Troubleshooting & Chromatographic Terms

Troubleshooting

- When you run into a problem, what are good steps to follow to solve the problem?
  - Ex: Trying to win a video game?
  - Ex: Trying to fix a printer issue?
  - Ex: Trying to fix a broken car?

Troubleshooting 101

- In Chromatography problems can arise from many sources
- Effective troubleshooting means
  1. Defining the problem
  2. Isolating the source (finding the cause)

- How should effective troubleshooting be done?
  - Logically, systematically, intelligently, experientially, collectively
  - This is true for all troubleshooting

Troubleshooting 101

1. **Don't add to the problem**
   - Think before you act
   - Once the problem is evident, make sure that your actions will not contribute to the problem: don't make things worse
2. **Define the Problem**
   - Identify the symptoms and accurately define the problem
   - The real cause may not be the most apparent thing
   - Only by identifying the true problem can you find the correct solution
   - Symptoms are not the same as problems
   - **SIMPLE THINGS FIRST**

Troubleshooting – Define the Problem

- What is the problem/symptom?
  - Runny nose
  - Cough
  - Aching joints
- These are all symptoms, the cause is the flu

Troubleshooting Instrumentation

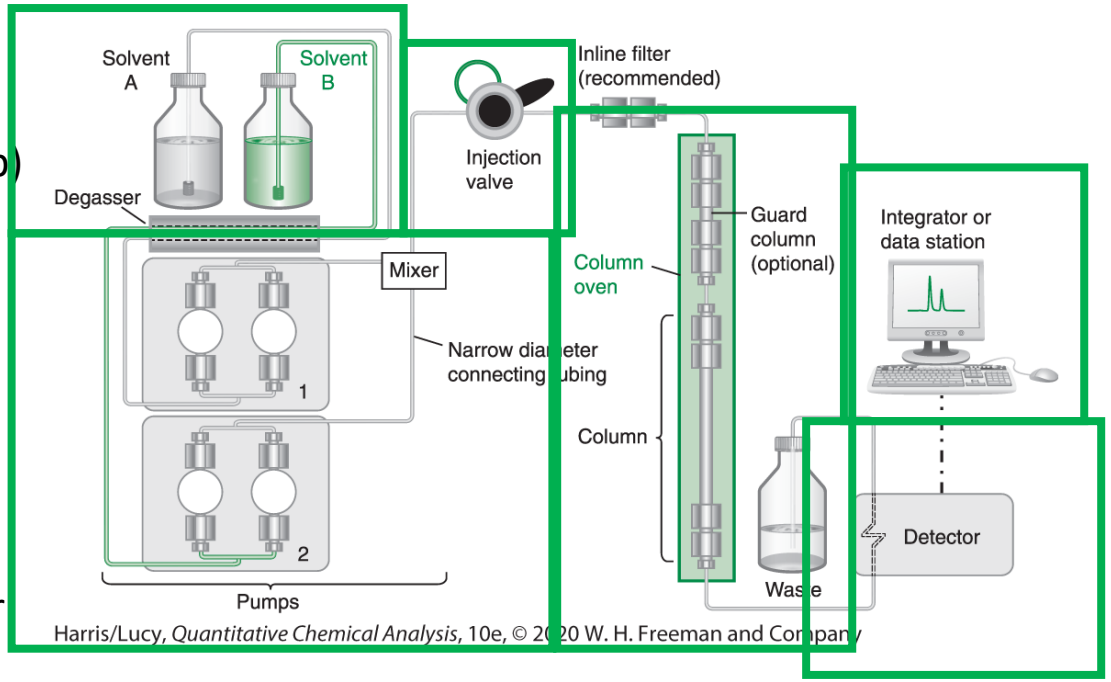
Need to know how each piece fits together to properly troubleshoot the instrument

The HPLC system consists of

- Solvent
- A solvent delivery system (pump)
- A sample injection valve
- A chromatographic column
- A detector
- A data system

