A Novel Method for Semi-Preparative Purification of Natural Compounds Using Isolation Gradients

Using the

Gilson GX-281

Purification System

GILSON®

Introduction:

- Preparative chromatography is often employed to purify large quantities of compounds
- Preparative columns (50.0 mm ID) are very expensive (>$5,000) and consumable instability associated with the column bed is a major concern
- Major cost is associated with synthesizing large (gram) quantities of a compound
- Time and labor are put forth in preparative synthesis to optimize the chemistry and minimize by-products that decrease overall yield of the compound of interest

Introduction, continued:

- Successful synthesis yield a small amount 2-5% of the overall mass to byproducts
- Generic gradient condition of 5 to 95% organic for all compounds
- Late eluting compounds can crash out of solution with low starting organics causing over-pressuring and diminished recoveries
- New approaches are being explored to separate the byproduct from the compound of interest.....

About Isolation Gradients

- *"Isolation Gradient"* offers an alternative separation technique for non-optimized synthetic schemes or natural product isolation (complex mixtures)
- *"Isolation Gradient"* :
  - Based on an analytical chromatographic analysis (LC/MS)
  - The compound of interest is identified and the (RT) retention time is implemented in an *"Isolation"* gradient template
  - The value represents the optimum separation condition in a minimal chromatographic window (shallow semi-preparative gradient)
  - The compound of interest is separated from all apposing interferents; column is also cleaned prior to the next injection, "non-isocratic"

Getting Started:

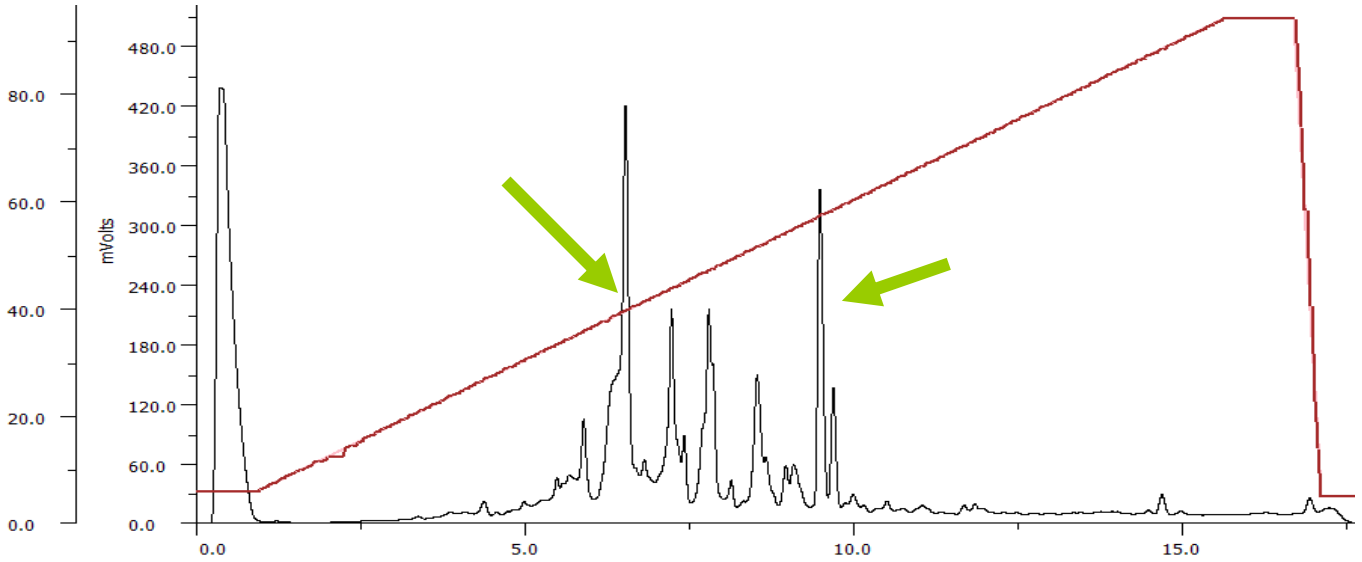
Steps to Perform:

