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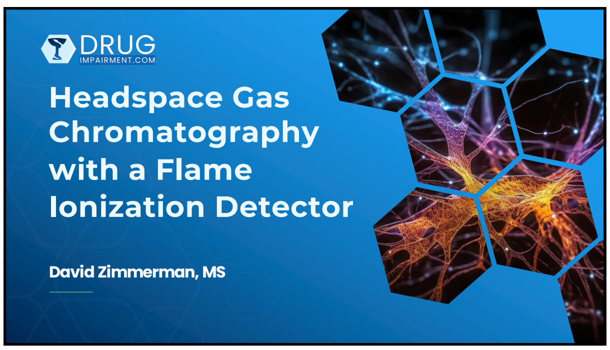
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Headspace Gas Chromatography with a Flame Ionization Detector
David Zimmerman, MS

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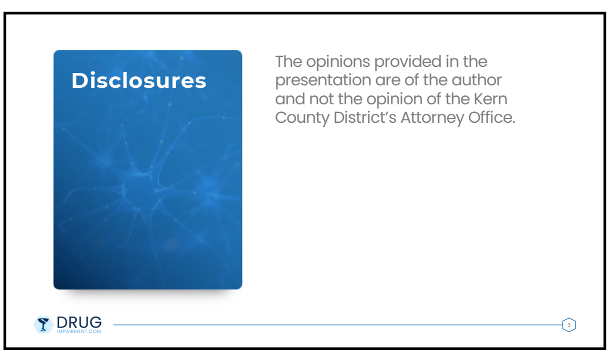
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The opinions provided in the presentation are of the author and not the opinion of the Kern County District's Attorney Office.
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Learning Objectives

1. Understand what headspace sampling is, how it is used, and why it is used in Forensic Toxicology
2. Understand what Gas Chromatography is and how to modify/develop a method
3. Understand what a Flame Ionization Detector is and why it is used in Forensic Toxicology

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Headspace Gas Chromatography with a Flame Ionization Detector

Instrument method used to analyze volatile sample

Headspace (HS) – Type of sampling

Gas Chromatograph (GC) – Separates the compounds

Flame Ionization Detector (FID) – Detects compounds

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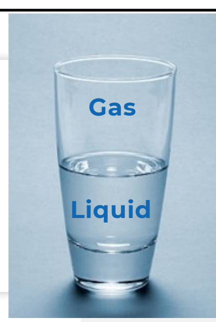
What is Headspace?

Glass Half Full or Half Empty?

Completely Full!

Headspace
Gas phase above a liquid sample in a sealed vial



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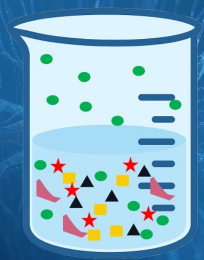
Why Sample the Headspace?

- Blood or Urine Matrix
- Lipids, phospholipids, electrolytes, water, protein, drugs, or alcohol
- Removes Interferents
- Dilute and shoot sample prep
- Only volatile compounds are sampled



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Henry's Law

Henry's Law Definition

At a constant temperature, the amount of a given gas that dissolves in a given type of volume of liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid.

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Henry's Law

Henry's Law Formula

$$P_i = K_h C_i$$

Where P_i = partial pressure of the gaseous solution
K_h= Heny's constant, solute-solvent pair
C_i = concentration of the dissolved gas

Relating this formula to alcohol analysis:
P_i= concentration of ethanol in the headspace
C_i= concentration of ethanol in the sample
K_h=constant for ethanol/water

