On-line Analysis of Flowing Streams using Micro-flow HPLC

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Gaithersburg, Sept, 2009

Outline

1) How we got into Microflow HPLC

- High Throughput Analysis (HTA) supports High Throughput Experimentation (HTE)

Review: *Chirality (*2009) *21*, 114-118

2) A Mobile Tool for Online HPLC Reaction Profiling

- the development of the AliquotMobile

Organic Process Research & Development (2007) 11, 870-876,

3) On Line Analysis of Flowing Streams Using Microflow HPLC

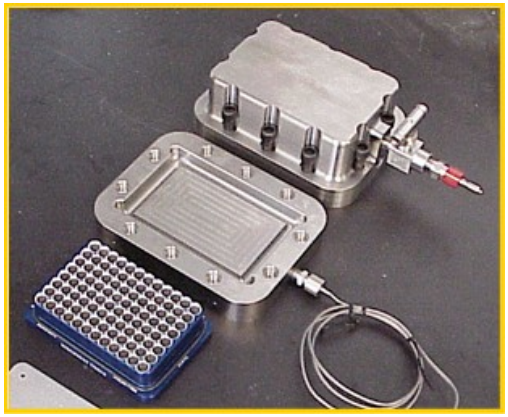
- Use of AliquotMobile for Flow Chemistry

Organic Process Research & Development (2009), 13, 1022-1025.

Who are we?

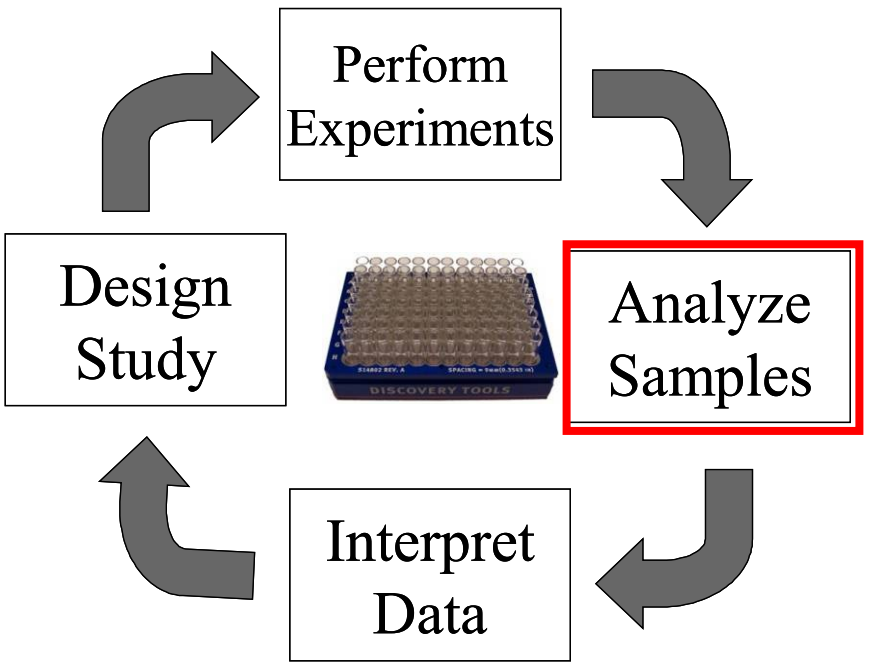
- Early Development Analytical Research (EDAR) – the analytical group supporting Merck process chemistry development
- Support chemistry development and release testing for active pharmaceutical ingredients (API)
- Capabilities
  - Small molecule analysis
  - Biologics analysis
  - Labeled compound analysis
  - Genotoxic impurity analysis
  - Advanced MS analysis
  - Other specialty analytical work: *metal, counter ions, residual solvents, titration*
- Solving complex analytical problems
- Regulatory quality requirements for safety studies and clinical trials
- Deployment of enabling analytical technologies and methodologies to end users

Increasing pace of drug discovery leads to demand for faster and more labor efficient process research techniques…



…increasing use of automation and parallel experimentation.

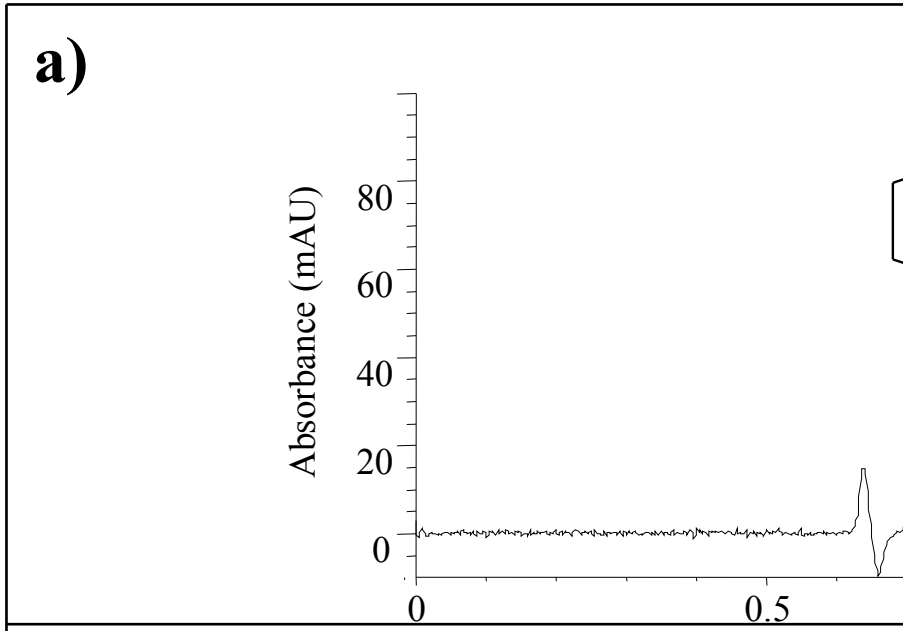
Higher and Higher Throughput…

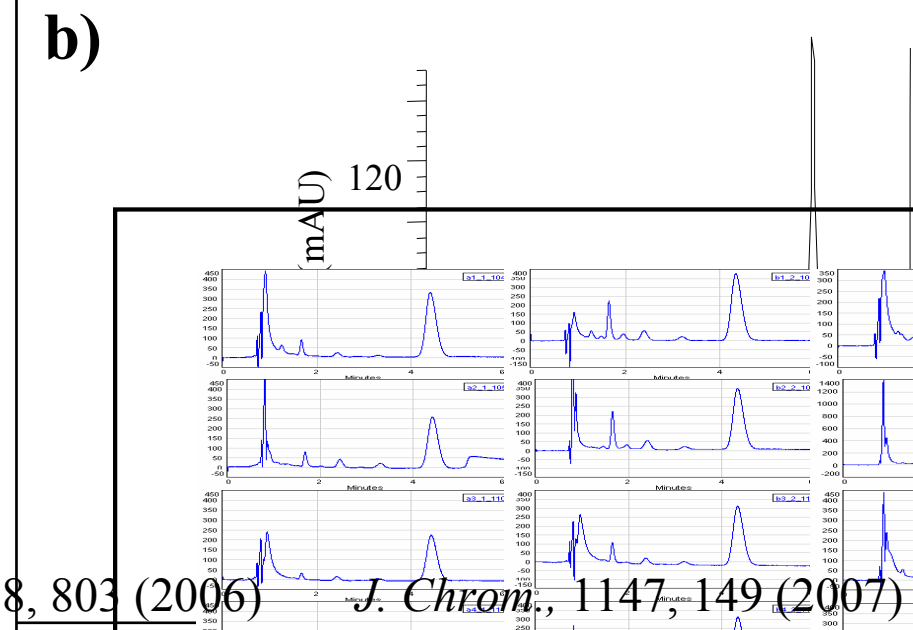


Conventional analysis soon becomes rate-limiting

We Need More Efficient Analytical Tools!

