GC x GC With Flow Modulation:
A Simple Approach to Resolving Complex Mixtures

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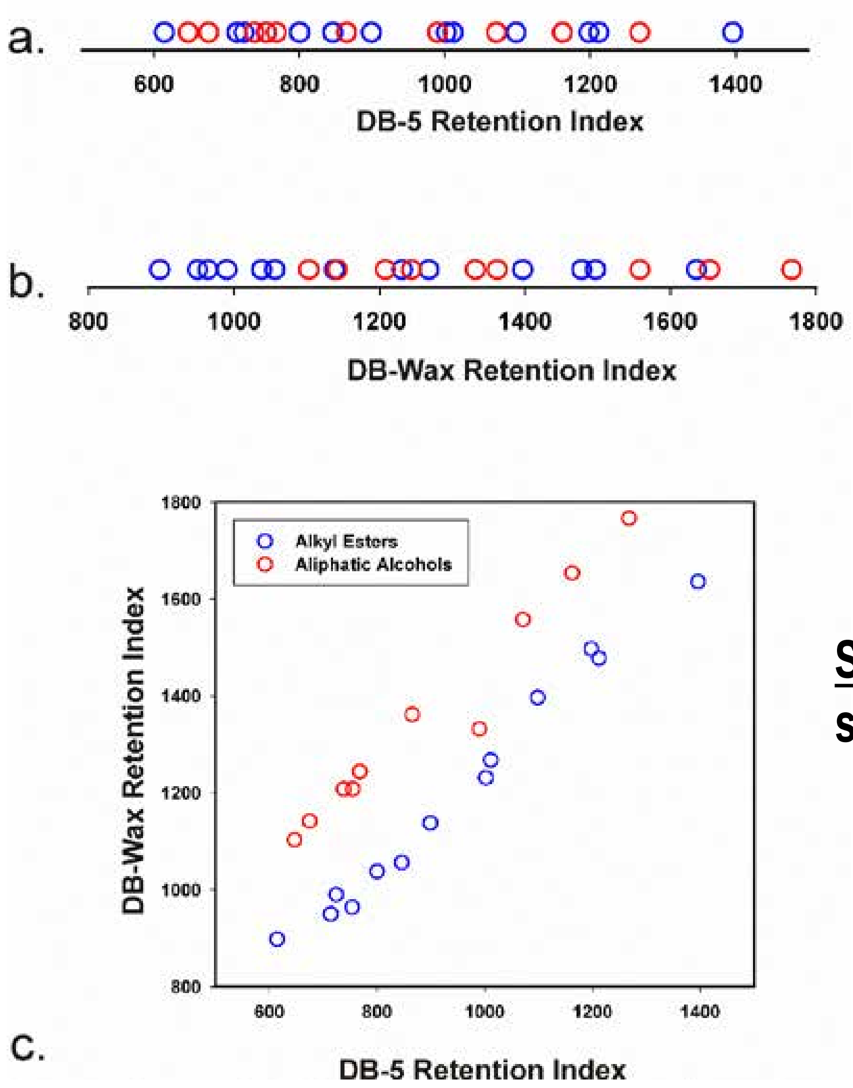
March 29, 2019

GC retention depends on both compound size and polarity: Group separations are difficult with a single column



<u>Flavor/Fragrance Compounds</u>

Alkyl Esters

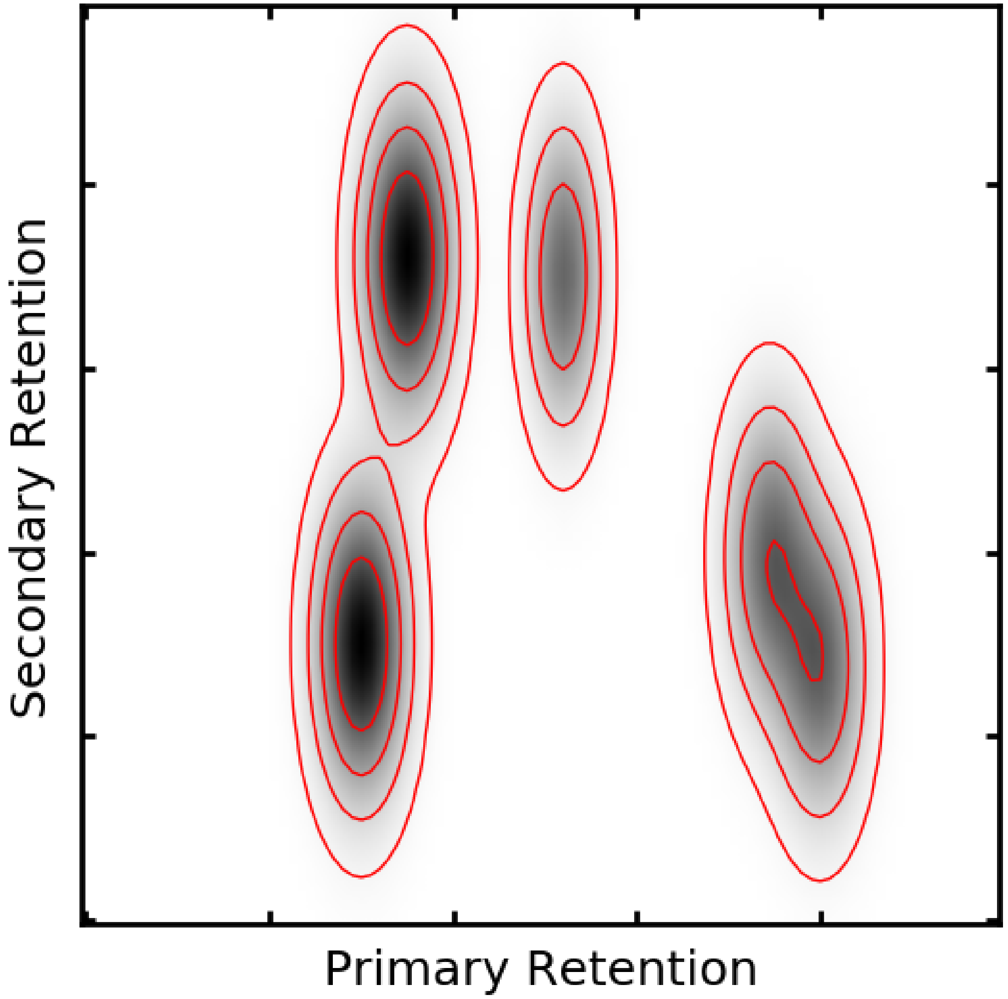
Aliphatic Alcohols

<u>Simultaneous</u> separation on two dissimilar stationary phases resolves the two groups

Retention Indices taken from K.L. Goodner, Food Science and Technology 41 (2008) 951-958.

Comprehensive 2-D Chromatography (GC x GC)

An experimental technique for separating mixtures on two stationary phases



Potential rewards with this approach…

Greater resolution

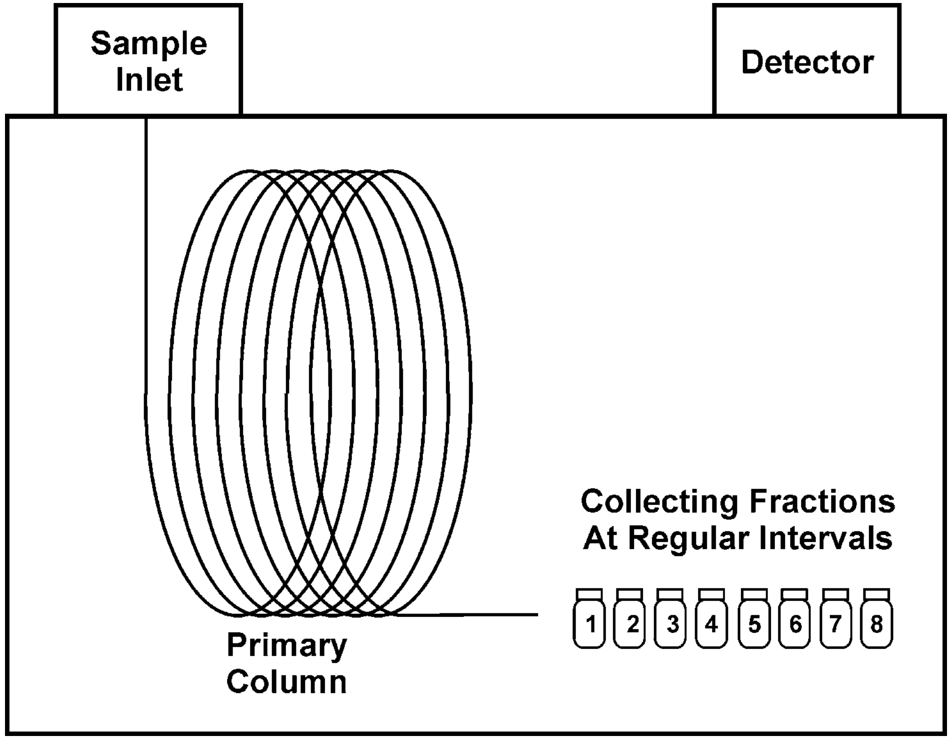
Enhanced qualitative information (i.e., group separations)

Increased sensitivity

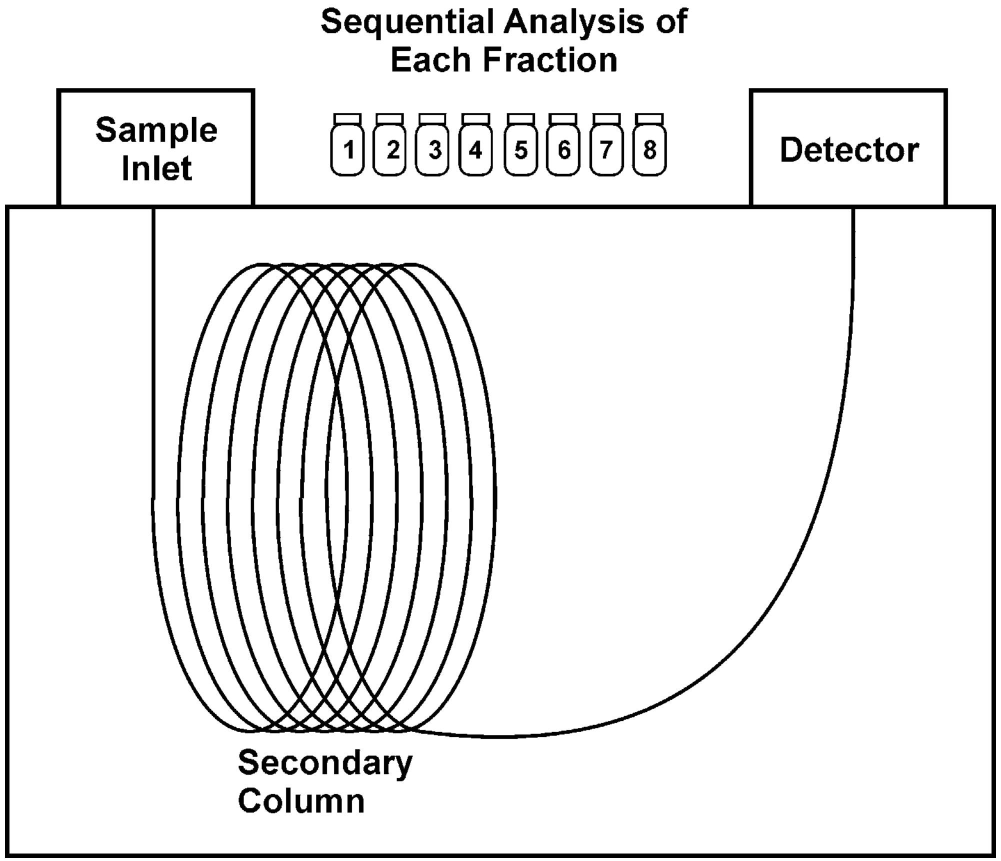
Can columns (essentially 1-D objects) be used to generate 2-D separations? Yes!!!!

A thought experiment about how this could be done (albeit in an insanely laborious fashion):

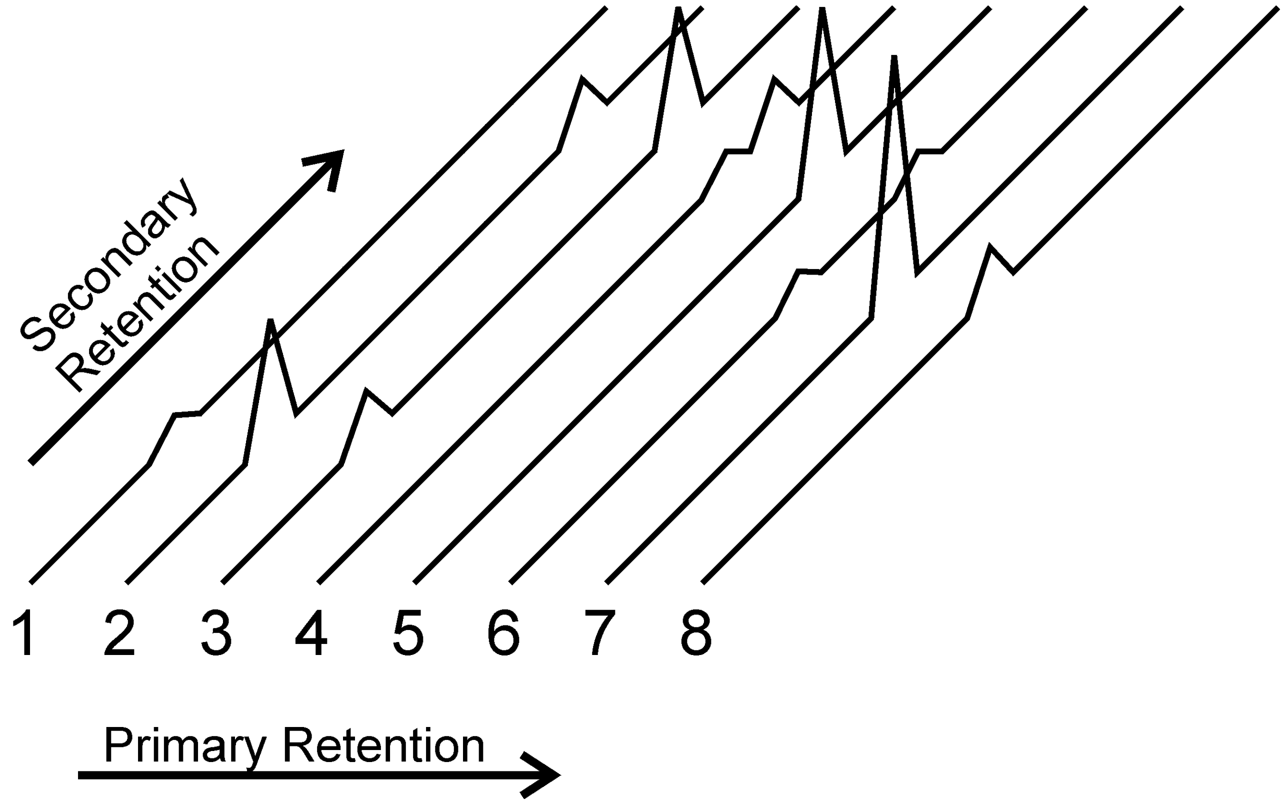
First, fractionate the sample on the primary column...



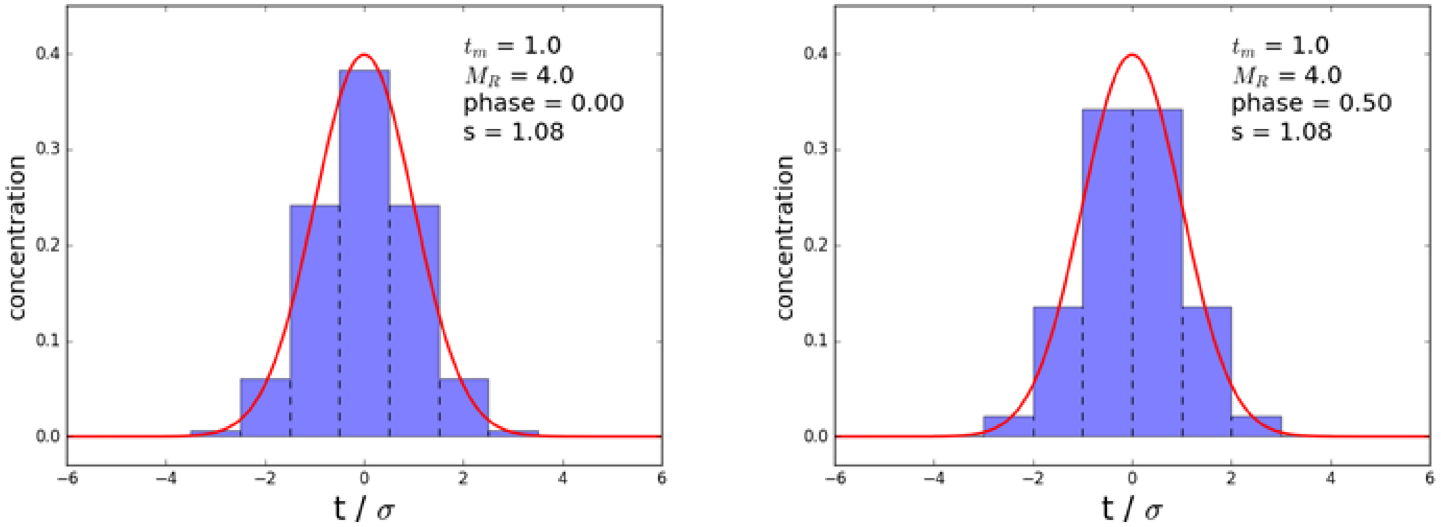
Then analyze each fraction independently on the secondary column that has a different stationary phase.



Then plot the chromatograms of each fraction side-by-side.



How small do we need to make the fractions to maintain the primary separation?



Collect at least 3 significant fractions from each peak to keep broadening to less than 15%.

Temperature programmed GC peaks are roughly 6 s wide (4s). You need to obtain a fraction every 2 s.

A 10 minute run, yields 300 fractions!!! This would be a nightmare to do by “hand”.

A Practical Solution = Comprehensive 2-D GC

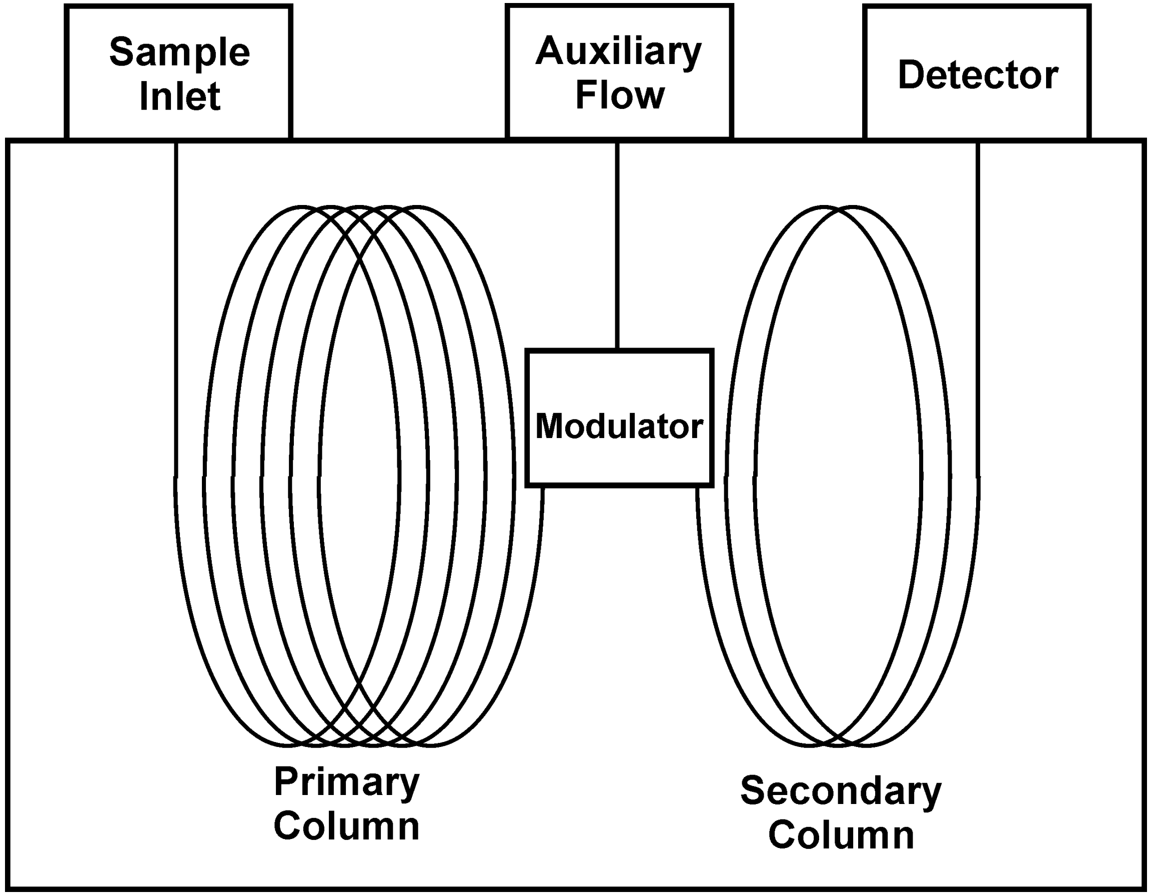
Don't store the fractions.

