AMGEN®
Pioneering science delivers vital medicines™

Application of Flow Reactors within Drug Discovery

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Thousand Oaks, California

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Drug Discovery Accelerating Technologies at Amgen

The function of the Medicinal Chemistry Research Technologies (MCRT) group at Amgen is to evaluate and advance tools for accelerated drug discovery, including:

- In silico target design tools
- Parallel and high-throughput synthesis tools
- High-throughput purification
- High-throughput characterization
- Reaction screening platforms

These tools must be:

- Effective
- Robust
- User-friendly (open access)

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Flow Chemistry as an Accelerating Technology

Potential for:

- Accelerated reaction rates
- Accessing supercritical temperature/pressure
- Increased safety (reactives, gases, pyrophoric catalyst handling)
- Precise control of conditions (screening, optimization)
- Ease of use through automation

However:

- Translating batch to flow is not “intuitive”
- Few user-friendly commercial tools available directed toward Med Chem scale (25-50 mg for in vitro studies, up to 100 g for intermediates or in vivo studies)

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ThalesNano© H-Cube™

Flow hydrogenation is advantageous due to relatively limited throughput, safety, and efficiency of batch processes.

Advertised features of H-Cube™:

- In situ H_2 production
- Adjustable parameters
  - Liquid flow rate
  - Temperature
  - "Full" H_2 flow mode (1 bar)
  - Controlled mode (metered H_2)
- User friendly interface
- Safe catalyst handling (CatCart™)

Images courtesy of Thalesnano Technology

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H-Cube™ with Autosampler

Gilson© liquid handler and ThalesNano© automation software:

- Permits walk-away use
- Experiments can be queued to facilitate screening

Image courtesy of Thalesnano Technology

See: Ladlow and Ley, *Adv. Synth. Catal*. **2007**, *349*, 535-538.

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ThalesNano© H-Cube™ at Amgen

Users have successfully performed:

- ✓ Aromatic nitro reductions
- ✓ Aliphatic nitro reductions
- ✓ Heteroaromatic ring saturation
- ✓ CBZ-hydrogenolysis
- ✓ *N*-Debenzylation
- ✓ Olefin reductions
- ✓ Azide reduction

- ➢ Case studies
- ➢ H-Cube™ characterization

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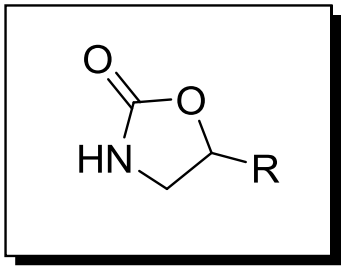
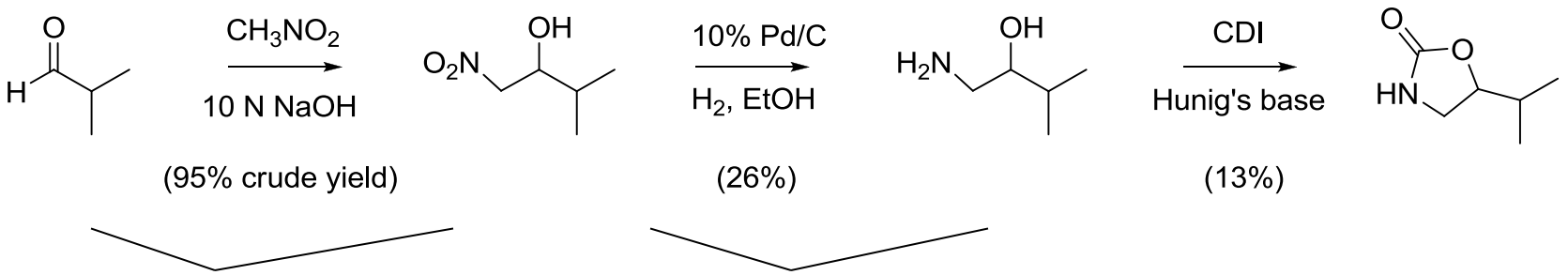
Oxazolidinone Synthesis



- Project team needed a series of 5-substituted oxazolidinones
- Limited commercial availability

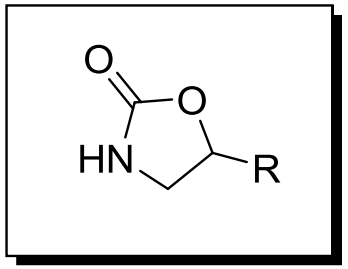
Original unoptimized Med Chem route:



- Henry reaction required tedious extractive workup

- Poor yield in reduction step due to impurities
- Chromatography required

Oxazolidinone Synthesis



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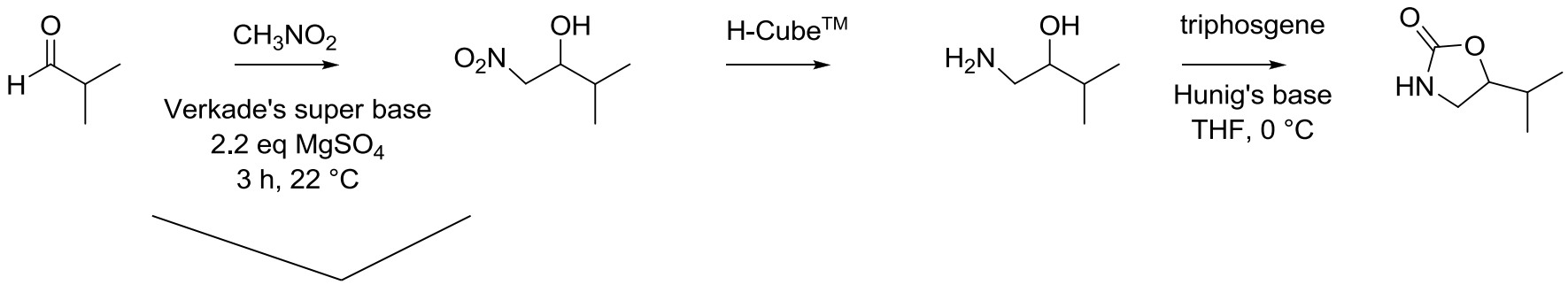
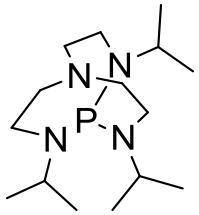
Parallel amenable route:



- Non-aqueous Henry requires only filtration through pad of silica
- Quantitative yield

Verkade's super base =



See: Verkade, J. G. *J. Org. Chem*. **1999**, *64*(12), 4298-4303.

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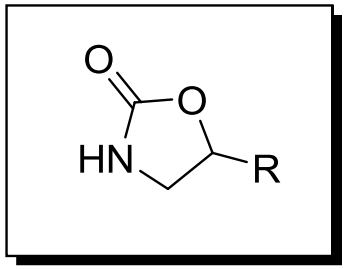
Oxazolidinone Synthesis



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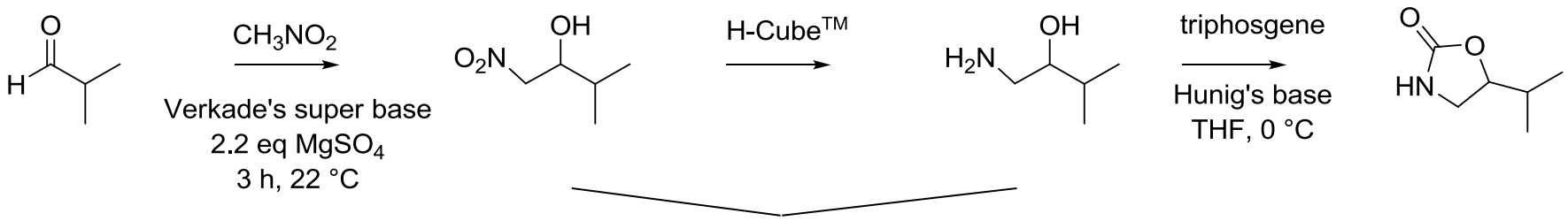
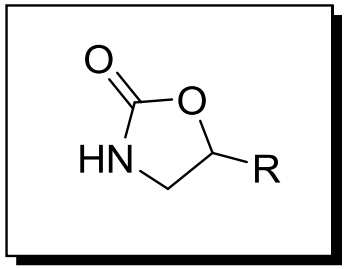


30 mm 10% Pd/C CatCart™
0.5 M substrate concentration
0.5 M AcOH in 1:1 EtOAc:EtOH
0.5 mL/min flow rate
40 °C, “full” H_2 mode
~4 mmol max. substrate per CatCart™

