Utilizing ELSD and MS as Secondary Detectors for Prep HPLC and Flash Chromatography

Tips and Techniques to Optimize ELSD and MS based Purification

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Utilizing ELSD and MS as Secondary Detectors for Prep HPLC and Flash Chromatography

Josh Lovell – Application Chemist

Teledyne ISCO

Joshua.Lovell@teledyne.com

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Outline

- Background and comparison of different available methods of detection
  - UV or UV-Vis
  - ELSD
  - MS
  - Alternate methods
- Getting the most from your UV or UV-Vis technique
- Choosing a Detector based on your application or compound class
- Benefits of an integrated ELSD solution
- Increasing efficiency and gaining information with Purlon MS module

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What Detection Options are Available and Useful for Purification

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Available Methods of Detection

- UV (200-400 nm) or UV-Vis (200-800 nm)
- Integrated ELSD




- Purlon Mass Spectrometer
- External Detector input (except NextGen)
  - Radiochemical detector
  - Fluorescence
  - Refractive Index (RI)

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UV and UV-Vis Detection

- Non-destructive technique
- UV (200-400 nm) or UV-Vis (200-800 nm) configuration
  - Both PDA detectors
  - Requires a chromophore for detection
  - Systems without baseline correction limits solvent choices
    - Baseline Correction available on NextGen Series
- Can choose to trigger collection or monitor up to 2 single wavelengths
- Entire UV spectrum is saved throughout the chromatogram
- All systems offer All-wavelength detection

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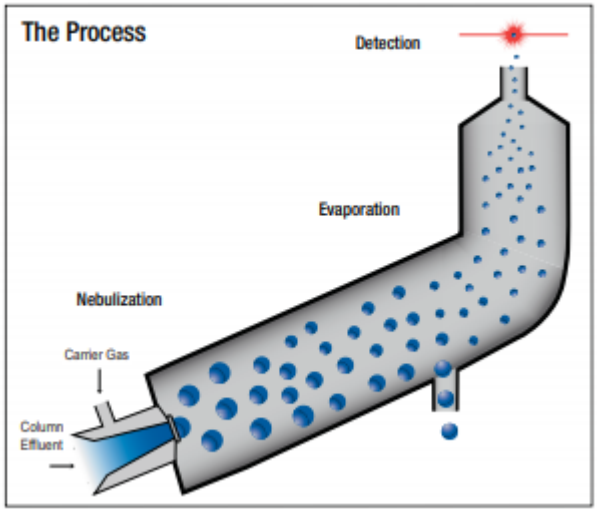
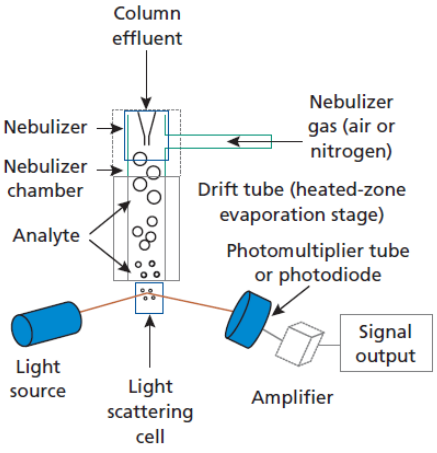
ELS Detection

- ELSD works by measuring the light scattered from solute particles remaining after nebulization of evaporation of the mobile phase.
- Nebulizer helps to spray the compound and mobile phase into the detector as droplets.
- As the droplets travels down the drift tube, the solvent evaporates, leaving semi-volatile and non-volatile particles.
- These particles are detected using a light source and sensor.





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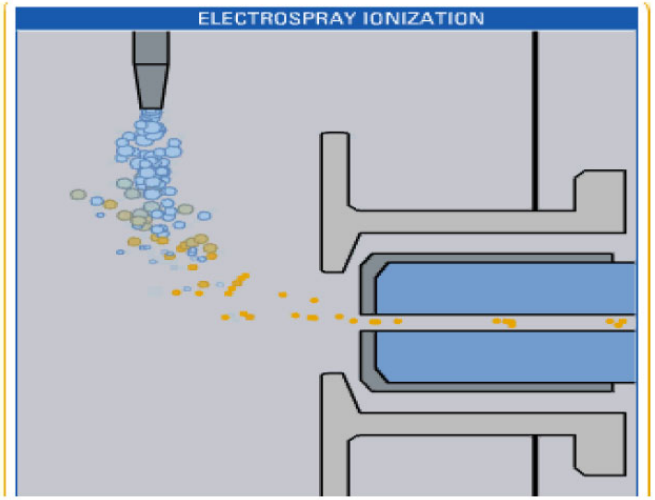
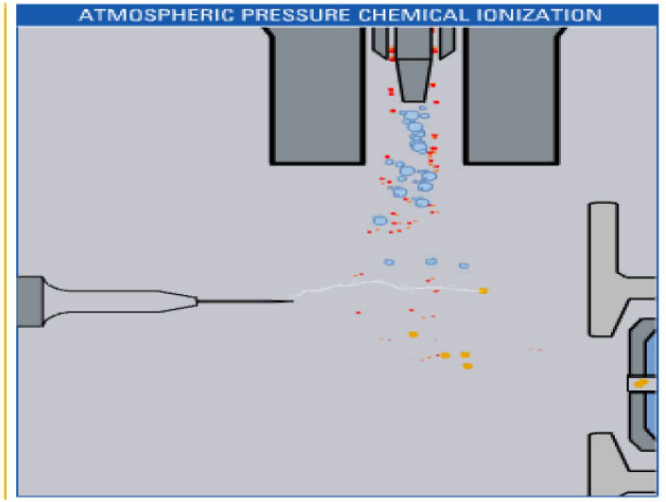
MS Detection

- ESI (Electrospray Ionization)
  - Ionization process that uses electrical fields to generate charged droplets and ions for analysis.
- APCI (Atmospheric Pressure Chemical Ionization)
  - Chemical ionization process where the solvent acts as CI reagent gas to ionize sample.
- Destructive technique
- Very sensitive (requires very little sample)
- Compounds must be ionize well to be detected





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Getting the most from your UV and UV-Vis Methods of Detection

Tips to maximize your UV and UV-Vis detection and optimize recovery

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NextGen 300/300+ Baseline Correction Feature

- Enables a short pre-run gradient to measure baseline absorbance
- Allows the system to subtract baseline from run.
- Expands detection abilities across all wavelengths, not limited by solvent UV cut-off
- Opens up other solvent alternatives not traditionally used in chromatography
  - Greener solvent alternatives
  - Solvents exhibiting different selectivity for more efficient separations

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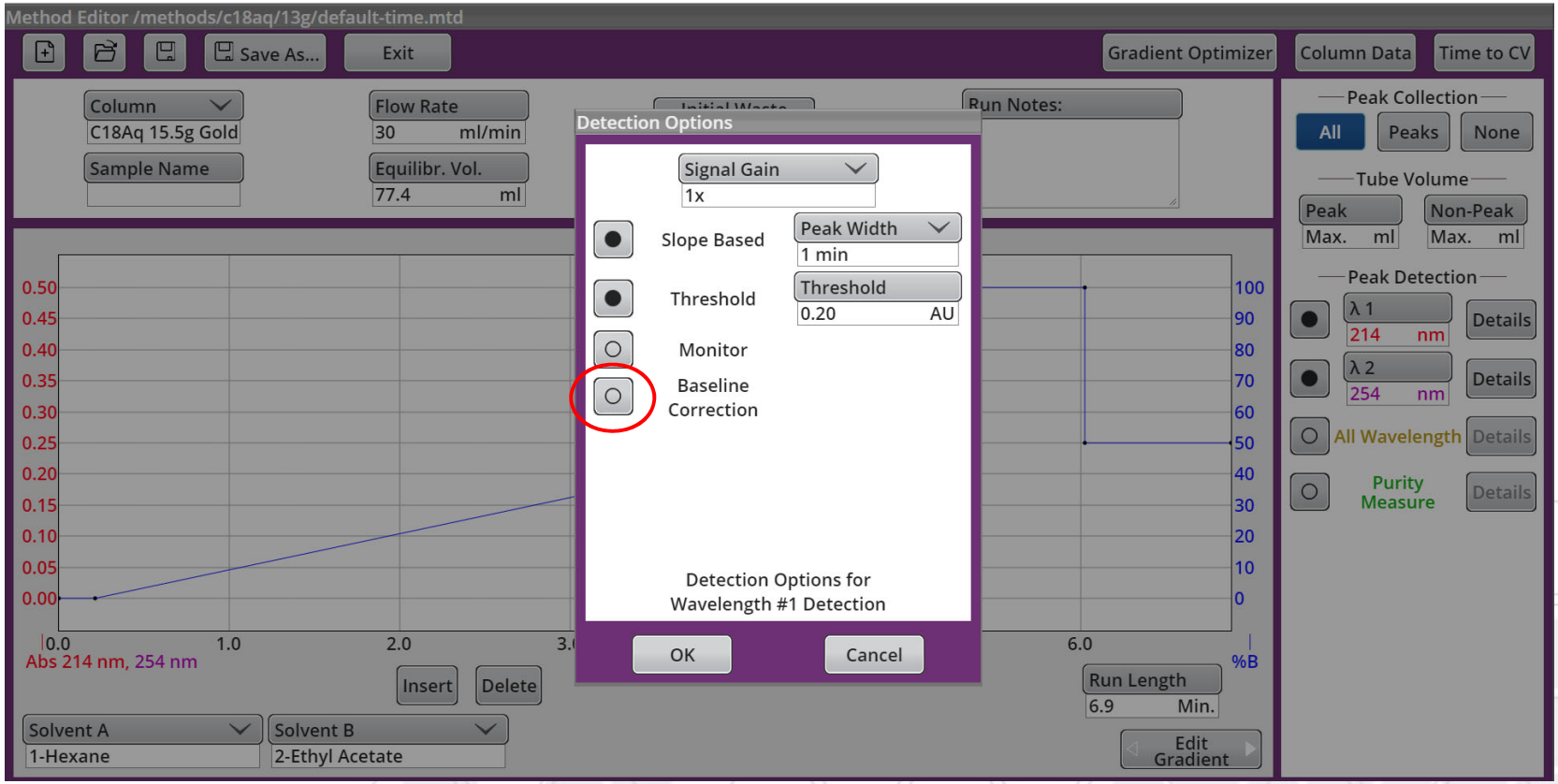
How to use Baseline Correction



