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NextGen update

Advanced CombiFlash Techniques

NextGen New Technologies

Agenda

- *Sample Introduction*
- *Detector Options*
- *PeakTrak Basics*
- *RFID Advantages*
- *Chromatography Options*

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CombiFlash NextGen Family

	NextGen	NextGen300+	NextGen300
Flow Rate	1-100 ml/min	1-300 ml/min	1-300 ml/min
Pressure psi (bar)	150 (10.3)	300 (20)	150 (10.3) 300 (20) optional
Pumping	Single HPLC	Dual syringe	Dual syringe
Detection	200-400 nm 200-800 nm ELSD MS	200-400 nm 200-800 nm ELSD MS	200-400 nm 200-800 nm ELSD MS
Injection Valve	No	Yes	Optional
Gradients	2 solvent binary	4 solvent binary with 3rd solvent modifier	4 solvent binary with 3rd solvent modifier
Level Sensing	No	Yes	Yes
Air Purge	No	Yes	Yes
RFID/Level Sensing	No	Yes	Yes
User Interface	12”	12” or 15”	12”

Specs cont.

	NextGen	NextGen300+	NextGen300
Sample Injection	Solid or liquid manual injection	Automated, self cleaning valve with injection through cartridge, syringe or directly on column	Solid or liquid manual injection. Optional automated valve
Solvent management	None	Active level sensing on inlet and waste	Active level sensing on inlet and waste
Dimensions (WxDxH)	14.1x17x26 in (36x43x66 cm)		
Weight	61 lb (27.7 kg) 74 lb (33.6 kg) w/ ELSD		
RFID	None	Column and racks	Optional column and racks
Certification	CE, NRTL	CE, NRTL	CE, NRTL

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Sample Introduction

Good Chromatography Starts at the Beginning

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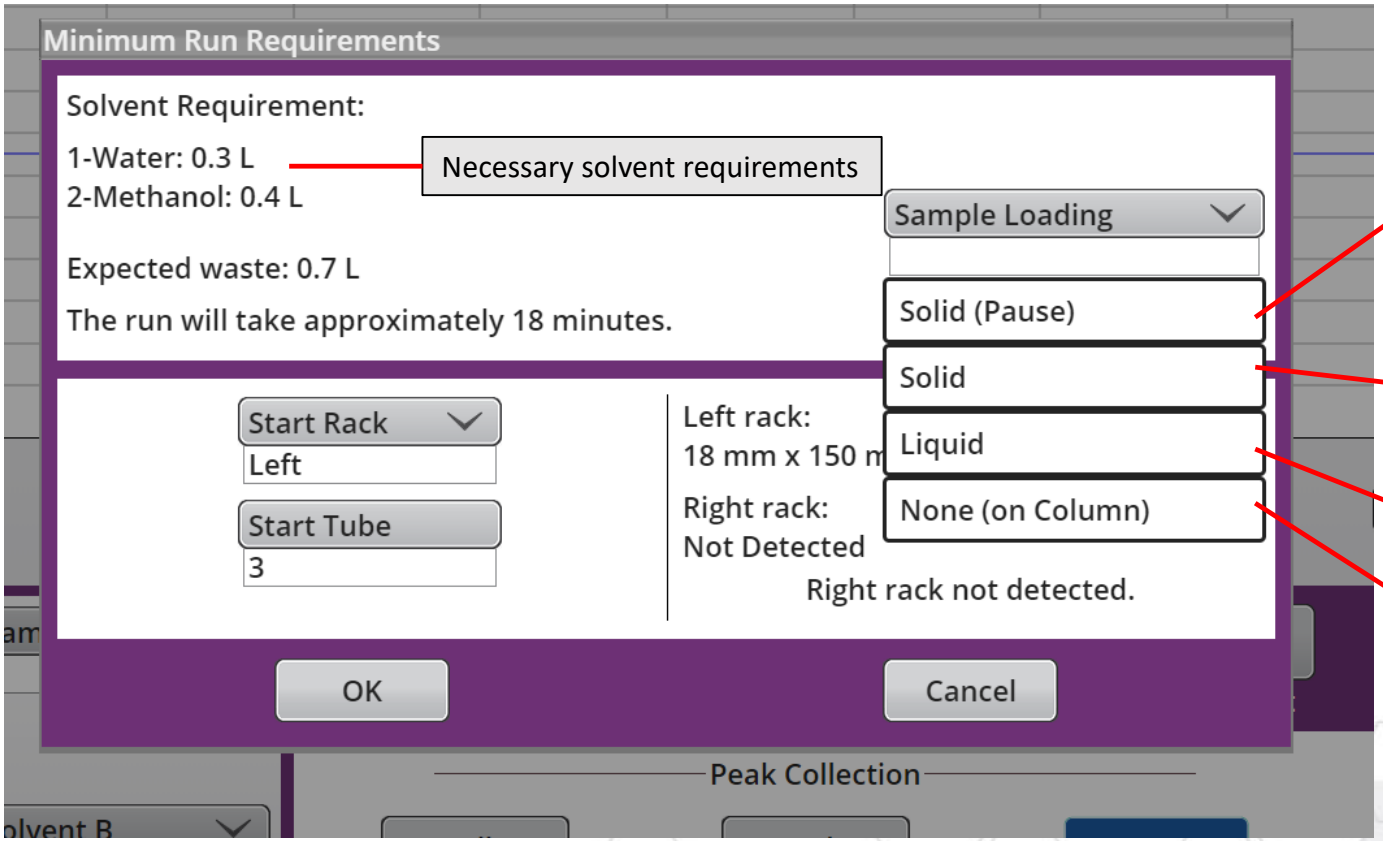
Injection Parameters



- Solid (Pause) goes through solid load cartridge but waits – this saves time for sample prep
- Solid goes through solid load cartridge and assumes sample is ready
- Liquid injection through top of injection valve
- Sample is on the column– may be a previous run or manually loaded

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Solid Loading

- User packed solid load cartridges
- Pre-packed solid load cartridges

- AN29 –
  - Loading Techniques
- AN88 –
  - C18 Loading Techniques
  - Celite packed cartridges
- AN82 –
  - Solid Load vs. Reservoirs

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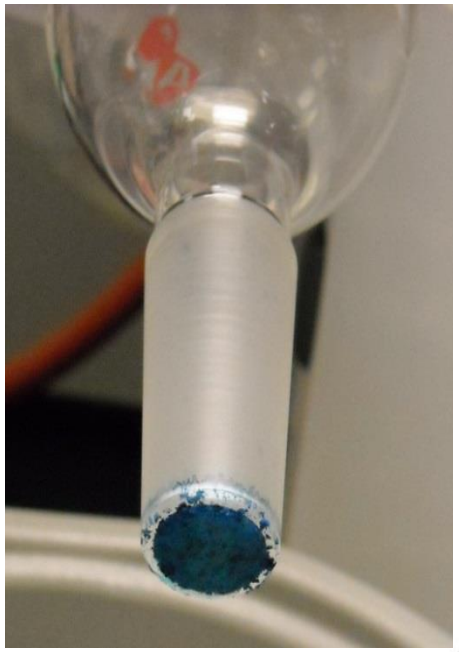
User Packed Solid Load Cartridges

- Weigh sample
- Determine amount of sorbent
  - 20% load for silica
  - 10% for Celite
- Dissolve sample completely
- Mix with silica
- Dry on rotary evaporator
- Place in cartridge
- Add top frit
- Insert solid load cartridge cap



Frit for 5 g solid load cartridge fits many 24/40 adapters commonly found in the United States and keeps silica and sample in the evaporation flask

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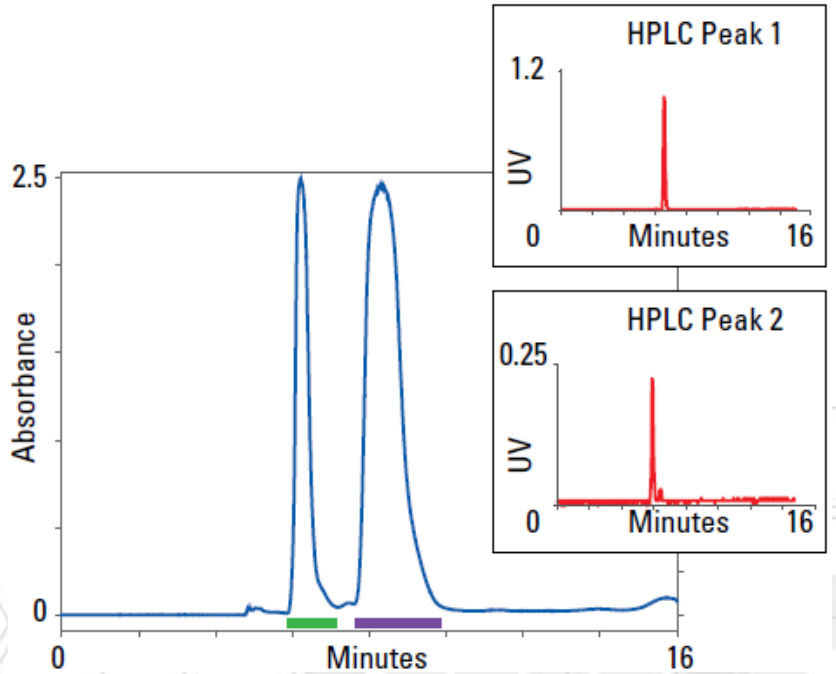
User Packed Solid Load Cartridges

- Best resolution
- Match sample size to silica for loading
- Allows use of strong solvents
- Act as pre-column
- Prevents clogs from precipitated sample
- Also called “empty” solid load cartridges



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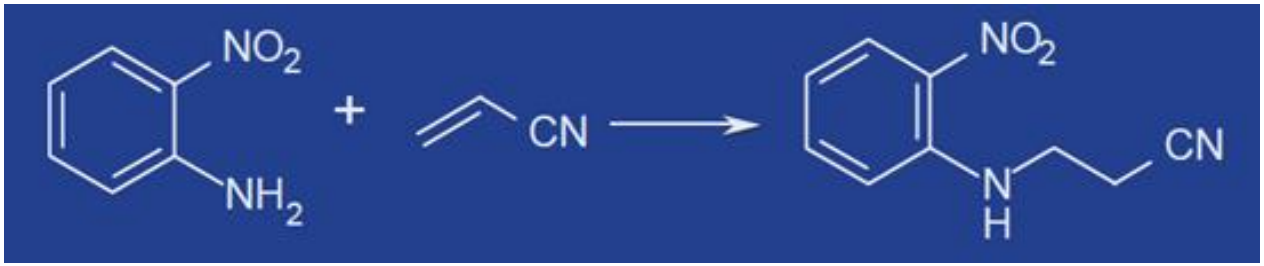
Comparison of Sample Loading Techniques for Silica Columns



- All runs used standard solvent method on a CombiFlash
- All runs used 2% sample load
- Solvents were hexane/ethyl acetate

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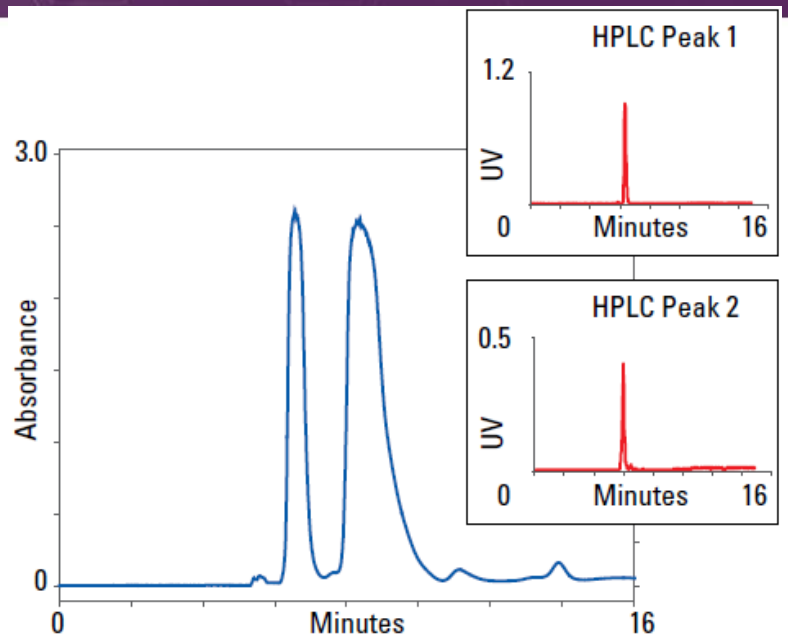
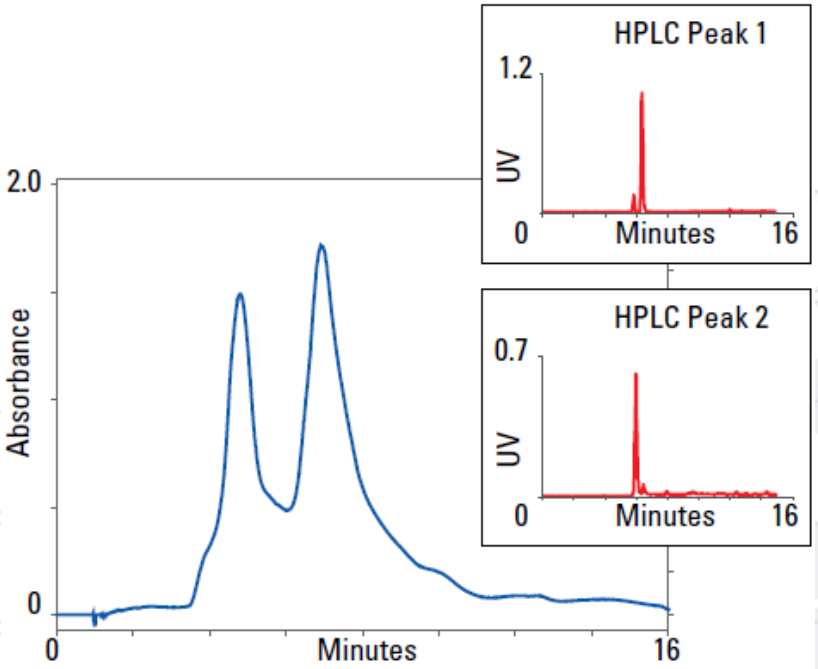
Pre-packed Solid Load Cartridge



Sample dried on cartridge



Sample not dried on cartridge

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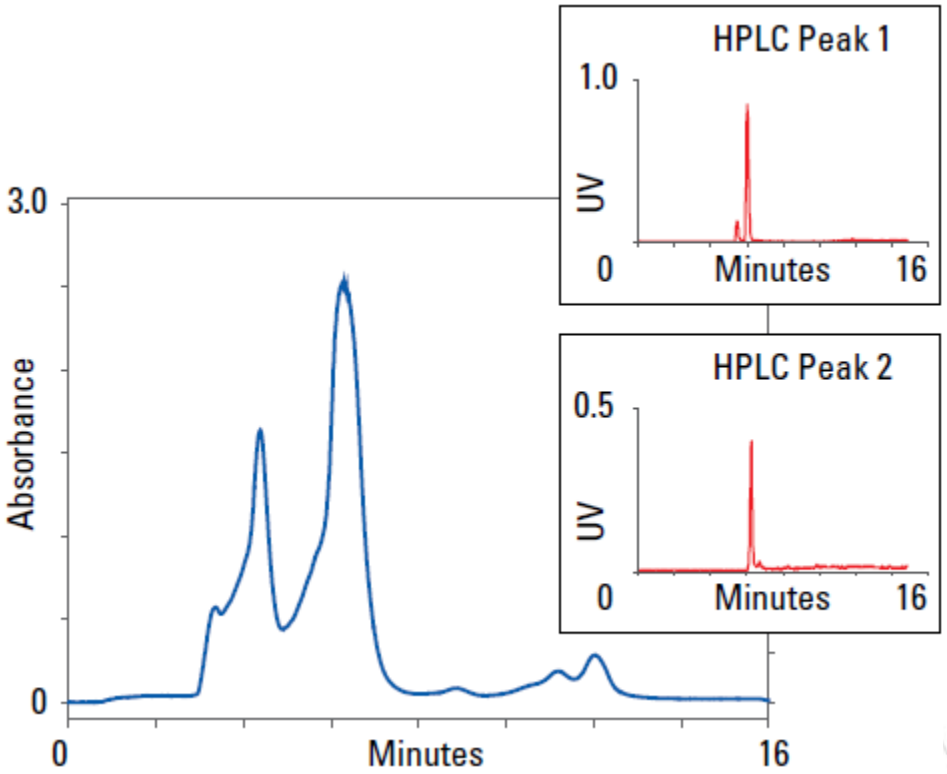
Liquid Loading