- Perform an analytical scout separation using a general gradient
- Identify the compound RT (retention time)
- Import or use the RT to determine the "Isolation Gradient" with the GX-281 Purification System
- Run "*Isolation Gradient*" w/ fraction collection

Isolation Gradient Technique:

- A basic and reliable purification technique useful:
  - When the compound of interest is in a complicated field and needs to be collected
  - When reducing solvent consumption is a factor
  - When building optimizing separation is necessary for effective fraction collection
- *Example using* acetone extract of Greek oregano
  - Shown to be a substantial source of active compounds, antioxidants (hydroxylated-aromatics)
  - A complicated chromatogram where specific components associated to anti-oxidant activity require isolation

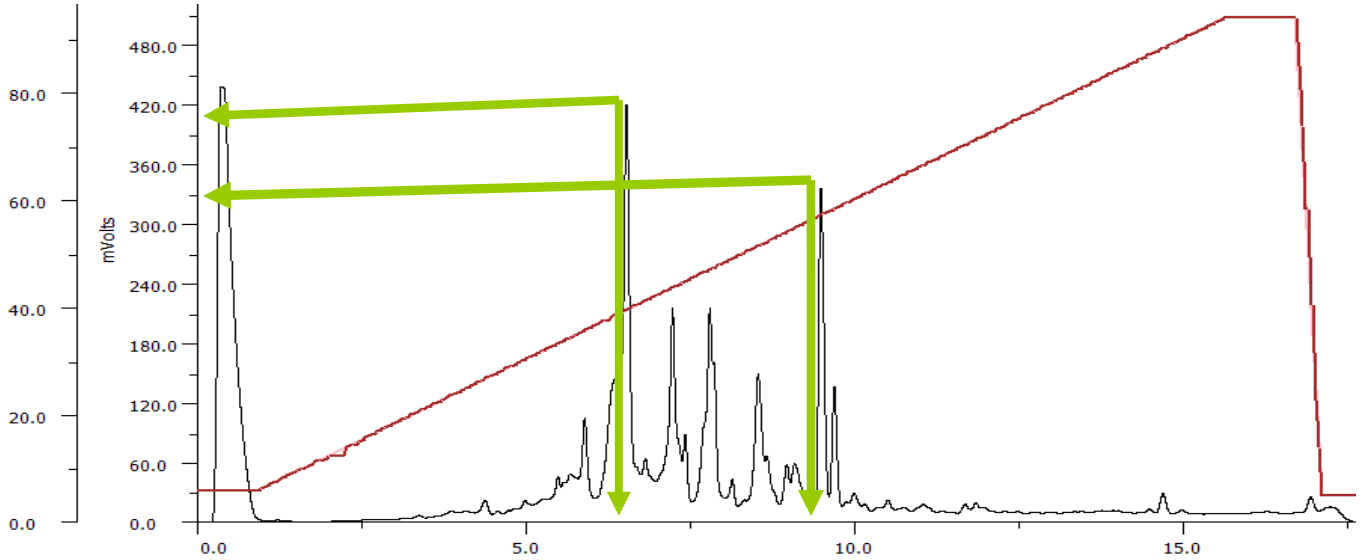
Analytical Scouting *"AS":*



Graph 1:

"AS" determined the "*Isolation gradient*" window that allowed the isolation of the active compound from a complicated array for semi-prep fractionation. The analytical sample was analyzed via UV and MS to finger print the desired compounds.