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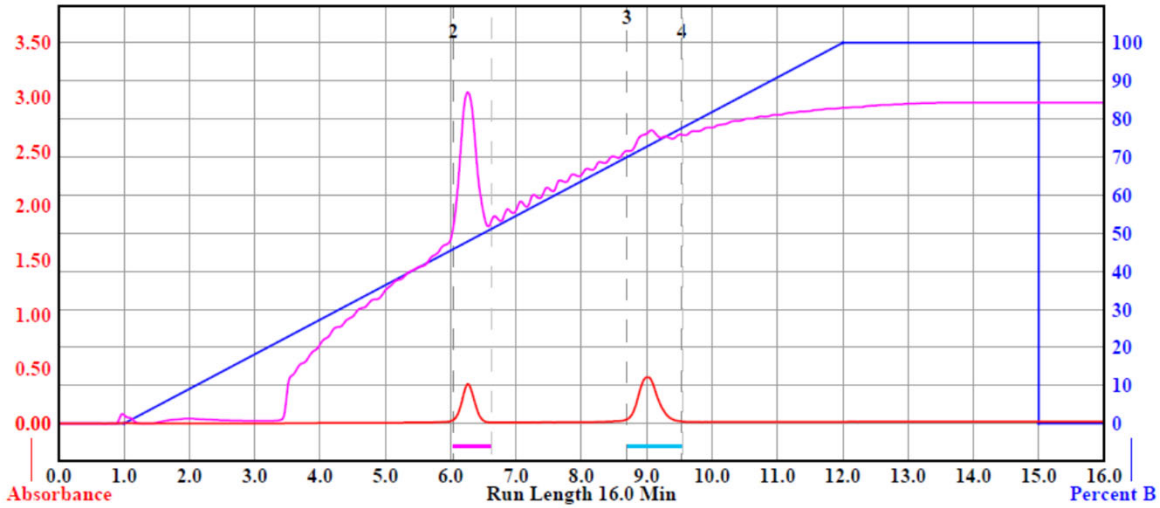
Baseline Correction Examples

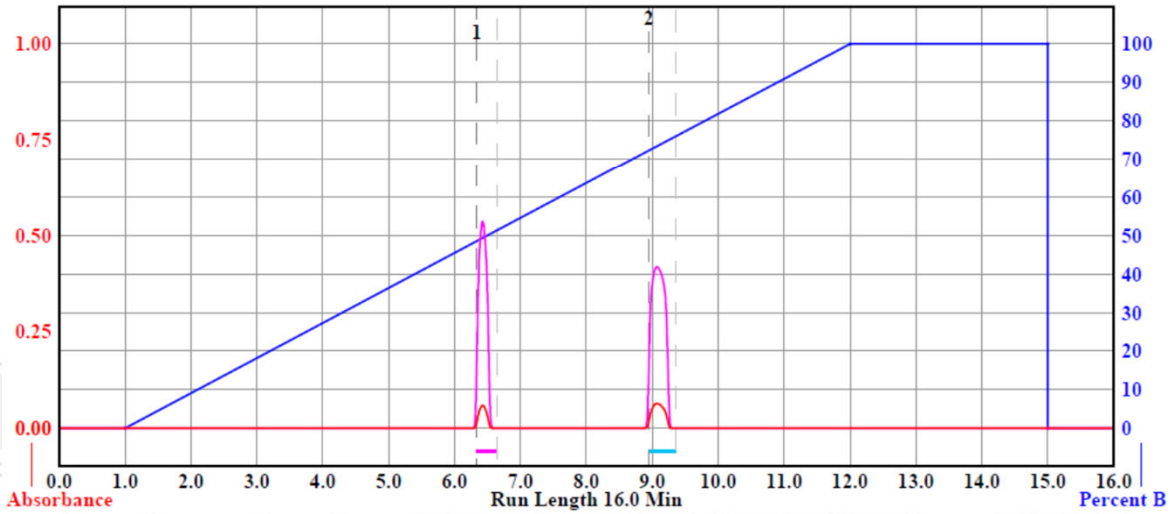


Without Baseline Correction and Ethyl Acetate as the B Solvent

@ 215 nm

With Baseline Correction and Ethyl Acetate as the B Solvent

@ 215 nm



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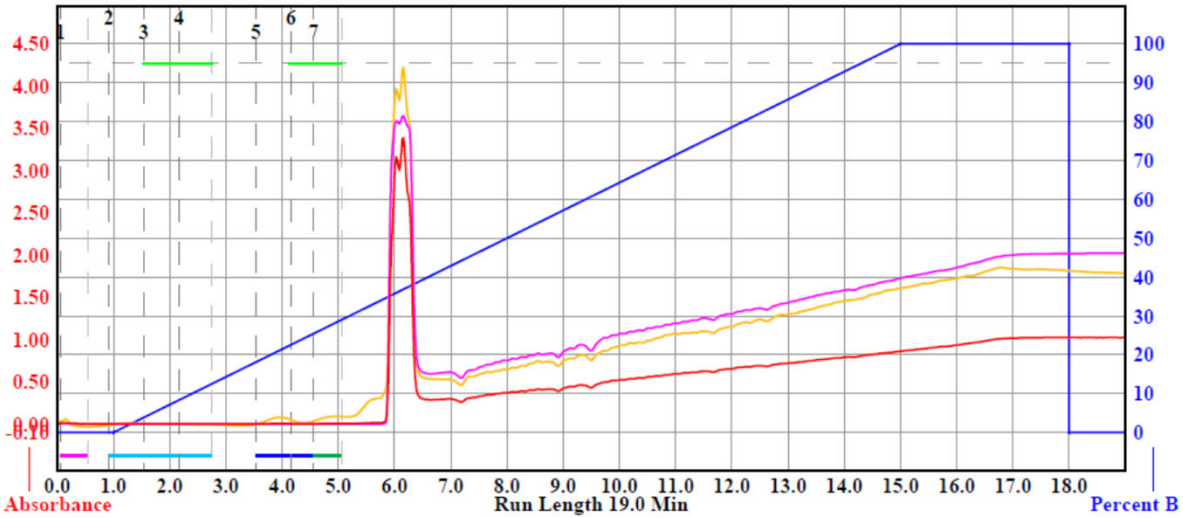
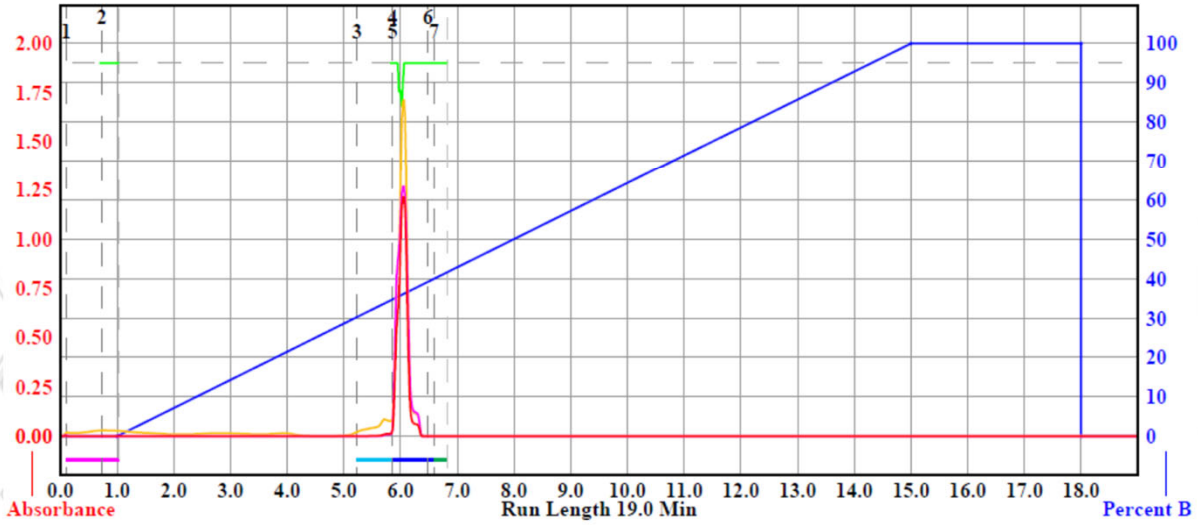
Baseline Correction Examples



Without Baseline Correction and Acetone as the B Solvent



With Baseline Correction and Acetone as the B Solvent

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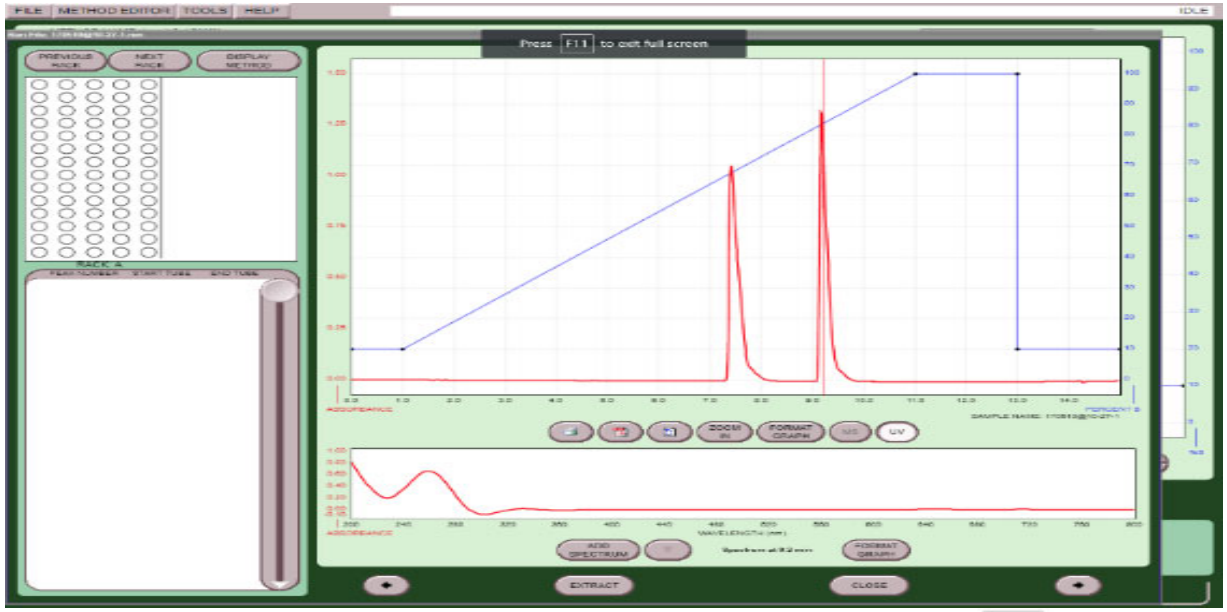
Optimizing UV or UV-Vis Settings for Maximized Recovery

- If using UV-Vis, and compound has a more strongly abosrobing wavelength outside the UV range leading to better detection sensitivity.
- Deviation from the optimal wavelength for a compound can significantly impact detection and thus fraction collection triggering and sample recovery.
- Finding the optimal wavelength for detection of your compound:
  - Use previous UV spectral data
    - Need to be aware of solvent and solvatochromatic effects due to gradient

Optimizing UV Settings for Maximized Recovery

- **<u>Or get the UV-Vis spectral data from small-scale scouting run using PeakTrak</u>**



  - PeakTrak allows you to pull up the UV or UV-Vis spectral data at certain time points. Allowing you to choose an optimal wavelength for detection for your unique compound in future runs and scale ups.
  - Minimizes solvatochromatic effects if using a gradient elution as the compound is already under similar solvent conditions as future runs.