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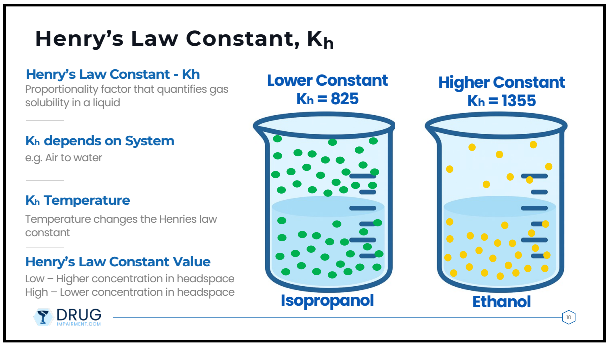
Henry's Law Constant, K_h
Henry's Law Constant - Kh
Proportionality factor that quantifies gas solubility in a liquid
K_h depends on System
e.g. Air to water
K_h Temperature
Temperature changes the Henries law constant
Henry's Law Constant Value
Low – Higher concentration in headspace
High – Lower concentration in headspace
Lower Constant
K_h = 825
Higher Constant
K_h = 1355
Isopropanol
Ethanol
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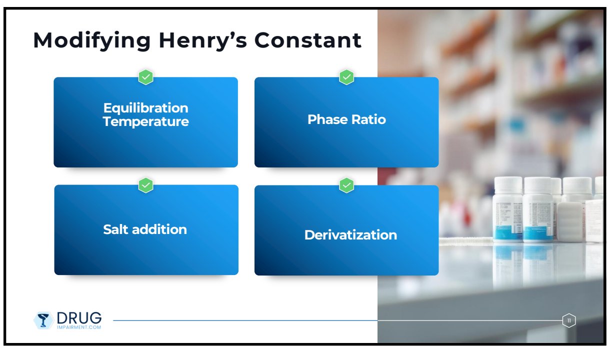
Modifying Henry's Constant
Equilibration Temperature
Phase Ratio
Salt addition
Derivatization
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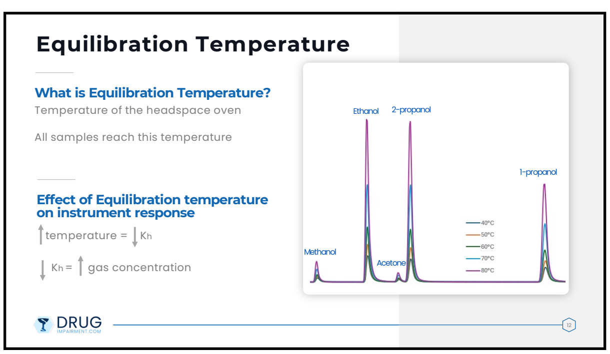
Equilibration Temperature
What is Equilibration Temperature?
Temperature of the headspace oven
All samples reach this temperature
Effect of Equilibration temperature on instrument response
↑temperature = ↓K_h
↓K_h = ↑gas concentration
Ethanol
2-propanol
1-propanol
Methanol
Acetone
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Acetaldehyde Production

Ethanol Oxidation

Ethanol gets oxidized in Acetaldehyde at elevated temperatures.

Proposed Reaction

$CH_3CH_2OH + Hb\text{-}O_2 \rightarrow CH_3CHO + Hb$

Smalldon, K.W. and Brown, G.A. (1973). "The Stability of Ethanol in Stored Blood Part II, The Mechanism of Ethanol Oxidation." Analytica Chimica Acta, 66 (1973) 285-290.

TABLE 2

BAC decrease after equilibration of blood samples at 50 °C and 60 °C.

Temperature (°C)	Equilibration time (minutes)	Blood alcohol concentration (%)	Blood alcohol concentration (%)
60	10	0.100	0.050
	50	0.099	0.048
	90	0.098	0.044
	120	0.091	0.040
50	10	0.100	0.050
	50	0.098	0.049
	90	0.095	0.047
	120	0.096	0.046

Chiarotti, M. and N. De Giovanni (1982). "Acetaldehyde accumulation during headspace gas-chromatographic determination of ethanol." Forensic Sci Int 20(1): 21-25.

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Phase Ratio, β

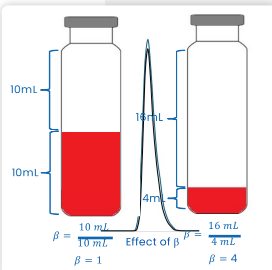
What is Phase Ratio?

Phase ratio is the volume of the gas divided by the volume of the liquid

$$\beta = \frac{V_{gas}}{V_{liquid}}$$

Effect of Phase Ratio

As β increases, larger volume of gas compared to liquid, the response of the volatile compound decreases.