Oxazolidinone Synthesis



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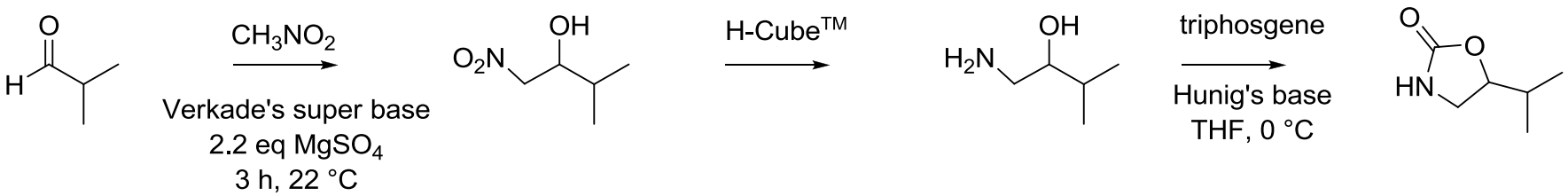

- ✓ Quantitative yields for first two steps
- ✓ No aqueous work-up required until the last step (overall >85% yield)
- ✓ Route was applied to a series of aldehydes to complete project

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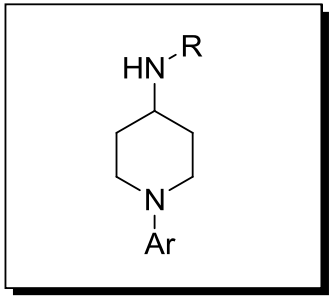
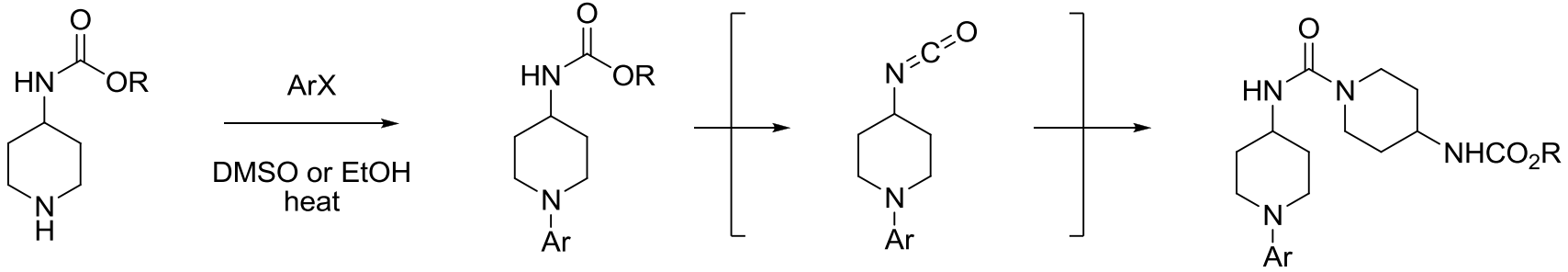
Screening library: Azide reduction

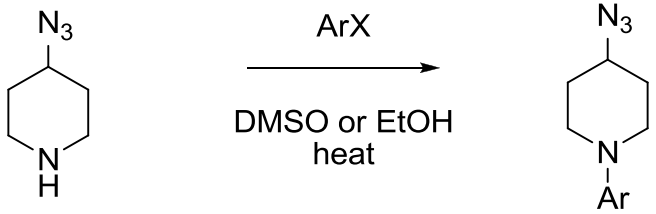


- Target-directed library of aminopiperidines
- CBZ- and Boc- protecting groups proved labile in SnAr



MW 200-234

- Azide afforded cleaner product
- Increased atom efficiency



MW 126

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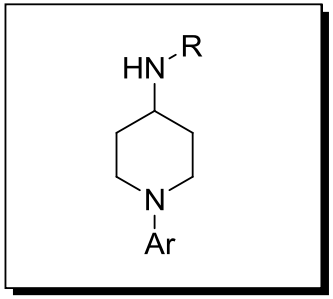
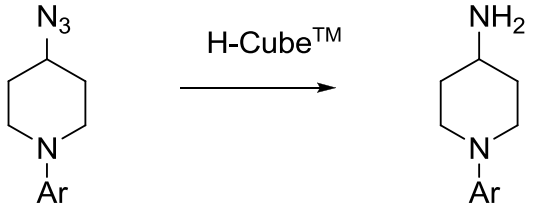
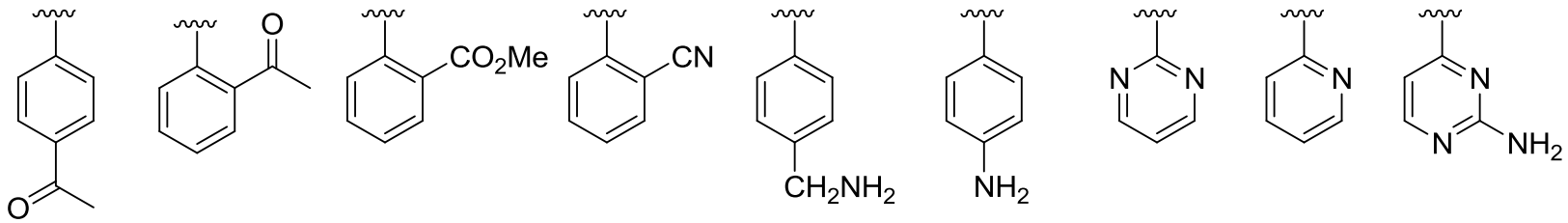
Screening library: Azide reduction



- CatCarts™ screened : Pd/C, Pt/C, Ir/C, $Pd(OH)_2/C$, Pt/C (S), $Pd\text{-}Cu/Al_2O_3$
- Solvents screened: THF and MeOH
- Conditions used: 0.04 M THF, 10% Pt/C, "full" H_2 mode, 1.0 mL/min, 30 °C



Ar =



Conversion (n=1)	>99%	>99%	>99%	>99%	>99%	>99%	>99%	89%	97%
Isolated yield	96%	>99%	>99%	87% *	>99% (from nitrile)	>99% (from nitro)	96%	79%*	52% **

*over-reduction by-products contributed to low yield

**poor recovery from catalyst

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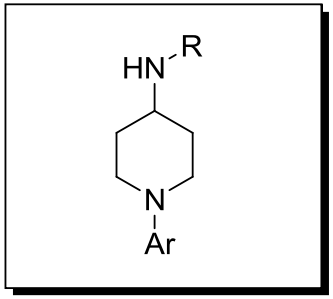
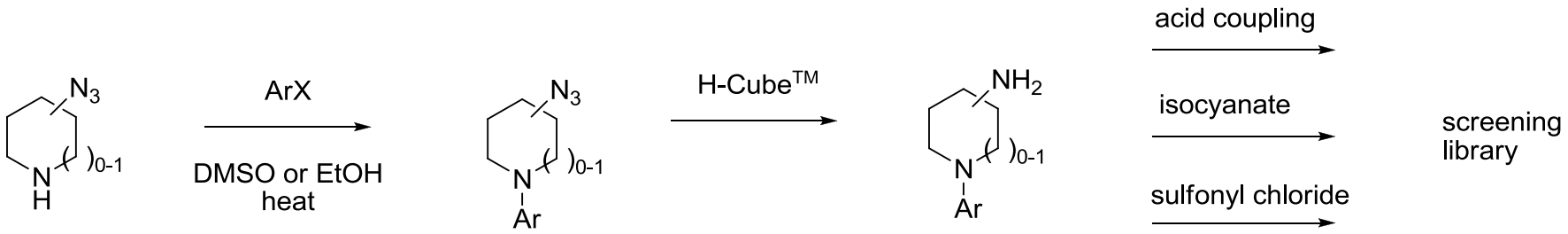
Screening library: Azide reduction





- Azide route is more atom efficient (lower mass starting material)
- H-cube™ reduction facilitates library synthesis
- Scale affords 120 library members per piperidine/pyrrolidine core
- Final libraries rapidly produced in 24 well plate format

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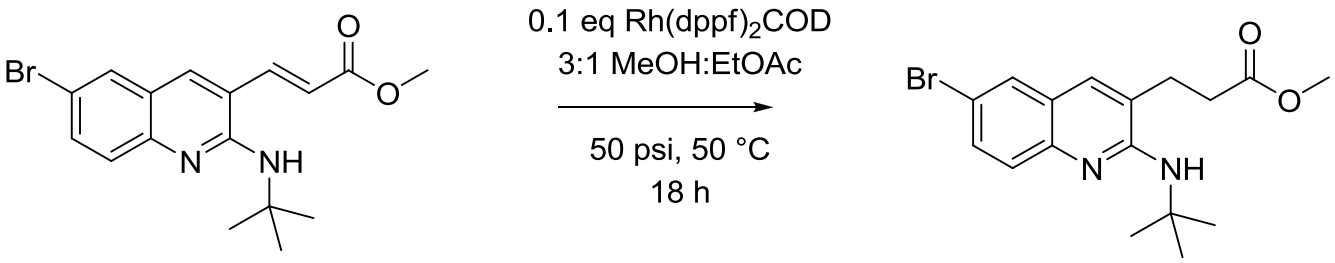
Selective reduction: Intermediate scale-up

Original batch route:



- Several days screening to identify best conditions
- Multiple batches run due to low solubility
- 88% yield after chromatography (5 g scale)

H-Cube™ continuous flow route?

