Measuring Hydrolytically Unstable Silanol for Toxicokinetic Studies:

Overcoming Inherent Instabilities with New Methodology

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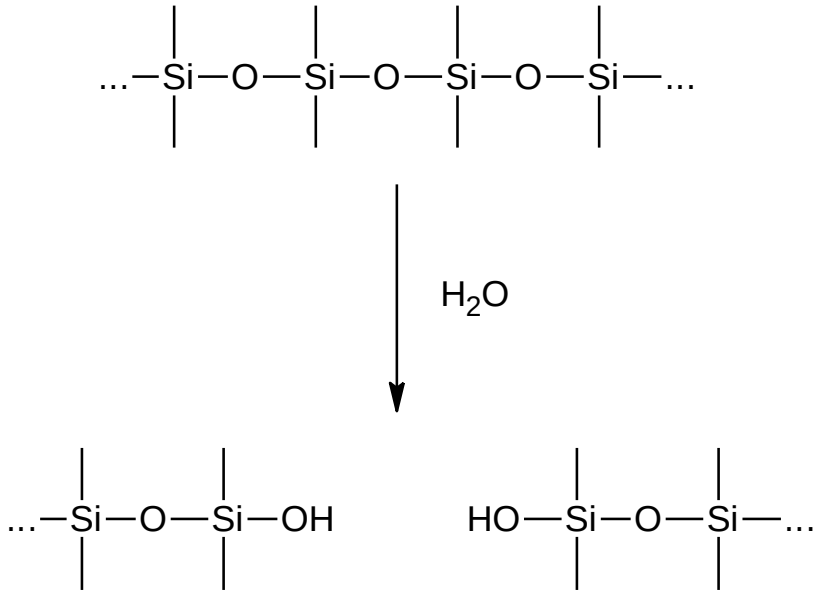
ENC NMR Software Symposium: April 10th, 2016

From Polydimethylsiloxane (PDMS) to Silanol

- PDMS used in: shampoos, conditioners, skin care, polish additives, waterproofing and many others

- Biological contact with D_4/D_5 = low molecular weight silanol functional materials

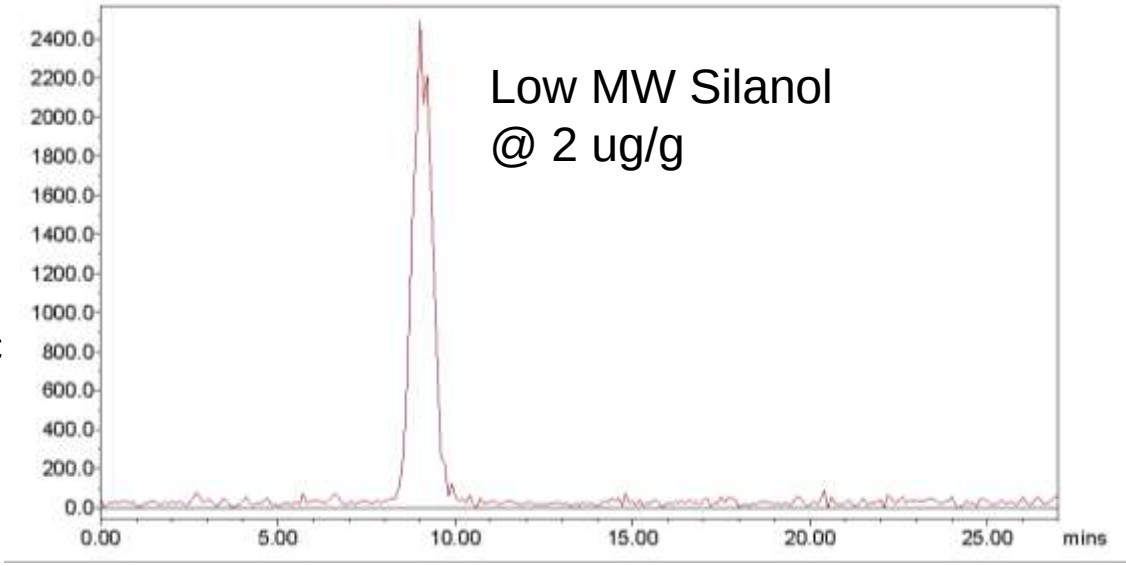
- Soil contact with siloxanes = low molecular weight silanol functional materials