Other important aspects to consider

- Sample and standard preparation



Chromatograms as Tools

- Used to identify and quantify

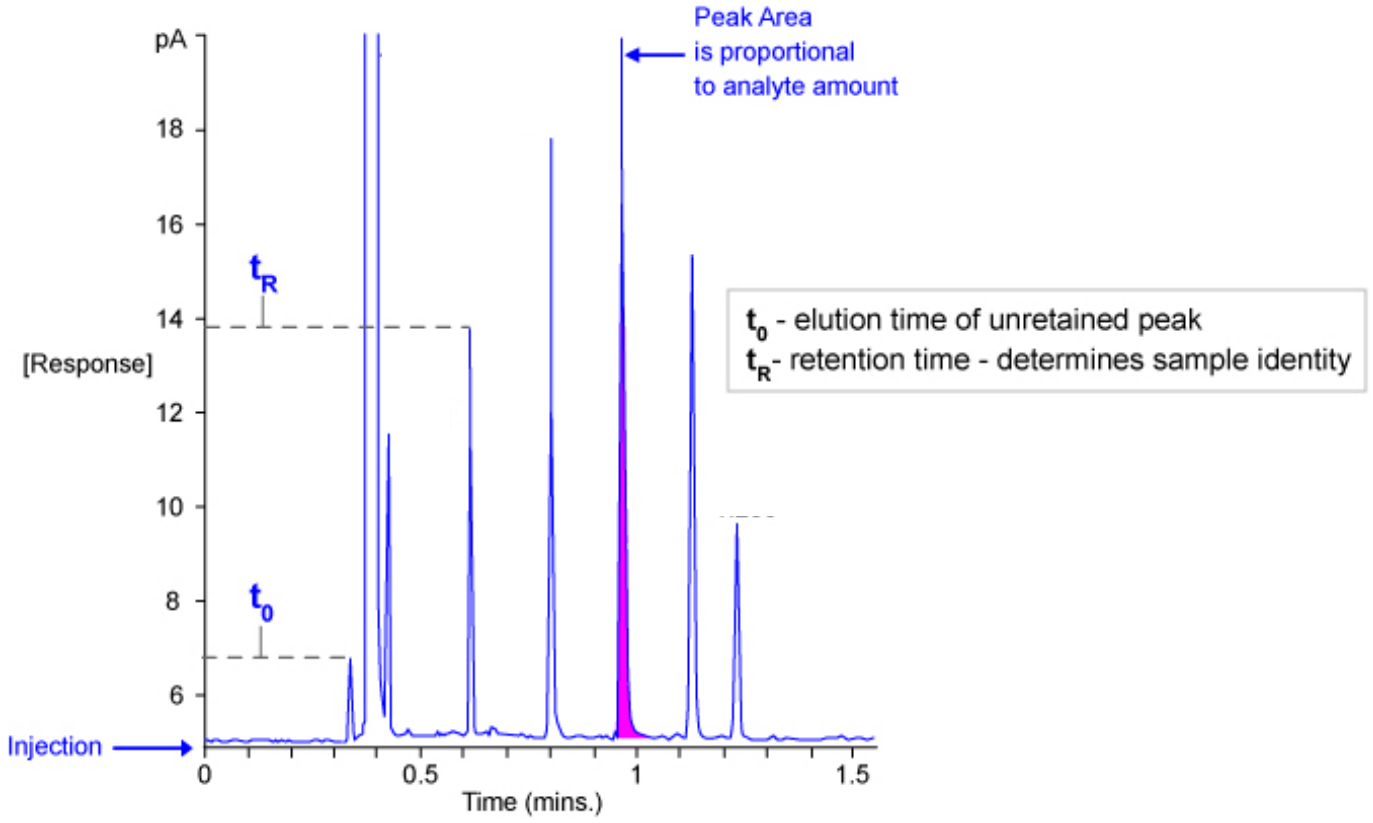


Image credit: ChromAcademy

Chromatograms as Tools

- Can also tell us about
  - The health of the column and the instrument
  - The suitability of the instrument & column for a particular analysis

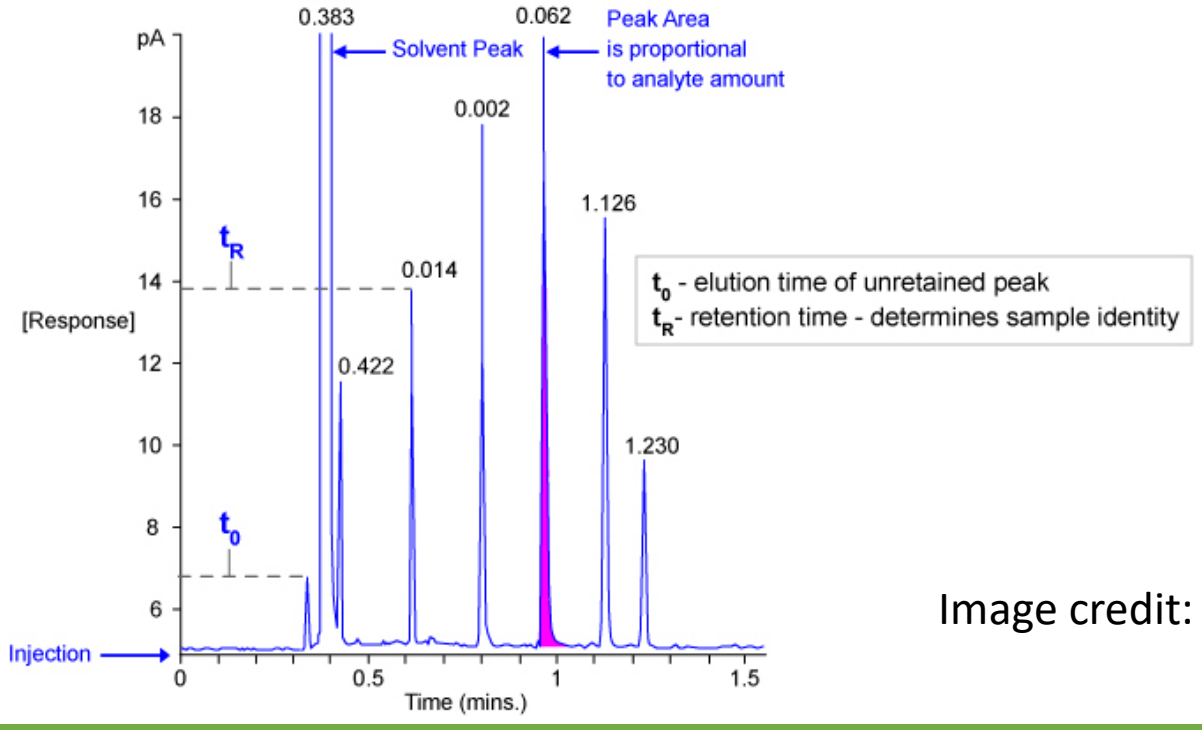


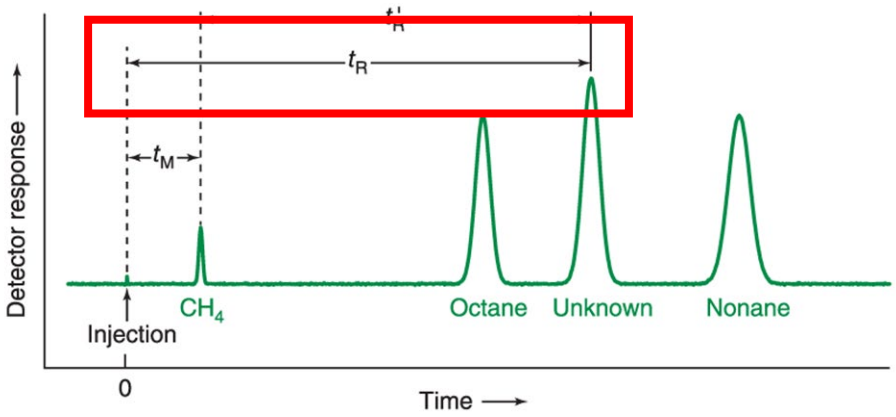
Image credit: ChromAcademy

Chromatography Terms

- We can describe a separation using a number of parameters including
  - The separating power of a column
  - The retention of a compound on a column
  - The degree of separation between two peaks
- These terms allow us to compare different analyses, columns, instruments, and methods