Multiparallel Microscale HPLC





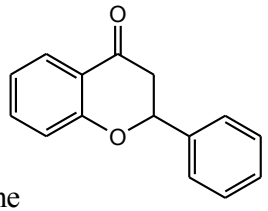
- Designed for high-throughput analysis
- 8 columns (0.3 mm i.d.) 16 pumps
- extremely low gradient dwell volume enables ultrafast gradients
- 0.5 – 2 h for analysis of 96 well plate

J. Liq. Chrom., 29, 2185 (2006) *Chirality*, 18, 803 (2006) *J. Chrom.*, 1147, 149 (2007)

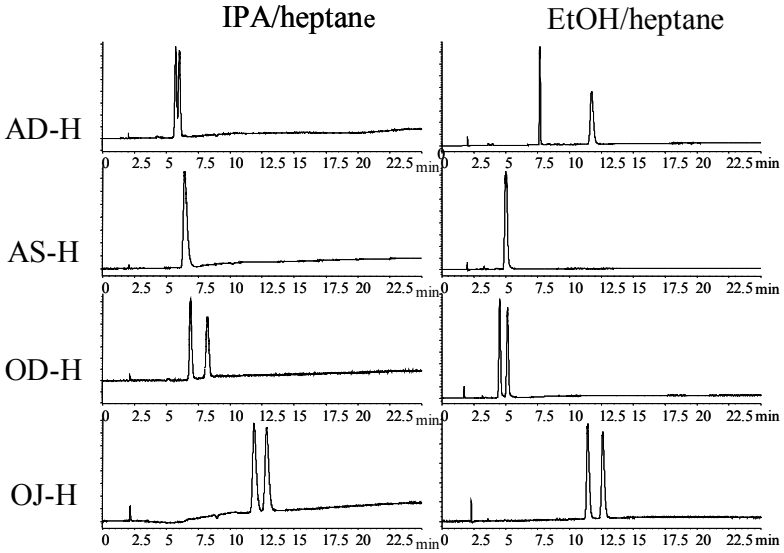
Multiparallel chiral HPLC method development

Multiparallel Chiral Method Development Screening Using an 8-channel Microfluidic HPLC System

Peter Sajonz*, Xiaoyi Gong; William R. Leonard Jr., Mirlinda Biba, and Christopher J. Welch*
Separation and Analysis Technologies, Merck and Co., Inc., Rahway, NJ 07065, USA

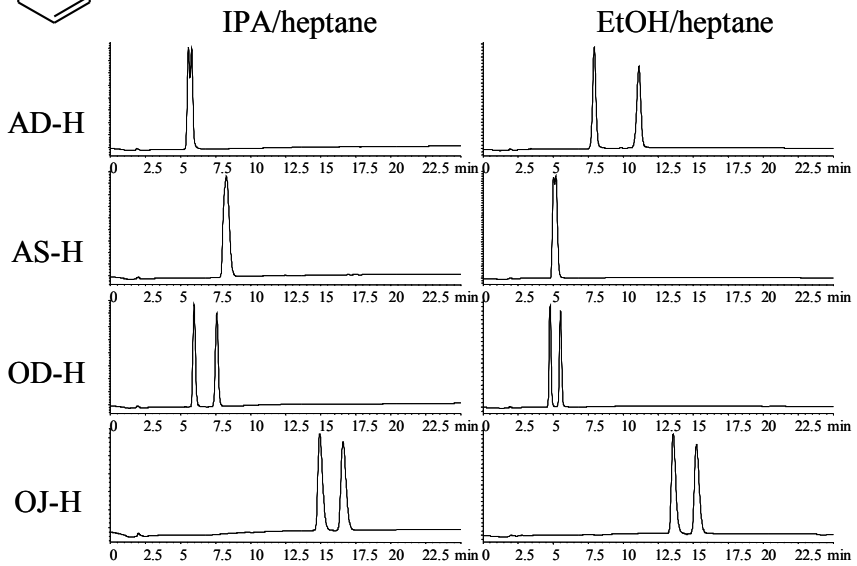


Eksigent Multiparallel Screening



30 minutes

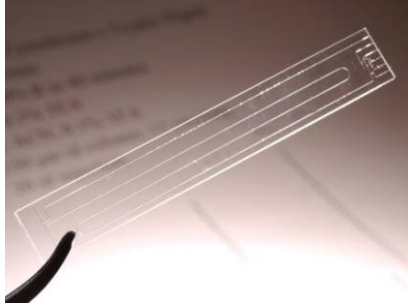
Agilent 1100 with Column Switcher



>6 hours

Chirality 18(10), 803-813, (2006)

Microscale?



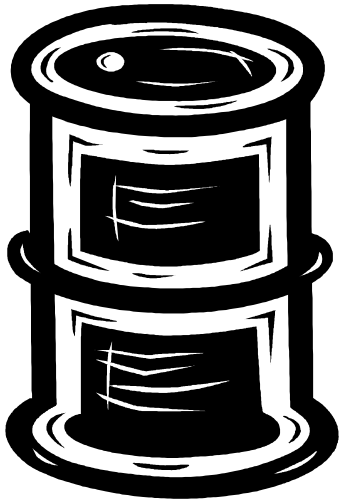
- Switch from 4.6 mm i.d. columns to 300 m i.d., and even smaller columns or chips
- Long advocated by many academics
- Becoming increasingly important in industrial research – e.g. proteomics
- Many advantages over conventional HPLC:
  - Multiplexing/High Throughput Analysis
  - More efficient use of valuable stationary phase- similar results using <0.5% of the material
  - Solvent savings/Green chemistry advantage

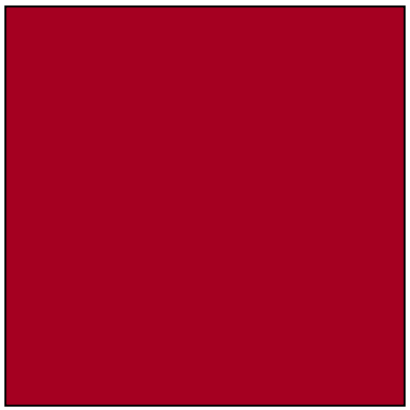
Analytical chromatography solvent savings one drop at a time

typical HPLC instrument ~1 mL/min

≈ ½ liter of waste per day

≈ 1 barrel of waste / year / instrument.





1.3 L

conventional chiral HPLC

5 mL

microflow chiral HPLC

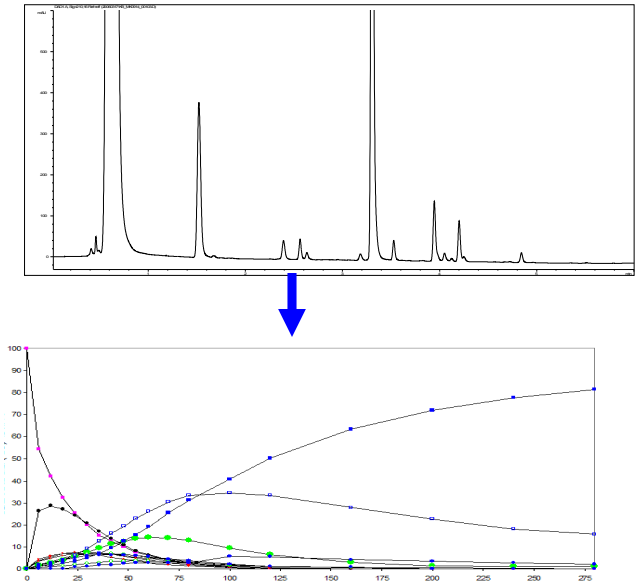
Drug Disc. World, Fall, 2007

Substantial solvent savings for a 96-well plate!

A Drive Toward Streamlined, User-Driven Technologies

Current state
manual samplings

HPLC run at another location
sometimes by another scientist
sometimes in another building



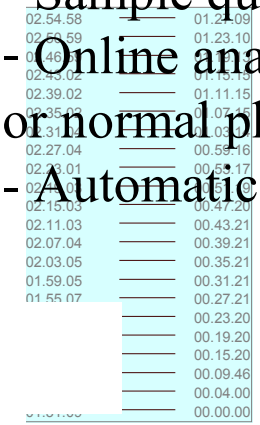
result to inform next step
in research

Can HPLC analysis be made more convenient for end users?