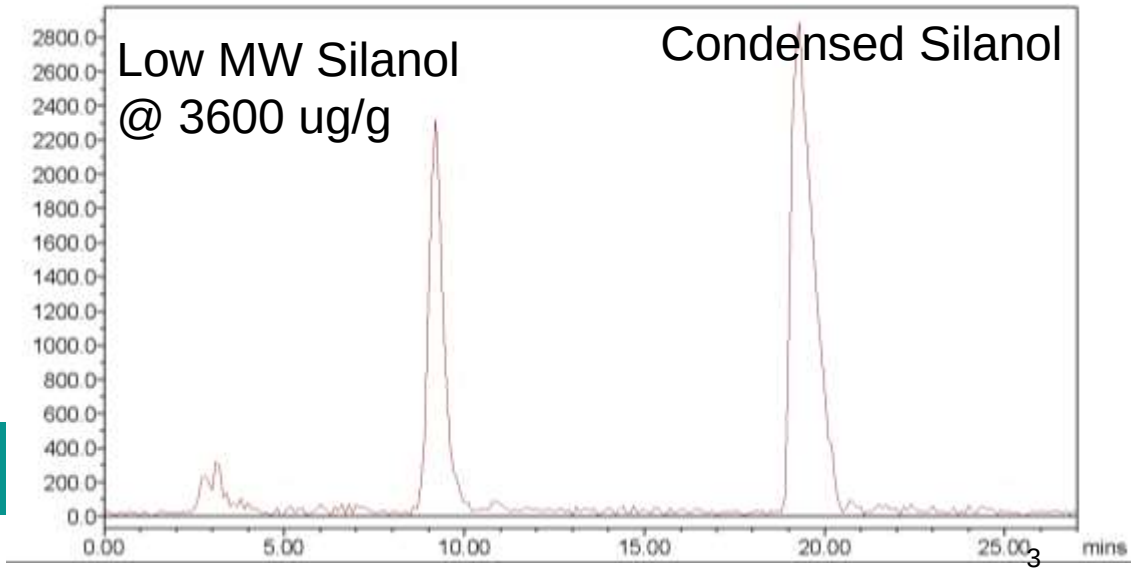


McMullin, T.S., Yang, Y., Campbell, J., Clewell, H.J., Plotzke, K., Andersen, M.E., Regul. Toxicol. Pharmacol., 74 (2016) S1-S13
Varaprath, S., McMahon, J.M., Plotzke, K.P., Drug Metab Dispos 31:206-204, 2003
Lehmann, R.G., Miller, J.R., Kozerski, G.E., Chemosphere 2000 Sep; 41 (5); 743-9

Pitfalls of Chromatographic Approach

- At concentrations in recovered urine:
  - Silanol condenses during chromatographic preparation
  - Phenomenon is noted in both GC (pictured) and LC





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Challenges of Low MW Silanol Analysis

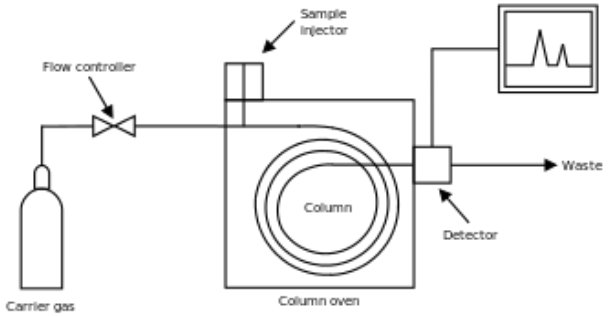
1. When we dose Low MW Silanol at 15 wt % in a medium…
   What is our confidence that it has not condensed?

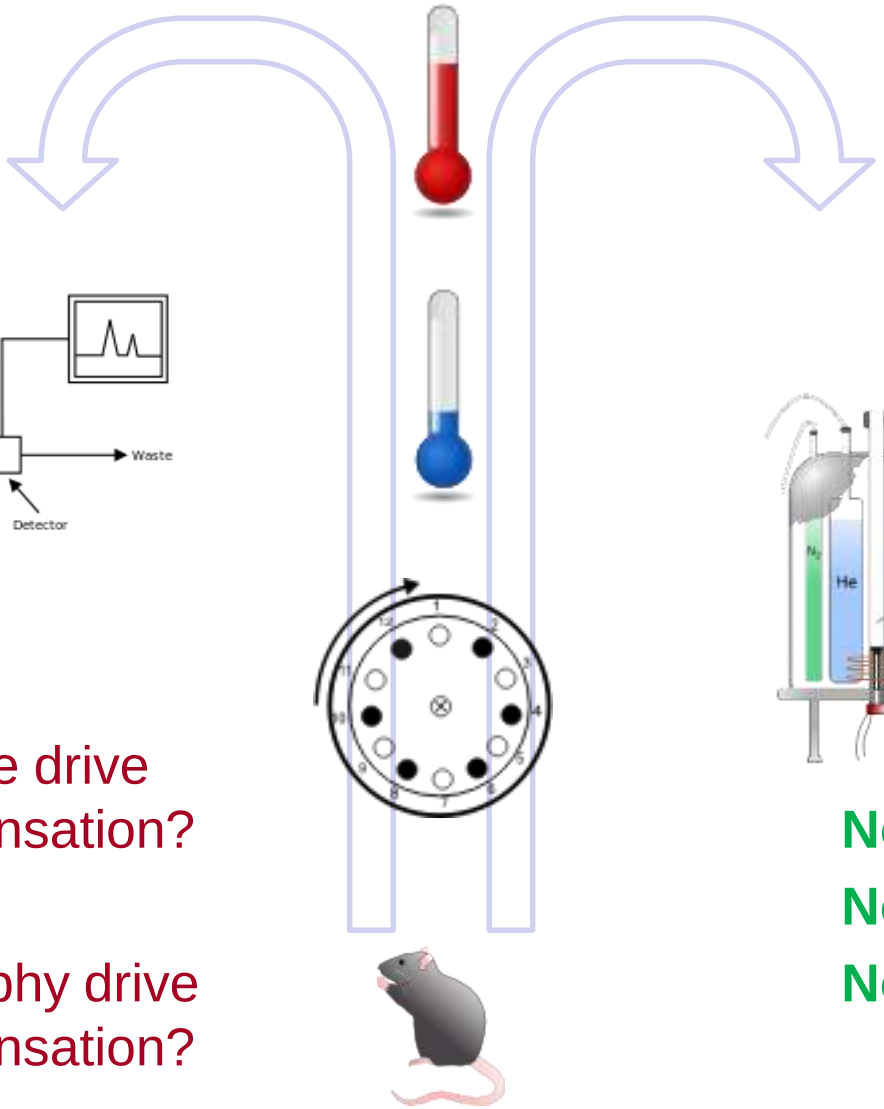
2. When we retrieve samples, are we collecting metabolites or equilibration products of metabolites?