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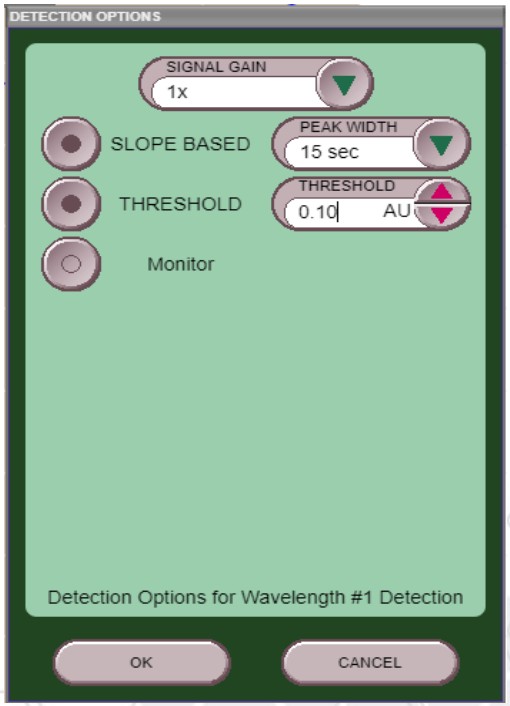
Sample Recovery Optimization via Detection Options

- Method Editor
  - Can adjust the threshold or slope-based fraction collection trigger settings.
  - Can monitor multiple single wavelengths



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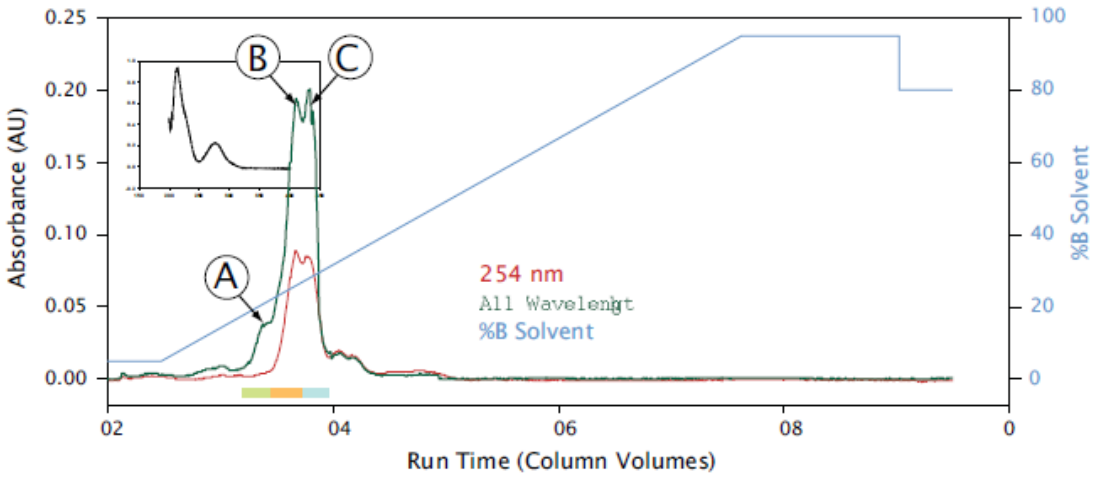
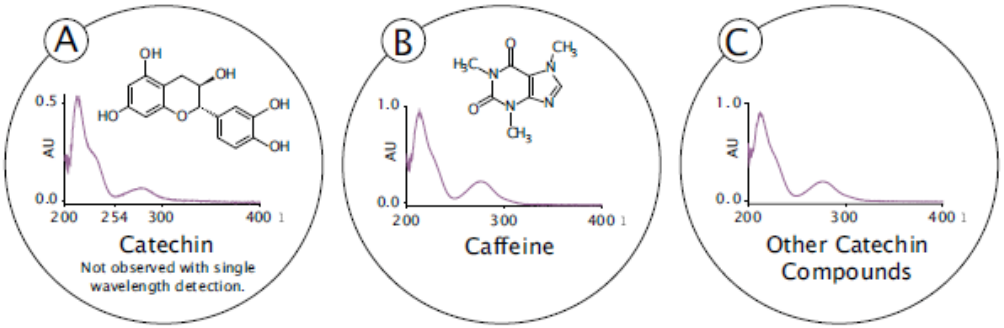
Benefits of All-Wavelength Detection

- All-Wavelength detection
  - Found in Method Editor
  - Select a λ range to monitor



- Differentiate between compounds that have overlapping single wavelengths



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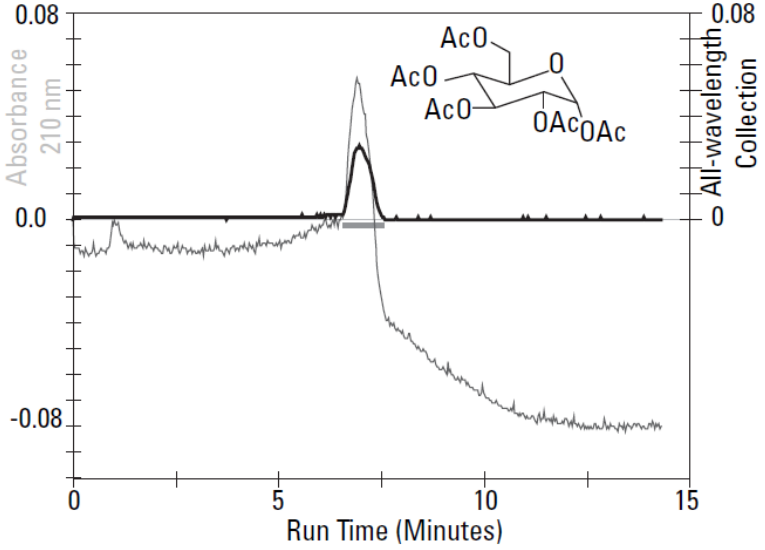
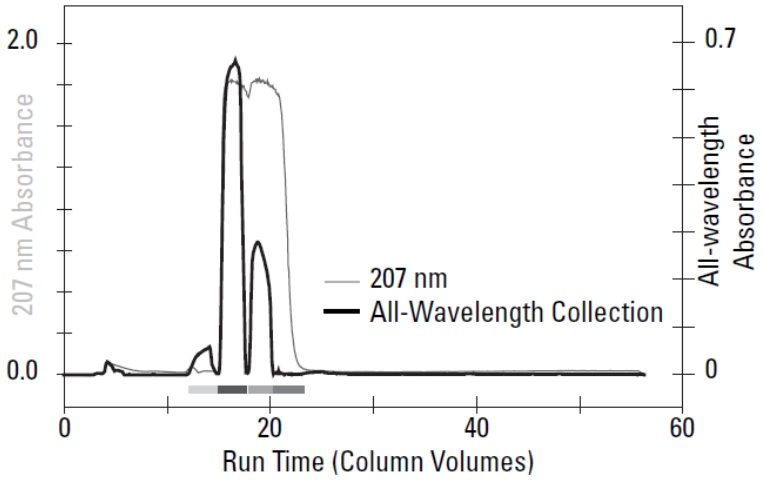
Benefits of All-Wavelength Detection

- Compensate for drifting baseline from solvent absorbance



- Separate peaks of closely eluting compounds that saturate a UV detector at a given wavelength.



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ELSD as a Method of Detection

Application and Compound Classes utilizing ELSD

Benefits of Integrated ELSD

Optimizing ELSD settings

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Compound Classes and Applications where ELSD Thrives

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Why Choose ELSD as a Detection Option

- Considered a universal detector, as it can detect compounds without chromophores, as long as they are semi- or non-volatile.
- Offers more uniform sensitivity of detection.
- Compounds that are non- or weakly-absorbing would stand greater ability of detection using ELSD vs. UV, increasing fraction collection accuracy and recovery.
- Limitations:
  - Destructive detection technique
  - Need to avoid non volatile mobile phase and/or additives
    - Avoid mineral acids and bases
    - Phosphate buffers
    - Can use TFA, ammonium formate, ammonium acetate, acetic acid, ammonium carbonate or ammonium hydroxide
  - Not able to detect volatile compounds

Survey of Applications of ELSD and analytical HPLC

- Natural Products
  - Unknown properties of compounds
- Small molecules without chromophores
- Polymers
- Lipids and fatty acids
- Carbohydrates
  - Invisible via UV or UV-Vis unless a chromophore is attached