Liquid loading shows reduced resolution when sample is dissolved in a strong solvent

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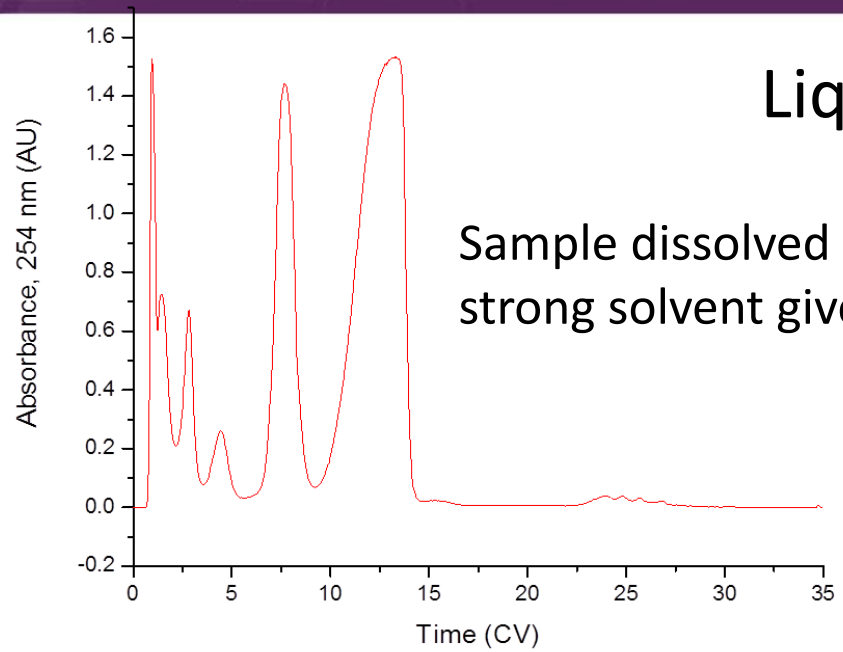
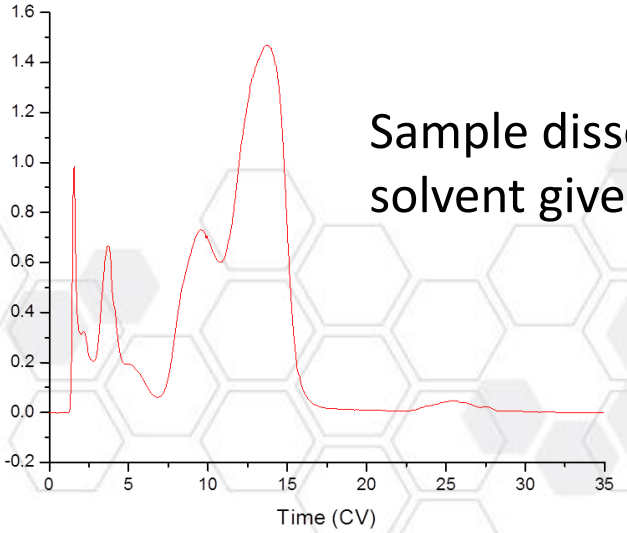
Liquid Injection Onto Column



Liquid injection is fast and easy

Sample dissolved in minimum strong solvent gives good resolution



Sample dissolved in strong solvent gives reduced resolution

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Sample Introduction Techniques

	Solid (Pause)	Solid	Liquid	None (Direct to Column)
Automated		✓		
Low Solubility	✓	✓		
Repeatable	✓	✓		
No carry over between runs	✓	✓		✓
100% sample transfer/ high recovery	✓	✓		
Minimal sample prep			✓	✓
Tight loading band *technique dependent	* ✓	* ✓	* ✓	* ✓
Filters and removes baseline material	✓	✓		
Improved separation w longer bed	✓	✓		
Increases life of reusable columns (C18)	✓	✓		

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Flash Column Load

Column Size	Easy separation Gold RediSep (ΔCV=6) : 20% loading	Easy separation (ΔCV=6) : 10% loading	Difficult separation (ΔCV=1): 1% loading
4 g	800 mg	400 mg	40 mg
12 g	2.4 g	1.2 g	120 mg
24g	4.8 g	2.4 g	240 mg
40 g	8.0 g	4.0 g	400 mg
80 g	16.0 g	8.0 g	800 mg
120 g	24 g	12 g	1.2 g
220g	44 g	22 g	2.2 g
330 g	66 g	33 g	3.3 g

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Detector Options

What you see is what you get

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Photo Diode Array Detector

- Variable Wavelength 200-400 nm
- Change Wavelength during the run
  - 2 wave length triggered collection
  - All wavelength triggered collection
- Purity Indicators
  - Dual Wavelength Monitoring
  - Purity Ratio

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Greater Dynamic Range

- NextGen has improved UV and UV-Vis detectors
  - Up to 4 AU dynamic range
- 0.1mm fixed path length flow cell
  - Fixed creates reliable and reproducible results
  - 0.1 mm keeps compounds on scale
  - 0.3, 0.5, 1 mm optional for increased sensitivity

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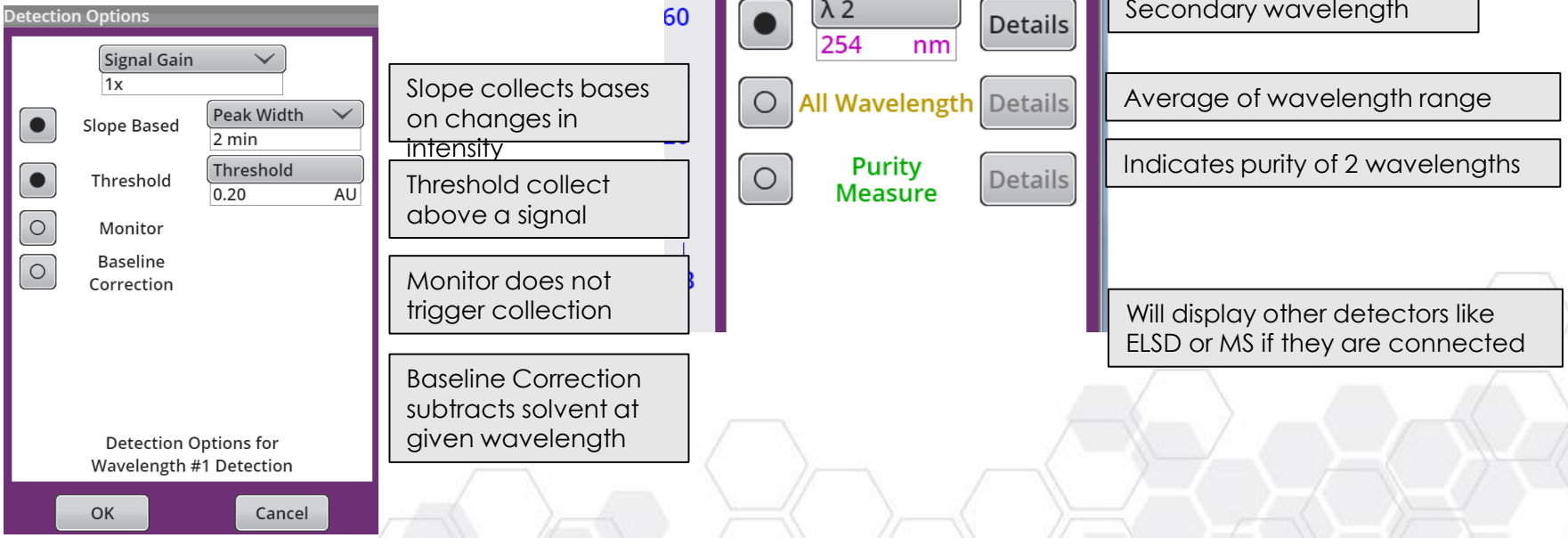
What You See – Detection Options

- Options allow for greatest sample recovery



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All Wavelength Detection

An average of signals from a given wavelength range

Increases UV signal from low UV absorber

Decreases UV signal from high UV signals to keep on scale

Average of signals

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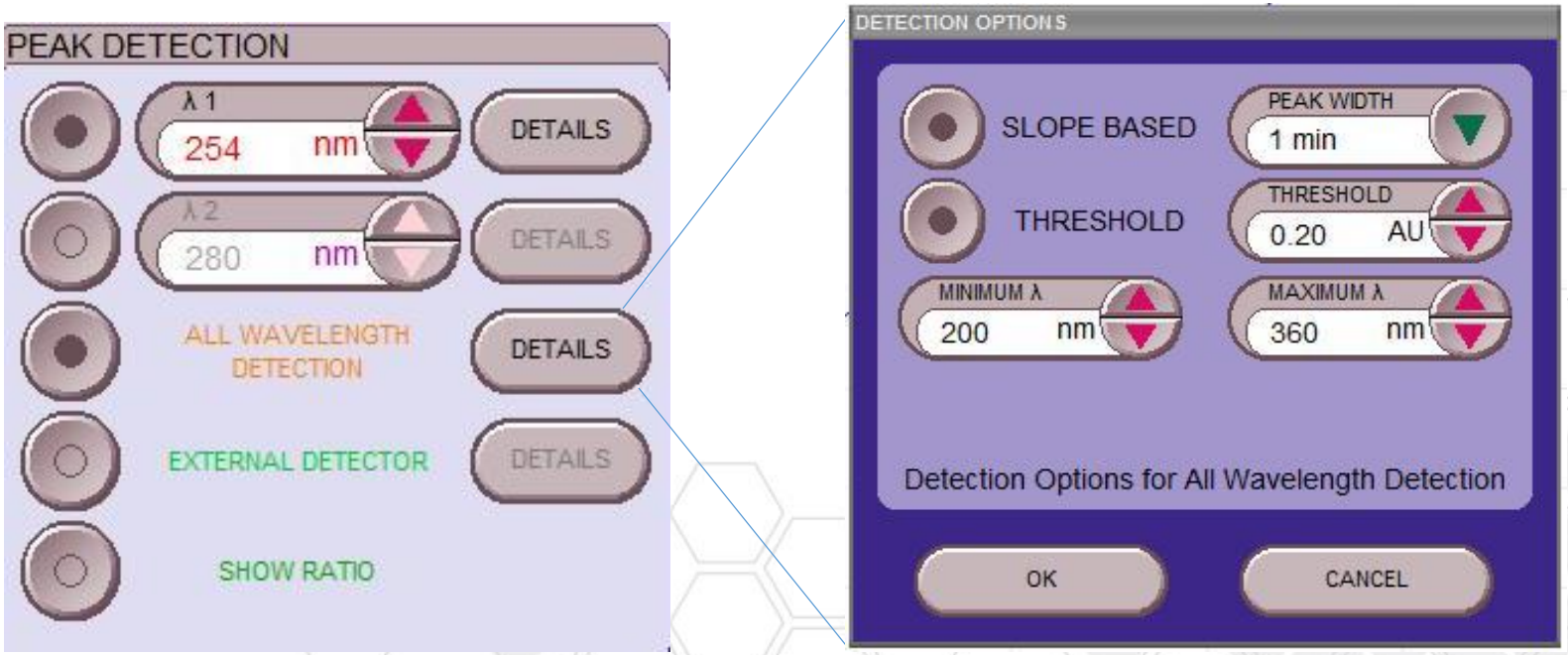
All Wavelength Collection
--Application Note 81

- All-wavelength collection is a technique that monitors all detector wavelengths in a user-defined range



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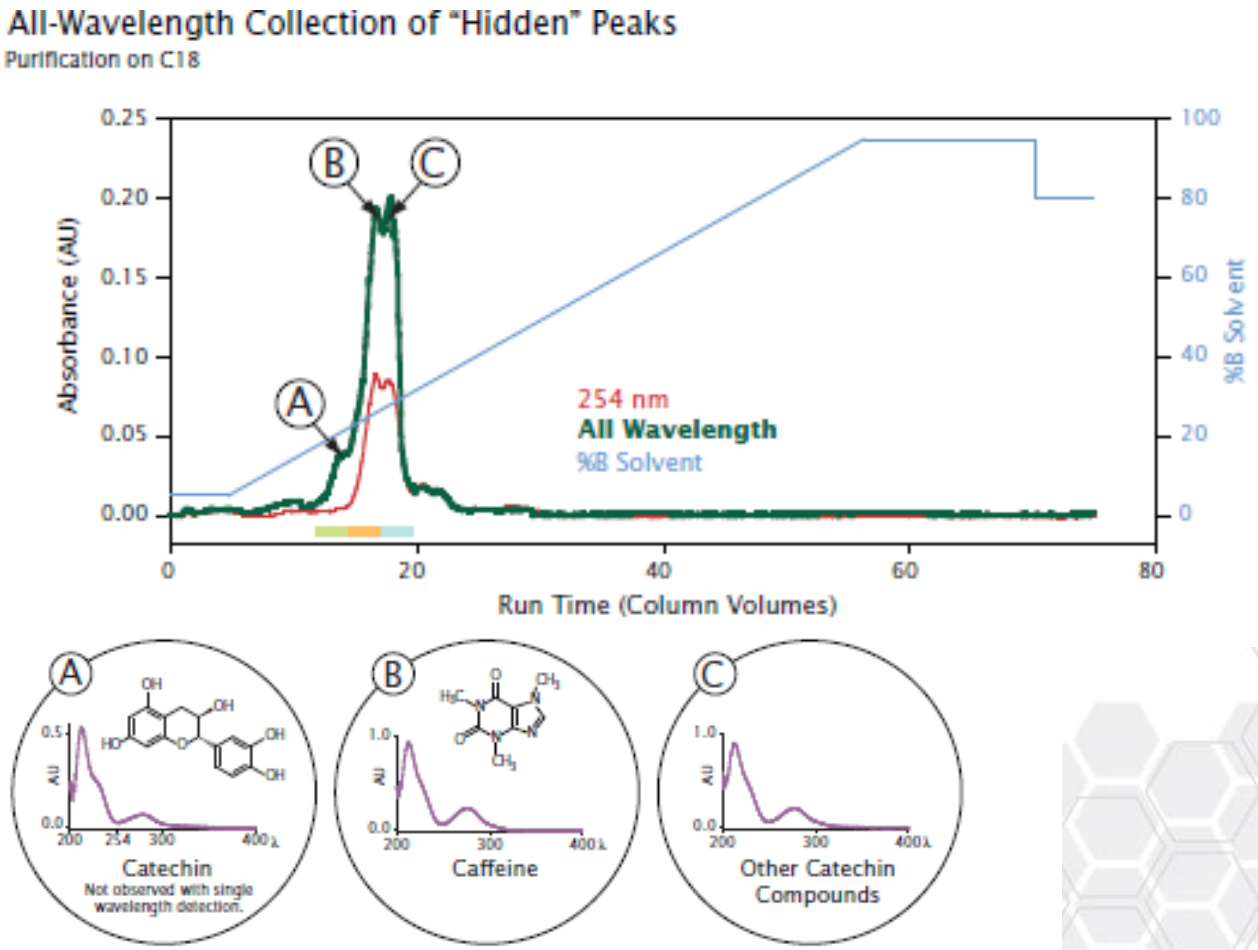
Purification of Green Tea Extracts Using All-Wavelength Collection



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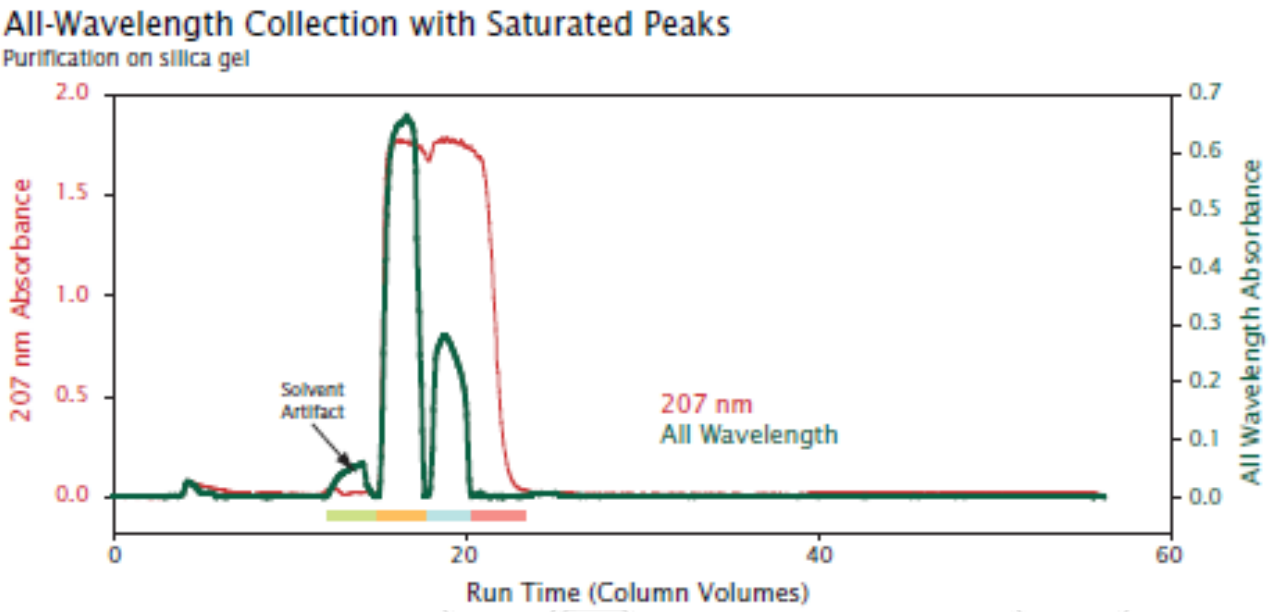
-- Compound A was not seen at 254 nm but was separated by All-wavelength

All-Wavelength Collection Saturated Peaks

- Trace at 207 nm was saturated.