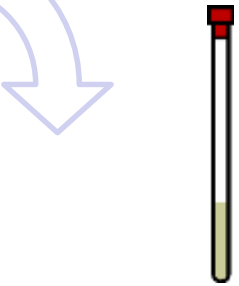
3. When we prepare samples for measurements, are we changing the equilibration conditions?

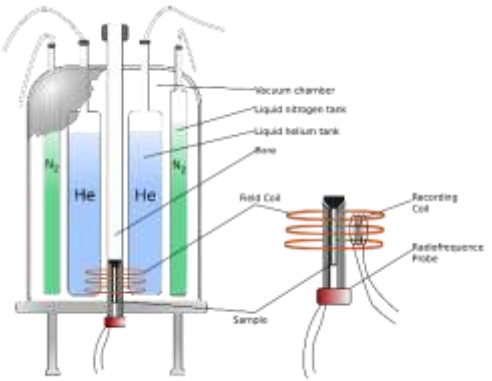
4. Do equilibration kinetics play a role during the course of the measurement?

Why NMR of Low MW Silanol?









Does phase change drive hydrolysis & condensation?

Does chromatography drive hydrolysis & condensation?

No phase change.

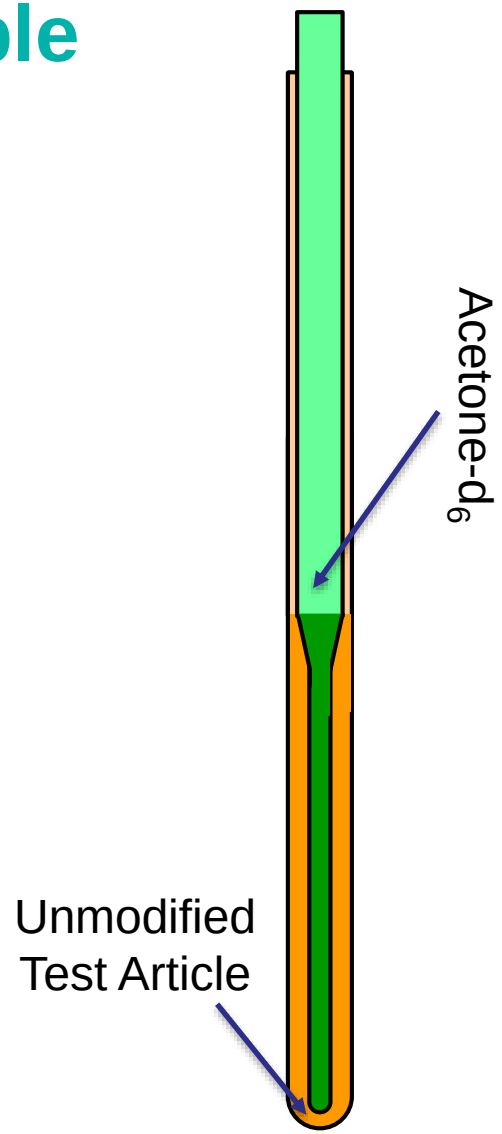
No dilution.

No heating.

Measuring an Unadulterated Sample

- Target test article = recovered rodent urine
- 5 mm coaxial tube
- External tube = test article