Analyze them immediately after collection!

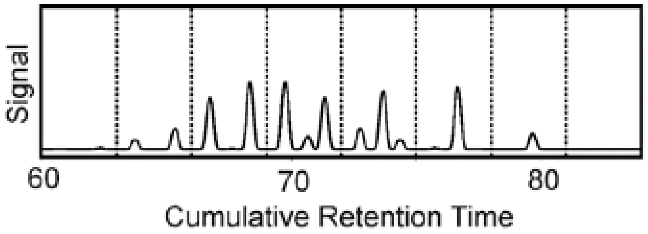
Perform a secondary separation every couple of seconds.

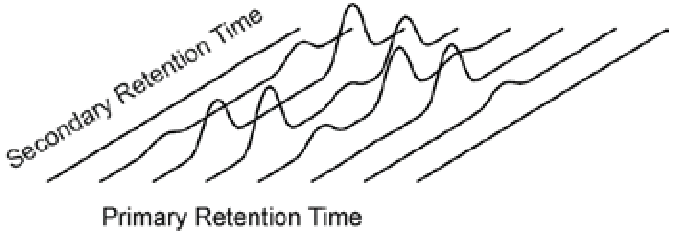
Ramp the oven temperature to remove the retention correlation.

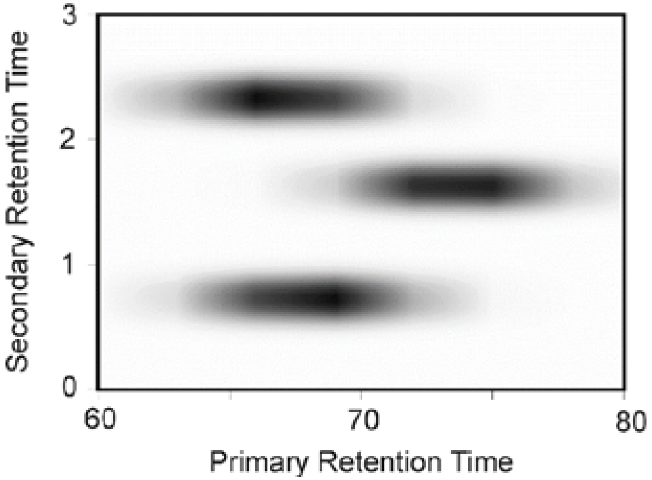
Detector must be fast!!!!



Converting a 1-D Signal Array to a 2-D Chromatogram







Note: Range of secondary retention times must be less than the modulation period to "keep the fractions separate".

Won’t the secondary separations have to be unreasonably fast/efficient?

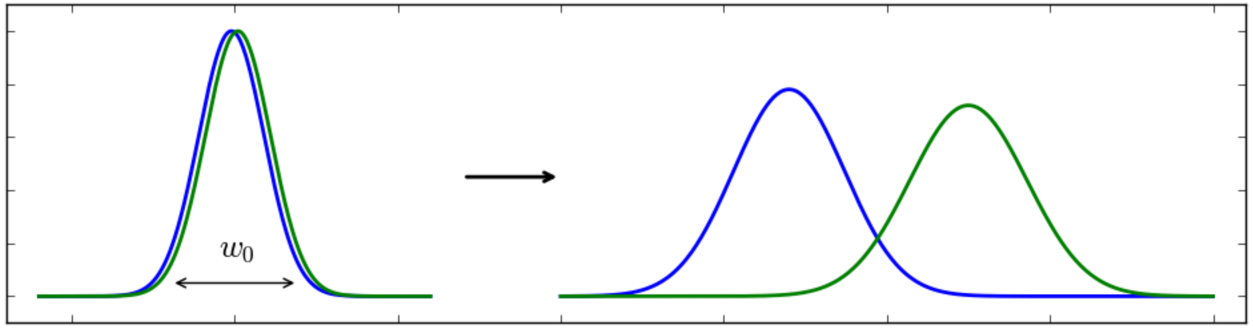
If we have a 2 s modulation period, then we only have a 2 s range of 2^{o} retention space to play with.

GC separations are normally conducted over many hundreds of seconds.

Will we need to invent new types of columns to separate peaks with sub-second widths?

The answer is “No”. Conventional narrow-bore GC columns have untapped potential for high speed separations.

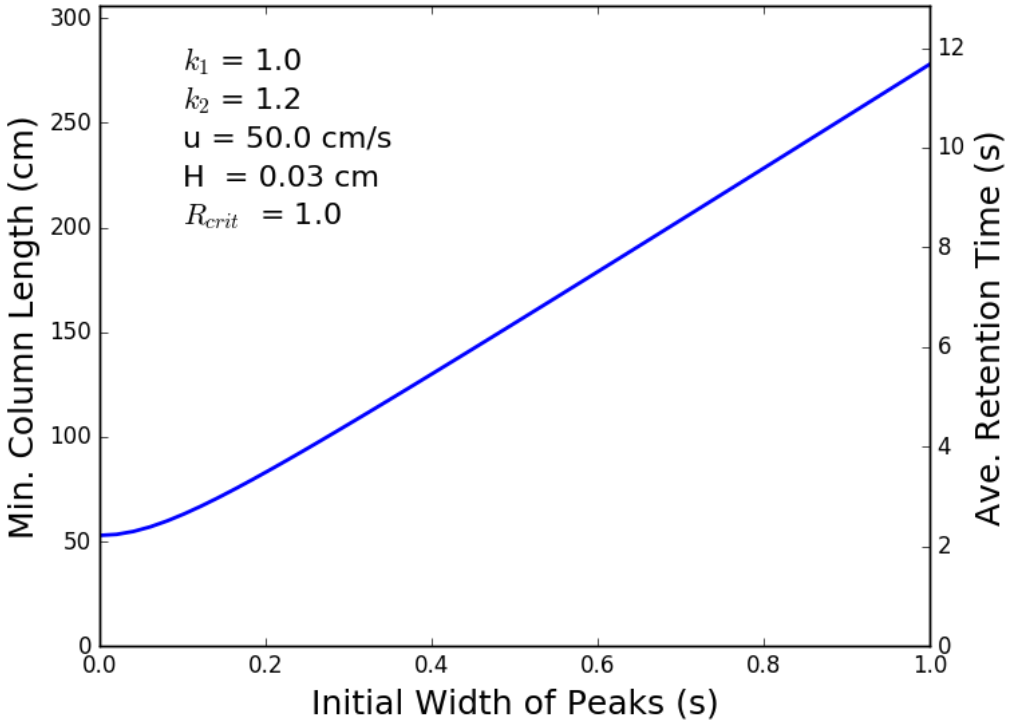
How fast can we separate two peaks?



$$t_R = \frac{L}{u}(1 + k)$$

$$w = \sqrt{w_0^2 + \frac{16\,H\,t_R^2}{L}}$$

$$R_S = \frac{2\Delta t_R}{w_1 + w_2}$$



The “Secret” of Modulation Success is Narrow Input Pulses

Decreasing the initial peak width decreases the amount of time required to separate two components…

until you reach the “speed limit” of the column, where on-column broadening dominates.

The “speed limit” of the column is determined by the number of theoretical plates (L/H) produced by the column/conditions.

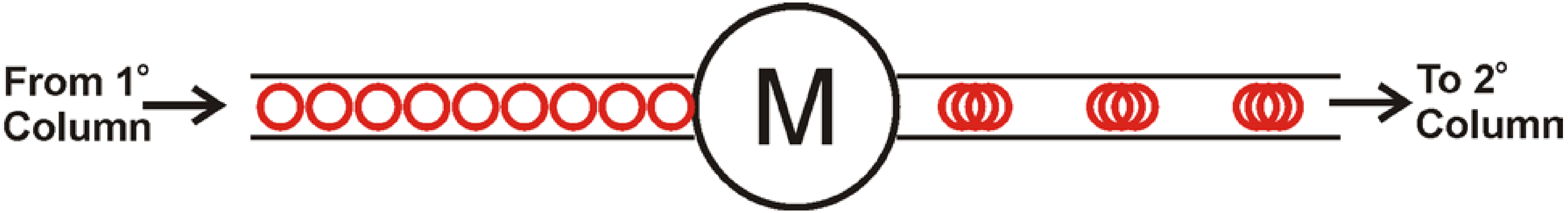
The Main Job of a GC x GC Modulator

Transfer primary effluent to the secondary column as a narrow pulse (< 100 ms), repeat every second or so.

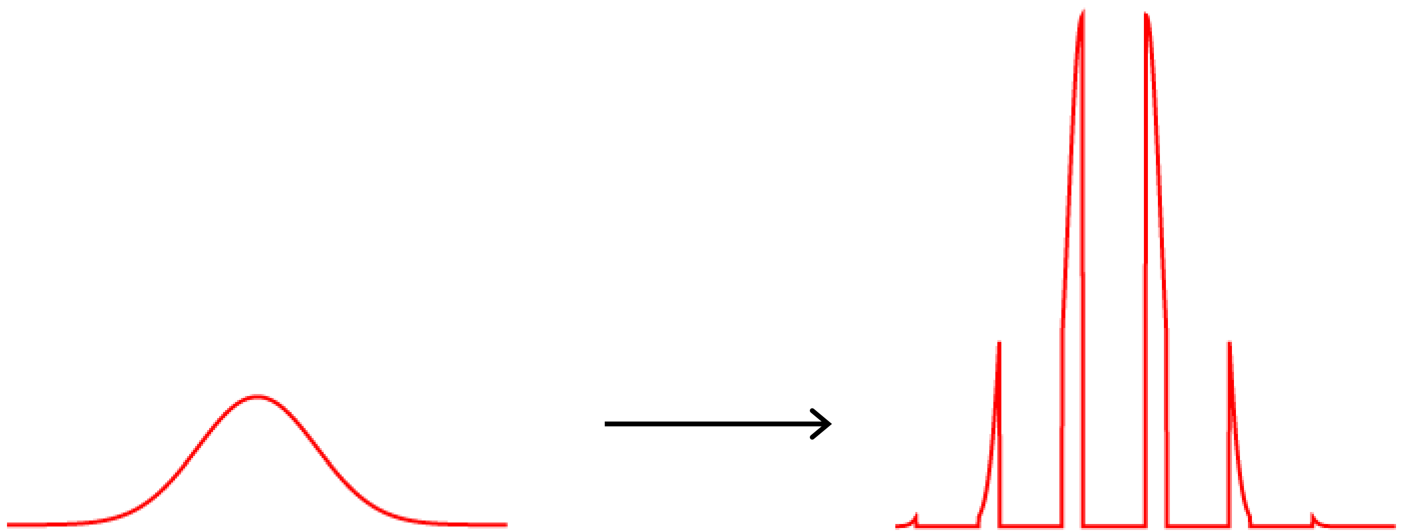
Do this thousands and thousands of times without failure.

Mechanisms of Modulation #1: Thermal Modulation

Concentrate Components As They Exit the Primary Column



Mechanisms of Modulation #1:
Concentrate Components As They Exit the Primary Column



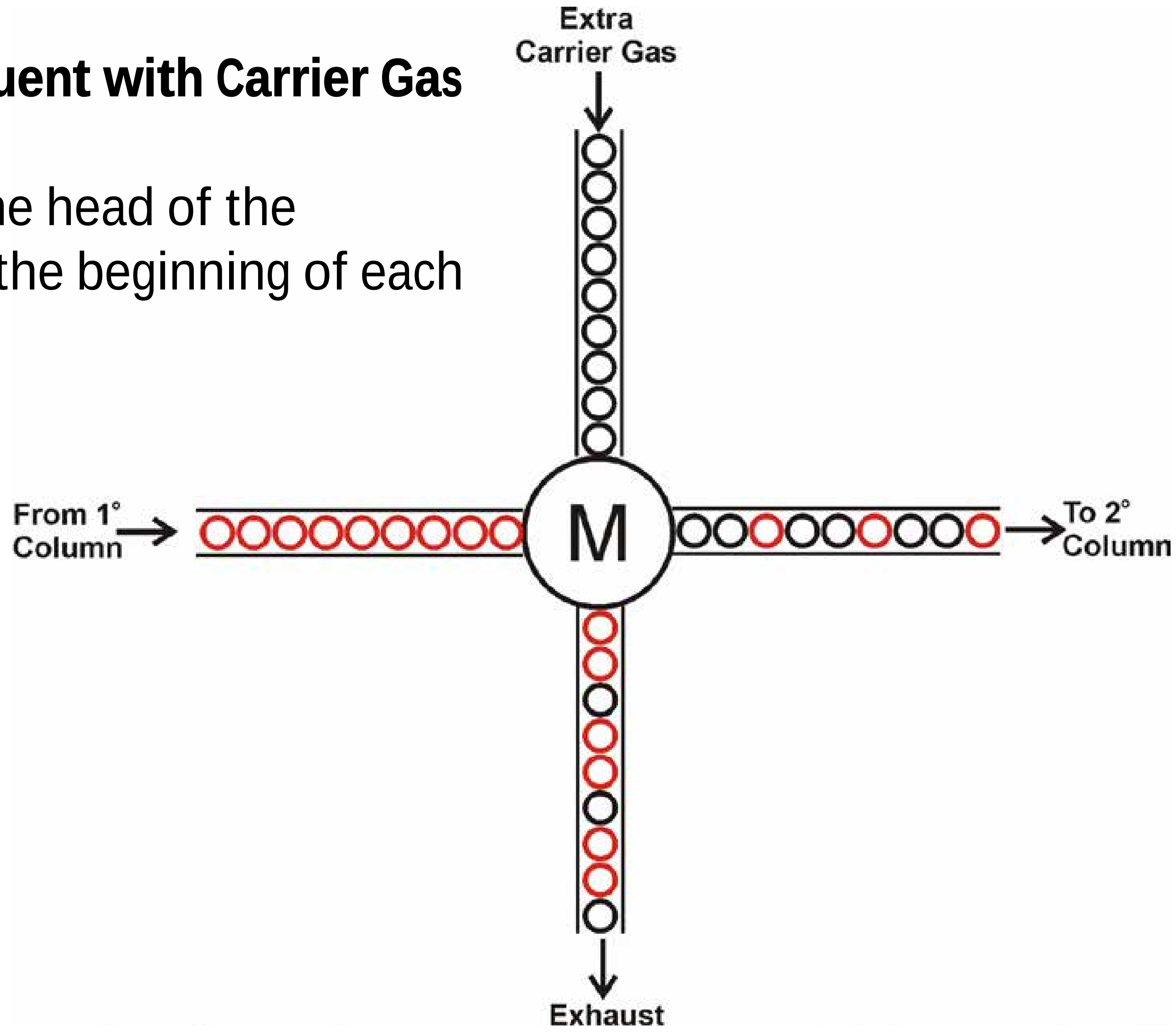
Pros:
- Increases component concentration
- Does not increase carrier flow load

Cons:
- Requires extreme temperature gradients
- <u>Not something that you throw together from stuff in your "junk" drawer</u>

Mechanisms of Modulation #2: Diverting Flow Modulation

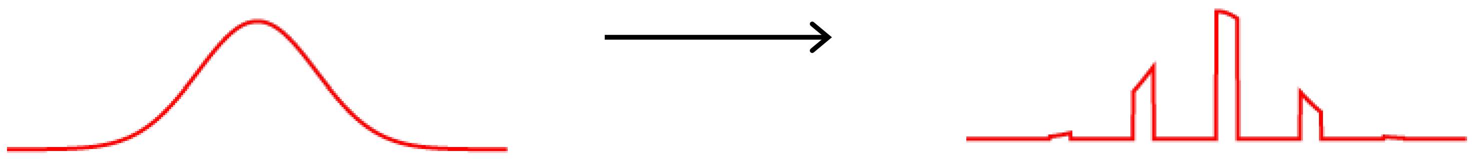
Replace Large Segments of Primary Effluent with Carrier Gas

Primary column effluent is diverted to the head of the secondary column for a brief interval at the beginning of each modulation period.



Mechanisms of Modulation #2: Diverting Flow Modulation

Replace Large Segments of Primary Effluent with Carrier Gas



Pros:

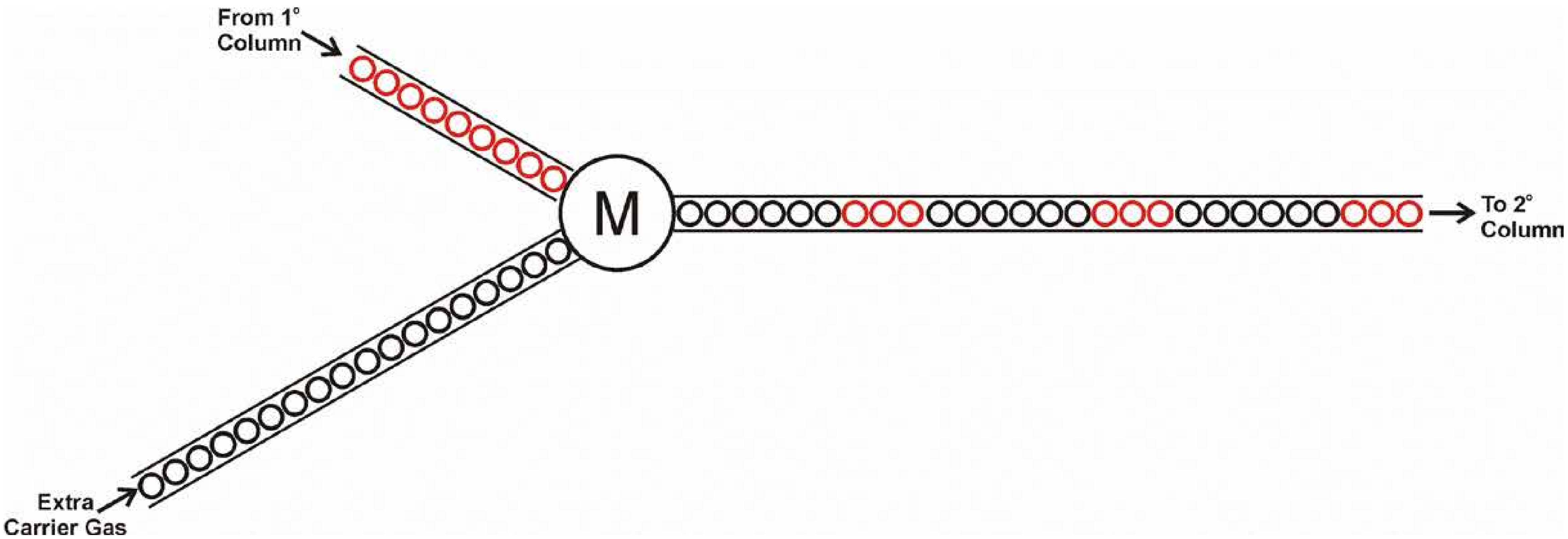
- Should be simple to implement
- Does not *necessarily* increase carrier flow load

Cons:

- Pulse width is related to transfer % (in a bad way)
- Low transfer % of primary effluent reduces sensitivity
- Requires additional pneumatics control module
- Introduces a potential source of leaks, activity, etc
- Under-sampling leads to loss in quantitative precision

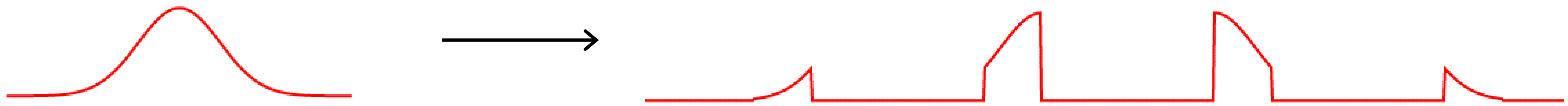
Mechanisms of Modulation #3: Differential Flow Modulation

Large segments of additional carrier gas are inserted between segments of primary effluent.