Retention Time

- Chromatogram is a graph of detector response (y) vs time (x)
- Retention time t_R
  - How long it takes for a compound to go from the injector to the detector



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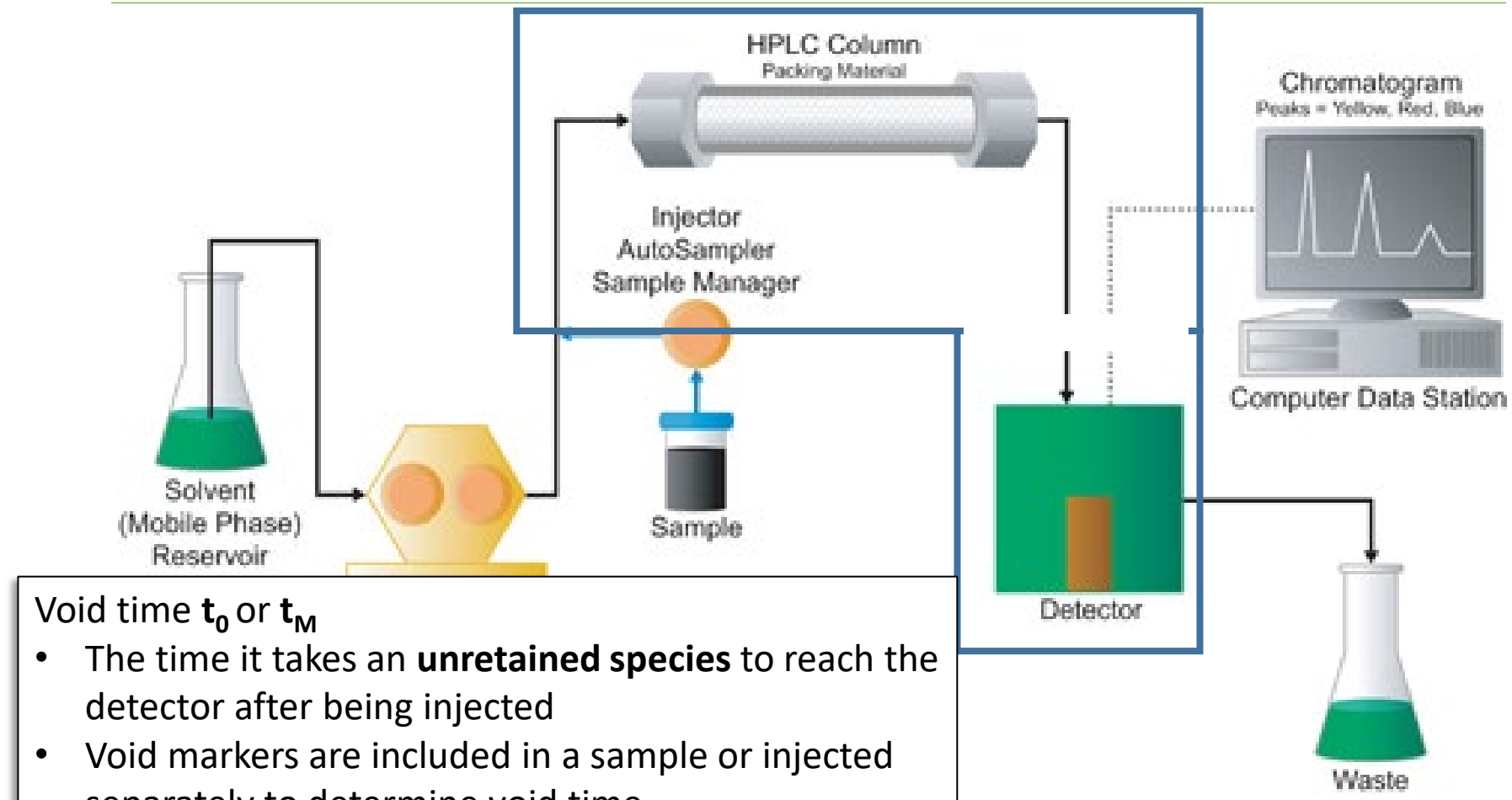
Mobile phase or an unretained solute (methane) travels through the column in the minimum possible time, t_M.

Void Time/Void Marker



Void time $\mathbf{t_0}$ or $\mathbf{t_M}$

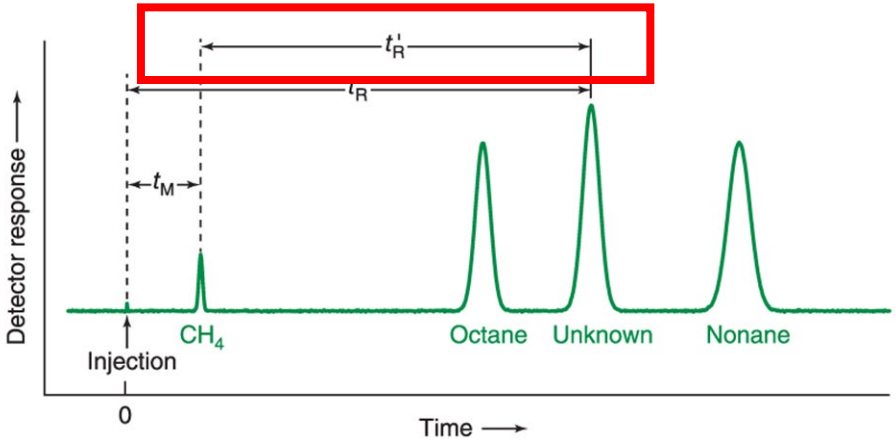
- The time it takes an **unretained species** to reach the detector after being injected
- Void markers are included in a sample or injected separately to determine void time

Image courtesy of Waters Canada

Retention Time: t_R

- Chromatogram is a graph of detector response (y) vs time (x)
- Retention time t_R
  - How long it takes for a compound to go from the injector to the detector
  - Elution time
- Adjusted retention time t'_R
  - The time it takes for a compound to elute, beyond that required for the void marker

$$t'_R = t_R - t_M$$



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Mobile phase or an unretained solute (methane) travels through the column in the minimum possible time, t_M.