- No concentration change
- No phase change
- No temperature change



Toxicokinetic NMR: Establishing Confidence

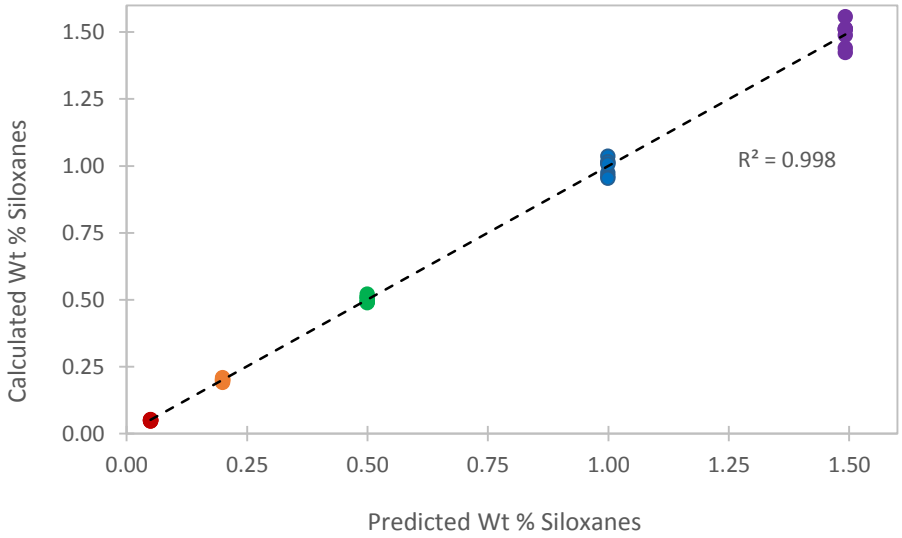
- As a new analytical tool, we needed to establish confidence
  - Concentration Linearity
  - Limit of Quantification
  - Molecular Selectivity
  - Test Repeatability
  - Repeatability
  - Sample Stability when Frozen
  - Hydrolysis and Condensation Kinetics

Profile of measures

	0.05 %	0.20 %	0.50 %	1.00 %	1.50 %
<1 h	F,K,T,C, Q,R	K,T,C,R	K,T,C,R	K,T,C,R	F,K,T,C, Q,R
F1, <1 h	F				F
F7, <1 h	F				F
F30, <1 h	F				F,C
11 h	K,T,C,R	K,T,C,R	K,T,C,R	K,T,C,R	K,T,C,R
17 h	K,T,C,R	K,T,C,R	K,T,C,R	K,T,C,R	K,T,C,R
27 h	K,T,C,R	K,T,C,R	K,T,C,R	K,T,C,R	K,T,C,R
114 h	K,T,C,R	K,T,C,R	K,T,C,R	K,T,C,R	K,T,C,R
127 h	K,T,C,R	K,T,C,R	K,T,C,R	K,T,C,R	K,T,C,R
161 h	K,T,C,R	K,T,C,M ,R	K,T,C,R	K,T,C,R	K,T,C,R

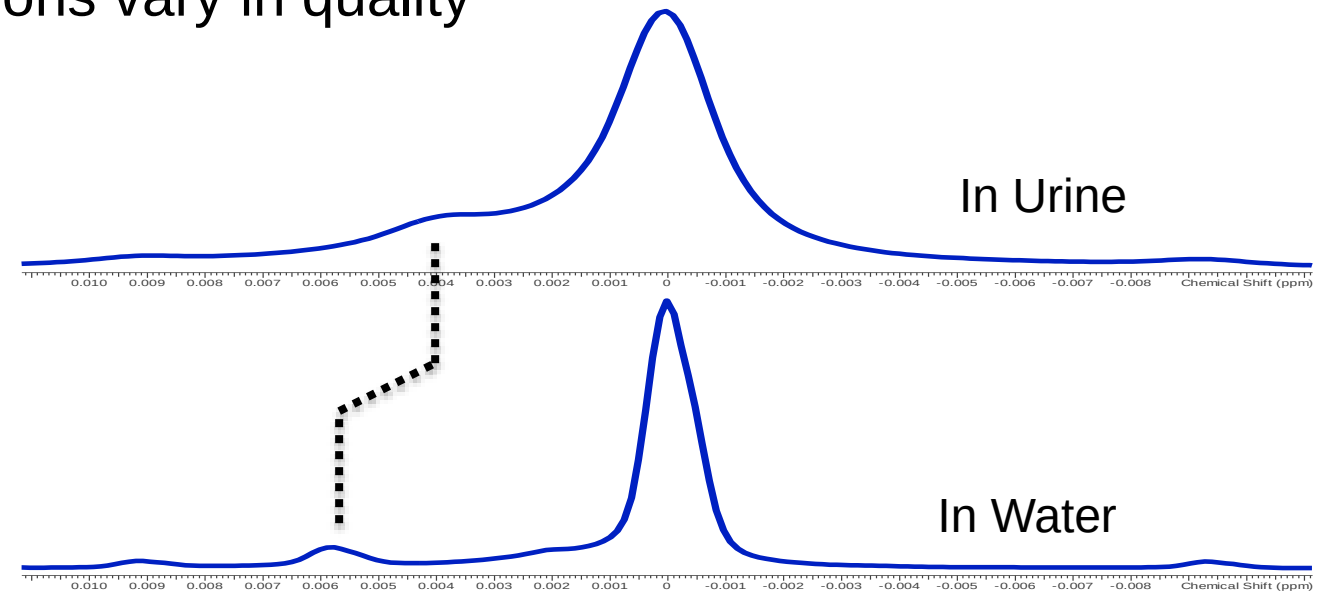
Concentration Linearity: Total SiMe

- Range
  - 0.05 to 1.5 wt %
  - Dosed into rodent urine
- Samples
  - 5 concentrations
  - 7 time points
- $r^2 = 0.998$
- Relative Error = 5 to 8 %