Mechanisms of Modulation #3: Differential Flow Modulation

Insert Even Larger Segments of Carrier Gas Between the Segments of Primary Effluent



Pros:
- Full transfer of primary effluent
- Should be simple to implement

Cons:
- Increased carrier load (can be bad for chromatography, bad for detector)
- Requires additional pneumatics control module
- Introduces a potential source of leaks, activity, etc

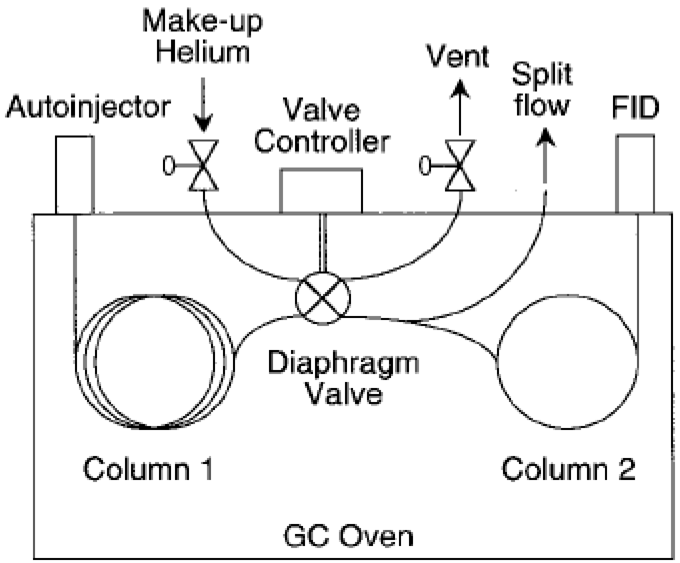
Diverting Flow Modulation

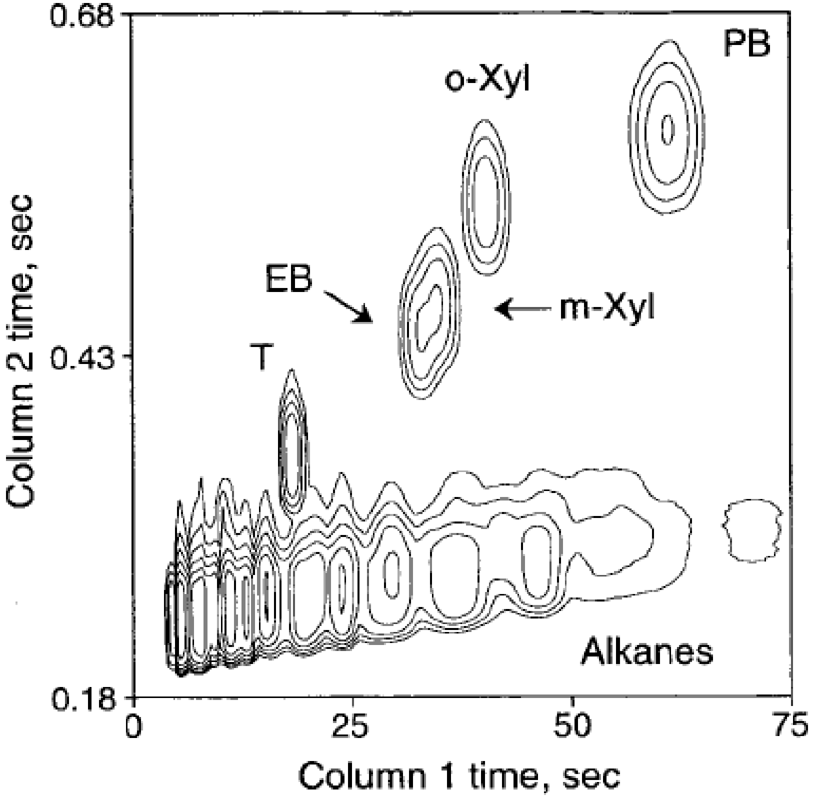
Anal. Chem. **1998,** *70,* 2796–2804

Comprehensive Two-Dimensional High-Speed Gas Chromatography with Chemometric Analysis

Carsten A. Bruckner, Bryan J. Prazen, and Robert E. Synovec*

Center for Process Analytical Chemistry, Department of Chemistry, Box 351700, University of Washington, Seattle, Washington 98195-1700



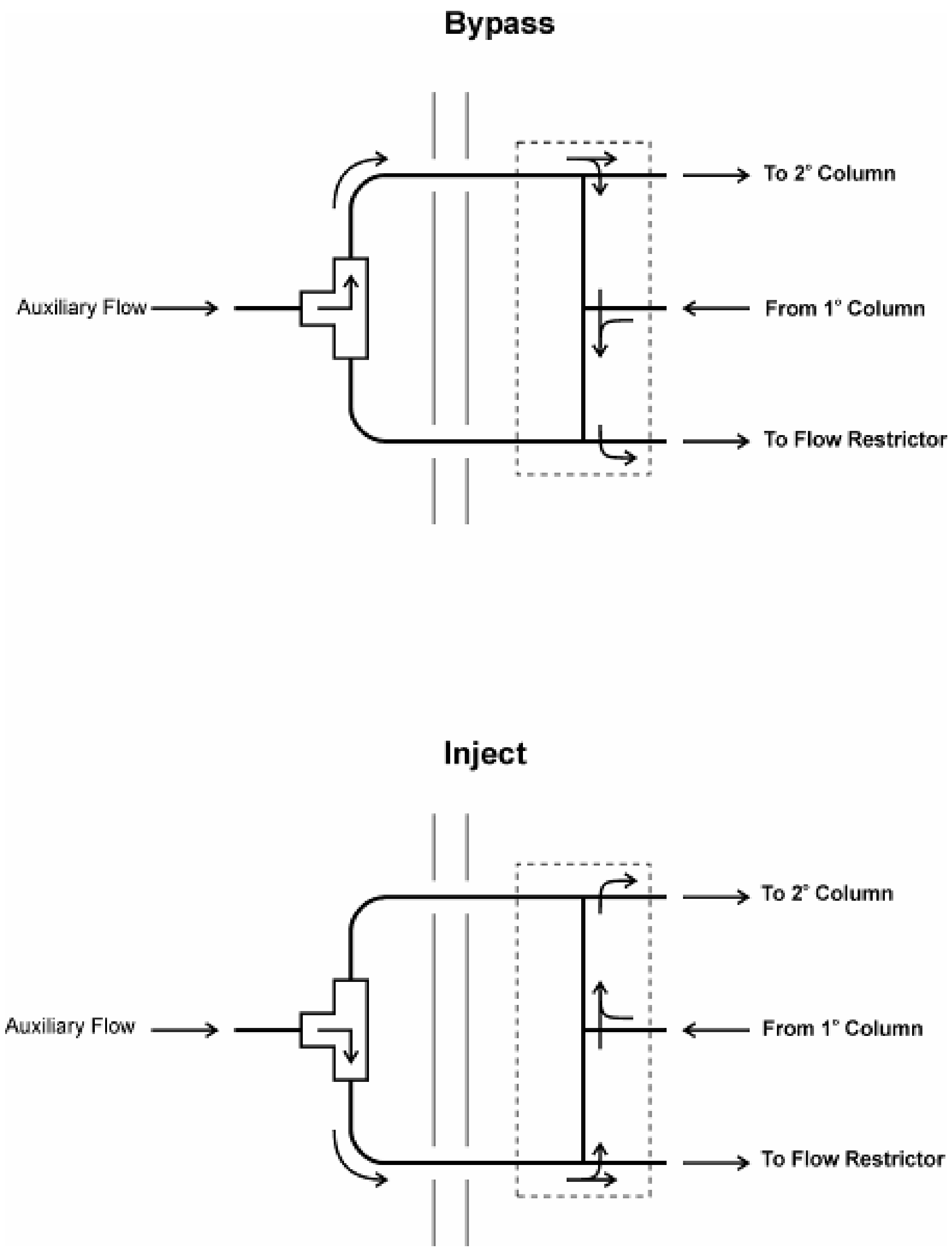


flu·id·ics

flo͞oˈidiks

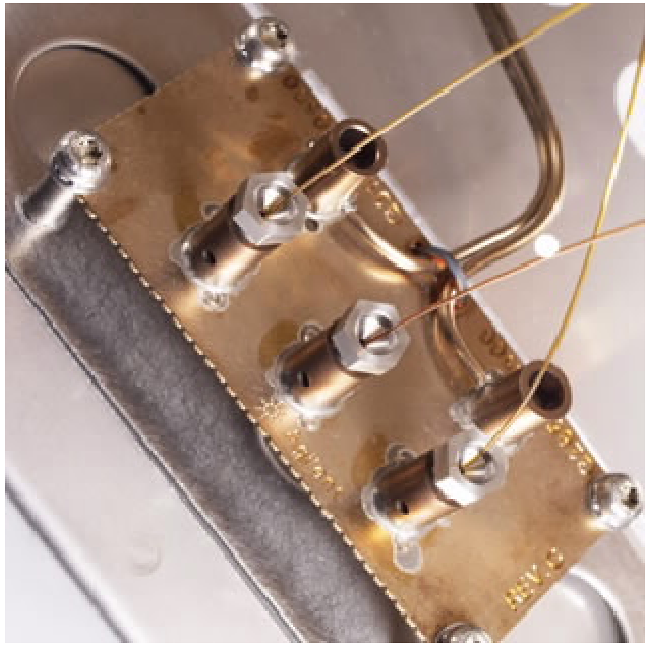
noun

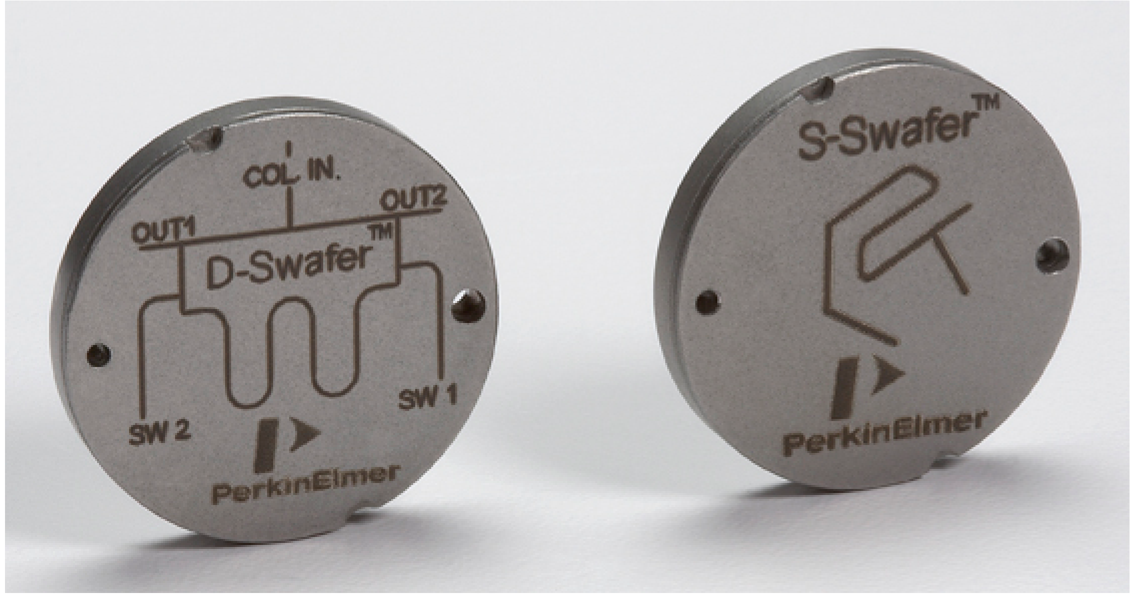
the study and technique of using small interacting flows and fluid jets for functions usually performed by mechanical devices.

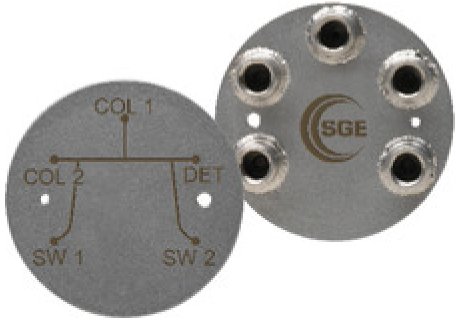


Pro Tip:
Understand the Deans Switch and you understand 90% of the logic behind fluidic modulators

Integrated Deans Switches Are Now Commercially Available







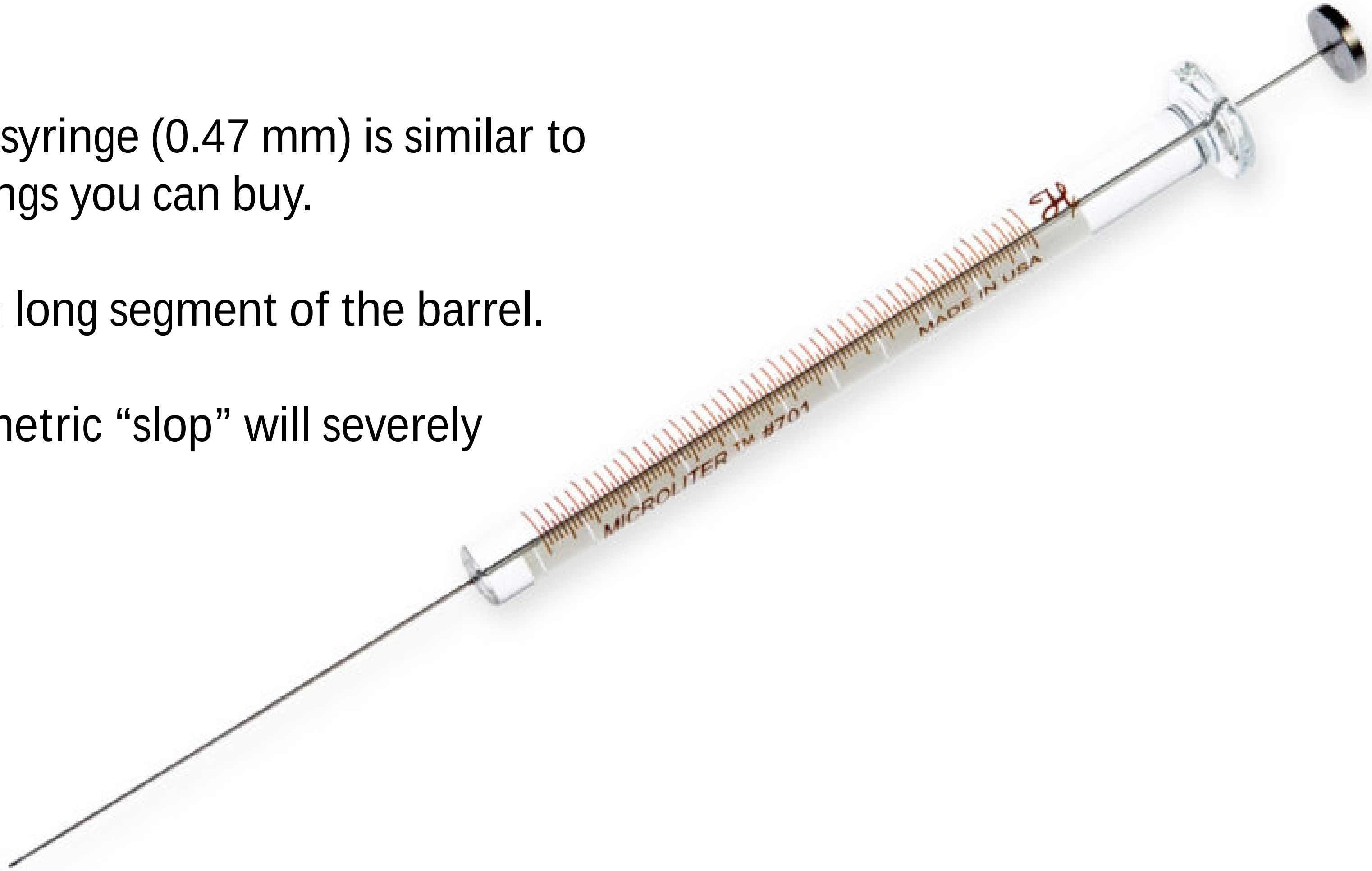
Volumetric Challenges of Diversion Modulation

A 50 ms wide peak moving at a flow rate of 1.0 mL/min represents 0.8 uL of gas

The id of the barrel of this syringe (0.47 mm) is similar to the ids of the smallest fittings you can buy.

A peak would fit in a 5 mm long segment of the barrel.

Bottom Line: A little volumetric “slop” will severely broaden your peaks



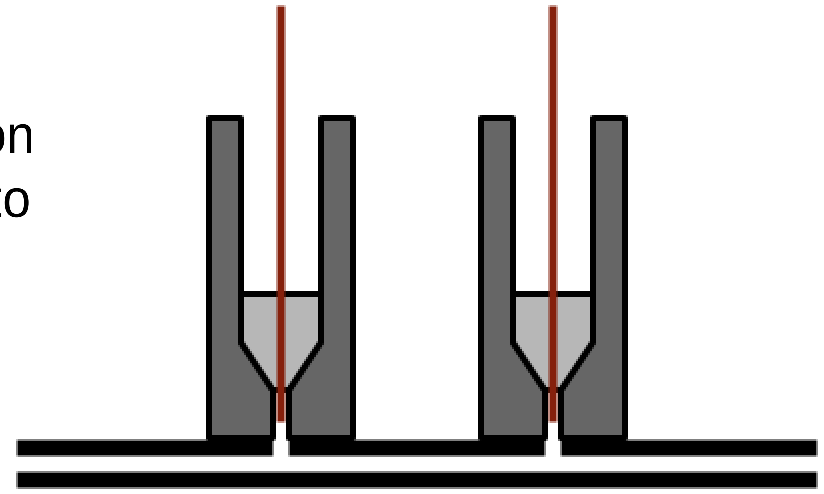
Planar Fluidics

Pros:

- Integration of junctions reduces the probability of leaks
- Creates a clean, simplified "look"
- Makes a physically robust device

Cons:

- Reduces adjustability
- Creates inherent limits to internal volume reduction
- Unswept volumes in critical locations are difficult to avoid.



Our Approach

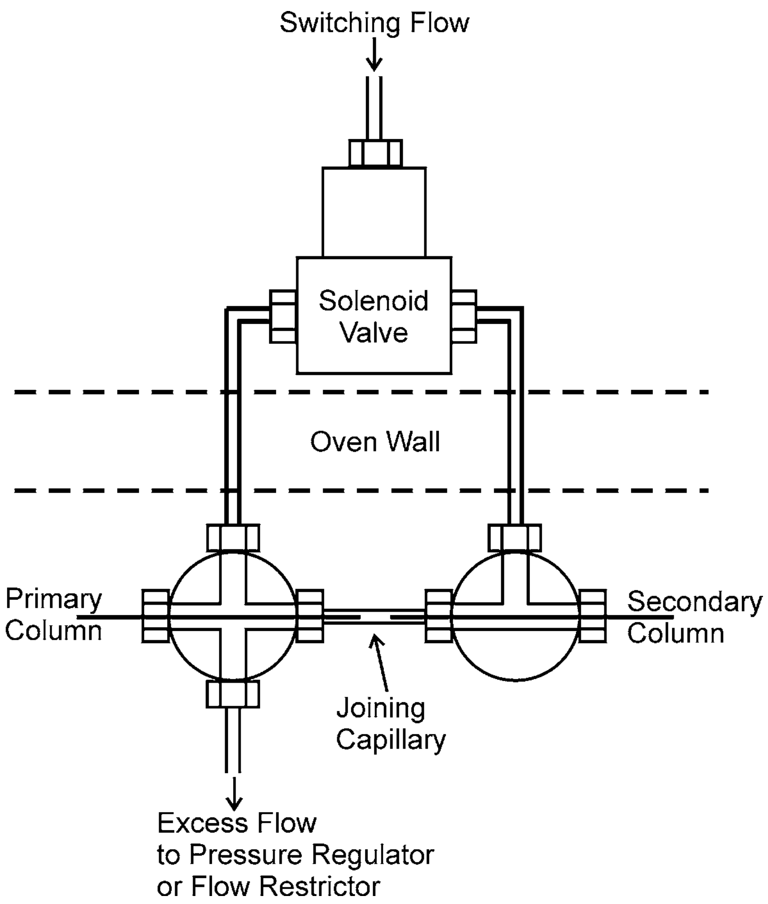
Focus on the part of the device most critical for high performance: the environment near the tips of the column.