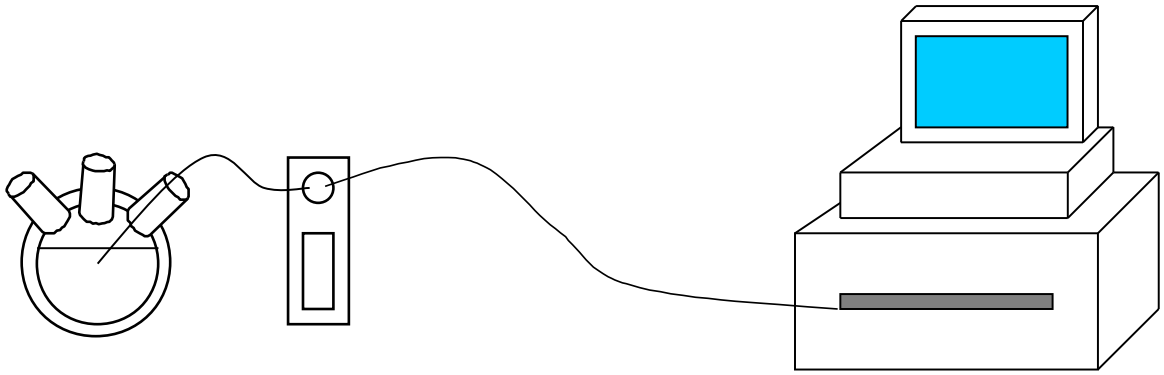
Point of Use HPLC - AliquotMobile

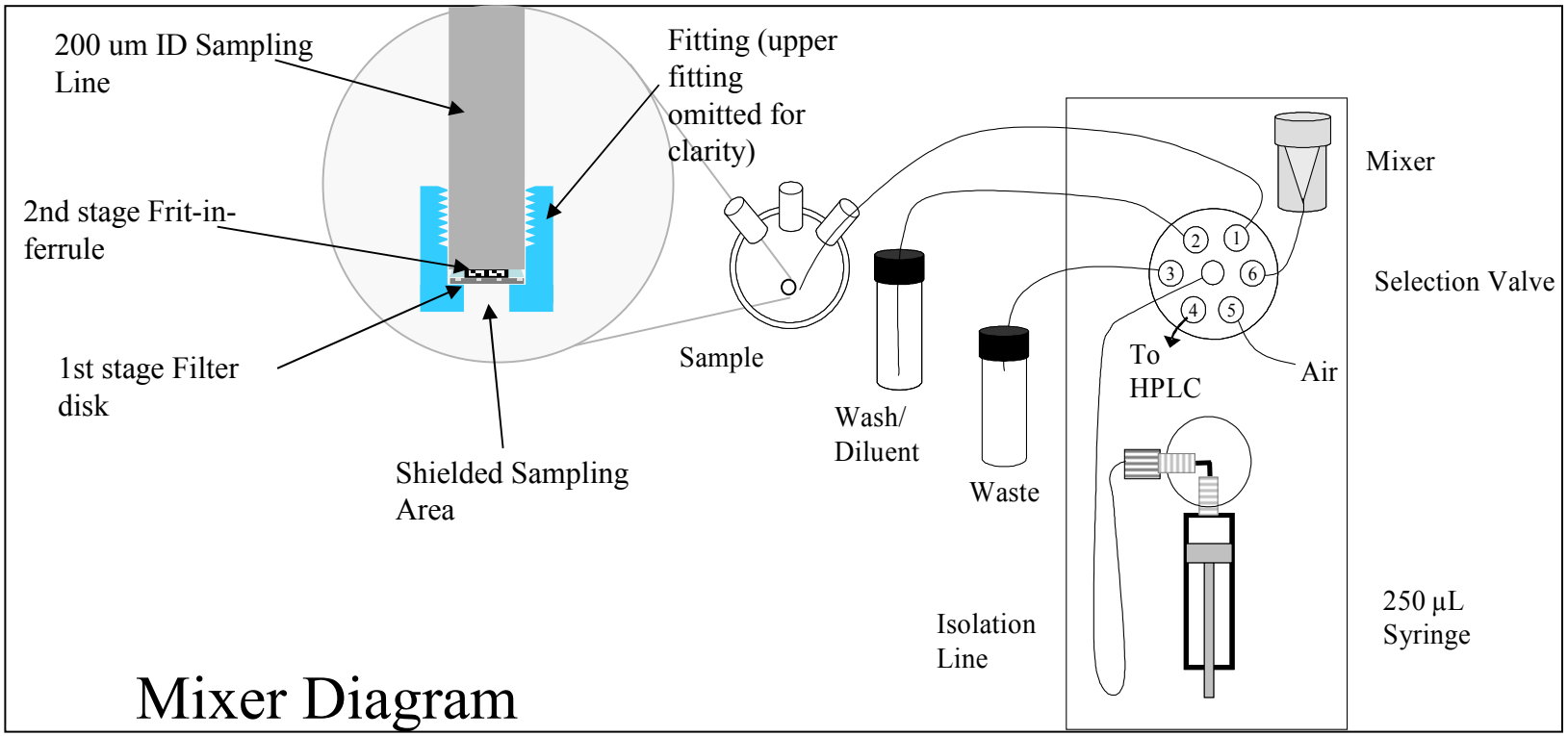
Features

- Withdraw aliquots over time
- Sample quenching and dilution – up to 100x
- Online analysis: chiral or achiral HPLC, reversed phase or normal phase.
- Automatic data processing to generate reaction profiles



