Optimized Instrument Parameters for a High Temperature HPLC System with Flame Ionization Detection

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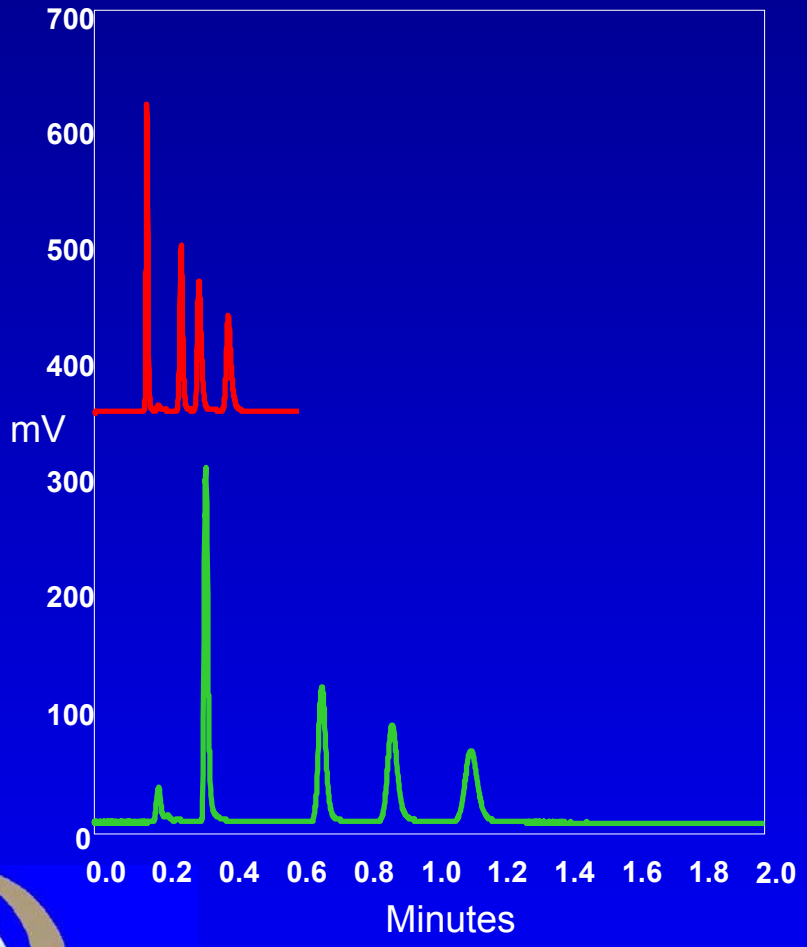
High Temperature Liquid Chromatography (HTLC) Advantages

The major advantages are

- Decrease analysis time (increase linear velocity)
- Increase efficiencies and resolution
- Tune selectivity with temperature
- Decrease organic solvent usage

Elution Strength Illustration: Separation of Steroids Using Water as the Mobile Phase



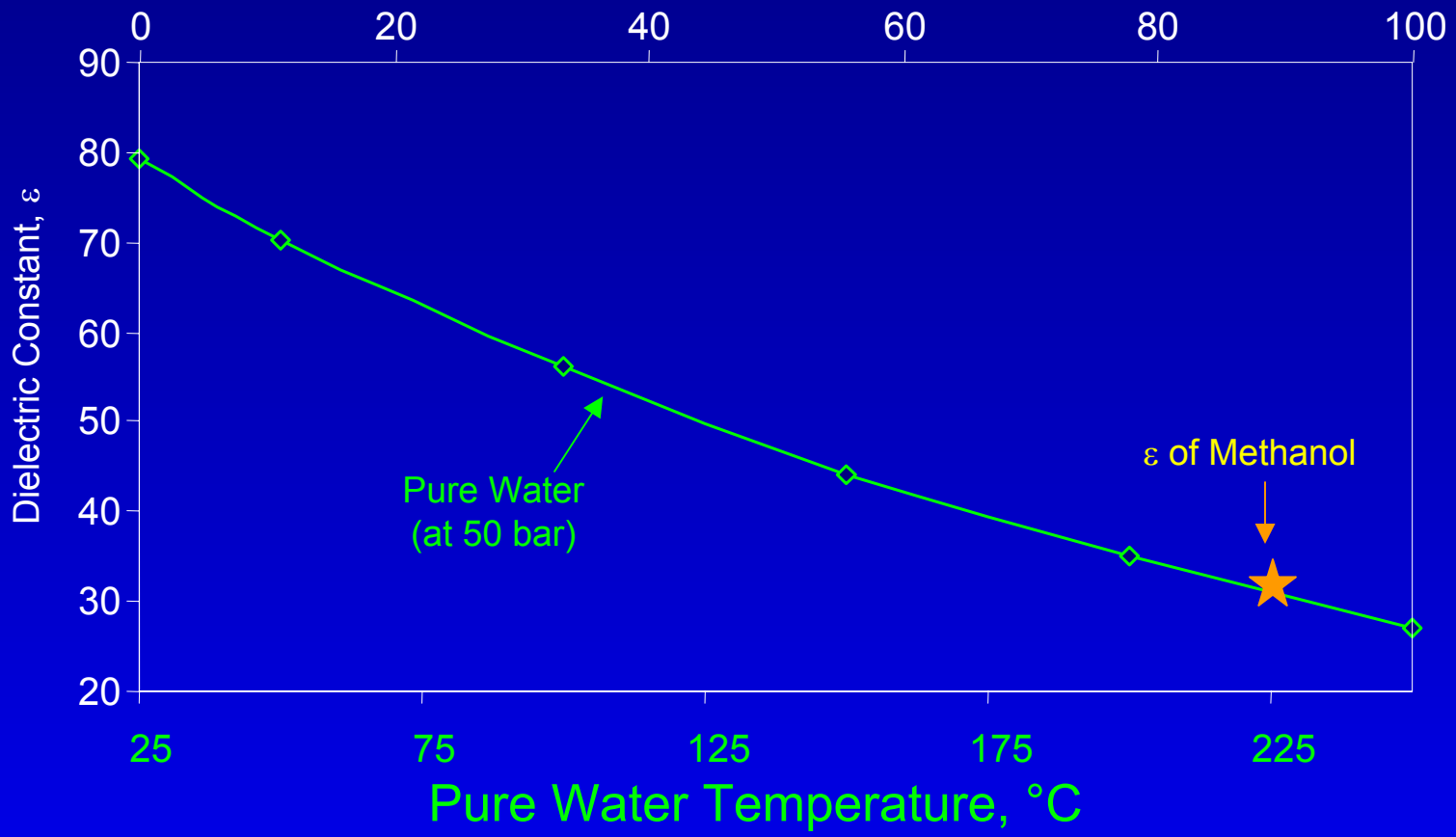
Column: ZirChrom PBD, 3 μm
100 X 4.6 mm
Detection: UV 254 nm