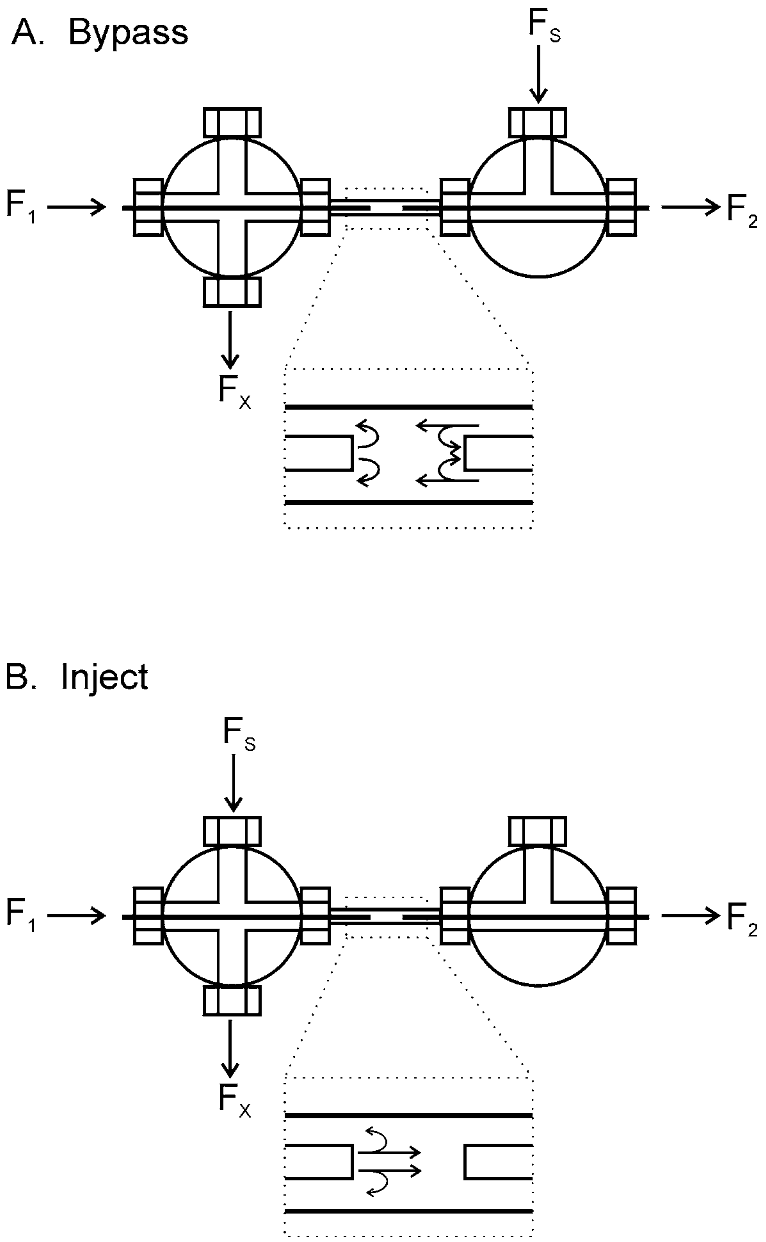
Build the device up from there.

This is the same design approach to a split/splitless inlet: get the environment around the sample transfer region right, the rest of the design become ancillary details.

Valve-Based Modulation: Diverting Type

A High Speed Deans Switch



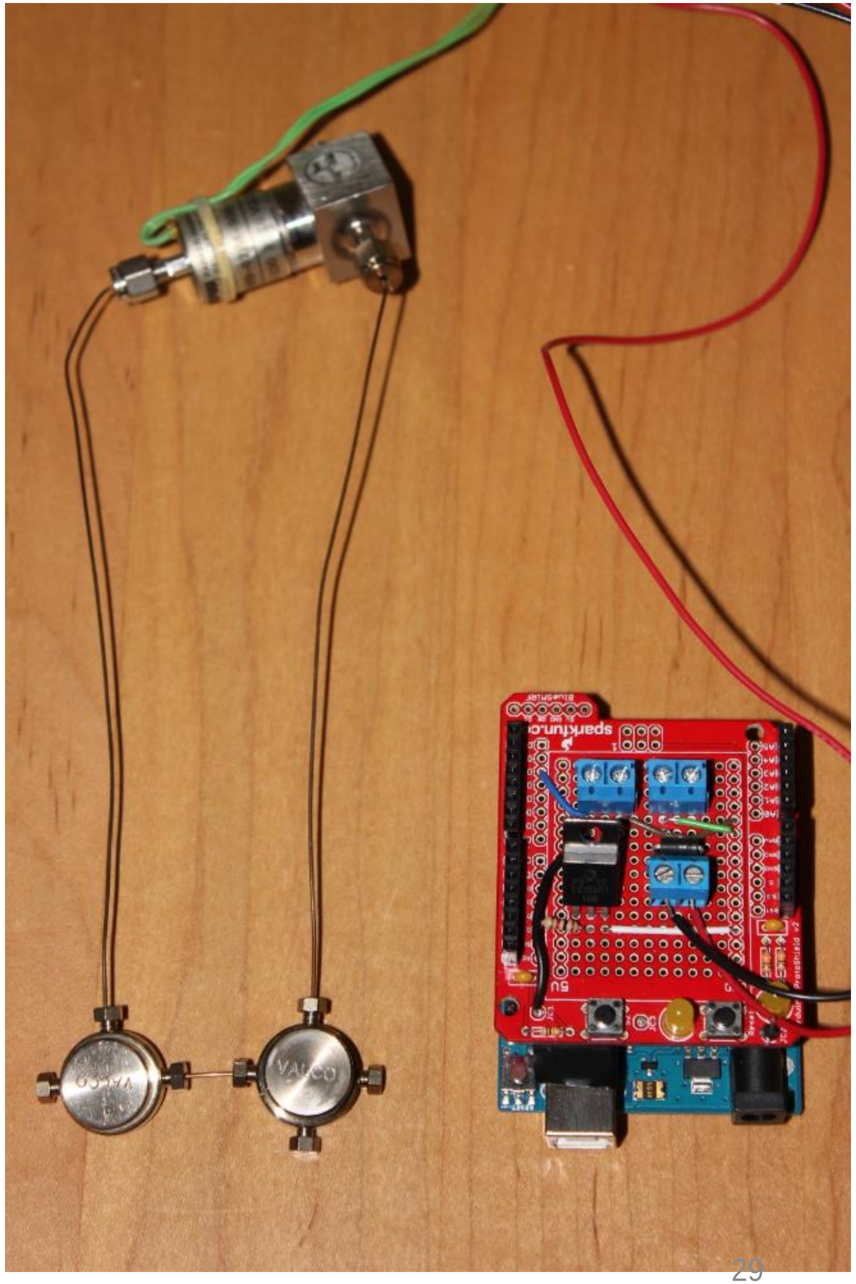


Ghosh, Bates, Seeley, and Seeley, J. Chrom. A 1291, 258, 2014.

A Simple Valve-Based Modulator:

3-port, 2-way Solenoid Valve
1/32” Valco Tee – ZT.5L
1/32” Valco Cross – ZX.5L
Restek MXT Deactivated Capillaries (0.53 mm ID)

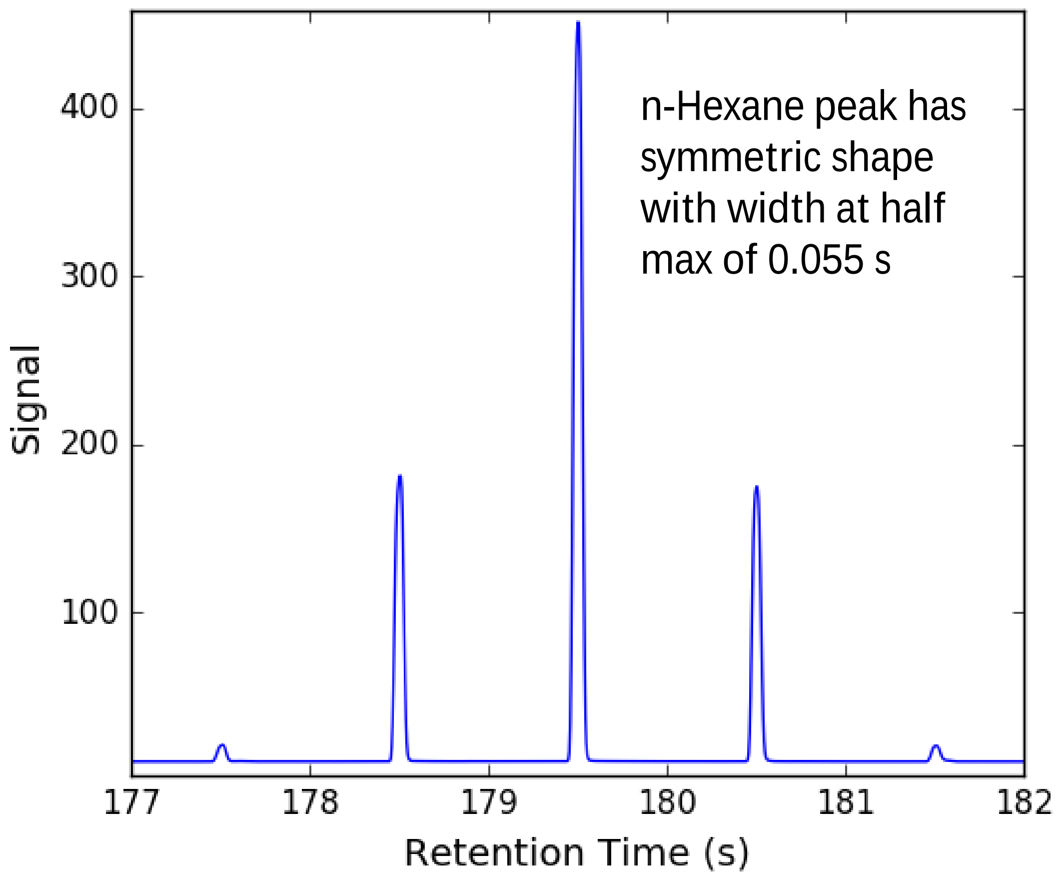
Actuation of the valve/modulator is controlled with a Arduino Uno Microcontroller Board using software written “in-house”



Diversion Modulation with the Joining Capillary Approach:

1.00 s modulation period
0.050 s injection time

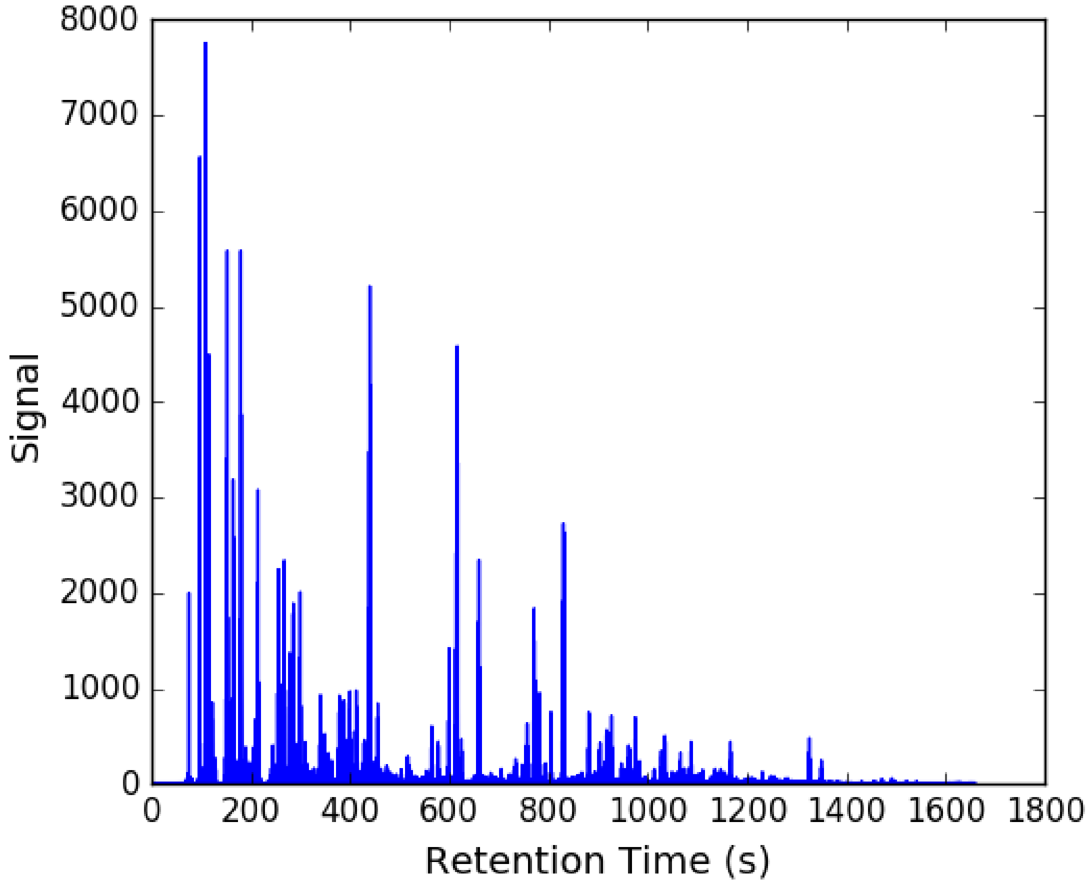
F_1 = 2.0 mL/min
F_2 = 1.8 mL/min

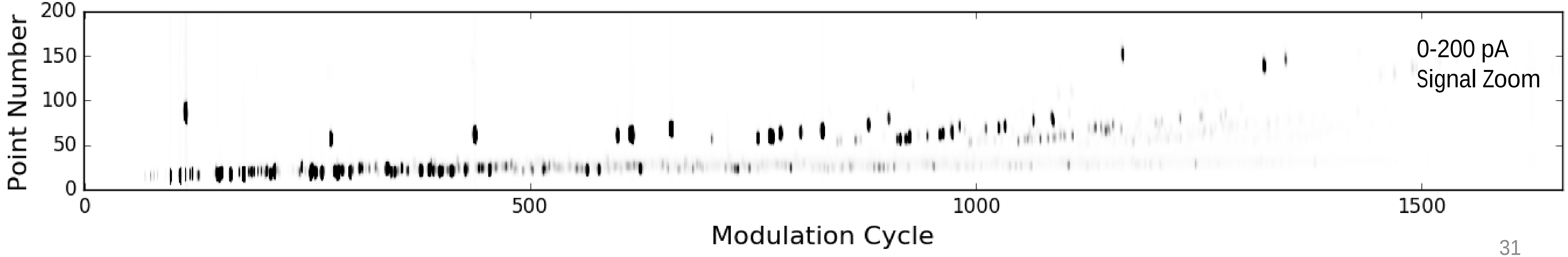


Diversion Modulation with the Joining Capillary Approach:

1.00 s modulation period
0.050 s injection time
F_1 = 2.0 mL/min
F_2 = 1.8 mL/min

Gasoline, 1 uL injected
1:50 split



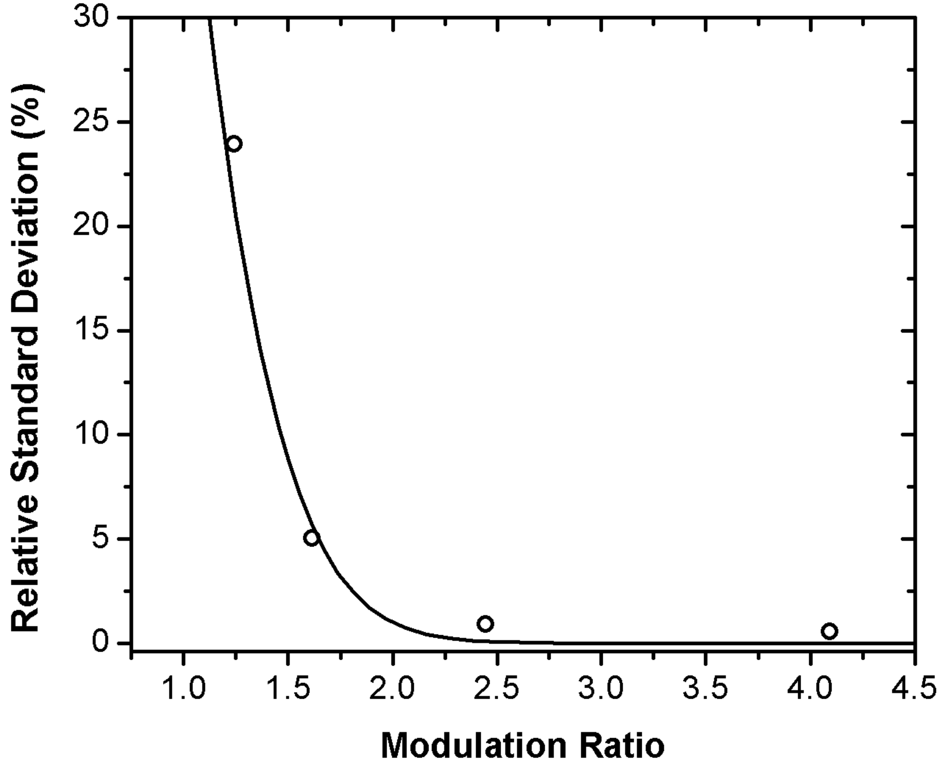


Note: 25 min separation

Diversion Modulation Provides Quantitative Results As Long as the Primary Peaks are not Under-Sampled:

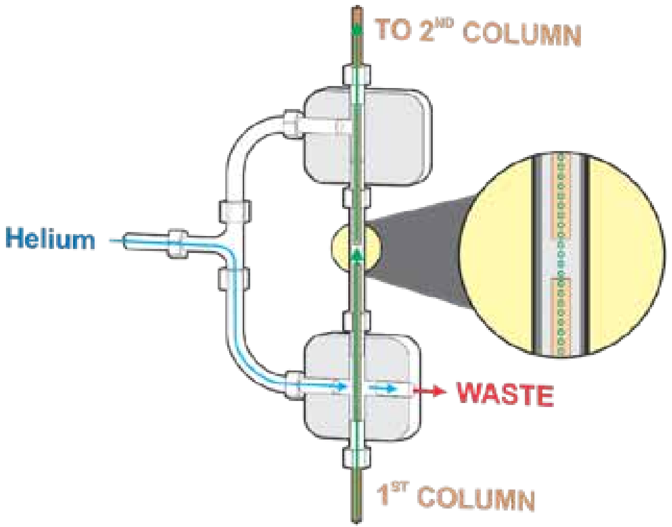
Low duty cycle modulation requires that modulation ratio is > 2.5.

Modulation ratio is $4s/P_m$. So keep the modulation period a little less than the peak widths at half maximum.