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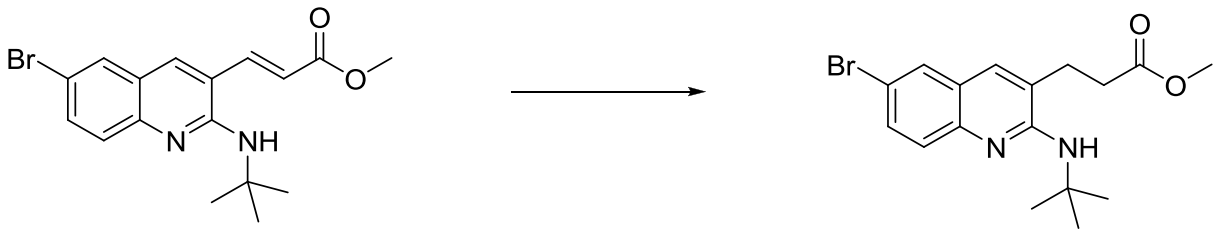
Selective reduction: Intermediate scale-up

H-Cube™ route:



- Screened 12 conditions, ~2 h using autosampler
- 5% Rh/C, THF, 80 bar, 30 °C, 2.0 mL/min gave best conversion/selectivity
- **85% yield**, @ 0.275 M in THF, 50 mg scale
- **60% yield**, @ 0.275 M in THF, 1.0 g scale
- Re-subjecting product mixture to reaction conditions increased side products due to over-reduction, decreased yield

What went wrong?...

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…and why is the H-Cube™ not getting greater use in Amgen Drug Discovery?

Amgen users have experienced:

- Incomplete reductions
- Over-reductions
- Poor reproducibility
- Poor recoveries

Problems may arise from:

- Fluid mechanics issues
- Insufficient hydrogen
- Catalyst deactivation
- Adsorption effects

Can we get a better understanding of these issues and achieve better success with the H-Cube™?

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Characterization of the H-Cube™

Using a simple model system (styrene reduction) examine:

- Run to run variability
- Hydrogen flow variability
- Dispersion effects

Develop a screening to scale-up (100 mg -10 g) workflow.

- Conserve material during screening
- Conserve time/volume during scale-up

Demonstrate workflow on a relatively “complex” model.

- 1 g scale hydrogenation of methyl 3-bromocinnamate
- Limit undesired side products, maximize yield

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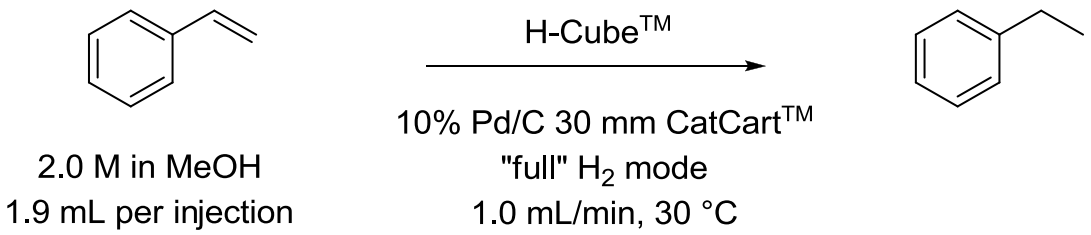
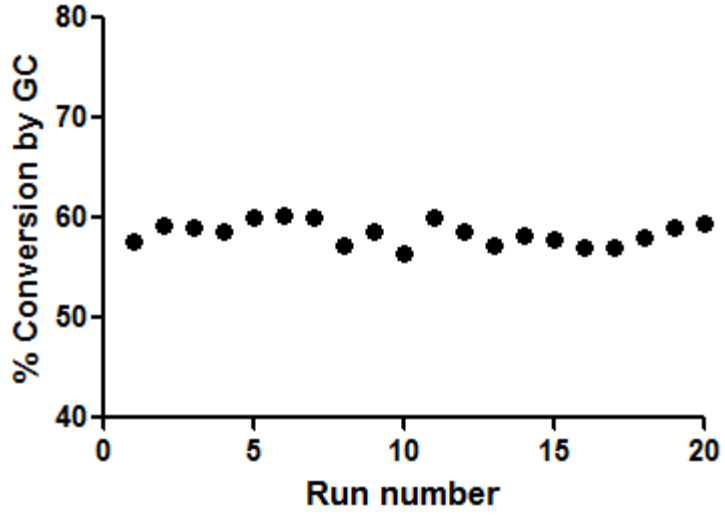
Run to run variability

- High concentration of substrate gives incomplete reduction
- CatCart™ was pre-conditioned (MeOH wash, 10 mL, in H_2 “full” mode)





➢ High reproducibility (56.4 – 60.2% conversion) over 20 sequential runs

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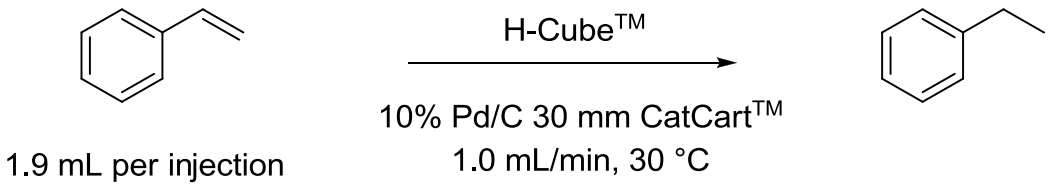
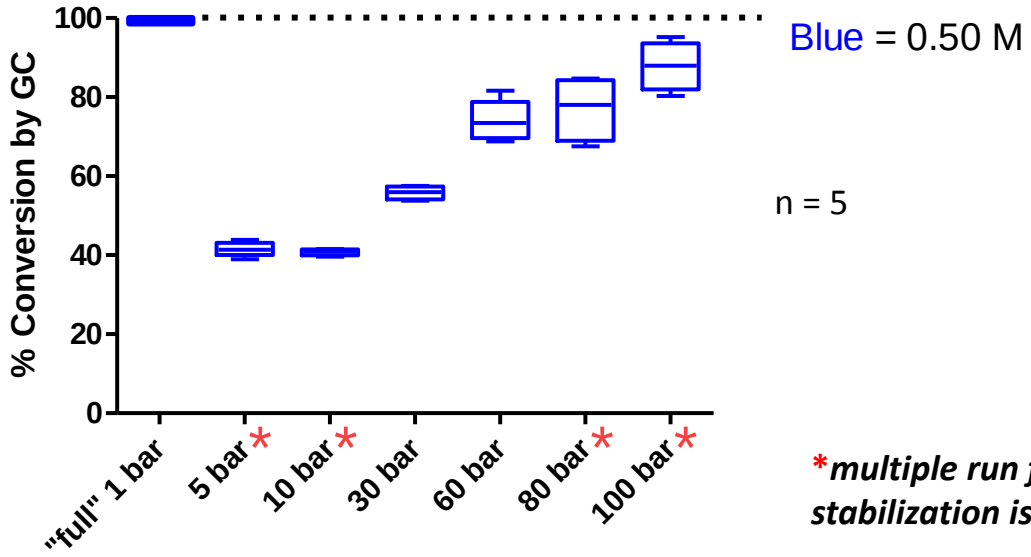
Variability Associated with Pressure Settings

- Styrene reduction was repeated @ 0.5 M in MeOH, varying pressures



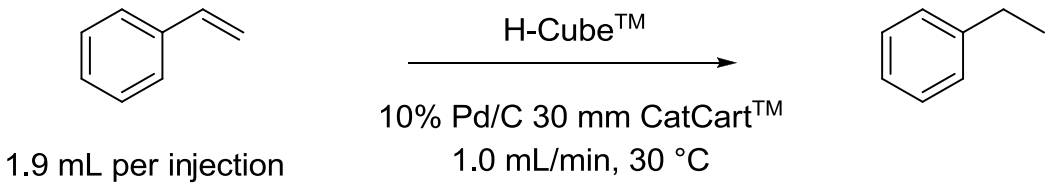


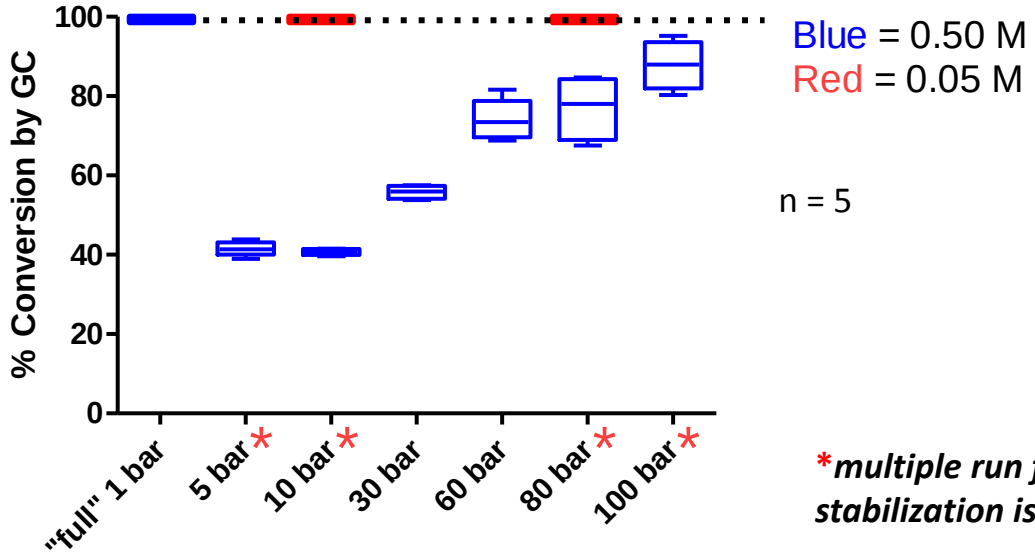
multiple run failures due to stabilization issues; data excluded