Flow Rate: 6.0 mL/min
Mobile Phase: Water
Temperature: 200°C

Flow Rate: 3.0 mL/min
Mobile Phase: 25:75 acetonitrile:water
Temperature: 50°C

Elution Order:
Uracil
Androstadienedione
Androstenedione
Epitestosterone

Solvent Polarity as a Function of Temperature



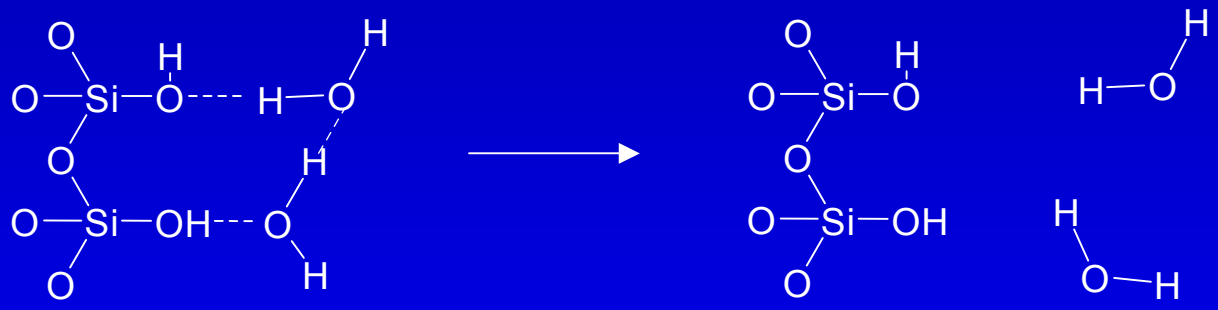
Data from Y. Yang et al. *J. Chromatogr. A* **810** (1998) 149.

Why Use Water as a Mobile Phase?

- Inexpensive
- Readily available
- Non-polluting
- Transparent to most detectors including UV, FID, and NMR (D_2O)
- Elution strength can be controlled by varying temperature

Temperature Affects of Hydrogen Bonding

- Increasing temperature-
  - Increases intermolecular distance
  - Weakens hydrogen bonds



Advantages of Using Flame Ionization Detection?

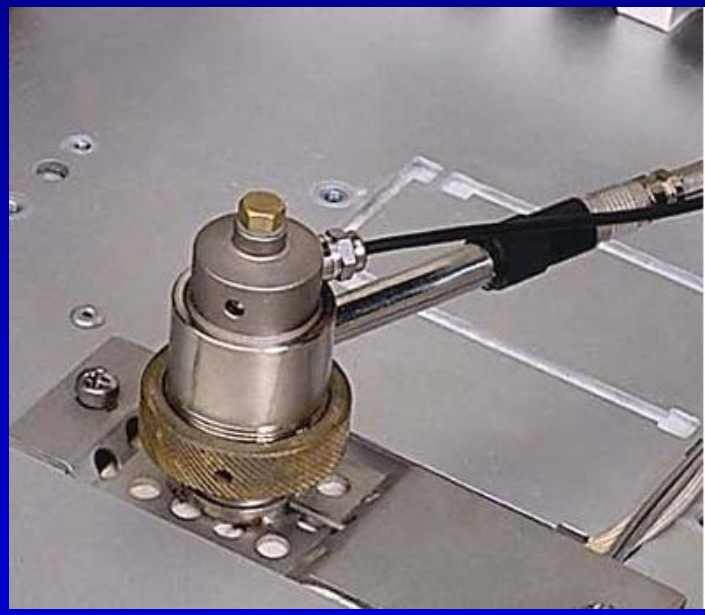
- Mass flow sensitive detector
- Response is proportional to the number of carbon atoms being burned
- Wide linear range, about 10^8
- Responds with high sensitivity to organic compounds

Instrumentation

- Aquachrom temperature programmable HPLC oven with FID
- Mobile phase preheater
- Split assembly
- Mobile phase pure water
- Eldex syringe pump
- Hypercarb™ column with stainless steel end fittings

FID Design

- Standard GC type design with grounded jet
- 0.020” internal diameter flame jet
- Temperature limit 400ºC