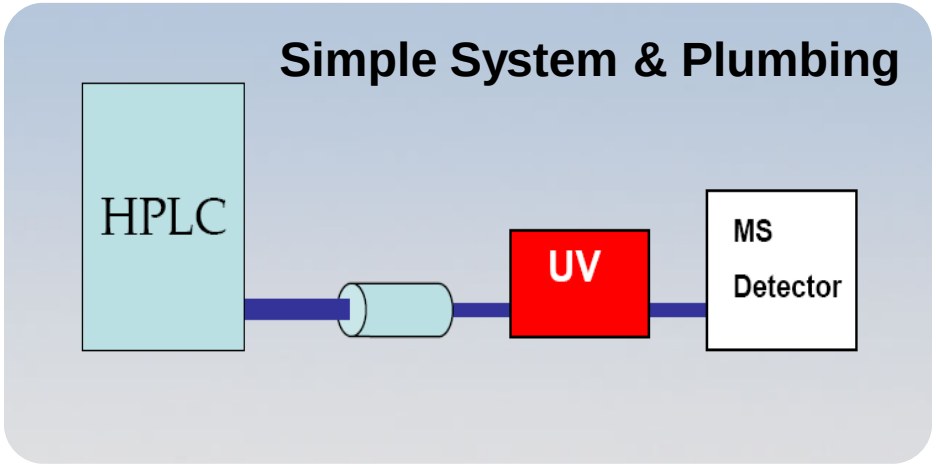
Analytical Scout – Determination of RT and Gradient



Graph 2: Analytical Analysis of active compounds Rosmarinic Acid and Carvacol, in Oregano. The analytical run determines the shallow gradient required for the isolation of specific compounds, based on the retention time and the slope of the analytical run the shallow "isolation gradient" will separate the compound of interest.