- Stabilization issues were more frequent below 30 bar and above 60 bar
- Variability increased with increasing pressure

Variability Associated with Pressure Settings

- The reduction was repeated @ 0.05 M, 10 bar and 80 bar only

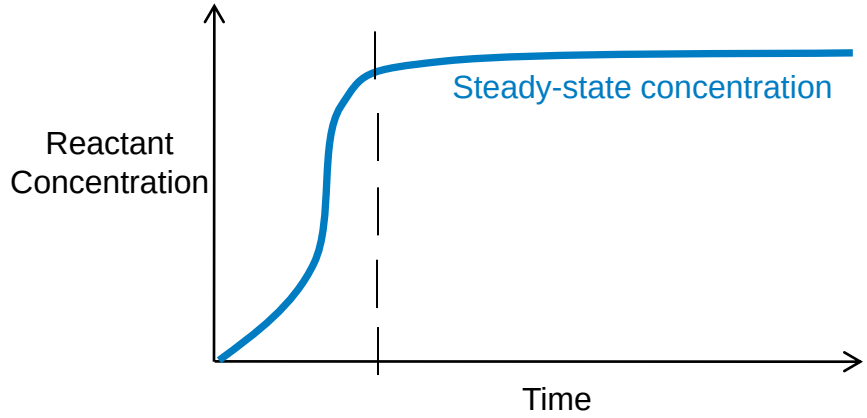




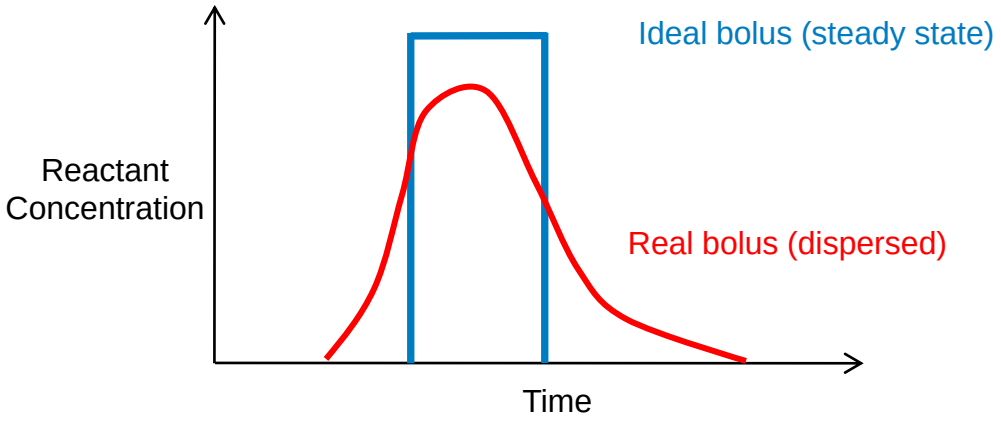
➢ At lower concentrations (recommended by ThalesNano©) variability is not observed.

Dispersion effects

Typical Process Research scale optimization examines parameters at steady-state (SS) concentrations:



Typical Med Chem Research scale uses bolus injections to conserve material, resulting in dispersion:



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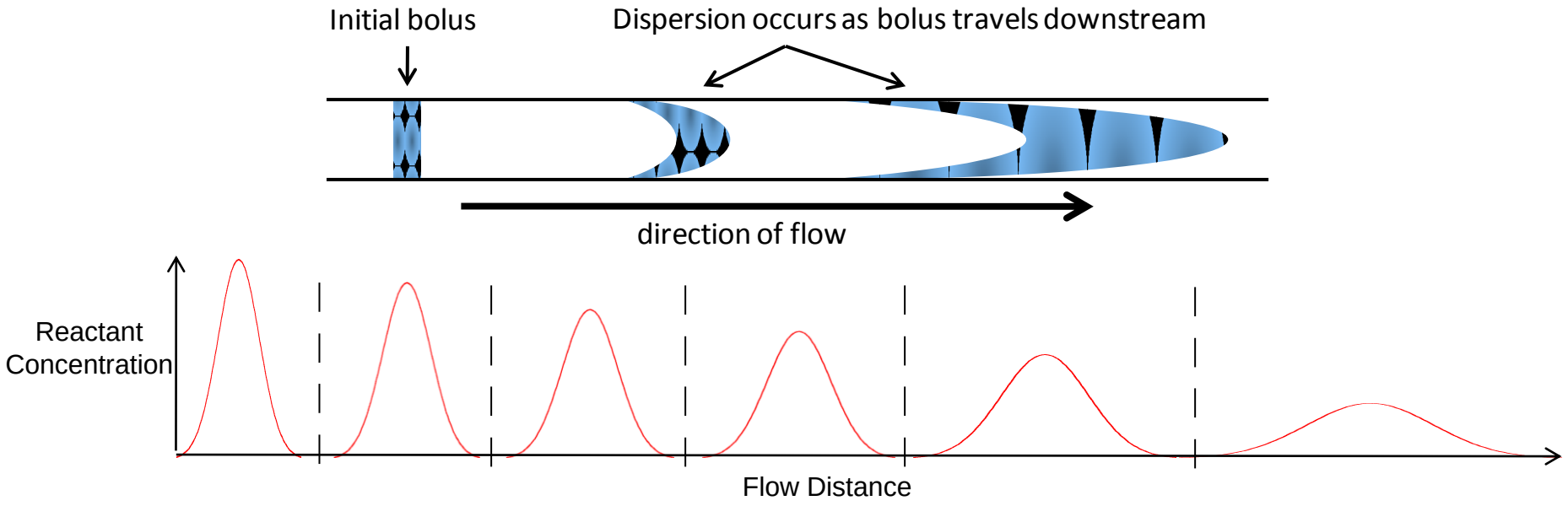
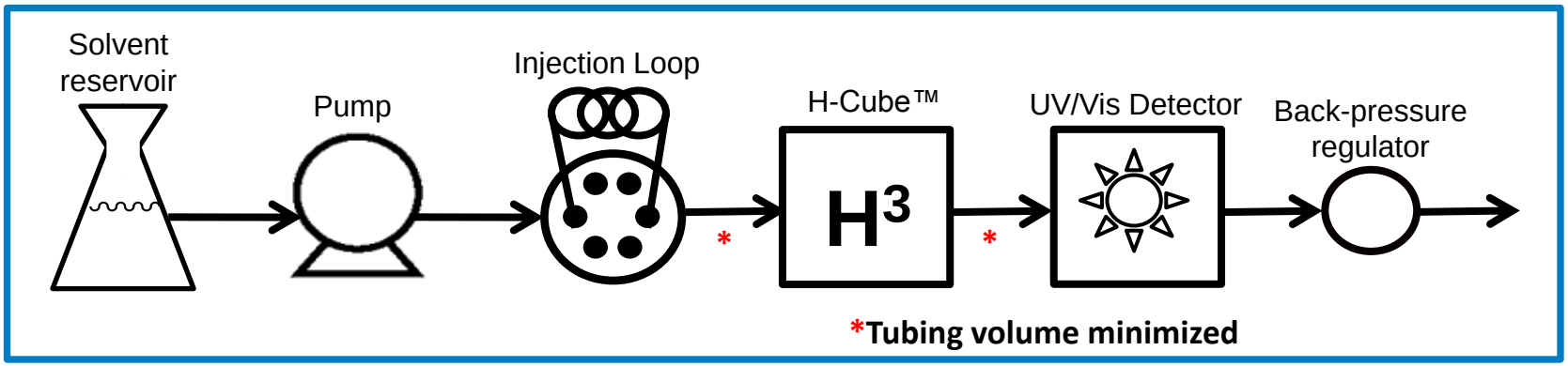
Dispersion effects

Factors that contribute to dispersion in a packed bed reactor:

- Flow rate
- Diffusion
- Channeling (effected by quality of the packed bed, frits, size/shape of cartridge)
- Particle size (large particle size contributes to dispersion, small size results in increased back pressure)
- Adsorption kinetics