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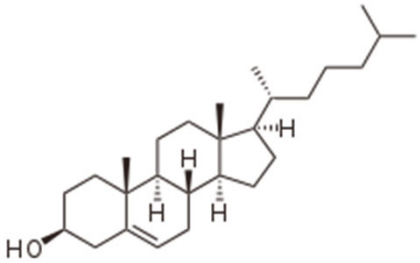
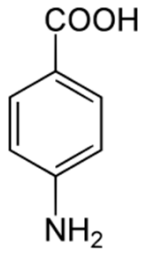
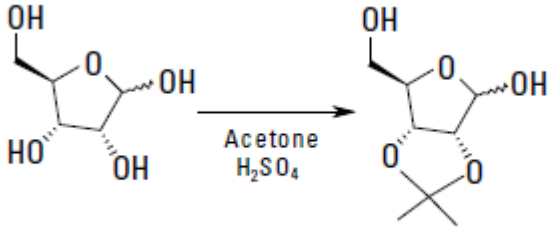
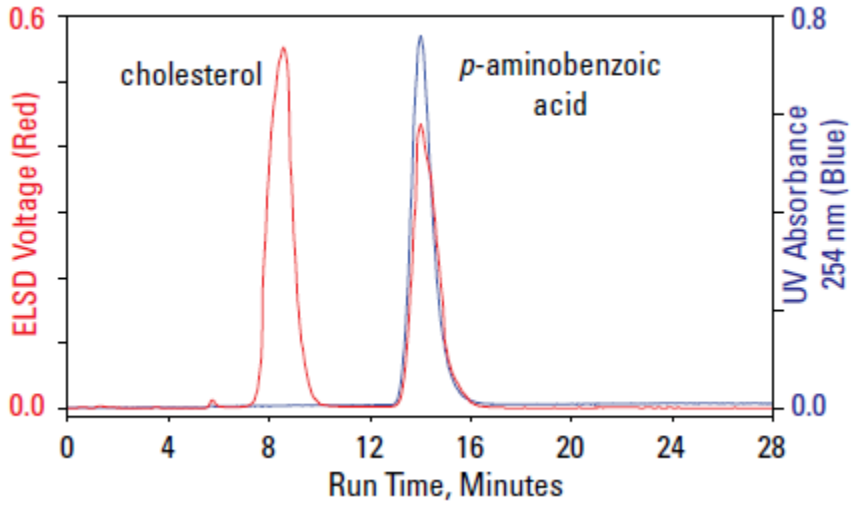
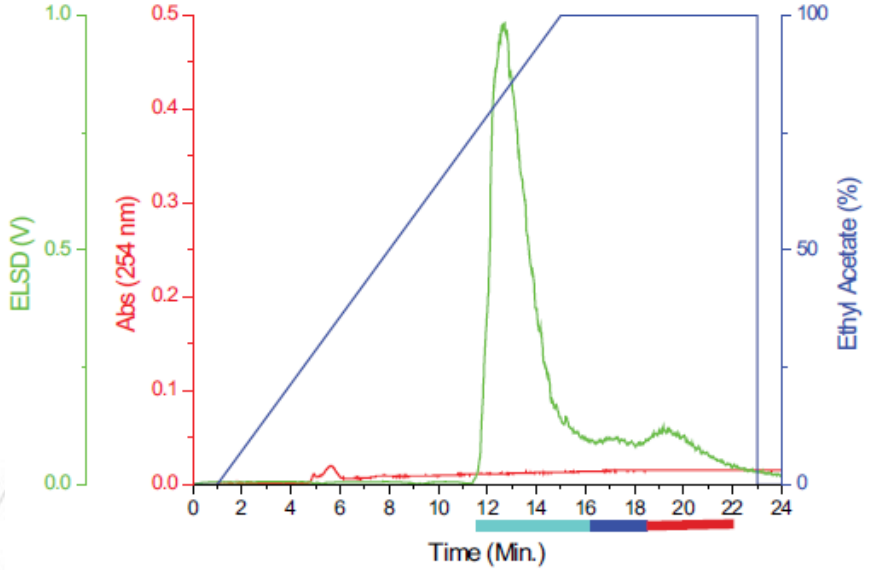
ELSD when Compounds are Undetectable using UV











Fraction 1: 692.1 mg

Fraction 2: 49.8 mg

Fraction 3: 37.6 mg

Total recovery: 821.6mg

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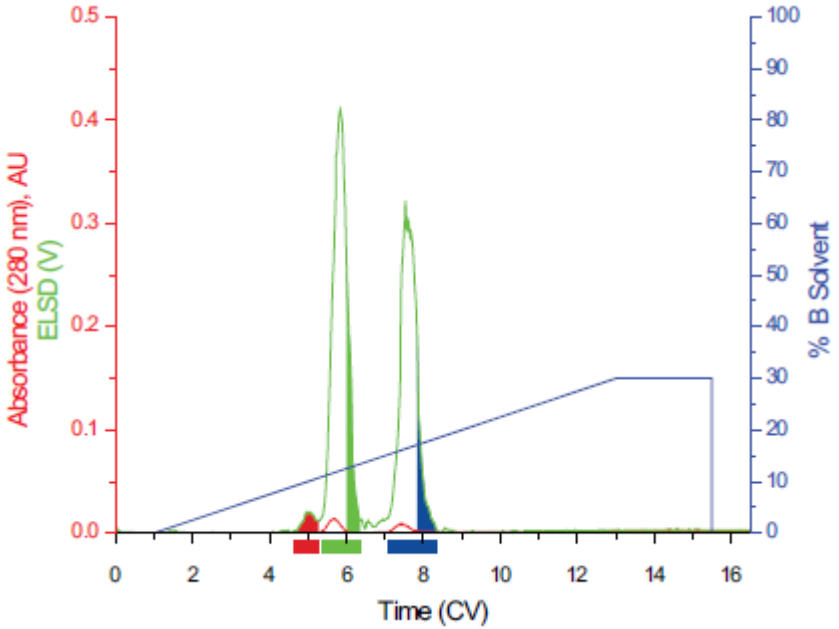
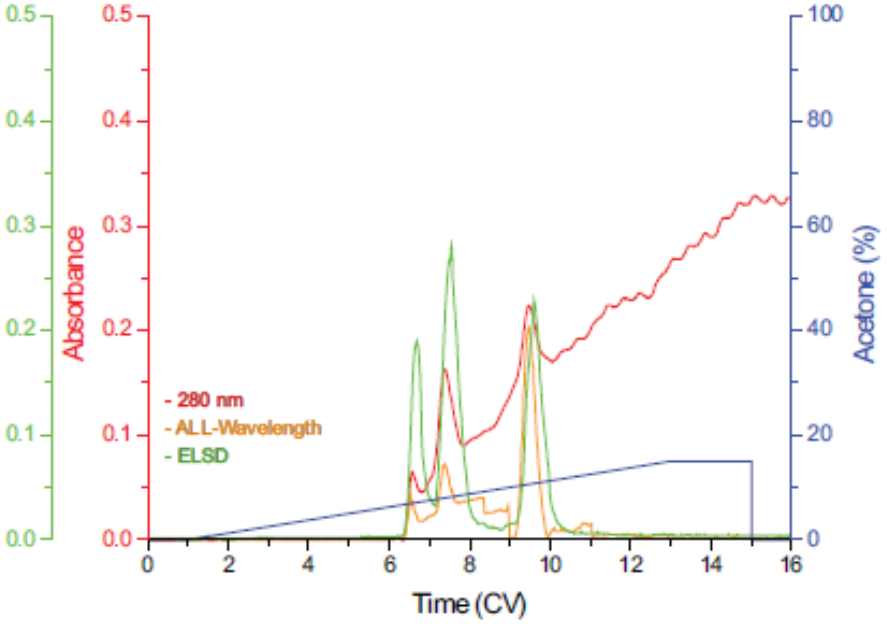
Using ELSD even when compound is UV detected





- Purification of Tocopherols
- Weakly UV absorbing
- Improved sample recovery due to increased sensitivity of ELSD over UV
- Able to use alternative solvent that interferes with UV signal

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Benefits of an Integrated ELSD Solution

Flash Solutions

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Benefits of an Integrated ELSD Flash System

- Active splitter pump to adjust to different flow rates.
- Seamless interaction between detector and software.
  - Ability to adjust ELSD settings during the run.
    - Dynamic Gain Adjustment in PeakTrak Method Editor for difficult to detect compounds.
  - Software monitors for any issues with the ELSD.
  - PeakTrak aligns ELSD and UV peaks to maximize sample recovery and accurate fraction collection.
- Smaller instrument footprint compared to external ELSD options.

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Benefits of an Integrated ELSD Solution

Prep HPLC Solutions

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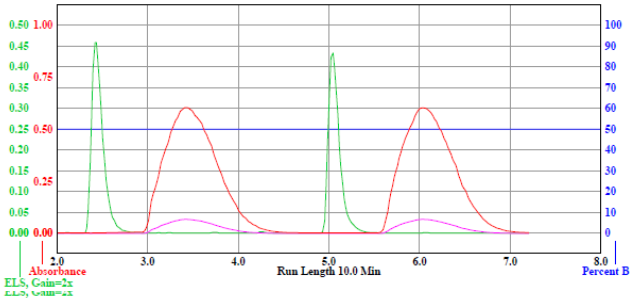
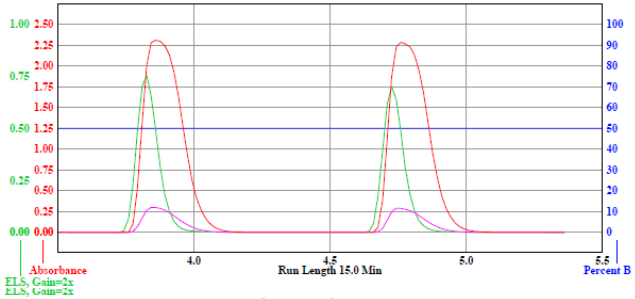
The Flow Rate Range Problem: Balancing Peak Overlap with Band Broadening

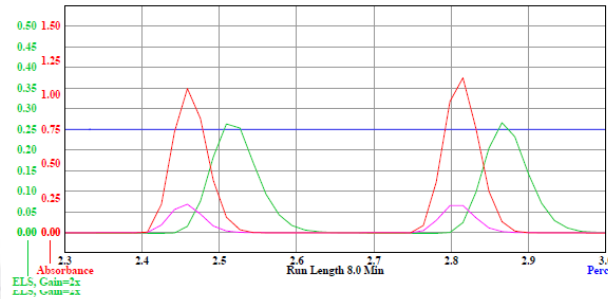
- Need more delay volume at higher flow rates to align UV and ELSD peaks (key for accurate peak cutting).
- Unnecessary delay volume results in peak broadening at lower flow rates
- If ELSD Peak is before the UV peak, then we can correct via software delay and still collect desired fraction.