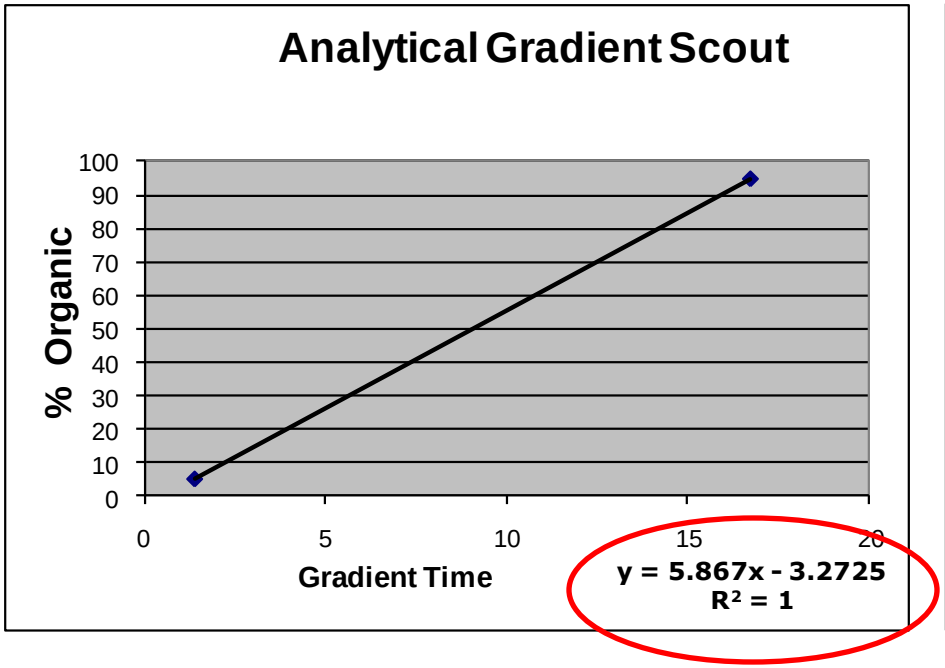
Analytical LC/MS Information

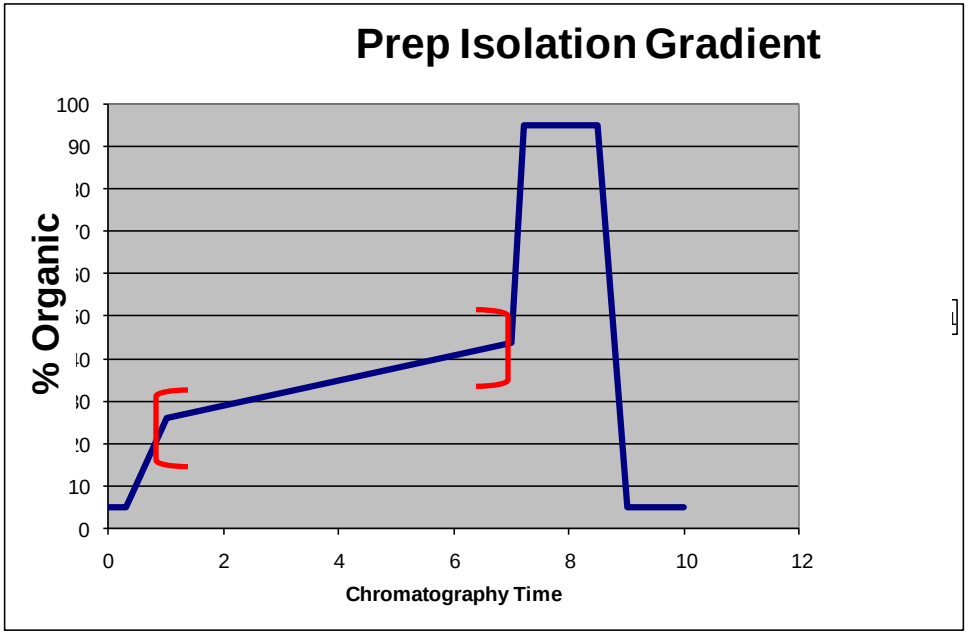
- Many MS Options
  - Single Quad
  - Dual Quad
  - Triple Quad
  - TOF



- Interested only in compound identification
- Column packing, pore size are identical to prep column

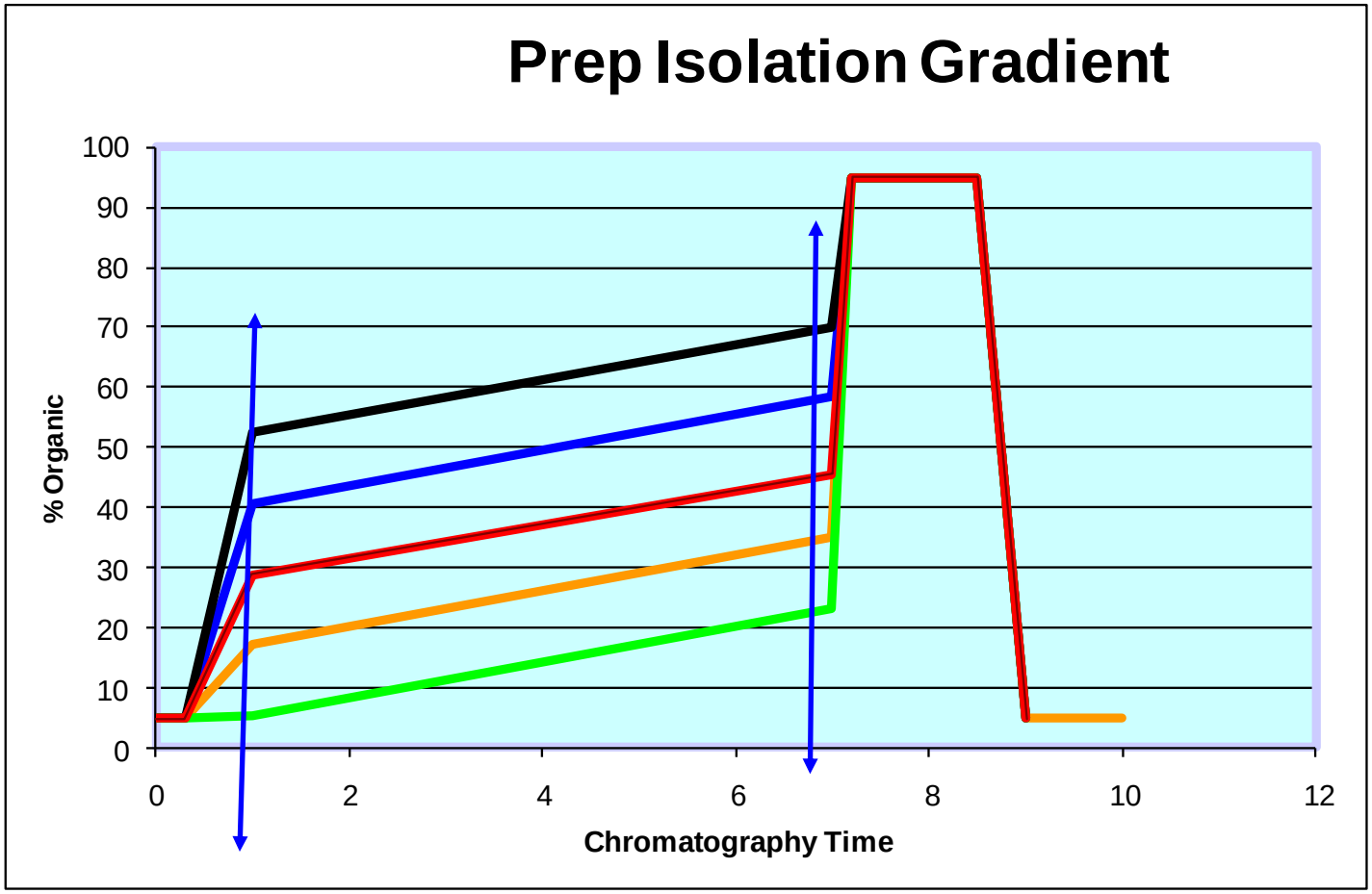
Determination of Preparative Isolation Gradient:





Linear equation calculated from the analytical run is used to determine the beginning and end % organic employed in the isolation gradient

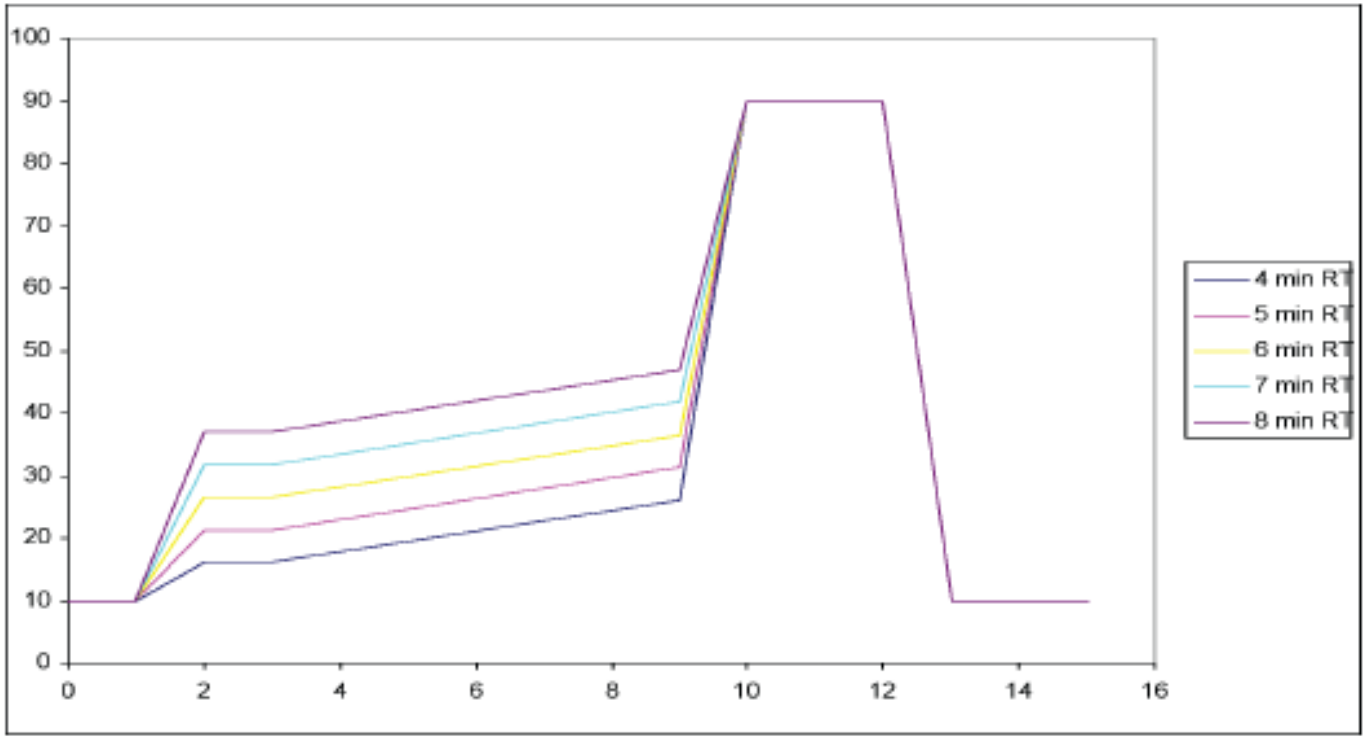
Various Isolation Gradients:



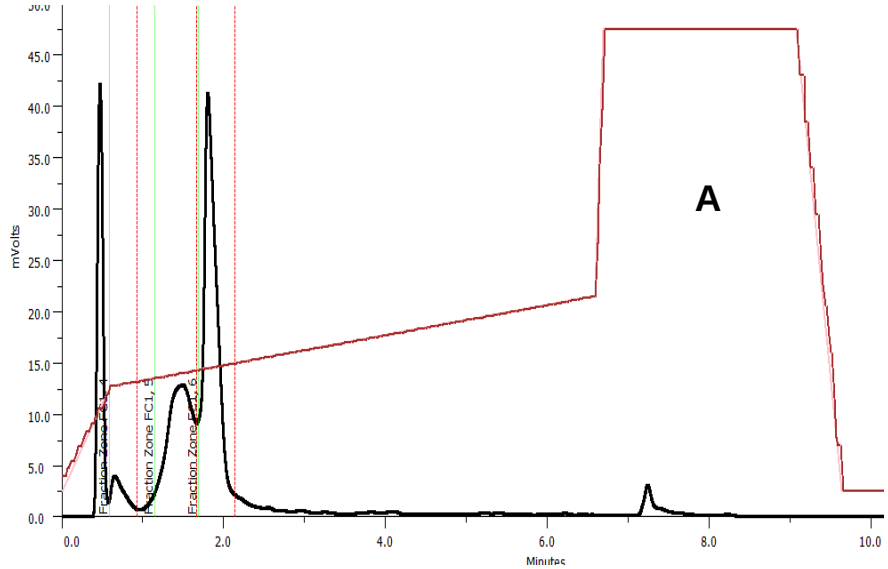
TRILUTION® LC Software Variables:

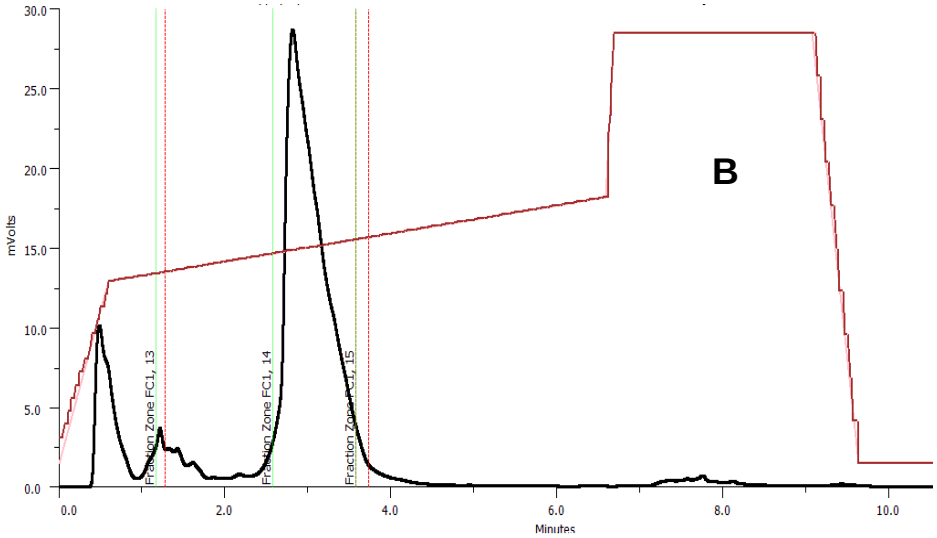
Start %B = ((Slope of Analytical Gradient) * (Peak RT) + (Intercept of Analytical Gradient)) * (Correction Factor) - (Active Gradient % Change/2)

End %B = ((Slope of Analytical Gradient) * (Peak RT) + (Intercept of Analytical Gradient)) * (Correction Factor) + (Active Gradient % Change/2)



Isolation Gradient of Oregano Extract:



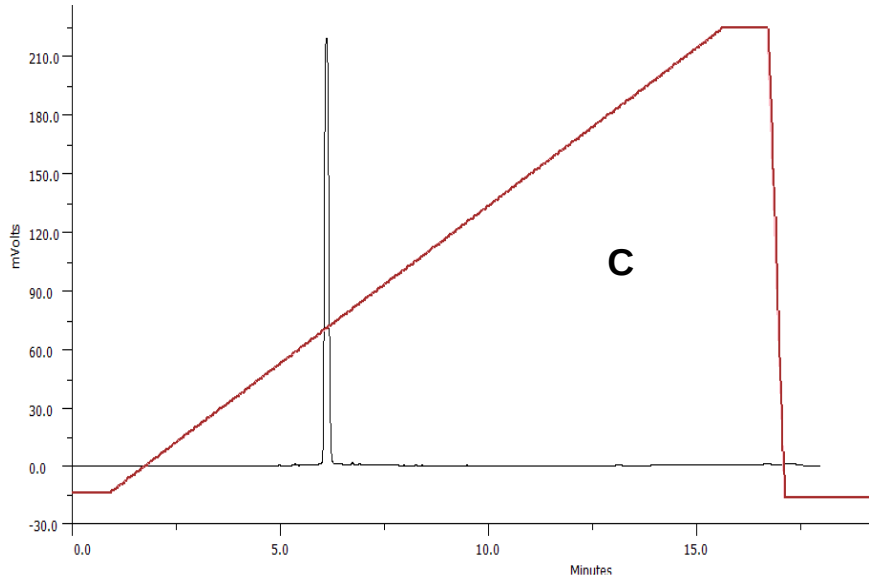


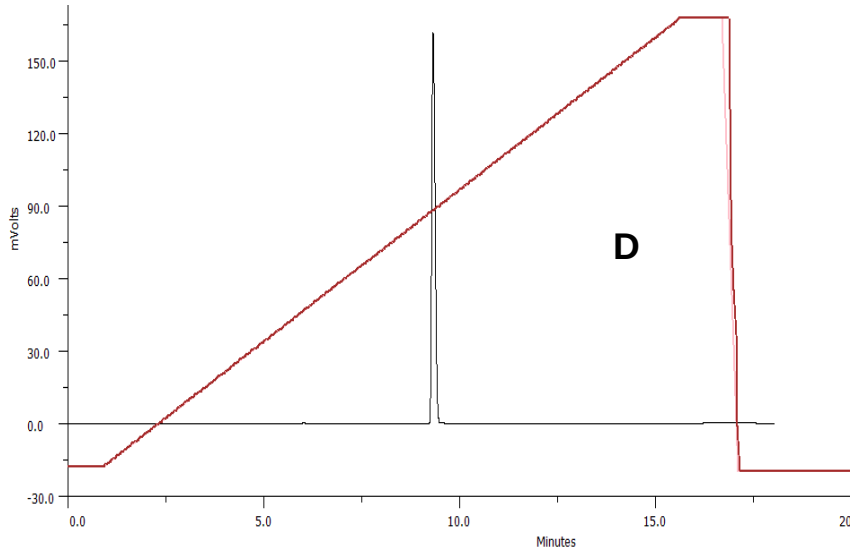
Chromatograms A & B: 50 mgs injected @ 30 mL/min