Characterization of dispersion in the H-Cube™



- For the characterization, a UV detector was added in-line
- Manual injections were performed using a sample loop
- Sample loop size was varied to accommodate different injection volumes

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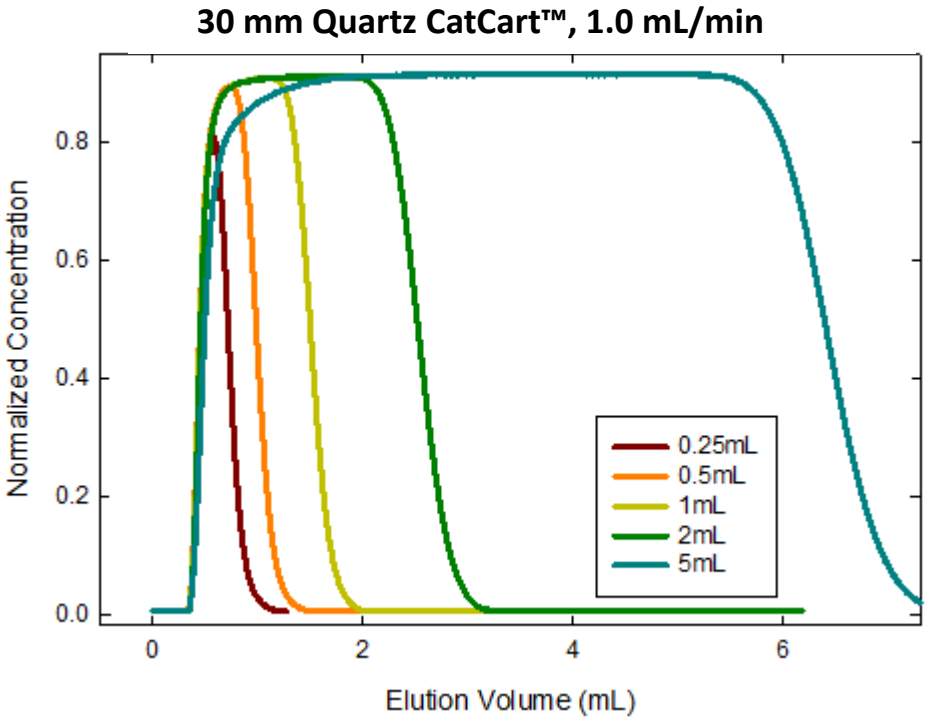
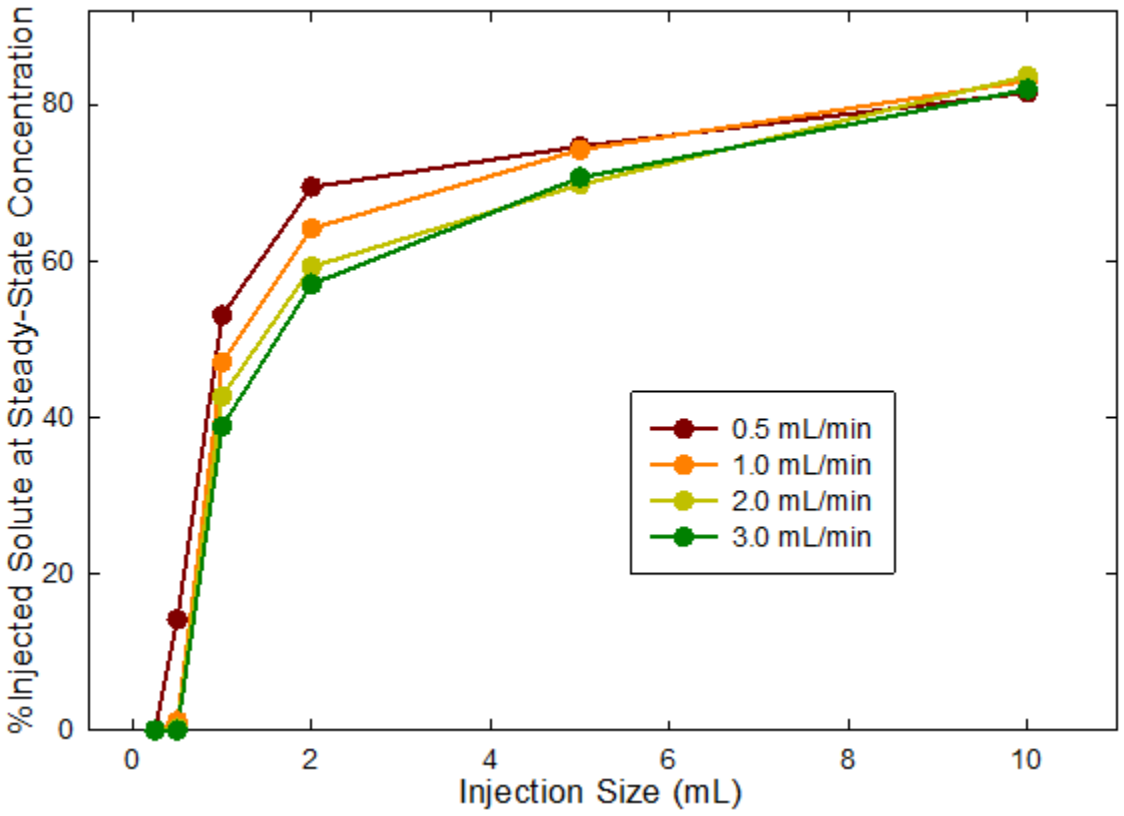
Dispersion by UV in the H-Cube™ Reactor

- Bolus injections of caffeine solution made through a 30 mm quartz CatCart™
- Injection volumes varied, constant flow rate, no H_2



- The H-Cube™ introduces dispersion.
- A steady-state concentration equal to that injected can be reached.
- ≥2 mL injection is needed to achieve significant steady-state.

Mass percent at Steady State (SS)



- 2 mL bolus gives ~60-70% mass at SS (flow rate dependent), in a 30 mm quartz CatCart™ (150 uL void volume)
- >2 mL bolus does not significantly increase mass percent at SS, relative to total volume injected

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Design of Experiment (DoE) Optimization of Model Styrene Reduction

Will optimization using 2 mL bolus injections translate to a SS continuous flow scale-up?

- DoE factors, 2 levels, n=2:

  Initial Concentration: 0.4 and 0.6 M

  Pressure setting: 40 and 80 bar

  Flow Rate: 0.4 and 1.0 mL/min

  Temperature: 20 and 35 °C

  Catalyst (10% Pd/C) Loading: 50 mg (micro) and 150 mg

- Initial concentration and pressure settings were the most critical factors*
- At 0.4 M and 80 bar: flow rate, cartridge loading, and temperature did not have significant effects
- Using the DoE optimized conditions ***>90% conversion*** was achieved upon scale-up (≥1 g) in continuous mode
- However, >3 g starting material was consumed during optimization