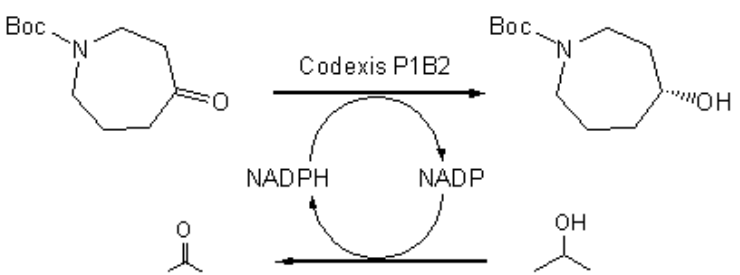
AliquotMobile and Express RT



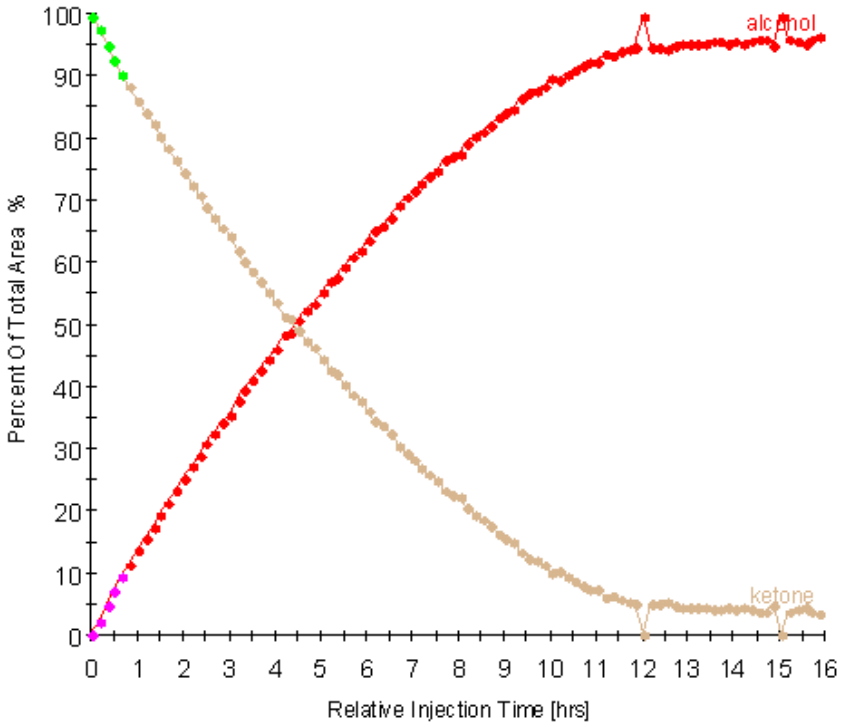


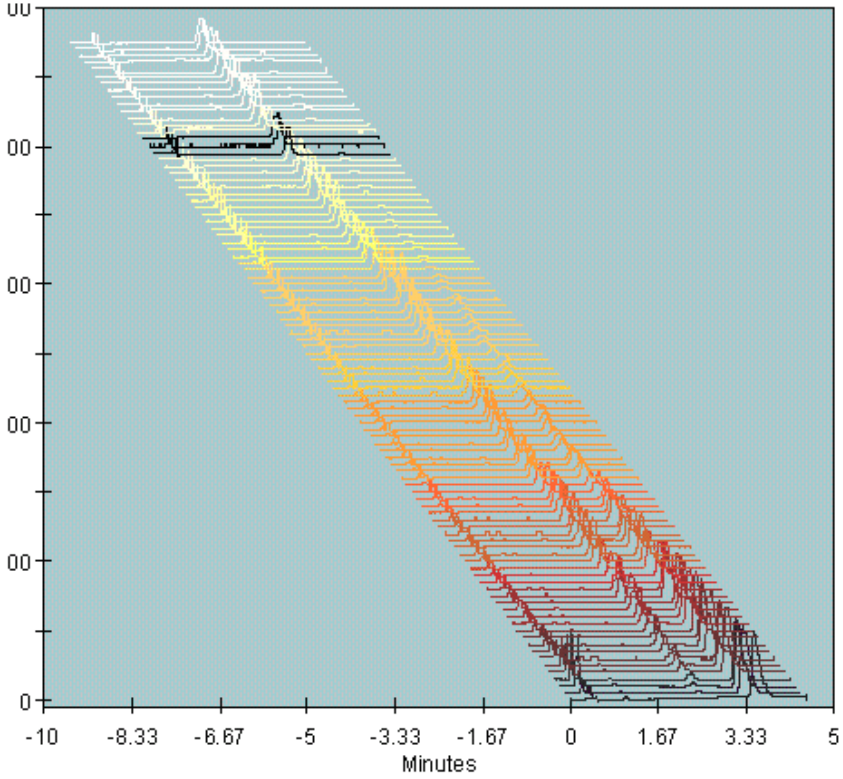
Mixer Diagram

Online HPLC Applications



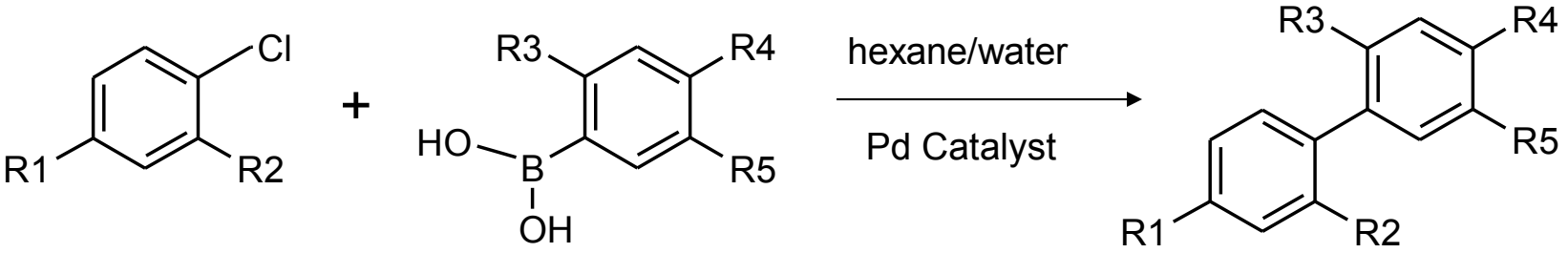
Aliquot Mobile

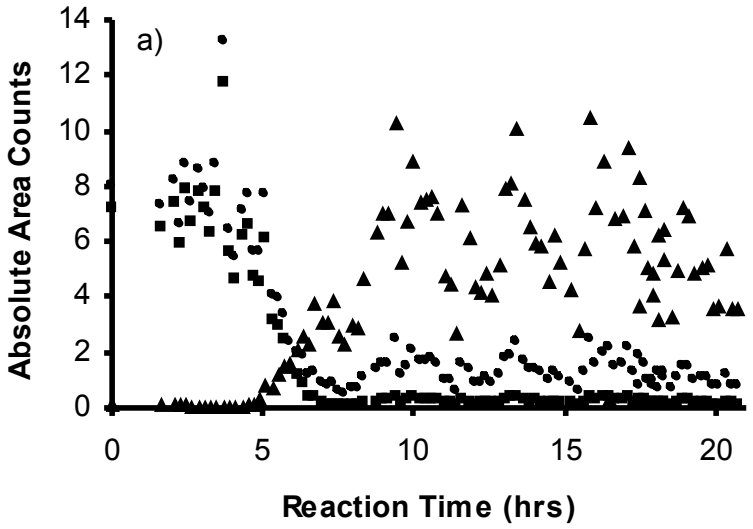


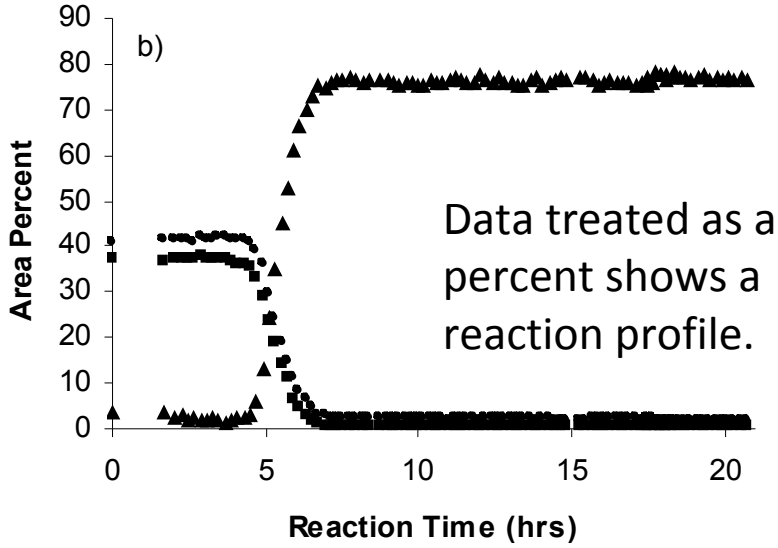


Chemist trained in about 20 minutes. Instrument ran unattended overnight (16 hours). Updated waterfall plot and reaction curve every 15 minutes.

On-line HPLC Profiling of Heterogeneous Suzuki Coupling



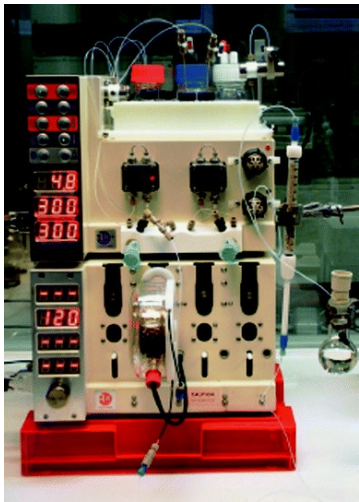




Data treated as area percent shows a clear reaction profile.

Can AliquotMobile Monitor Flow Reactions?