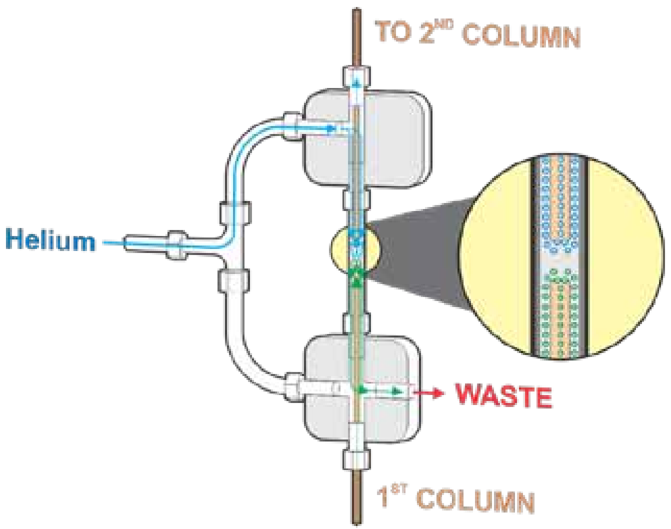


LECO
EMPOWERING RESULTS

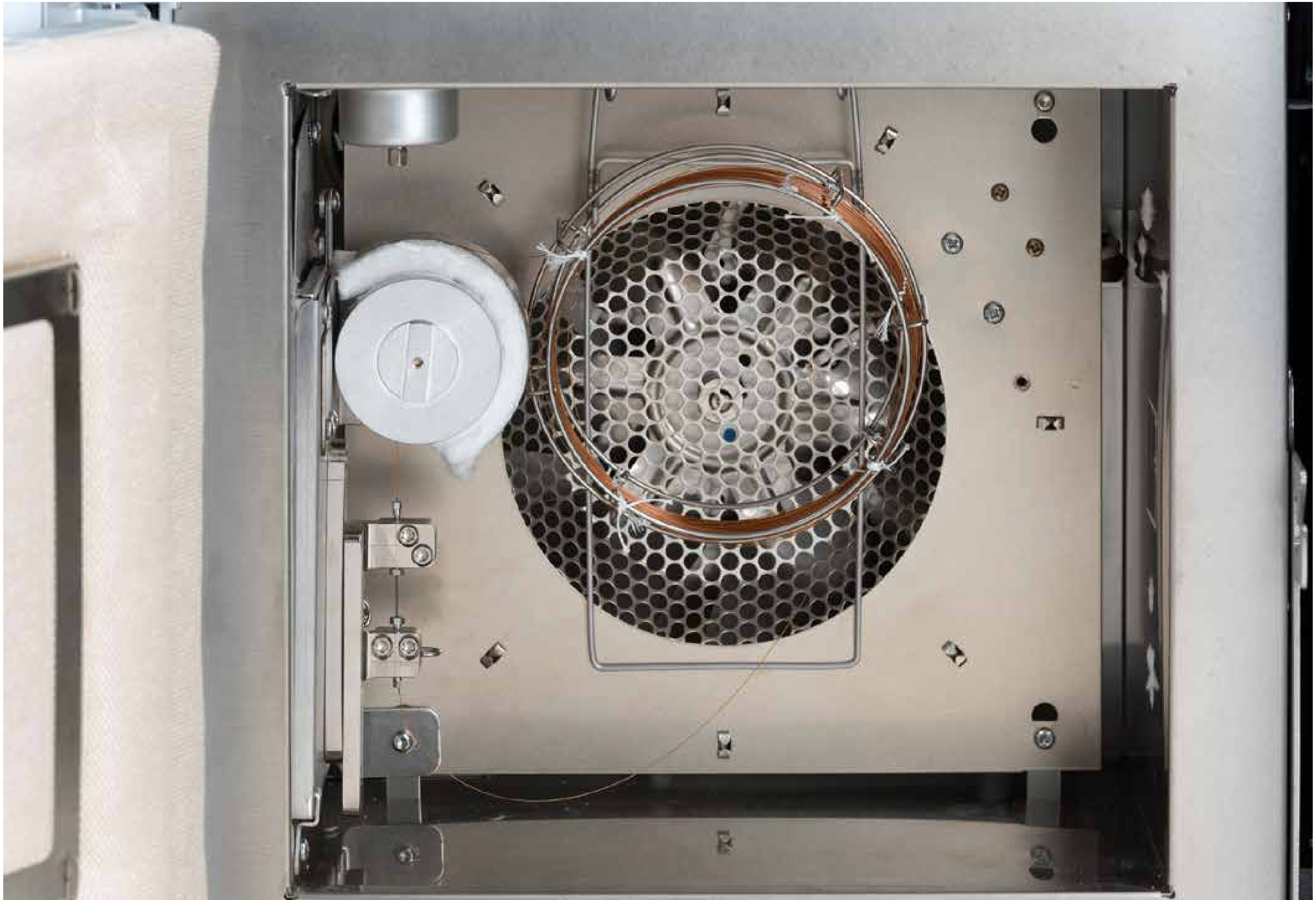
FLUX
GC×GC



Inject Mode

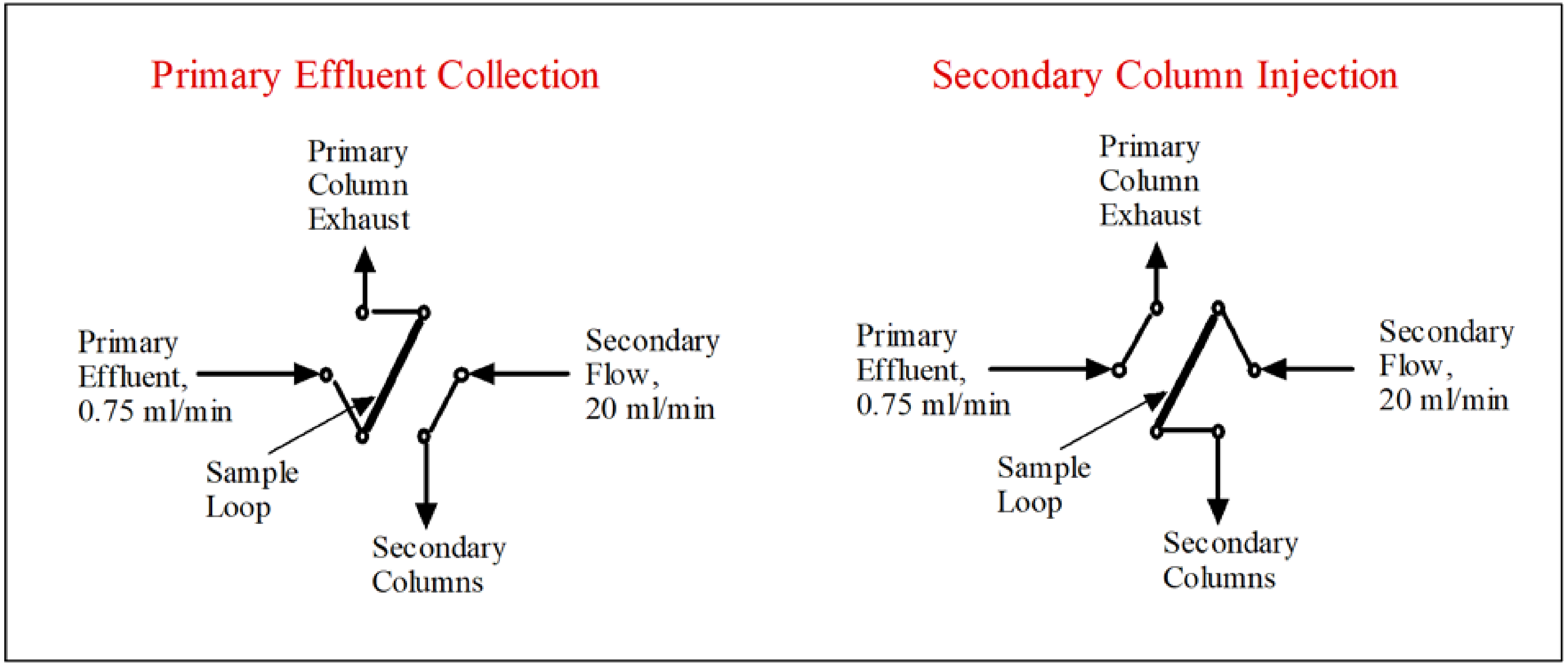


Divert Mode



Differential Flow Modulation

Differential Flow Modulation



Higher duty cycles possible (d = 0.9).
Diaphragm valves impose temperature limitations.
Higher secondary column flows are required.

Anal. Chem. **2000,** *72,* 4346−4352

Comprehensive Two-Dimensional Gas Chromatography via Differential Flow Modulation

John V. Seeley,* Frederick Kramp, and Christine J. Hicks

Department of Chemistry, Oakland University, Rochester, Michigan 48309-4477

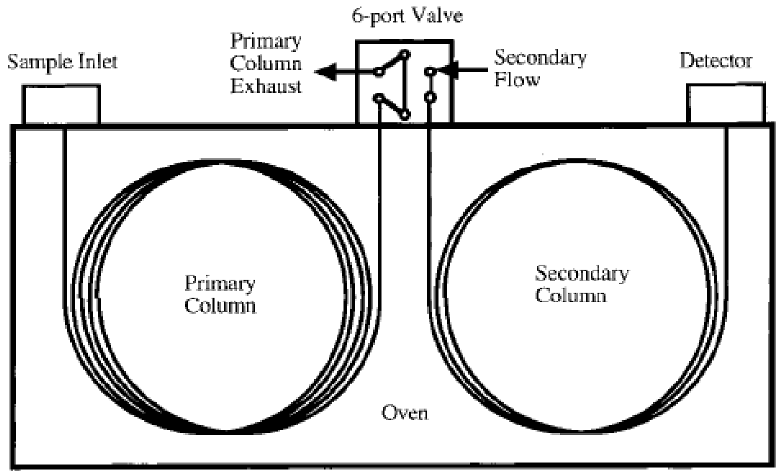
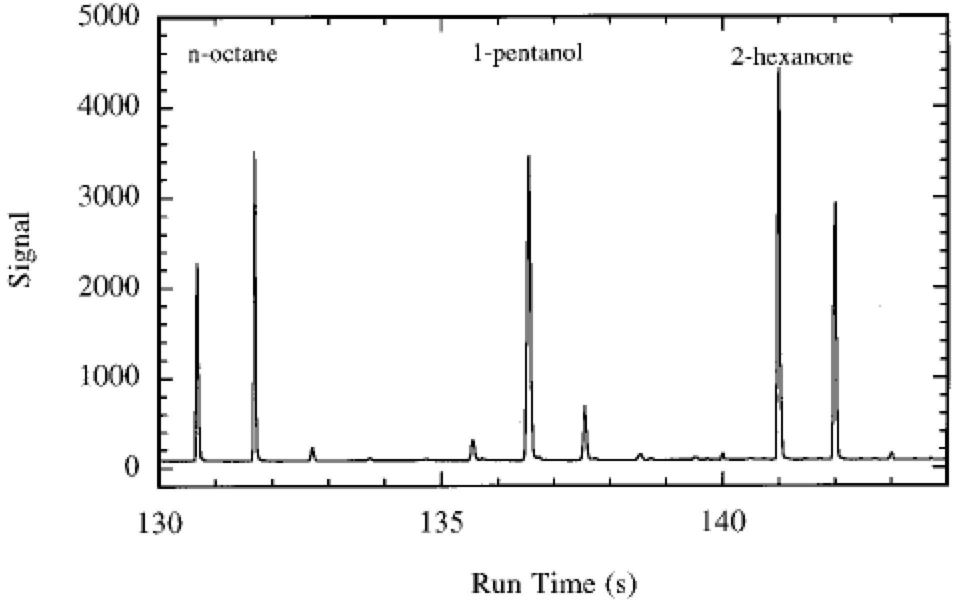
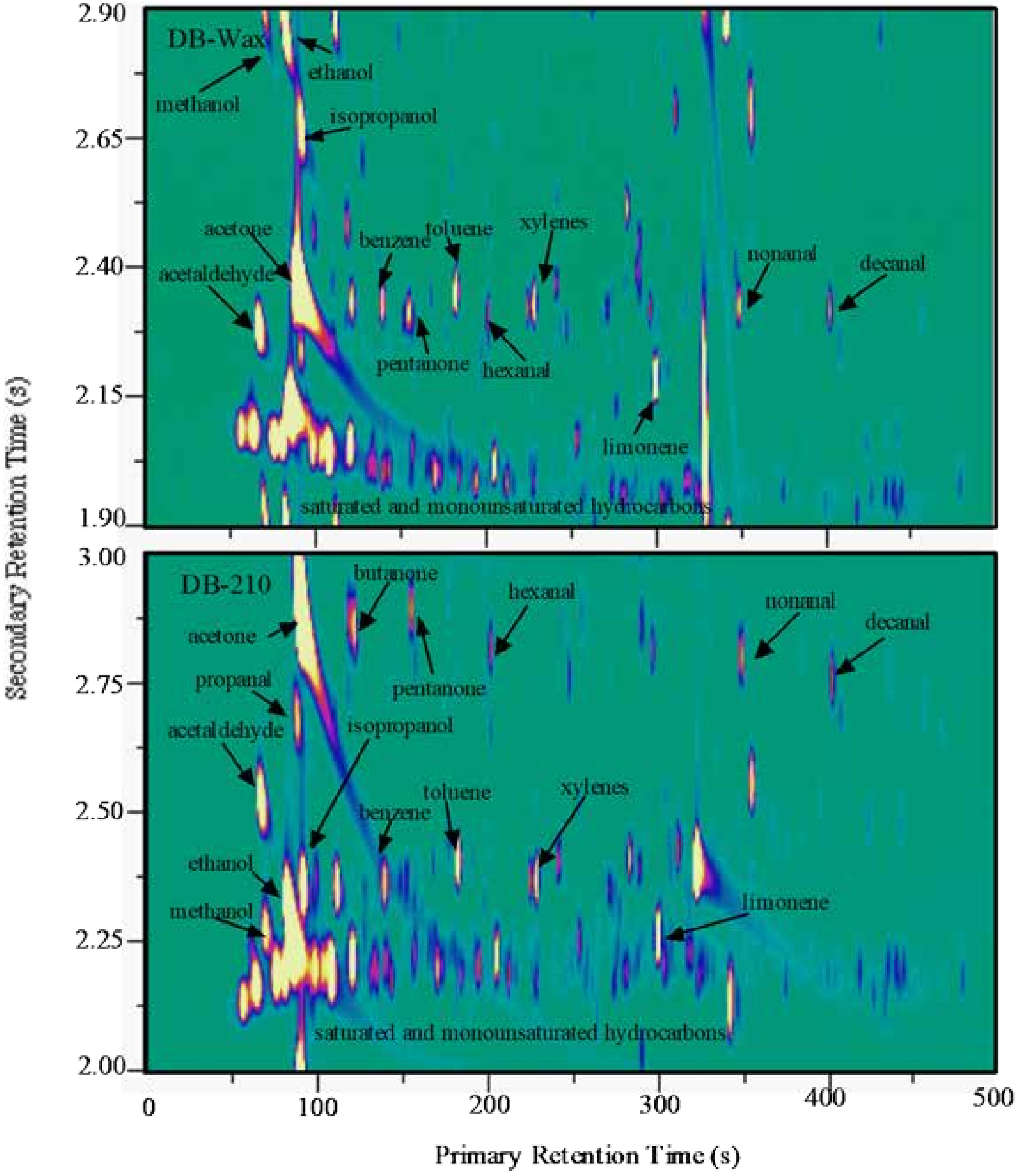


Figure 1. Schematic of a differential flow GC × GC system.



Breath



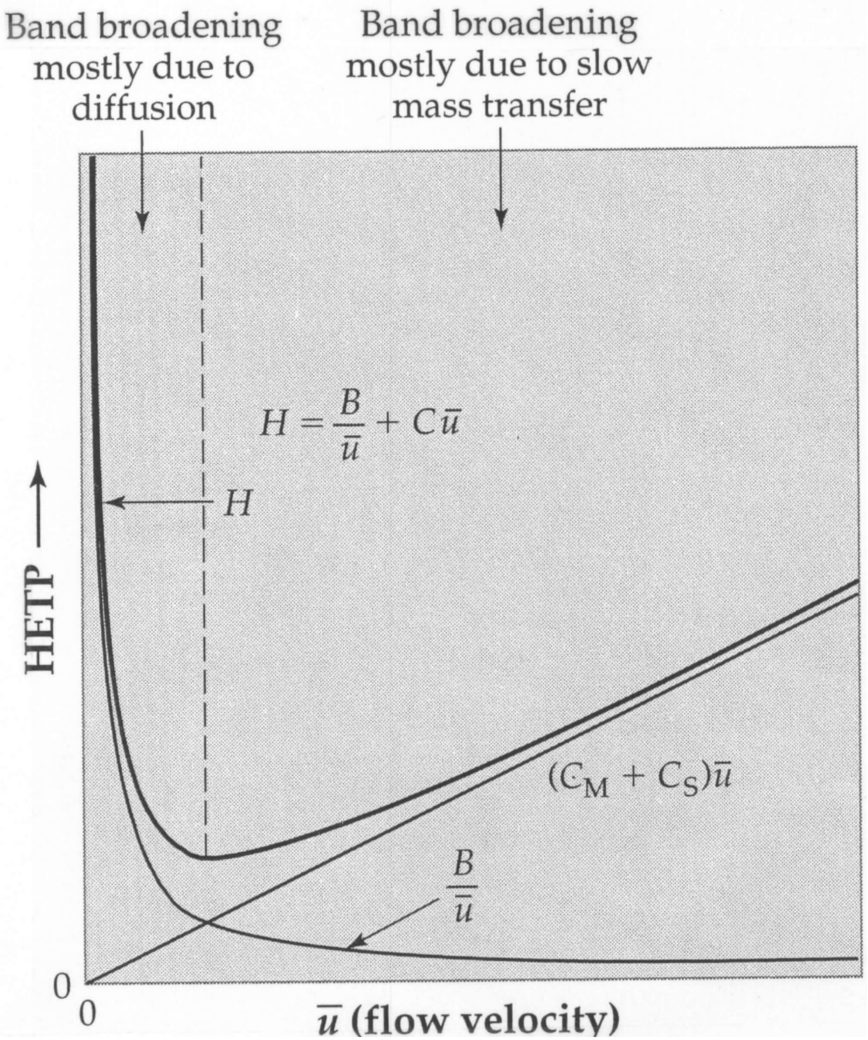
1.5 L sampled
Full Scale = 500

Digression on Flow Optimization in the Secondary Column

Why?

Because with differential flow modulation, the width of the input pulse decreases with increasing secondary column flow.

Be Careful with the van Deemter Plot!



<u>What this plot used to mean to me:</u>

- There is an optimal flow (normally around 1 mL/min) that minimizes plate height.
- Operating at this flow produces the narrowest peaks.
- Peaks will be much broader at flows higher than the optimal flow.

Image taken from Rubinson and Rubinson, "Contemporary Chemical Analysis"

Don't over-interpret the van Deemter Plot!

$$w = \sqrt{w_0^2 + \frac{16\,H\,t_R^2}{L}}$$

H is just one of several factors that affect w.

For set values of L and w_0, minimizing H minimizes peak width.

A GCxGC secondary separation seeks to minimize peak width for a given range of t_R. L can be adjusted.

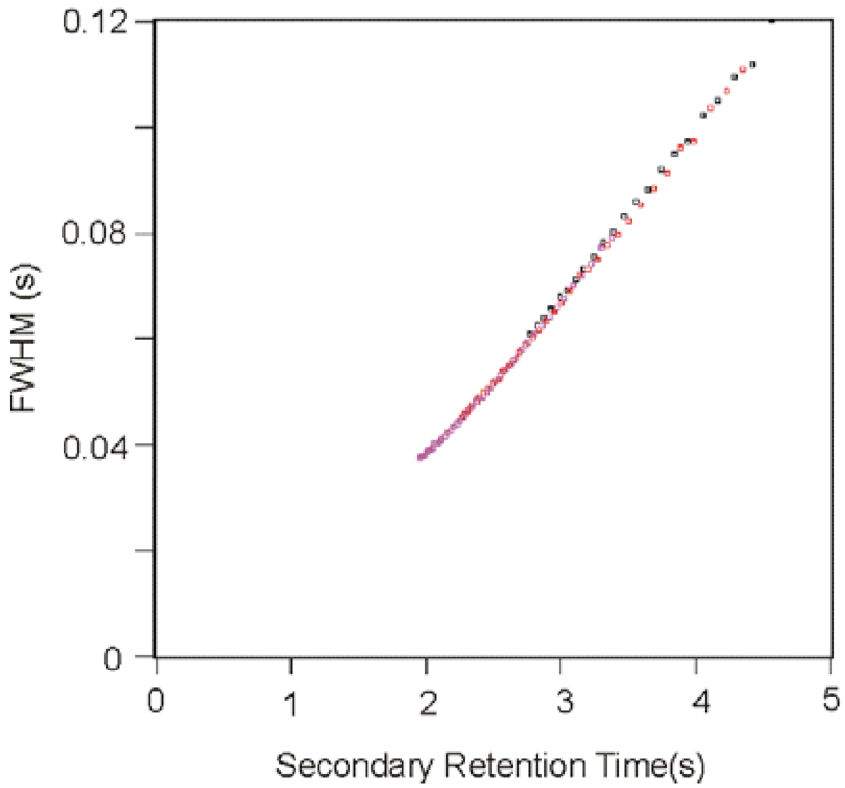
Under differential flow modulation conditions, increasing F_2 decreases w_0 but also increases H (because it increases u).