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Salt Addition

Salt Addition

Salting out effect

Increases the ionic strength of the solution, causing other compounds to interact less with solution

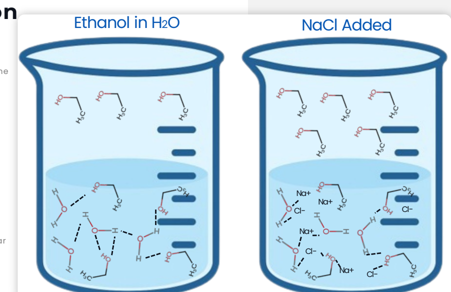
If using 20 to 40% wt/wt will maintain ionic strength

Effect

Not the same for all compounds

Low K values experience less change

Volatile polar compounds in polar matrices will experience larger shifts



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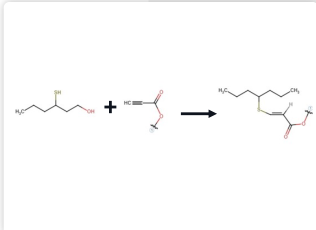
Derivatization

What is Derivatization?
Sample treated with a compound to form a product with a decreased K value.

Examples

Esterification or acetylation are some way to derivatize a compound

Fatty acids – alcohols - esterification



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Type of Headspace Autosamplers

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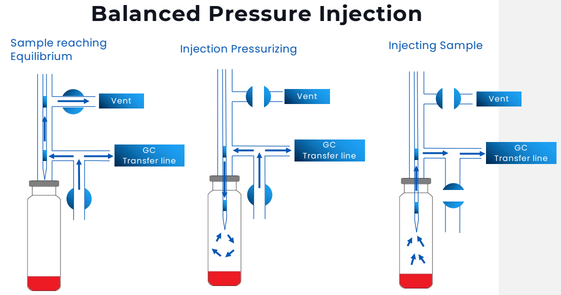
Balanced Pressure Injection



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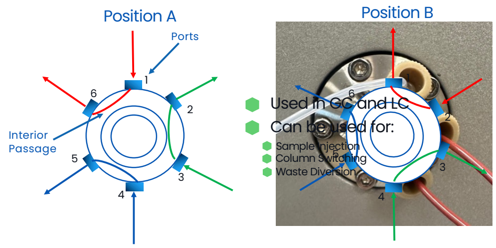
6 port Valve



- Used in GC and LC
- Can be used for:
  - Sample Injection
  - Column Switching
  - Waste Diversion

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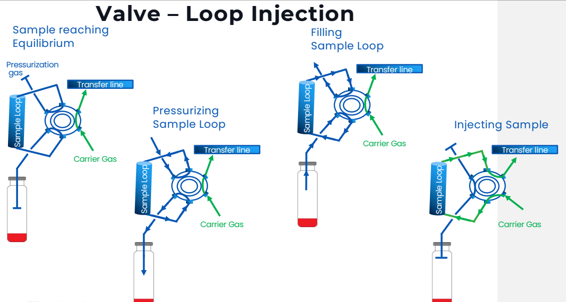
Valve – Loop Injection



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Headspace Recap

- **Headspace** – Gas phase above the liquid sample
- **Henries Law** – Explains why headspace sampling works
- **Only volatile Compounds are analyzed**
- **Volatility can be Modified**
  - Salt addition
  - Temperature
  - Phase ratio
  - Derivatization
- **Sampling** – Multiple routes to sample headspace

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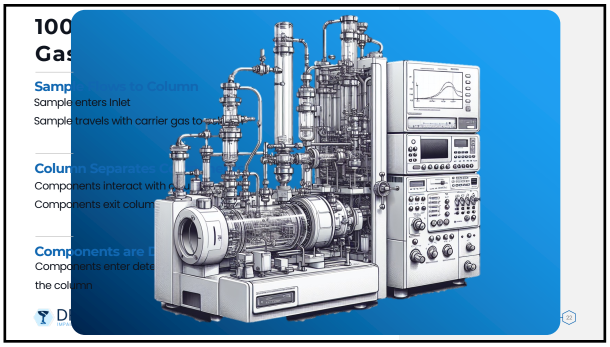
Sample enters Inlet
Sample travels with carrier gas to