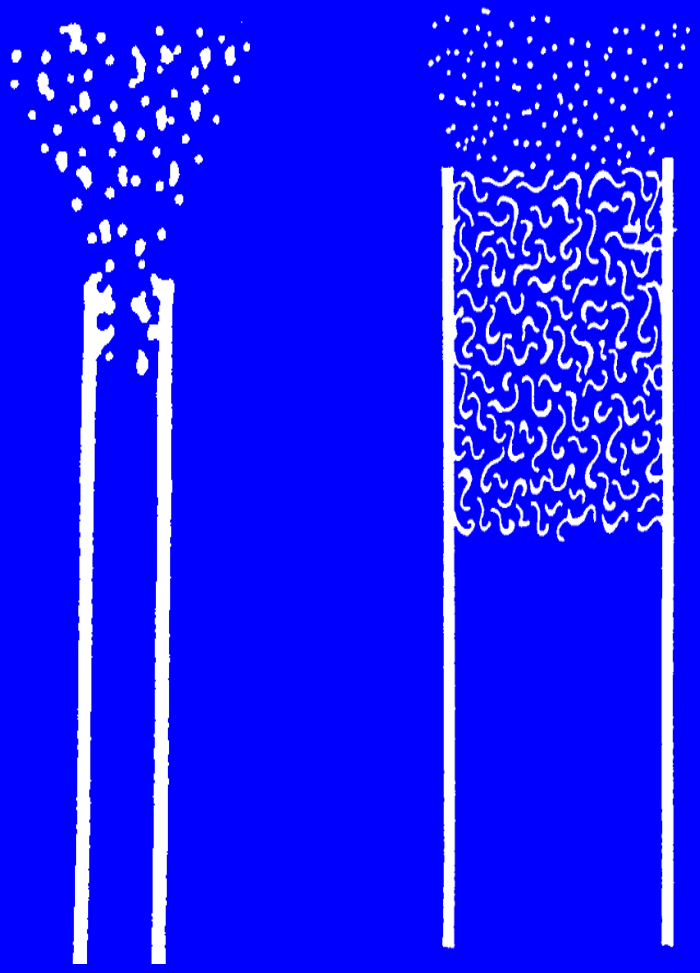


Optimization Parameters

- Detector interface
- FID conditions
- LC column sizes
- Pump flow rate

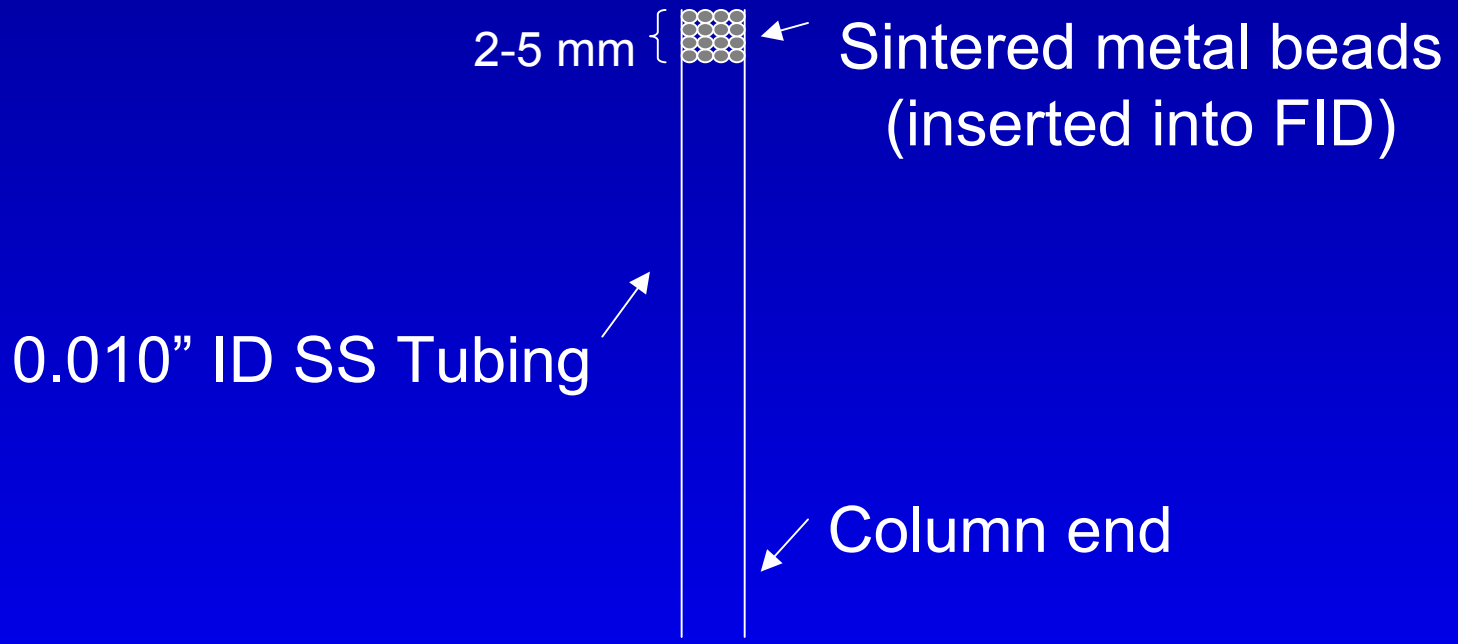
Restrictor Types for Superheated Water

- Stainless steel (silica degrades)
- Create backpressure to keep water in liquid form
- Pressure drop short distance (heated zone)
- Introduce water into the flame in aerosol