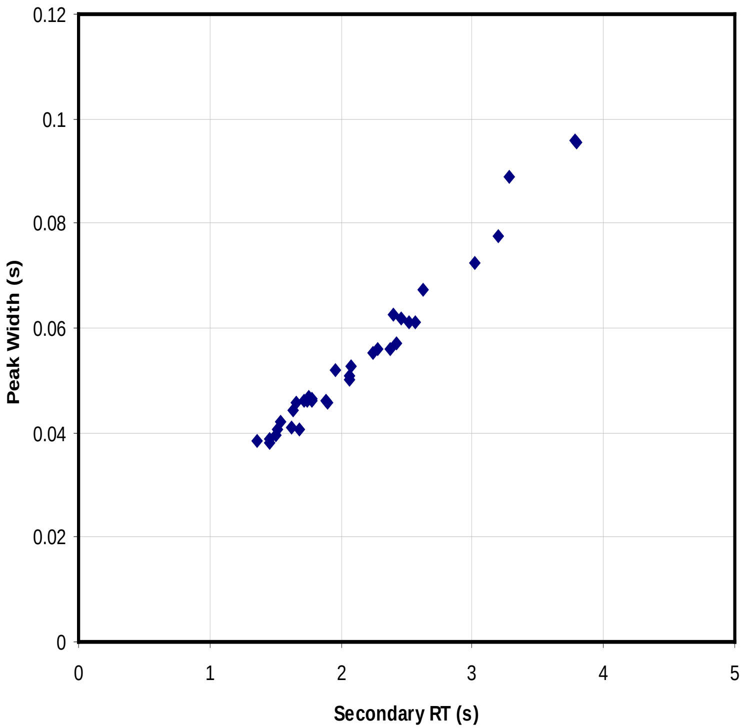
However, as F_2 is increased, L can be increased while maintaining the same retention times.

At high flows, $H \approx Cu$. So increasing L at the same rate as F_2 keeps the H/L ratio essentially constant.

Comparing High Flow Modulation To Thermal Modulation

Wax secondary columns

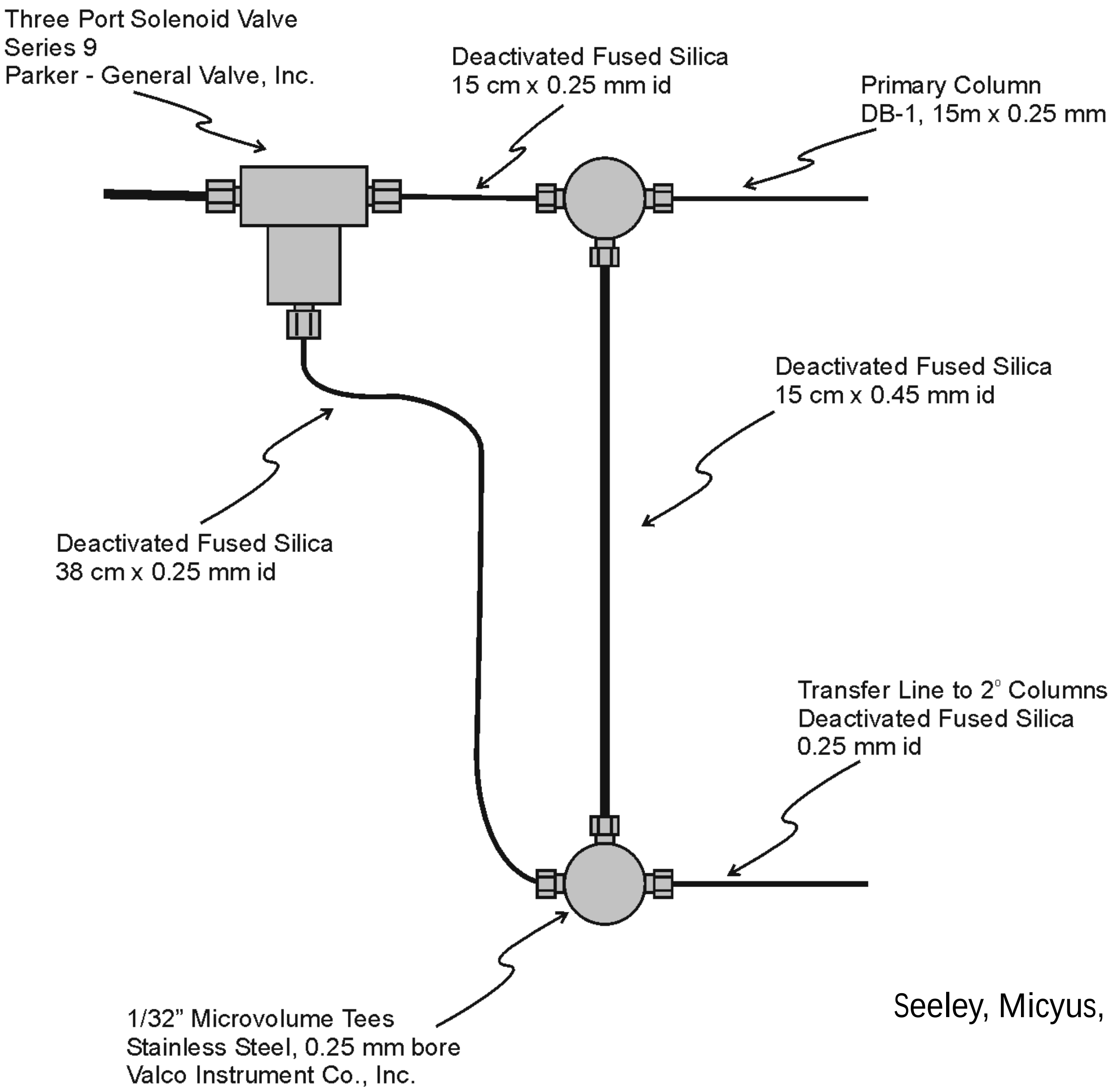




F = 10.5 ml/min
L = 500 cm
id = 0.25 mm
1500 pl/m

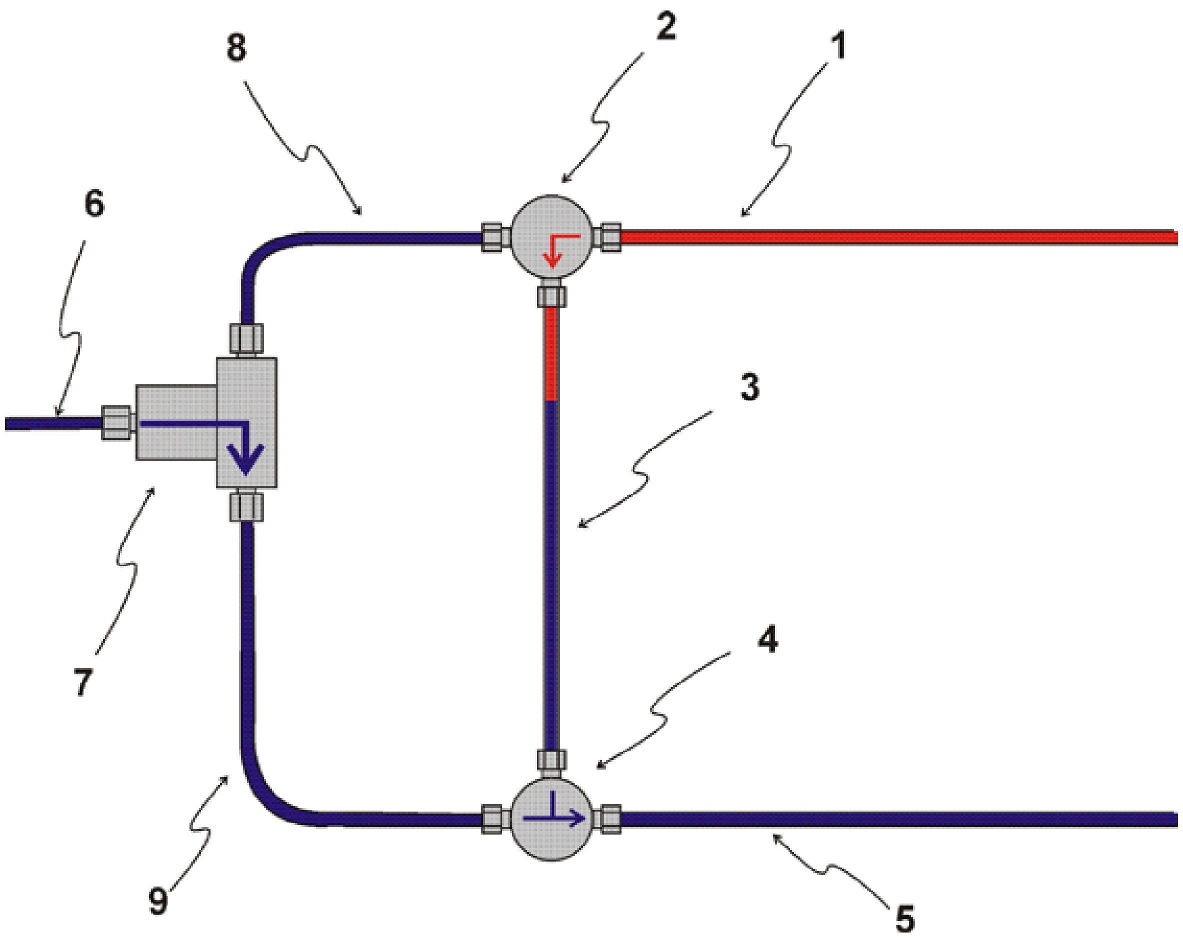
F = 0.80 ml/min
L = 100 cm
id = 0.1 mm
8800 pl/m

A Simple Differential Flow Modulator

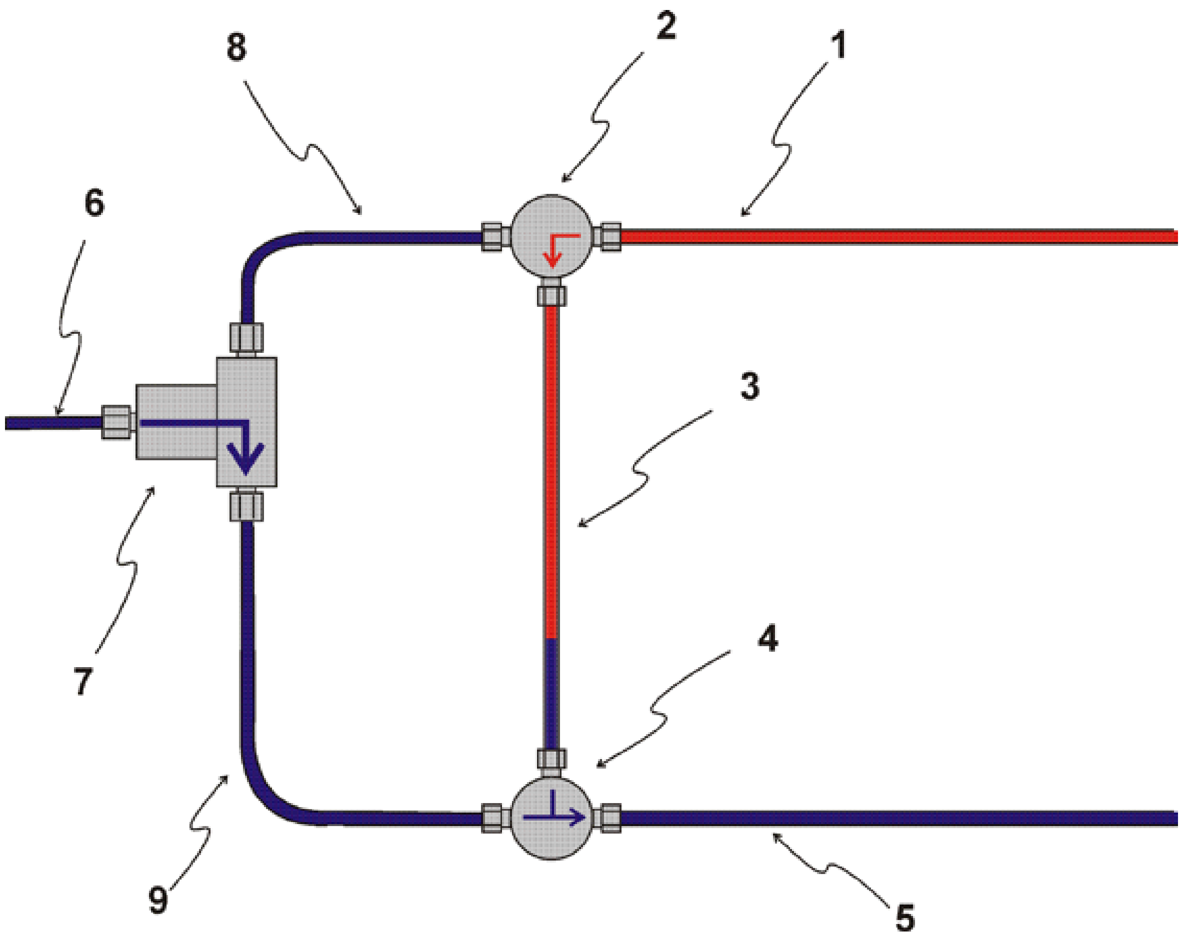


Seeley, Micyus, McCurry, Seeley, Am. Lab. News, 38, 24, 2006.