5 mL/min



30 mL/min



125 mL/min

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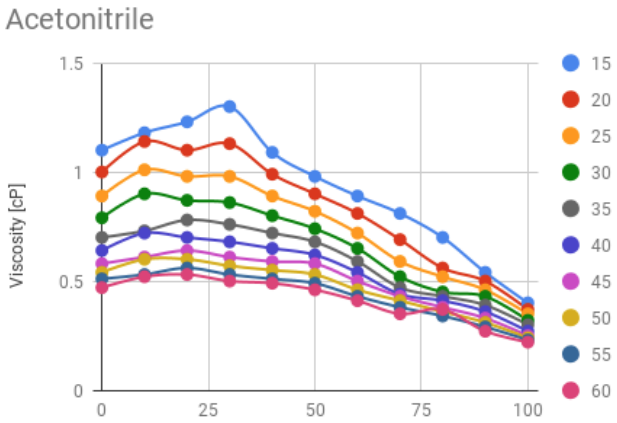
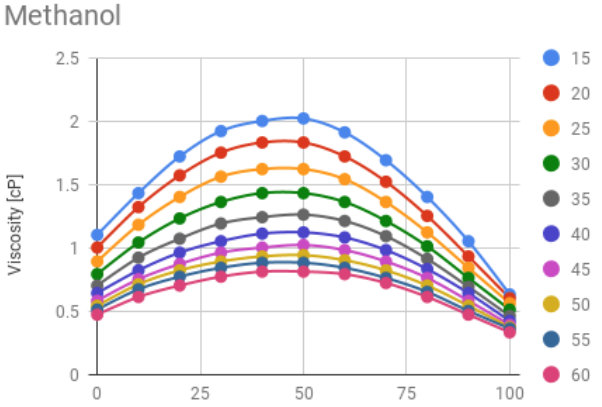
The Problem of Changing Viscosity with Gradient Profiles

Acetonitrile-water mixture



Methanol-water mixture



- Gradient composition results in differing viscosity across gradient range.
- Different viscosities for different solvent compositions (Acetonitrile and Methanol have very different profiles with water).
- With a passive splitter, this results in different delay times between ELSD and UV because the split ratio (dependent on pressure) varies with viscosity (which affects back pressure).

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http://www.chromatographer.com/mobile-phase-viscosity/

Other Issues with Current Non-Integrated Prep HPLC ELSD Solutions

- Preparative HPLC systems offer a very wide range of flow rates 1 mL/min to over 100 mL/min.
- For Prep HPLC, use of active splitter pump is prohibitive because of increased pressure requirements.
- System usually set up for a small flow rate range, and if changing column sizes, need to manually change splitter and delay tubing loop.
- Software correction is limited to known delay time or volume.
- No known solutions for changing viscosity resulting in UV and ELSD misalignment.

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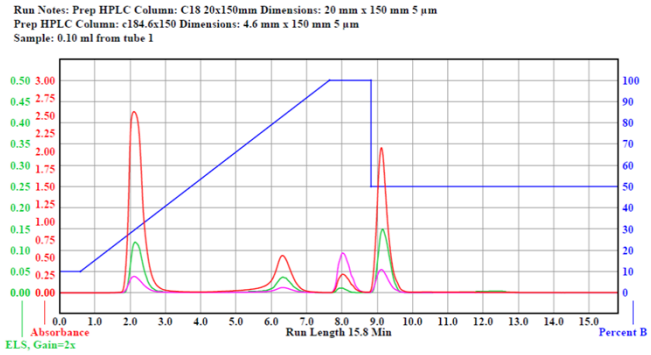
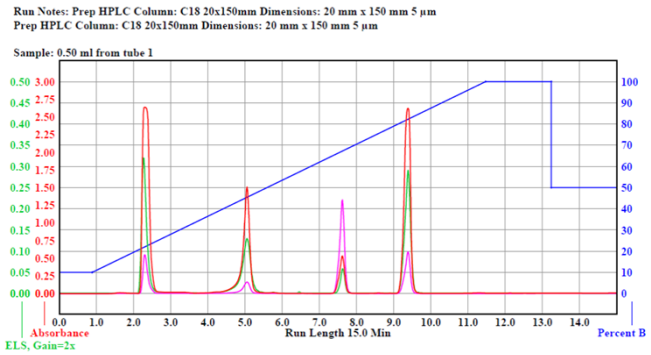
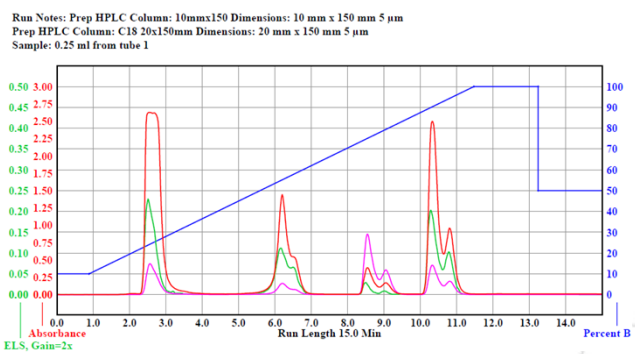
The Range of Automated Peak Overlap with ACCQPrep ELSD



4.6 mm x 150 mm @ 2 mL/min

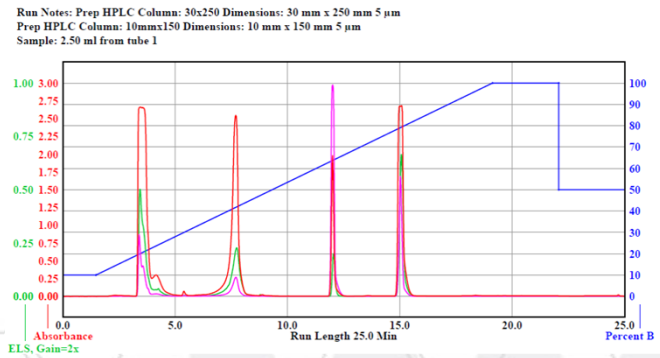


20 mm x 150 mm @ 20 mL/min



10 mm x 150 mm @ 5 mL/min

Peak overlap between ELSD and UV at flow rates from 2 to 125 mL/min



30 mm x 150 mm @ 42.5 mL/min

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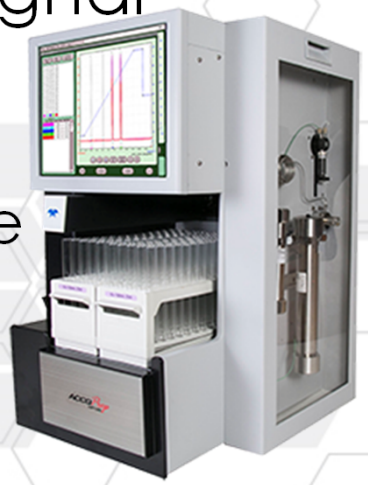
4-component test mix to confirm accurate alignment of ELSD and UV signals throughout the gradient

Solving these problems with ACCQPrep ELSD and PeakTrak

- PeakTrak recognizes flow rate for run and selects the appropriate passive splitter path.
  - Minimizes unnecessary band broadening.
  - Maximizes UV and ELSD signal overlap with optimal delay loop for flow rate.
  - Optimized split ratio to ELSD for improved sensitivity at different flow rates while minimizing unnecessary sample loss via ELSD.
- PeakTrak further improves the UV/ELSD signal overlap with changing gradient solvent composition.
  - Maximizes peak overlap which leads to more accurate fraction cutting and collection.



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Optimizing ELSD Settings

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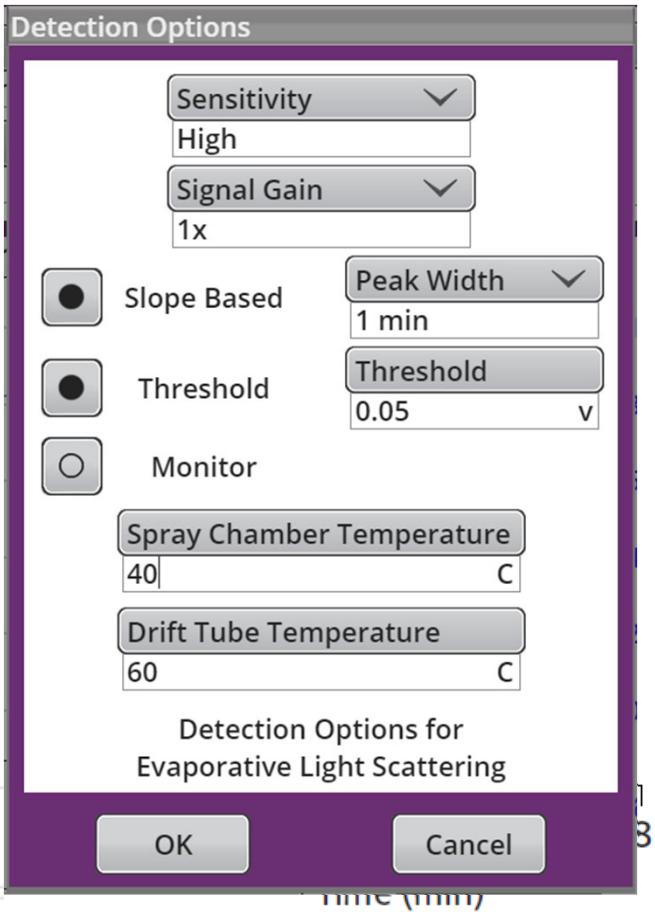
Effect of Solvent Choice and Modifier on ELSD Signal to Noise

- Noise increases with less volatile solvents and modifiers added.
- Normal phase or reverse phase applications
- Can adjust sensitivity from Normal to High
  - Prep HPLC usually keep on high when using reverse phase
- Adjust spray chamber or drift tube temperature
  - May improve ability to see semi-volatile components by lowering temperatures
  - This could also allow more solvent particles to travel to detector rather than evaporating out. (RP especially)



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MS as a Method of Detection

Application and Compound Classes utilizing MS

PeakTrak MS Integration Features

Examples of Mass Directed Purification

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Compound Classes and Applications using MS

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Choices for MS Method Development

- Ionization Method
  - APCI—Atmospheric Pressure Chemical Ionization
  - ESI—Electrospray Ionization
- Carrier solvent
  - Dilutes sample
  - Necessary to help ionize compounds for MS detection
  - Requires solubility with your mobile phase system
    - MeOH or Acetonitrile with RP
    - DCM a good choice for NP

Compound classes and MS Method Development

- ESI
  - Reverse phase
  - Compounds easily charged in solution
    - Proteins
    - Peptides
    - Oligonucleotides
    - Heteroatom containing compounds
- APCI
  - RP or Normal
  - Small
  - Polar to Nonpolar compounds
    - PAH (Polycyclic Aromatic Hydrocarbons)
    - Fatty Acids
    - Steroids