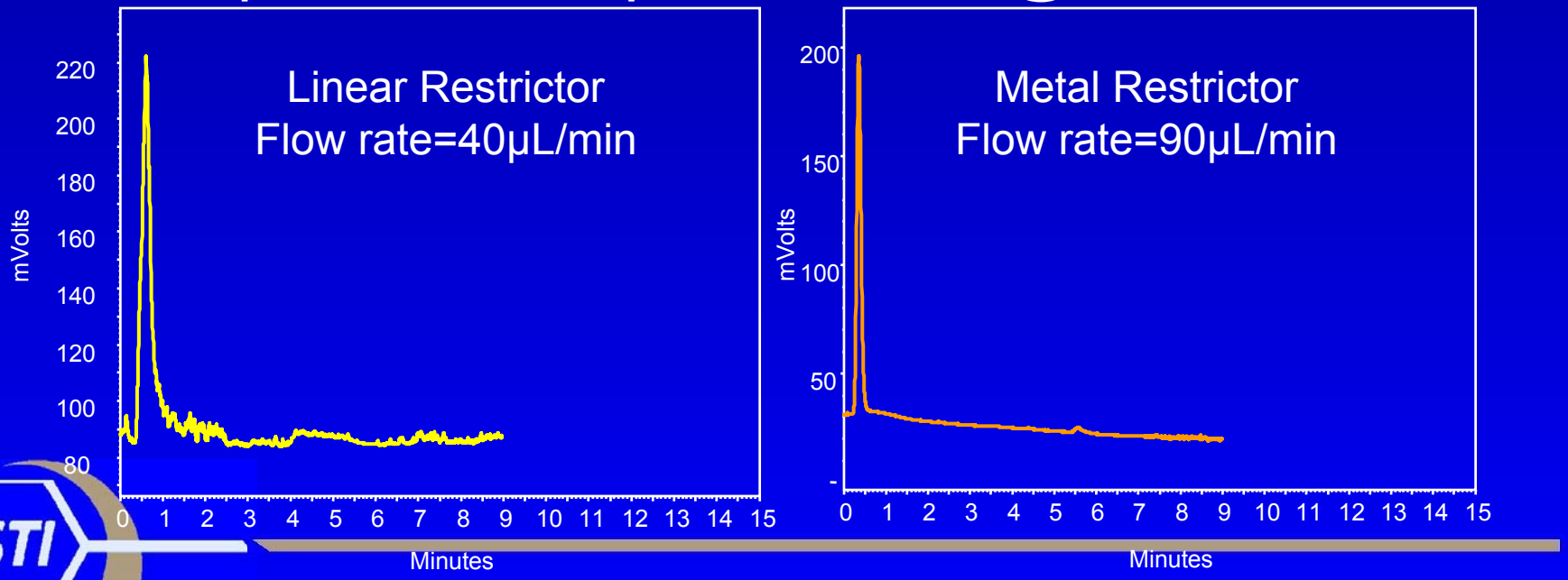
Selerity Restrictor

- Introduce water into flame in aerosol form



Linear vs. Metal Restrictor

- Direct into flame jet (no column)
- Methanol
- Temperature ramp: 50-200°C @ 20°C/min.



Factors Affecting Sensitivity

- Volume of water and sample introduced into FID
- FID temperature
- Restrictor position
- Detector gases

Superheated Water Flow into Flame

- Started with SFC-FID conditions
- Direct connection from injector to FID (eliminate column interference)
- Oven program from 50 to 200ºC