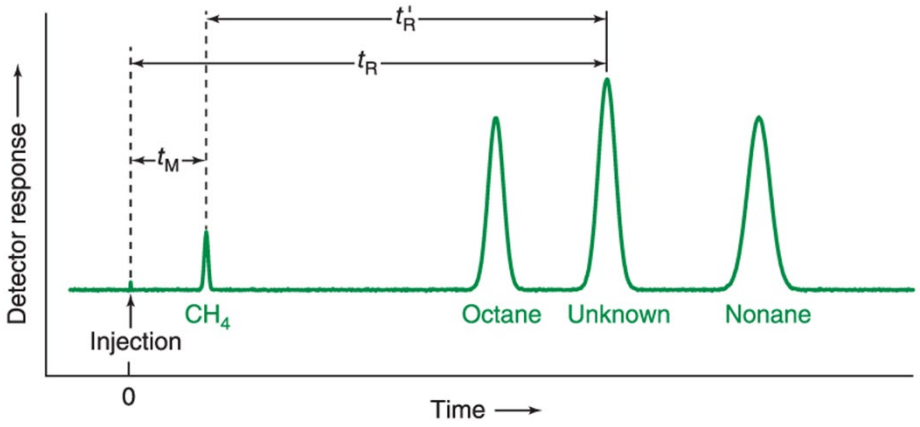
Retention Factor: *k*

- Retention factor
  - Used to describe the degree of retention for a compound

$$k = \frac{t_R - t_M}{t_M} = \frac{t'_R}{t_M}$$

  - As $t_R \uparrow$, $k \uparrow$

.



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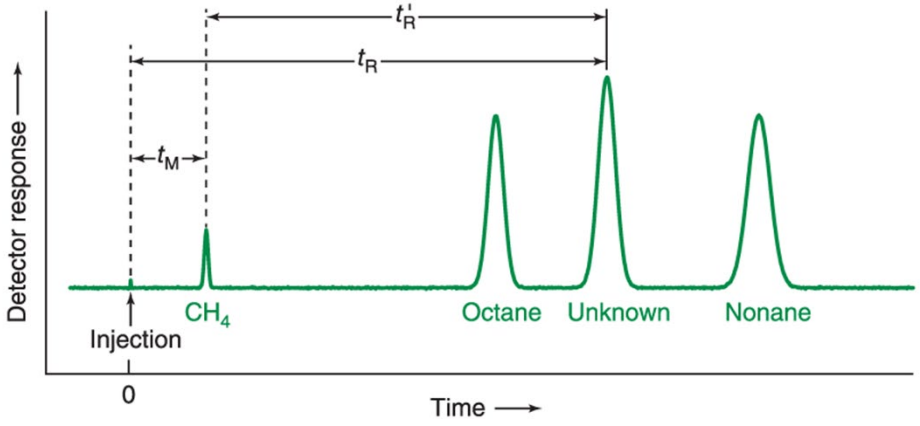
Selectivity or Separation Factor: α

- **α** is the ratio of the adjusted retention times for two solutes.
- Provides relative selectivity of compounds
  - Often used as a criteria for a method

$$\alpha = \frac{t'_{R2}}{t'_{R1}} = \frac{k_2}{k_1}$$

where component 2 is eluted later than component 1

Figure 23-7



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Practice Problem 2

- Compounds A and B have retention times of 2.142 and 2.193 min, respectively, on a 15-cm column.
- An unretained species passes through the column in 0.864 min.
- The peak widths for A and B are 0.051 and 0.052 min, respectively.
- Calculate:
  - The separation factor/selectivity for the separation

$$\alpha = \frac{t'_{R2}}{t'_{R1}} = \frac{k_2}{k_1}$$

Selectivity or Separation Factor: α

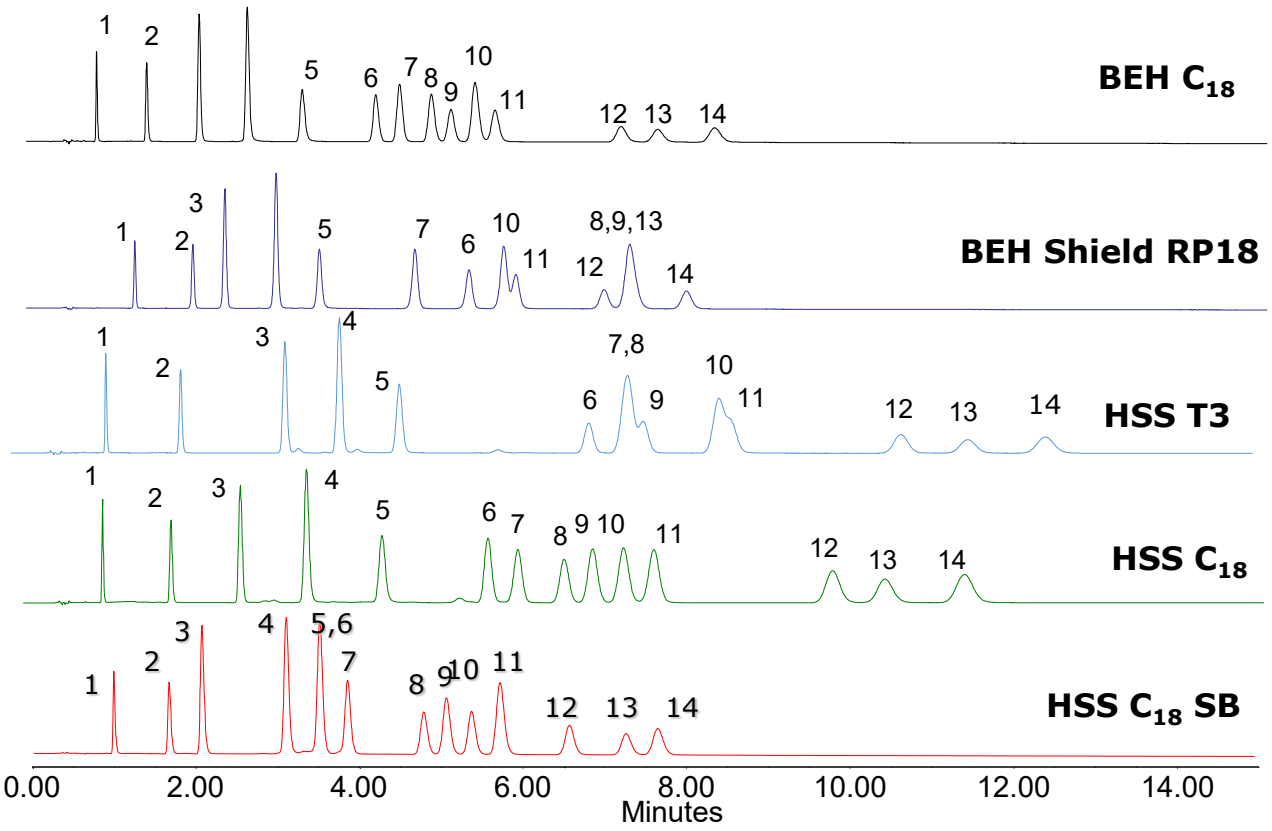
- The term selectivity is also used qualitatively to mean the elution order and relative degree of separation for a set of compounds

