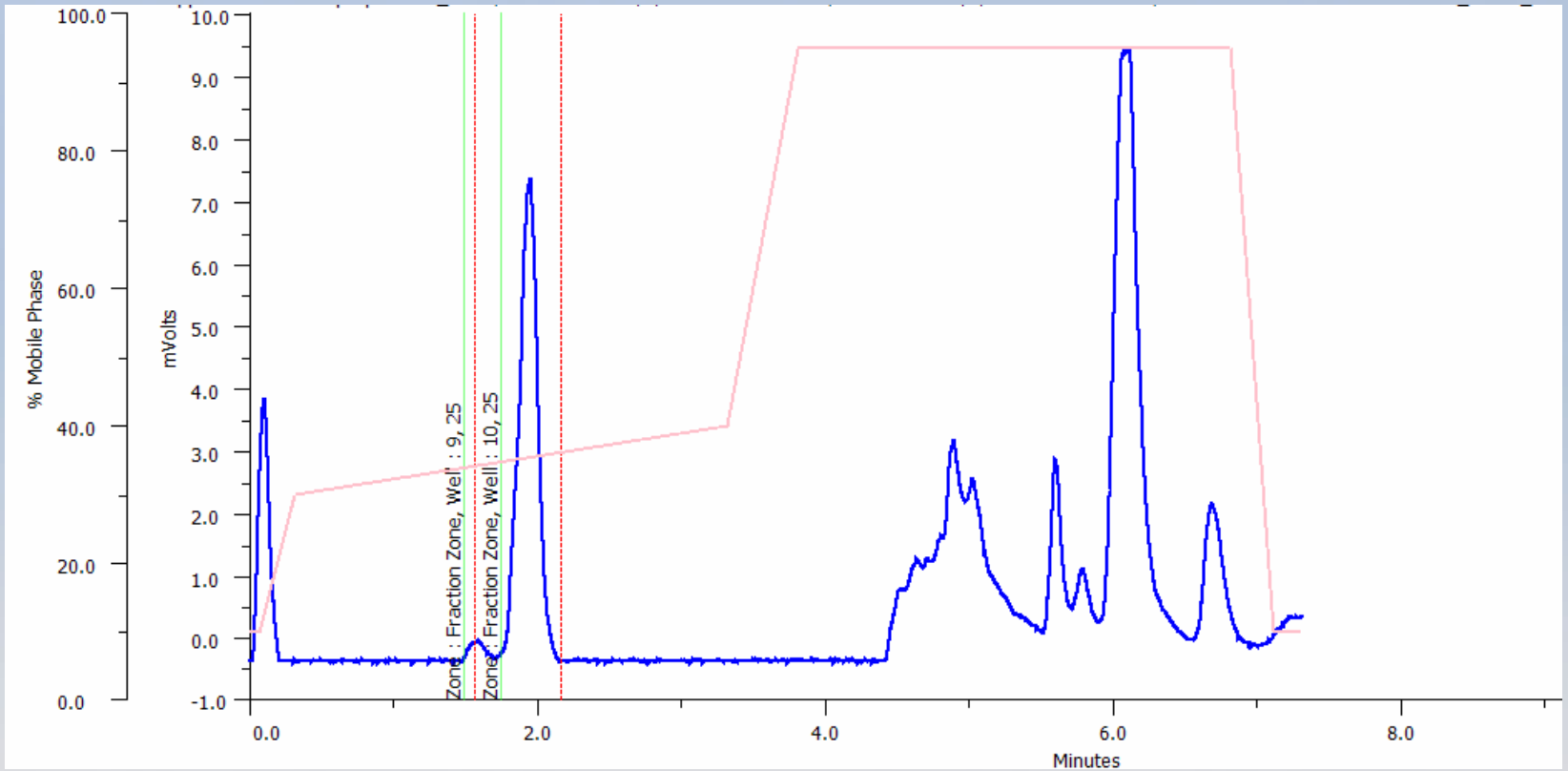
Graph 2: Example of the gradient profile and optimization of the analyte window, based on the window that the compound falls within is then stretch out to make a much shallower gradient to isolate the compound of interest for collection and purification