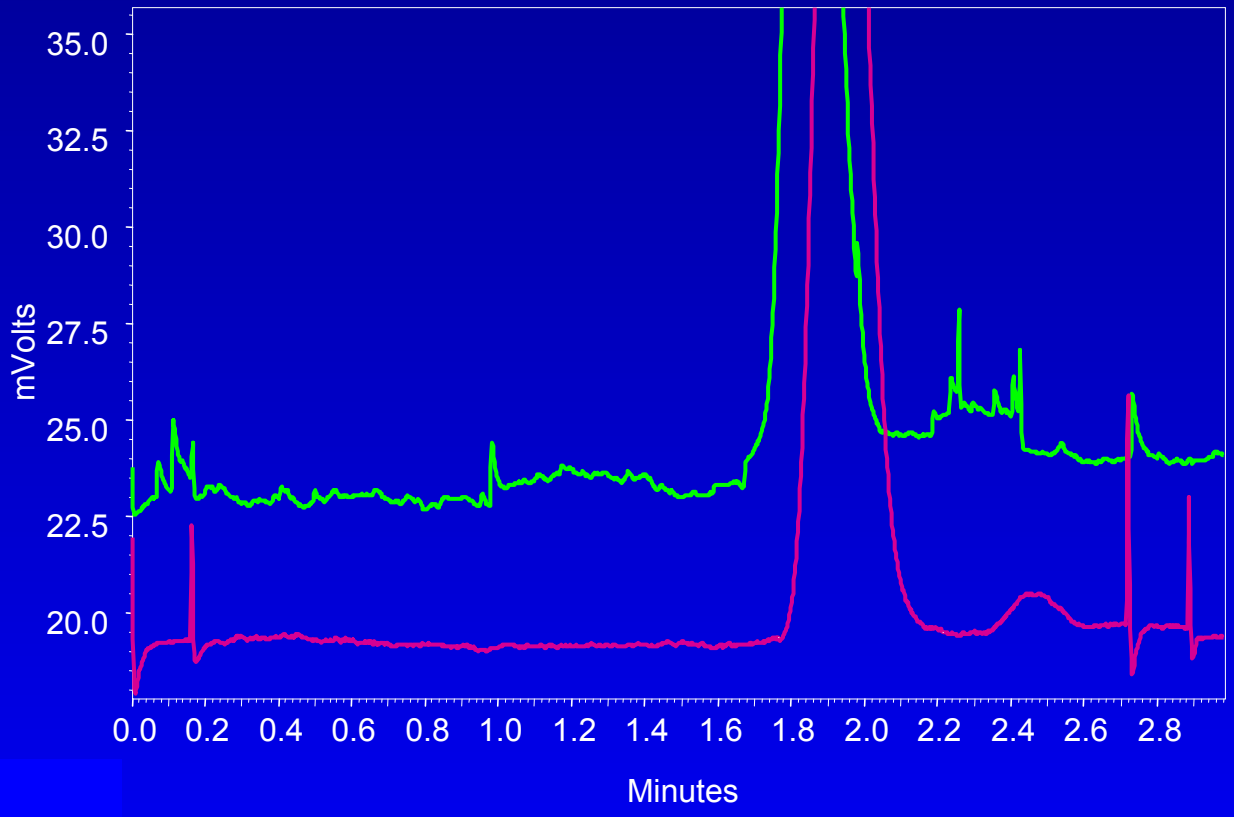
Results

- Maximum amount of water flow equals 100 µL/min

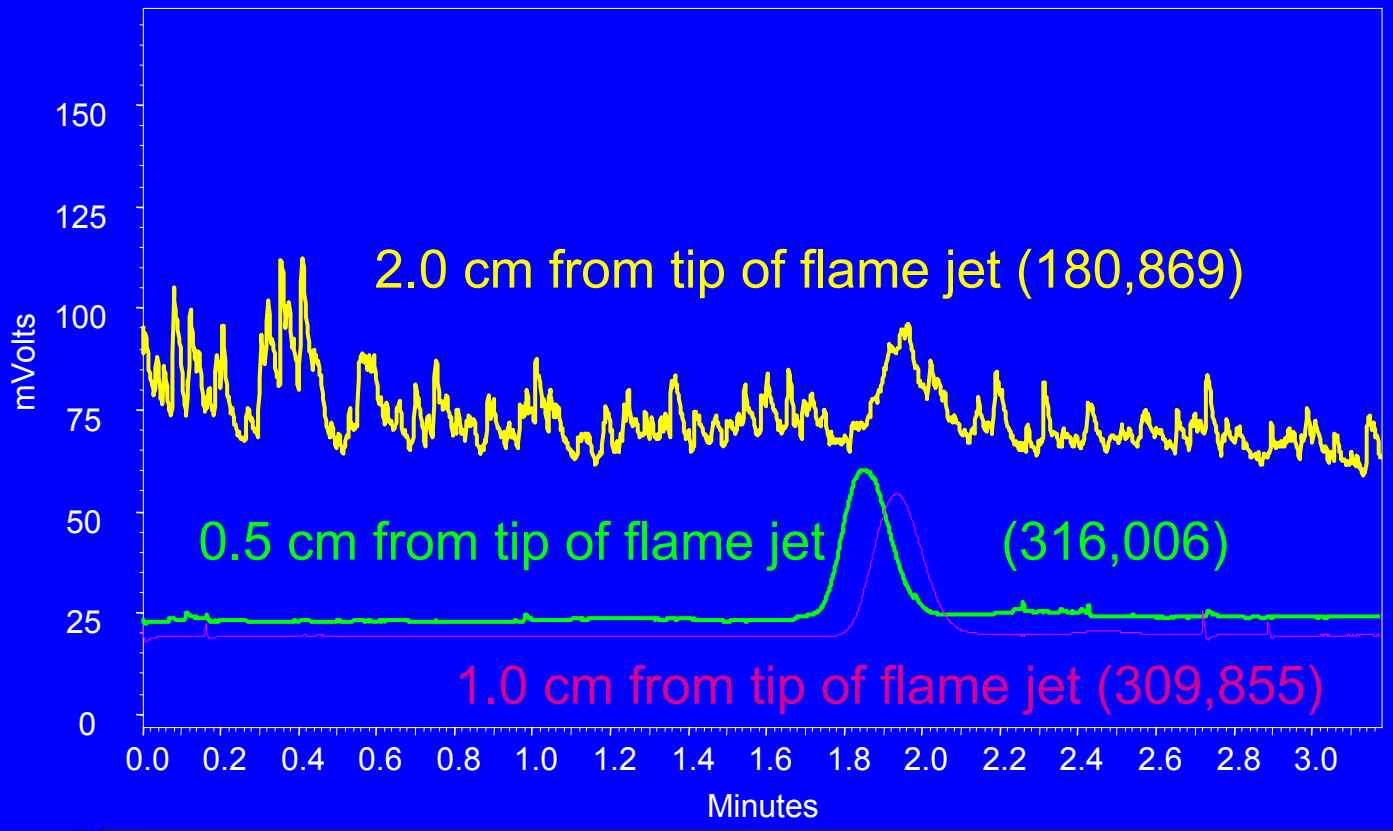
FID Temperature

- FID temperature must be above 375°C
- No significant change in sensitivity or peak shape at FID temperatures of 375 and 400°C

Baseline Difference Between 0.5 cm and 1.0 cm



Distance of Restrictor From Tip of Flame Jet

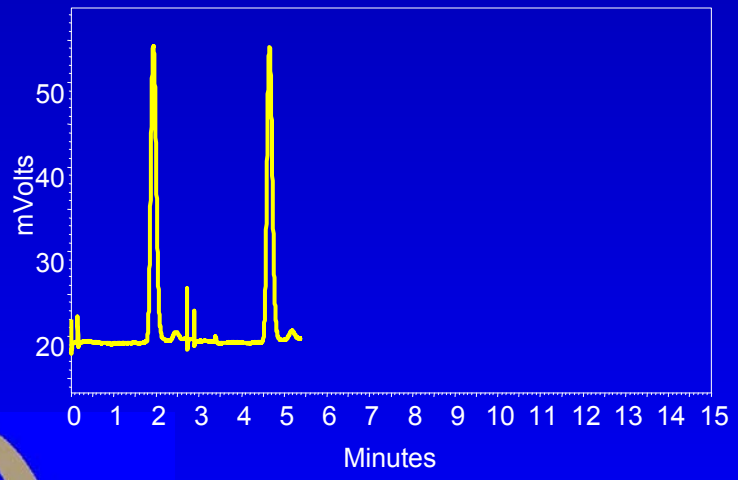


Detector Gas Flow Rate (H_2)

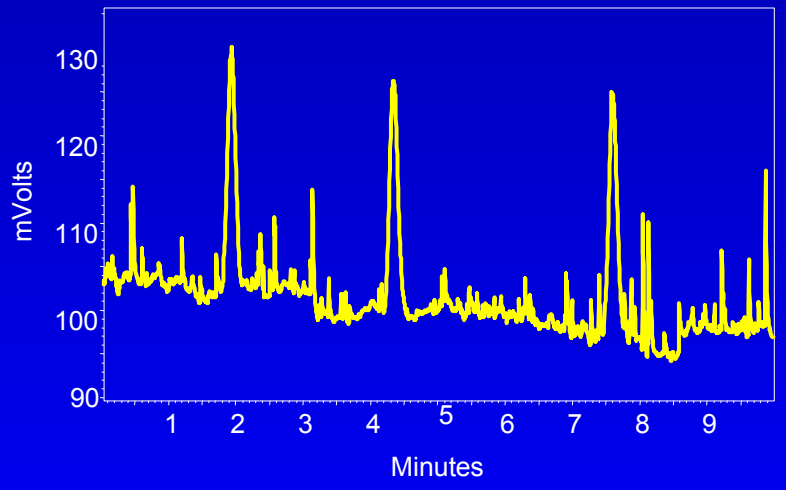
- Oven program 50 to 200°C

H_2 flow rate	Area	Variance
75 mL/min	316,006	Reference
100 mL/min	283,637	10 %
115 mL/min	237,095	25 %