Selectivity Changes - Example

Conditions :
Columns: 2.1 x 100 mm

- We would say the selectivity is changes for these different columns
  - Meaning the elution order and/or relative degree of separation

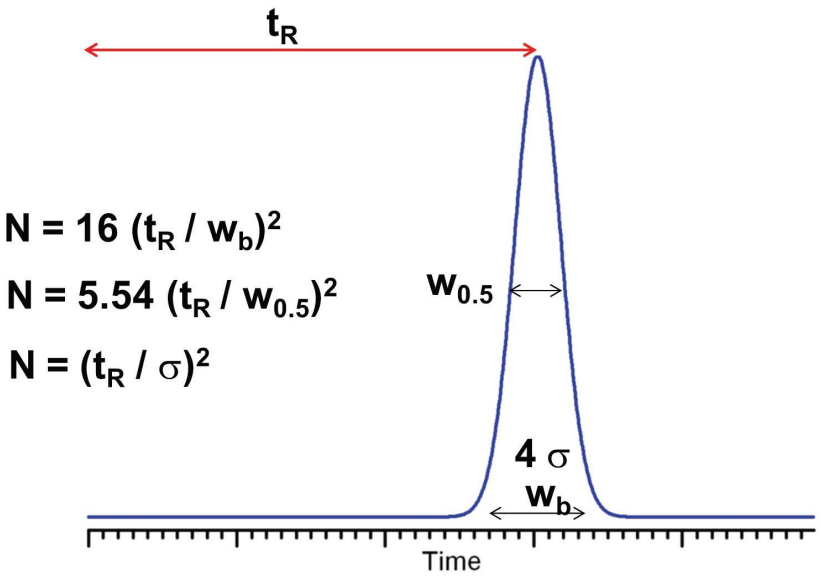
4. 1,3-DNB
5. NB
6. Tetryl
7. TNT
8. 2-Am-4,6-DNT
9. 4-Am-2,6 DNT
10. 2,4-DNT
11. 2,6-DNT
12. 2-NT
13. 4-NT
14. 3-NT



Courtesy of Waters Canada

Efficiency or Plate Count

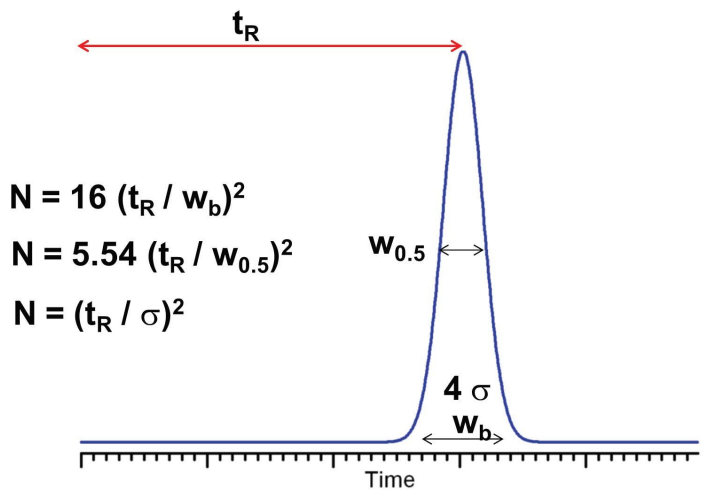
- N = the efficiency of a column
  - A gauge of how well the column is performing
- Should stay constant
  - If changes perhaps the column is damaged or not connected properly
- $W_{0.5}$ is easiest to measure
  - Esp if baseline separation not achieved



http://www.sepscience.com/Techniques/LC/Articles/317-/HPLC-Solutions-32-Back-to-Basics-4-Efficiency