Hexane:Acetone gradient

- All-Wavelength Collection triggers collection accurately to minimize cross contamination
- Solvent Artifact is seen until the algorithm eliminates the signal



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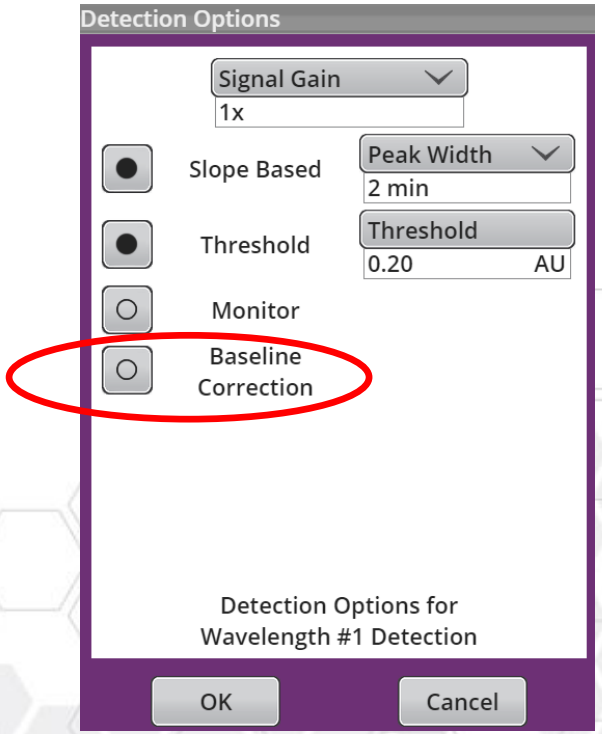
Baseline correction

- Solvent is subtracted from the base line
- See more of the compound
- Faster response time and increased recovery of compounds



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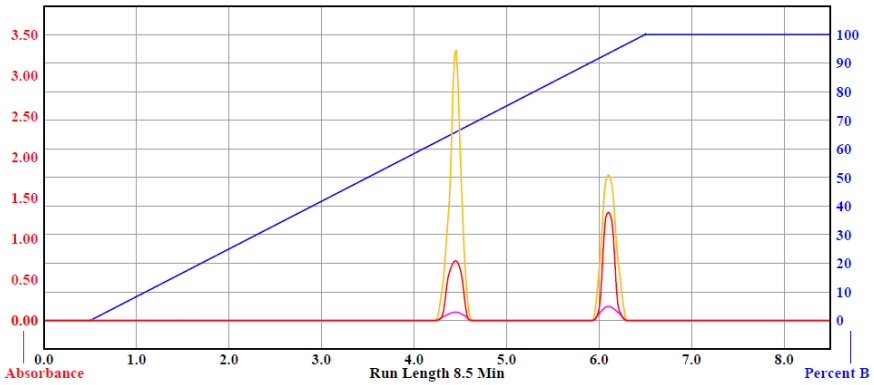
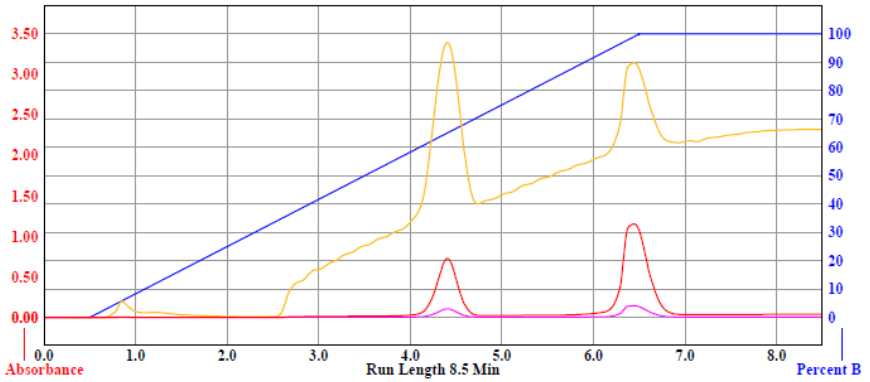
Comparison Baseline Correction

Hexane/Ethyl Acetate gradient.
Yellow All Wavelength detection 200-300 nm





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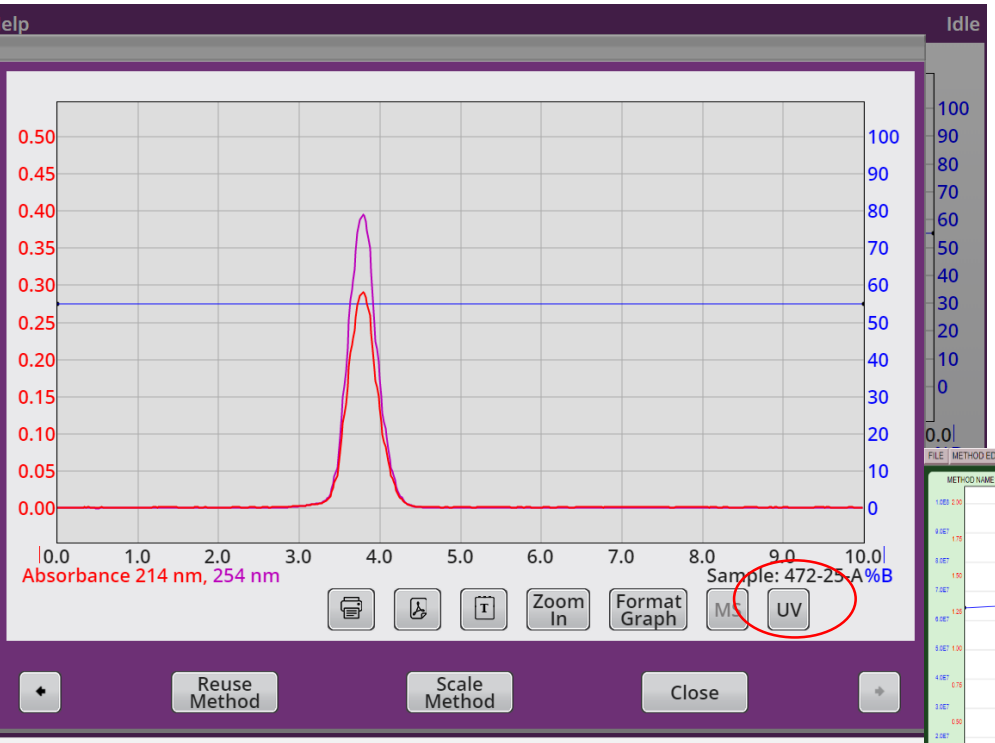
Spectral Data



View Spectral Data
--During run
--Post run

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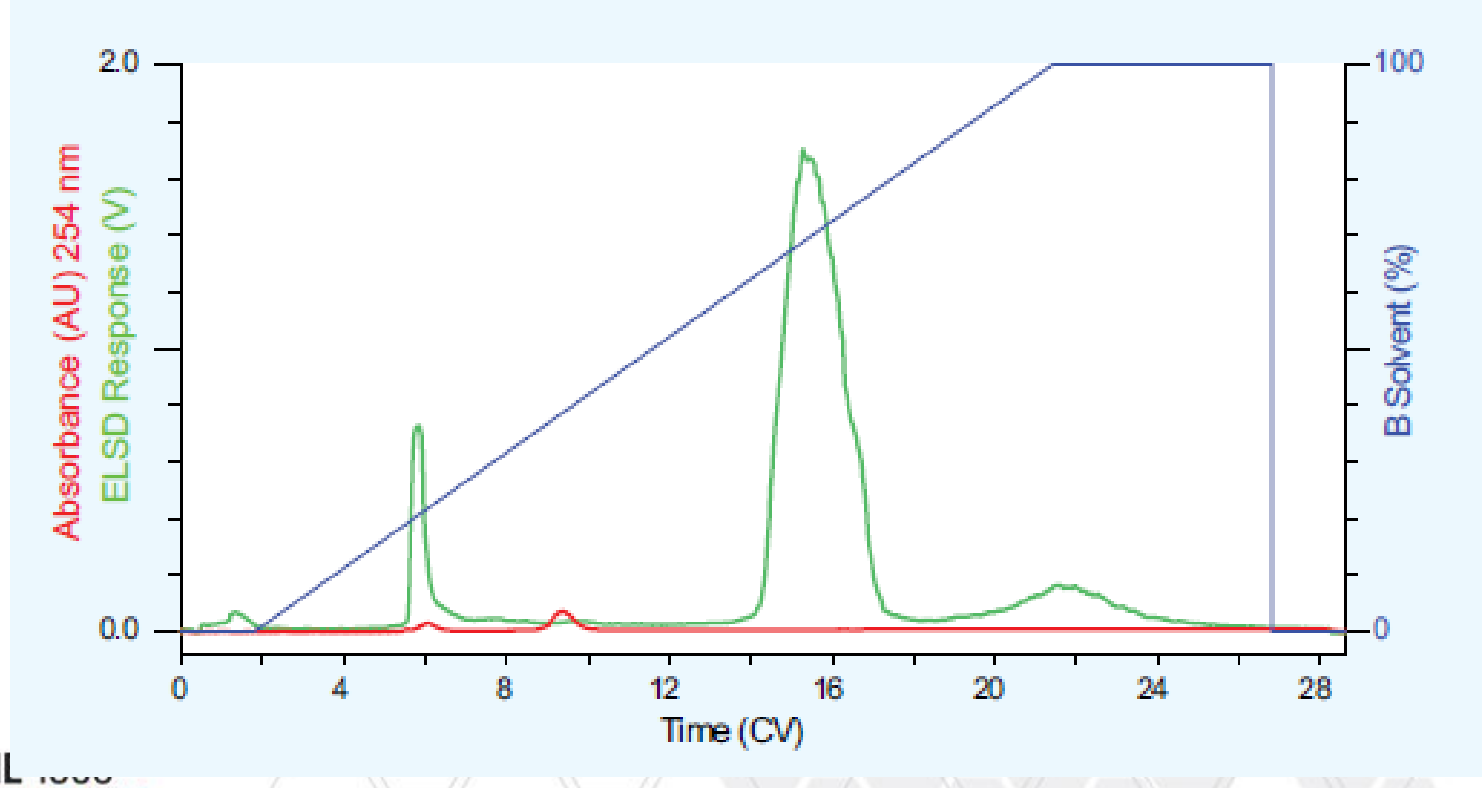
Detection Options

- Photo diode array
- Variable wavelength detection (200-400 nm) standard
- Visible wavelength detection (200-800 nm)
- All-Wavelength Detection
- ELSD integrated in unit
- Mass directed collection
  - “S” up to 1200 Daltons
  - “L” up to 2000 Daltons

Integrated ELSD
Detection of non-UV Absorbing Compounds
--Application Note 80

- Reaction mixture where product has no UV chromophore



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ELSD Specs

	Internal ELSD
Gas Inlet Pressure	60-70 psi
Gas Consumption	<2.5 SLPM
Spray Chamber Temperature	Setting range 10-60 °C
Drift Tube Temperate	Setting range 30-90 °C
Split Flow Rate	0.75 ml/min 5-30 ml/min Increases from 0.75 to 1.5 ml/min 30-60 ml/min 1.5 ml/min 60-300 ml/min
Sensitivity	High & Normal settings

ELSD detection is not recommended for flow rates below 5 ml/min

ELSD can be turned off for any runs below this recommendation.

-Note flash columns ≥ 4 grams are optimal over 15 ml/min.

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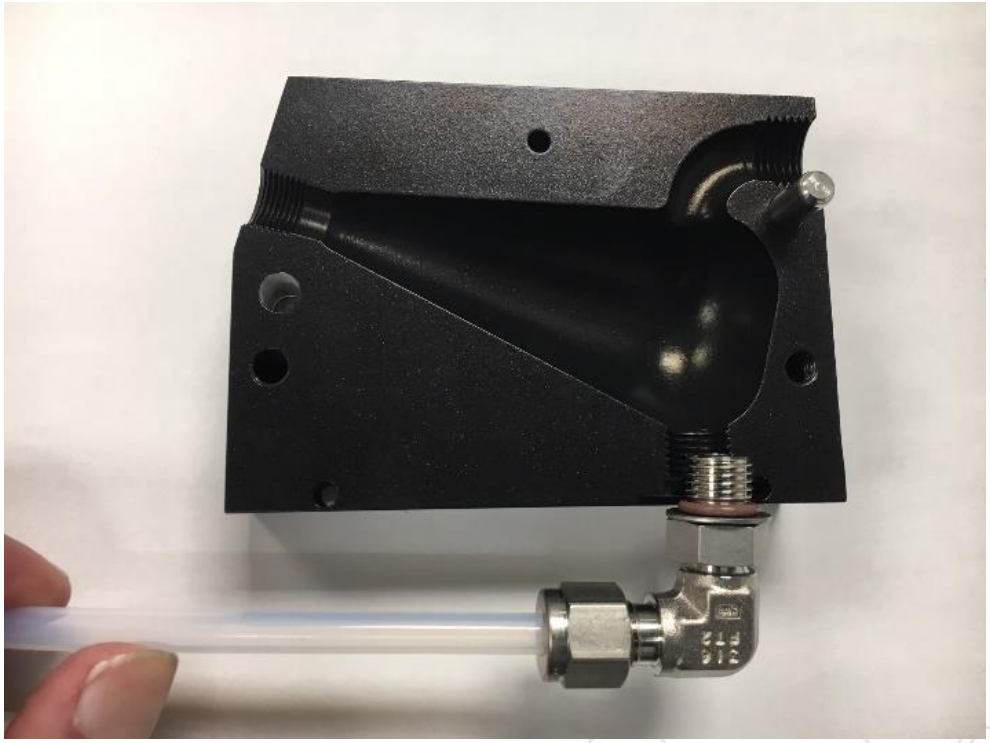
New ELSD Spray Chamber



- New spray chamber compatible with aqueous buffers
- Design is simple to clean
- Offers High and Normal sensitivity software selection