Molecular Selectivity: Isolating principle silanols

- In water – near baseline resolution
- In Urine –
  - Two major silanol species converge
  - All signals broaden
  - Shim solutions vary in quality



Limit of Quantification

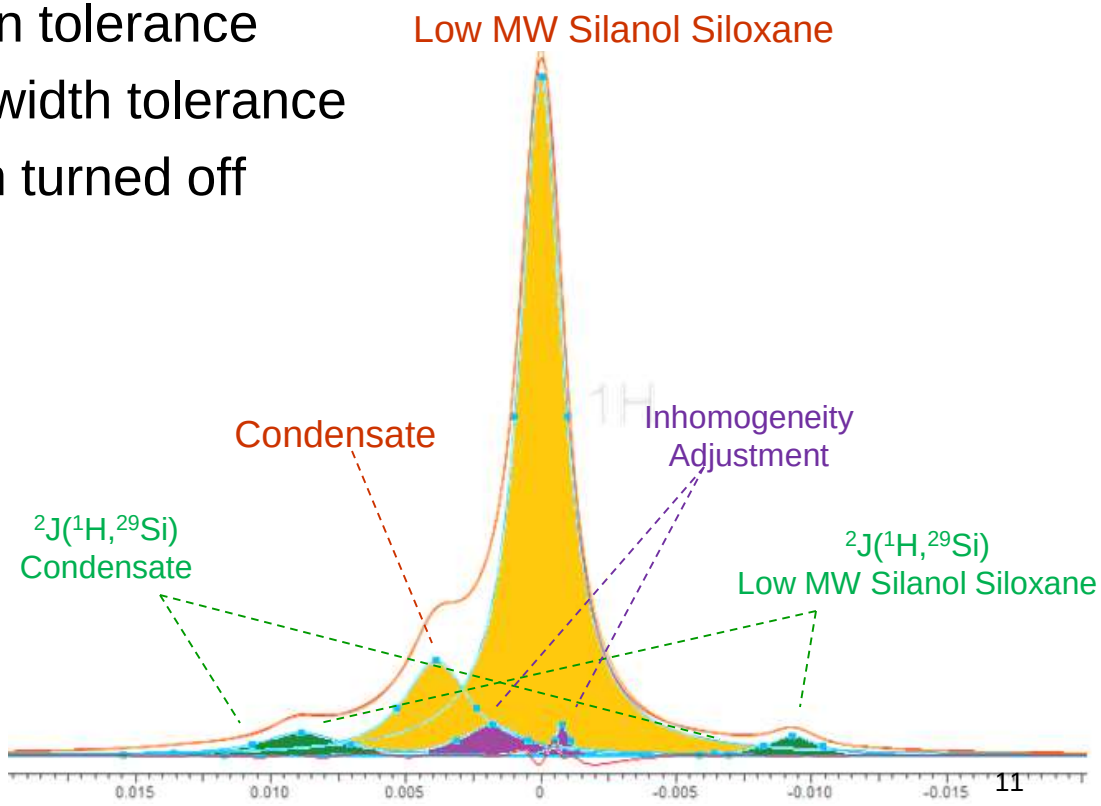
- Commonly 10x RMS noise approximates LoQ
- Bad shims & high convolution limit this approximation
- Further complicated by kinetic evolution
  - 1.5 wt % silanol measured 7 times @ 18-19 h
  - 1.5 wt % silanol measured 3 times @ 0.5-1 h
  - 0.05 wt % silanol measured 7 times @ 0.5, 11, 17, 27, 114, 127, & 161 h

Concentration	Relative error	Method
>400 ppm	± 5-8* %	Measured on 1.5 wt % silanol, low MW signal
55 ppm	± 30 %	Measured on 0.05 wt % silanol, condensed silanol signal
1 ppm	Unknown	S/N extrapolation from 1.5 & 0.05 wt % prep samples

* - the designed study measures 1 %, but takes advantage of an unreasonable shim solution

Peak Detection: Deconvolution Solution

- Assume similar peak shapes
- If deconvolution is good, peak heights define concentration
- Deconvolution package
  - 8 fitted points
  - 0.25 Hz of peak position tolerance
  - 0.04 to 2.5 Hz of peak width tolerance
  - Non-Lorentzian fraction turned off