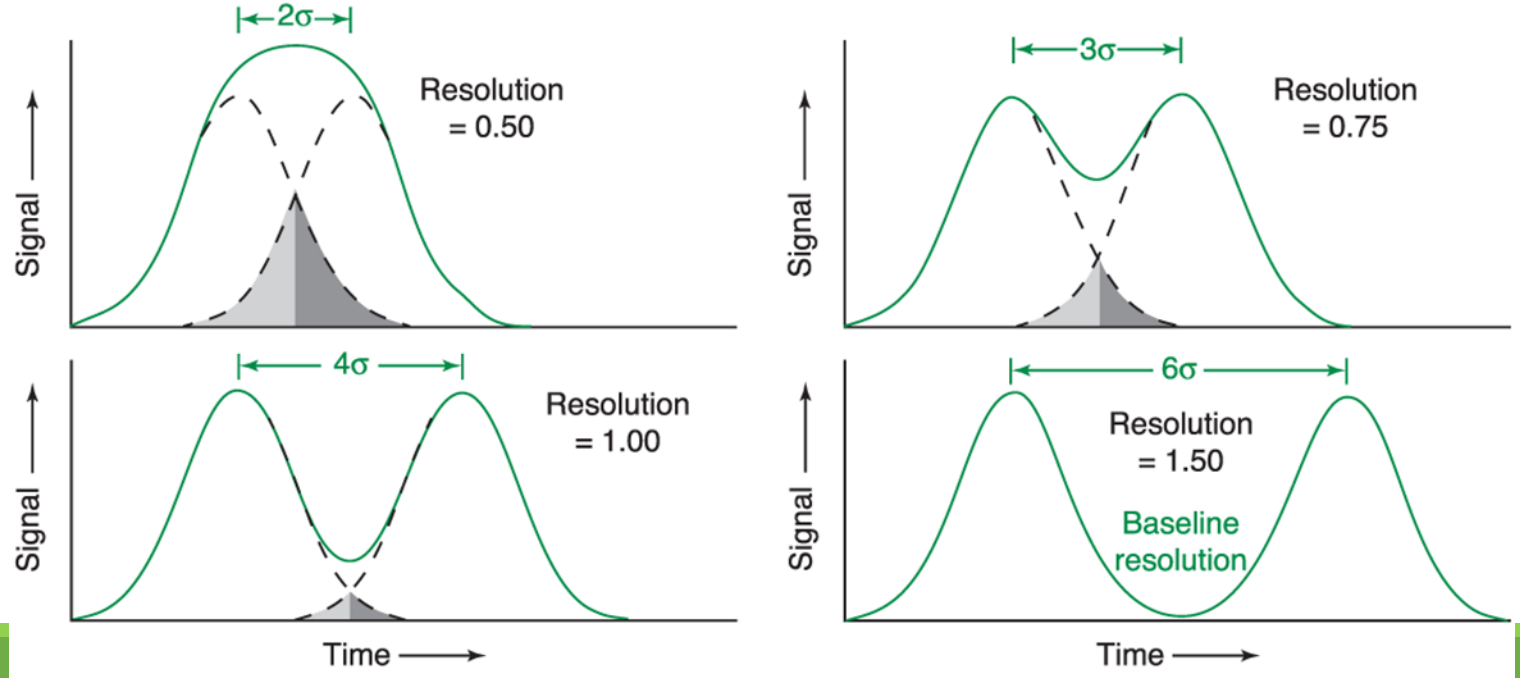
Practice Problem 3

- Compounds A and B have retention times of 2.142 and 2.193 min, respectively, on a 15-cm column.
- An unretained species passes through the column in 0.864 min.
- The peak widths at base for A and B are 0.051 and 0.052 min, respectively.
- Calculate:
  - The efficiency for the last peak



Resolution Between Peaks

- $Resolution\ (Rs) = \frac{2*|t_{RB} - t_{RA}|}{W_A + W_B}$
- Separation at **base** of peaks
- Baseline resolution is ideal
  - Where the peaks touch down between each other (Rs = 1.5)
- Many methods have a minimum resolution requirements



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Practice Problem 4

- Compounds A and B have retention times of 2.142 and 2.193 min, respectively, on a 15-cm column.
- An unretained species passes through the column in 0.864 min.
- The peak widths for A and B are 0.051 and 0.052 min, respectively.
- Calculate:
  - $Resolution\ (Rs) = \frac{2*|t_{RB} - t_{RA}|}{W_A + W_B}$

Selectivity vs Resolution

- Both take into account the separation of the peaks at their apex
  - Only resolution includes the width of the peaks

Tailing Factor

- In an ideal world peaks are symmetrical
- In HPLC “tailing” can be problematic
  - Decreases resolution
  - USP tailing calculated by software
  - Many methods have a USP Tailing factor pass/fail criteria

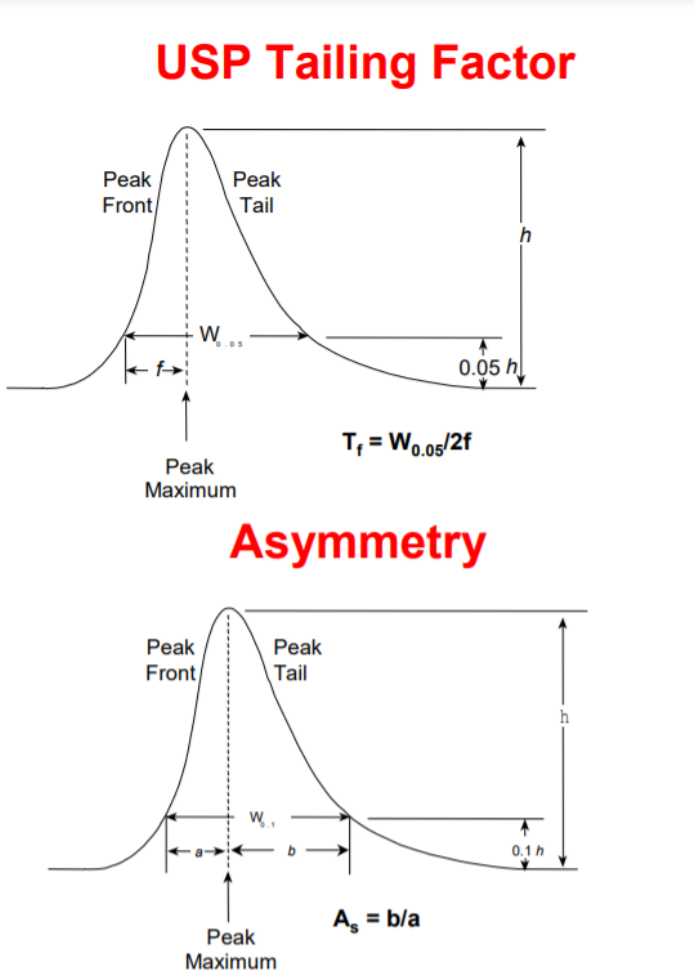
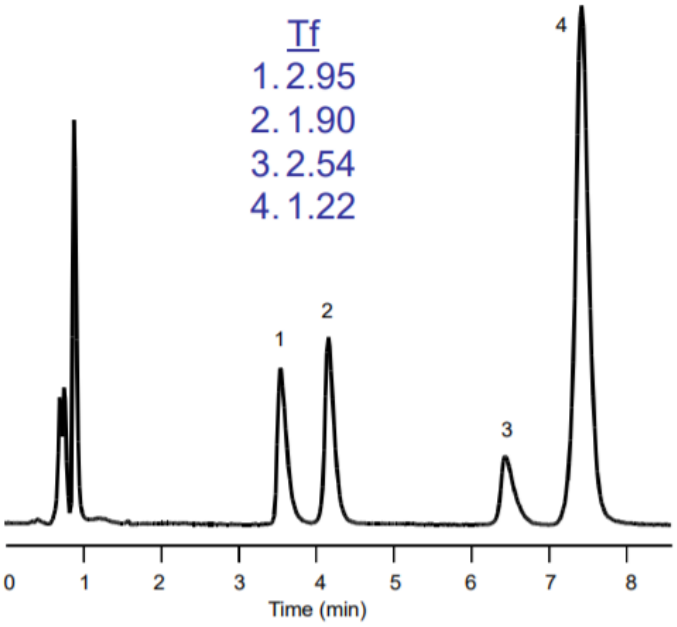