Results from the preparative isolation gradient

Rosmarinic acid (A) and Carvacol (B)

Re-Injection of Collected Fractions from Isolation Gradient:

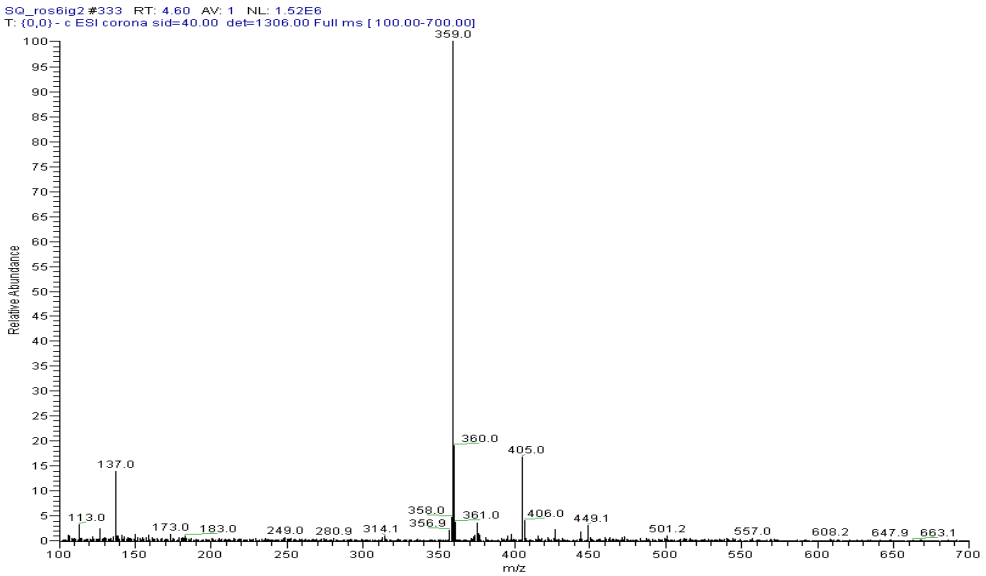




Chromatograms C & D: >95% recovery

Reinjections of the collected fractions from prep isolation gradient for: Rosmarinic acid (A) and Carvacol (B)

MS evaluation of Collected Fraction from Isolation Gradient:



Graph 3: Represents the MS for Rosmarinic acid (MH^- 359) collected from the preparative isolation gradient. A 20 uL injection was introduced to the MS, 4.6 x 50 mm Luna, C-18, 2 mL/min. Carvacol does not give a MS spectrum.

Summary:

- Isolation gradient chromatography offers an additional option based on the RT and % organic of the peak of interest within a complicated chromatogram
- The compound RT information from the analytical general gradient is used to determine gradient of the semi-preparative purification run; TRILUTION LC variables add flexibility
- Optimizing the gradient selection for the compound of interests' retention time can greatly increase recovery as well as reduce maintenance issues

Summary, continued:

- Narrow isolation gradients provide benefits of mass based purification such as reduced fractions
- Narrow isolation gradients produce excellent purification and recovery of compounds from complex chromatographic analytical scouts; recoveries > 95%
- Isolation gradients allow for optimized chromatography and greater separations from interfering components; >95% recovery
- Analytical scouting with MS analysis offers the information required for semi-preparative separations