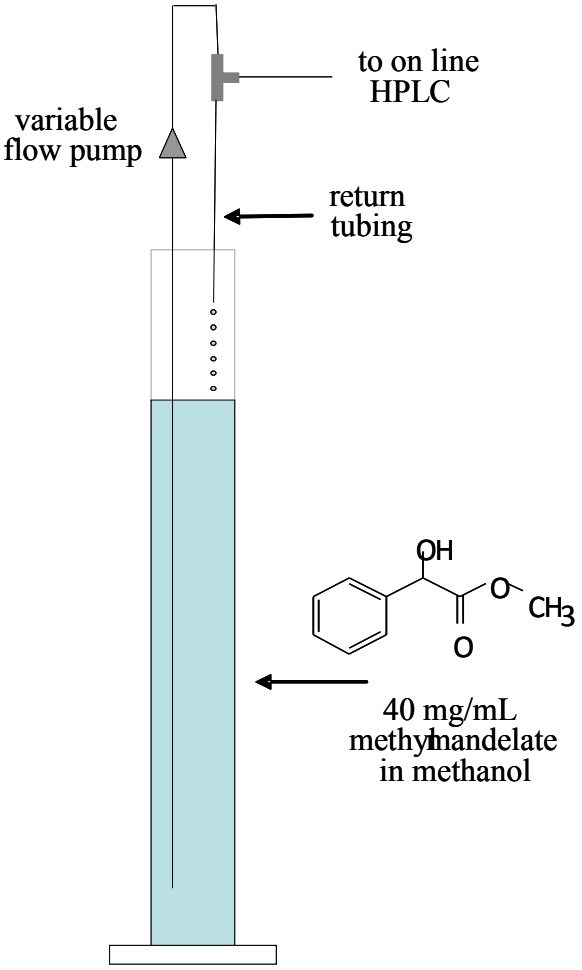
- Many possible advantages
  - No manual sampling or sample preparation required
  - Continuous monitoring enables real time detection of flow chemistry reactor issues (flow rate/temperature fluctuation, bubbles, clogging, etc)
  - Continuous quality control
  - Walk away reaction optimization
  - Online HPLC suitable for studying complex reaction systems with chromatographic resolution



Many Applicable Analytical Techniques

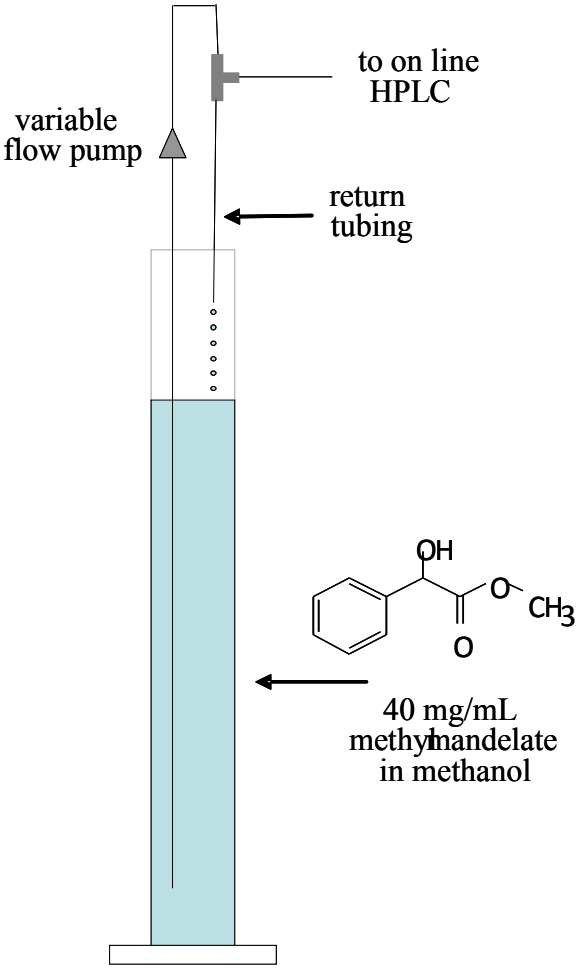
- Online FTIR
  - LaPorte, T.L, Hamedi, M., DePue, J.S., Shen, L., Watson, D., Hsieh, D. *Org. Proc.R&D.* **2008**, *12*, 956-966.
- Online Raman
  - Leung, S.A., Winkle, R.F., Wootton, R.C.R., deMello, A.J. *Analyst* **2005**, *130*, 46-51.
- Online sensors
  - **UV**: Baru, M.B., Mustaeva, L.G., Gorbunova, E.Y., Vagenina, I.V., Kitaeva, M.A., Cherskii, V.V. *J. Peptide Res.* **1999**, *54*, 263-269.
  - **Visible**: Criado, A., Cardenas, S., Gallego, M., Valcarcel, M. *Talanta* **2000**, *53*, 417-423.
  - **MS**: Marta, C.T., Elders, N., Krabbe, J.G., Kool, G., Niessen, W.M.A., Orru, R.V.A., Irth, H. *Anal. Chem.* **2008**, *18*, 7121-7127.
  - **NMR**: J. Bart, A.J. Kolkman, A.J. Oosthoek-de Vries, K. Koch, P.J. Nieuwland, H.J.W.G. Janssen, J.P.J.M van Bentum, J.A.M. Ampt, F.P.J.T. Rutjes, S.S. Wijmenga, H.J.G.E. Gardeniers, A.P.M. Kentgens *J. Am. Chem. Soc.* **2009**, *131*, 5014-5015.

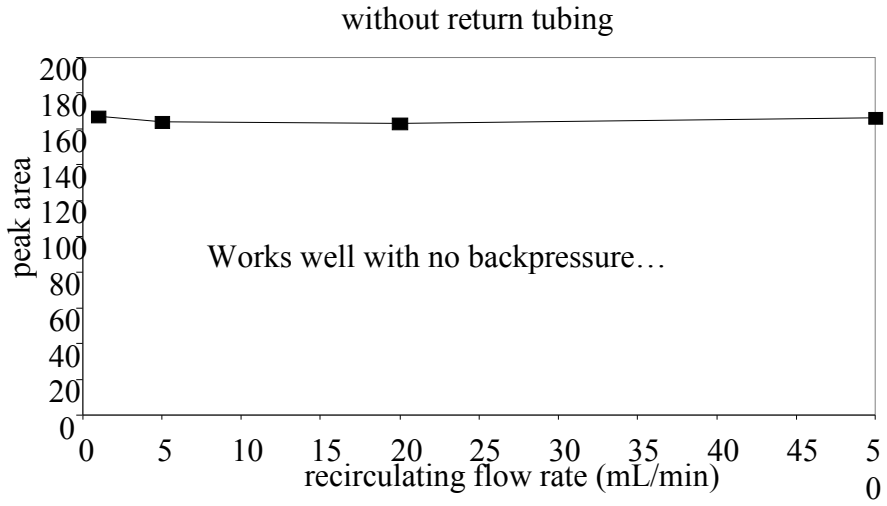
Accurate Sampling from Pressurized Stream?

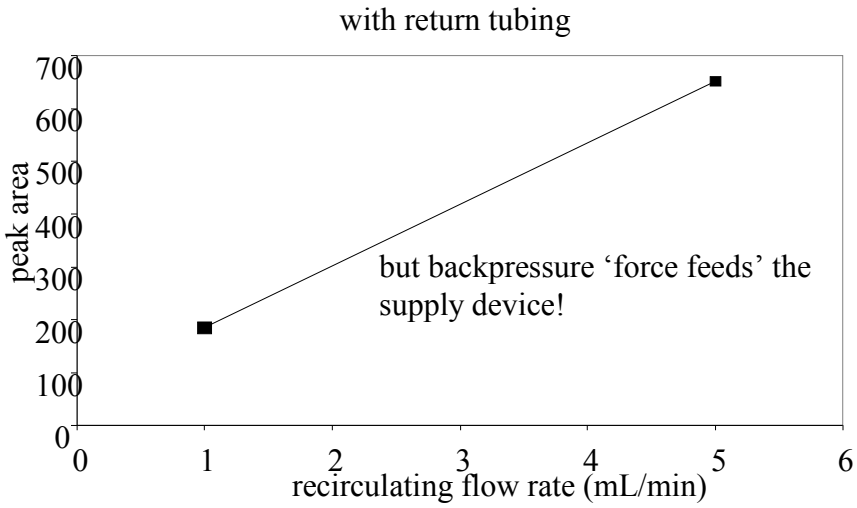


- The AliquotMobile has been demonstrated to work well for batch chemistry, but it was not designed to sample from pressurized systems.
- Most flow reactions are carried out under pressure.
- Would this be an issue?