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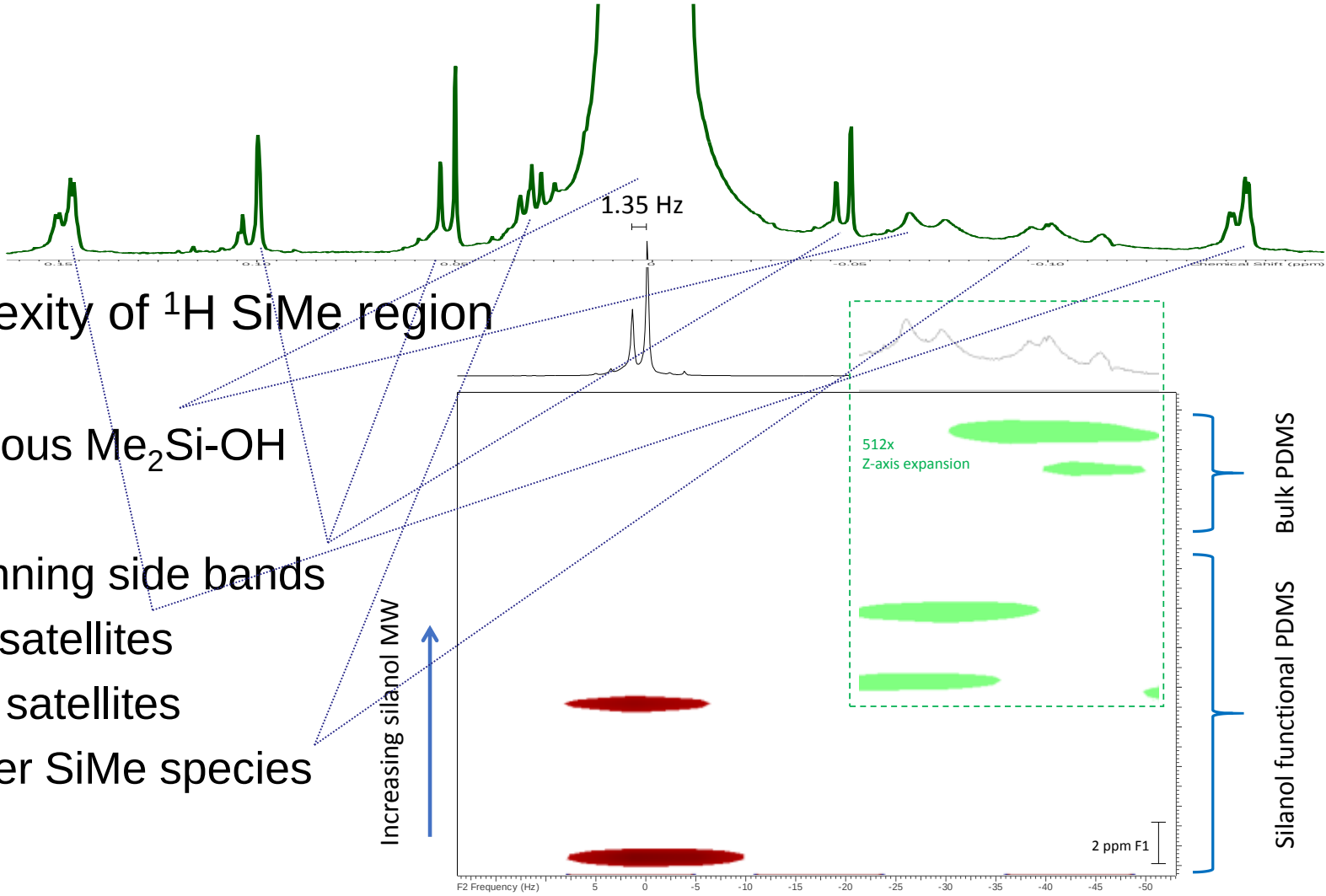
Molecular Selectivity: Authentic vs Artifact

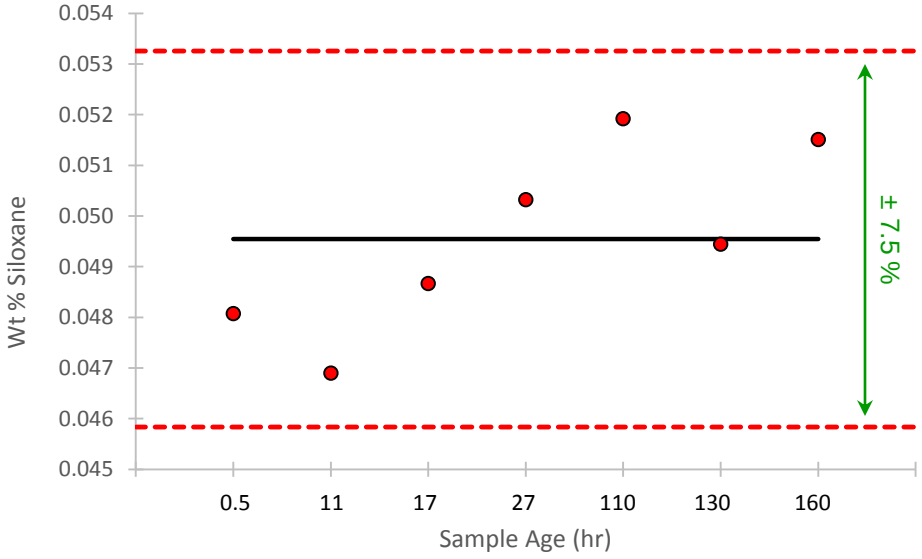
- Complexity of ^{1}H SiMe region
  - Various Me_2Si-OH
  - Spinning side bands
  - ^{13}C satellites
  - ^{29}Si satellites
  - Other SiMe species