*based on JMP software analysis

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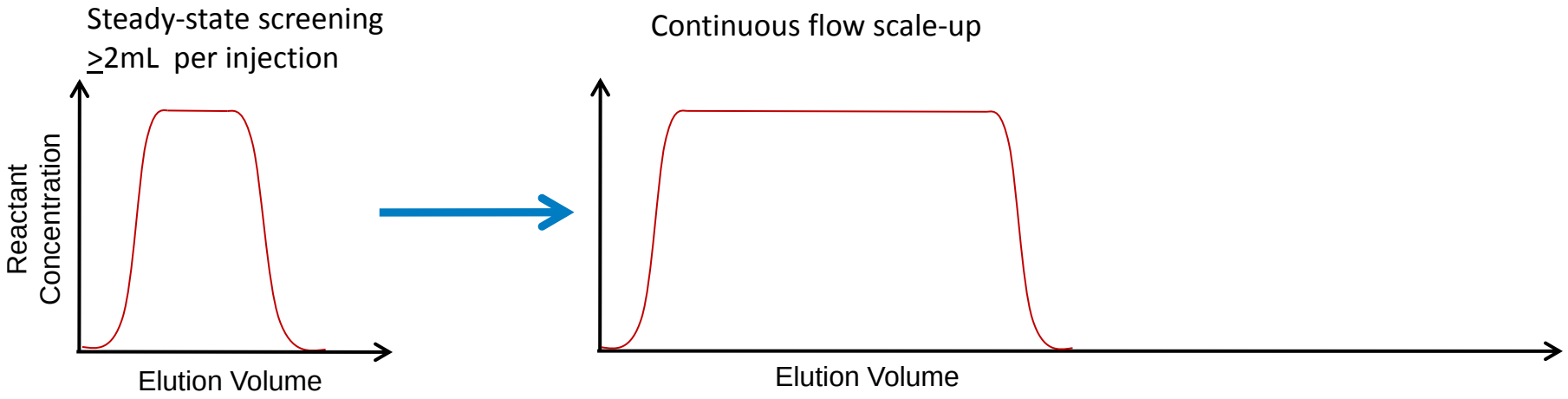
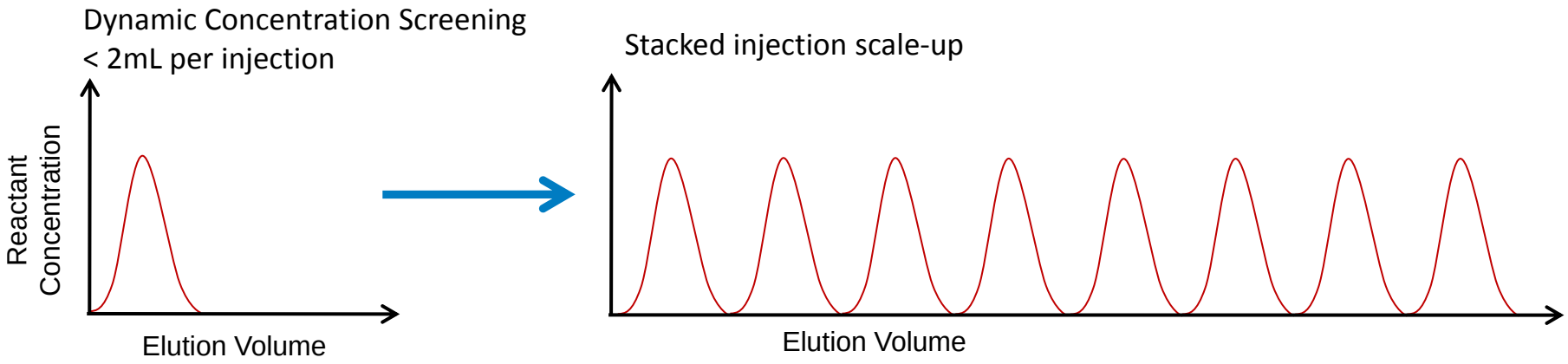
Scale-up in Continuous Mode vs. Stacked Injections

"Process scale" optimization: conserves time/volume during scale-up



"Med Chem scale" optimization: conserves material during screening



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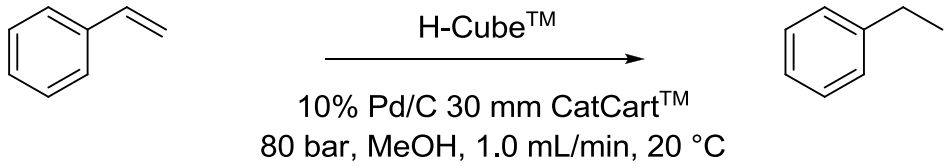
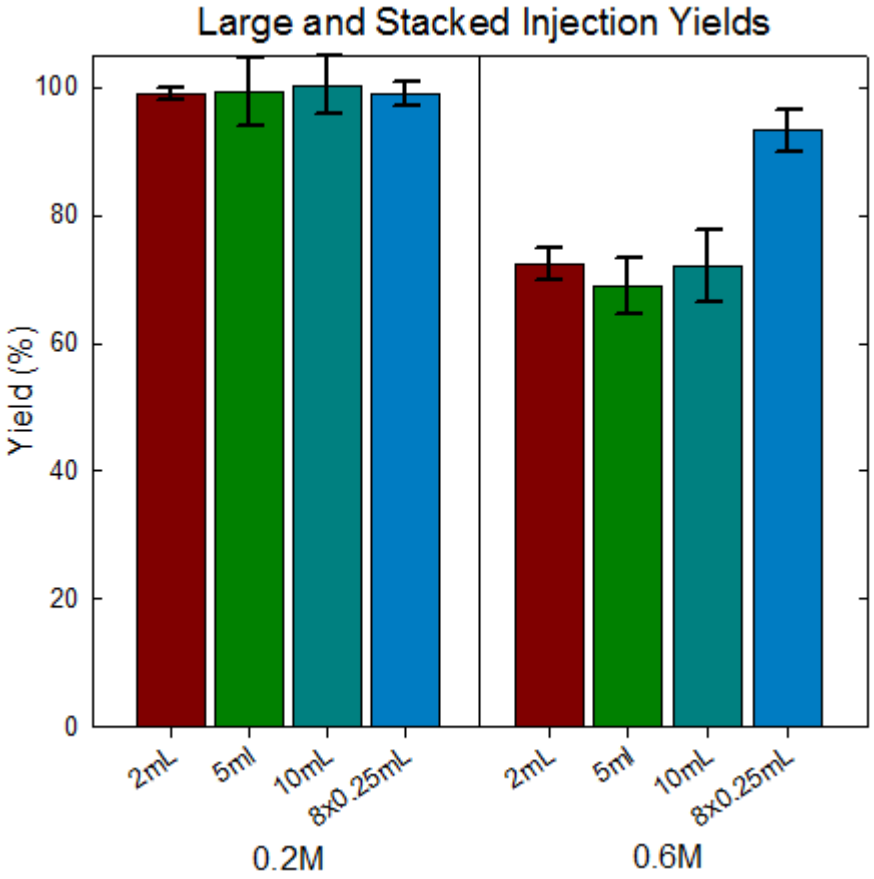
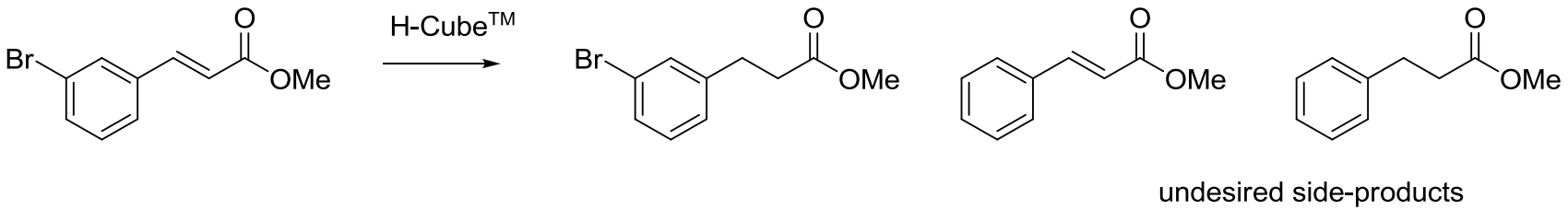
Scale-up in Continuous Mode vs. Stacked Injections





- Styrene reduction yield unaffected by increasing injection volume
- High yield at 0.2 M achieved with stacked injections (0.25 mL)
- At 0.6 M, stacked injections increased yield ~15%

More “Complex” Scenario: Adsorption Effects



- Goal: 1 g scale, high yield, low impurity profile.
- Minimal catalyst screen showed 5% Rh/C CatCart™, THF as best option.
- DoE optimization, 2.0 mL injections, 3 factors (n=3 for each):
  - **Initial conc.**: 50 and 250 mM
  - **Pressure**: “Full” and 50 bar
  - **Flow rate**: 0.5 and 2.0 mL/min
- DoE optimized conditions (50 mM, “full” mode, 2.0 mL/min, 30 °C)
  - afford **95%** yield in 2 mL injection (24 mg reactant)
  - afford **60%** yield in continuous mode (1 g reactant)

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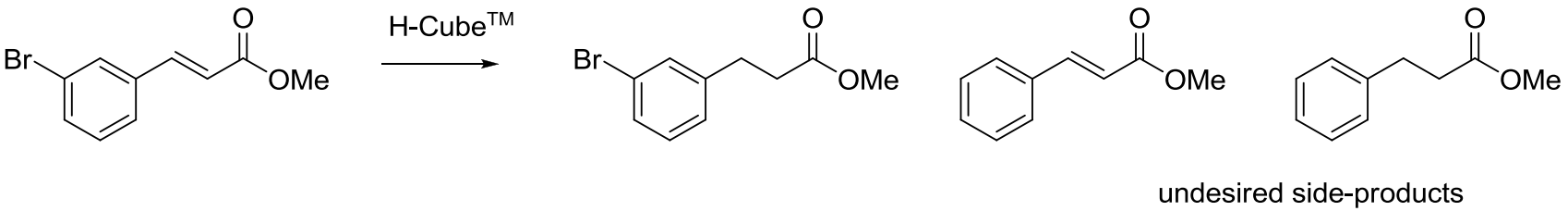
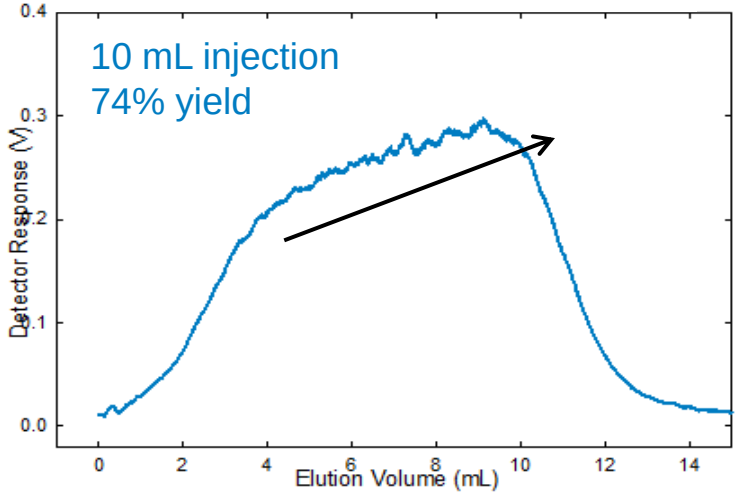
More “Complex” Scenario: Adsorption Effects