Preparative Purification:

- Although the analytical scouting runs will give you all the information that you'll need to optimize your preparative run with purified fractions collected; variability in the preparative run needs to be available so that researches can just input the gradient range and active running time required

- This is available through an interactive application screen including bar code identification

Selecting a Preparative Gradient:



Graph 3: Based on the analytical data acquired from the scout chromatographic run, a 30-40% organic mobile phase would facilitate the optimum separation, for our compound of interest, activating a shallow gradient for the specific compound relative to all other compounds within our chromatogram allows for an easy and complete fraction collection of our compound within minimal fractions, 21.5 x 50 mm Luna C18, 25 mL/min, 500 ul injection.

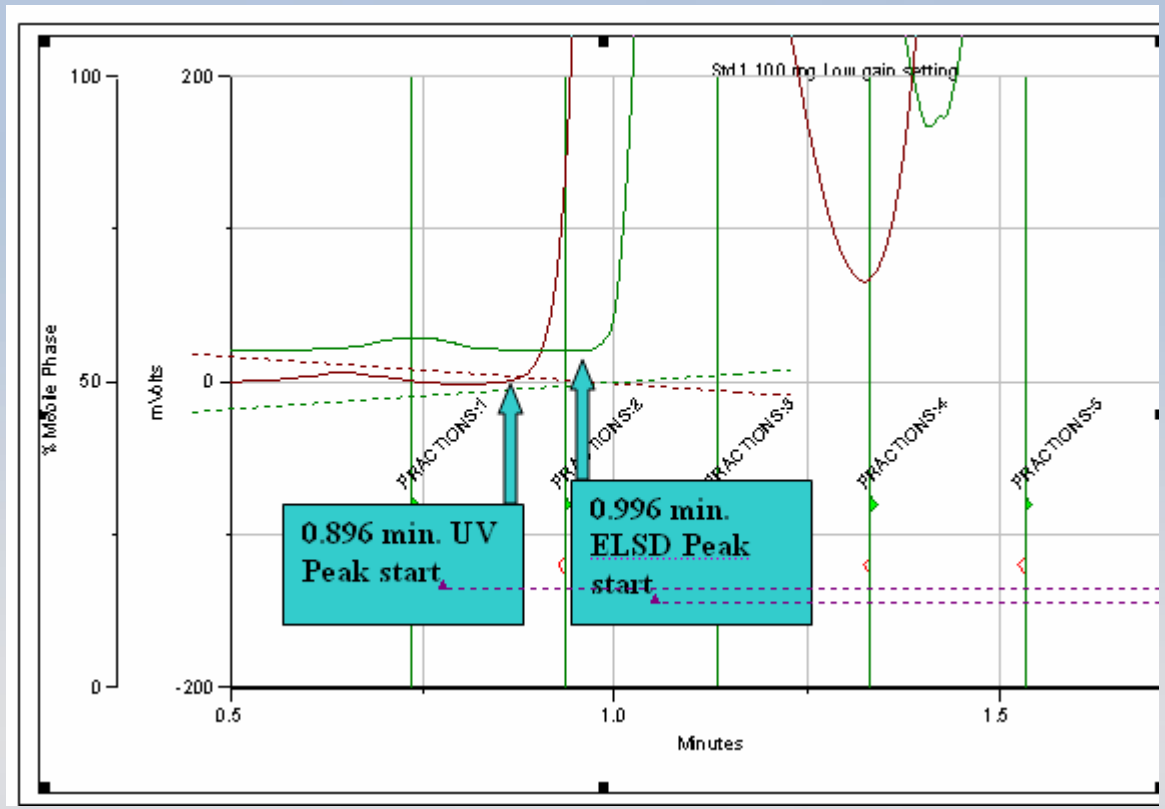
Collection of Fractions:

- Whenever the flow is split to two detectors and only a small amount of the flow is going to the destructive detector (MS,ELSD), a lag between the two signal will prevail