Using the Right Conditions…

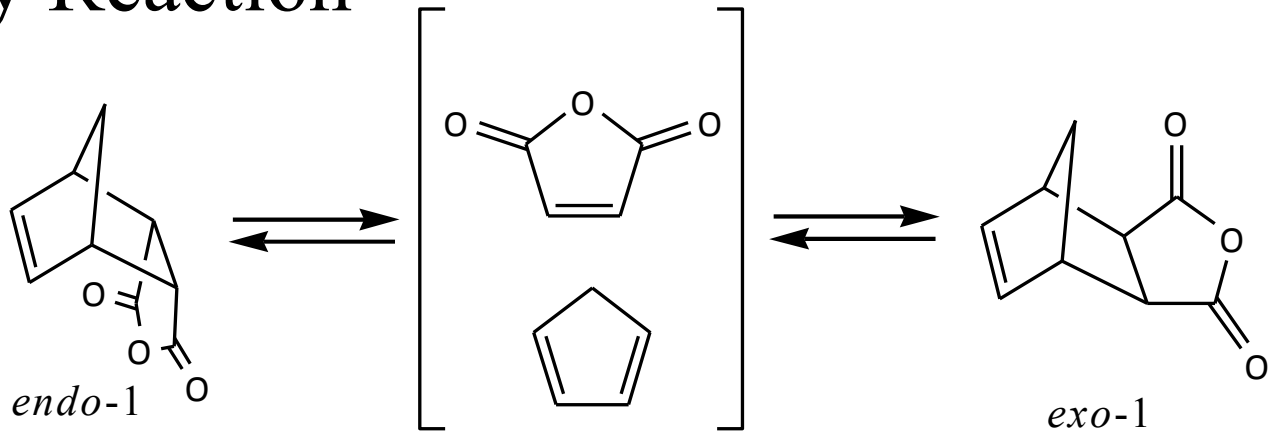






The instrument works fine sampling from a flowing stream when the backpressure is kept low. Downstream constrictions should be kept to a minimum.

Online Sampling of a Thermal Isomerization Flow Chemistry Reaction

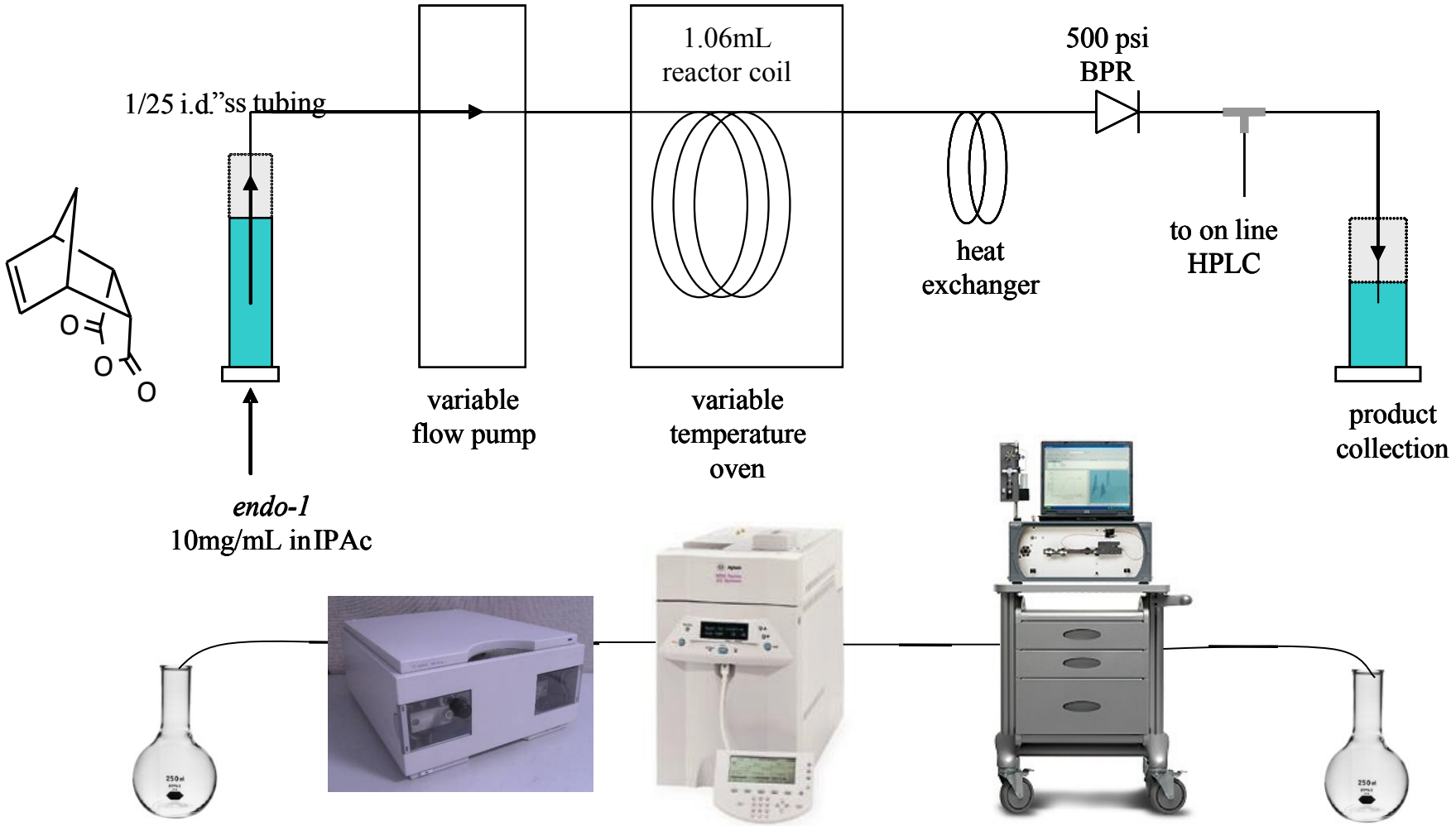


endo-1 *exo*-1

(*cis*-*endo*-5-norbornene-2,3-dicarboxylic anhydride)

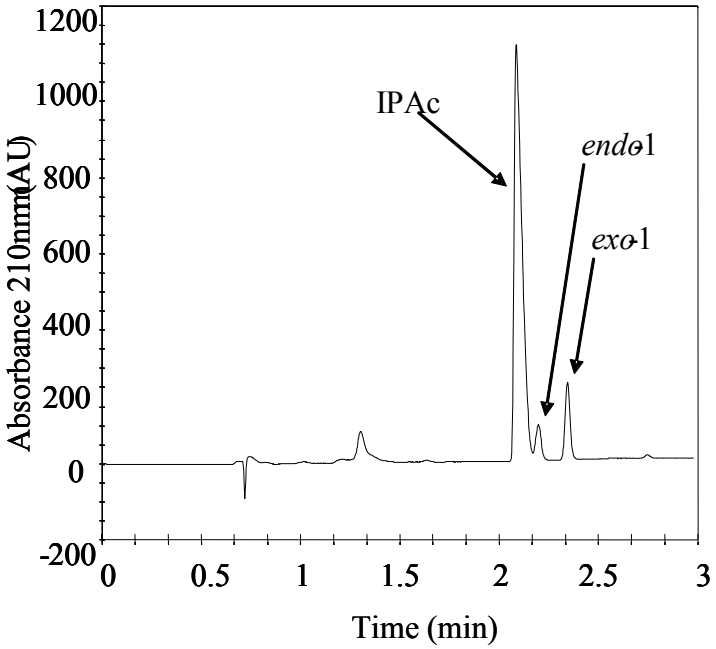
- Classic Diels-Alder reaction
- *endo* is the thermodynamic product
- Desired *exo* can be obtained by thermal isomerization
- Flow chemistry allows operation above the boiling point of the solvent if a backpressure regulator is used.