Integrated ELSD available on all CombiFlash models

- No additional lab space required
- Available on NextGen 300+, 300, 100

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Two Modes of Operation

- Normal Sensitivity
  - Set to ½ of Lumen sensitivity
  - Gain default set to 2X
    - Results in same peak magnitude as current response
    - Ability to lower the sensitivity for strong signals
- High Sensitivity
  - Set to 6X Lumen sensitivity
  - Ability to cut peaks of <5mg samples

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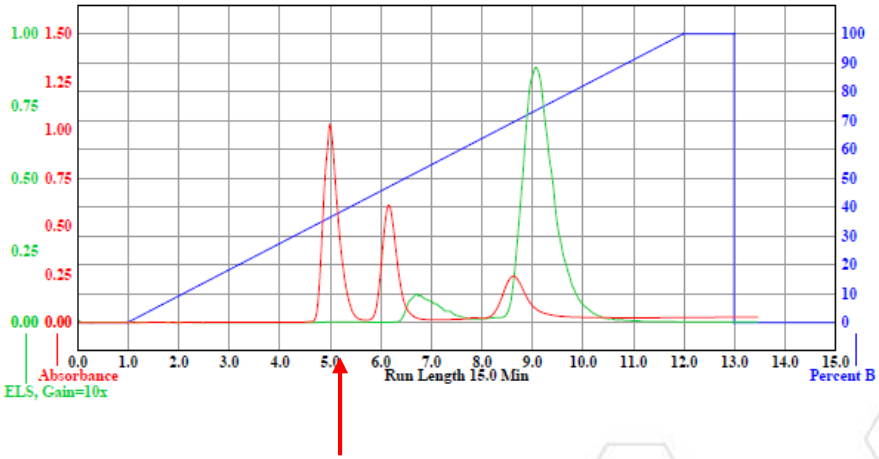
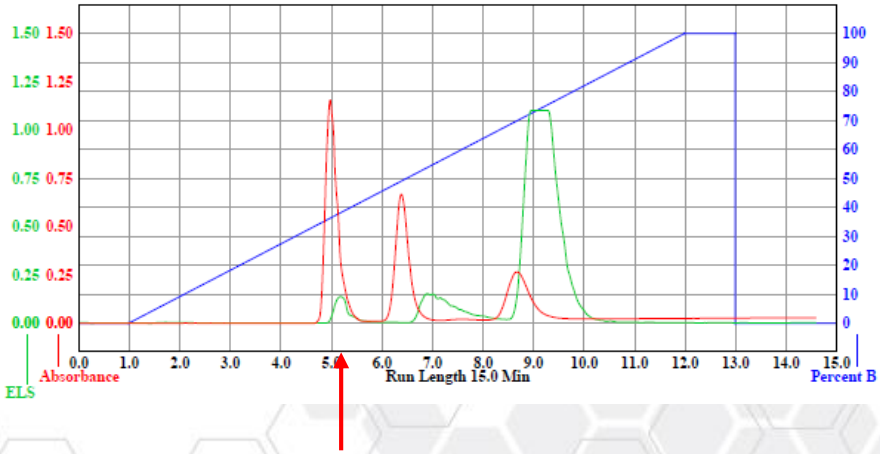
ELSD Sensitivity Selection

Normal Sensitivity ELSD Signal



High Sensitivity ELSD Signal



0.5gm of Test Mix A.

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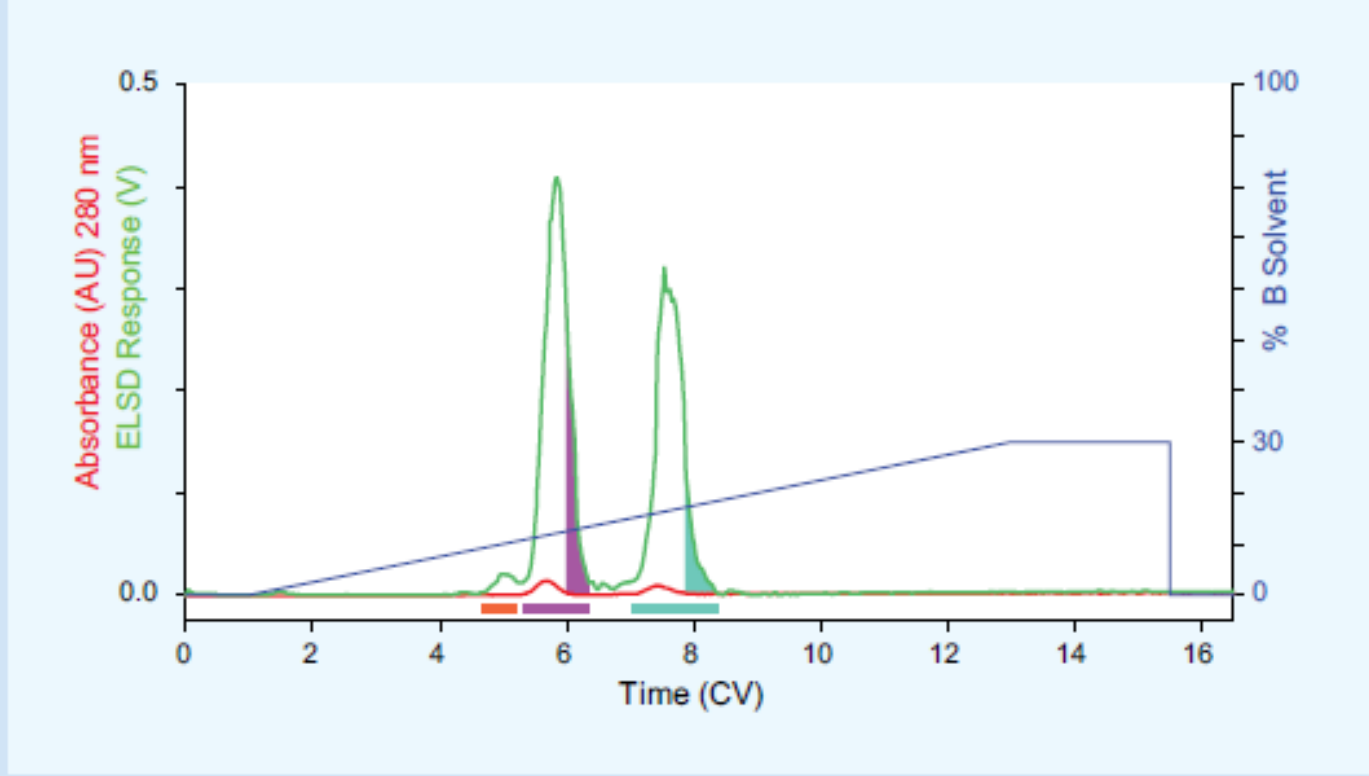
Improved Recovery over UV Detection

- Shaded areas on peaks denote peak area detected by ELSD but not with UV



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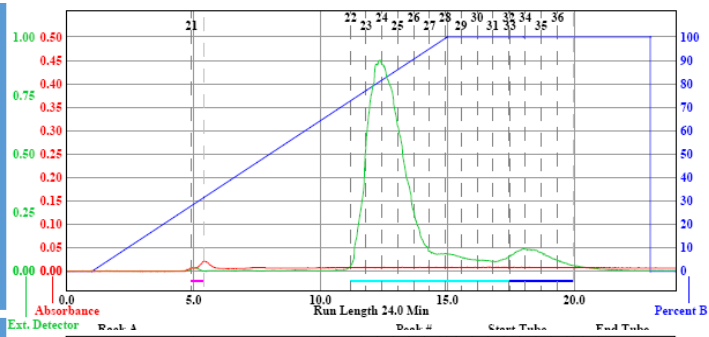
Improved Recovery over Competitors

A sample of 2,3-isopropylidene-D-ribofuranose was synthesized and the reaction mixture (1.0 mL) was purified on a 40g Redi*Sep* Rf silica column (PN 69-2203-340) using the default gradient on each flash system. Gradient was hexanes/ethyl acetate.

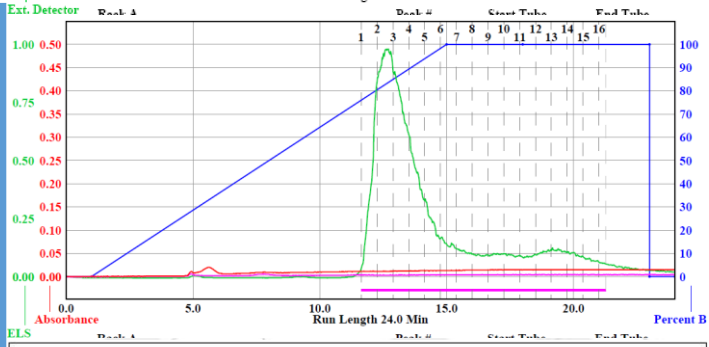
CombiFlash NextGen ELSD

Tubes 22-26: 691.4mg
Tubes 27-32: 45.6mg
Tubes 33-36: 84.6 mg
Total recovery: 821.6mg



CombiFlash Rf Lumen with internal ELSD

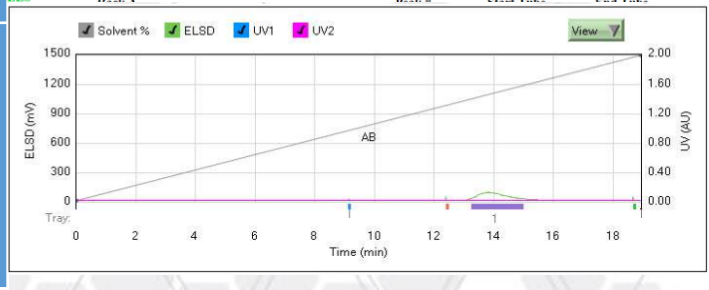
Tubes 1-8: 692.1mg
Tubes 27-32: 49.8mg
Tubes 33-36: 37.6 mg
Total recovery: 779.5mg



Competitive flash system with ELSD

Tubes 4-7: 444.6 mg

Total recovery: 444.6 mg



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CombiFlash PurIon MS

- Available on all Combiflash NextGen, EZPrep, ACCQPrep models
- A fully integrated operation in PeakTrak software
  - Simple to operate
- Mass directed purification
- Compound verification
- UV/Vis and ELSD detection along with MS

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Purlon Module Specs

	"S" Model	"L" Model
Upgrade available	Rf+, Lumen, EZPrep, ACCQPrep, NextGen	
Dimensions (WxHxD)	11x26x22 in (28x66x56 cm) MS and FI 9x10x18 in (23x26x46 cm) Roughing Pump	
MS Detection	10-1200 Dalton 1 Dalton resolution	10-2000 Dalton 1 Dalton resolution
Ionization Mode	Positive and Negative Automatic Switching	
Capillary Cone Removal	Does not require vacuum shutdown	
Probes	ESI or APCI	
Sample injection	Direct Injection for MS Method Development	

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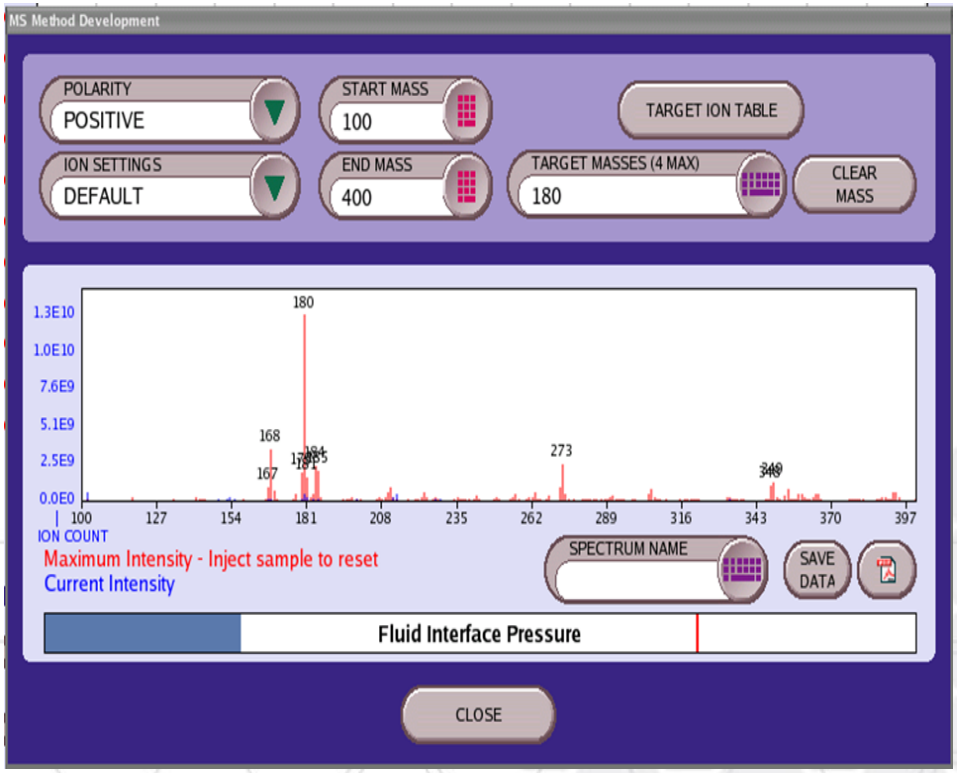
Benefits to the End User

- Determine presence of co-eluting impurity
  - Quickly determine that additional purification needed
- Collect only on target mass
  - Increase throughput
    - Shorten run times by terminating purification after target has eluted
    - Lower solvent consumption and costs
  - Eliminate the need for post-purification analysis
    - No more TLC
    - No post-run LC/MS
- Collect over a mass range
  - Eliminate unwanted masses
  - Useful in Natural Product Research

Terminate On Target

- Use MS Method Development screen
- Select desired masses



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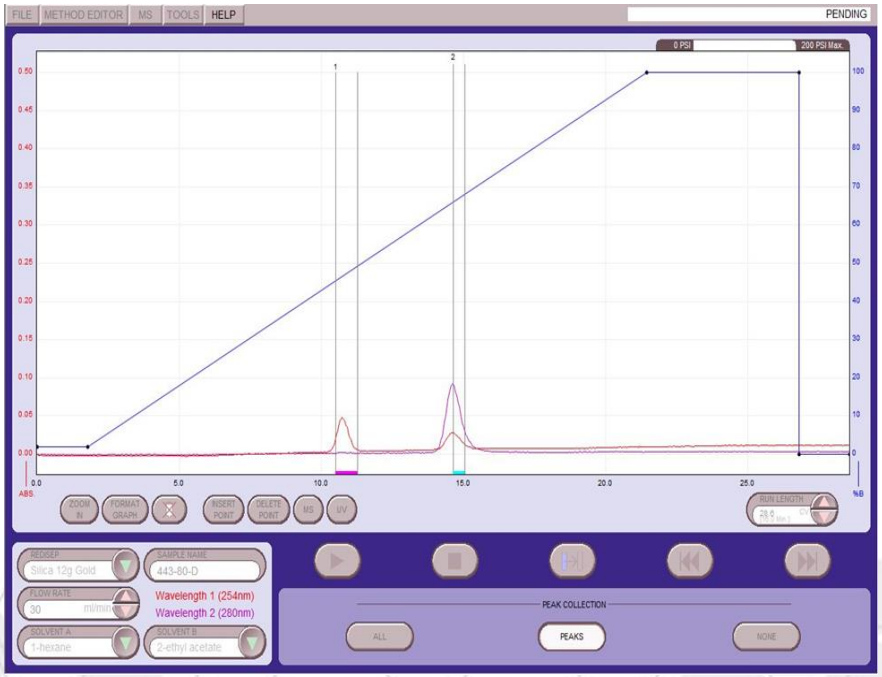
Sample run