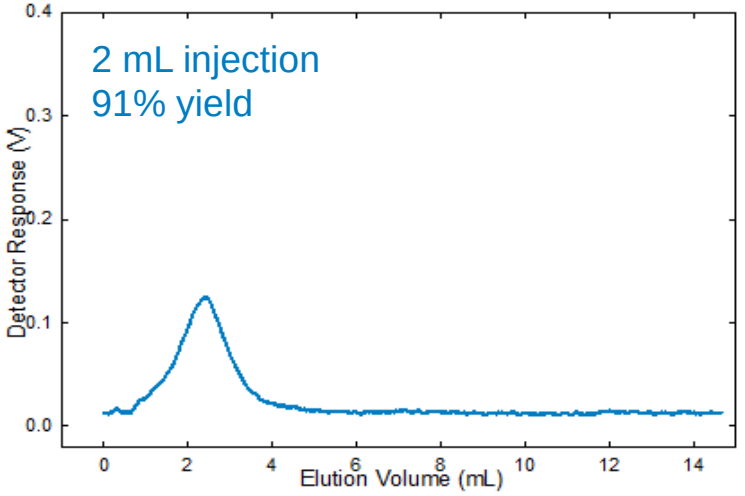


- Deactivation observed over 10 mL injection
- Increasing temperature to 60 °C increased yield ~10%
- Catalyst recovers after washing

Increase in %reactant over time



Same CatCart™ after 30 mL THF flush



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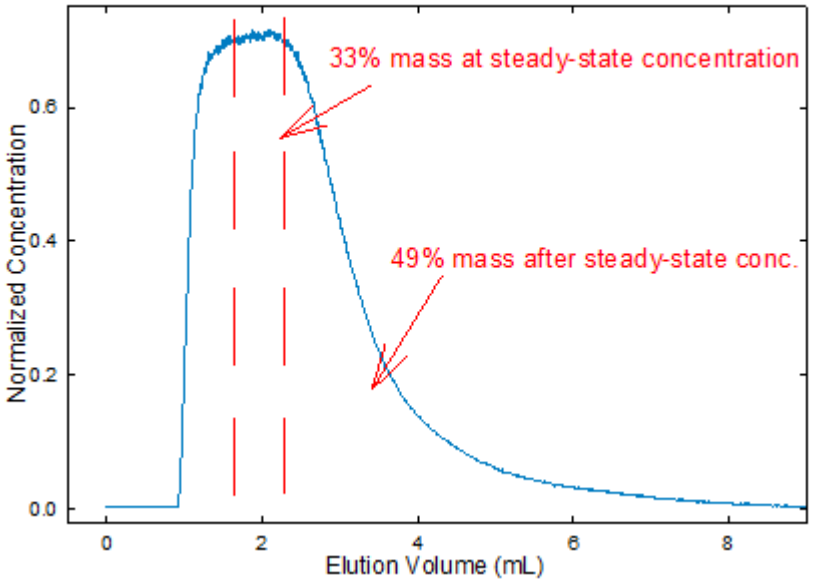
Effect of Chemotype and Catalyst on Adsorption

50 mM methyl 3-Br cinnamate THF, 2 mL at 2.0 mL/min, no H2



Starting Material	30 mm CatCart™	%Mass at Steady-State
Cinnamate	5% Rh/C	33
Styrene	5% Rh/C	40
Cinnamate	10% Pd/C	37
Styrene	10% Pd/C	49

- Mass delivered at the steady-state concentration is chemotype and catalyst dependent.
- Study of these adsorption effects is in progress.

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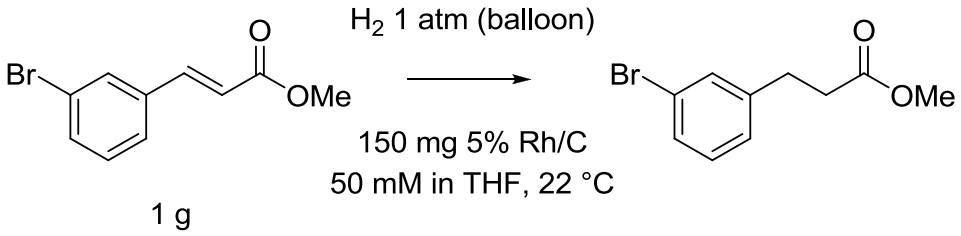
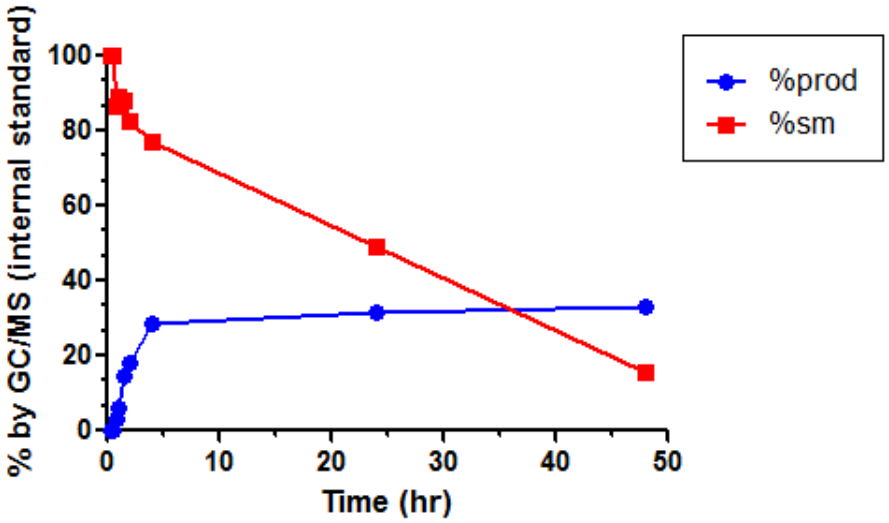
Comparison to Batch



1 g



- **33%** yield after 48 hr in batch mode, with **51%** by-products
- **60%** yield in 45 min in continuous flow, **<1%** by-products

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Conclusions

What we've recommended to our colleagues…

- *For simple reductions* on large scale (1-10 g), the stand alone H-Cube can be used with 2 mL injections for steady state reaction optimization prior to continuous flow scale-up.
- For the typical Med Chem application (<1 g), use dynamic screening followed by stacked injections for best results.
- Scale in batches, using multiple CatCarts, when catalyst deactivation may be an issue.
- Use the controlled mode feature with caution, with the understanding that higher pressures lead to higher variability and failed runs due to instability.

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Thank you

At Amgen :

- **David Wernick** (UCLA intern)
- **Callie Bryan** (Medicinal Chemistry Research Technologies)
- John Eschelbach (Discovery Analytical Sciences)
- Jim Petersen (Research Automations Technologies)
- Alan Allgeier (Catalysis and Hydrogenation Group)
- Peter Grandsard
- Randall Hungate

At ThalesNano:

- Richard Jones
- Laszlo Urge
- Paul Whittles
- Alan Boyle

RSC/SCI Symposium coordinators and speakers

Glaxo SmithKline

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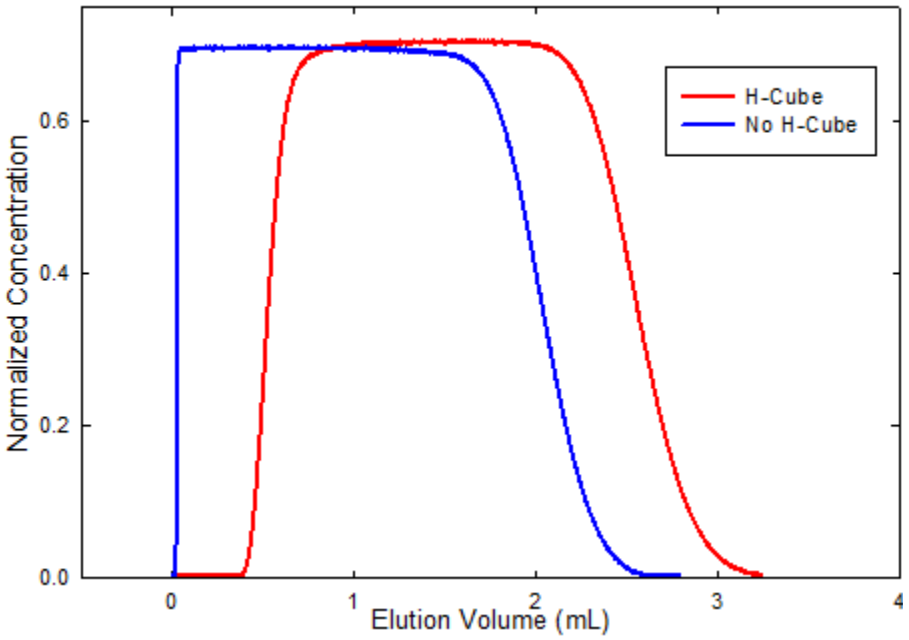
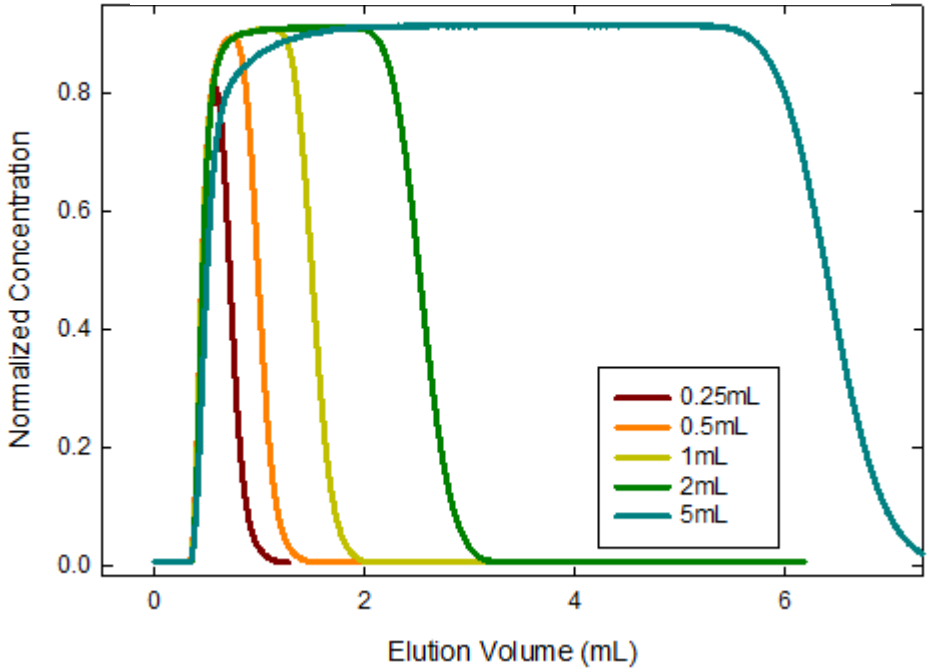
Dispersion by UV in the H-Cube™ Reactor

1. Bolus caffeine solutions, with and without the H-Cube™ (quartz CatCart™)
2. Injection volumes were varied

Elution profiles, 2 mL bolus, 0.5 mL/min



30 mm Quartz CatCart, 1.0 mL/min



- The H-Cube™ introduces dispersion.
- A steady-state concentration equal to that injected can be reached.
- 2 mL or larger injection is needed to achieve significant steady-state.

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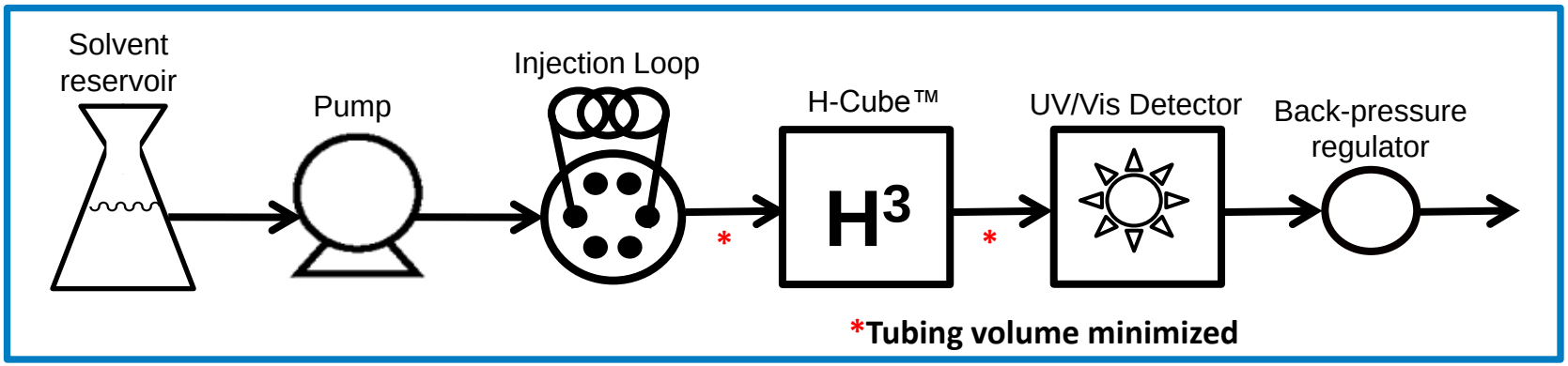
Characterization by UV



- UV detector was added in-line to follow the course of styrene reduction and examine dispersion effects
- $\varepsilon_{styrene} >> \varepsilon_{ethylbenzene}$
- An increase in absorbance is associated with a decrease in product formation
- Under styrene reduction conditions *where hydrogen is limiting*, UV monitoring affords an indirect measure of hydrogen production

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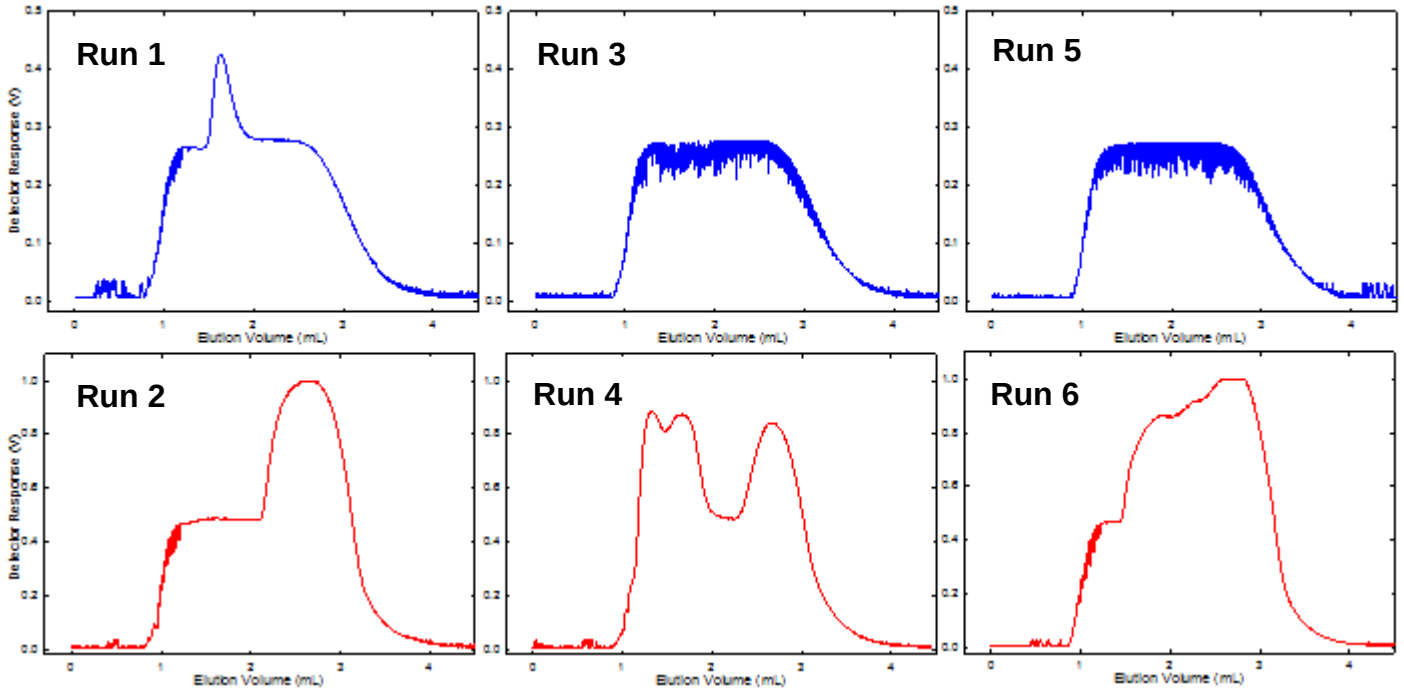
Hydrogen flow variability by UV

- 2 mL injections @ 0.2 M or 0.6 M styrene in MeOH
- 80 bar, 1.0 mL/min, 30 mm 10% Pd/C, 30 °C
- Monitored at 265 nm



0.2M

0.6M

➢ Reaction rate drops significantly at several points, suggesting interruption of hydrogen flow.