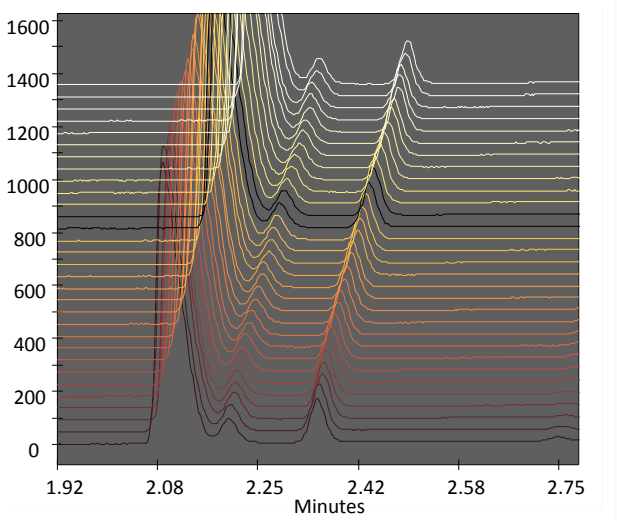
Flow Reactor Setup

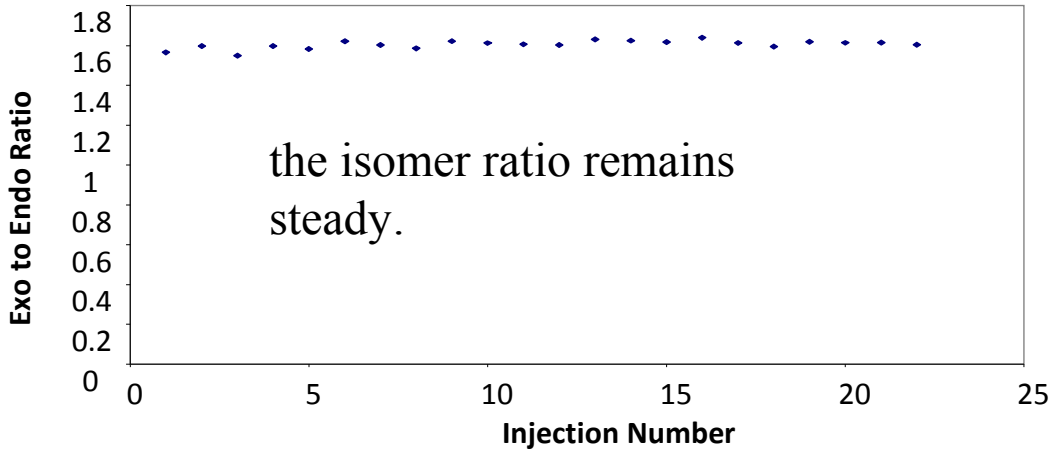


No specialized equipment needed!

Online HPLC Results



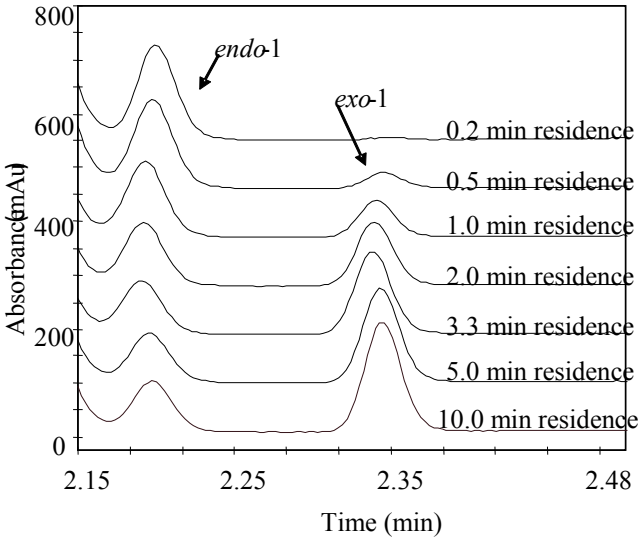


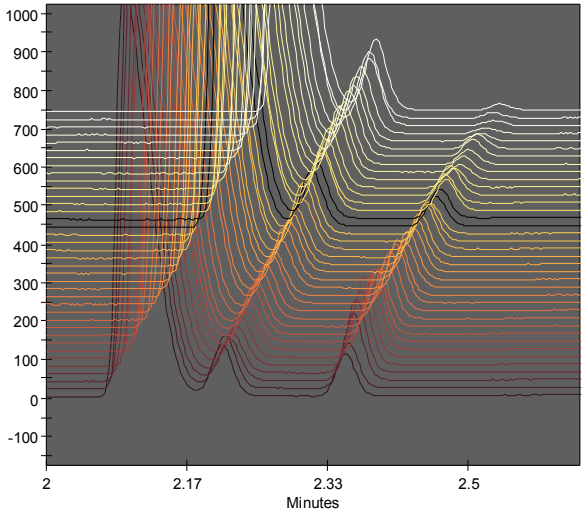


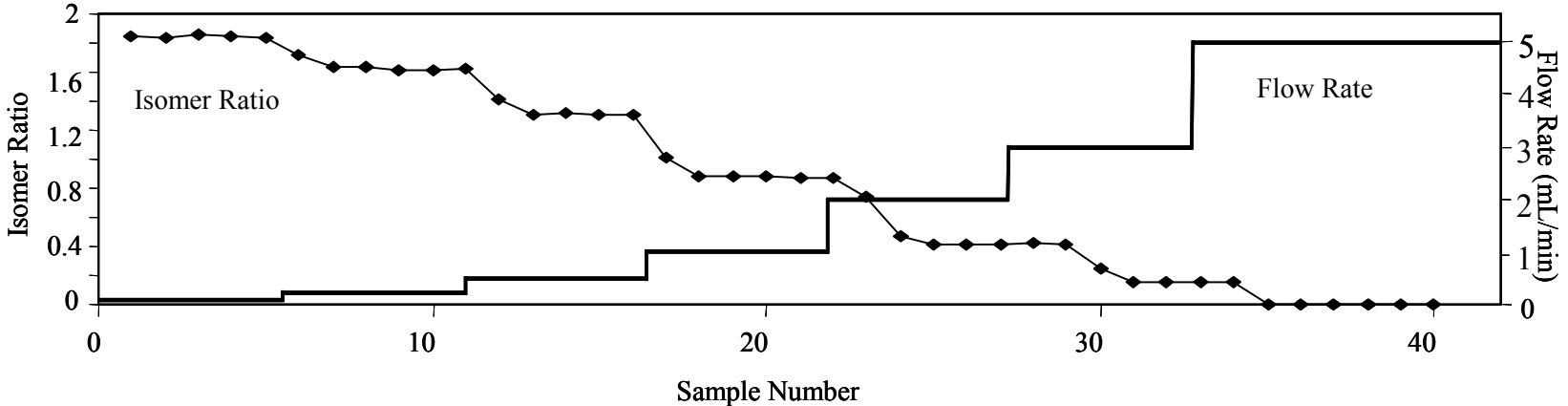
Good reproducibility from a flowing stream at steady reaction conditions.

Using Online HPLC in Flow Reaction Development

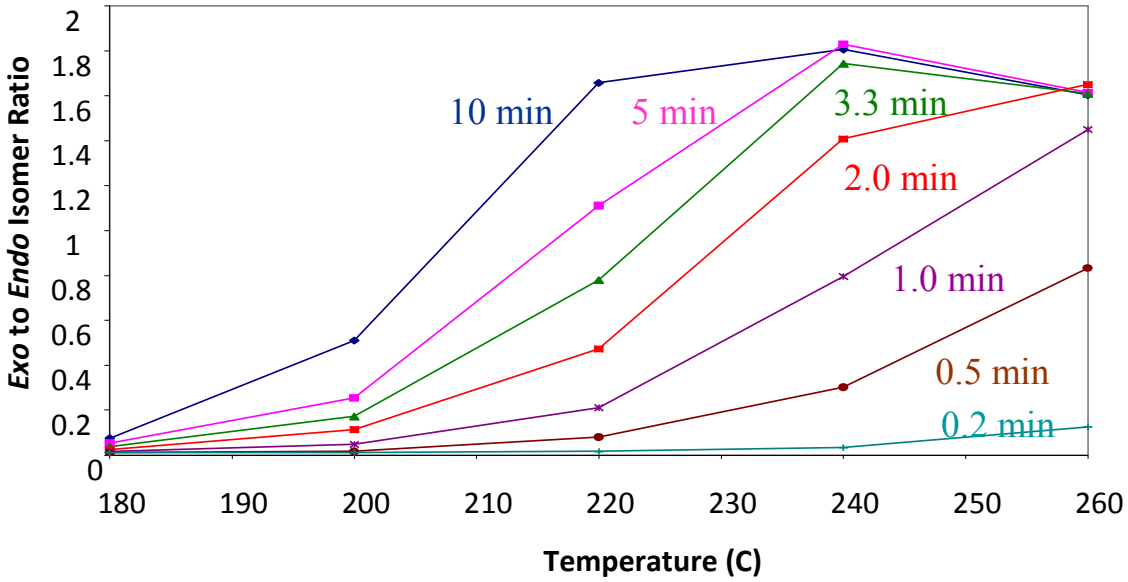
- Pump and ov
- Residence tin stayed the same.