100 mL/min

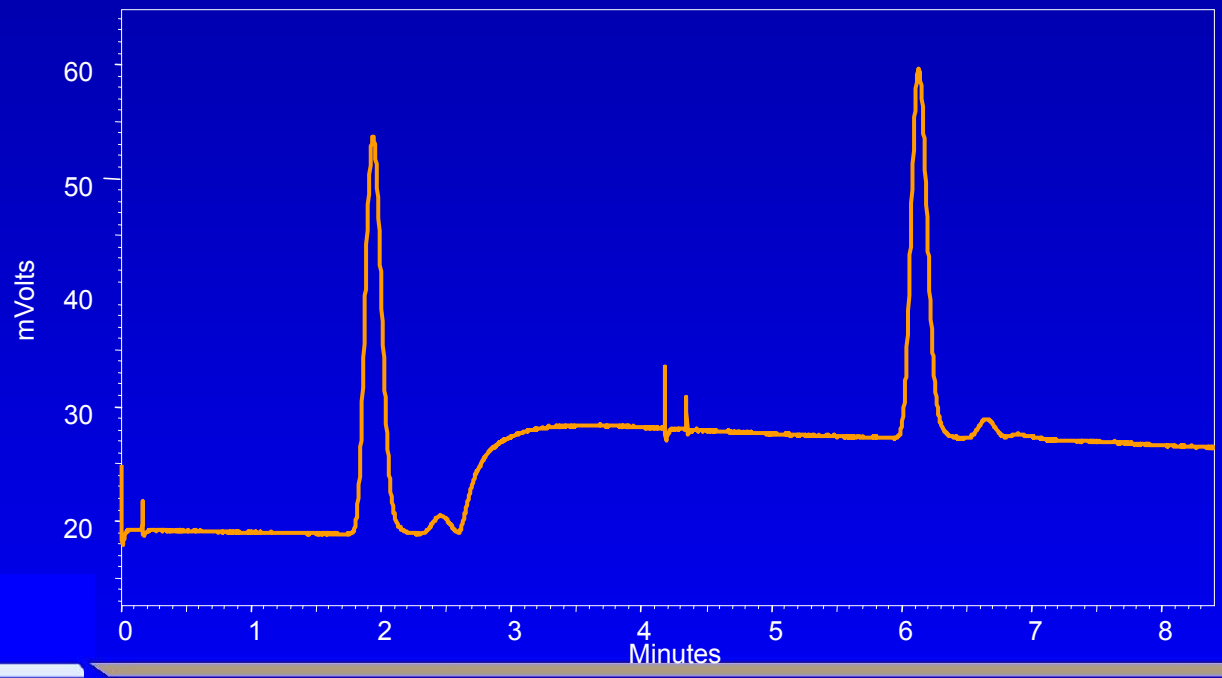


115 mL/min



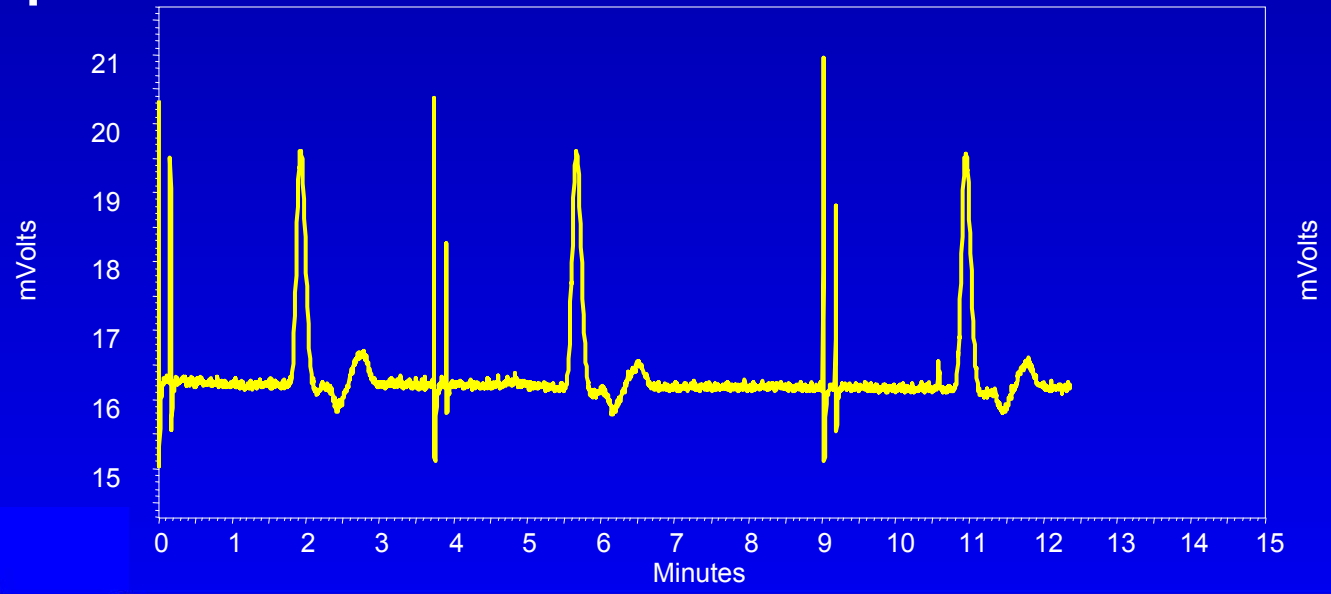
Effect of Make-up Gas on Sensitivity (Nitrogen)