Image: https://www.agilent.com/cs/library/eseminars/Public/secrets%20of%20good%20peak%20shape%20in%20hplc.pdf

Examples of Tailing



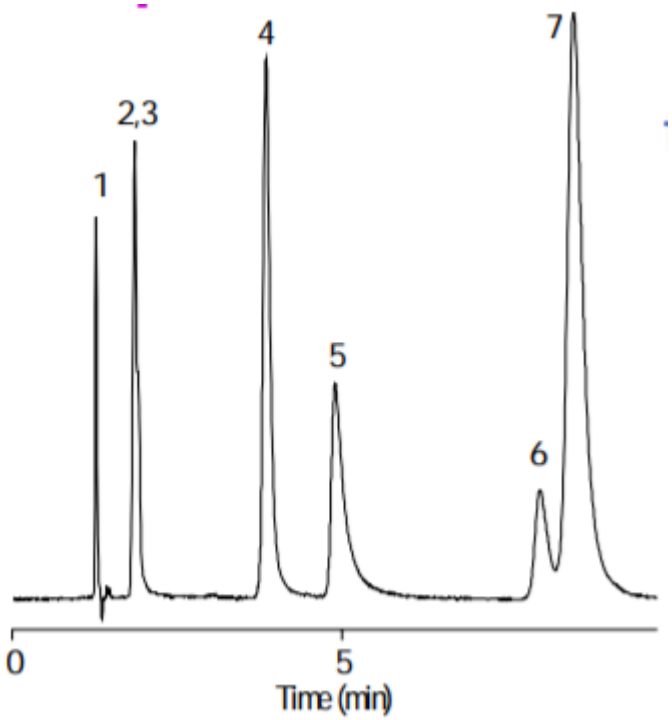
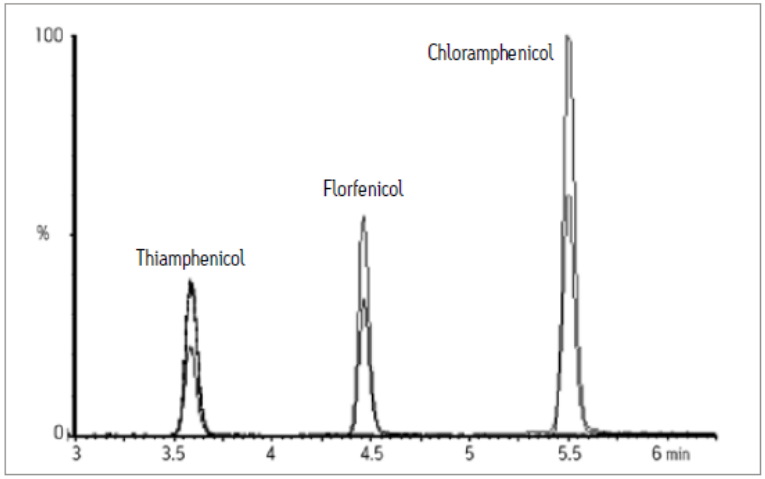


Image: https://www.agilent.com/cs/library/eseminars/Public/secrets%20of%20good%20peak%20shape%20in%20hplc.pdf

Applications to Troubleshooting

- We can compare these terms to monitor for changes and evaluate methods for success
  - Changing retention times may indicate problems with the flow rate, column, or mobile phase
  - Changing resolution may indicate problems with tubing and/or columns
  - Changing peak areas may indicate a problem with the injector, samples, or standards
  - Changing efficiency may indicate the column is aging and in need of replacement
  - Unacceptable values may indicate the instrument is not suitable or that the method is not fit for purpose

Troubleshooting - Chromatograms



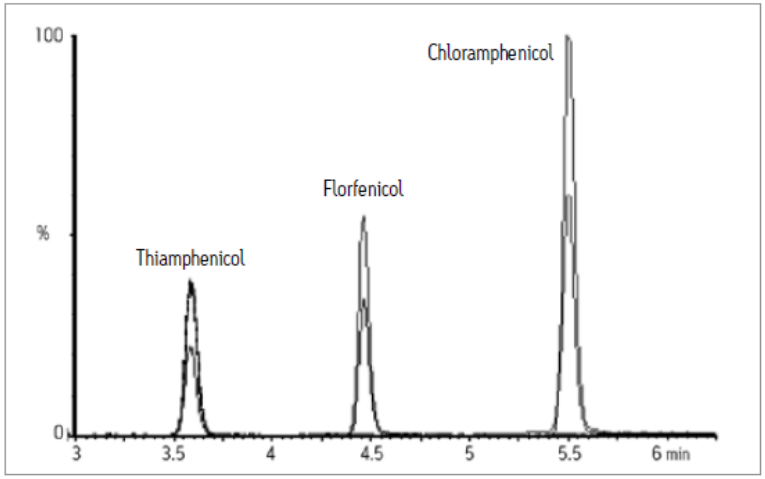
"My peak areas aren't the same"

- Define the Problem
  - All of the peaks or just some?
  - How many injections?
  - How significant?
- *All peak areas for second injection were double the first*