- Weak UV absorbance
- Run entire program



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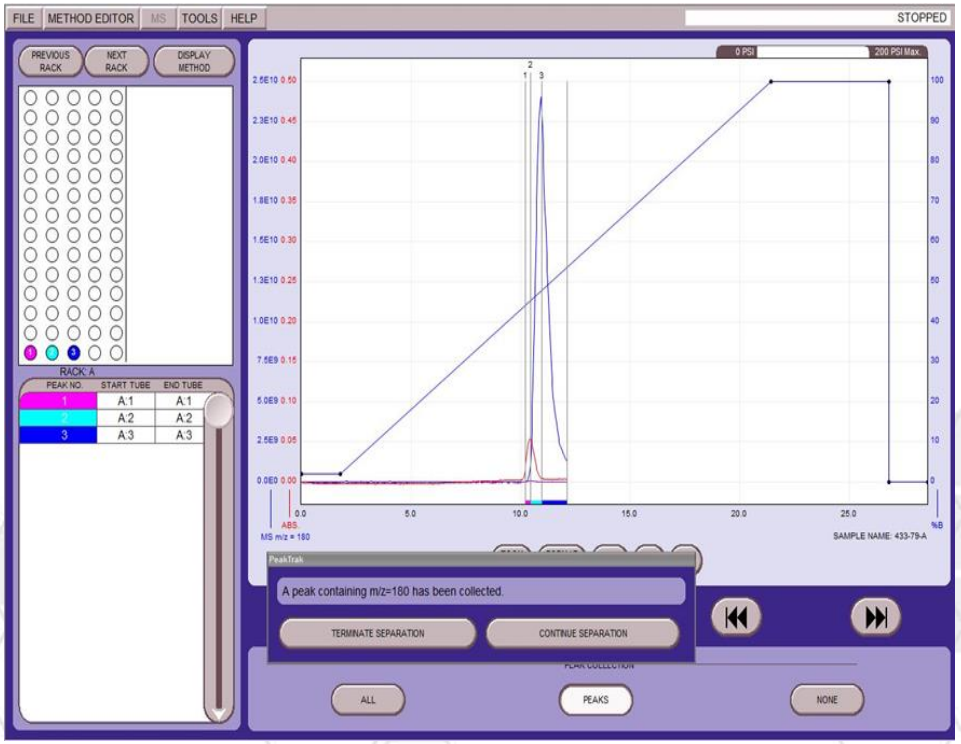
Terminate on Target

- Terminate run after peak with m/z=180 Da collected
- Collection with UV and MS
- Time and solvent saved



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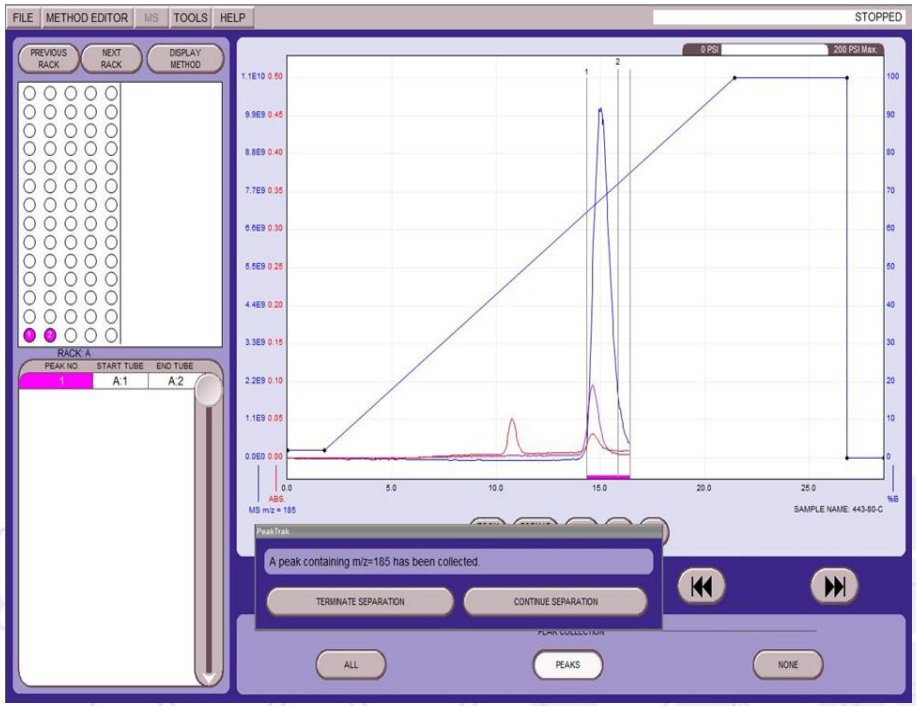
Terminate on Target

- Second peak collected, terminate on m/z = 185 Da
- Collection on SIM (single ion monitoring) 185 Da



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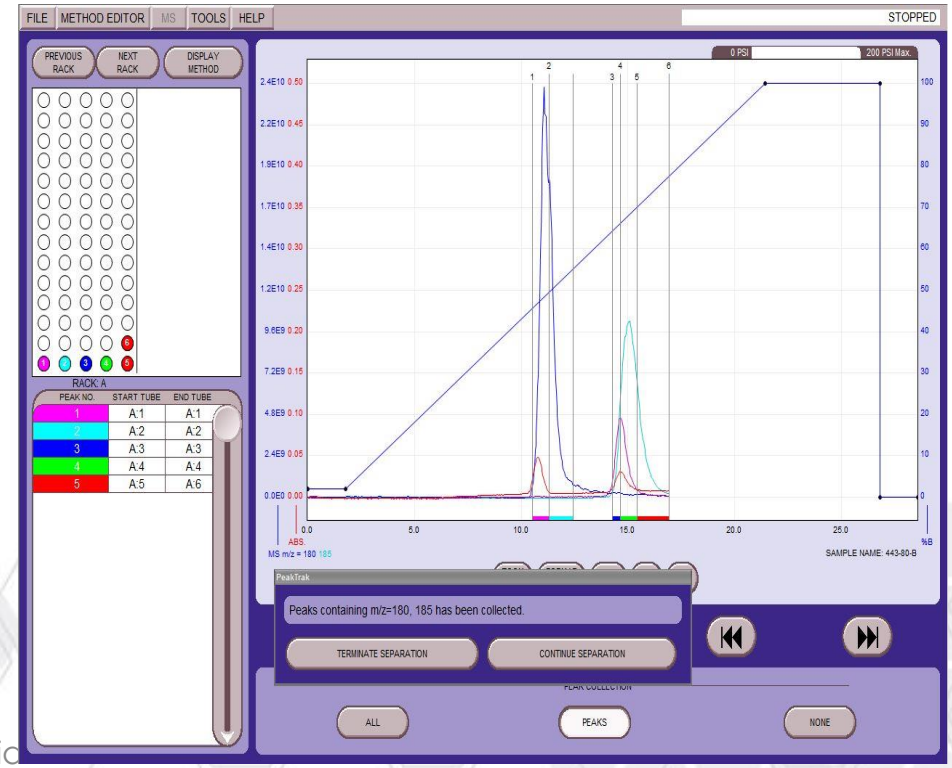
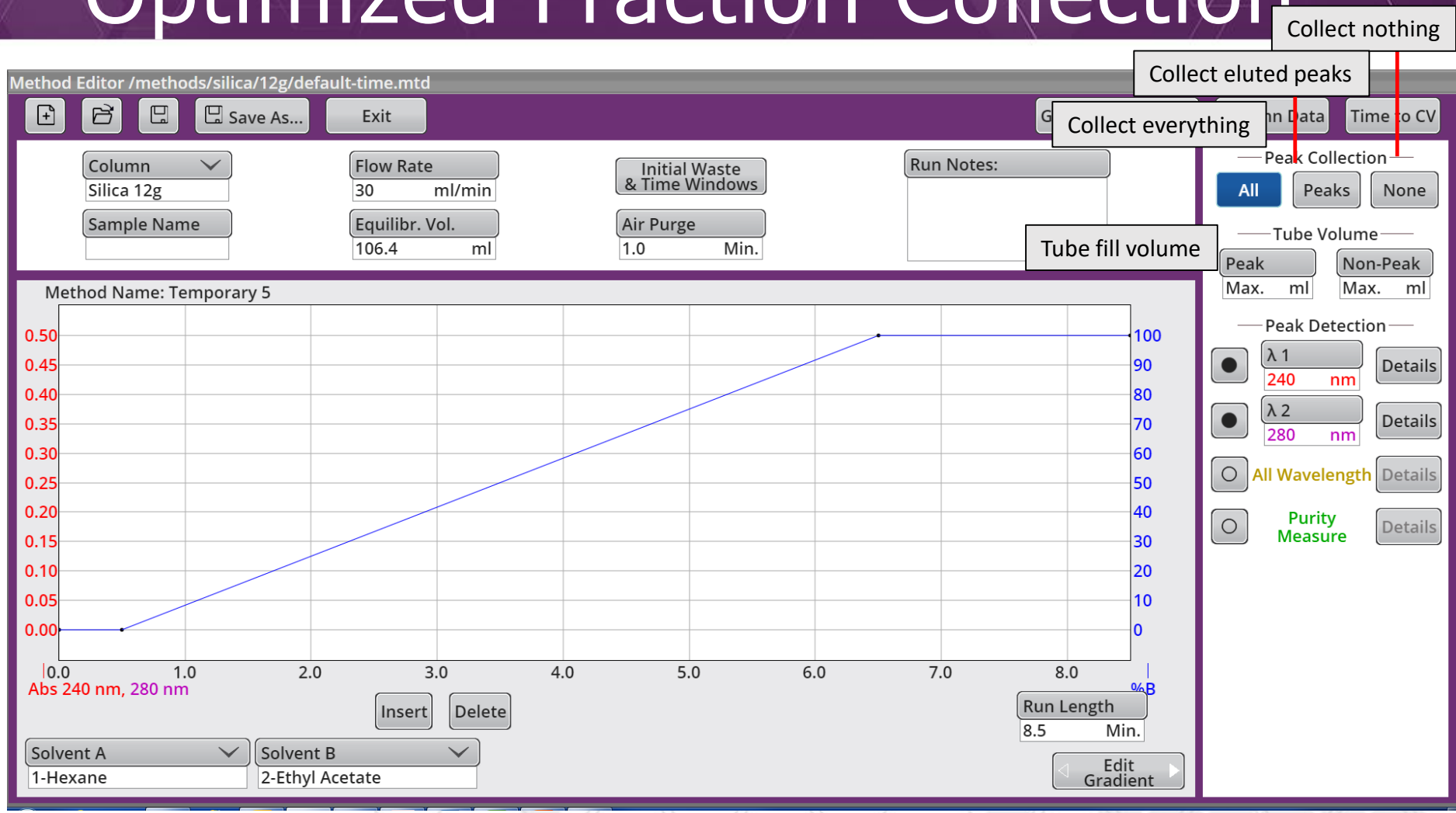
Terminate on target

- Terminate run after m/z 180 AND 185 Da collected.
- Up to 4 SIM or 3 SIM and 1 XIC (extracted ion current)



What You Get – Optimized Fraction Collection



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Peak Parameters

- Slope Based
  - Peaks will be recorded if the slope algorithm indicates a peak within the Peak Width setting
- Peak Width
  - Average peaks are detected based on a range from 0.2 to 2 times the peak width at baseline.
- Threshold
  - Collects peaks if the absorbance unit (AU) set is exceeded.
- Select both Slope and Threshold as an OR operation
- Monitor
  - Detection signal will be displayed but will not trigger collection