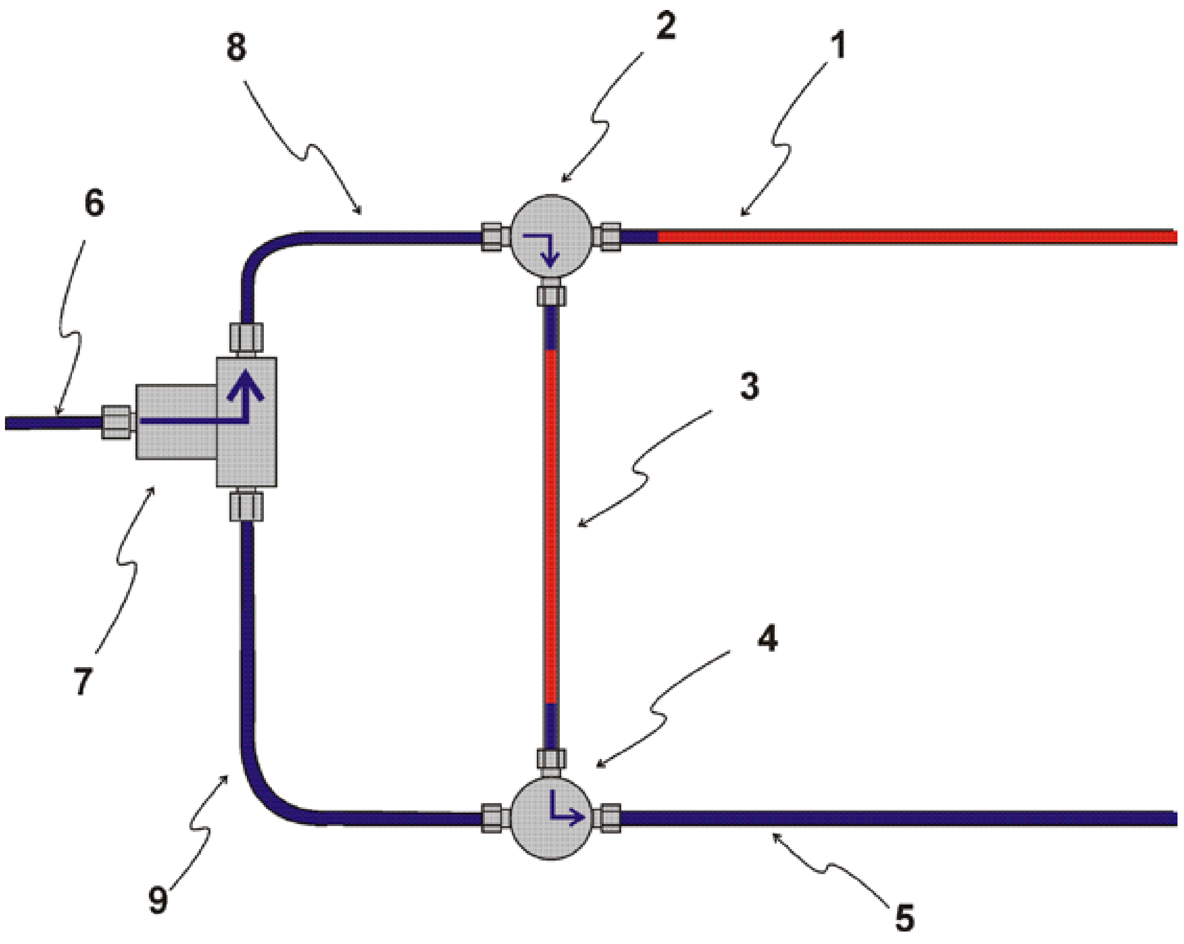
Loading The Sample Loop



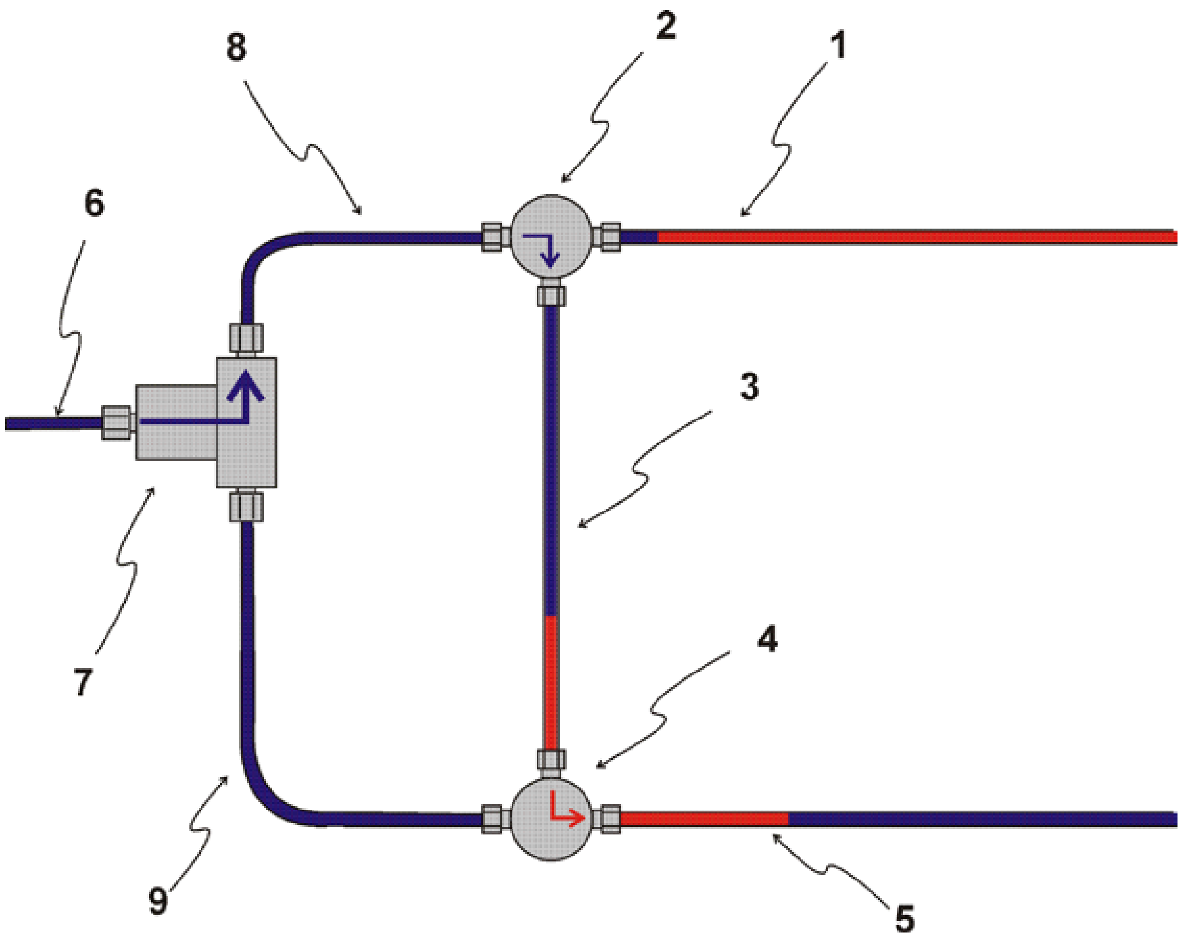
Loading The Sample Loop



Flushing The Sample Loop



Flushing The Sample Loop



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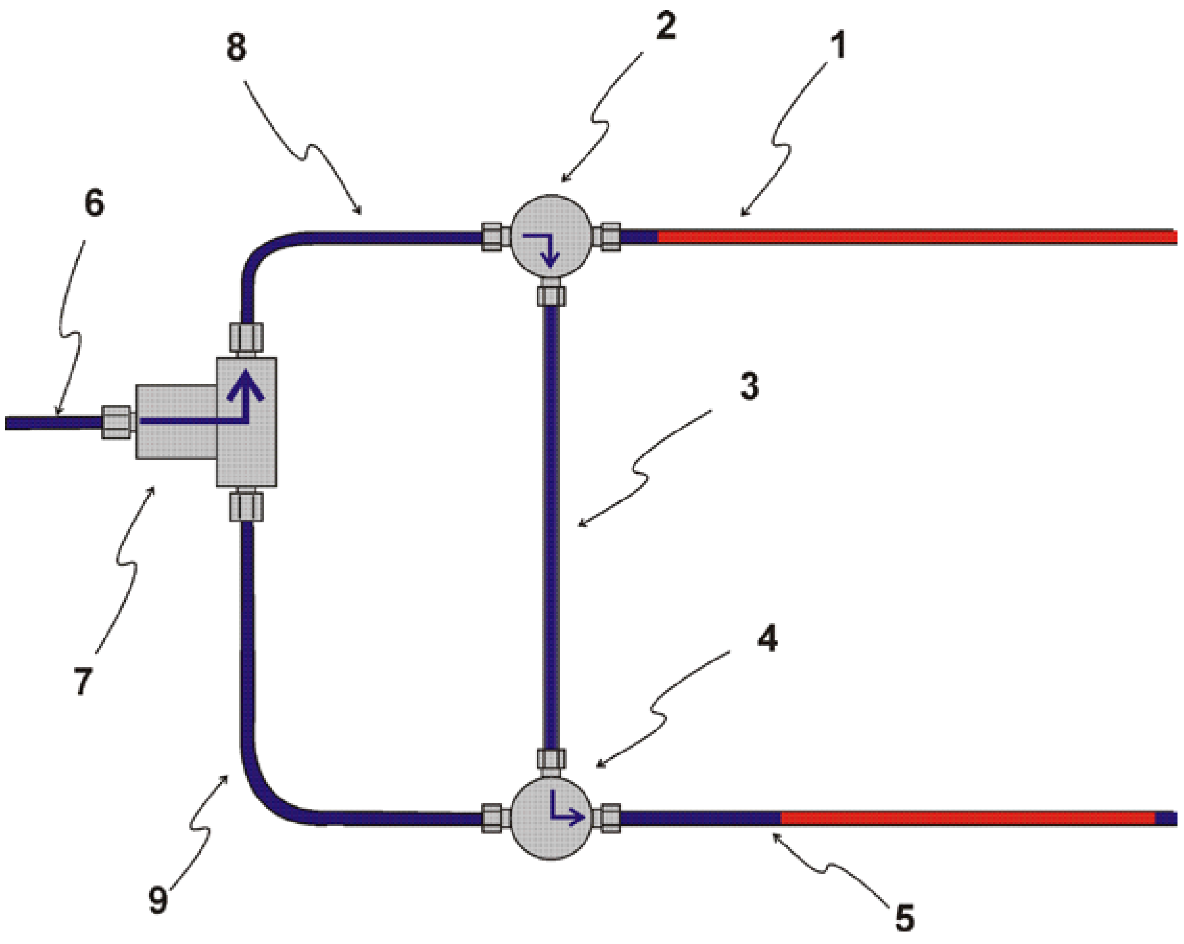
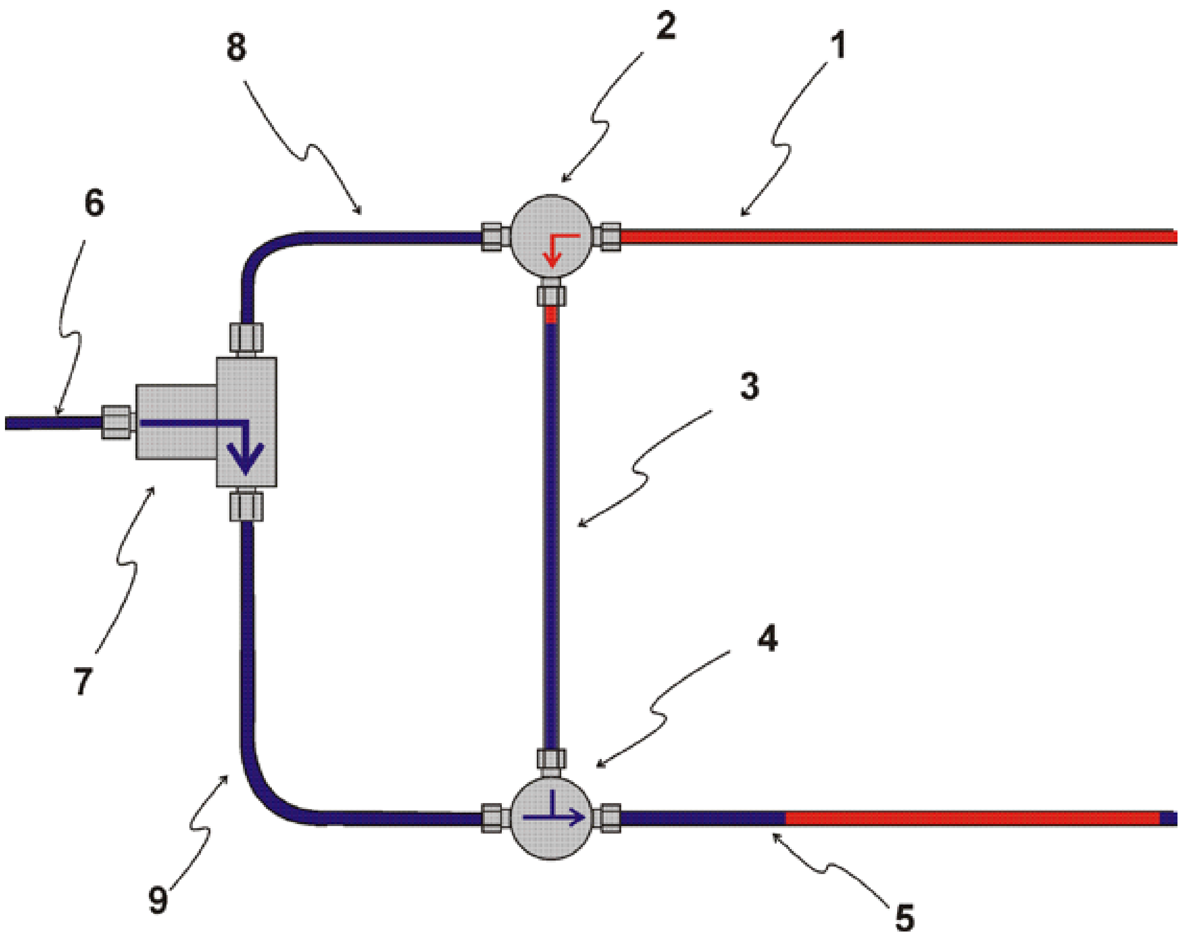
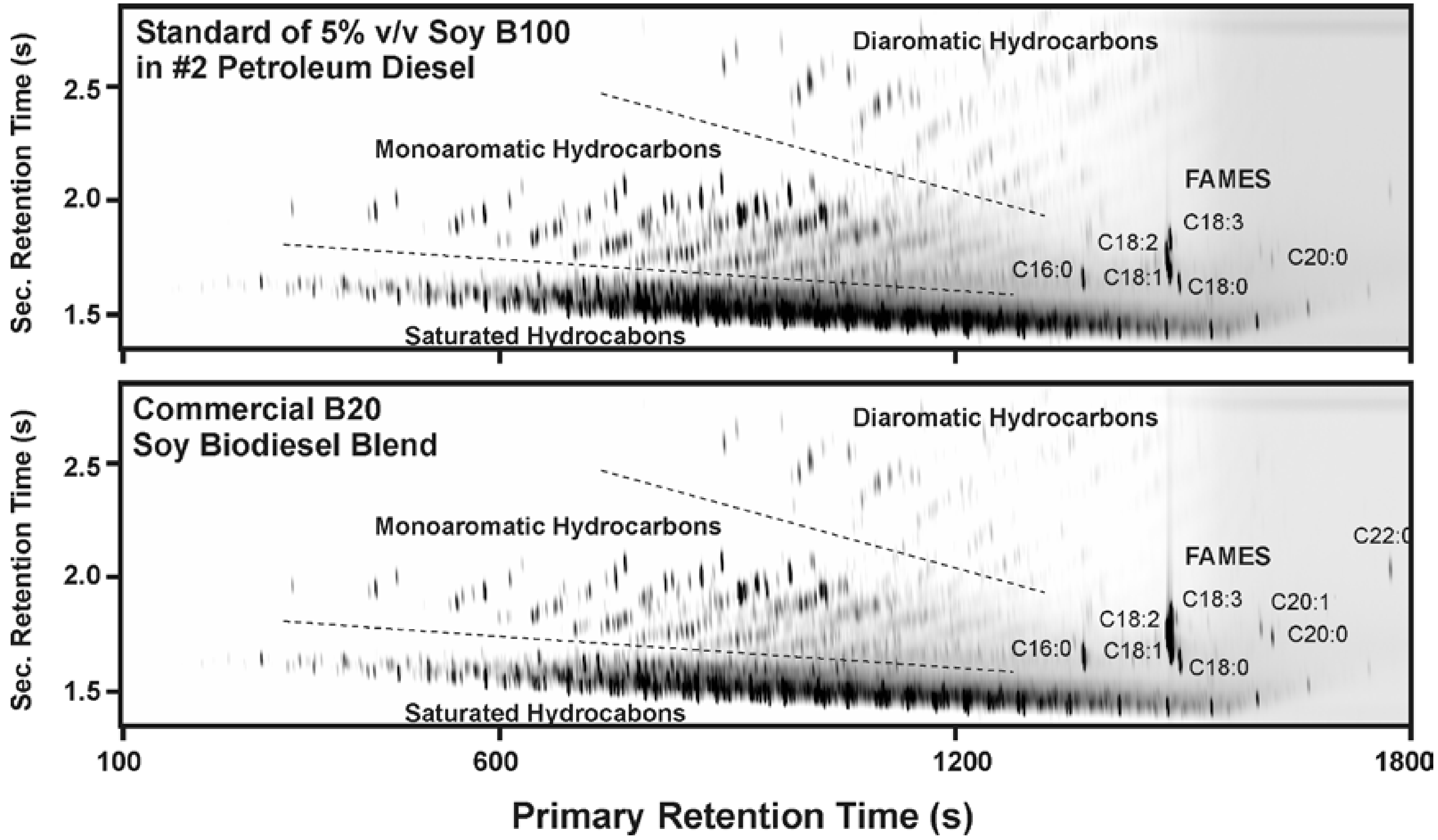


Loading The Sample Loop



Differential Flow GCxGC Analysis of Biodiesel Blends



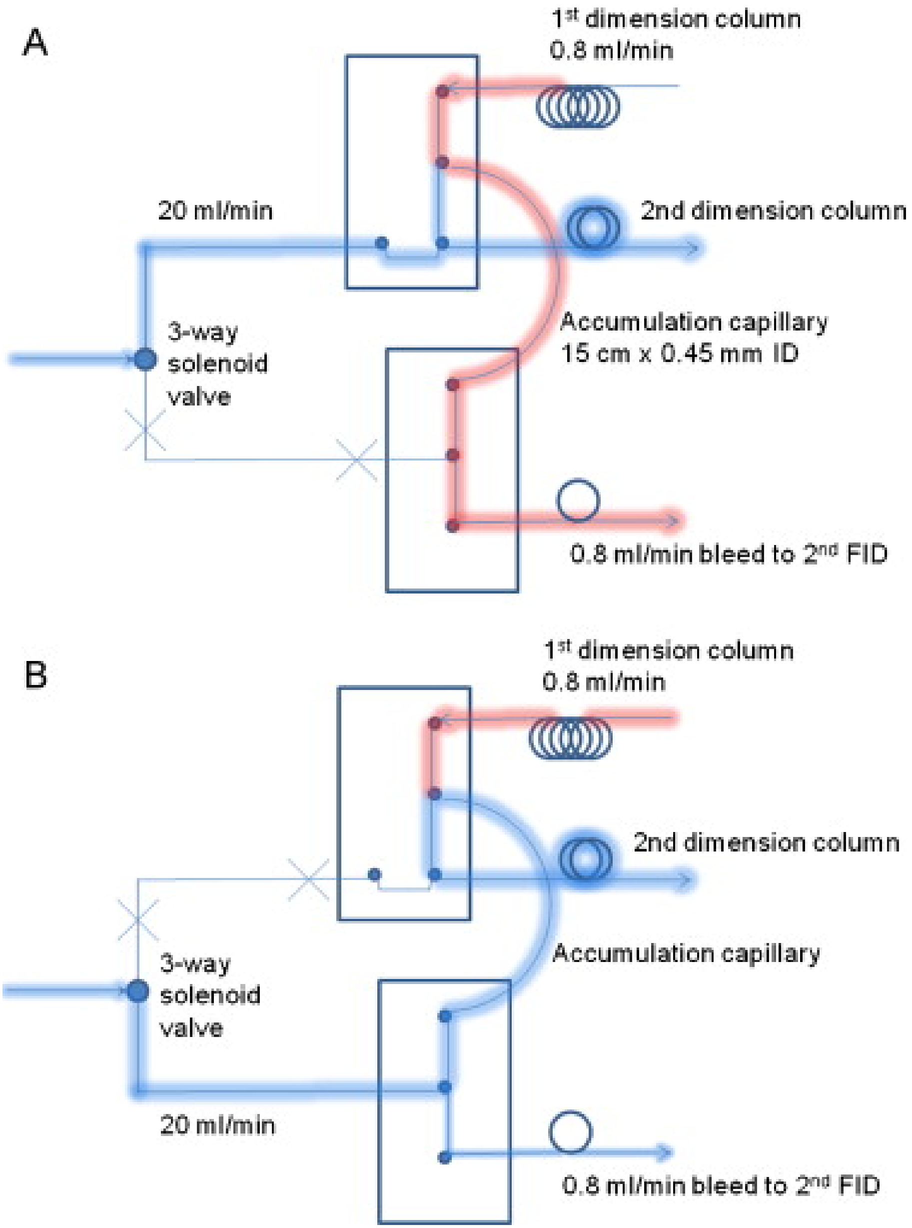
Seeley JV, Seeley SK, Libby EK, McCurry JD
Journal of Chromatographic Science, 45, 650-656, 2007.

The Reverse Flush Modulator:

An Improved Fluidic Modulator
For Differential Flow Modulation

James F. Griffith, William L. Winniford,
Kefu Sun, Rob Edam, Jim C. Luong

Journal of Chromatography A
Volume 1226, 2012, 116–123



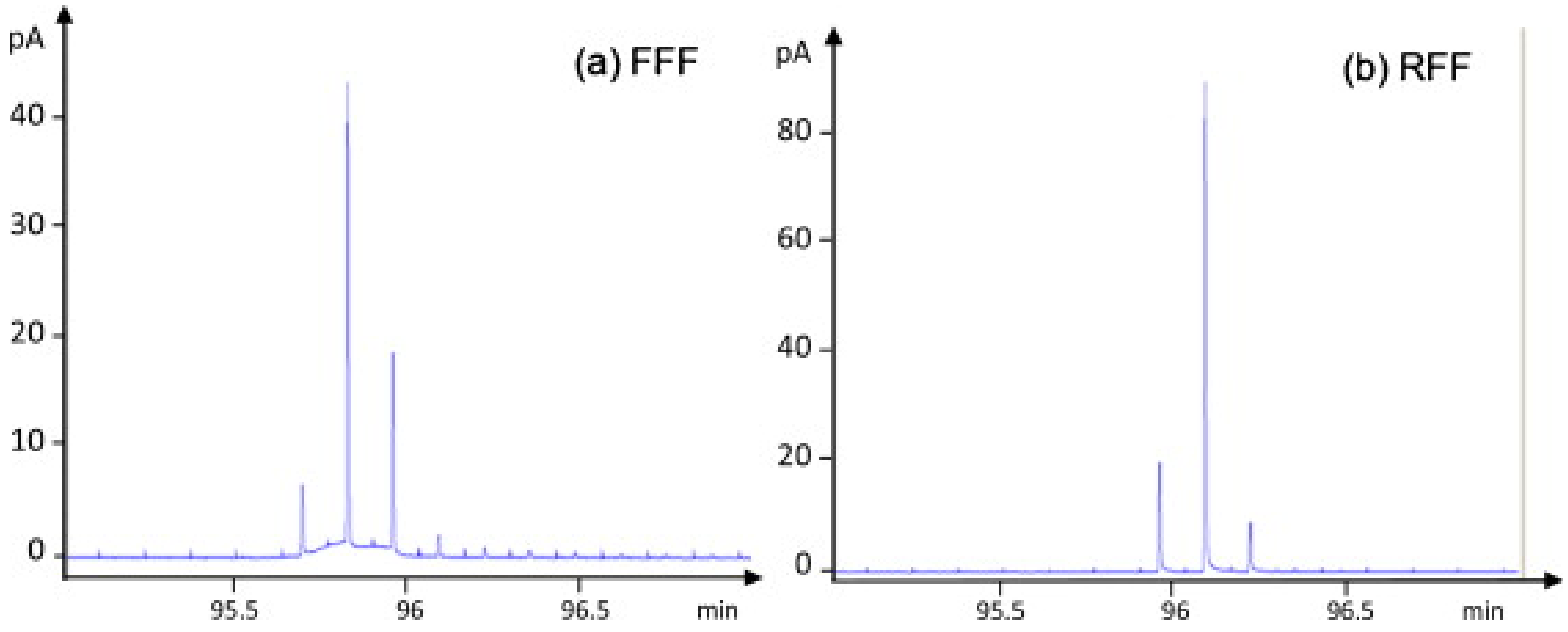


Fig. 3. Intensities of the modulated peaks of nC32 with a 1-plate FFF (a) and a 1-plate RFF (b).

Chloé Duhamel, Pascal Cardinael, Valérie Peulon-Agasse, Roger Firor, Laurent Pascaud, Gaëlle Semard-Jousset, Pierre Giusti, Vincent Livadaris

Comparison of cryogenic and differential flow (forward and reverse fill/flush) modulators and applications to the analysis of heavy petroleum cuts by high-temperature comprehensive gas chromatography ☆

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http://dx.doi.org/10.1016/j.chroma.2015.01.095

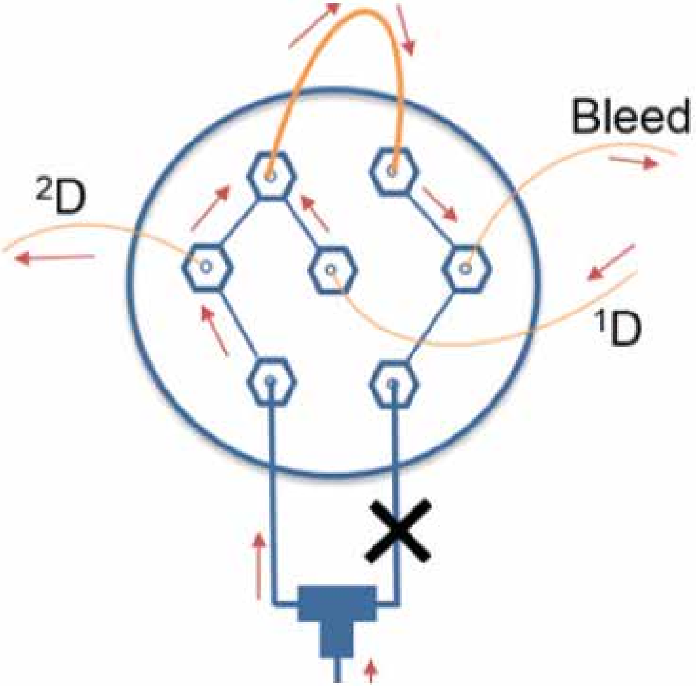
How the Reverse Flush Modulator Improves the “Two Tee” Modulator

It doesn’t rely upon pressure pulses.