Information Rich Purification

Examples of Optimization of ESI Mass-Directed Fractionation

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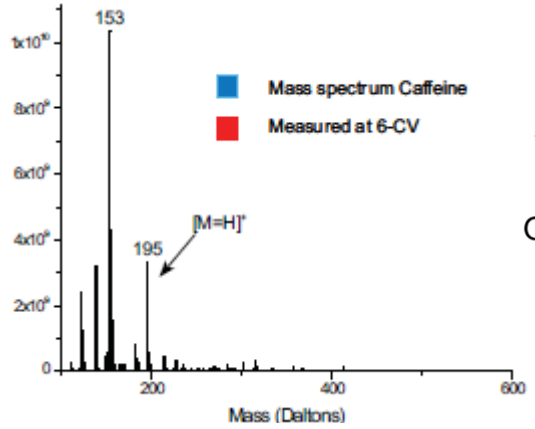
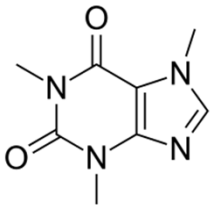
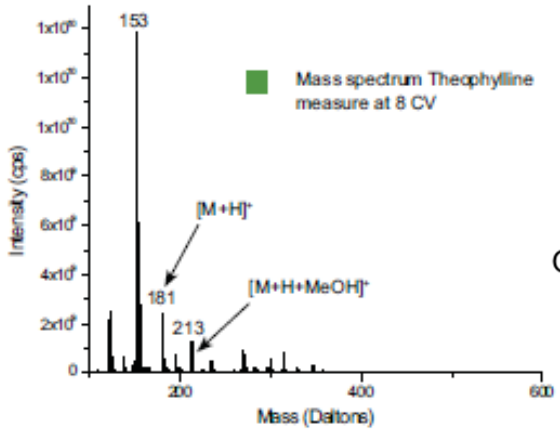
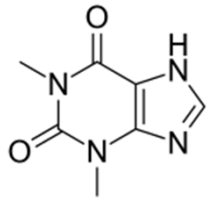
MS Directed Fractionation using Extracted-Ion Current (XIC)

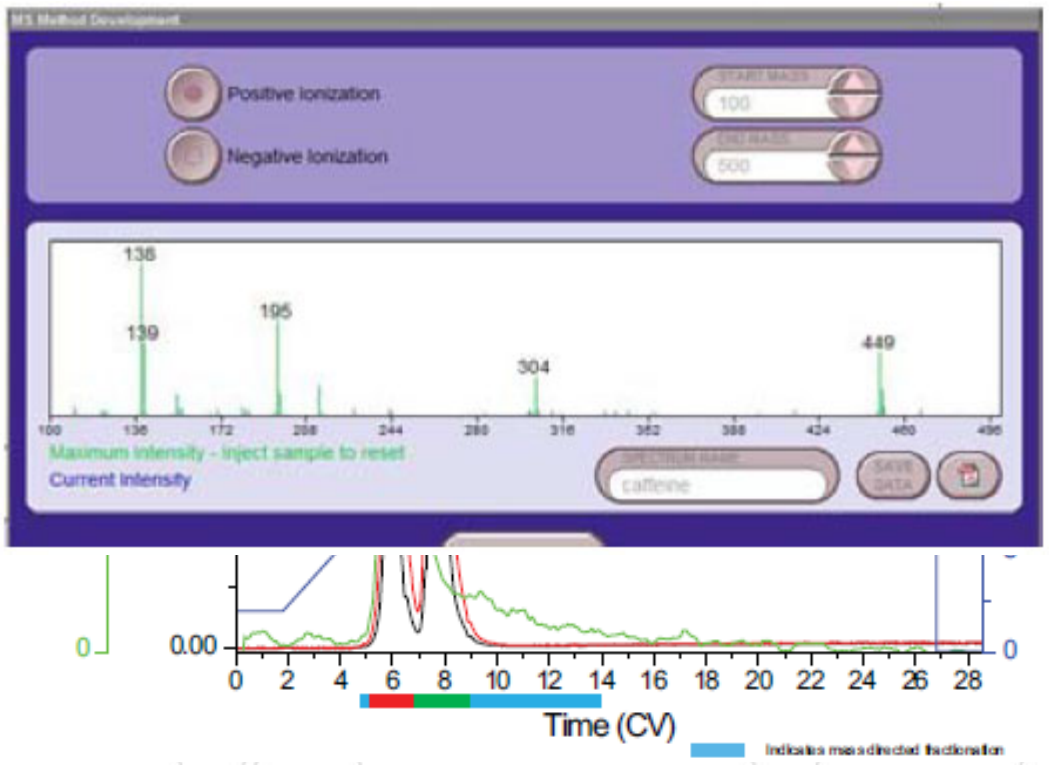








Separation of caffeine and theophylline using single ion current (SIC)
ESI Probe in Positive Mode
Carrier solvent 0.1% Formic acid in MeOH
m/z range trigger set to 195



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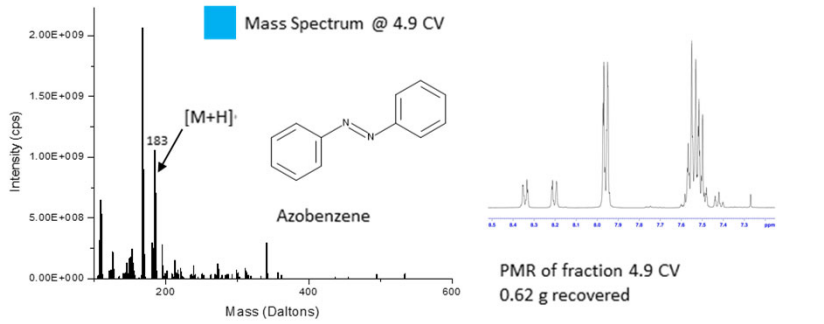
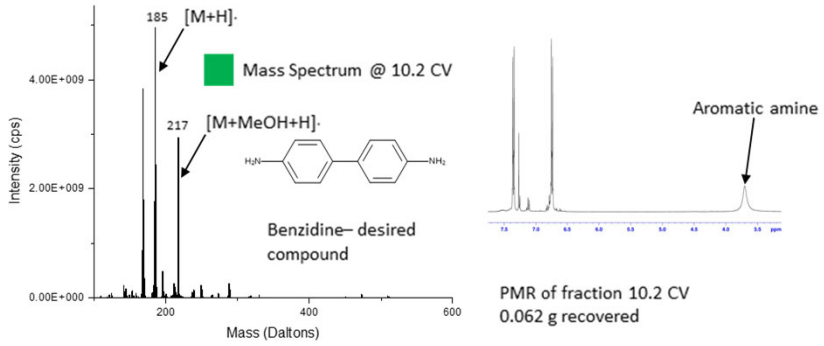
MS Directed Fractionation using Extracted-Ion Current (XIC)





0.0821 g collected @ 4.2 CV

0.0621 g- manually moved fraction collector based on MS trace collected @6.5 CV

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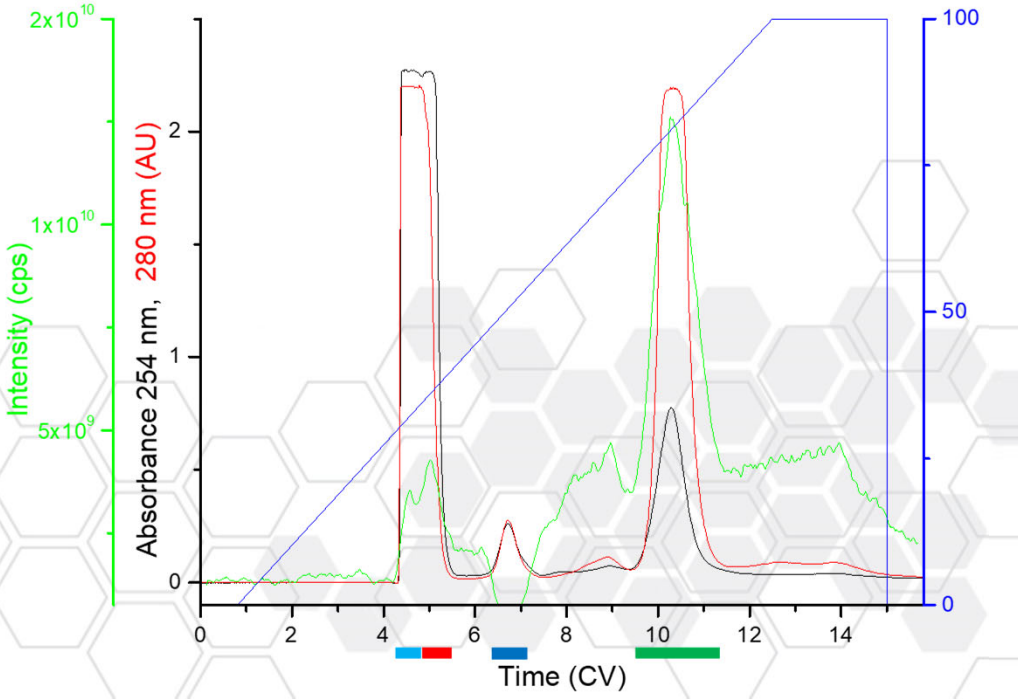
Separation of reaction mixture of Synthesis of Benzidine using Extracted-ion current (XIC)
ESI Probe in Positive Mode
Carrier solvent 0.1% Formic acid in MeOH
m/z trigger set to 175-300 Da
Major peak is actually an oxidation side-product



Information Rich Purification

Examples for optimization of APCI Mass-directed Fractionation

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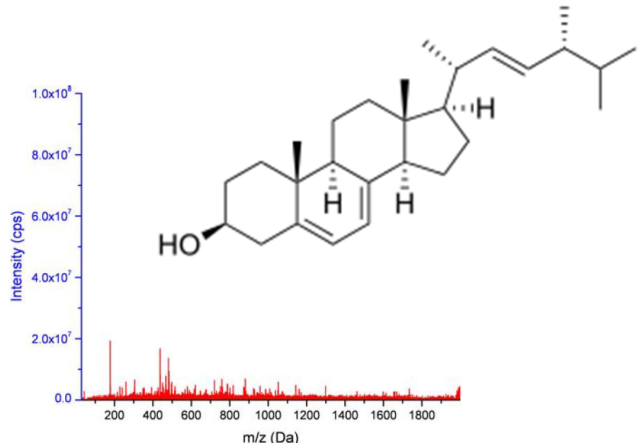
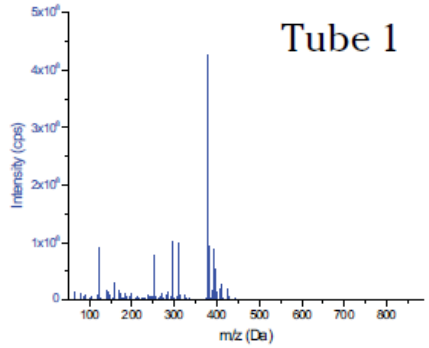
MS Method Development
Choosing APCI over ESI