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PeakTrak Basics

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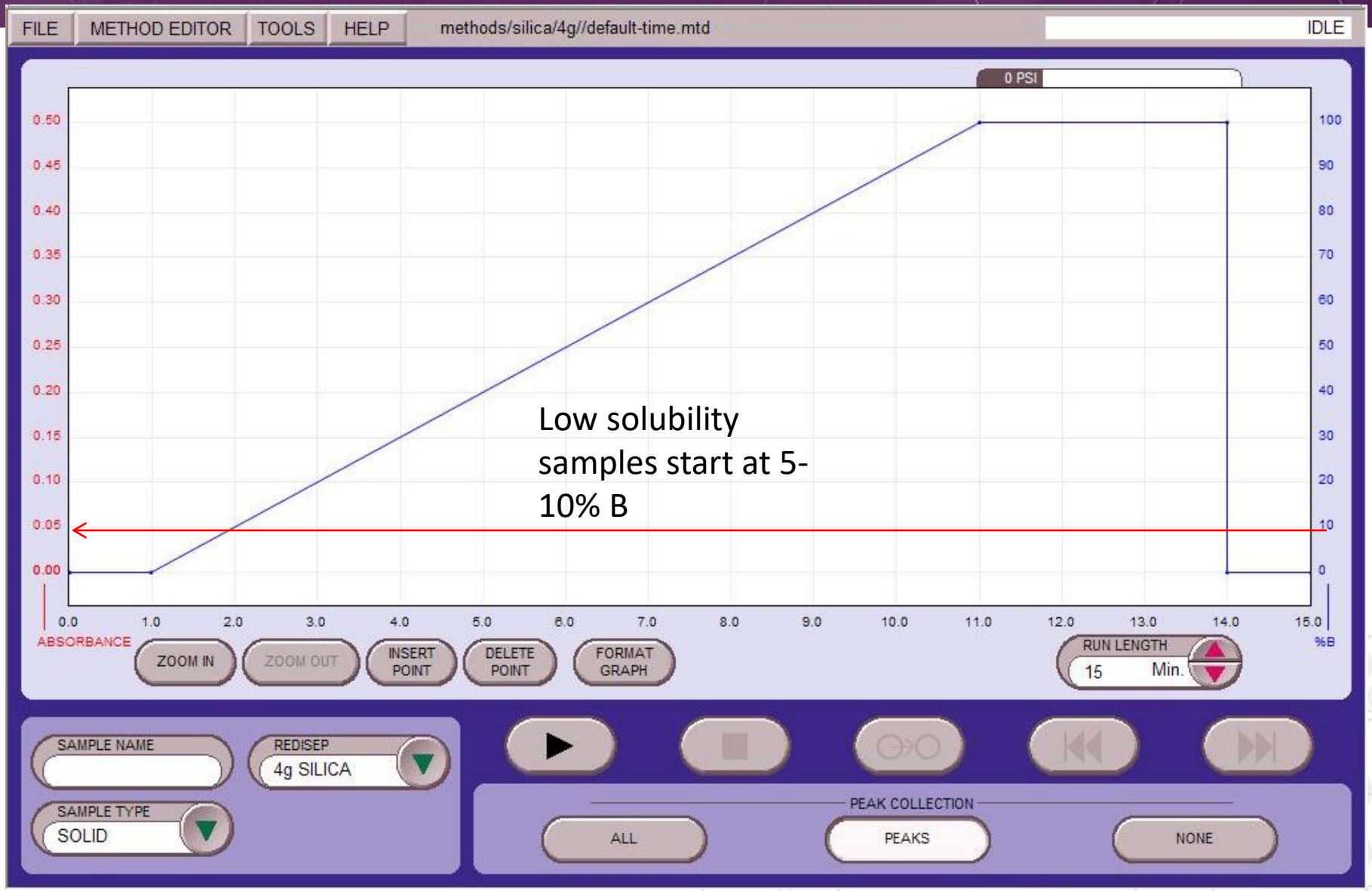
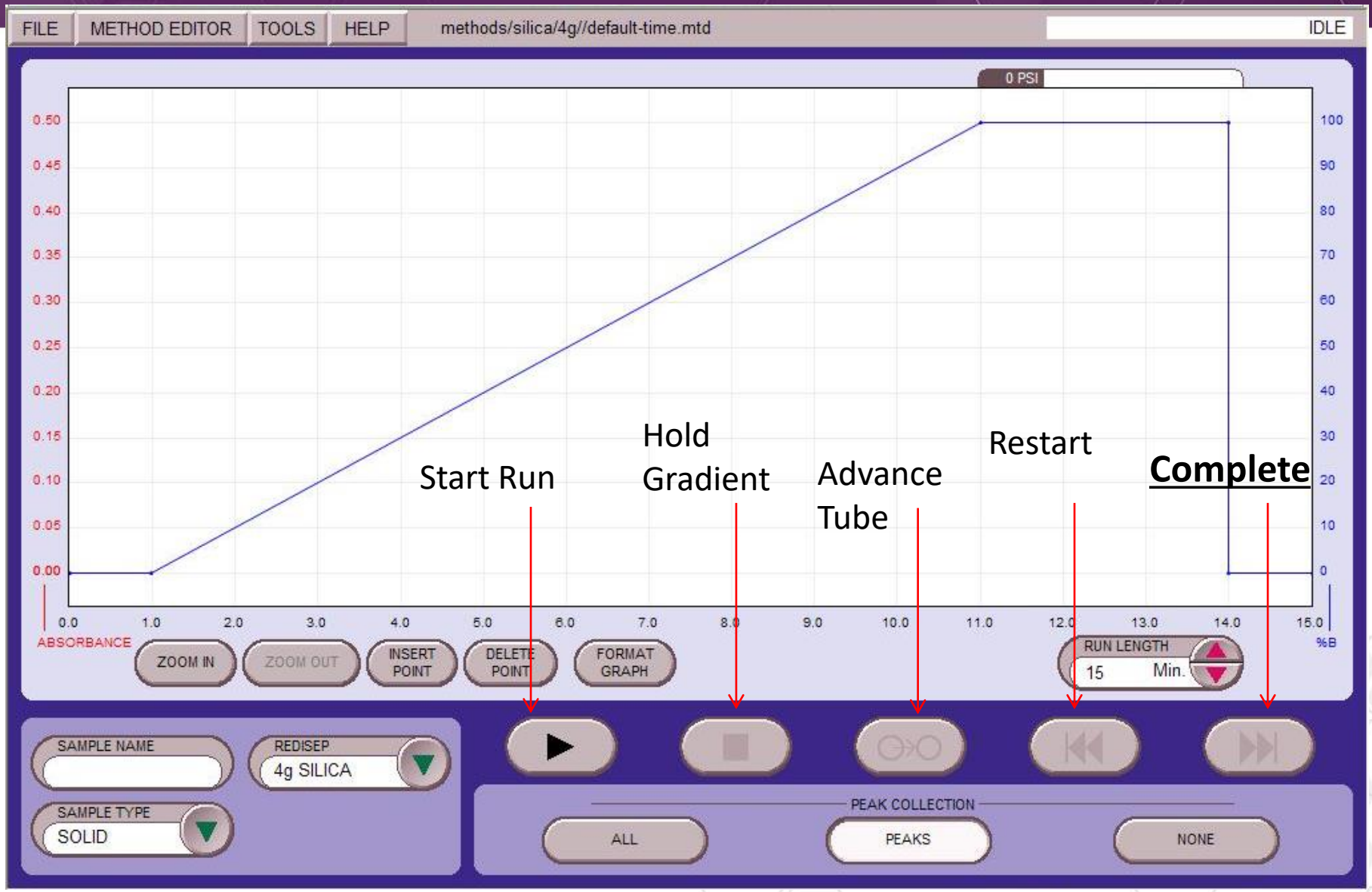
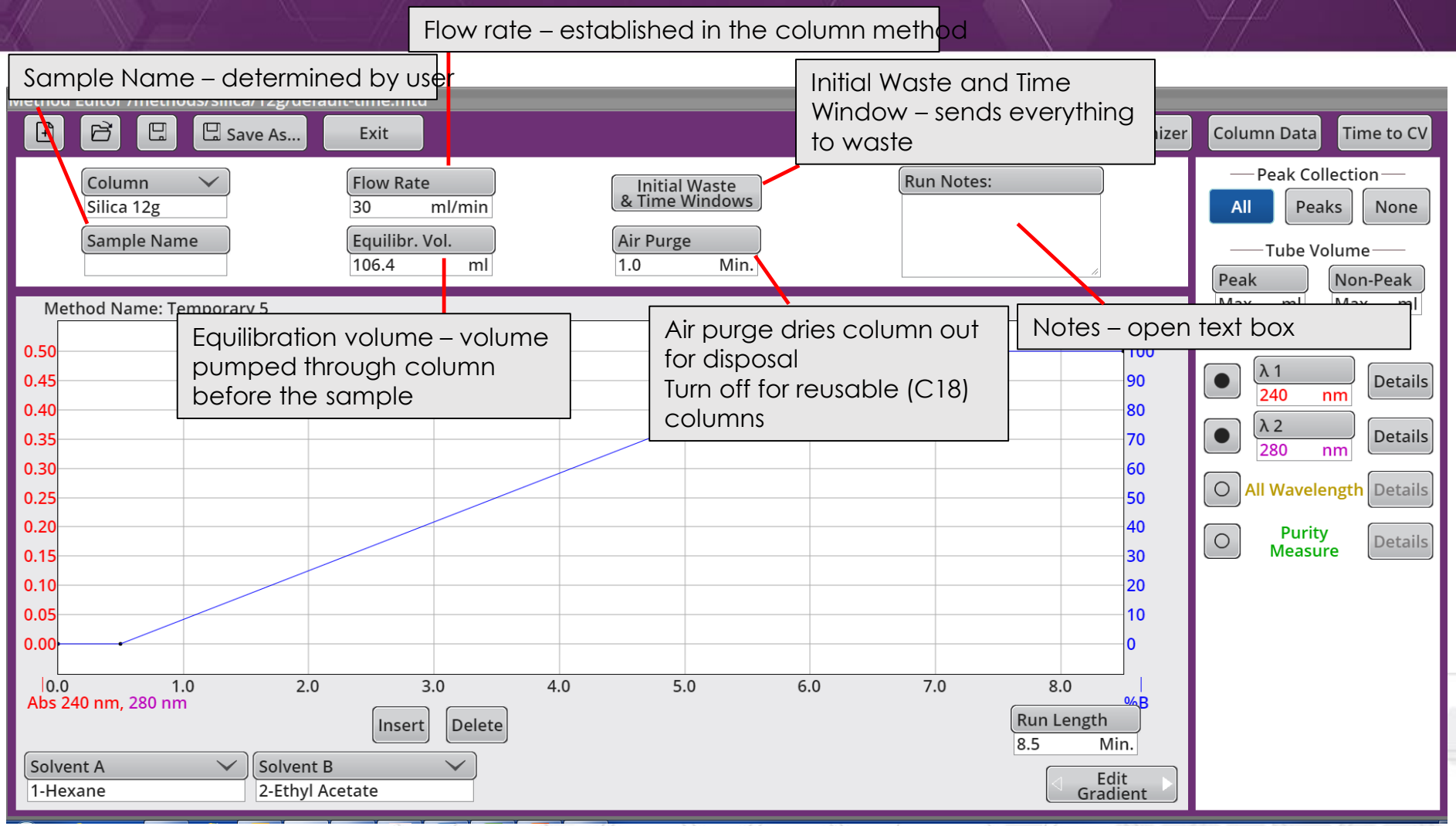
Basic PeakTrak Run Screen



PeakTrak Main Screen



PeakTrak Method Editor



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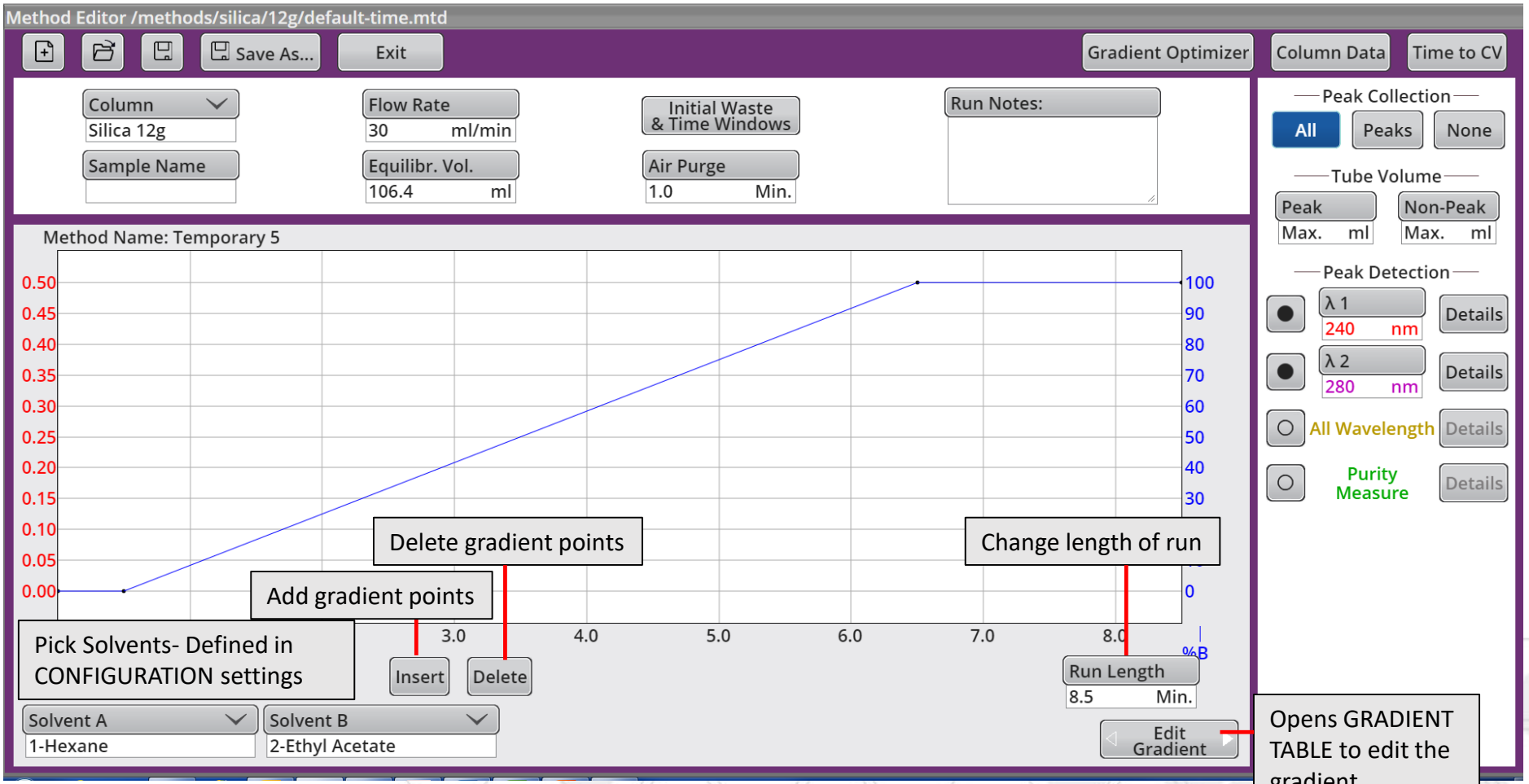
PeakTrak Method Editor



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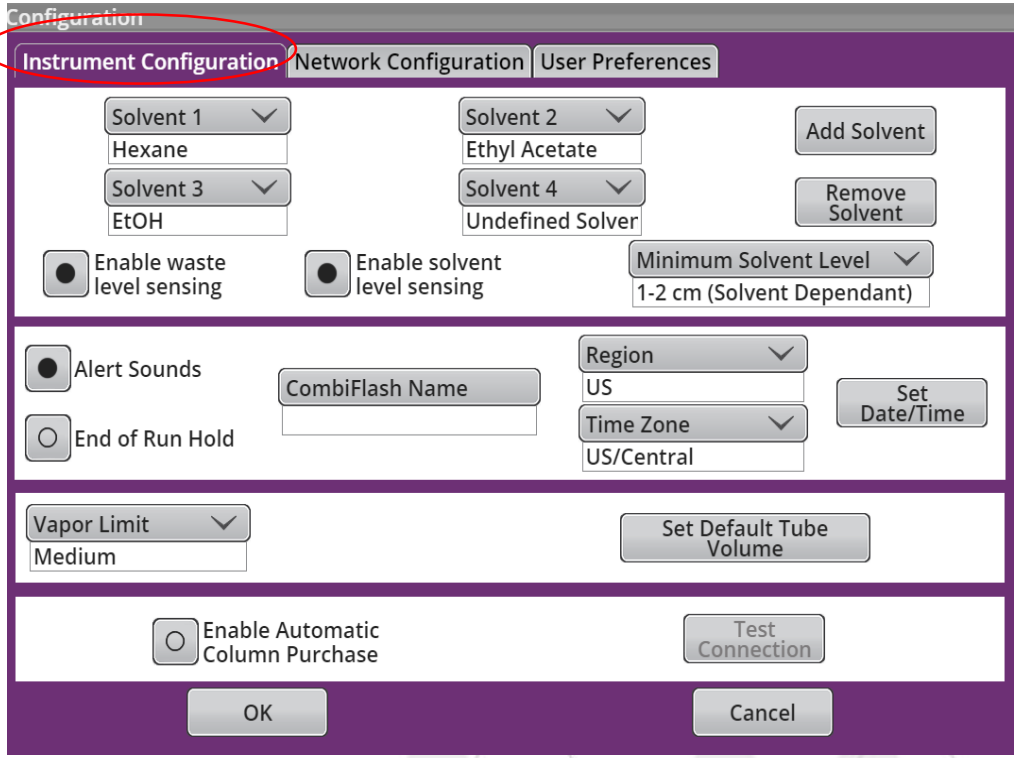
Behind the scenes- Configuration of CombiFlash

- Select solvent per inlet line
- Add and remove solvents
- Enable solvent sensing (300+)
- Set solvent detect level (300+)
- Turn on audible alarms
- Name the CombiFlash
- Set the time zone
- Set vapor limit
  - high/med/low/off
  - optional
- Set default tube volume



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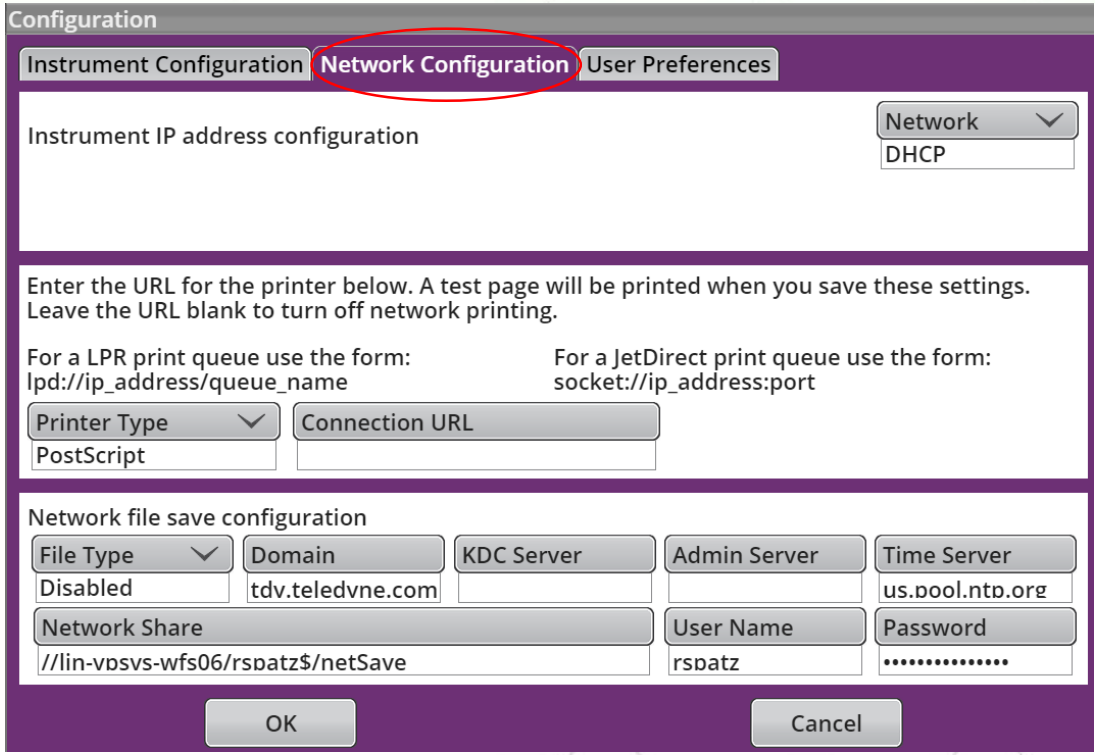
Network Configuration

- View the unit from desktop
- Print via network printer
- Auto save to network
- Need to work with local IT department
- TN 28 Networking Guidelines