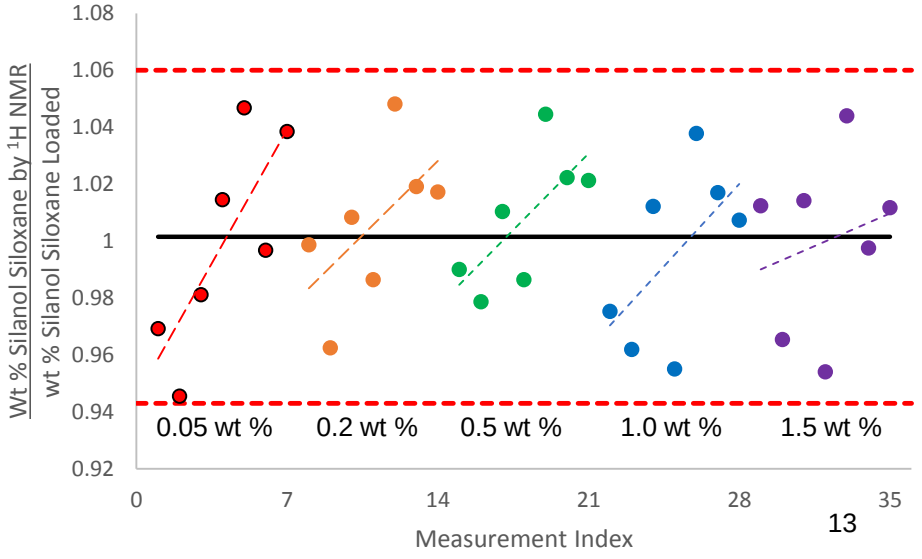


Test Repeatability: Total SiMe

- 5 samples (0.05 to 1.5 wt %)
- 7 time measurements (0.2 to 165 h)

- Relative error ranges from 5 to 8 % per subset
- Water may be evaporating over a week yielding SiMe increase
- SiMe phase separation not detected

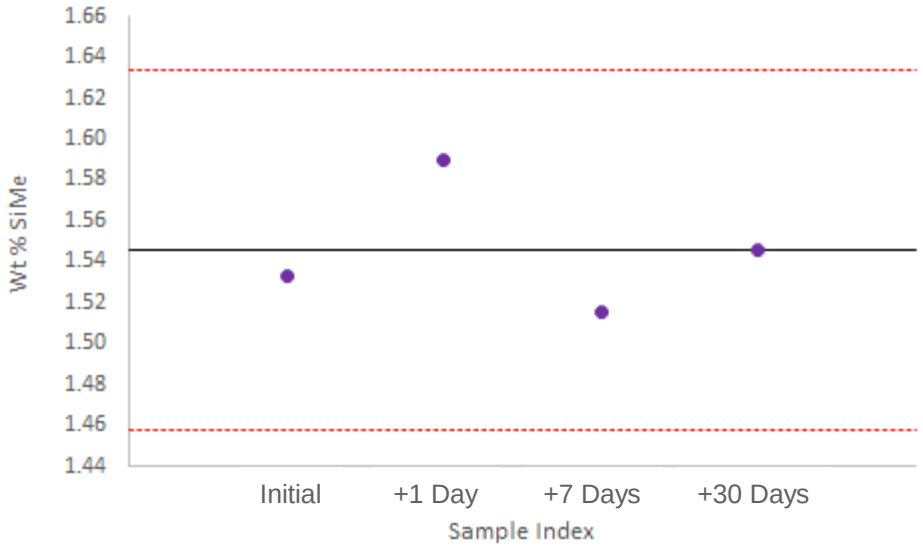




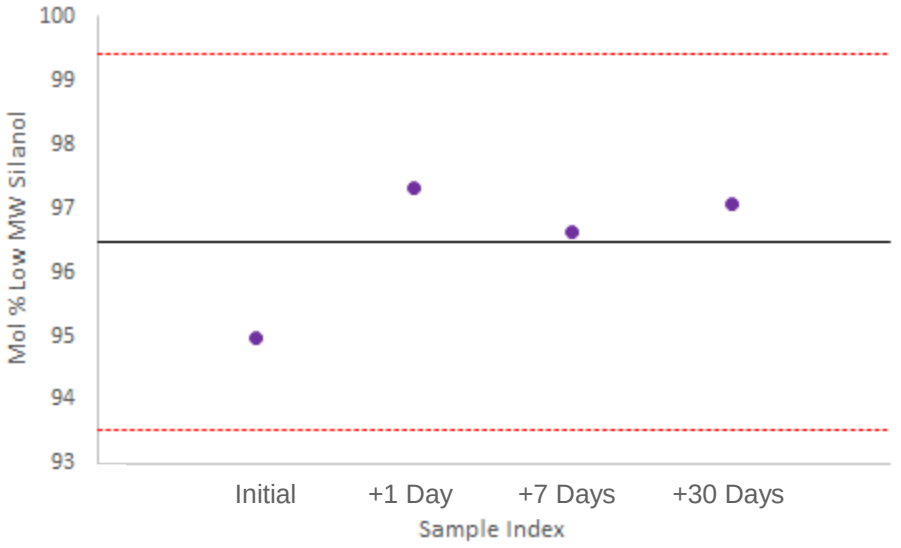
Low MW Silanol Stability @ -70 º C

- Prepared at 1.492 % low MW silanol
- Initial; +1 day frozen; +7 days frozen; +30 days frozen