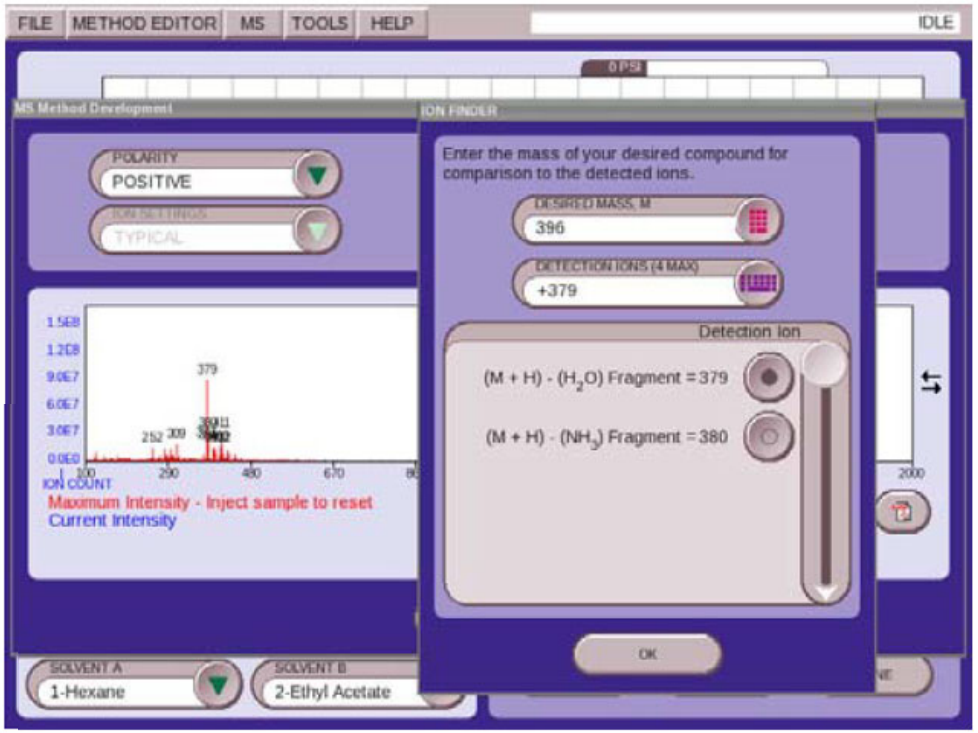


ESI



APCI

Purification of Ergosterol using single ion current (SIC)
APCI
Carrier solvent 0.1% Formic acid in MeOH
Ergosterol mass of 396.3 Da
Ion Finder suggested 379 Da due to loss of H_2O from $[M+H]^+$



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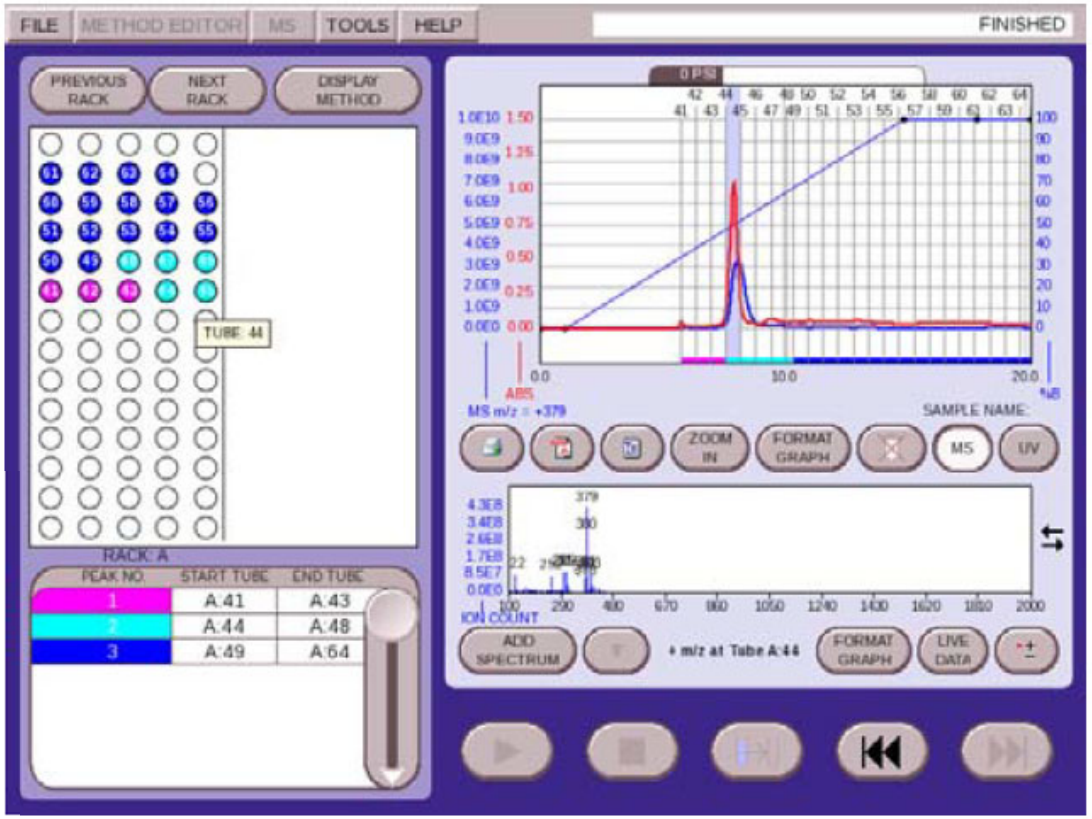
Information Rich Purification: Comparing Collected Fractions

- Collected fractions after purification of ergosterol
- PeakTrak allows you to compare MS of each tube in order to confirm whether to combine of not

Just select the tube in the rack map to show the MS for that fraction



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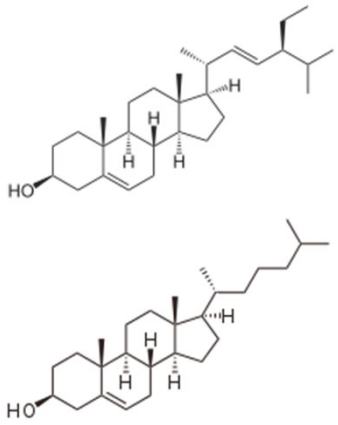
Information Rich Purification: Multiple MS Traces



Purification of a mixture of stigmasterol and cholesterol
APCI
Carrier solvent: DCM

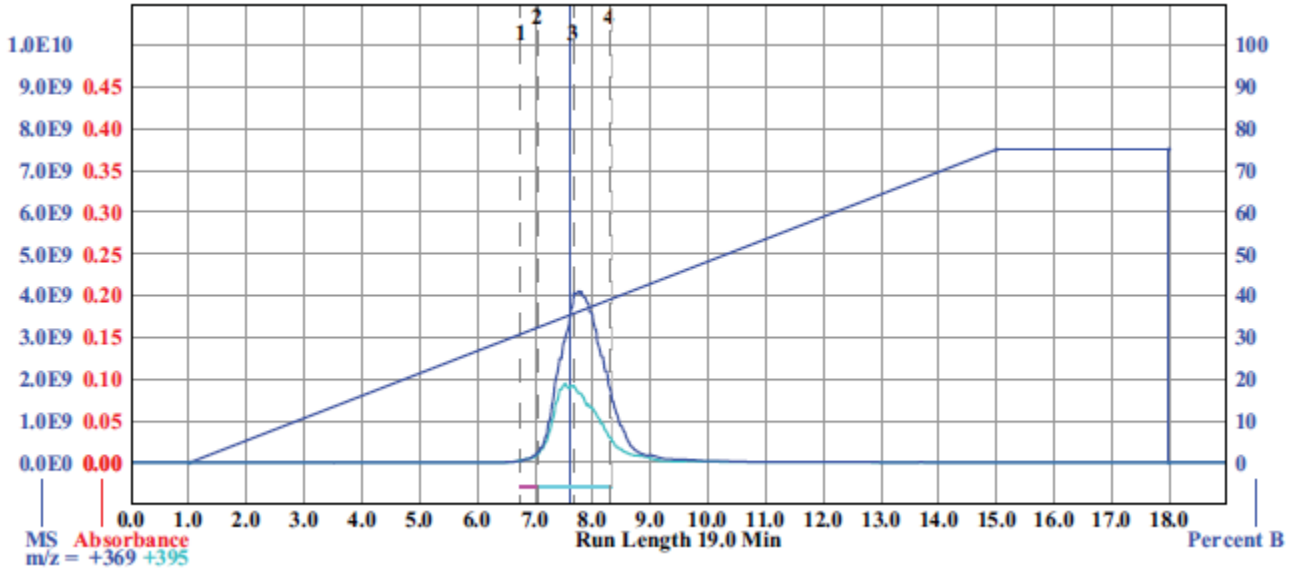
- Stigmasterol mass of 412.4 Da

Ion Finder suggested 379 Da due to loss of H_2O from $[M+H]^+$

- Cholesterol mass of 386.4 Da

Ion Finder suggested 369 Da due to loss of H_2O from $[M+H]^+$

Overlap of both MS shows the compound co-eluted



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Information Rich Purification