- 8.4% loss of area without nitrogen



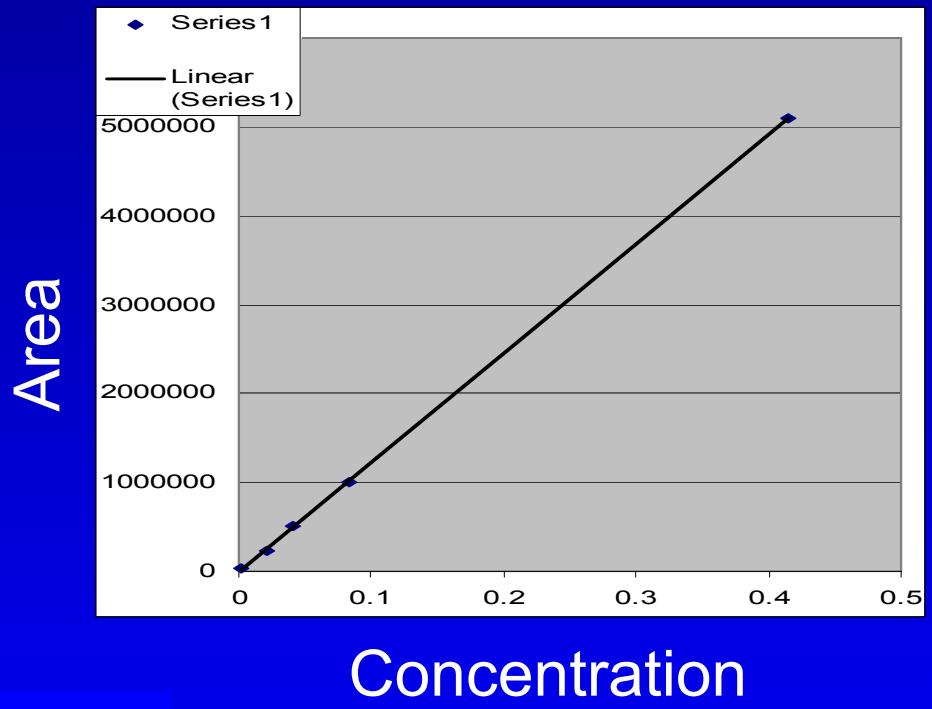
Sensitivity

- 10.4 ng of methanol on column (Area=28,302)
- Optimum conditions



Detector Linearity

Concentration Range= 0.00208-0.415 mg/mL



Regression Coefficient

R^2=0.99996159

Traditional LC Column Sizes and Optimal Pump Flow Rates

- 1.0 mm ID
  - Optimum flow = 50 µL/min
  - Superheated water into flame 50-100 µL/min
- 2.1 mm ID
  - Optimum flow = 200 µL/min (must split flow)
  - Up to 1.0 mL/min with split
- 4.6 mm ID
  - Optimum flow = 1.0 mL/min
  - 10% sample elutes to FID
  - Use concentrated samples