Troubleshooting 101

3. **<u>Gather information</u>**
   - What is and is not affected?
   - Do I know and understand these things?
   - What was happening before the problem happened?
     - What events transpired
   - Give information that is accurate, detailed and all inclusive
   - Don’t leave something out because you think it does not matter
   - Review critically and carefully any hard data the supports the existence of the problem

Troubleshooting - Chromatograms

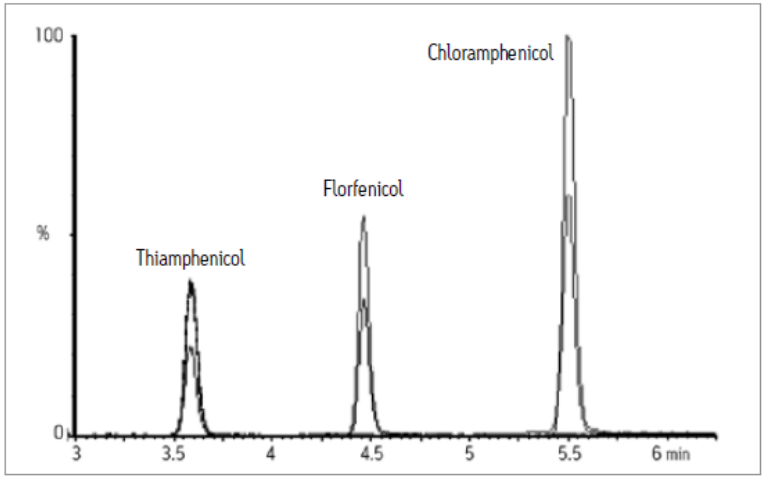


- *Peak areas for second injection were double the first*
- Gather information
  - What is the standard? Or sample?
  - Same instrument? Manual or Autosampler?
  - Same day? Right after each other?
  - Retention times unchanged
  - No new peaks

Troubleshooting 101

4. **<u>Narrow the cause down</u>**
   - Rule #1 – consider the simple things first
   - Eliminate potential causes that would not apply
   - To investigate the issue change or consider ONE thing at a time

Troubleshooting – Chromatograms

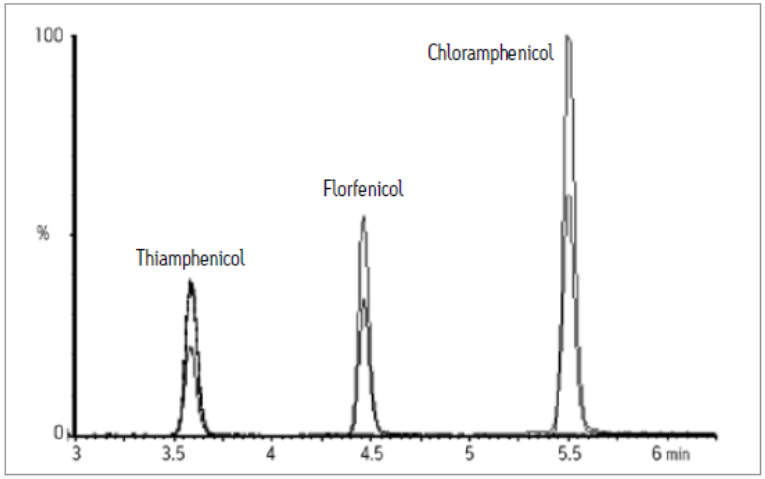


- Potential causes
  - User error with needle (manual injector)
    - Variability in needle fill
    - Needle not fully seated in injector
  - Injector malfunction (autosampler)
  - Wrong standard injected

Troubleshooting 101

5. **<u>Try the options</u>**
   - What is left to try & does it make sense to try it?
   - Keep the problem and what is and is not affected in the front of your mind
   - Get help
     - Choose your help wisely!!!

Troubleshooting – Chromatograms

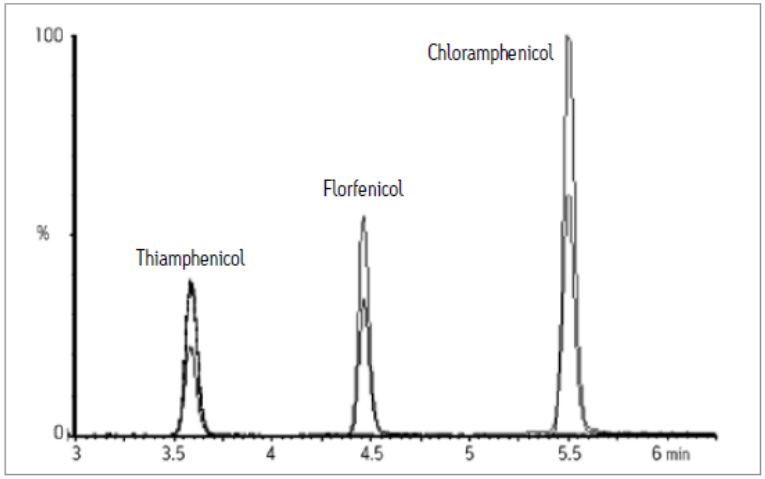


- Information: Autosampler, making multiple injections in series
- Options: Wrong standard injected, autosampler malfunction
- Try: Review injection instructions – did you inject from the same vial twice?
  - Oh! The method says vial 1 then vial 2!

Troubleshooting 101

6. **<u>Evaluate success</u>**
   - Did your solution work?
   - Go tell your boss what happened and how you took care of it: Congratulations - you are gaining experience and prestige!

Troubleshooting – Chromatograms



- Updated method to inject twice from the vial 1 before moving to vial 2.
  - Problem solved

Troubleshooting 101 - Summary

1. Don’t add to the problem

2. Define the Problem

3. Gather information

4. Narrow it down

5. Try the options

6. Evaluate success

Note: If you are not successful, you may have to repeat all 6 steps, or you may not. Do I need to repeat from step 2? from step 3? From step 5?

Summary

- Troubleshooting
  - Should be performed in a linear, logical fashion
    - Know the general steps discussed
  - Keep it Simple
- These key terms discussed are important and will be used routinely in industry