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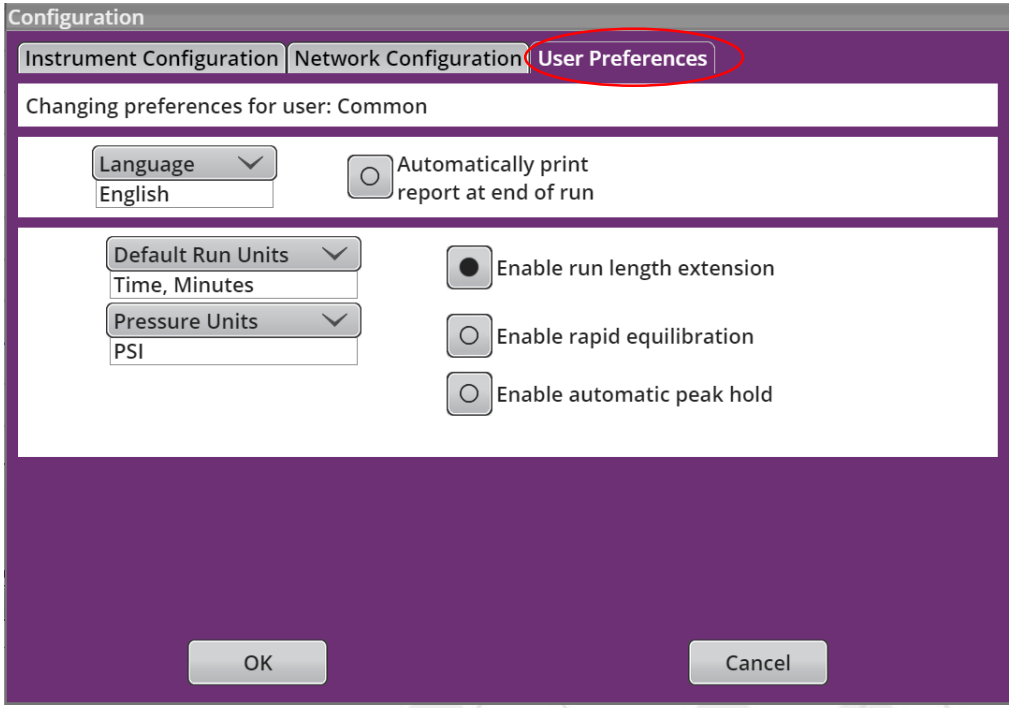
User Preferences

- Set Language
- Auto print function
- Default display units
- Enable peak hold to extend the run
- Enable rapid equilibration
- Enable auto peak holds
- Pressure in psi or bar



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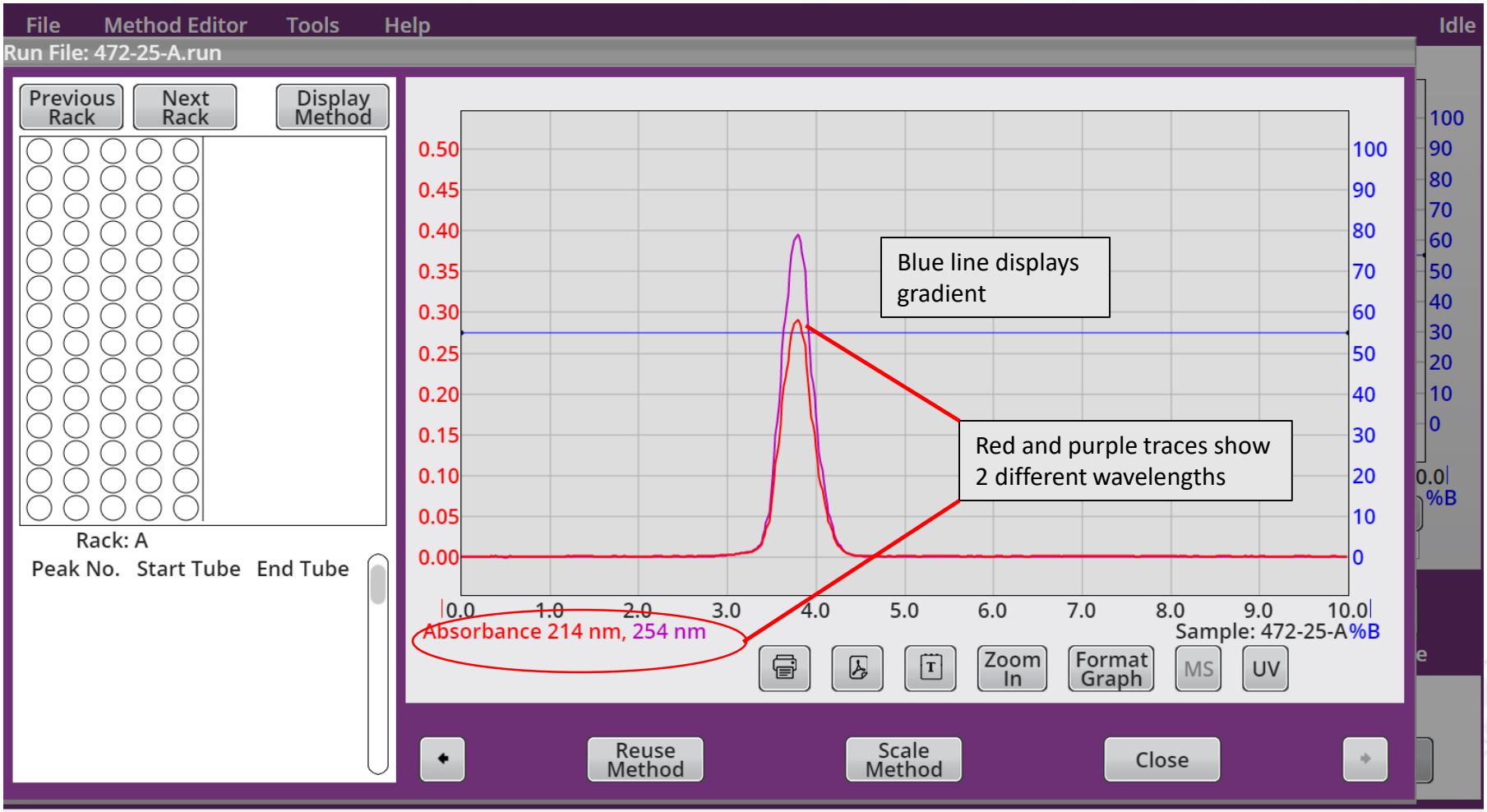
Post Run – View Separations



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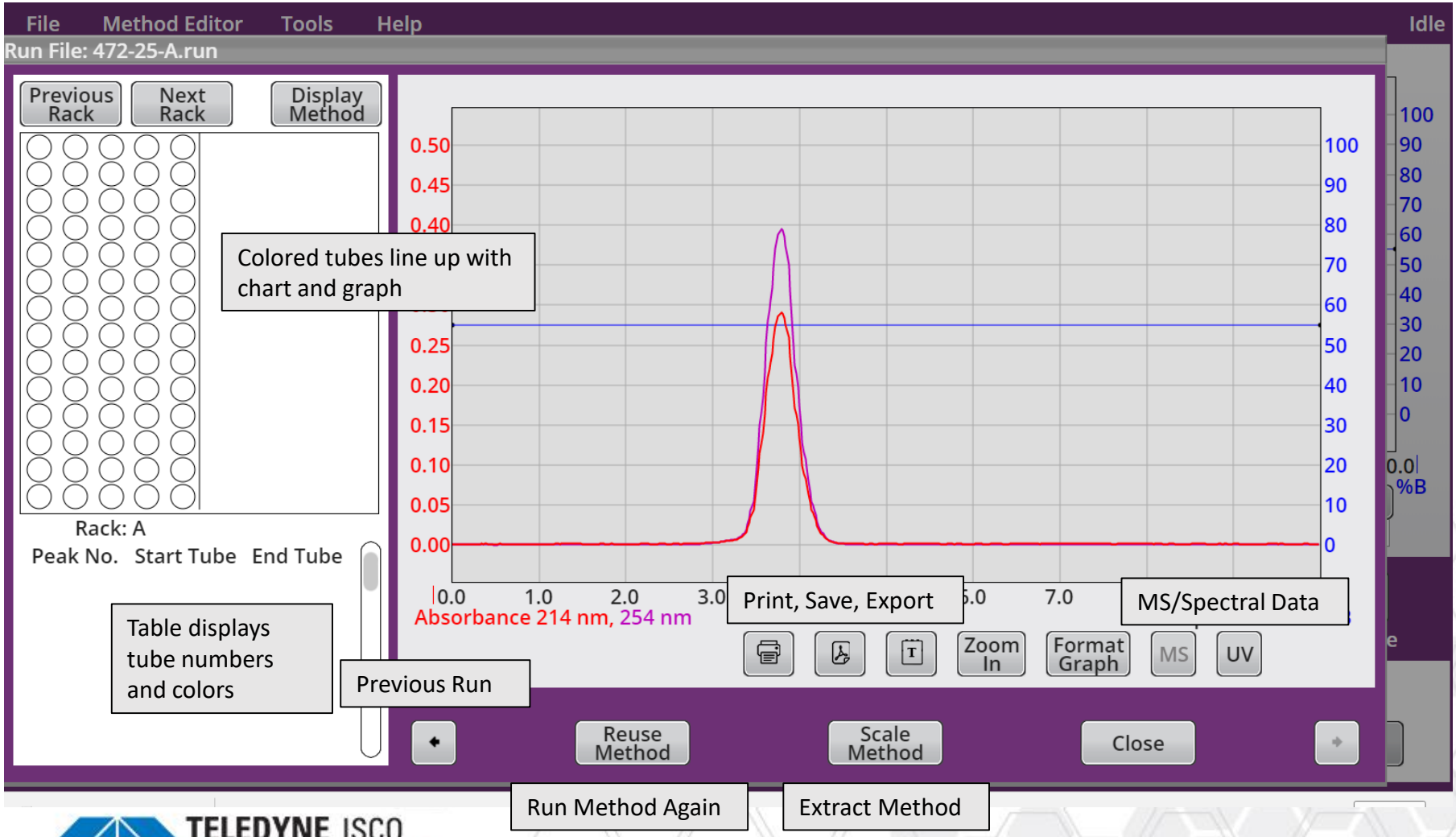
View Separations



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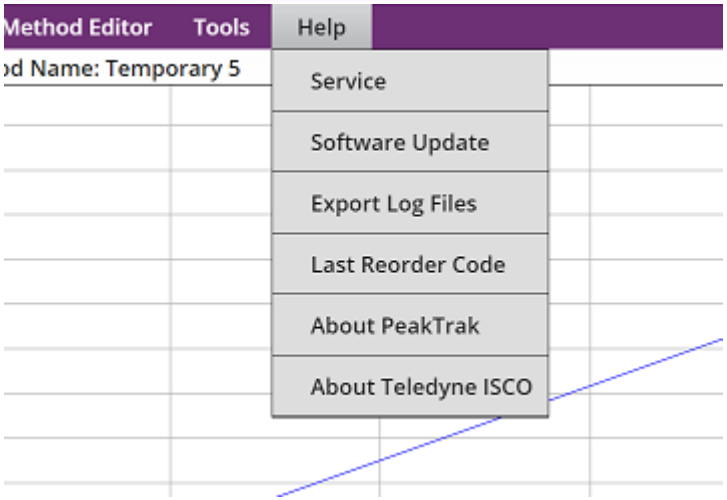
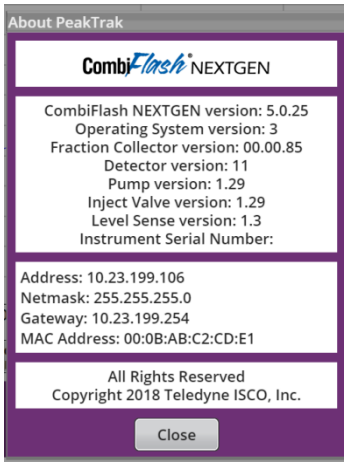
Help Window





- Service – access the service functions
- Software Update –update from a USB drive
- Export Log Files – export to troubleshoot any software bugs
- Last Reorder Code – active for Column Plan (300+)
- About PeakTrak – tells the software version
- About Isco – Contact Teledyne Isco

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RFID Technology

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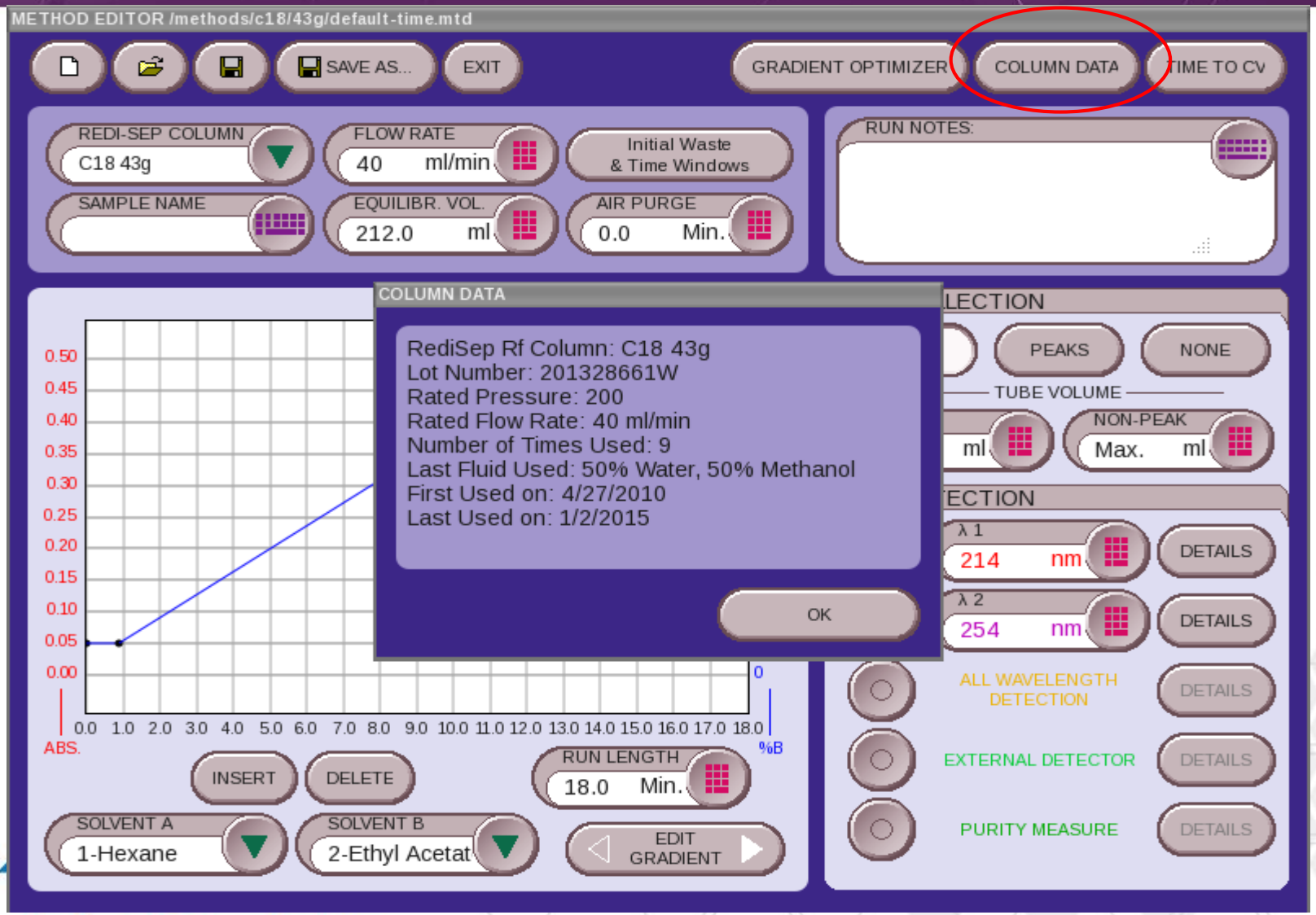
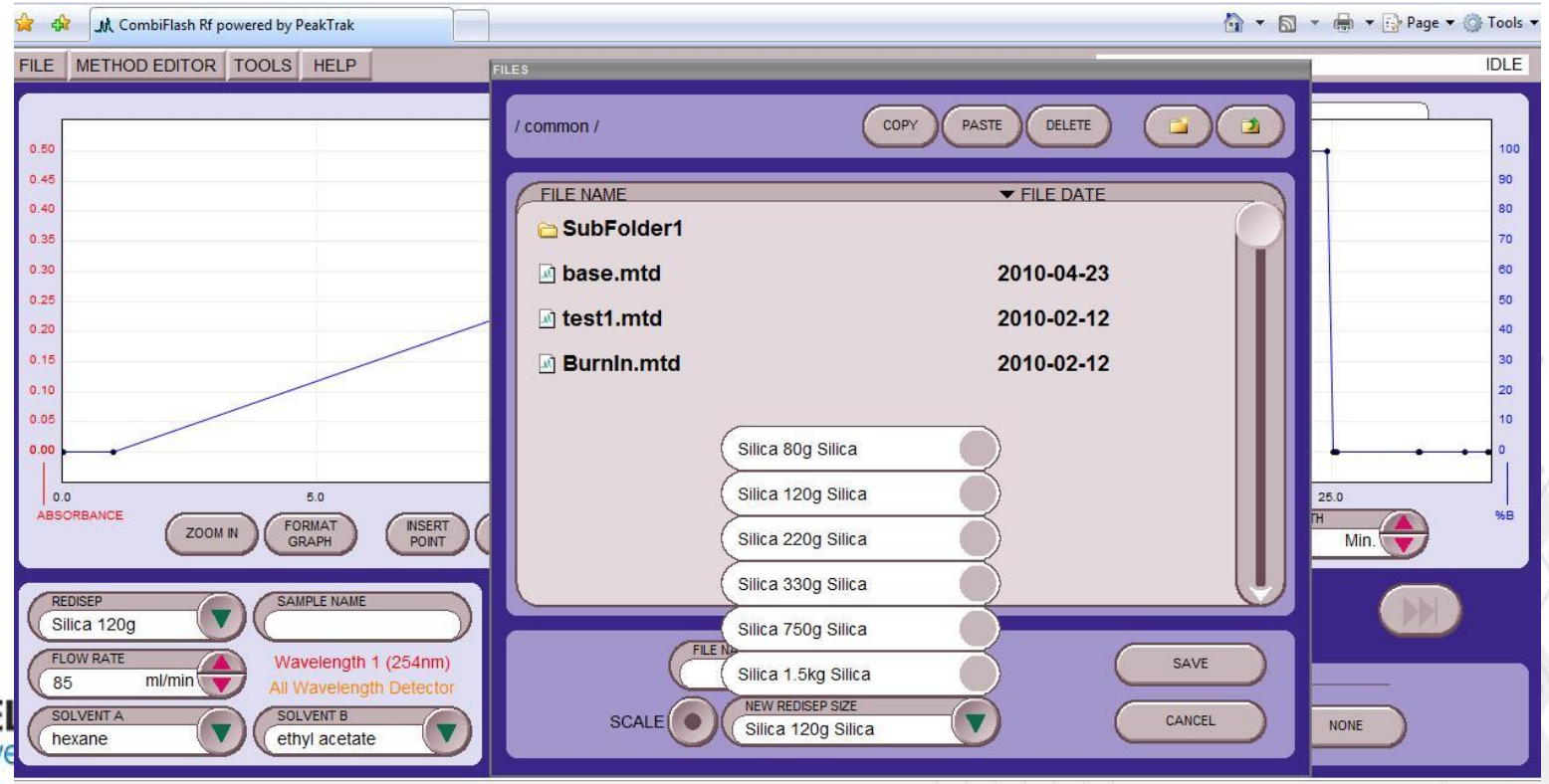
RediSep Information