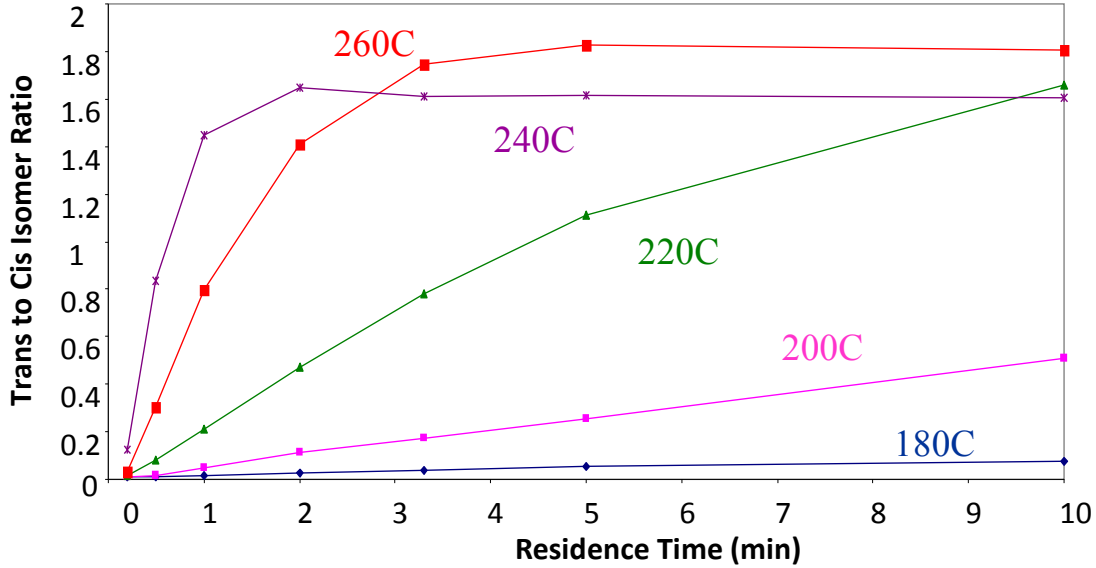




Single Parameter Reaction Optimization

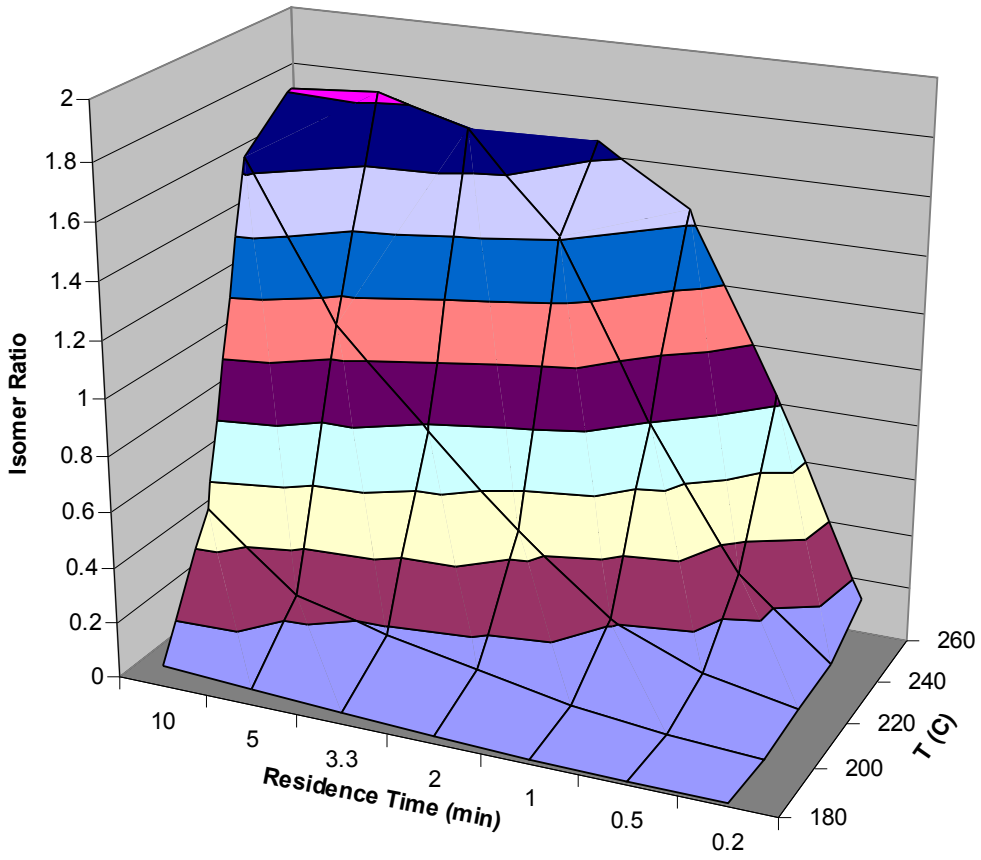


240^{0}C Optimal Temperature



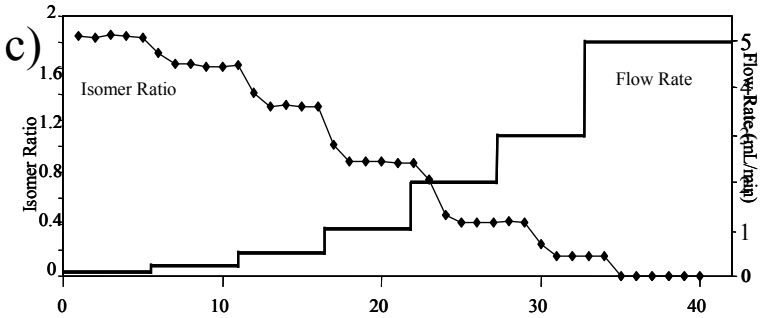
5 Minute Optimal Residence Time

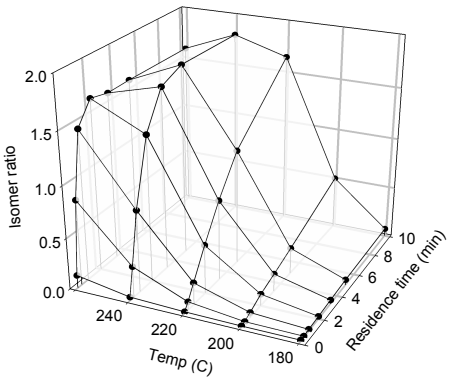
Creating a 3-D Reaction Profile



Future Directions

- Full computer control of temperatures and flow rates
  - One-button reaction profiling
- Automated integration with feedback control:
  - Instrument scans response surface, and drives toward optimum.
  - Maintain the reaction within set boundaries
  - Process safeguarding: If results look bad, and cannot be improved, stop the flow and set an alarm
- Pressurized reaction sampling
  - Allows in-line sampling at any point within the flow reactor setup.
  - Reaction monitoring of vessels under pressure (especially hydrogenation).





tub

Acknowledgements

- Chris Welch, Xiaoyi Gong, Wes Schafer, Mirlinda Biba, Zainab Pirzada, Tanja Brkovic, Fiona Lin, Zhihong Ge
- Sarah Dolman, Jason Nyrop, Charles Orella, Greg Hughes, Birgit Kosjek, Hallena Rogers

Backup slides

On-Line Microfluidic HPLC Capabilities

- Minimum cycle time: 3 minutes
- Sampling volume: as low as 18 µL
- Pre-programmed sample dilution factors: 1, 2, 3, 10, 30, 100, 300, 1000
- Wetted parts: Fluoropolymers (FEP, PFA, CTFE), fused silica, 316SS, PEEK
- pH range: 1–12
- Most columns available in 0.3 mm id format
- Real-time reaction monitoring