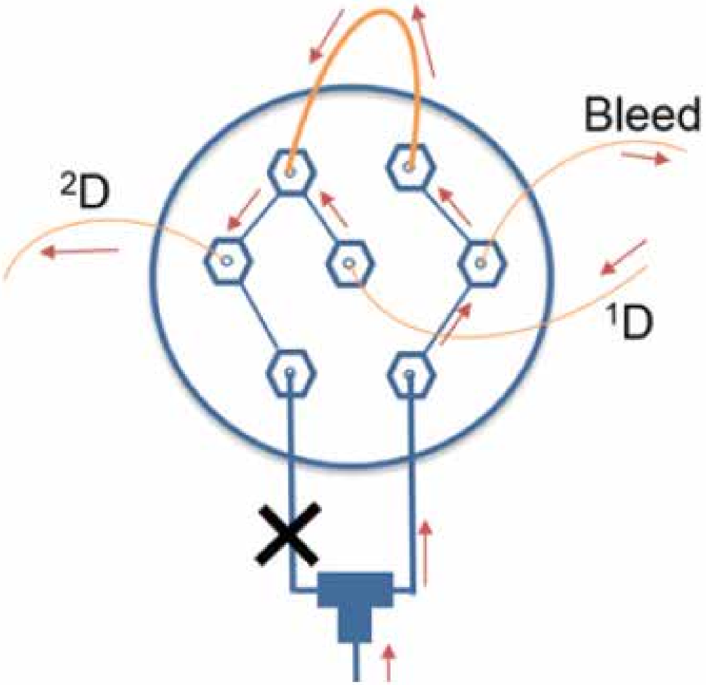
Potentially easier to optimize and to translate to new conditions.

Back flushing means the full loop need not be flushed.

Better at diminishing the impact of poor “tuning”.



Fill mode



'Flush' mode

The valve-based Insight modulator uses differential flows to simply 'fill' and 'flush' a sample loop – meaning low running costs for routine GC×GC and none of the logistical issues associated with liquid cryogen.

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The multi-mode modulator: A versatile fluidic device for two-dimensional gas chromatography

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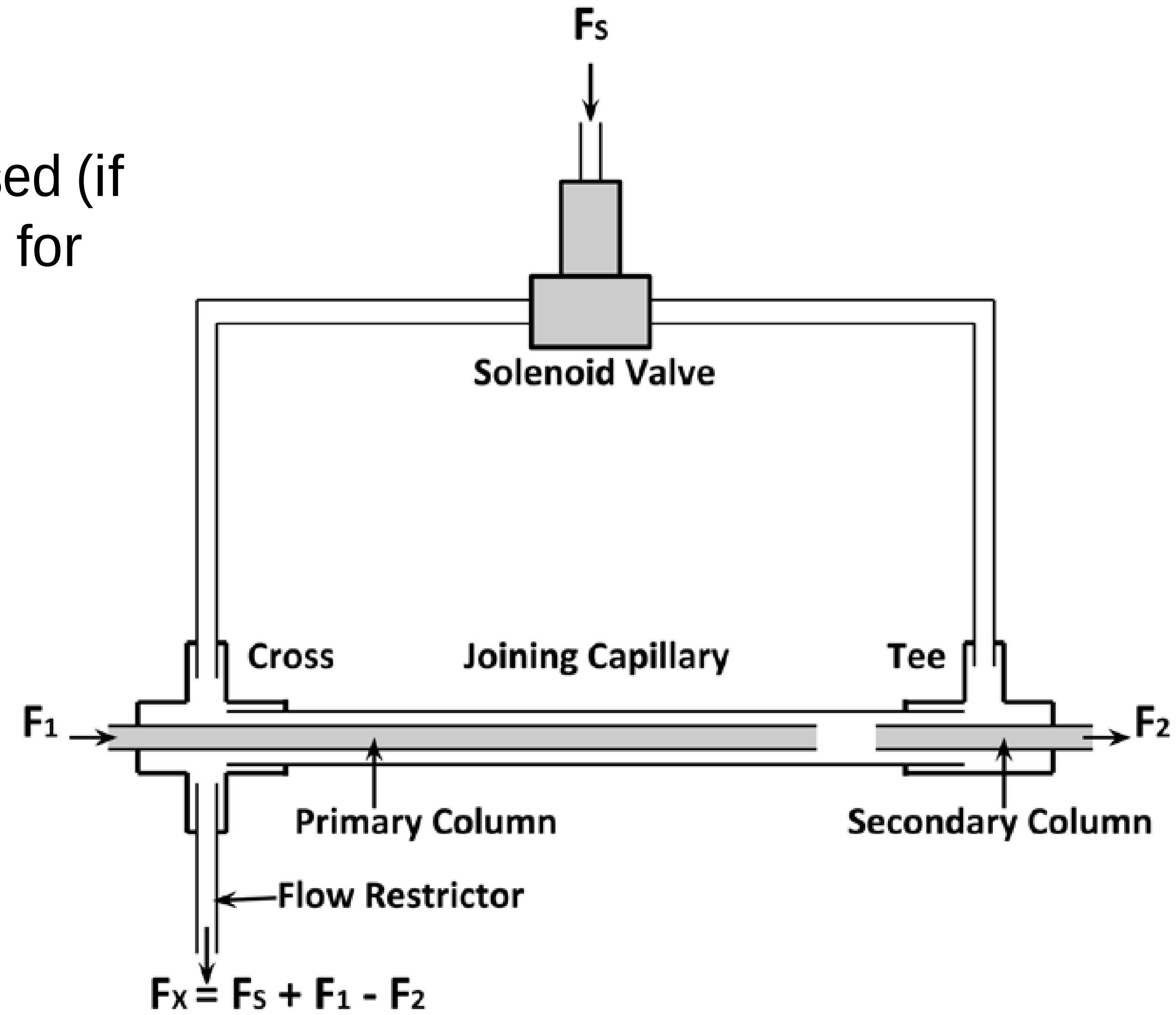
What's the Big Point? The same hardware can be used to perform diverting and differential flow. So the choice between Diverting vs Differential Flow is run-time choice not a hardware choice. This is much like (in more ways than one) choosing between a split vs split-less injection.

- Increase the size of the joining capillary
- Offset the junction area
- Now the joining capillary can be used (if you wish) to store primary effluent for differential flow modulation.

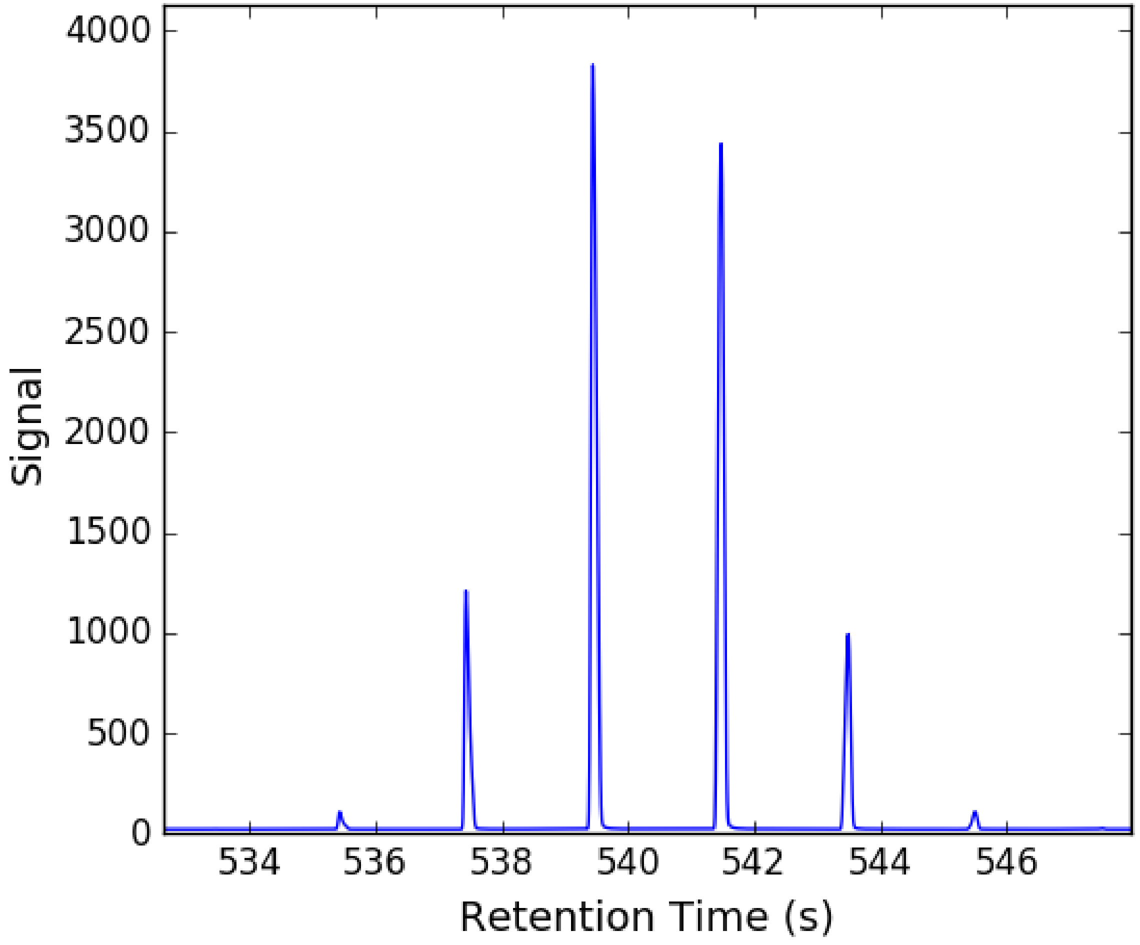


GC x GC Separations:

2.00 s modulation period
0.150 s injection time

F_1 =0.88 mL/min
F_2 = 20.0 mL/min

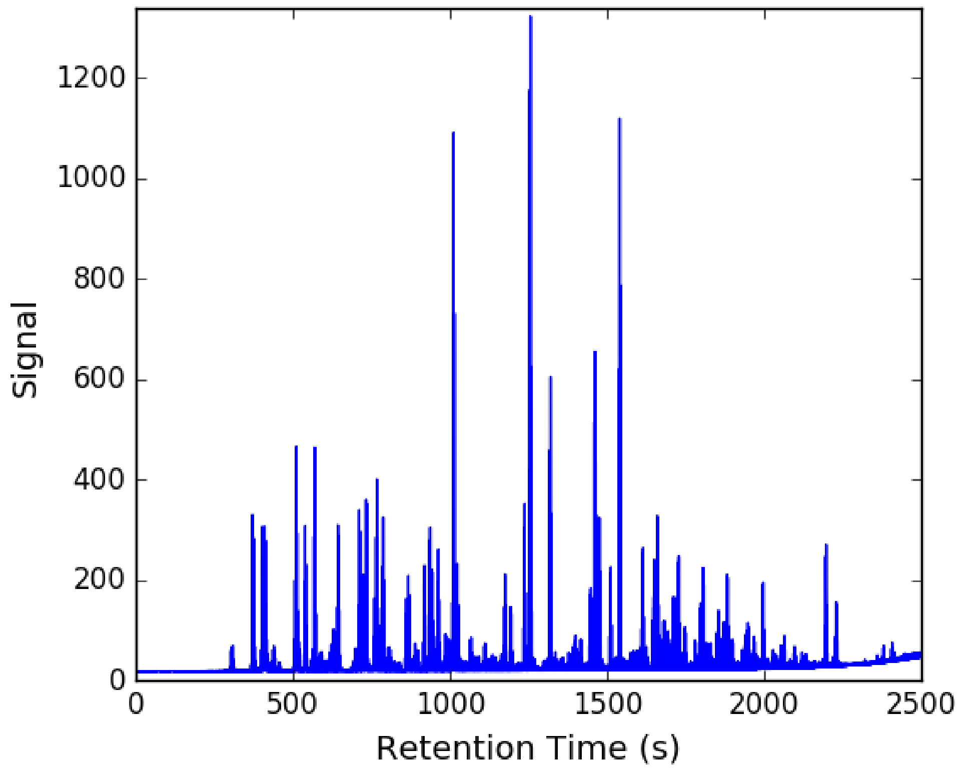
Alkane peak has minimal tailing with width at half max of 0.103 s

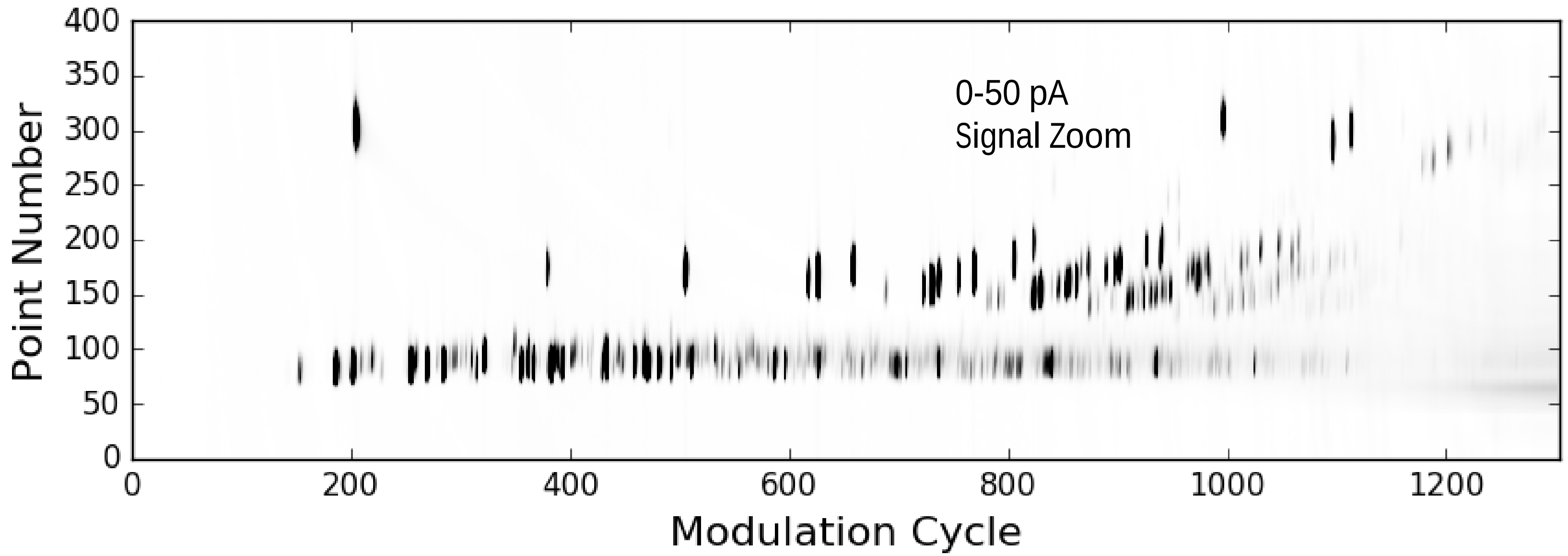


Differential Flow Moduation:

2.00 s modulation period
0.150 s injection time
F_1 = 0.88 mL/min
F_2 = 20.0 mL/min

Gasoline, <1 uL injected
1:100 split





Note: 20 min separation

Limits of Modulator Performance

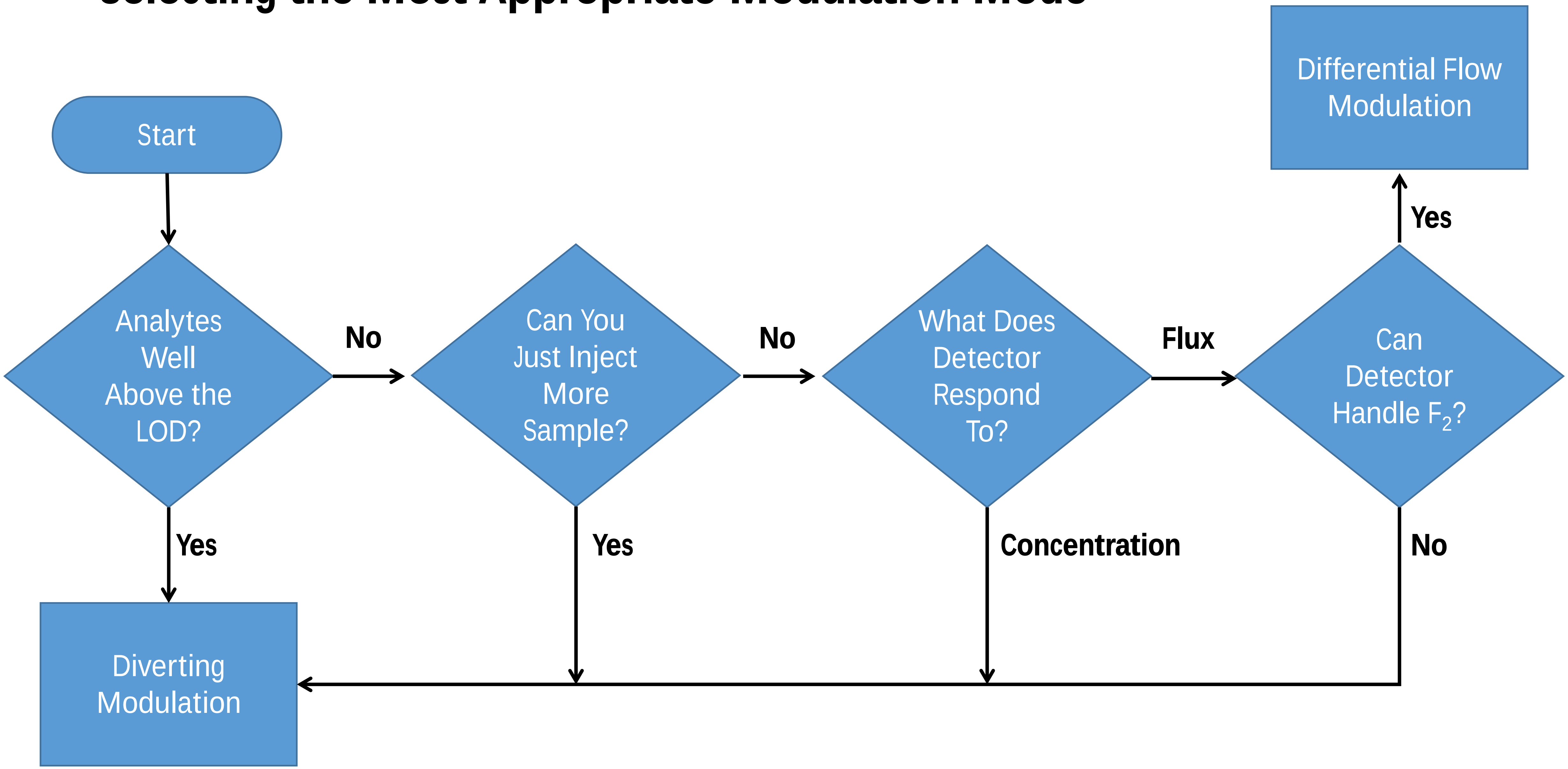
	Thermal	Diverting	Differential Flow
Min. Pulse Width	NL	t_{inj}	$(F_1 / F_2)\ P_M$
Max. 2º Peak Capacity	NL	P_M / t_{inj}	F_2 / F_1
Transfer Fraction	1	t_{inj} / P_M	1
Max. Conc. Enhancement	NL	1	1
Max. Flux. Enhancement	NL	1	F_2 / F_1

NL = No *Inherent* Limit Imposed By Modulator

Qualitative Comparison of Flow Modulator Classes

	Diverting	Differential Flow
Flow Flexibility	ü	
Column Flexibility	ü	
Detector Flexibility	ü	
P_M Flexibility	ü	
“Turn-off” w/o Dilution	ü	
Low Tailing Risk	ü	
Quantitative Precision		ü
Flux Enhancement		ü

Selecting the Most Appropriate Modulation Mode



Summary

Flow modulation is a simple way to generate GC x GC separations

Flow modulation always involves one or more compromises

- **Diverting mode:** transfer % vs. initial pulse width

- **Differential flow mode:** secondary column flow vs. initial pulse width

In many (but not all) cases, these compromises do not significantly diminish the utility of the resulting separation.

Mixed-modes of flow modulation are possible, and probably the future of flow modulation.

	Low Xfer		High Xfer	
Diverting	⟵		⟶	Differential
	Low F2		High F2	