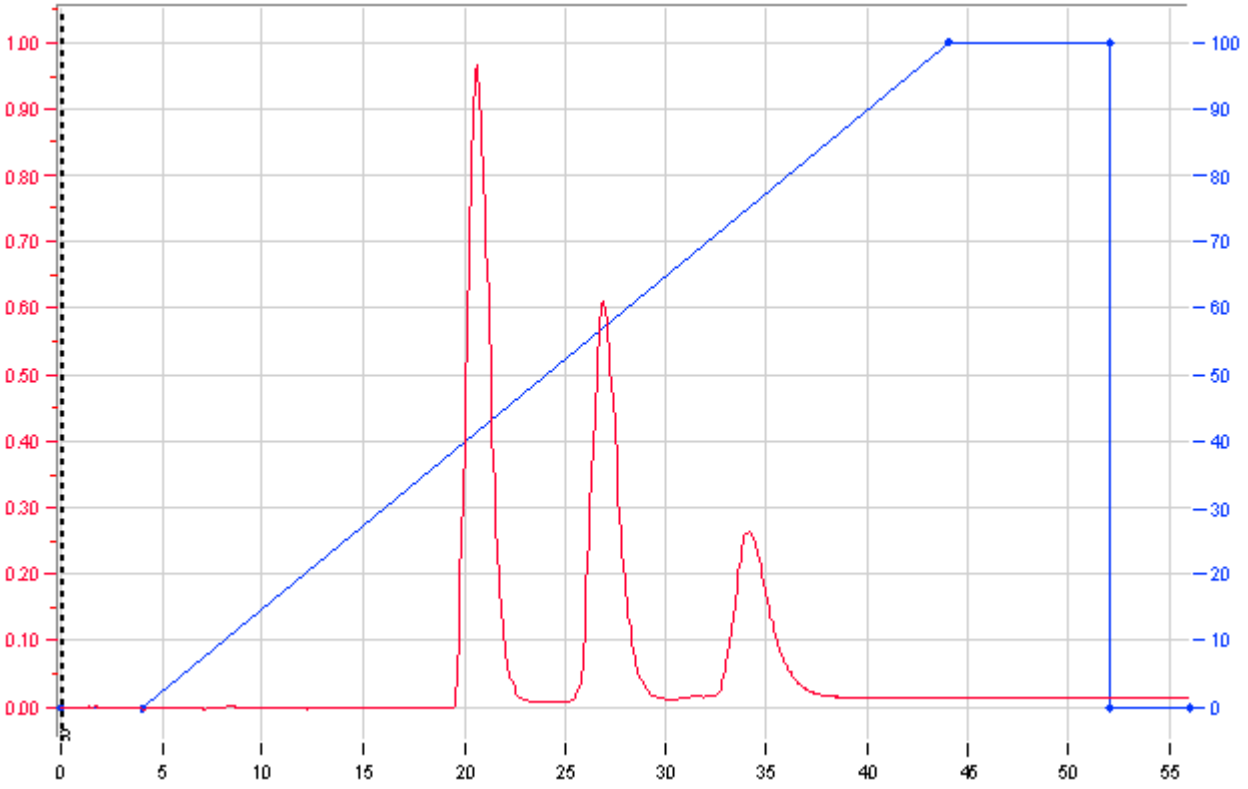


Scale Up from Milligrams to Grams

- Easily convert 4 g column method to 330 g column method on the Rf and up to 3 kg on Torrent
- Open method to be scaled
- Go to “File” then “Save method as…”



4g column



Sample Name: 050726@14-19-1
Date: 26 Jul 2005
RediSep Column: 4 g
Run length: 56.0 CV

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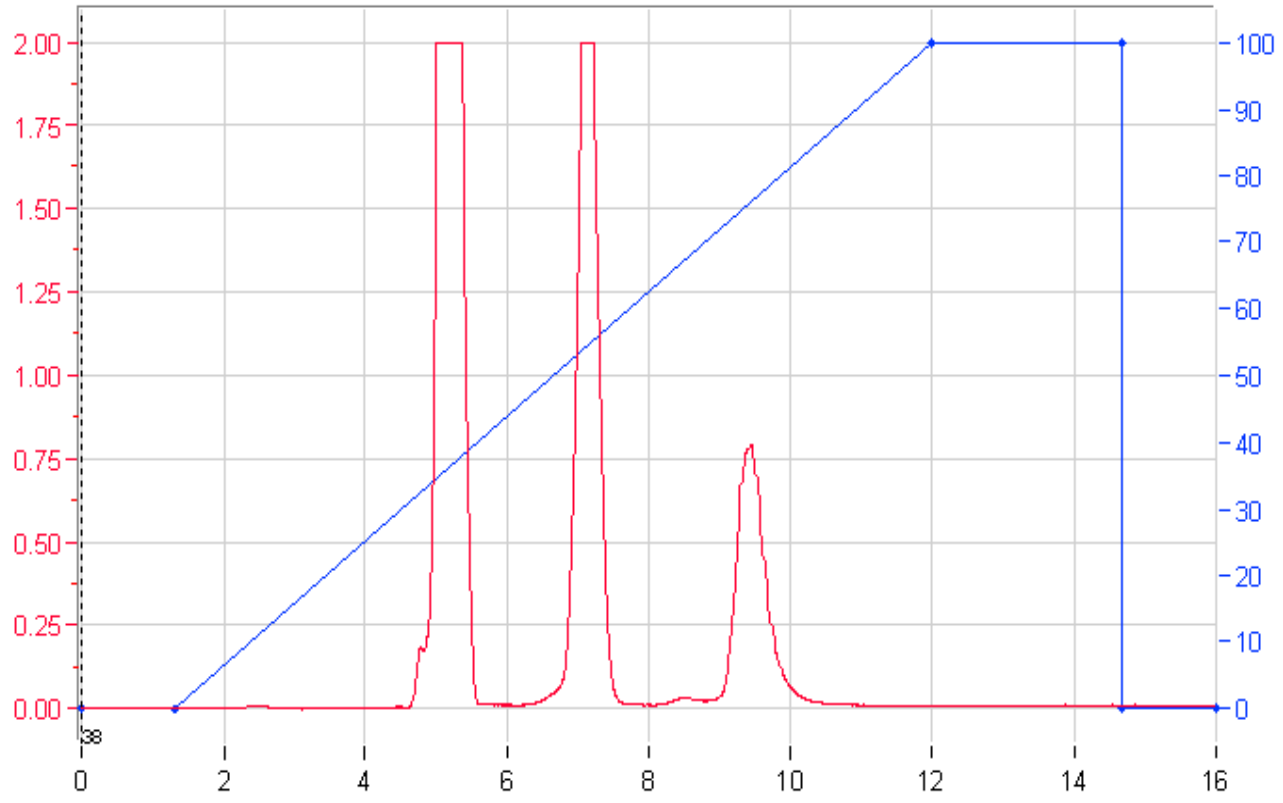
Scaled to 330g Column



RediSep Column: 330 g
Sample mass: 5.9g
Run length: 16.0 CV

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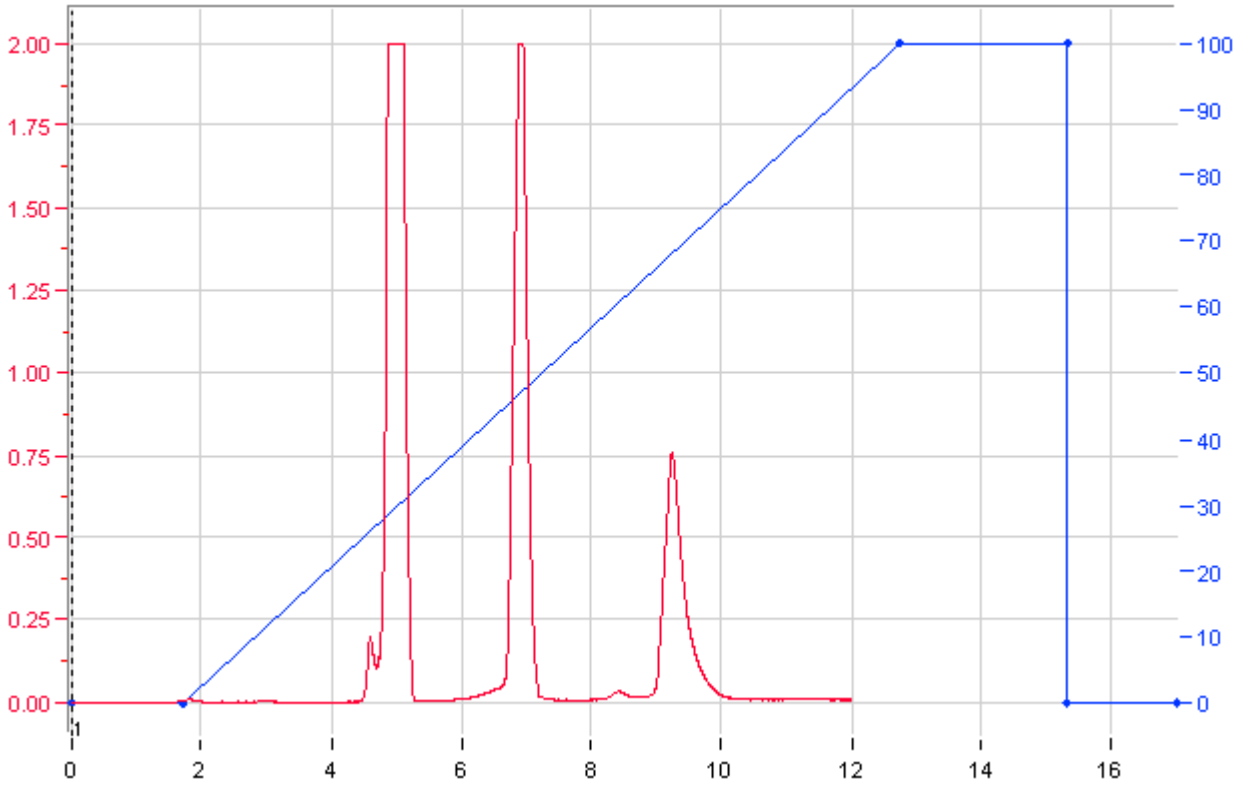
Scaled to 1.5 kg Column



RediSep Column: 15000g
Sample mass: 27g
Run length: 17.0 CV

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Load Method from Column

FILE

IDLE

FILES

RENAME COPY PASTE DELETE

Keyword

Search

	FILE DATE
7-1.run	2017-03-10
l-1.run	2017-03-09
170309@13-48-1.run	2017-03-09
170309@13-34-1.run	2017-03-09
170309@13-17-1.run	2017-03-09
170309@13-04-1.run	2017-03-09
170309@12-56-1.run	2017-03-09
170309@12-46-1.run	2017-03-09

0.10 0.05 0.00 0.0 ABS.

100 90 80 70 60 50 40 30 20 10 0 18.0 %B

REDI C18

FLOW 40

SOLV 1-H

FILE TYPE ALL FILES (*.*)

FILE NAME

OPEN

CONTINUE LAST RUN ON DETECTED COLUMN

LOAD PREVIOUS RUN FROM DETECTED RACK

CANCEL

Load Method from Rack

FILE

IDLE

FILES

RENAME COPY PASTE DELETE

Keyword

Search

FILE DATE

7-1.run	2017-03-10
1-1.run	2017-03-09
8-1.run	2017-03-09
170309@13-34-1.run	2017-03-09
170309@13-17-1.run	2017-03-09
170309@13-04-1.run	2017-03-09
170309@12-56-1.run	2017-03-09
170309@12-46-1.run	2017-03-09

FILE TYPE

ALL FILES (*.*)

FILE NAME

OPEN

CONTINUE LAST RUN ON DETECTED COLUMN

LOAD PREVIOUS RUN FROM DETECTED RACK

CANCEL

0.10 0.05 0.00 0.0 ABS.

100 90 80 70 60 50 40 30 20 10 0 18.0 %B

C18

40

Chromatography Options

ACCQPrep HP150
Prep HPLC

EZPrep
Hybrid Prep/Flash LC

NextGen
Flash LC

Torrent
Proccess Flash LC

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LC Comparisons

LC Method	Teledyne ISCO	Particle Size	Flow Rate Range	Back Pressure	Capacity
Process Flash	Torrent/ NextGen	20-60 μ	0.3-1 L/min	25-75 PSI	g-100's g
Flash	NextGen/ EZPrep	20-60 μ	>18 mL/min	10-50 PSI	mg-g
Prep HPLC	EZPrep/ ACCQPrep	5-20 μ	3-100 mL/min	200 – 2000 PSI	10-500 mg
Analytical HPLC	NA	5 u	1 mL/min	500- 6000 PSI	<5 mg
UHPLC	NA	1.3-3 μ	0.1-0.5 mL/min	>6000 PSI	----

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Thank you!!

Guidelines & Tactics for Flash Chromatography

Free download

www.teledyneisco.com/flashguide

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