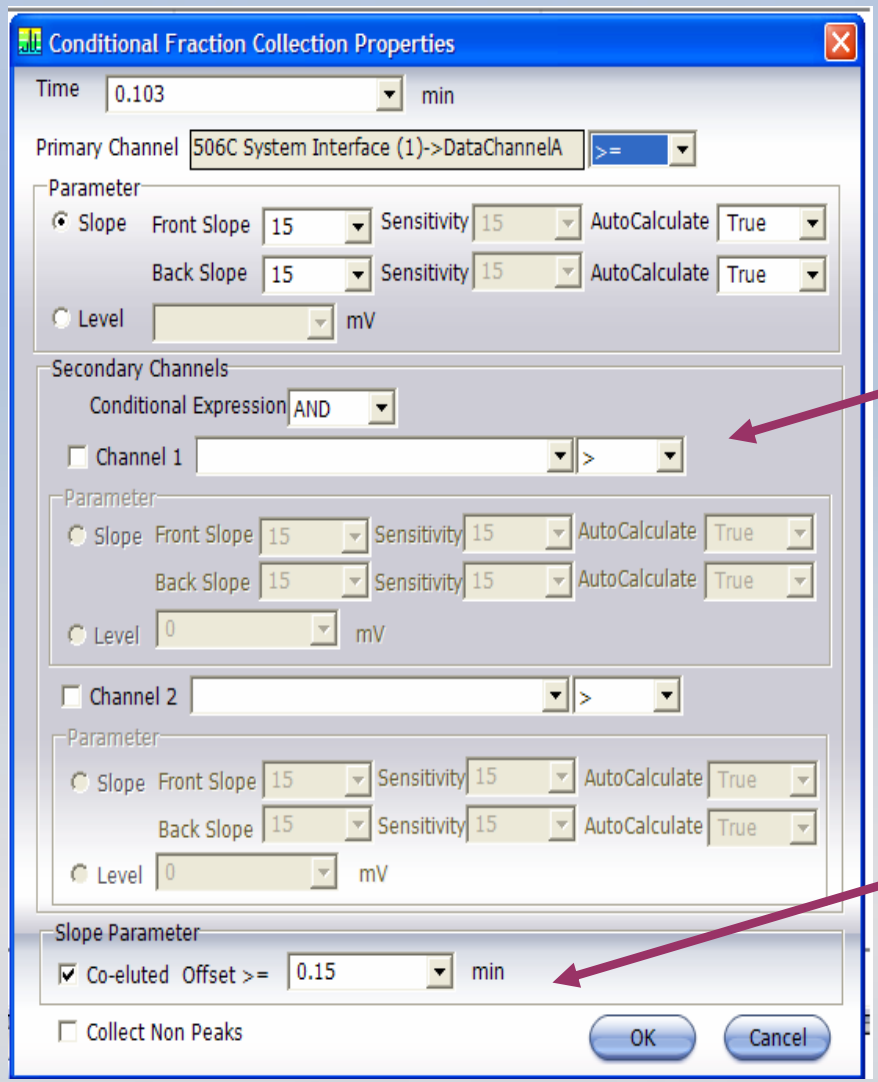
- Allowing the software to compensate for a delay relative to fraction collection is imperative for optimum recovery and intelliFC within a system

- Under “Conditional Logic FC” the software can take into account this time lag and collect appropriately in addition to collection based on advanced conditional logic e.g. second channel conditional logic, *and/if logic*

ELSD & UV For Fraction Collection



Conditional Logic Fraction Collection:

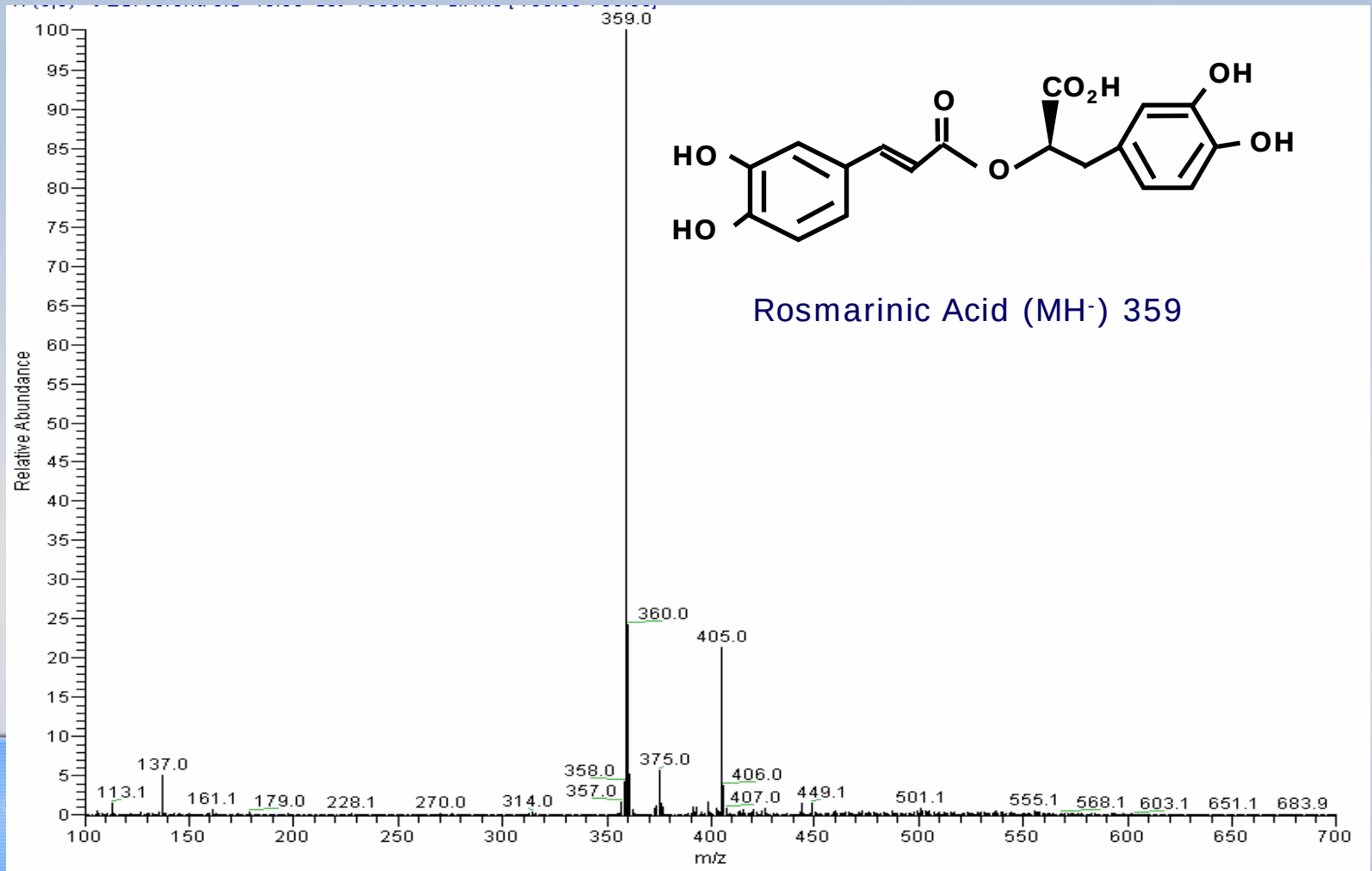


Secondary Channel Logic is available

Co-elute offset set the variance between the PREPELSD vs. the UV signal for active fraction collection

Confirming the Quick Prep:

- Since the system can also inject analytically as we saw with the scout run we can re-inject the fraction and confirm the collection of the compound of interest, recovery ~ 96% purity



Rosmarinic Acid (MH^-) 359

Summary and Conclusion:

- The system presented offers an alternative to more complex systems involving MS analysis for preparative LC, via UV/ELSD
- Understanding that a preparative purification may exhibit a more complicated separation than wished; the system presented in this application allows researchers to consider other relatively simplistic approaches to purification
- The flexibility of the system will allow for analytical scouting of the optimum gradient window and therefore answer the preparative gradient required for fraction collection of the compound of interest in greater concentration/fraction