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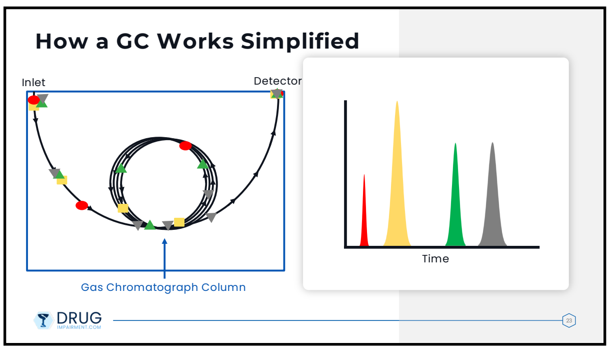
How a GC Works Simplified
Inlet
Detector
Gas Chromatograph Column
Time
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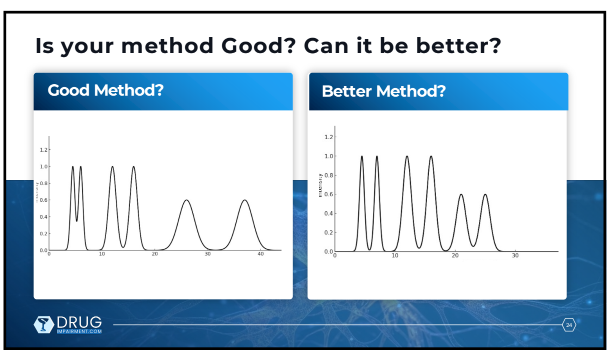
Is your method Good? Can it be better?
Good Method?
Better Method?
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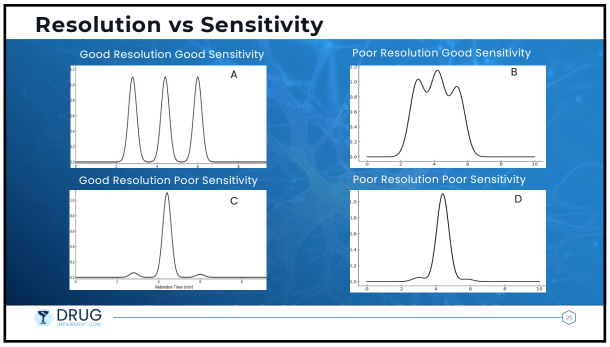
Resolution vs Sensitivity
Good Resolution Good Sensitivity
A
Poor Resolution Good Sensitivity
B
Good Resolution Poor Sensitivity
C
Poor Resolution Poor Sensitivity
D
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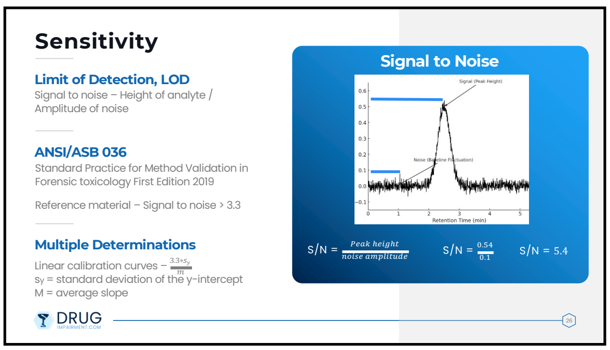
Sensitivity
Limit of Detection, LOD
Signal to noise – Height of analyte / Amplitude of noise
ANSI/ASB 036
Standard Practice for Method Validation in Forensic toxicology First Edition 2019
Reference material – Signal to noise > 3.3
Multiple Determinations
Linear calibration curves – $\frac{3.3 * s_y}{m}$
s_y = standard deviation of the y-intercept
M = average slope
Signal to Noise
Signal (Peak Height)
Noise (Baseline Fluctuation)
Retention Time (min)
S/N = $\frac{Peak\ height}{noise\ amplitude}$
S/N = $\frac{0.54}{0.1}$
S/N = 5.4
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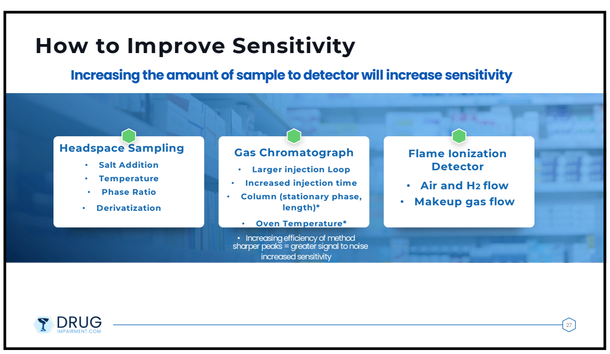
How to Improve Sensitivity
Increasing the amount of sample to detector will increase sensitivity
Headspace Sampling
• Salt Addition
• Temperature
• Phase Ratio
• Derivatization
Gas Chromatograph
• Larger injection Loop
• Increased injection time
• Column (stationary phase, length)*
• Oven Temperature*
• Increasing efficiency of method sharper peaks = greater signal to noise increased sensitivity
Flame Ionization Detector
• Air and H_2 flow
• Makeup gas flow
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Resolution