Split Assembly

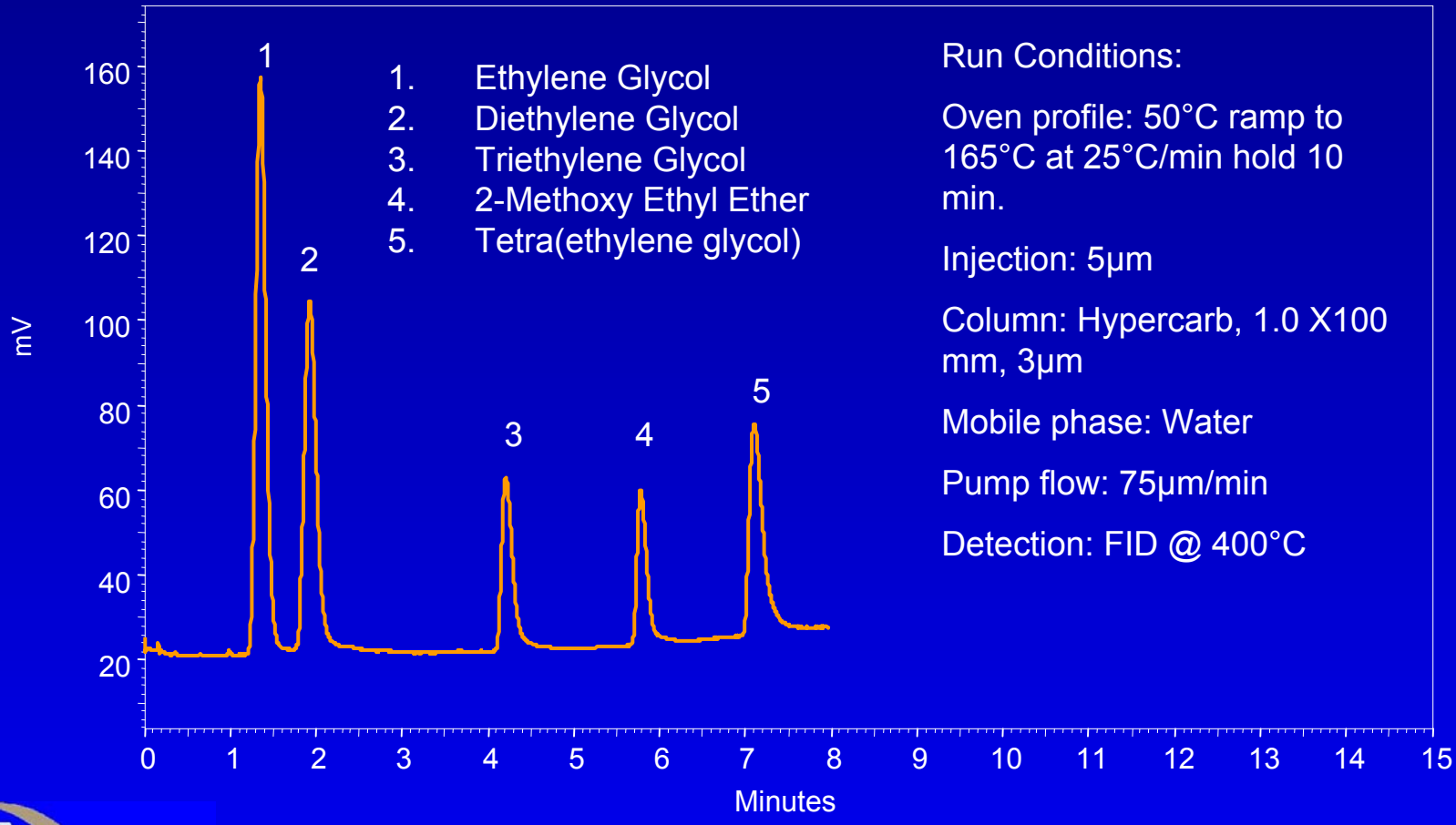
- 40 µm ID fused silica

Flow Rate vs. Area (2.1mm ID Columns)

Water flow rate	Area	Theoretical split	Length split tubing(40 µm ID)
200 µL/min	2,880,393	50%	25 cm
300 µL/min	1,523,720	67%	20 cm
400 µL/min	1,381,774	75%	10 cm
500 µL/min	1,274,604	80%	10 cm
600 µL/min	1,056,077	83%	10 cm
700 µL/min	938,846	86%	5 cm
800 µL/min	648,870	88%	5 cm
900 µL/min	560,189	89%	5 cm
1000 µL/min	562,955	90%	5 cm

1 mg/mL Methanol

Glycols on 1.0mm ID Hypercarb™



1. Ethylene Glycol
2. Diethylene Glycol
3. Triethylene Glycol
4. 2-Methoxy Ethyl Ether
5. Tetra(ethylene glycol)

Run Conditions:

Oven profile: 50°C ramp to 165°C at 25°C/min hold 10 min.

Injection: 5µm

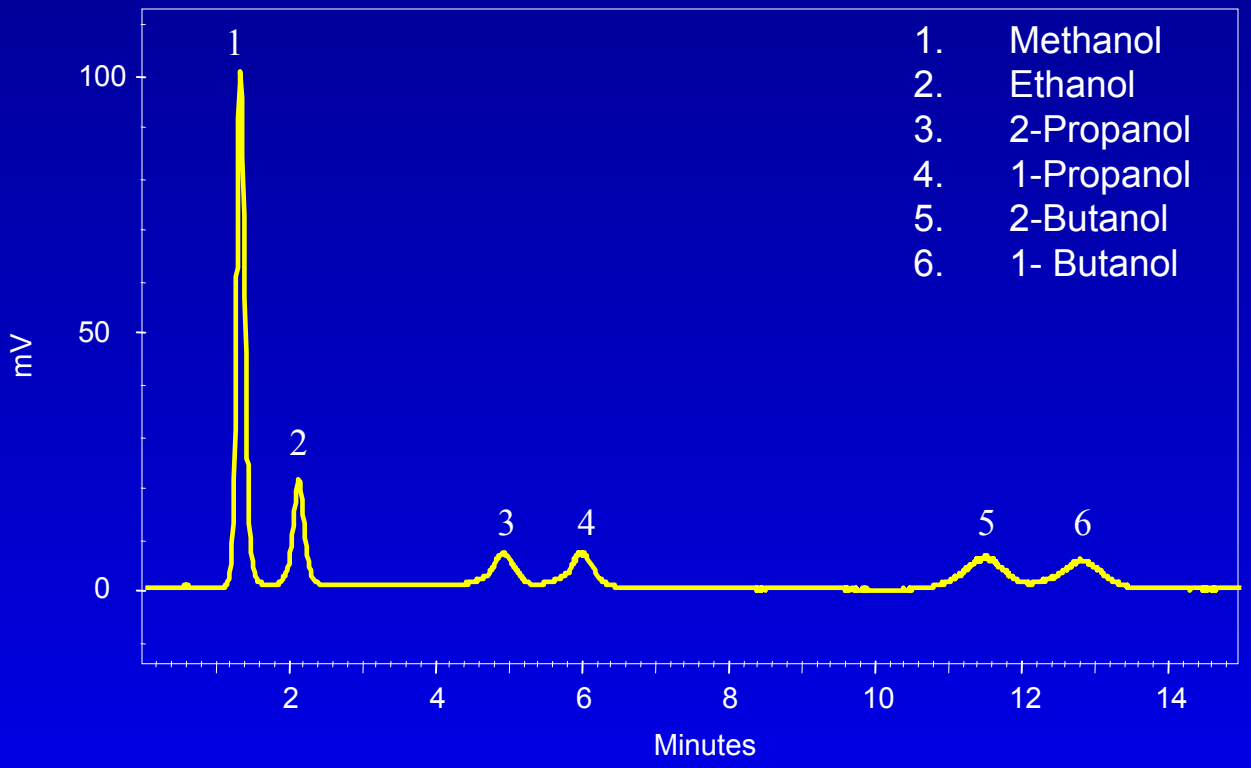
Column: Hypercarb, 1.0 X100 mm, 3µm

Mobile phase: Water

Pump flow: 75µm/min

Detection: FID @ 400°C

Alcohols on a 2.1mm ID Polymer Column



Run Conditions:

Oven profile: 50°C ramp to 135°C at 10°C/min hold 10 min.

Column: Jordi DVB, 2.1 X100 mm, 5µm

Mobile phase: 0.025%TFA

Split flow: 40µm ID fused silica back pressure

Pump flow: 0.500 mL/min

Detection: FID @ 400°C

Conclusion

- Optimum conditions depend on separation goals
- Sensitivity: use low hydrogen flow, low pump flow rates and 1.0 mm columns
- Fast analysis or dual detection: use higher hydrogen flow rates, higher pump flow rates, larger columns and split flow

Acknowledgements

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