- Acceptable freeze/thaw stability
- Acceptable 30 day storage
- Relative purity of low MW silanol >90 %



Measured at 1.55 ± 0.09 % silicone

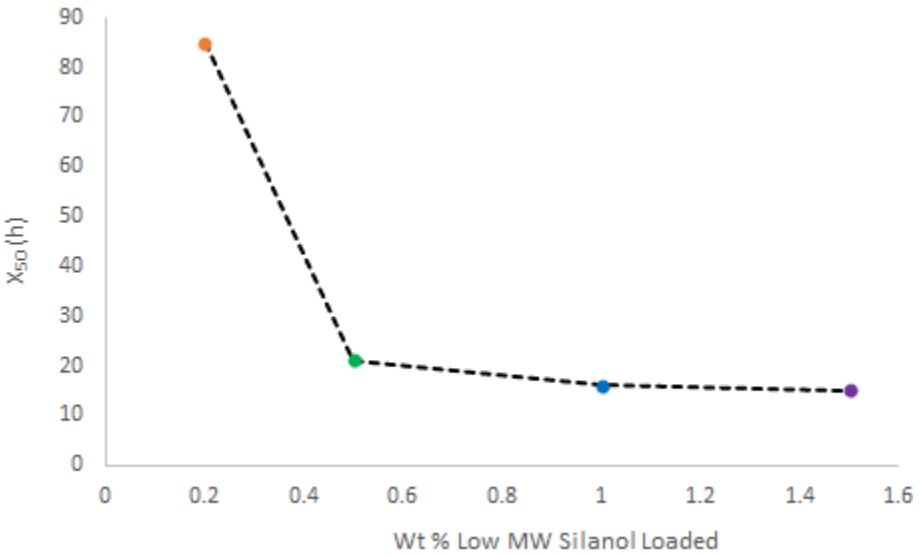


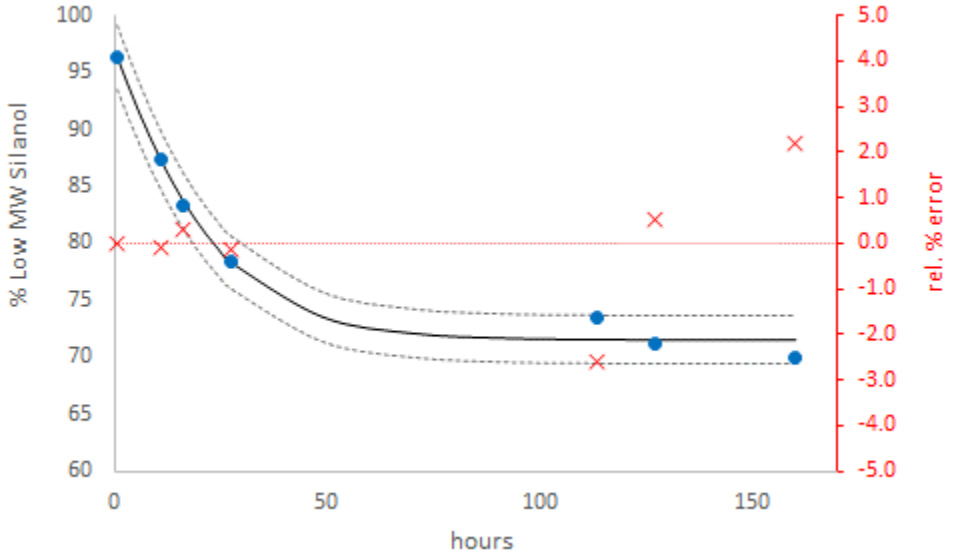
Low MW silanol relative to condensate = 96.5 ± 2.9 %

Equilibration Kinetics

- Reaction follows simple: $A + B \underset{k_r}{\overset{k_f}{\rightleftharpoons}} C + D$

- Significant change in X_{50} between 0.2 & 0.5 wt %
- Silanol condensation % is negligible @ 0.05 wt % dose
- Condensation % @ T_∞ increases (up to 34 %) with silanol loading





^{1}H NMR Capability Summary

- Linear SiMe detection between 0.05 & 1.5 wt %
- Repeatability = 5 to 8 % relative
- Limit of Quantification = 2 to 50 ppm, resolution limited
- Limit of Detection = 2 to 14 ppm, sensitivity limited
- Cryogenic stabilization demonstrated
- Low MW condensation equilibration kinetics characterized
- Synthetic standards & control spiking experiments not required

Acknowledgements

Jean Domoradzki, Ph.D., D.A.B.T.

Jacob Sushynski, B.S.

Greg Cushing, Ph.D.

Deb McNett, B.S.