Measurement of the degrees of separation between two advancement peaks.

Baseline peak width

$$R_s = \frac{(t_{r2} - t_{r1})}{0.5(w_1 + w_2)}$$

t_{r2} = Retention time peak 2
t_{r1} = Retention time peak 1
w_1 = peak 1 width
w_2 = peak 2 width

Half Height peak width

$$R_s = \frac{(t_{r2} - t_{r1})}{0.85(w_{1(0.5)} + w_{2(0.5)})}$$

t_{r2} = Retention time peak 2
t_{r1} = Retention time peak 1
$w_{1(0.5)}$ = peak 1 width at half height
$w_{2(0.5)}$ = peak 2 width at half height

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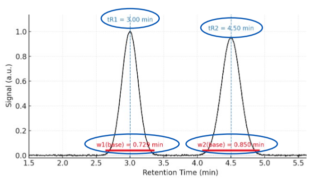
Resolution - Calculation



$$R_s = \frac{(T_{r2} - T_{r1})}{0.5(w_1 + w_2)} \qquad R_s = \frac{(4.50\ \text{min} - 3.00\ min)}{0.5(0.729\ min + 0.850\ min)} \qquad R_s = 1.9$$

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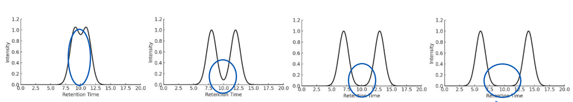
Increasing resolution



Resolution Increasing

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Fundamental Resolution Equation

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{k_2 + 1}\right)$$

Where
R_s = Resolution
N = Theoretical number of plates
α = Selectivity
k = Retention

Block 1 - Efficiency $\left(\frac{\sqrt{N}}{4}\right)$
- Length of Column
- Diameter of column
- Carrier gas type
- Linear velocity of carrier gas

Block 2 - Selectivity $\left(\frac{\alpha - 1}{\alpha}\right)$
- Stationary Phase
- GC oven Temperature

Block 3 - Retention $\left(\frac{k_2}{k_2 + 1}\right)$
- Inner Diameter of Column
- Film thickness of Column
- GC Oven Temperature

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Fundamental Resolution Equation
Block 1 - Efficiency

$$\left(\frac{\sqrt{N}}{4}\right)$$

N – Theoretical Number of Plates

What is N, the theoretical number of plate?

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Number of Theoretical Plates (N)

Concept Introduced in the 1930's
Developed for Fractional Distillation. Later applied to chromatography

Theoretical Plate
Represents a single equilibrium step between compound and stationary phase

Can calculate N
2 formulas – base of peak and half height

To Remember
Theoretical concept, no actual plates exist

Compounds have different theoretical plates on the same column

$$N = 16\left(\frac{T_r}{W_b}\right)^2$$

$$N = 5.54\left(\frac{T_r}{W_{\frac{1}{2}}}\right)^2$$

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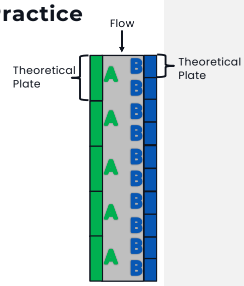
Theoretical Plates in Practice

Compound A
Number of Theoretical Plates = 5

Compound B
Number of Theoretical Plates = 10

Number of Theoretical Plates
Number of Theoretical Plates = 10,000's or 100,000's