Purifying Peptides using Prep HPLC

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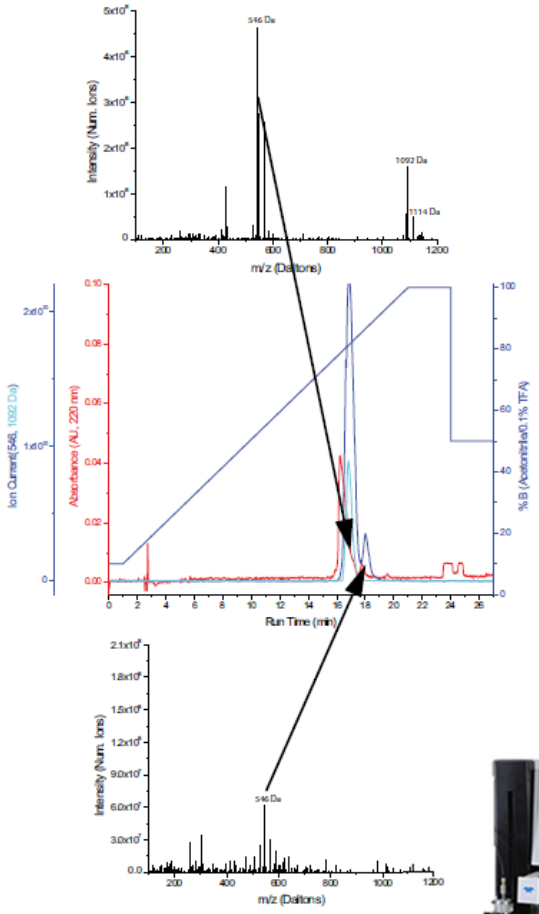
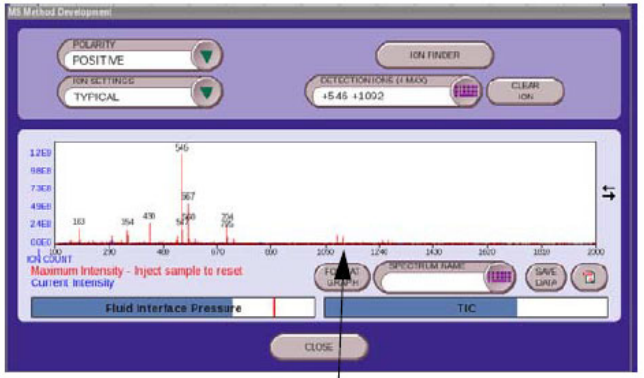
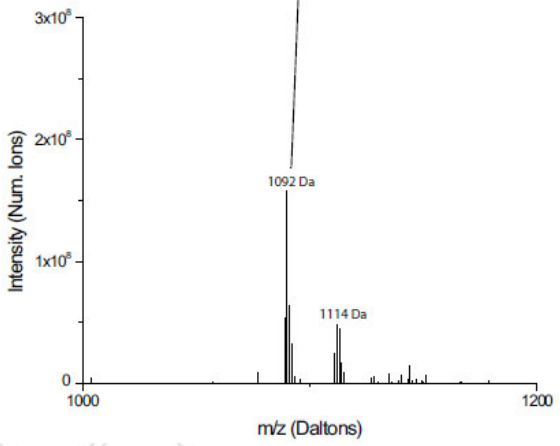
Purifying Peptides using Prep HPLC

Purification of Crude Peptide using single ion current (SIC)
ESI
Carrier solvent 0.1% Formic acid in MeOH
Peptide HNWYPAAPH mass of 1091.5 Da
Visible ions of $[M+H]^+$ of 1092 Da; $[M+Na]^+$ of 1114 Da; and doubly-charged $[M+2H]2^+$ of 546 Da







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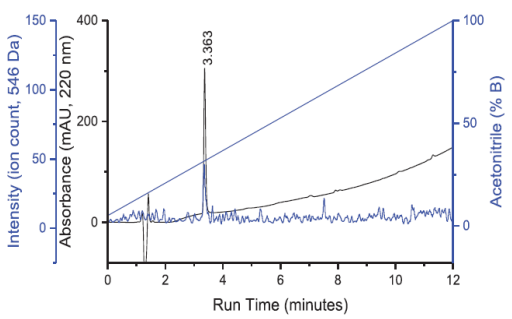
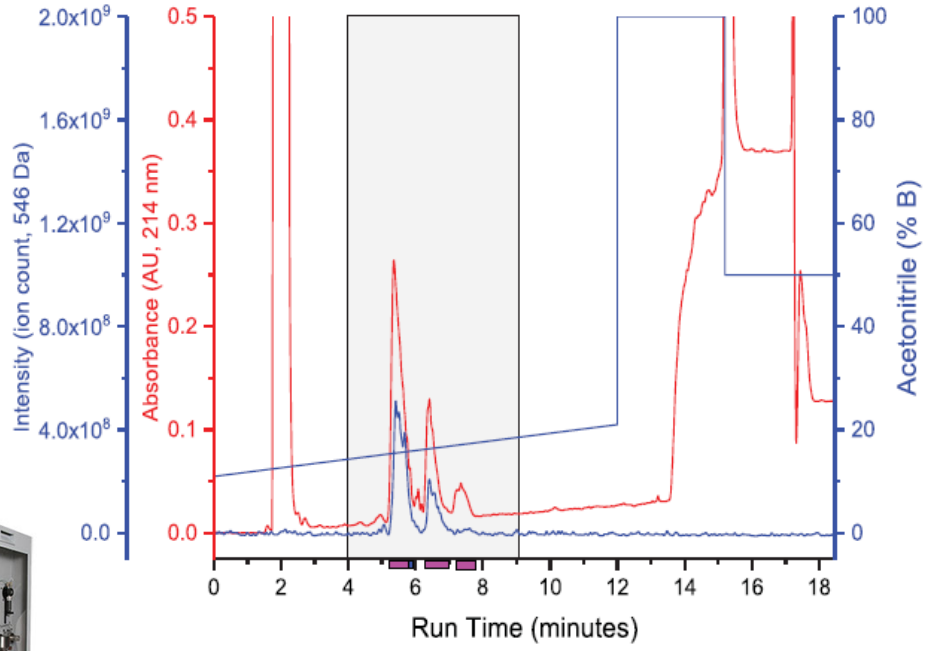
Purifying Peptides using Prep HPLC

Purification of Crude Peptide using single ion current (SIC)
ESI
Carrier solvent 0.1% Formic acid in MeOH
Peptide HNWYPAAPH mass of 1091.5 Da
Visible ions of $[M+H]^+$ of 1092 Da; $[M+Na]^+$ of 1114 Da; and doubly-charged $[M+2H]2^+$ of 546 Da





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Terminate on Target

Saving Solvent and Time

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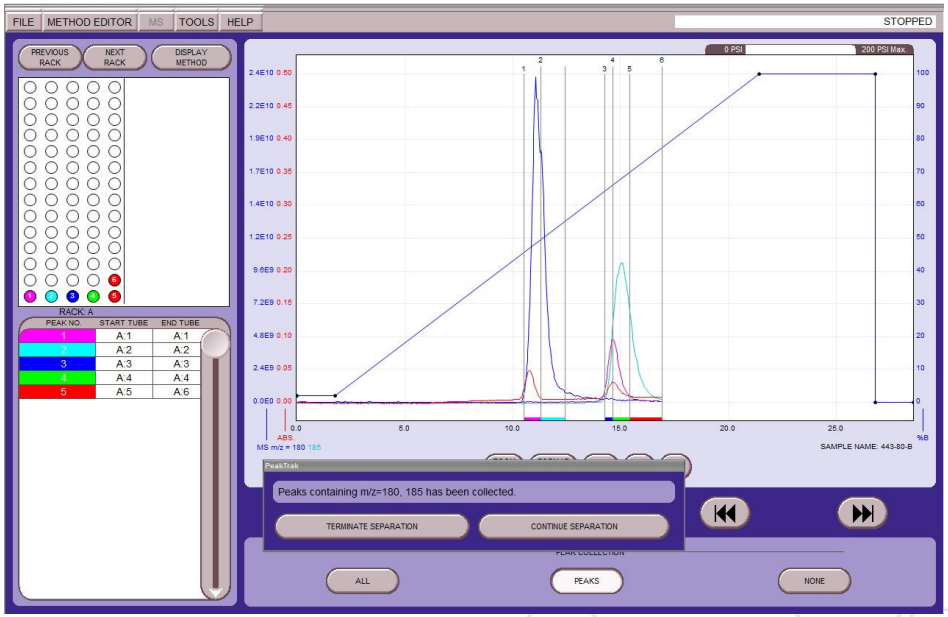
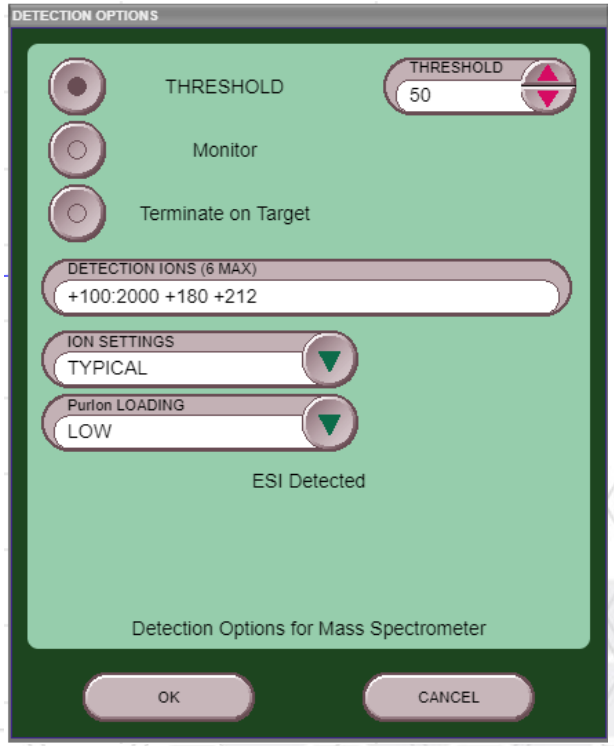
Smart MS Fraction Collection: Terminate on Target

Can select multiple masses to monitor or collect on (up to 4)
Can Terminate on Target for some or all masses
Won't stop run until all terminate on Target Masses are selected





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Summary

- Described the available methods of detection including UV, UV-Vis, ELSD, and MS.
- Discussed tips on how to get the most from your UV or UV-Vis detector.
- Revealed the benefits and convenience that an integrated ELSD detector offers.
- Showed examples of compounds and applications where ELSD or MS offered a more suitable detection technique.
- Examined different features and settings available on PeakTrak to improve purification with different detector options.

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Teledyne Isco Chromatography Systems

NextGen 300+

NextGen 300

NextGen

EZ Prep

Purlon

ACCQPrep

Prep HPLC

Torrent

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Guidelines & Tactics for Flash Chromatography

For your free copy, visit:

www.teledyneisco.com/en-us/chromatography

And then click on “Flash Guide”

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Upcoming Webinars

- "What to Do When Things Go Wrong in LC"
  - March 19 by Jack Silver

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Questions?

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Introduction to Flash Chromatography

Josh Lovell – Application Chemist
Teledyne ISCO
Direct: (402) 465-2018
Joshua.Lovell@teledyne.com

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Thank You

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