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Fundamental Resolution Equation Block 1 - Efficiency

$$\left(\frac{\sqrt{N}}{4}\right)$$

N – Theoretical Number of Plates

Length of Column
N = 5000 with 15m column
N= 10000 with 30m column
Does not double resolution

Stationary Phase
Changes the # of equilibration steps

Linear Gas Velocity
Faster gas flow decreases Number of Theoretical plates

Carrier Gas Type
Changes the # of equilibration steps

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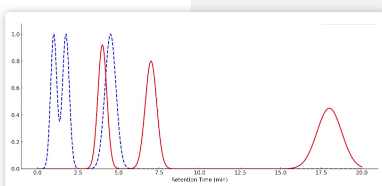
Modifying Block 1 – Column Length

Blue – Shorter Column
- Peaks 1 and 2 Overlap
- Total run time ~5 minutes

Red – Longer Column
- No peak overlap
- Peak Broadening – lower sensitivity
- Longer run time



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Fundamental Resolution Equation
Block 2 - Selectivity

$$\left(\frac{\alpha - 1}{\alpha}\right)$$

$$where\ \alpha = \frac{k_2}{k_1}$$

k_1 and k_2 are the Retention factor, k, for the peaks 1 and 2

Temperature of GC Oven

Can either increase or decrease selectivity depending on the compounds of interest

Stationary Phase

Example – Change from Agilent DB-ALC 1 to a DB-ALC2 column

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Retention Factor, k

What is Retention Factor?

Ratio of time solute spend in the stationary phase vs the carrier gas

What influences k?

Compounds that interact longer with the column will have a higher retention factor

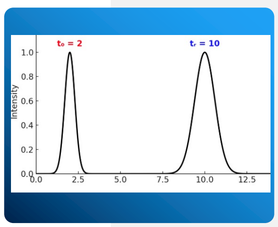
Formula

$$k = \frac{(t_r - t_0)}{t_0}$$

Where
t_r = retention time of peak
t_0 = void time

Target Retention Factor

First eluting compound > 1.0
Target 1.5
k>1.5 is wasted analysis time



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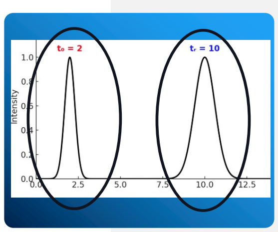
Retention Factor, K, Calculation

$$k = \frac{(t_r - t_0)}{t_0}$$

$$k = \frac{(10 - 2)}{2}$$

$$k = 4$$



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Selectivity Calculation, $\alpha = \frac{k_2}{k_1}$

Retention Factor

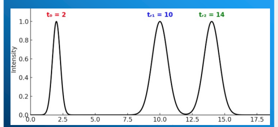
$$k_1 = \frac{(t_{r1} - t_0)}{t_0} \qquad k_2 = \frac{(t_{r2} - t_0)}{t_0}$$

$$k_1 = \frac{(10 - 2)}{2} \qquad k_2 = \frac{(14 - 2)}{2}$$

$$k_1 = 4 \qquad k_2 = 6$$

Selectivity

$$\alpha = \frac{k_2}{k_1} \qquad \alpha = \frac{6}{4} \qquad \alpha = 1.5$$



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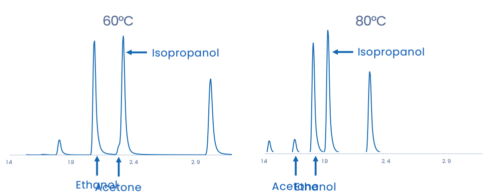
Selectivity – Oven Temperature Adjustment



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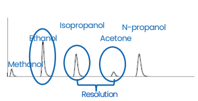
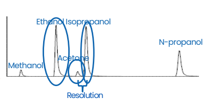
Selectivity – Stationary phase

Column DB-ALC 1

Column DB-ALC 2





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Fundamental Resolution Equation
Block 2 - Selectivity

$$\left(\frac{\alpha - 1}{\alpha}\right)$$

$$where\ \alpha = \frac{k_2}{k_1}$$

1st Method development
Largest influence in resolution

Selectivity change from 1.1 to 1.5
267% increase in resolution

Selectivity is between 2 peaks

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Fundamental Resolution Equation
Block 3 – Retention (capacity)

$$\left(\frac{k_2}{k_2 + 1}\right)$$

Where k_2 is the retention factor

Retention Factor, K
Same retention factor previously discussed

Temperature
Lower column temperature increases retention

Carrier Gas Type and Velocity
Higher velocity lower retention factor

Stationary Phase
Different stationary phase

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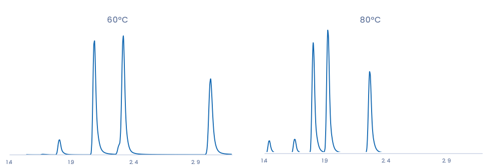
Retention – Temperature Adjustment



Change in retention varies depending on the compound properties

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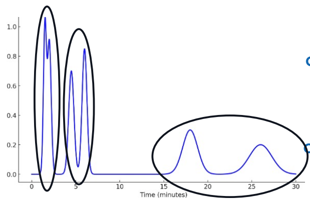
Modifying Retention



Increase theoretical number of plate
Improve resolution for peak 1 and 2 and 3 and 4
Increase run time and peak 5,6 will be broader

Change Selectivity – Stationary Phase
Might lead other peaks overlapping
Current method is close, just needs slight change

Change Retention – GC Oven Temperature
Increase retention for peak 1, 2, 3, and 4
Decrease retention for peaks 5 and 6

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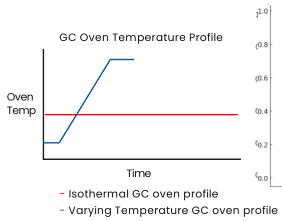
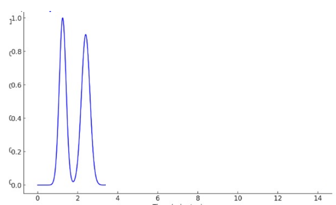
GC Oven Temperature Ramp





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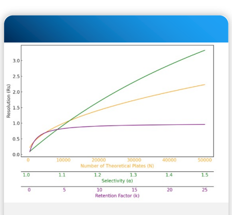
Resolution Recap



Efficiency Selectivity Retention

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{k_2 + 1}\right)$$

Selectivity – Largest influence on resolution
Efficiency – Increasing N increases method run time
Retention – Similar to selectivity
- GC Oven temperature profile

How to Modify

Selectivity
- Stationary Phase
- GC Oven Temperature
- Carrier Gas type

Efficiency
- Column Length
- Stationary Phase
- Carrier gas type
- Linear velocity carrier gas

Retention
- Stationary Phase
- GC Oven Temperature
- Carrier Gas type
- Linear Velocity carrier gas

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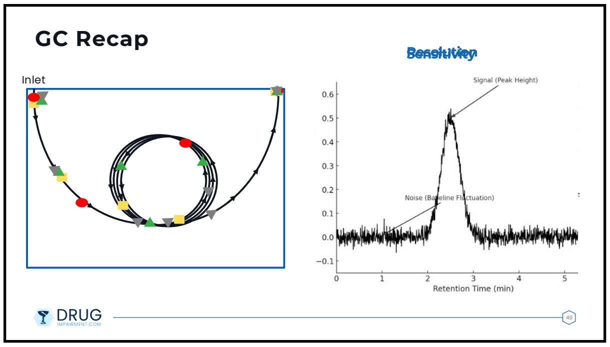
GC Recap
Inlet
Signal (Peak Height)
Noise (Baseline Fluctuation)
Retention Time (min)
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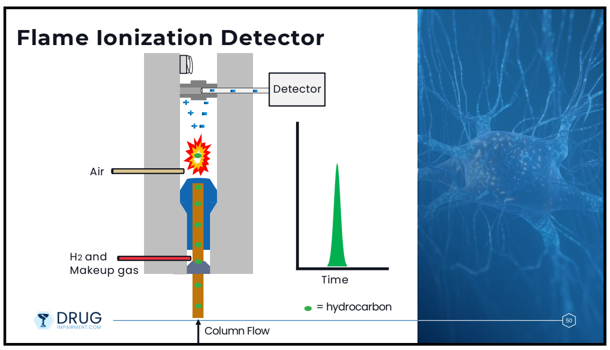
Flame Ionization Detector
Detector
Air
H2 and
Makeup gas
Time
= hydrocarbon
Column Flow
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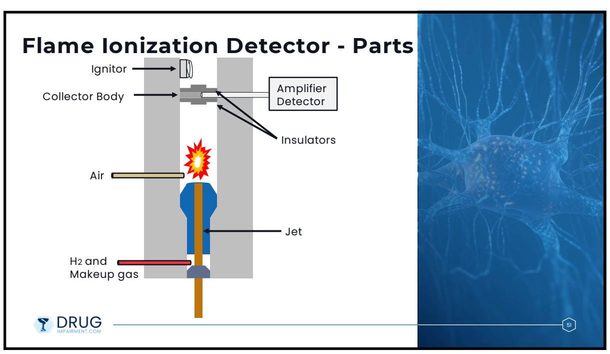
Flame Ionization Detector - Parts
Ignitor
Collector Body
Amplifier
Detector
Insulators
Air
Jet
H2 and
Makeup gas
DRUG

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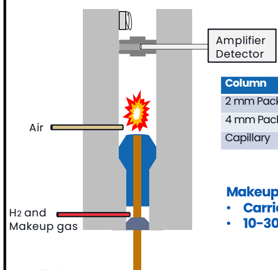
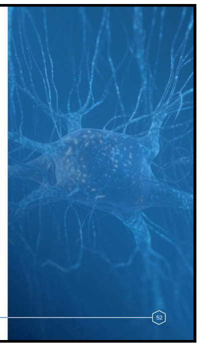
Makeup Gas



Column	Flow to Detector
2 mm Packed	20-30 mL/min
4 mm Packed	50-60 mL/min
Capillary	1-2 mL/min

Makeup Gas
- **Carrier Gas or Nitrogen**
- **10-30 mL/min**



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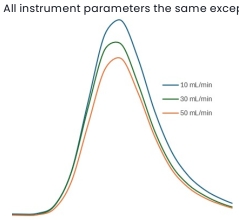
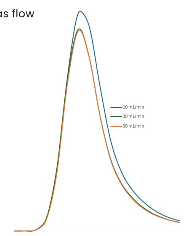
Makeup Gas Test

- All instrument parameters the same except makeup gas flow



Acetone



Isopropanol

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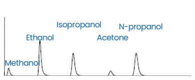
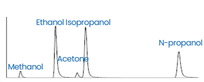
FID Benefits and Limitation

Benefits
- Sensitive
- Repeatable
- Wide detection range
- Simple to maintain
- Cheap

Limitations
- Hydrocarbon analytes
  - Cannot detect inorganic compounds
  - Poor response for compounds containing oxygen sulfur or halogens
- No structural information
- Cannot distinguish if compounds are overlapping





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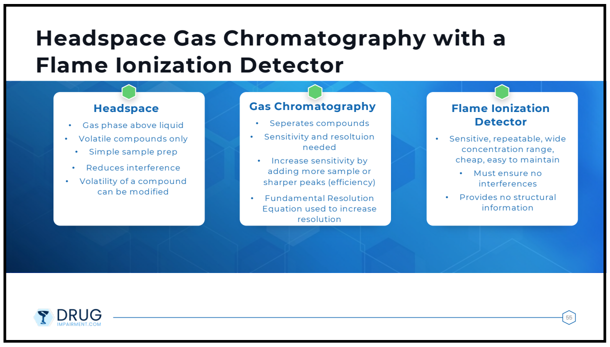
Headspace Gas Chromatography with a Flame Ionization Detector
Headspace
Gas phase above liquid
Volatile compounds only
Simple sample prep
Reduces interference
Volatility of a compound can be modified
Gas Chromatography
Seperates compounds
Sensitivity and resoltuion needed
Increase sensitivity by adding more sample or sharper peaks (efficiency)
Fundamental Resolution Equation used to increase resolution
Flame Ionization Detector
Sensitive, repeatable, wide concentration range, cheap, easy to maintain
Must ensure no interferences
Provides no structural information
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